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The invasion of the colonial ascidian, *Didemnum vexillum*, into south shore bays of Long Island, New York — Feeding and metabolic characteristics

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Abstract of the Thesis

The invasion of the colonial ascidian, *Didemnum vexillum*, into south shore bays of Long Island, New York — Feeding and metabolic characteristics

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The invasive ascidian, *Didemnum vexillum*, has recently spread into south shore bays of Long Island, New York including Great South Bay and Shinnecock Bay. In order to determine the impact this invasive species will have on these bays, colonies from Shinnecock Bay were studied, due to easiest accessibility. *D. vexillum* currently forms extensive mats on the pillars of Ponquogue Bridge located in Shinnecock Bay. These mats have the potential to drastically alter the structure of the ecosystem; an effect which has been observed in other waters that the species has invaded. Thus, in order to predict the impact *D. vexillum* may have on predator-prey relationships in Shinnecock Bay, clearance rates (L g_{dw}^{-1} h^{-1}) on total chlorophyll-*a* and picoplankton were measured, both in the laboratory and in the field, from May, 2009 through October, 2009. In addition, during the clearance rate experiments conducted in the laboratory, oxygen consumption was measured. Field growth rates of *D. vexillum* colonies were also estimated. Using the growth and oxygen consumption rates, as well as literature values of ammonia excretion and absorption efficiency, I calculated how much energy (g_{dw}^{-1} h^{-1}) the species needed to consume to support the observed growth. Clearance rates from both laboratory and field experiments were always very low, and most were not statistically significant from zero, suggesting *D. vexillum* did not feed during the experiments. Five out of the seven oxygen consumption data were statistically significant from zero; however the values were lower than most other ascidians, suggesting the colonies of *D. vexillum* were limiting their energy expended by not feeding on suspended particles in the water column. Estimated clearance rates needed to support the growth rates were much higher than the measured clearance rates and were within the range of values for other colonial ascidians. However, these estimates may be lower than what actual clearance rates of *D. vexillum* are due to low growth and oxygen consumption rates.

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List of Symbols and Abbreviations

º — degrees

$ — dollars

´ — minutes

% — percent

± — plus or minus

² — squared

A — energy absorbed

C — Celsius

CR — clearance rate

cm — centimeters

d — day

dw — dry weight

F_0 — chlorophyll-*a* concentration at time 0

F_t — chlorophyll-*a* concentration at time t

FL — fluorescent parameter

FSC — forward scatter

g — grams

GF/F — glass fiber/fine

h — hour

J — joules

kJ — kilojoules

km — kilometers

L — liters

M — volume

m — meters

mg — milligrams

ml — milliliters

mm — millimeters

N — North

nm — nanometers

O_2 — oxygen

P — production

pH — potential of hydrogen

ppt — parts per thousand

PVC — polyvinyl chloride

R — metabolic rate

SD — standard deviation

t — time

U — ammonia excretion

µg — micrograms

µm — micrometers

µmol — micromole

W — West

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Introduction

Didemnum vexillum is an invasive benthic tunicate that has been called an "ecosystem engineer" due to its ability to drastically change the predator-prey dynamics of ecosystems that it invades (Wallentinus and Nyberg, 2007). Species diversity has been reduced by *D. vexillum* in locations such as Georges Bank (off the coast of New England), where *D. vexillum* was first found in 1998 (Bullard et al., 2007a). The species currently covers between 50 and 90% of a 230 km^2 area there (Bullard et al., 2007a). In addition, the invasion of *D. vexillum* into Shakespeare Bay, New Zealand has caused $807,000 of damage to the *Perna canaliculus* (green mussel) fishery over five years (Sinner and Coutts, 2003).

Thus, it is important to study the invasion of *Didemnum vexillum* into south shore bays of Long Island (New York) in order to predict the impact this invasive species will have on these ecosystems. Due to accessibility, colonies from Shinnecock Bay were studied. Since *D. vexillum's* initial invasion into Shinnecock Bay in 2004, the species has formed extensive mats on the pillars of Ponquogue Bridge (40º50.52´ N, 72º29.98´W) (Bullard et al., 2007a). These mats cover native organisms that reside on the pillars, and outcompete them for space, thus reducing species diversity. If this ascidian continues to spread throughout Shinnecock Bay and other south shore Long Island bays, it could greatly alter the ecosystem structure of the bays, directly through smothering organisms, and through indirect means such as trophic cascade. In addition, *D. vexillum* may possibly benefit certain organisms such as *Zostera marina* (eelgrass) by filtering particles out of the water column, thus allowing more light to reach the plant which could promote greater growth. At the same time it may harm other suspension feeders if food is a limiting factor within the ecosystem.

In order to predict ecological effects of *Didemnum vexillum* in south shore Long Island bays, its clearance rates and particle size selection need to be known. While clearance rates of several species of ascidians are known, there is no literature on the specific clearance rate of *D. vexillum* to date (Petersen, 2007). Thus, clearance rate experiments using colonies from Ponquogue Bridge were conducted both in the laboratory and in the field from May through October, 2009. At the same time, oxygen consumption was measured, as well as growth rates, to determine metabolic characteristics of *D. vexillum*.

Background

Origin and Distribution

While it is known that *Didemnum vexillum* is an invasive species to the United States, its origin is unknown. Recent reports point to Japan as its native origin because a museum sample from Mutsu Bay, collected in 1926, appears to be *D. vexillum* (Lambert, 2009). How the species spread to other countries is still under debate. The mode of transport is most likely boats and ships, both recreational and commercial, since the species has been found within ship hulls as well as attached to the bottoms of boats. *D. vexillum* probably fouled barges as opposed to fast moving ships which move too quickly for *D. vexillum* to remain attached (Lambert, 2009). Some believe it may have entered new locations via boats carrying fouled oysters from Japan (Lambert, 2009). This is likely not the case since *D. vexillum* became apparent many years after the importation of oysters from Japan stopped in the 1960s (Lambert, 2009). Once *D. vexillum* invades an area it thrives because it is a strong spatial competitor (Lambert, 2009).

The first official documentation of *Didemnum vexillum* in the United States occurred in the Damariscotta River, Maine, in 1993, with anecdotal reports dating back to 1988 (Valentine et al., 2007a). However, it is likely that this invasive species has been there since the 1970s based on reports from oystermen in Maine (Bullard et al., 2007a). In addition, photographs from 1982 show many colonies of *D. vexillum* on oyster aquaculture nets in the Damariscotta region (Dijkstra et al., 2007). Initial observations of *D. vexillum* were few, due to small and isolated populations of *D. vexillum*, but as the population began to grow in the 1990s the species rapidly increased in both population size and distribution (Bullard et al., 2007a). Along the east coast of the United States, *D. vexillum* is found on hard substrates in the majority of subtidal ecosystems from Eastport, Maine to Shinnecock, New York, a 750 km range (Carman and Roscoe, 2003). *D. vexillum* is also found along more than 800 km of the west coast of the United States where it was first observed in San Francisco Bay in 1993 (Bullard et al., 2007a). It has since extended its range so that it is currently found from Humboldt Bay to Port San Luis, California, as well as at locations within the Puget Sound, Washington (Bullard et al., 2007a). *D. vexillum* is also found in Canada, Europe, and New Zealand, which is the only country in the southern hemisphere with documented reports of *D. vexillum* (Bullard et al., 2007a; Coutts, 2002; Lambert, 2009). Ranges in both the United States and abroad are continuing to increase.

Colonies of *Didemnum vexillum* are most commonly observed in shallow subtidal locations, but have been observed at depths in excess of 81 m (Bullard et al., 2007a). Such sites include Georges, Stellwagen, and Tillies Banks off the coast of New England. Colonies are also found along the bottom of the Long Island Sound (35 m) (Bullard et al., 2007a). At Georges Bank, where the water column is mixed year round and thus food is not limiting, *D. vexillum* dominates the seafloor (Bullard et al., 2007a; Valentine et al.,

2007b). Most other invasive ascidians along the coasts of the United States favor shallow waters and are not found in deep waters. This suggests that *D. vexillum* is more successful in deeper, cooler waters than other invasive ascidians in the same areas (McCarthy et al., 2007). *D. vexillum* might be unable to survive the temperature extremes of shallower waters and thus is found at deeper sites which have cooler temperatures in the summer and warmer temperatures in the winter (Osman and Whitlatch, 2007). During warmer summers, the growth rate of *D. vexillum* decreases, but it increases during colder summers (McCarthy et al., 2007). In addition to being found subtidally and in deeper waters, *D. vexillum* has also been observed to survive air exposure during spring tides in the lower intertidal zone in locations such as a tide pool at Sandwich, Massachusetts during the spring, summer, and fall (Bullard et al., 2007a). In New York, colonies on Ponquogue Bridge in Shinnecock Bay were observed above the low tide mark in the fall of 2009 (personal observation).

As demonstrated by the current geographic range, *Didemnum vexillum* most commonly grows in temperate environments and can be found in waters with temperatures ranging from -2ºC to over 24ºC, but does not thrive in either of the temperature extremes (Bullard et al., 2007a). Presently, *D. vexillum* has not been observed south of New York, probably due to the fact that summer water temperatures become too high for the species to survive. Salinity also greatly affects growth and viability of *D. vexillum.* Growth rates are significantly higher in high salinity (26-30 ppt) areas, while in lower salinity areas its health deteriorates (Bullard and Whitlatch, 2009). This is to be expected since the majority of ascidians favor high salinity areas.

Didemnum vexillum is found on many hard substrates and can occasionally be observed on soft substrates, such as sand, as long as they are not completely buried (Bullard et al., 2007a). Natural hard substrates for this species include gravel and subtidal rocks, but it is more often observed on human-made substrates such as submerged ropes, tires, boat hulls, sides and bottoms of docks, pilings and pillars (Bullard et al., 2007a). In Shinnecock Bay, *D. vexillum* is found on a human-made hard substrate, the pillars of the Ponquogue Bridge.

