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Nearshore Benthic Oxygen Dynamics in Lake Michigan

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NEARSHORE BENTHIC OXYGEN DYNAMICS IN LAKE MICHIGAN

by

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ABSTRACT

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by

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Under the Supervision of Professor Harvey Bootsma

The intense colonization of the Laurentian Great Lakes by dreissenid mussels has profoundly changed ecosystem processes, particularly benthic oxygen dynamics. Dissolved oxygen concentrations in mussel beds, sloughed *Cladophora* mats, and sediment indicate that hypoxia forms and disappears in some substrata (ephemeral *Cladophora* mats), while occurring consistently in others (depositional areas of sloughed *Cladophora*). Dissolved organic carbon concentrations are high (mean: 143 ± 28 ppm) in depositional *Cladophora* mats but lower (< 10 ppm) in most other environments. Field sampling and laboratory experiments suggest that under conditions of low water velocity and thick *Cladophora* cover, hypoxia may develop atop and within mussel beds. Sloughed *Cladophora* areal respiration was ~80% of mussel assemblage areal respiration and shallow anoxia developed within 3-6 hours of algal incubation. Vertical mixing rates in mussel beds with attached *Cladophora* were inferred from measurements of oxygen flux and vertical gradients. The diffusion coefficient into mussel beds ranged from 6.9 x 10^{-4} to 2.4 x 10^{-3} cm^2 s^{-1}.

Respiration rates of the quagga mussel (*Dreissena rostriformis bugensis*) were determined for the shallow and profunda morph phenotypes from *in situ* and laboratory

experiments under a range of temperature (4-20°C), shell size, and food availability conditions. Temperature-normalized oxygen consumption was significantly lower for the hypolimnetic profunda phenotype. Mass-normalized respiration rates were inversely related to mussel size, and the slope of this relationship increased with temperature. Mussels adjusted their oxygen consumption in response to food enhancement and deprivation, lowering their respiration to a basal metabolic rate 18 hours after food deprivation. In response to decreasing ambient oxygen, quagga mussels exhibited first-order reaction kinetics, with mass-normalized respiration rate at a dissolved oxygen concentration of 0.10 mg L^{-1} being 1% of that at saturation. Using published data on quagga mussel energy budgets and respiratory quotients, oxygen consumption rates were converted to organic carbon consumption rates. Using these values, along with data on mussel biomass and size frequency distribution in Lake Michigan, it is estimated that quagga mussels consume 15% of annual phytoplankton production. Dreissenids appear to exert significant direct and indirect influences on benthic oxygen dynamics with implications extending up the food web.

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“A skillful limnologist can probably learn more about the nature of a lake from a series of oxygen determinations than from any other kind of chemical data.”

-G.E. Hutchinson

1. Introduction

Understanding the mechanisms that control benthic dissolved oxygen concentrations has broad scientific implications. An understanding of dissolved oxygen distribution and dynamics can provide critical insights into benthic production and metabolism (Carlton and Wetzel, 1986; Stoeckmann and Garton, 1997) and biogeochemical processes (Carlton et al., 1989; Archer and Devol, 1992; McManus et al., 1997). For example, in addition to directly reflecting the metabolic processes of photosynthesis and respiration, dissolved oxygen strongly influences the cycling of numerous elements in aquatic systems, including nitrogen, phosphorus, and iron (Rysgaard et al., 1994; Hupfer and Dollan, 2003; Turner, 2010). Dissolved oxygen also has a strong influence on the distribution and behavior of aquatic biota, from fish to bacteria. Despite the tremendous influence of benthic dissolved oxygen (DO) on a variety of benthic processes, changes to benthic dissolved oxygen dynamics as a result of the intense colonization of dreissenid mussels and related growth and deposition of nuisance *Cladophora* algae has not been well studied. This paper will address two aspects of nearshore dissolved oxygen dynamics in Lake Michigan: benthic oxygen dynamics in relation to substratum type and oxygen consumption by quagga mussels (*Dreissena rostriformis bugensis*)**.**

Colonization of the Laurentian Great Lakes by mussels has profoundly changed nearshore nutrient cycling (Arnott and Vanni, 1996; Bruesewitz et al., 2006; Bootsma and Liao, 2013) and food web interactions (Fahnenstiel et al., 2010a; Kerfoot et al., 2010; Vanderploeg et al., 2012). Dreissenids appear to have become a major conduit for nutrient and energy flow in the Great Lakes (Madenjian, 1995; Stoeckmann, 2003; Hecky et al., 2004; Nalepa et al., 2009; Higgins and Vander Zanden, 2010). It is important to determine the fate of the nutrients and energy that flow through mussels, and oxygen dynamics are a mechanism for quantifying energy flow (Stoeckmann and Garton, 1997).

Nutrients ingested by mussels are deposited as feces and pseudofeces in mussels beds, where bed structure traps these and other settled material, preventing re-suspension and effectively restricting access to upper trophic levels (Hecky et al., 2004; Nalepa et al., 2009). The fate of material in the bed will be determined in part by redox conditions within mussel assemblages. A low oxygen environment beneath dense assemblages of respiring dreissenids may increase the probability of sediments going anoxic, influencing a number of lake-wide biogeochemical processes including phosphorus recycling and nitrification/denitrification. The hypoxic and potentially anoxic levels at the base of beds may lead to the release of iron-bound phosphorus as is hypothesized to be occurring in the anoxic intestinal tracts of mussels (Hecky et al., 2004), and was found in microcosm experiments of mussels atop sediment (Turner, 2010). On continental margin sediment at low bottom water oxygen concentrations, phosphate flux out of sediment is elevated (McManus et al., 1997). Furthermore, the hypoxic conditions at the base of mussel beds and other substrates may lead to nitrogen loss from denitrification as was seen in field sampling (Bruesewitz et al., 2006) and laboratory mesocosms (Bruesewitz et al., 2008) and in layers of anoxic sediments (Carlton et al., 1989).

Oxygen dynamics can be used to better understand processes of whole lake productivity and eutrophication (Charlton, 1980; Burns et al., 2005; Edwards et al., 2005a). Charlton (1980) found that oxygen in the hypolimnion depends upon hypolimnion thickness, temperature, and lake productivity. Hypolimnetic depletion, sensitive to sediment oxygen demand (Edwards et al., 2005a), has the potential to be influenced by mussels, but very little research has identified how dreissenids directly influence the oxygen condition within their immediate vicinity. On a lake-wide scale, large *Microsystis* blooms have been tied to mussel filtration (Vanderploeg et al., 2001; Bykova et al., 2006). Further, although a suite of problems contributes to Lake Erie's

hypoxic events (Hawley et al., 2006; Vanderploeg et al., 2009), the role of mussels in promoting hypoxic conditions should not be overlooked.

Within Lake Michigan and some of the other Laurentian Great Lakes, a phenomenon closely linked to dissolved oxygen dynamics is avian botulism. While botulism outbreaks have been observed in the Great Lakes for over a half century (Monheimer, 1968; Brand et al., 1983), and *Clostridium botulinum* DNA has been detected in sediments dating from the early 1900s (M. Edlund, personal communication), they appear to have become more frequent in the last decade (Pérez-Fuentetaja et al., 2006; Pérez-Fuentetaja et al., 2013), especially in Lake Michigan (Lafrancois et al., 2011). Botulism poisoning results from a powerful neurotoxin produced by the obligate anaerobic bacterium *Clostridium botulinum* (Fenchel and Finlay, 1995; Rocke and Bollinger, 2007). The timing and location of the anoxic events that allow the survival and growth of *C. botulinum* in Lake Michigan have been difficult to establish, but it has been suggested that the development of anoxia in mussel beds, in combination with the delivery of organic material to these beds, may produce the conditions necessary for the growth of *C. botulinum* and be tied to outbreaks of Type E botulism (Getchell and Bowser, 2006). Coincident with the establishment of dreissenids has been the resurgence of *Cladophora* growth in the Great Lakes (Higgins et al., 2008; Auer et al., 2010; Tomlinson et al., 2010; Depew et al., 2011). The high nutrient, low oxygen environment of sloughed *Cladophora* beds may provide an ideal environment for *C. botulinum* vegetative cell growth and toxin production. Furthermore, the thick *Cladophora* cover blanketing dense mussel assemblages may increase the likelihood of mussel beds going anoxic and create the high nutrient, low oxygen environments favorable for toxin production.

Mussel colonization and related benthic algal growth has created new habitat for organic decomposition, which had previously occurred in the water column and mussel-free substrata. Increased benthic organic loads in concert with increased water clarity and light flux has

promoted nearshore benthic algal growth, with *Cladophora* biomass reaching the nuisance levels seen in the 1960s and 1970s (Bootsma and Liao, 2013). In a study along the Lake Ontario shoreline, dreissenids were found to excrete more phosphorus than is required to sustain *Cladophora* growth (Ozersky et al., 2009). In a marine bivalve community, benthic microalgal abundance increased by a factor of 4-8 in the presence of mussels (Pfister, 2007).

A comparison of oxygen concentrations and nutrients (dissolved organic carbon) between mussel beds, sloughed *Cladophora* mats, and sediment will indicate whether dreissenids have altered benthic oxygen and biogeochemical processes linked to dissolved oxygen, directly or via their effects on benthic algal growth. Furthermore, a better understanding of substrata differences in dissolved oxygen may help identify the temporal and spatial frequency of the low DO, high nutrient substrates that are potential hot-spots of botulinum toxin production.

1.1 Benthic oxygen dynamics

Much of the research investigating benthic oxygen dynamics has examined the sediment-water interface. Oxygen uptake rates and distribution of oxygen in sediment (Revsbech et al., 1980; Jørgensen and Revsbech, 1985; Jørgensen and Des Marais, 1990; Rasmussen and Jørgensen, 1992), is measured in an effort to model oxygen flux through the boundary layers overlying sediment (Hall et al., 1989; Archer and Devol, 1992). Although models of sediment-water oxygen exchange are not completely transferable to mussels beds, the general principles can be used to understand oxygen dynamics in and around mussel beds. Processes in sediments, biofilms, and microbial communities are strongly influenced by the presence of hydrodynamic boundary layers (Kuhl and Revsbech, 2001). Boundary layers can limit mass transfer between the water column and the benthic matrix. The benthic boundary layer (BBL) is defined in many ways, most often related to the spatial scale of the properties being studied (Figure 1). In general, the BBL is the

part of the marine or aquatic environment directly influenced by the presence of the interface between the sediment bed and overlying water (Dade et al., 2001).

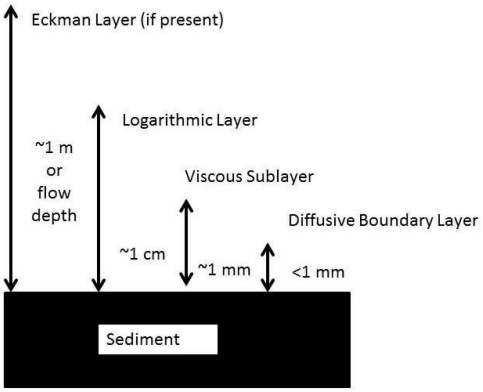


Figure 1. Idealized representation of the components of the BBL. Adapted from (Boudreau and Jørgensen, 2001).

While much of the vertical transport in the BBL is a result of turbulence, when turbulence and waves die out the region is dominated by viscous transport. The viscous sublayer is a thin, centimeter-scale layer that exhibits the largest changes in velocity and exists at the lower part of the BBL (Dade et al., 2001). At the very surface of the sediment-water interface is a film of water only tenths of a millimeter thick called the diffusive boundary layer (DBL). The DBL can be thought of as a blanket covering the entire sediment surface through which oxygen moves (Jørgensen, 2001). Microgradient profiles of oxygen within the BBL have demonstrated a transition within the boundary layer between the bulk water above, where eddy diffusion dominates particle movement in the viscous sublayer, to molecular diffusion within the DBL. As the layer at the point of contact between sediment and water, the DBL has been well studied when attempting to characterize benthic oxygen uptake and fluxes across the (Hall et al., 1989; Jørgensen, 2001).

The thickness of the DBL is unstable, affected by the turbulent flow in the overlying water. Mass transport of solutes in the DBL transitions from movement controlled by advective

motion (turbulence) to molecular diffusion as the sediment-water interface is approached (Kuhl and Revsbech, 2001). Studies comparing model and *in situ* chamber experiments over sediment have found that the rate of oxygen uptake is dependent on DBL thickness, such that a thicker sublayer results in a reduced uptake rate (Hall et al., 1989).

The physical parameters of the DBL outlined above effectively create a partial chemical barrier with a steep, linear gradient between bulk water and solid surfaces, which can present a major resistance to transport. If the solid surface has a high enough oxygen uptake rate from decomposing plant or animal material, the DBL can be anoxic even if the above water is oxygenated, creating thin areas of anoxia on the sediments (Jørgensen and Revsbech, 1985). This same chemical barrier can be expected to form in and around mussel beds, where the dense physical structure of the mussel assemblage traps nutrients and organic carbon in the form of feces, pseudofeces and settling seston, and restricts water movement.

A number of studies have highlighted the ability of benthic models to reliably simulate DBL hydrodynamics and oxygen flux, using microelectrodes to capture fine resolution changes in oxygen (Revsbech et al., 1980; Hall et al., 1989; Rasmussen and Jørgensen, 1992). In this study I will use a micro-optode system. Optodes are relatively easy to construct, robust, highly sensitive to small changes in oxygen concentrations, do not consume oxygen, are not dependent on flow velocity, or disrupted by changes in salinity and pH (Klimant et al., 1995; Klimant et al., 1997; Kuhl and Revsbech, 2001). These qualities allow for the use of optodes in a variety of media and offer an appealing alternative to microelectrodes for environmental sampling. Despite the suitability of optodes for measurements in this study, response time appears to be the major area where microelectrodes are unmatched, with some well-designed electrode sensors exhibiting response times of around 0.1 seconds (Tengberg et al., 2006). A study evaluating the responses of optical oxygen sensors and electrodes to temporal changes in DO concentrations found that the

optode signal responds more quickly than the electrode signal when O_2 is rapidly increasing in a sample, but responds less quickly than the electrode when O_2 is rapidly declining (Glazer et al. 2004).

2. Nearshore benthic oxygen dynamics in Lake Michigan in relation to substratum type

2.1 Purpose of study

This chapter describes a series of field investigations and laboratory experiments comparing oxygen and dissolved organic carbon (DOC) concentrations between mussel beds, sloughed *Cladophora* mats, and bare sediments. The purposes of this study were (1) to determine whether dreissenid mussels have directly or indirectly (via their effects on *Cladophora*) altered benthic oxygen dynamics, (2) determine the effects of sloughed *Cladophora* mats on benthic oxygen, and (3) identify low dissolved oxygen (DO), high nutrient substrata that are potential hot-spots of botulinum toxin production. Pinpointing these environments will allow for targeted sampling of the locations and time periods most likely to be associated with toxin production.

The measurement of oxygen concentrations and dynamics in mussel beds is challenging due to the small spatial scales over which DO gradients likely occur. Mussel bed thickness is generally on the order of 1 to several cm, which means that within these beds significant DO gradients likely occur over scales of several mm or less. These challenges in measuring DO at the base of the bed have limited the ability of benthic oxygen models to accurately capture flux into and out of mussel assemblages, particularly when beds are covered by thick algal cover. To overcome this problem, in this study I utilized a fine-tipped DO probe to measure near-bed oxygen.

2.2 Avian botulism

Bird die-offs from avian botulism have occurred in the Great Lakes for many years as relatively infrequent events (Monheimer, 1968; Brand et al., 1983). In Lake Michigan, Type E avian botulism was first reported in 1963 and subsequently occurred every few years through 1983 (Rocke and Bollinger, 2007). Recently the occurrence and intensity of outbreaks have increased.

Beginning in 2006 there was a resurgence of mortality events, and die-offs have been observed with regularity every year since then. Since 2006 over 4,000 birds have died, 760 of them common loons, a state threatened species in Michigan. Costs and logistics prevent the testing for botulism toxin in every dead bird found in the lakeshore. Once botulism toxin has been established as the cause of mortality for a subset of individuals from each species, all birds found dead during the beach monitoring season (June-November) are assumed to have died from the toxin unless another cause of death is determined.

Clostridium botulinum is a naturally occurring, cold tolerant, anaerobic bacterium widely distributed in sediment (Fenchel and Finlay, 1995; Rocke and Bollinger, 2007). It produces dormant spores that can survive extreme conditions over long periods of time. Dormant spores germinate into vegetative cells that grow under a temperature range of 6-41°C, with optimal growth temperature ranging from 25-32.5°C (Grecz and Arvay, 1982; Peck, 2009). The nutrient requirements for vegetative cell growth in the environment have not been well studied (S. Haack, personal communication) but food models suggest that growth depends on the protein content of the environment in which it is growing (Long and Tauscher, 2006), with the minimum required compounds needed for the germination of spores of *C. botulinum* type-E being L-alanine, glucose, lactic acid, and bicarbonate (Ando and Iida, 1970). Cell multiplication is limited to environments with a pH above 5.0 and NaCl concentrations below 5% (Peck, 2009). Once vegetative cell threshold has been reached, cells produce botulinum neurotoxin. The botulinum Type E toxin (BoNT/E) is the serotype associated with most botulism outbreaks in the Great Lakes. This toxin is one of the most poisonous substances known (Arnon et al., 2001), affecting the vertebrate nervous system causing muscle paralyses and eventually death by drowning, predation, asphyxiation, or dehydration.

