FINAL REPORT

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Project title: Intensive culture of oysters, *Crassostrea virginica*, in the Chesapeake Bay: Development of flow models to predict optimum site selection for off-bottom culture.

Submitted to:
Steve Olson
National Coastal Resources Research and Development Institute
National Oceanic & Atmospheric Administration
528 SW Mill, Suite 222
P.O. Box 751
Portland, Oregon 92207

Prepared by:
Mark Luckenbach and Roger Mann
Virginia Institute of Marine Science
College of William & Mary
Gloucester Point, VA 23062

Roger Mann
Roger Mann
Principal Investigator
(804) 642-7360

Mark Luckenbach
Mark Luckenbach
Co-Principal Investigator
(804) 787-5816

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Abstract

Intensive off-bottom culture of oysters, *Crassostrea virginica*, is emerging as an alternative to a collapsed wild oyster fishery along the mid-Atlantic coast of the U.S. The Virginia Institute of Marine Science Oyster Aquaculture Program has been actively involved in the development of techniques which have led to rapid growth in oyster aquaculture in Chesapeake Bay.

Our approach towards growing oysters in disease endemic areas is predicated on rapid growth to market size, in effect out running the diseases. Thus, the selection of good growing sites is critically important. In this project we sought to improve site selection capabilities for oyster aquaculture by developing an approach based upon the potential of the local food supply to support rapid growth. Based upon field experiments using hatchery-spawned, single cohort animals, we initially developed *a posteriori* models to explain variation in oyster growth between sites. Subsequently, these models provided the basis for making *a priori* predictions of oyster growth rates, which we then tested in field experiments conducted at a large number of sites in Virginia.

Initial tests of the model revealed some success, but also identified some areas of concern: high current velocities and dinoflagellate blooms. Field experiments which manipulated water flow through groups of cultured oysters were used to clarify the relationship between current velocity and oyster growth rate. A series of studies on the effects of dinoflagellate blooms on oysters (conducted in this and a concurrent project) revealed species-specific effects of dinoflagellates on oyster growth and survival, and pointed to the need to apply this model cautiously in areas which experience blooms.

In conjunction with another research project (funded by VA Center for Innovative Technology), we produced triploid (3N) oysters and compared their growth rates in the field to normal diploid (2N) oysters. Although triploid oysters generally grew slightly faster than diploid oysters in the field, this improved performance was not sufficient to offset the hatchery losses associated with their production.

The approaches we developed have contributed significantly to our ability assist in site selection for a growing oyster aquaculture industry in the mid-Atlantic. Widespread dissemination of the results at scientific meetings and aquaculture workshops have made the approach of evaluating sites based, in part, upon food flux available to the emerging industry.

Introduction

In response to the continuing decline of natural populations of *Crassostrea virginica* along the mid-Atlantic coast of the U.S., intensive off-bottom culture of oysters is emerging as an alternative fishery. This activity employs hatchery-reared seed and is predicated on rapid growth to market size to avoid disease-related mortality. Crucial elements of this strategy are (*i*) the selection of good broodstocks, (*ii*) the timing of spawning to reduce disease exposure, (*iii*) the identification of good growing sites, and (*iv*) the use of proper off-bottom containment to provide for predator protection and support rapid growth. Additionally, experiments at VIMS have investigated the value of manipulating the chromosome number of oysters; that is, producing sterile 3N or triploid oysters (as opposed to normal 2N or diploid).

Proper site selection is often identified as the most important component of bivalve aquaculture (Manzi and Castagna 1989). In the case of oyster culture in this region, it is absolutely critical to achieving the requisite rapid growth. Since considerable investment may be associated with establishing a grow-out site, it is especially important growers select sites which will support rapid growth. Further, regulatory agencies which are charged with permitting use of nearshore waters are in need of guidelines for establishing the suitability of proposed culture sites.

In response to these needs we initiated this project to improve site selection capabilities for oyster aquaculture, based upon the potential of the local food supply to support rapid growth. Taking an experimental approach using hatchery-spawned, single cohort animals, we initially developed *a posteriori* models to explain variation in oyster growth between sites. Subsequently, these models provided the basis for making *a priori* predictions of oyster growth rate. In addition, a concurrent project, funded by Virginia's Center for Innovative Technology (CIT), allowed us to compare the performance of triploid and diploid oysters under identical culture conditions and to relate that performance to our food supply model. In a later addition to this project, we received support from the National Marine Fisheries Service Saltonstall-Kennedy Program (NMFS-SK) to evaluate the effects of dinoflagellate blooms on oyster feeding, growth and survival and to incorporate them into our modelling efforts. A NOAA National Estuarine Research Reserve System (NOAA-NERRS) project investigating the effects of flow rate on oyster feeding is designed to further improve our model development.

Background

Feeding, flow and growth of oysters

A number of factors may affect growth rates of bivalves in general and oysters specifically. Growth of oysters usually correlates positively with temperature (e.g. Dame 1972; Brown 1988) though high summertime temperatures may contribute to reduced growth (Malouf and Breese 1977). While intra-annual temperature variation may be a dominant factor affecting seasonal growth patterns within a habitat, over the ranges of habitats and latitudes covered by oyster culture in Virginia it is unlikely to explain significant variation between growth rates at various sites.

Salinity, both means and variations, affects oyster growth rates. In particular, at low salinity (below ~ 10 ppt) growth rates are much reduced. Insofar as off-bottom culture is predicated on rapid growth to market and oysters in low salinity are afforded at least a partial refuge from disease, we have focussed our efforts for this study in explaining variations in growth between sites above 15 ppt annual average salinity. While the design described below will permit the incorporation of a salinity term in the model, it is not anticipated to be of major importance. Dissolved oxygen concentration is of potential importance in explaining variation between growth rates at various sites if levels fall below threshold values. In Chesapeake Bay high summertime temperatures and biological oxygen demand contribute to seasonal low dissolved oxygen events (Officer et al. 1984). Spatial variability in the duration and intensity of such events between culture sites may contribute to observed variations in growth rates between sites during summer months, but small fluctuations at other times are unlikely to have significant effects.

The supply of food may be an important determinant of growth rates in oysters (Hall 1984; Brown 1988; Brown and Hartwick 1988 a,b) and other bivalves (Widdows et al. 1979; Incze et al. 1980; Frechette and Bourget 1985; McDonald and Thompson 1985). Characterization of food supply, however, poses a particularly vexing problem.

Crassostrea virginica selects particles on the basis of particle size (Haven and Morales-Alamo 1970), organic content (Newell and Jordan 1983) and algal species (Shumway et al. 1985). Though recent investigations are beginning to elucidate the dietary components (e.g. lipids, carbohydrates, proteins and sterols) which contribute to variations in oyster growth, much is still unknown about what constitutes a good diet for oysters. In contrast, relatively simple characterization of food as particulate organic matter (POM) has sufficed to explain much variation in growth rates for mussels (Widdows et al. 1979; 1984).

Growth rates of *Crassostrea gigas* have been shown to correlate with food supply by Brown and colleagues (Brown 1988; Brown and Hartwick 1988 a,b), who used point density estimates of algal standing stocks as food supply indicators. Their estimates of food supply did not, however, incorporate water flow rates which have been shown to affect *C. gigas* growth (Malouf and Breese 1977). The incorporation flow rates into estimates of food supply would seem necessary since water velocity and food concentrations interact to determine food supply and feeding efficiency (Kirby-Smith 1972; Wildish et al. 1987; Cahalan et al. 1989). Food supply, estimated as tidal volume X food concentrations (POM or chlorophyll), have been used to determine carrying capacities or production limits of mussel culture (Incze and Lutz 1980; Incze et al. 1981; Rosenberg and Loo 1983; Carver and Mallet 1990), but not specifically to explain variation in growth rates between sites. In a multivariate analysis of factors affecting *C. gigas* growth rates between 10 sites in British Columbia, Brown (1988) found that static food concentration measures explained significant variation, but he failed to incorporate water flow estimates that would likely have increased the discrimination between sites. In the shallow, well-mixed portions of Chesapeake Bay where we have observed growth differences over small scales (m's to km's), we anticipate that the flow velocity component of food supply has a dramatic affect upon growth rates.

Seston characterization

Unless otherwise noted all samples for characterization of seston were taken 0.5 m below the water surface. Clean glass jars with plastic screw caps were dipped into the water by hand, then the tops removed. Replicate samples were taken 5 min apart for all components of the seston. For suspended solids 500 ml, for chlorophyll *a* and phaeophytin 250 ml, and for particulate organic carbon (POC) and particulate organic nitrogen (PON) 100 ml of seawater were collected. Samples were immediately placed on ice and transported to the laboratory where all samples were filtered within 6 hours and filters were frozen until further processing.

Samples for determination of suspended solids were filtered onto pre-ashed and pre-weighed glass fiber filters. These filters were dried and weighed to determine total suspended particles, then ashed and re-weighed to determine organic and inorganic components. Chlorophyll *a* and phaeophytin concentrations were determined by extracting the filters in 2 -3 ml of 90% acetone and determining concentrations using a Milton Roy Scanning Spectrophotometer and standard procedures (Strickland and Parsons 1972). Samples for POC and PON were subdivided into 4 25-ml subsamples before filtration onto 13 mm glass fiber filters. Each filter was then individually analysed for POC and PON on a Carlo Erba CHN analyzer.

Flow measurements

Central to our objective of determining the quantity of food available to oysters at a particular location is estimating the time-averaged water flow past the oysters. Since tidal currents vary considerably over the semi-diurnal tidal cycle, a minimum of one tidal cycle (12 hrs) and preferrably longer is required to obtain such an estimate. While this could be achieved with a variety of different types of current meters attached to data loggers, such instrumentation is expensive and thus inconsistent with our needs to (*i*) measure flow at numerous locations simultaneously during model development and (*ii*) provide the shellfish culture industry with an easily accessible test.

Thus, we used the dissolution rate of chlorine tablets as a means of estimating integrated water flow over 48 hr periods. Dissolution rates of other materials (primarily gypsum) have been used by other investigators (e.g., Doty 1971) to estimate actual or relative flow rates; however, these materials often give unreliable results for time periods greater than a few hours because pieces may break off. We have found that three inch diameter chlorine tablets, commercially available for swimming pool disinfection, provide a reliable and repeatable means of obtaining dissolution rates in the field over time scales of hours to days.