The physical appearance of *Didemnum vexillum* varies depending on location. This colonial tunicate ranges in color from pink, pale orange, to tan. It is composed of many zooids, ranging from colorless to pale yellowish-orange, that are 0.2 mm wide and 1 mm long (Kott, 2004). *D. vexillum* has sparse, calcareous spicules mostly found in the upper layer of the tunic (Lambert, 2009). Spicule growth is affected by salinity (Lambert., 2009). Colonies in high salinity areas have more dense spicules, while *D. vexillum* has less dense spicules in low salinity areas and becomes almost aspiculate (Lambert, 2009). The growth form of *D. vexillum* is also habitat dependent. Key factors possibly regulating the growth form include the type of habitat, amount of available space, and current velocities (Bullard et al., 2007a). Mat-like forms are typically observed in areas where there are strong currents, while rope-like forms up to 1 m long are found in areas with weaker currents (Bullard et al., 2007a). This is true of the field site in Shinnecock Bay. This location is close to Shinnecock Inlet and thus there are strong tidal currents which promote the growth of the mat-like forms that are observed on the pillars of the bridge (Figure 1).

Figure 1. Mat-like colony of *Didemnum vexillum* on a pillar of Ponquogue Bridge in October, 2009.

How far *Didemnum vexillum* can extend its range, recover from seasonal die-backs, and dominate areas it has already invaded, is greatly influenced by reproduction. This invasive ascidian can reproduce sexually and brood its larvae, a characteristic of all colonial ascidians (Bullard et al., 2007a). Panktonic larvae are released through the cloacal siphon over several months of the year when water temperatures are between 14 and 20ºC (Lambert, 2009). The appearance of newly settle juveniles varies with geographic location (Valentine et al., 2007a). On the northeast coast of the United States, most juveniles are found between July and November, while on the coast of California, peak settlement occurs between July and August (Bullard et al., 2007a).

In addition to sexual reproduction, *Didemnum vexillum* can reproduce asexually via fragmentation to create new colonies (Bullard et al., 2007a). Rope-like colonies with large, fragile lobes are especially prone to fragmentation (Bullard et al., 2007a). Fragments can be transported significant distances before reattaching because they can survive in the water column for up to four weeks (Bullard et al., 2007b). Fragmentation may result in a greater probability of survival due to the possibility that reattached lobe fragments are less likely to be harmed by predators or competition compared to newly settled larvae, thus greatly enhancing the dispersal potential of *D. vexillum* (Marshall and Keough, 2003). It has been observed in New Zealand that colonies attached to the bottom of barges have broken off and reattached to and grown on benthic substrates located on the seafloor (Coutts, 2002). Fragments of *D. vexillum* are able to re-attach within six hours of contact with a hard substrate (Lengyel et al., 2009). Another advantage of fragmentation is that fragments can carry brooded larvae which can lead to further dispersal (Bullard et al., 2007a). This behavior may explain why *D. vexillum* is so dominant in Georges Bank, where numerous scallop dredging operations resulted in much fragmentation (Bullard et al., 2007a).

Ecological Interactions

With the aid of fragmentation, *Didemnum vexillum* now overgrows many organisms including macroalgae, bryozoans, sponges, hydroids, scallops, anemones, mussels, tubiculous polychetes, crustaceans, as well as other ascidians (both colonial and solitary) in areas it has invaded (Bullard et al., 2007a). While the abundance of *D.*

vexillum may be limited when competitors are present, the species can still overgrow or displace the majority of other species competing for the same space (Osman and Whitlatch, 2007). Within certain areas, up to 75% of benthos in Long Island Sound and Georges Bank are covered by *D. vexillum* (Lengyel et al., 2009; Whitlatch and Osman, 2009). In Long Island Sound (New York), the only two species that *D. vexillum* did not overgrow were *Astrangia poculata* (northern star coral) and *Ceriantheopsis americana* (cerianthid anemone) (Bullard et al., 2007a).

Mat-like colonies of *Didemnum vexillum* have dramatic ecological effects by changing the habitat complexity of the seafloor from a three-dimensional system to a two-dimensional system (Mercer et al., 2009). They limit food delivery to other benthic animals and reduce settlement of other organisms. In addition, biogeochemical cycling of many elements and nutrients may be altered by *D. vexillum* colonies on the seafloor. The mats may decrease oxygen exchange between the water and sediments, which may further alter distribution of other species (Mercer et al., 2009).

Mats of *Didemnum vexillum* can cover the siphons of epifaunal and infaunal bivalves within a few weeks, thus starving them and possibly resulting in death (Bullard et al., 2007a). *D. vexillum* has been observed on *Mytilus edulis* (blue mussels), *Crassostrea virginica* (eastern oysters), and *Placopecten magellanicus* (sea scallops) (Bijkstra et al., 2007). Thus, *D. vexillum* can pose a significant threat to the aquaculture industry, which has already been observed in Shakespeare Bay, New Zealand. There, *D. vexillum* caused damage by directly smothering the green mussels as well as fouling the aquaculture nets and bags, thus restricting water movement and food delivery to mussels (Carver et al., 2003).

Although *Didemnum vexillum* harms some species, it may also help other species by providing a new habitat that gives protection from epibenthic predators, such as for crabs. Crabs were typically observed within the crevices of colonies of *D. vexillum* on the pillars of Ponquogue Bridge. In addition, *D. vexillum* may also benefit species such as eelgrass by filtering the water column of particles, thus allowing more light to reach the bottom (Mercer et al., 2009).

It was first thought that *Didemnum vexillum* may be protected from predation through chemical defenses (Bullard et al., 2007a). Although this has not been directly observed in *D. vexillum*, other *Didemnum* species use chemical defenses (Vervoort et al., 1998; Pisut and Pawlik, 2002). For example, *Didemnum conchyliatum*, which can be found in Caribbean waters, utilizes the chemical isolate "didemnimide D" (Vervoort et al., 1998). This toxin is very potent and deters feeding by fish at very low concentrations. However, recent reports determined that *D. vexillum* does not contain potent anti-predator secondary metabolites (Lambert, 2009). Instead, it is believed that generalist fish predators are deterred by the low surface pH of the tunic which ranges from 2 to 3 (Bullard et al., 2007a). The acidic nature of cell lysates is caused by an intracellular reducing system, which is commonly found in ascidians (Coutts, 2002). The acidic nature of *D. vexillum* may also deter larval settlement. Substrates that used to be settling grounds for larvae may become dominated by *D. vexillum* when it invades an area. Larvae are unlikely to settle on the tunic of *D. vexillum* because studies have shown that when pH is less than 6.75, abnormal development, as well as mortality, occurs in molluscan larvae (Calabrese and Davis, 1966). Thus, the available space for larval settlement may be reduced due to the invasion of *D. vexillum*.

It appears that adult and juvenile *Didemnum vexillum* are not significantly affected by predators and are able to flourish where other ascidian invaders cannot, because of high predation on new recruits and young juveniles (Osman and Whitlatch, 2007). However, in predation experiments, *D. vexillum* recruits were eaten by at least one predator (which was not identified) (Osman and Whitlatch, 2007). In addition, degenerating colonies, which are observed during periods of extreme temperatures, are preyed upon by *Littorina littorea* (common periwinkle) (Valentine et al., 2007a). Photographs taken in British Columbia displayed a large seastar and sea urchins feeding on *D. vexillum* (http://woodshole.er.usgs.gov/project-pages/stellwagen/didemnum/index.htm). A chiton has also been seen feeding on *D. vexillum* in New Zealand (Lambert, 2009)

Feeding Characteristics

Like most ascidians, *Didemnum vexillum* is a benthic suspension feeder using a branchial basket and a mucus net to collect particles (Bone et al., 2003). Seawater and particles are brought into each individual zooid via its oral siphon (Valentine et al., 2007a). The mucus net is composed of very fine filaments, 10-40 nm thick, and consists of rectangular meshes that are 0.2-0.5 μm wide and 0.5-2.2 μm long (Bone et al., 2003). The mucus net of *D. vexillum* can filter particles as fine as 0.6 μm, significantly smaller than the size most bivalves can efficiently filter (Riisgaard and Larsen, 2001). The mucus net is transported along the inner side of the branchial basket after being produced by the endostyle (Werner and Werner, 1954). Cilia, which line the stigmata openings of the branchial basket, then pump water through the inhalant siphon (Peterson, 2007). A food string forms along the dorsal lamina as a result of particles becoming trapped in the mucus net (Peterson, 2007). This food string is drawn to the esophagus and enters the digestive tract. Fecal pellets leave the zooid through the cloacal siphon, into the cloacal canal system, and finally into cloacal apertures on the surface of the colony (Valentine et al., 2007a). *D. vexillum* has been observed to be non-selective with respect to size of suspended particles, as is the case for other mucus web feeders (Randløv and Riisgård, 1979; Robbins, 1983, 1984; Klumpp, 1984; Stuart and Klumpp, 1984; Bingham and Walters, 1989; Ribes et al., 1998). This ascidian's high efficiency in collecting small particles may potentially help to decrease abundance of picoplankton, including *Aureococcus anophagefferens*.

Aureococcus anophagefferens was first observed in the mid-Atlantic coastal waters of the United States in 1985 (Bricelj and Lonsdale, 1997). *A. anophagefferens* has been observed in Long Island bays (New York), Narragansett Bay (Rhode Island), and Barnegat Bay (New Jersey) (Bricelj and Lonsdale, 1997) and recently found in waters off of Delaware, Maryland, and Virginia (Gobler et al., 2005). Since then, the greatest reoccurrence of *A. anophagefferens*, a picoplanktonic alga about 2 μm in diameter, has occurred in Long Island bays (Bricelj and Lonsdale, 1997). Blooms of *A. anophagefferens* in shallow estuaries are promoted by elevated salinities, reduced flushing rates, high dissolved organic matter and low dissolved inorganic nitrogen, and light attenuation (Gobler et al., 2005). The density of these blooms can become greater than 10^6 cells ml^{-1} and can last for several months throughout late spring and summer

(Bricelj and Lonsdale, 1997). These blooms are not harmful to humans; however, they have negative impacts on marine organisms. At concentrations as low as 20,000 cells ml^{-1}, sublethal effects, including reduced abilities to feed, grow, and reproduce, are exhibited by adult suspension-feeding bivalves, while death occurs at densities of about 10^6 cells ml^{-1} (Bricelj and Lonsdale, 1997). Other benthos, including *Zostera marina*, also suffer as a result of *A. anophagefferens* limiting light penetration to deeper depths by up to 50%. Loss of *Z. marina* beds can impair economically important marine organisms such as scallops, hard clams, and possibly blue mussels, which are both ecologically and environmentally important in Long Island waters (Bricelj and Lonsdale, 1997).

