Coastal bivalve aquaculture carbon cycling, spatial distribution and resource use in Virginia, USA and Baja California, Mexico

Kyle Aaron Emery New Egypt, NJ

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Abstract

Aquaculture is a global industry responsible for over half of the world's fisheries production. A major component of this industry is shellfish aquaculture. Shellfish aquaculture is prevalent in many nearshore marine ecosystems and has effects on both the system structure and processes. These effects on coastal environments have both ecological and economic impacts with policy and management implications. This thesis explores several aspects of shellfish aquaculture in coastal Virginia, USA and Baja California, Mexico using carbon budgeting, geographic information systems (GIS) analysis and stable isotope analysis.

The impact of hard clam (*Mercenaria mercenaria*) aquaculture on carbon cycling in a tidal inlet was evaluated for Cherrystone Creek, a small tributary of the Chesapeake Bay. The fluxes and pools of organic and inorganic carbon driven by clam aquaculture were of a similar magnitude to system processes such as water column production and carbon burial. Clam consumption is likely supported by production from outside of the system imported daily through tidal exchange. CO₂ production is also enhanced through clam respiration and calcification. A large amount of carbon (135 Mg C yr⁻¹) is removed annually through harvests in the clam shell and tissue material. The carbon associated with these withdrawals is generally not returned to the system. Intensive shellfish aquaculture alters coastal carbon cycling through the addition of large fluxes and pools of organic and inorganic carbon.

An analysis of annual aerial images of the ocean side lagoons of the Virginia Coastal Reserve (VCR) in GIS was conducted to identify the temporal and spatial trends of clam aquaculture for the period 2002 – 2012. Aquaculture of the hard clam has increased annually, even while state harvest numbers have remained relatively stable. The number of clam beds has grown by about 250 beds per year from 1,180 in 2002 to 4,430 in 2012. This increase

corresponds to over 1 km² of new clam farms in the VCR. Clam farms were not randomly located or based solely on bottom area available for leases that allow aquaculture. They were located in shallow water adjacent to inlets and channels. Using spatially explicit data for the VCR, constraints related to bathymetry, water residence time and the sediment grain size of clam farms were determined and used to predict potential areas for future aquaculture expansion.

Clam farms are most likely to be found in locations with shallow depths (0 - 2.5 m below mean sea level), short water residence times (<0.5 hours - 108 hours) and sandy sediments (40 - 90%).

Clam aquaculture in the VCR currently occupies 1.8 km² with a potential habitable zone of 120.9 km² remaining, indicating the potential for future expansion with an unlikely spatial limitation.

Given the good water quality of these coastal lagoons and the high flushing rate, it is also unlikely that aquaculture will encounter resource limitations in the near future.

Lastly, shellfish aquaculture resource use was explored with a stable isotope analysis of Pacific oysters (*Crassostrea gigas*) and their potential food resources in Bahía San Quintín, Baja California, Mexico. The stable isotopes ratios of hydrogen (²H/¹H) and carbon (¹³C/¹²C) were measured for oysters, seagrass (*Zostera marina*) and macroalgae (*Ulva* spp.) and calculated for phytoplankton. These values were used in a Bayesian mixing model to estimate a posterior distribution of resource use. There was no seasonal effect on resource use as upwelling conditions typical of the region were below average prior to sampling events. However, there was a strong spatial gradient in the system. Phytoplankton were the primary resource (median values 67 and 79%) for oysters nearest to the mouth of the bay while macroalgal importance increased (43 and 56%) for oysters in the upper reaches of the system. The mixed resource use of the oysters highlights their ability to adapt to different locations and resource availability, potentially allowing for a higher system carrying capacity.

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Introduction

Aquaculture is a globally expanding industry that contributes to feeding an increasing global population. While aquaculture is often considered a more sustainable method of seafood production than capture fisheries, there are nevertheless impacts on coastal systems. Shellfish cultivation is one of the largest sectors of aquaculture and is predominantly carried out in nearshore marine environments. Coastal shellfish operations are often very intensive, involving dense assemblages over extended areas. In North America shellfish aquaculture occurs on both the east and west coasts as well as in the Gulf of Mexico. Commonly farmed species include the hard clam (*Mercenaria mercenaria*), the eastern oyster (*Crassostrea virginica*) and the Pacific oyster (*Crassostrea gigas*). These filter-feeding bivalves can have significant effects on their resident ecosystems. This thesis explores several methods of evaluating shellfish aquaculture trends and resource use in intensively farmed coastal systems.

The first chapter quantifies the fluxes and pools of both organic and inorganic carbon related to hard clam aquaculture in Cherrystone Inlet, Virginia. The coastal zone is prominent in global carbon cycling, processing carbon of both terrestrial and marine origin. To better constrain changing coastal carbon fluxes, it is important to estimate and constrain fluxes associated with shellfish aquaculture relative to better understood estimates of pelagic and benthic production and respiration, carbon burial, etc. Estimates of carbon fluxes associated with clam aquaculture were derived from harvest data, clam farm coverage, clam weight and length measurements, and clam physiological relationships. The clam related fluxes were compared to estimates of system processes. As expected, the magnitude of carbon cycling associated with clam aquaculture is large and comparable to system-scale processes in a heavily farmed tidal inlet. These results indicate shellfish aquaculture may be a dominant feature of nearshore carbon

cycling when carried out at high densities and over a large spatial extent. Including shellfish aquaculture in carbon budgeting will improve our understanding of coastal carbon cycling and potentially impact future carbon credit scenarios.

The second chapter identifies the trends and spatial extent of hard clam aquaculture in the Virginia Coast Reserve (VCR). The amount of clam farms and their locations within the VCR may have important implications for the system's carrying capacity. As shellfish aquaculture expands there is the potential for space or resource limitation. Although the VCR is a large coastal system, it is unlikely that the entirety of the VCR is suitable for clam aquaculture. The VCR consists of shallow coastal lagoons with good water quality, low water residence times, and sandy sediments. An analysis of aerial images from 2002 – 2012 was conducted with GIS to determine trends in the number of clam beds over time and to identify the areas where clam aquaculture is prevalent. There was a significant increase in clam aquaculture over the studied time period. The locations of clam aquaculture were not randomly distributed as most sites were in shallow waters adjacent to channels and inlets in the ocean-side portions of the lagoons. Environmental characteristics (bathymetry, water residence time, sediment grain size) of the clam farms were quantified and used to determine areas within the VCR that may serve as potential suitable habitat for expanding clam aquaculture. This type of information may prove useful to shellfish farmers, managers, and the public in considering how areas of the VCR may be used in the future.

The third chapter uses analysis of stable isotope ratios to determine the food resource use of aquacultured Pacific oysters located in Bahía San Quintín, Baja California, Mexico. This bay is a reverse estuary, where salinity increases up the bay due to high evaporation and little to no inputs of freshwater. Another interesting feature of this system is that it experiences seasonal

upwelling. The western arm of this bay has a large oyster aquaculture industry which extends from near the mouth of the bay into the upper reaches. The stable isotope ratios of hydrogen (²H/¹H) and carbon (¹³C/¹²C) were measured for the oysters and for the potential food resources of seagrass (Zostera marina) and macroalgae (Ulva spp.). Isotope values for phytoplankton were calculated from the dissolved inorganic carbon and hydrogen isotope values measured from seawater samples. A Bayesian mixing model was used to estimate the posterior distributions of resource use for the three potential end members. Upwelling was minimal over both sampling periods, therefore a seasonal effect in resource use was not observed. However, as hypothesized, there was a spatial gradient in resource use. Oysters at the site nearest to the mouth of the bay predominantly used phytoplankton. The oysters at the upper site used a greater proportion of macroalgae. This study highlighted the utility of the hydrogen stable isotope in aquatic food web studies and also provided insight into resource use by filter-feeding shellfish. The oysters of this study appear adaptable to changing resource availability and the system as a whole may have a greater carrying capacity given that phytoplankton is not the only food resource used by the oysters.

This thesis explores interactions of intensive shellfish aquaculture and coastal ecosystems. Intensive shellfish aquaculture has numerous implications for the coastal systems where farms are prevalent. Developing an understanding of how shellfish aquaculture interacts with the carbon cycle is a necessary measure in determining how aquaculture can alter system-scale processes, such as coastal carbon cycling. Intensive shellfish culture can grow rapidly over short periods of time, while requiring sufficient conditions and resources from the natural environment to succeed. Environmental data may be used to predict locations suitable for intensive aquaculture, which can provide managers with useful information and can be used in

determining the carrying capacity of a system. Stable isotope analysis in coastal systems and aquaculture research is also useful in determining how the industry interacts with the ecosystem while also having implications for the carrying capacity with respect to resource availability. Shellfish aquaculture is a growing industry and has system-scale implications. This thesis details how shellfish aquaculture interacts with the surrounding ecosystem and how the tools available for scientists and managers can be used to assess these implications and plan for future expansions.

$\label{lem:chapter 1} The \ \text{effect of intensive hard clam} \ (\textit{Mercenaria mercenaria}) \ \text{aquaculture on carbon cycling}^a$

^a To be submitted for publication with the following co-authors: Jennie Rheuban, Michael Pace, Anna Murphy, Iris Anderson and Karen McGlathery

Abstract

The addition of intensive shellfish aquaculture operations to the coastal zone potentially impacts carbon cycling through the creation of new pathways and alterations of existing stocks and flows. Cherrystone Inlet is a small (6 km²) tidal inlet that opens into the Chesapeake Bay that has had large increases in hard clam (*Mercenaria mercenaria*) aquaculture. In this study we estimate fluxes associated with clams and compare these to other carbon cycling processes such as gross primary production and respiration. Clam consumption approaches 1000 Mg of C per year and is about 30% of water column gross primary production (3483 Mg C) and respiration (2950 Mg C). Clams affect CO₂ exchange through the processes of respiration and calcification. As filter feeders they consume a majority of the organic matter in the water column, 58% of which is subsequently respired. Annual harvests of the clams results in removals of both organic (45 Mg C) and inorganic (90 Mg C) carbon in the tissue and shell material, respectively. Future research is needed to develop more complete carbon budgets of systems both with and without aquaculture to better constrain impacts on coastal carbon cycling and aid management of shellfish operations.

Introduction

The growing use of coastal waters for aquaculture potentially changes key biological and chemical characteristics of these diverse systems. Global aquaculture, and more specifically mariculture, has increased annually with a large portion of harvests being mollusks (Campbell and Pauly 2013, FAO 2014). Shellfish aquaculture is also increasing in the United States (USDA 2005). The most common nearshore shellfish aquaculture species in the United States are eastern and pacific oysters (*Crassostrea virginica* and *gigas*, respectively) and hard clams (*Mercenaria mercenaria*). In the Chesapeake Bay region of Virginia, aquaculture of the hard clam produces around 200 million clams each year, with 2013 being the largest year in recent record (Hudson and Murray 2014). Independent observations support a growing production. Clam aquaculture in the ocean-side lagoons of the Virginia Coast Reserve has expanded 375% over the past decade based on analysis of aerial images (Emery Ch. 2).

Clam aquaculture has the potential to affect nearshore coastal environments and their ecology through water filtration, alterations of biogeochemical cycling and the provisioning of habitat (Grizzle et al. 2001, Powers et al. 2007, Dame 2012). Clam aquaculture occurs in subtidal and inter-tidal marine environments generally to depths of 2 m below mean sea level (Arnold et al. 2000, Emery Ch. 2). These shellfish are thus able to couple the benthic and pelagic environments in coastal waters, enhancing the exchange of organic matter and nutrients (Dame et al. 1989, Grizzle et al. 2001 Dame 2012). Hard clams are filter feeders that utilize suspended forms of organic matter generally composed of micro- and macroalgae (Secrist 2013, Hondula and Pace 2014). High stocking densities of clams may deplete food resources for both the clams and other organisms (Dame and Prins 1998, Byron et al. 2011a, Byron et al. 2011b, Guyondet et al. 2013). Nutrient dynamics are also altered by the presence of dense shellfish operations. The

release of labile nutrients through clam excretion, egestion and pseudofeces formation may enhance the growth of macroalgae (Powers et al. 2007, Murphy et al. In Review). Alternatively, aquacultured shellfish can reduce nutrients through filter feeding, leading to a subsequent improvement in water quality (Bricker et al. 2014, Rose et al. 2014).

The addition of shellfish aquaculture may alter fluxes of carbon in nearshore marine ecosystems, affecting both organic and inorganic carbon. With respect to organic carbon, filter feeders may draw down particulate organic carbon stocks. Some of this organic carbon is exported with shellfish harvests in tissue or shell material while some is transferred to the benthos through biodeposition where it is buried, mineralized or re-suspended. Most of the fluxes of organic carbon with respect to intensive hard clam aquaculture have yet to be quantified. Fluxes of inorganic carbon are also altered through the addition of shellfish aquaculture, primarily due to respiration and shell calcification. Calcification occurs when dissolved inorganic carbon is fixed as calcium carbonate shell material. This process also results in production of carbon dioxide (CO₂) (Hily et al. 2013). Shell removal through harvests represents a flux out of the system as does any net CO₂ gas transfer to the atmosphere. Similarly, the fluxes of inorganic carbon related to intensive hard clam aquaculture have been minimally described. Quantifying the additional and altered carbon fluxes associated with shellfish aquaculture can be used to gauge the overall response of a system to increased aquaculture as well as the potential carrying capacity.