Factors associated with botulism outbreaks and toxin gene detection have been evaluated in various contexts. Recent studies have shown that botulism outbreak events on Lake Michigan most frequently occurred in years with low mean water levels and higher than average surface water temperatures (Lafrancois et al., 2011). In a spatial survey of Lake Erie, lower DO, pH, and redox potential near the sediment was associated with higher specific conductance and type E toxin gene in sediments (Pérez-Fuentetaja et al., 2006). In wetlands, pH, redox potential, and salinity measured just above the sediment-water interface were associated with the risk of botulism outbreaks (Rocke and Samuel, 1999). Wetlands experiencing botulism events had greater percent organic matter in the sediment and lower redox potential in the water than non-outbreak wetlands (Rocke et al., 1999; Rocke and Samuel, 1999). Rocke et al. (1999) found that an increase in invertebrate biomass was associated with the probability of botulism in sentinel mallards. This may result in part from the tendency of benthic organisms to carry significantly higher levels of Type E spore DNA than occurs in sediments (Pérez-Fuentetaja et al., 2011).

2.3 Methods

2.3.1 Study sites

Field sampling was conducted via SCUBA diving at Sleeping Bear Dunes National Lakeshore, a unit of the National Park Service located along the northwest coast of Michigan's Lower Peninsula, from July – October 2012. DO profiling was conducted at: Good Harbor Bay - 10 m depth (GH10m, 44° 58.899'N, 85° 49.852'W), Good Harbor Bay - 20 m depth (GH20m, 44° 58.465'N, 85°48.872'W), and South Manitou Island - 10 m depth (SMI, 45°00.665'N, 86°05.485'W) (Figure 2). Good Harbor Bay was selected as a sampling site because of the presence of rocky shoals covered with dreissenids, relative accessibility by boat from a harbor, and proximity to a beach of high avian botulism mortality. Preliminary qPCR screening by the U.S. Geological Survey (USGS) indicated that Good Harbor Bay features locations with

relatively high levels of *Clostridium botulinum* DNA, suggesting a possible location for BoNT/E production and transmission.

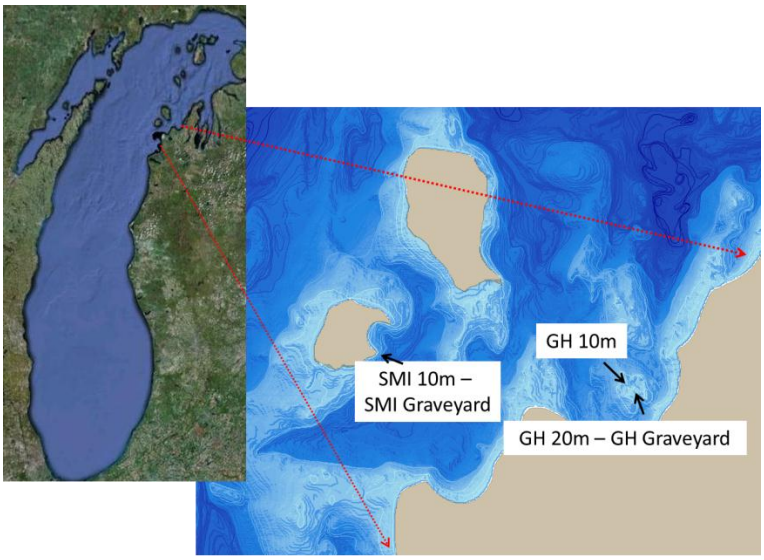


Figure 2: Location of dissolved oxygen profiling sites in northern Lake Michigan at Sleeping Bear Dunes National Lakeshore. The name of the site and the depth are indicated.

Within these sites, four environments were explored: rocky shoals, bare sand, benthic mud, and sloughed *Cladophora*. Rocky shoals are areas of large boulders almost completely covered by dense mussel assemblages with *Cladophora* growing atop. Space between the boulders ranges from 1/3 m to > 3 meters and boulders are grouped in clusters. Between the boulders areas of sand largely remain bare. Occasionally sandy areas are covered in mats of sloughed *Cladophora* that have been observed to be up to a 1/2 m thick. These mats, hereafter referred to as ephemeral *Cladophora* mats, appear to quickly form from senesced *Cladophora* and rapidly break apart and disappear following large storm and wind events.

Benthic mud was collected by a Ponar dredge from GH20m. Benthic mud was black/gray in color and covered by a thin (2 mm) green and brown layer that appeared to be decomposed algae.

Two areas of thick, sloughed *Cladophora* deposition, termed the Graveyard, were explored at SMI and GH20m. Graveyards were comprised of densely packed, decomposing

Cladophora with a thin layer of sand and bacteria atop (Figure 3). Beneath the green surface layer, material was black and fluffy. Disturbing the mat released bubbles and visibility was quickly reduced until material settled. Emerging from the mat, dive gear and equipment smelled strongly of decomposing organic material. Benthic invertebrates, primarily chironomids, were frequently found in the top layer of the Graveyards and gobies were seen swimming along the top of the mat. Exploratory dissolved oxygen profiling in 2006 identified zones of anoxia 6 cm into these algal mats at SMI (B. Lafrancois and H. Bootsma, unpublished).

Figure 3: *Cladophora* mat at the SMI Graveyard. Note the thin bacterial layer atop decomposing algae.

Graveyard sites at South Manitou Island -10m depth (SMI Graveyard) and Good Harbor Bay -20m depth (GH Graveyard) were identified in this study. The SMI graveyard, located at the southeast corner of South Manitou Island, is less than 100 m offshore and can be accessed easily by SCUBA diving from shore. For this study, the SMI Graveyard was investigated at a depth of 10-12 m but likely extends into deeper water following the sloping bathymetry away from the island. Due to the graveyard's proximity to shore, thick mats of sloughed *Cladophora* are found throughout the summer on adjacent beaches. Dead birds have turned up on the shoreline close to the SMI Graveyard, warranting further investigation of these mats as possible toxin hot-spots. The low oxygen environment created from the decomposing algae may provide the necessary habitat for toxin production.

The Good Harbor Graveyard is a *Cladophora* depositional area 20-35 m deep in the southeast corner of GH20m. Located at the southern end of a shoal, this deep basin is surrounded by shallower substrate, creating an area where sloughed *Cladophora* and other particulate material can deposit and accumulate over time. Exploratory sampling with a Ponar dredge, video camera tows, and SCUBA diving indicated an area of length ~250 m east-west and ~150 m north-south, with mats of senesced *Cladophora* up to one meter thick.

The bathymetry surrounding both Graveyards creates depositional areas favorable to the accumulation of decomposing *Cladophora*. Monthly sampling at both sites indicates that the SMI Graveyard may be more stable and consistent, with mats of sloughed *Cladophora* identified during every monthly sampling event. Unlike the GH Graveyard, where a thick *Cladophora* mat that had been seen during every monthly sampling event May-October 2012, could no longer be found by video camera tows and ponar samples in November 2012.

2.3.2 Field Sampling

Dissolved oxygen profiles were conducted in a variety of substratum types (summarized in Table 1). At GH20m, Ponar grab samples and sloughed *Cladophora* mats (GH Graveyard) were profiled. At SMI, sloughed *Cladophora* mats (SMI Graveyard) were the only substrate profiled. At GH10m, environments profiled included: mussel assemblages with attached *Cladophora*, ephemeral mats of *Cladophora* deposited after a mid-season sloughing event, sediment, benthic mud, sloughed *Cladophora* placed in PVC tubes as part of an experiment investigating algal decomposition and the potential for toxin production, and the sediment beneath the tubes. The *Cladophora* in PVC tubes was part of a field experiment investigating the hypothesis that conditions within and beneath sloughed *Cladophora* mats promote anoxia, *C. botulinum* cell growth, and toxin production (B. Lafrancois and H. Bootsma, personal communication). Sloughed *Cladophora* was placed in tubes, creating “decomposing mats,”

similar in structure and thickness to mats found in the graveyards. Dissolved oxygen in the *Cladophora* and in the sediment beneath the tubes were tracked monthly during the summer and fall. Experiments were intended to mimic naturally occurring sloughed *Cladophora* mats, which tend to form and disperse quickly. Profiled substratam were chosen to determine their potential as environments suitable for *C. botulinum* toxin production. It is probable that decaying mats of *Cladophora* contain the high nutrient and low oxygen requirements for *C. botulinum* vegetative cell growth.

For all future discussions, hypoxia is defined as DO levels < 2 mg L^{-1}. In profiles into mussel covered rocks, once resistance to profiling was felt, suggesting contact with the rock, profiling ceased and *Cladophora* was moved aside to determine the thickness of the algal layer atop and height of the mussel assemblage beneath. When hypoxia was identified above and within assemblages, depth averaged water velocities for dates and times of field sampling at GH10m were obtained from the Great Lakes Coastal Forecasting System (GLCFS) point query tool (glos.us/data-tools/point-query-tool-glcfs).

Table 1. Location and substrate type of field dissolved oxygen profiling from repeated sampling events, occurrence of hypoxia (H) and/or anoxia (A) noted, depth into substrate of (H) and (A), and results of DOC analyses of material (number of DOC sampling dates shown in parentheses). A range of depths into substrate when H and A were identified indicates the results from multiple profiles. DOC samples collected in top 1 cm of *Cladophora* mat and sediment. For direct comparisons between DOC and DO profiles see Figures 9, 10, 13. All substratum types except those collected by Ponar dredge were profiled *in situ*.

Location (depth)	**Substratum type**	**Hypoxia /anoxia?**	**Depth into substrate (mm)**	**DOC (mg L^{-1}) mean ± SD**
Good Harbor Bay (10 m)	Sediment beneath ephemeral sloughed *Cladophora* mat	Yes	14 (H) 20 (A)	3.8* (1)
	Base of mussel bed with sparse attached *Cladophora* atop.	No	---	------
	Atop mussel bed + base of mussel bed with thick attached *Cladophora* atop.	Yes	41-64 (H)	2.9 ± 0.4 (5)

	Sloughed Cladophora in PVC (algal deposition experiment)	Yes	1-20 (H) 16-35 (A)	16.6 ± 4.4 (6)
	Sediment beneath PVC *Cladophora*	Yes	1-27 (H)	6.4 ± 2.3 (4)
	Sediment not under tubes (control sediment)	Yes	4-12 (H)	7.5 ± 1.9 (3)
Good Harbor Bay (20-30m)	Sediment (collected via Ponar)	Yes	2 (H)	7.5 ± 3.6 (4)
South Manitou Island (10 m)	*Cladophora* deposition	Yes	1-2 (H) 12 (A)	90.4 ± 52.9 (3)
'SMI graveyard'	Sediment atop detritus	DNP	---	143 ± 27.8 (2)
Good Harbor Bay (20 m)	*Cladophora* deposition	Yes**	1 (A)	6.8 ± 0.71 (4)
'GH Graveyard'	Sand atop *Cladophora* deposition	Did not profile	---	3.7 ± 0.99 (2)

*Sediment beneath ephemeral mat, only one sample taken
**Sample probed but no profiles taken

2.3.3 Measuring dissolved oxygen: optical oxygen systems

Accuracy in measuring dissolved oxygen is a scientific priority and researchers utilize a variety of techniques to measure DO flux across the sediment-water interface and within the benthic boundary layer. For all field and laboratory experiments I used a micro-optode system to measure DO because of its sensitivity to small changes in oxygen concentrations and compatibility for use underwater. Optical fluorescence quenching optode systems, also known as optical oxygen sensors, are a relatively new method for measuring dissolved oxygen. Optical sensors allow for a high degree of resolution and sensitivity while providing real-time and continuous profiles of dissolved oxygen without measurement-induced consumption of oxygen. Optical oxygen systems capitalize on the ability of selected substances to act as dynamic luminesce quenchers; ruthenium complexes are often used as the oxygen-sensitive indicator dye (Tengberg et al., 2006). Ruthenium is insoluble in water, can be immobilized in a polymer, and

will not leach into the sample (Klimant et al., 1995). As the ruthenium complex is illuminated with blue light, it becomes excited and emits a red luminescent light with a lifetime (intensity) dependent on the ambient oxygen concentration (Tengberg et al., 2006). Optodes use single-strand fiber-optics that collect and direct light signals between the sensor tip and the measuring system (Kuhl and Revsbech 2001). An underwater housing and a manual micromanipulator, allowing for profiling in increments as small as 1 mm, were constructed for the micro-optode system (Figure 4). The probe was recalibrated before most sampling events.

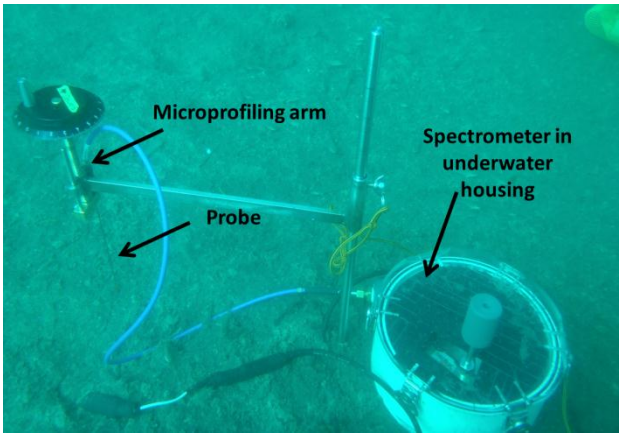


Figure 4: Dissolved oxygen microprofiler atop *Cladophora* mat at Graveyard site.

2.3.4 Subsamples for qPCR and DOC

On most sampling dates subsamples of material were taken for dissolved organic carbon (DOC) and qPCR analyses. Samples for qPCR were tested for presence/absence and quantification of the BoNT/E gene at the USGS-Michigan Water Science Center. Analyses were still being conducted at the time of writing and results from qPCR tests will be addressed in a subsequent paper. Forty milliliters of the top 1cm of benthic material (sediment, filamentous *Cladophora* in decomposing mats, sediment/biodeposits around mussel assemblages) were collected via syringe after DO profiling and samples were split evenly for qPCR and DOC analyses. Initial DOC sample processing occurred on the same day as collection: contents of each syringe were filtered (ashed, 0.70 μm Whatman GF/F), extracting from 0.6 to 6 mL of pore water

(filtrate) which was refrigerated. The following day, filtrate was shipped overnight to the UWM-School of Freshwater Sciences and carbon content analyzed using a Shimadzu TOC-5000 Total Organic Carbon Analyzer equipped with an ASI 5000 Auto Sampler. Samples for qPCR were stored frozen until analysis.

Although a set amount of material was collected to allow for quantitative comparisons between types of substrate, organic artifacts, particularly in material around mussel beds, could have confounded results and make comparisons between substrate types difficult. Regardless, DOC results are still useful when paired with DO values to make a general assessment of a substrata's likelihood of being a location of BoNT/E production.

2.3.5 Laboratory incubations

Dissolved oxygen concentration within various substrata will be a function of two factors: respiration rate within these substrata, and the oxygen flux from overlying water, which is determined largely by substratum porosity. In order to determine the importance of the first factor, lab experiments were conducted to measure the respiration rate in various substratam. The substrata used were sediment and biodeposits from around mussel assemblages collected from GH10m, and sloughed *Cladophora* from GH10m and the GH Graveyard. Samples were stored at 7°C until experimentation, which was conducted within 24 hours of collection. Incubations of sloughed *Cladophora* from GH10m were performed in triplicate, while those with *Cladophora* from the GH Graveyard in duplicate. Only one incubation of sediment and biodeposits from between mussels was conducted because of a shortage of material collected during sampling. For all experiments, the control was lake water collected from the same location as the experimental substratum material. Incubations were performed in either 250 mL bottles (GH10m samples) or 6 cm diameter sediment core tubes (GH Graveyard), filled 2/3 full with sloughed *Cladophora* and topped with lake water. For the incubations of sloughed *Cladophora* collected from GH10m, DO

profiles in the algal mat were measured over time, starting immediately after set up of the experimental chambers, to determine the time required for hypoxic or anoxic conditions to develop in the material. The incubations of *Cladophora* from the GH Graveyard and GH10m were assessed for total mat respiration. Dissolved oxygen measurements were taken in the overlying water and into the algal mat every 2-4 hours. The change in DO was integrated over depth and an areal respiration rate (mg O_2 m^{-2} hr^{-1}) for the incubated material was calculated with the following equation:

$$R = (C_{T2}\text{-}C_{T1})/ \text{(surface area of the mat)}/ (\Delta T) \qquad (1)$$

R = respiration rate, C_{T2} = the total oxygen concentration in the chamber at T_2 integrated from changes in DO concentration by depth, C_{T1} = the total oxygen concentration in the chamber at T_1 integrated from changes in DO concentration by depth. ΔT = the change in time from T_2 to T_1. Integration was determined by multiplying the DO concentration at each profile point by the volume of the profiled layer. This calculation was done for each point in the profile (both the overlying water and into the benthic material) and all points were summed to determine total chamber oxygen consumption. This integration was determined for each time point where a DO profile was recorded. The majority of oxygen consumption occurred between the first two time points and is the value of C_{T2}-C_{T1} used in Equation 1.Although the respiration rate was calculated for each time interval, the respiration rate for only the first time interval is reported.

During the course of experimentation, incubations were kept near *in situ* temperatures either by placement in a bath of ice-water (GH10m) or in a refrigerator (Pit). Efforts were taken to minimize disturbances during DO profiling. Samples were kept at the ambient lake bottom temperatures at the time of collection: 16°C for the GH10m samples and 12°C for the GH Graveyard samples. The overlying water atop the algal mat was gently stirred before sampling to

ensure that dissolved oxygen was homogeneously distributed. Subsamples of material not used in incubations were inspected for invertebrates.