Another picoplankter that *Didemnum vexillum* may feed on is phycoerythrin-containing *Synechococcus* sp.. The phycoerythrin in these coccoid cyanobacteria serve as accessory pigments during photosynthesis in addition to functioning as a nitrogenous reserve (Campbell et al., 1983). It is easy to identify these cells using a flow cytometer because instead of autofluorescing red like other chlorophyll-containing cells, they autofluoresce orange (Campbell et al., 1983). Populations of *Synechoccus* that contain phycoerythrin typically dominate the smaller picoalgae size range, especially at the start of the summer when there is a change from larger to smaller algal cells. *Synechoccus* populations remain high until late fall or early winter in south shore estuaries of Long Island (New York) (Campbell et al., 1983).

Methods

Clearance Rates and Oxygen Consumption of *Didemnum vexillum* in the Laboratory

In order to determine how *Didemnum vexillum* may change the ecosystem structure in south shore bays of Long Island, clearance rates and oxygen consumption were measured in the laboratory. Samples of *D. vexillum* were collected from Ponquogue Bridge (40º50.52´ N, 72º29.98´W) in Shinnecock Bay (Long Island, New York) seven times, during low tide, from May to July 2009 (Table 1). A paint scraper was used to detach the colonies from the pillars of the bridge. The size of the colonies taken depended on the health of the colonies, but ranged from 2.59 to 16.46 g dry weight (g_{dw}) with an average area of 91 cm^2. Samples were placed in coolers with water from Shinnecock Bay and were transported to Stony Brook University's Blue Point Facility in West Sayville, New York.

Table 1. Sampling dates and environmental conditions

Date	°C in Field	°C at Lab	Salinity in field	Salinity at lab	Starting chl-*a* (μg L^{-1})
21-May-09	17	17	30	26	8
3-Jun-09	17	19	31	23	6
10-Jun-09	18	20	27	24	12
18-Jun-09	18	21	30	20	15
25-Jun-09	20	20	30	22	19
2-Jul-09	20	21	27	22	7
24-Jul-09	22	24	27	22	12

Once samples were brought to the laboratory at Blue Point, each colony was placed on a plastic petri dish (diameter 9.4 cm), which was supported by a plastic tripod (height 3.5 cm). These tripods were then placed inside cylindrical 1.4 L acrylic chambers (height 12.7 cm, diameter 14.5 cm) (Figure 2). These chambers were placed on top of water-driven magnetic stirrers whose bases were attached to the bottoms of sea tables. Magnetic stir-bars were placed in the center of each chamber underneath the plastic tripod. Seven or eight chambers were set up this way, with an additional two or three (so that the total number of chambers was 10) having the same set up, but with no colony of *Didemnum vexillum*, thus acting as the control chambers.

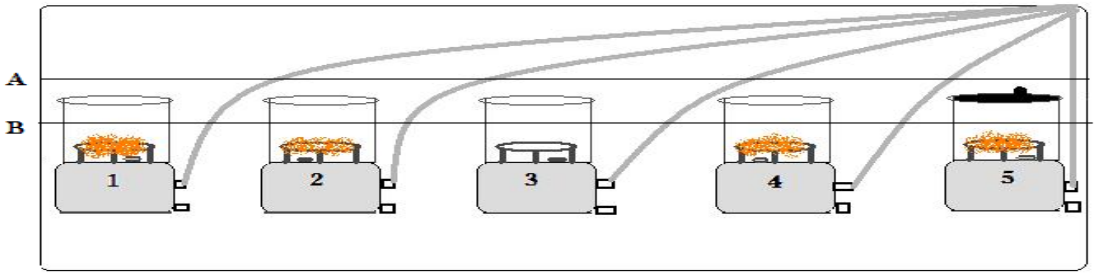

Figure 2. Diagram of chambers on top of magnetic stirrers in a sea table at Stony Brook University's Blue Point facility. Lines going to each chamber represent water being carried from head tanks via tubing into each magnetic stirrer. Line A shows the water level above the tops of the chambers for oxygen consumption experiments and line B shows the water level below the tops of the chambers for clearance rate experiments. Chamber 3 is a control. Chamber 5 shows the lid on the chamber for oxygen consumption experiments.

Samples were left overnight in running seawater, which was pumped in from Great South Bay (Pondmaster Model 18B Utility Pump) and filtered through 80 μm mesh (to prevent predation by zooplankton). This water flowed into the sea table via tubing attached to each magnetic stirrer, thus powering the stirrers. The water level in each sea table was at line A in Figure 2 so water in the chambers freely exchanged with water coming into the sea tables from Great South Bay.

Clearance rate experiments were conducted the day after collection using the same setup described above, except the water level was dropped from level A to level B so that water in the chambers did not exchange with water in the sea table during the experiment. The laboratory was kept dark for these experiments and the sea tables were

covered with black plastic to minimize photosynthesis within the chambers. Once the water level in the sea table was lowered to line B, duplicate 30 ml samples of seawater were taken from each of the ten chambers at the start of the experiment. Water samples were immediately filtered through glass fiber filters (Whatmann GF/F, 25 mm diameter). The filters were placed in glass test tubes on ice and 7 ml of acetone was added to each test tube to extract chlorophyll from the filters. Additional samples were taken periodically throughout the experiment and final samples were taken at either three or four hours from the start time.

The filters in acetone were brought back to the laboratory at Stony Brook University and placed in the freezer at -20ºC for 24 to 48 hours. Chlorophyll-*a* levels were then measured for each tube using a fluorometer (Turner 10-AU) and chlorophyll-*a* concentrations were calculated as described in Arar and Collins (1997). Clearance rates were then calculated using the formula:

$$CR = \frac{M}{nt}\left[\left(\ln\frac{F_0}{F_t}\right) - \left(\ln\frac{C_0}{C_t}\right)\right]$$

where M is the volume of the chamber, n is the weight of the colony, t is the elapsed time after the start of the experiment, F_0 is the concentration of chlorophyll-*a* in the experimental chambers at time 0, F_t is the concentration of chlorophyll-*a* in the experimental chambers at time t, C_0 is the concentration of chlorophyll-*a* in the control chambers at time 0, and C_t is the concentration of chlorophyll-*a* in the control chambers at time t.

In four of the seven experiments, 1 ml samples were taken from each chamber at the start and end of the experiment and placed in vials containing 1 ml glutaraldehyde to preserve the samples for flow cytometry. Preserved samples were analyzed using events (overall number of particles), forward scatter (amount of light scattered in the forward direction as a laser strikes the cell, whose magnitude is roughly proportional to the size of the cell), side scatter (light scattered at larger angles due to the granularity and structural complexity inside the cell), FL2 (emissions wavelengths of 564-606 nm; phycoerythrin), and FL3 (emissions wavelengths of 650-680 nm; chlorophyll-*a*) using a flow cytometer (BD FACSCalibur). Each sample was run through the flow cytometer for five minutes or until 100,000 events were recorded. Based on the elapsed time and the number of events, events per second was calculated for each sample. Events per microliter was then determined based on the rate at which the sample was being taken into the flow cytometer. Each sample was graphed and analyzed using WinMDI 2.9. Density plots of forward scatter versus chlorophyll-*a* and forward scatter versus phycoerythrin were used to examine different fluorescent signals (Figure 5). Each graph was broken into regions based on differing size and fluorescence, to account for different types of particles as well as noise (the least fluorescing group of particles). Since DNA dyes were not used, it is likely that the low fluorescing particles within the region termed “noise” are pieces of detritus and heterotrophic microorganisms, and thus were excluded from the calculations of clearance rates. For each sample, the total percent change over time and the percent change for specific regions were determined for each chamber. Clearance rates were then calculated, as described above, for the different regions, as well as total events not considered noise. Clearance rates were then compared amongst the different regions to

determine if *Didemnum vexillum* displayed any selectivity towards certain groups of particles.

After the clearance rate experiment was completed, the experimental chambers were used to measure oxygen consumption by *Didemnum vexillum*. Seawater from Great South Bay continued to flow into the sea tables and a taller standing pipe was put in to allow the water level to rise to line A in Figure 2, thus flushing the chambers with fresh sea water for a minimum of one hour before the oxygen consumption experiment began. Some light was allowed into the laboratory but the sea tables were still under a black plastic covering. The chambers were set up the same as for the clearance rate experiment but this time they had lids on them and the water level was above the tops of the chambers (line A in Figure 2). These lids had small holes (0.5 cm) in them that were closed off with a plug except for when sampling. Water samples (10 ml) were taken by syringe from each chamber at time 0, 1 hour, and 2 hours. Oxygen concentrations in samples were determined using a microwinkler technique modified from Carpenter (1965) and developed by R. A. Aller (SoMAS). The analysis was done immediately after taking the samples and completed within 15 minutes.

Upon the completion of Winkler titrations, *Didemnum vexillum* colonies used in the experiment were brought back to the laboratory at Stony Brook University. Shells and organisms, such as crabs, living within *D. vexillum* were removed using forceps. *D. vexillum* were then placed on aluminum foil trays and put into a 40ºC oven for four to five days before measuring their dry weights. Areas of each colony were measured from digital pictures taken before each experiment (using Image J). Areas were calibrated by having an object with a known area in each of the pictures of *D. vexillum*. Calculated areas were then divided into the corresponding weights of each colony to determine an index of thickness. This index of thickness was used to estimate percent growth in the field over a given time period.