The objective of this study was to estimate the carbon fluxes associated with hard clam aquaculture in a heavily farmed tidal inlet. We hypothesized that clam aquaculture alters existing pathways and creates new carbon flow pathways for both organic and inorganic forms of carbon. The fluxes of carbon related to clam aquaculture were estimated for Cherrystone Inlet, a small

Chesapeake Bay tributary located in the Virginia portion of the Delmarva Peninsula. The magnitudes of these fluxes related to clams were compared to estimates of carbon fluxes such as primary production that would occur in the system even in the absence of clam aquaculture.

Methods

Site Description

Cherrystone Inlet (Figure 1), a shallow tidal creek utilized for shellfish aquaculture, is located on the western side of the Delmarva Peninsula, near Cheriton, VA (37.2918° N, 76.0163° W). It is a 6 km² inlet which opens into the eastern side of the Chesapeake Bay (Condon 2005, Robinson et al. 1998). The inlet volume is 15.4 km³ (Kuo 1998). Cherrystone Inlet experiences a tidal range of about 0.7 meters (NOAA 2009) and has an annual salinity range of 16 – 23 parts per thousand (Robinson & Gallagher 1999). Hard clam aquaculture is prevalent, occurring along a majority of the shoreline (Figure 1).

Cherrystone Inlet Clam Data

Total hard clam harvests in term of numbers of individuals were obtained for Cherrystone Inlet in the year 2012 from local growers. Harvested clams were categorized by growers based on commercial sizes as; button clams (small), little neck clams (medium), and middle neck clams (large). The standing stock of clams in Cherrystone Inlet was estimated using aerial image analysis (as in Emery Ch. 2). Clam beds are readily visible in photographs taken at low tide for the annual Submerged Aquatic Vegetation Survey conducted by the Virginia Institute of Marine Science. Each clam bed contains around 50,000 clams and is covered with a protective antipredator netting which appears as a black rectangle and measures 72 m² (Figure 2) (Luckenbach and Wang 2004, Murphy et al. In Review). The total number of clam beds in 2012 was counted

from the photos and multiplied by 50,000 to estimate the total clam aquaculture population for the inlet. Clam aquaculture aerial coverage was also determined for 2001 and 2003 – 2012 and combined with prior estimates for 1990 – 1997 (Woods 2001) to evaluate trends in Cherrystone Inlet. The proportion of harvested clams in each size category was used to estimate the standing stock of clams in Cherrystone Inlet by size category. A sampling of hard clams was conducted to determine average lengths and weights for the shells in each size class. Cleaned shells were dried at 60° C for 48 hours before weighing.

Organic Carbon Flux Calculations

Calculations for fluxes of organic carbon that would occur in the absence of clam aquaculture included primary production and respiration in the water column, the available supply of organic carbon in the inlet, and the burial of organic carbon. Importantly, these fluxes are not free of influence from clam aquaculture. Fluxes dependent on clam aquaculture include clam consumption, respiration, biodeposition, and removal through harvests. Benthic fluxes of organic carbon were excluded because hard clams are filter feeders and therefore dependent on water column organic carbon, so processes related to the benthos, such as benthic respiration and production, were not included. When possible, estimates were scaled by clam growing season with May – October representing the growing season and November – April representing the low or no growth season (Hibbert 1977). Water column production on an annual basis was calculated with gross primary production measurements using light:dark bottle incubation in Cherrystone Inlet (Reay et al. 1995). Values were scaled to represent the entire inlet. Production estimates are useful for determining the renewal of organic carbon within the system, but to estimate the carbon available at any given time for filter feeding we used measurements of particulate organic carbon from Kuo (1998). These values were scaled to represent the entire inlet.

Three fates of organic carbon were considered in this study. First, carbon deposited from the water column to the sediments may be respired, resuspended or buried. Estimates of particulate organic carbon burial for the Chesapeake Bay from Roden et al. (1995) were scaled to Cherrystone Inlet. Water column respiration of organic carbon was measured by Kemp et al. (1997) for the lower Chesapeake Bay using light:dark bottle incubations and was applied to Cherrystone Inlet. Lastly, consumption of organic carbon by clams was calculated using the filtration rates of hard clams and the production estimates given above. Equations using water temperature and shell length (Hibbert 1977) were applied to the three size classes of clams. The average lengths measured in this study and the estimated number of individual clams in each size class as determined from the image analysis were used to calculate the volume of water filtered by all clams in Cherrystone Inlet for the growth and no growth seasons. The volume filtered in one year was multiplied by the estimated production (Reay et al. 1995) to calculate the amount of carbon consumed by all clams in Cherrystone Inlet.

There are several fluxes of organic carbon that occur following consumption by clams in relation to the stock of organic carbon in Cherrystone Inlet. Clam biomass was estimated using the organic matter contents of shell (1.9%) (Price 1976) and an ash-free dry weight relationship for clam tissue (Condon 2005). Shell organic carbon was calculated for each size class using measured shell weights and the number of clams in Cherrystone Inlet. Tissue organic carbon was calculated using an ash-free dry weight relationship determined for clams in Cherrystone Creek (Condon 2005), measured shell lengths, and the number of clams in Cherrystone Inlet. These two values were combined for an estimate of total organic carbon in the Cherrystone Inlet clam stock. Assuming the clam organic carbon stock is maintained at a steady state, we estimated the fates of organic carbon consumed by clams in respiration, biodeposition, and removal through harvests.

Respiration was estimated using water temperature and shell length based equations (Hibbert 1977) for the growth and no growth periods of the year. Water temperatures from the Chesapeake Bay Program (CBP 2012), measured shell lengths, and the number of clams in Cherrystone Inlet were used in this calculation. The amount of organic carbon removed annually in shell and tissue material due to clam harvests was calculated as above using the same methods but based on the number of clams harvested instead of the number of clams in the inlet.

Biodeposition (feces + pseudofeces) was calculated by subtracting the carbon utilized for annual respiration and assimilation (harvested organic carbon) from the consumption value.

Inorganic Carbon Flux Calculations

Estimated fluxes of inorganic carbon that would occur regardless of clam aquaculture were constrained to the stock of inorganic carbon of the inlet and the flux to the atmosphere. These, however, may be altered by the presence of clam aquaculture. Clam dependent fluxes included calcification and removal of shell from the inlet stock due to harvests. Benthic fluxes of inorganic carbon were excluded. The stock of inorganic carbon in Cherrystone Inlet was calculated using dissolved inorganic carbon concentrations from the lower York River estuary of the Chesapeake Bay (Raymond and Bauer 2001) scaled to the volume of the inlet. The flux of carbon to the atmosphere, or net CO₂ efflux, was estimated from an annual range for the lower York River estuary (Raymond et al. 2000) scaled to Cherrystone Inlet.

Calcification is the process by which calcium carbonate is formed and deposited as shell material. This value was calculated using a hard clam calcification rate (Waldbusser et al. 2010), minimum and maximum hard clam growth rates (Fritz and Haven 1981), a shell length to total wet weight relationship for Cherrystone hard clams (Condon 2005), and measured shell lengths. Calcification in the system by other organisms (i.e. eastern oysters) was assumed negligible. The

production of CO₂ associated with calcification was determined using the relationship given by Frankignoulle et al. (1994) and water temperatures (CBP 2012). These data allow calculation of ψ, the fraction of CO₂ produced for each mole of calcium carbonate produced. The stock of inorganic carbon in shell material in Cherrystone Inlet was determined using the number of clams in the inlet, measured shell weights, and the inorganic carbon content of shell (Price 1976, Doering et al. 1987, Bouillion et al. 2011). The same method was applied in determining the amount of inorganic carbon removed annually from the inlet by using harvest numbers.

Results

Hard clam harvests in Cherrystone Inlet in 2012 totaled 30,057,361 individuals. A total of 2,514 clam beds were identified in the aerial images from 2012 which at a density of 50,000 clams per bed indicated a clam population of 125,700,000 individuals (may overestimate if high mortality year). Overall clam aquaculture coverage in Cherrystone Inlet has significantly increased since 1989 (Figure 3, $R^2 = 0.77$, p < 0.001) with an annual clam bed growth rate of about 104 beds. Of the clams harvested in 2012 from Cherrystone Inlet 4.9% were button clams, 43.2% were little necks and 51.9% were middle necks. Average shell lengths ranged from 38.5 to 56.1 mm and shell weights from 10.7 to 34.9 g (Table 1).

Organic carbon fluxes of production, respiration and burial were estimated using data from or adjacent to Cherrystone Inlet. The fixation of carbon through water column photosynthesis resulted in an estimated flux of 3,274 Mg Carbon (C) yr⁻¹ to particulate organic carbon (Figure 4). The organic carbon available to filter feeding consumers was 30 Mg C based on average POC values (Figure 4). An estimated 347 Mg C yr⁻¹ is buried through natural sedimentation processes (Figure 4). We did not consider other benthic processes, but some

amount of carbon is respired in or re-suspended from the sediments. Water column respiration was estimated at 2950 Mg C yr⁻¹ (Figure 4). These processes occur regardless of the presence of aquaculture, but the magnitude of the fluxes may be altered by clam aquaculture.

There are several organic carbon pathways dependent on clam aquaculture. The standing stock of clams in Cherrystone Inlet was assumed constant, with annual harvests being replaced by new juvenile clams. Consumption of organic matter by clams was 997 Mg C yr⁻¹, or 30.5% of the annual gross primary production of Cherrystone Inlet (Figure 4). Annual clam respiration was 575 Mg C (Figure 4). The amount of organic carbon in the shell and tissue material of all clams in the inlet was 189 Mg C with 45 Mg C being removed annually through harvests (Figure 4). By assuming a constant stock of clams in the inlet, 45 Mg C represents the annual production of clams, although this figure underestimates losses due to in situ mortality. These calculations allowed for an estimation of biodeposition (feces + pseudofeces) in which respiration and production were subtracted from consumption, resulting in 384 Mg C yr⁻¹ transferred to the benthos (Figure 4).

Fluxes of inorganic carbon were estimated for Cherrystone Inlet using data from adjacent locations. The standing stock of dissolved inorganic carbon in the inlet was estimated from the nearby York River estuary. This pool of carbon, 198 Gg C, is three orders of magnitude larger than all other pools or fluxes of carbon considered in this study (Figure 4). Assuming similar CO₂ concentrations as the York River, an estimated 32 – 62 Mg C yr⁻¹ are released to the atmosphere from Cherrystone Inlet (Figure 4).

The addition of clam aquaculture to Cherrystone Inlet creates additional fluxes and pools of inorganic carbon. Based on the number of clams in the inlet, we estimated that 375 Mg C is stored as calcium carbonate in shell material (Figure 4). Annually, 90 Mg C is removed as shell

through harvests (Figure 4). Using annual growth estimates, an estimated 528 Mg of DIC is utilized for shell building (i.e. calcification) (Figure 4). However, calcification also results in the production of CO₂. The ratio of CO₂ to CaCO₃ production averaged 0.6 based on water temperature during the growing season of May – October. This flux added 317 Mg C yr⁻¹ to the inlet's inorganic carbon stock (Figure 4).

Discussion

Cherrystone Inlet

Hard clam aquaculture in Cherrystone Inlet grew consistently over the past two decades (Figure 2) with an average increase of 104 clam beds per year. In 2012 over 30 million clams were harvested, which was a significant component of the total Virginia harvest (214 million clams) (Hudson and Murray 2014). With a clam aquaculture operation of this magnitude, ecological effects were not only expected in Cherrystone Inlet, but observed. For example, measurements of nutrient flux (ammonium and phosphate) increased over 100-fold at clam sites compared to reference sites and significant support of macroalgal growth was also found at clam sites (Murphy et al. In Press). Shellfish aquaculture in general affects the local environment, but in many cases those effects are minimal compared to fish aquaculture or even beneficial (Crawford et al. 2003, Dumbauld et al. 2009, Rose et al. 2014). In this study we sought to estimate how shellfish aquaculture alters carbon cycling at the scale of an entire inlet.

Carbon Fluxes

The fluxes of both organic and inorganic carbon initiated by clam aquaculture, along with new carbon pools, indicate large changes to coastal carbon cycling in comparison with non-aquacultured systems. Prior research has analyzed the rates of consumption, assimilation, respiration and biodeposition of organic carbon by shellfish (Tenore and Dunstan 1973, Hibbert

1977, Doering et al. 1987). The effects of shellfish on carbon cycling have been explored in several studies, especially with respect to CO₂ generation (Chauvaud et al. 2003, Waldbusser et al. 2010, Mistri and Munari 2012, Mistri and Munari 2013). Carbon is also removed from coastal waters through the harvest of shellfish (Tang et al. 2011). With respect to Cherrystone Inlet, we compared carbon fluxes resulting from clam aquaculture to the magnitude of other carbon fluxes in the system that, although not independent of the clam aquaculture, would occur in its absence.