The rate of weight loss of these tablets in flowing water is proportional to the mean and fluctuating components of velocity (current speed and turbulence, respectively) and to water temperature. At constant temperature, the comparison of dissolution rates between tablets at different locations provides an estimate of **relative** flow rates at the two locations. While relative flow rates alone were sufficient for developing and testing the model in our area, we calibrated

tablets to determine the relationship between dissolution rate and **actual** flow speeds to make the approach broadly usable. Further, we sought to clarify the relationships between dissolution rate and intergrated water flux (e.g., how does dissolution rate over 5 hrs @ 20 cm/s compare to 10 hrs @ 10 cm/s?), and between dissolution rate and salinity.

To determine the effect of salinity on dissolution rate of tablets, pre-weighed chlorine tablets were placed into 20 L buckets containing water at 0, 10, 20 or 30 ppt sea salts. Three replicates for each salinity were set up and all buckets maintained in a water bath at 20 ± 1 °C for five days. Tablets were removed daily, blotted dry, weighed and returned to the buckets. Water in all buckets was changed daily.

Calibration of the tablets was conducted in a 5-m long recirculating seawater flume. The flume, which has been described in detail in Orth et al. (1994), permits control of flow speed and water temperature. Three replicate tablets were suspended above the flume bed in mesh bags and dissolution rates at flow speeds of 5, 10, 15, 20 and 25 cm/s were determined. The effect of total volume flux of water (rather than flow speed) on tablet dissolution was determined by comparing weight loss at flow speed and time combinations which yielded equal flux (e.g., 25 cm/s x 1 hr and 5 cm/s x 5 hrs).

Development of *a posteriori* models

Our primary objective was to develop an approach towards predicting average growth rates for oysters at a given site. The first step towards achieving this was to develop a model to explain (after the fact) variation in growth rates between sites. We made three important assumptions at the outset: (*i*) that it was variation in average growth between sites, not in growth between oysters within a site that was of concern; (*ii*) that we were not concerned with intra-annual variation in growth rate, which is largely attributable to temperature and which occurs at all sites in Virginia; and (*iii*) that variation in annual growth rate between sites would be driven by variations in growth during the peak growing seasons (spring and fall).

With these caveats in mind we designed our experiments to explain observed variation in mean growth rate of oysters over 1 month periods during spring and fall. The general design for all experiments was as follows. All oysters used in an experiment were from a single cohort, spawned in the VIMS oyster hatchery and maintained in off-bottom culture until use. Fifty juvenile oysters were randomly selected for deployment at each site; each oyster was measured for shell height and shell width and numbered with paint on the shell. The oysters were placed in a plastic mesh bag and deployed on fixed racks at selected sites 0.5 m below the surface at MLW. After 1 month the oysters were retrieved and re-measured. During the 1 month deployments we measured seston characteristics and flows on spring and neap tidal cycles. On two successive days (during both the spring and neap series) water samples were taken on mid-flood and mid-ebb tides for characterization of seston. Replicate chlorine tablets were deployed at the same level in the water column as the oysters for approximately 48 hrs.

The first of these experiments was conducted in the fall of 1990 and served as the basis for

developing a preliminary explanatory model against which we could test predictions about future growth rates. In that experiment oysters were deployed at six stations located along Virginia's Eastern Shore (Fig. 1) and considerable variation was observed in average growth rate between the stations (Fig. 2). We explored numerous *a posteriori* multiple regression models and found the one depicted in Fig. 3 to provide the best fit with the fewest variables. We then conducted numerous field experiments to test the validity of applying this *a posteriori* model in an *a priori* manner to predict oyster growth rates.

Testing *a priori* predictions

We anticipated at the outset of this project that the process of testing and refining the predictive capabilities of our model would proceed as follows. First, we would repeat the experiment described above in different years, locations and with different oyster cohorts. Data collected on seston composition and water flow during these experiments would be used in the formula developed from the previous experiment (as in Fig. 3) to predict oyster growth rates over the one month deployment period. These predicted growth rates would then be compared to observed growth rates to assess the efficacy of the model. If there was poor correlation between predicted and observed growth rates (and we expected there might be initially), we would combine datasets from the different experiments and conduct more *a posteriori,* exploratory multiple regression, followed by more experiments to test predictions. In this iterative manner we expected to blindly refine the model to the point that it was generally applicable across locations and years. In reality what occurred was that during the initial test the model performed well in most cases and where it did not we were able to formulate specific hypothesis for why it failed. As indicated in the Results section, our initial test suggested that the impacts of very high current velocities and dinoflagellate blooms needed to be incorporated into the model. Thus, rather than blindly repeating the exploratory modeling phase of the work, we were able to focus on more product refinements.

Initial model tests were performed in the fall of 1991 at 10 stations in the manner described above. Four of the stations were the same as those used to develop the model and six were new (see Table 1). This experiment was conducted without modifications to the original design.

Figure 1. Station locations.

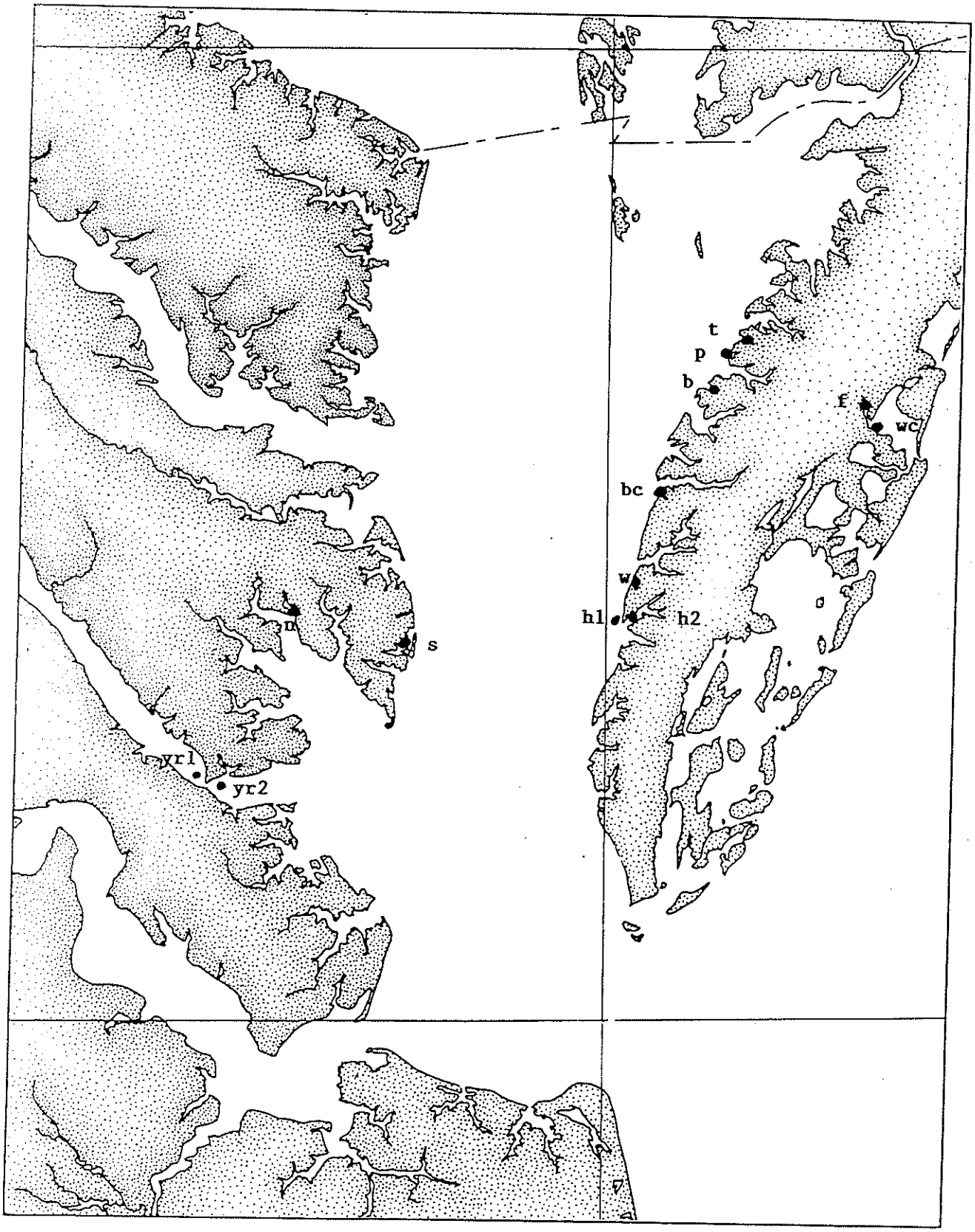


Figure 2. Mean shell growth by station in initial experiment (n=50 oysters/station), station labels are given in Table 1.

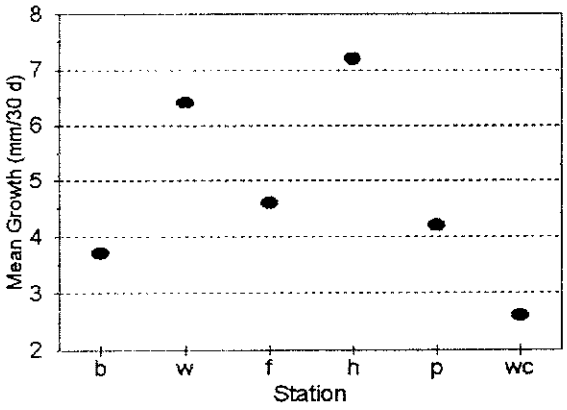


Figure 3. *A posteriori* multiple regression model for mean growth in shell height. Station labels given in Table 1.

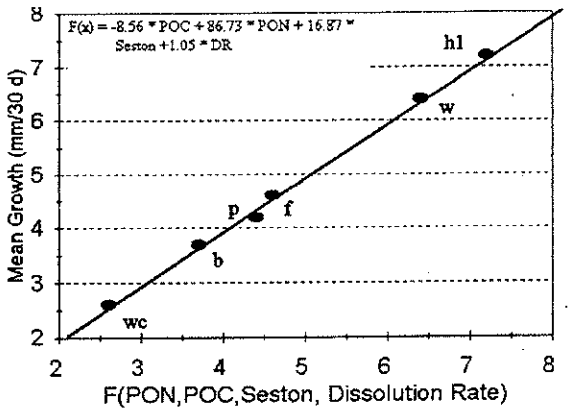


Table 1. Station abbreviations.