Clearance Rates of *Didemnum vexillum* in the Field

Experiments were conducted twice in the field in order to determine *in situ* clearance rates of *Didemnum vexillum*, and thus determine if any laboratory variables, such as handling the species or differences in salinity between the field and the laboratory affected clearance rates. Experiments started at low tide, at Ponquogue Bridge on September 13, 2009 (23.2°C, 27 ppt) and October 10, 2009 (15.2°C, 35 ppt). PVC pipe (10.2 cm diameter), with rope attached to it, was secured to a plastic gallon bag on one end. The volume of the chamber and plastic bag was 4 L. The purpose of the flexible gallon bag was to promote mixing within the chambers as the bag was moved by water currents, to keep particle suspensions homogeneous. A 30 cm by 25 cm piece of neoprene (1.2 cm thick), with a cutout a little smaller than the diameter of the PVC pipe, was held against an area of the pillars where a colony of *D. vexillum* existed. The PVC pipe was put against the neoprene so that the openings lined up and the rope from the PVC pipe was wrapped around the pillar to hold the neoprene and experimental chamber in place (Figure 3). This was done three times over colonies of *D. vexillum* and twice

over areas with no *D. vexillum* or other macrobenthic fauna (controls). The PVC pipe had a small hole (0.6 cm diameter), in which a small piece of tubing (0.5 cm outer diameter, 0.4 cm inner diameter) was inserted to remove seawater samples from the chamber. On the outside of the PVC pipe, the tubing was clamped off to ensure that water did not enter the chamber. Water samples (30 ml) were taken through the tubing at times 0, 1.5 hours, and 3 hours. The samples were put into opaque containers and placed on ice in coolers and brought back to the laboratory to be analyzed for chlorophyll-*a* through fluorometry as described above. During the experiment on October 10, 2009, 1 ml samples were taken at times 0 and 3 hours, preserved with glutaraldehyde, and returned to the laboratory for flow cytometer analysis as described above. The three colonies that were enclosed under the PVC pipe and bag were scraped off the pillars and brought back to the laboratory to be dried and weighed as previously described.

Figure 3. *In situ* filtration chamber, with plastic bag attached to it, is held onto the pillar of Ponquogue Bridge by rope. Tube for sampling can be seen on top of the PVC pipe.

Statistical Analysis for Laboratory and Field Experiments

Dixon's test was used to determine if any outliers existed from measurements of clearance rates (based on fluorometer and flow cytometer measurements) and oxygen consumption (Sokal and Rohlf, 1981). Unpaired t-tests were conducted to determine if clearance rates and oxygen consumption were statistically significant from zero as well as to determine if any selectivity occurred between different groups of phytoplankton.

Estimating Clearance Rates of *Didemnum vexillum* Using Measured Growth and Respiration

Other researchers who have worked with *Didemnum vexillum* have stated that the species does not respond well to handling (Robert Whitlatch, University of Connecticut; pers. com.). Although handling was minimal, it may still have been detrimental, causing colonies to stop filtering, and thus affecting the clearance rates of *D. vexillum*. So in addition to experiments designed to measure clearance rates directly, separate estimations of clearance rates were made based on measured growth and respiration rates, as well as literature values of absorption efficiency and excretion. The following energy budget was used:

$$P = A - (R + U)$$

where P is production (growth), A is energy absorbed, R is metabolic rate (μmol h^{-1}), and U is net ammonia excretion (μmol h^{-1}) (Hawkins et al., 1985).

Calculations were based on a one week period from June 18, 2009 to June 25, 2009 when *Didemnum vexillum* colonies exhibited relatively high growth rates and laboratory respiration rates on both dates were significantly different from zero. Three calculations were made to estimate a range of clearance rates based on different estimates of excretion rates and energy contents of ascidian tissues. Growth was calculated from the difference in the index of thickness (g_{dw} cm^{-2}) and was measured as growth in g g_{dw}^{-1} d^{-1}. Percent cover of *D. vexillum* colonies remained relatively constant during this time, thus calculations for growth were based only on the difference in thickness and not percent cover. The change in biomass was multiplied by the energy content of ascidian tissue. A high estimate of the energy content of ascidians of 14.1 kJ g_{dw}^{-1} was used for the calculation of the high clearance rate (McClintock et al., 2004). An energy content of 9 kJ g_{dw}^{-1} was used for the median clearance rate and 5 kJ g_{dw}^{-1} was used for a minimum clearance rate value (Kowalke et al., 2001). The resulting product is growth rate expressed in kJ g_{dw}^{-1} d^{-1}.

Energy absorbed is the product of absorption efficiency and ingestion rate. It was assumed that ingestion rate equals clearance rate since all particles entering the pharynx become trapped in the mucus net for most ascidians (Petersen, 2007). Absorption efficiency was estimated at 70% for *Didemnum vexillum* based on values for other benthic suspension feeders that typically ranged between 60 and 70% (Rosenberg and Loo, 1983).

Respiration rates were derived from oxygen consumption on June 18, 2009 and June 25, 2009. The values of oxygen consumption on these two dates were averaged together and then multiplied by the oxycaloric coefficient of 0.45 J μmol O_2^{-1} so that values could be expressed in J g_{dw}^{-1} d^{-1} (Widdows, 1978).

Ammonia excretion rates were not measured during the experiments, and thus values from literature were used. A value of 16.00 J g_{dw}^{-1} d^{-1} was used for the calculation of the maximum value of clearance rate and a value of 5.04 J g_{dw}^{-1} d^{-1} was used for the calculation of the median value (Jiang et al., 2008). These values were based on a range of ammonia excretion rates for *Styela clava* at a water temperature within the range of the water temperature at the laboratory between June 18, 2009 and June 25, 2009. An ammonia excretion rate of 0 J g_{dw}^{-1} $d^{-1}$was utilized, eliminating excretion energy, in order to obtain a minimum clearance rate.

Results

Clearance Rates and Oxygen Consumption of *Didemnum vexillum* in the Laboratory

Based on fluorometer measurements, clearance rates ranged from -0.01 to 0.04 L h^{-1} g_{dw}^{-1} for the seven laboratory experiments (Table 2). Negative numbers indicate higher measurements of chlorophyll-*a* at time t compared to time 0. Only two of the estimates (June 10, 2009 and July 24, 2009) were statistically significant from zero (with an alpha of 0.05). This was mostly due to the fact that clearance rates were low in the experimental chambers, and not due to high clearance rates in the control chambers. Clearance rates based on fluorometer readings appear to be inversely proportional to changes in the salinity of seawater (Table 1) from Shinnecock Bay to Great South Bay (Figure 4, Table 3). The equation for the line is $CR = (a + b)(\exp(c)(\Delta S))$ where CR is clearance rate and ΔS is change in salinity.

Table 2. Average clearance rates for experimental chambers (corrected for control chambers) in L $h^{-1}g_{dw}^{-1}$ for seven laboratory experiments based on fluorometer measurements

Date	Average CR (L h^{-1} g_{dw}^{-1})	SD	t-value	t-critical	Significance
21-May-09	0.02	0.04	1.46	1.89	not significant
3-Jun-09	-0.01	0.01	-1.80	1.94	not significant
10-Jun-09	0.04	0.04	2.47	1.94	significant
18-Jun-09	-0.01	0.02	1.56	1.94	not significant
25-Jun-09	-0.01	0.01	-2.03	1.94	not significant
2-Jul-09	0.00	0.02	0.13	1.94	not significant
24-Jul-09	0.01	0.01	3.21	1.94	significant

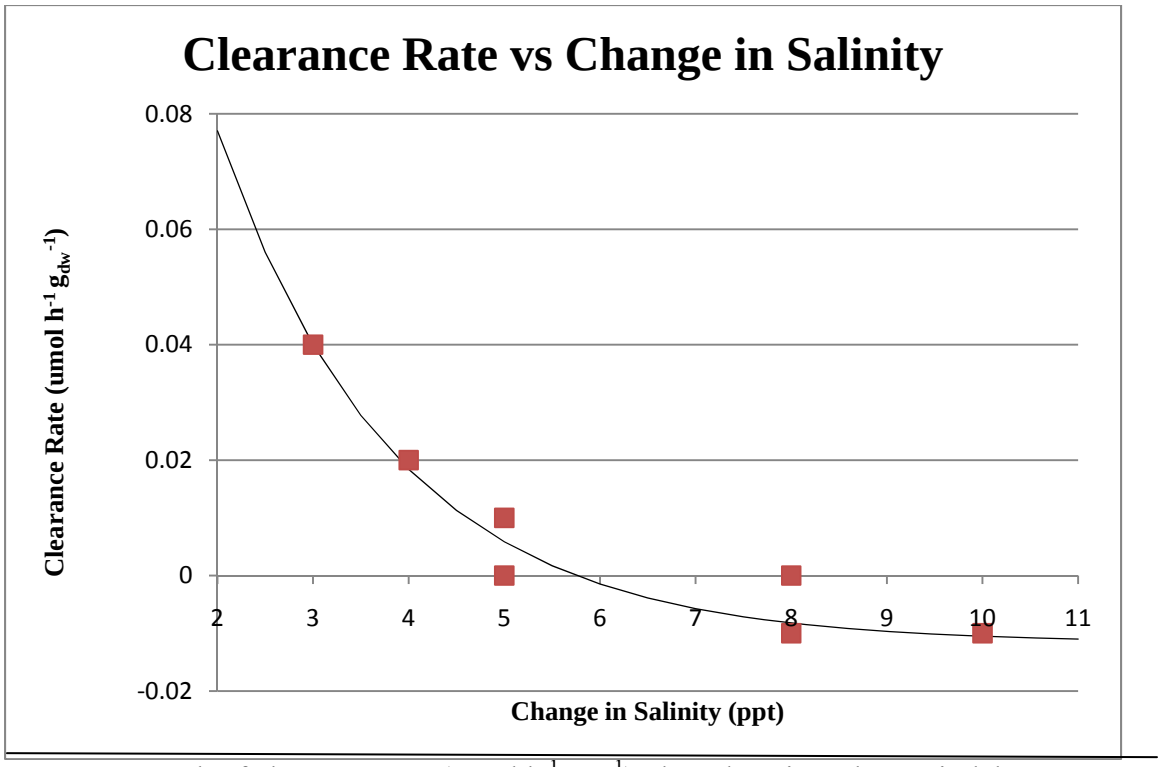

Figure 4. Graph of clearance rate (µmol h^{-1} g_{dw}^{-1}) plotted against change in laboratory salinity (ppt).

Table 3. Line parameters for graph of clearance rate vs change in laboratory salinity. Equation for line is CR = (a + b) (exp(c(ΔS)). df=4 r^2=0.97 alpha=0.05

	Estimate	Standard Error	t-value	p-value
a	-0.0117	0.0033	-3.5323	0.0242
b	0.2616	0.0872	2.9991	0.0400
c	-0.5406	0.1131	-4.7776	0.0088

Flow cytometer data were also used to calculate clearance rates for four of the dates. Data from forward scatter versus chlorophyll-*a*, excluding noise, was used (Figure 5A). This gave values of clearance rates that ranged from 0.00 to 0.02 L h^{-1} g_{dw}^{-1}; however, none of these estimates was statistically significant from zero (alpha of 0.05) (Table 4). Clearance rates were then calculated from phycoerythrin versus chlorophyll-*a* data from the flow cytometer (Figure 5B). Values ranged from -0.01 to 0.02 L h^{-1} g_{dw}^{-1}. The May 21, 2009 experiment showed the greatest clearance rate and was the only value that was statistically significant from zero. Flow cytometer data also indicated that *Didemnum vexillum* is not selective, as clearance rates of different size and different fluorescing properties were not statistically different from one another (alpha of 0.05).