The addition of clam aquaculture to the system initiated several new fluxes of carbon. The clams consume 29% of annual production, which is respired, biodeposited, or assimilated. The carbon lost through harvests is not returned to the system. Interestingly, the amount of organic carbon lost annually through biodeposition and clam harvests (429 Mg C) is very close to the annual net production of organic carbon within the water column (533 Mg C).

Evasion of CO_2 to the atmosphere from super-saturated waters may occur in the absence of clam aquaculture, but is likely enhanced due to CO_2 released from respiration and calcification processes (Chauvaud et al. 2003, Mistri and Munari 2012). Note we estimate CO_2 is lost to the atmosphere despite net water column autotrophy (GPP > R, Figure 4). In addition to clam produced CO_2 , the source of excess CO_2 is likely benthic respiration which we did not estimate. The largest carbon pool in Cherrystone Inlet is dissolved inorganic carbon. The addition of clams results in calcification of some of the DIC stock yielding CO_2 as a by-product (Frankignoulle et al. 2004). In addition, a significant flux of carbon out of the system occurs with clam harvests, as the shell material is likely not returned to the system.

Additional Carbon Pathways

This analysis focuses only on within-system fluxes of carbon, neglecting large exchanges with the Chesapeake Bay as well as some of the carbon pools in Cherrystone Inlet. For example

we did not consider standing stocks or cycling of dissolved organic carbon (DOC) because particulate organic matter is the primary food source of the clams (Grizzle et al. 2001) and clam effects on DOC are likely small. Cherrystone Inlet receives DOC inputs from the terrestrial environment and marshes, and there is a large exchange with the Chesapeake Bay as the tidal prism represents 37.7% of the inlet volume (Kuo et al. 1998). Regardless of the net import or export of DOC with the bay, our estimates of clam consumption, respiration, biodeposition and calcification would not change.

Tidal exchange carries POC into and out of the Inlet, therefore the Chesapeake Bay may represent a net POC source. Tidal exchange, however, is an important indicator of resource availability, not use (Carver and Mallet 1990). Based on our estimates, the current Cherrystone Inlet clam population filtered 19-32% of the inlet volume or 50-84% of the time averaged tidal prism daily, depending on the growth season. We do not have annual net primary production by phytoplankton to assess against clam consumption but it is possible that production in the inlet cannot alone sustain the current clam population. Inputs from tidal exchange and use of other forms of organic matter may supplement inlet primary production as food sources for the clams.

Sources of organic carbon other than phytoplankton production should be considered as contributors to supporting clams as well as the inlet's organic carbon stock. Benthic microalgae production was not considered although some of this material may be represented in the particulate organic carbon pool due to resuspension. The production of macroalgae was also not included, even though this material can contribute to the particulate pool and its growth is often significantly enhanced with the presence of shellfish aquaculture (Powers et al. 2007, Secrist 2013, Murphy et al. In Press). There is an annual input of carbon to the system in the form of seed clams that was not considered, but this input of carbon is small relative to the current clam

population and the annual harvest of clams. The size of seed clams is generally 12 mm (Castagna 2001) and the annual carbon input is on the order of 1-2 Mg C yr⁻¹.

Uncertainty

The limited data available for carbon flux estimations in this study make assessing uncertainty difficult. The carbon fluxes directly related to clam aquaculture are the most certain of the estimates made in this study. Established relationships for hard clam growth (Fritz and Haven 1981, Condon 2005), filtration (Hibbert 1977), respiration (Hibbert 1977), calcification (Waldbusser et al. 2010) and shell and tissue content (Price 1976) were used with direct measures of shell lengths and weights for different size classes of clams from Cherrystone Inlets. Hard clam harvest and standing stock estimates were also made directly in this study. With respect to organic carbon fluxes, estimates of consumption, respiration, tissue and shell harvests and the shellfish stock due to measurement uncertainty, stock variation and inter-annual factors such as temperature fluctuations. The estimate of biodeposition is dependent on a number of other factors not available for this system, such as suspended sediment concentrations, and therefore was made using a more qualitative approach. Water column production (GPP) and the organic stock of the inlet were calculated from earlier studies in Cherrystone Inlet and may not be representative of current conditions. Respiration (R) in the water column was extrapolated from Chesapeake Bay measurements and may include the uncertainties described above in addition to that associated with making such extrapolations. With respect to inorganic carbon, the estimates of calcification, CO₂ production, shell harvest, and shell stock face the same potential error associated with measurement uncertainty, stock variation and inter-annual factors such as temperature fluctuations. Cherrystone Inlet inorganic carbon and atmospheric flux are not site-specific are also extrapolated values.

System Scale Effects

Alteration of carbon cycling associated with shellfish aquaculture is potentially large and the results of this study identify the magnitude of changes. The consumption of organic matter by shellfish highlights a new pathway for carbon that may limit other species in the system and potentially the aquaculture industry itself. The available resources and the quantity utilized by shellfish is important information for managers and those looking to assess the carrying capacity of a given system with respect to shellfish aquaculture (Guyondet et al. 2013, Filgueira et al. 2014). The added CO₂ resulting from shellfish respiration and calcification may enhance CO₂ emissions from the system (Chavaud et al. 2003, Mistri and Munari 2012, Mistri and Munari 2013). Lastly, the large amount of organic and inorganic carbon lost from the system due to annual harvests may shift future system production and exchange with the greater Chesapeake Bay and/or terrestrial and marsh environments.

Consistent withdrawals of calcium carbonate through harvests can reduce alkalinity thereby increasing the potential for acidification (Waldbusser et al. 2013). The transfer of carbon due to aquaculture out of coastal systems and into the human food production system remains largely unquantified. In particular, the fate of shells is generally unknown. In some cases shells are returned to coastal systems for aquaculture or restoration purposes (Piazza et al. 2005). However, shells are also largely disposed of on land, representing a potentially long-term carbon sink (NRC 2010). Given the impacts of aquaculture estimated in this study, developing improved carbon budgets for systems with and without shellfish aquaculture is warranted to improve understanding of coastal carbon cycling (Doney 2010, Cai 2011, Bauer et al. 2013, Laruelle et al. 2014, Gruber 2014, Filgueira et al. 2015).

Conclusions

Because of expansion, impacts of shellfish aquaculture will likely increase. Our analysis illustrates that the introduction of shellfish aquaculture generates new and substantial organic and inorganic carbon fluxes, especially in local inlets with intensive production. These new pathways for carbon transfers are of a sufficient magnitude to indicate substantial acceleration of carbon cycling. Future research to establish carbon budgets in the presence and absence of shellfish aquaculture will improve understanding of shellfish aquaculture effects on carbon cycling and provide a better basis for management.

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Tables

Table 1.1: Average shell length (cm), average shell dry weight (g) for the three size classes of hard clams sampled from Cherrystone Inlet. (n) indicates the number of clams sampled for each specific measurement.

Shellfish	Shell Length (cm) ± SE (n)	Shell Dry Weight (g) ± SE (n)
Hard Clams		
Small	38.5 ± 0.5 (25)	$10.72 \pm 0.33 (40)$
Medium	45.7 ± 0.7 (23)	17.45 ± 0.86 (23)
Large	$56.1 \pm 0.8 (25)$	34.90 ± 1.14 (36)

Table 1.2: Methods and sources for the Cherrystone Creek carbon budget calculations.

Abbreviations are: CC = Cherrystone Creek, C = carbon, OC = organic carbon POC = particulate organic carbon, DIC = dissolved inorganic carbon GPP = gross primary production, Rp = pelagic respiration, AFDW = ash-free dry weight, TWW = total wet weight LOI = loss on ignition

Flux/Pool	Method	Sources
CC organic C stock	CC POC in g/m3 scaled up	Kuo 1998 (POC and volume)
CC OC burial	Chesapeake POC burial in mol C m-2 yr-1 converted and scaled up	Roden et al. 1995 (POC), Condon 2005 and Robinson et al. 2007 (CC Area)
WC respiration	Chesapeake R (Rp) converted from O2 to C and scaled up	Kemp et al. 1997 (respiration), Condon 2005 and Robinson et al. 2007 (CC Area)
WC production	CC annual GPP scaled to entire inlet	Reay et al. 1995 (CC GPP), Condon and Robinson et al. 2007 (CC area)
Clam consumption	Filtration rate for 3 sizes times number of clams for each size in CC. Volume filtered times winter and summer production rate. Summed and scaled up	Hibbert 1977 (Filtration rate equation, growth seasons), Reay et al. 1995 (CC GPP), Kuo et al. 1998 (CC Volume), My data (# clams, sizes)
Clam OC stock	Shell weights for 3 sizes of clams times number of clams in each size class in CC times organic C in shell and summed. AFDW from length for 3 size classes times # clams in CC in each size class. Sum tissue OC with shell OC	This paper, Price 1976 (shell and tissue organic matter from LOI), Condon 2005 (CC AFDW shell length relationship)

Clam respiration	CC Temp data, shell lengths and # of clams to calculate R, convert from O2 to C and scale up	This paper, CBP (water temp), Hibbert 1977 (Respiration equation, growth seasons)
Clam biodeposition	Consumption - tissue/shell stock (assimilation) - respiration = (Feces + pseudofeces)	This paper
Tissue/shell OC harvests	AFDW from shell length for each size class times # clams in CC. Number of clams times shell weights for each size class times # clams in CC times shell OC. Sum both	This paper, Condon 2005 (AFDW equation), Price 1976 (tissue and shell organic matter from LOI)
Atmospheric exchange	Scale up annual range to CC area	Raymond et al. 2000 (CO2 evasion for lower York), Condon 2005 and Robinson et al. 2007 (CC Area)
DIC stock	Chesapeake DIC in micromol converted and scaled up.	Raymond and Bauer 2001 (DIC concentration York River estuary), Kuo 1998 (CC volume)
Calcification	Shell growth rate min and max used for growing season (May-Oct). Use in equation for TWW for each size class. Scale to CC. Convert TWW growth to C added	This paper, Condon 2005 (TWW equation), Fritz and Haven 1981 (Clam growth rate), Waldbusser et al. 2010 (Calcification rate)
CO2 production	Find average annual ψ value for CC and multiply by Calcification	This paper (calcification), Frankingoulle et al. 1994 (Psi equation 0.6 for growing season), CBP (water temp)
Shell IC stock	Number of clams in CC by size class times shell C content summed	This paper

Shell IC harvests	Number clams harvested in CC by size class times shell C content and summed	This paper
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Figures

Figure 1.1: Cherrystone Inlet (black star) is located on the western side of the Delmarva

Peninsula and opens into the Chesapeake Bay. Aquaculture clam beds from 2012 are outlined in black.

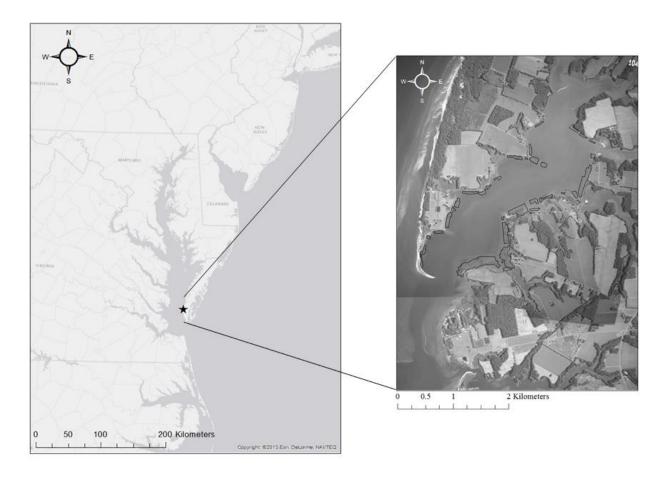


Figure 1.2: Hard clam aquaculture beds in Cherrystone Creek photographed by the Virginia Institute of Marine Sciences annual Submerged Aquatic Vegetation survey. Dark gray to black rectangles are active clam beds.

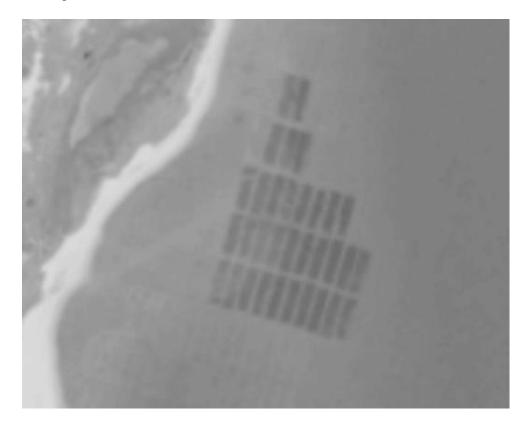


Figure 1.3: Long-term trends (1989 - 2012) in active aquaculture clam beds in Cherrystone Inlet based on areal photograph analysis for the years 2001 - 2012 combined with data for aquaculture coverage from 1989 - 1997 from Woods (2001).