Abbreviation	Station
b	Butcher Creek
p	Pungoteague Creek
wc	Wachapreague Channel
w	Westerhouse Creek
h1	Hungars Creek site 1
h2	Hungars Creek site 2
f	Finney Creek
yr1	York River site 1
yr2	York River site2
bc	Bull Cove
n	North River
t	Tarkhill Creek
s	Stutts Creek

1. Effects of dinoflagellate blooms

Blooms of dinoflagellates and other harmful phytoplankton have been shown to cause bivalve mortalities in other studies. Apart from some of the anecdotal evidence, there are no reports of oyster mortalities in Chesapeake Bay associated with algae blooms. Assessing sublethal effects of dinoflagellate blooms on oysters can be difficult. Oyster growth rates vary widely from one location to another in response to such environmental conditions as salinity, temperature, flow regime and food supply. Thus, comparing growth rates between bloom and non-bloom sites and attributing observed differences to dinoflagellates has been problematic. We sought to evaluate these effects within the context of our growth model and at the same time provide refinements to the model based upon observed dinoflagellate effects.

Our approach in this study was to deploy oysters initially during August and September, 1992, from a single cohort into 6 sites where blooms were expected and 6 where they were not. Water samples, aerial reconnaissance and information on past blooms lead us to select these 12 sites for comparisons of survival and growth in field bloom and non-bloom sites (see Table 2).

Table 2. Field sites for deployment of oysters during late summer bloom. *No bloom* indicates that visual inspection, *in vivo* fluorescence and microscopic examination never indicated bloom levels of dinoflagellates in the vicinity; *Bloom* indicates that one or more of these suggested bloom, at least, close to the oysters.

Location	Bloom status
York River, site 1	Bloom
York River, site 2	Bloom
The Gulf	Bloom
Hungars Creek, site 1	Bloom
Hungars Creek, site 2	Bloom
Westerhouse Creek	Bloom
Bull Cove	No bloom
Butchers Creek	No bloom
Pungoteague Creek	No bloom
Tarkill Creek	No bloom
Stutts Creek	No bloom
North River	No bloom

Observations in the vicinity of these sites during the deployments indicated at least the intermittent presence of red water near 6 of the sites. Actual durations of exposure, intensity of the blooms and complete species composition are unknown, but the dominant dinoflagellates in all bloom samples was *Cochlodinium heterolobatum*, *Gyrodinium* sp. and *Gymnodinium* sp.

Frequent monitoring of the sites throughout the experiment supported the expectation that the No Bloom sites were not impacted by dinoflagellate blooms. The Bloom sites appeared to have at least some exposure to obvious blooms. Where water samples were available, the late summer blooms were dominated by *Cocholodinium*, *Gyrodinium* and *Gymnodinium*. The actual frequency and duration of exposure to bloom water could not be quantified for any of the sites.

Fifty juvenile oysters from a 1991 hatchery-produced cohort were first deployed in floats at each site during June 1992. When late summer blooms were noted beginning in August, oysters were retrieved, measured and returned to the sites. On September 9-10, 1992, samples were taken as we have described in previous reports for estimating food flux. At that time samples were also taken for disease assays. Two weeks later these test results revealed that this cohort was infected with MSX (*Haplosporidium nelsoni*); concurrently significant mortality was noted at most of the sites (Table 3). Thus on 10/1/92 through 10/2/92 we terminated this experiment and deployed a new cohort of oysters which tested negative for disease. The entire experiment was repeated through October at all 12 of the sites.

Blooms persisted during the first half of October 1992 throughout much of the lower Chesapeake Bay; however, they were neither as intense nor as widely distributed as in the previous month. The oysters at the two York River sites were clearly exposed to bloom water at numerous times during the first two weeks of October. Bloom water generally moved up and down the river with the tides, usually impacting oysters on ebb tides. Bloom exposures at the other field sites are more difficult to confirm. A large bloom persisted near the mouth of Hungars Creek and red water was occasionally observed to move into the test sites. Likewise, red water was observed on two occasions in Westerhouse Creek during the first two weeks of October. Logistically, we were unable to quantify the frequency and duration of exposure at these sites. We did not observe obvious blooms in Bulls Cove during October, but did so during the earlier September deployment. No red water was ever noted in the vicinity of the North River, Stutts Creek, Butchers Creek, Pungoteague Creek or Tarkill Creek during the months of September or October.

Table 3. Percent mortality by location during the September deployment. Mortalities are presumed to be largely the result of infection by *Haplosporidium nelsoni*.

Location	% Mortality
York River, site 1	16%
York River, site 2	4%
The Gulf	38%
Hungars Creek, site 1	14%
Hungars Creek, site 2	30%
Westerhouse Creek	22%
Bull Cove	28%
Butchers Creek	34%
Pungoteague Creek	36%
Tarkill Creek	32%
Stutts Creek	2%
North River	14%

On Oct. 5-7, 1992, and Oct. 13-14, 1992, field measurements were again taken for estimation of food flux. All oysters were removed on Nov. 4, 1992, measured and the experiment terminated. The oysters at The Gulf and Tarkill Creek were lost during the experiment for reasons unrelated to dinoflagellates. No mortality was observed at any of the other sites.

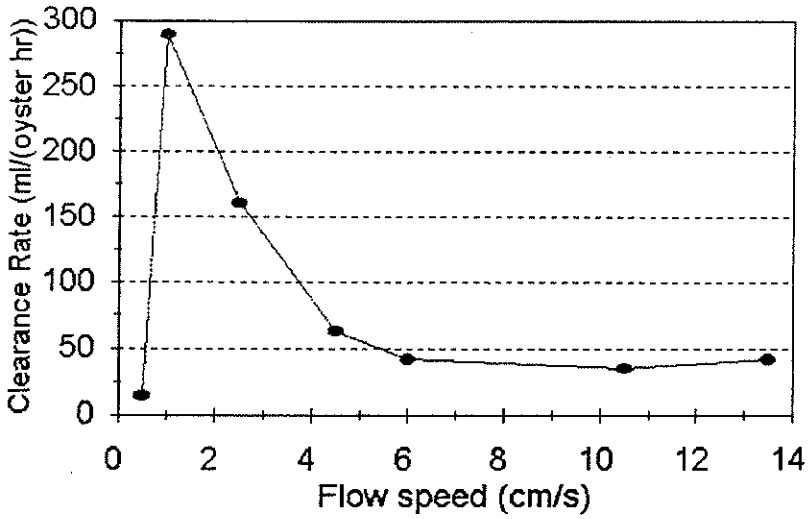
Values for POC, PON, Total Seston and dissolution rate of chlorine tablets were put into the multiple regression model (Fig. 3) and predicted and observed growth rates compared.

2. Effects of flow speed

Results from our initial test of the model (see Fig. 11 in the Results section) led us to hypothesize that the positive, linear relationship between flow speed and growth in our model was incorrect. In particular, we noted that high predicted growth rates at the York River sites during that experiment were largely the result of high flow velocities and speculated that above some flow speeds there might, in fact, be a negative influence of flow on growth. This was consistent with some recently published work by Grizzle et al. (1992) which indicated that oyster growth

was a non-monotonic function of current speed, with growth rates initially increasing with increasing flow speed then declining at higher flow speeds. It was also consistent with some work we were conducting (with support from NOAA-NERRS) in the seawater flume to examine the relationship between flow speed and oyster grazing rates (Fig. 4).

Figure 4. Clearance rate vs flow speed from flume experiments.



There remained a serious discrepancy between these results and those of previous model performance which suggested a positive effect of flow rate over a wide range of flows. We designed the following experiment as a means of field testing the effects of flow speed over a range which spanned both sides of the peak in the above graph. We modified our basic experimental design by placing oysters in floats near the surface in two arrangements: one was inside the standard ½ inch mesh bag and the other was inside multiple layers of mesh to reduce water flow. Oysters were also placed in ½ inch mesh bags on fixed racks 25 cm above the sediment surface where flow rates are lower than at the surface. Three experimental locations were used in a balanced design (Table 4). The experiment was established in May 1993, and flow, seston and growth measurements were made in June, July and Sept. 1993. Seston samples were taken hourly for 12 hours (to span a full tidal cycle) during spring and neap tidal phases during each month samples were taken. Surface water samples were collected as previously described and bottom water samples were collected using a Nansen bottle. Chlorine tablets were deployed inside the mesh with the oysters to provide estimates of water flow within each treatment.

Table 4. Experimental design for flow manipulation experiment

Location	Treatment	# Oysters
Gloucester Point	Surface, unrestricted flow	50
	Surface, restricted flow	50
	Bottom, unrestricted flow	50
Westerhouse Creek	Surface, unrestricted flow	50
	Surface, restricted flow	50
	Bottom, unrestricted flow	50
Hungars Creek	Surface, unrestricted flow	50
	Surface, restricted flow	50
	Bottom, unrestricted flow	50

Results

Triploid oysters

The percent triploidy induced varied considerably as we tested different protocols (Table 5). One spawn on 7/16/91, using the protocols outlined in the Methods section, yielded particularly good results. Embryonic mortality resulting from the treatment with cytochalasin B was always high, often in excess of 75% of the pre-treatment egg count. This level of embryo loss is generally not a problem in *Crassostrea gigas*, where induction of triploidy is a common hatchery practice. However, it poses a real problem for commercial production of triploid *C. virginica*, which has lower fecundities and for which wild broodstocks are increasing rare.

Table 5. Production of triploid oyster seed.

Spawn Date	%Triploidy	# Oyster Seed
3/23/90	80	1,500
5/7/91	50	3,500
7/16/91	96	10,000
7/30/91	15	10,000
8/6/91	15	12,000
8/20/91	5	5,000
9/17/91	43	5,000

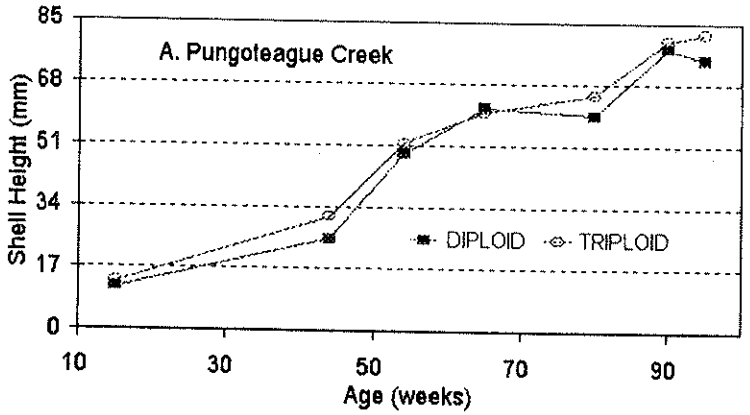
The three groups from our 1990 and 1991 spawns which yielded the greatest percent triploidy were chosen for use in field grow out trials at seven locations (Table 6). Of these three groups the 7/16/91 spawn was our best batch in terms of % triploidy, total numbers of oysters and availability of an appropriate control group. For this group we produced an untreated, diploid group from the same spawn which was treated identically in all other respects to the treated group.