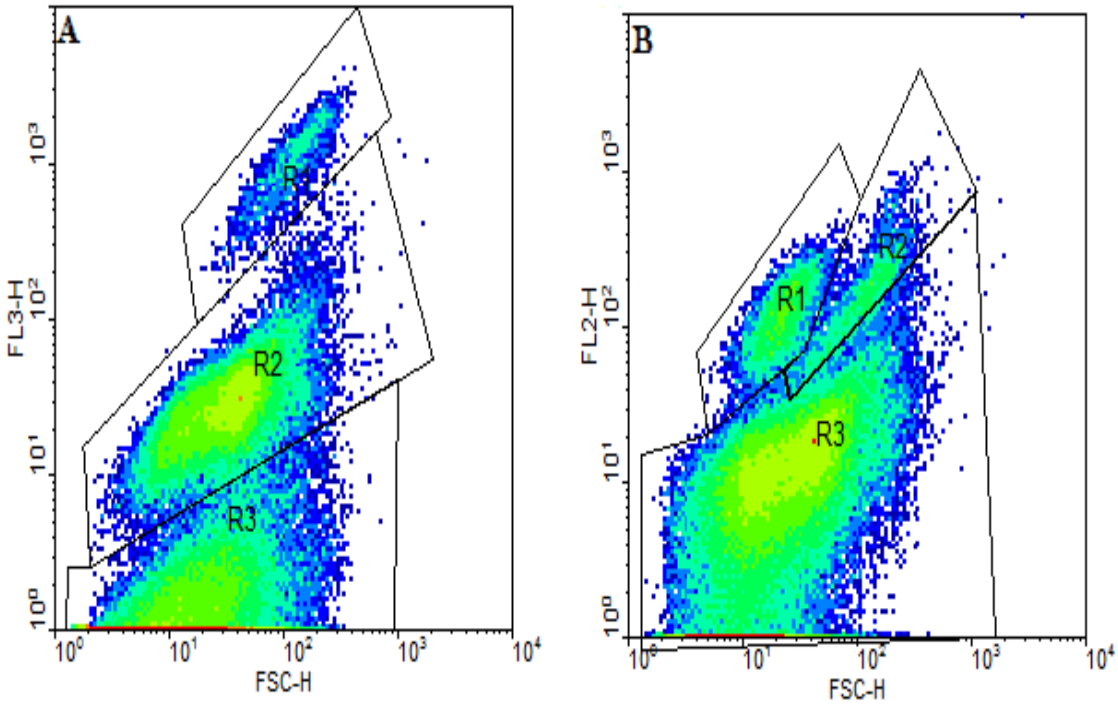

Figure 5. A is an example of a graph of forward scatter (which is a function of particle size) and FL3 (chlorophyll-*a*) for the experiment on July 2, 2009 while B is an example of a graph of forward scatter and FL2 (phycoerythrin) for the experiment on June 25, 2009. Both are broken into three different regions. R1 and R2 were used in the calculations of clearance rate. R3 was considered noise and thus was excluded from the calculations.

Table 4. Average clearance rates (L h^{-1} g_{dw}^{-1}) for four laboratory experiments based on flow cytometer analysis using non-noise data from forward scatter versus chlorophyll-*a* and non-noise data from forward scatter versus phycoerythrin

	Forward Scatter vs Chlorophyll-*a* non-noise				
Date	Average CR L h^{-1} g_{dw}^{-1}	SD	t-value	t-critical	Significance
21-May-09	0.02	0.04	1.70	1.89	not significant
25-Jun-09	0.02	0.04	1.36	1.94	not significant
2-Jul-09	0.00	0.01	0.11	1.94	not significant
24-Jul-09	0.00	0.04	0.12	1.94	not significant

	Forward Scatter vs Phycoerythrin non-noise				
Date	Average CR L h^{-1} g_{dw}^{-1}	SD	t-value	t-critical	Significance
21-May-09	0.02	0.01	4.16	1.89	significant
25-Jun-09	0.00	0.05	-0.09	1.94	not significant
2-Jul-09	0.00	0.02	0.38	1.94	not significant
24-Jul-09	-0.01	0.03	-0.72	1.94	not significant

Values of oxygen consumption ranged from -2.37 to 14.34 µmol h^{-1} g_{dw}^{-1} (Table 5). Of the seven values, only two were not significantly different than zero (June 10, 2009 and July 2, 2009), which were the two smallest values. Negative numbers indicate that higher concentrations of oxygen were measured at time t compared to time 0. Of the significant values, oxygen consumption by *Didemnum vexillum* was greatest on July 24, 2009 (14.34 µmol h^{-1} g_{dw}^{-1}) and lowest on May 21, 2009 (2.90 µmol h^{-1} g_{dw}^{-1}). Oxygen consumption appears to increase with increases in temperature (Figure 6.)

Table 5. Oxygen consumption in µmol h^{-1} g_{dw}^{-1} for the seven laboratory experiments

Date	Average Oxygen Consumption (µmol h^{-1} g_{dw}^{-1})	SD	t-value	t-critical	Significance
21-May-09	2.90	1.65	4.64	1.94	significant
3-Jun-09	4.84	1.28	10.00	1.94	significant
10-Jun-09	2.34	2.89	1.99	2.02	not significant
18-Jun-09	6.45	2.23	5.79	2.35	significant
25-Jun-09	4.71	1.68	6.85	2.02	significant
2-Jul-09	-2.37	1.40	-3.78	2.13	not significant
24-Jul-09	14.34	14.25	2.25	2.13	not significant

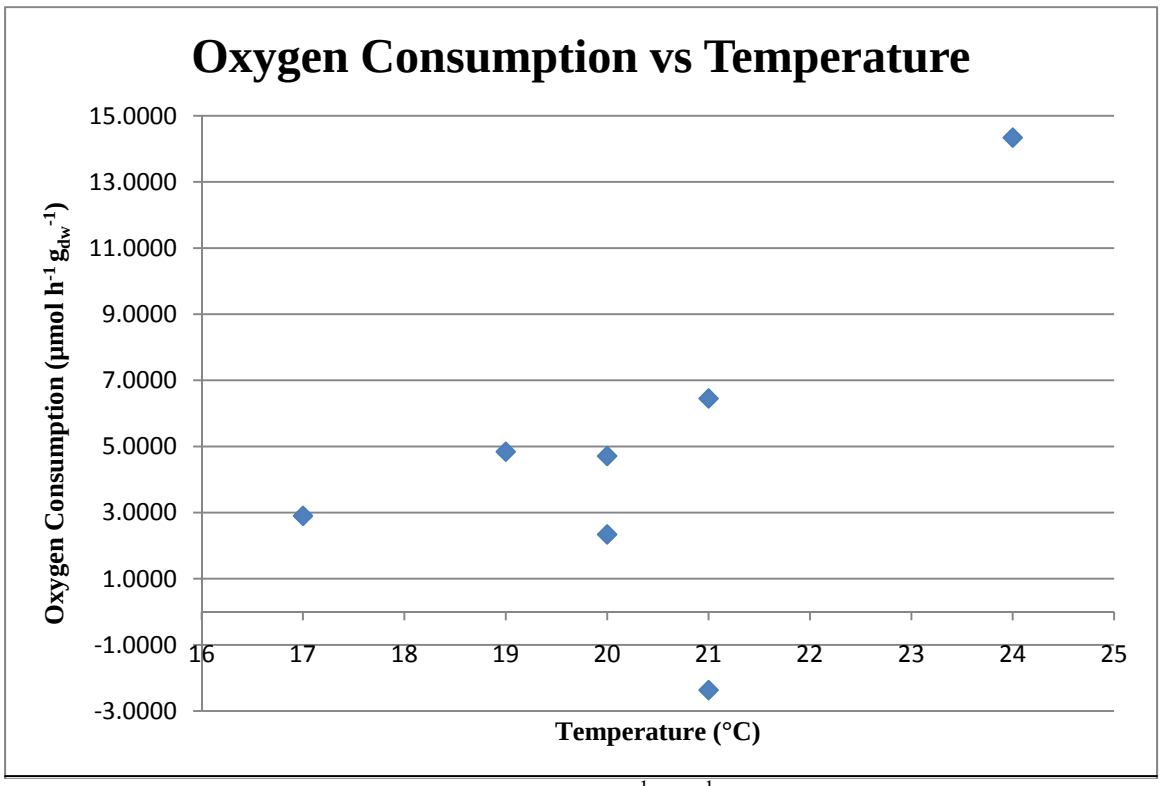

Figure 6. Graph of oxygen consumption (µmol h^{-1} g_{dw}^{-1}) plotted against temperature (°C).

Index of thickness values were averaged together for each of the seven laboratory experiments (Table 6). The thickest colonies were found on June 25, 2009 and had an index of thickness of 0.10 g_{dw} cm^{-2} while the thinnest colonies were collected on July 2, 2002 and had a thickness of 0.07 g_{dw} cm^{-2}.

Table 6. Index of thickness averaged for colonies used in experiments

Date	Index of Thickness (g_{dw} cm^{-2})
21-May-09	0.09
3-Jun-09	0.09
10-Jun-09	0.07
18-Jun-09	0.08
25-Jun-09	0.10
2-Jul-09	0.07
24-Jul-09	0.09

Clearance Rates of *Didemnum vexillum* in the Field

The field experiment conducted on September 13, 2009 yielded a clearance rate of 0.22 L h^{-1} g_{dw}^{-1} ± 0.41 based on fluorometer measurements of chlorophyll-*a* (t-value = 0.92; t-critical = 2.92). During the field experiment on October 10, 2009, 0.07 L h^{-1} g_{dw}^{-1} ± 0.10 was calculated as the clearance rate from the fluorometer readings (t-value = 0.98; t-critical = 6.31). Both of these values, however, were not statistically significant from zero (alpha of 0.05).