To assess the potential influence of near-bottom current velocity on dissolved oxygen in mussel / *Cladophora* beds, one experiment was conducted in which DO profiles were measured in a bed consisting of mussels within an artificial *Cladophora* mat. Shallow morph mussels were collected from a depth of 9 m at Atwater Bay (near Milwaukee). Shells were scrubbed to remove attached algae and maintained in aerated buckets at 10°C. Water was exchanged from the buckets every 1-2 days and replaced with water from the Milwaukee inner harbor. Mussel assemblages were constructed using a cement tile base (20 x 9.5 x 1.2 cm) topped with a thin piece of PVC (20 x 9.5 x 0.5 cm) with holes cut on a 1.5 cm grid. Marabou chicken feathers, replicating *Cladophora* strands, were glued into the holes on the tile (Figure 5). Tiles with attached feathers were soaked for 24 hours prior to experimentation to remove all residues from the glue.

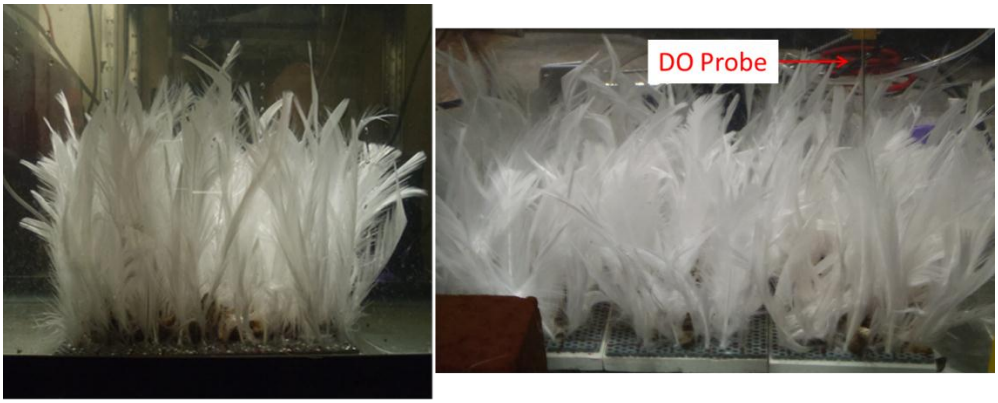


Figure 5: Experimental 'mussel assemblage' set-up in the aquarium (left). Side-view of bricks replicating a large rock coved by mussels (right). Arrow indicates location of dissolved oxygen probe.

Feather density approximately replicated *in situ Cladophora* in mid-summer, at the peak of algal growth when density is thickest. Feathers were used instead of *Cladophora* to avoid the effects of algal photosynthesis and/or respiration on oxygen dynamics, allowing for a direct assessment of the effect of near-bottom flow on dissolved oxygen. Space was left between feathers to ensure that mussels could be placed atop the brick at dreissenid densities replicating

4,500 mussels · m^{-2}, close to estimated nearshore areal densities of 5,000 mussels · m^{-2} (H. Bootsma, unpublished). Three tiles were prepared with similar feather coverage and placed side-by-side in the aquarium to simulate a mussel bed with *Cladophora* atop (Figure 6).

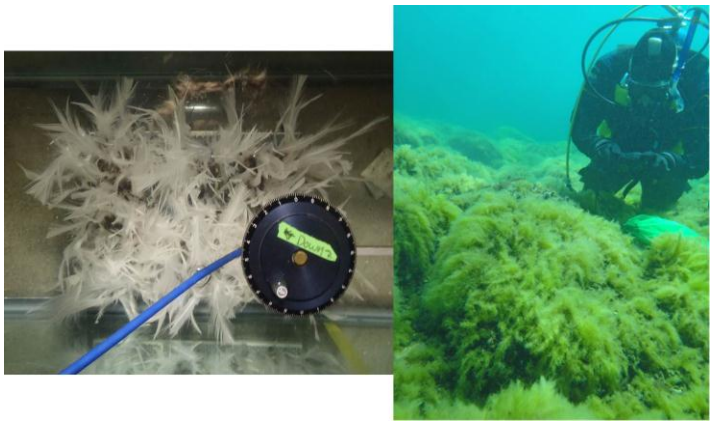


Figure 6: Birds-eye view of assemblage set-up with DO system profiler in place (left). Comparison with *Cladophora* covered rock, Good Harbor Bay 5m, August 2011 (right).

A 50 gallon aquarium was fitted with a recirculating pump, creating water velocities close to *in situ* conditions. Velocity was measured with a Vectrino Acoustic Doppler Velocimeter (Nortek AS) and ranged from 0.64-3.28 cm sec^{-1} within the water column when the pump was on, to 0.16-0.83 cm sec^{-1} when the pump was off (Figure 7). Experimental values were at the low end of the mean (4.66 ± 5.29 cm s^{-1}) depth averaged water velocities for GH10m from June-November 2012 but close to the mode (1.56 cm s^{-1}) and median (2.96 cm s^{-1}). Experiments were conducted with the pump on and off to assess the potential for anoxia to develop within a mussel bed under the two flow conditions.

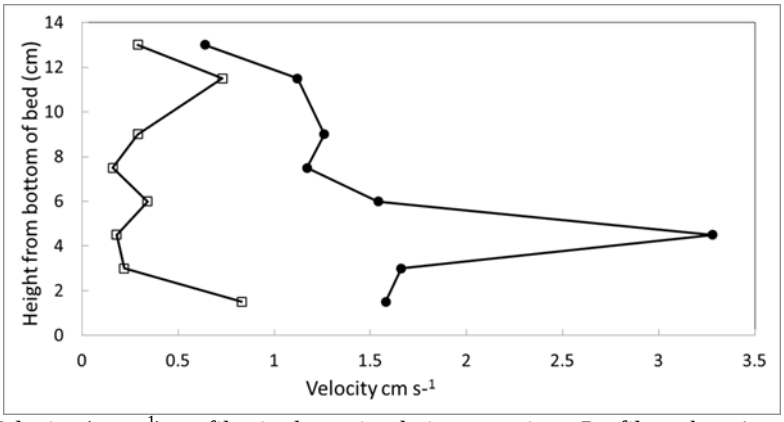


Figure 7: Velocity (cm s^{-1}) profiles in the recirculating aquarium. Profiles taken 4 cm in front of the mussel assemblage with the pump off (□, median: 0.29 cm s^{-1}) and on (●, median: 1.4 cm s^{-1}).

Prior to experimentation, water in the aquarium was aerated for three hours ensuring oxygen saturation at the start of each experimental run. Tiles were placed perpendicular to the direction of flow (Figure 8), except for one trial with the pump off, when tiles were placed side-by-side parallel to water movement. Dissolved oxygen profiling was conducted along a linear transect along the direction of the current, bisecting the middle of the assemblage where feather density was thickest and mussels were most protected from faster water velocities along the side of the aquarium. Once mussel assemblages were placed in the aquarium, a DO profile was measured from the top of the assemblage to its base. Following the profile measurement, the probe was left in place at a point 2 cm above the base, recording DO every 20 seconds for the duration of the experiments, which lasted from 5 – 16 hours. At the end of each experiment, DO profiles were measured along a transect through the middle of the assemblage.

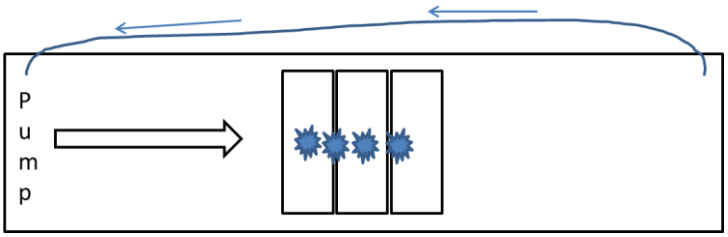


Figure 8: Birds-eye view of experimental setup in aquarium. Black arrow indicates direction of water flow in aquarium. Blue arrows indicate direction water pumped out of aquarium and into recirculating pump. Black squares indicate mussel assemblages and stars are locations of profiles.

2.4 Results

2.4.1 *In situ* measurements

As expected based on preliminary sampling in previous field seasons, profiles into *Cladophora* mats at the SMI Graveyard revealed hypoxic conditions only 1-2 mm into the substrate and anoxic conditions at 5 mm into the mat on August 20, 2012 (Figure 9). The spike in oxygen at the base of the profile on September 25, 2012 may be from the introduction of oxygen into the mat while profiling.

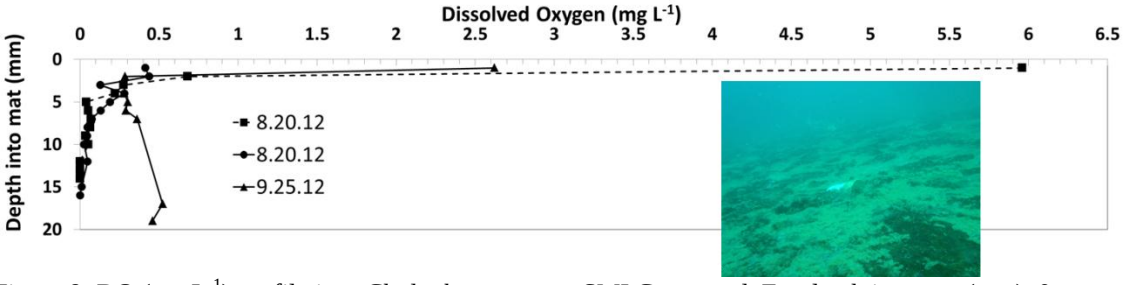


Figure 9: DO (mg L^{-1}) profile into *Cladophora* mats at SMI Graveyard. For depth into mat (mm), 0 mm = mat-water interface. Profiles are from duplicate profiles on August 20, 2012 (●, ■) and a profile on September 25, 2012 (▲). Mean (±SD) DOC concentration (ppm) samples collected atop profile on September 25, 2012 (■) indicated.

The thickness of depositional *Cladophora* and filamentous nature of the decomposing algae at the GH Graveyard, which created turbid conditions with visibility reduced to 1-2 feet when agitated, rendered profiles with the micromanipulator difficult to obtain. In lieu of profiling with the micromanipulator, the DO probe was slowly inserted into the mat by hand. Several of these 'profiles by hand' revealed anoxic conditions just 1-2 mm into the *Cladophora*. Interestingly, DOC levels within this environment were much lower than in the SMI Graveyard but comparable to values in sediment from Ponar grabs of benthic mud from the same location collected earlier in the season (Table 1). Within Ponar grabs from GH20m, hypoxia was observed 2 mm into the substrate (Figure 10).

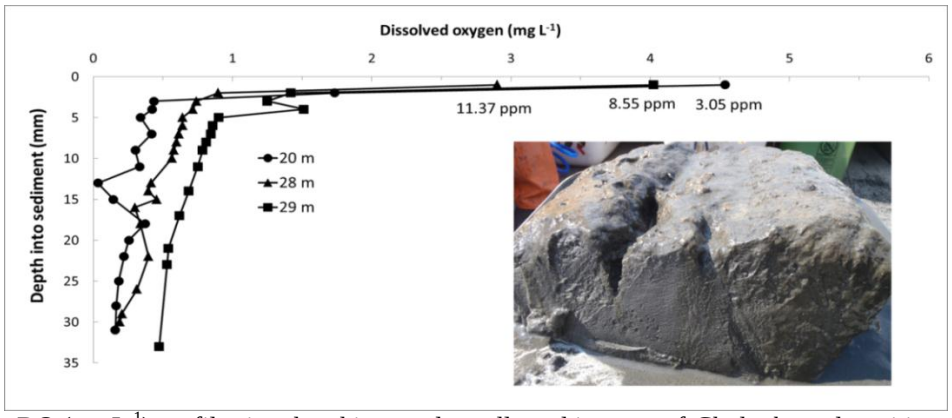


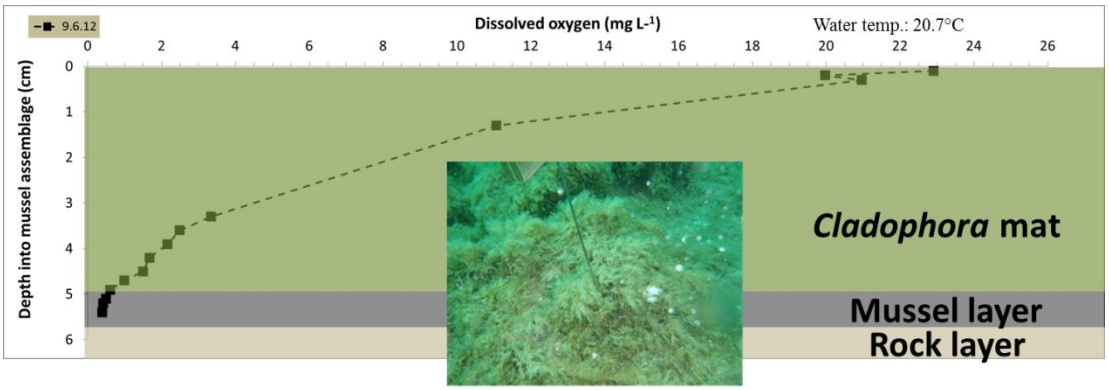
Figure 10: DO (mg L^{-1}) profiles into benthic samples collected in areas of *Cladophora* deposition at Good Harbor Bay 20m with Ponar dredge. For depth into sediment (mm), 0 mm = sediment-air interface. Ponar depths: 20 m (●), 28 m (▲), 29 m (■). DOC (ppm) concentrations of pore water from top 2 cm listed at top of graph beneath corresponding profile.

As hypothesized, mussel assemblages contained areas of hypoxia and near anoxia but only during two days in early September when there was with a thick growth of *Cladophora* atop the mussels. Profiling was conducted atop mussel beds from early August-late September, but low DO values were observed only during a period preceding *Cladophora* senesce and sloughing, when bottom water was warm (20°) and water velocity low (0.40 cm s^{-1} – 2.23 cm s^{-1}) (Figure 11). Within the first 1-3 cm into the *Cladophora* canopy, DO spiked, likely the result of photosynthesis, then quickly dropped to low levels, eventually reaching 0.40-1.64 mg L^{-1} at the base of the assemblage. Mussel assemblages were estimated to be ~1 cm high, expressed as the bottom 1 cm of profiles.

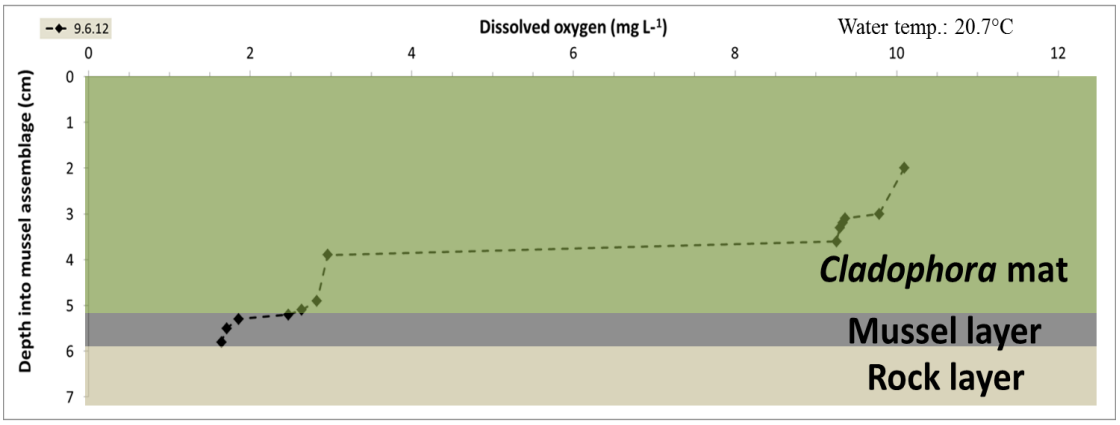
No oxygen gradient was seen during profiling before and after these two days (Figure 12). On one of the sampling dates when water velocity was low (1.92 cm s^{-1}), *Cladophora* was removed from atop the assemblage 20 minutes prior to profiling (12a) to assess oxygen conditions at the base of the assemblage without the presence of an algal mat limiting mixing between the assemblage and overlying water; no oxygen gradient was seen. Hand probing into the base of mussel beds at GH10m on multiple dates in October revealed high DO conditions at

the base of beds. Mussel assemblages were almost completely bare of *Cladophora* during these profiles.

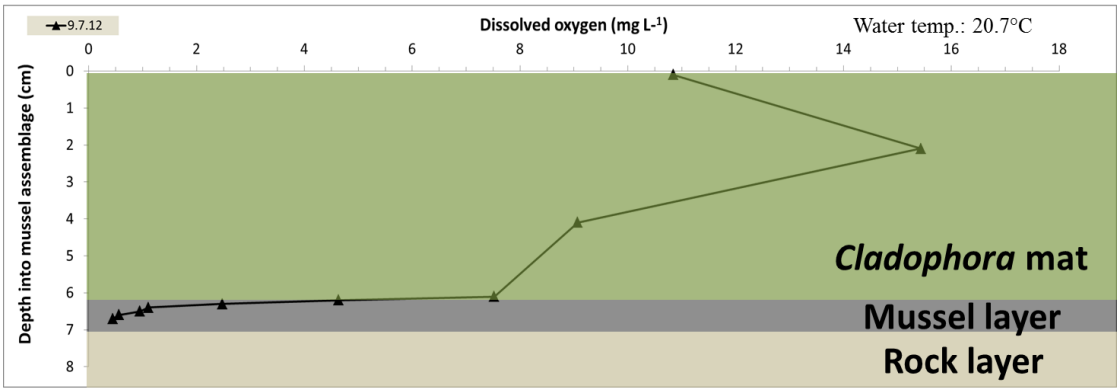
11a.



11b.



11c.



11d.