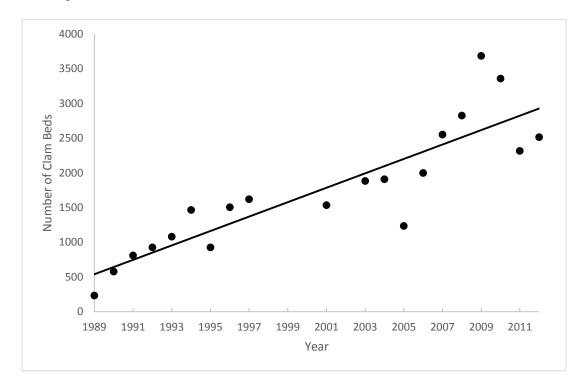
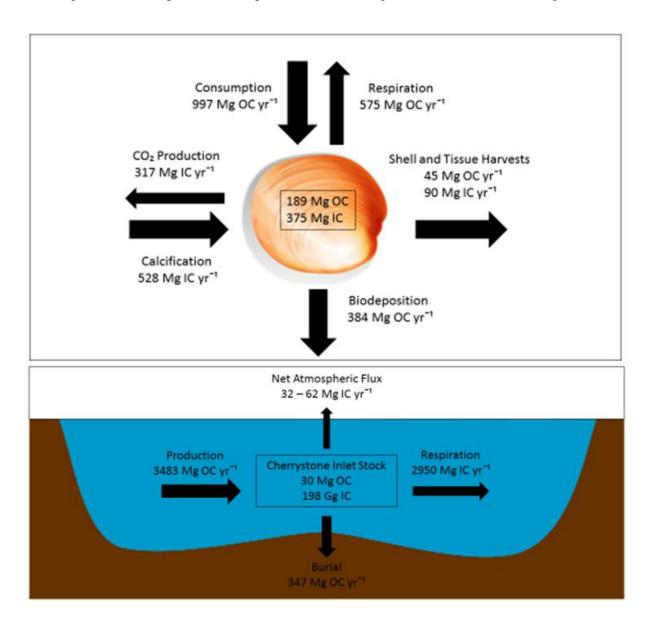


Figure 1.4: The fluxes (arrows) and pools (boxes) of organic and inorganic carbon in Cherrystone Inlet considered in this study with associated values. Values in the top box are associated with clam aquaculture and values in the bottom box are system values that would occur regardless of the presence of aquaculture. OC = organic carbon and IC = inorganic carbon.



Chapter 2

Trends in hard clam (*Mercenaria mercenaria*) aquaculture in the Virginia Coast Reserve (USA) in relation to potential suitable area^b

^b To be submitted for publication with the following co-authors: Michael Pace, David Richardson, Patricia Wiberg, Joel Carr and John Porter

Abstract

Shellfish aquaculture is rapidly expanding globally in coastal systems, the rates of which are not well documented nor are the locations of suitable habitats. In Virginia (USA), hard clam (Mercenaria mercenaria) aquaculture is prevalent in nearshore environments and is also increasing, specifically in the area known as the Virginia Coast Reserve (VCR). An aerial image analysis was conducted to determine changes in the number of active individual clams beds over the years 2002 to 2012. Beds increased from 1,181 to 4,431 during this time. We estimate total areal coverage of clam farms in 2012 at 1.81 km² which represents 0.35% of the VCR. Locations of clam farms were compared to environmental parameters to determine depth (0.13 - 2.60 m)below MSL), water residence time (0.4 - 108 hours) and grain size (40 - 90% sand) ranges that could be used to identify areas of the VCR potentially suitable for clam aquaculture. Of the 512 km² of the VCR considered in this study, 121 km² or 24% is suitable for clam farming based on these metrics. Of all clam farm area in 2009 and 2012, 65% overlapped with the established constraints. The methods and environmental parameters used in establishing clam farm criteria for the VCR may be applied, with additional parameters, to other systems with shellfish aquaculture.

Introduction

Aquaculture is a fast growing industry that is a potentially sustainable alternative to capture fisheries (Subasinghe et al. 2009, Bostock et al. 2010). Global aquaculture production in 2012 was worth an estimated \$144 billion, of which a significant portion was shellfish (FAO 2014). In addition to economic benefits, bivalve culture also interacts with the local ecosystem, providing a number of ecosystem services (Gallardi 2014). These may include increased water clarity due to nutrient and organic matter removal (Dame et al. 1989, Dame 2012), enhanced benthic-pelagic coupling (Dame 2012), shoreline protection (Scyphers et al. 2011) and habitat (Powers et al. 2007). As important aquaculture species, bivalves also provide nutrition to humans.

In the United States, shellfish aquaculture occurs along both the Pacific and Atlantic coastlines including the Gulf of Mexico. In the Mid-Atlantic region, Virginia is a large producer of hard clams (*Mercenaria mercenaria*) (Hudson and Murray 2014). Clam aquaculture production in the state of Virginia has been consistent over the period 2005 – 2013 with around 180 million individuals harvested annually (Hudson and Murray 2014), but the spatial distribution of this production is less clear. Clam aquaculture occurs in many of the more saline tributaries of the Chesapeake Bay. Clam aquaculture is also prevalent in the ocean-side coastal lagoons of the Virginia Coast Reserve (VCR) (Orth et al. 2006, Orth et al. 2010), yet the total number of clam beds and area of bottom-ground utilized remains unquantified.

Determining the aerial coverage of clam aquaculture is important for several reasons. With increasing aquaculture usage of lagoon bottom conflicts may develop over suitable habitat, especially with the expansion of restored seagrass meadows (Woods 2001, Orth et al. 2006, Orth et al. 2010). It is also important to quantify environmental conditions supporting production. The

extent of clam aquaculture can aid in quantifying effects of this industry on coastal ecosystems including the alteration of carbon cycling, accelerated nutrient cycling, altered primary production, and increased water clarity (Ferreira et al. 2007, Dame et al. 2012, Guyondet et al. 2013, Rose et al. 2014, Emery et al. In Prep, Murphy et al. In Press). Quantifying trends in the number of active clam beds is also important for understanding the economics and dynamics of the industry within the VCR. With shellfish aquaculture increasing in Virginia's coastal lagoons there is a growing need to determine carrying capacity, which can only be accomplished if there is a clear understanding of the magnitude and distribution of shellfish aquaculture (Aguirre-Muñoz et al. 2001, Byron et al. 2011a, Guyondet et al. 2013, Saurel et al. 2014).

In other systems the importance of quantifying coverage of shellfish aquaculture has been demonstrated for both bottom-ground coverage (Carswell et al. 2006) and ecosystem carrying capacity (Byron et al. 2011a). In Baynes Sound on Vancouver Island, British Columbia, Canada several species of clams (*Prothaca staminae*, *Nuttallia obscurata*, *Tapes philippinarum*) are cultured using anti-predator netting (Carswell et al. 2006). These nets, which are also used in VCR clam aquaculture, are visible in aerial photographs (Carswell et al. 2006), especially when fouled with macroalgae (Powers et al. 2007, Saurel et al. 2014). A spatial analysis of these clam beds, which considered a time-series evaluation of coverage, site characteristics, and the potential impact on shorebirds, was undertaken to determine the footprint of aquaculture in nearshore environments (Carswell et al. 2006, Bendell and Wan 2011). In the coastal bays of Rhode Island, estimates of shellfish aquaculture were used along with a model to determine carrying capacity (Byron et al. 2011a, b). Carrying capacity analysis generally requires biomass estimates (Byron et al. 2011a, b), which can be obtained from harvest reports, but harvests are often inaccurate due to underreporting (MCZM 1995, Watson & Pauly 2001, J. Wesson,

Personal Communication). Biomass estimates based on area and stocking density are more accurate and with aerial images are easily replicated from year to year.

A temporal and spatial analysis of clam beds is useful for determining how aquaculture is changing and the importance of site locations. Given the relatively high water quality and shallow depth of VCR lagoons (McGlathery et al. 2007), this location appears highly suitable for clam aquaculture. While clam bed locations may also be partially controlled by extrinsic factors, such as bottom lease availability or farmer choices, observations of existing beds indicated they were non-randomly distributed and that the number of active areas was far lower than the number of available areas for leasing. We hypothesized a significant increase in the number of clam beds over the last decade. We also hypothesized that the locations of these clam beds relate to spatially explicit lagoon characteristics, such as depth, water residence time and sediment grain size. In this study we quantified trends in clam beds over time and the relationship of clam farm location to bathymetry, water residence time and sediment grain size using methods that can be applied generally to other systems with shellfish aquaculture.

Methods

Study Site

This study was conducted at the Virginia Coast Reserve Long Term Ecological Research site on the Eastern Shore of Virginia in the ocean side lagoons of the Delmarva Peninsula (Figure 1). The area of the VCR in question consists of relatively shallow coastal lagoons (mean low water to -2 m) (Oertel 2001) and occupies an area of 511.6 km² (mean sea level and below) from Quinby Inlet (37.4668° N, -75.6663° W) in the north to Fisherman's Island (37.0960° N, 75.9580° W) in the south. Lagoons have low nutrient levels (McGlathery et al. 2001), short

water residence time (Safak et al. 2015), and periodically high levels of sediment resuspension (Lawson et al. 2004, Mariotti et al. 2010). Historically, the VCR had a large bay scallop (*Argopecten irradians*) fishery that collapsed in the 1930's due to seagrass decline. During the late 1990's and early 2000's clam farming operations developed in the lagoons (Orth et al. 2002, Orth et al. 2006, Orth et al. 2010). Clam aquaculture is carried out by planting dense assemblages of hatchery clams on the lagoon bottom and covering these beds with anti-predator netting. Clams grow in situ for approximately eighteen to twenty-four months and are then harvested.

Aquaculture Site Identification and Coverage Trends

Aerial images from the annual Virginia Institute of Marine Sciences Submerged Aquatic Vegetation survey were used to enumerate clam beds on the lagoon bottom (Figure 2) (Orth et al. 2010). Active clam beds appear as dark rectangles due to macroalgal fouling of the anti-predator netting (Powers et al. 2007, Saurel et al. 2014, Murphy et al. In Press) while inactive beds appear as faint rectangular outlines of the same dimensions. Images from 2002-2012 were analyzed in ArcMap 10.1 (ESRI) to identify the locations of clam aquaculture within the VCR. A new shapefile was created for each year to allow for delineation of clam farms (aggregations of beds as in Figure 2) into individual polygons using the create features function. VCR-wide delineation of clam beds was completed for the years 2003, 2009 and 2012, based on the availability of georectified images. A partial delineation was completed for the geo-rectified photographs of the remaining years with clam beds from missing segments counted, but not delineated, from nongeo-rectified photographs. The number of active individual clam beds at each farm were counted for each annual survey from 2002 - 2012. The area of each polygon was recorded for the fully delineated years. A linear regression between the number of active clam beds in the VCR and time was used to observe the trend in coverage over the 2002-2012 time period. The total ground

area utilized by active clam aquaculture beds in the VCR was determined by multiplying the number of active beds by 72 m², the standard bed size as determined by the anti-predator nets (Luckenbach and Wang 2004). The total area directly impacted by clam aquaculture (active beds, inactive beds, space between beds = clam farm) was calculated by summing the area of the delineated polygons for 2003, 2009 and 2012. Clam bed locations based on the aerial survey of 2012 were ground-truthed in July 2014 using a hand-held GPS (Figure 3).

Aquaculture Spatial Analysis

A spatial analysis of clam aquaculture in the VCR was conducted to determine several environmental characteristics of farms and to identify areas that, meeting these conditions, may be suitable for future culture. The clam bed shapefile from 2009 was used to best match bed locations with other available data. The first parameter analyzed was the bathymetry of the clam farms. A raster layer of VCR bathymetry data (depth below mean sea level (MSL)) from Richardson et al. (2014) was used to determine the range of depths covered by all 64 clam farms in 2009. This was achieved by using the zonal statistics tool to calculate the minimum and maximum depths for each of the farms. The average of all the minimum depths and all of the maximum depths was calculated. The standard deviation of the minimums was subtracted from the average of the minimums and the standard deviation of the maximums was added to the average of the maximums to develop a range of depths, encompassing uncertainty, utilized by clam aquaculture. This range was then used in a conditional analysis in ArcGIS to create a layer of the VCR area that fell within this depth range.

The second variable analyzed was water residence time. A raster file of water residence time (hours) was created from data on the modeled release of neutrally buoyant tracer particles and a power relationship derived from distance to the nearest inlet times root mean squared

velocity (Safak et al. 2015). In addition to inlet proximity, Safak et al. (2015) found that wind forcing, tidal phase of release and bay geomorphology exerted the most control on residence time estimates. The zonal statistics tool was used to calculate the minimum and maximum water residence time for each of the 64 clam farms from 2009. The average of all the minimum water residence times and of all the maximum water residence times was calculated. The standard deviation of the minimums was subtracted from the average of the minimums and the standard deviation of the maximums was added to the average of the maximums to develop an average range of water residence time, error included, for the clam aquaculture sites. This range was used in a conditional analysis in ArcGIS, as above, to create a new layer of the VCR area where the water residence time fell within this range. The area of clam beds that overlapped with this range was calculated.