Flow cytometer measurements were made for the field experiment on October 10, 2009. From these data, a clearance rate of -2.08 L h^{-1} g_{dw}^{-1} ± 0.26 was found for non-noise forward scatter versus chlorophyll-*a* (t-value = -11.23; t-critical = 6.31). For non-noise forward scatter vs phycoerythrin, a clearance rate of -2.14L h^{-1} g_{dw}^{-1} ± 0.34 was determined (t-value = -9.25; t-critical = 6.31). Neither of these values was statistically significant from zero (alpha of 0.05). For the forward scatter versus chlorophyll-*a*, no selectivity occurred between different regions (Figure 5). When forward scatter vs phycoerythrin was broken down into two regions (excluding noise), the clearance rates of the different regions were statistically different from one another (alpha of 0.05). However, clearance rates for each region were not statistically significant, in addition to the fact that both groups were experiencing an increase in particles, not a decrease during the field incubation.

Estimated Clearance Rates of *Didemnum vexillum* Using Measured Growth and Respiration

Calculated clearance rates, based on an energy budget using measured growth and respiration, gave much higher estimates of clearance rates, compared to those measured in the laboratory and during the field study. A minimum clearance rate of 0.32 L g_{dw}^{-1} h^{-1} was calculated for *Didemnum vexillum* to maintain the growth and respiration rates measured during the experiments. This value assumes that zero energy is used for ammonia excretion. A median value of 0.52 L g_{dw}^{-1} h^{-1} was calculated. This assumes average excretion energy and average energy content (values based on averages for other

ascidians). The high value for clearance rate of *D.* vexillum was calculated to be 0.77 L g_{dw}^{-1} h^{-1}. High excretion energy, as well as a high energy content (values based on highs for other ascidians), was assumed.

Discussion

Clearance Rate and Oxygen Consumption of *Didemnum vexillum* in the Laboratory

The main goals of this study were to measure clearance rates of *Didemnum vexillum* as well as determine if the species is particle selective in order to predict its possible effects on south shore bays of Long Island. However, only three statistically significant values of clearance rates were obtained. Two of these values were from fluorometer data, 0.04 L h^{-1} g_{dw}^{-1} ± 0.04 on June 10, 2009 and 0.01 L h^{-1} g_{dw}^{-1} ± 0.01 on July 24, 2009, and the third was from flow cytometer data, 0.02 L h^{-1} g_{dw}^{-1} ± 0.01 on May 21, 2009. These three values, even though statistically significant, are very low, making it appear that *D. vexillum* was not really feeding in the experiments. Fluorometer and flow cytometer estimates were never statistically significant on the same day, thus lowering the validity of these values. Thus, it appears that *D. vexillum* was feeding minimally, if at all, in the laboratory.

The average value of oxygen consumption based on statistically significant values was 7.90 ml O_2 h^{-1} g_{dw}^{-1} (0.25 mg O_2 h^{-1} g^{-1}). This value was lower than literature values of oxygen consumption for other ascidians (Table 7). Thus, while oxygen consumption was occurring during the laboratory experiments, and the animals were alive, they may have limited their energy expended by not feeding on suspended particles. It should also be noted that the values obtained in this study are lower compared to literature values because the colonies of *Didemnum vexillum* may have been at rest, thus lowering their oxygen consumption, as seen in bivalves (Kraus and Doeller, 1988).

Table 7. Measured oxygen consumption of other ascidians compared with *Didemnum vexillum*

Species	Oxygen Consumption mg O_2 h^{-1} g_{dw}^{-1}	Temperature °C	Reference
Phallusia mammillata	0.57 - 1.0	15	Fiala-Medioni, 1979
Styela clava	0.60 ± 0.37	15	Riisgård, 1988
Halocynthia papillosa	1.39 ± 0.43	12-22	Coma et al., 2002
Didemnum vexillum	0.25	17-24	This study

Didemnum vexillum may have saved energy because it stopped consuming due to disturbance from handling. While care was taken in the transportation of *D. vexillum* from Ponquogue Bridge to the laboratory at Blue Point, and minimal handling occurred

during the removal of the colonies from the pillars of the bridge, the removal of the colonies from the bridge may have been enough to disturb it.

Didemnum vexillum may have also not done well in the laboratory due to the lower salinity of the seawater entering the laboratory from Great South Bay. *D. vexillum* typically experiences its highest growth rate when salinities range from 25 to 30 ppt, and then gradually experiences a decline in its health (Bullard and Whitlatch, 2009). During the seven sampling dates at Ponquogue Bridge, only June 3, 2009 was not within this range (34 ppt) (Table 1). However, at the Blue Point laboratory, only on one day of experiments, May 21, 2009, was the salinity within the range that *D. vexillum* typically favors and grows in. Feeding still did not appear to occur on this date, showing that the drop in salinity may have harmed the species even though the salinity at the laboratory was within the range that *D. vexillum* favors. This trend is shown in Figure 4. However, the appearance of the species had deteriorated even before it was put into the sea table at the laboratory in terms of a reduction in the vibrancy of color of the colonies. This leads one to believe that it was not the reduction in salinity alone that caused *D. vexillum* to feed minimally, if at all, in the laboratory.

Other studies have shown that high concentrations of particles may negatively affect clearance rates of suspension-feeding tunicates (Petersen and Riisgård, 1992, Petersen et al., 1995, and Petersen et al., 1999). Armsworthy et al., (2001) determined that high concentrations of particles caused squirting (pushing water out of their bodies) to increase. This altered the transport of the mucus net, although this did not affect the clearance rates (up to a concentration of 7.4×10^7 cells ml^{-1}). Other studies have found an inverse relationship between suspended particle concentrations and filtration rates of ascidians starting at concentrations as low as 1×10^3 to 2×10^5 cells ml^{-1} (Fiala-Medioni, 1979; Robbins, 1983, 1984; Petersen and Riisgård, 1992; Petersen et al., 1995). Particle concentrations in Great South Bay can be very high at times, especially in a place such as a marina, which is where Stony Brook University's Blue Point facility is located. At marinas, boat propellers stir up bottom sediment which results in higher concentrations of suspended sediment. However, based on flow cytometer measurements, particle concentrations did not exceed the starting concentrations at which other researchers have seen an inverse relationship between suspended particle concentrations and filtration rates. What can be said though, is that chlorophyll-*a* concentrations during the field experiments in Shinnecock Bay, which averaged 4.12 $\mu g\ L^{-1}$, were much lower than chlorophyll-*a* concentrations at Stony Brook University's Blue Point facility, which averaged 11.30 $\mu g\ L^{-1}$.

During the three experiments in which clearance rates were statistically significant, no selectivity occurred between different size and different fluorescing particles. This was expected since ascidians as a group have been seen to be non-selective (Fiala-Médioni, 1987). In addition, *Didemnum vexillum* has the ability to feed on particles as small as *Aureococcus anophagefferens* and thus may be capable of reducing brown tides that are frequent in Long Island bays (Riisgaard and Larsen, 2001). However, the toxins of *A. anophagefferens* may harm *D. vexillum* like they do other benthic suspension feeders, causing a reduction in feeding, reproduction, and growth, and possibly lead to death. If *D. vexillum* is not harmed by these toxins, and is able to feed on *A. anophagefferens*, it may help other benthic suspension feeders and seagrasses which are harmed both directly and indirectly by *A. anophagefferens.* However, further studies

need to be conducted to determine the relationship between the *D.vexillum and A. anophagefferens* and what impact they may have on one another.

Clearance Rates of *Didemnum vexillum* in the Field

In order to measure ambient clearance rates of *Didemnum vexillum* colonies in Shinnecock Bay, two field experiments were conducted. These experiments helped to alleviate the variables listed above of bringing the colonies back to the laboratory. While clearance rates were still not statistically significant from zero, field experiments have potential to work in the future. Due to time restraints, only five chambers were set up on the pillars, three with *D. vexillum*, and two as controls. Future studies with more chambers are suggested so that results may be statistically significant. Also, attaching these chambers to a cylindrical pillar, may still have allowed water to enter, even with the neoprene acting as a seal. In addition, the placement of chambers on continuous colonies of *D. vexillum* (which wrapped around the circumference of the pillars) may have still disturbed the part of the colony under the PVC pipe, even though that specific part of the colony was not disturbed. While *D. vexillum* was only found on pillars at this location, field experiments may work better in shallow areas where the species is found on rocks on the seafloor. In that situation, an enclosure can be put around rocks with a small opening for sampling that is closed, except for when seawater samples are taken. This would better ensure a closed system as well as not disturb any part of the colony. Another option would be to set up recruitment panels to collect larvae of *D. vexillum*. Once *D. vexillum* colonizes these panels, they can either be brought back to the laboratory for experiments or can be enclosed in the field for *in situ* field experiments. In either case, the colonies would not have to be touched, minimizing disturbance to the species.

Estimated Clearance Rates of *Didemnum vexillum* Using Measured Growth and Respiration

The estimates of clearance rates, based on measured respiration and growth rate, show that *Didemnum vexillum* must be filtering much faster than was measured in the clearance experiments. The estimated clearance rates are within the range of other colonial ascidians, but lower than the clearance rates of solitary ascidians (Table 8). It is probable, however, that the clearance rates of *D. vexillum* are actually higher than the estimated values, even the high estimated value, at certain times. The growth rate used for the estimated clearance rate value was computed during June, 2009 when the colonies were already established on the pillars of Ponquogue Bridge. The percent cover of the species on the pillars was not changing at this time, limiting growth to increase in thickness. However, when *D. vexillum* first comes back after a winter or summer die-back, their growth rates are probably much higher than the 3% increase per day that was measured in June, 2009. Other studies have shown growth rates as high as six times this percentage, at about 18% increase per day; this growth rate was much higher than growth exhibited by *Botrylloides and Botryllus* (McCarthy et al., 2006). Thus, if *D. vexillum* can grow this rapidly, it is probable that its clearance rate is proportionately higher than was estimated in this study. Using a medium value of growth at 9% increase per day,

estimates of clearance rates were in fact much higher: ranging from a minimum of 0.79 L h^{-1} g_{dw}^{-1}, a medium of 1.36 L h^{-1} g_{dw}^{-1} , and a maximum of 2.02 L h^{-1} g_{dw}^{-1}. These values are still well within the range of other colonial ascidians, but may still be on the low end since calculations were made using what may have been a resting metabolic rate for the species. Since all this evidence points to *D. vexillum* feeding more than what was seen at the laboratory, it is probable that the handling of the colonies caused the species to slow their feeding rates to minimal values, if any at all.