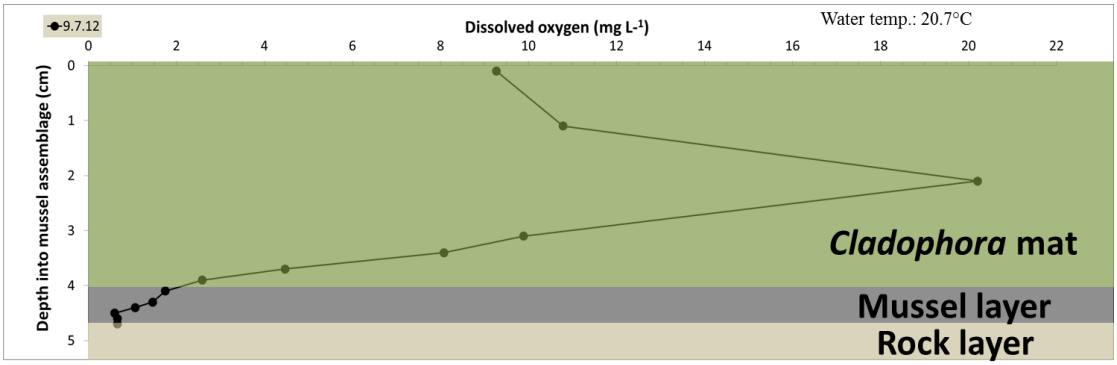
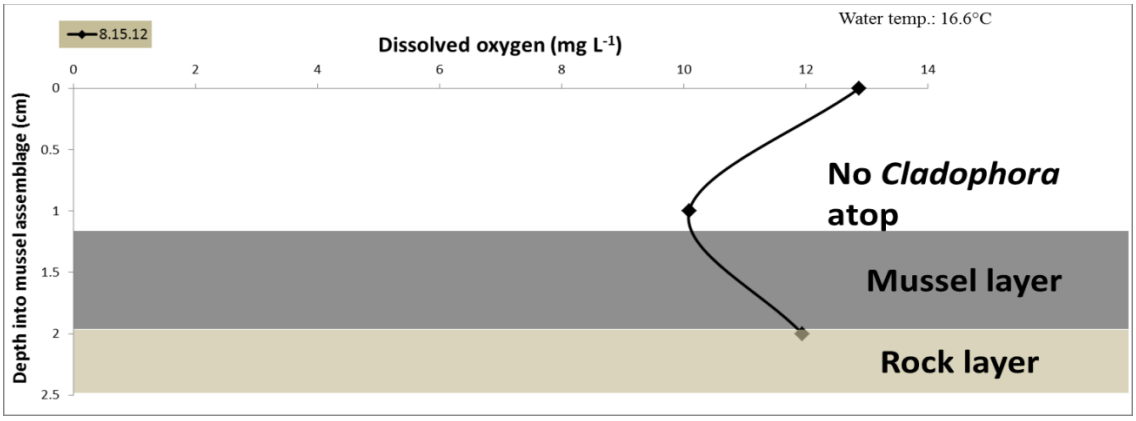


Figure 11: DO (mg $^{L-1}$) profile into mussel assemblage covered in thick *Cladophora*. For depth into assemblage (mm), 0 cm = *Cladophora*-water column interface. Profiles are from two sampling dates, with replicates on each date: (11a, b) September 6, 2012 (■, ♦) and (11c, d) September 7, 2012 (▲, ●). Profile on September 6, 2012 (11b.) started into the mat. Depth averaged water velocity during hours profiled: 2.23 cm s^{-1} (■, ♦) and 0.40 cm s^{-1} (▲, ●). Spike in DO 1-3 cm into substrate is likely the result of photosynthesis within the top layer of the mat.

12a.



12b.

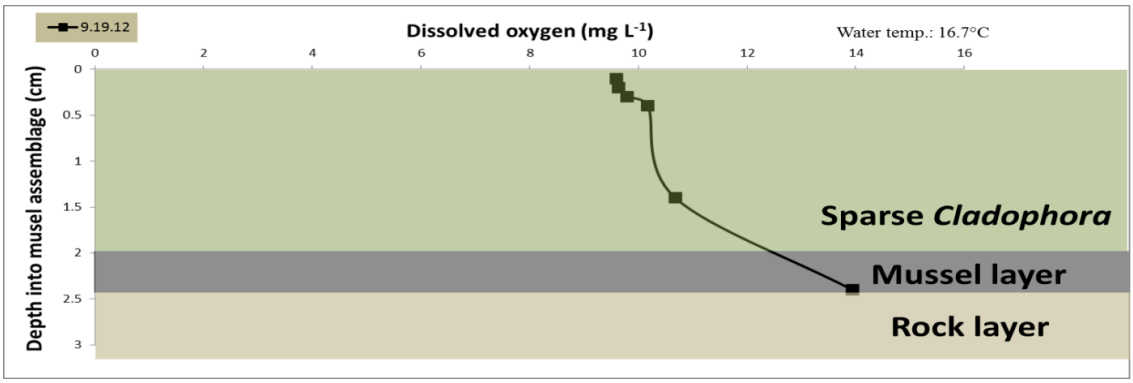


Figure 12: DO (mg L-1) profile into mussel assemblage after *Cladophora* picked from top of assemblage (12a.) and sparse *Cladophora* (12b.). Profiles are from two sampling dates: August, 15 2012 (■) and September 19, 2012 (■). Depth averaged water velocity during hours profiled: 1.92 cm s^{-1} (12a) and 3.43 cm s^{-1} (12b).

Profiles in ephemeral sloughed *Cladophora* mats at GH10m (Table 1) and within the PVC tubes of sloughed *Cladophora* (Figure 13) revealed hypoxia at depths of 14 mm and between 1-20 mm, respectively. The ephemeral sloughed *Cladophora* mats were seen only once at GH10m and had thinned significantly by the next dive two weeks later.

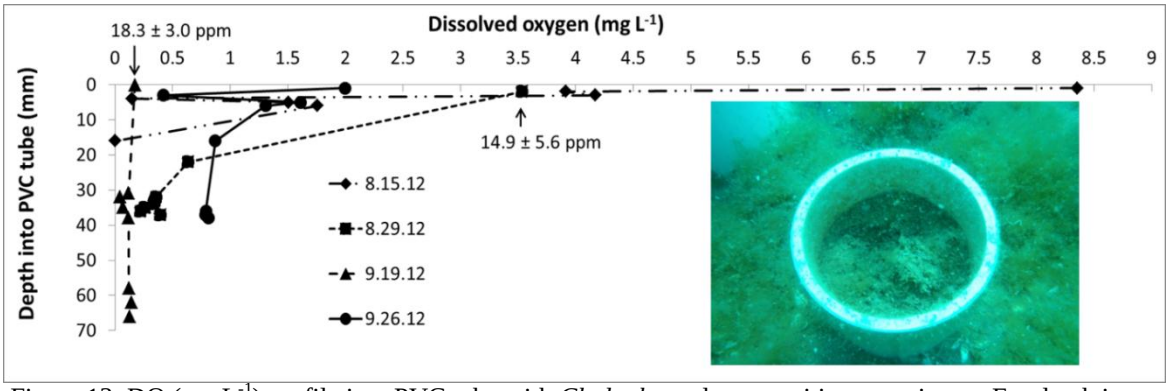


Figure 13: DO (mg L^{-1}) profile into PVC tube with *Cladophora* decomposition experiment. For depth into tube, 0 mm = *Cladophora*-water interface. Profiles from sampling on four dates: August 15, 2012 (♦), August, 29, 2012 (■), September 19, 2012 (▲), September 26, 2012 (●). Mean (± SD) DOC concentration (ppm) from triplicate samples collected on August 29, 2012 and September 19, 2012 indicated on graph.

Within sediment, hypoxia was seen at shallower depths underneath the PVC tubes than in profiles in sediment not under tubes (control sediment) (Table 1, Figure 14). Neither environment had areas of anoxia, even in one profile that went 6 cm into the substrate. Sediment beneath the PVC tubes was black in color suggesting the presence of decomposing material, although mean DOC concentrations for both control and PVC sediment were comparable (Table 1).

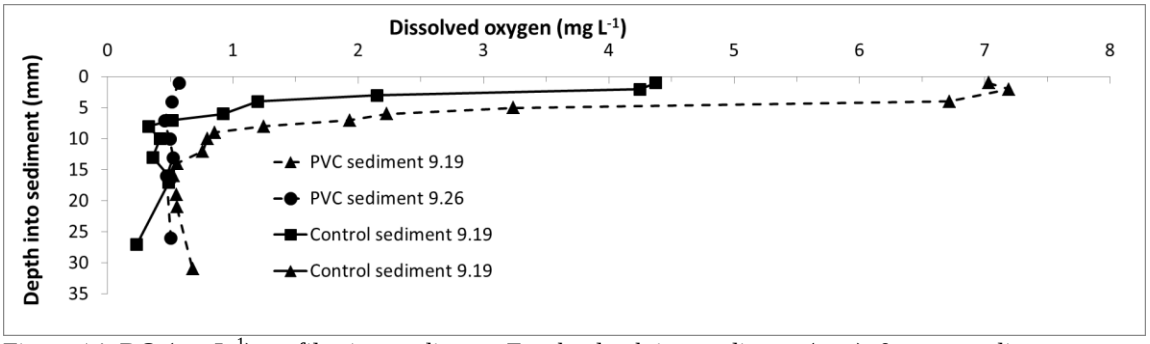


Figure 14: DO (mg L^{-1}) profiles into sediment. For the depth into sediment (mm), 0 mm = sediment-water interface. Sediment beneath PVC *Cladophora* tubes on two sampling dates: September 19, 2012 (- - ♦ - -) and September 26, 2012 (- - ●- -) and control sediment on: September 19, 2012 (profile one ■, profile two ▲).

2.4.2 Laboratory: sediment and sloughed *Cladophora* incubations

Areal respiration rates for laboratory incubations of *Cladophora* and sediment/biodeposits collected around assemblages were compared to *in situ* mussel assemblage respiration rates (Figure 15). Sloughed *Cladophora* from ephemeral mats at GH10m respired at rates similar to sloughed *Cladophora* from the GH Graveyard, both respiring at a rate ~80% of the mussel assemblage respiration rate. Sediment respiration was one-third of mussel assemblage respiration. Samples for DOC analyses were collected on only two of the four substrate types, with relatively similar concentrations for the sloughed *Cladophora* and sediment around mussel assemblages. Decomposing *Cladophora* from the SMI Graveyard was found to have DOC concentrations 21 times higher than *Cladophora* at the GH Graveyard (Table 1), despite similarities in appearance and mat structure. Within most *Cladophora* mat samples, anoxia was seen at a depth of 3 mm within 3-6 hours of the start of incubations.

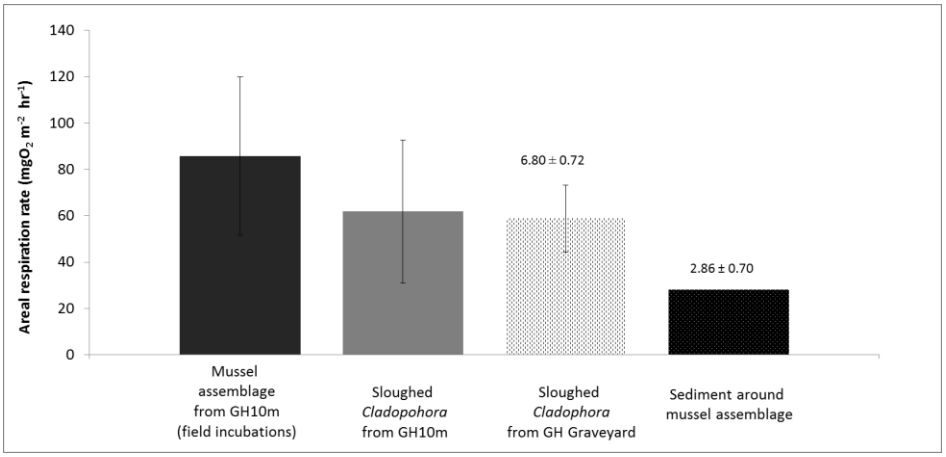


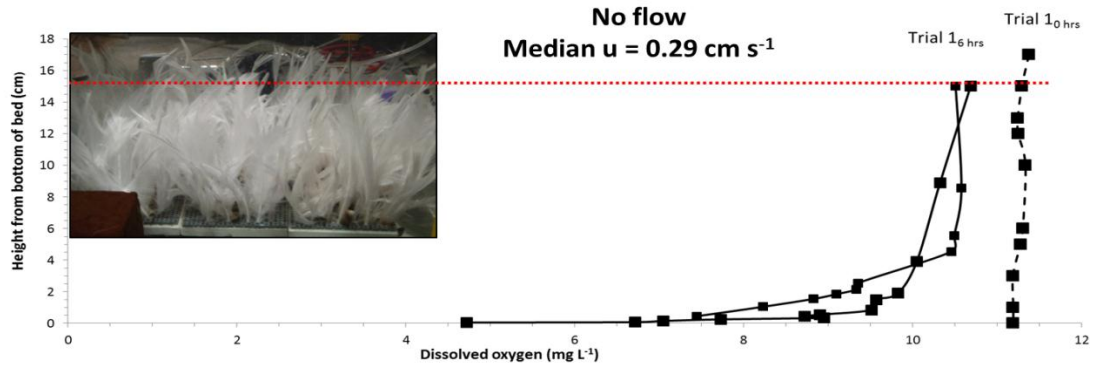
Figure 15: Mean (± SD) areal respiration rates from substrate incubations. Only one incubation of sediment collected from between mussels. All incubations were conducted in the laboratory with field collected samples, except mussel assemblage incubations which were conducted *in situ*. Mean density of mussel incubations: 5545 mussels·m^{-2}. Numbers atop bars indicate mean (± SD) DOC (ppm) of material that was analyzed for dissolved organic carbon.

2.4.3 Laboratory: the influence of algal cover and current on DO in mussel beds

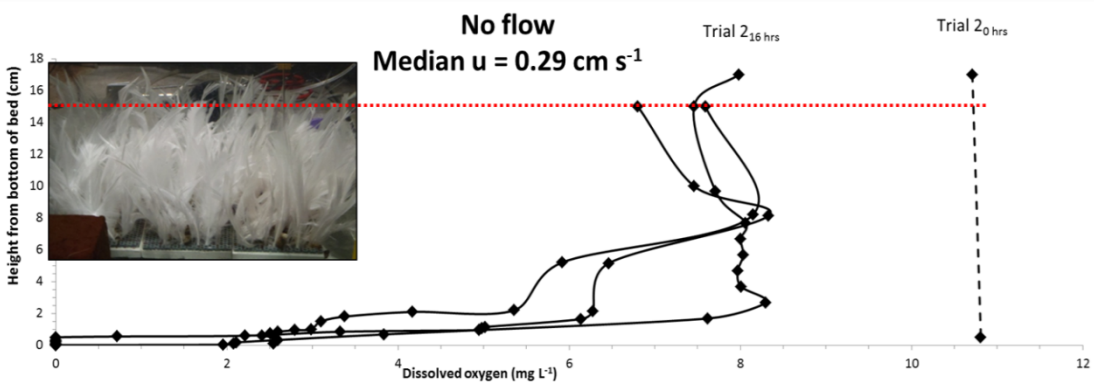
Oxygen measurements made in the mussel bed overlain by artificial *Cladophora* suggest there is potential for a hypoxic layer to form within a densely packed assemblage, but only under

conditions of low (median: 0.29 cm s^{-1}) water velocity (Figure 16a,b). When the pump was on and water was circulating, no oxygen gradient developed in the mussel assemblage (Figure 16c).

16a.



16b.



16c.

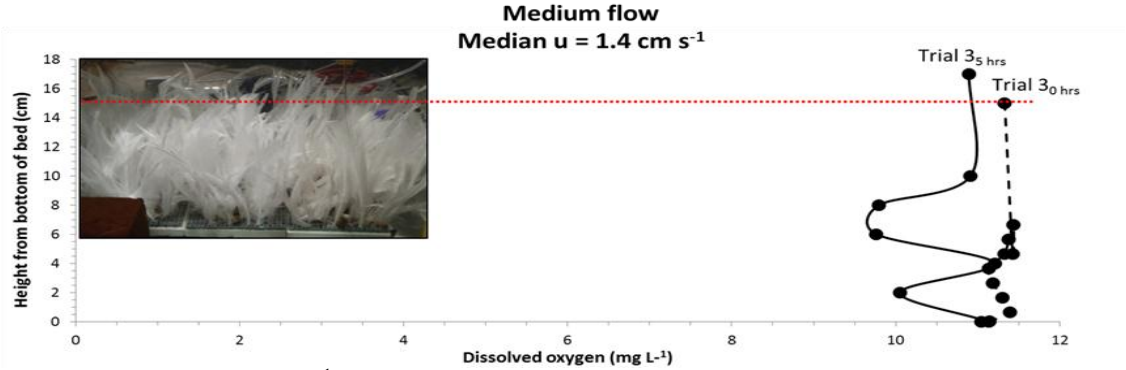


Figure 16: DO profiles (mg L^{-1}) for trials with no flow (16a, b) and medium flow (16c). Dissolved oxygen profiles (mg L^{-1}) into mussel bed (cm) taken at the beginning (------) and end (—) of trials one (6 hours) and three (15 hours). For trial two (5 hours), there was no initial profile, but ambient dissolved oxygen was recorded at the top and bottom of the mussel bed (- -♦- -), and end profiles were taken (—♦—). Multiple profiles at the end of each experiment were taken at points along a linear transect through the middle of the assemblage. Top of '*Cladophora*' indicated (----------).