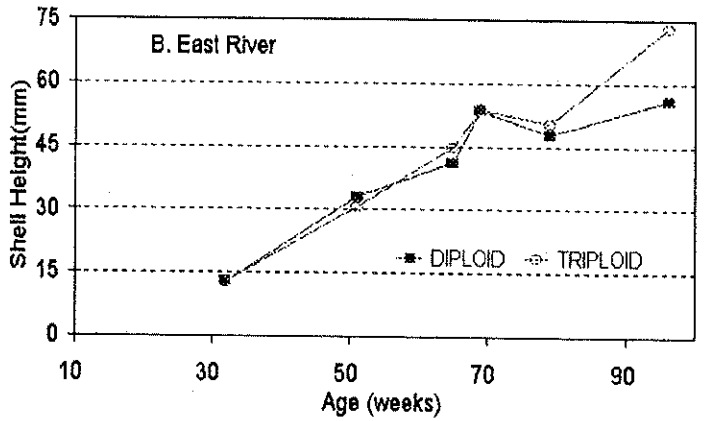
Table 6. Field tested triploid oysters. Spawn date, % triploidy, number of seed and deployment site are noted.

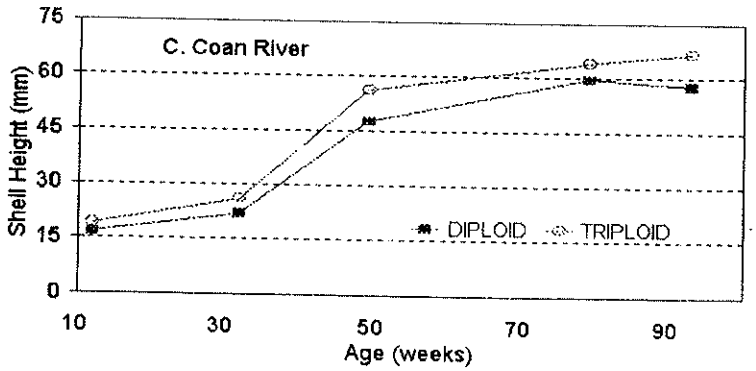
Spawning Date	% Triploidy	Total Number	Location(s)
3/23/90	80	1,500	1. Pungoteague Cr.
5/7/91	50	3,500	1. Pungoteague Cr. 2. Butchers Cr. 3. Bradfords Bay 4. Taskmaster Cr.
7/16/91	96	10,000	1. Pungoteague Cr. 2. Coan River 3. Moran Cr. 4. East River

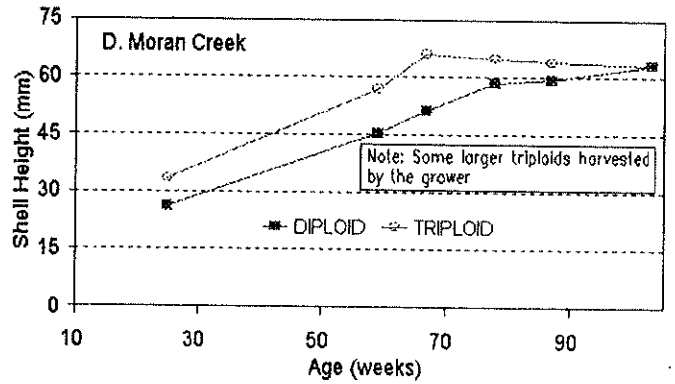
Growth in shell height over a 2 year period for the triploid and diploid oysters spawned on 7/16/91 revealed that triploid oysters were always greater than or equal in size to diploids at all four locations (Fig. 5). On dates at some locations, observed differences in shell height were statistically different, on others they were not. We believe these trends to be biologically significant (that is, significant to for oyster aquaculture) because (1) these measures, made in one dimension, under estimate the overall differences we observed in three dimensions, (2) meat weights are greater in triploids, especially after spawning by diploids, and (3) even small differences in size can have important consequences for the oyster culturist. Nevertheless, the extremely high cost (in terms of larval loss) associated with the production of triploid *Crassostrea virginica* clearly make their use prohibitively expensive at this stage.

Figure 5. Growth of triploid and diploid oysters from a single cohort at four locations.









Flow measurements

The dissolution of chlorine tablets provided reliable consistent indicators of water flow. Variation between dissolution rates among replicate tablets was low (Fig. 6) and varied little with salinity (Fig. 7). Dissolution rate varied linearly with flow speed (Fig. 8), and weight loss was varied with total volume flux of water for different flow speeds (Fig. 9).

Figure 6. Comparison of weight loss between replicate tablets.

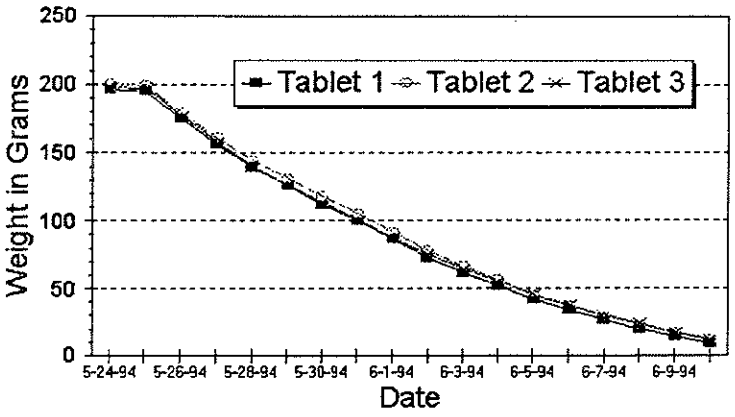


Figure 7. Weight loss over time in still water at varying salinity.

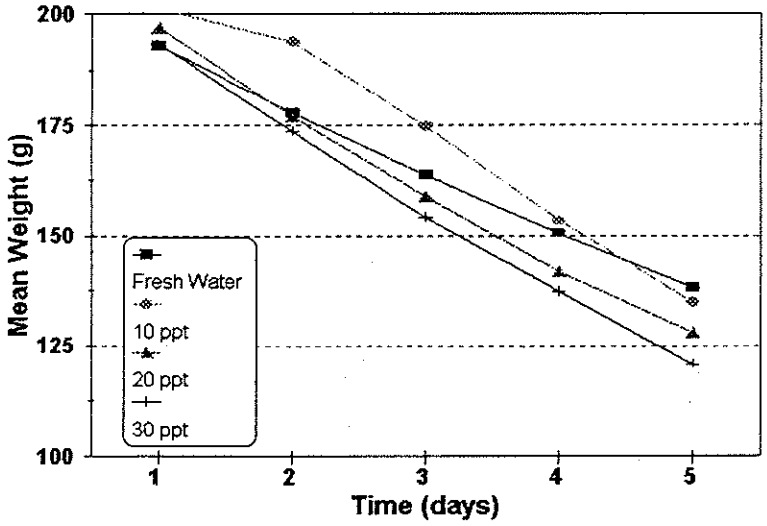


Figure 8. Mean dissolution rate vs flow speed at 20 ppt.

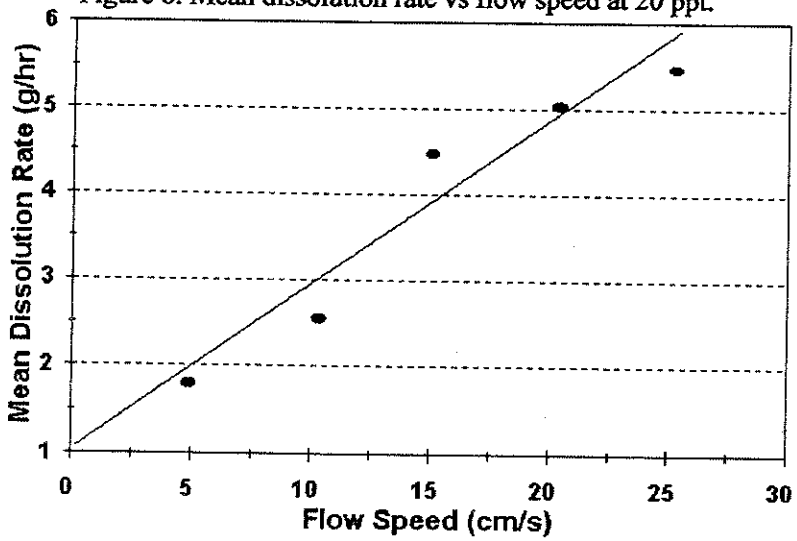
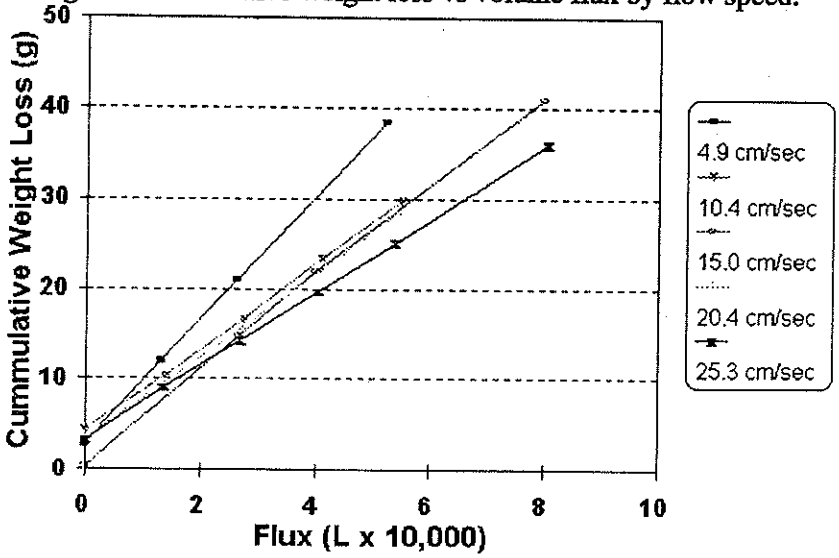


Figure 9. Cumulative weight loss vs volume flux by flow speed.