The third variable analyzed was sediment grain size using a raster layer of surface sediment grain size fractions from Wiberg et al. (In Review). Their analysis estimated the sediment fraction less than 63 µm, between 63 and 125 µm, and above 125 µm using grain size measurements from transects within the lagoons and root mean squared velocities. The zonal statistics tool was used to calculate the minimum and maximum percent of sediment over 125 µm (sand) for each of the 64 clam farms. The average of all the minimum sand fractions and of all the maximum sand fractions was calculated. The standard deviation of the minimums was subtracted from the average of the minimums and the standard deviation of the maximums was added to the average of the maximums to develop an average range of percent sand, error included, for the aquaculture clam farms. This range was used in a conditional analysis, as above, to create a new layer of the VCR area where the percent sand fell within this range. The area of clam beds that overlapped with this range was calculated. A conditional analysis was then

run for the depth, water residence time and sand fraction ranges to determine the area of the VCR that met all three conditions.

As an independent measure, the depth range of aquacultured clams in the VCR was also compared to a published depth range of 0-1.8 m below mean sea level (Arnold et al. 2000). This was the only hard clam aquaculture variable independent of the VCR that could be applied to this analysis. Other constraints, such as water chemistry and suspended sediment concentrations, which could be garnered from the literature were not available at a fine spatial resolution in the VCR, thereby preventing such analysis. Independent range estimates of variables that are known at a fine resolution for the VCR, such as water residence time, are too system specific and variable to be applied here, especially in the case of systems with significant freshwater inputs (Dame and Prins 1998). The percentage of clam bed area that fell in both depth ranges was calculated along with the difference in total VCR area for each depth range. The depth range from Arnold et al. (2000) was also applied to an analysis with water residence time and grain size. In all analysis, the results of the independent depth data did not differ greatly from the analysis using VCR derived depth data, hence the results using the Arnold et al. (2000) depth range are not presented.

After establishing the spatial distribution of the abovementioned environmental variables the resulting potential habitat areas were then further restricted by removing locations which overlapped with the Baylor Survey Grounds. These areas are managed by the state of Virginia for public oyster harvesting (Mann et al. 2009). These grounds are not available for lease or use by large shellfish aquaculture operations and therefore are excluded from the range of potential future farm sites. The outputs of these analyses were compared to calculate the potential area available for clam aquaculture given the various restrictions. These outputs were then analyzed

to determine the amount of area utilized by clam aquaculture in 2009 and 2012 captured by the given constraints. The 2012 clam sites were analyzed to determine how these conditions for farm placement hold over time.

Results

Bed Locations and Temporal Trends

Clam beds in the VCR are located on the landward side of the barrier islands (Figure 4). The beds are often adjacent to deeper channels. Clam aquaculture does not occur in the inner part of the VCR lagoons close to the mainland (Figure 4). Farms that previously appeared in these locations did not persist over time. Beds are located in bottom areas leased by the State of Virginia. Potential leased area is far greater than the current extent of clam beds.

The number of active clam beds increased from 1,181 in 2002 to 4,431 in 2012. This trend was significant ($R^2 = 0.72$, p < 0.001) (Figure 5). The 375% increase in active clam beds corresponded to an areal increase of 234,000 m² of active clam beds. The change in area directly impacted by clam aquaculture, given as total polygon or farm area and not solely active beds, increased from 387,961 m² in 2003 to a total current impacted area (as of 2012) of 1,812,223 m². The estimates of total farm area in 2009 and 2012 were used in the following analyses.

Spatial Analysis of Clam Farms

The constraints of depth, water residence time and grain size (Table 1) were used to determine the area of the VCR potentially suitable for clam aquaculture (Table 2). The area impacted by clam aquaculture (active beds, inactive beds, space adjacent to beds) in 2009 was 1.56 km², or roughly 0.3% of the total VCR lagoon area. The depth range of these clam beds

(average min. – SD and average max. + SD) ranged from 0.13 - 2.60 m below MSL. The area of the VCR meeting this depth criteria was 374 km² (Figure 6), or 73% of the study region. Overlap between the 2009 clam beds and the area of the VCR of the same depth range was 1.44 km^2 , indicating that 92.3% of the 2009 clam beds were represented by the 0.13 - 2.60 m depth range.

The range (average min. – SD and average max. + SD) of water residence time in the 2009 clam beds was 0.4-108 hours. The minimum value of 0.4 hours was the absolute minimum water residence time value measured and was used because the average of the minimums minus the standard deviation was negative. Four clam farms were removed in the determination of this range because the maximum water residence times associated with those farms were an order of magnitude higher than all other farms. These sites were located in the upper portion of Magothy Bay where the modeled residence times are exceptionally high due to restricted tidal exchange (Safak et al. 2015). The area of the VCR met by the water residence time conditions was 248 km², or 48% of the study region. There was an overlap of 1.25 km² or 80% of the 2009 clam sites.

The range (average min. – SD and average max. + SD) of the sediment fraction being greater than 125 μ m in the 2009 clam beds was 0.40 – 0.90, i.e. 40 – 90% of the sediment was considered sand. The area of the VCR that met this condition was 329 km² or 64% of the study region. Overlap between the 2009 clam beds and this grain size range was 1.41 km² or 90% of the clam sites.

A conditional analysis of potential clam bed locations based on depth, water residence time, and grain size restrictions resulted in 186 km² of the VCR satisfying all three conditions (Figure 7). The combination of these three variables restricts potential area in the VCR to 36% of the analyzed region. Removal of the areas within the VCR known as the Baylor Survey Grounds

reduced the potential area available for hard clam aquaculture. With respect to depth only, the area available with the Baylor Survey Grounds removed was 236 km² (Figure 8A). This accounts for 46% of the area of the VCR study region. The area available with the combination of depth, water residence time, grain size and the Baylor Survey Grounds removed was 121 km² (Figure 8B). This was equivalent to 24% of the VCR study region.

A conditional analysis was conducted to determine the area of the 2009 clam beds that overlapped with the depth, water residence time and grain size criteria with removal of the Baylor Survey Grounds. Using the VCR constrained variables, there was an overlap of 1.01 km² with the 2009 clam beds, representing 65% of their total area that year. In other words most clam beds fell within the constrained area but not all. The area impacted by clam beds in 2012 increased to 1.81 km². The depth, water residence time and grain size ranges with the Baylor Survey Grounds removed overlapped with 1.17 km² of the 2012 clam beds. This indicated a 65% match between the 2012 clam bed locations and the constraints set using the 2009 clam beds locations.

Discussion

Temporal Trends

Aquaculture of the hard clam in the Virginia Coast Reserve increased significantly during the period 2002 – 2012 (Figure 5). The number of actively farmed 72 m² clam beds grew by 3,250 during this period and total area impacted by clam aquaculture increased by over 1.4 km². With around 50,000 clams per bed (Luckenbach and Wang 2004), VCR aquaculture farms in 2012 held an estimated 221,550,000 hard clams. Although the number of active clams beds increased from 2002 – 2012, there were some years with lower coverage than prior years

followed by years with larger increases (Figure 5). This cyclical pattern was similar to up and down trends observed in the number of aquacultured hard clams planted and sold in the state of Virginia (Hudson and Murray 2014). This pattern is also evident in the number of full- and part-time jobs provided by the Virginia hard clam aquaculture industry (Hudson and Murray 2014). While hard clam aquaculture appears to be growing steadily in the VCR, there is some interannual variability related to growing and economic conditions. Reported harvests of aquacultured hard clams for the state of Virginia from 2005 to 2013 did not increase and were generally in the range of 150 to 200 million clams, with 2013 being the largest harvest year at 214.4 million clams (Hudson and Murray 2014). The increasing trend in the VCR portion of Virginia suggests this area may be contributing more to total production over time. Also, there is a possibility that state-wide production is increasing but is under reported. The water quality of the coastal lagoons (McGlathery et al. 2007) may be more favorable to growers than other locations within the state.

Spatial and Environmental Analysis

The location of many of the clam farms in the VCR is in the eastern portions (toward the ocean) of the coastal lagoons adjacent to channels, yet still in shallow waters (Figure 4). Farms near the mainland occasionally appeared in aerial images but did not persist over time. GIS data on bottom-ground leases in the VCR, required for the grow-out method of these farms, indicated that leased areas are more widespread than the clam farms (Berman et al. 2004). It is thus likely that certain environmental factors, in addition to lease locations, influenced the locations of these farms. In this study we sought to characterize the clam aquaculture sites in relation to bathymetry, water residence time, and grain size to assess the potential for continued expansion of hard clam aquaculture. The characteristics of hard clam aquaculture sites in the VCR obtained

through GIS analysis may also be used as a guiding tool for site selection in other systems, as with the parameters analyzed by Arnold et al. (2000). However, it is important to consider that social factors, such as clam farmer choices (i.e. depth willing to plant), may also play an important role in determining farm locations.

Bathymetrically, wild hard clams thrive from the intertidal zone to depths of 12 meters (Harte 2001). Aquaculture of this species, however, is generally constrained to the shallow coastal zone for ease of access. Arnold et al. (2000) noted that hard clam aquaculture generally occurs to depths of 1.8 m. Hard clam aquaculture in the VCR was distributed over a wider range from 0.13 to 2.60 m below mean sea level, with some locations reaching into more extreme shallows or depths. The large tidal range of the VCR (1.2 m) allows farming at greater depths, but may restrict management and harvesting of such sites to low tide. The mean depth of the 64 clam beds in 2009 was 0.98 m below mean sea level. This depth permits the clams an extended period of daily submergence consistent with improved growth and survivorship under subtidal vs. intertidal conditions (Eversole et al. 1990, Walker and Heffernan 1990, Grizzle et al. 2001).

Water residence time, the second variable analyzed in this study, is an important factor in bivalve survival because it influences water quality, food availability, and substrate type (Grizzle et al. 1992, Grizzle et al. 2001, Guyondet et al. 2013). Residence time is system specific and can vary greatly among locations with bivalve populations (Dame and Prins 1998). Water residence time for the VCR is short along the eastern portion of the coastal lagoons (< 6 hours) and greatly increases towards the mainland (> 1000 hours) (Safak et al. 2015). The range of water residence determined time for hard clam aquaculture sites in the VCR was 0.4 to 108 hours. The large range is due to the presence of several farms in regions where the water residence time sharply increases. The mean water residence time for the clam sites analyzed was 32 hours, which is

reflective of the majority of sites being located adjacent to channels and inlets (Figure 3). Water residence time was the most restrictive of the conditions applied to the VCR in determining potentially habitable area (Table 2).

Grain size was analyzed as a parameter that could be used for determining future hard clam aquaculture sites. Hard clams have higher growth rates in sandy sediments compared to silts and muds (Pratt and Campbell 1956, Rhoads and Panella 1970, Greene 1979, Grizzle and Morin 1989). Sandy environments are preferred because these sediments are generally more oxygenated with less hydrogen sulfide than muds or silts (Grizzle et al. 2001). The potential for sediment resuspension is also important for the survival of filter feeding bivalves. Muds and silts are more easily re-suspended than sand, and re-suspended particles can inhibit hard clam feeding. Studies have observed both reduced feeding and growth of hard clams with suspended sediment concentrations above threshold values (Bricelj et al. 1984, Bricelj and Malouf 1984, Turner and Miller 1991). For the clam farms in the VCR sand generally ranged from 0.40 to 0.90 as a proportion of total sediment composition (Wiberg et al. In Review). On average, hard clam farms in the VCR have a sediment fraction that is 2/3 sand.

The total water area considered for this study was 511.6 km², representing the southern portion of the VCR where clam aquaculture is prevalent. This area extends from the southern tip at Fisherman's Island to the northern extent of hard clam aquaculture at the northwest end of Quinby Inlet (Figure 4). Based on 64 hard clam aquaculture farms in 2009 in combination with existing environmental data, we established criteria for determining potential areas for new farms. These criteria were used with the clam farms identified in 2009 and 2012 to determine the farm area captured by the given conditions. Clam aquaculture impacted 1.56 km² and 1.81 km² of bottom ground in 2009 and 2012, respectively. No one condition greatly reduced the potential

ground available for hard clam aquaculture, but the combination of bathymetry, water residence time, and grain size indicated that 186 km² satisfy the three constraints (Figure 7). The addition of the Baylor Survey Grounds (restricted for public oyster harvest) as a non-environmental constraint reduced the potential available area satisfying these conditions to 121 km² (Figure 8B).