These same results can been seen from continuous DO readings taken 2 cm off the base of the mussel bed, just above the mussel bed (Figure 17). Despite efforts to create a vertically uniform flow, water velocity spiked at 3.28 cm s^{-1} at 4.5 cm from the base of the aquarium, just above the mussels (Figure 7). Higher velocity atop the assemblage likely increased mixing with the overlying water and prevented the formation of an oxygen gradient.

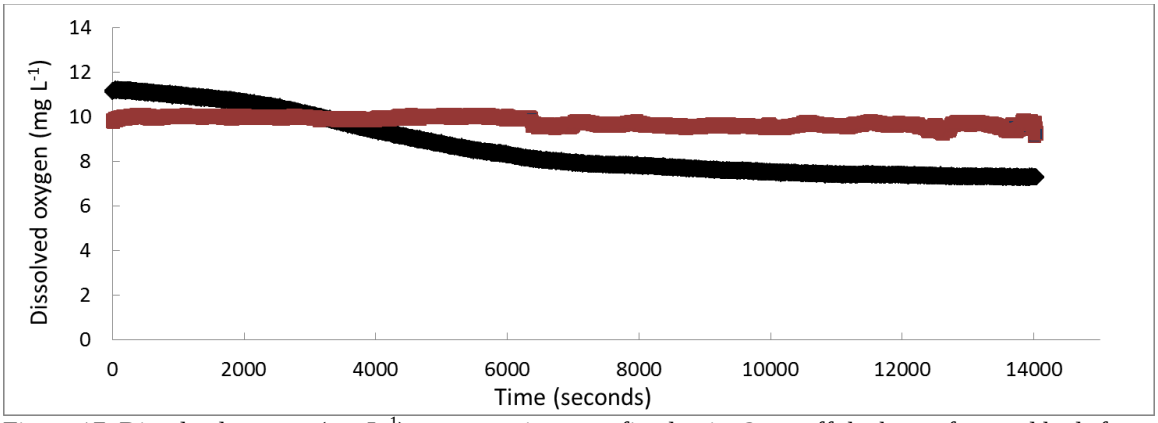


Figure 17. Dissolved oxygen (mg L^{-1}) concentrations at a fixed point 2 cm off the base of mussel beds for trials with pump off (●) and pump on (●). Fluctuations starting at 7000 seconds for trial with pump on, likely the result of mussels hitting probe, disrupting optode transmission.

2.5 Discussion

2.5.1 Hypoxia in benthic substrates

Based on the results reported here, hypoxia appears to quickly form and disappear in some substrata, while occurring consistently across the sampling season in other substrata. Ephemeral sloughed *Cladophora* mats at GH10m, which formed and then dispersed within 3-4 weeks in mid-summer, were anoxic within 2 cm of their upper surface. Meanwhile, shallow areas of anoxia in decomposing *Cladophora* accumulating in depositional areas at the SMI and GH Graveyards were observed on all sampling dates. In the PVC tube *Cladophora* decomposition experiment, hypoxia and anoxia were found at 1.6 cm into the loose *Cladophora* within four weeks after the start of the experiment, and likely occurred much earlier before the site was revisited. In the incubations of sloughed *Cladophora*, shallow anoxia developed within six hours of the start of

incubations, suggesting the ability of anoxia to rapidly develop if mats are restricted from mixing with overlying water. Consistently hypoxic and anoxic conditions in sloughed *Cladophora* mats and SMI Graveyard DOC concentrations almost 20 x larger than in sediment, support the hypothesis that depositional *Cladophora* may provide the anaerobic, high nutrient environment necessary for *C. botulinum* toxin production. Shallow anoxia suggests that, should toxin production occur, it may be at a shallow enough depth that invertebrates burrowing at the top of the mat could be exposed to the toxin, and that relatively minor hydrodynamic events may be able to resuspend toxin-enriched sediments and biota.

Direct comparisons between DOC concentrations and published nutrients requirements for *C. botulinum* vegetative cell growth and toxin are difficult. Literature on *C. botulinum* type E details the types of compounds necessary for the germinations of spores (glucose, L-alanine, bicarbonate) and concentrations for optimum germination (50 mmol L^{-1}) under laboratory conditions for food models (Ando and Iida, 1970; Plowman and Peck, 2002), but nutrient growth requirements for *C. botulinum* in the environment have not been evaluated. Although the food models may be transferrable to the environment, the parameters measured in a laboratory at a scale greater than a few millimeters may be unrelated to what bacteria experience in the environment (S. Haack, personal communication). The micro-scale measurements conducted in this study may be the first in this regard. Of note, DOC concentrations within the GH Graveyard were lower than at the SMI Graveyard; an obvious explanation for these differences is not apparent but may be due in part to differences in the time that sloughed *Cladophora* had accumulated at each site. Monthly sampling at both graveyards indicates that mats at SMI may be present more consistently, allowing for the accumulation of organic material and a resultant higher DOC concentration in the top centimeters of the mat.

Profiles into ponar grab samples of benthic mud from GH20m revealed areas of hypoxia--and in one instance anoxia—similar to *in situ* profiling in the SMI and GH Graveyards. It is likely that benthic mud goes anoxic at shallow depths, although that was not seen in profiling. This mischaracterization of *in situ* oxygen conditions may be from the introduction of oxygen into Ponar grab samples as they were brought to the surface, particles in the mud interfering with the optode probe's light transmission, or a loss of probe calibration; for these reasons, near anoxic profiles in benthic mud can likely be considered anoxic. Ponar grabs were collected across a 10 m depth gradient comprising three distinct sampling points. This suggests homogeneity in oxygen conditions across the substrate. Although we dove only one section of the GH Graveyard, we can assume that the area sampled accurately characterizes this and possibly other nearshore depositional areas.

A relatively high areal respiration rate, about 80% of mussel assemblage respiration, suggests oxygen consumption in decomposing *Cladophora* may be high enough to deplete DO within mats if conditions are calm and mats remain stable and unmixed with overlying water. Throughout the sampling season, large numbers of gobies, which are known to feed on benthic invertebrates and frequently found in the stomachs of botulism-affected birds, were spotted by divers at both Graveyards and appeared at high densities in minnow traps during bimonthly sampling. Benthic invertebrates including oligochaetes, nematodes, and amphipods have been found to carry high levels of *C. botulinum* spores in other lakes, with chironomids containing the highest concentration at 1×10^5 copies of spore DNA mg^{-1} (Pérez-Fuentetaja et al., 2011; Pérez-Fuentetaja et al., 2013). Chironomids can live in low oxygen conditions and are abundant in organic-rich sediment. They were the only benthic invertebrate found living within depositional *Cladophora* at the Graveyards. In Lake Erie, nearshore (mean depth = 6.6 ± 1.4m) benthic organisms were found to carry higher concentrations of spore DNA than offshore (mean depth =

17.4 ± 1.1 m) organisms (Pérez-Fuentetaja et al., 2013). Future benthic sampling should target the SMI and GH Graveyards, and other depositional sites to investigate the frequency of resuspension events that might make toxin more available to higher trophic levels through the dispersion of bacteria and benthic-feeding invertebrates. Although only two *Cladophora* deposition sites were identified in this study, high resolution benthic mapping (B. Lafrancois, personal communication) identified other nearshore areas with bathymetry that suggests the potential for algal deposits. Deep (> 60 m) holes, located relatively close to shore may create areas where sloughed *Cladophora* can collect as thick mats and decompose. Should these areas persist for a long enough time for vegetative cell growth and toxin production, seasonal storm events that resuspend bottom material may 'flush' out these areas making the material within depositional areas available to birds feeding on floating mats and *Cladophora* deposited on shore. Depositional areas located adjacent to shallow shoals and close to shore are bathymetric features unique to the northern parts of the lake and may explain why recent botulism outbreaks are more common in northern Lake Michigan than in the southern region. Ground truthing of recently completed benthic mapping will be necessary to determine if areas of similar bathymetry accumulate *Cladophora* of the same depth and temporal frequency as the SMI and GH Graveyards.

Despite the black, organic appearance of sediment beneath PVC tubes, oxygen penetration beneath tubes was similar to profiles in sediment not under tubes, as were DOC concentrations. In both locations, sediment dissolved oxygen concentration within the upper 30 mm never fell below 0.23 mg L^{-1}. A seen in the laboratory incubations, sloughed *Cladophora* can rapidly go anoxic, particularly if restricted from mixing with overlying water, such as the conditions in the PVC tubes. The high respiration rate of *Cladophora* and restricted mixing likely accounts for areas of shallow anoxia in the PVC tubes. To measure oxygen concentrations in the

sediment beneath the PVC, the tubes had to be moved aside to identify the change in substrate from *Cladophora* to the sediment beneath. Despite efforts to restrict disturbance of the top layer of sediment, oxygen may have been introduced to the top millimeters of sediment, confounding profile results. It is likely that the black color of the substrate was caused by real anoxia even though the measurements did not reflect this.

Although I did not identify areas of anoxia in sediment under the PVC tube and sediment not under tubes, I cannot say with confidence that these areas are not anoxic. A slight loss of probe calibration could have mischaracterized anoxic areas as hypoxic and/or sediment particles against the tip of the sensor may have interfered with the light transmission necessary for an accurate reading. Both of these issues could misrepresent anoxic areas as hypoxic. Other studies examining oxygen penetration into marine and aquatic sediment have observed anoxic conditions within the upper 10 mm (Jørgensen and Revsbech, 1985; Carlton and Wetzel, 1986; Carlton et al., 1989; Archer and Devol, 1992), a substrate depth where I expected to find anoxia but only identified hypoxia in my sampling. In sediment cores from Lake Superior, oxygen consumption from sediment ranged from 4.42 to 10.01 mg O_2 m^{-2} hr^{-1} (Carlton et al., 1989). Although lower than the sediment respiration rate observed in this study, the difference may be explained by the organic content of material being sampled. The Lake Superior study found a positive correlation between oxygen consumption and particulate organic carbon content in the upper 1 cm of cores. Sediment collected around mussel beds is likely to be more organically rich than Lake Superior benthic sediment owing to the nutrient rich environment around the bed created from mussel egestion (Conroy et al., 2005; Ozersky et al., 2009; Bootsma and Liao, 2013). The areal respiration rate of sediment around mussel beds was one-third of mussel assemblage respiration, indicating the contribution of the periphyton, feces, and pseudofeces living within mussel assemblages to total assemblage oxygen consumption. Although the contribution of sediment and

biodeposits is significant, it appears that most of the respiration is due to mussels. Furthermore, mussel assemblages reduce the mixing that occurs above rocks and sediment. A higher respiration rate and reduced mixing with overlying water suggests hypoxia may be more likely to occur in mussel beds than in organic sediment on top of uncolonized rocks. Future research should conduct *in situ* incubations that attempt to isolate sediment and detrital respiration from that of mussels.

In mussel assemblages, the presence of hypoxic water occurred only during a period when there was a thick *Cladophora* mat associated with the assemblage, warm water temperatures, and low bottom current, suggesting that the temporal frequency of hypoxia may be limited to periods of the simultaneous occurrence of specific physical and environmental conditions that prevent mixing between assemblages and overlying water. It appears that a thick *Cladophora* canopy atop mussel assemblages exerts a smothering effect on dreissenids, even during the day when the mat is photosynthesizing. The mat appears to limit oxygen transfer and mixing between the mussels and overlying water.

Great Lakes Coastal Forecasting System depth-averaged bottom velocities from GH10m indicate periods where velocity was less than 1.0 cm s^{-1}, which may be low enough to allow the formation of a hypoxia layer atop the mussel beds. Future field sampling on more occasions with thick *Cladophora* atop mussels is necessary to determine if hypoxic conditions are persistent throughout the period of dense algal cover, which can exist for several months in the summer. Continuous DO measurements at the base of a bed over multiple days during periods of thick algal cover would assess the potential for hypoxia development during the night when *Cladophora* is respiring and capture mussel bed DO under a range of bottom velocities.

Experiments in the aquarium confirm *in situ* findings of DO gradients atop mussel beds only under conditions of low water velocity and restricted mixing by a *Cladophora* canopy. The

use of an inorganic material to recreate the algal canopy eliminates the effect of overnight *Cladophora* respiration, potentially underestimating the likelihood of hypoxia formation by not including an oxygen consuming component of the mussel assemblage. Initial *in situ* and experimental findings suggest that the base of mussel beds may provide the anoxic conditions necessary for vegetative cell growth and toxin formation, but further research into the temporal frequency of conditions promoting anoxia are necessary

Future aquarium experiments should consider the size, bed structure, and thickness of algal cover. Creating a bed that is wider, longer, and taller, would more accurately replicate large boulders covered in mussels, the bottom structure of Lake Michigan's rocky nearshore. Trials in a larger aquarium or flume would accommodate a longer mussel bed and allow for testing under a range of velocities. Furthermore, in a large flume the assemblage would be less impacted by water movement along the sides. Feathers were the material most qualitatively similar to filamentous *Cladophora* in size, branching structure, and movement in water. The use of larger tiles as the mussel beds' base would allow more surface area for feather attachment and create a more dense algal cover than what was achieved in this experiment. An algal layer similar in thickness to *in situ* conditions is critical to achieving the restricted mixing between mussels and overlying water that was seen in the lake.

Although it is difficult to reach a conclusion on the exact conditions necessary to promote hypoxia (as I only identified low oxygen concentrations above and within assemblages on two sampling dates), results from laboratory experiments support the conclusion that hypoxia may occur during periods of thick algal cover and low water velocity. It appears that physical structure is largely responsible for creating hypoxic near-bed oxygen conditions, but biological processes, such as areal respiration of dense mussel colonies, may promote within assemblage oxygen depletion in concert with the effects of assemblage structure and near bottom water velocity.

The implications of mussel bed DO conditions on nutrient cycling remain unresolved. Although it appears that low oxygen conditions are created atop and within beds, the frequency of these occurrences still needs to be determined. While mussels are a direct source of dissolved nitrogen and phosphorus through excretion (Arnott and Vanni, 1996), the infrequent occurrence of near bottom oxygen anoxia may not influence N and P cycling. Further experiments that pair DO profiling with N and P flux measurements are necessary to make conclusions on the implications of mussel bed oxygen on nutrient cycling.

2.5.2 Benthic oxygen flux model

Vertical mixing rates at the base of the benthic boundary layer have a strong influence on the sediment-water flux of various solutes, including oxygen and dissolved nutrients. They also regulate the supply of phytoplankton from the water column to filter feeding dreissenids (Ackerman, 1999; Ackerman, 2013). Direct, *in situ* measurement of these mixing rates is difficult due to the small spatial scales over which they occur, and the challenge of making flow measurements within complex matrices such as mussel beds and macroalgae. However, mixing rates can be inferred from measurements of solute fluxes and vertical gradients. Dissolved oxygen concentrations can be input into a simple benthic flux model to determine the diffusion coefficient within a mussel bed. Recent models describing the diffusion of oxygen across sediment expand on work by Bouldin (1968) that classified a variety of oxygen exchange scenarios.

The vertical flux, J, of O_2 into a mussel bed can be described by Fick's First Law of Diffusion (Jørgensen and Revsbech, 1985):

$$J = -D\,(dC/dz) \qquad (2)$$

Where D = the diffusion coefficient of oxygen into the mussel bed, C = O_2 concentration, and Z = depth (Z = 0 at the mussel-sediment interface). The flux can be expressed in terms of changes in O_2 concentration across the DBL as:

$$J = D\ (C_w\text{-}C_o)/Z_\delta \qquad (3)$$

Where C_W = O_2 concentration at the top of the mussel bed, C_O = O_2 concentration at the mussel-sediment interface, and Z_δ = thickness of the effective DBL (the height of the mussel bed). Within most of the DBL, the oxygen concentration gradient is steep and linear under stirred conditions.

Results from *in situ* and laboratory experiments captured DO at the base of the bed and atop the mussel assemblage and were combined with temperature-dependent areal respiration rates (Chapter 3). Although this requires the combination of measurements from different sampling dates, mussel densities for the areal respiration measurements and DO profiles were measured at the same location, GH10m. Mean mussel density under incubations chambers was 5545 ± 2179 mussels · m^{-2}, this is comparable to a 2012 mean mussel density at GH10m of 4439 ± 1206 mussels · m^{-2}. A diffusion coefficient was determined using a rearranged equation 3:

$$D = (J^* Z_\delta)/ (C_w\text{-}C_o) \qquad (4)$$

This model assumes zero-order reaction kinetics, although findings indicate mussel assemblages exhibit first-order kinetics (Chapter 3.4.5). Despite this, a zero-order model was utilized for its simplicity in application. The assumption of a zero-order relationship results in an estimate of D that is greater than those that would be determined using a first-order model as it does not account for a smaller vertical flux (J) in response to decreasing ambient oxygen. More refined calculations should consider a first-order model. There is the possibility that within the environment a transition between zero-order and first-order reactions occurs, in which case a mixture of models could be considered (Bouldin, 1968). In all calculations in mussel beds, Z_δ was assumed to be 1 cm.