Tests of model predictions

1. Test of *a priori* predictions

The initial test of the model as a predictive tool provided mixed results. A wide range in growth rates was observed at the ten stations over the one month duration of this experiment (Fig. 10). For six of the ten stations tested the model performed well, giving predicted growth rates close to observed. However, in four of the ten cases predicted values were considerably higher than observed growth rates (Fig 11). When we examined the parameter values in the

Figure 10. Mean shell growth by station (n= 50 oysters).

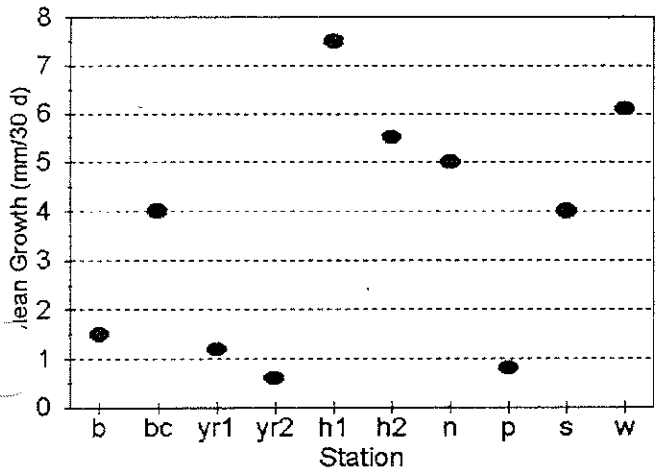
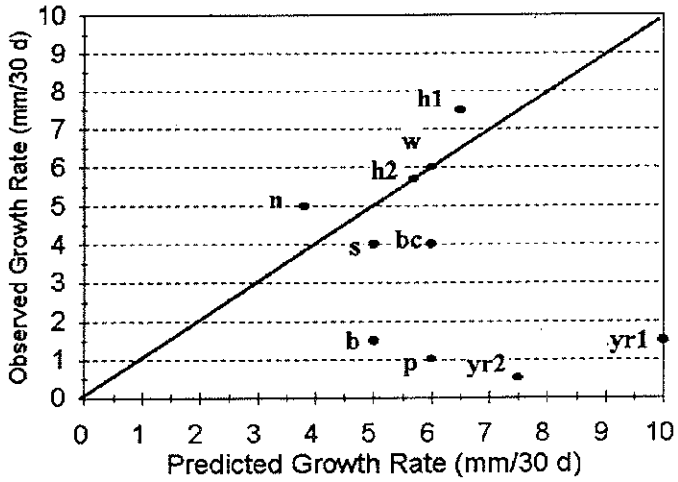


Figure 11. *A priori* test of model predictions.

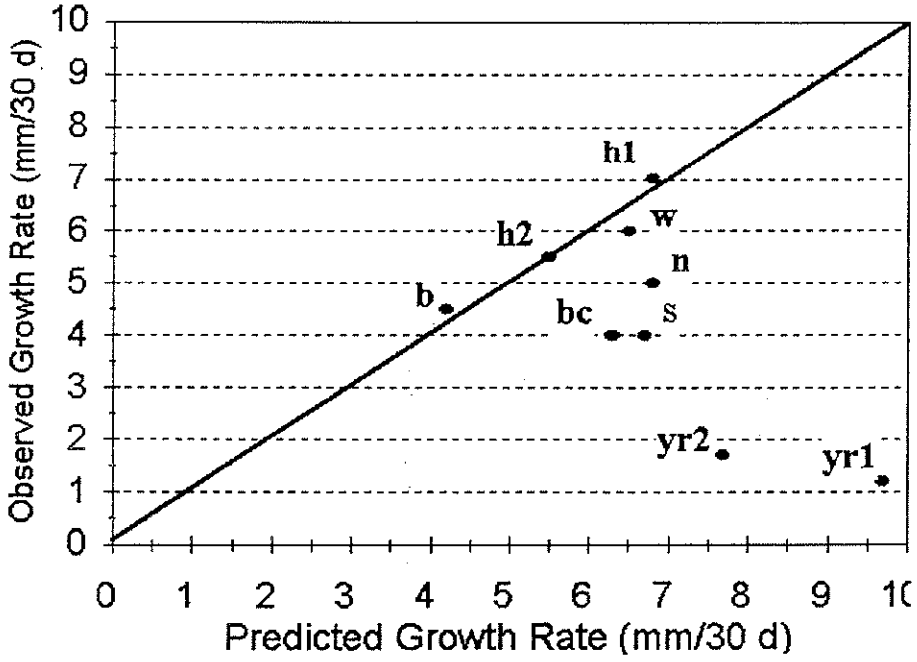


model responsible for the high predicted growth rates, two patterns were evident. At the Pungoteague (p) and Butcher (b) Creek stations very high PON values were responsible for the high predicted values; at the two York River stations (yr1 and yr2) it was high flow rates which contributed most prominently to the high predicted values. Anecdotal evidence from observations by technicians and collaborating oyster culturists suggested that the Pungoteague and Butcher Creek sites, which are separated by approximately 3 km, experienced a dinoflagellate bloom during the course of this experiment. At the time we were not alerted to the potential for blooms in the area and failed to quantify its presence. However, this observation did lead us to conduct the field experiment detailed in the next section and a series of laboratory experiments (under a project funded by NOAA-SK) on dinoflagellate effects on oysters (Luckenbach et al. 1994; Sellner et al. 1994). The discrepancy between predicted and observed growth rates at the York River sites led us to investigate in greater detail the effects of water flow on oyster growth rates in the field (described below) and in a series of flume experiments supported by NOAA-NERRS (Harsh 1995).

2. Effects of dinoflagellates

The initial experimental deployment of oysters into sites with and without blooms had to be abandoned because of mortalities attributable to *Haplosporidium nelsoni* (MSX) [see Table 3 and p. 13]. MSX is known to affect the growth rate of infected oysters, so any attempt to evaluate the effects of food flux in the context of this study would have been confounded. Our secondary deployment of oysters into the *Bloom* and *No bloom* sites in October of 1992 was largely successful, with oysters in the York River clearly exposed to dinoflagellate blooms and oysters at the other *Bloom* sites apparently exposed intermittently. The fit of predicted to observed mean growth in this experiment was good at most sites except for the two York River stations (Fig. 12). These two sites, which experienced the most frequent bloom exposure, had growth rates which were much below those predicted by the model. The unconfirmed implication of this experiment is that oyster growth at sites with frequent exposure to this late summer bloom experienced much reduced growth.

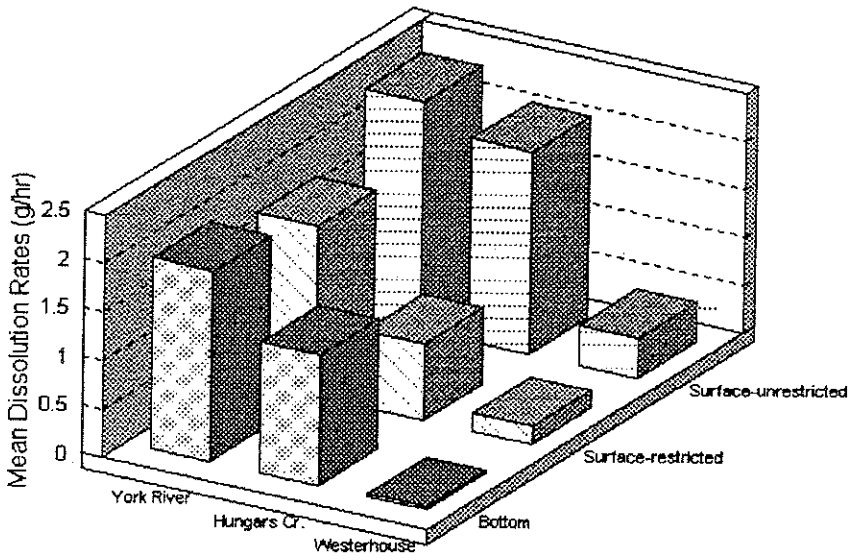
Figure 12. Observed and predicted growth in *Bloom* and *No Bloom* locations.



3. Effects of flow speed

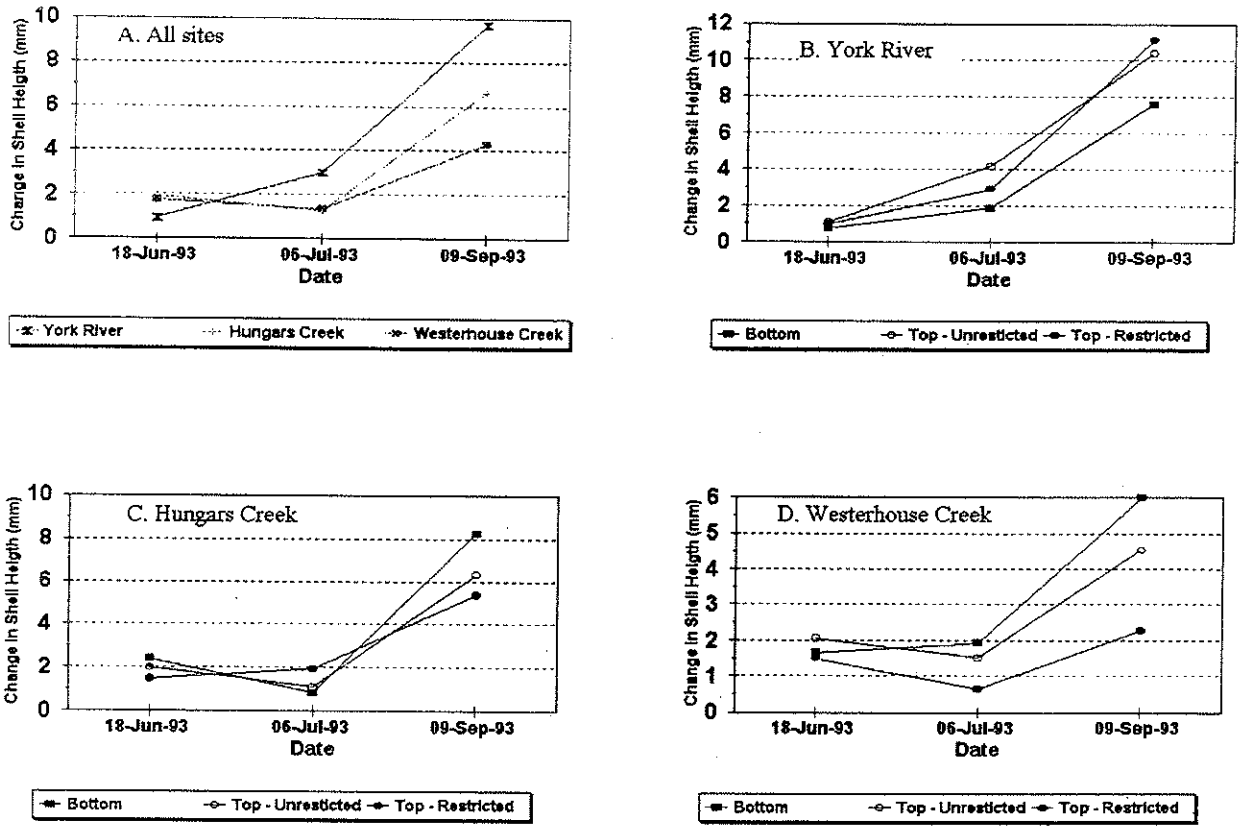
In the experiment designed to modify water flow past the oysters, flow speeds varied between sites and treatments (Fig. 13). The surface restricted flow and the bottom treatment were always less than the unrestricted flow treatment, but the relationship between the bottom and surface restricted varied between sites.