Table 8. Clearance rates of other ascidians, both colonial and solitary, in L g_{dw}^{-1} h^{-1}

Species	Clearance Rate (L g_{dw}^{-1} h^{-1})	Reference
Colonial:		
Aplidium altarium	2.60	Koike and Suzuki, 1996
Didemnum molle	2.00	Koike and Suzuki, 1996
Didemnum cf.albopunctatum	3.60	Koike and Suzuki, 1996
Lissoclinum bistratum	0.18	Koike and Suzuki, 1996
Lissoclinum voeltzkowi	0.07	Koike and Suzuki, 1996
Solitary:		
Ciona intestinalis	7.1	Petersen and Riisgård, 1992
Ascidia astra	1.1 to 2.0	Hecht, 1916
Molgula manhattensis	6.2	Jørgensen, 1949
Styela clava	5.8	Homes, 1973

Range of *Didemnum vexillum* Along the East Coast of the United States

Bays along the south shore of Long Island are currently the southern boundary for *Didemnum vexillum*. Colonies of *D. vexillum* found north of Long Island in shallow waters typically experience a die-back during the winter when water temperatures get too cold (Valentine et al., 2005). This was seen in the population at Ponquogue Bridge, which died back in the middle of February, 2009 when surface seawater temperatures were just above 0ºC. The colonies at Ponquogue Bridge experienced an additional die-back in the summer, which has not been documented in populations north of Long Island. The summer die-back occurred in August, 2009 when surface water temperatures exceeded 23°C, with the colonies reappearing on the pillars in the middle of September 2009 when water temperatures began to drop below 24ºC. Right before both die-backs the colonies' color faded from an orange to a brown-green color and fecal pellets collected in the cloacal canals of the colonies. While one could see the colonies deteriorating, the percent cover of the species on the pillars remained relatively constant (about 50 to 75% of the area from the low tide mark to about 1 m below) and then they rapidly died-back within a week. Their reappearance was equally rapid; the animals went from 0% cover to 25 to 50% cover of the pillars from the low tide mark to about 1 meter below within a couple of weeks. Thus, it is unlikely that *D. vexillum* will be able to successfully colonize many shallow south shore Long Island waters, where seawater temperatures are warmer for more of the year and will probably act to limit the invasion of *D. vexillum* by causing multiple die-backs. However, the species may be able to

invade deeper areas where they are not exposed to extreme warm temperatures. Colonies found in Georges Bank do not experience a winter die-back probably because they are not exposed to the extreme cold temperatures (Valentine et al., 2007a). Thus, the same may be true for warm temperatures.

Conclusion

It is hard to predict the total impact *Didemnum vexillum* will have on Shinnecock Bay and Great South Bay if the population expands since it appears that the species was possibly limiting its energy expended by not feeding on suspended particles, both in the laboratory and in the field during experiments. However, it was observed that *D. vexillum* was growing in mats on the pillars of Ponquogue Bridge between April and July of 2009, and from August, 2009 until February, 2010. So while it can currently not be predicted if *D. vexillum* will outcompete other species for food, it has already been seen that the species is outcompeting other organisms for space, and this alone has the capacity to alter predator-prey interactions within the ecosystems of south shore bays of Long Island.

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Appendix 1:Tables 9-34

Table 9. Clearance rates, uncorrected for the controls, based on fluorometer measurements from laboratory experiment on May 21, 2009 (control chambers are bold and italicized)

	Clearance Rates	
Chamber	L h^{-1}	L h^{-1} g_{dw}^{-1}
1	***0.23***	***N/A****
2	0.12	0.01
3	0.89	0.19
4	0.23	0.03
5	0.19	0.02
6	0.45	0.13
7	-0.02	-0.00
8	0.09	0.01
9	0.62	0.07
10	***0.14***	***N/A****
Averages:	0.29	0.06

***** indicates no value since control chambers did not contain *Didemnum vexillum*

Table 10. Clearance rates, uncorrected for controls, based on forward scatter vs chlorophyll-*a* non-noise flow cytometer analysis from laboratory experiment on May 21, 2009 (control chambers are bold and italicized)

	Forward Scatter vs Chlorophyll-*a* Non-noise	
Chamber	L h^{-1}	L h^{-1} g_{dw}^{-1}
1	***-0.13***	***N/A****
2	0.12	0.01
3	0.00	0.00
4	0.28	0.04
5	-0.28	-0.04
6	0.16	0.05
7	0.02	0.00
8	0.17	0.02
9	0.21	0.02
10	***0.00***	***N/A****
Averages:	0.06	0.01

* indicates no value since control chambers did not contain *Didemnum vexillum*

Table 11. Clearance rates, uncorrected for controls, based on forward scatter vs phycoerythrin non-noise flow cytometer analysis from laboratory experiment on May 21, 2009 (control chambers are bold and italicized)

Forward Scatter vs Phycoerythrin Non-noise		
Chamber	L h^{-1}	L h^{-1} g_{dw}^{-1}
1	*0.02*	*N/A**
2	**0.26**	**0.03**
3	0.15	0.02
4	0.13	0.02
5	0.01	0.00
6	0.17	0.05
7	0.17	0.02
8	0.35	0.03
9	0.36	0.04
10	***0.09***	***N/A****
Averages:	0.17	0.03

* indicates no value since control chambers did not contain *Didemnum vexillum*

Table 12. Oxygen consumption rates, uncorrected for controls, from laboratory experiment on May 21, 2009 (control chambers are bold and italicized)

	Oxygen Consumption	
Chamber	µmol O_2 h^{-1}	µmol O_2 h^{-1} g_{dw}^{-1}
1	***28.18***	***N/A*****
2	43.40	4.44
3	34.48	7.31
4	56.88	7.22
5	43.58	5.87
6	39.55	11.47
7	49.70	4.88
8	N/A**	N/A**
9	74.55	8.18
10	***26.43***	***N/A****
Averages:	44.08	7.05

* indicates no value since control chambers did not contain *Didemnum vexillum*

Table 13. Clearance rates, uncorrected for the controls, based on fluorometer measurements from laboratory experiment on June 3, 2009 (control chambers are bold and italicized)

	Clearance Rates	
Chamber	L h^{-1}	L h^{-1} g_{dw}^{-1}
1	***0.01***	***N/A****
2	0.09	0.01
3	0.20	0.03
4	0.05	0.01
5	-0.03	-0.00
6	-0.02	-0.00
7	0.22	0.02
8	N/A**	N/A**
9	0.14	0.02
10	***0.11***	***N/A****
Averages:	0.09	0.01

* indicates no value since control chambers did not contain *Didemnum vexillum*
** indicates no values because chamber was not used

Table 14. Oxygen consumption rates, uncorrected for controls, from laboratory experiment on June 3, 2009 (control chambers are bold and italicized)

	Oxygen Consumption	
Chamber	µmol O_2 h^{-1}	µmol O_2 h^{-1} g_{dw}^{-1}
1	***0.18***	***N/A****
2	45.50	3.89
3	29.40	4.89
4	22.93	5.29
5	23.98	3.02
6	61.78	5.22
7	55.13	5.09
8	N/A**	N/A**
9	46.20	6.49
10	**2.45**	**N/A***
Averages:	31.93	4.84

* indicates no value since control chambers did not contain *Didemnum vexillum*
** indicates no values because chamber was not used

Table 15. Clearance rates, uncorrected for the controls, based on fluorometer measurements from laboratory experiment on June 10, 2009 (control chambers are bold and italicized)

	Clearance Rates	
Chamber	L h^{-1}	L h^{-1} g_{dw}^{-1}
1	***-0.00***	***N/A****
2	0.37	0.09
3	0.11	0.02
4	0.12	0.01
5	***-0.04***	***N/A****
6	0.19	0.06
7	0.00	0.00
8	***-0.14***	***N/A****
9	0.05	0.01
10	0.14	0.01
Averages:	0.09	0.03

* indicates no value since control chambers did not contain *Didemnum vexillum*

Table 16. Oxygen consumption rates, uncorrected for controls, from laboratory experiment on June 10, 2009 (control chambers are bold and italicized)

	Oxygen Consumption	
Chamber	µmol O_2 h^{-1}	µmol O_2 h^{-1} g_{dw}^{-1}
1	***2.10***	***N/A****
2	-4.38	-1.06
3	12.25	2.31
4	-34.30	-3.20
5	***32.90***	***N/A****
6	26.95	8.15
7	26.43	5.67
8	***0.35***	***N/A****
9	1.93	0.25
10	24.50	2.33
Averages:	8.87	1.81

* indicates no value since control chambers did not contain *Didemnum vexillum*

Table 17. Clearance rates, uncorrected for the controls, based on fluorometer measurements from laboratory experiment on June 18, 2009 (control chambers are bold and italicized)

	Clearance Rates	
Chamber	L h^{-1}	L h^{-1} g_{dw}^{-1}
1	***0.05***	***N/A****
2	***0.07***	***N/A****
3	0.05	0.01
4	0.10	0.01
5	*-0.10*	*-0.02*
6	0.08	0.03
7	-0.04	-0.01
8	*0.01*	*0.00*
9	***0.01***	***N/A****
10	-0.16	-0.03
Averages:	0.01	-0.00

* indicates no value since control chambers did not contain *Didemnum vexillum*

Table 18. Oxygen consumption rates, uncorrected for controls, from laboratory experiment on June 18, 2009 (control chambers are bold and italicized)

	Oxygen Consumption	
Chamber	µmol O_2 h^{-1}	µmol O_2 h^{-1} g_{dw}^{-1}
1	***20.13***	***N/A****
2	***15.23***	***N/A****
3	16.98	4.62
4	-8.93	-0.71
5	*64.75*	*11.92*
6	43.40	16.75
7	45.15	10.87
8	*46.90*	*7.00*
9	***-27.30***	***N/A****
10	45.33	7.62
Averages:	26.16	8.30

* indicates no value since control chambers did not contain *Didemnum vexillum*

Table 19. Clearance rates, uncorrected for the controls, based on fluorometer measurements from laboratory experiment on June 25, 2009 (control chambers are bold and italicized)