DO atop the mussel assemblage and at the base of the bed for profiles under thick *Cladophora* were used in Equation 4, to determine a within-bed diffusion coefficient for the periods when algal cover may have restricted mixing with the overlying water. Diffusion coefficients ranged from 6.9×10^{-4} to 2.4×10^{-3} cm^2 s^{-1}. A diffusion coefficient was determined for the canopy atop the mussel assemblage using the same equation set-up and profiled values for DO in the algal mat, with variable heights (Z_δ) depending on the thickness of the *Cladophora* canopy. Coefficients in the algal mat ranged from $3.04 - 7.47 \times 10^{-4}$ cm^2 s^{-1}. Both diffusion coefficient ranges are 2-3 orders of magnitude smaller than diffusivity values derived from a particle image velocimetry (PIV) system for the mussel-water interface in coastal sites 10-20 m in depth (Q. Liao, personal communication). The PIV system, which is an optical, 2-dimension method of measuring velocity, is utilized above mussel beds and *Cladophora* where flow is turbulent (Liao et al., 2009). In this study, the measurements used to calculate diffusivity coefficients were made in both the protective *Cladophora* canopy atop the bed and within the tightly packed shells of the assemblage. Isolation from turbulent forces provided by these physical structures likely reduces turbulent diffusion within the bed, although a calculated diffusivity higher than the molecular diffusion of oxygen in water [1.80×10^{-5} cm^2 s^{-1} at water 20°C (Han and Bartels, 1996)] indicates an advective mechanism within the bed stronger than molecular diffusion. The lack of a DO gradient atop mussel beds when *Cladophora* cover was sparse suggests that turbulent forces may control DO flux atop assemblages during periods of high bottom water velocity and/or sparse algal cover, but once mussel assemblages are restricted from mixing with the overlying water, weaker within-bed advective forces control oxygen flux.

3. Oxygen consumption by quagga mussels (*Dreissena rostriformis bugensis*) in response to environmental variables

3.1 Background

Since the invasion and colonization of the zebra mussel (*Dreissena polymorpha*) in the Laurentian Great Lakes, a significant amount of research has gone into understanding mussel physiology, specifically metabolism and oxygen demands. Mussel metabolism informs processes of nutrient cycling and sequestration (Arnott and Vanni, 1996; Cha et al., 2011; Evans et al., 2011; Bootsma and Liao, 2013), bioenergetics (McMahon, 1996), feeding (Sprung, 1995a; Baldwin et al., 2002), changes in species composition (Nalepa et al., 2009) and overall ecosystem engineering in the nearshore (Hecky et al., 2004). Respiration, an aspect of mussel physiology that underpins all of the aforementioned processes, has been well investigated in zebra mussels (Quigley et al., 1993; Aldridge et al., 1995; Thorp et al., 1998; Fanslow et al., 2001) but few studies have examined oxygen consumption in quagga mussels.

The almost complete displacement of zebra mussels by their congener, the quagga mussel (*Dreissena rostriformis bugensis*) in much of the Great Lakes (Mills et al., 1993; Patterson et al., 1995; Mills et al., 1996; Nalepa et al., 2009), has necessitated an understanding of quagga mussel physiology to better determine the ecological impacts of this relatively new invader. Data on quagga mussel respiration are limited. A few laboratory studies have examined quagga mussel respiration rates, but these were done under a limited range of environmental conditions (Stoeckmann, 2003; Turner, 2010) and only one study has used the profunda phenotype (Nalepa et al., 2010). In that study, rather than measuring respiration directly, rates were approximated from the relationship between electron transport activity (a measure of metabolic activity) and respiration.

Recent surveys conducted on Lake Michigan at depths of 16 m to > 90m found quagga mussels in even the deepest parts of the lake, with increasing densities at most depth intervals (Nalepa et al., 2010). Almost all quagga mussels collected in those surveys were of the profunda morph phenotype, which is found primarily in hypolimnetic waters (Nalepa et al., 2010). The profunda type is a genetically similar but morphologically distinct taxon from the epilimnetic shallow morph quagga mussel (Spidle et al., 1994; Claxton and Mackie, 1998). This phenotype is particularly suited to living in the soft substrate of deep waters, possessing a long incurrent siphon that can be extended upward to overlying water while one valve remains in contact with the sediment (Vanderploeg et al., 2010). Given the expanding population of quagga mussels at depths supporting the profunda phenotype, it is important to quantify any metabolic differences between shallow and profunda morph mussels.

The refinement of quagga mussel oxygen consumption rates tested under a variety of environmental conditions can be used to more accurately quantify quagga mussel feeding, nutrient cycling, bioenergetics, and inform predictions of future ecological changes with the continued expansion of *D. rostriformis bugensis*. In bivalves, respiration represents a large proportion of consumed energy (Stoeckmann and Garton, 1997) and respiration measurements can be used in determining how mussels may be altering energy flow in the Great Lakes.

3.2 Purpose of study

Mussels play an integral role in benthic oxygen dynamics and there is a demonstrated need to better understand the factors that influence oxygen consumption by mussels. The purpose of this study was to determine how four environmental variables – temperature, mussel size, food supply, and dissolved oxygen concentration – influence quagga mussel respiration rate, to determine how oxygen consumption of shallow and profunda morphs may differ, and to use the

resultant measurements to assess the role of the quagga mussel as an energy conduit in the Lake Michigan food web.

3.3 Methods

Experiments investigating respiration response to temperatures were conducted through both *in situ* incubations and laboratory experiments. All other environmental parameters were tested in the laboratory.

3.3.1 Laboratory mussel respiration experiments

Collection and Maintenance

Shallow morph mussels were collected from Atwater Bay from 2-21 days before experimentation. Immediately after collection, mussel shells were gently scrubbed to remove attached algae and maintained in aerated buckets in the dark in a refrigerated room at temperatures specific to each experiment. Water was exchanged from the buckets every 1-2 days and replaced with water from the Milwaukee inner harbor, hereafter called harbor water. Sub-samples of harbor water were analyzed once a week for carbon content to determine the concentration of food the mussels were receiving. Harbor water was filtered (ashed, 0.70 μm Whatman GF/F), and filters were wrapped in tin discs (30 mm), compressed, and combusted. Seston carbon concentrations were determined using the calibrated beam strength of the mass spectrometer (Finnigan MAT delta S SIR-MS, with elemental analyzer front end and ConFlo II interface), calculated as μg C L^{-1} (Ngochera and Bootsma, 2011). Over six sampling points from November-December 2011, during the time period most laboratory experiments were conducted, carbon concentration of harbor water ranged from 161-232 μg C L^{-1}, mean 190 ± 12 μg C L^{-1}. These concentrations are comparable to in-lake conditions at Atwater Bay 10m, where seston concentrations at 2 m off the bottom ranged from 73–272 μg C L^{-1}, mean 146 ± 12 μg C L^{-1} over eighteen points of seasonal sampling from May-November. Hypolimnetic carbon concentration

was determined from samples collected at ~50 m from a 70 m site off Atwater Bay. At three sampling points from June-July 2012, hypolimnetic water was collected with a Niskin bottle and filtered, the carbon content was analyzed.

Unless noted, all experiments were conducted using shallow morph mussels. Chambers of aerated water only (without mussels) from each temperature treatment were used as controls. At the conclusion of each experiment, all mussels were counted and their lengths determined. Throughout this study respiration rates were normalized to dry weight, using length-weight relationships specific to the season and location of mussel collection.

3.3.2 Effect of temperature on oxygen consumption

Mussels between 15 and19 mm were transferred from the aerated containers at maintenance temperature (8°C) to aerated 4 L bottles of harbor water at experimental temperatures: 4, 10, 14, and 20°C, which reflects the range of nearshore bottom water temperatures at Atwater Bay from May-November (4-24°C). Following a three-hour exposure to the experimental temperature, mussels were transferred into 190 mL triplicate chambers for each temperature treatment and filled with water from the aerated 4 L bottles. Each chamber contained 23-26 mussels, equivalent to an areal density of 78 mussels · m^{-2}. Although lower than an estimated nearshore areal density in Lake Michigan of ~5,000 mussels · m^{-2}, this experimental density captured changes in DO over a short time without completely depleting oxygen in the chamber, which could have confounded respiration rates. During the pre-experiment exposure period, chambers were checked to ensure that mussels were opening shells and extending siphons. Initial DO was measured with a Clark-type polarographic oxygen electrode (YSI model 600R sonde) and a YSI 650MDS display data logger. Incubations ran for 2 hours, and a final DO was recorded at the end. Three experiments were conducted over an eight day period: the first trial two days after mussel collection, the second trial eight days after mussel collection, and the

third trial nine days after mussel collection. Time between collection and experimentation allowed mussels to habituate to harbor water food concentrations. Results of a two-way ANOVA indicated no significant difference in respiration across the three dates ($p = 0.08$), using a significance level of 0.05.

In July 2012, a similar experimental setup was used to measure the effect of temperature on oxygen consumption by profunda morph quagga mussels. Eight days before experimentation, mussels were collected with a Ponar dredge at a 55 m site off Atwater Bay and individuals between 15-25 mm were gently scrubbed to remove attached algae. Mussels were maintained in aerated buckets at 8°C with daily exchange of harbor water. Three days before experimentation, mussels were transferred into aerated 4 L bottles of harbor water and exposed to experimental temperatures: 4, 10, 14, and 20°C. During the three day exposure period, habituation water continued to be refreshed daily with harbor water. Prior to experimentation, for each temperature treatment, mussels were transferred into triplicate 190 mL chambers and filled with water from the aerated 4 L bottles. Each chamber contained 23-26 mussels. Initial DO was measured with an optical dissolved oxygen sensor (NeoFox Sport micro-optode system). Incubations ran for 2 hours, and a final DO was recorded at the end.

3.3.3 Effect of food deprivation on oxygen consumption

Following completion of the temperature-respiration experiments, shallow morph mussels were returned to one bucket and habituated at 11°C with a daily exchange of harbor water. A food deprivation experiment was conducted 15 days after *in situ* collection. Three days before experimentation the organic carbon content of harbor water was analyzed daily. On the day of experimentation, two hours after being provided with fresh harbor water, mussels were transferred to quadruplicate chambers of aerated, dechlorinated tap water filtered (70 μm Whatman GF/F) to remove all nutrients. Four chambers with mussels sized 15-21 mm with 24-26

mussels/chamber were prepared. The initial and final DO was measured with a Traceable® Digital Oxygen Meter. Final DO readings were taken following two hours of closed chamber respiration. After the final reading, mussels were kept with their chamber group and transferred to a fresh supply of aerated, filtered water at experiment temperatures in five-gallon buckets. The cycle of closed-chamber respiration experiments followed by a transfer to a chamber of aerated, filtered water was repeated for a total of five respiration incubations at: 1, 3, 17, 24, 49, and 73 hours from feeding. These times are the mid-point of two-hour incubations and indicate the time since mussels were food deprived. DO consumption in a control chamber filled with aerated, filtered water was also measured at each time point.

3.3.4 Combined effect of mussel size and temperature on oxygen consumption

The effect of mussel size on oxygen consumption was tested at two temperatures (6°C and 17°C). Mussels were collected 21 days before experimentation and their shells were gently scrubbed to remove attached algae upon return to the lab after collection. Mussels were maintained in aerated buckets at 8°C. Twenty-four hours before the start of the experiment, water temperature was lowered to 6°C. One hour prior to measuring respiration rate, mussels were transferred into triplicate 190 mL chambers by shell length size class: 5-10 mm, 11-15 mm, 16-19 mm, ≥20 mm. Initial DO was measured with a Traceable® Digital Oxygen Meter. Incubations ran for 2 hours, and a final DO was recorded at the end. Following experimentation, mussels were kept with their chamber group in individual flow-through containers in aerated buckets of harbor water and fed a fresh supply of harbor water. Buckets were stored at 17°C in preparation for the next experiment. After 24 hours of temperature habituation, the same setup and experiment was performed at 17°C; mussels were kept in the same chamber groups as the 6°C treatment.

3.3.5 Effect of food enhancement on oxygen consumption

In May 2012 mussels were collected from Atwater Bay. Their shells were scrubbed to remove attached algae and mussels were habituated at 9°C for eight days prior to experimentation. A daily exchange of harbor water ensured mussels were well fed before experimentation. During experiments, mussels were fed various dilutions of a mixed algal assemblage consisting primarily of chlorophytes and diatoms. The mixed algal assemblage--grown as stock for yellow perch first feeding--was started in 1997 with mixed pond water. Three times a year, 1-3 L of powered alfalfa meal was added to the algal mixture, which was kept aerated at 18-22°C under continuous light 1 m above the tank. Dilutions of the mixed algal assemblage resulted in four different food concentrations, with organic carbon concentrations of 169, 228, 455, and 5504 μg L^{-1}. These concentrations were chosen to simulate a range of conditions, from those in Lake Michigan during mid to late-stratification periods to more eutrophic conditions. Food treatments were prepared prior to the experiment and stored in 45 L carboys which were kept at 10°C, the temperature used during experiments. Experimental mussels were placed into aerated 5 gallon buckets (40 mussels per bucket) filled with 13 liters of food enhanced water. Water from each bucket was exchanged every 8 hours with a fresh supply of food enhanced water. The exchange period and volume of water that mussels were exposed to was chosen to replicate clearance rates of 1.0 L $mussel^{-1}$ hr^{-1}. Although slightly higher than published clearance rates that range from 0.2 to 0.4 L $mussel^{-1}$ hr^{-1} for quagga mussels (Ackerman, 1999; Baldwin et al., 2002), feeding at a high rate ensured that mussels were well fed during experimentation. Following 24 hours of feeding, mussels from each treatment were transferred to smaller experimental chambers filled with water from the buckets within which they had been feeding. Chambers of mussel-free aerated water from each food treatment were used as controls. Mussels were habituated for ten minutes in the chambers, and they were

observed to ensure that siphons were opened before the initial DO was measured with an optical sensor. Following two hours of closed chamber respiration, final DO readings were recorded.

The experiment was repeated in July 2012 with a narrower range of food concentrations and the inclusion of a modified control treatment of filtered, dechlorinated tap water. Experimental setup and procedures followed the May protocol with the exception of the preparation of the food treatments. Stock algae (*Scenedesmus quadricauda)* cultured in the lab were added to 45 L carboys of lake water to achieve the desired food concentrations. A sub-sample of the water from each treatment was analyzed for particulate organic carbon. Carbon concentrations in the four treatments were: 28, 143, 358, 843, and 1498 μg L^{-1}. As in the May experimentation, mussels were given fresh, food enhanced water every eight hours and chambers of aerated water only (without mussels) from each food treatment were used as controls.

3.3.6 Effect of decreasing oxygen on respiration rate

Mussels were kept at 11°C for ten days prior to experimentation with a daily exchange of harbor water, after which they were transferred to a 500 mL chamber filled with aerated harbor water. Mussel density in the chamber was equivalent to 86 mussels · m^{-2}. A magnetic stir bar was placed in the chamber and the chamber was placed atop a stir plate, ensuring that water above the mussel bed remained mixed throughout the experiment. Plastic mesh placed mid-way in the water column kept mussels suspended above the stir bar. An optical DO probe and a temperature probe were inserted into the chamber through stoppers fitted into the chamber lid, allowing for continuous DO monitoring while keeping the chamber gas-tight. The chamber was left undisturbed for 24 hours. DO was recorded at 20 second intervals, allowing for a continuous measurement of respiration rate (slope of the DO vs. time relationship), which could be compared with DO concentration.

3.3.7 Measuring community metabolism: *In-situ* benthic oxygen chambers

Benthic respiration incubations determining oxygen consumption in shallow morph quagga mussels were conducted at a nearshore (10 m depth) site in Good Harbor Bay (Sleeping Bear Dunes National Lakeshore), Lake Michigan (44° 58.899' N, 85 °49.852' W) from June to October 2011 and June 2012 under a range of temperatures (7-22°C). An additional respiration experiment was conducted at Atwater Bay (43° 05.719’ N, 087° 51.875’ W) in October 2011. Atwater Bay is a rocky, nearshore site, 5 km north of the Milwaukee Harbor, similar in depth, bathymetry, and mussel density to Good Harbor Bay.

Flux chambers 7.25 cm tall and 4.75 cm in radius, covering a surface area of 71 cm^2 and equipped with manually operated stir paddles, were constructed from gray PVC tubing. The use of opaque PVC ensured that all incubations occurred in the dark. A 5 cm tube opening at the top was fitted to accommodate a YSI 600R sonde attached to a YSI 650MDS display data logger used to measure dissolved oxygen changes within the chamber; a custom-built underwater case allowed use of the probe and logger at depth, allowing a SCUBA diver to continuously monitor DO within the chamber. A circular ring around the mussel assemblages was scraped clear, allowing a tight fit between the chamber and rock. *Cladophora* algae growing atop the mussel assemblage was removed by hand, taking care to not dislodge any mussels. The chamber was placed over the mussel bed and a neoprene skirt and weighted neoprene sock were fitted around the base of the chamber, forming a tight seal that prevented gas and water exchange between the chamber and the ambient water column. After placement, the chamber was stirred and then left to stabilize for ~2 minutes, when initial DO measurements were recorded manually. The data logger also recorded DO continuously at 30 second intervals. Chambers were left for 20-30 minutes and stirred gently at 10 minute intervals during the experiment. A final DO reading was manually recorded before the chamber was removed. Mussels from beneath the chamber were collected and returned to the laboratory, where their number, length, and dry weight were determined. Dry

weights of mussels for all field experiments were determined by drying the shell-free tissue at 50°C for 24 hours.

3.4 Results

3.4.1 Effect of temperature on oxygen consumption

Respiratory rates from laboratory chamber experiments were similar to rates determined from field incubations (Figure 18). A strong linear relationship was observed between respiration rates (R) and temperature (T) for both profunda and shallow morph mussels (Figure 19).