Figure 13. Dissolution rate vs location and treatment.



Oyster growth varied between locations and across treatments in a location (Fig. 14 A-D). Variation between treatments was not constant across locations, with Hungars Creek and the York River exhibiting greatest growth rates in surface restricted floats, and Westerhouse Creek with greatest growth rates in the bottom unrestricted treatment.

Figure 14. Mean growth of oysters during flow manipulation experiment for all sites (A) and in each treatment at each site (B-D).



Time series of total seston , PON and POC (Fig. 15) all reveal variations between surface and bottom waters and between sites. Also, there was considerable short-term variation in seston composition over the twelve hour sampling periods.

Mean values for seston quantity and composition, together with mean dissolution rates, from this experiment were used in the regression model (SHELL GROWTH = -8.56*POC + 86.73*PON + 16.87*SESTON + 1.05*DISSOLUTION RATE) to predict growth rates under these treatment conditions. The concordance between predicted and observed growth rates (computed over the entire 4 month deployment but reported as growth/30 d to remain consistent with earlier presentations) is extremely good (Fig. 16) indicating that the model performed well under these circumstances.

Figure 15. Time series of suspended sediment, PON and POC in surface (solid line) and bottom (dashed line) samples at three locations. (Sample taken in June.)

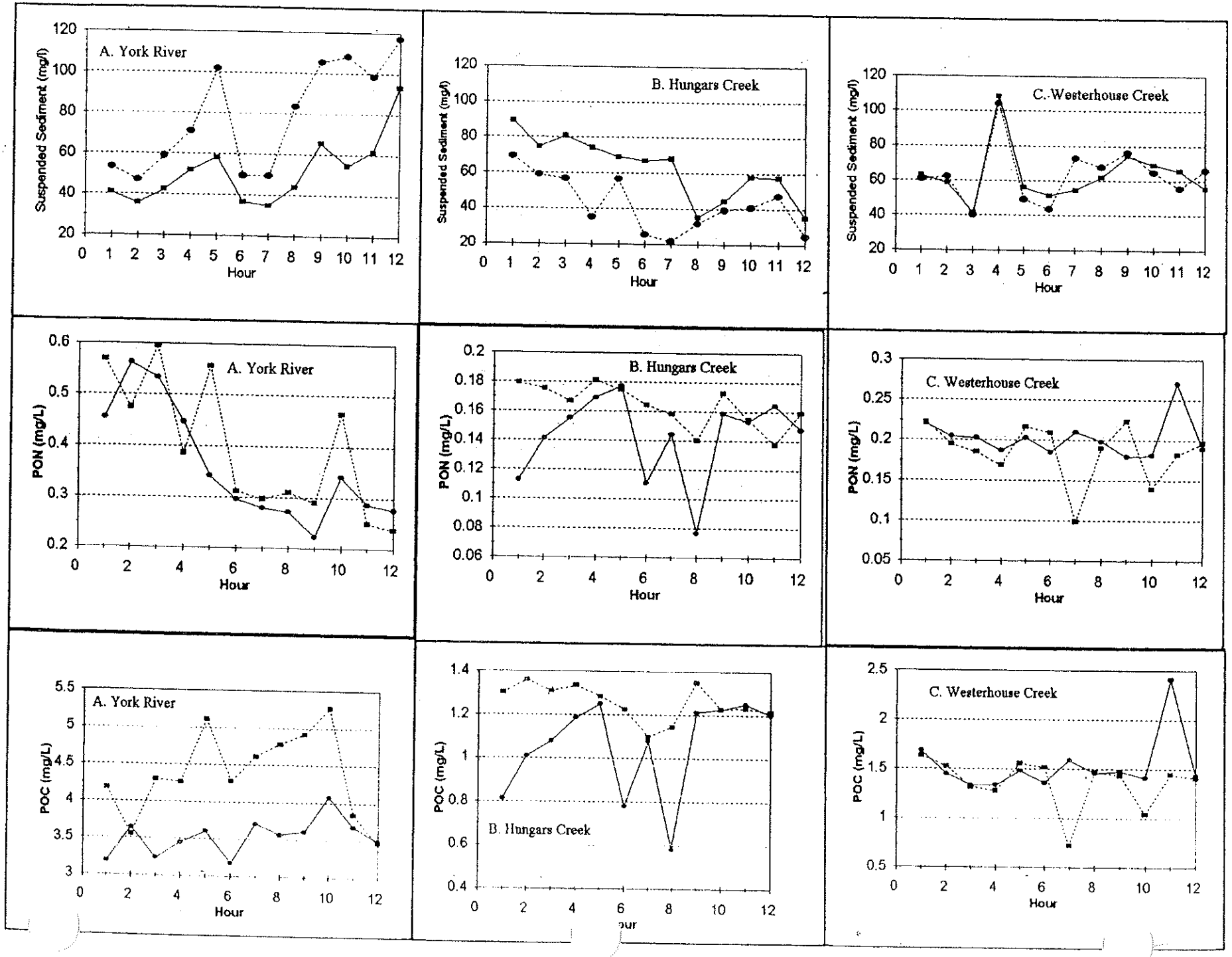
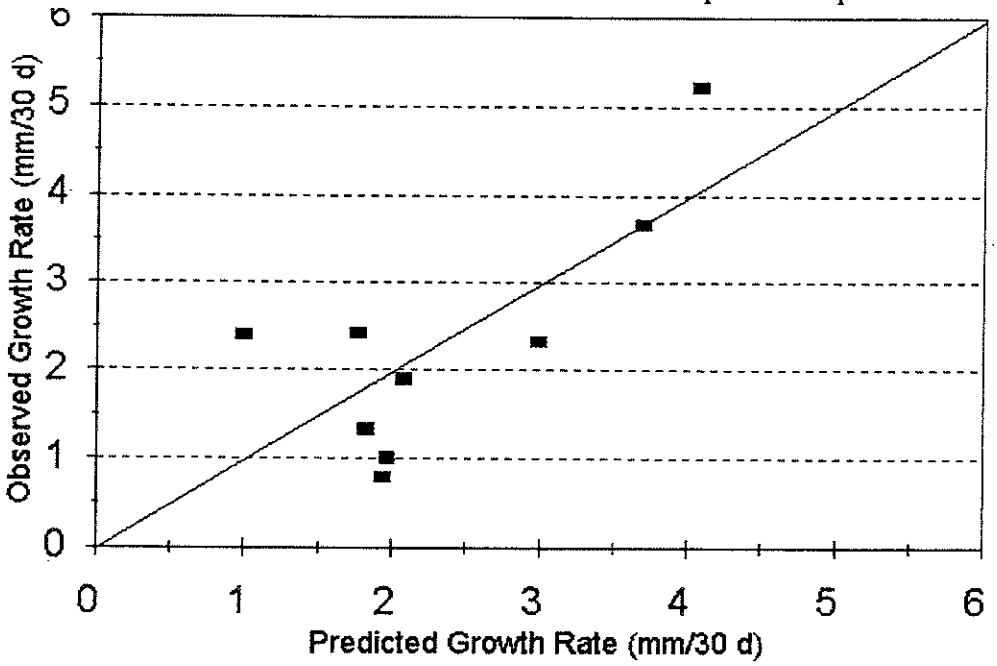


Figure 16. Performance of the model in the flow manipulation experiment.



Summary of model performance

The food flux model has proven thus far to be a useful tool for predicting **potential** growth rates of cultured oysters in the field. Within the context of the experimental conditions under which we have tested it, the predictions provided by the model have generally given good predictions of actual observed growth rates. In those instances where the model has failed to correctly predict actual growth rates, explanations seem apparent.

First, in the case of high predicted and low observed growth rates at stations yr1 and yr2 (see Fig. 11) it seemed apparent applying the model in areas with very high flow rates was a problem. We initially suspected that a dependence of filtration efficiency on flow velocity (Fig. 4) might require a modification of the model to include a non-linear relationship between flow velocity and growth rate. However, the good performance of the model in the flow modification experiment and subsequent flume experiments have revealed the reason for this discrepancy. We now understand that the relationship between flow speed and filtration rate depicted in Fig. 4 and a similar relationship described by Grizzle et al. 1992 between flow speed and growth rate reflect biomechanical and physiological processes in the feeding by individual

oysters. Within groups of oysters (e.g., a natural oyster reef or bag of cultured oysters) where food depletion can become an issue, the relationship between **average growth within the group** and flow speed is positive over a much wider range of flows. We have not quantified this relationship, but it is clear that it will depend upon oyster density, seston levels and turbulence characteristics as well as flow speed. At the present time it appears that the simple linear relationship in the original model works well. The poor performance of oysters at sites yr1 and yr2 turned out to be related to mechanical disturbance of oysters at higher flows (i.e., they got rolled around in the bags). When we secure oysters in the bags better, as was done in the flow manipulation experiment, observed growth rates match predicted values at high flow speeds (Fig. 16).

The second set of conditions under which the model did not perform well is in the presence of dinoflagellate blooms (Figs. 11 and 12). The presence of dinoflagellate blooms give high PON values which lead the model to predict high growth rates; however, bloom-forming dinoflagellate species do not necessarily constitute good food for oysters. In fact, we have found species-specific impacts of dinoflagellates on oyster feeding, growth and survival of cultured oysters in Virginia (Luckenbach et a l. 1993; Sellner et al. 1995). The implication of these findings is that microscopic inspection of phytoplankton samples should be a routine part of ongoing oyster culture operations and that caution must be exercised in predicting growth rates to ensure that dinoflagellate blooms do not bias predictions.