This study focused only on available (meaning measured and/or modeled) environmental parameters for the given geographic range. There are additional metrics from the VCR which could be applied to smaller regions (i.e. individual lagoons), but none that cover the entire area of this study (Figure 4). Additional local variables which could be applied include stocking density, suspended sediment concentrations, water velocity and water chemistry. These additional variables may improve prediction of the habitable range of hard clams or other popularly farmed shellfish, such as the eastern oyster (Crassostrea virginica). In large scale aquaculture operations shellfish are stocked at very high densities. However, density can negatively affect growth (Peterson and Beal 1989, Eversole et al. 1990). Suspended sediment concentrations can also be useful in determining the potential success of an aquaculture site because growth or feeding inhibition occurs at concentrations above threshold values (Bricelj et al. 1984, Bricelj and Malouf 1984). Water velocity is an important factor that could reduce potential farm area as sites of high flow speeds can restrict feeding and therefore growth (Turner and Miller 1991, Grizzle et al. 1992, Judge et al. 1992, Grizzle et al. 2001). Habitable area for hard clams can also be controlled by salinity and dissolved oxygen levels (Arnold et al. 2000). The minimum salinity for hard clam survival is 12.5 parts per thousand (ppt) while the upper limit is > 35 ppt, with an ideal range of 20 – 30 ppt (Castagna and Chanley 1973, Malouf and Bricelj 1989, Grizzle et al. 2001). Dissolved oxygen levels should exceed 1.0 mg/l otherwise

negative physiological effects occur (Malouf and Bricelj 1989, Arnold et al. 2000). With respect to temperature, maximum hard clam growth occurs from 20 - 25 °C, although the absolute temperature range suitable for growth is 7 - 31 °C (Ansell 1968, Laing et al. 1987, Grizzle et al. 2001).

Although salinity, oxygen and temperature within the VCR typically fall well within the range supporting growth of hard clams, the incorporation of additional data would likely permit greater restriction of the predicted suitable farming range. However, these factors are unlikely to substantially reduce the large possible range of clam aquaculture compared to current usage. While Bendell and Wan (2011) found that space can limit shellfish aquaculture, the VCR industry will not become space limited in the foreseeable future based on environmental criteria. Resource availability is another potential limitation to aquaculture expansion. The ecological carrying capacity, as opposed to space, concerns the productivity of the entire system. In larger systems shellfish aquaculture does not approach a system's carrying capacity (Byron et al. 2011a), however, the shellfish may still exert an effect on their local ecology and food supply (Guyondet et al. 2013, Filgueira et al. 2014, Saurel et al. 2014). Further research within the VCR is necessary to determine the effects of the clam industry on the ecosystem. A growing oyster aquaculture industry and the potential return of the bay scallop (Argopecten irradians) will need to be considered as these species are also filter feeders (Bosch et al. 2010, Orth and McGlathery 2012, Hudson and Murray 2014). The interaction between hard clam aquaculture and restored eelgrass (Zostera marina) within the VCR may also become relevant as both continue to expand (Orth et al. 2006, Orth et al. 2010), further highlighting the need to explore the spatial and resource limitations of the VCR with respect to shellfish aquaculture.

Conclusions

Hard clam aquaculture has increased significantly in the VCR over the last decade, as determined from an aerial image analysis. Aquaculture sites are located near channels or inlets and in sandy sediments, even though there are shellfish leases available over a more widespread area. Clam farming occurs in regions of relatively shallow bathymetry, short water residence time and a large sand sediment fraction. By isolating regions of the VCR meeting these conditions and removing the areas occupied by the public oyster harvesting Baylor Survey Grounds, we identified a range of potential aquaculture area for the hard clam. It is unlikely that clam aquaculture will become spatially limited within the VCR in the near future; however, there remains the need to determine ecosystem impacts of clam aquaculture. The methods applied in this study may be applicable to other systems with shellfish aquaculture provided adequate data availability and analytical tools.

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Tables

Table 2.1: Environmental parameters and values used to model possible locations of clam aquaculture in the VCR. Depth was determined from locations of 2009 clam beds in the VCR or the depth range of Arnold et al. (2000). Water residence time and the sediment fraction greater than 125 μ m (sand) were determined from the 2009 clam beds in the VCR.

Parameter	Minimum	Maximum
Depth (m below MSL)	0.13	2.60
Depth (m below MSL) (Arnold et al. (2000))	0.00	1.80
Water Residence Time (hours)	0.41	107.80
Sediment Fraction Greater than 125 μm	0.40	0.90

Table 2.2: Measured area of the portion of the VCR studied and the 2009 and 2012 clam beds. Also included are areal estimates of the VCR available for clam aquaculture given the various constraints applied.

Location	Constraint	Area (km²)
VCR	None	511.6
2009 Clam Beds	None	1.56
2012 Clam Beds	None	1.81
VCR	Depth Only	373.9
VCR	Water Residence Time Only	247.7
VCR	Grain Size Only	328.9
VCR	Depth and Baylor Grounds Removed	235.9
VCR	Depth, Water Residence Time, Grain Size	186.0
VCR	Depth, Water Residence Time, Grain Size, Baylor Grounds Removed	120.9

Figures

Figure 2.1: A site map of the US East Coast including the Chesapeake Bay and Delmarva Peninsula with an inset of the Virginia Coast Reserve study site.



Figure 2.2: Aerial image from the 2012 Virginia Institute of Marine Sciences annual seagrass survey. Active clam beds are visible as dark rectangles due to macroalgal fouling of anti-predator cover netting. Each clam bed measures 72 m².

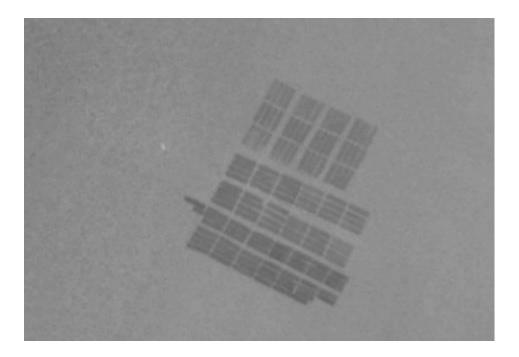


Figure 2.3: The locations of clam beds (red polygons) in 2012 as determined by GIS image analysis and the GPS locations of clam farms in Magothy Bay in 2014 (yellow points). One new site is present in the northern portion of the bay and there is one fewer site just south of the new farm.



Figure 2.4: The distribution of clam aquaculture sites in 2012 within the VCR. Clam farms are highlighted in black and at present occupy a small amount of coastal lagoon area.

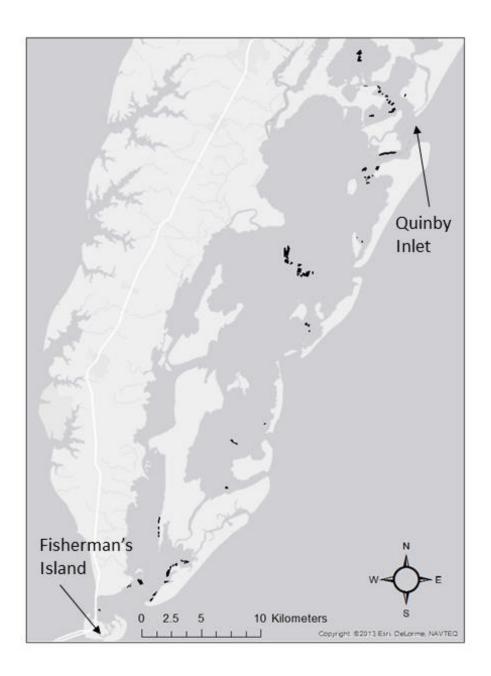


Figure 2.5: The number of individual clam beds per year in the VCR. There is an average increase of about 250 beds per year (y = 248.5x - 496245; $R^2 = 0.72$).

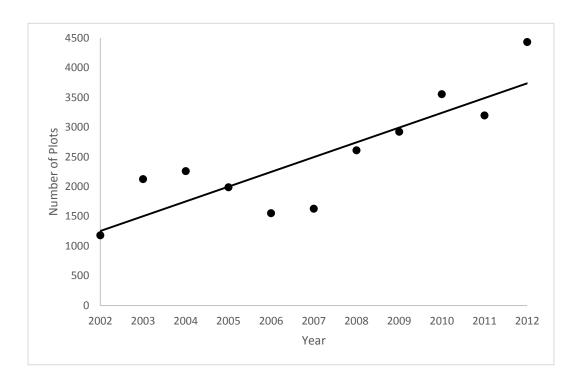


Figure 2.6: Area of the VCR which meets the depth constraints (average min. and max. \pm SD) determined from the 2009 clam beds.

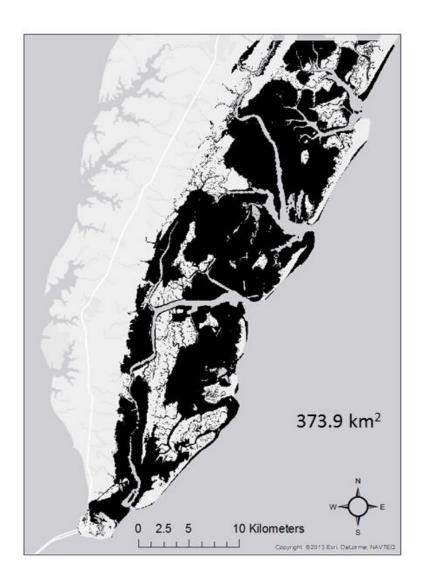


Figure 2.7: Area of the VCR which meets the VCR depths, water residence time, and grain size constraints (average min. and max. \pm SD) set by the 2009 clam beds.

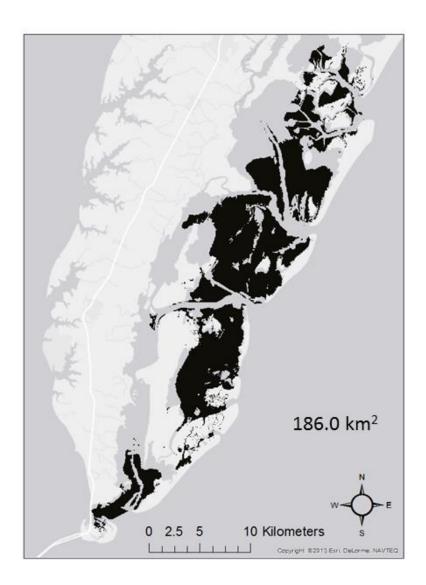
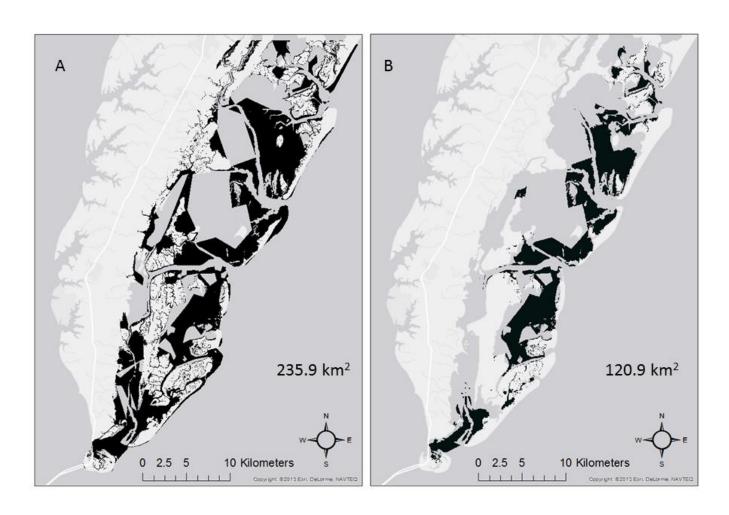


Figure 2.8: Analyses with the public Baylor Survey Grounds removed from potential clam aquaculture sites. A. Area of the VCR which meets the depths (average min. and max. \pm SD) determined from the 2009 clam beds. B. Area of the VCR which meets the VCR depths, water residence time, and grain size constraints (average min. and max. \pm SD) set by the 2009 clam beds.



Chapter 3

Resource use of an aquacultured oyster (*Crassostrea gigas*) in the reverse estuary Bahía San Quintín, Baja California, México^c

^c Submitted for publication with the following co-authors: Grace Wilkinson, Victor Camacho-Ibar, Michael Pace, Karen McGlathery, Jose Sandoval-Gil, Julieta Hernández-López

Abstract

Shellfish aquaculture is prominent in many coastal and estuarine environments and has both ecological and economic effects. Bahía San Quintín is a reverse estuary in Baja California, Mexico where Pacific oysters (Crassostrea gigas) are cultivated. While oysters likely feed heavily on phytoplankton especially during upwelling periods, we hypothesized that other forms of organic matter such as seagrass (Zostera marina) and macroalgae (Ulva spp.) are used by the oysters, especially in the most inshore portions of the bay. We measured the carbon and hydrogen stable isotope composition of oysters and their potential food resources and applied a Bayesian mixing model to evaluate resource use. Hydrogen isotopes provided a large separation between potential food resources. Although we did not find any strong seasonal effects due to upwelling, there was a strong spatial gradient in resource use. Phytoplankton were most important at a lower (oceanic) site (median resource use for two sampling times: 67 and 79%) and decreased up the estuary as macroalgae became more important (44 and 56%). At all sites for both sampling times seagrass was unimportant for oysters. The gradient of high phytoplankton use at the lower site to increased macroalgal use at the upper site is likely due to available resource biomass. Results indicate the adaptability of oysters to varying resource availability and the possibility of a higher system carrying capacity given multiple potential food sources. This study also highlights the utility of hydrogen isotopes in estuarine food web research.