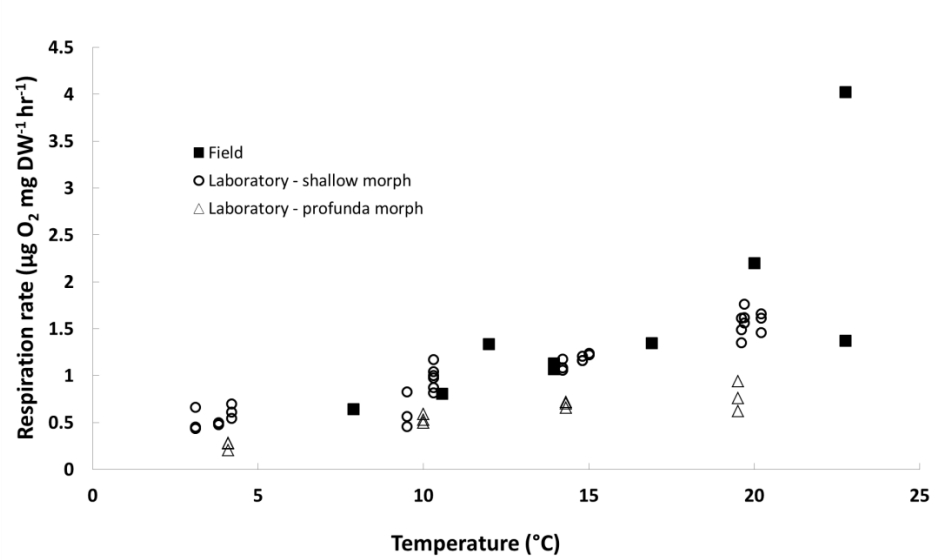


Figure 18: Relationship between respiration rate (µg O_2 mg DW^{-1} hr^{-1}) and temperature (°C) for all field (■) and laboratory experiments. Laboratory experiments tested both phenotypes: shallow (□) and profunda (Δ). Laboratory values for all three trials with shallow morph mussels and the sole trial with profunda morph mussels are included.

The relationships are: R= 0.0642*(T) + 0.2617 (r^2 = 0.90, $p < 0.001$) for shallow morphs and R = 0.034*(T) + 0.1586 (r^2 = 0.84, $p < 0.001$) for profunda morphs. Results of an ANCOVA suggest a significant effect of both temperature ($p < 0.001$) and phenotype ($p < 0.001$) on respiration, as well as a significant interactive effect ($p < 0.001$), indicating that the temperature responses of the two morphs are significantly different. A summary of the mean respiration rates at each temperature treatment for shallow and profunda morph quagga mussels is listed in Table 2.

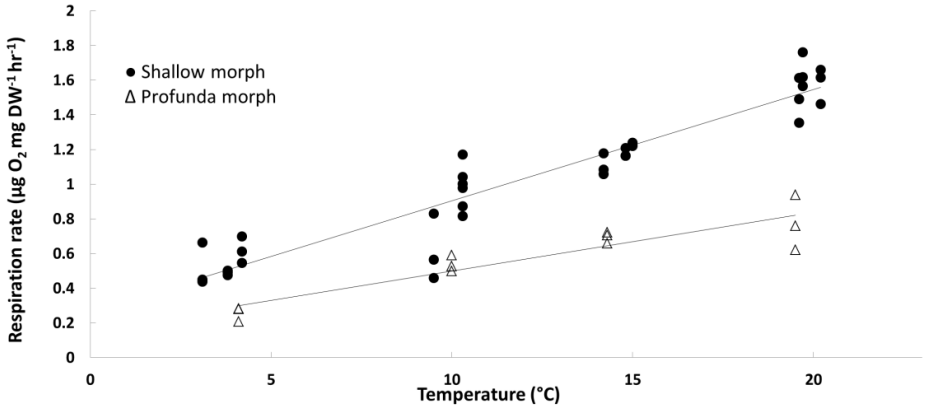


Figure 19: Relationship between respiration rate (R, µg O_2 mg DW^{-1} hr^{-1}) and temperature (T, °C) for all experimental trials with quagga mussels by phenotype: shallow (●) and profunda (Δ). Regressions: R= 0.0642*(T) + 0.2617 (r^2 = 0.90, $p < 0.001$) for shallow morph; R = 0.034*(T) + 0.1586 (r^2 = 0.84, $p < 0.001$) for profunda morph.

Table 2: Mean (± SD) oxygen consumption for laboratory experiments with shallow and profunda morph quagga mussels. Number of trials listed in parentheses.

	Shallow morph	Profunda morph
Temperature (°C)	Respiration rate (µg O_2 mg DW^{-1} hr^{-1})	Respiration rate (µg O_2 mg DW^{-1} hr^{-1})
4	0.54 ± 0.07 (3)	0.26 ± 0.04 (1)
10	0.86 ± 0.12 (3)	0.54 ± 0.05 (1)
14	1.17 ± 0.03 (3)	0.70 ± 0.03 (1)
20	1.57 ± 0.11 (3)	0.77 ± 0.16 (1)

3.4.2 Effect of food deprivation on oxygen consumption

Respiration rates dropped off between hours 4 and 18, maintaining a steady rate through the remainder (74 hours) of the experiment (Figure 20). There is a significant effect of time from final feeding on respiration rate ($p < 0.001$). Results of a pair-wise Tukey's HSD suggest no significant difference between hours 2 and 4 ($p = 0.44$), a significant difference between hours 4 and 18.5 ($p < 0.001$), and no significant difference between time points thereafter ($p = 1$).

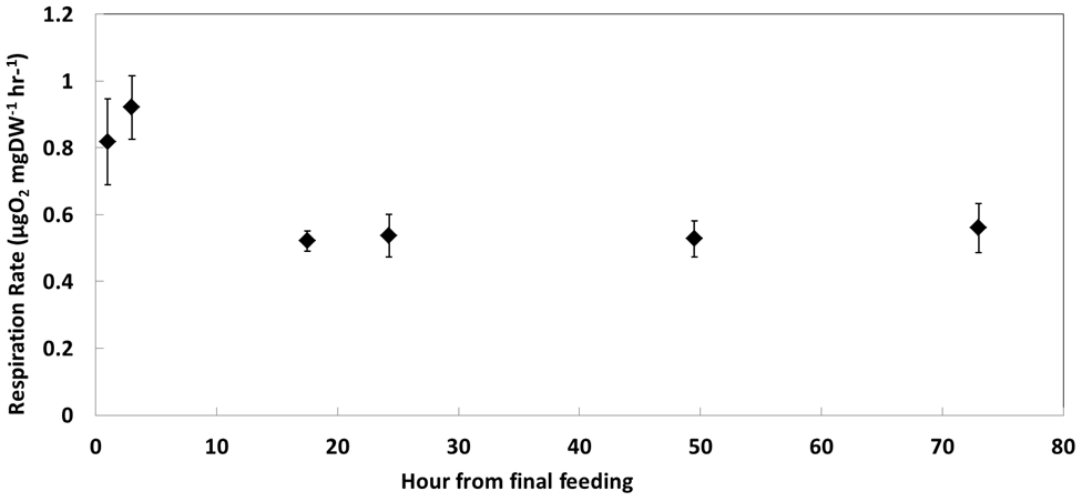


Figure 20: Relationship between respiration rate (µg O_2 mg DW^{-1} hr^{-1}) and food deprivation (hours from final feeding) for shallow morph mussels acclimated and experimented at 11°C. Points are mean (± SD) respiration rates for triplicate incubations. The experimented was terminated after 74 hours.

3.4.3 Effect of mussel size and temperature on oxygen consumption

Respiration rates were significantly higher at 17°C than at 6°C for all size classes. There is a significant relationship between size and respiration ($p < 0.001$), and temperature and respiration ($p < 0.001$), and a significant interaction between size and temperature ($p < 0.001$). The difference in temperature response narrowed with increasing mussel shell length. At the smallest size class (5-10 mm) the difference in mean respiration rate between temperatures was over six times larger than the difference in respiration between temperature treatments at the largest size class (≥ 20mm) (Figure 21). The relationship between respiration (R) and size (S, shell length) was best expressed as a 2nd order polynomial relationship for both temperature treatments. The relationships are $R = 0.0043*(S)^2 - 0.1669*(S) + 2.0612$ ($r^2=0.99$) for 6°C and $R = 0.0005*(S)^2 – 0.2466*(S) + 3.7171$ ($r^2=0.98$) for 17°C. The Q_{10} for the difference between the temperature treatments was 2.0 ± 0.05 for each size class except for the largest sized mussels, which had a Q_{10} of 1.32.

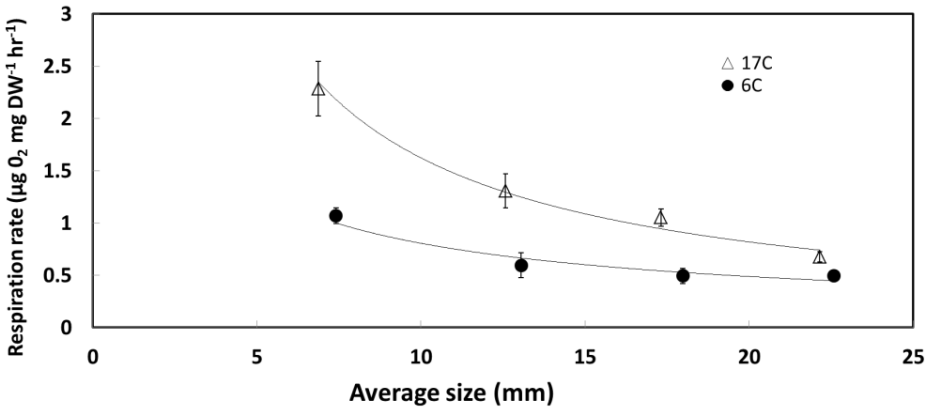


Figure 21: Relationship between respiration rate (µg O_2 mg DW^{-1} hr^{-1}) and temperature for treatments at 17°C (Δ) and 6°C (●) by shell length size class: 5-10 mm, 11-15 mm, 16-19 mm, ≥20 mm for shallow morph mussels. Points are average size of mussel in each class and are mean (± SD) respiration rates for triplicate incubations. Relationships: $R = 0.0043*(S)^2 - 0.1669*(S) + 2.0612$ (r^2=0.99) for 6°C and $R = 0.0005*(S)^2 – 0.2466*(S) + 3.7171$ (r^2=0.98) for 17°C.

3.4.4 Effect of food enhancement on oxygen consumption

The change in respiration rates with increasing food concentrations followed a similar curve for both trials through a range of particulate carbon concentrations (Figure 22). Peak respiration occurred at a concentration of 358 µg C L^{-1}. To give an in-lake context for the seston concentrations used in this study, seasonal particulate carbon concentration from nearshore water samples collected from 2 m off the bottom at Atwater Bay from May-October, ranged from 73-272 µg C L^{-1}, with a mean of 147 µg C L^{-1}. In the hypolimnion, the seston concentration ranged from 46.1- 63.8 µg C L^{-1} with a mean of 55.5 µg C L^{-1} (Driscoll, personal communication).

Results indicated that quagga mussel respiration rate increases as food concentration increases above that typically observed in nearshore Lake Michigan up to a threshold food concentration of approximately 360 µg C L^{-1}, beyond which respiration rate declines. The lowest respiration rate of 0.30 µg O_2 mg DW^{-1} hr^{-1}, which falls below the mean *in situ* respiration rate measured for nearshore mussels, occurred at a particulate C concentration of 5504 µg C L^{-1}, which is 37 times greater than the mean nearshore particulate carbon for May-October. Across all sampling points respiration rates did not directly increase in proportion to food concentration,

although proportionality was seen between food concentrations of 169 and 357 µg C L^{-1} when both respiration and carbon concentration approximately doubled.

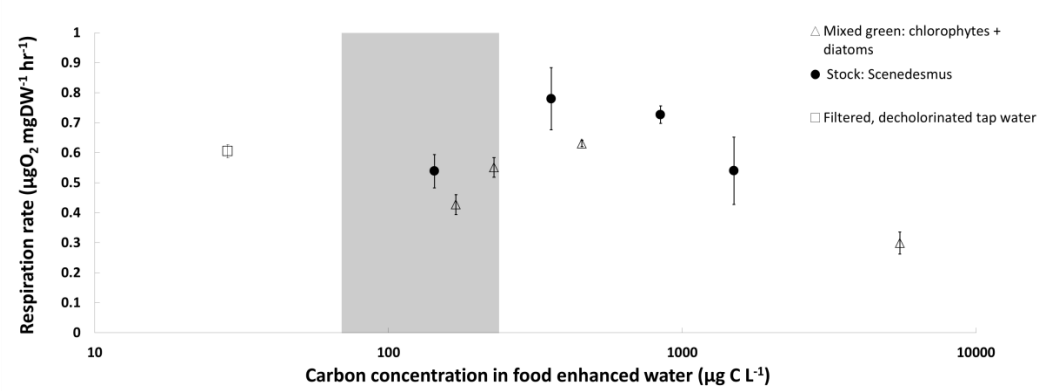


Figure 22: Relationship between respiration rate (µg O_2 mg DW^{-1} hr^{-1}) and particulate carbon concentration (µg C L^{-1}) in food enhanced water for shallow morph mussels. Trials were conducted in May with a food source of mixed green algae (Δ) and July with a food source of stock *Scenedesmus* sp. (●) 2012 at an experimental temperature of 10°C. In July, a modified control treatment of filtered, dechlorinated tap water (□) was included. The gray box indicates the range of seasonal (May-November 2010-12) nearshore particulate carbon concentrations.

3.4.5 Effect of decreasing oxygen on respiration rate

Shallow morph quagga mussels adjusted their respiration rate in response to decreasing ambient dissolved oxygen conditions (Figure 23). A plot of the natural log of dissolved oxygen concentration against time resulted in a near-linear relationship, indicating a first-order relationship between dissolved oxygen concentration (DO) and respiration rate (R), which is defined as $DO = 0.0779*(R)^2 - 0.1188*(R) + 0.0642$ (r^2=0.96).

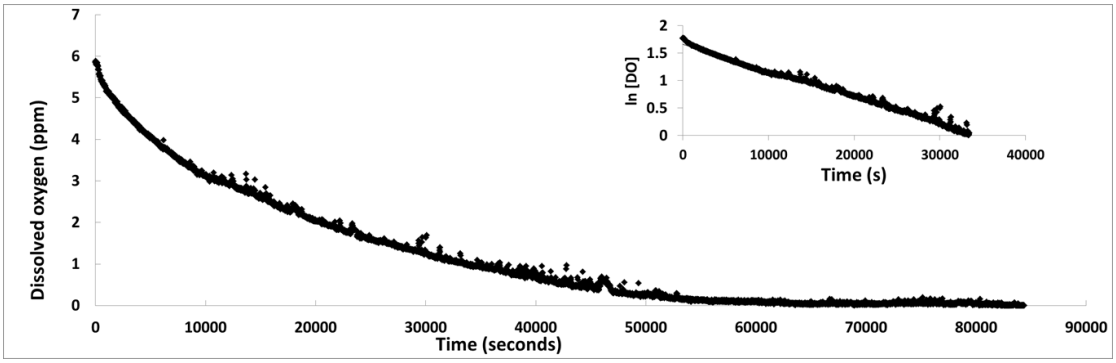


Figure 23: Shallow morph mussel oxygen consumption (ppm) in a closed container over 23.25 hours. Habituation and experimental temperature were 11°C. Inset: the relationship between the natural logarithm

of dissolved oxygen concentration (ppm) in a closed container over time (seconds), a near-linear slope (r^2=0.99) of this relationship time indicated first-order reaction kinetics.

3.4.6 *In situ* community metabolism

Benthic chamber experiments to determine community metabolism of mussel assemblages were conducted from June-October 2011 and June 2012 (Figure 17). Respiration rate for all incubations conducted at SLBE and Atwater Bay increased with temperature (r^2 = 0.49), although the relationship was not statistically significant (p = 0.77).

3.5 Discussion

3.5.1 Effect of temperature and mussel size on respiration

Despite differences that may have existed between lab and field conditions, including food supply, food composition, mussel density, current velocities, and vertical mixing rates, the respiration – temperature relationship observed for *in situ* measurements was very similar to that observed for lab experiments, suggesting that temperature is a dominant factor regulating quagga mussel respiration rate. Indeed, 55% of the variability in in-lake respiration rates could be accounted for by temperature. The significance of temperature as a factor regulating dreissenid respiration has been observed in a number of other studies (Aldridge et al., 1995; Fanslow et al., 2001; Alexander and McMahon, 2004).

There were significant differences in the temperature response of shallow and profunda morph quagga mussel respiration based on temperature for both the shallow and profunda phenotype. Laboratory mean respiration rates of both phenotypes were comparable to published respiration rates of 1.45 μg O_2 mg DW^{-1} hr^{-1} and 0.95 μg O_2 mg DW^{-1} hr^{-1} at 10.0°C and 5.0°C for shallow morph mussels collected at a depth of 4-5 m in Lake Erie (Stoeckmann, 2003) and respiration rates of 0.48 μg O_2 mg DW^{-1} hr^{-1} and 0.23 μg O_2 mg DW^{-1} hr^{-1} at comparable temperatures for profunda morph mussels collected in Lake Michigan (Nalepa et al., 2010). Q_{10}

values for mean respiration rates for both phenotypes at cool and warm temperatures were within the range of values reported for *D. polymorpha* (McMahon, 1996).

Profunda morph quagga mussels' lower respiration rate across all temperatures suggests that this phenotype either has a lower basal metabolic rate or it allocates more energy resources towards other metabolic needs, such as growth or reproduction. A lower metabolic rate results in a lower food maintenance requirement, an advantage in the food-scarce hypolimnion. Furthermore, a smaller temperature slope for profunda morph suggests less flexibility with regard to temperature response, a trait suited to the consistent bottom temperatures of the hypolimnion. Shallow morph quagga mussels' more flexible temperature slope, on the other hand, suggests adaptability to changing temperature conditions, an advantage in the nearshore, where bottom temperatures can quickly change following an upwelling event. Shallow morph mussels appear to be more metabolically active even at low temperatures, necessitating higher food maintenance requirements met by the relatively food-rich nearshore waters. It appears that both phenotypes' metabolic demands are well suited to their respective environments.