Several other important caveats must be heeded in the application of this model.

(1) This model predicts **average** growth rates at a site. It makes no predictions about variances in growth rate among oysters at a location.

(2) This model predicts **potential average** growth rates at a site based upon food flux estimates. It does not account for many factors which may reduce growth rates below this potential (e.g., dinoflagellate blooms, disease, other aspects of water quality, or poor handling techniques by growers). Complimenting this model with visual inspection of phytoplankton assemblages and disease monitoring seems prudent.

(3) This model was developed to predict **potential average seasonal** growth rates during times of good oyster growth (spring and fall). It is not expected to apply at times when food supply is not a limiting factor for growth (e.g., especially cold winters or hot summers in the mid-Atlantic region).

In those cases where it is valuable for an oyster aquaculturist to be able to evaluate potential growing areas prior to establishing them, this approach may be employed to estimate the potential average peak seasonal growth rates at alternative sites. Under our controlled experimental conditions, the model has done a good job of predicting actual observed growth

rates; under more typical culture conditions it might be expected to at least provide estimates of **relative potential average seasonal** growth rates between sites.

Dissemination and use of the results

Meetings and workshops

Results from various stages of this work have been presented at numerous scientific meetings and at workshops for oyster aquaculturists. These forums gave us the feedback necessary to focus the work on areas of real need and provided us a means of disseminating the results. A summary of the meetings and presentations follows.

1. 12th Annual Shellfish Biology Meeting, Milford, CT, Feb. 1992. "Toward a model for predicting oyster growth rate in the field." This meeting is attended by research scientists, Sea Grant extension personnel and shellfish aquaculturists from the Northeast and mid-Atlantic states.

2. Joint meeting of the Estuarine Research Federation and the European Coastal Sciences Association, Plymouth, U.K., Sept. 1992. "Development of a food flux model for predicting growth rates of oysters in the field" Attended by U.S. and European marine scientists.

3. National Shellfisheries Association, Portland, OR, June 1993, "'Non-toxic' dinoflagellate bloom effects on oyster culture in Chesapake Bay" Attended by shellfisheries biologists and aquaculturists.

4. National Shellfisheries Association, Charleston, SC, Apr. 1994, "Effects of water flow on feeding in a nonsiphonate bivalve." Attended by shellfisheries biologists and aquaculturists.

5. Workshop on Oyster Aquaculture, Gloucester Point, VA, Nov. 1994. This full day workshop on oyster aquaculture was organized by Luckenbach and supported with NCRI funds. The workshop covered a broad range of topics related to oyster aquaculture, including site selection criteria. A list of registered attendees is included in the Appendix and brochures detailing the workshop are submitted with this report. The workshop was very well attended (236 registered attendees) and we continue to get requests to hold additional ones.

6. Seminar on Oyster Aquaculture, Melfa, VA, May 1995. This evening seminar, sponsored by the Eastern Shore Chamber of Commerce, attracted over 75 attendees from local communities where the economy is very dependent upon the seafood business. An introduction to the techniques of oyster aquaculture, including findings from this study, was presented.

7. VIMS sponsored workshops on oyster aquaculture, including site selection criteria, have been held on several occasions for groups ranging in size from 25 - 50 peoples. These workshops have been offered in direct response to requests for information from the public; they include some lecture and hands-on demonstration of techniques and usually last about ½ day. Dates and locations of workshops held to date are:

15 Apr 93 -- Gloucester Point, VA
22 Mar 94 -- Wachapreague, VA
7 Apr 94 -- Gloucester Point, VA
26 May 94 -- Lancaster, VA

We expect to hold similar workshops in the future as they provide an efficient means for us to disseminate results from our research on oyster aquaculture to current and prospective growers.

Direct requests

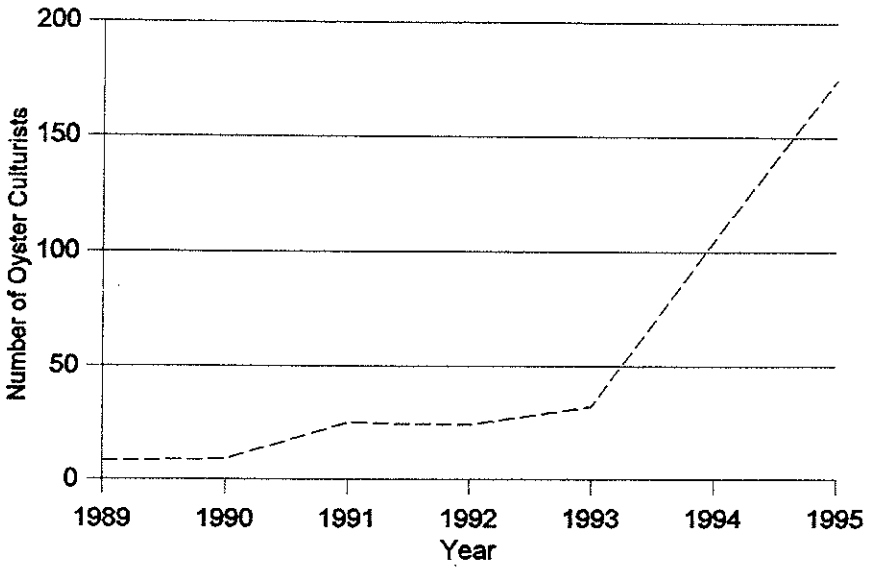
In the case of most requests for site evaluations (generally by "oyster gardeners"), we have been recommending that growers use the chlorine tablet method for assessing water flow as a first cut approach. The growth of oyster aquaculture (see Fig. 17, below) beyond our expectations has clearly lead to limitations in our ability to utilize this method to the benefit of most growers. Since the nutrient and chlorophyl analyses are beyond the capabilities of growers, it may be possible for them to contract for these services with private laboratories in the region. However, in many cases it seems sufficient for small-scale oyster growers to test flow rates before selecting their growing sites.

Requests to apply this method to testing alternative locations for large-scale commercial operations have come to us from 3 growers. Mr. Odis Cockrell (Middle Bay Marine Construction, Hwy 200, Box 133, Burgess, VA 22432), Mr. Joe Peirson (P.O. Box 222, Eastville, VA 23347) and Mr. Jeff Gardner (Shellfish For You, 227 Shore Dr., Waverly, RI 02891). In each case we collected water samples from two potenital growing locations and deployed chlorine tablets for two-day, time-averaged flow estimates. We supplied to the growers our results indicating which of the two locations had higher predicted potential average growth rates.

Evaluating the Impact of the Project

The increasing popularity of oyster aquaculture in Virginia has lead to a deluge of requests for extension services by the VIMS Oyster Aquaculture Program staff, including site evaluation. Figure 17 shows the growth in numbers of people involved in off-bottom oyster aquaculture during the past 5 years. Though much of this activity reflects the increasing popularity of "oyster gardening"--ie, small-scale, non-commercial culturing--a growing number of larger commercial ventures and marketing co-ops are also developing. Site evaluation for all of the new sites established each year was beyond the scope of this project and the capabilities of our program. Further, many oyster culturists either do not need or do not want a site evaluation conducted; often an individual must make do with limited available waterfront for oyster culture.

Figure 17. Participation in Oyster Aquaculture in Virginia, 1989 - 1995



The increase in oyster aquaculture activity in Virginia within the last few years cannot be attributed to any single factor. The continued demise of wild oyster stocks has certainly had a lot to do with the increasing popularity of oyster culture. Most important, we believe, has been the demonstrated success of culture efforts during the early 1990's . In Virginia, until the past two years, all private oyster aquaculture was conducted in collaboration with the VIMS Oyster Aquaculture Program. Much of what we have learned about site selection during the process of conducting this work has contributed to our success and to the growth in oyster culture, even though that has not always come through the direct application of the growth model.

The workshops which we have held to disseminate the results of our findings on all aspects of oyster culture have done much to promote the growth of oyster aquaculture in Virginia and neighboring states. Oyster aquaculture is certain to continue to grow in Virginia over the next few years and we will do our best to track that growth. It is not possible to quantify what portion of this growth is directly related to this work, but as pointed out earlier proper site selection is the most important component of bivalve aquaculture (Manzi and Castagna 1989).

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Appendix I. Attendees at oyster culture workshop, November 19,1994.

Name	Address
Abbott, David	P.O. Box 36, Burgess, VA 22432
Abbott, Joyce M.	P.O. Box 36, Burgess, VA 22432
Ainslee, Jack	814 H Greenbrier Circle, Chesapeake, VA 23320
Ambach, Dwight	P.O. Box 26, Susan, VA 23163
Applin, Paul	Gum Point Oyster Co., P.O. Box 100, Whitemarsh, VA 23183.
Armentrout, Russell	Route 2, Box 3640, White Stone, VA 22578
Arnold, Thomas	8175 Little England Road, Hayes, VA 23072
Atkison, Lee R.	1140 #G East Ocean View, Norfolk, VA 23503
Baldwin, Jeff	1340 Bartlett Rd., Richmond, VA 23231
Baxter, Laura H.	Icehouse Cove, 54322 White Hall Road, Zanoni, VA 23191
Beard, Don	Route 1, Box 1192, Weems, VA 22576
Beers, Pat	Route 1, Box 7645, White Stone, VA 22578
Beers, Paul	Route 1, Box 7645, White Stone, VA 22578
Birdsall, Frank	Route 2, Box 3640, White Stone, VA 22578
Birtman, Byron	Route 1, Box 226C, Hague, VA 22469
Bisek, Walter	2153 Windward Shore Drive,Virginia Beach, VA 23451
Bloxom, Robert S., Jr.	P.O. Box 27, Mappsville, VA 23407
Bower, David	4600 David's Mill Dr., Chesapeake, VA 23321
Bower, Jeannie	Sea Coast Manufacturing, P.O. Box 370, Atlantic, VA 23303
Boyd, Cal	Route 1, Box 2750, Reedville, VA 22539
Boyd, Jan	Route 1, Box 2570, Reedville, VA 22539
Brock, Frasia H.	12201 Roane Ave., Gloucester, VA 23061
Broderson, Ted	P.O. Box 524, North, VA 23128
Brown, Charlie	1034 Crowhill Rd., Beaufort, NC 28517
Brown, Roger	3501 Cardinal Lane, Portsmouth, VA 23703
Brunk, Peter A.	3501 Cardinal Lane, Portsmouth, VA 23703
Bryson, A.Y.	P.O. Box 515, North, VA 23128
Budwell, Leigh	Box 6, Cobbs Creek, VA 23035
Byrd, Ronnie	Sea Coast Manufacturing, P.O. Box 370, Atlantic, VA 23303
Calvo, Gustavo	8998 Unionville Road, Easton, MD 21601
Cannon, Rob	9519 Blackburn Dr., Burke, VA 22015
Cashwell, Rudy	VIMS Eastern Shore Lab, Wachapreague, VA 23480
Chewning, Rush W.	Route 2, Box 1854, Heathsville, VA 22473
Chowning, Randy	9503 Bonnie Dale Road, Richmond, VA 23229
Christie, John S.	Route 2, Box 2785, Reedville, VA 22539
Christie, June	Route 2, Box 2785, Reedville, VA 22539
Chu, Fu Lin	VIMS, P.O. Box 1346, Gloucester Point, VA 23062
Cockrell, Odis	Middle Bay Marine Construction, Inc., Burgess, VA 22432
Conner, William A.	1806 Rosemont Lane, Hayes, VA 23072
Constantin, Georgetta	VIMS, P.O. Box 1346, Gloucester Point, VA 23062