	Clearance Rates	
Chamber	L h^{-1}	L h^{-1} g_{dw}^{-1}
1	0.22	0.02
2	***0.03***	***N/A****
3	0.02	0.00
4	0.03	0.00
5	***0.11***	***N/A****
6	-0.04	0.00
7	***0.18***	***N/A****
8	-0.11	-0.01
9	0.05	0.00
10	0.08	0.01
Averages:	0.06	0.00

* indicates no value since control chambers did not contain *Didemnum vexillum*

Table 20. Clearance rates, uncorrected for controls, based on forward scatter vs chlorophyll-*a* non-noise flow cytometer analysis from laboratory experiment on June 25, 2009 (control chambers are bold and italicized)

	Forward Scatter vs Chlorophyll-*a* Non-noise	
Chamber	L h^{-1}	L h^{-1} g_{dw}^{-1}
1	-0.05	-0.00
2	**0.15**	***N/A****
3	0.96	0.07
4	1.11	0.09
5	***0.84***	***N/A****
6	0.22	0.02
7	**0.17**	***N/A****
8	0.29	0.04
9	0.18	0.01
10	0.05	0.01
Averages:	0.39	0.03

* indicates no value since control chambers did not contain *Didemnum vexillum*

Table 21. Clearance rates, uncorrected for controls, based on forward scatter vs phycoerythrin non-noise flow cytometer analysis from laboratory experiment on June 25, 2009 (control chambers are bold and italicized)

	Forward Scatter vs Phycoerythrin Non-noise	
Chamber	L h^{-1}	L h^{-1} g_{dw}^{-1}
1	-0.04	-0.00
2	***0.13***	***N/A****
3	0.98	0.07
4	1.08	0.09
5	***0.85***	***N/A****
6	0.21	0.02
7	***0.10***	***N/A****
8	0.22	0.03
9	0.01	0.00
10	0.01	0.00
Averages:	0.36	0.03

* indicates no value since control chambers did not contain *Didemnum vexillum*

Table 22. Oxygen consumption rates, uncorrected for controls, from laboratory experiment on June 25, 2009 (control chambers are bold and italicized)

	Oxygen Consumption	
Chamber	µmol O_2 h^{-1}	µmol O_2 h^{-1} g_{dw}^{-1}
1	50.93	3.55
2	***9.63***	***N/A****
3	41.30	3.13
4	57.23	4.67
5	***1.23***	***N/A****
6	64.93	4.97
7	***-14.00***	***N/A****
8	-37.98	-4.63
9	45.33	2.75
10	59.68	8.81
Averages:	27.83	3.32

* indicates no value since control chambers did not contain *Didemnum vexillum*

Table 23. Clearance rates, uncorrected for the controls, based on fluorometer measurements from laboratory experiment on July 2, 2009 (control chambers are bold and italicized)

	Clearance Rates	
Chamber	L h^{-1}	L h^{-1} g_{dw}^{-1}
1	0.09	0.01
2	0.06	0.01
3	0.13	0.01
4	0.04	0.01
5	0.02	0.00
6	***0.03***	***N/A****
7	0.11	0.02
8	0.07	0.01
9	***0.04***	***N/A****
10	***-0.03***	***N/A****
Averages:	0.06	0.01

* indicates no value since control chambers did not contain *Didemnum vexillum*

Table 24. Clearance rates, uncorrected for controls, based on forward scatter vs chlorophyll-*a* non-noise flow cytometer analysis from laboratory experiment on July 2, 2009 (control chambers are bold and italicized)

	Forward Scatter vs Chlorophyll-*a* Non-noise	
Chamber	L h^{-1}	L h^{-1} g_{dw}^{-1}
1	-0.10	-0.01
2	0.02	0.00
3	0.03	0.00
4	0.07	0.02
5	0.18	0.01
6	***0.03***	***N/A****
7	0.02	0.00
8	-0.08	-0.01
9	***0.06***	***N/A****
10	***-0.05***	***N/A****
Averages:	0.0186	0.00

* indicates no value since control chambers did not contain *Didemnum vexillum*

Table 25. Clearance rates, uncorrected for controls, based on forward scatter vs phycoerythrin non-noise flow cytometer analysis from laboratory experiment on July 2, 2009 (control chambers are bold and italicized)

	Forward Scatter vs Phycoerythrin Non-noise	
Chamber	L h^{-1}	L h^{-1} g_{dw}^{-1}
1	-0.11	-0.01
2	0.02	0.00
3	0.02	0.00
4	0.07	0.02
5	0.17	0.01
6	***-0.12***	***N/A****
7	0.02	0.00
8	-0.08	-0.01
9	***0.07***	***N/A****
10	***-0.03***	***N/A****
Averages:	0.00	0.00

* indicates no value since control chambers did not contain *Didemnum vexillum*

Table 26. Oxygen consumption rates, uncorrected for controls, from laboratory experiment on July 2, 2009 (control chambers are bold and italicized)

	Oxygen Consumption	
Chamber	µmol O_2 h^{-1}	µmol O_2 h^{-1} g_{dw}^{-1}
1	44.80	5.67
2	17.50	2.84
3	-0.35	-0.03
4	17.50	4.22
5	14.00	1.14
6	***-1.05***	***N/A****
7	73.50	13.71
8	-4.38	-0.61
9	***27.48***	***N/A****
10	***25.20***	***N/A****
Averages:	21.42	3.85

* indicates no value since control chambers did not contain *Didemnum vexillum*

Table 27. Clearance rates, uncorrected for the controls, based on fluorometer measurements from laboratory experiment on July 24, 2009 (control chambers are bold and italicized)

	Clearance Rates	
Chamber	L h^{-1}	L h^{-1} g_{dw}^{-1}
1	0.04	0.00
2	***0.01***	***N/A****
3	0.10	0.03
4	0.01	0.00
5	0.15	0.01
6	0.09	0.02
7	***0.01***	***N/A****
8	0.07	0.01
9	***0.03***	***N/A****
10	0.07	0.01
Averages:	0.06	0.01

* indicates no value since control chambers did not contain *Didemnum vexillum*

Table 28. Clearance rates, uncorrected for controls, based on forward scatter vs chlorophyll-*a* non-noise flow cytometer analysis from laboratory experiment on July 24, 2009 (control chambers are bold and italicized)

	Forward Scatter vs Chlorophyll-*a* Non-noise	
Chamber	L h^{-1}	L h^{-1} g_{dw}^{-1}
1	0.10	0.01
2	**0.14**	***N/A****
3	-0.14	-0.04
4	-0.18	-0.03
5	*0.04*	*0.00*
6	0.02	0.00
7	***-0.28***	***N/A****
8	-0.06	-0.01
9	***0.04***	***N/A****
10	-0.03	0.00
Averages:	-0.04	-0.01

* indicates no value since control chambers did not contain *Didemnum vexillum*

Table 29. Clearance rates, uncorrected for controls, based on forward scatter vs phycoerythrin non-noise flow cytometer analysis from laboratory experiment on July 24, 2009 (control chambers are bold and italicized)

	Forward Scatter vs Phycoerythrin Non-noise	
Chamber	L h^{-1}	L h^{-1} g_{dw}^{-1}
1	0.11	0.01
2	***0.13***	***N/A****
3	-0.12	-0.04
4	-0.18	-0.03
5	*0.05*	*0.00*
6	0.02	0.00
7	***-0.28***	***N/A****
8	-0.06	-0.01
9	***0.05***	***N/A****
10	-0.02	0.00
Averages:	-0.03	-0.01

* indicates no value since control chambers did not contain *Didemnum vexillum*

Table 30. Oxygen consumption rates, uncorrected for controls, from laboratory experiment on July 24, 2009 (control chambers are bold and italicized)

	Oxygen Consumption	
Chamber	µmol O_2 h^{-1}	µmol O_2 h^{-1} g_{dw}^{-1}
1	36.58	3.53
2	***12.95***	***N/A****
3	57.75	16.66
4	45.50	8.16
5	23.45	2.07
6	68.95	14.61
7	***-24.85***	***N/A****
8	26.60	5.06
9	***-7.53***	***N/A****
10	77.70	7.55
Averages:	31.71	8.23

* indicates no value since control chambers did not contain *Didemnum vexillum*

Table 31. Clearance rates, uncorrected for the controls, based on fluorometer measurements from field experiment on September 13, 2009 (control chambers are bold and italicized)

	Clearance Rates	
Chamber	L h^{-1}	L h^{-1} g_{dw}^{-1}
1	0.65	0.37
2	***0.59***	***N/A****
3	1.40	2.17
4	1.25	0.50
5	***0.39***	***N/A****
Averages:	0.78	1.01

* indicates no value since control chambers did not contain *Didemnum vexillum*

Table 32. Clearance rates, uncorrected for the controls, based on fluorometer measurements from field experiment on October 10, 2009 (control chambers are bold and italicized)

	Clearance Rates	
Chamber	L h^{-1}	L h^{-1} g_{dw}^{-1}
1	-0.16	-0.21
2	-0.16	-0.16
3	-0.12	-0.08
4	***0.00***	***N/A****
5	***-0.10***	***N/A****
Averages:	-0.11	-0.15

* indicates no value since control chambers did not contain *Didemnum vexillum*

Table 33. Clearance rates, uncorrected for controls, based on forward scatter vs chlorophyll-*a* non-noise flow cytometer analysis from field experiment on October 10, 2009 (control chambers are bold and italicized)

	Forward Scatter vs Chlorophyll-*a* Non-noise	
Chamber	L h^{-1}	L h^{-1} g_{dw}^{-1}
1	0.23	0.30
2	0.13	*0.13*
3	1.51	1.07
4	***1.96***	***N/A****
5	***N/A******	***N/A******
Averages:	0.96	0.50

* indicates no value since control chambers did not contain *Didemnum vexillum*
***indicates no value because sample was not taken from chamber for flow cytometer analysis

Table 34. Clearance rates, uncorrected for controls, based on forward scatter vs phycoerythrin non-noise flow cytometer analysis from field experiment on October 10, 2009 (control chambers are bold and italicized)

	Forward Scatter vs Phycoerythrin Non-noise	
Chamber	L h^{-1}	L h^{-1} g_{dw}^{-1}
1	0.12	0.16
2	*0.09*	*0.09*
3	1.54	1.09
4	*1.94*	*N/A**
5	*N/A***	*N/A****
Averages:	0.92	0.45

* indicates no value since control chambers did not contain *Didemnum vexillum*
***indicates no value because sample was not taken from chamber for flow cytometer analysis