Keywords

Shellfish aquaculture, Bayesian mixing model, *Crassostrea gigas*, carbon, hydrogen, stable isotopes

Introduction

Aquaculture is a rapidly growing industry providing seafood production that is nearly half the amount of capture fisheries (FAO 2014). In nearshore environments, such as estuaries and coastal lagoons, aquaculture is largely of mollusks (Campbell and Pauly 2013, Gallardi 2014). Filter-feeding bivalves remove suspended particles from the water column and models of resource use indicate that phytoplankton are generally their primary food resource (Newell 1988, Riera 2007, Leal et al. 2008, Dame 2012, Guyondet et al. 2013, Filgueira et al. 2014, Saurel et al. 2014). However, phytoplankton may not be the sole or primary resource of aquacultured bivalves in habitats with low phytoplankton populations (Dubois et al. 2007, Secrist 2013, Hondula and Pace 2014).

Resource use by consumers (e.g. oysters) can be highly variable and is dependent on system characteristics including food quantity and quality (Phillips et al. 2014) and also the species' food selectivity (Rosa et al. 2013). Stable isotope analysis has the potential to provide quantitative estimates of resource use by aquatic consumers and to identify important spatial and temporal gradients in feeding (Fertig et al. 2014, Middleburg 2014, Phillips et al. 2014). These gradients may arise from several factors including spatial variation in system properties (Deegan and Garritt 1997, Kanaya et al. 2007, Como et al. 2012, Marchais et al. 2013, Kopp et al. 2014, Vinagre and Costa 2014) and seasonal changes to resource availability and/or isotope values (Page and Lastra 2003, Baeta 2009, Soares et al. 2014). However, the use of stable isotopes may also be complicated because of spatial and temporal dynamics (Peterson 1999). For example, seasonal coastal upwelling may alter nitrogen inputs (Camacho-Ibar et al. 2003, Hernández-Ayón et al. 2004, Zertuche-González et al. 2009) leading to differences in the isotopic

composition of resources and consumers in adjacent systems (Liu and Kaplan 1989, Sigman et al. 2005).

The use of δ^{13} C and δ^{15} N is a common approach in determining resource use in estuarine food webs (Bouillon et al. 2011). However, variability in nitrate supply due to upwelling events (Camacho-Ibar et al. 2003), variable trophic fraction (Post 2002) and internal processes of N-cycling such as denitrification (Fourqurean et al. 1997) and nitrification (Peterson and Fry 1987) may complicate the use of δ^{15} N. An alternative method is to use hydrogen stable isotopes (δ^2 H) because trophic fractionation is minimal and there are often large differences in 2 H values between resources (Solomon et al. 2009, Cole et al. 2011, Hondula et al. 2013, Wilkinson et al. 2013). While the application of δ^2 H in the study of aquatic food webs is relatively common in freshwater ecosystems, its application in estuarine ecosystem studies is scarce, despite its potential utility (Hondula et al. 2013, Hondula and Pace 2014).

In this study we quantified resource use of an aquacultured bivalve, the Pacific oyster (*Crassostrea gigas*), in the reverse estuary Bahía San Quintín. In this system temperature and salinity increase towards the upper bay due to high evaporation and the absence of freshwater inputs. There is also little to no terrestrial inputs of organic matter. Bahía San Quintín also experiences wind-induced coastal upwelling which seasonally affects nutrient availability. We estimated resource use with a Bayesian mixing model using δ^2H and $\delta^{13}C$ for an aquacultured oyster and three potential resources (phytoplankton, seagrass, macroalgae). We expected phytoplankton to be an important resource because seasonal upwelling promotes phytoplankton growth. However, we hypothesized that the use of phytoplankton by oysters would vary by location, with greater use near the mouth of the bay and reduced use in the upper reaches where seagrasses and macroalgae are more abundant.

Methods

Site Description

Bahía San Quintín is on the Pacific coast of Baja California, Mexico (30° 25' N, 115° 59' W) (Figure 3.1). Due to low annual precipitation and minimal freshwater inputs, water temperature and salinity increase with distance away from the mouth of the estuary, hence the reverse estuary classification. Annual water temperatures and salinity at the mouth of the lagoon range from 11-22 °C (Alvarez-Borrego and Alvarez-Borrego 1982) and 33.3-34.0 ppt (Camacho-Ibar et al. 2003), respectively. There is a tidal range of up to 2.5 m in spring tides at this site (Delgado-González et al. 2010), while average depth is 2 m below mean sea level. Off the northwestern Baja California coast, winds promote favorable upwelling conditions throughout the year, however, upwelling events intensify from April to June (Zaitzev et al. 2003). The dominant primary producers, apart from microalgae, include the seagrass *Zostera marina* and the macroalga *Ulva* spp. (Zertuche-González et al. 2009). A large aquaculture industry that raises suspended (hanging line and floating bag) Pacific oysters (*Crassostrea gigas*) operates in the western arm of this system (Figure 3.1).

Sample Collection and Analysis

Samples were collected in November 2013 and June 2014 to include both non-upwelling and upwelling conditions. November samples were obtained at a lower site (Lower) near the mouth of the bay and an upper site (Upper) near the top of the western arm (Figure 3.1). June samples were obtained at the same Lower and Upper sites with the addition of a central site (Mid) located between the other two sites (Figure 3.1). In November and June, fifteen oysters (*Crassostrea gigas*) were taken from aquaculture grow-outs and pooled into five groups of three for analysis. The adductor muscle from each oyster was removed and rinsed with deionized

water before drying at 60°C for 48 hours. Dried muscle tissue was ground with a mortar and pestle to homogenize the sample and stored in 20 ml borosilicate vials prior to analysis. Macroalgae (Ulva spp.), seagrass ($Zostera\ marina$), and phytoplankton were considered as potential resources. Three replicates of seagrass and macroalgae were collected as grab samples in November and June from each site. The seagrass and macroalgae were cleared of epiphytic material and rinsed with deionized water prior to drying at 60°C for 48 hours. Samples were then ground to homogenize and stored in 20 ml borosilicate vials until isotopic analysis. Stable isotope analysis for all solid samples (oysters, seagrass and macroalgae) was completed by the Colorado Plateau Stable Isotope Laboratory (CPSIL, www.isotope.nau.edu) following the procedures reported in Doucett et al. (2007). Values are reported in per mil (%) notation and are relative to the international standards Vienna Pee Dee Belemnite (VPDB) for δ^{13} C and Vienna Standard Mean Ocean Water (VSMOW) for δ^{2} H.

A site-specific phytoplankton isotopic value was calculated from measurements of inorganic substrates (water and dissolved inorganic carbon) measured at each site. Water samples from each site were filtered using 47 mm diameter cellulose acetate membrane filters (nominal pore size $0.8\mu m$, Whatman). Filtered water for hydrogen isotope analysis ($\delta^2 H_{water}$) was stored in 20 ml borosilicate vials with no headspace and refrigerated. Analysis was completed at the University of California Davis Stable Isotope Facility (www.stableisotopefacility.ucdavis.edu). Filtered water for isotopic analysis of dissolved inorganic carbon (δ^{13} C-DIC) was preserved with 1 ml mercuric chloride to halt biological activity and stored in 40 ml amber borosilicate vials with black butyl rubber septa and no headspace and refrigerated until analysis. November samples were analyzed at CPSIL and June samples were analyzed at the Boston University Stable Isotope Laboratory (www.bu.edu/sil).

Seawater δ^2 H and δ^{13} C-DIC were analyzed at different laboratories, but values were consistent with prior surface ocean measurements (Bidigare et al. 1997, Lécuyer et al. 1998). Calculation of Phytoplankton Isotopic Composition

The phytoplankton isotopic value was calculated using the inorganic measurements from each site and discrimination values for each isotope. The $\delta^{13}C$ of phytoplankton ($\delta^{13}C_{phyto}$) was calculated as

$$\delta^{13}C_{Phyto} = \delta^{13}CO_2 - \varepsilon_C$$
 [1]

where $\delta^{13}C$ of the aqueous CO_2 ($\delta^{13}CO_2$) was calculated from the $\delta^{13}C$ -DIC (Zhang et al. 1995) and a marine algae fractionation term $\mathcal{E}_C = -16.3 \pm 0.7\%$ (Laws et al. 1995). Values of \mathcal{E}_C can vary with environmental conditions and species (Hinga et al. 1994). However, the mean \mathcal{E}_C value from Laws et al. (1995) was based on samples taken in the equatorial Pacific and is similar to values from other studies (Bidigare et al. 1997, Popp et al. 1998). The δ^2H of phytoplankton (δ^2H_{phyto}) was determined in a similar manner using equation 2,

$$\delta^{2H_{Phyto}} = \delta^2 H_{Water} - \varepsilon_H$$
 [2]

measured values of $\delta^2 H_{water}$ and a fractionation term $\epsilon_H =$ -157.5 \pm 16.5% (Hondula and Pace 2014). Isotopic values of the other resources (seagrass and macroalgae) used in the model came from the direct measurements of those materials as described above.

Bayesian Mixing Model

A Bayesian mixing model written in R and JAGS (Just Another Gibbs Sampler) was used to estimate resource fractions of phytoplankton, macroalgae and seagrass assimilated by aquacultured oysters. This mixing model, modified from Wilkinson et al. (2013), incorporates all

of the isotope data, prior information and uncertainty to produce posterior estimates of resource fractions in the following equations.

$$\delta^{13}C_{oysters} = (\phi_z \times \delta^{13}C_z) + (\phi_{ma} \times \delta^{13}C_{ma}) + (\phi_{phyto} \times \delta^{13}C_{phyto}) + \Delta C$$

$$\delta^2 H_{oysters} = (\phi_z \times \delta^2 H_z) + (\phi_{ma} \times \delta^2 H_{ma}) + (\phi_{phyto} \times \delta^2 H_{phyto}) \times (1 - \omega) + \omega \times \delta^2 H_{water}$$

$$1 = \phi_z + \phi_{ma} + \phi_{phyto}$$

In this system of equations ϕ is the fraction each given end member (z = seagrass, ma = macroalgae and phyto = phytoplankton) contributed to the measured oyster isotope values. Resource fractions are estimated in the model and given an uninformed prior distributions that are center log ratio transformed (Semmens et al. 2009; Solomon et al. 2011). In eq. 3, the term ΔC is the trophic fraction for carbon by the oysters. A value of $\Delta C = 1.05\% \pm 0.75$ was used from a similar shellfish study using aquacultured hard clams (*Mercenaria mercenaria*) (Hondula and Pace 2014). In eq. 3, the ω term accounts for the contribution of environmental water to oyster organic matter and was set at $\omega = 0.15 \pm 0.09$ as determined by Hondula and Pace (2014) for hard clams.

Results

Consumer and Resource Isotope Values

The end members were well separated in $\delta^{13}C$ and $\delta^{2}H$ isotope space (Figure 3.2). Seagrass mean $\delta^{13}C$ values ranged from -9.35 to -7.65‰ and were the most positive of the samples collected (Supplementary Table 3.1). Seagrass mean values of $\delta^{2}H$ were more variable across sites and ranged from -94.65 to -76.40‰ (Supplementary Table 3.1). Macroalgae mean values of $\delta^{13}C$ ranged from -13.86 to -11.68‰ and $\delta^{2}H$ from -206.12 to -174.16‰ (Supplementary Table 3.1). DIC $\delta^{13}C$ ranged from -0.68 to 1.34‰ and $\delta^{2}H$ from -2.30 to 0.00‰

(Supplementary Table 3.1). The mean phytoplankton end member isotope values calculated for each site ranged from -26.1 to -24.5% for δ^{13} C and from -159.8 to -157.5 for δ^{2} H (Supplementary Table 3.1).

Oyster mean δ^{13} C values were more negative at the Lower site than the Upper site in both November and June and also differed by about 2-3‰ on both occasions, with a total range of -20.32 to -17.36‰ across sites and seasons (Supplementary Table 3.1). Uncorrected oyster mean δ^2 H values did not vary substantially by site and fall within a small range of -151.42 to -147.53‰ (Supplementary Table 3.1). Prior to plotting, oyster carbon and hydrogen isotope values were corrected for trophic fraction and environmental water, respectively. Corrected oyster isotope values fell on or adjacent to the mixing line between the phytoplankton and macroalgae end members for each sampling site and time (Figure 3.2).