Cookson, Ryan	VIMS, P.O. Box 1346, Gloucester Point, VA 23062
Craig, Seth	1300 Thornton St., Fredericksburg, VA 22401
Craig, Judith	1300 Thornton St., Fredericksburg, VA 22401
Crewe, James I.	884 Lacon Dr., Newport News, VA 23602
Crewe, Linda	P.O. Box 412, Wicomico, VA 23184
Croonenberghs, Robert B.	Division of Shellfish Sanitation, Richmond, VA 23219
Davick, Kelly	P.O. Box 10, Wicomico Church, VA 22579
DeMarco, Andrew	3596 Krista Lane, Hayes, VA 23072
De Marco, George	P.O. Box 604, Mathews, VA 23109
Dickenson, Tanyua L.	Rappahannock Record, P.O. Box 400, Kilmarnock, VA 22482
Dreher, Bob	Route 2, Box 1330, Lancaster, VA 22503
DuPaul, William	VIMS, P.O. Box 1346, Gloucester Point, VA 23062
Edmonds, John	3165 Holly Cliff Lane, Portsmouth, VA 23707
Edmonds, Shela	3165 Holly Cliff Lane, Portsmouth, VA 23707
Ellis, Aubrey	726 Yarmouth St. Norfolk, VA 23510
Farlow, Wayne	402 Kemp Lane, Chesapeake, VA 23325
Fauber, D.W.	P.O. Box 266, 2496 Jacobia Lane, Cape Charles, VA 23310
Fortin, Jane	Route 2, Box 1515H, Lancaster, VA 22503
Fortin, Roger T.	Route 2, Box 1515H, Lancaster, VA 22503
Foster, Neal	no address
Fowler, Tom	6213 Winthrop Dr., Raleigh, NC 27612
Fox, George	HCR 75, Box 636, Redart, VA 23076
Fridley, David	VIMS, P.O. Box 1346, Gloucester Point, VA 23062
Gardner, Jeff	Shellfish For You, 227 Shore Dr., Waverly, RI 02891
Gonsowski, Bill	1469 Alaska Rd., Woodbridge, VA 22219
Gonsowski, Ed	1469 Alaska Rd., Woodbridge, VA 22219
Graffy, Richard	Route 1, Box 637, Lancaster, VA 22503
Greene, Kathleen	VIMS, P.O. Box 1346, Gloucester Point, VA 23062
Greene, Terrell	P.O. Box 476, Wicomico Church, VA 22579
Gregg, W.E.	P.O. Box 69, Wicomico Church, VA 22579
Gregory, Jack	Box 660, Cobbs Creek, VA 23035
Gregory, Wanda Cook	Box 660, Cobbs Creek, VA 23035
Griffith, M.B.	P.O. Box 293, Port Haywood, VA 23138
Hallett, David	Route 1, Box 805, Weems, VA 22576
Hanchey, Howard	Seminary Post Office, Alexandria, VA 22304
Harding, John	P.O. Box 44, Burgess, VA 22432
Harding, Karen	P.O. Box 44, Burgess, VA 22432
Hargis, William	Virginia Institute of Marine Science, Gloucester Point, VA 23062
Hatch, John L.	Box 7775, Haynes Drive, Gloucester, VA 23061
Hawkins, Wayne	CIT Tower, Suite 600, 2214 Rock Hill Road, Herndon, VA 22070
Hawkins, Ms.	CIT Tower, Suite 600, 2214 Rock Hill Road, Herndon, VA 22070
Hayes, George	VIMS, P.O. Box 1346, Gloucester Point, VA 23062
Hays, Patricia	P.O. Box 111, Belmont Dr., Mollusk, VA 22517

Hays, Raymond	P.O. Box 111, Belmont Dr., Mollusk, VA 22517
Hearns, Charlie	RR2, Box 1516, Lancaster, VA 22503
Hearns, Elizabeth	RR2, Box 1516, Lancaster, VA 22503
Heath, Mark	P.O. Box 565, Eastville, VA 23347
Hecht, Joe C.	RR2, Box 1546, Lancaster, VA 22503
Heinemann, Jack	7140 Ware River Dr., Gloucester, VA 23061
Henderson, Alfred	P.O. Box 437, Kilmarnock, VA 22482
Henderson, Franklin D.	P.O. Box 186, Irvington, VA 22480
Hobt, Murrell	73 Merrimac Trail, Apt 10, Williamsburg, VA 23185
Holton, Linwood	6010 Claiborne Rd., McLean, VA 22101
Hopton, Dave	P.O. Box 186, Irvington, VA 22480
Humphries, Hank	VIMS, P.O. Box 1346, Gloucester Point, VA 23062
Hurlock, Tamara	Wachapreague, VA 23480
Hutchen, Ellet	Route 2, Box 1179, Lancaster, VA 22503
Hutson, Robert	Ocean Products Research, Diggs, VA 23045
Hutzel, Abe	P.O. Box 143, Kinsale, VA 22488
Ilyama, Laura	2480- 16th St., N.W., Apt. 713, Washington, DC 20009
Johnson, J.Wallace	Route 1, Box 142, Callao, VA 22435
Jones, L. Clarke, Jr.	P.O. Box 15539, Richmond, VA 23227
Jones, JoAnne	19324 Herring Creek Rd., Ruther Glen, VA
Kaattari, Stephen	VIMS, P.O. Box 1346, Gloucester Point, VA 23062
Kelsey, Andrew	Route 2, Box 1770, White Stone, VA 22578
Kelsey, Ann R.	Route 2, Box 1770, White Stone, VA 22578
Kelsey, Fred	P.O. Box 88, Merry Point, VA 22513
Kemp, Skip	NC Sea Grant, P.O. Box 3146, Atlantic Beach, NC 28512
Kern, Raymond	4121 Park St., Hayes, VA 230072
Kupiec, John	P.O. Box 780, Mathews, VA 23109
Kurkowski, Ken	Stubbs Seafood, P.O. Box 1236, Chincoteague, VA 23336
Lambert, Morton T.	511 Latane Dr., Richmond, VA 23236
Latell, Margaret	3325 Prince Charles Court, Falls Church, VA 22044
Lawson, Dee	P.O. Box 888, Mathews, VA 23109
Lawson, Dunbar	P.O. Box 888, Mathews, VA 23109
Layer, Carol	P.O. Box 130, North, VA 23128
Layer, Joseph	P.O. Box 130, North, VA 23128
Lee, Fred	Route 3, Box 1439, Lancaster, VA 22503
Leggett, A. Thomas, Jr.	P.O. Box 412, Wicomico, VA 23184
Leonard, Kathy	VMRC, 2600 Washington Ave., Newport News, VA
Lester, Elizabeth	106 Rosemount, Williamsburg, VA 23188
Li, Tong	VIMS, P.O. Box 1346, Gloucester Point, VA 23062
Linton, Andy	Sea Coast Manufacturing, P.O. Box 370, Atlantic, VA 23303
Littlefield, Arthur	P.O. Box 10, Wicomico Church, VA 22579
Liverman, Donald L., Sr.	VMRC Associate Member, Newport News, VA 23607-0756
Long, Daniel	2482 Jacobia Lane, Cape Charles, VA 23310

Travelstead, Jack	VMRC, 2600 Washington Ave., Newport News, VA 23607
Trice, Ashton L.	1000 Ashbridge Place, Richmond, VA 23233
Trice, Hap	1000 Ashbridge Place, Richmond, VA 23233
Tye, Julia D.	P.O. Box 1070, Suffolk, VA 23434
Tye, Tom	P.O. Box 1070, Suffolk, VA 23434
Van Horn, Donald	General Delivery, Susan, VA 23163
Van Horn, Rodger	General Delivery, Susan, VA 23163
Volety, Aswini	VIMS, P.O. Box 1346, Gloucester Point, VA 23062-1346
Wade, Alton S.	402 Kemp Lane, Chesapeake, VA 23325
Wallace, Wendell	Route 1, Box 875, Weems, VA 22576
Warner, B. Henry	6400 Garnett Dr., Chevy Chase, MD 20815
Wesley, Edward L.	12676 River Road, Richmond, VA 23233
Wesson, Jim	VMRC, 2600 Washington Ave., Newport News, VA 23607
Wheeland, Hoyt	Piankatank Project, P.O. Box 1425, Tappahannock, VA 22560
White, John T., III	1000 S. Columbus St., Arlington, VA
Wiggins, William A.	P.O. Box 460, Wicomico Church, VA 22579
Williams, Armistead	12 Bayberry Lane, Williamsburg, VA 23185
Williams, Robert L.	7495 Wilson Farm Lane, Gloucester, VA 23061
Wilson, Hugh	4090 Dogwood Dr., West Point, VA 23181
Winfield, Gordon	Route 1, Box 815, Lancaster, VA 22503
Wissel, Kevin	1009 Front St., Beaufort, NC 28516
Wright, Judson	3501 Cardinal Lane, Portsmouth, VA 23703
Yashiro, Joshua	Happy World America Inc., Elizabeth, NJ 07207-0905
Zierow, Rolff	P.O. Box 840, Mathews, VA 23109