Mixing Model Results

As indicated by the triangles in Figure 3.2, seagrass was not a significant resource to oysters at any site in both November and June (Figure 3.3). The median values of the posterior distributions of resource use for seagrass ranged from 0.03 to 0.07 (Figure 3.3). While phytoplankton was the most important resource (medians: 0.38-0.79) in all but one model run, its importance was diminished at the Upper site (Figure 3.3). Phytoplankton resource use decreased with increasing distance from the mouth of the estuary. The use of macroalgae by the oysters increased from 0.13-0.23 at the Lower site to 0.44-0.56 at the Upper site (Figure 3.3).

Discussion

Spatial patterns of resource use

Aquacultured oysters at all sites in Bahía San Quintín assimilated multiple food resources. There was a strong spatial pattern in resource use within the estuary with phytoplankton and macroalgae as the primary resources for oysters. At the Lower site, phytoplankton were the primary resource for aquacultured oysters, contributing 68 – 79% (Figure 3.3). Results from the Mid site were similar with a median of 59% and overlapping distributions (Figure 3.3). The Upper site, however, was more variable and the mixing model indicated lower use of phytoplankton and greater use of macroaglae, with medians ranging from 44 to 56% (Figure 3.3). Macroalgae were also important resources at the lower site, contributing 13 – 23% (Figure 3.3). Phytoplankton are abundant throughout Bahía San Quintín (Millán-Núñez et al. 2004, Gracia-Escobar et al. 2014) and are typically an important food source to the aquacultured oysters. *Ulva* spp. is an important source of organic matter and prior research indicated this alga is highly abundant in the western arm of the bay (Zertuche-González et al. 2009). Our results are also consistent with a shift in the contributions by phytoplankton and *Ulva* spp. to the sediment organic carbon pool in subtidal seagrass meadows from 56% and 16% respectively at a station in the south of the lagoon to 38% and 47% respectively at a station near our Upper site as reported by Jorgensen (2006).

Seagrass was not important as a resource to oysters for any of the sites or seasons sampled (Figures 3.2 and 3.3), despite its notable seasonal productivity patterns in Bahía San Quintín (Cabello-Pasini et al. 2003). Although *Z. marina* is a significant component of the benthic and intertidal environments in Bahía San Quintín (Ward et al. 2003), it is composed of more refractory carbon related to structural tissue (Cabello-Pasini et al. 2004) than phytoplankton and macroalgae, which likely limits its trophic role (Klumpp et al. 1992). The

same species of seagrass was also unimportant in supporting hard clam aquaculture in the Virginia Coast Reserve, USA (Hondula and Pace 2014).

Seasonal patterns of resource use

Bahía San Quintín opens into the Eastern Pacific ocean and is subject to seasonal coastal upwelling. Typically, this leads to seasonal changes in nutrient inputs with enhanced primary production occurring during the upwelling season (Millán-Núñez et al. 1982, Camacho-Ibar et al. 2003, Ribas-Ribas et al. 2011). The purpose of sampling during November and June was to contrast resource use during upwelling and non-upwelling conditions, respectively. Sampling occurred in the months after peak upwelling and non-upwelling conditions to ensure that tissue isotope values would be reflective of those conditions given the longer turnover time of muscle tissue (Yokoyama et al. 2005, Thomas and Crowther 2014). However, upwelling conditions off Bahía San Quintín were weaker than normal prior to June sampling with upwelling indices 28-63 m³/second/100 m coastline below April and May averages for 2011-2013 (NOAA 2014). Therefore, differences in environmental conditions between November and June were likely small. An increase in phytoplankton availability and potential importance as a food item at all sites was expected in June due to upwelling, but the opposite occurred with macroalgae becoming a more important food source at the Lower and Upper sites in June (Figure 3.3). *Ulva* spp. also responds to upwelling nutrient supply and its biomass tends to peak in summer months in estuaries of the northeastern Pacific region (Hessing-Lewis and Hacker 2013). Were stronger upwelling conditions present, there potentially would have been a greater shift in resource use (Page and Lastra 2003).

Summary and Implications

Although we were unable to detect strong seasonal effects in oyster resource use due to a weak upwelling event, there was a clear spatial gradient in resource use from phytoplankton to macroalgae moving up the bay from the mouth which was present in both November and June (Figure 3.3). Generally, resource use by estuarine consumers varies based on location due to freshwater/terrestrial inputs, changes in primary producers, temperature and salinity, and other factors (Deegan and Garritt 1997, Doi et al. 2005, Oczkowski et al. 2010, Como et al. 2012, Marchais et al. 2013, Kopp et al. 2014). Locally for Bahía San Quintín, this variation in resource use between the Lower and Upper sites was probably controlled by resource availability. Phytoplankton production and biomass decrease from the mouth of the estuary towards the upper reaches (Millán-Núñez et al. 1982, Gracia-Escobar et al. 2014), while *Ulva* spp. biomass and abundance increase, especially in the western arm (Ward et al. 2003, Zertuche-González et al. 2009, Jorgensen et al. 2010).

Our isotope data and modelling interpretation for Bahía San Quintín indicate that the aquacultured oysters did not rely solely on phytoplankton. The hydrogen and carbon isotopes clearly distinguished potential resources from one another. Our model results are supported by the consumer data that fall within the mixing polygon between the well-constrained phytoplankton and macroalgae end members and far from seagrass. Thus, our results avoid several issues with isotope mixing models highlighted by Fry (2013). In addition, prior studies indicate use of macroalgae by both cultured clams (Hondula and Pace 2014) and Pacific oysters (Dubois et al. 2007, Lefebvre et al. 2009). Interestingly, the dominant macroalgal in this study, *Ulva* spp., is an important dietary resource to marine invertebrates, increasing productivity, reducing mortality, and stimulating feeding and protein intake when studied as a dietary additive or intervention for aquacultured seafood (Cyrus et al. 2014, Lange et al. 2014).

Our results indicated the importance of both phytoplankton and macroalgae as resources to the cultured oysters. As the use of coastal waters for bivalve aquaculture increases, so does the potential for these systems to reach or exceed their carrying capacity, estimates of which are generally based on phytoplankton or seston availability, the organisms' filtration rate, and the amount and spatial distribution of the shellfish (Dame and Prins 1998, Delgado-González et al. 2010, Guyondet et al. 2013, Filgueira et al. 2014, Saurel et al. 2014). The oysters in Bahía San Quintín have two potential food sources in phytoplankton and macroalgae that contribute to the system's carrying capacity and provide some resilience to changing conditions and resource availability.

Stable isotopes are often used to identify consumer resource use. By using two isotopes that provided a large distinction between possible end members (δ^2H and $\delta^{13}C$), we were able to discern the relative importance of various resources. Carbon isotopic composition distinguished seagrass and phytoplankton while hydrogen isotopic composition distinguished seagrass, phytoplankton and macroalgae. This study highlights the utility of hydrogen isotopes in estuarine food web research.

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Figures

Figure 3.1: Map of Bahía San Quintín and the three oyster farm locations (stars) used in this study. U = Upper site, M = Mid site, and L = Lower site

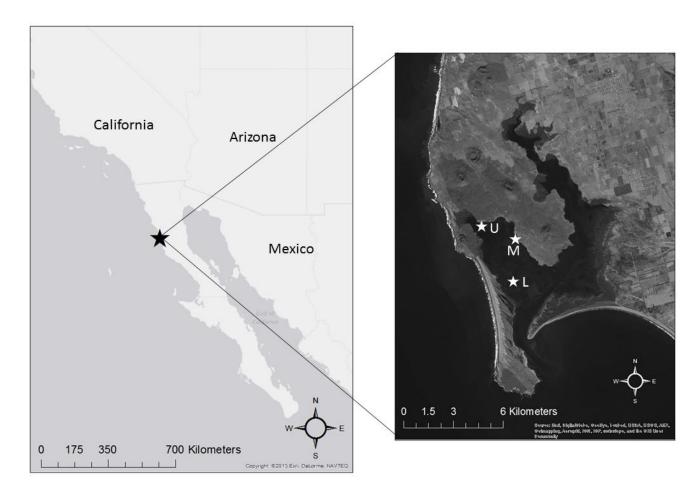


Figure 3.2: Consumer and end member δ^2H and $\delta^{13}C$ isotope values. Uncorrected oyster values (grey) are initial isotope results whereas corrected oyster values (black) have been adjusted for trophic fractionation (carbon) and dietary water (hydrogen) and are the value used in the model. Error bars represent standard deviation, $\delta^{13}C$ error bars are smaller than symbols. A) Lower site November 2013. B) Upper site November 2013. C) Lower site June 2014. D) Mid site June 2014. E) Upper site June 2014

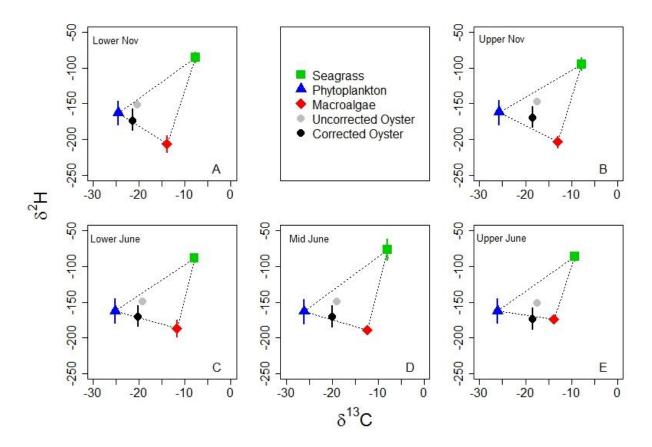
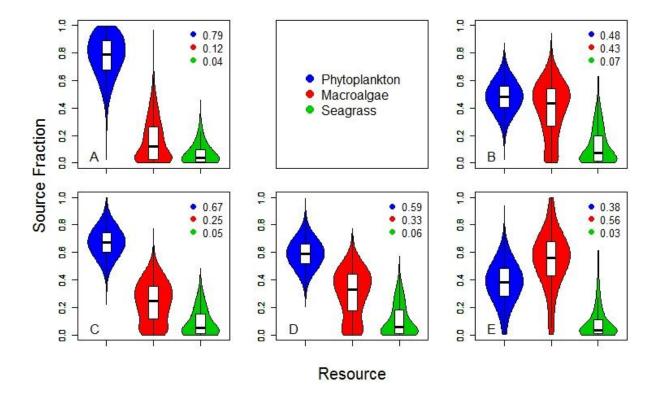


Figure 3.3: Posterior distribution of the source fraction estimates from the Bayesian mixing model. Width of violin plots represents frequency. Median values are reported for each source considered. Box-plots (white areas within violins) display median, 25th and 75th percentiles. Phytoplankton are displayed in blue, macroalgae in red, and seagrass in green. A) Lower site November 2013. B) Upper site November 2013. C) Lower site June 2014. D) Mid site June 2014. E) Upper site June 2014



Supplemental Material

Supplemental Table 3.1

Hydrogen ($\delta^2 H$) and carbon ($\delta^{13} C$) mean isotope values for the organic material sampled at all sites November 2013 and June 2014. For the inorganic material (seawater) the mean $\delta^{13} C$ for total dissolved inorganic carbon is presented. This value was used to calculate $\delta^{13} C$ of the CO_2 component used for the phytoplankton $\delta^{13} C$ estimation.

Organic Material	Site	Month	Mean δ ¹³ C (SD) ‰	Mean δ ² H (SD) ‰
Oysters	Lower	Nov	-20.32 (0.25)	-151.42 (2.74)
Oysters	Upper	Nov	-17.45 (0.16)	-147.53 (2.85)
Oysters	Lower	June	-19.19 (0.31)	-149.17 (3.72)
Oysters	Center	June	-18.92 (0.15)	-148.78 (4.63)
Oysters	Upper	June	-17.36 (0.10)	-151.50 (2.52)
Macroalgae	Lower	Nov	-13.86 (1.10)	-206.12 (11.83)
Macroalgae	Upper	Nov	-12.93 (0.57)	-203.15 (8.00)
Macroalgae	Lower	June	-11.68 (0.59)	-186.59 (11.50)
Macroalgae	Center	June	-12.38 (0.60)	-188.66 (3.16)
Macroalgae	Upper	June	-13.81 (0.70)	-174.16 (1.39)
Seagrass	Lower	Nov	-7.65 (0.52)	-85.46 (7.66)
Seagrass	Upper	Nov	-7.83 (0.33)	-94.65 (9.16)
Seagrass	Lower	June	-7.96 (0.19)	-88.62 (5.72)
Seagrass	Center	June	-8.04 (0.21)	-76.40 (15.10)
Seagrass	Upper	June	-9.35 (0.75)	-86.03 (6.69)
Inorganic	•••		512 0 (07) 0(53(57.) 64
Material	Site	Month	Mean δ ¹³ C (SD) ‰	Mean δ²H (SD) ‰
Seawater	Lower	Nov	1.34 (NA)	-2.30 (0.48)
Seawater	Upper	Nov	-0.03 (NA)	0.00 (0.36)
Seawater	Lower	June	0.37 (0.01)	-1.61 (0.25)
Seawater	Center	June	-0.68 (0.12)	-2.09 (0.49)
Seawater	Upper	June	-0.67 (0.10)	-1.78 (0.10)