Similar respiration rates for field and laboratory incubations suggest that even though field incubations incorporate the oxygen consumption of all organic material in and around the mussel assemblage—periphyton, mussel feces and pseudofeces, sediment, invertebrates-- mussels are responsible for almost all of the respiration observed in *in situ* chambers in the dark, as was discussed in Chapter 2. Mussels' dominance of chamber respiration suggests that benthic oxygen dynamics may be heavily influenced, if not controlled by, dreissenids in densely colonized areas. The contribution of *Cladophora* to areal respiration should be determined in future studies. A comparison of night/dark areal respiration with *Cladophora* attached to mussels is one method of isolating the contribution of algae.

Mussel size and the interaction of mussel size and temperature had a significant effect on respiration. Biomass-normalized oxygen consumption decreased with increasing shell length, consistent with published results of size class studies of zebra mussel respiration (Quigley et al., 1993; Summers et al., 1996). Many bivalve physiological processes are size-dependent, with exponential changes in feeding and oxygen consumption based on size (Young et al., 1996). In zebra mussels, total food consumption and pseudofeces production varied by more than an order of magnitude when scaled by size (Young et al., 1996). Our finding of a non-linear relationship between mussel size and respiration reinforces the need to utilize size-scaled respiration values when determining large-scale impacts of dreissenids.

For both temperature treatments there was a significant difference between the respiration rates of the smallest mussels and every other size class. Smaller mussels appear to be more responsive to temperature changes. For the smallest measured size class (5-10 mm), the respiration rate at 17°C was over 2 times greater than that at 6°C. By contrast, mussels greater than 20 mm respired at similar rates at 17 and 6°C. Furthermore, it appears that at 6°C, respiration rate changes little with length beyond 13 mm. These findings suggest that at cooler temperatures, once mussels pass a shell length threshold of about 13 mm, they establish a stable respiration rate, perhaps related to the increased reproductive abilities of larger mussels and simultaneous decreased investment in somatic growth. In *D. polymorpha*, reproductive effort and output increases with shell length and body size (Sprung, 1995b; Stoeckmann and Garton, 1997), and there is a correlation between mean oocyte numbers and shell length (Neumann et al., 1993). Considering the cool water preference of *D. rostriformis bugensis*, the establishment of a stable basal metabolic rate may coincide with the energetic shift from growth to respiration at a certain size threshold. As temperatures increases, it becomes more critical to account for the effects of size on respiration, an important consideration for bioenergetic models.

In a closed chamber, oxygen consumption for shallow morph mussels displayed first-order reaction kinetics, indicating metabolic adaptability to decreasing ambient dissolved oxygen. It appears that quagga mussels are able to adjust their respiration when faced with short-term exposure to low dissolved oxygen conditions. These results are similar to published findings that zebra mussels are unable to maintain a constant respiration rate when exposed to low oxygen levels (Quigley et al., 1993). Studies examining the effect of acclimation temperature and experimental temperature on respiration of mussels in hypoxic waters found that *D. polymorpha* are poor respiration regulators when warm-acclimated but increase their regulatory ability when cold-acclimated (Matthews and McMahon, 1999; Alexander and McMahon, 2004). *D. rostriformis bugensis'* occurrence in profundal regions suggests better tolerance to hypoxic conditions than zebra mussels (McMahon, 1996). Under conditions of long-term exposure to hypoxia, LT_{50} in *D. polymorpha* ranged from 2-3 days at 25°C and 35-41 days at 5°C (Matthews and McMahon, 1999). In large oligotrophic lakes, where the epilimnion and hypolimnion remain well oxygenated throughout the year (Wetzel, 2001), quagga mussels are rarely exposed to conditions of anoxia or even hypoxia. In the present study, exploratory dissolved oxygen profiling around dense mussel assemblages covered in thick *Cladophora* during conditions of warm bottom water temperatures and low current velocities revealed areas of water with DO concentrations of less than 2.0 mg L^{-1} (Chapter 2), suggesting the potential for ephemeral hypoxic conditions to develop atop mussel beds (Tyner, unpublished). Should these periods remain brief, *D. rostriformis bugensis*, appears able to withstand the short-term stress by adjusting its metabolic rate.

3.5.2 Effect of food supply on respiration

In suspension-feeding bivalves, the gills are used for respiration and feeding. When food is absent, pumping rate is low and respiration decreases to a basal metabolic rate; with increasing

food concentration, respiration increases to an active metabolic rate (Madon et al., 1998; Stoeckmann and Garton, 2001). I found that under conditions of food deprivation, mussels responded by dropping to a stable metabolic rate between 4 and 18.5 hours post-feeding. They maintained this rate for three days after their last feeding. These findings suggest that quagga mussels are able to adjust their metabolism in response to food availability, promoting survival during long periods of low food supply. Similar results have been reported for the zebra mussel, which was observed to exhibit 50% mortality after 352 days of food deprivation at 15°C and 67% mortality after 774 days at 5°C (McMahon, 1996).

Nalepa et al. (2010) conclude that the lower respiration rates of profunda morph mussels indicate the phenotypes' lower metabolic costs and suitability for food-scarce offshore waters. Interestingly, I found that the mean respiration rate of food-deprived shallow morph mussels from 18-74 hours post feeding at an experimentation temperature of 11°C was 0.54 ± 0.02 μg O2 $mgDW^{-1}$ hr^{-1}, nearly identical to the profunda respiration rate of 0.53 μg O2 $mgDW^{-1}$ hr^{-1} at 11°C, predicted by the relationship between respiration and temperature for profunda mussels (Figure 19). This suggests that shallow morph mussels under conditions of food deprivation may be able to adjust their metabolism in a similar manner as profunda morph mussels have adjusted to the low food availability of the hypolimnion. However, for a given temperature shallow morph mussel's higher metabolic rate than profunda mussels (Figure 19) may confound the effect of metabolic depression under food deprivation seen at an experimental temperature of 11°C, rendering a low-food environment problematic for shallow morph mussels. Conducting the food deprivation experiment under a range of temperatures is necessary to determine if this metabolic similarity between food-deprived shallow morph mussels and profunda morph mussels holds true for all experimental temperatures. Overall it appears that quagga mussel's low respiration rate and

relatively high assimilation efficiency (Stoeckmann and Garton, 1997; Baldwin et al., 2002) are suited for growth and reproduction at low food levels (Nalepa et al., 2009).

Quagga mussels respond to food-enhanced water by increasing their respiration up to a maximum rate, after which oxygen consumption drops with increasing seston concentration. This maximum rate is likely tied to the point where pseudofeces production can no longer keep up with the increasing organic load. In bivalves under conditions of high turbidity, pseudofeces are formed as a mechanism to clear the gills for respiration and filter-feeding (Alexander et al., 1994). Mussel's respiratory response to increasing food concentrations was the same in experiments conducted in May and July. Peak respiration occurred at a particulate C concentration of 358 μg C L^{-1}. This is 2.5 times greater than the mean nearshore particulate C concentration of 147 μg C L^{-1}, suggesting that the nearshore mussel community persists at suboptimal food concentrations in Lake Michigan, and would consume more phytoplankton if it were available. Whether or not this is true for the profundal community cannot be determined with the data at hand, but considering the low particulate C concentrations in the hypolimnion (Driscoll, personal communication) it is likely that these mussels would benefit from any increase in food supply, despite the fact that they have a lower metabolic rate. Indeed, the apparent decrease in dreissenid density at depths within the upper hypolimnion between 31 and 50 m (Nalepa et al., 2010), suggests that mussel density at these depths has exceeded that which can be supported by current phytoplankton concentrations.

Our results are similar to published findings that seston concentration has a significant effect on respiration, with respiration peaking and then decreasing as food concentration increased (Madon et al., 1998). Acute high food concentrations appear to depress respiration in the same way that respiration is depressed with increased turbidity (Alexander et al., 1994; Summers et al., 1996). Under a scenario of increased food availability *and* particulate matter

suspension, respiration decreases, although not significantly, as the ratio of inorganic: organic material increases (Madon et al., 1998). Under conditions of enhanced food supply that is not accompanied by additional recalcitrant inorganic matter, as occurred in our experiments, it appears that there remains an incipient limiting threshold beyond which mussels do not benefit from increased food concentration, likely due to the filtration capacity of the gills being exceeded. In the lake, increased turbidity following a storm event may present a scenario where mussels respond to a spike in seston concentrations by initially increasing their respiration while feeding in food-enhanced waters.

Seston composition and quality have been found to influence oxygen consumption (Fanslow et al., 2001; Stoeckmann and Garton, 2001) and deserve consideration when evaluating lake-wide metabolic responses to increased food availability. I found a similar relationship between food and respiration under two experimental conditions with different food types (mixed algal assemblage and single species cultured algae), suggesting that the relationship may be applicable under a broad range of food conditions.

3.5.3 Estimating carbon consumption at the ecosystem scale

A number of recent changes in Lake Michigan have been attributed to dreissenid mussels. These include increased water clarity, lower plankton concentrations, declines in the benthic amphipod, *Diporeia* sp., and declines in forage fish abundance (Hall et al., 2003; Madenjian et al., 2006; Fahnenstiel et al., 2010a; Vanderploeg et al., 2012). All of these changes are ultimately attributable to the ability of dreissenids to filter out large amounts of plankton and alter energy flow in lakes.

Quantifying the impact of dreissenids on altering energy flow is a research priority. Many recent studies have addressed this need (Hecky et al., 2004; Nalepa et al., 2009; Higgins and Vander Zanden, 2010; Ryan et al., 2013), often by modeling the impacts of mussels on seston

consumption (Madenjian, 1995; Stoeckmann and Garton, 1997; Fanslow et al., 2001). Because oxygen consumption represents a large fraction of the energy budget of dreissenids (Stoeckmann and Garton, 1997), our respiration measurements allow for another method by which energy flow through dreissenids can be estimated, with spatio-temporal variations in temperature, food supply, and mussel size distribution being accounted for.

While many studies have examined zebra mussel feeding under conditions that simulate *in situ* current velocities (Ackerman, 1999; Ackerman et al., 2001; Vanderploeg et al., 2010), it can be difficult to completely replicate benthic conditions in the laboratory. Ackerman et al. (2001) found that clearance rates of *D. polymorpha* on a reef in western Lake Erie were 40% lower than rates observed in the laboratory, likely due to the complexity of digesting natural seston versus more easily digestible algal cultures used in laboratory studies. Simple laboratory-derived filtration estimates often do not account for the contribution of refiltration in community metabolism, thereby overestimating the grazing impact of dreissenids (Yu and Culver, 1999). In determining the impact of dreissenids mussels on plankton, the utility of volumetric models that use lake volume and per unit area clearance rates is limited to shallow well mixed lakes (Ackerman, 1999).

Considering these limitations, oxygen consumption--which can be considered an index of how much mussels are *actually* eating as opposed to how much they are able to eat under ideal conditions--may be a useful tool in determining dreissenid organic C consumption and quantifying impacts of mussels on phytoplankton production and biomass in large oligotrophic lakes that experience seasonal stratification. Furthermore, oxygen consumption integrates energy requirements over a given amount of time, as opposed to direct measurements of clearance rate, which may change over short time intervals and are difficult to measure *in situ.*

I estimated annual oxygen consumption by quagga mussels in the southern basin of Lake Michigan. Separate estimates were made for the profundal and nearshore zones, with the boundary between these two zones being operationally set at a depth of 15 m. This depth was also assumed to separate the shallow morph from the profunda morph, which is supported by the observations of Nalepa et al. (2010). The boundary of the Southern Basin followed those defined by Nalepa et al. (2010). Estimates of Southern Basin area were based on GIS data and calculations from C. Houghton (unpublished). Nearshore mussel biomass and size frequency distribution were obtained from 2012 seasonal (May-November) sampling at Atwater Bay and scaled to an estimate of 24% nearshore hard substrate (Brooks et al., 2012). Profundal biomass and size frequency distribution were derived from Nalepa et al. (2010). Nearshore biomass was estimated from 2012 monthly sampling at Atwater Bay. Profundal zone depth-specific temperatures were obtained from Nalepa et al. (2010) and for the nearshore zone I used temperatures measured continuously by a sonde deployed at a depth of 9 m in Atwater Bay in 2011-12. Parameters used for calculations are shown in Table 3.

Oxygen consumption was converted to calories using an oxycaloric coefficient of 3.38 calories · mg O_2^{-1} (Crisp, 1984). Using an estimate of 88% of zebra mussel energy utilized in oxygen consumption (Stoeckmann and Garton, 1997), calories invested in oxygen consumption were converted to a total yearly caloric demand for mussels. Finally, caloric demand was converted to annual carbon consumption using a conversion factor for phytoplankton of 11.40 calories · mg carbon^{-1} (Platt and Irwin, 1973). Combining the above relationships, respiration of 1 mg O_2 is equivalent to consumption of 0.337 mg organic C. Size- and temperature-specific respiration rates were used in all calculations.

Table 3: Parameters used for carbon consumption estimates by depth zone in the southern basin of Lake Michigan. Southern basin area approximated to be 1.9 x 10^{10} m^2, and nearshore estimated to be 7.55 % of southern basin area.

Depth zone (m)	Relative area (%)	Percent of the population by size interval. Size categories based on shell length.				*Dreissena* biomass (g m^{-2})	Average monthly temp.(°C)[+]
		0-10 mm	10-15 mm	15-20 mm	20+ mm		
0-15	24	----------NA*----------				50.4	monthly**
16-30	22.6	97.1	2.1	0.5	0.3	3.5	6-20
31-50	11.6	55.6	19.6	18.3	6.5	43.9	<5-11
50-90	32.2	79.1	18.5	2.1	0.3	15	<5
≥ 90	33.6	95.1	3.3	0.2	0.5	1	<5

[+] Temperature-specific respiration used in all calculations. See Nalepa et al. (2010) for depth-specific temperatures by month used for zones 16 - ≥90 m

*Size distribution not applied to nearshore mussels as it comprises a relatively small area of the entire southern basin. All nearshore mussels assumed to be 15-19 mm for size-specific respiration calculation.

**Nearshore monthly temperature values (°C) obtained from Atwater Bay 10m bottom sonde: Dec-March 3.3, April 7.1, May 7.7, June 9.3, July 17.1, Aug 17.01, Sept. 14.0, Oct. 9.3, Nov. 7.1.

The rate for annual quagga mussel carbon consumption in southern Lake Michigan was estimated to be 4.13 x 10^8 kg C yr^{-1}, 10% of that total can be attributed to nearshore mussel consumption. Total population consumption was strongly influence by size-specific respiration. Throughout the season, the smallest sized mussels had the highest proportion of population seston consumption, similar to findings by Stoeckmann and Garton (1997) on Lake Erie. The estimated carbon consumption is put in perspective by comparisons with other carbon flux rates, including phytoplankton production and biomass obtained from Fahnenstiel et al. (2010) and estimated settling rates (Eadie et al., 1984).

Using recent estimates of net phytoplankton production in southeastern Lake Michigan (Fahnenstiel et al., 2010b) weighted to southern basin depth zone volumes in Vanderploeg et al. (2010), and our carbon consumption estimate, mussels consume 15% of southeastern Lake Michigan annual net primary production. This approximation assumes that the rates of daily areal primary production for the period of late stratification extend over the winter isothermal period

(January-March) when no sampling was conducted (Fahnenstiel et al., 2010b). This consumption estimate is at the low end of published results examining large-scale impacts of mussel feeding on algal production. Based on models from the western basin of Lake Erie, zebra mussels remove from 26 ± 10% (Madenjian, 1995) to 53% (Boegman et al., 2008) of primary production. When Boegman et al. (2008) applied their model to a fully mixed water column, mussels consumed 77% of western basin algal growth (Boegman et al., 2008). Hydrodynamics play a significant role in algal delivery to mussel beds (MacIsaac et al., 1999; Ackerman, 2013). Models that examined vertical mixing in linking surface production to benthic feeding reported a range of 8% to 67% daily consumption of algal biomass in the water column based on the magnitude and shape of the diffusivity profile (Edwards et al., 2005b). A lower percent annual consumption in Lake Michigan may be explained by both a more strongly stratified and longer period of stratification in Lake Michigan than Lake Erie, isolating benthic mussels from phytoplankton production in the euphotic zone.

I compared my estimates of mussel feeding rate with recent measurements of offshore phytoplankton biomass in Lake Michigan (Fahnenstiel et al., 2010a). This comparison indicates that quagga mussels consume 3.19% of annual mean surface mixed layer (SML) phytoplankton and 2.18% of the SML+ deep chlorophyll maximum. An assumption incorporated into this calculation is that mean SML carbon concentrations for the late stratified period extended into the winter.

Finally, annual carbon consumption was compared to estimated settling rates in the southern basin of Lake Michigan (Eadie et al., 1984) adjusted for recent decreases in phytoplankton production (Fahnenstiel et al., 2010b). A rate for annual areal organic carbon flux for Lake Michigan was estimated from four sediment traps located at 35 m, below the thermocline in > 50 m of water (Eadie et al., 1984). This value was scaled to the change in

southeastern Lake Michigan offshore phytoplankton production between surveys in 1988-87 and 2007-08 (Fahnenstiel et al., 2010b). The rate of areal carbon flux was applied to the area of the southern basin (Table 3) to determine annual estimates for organic C carbon settling before and after the recent decrease in phytoplankton production. Results from this analysis suggest that quagga mussels are consuming 94% of annual settled organic C without the adjustment for the recent decrease in phytoplankton production and 160% of annual settled organic C with the adjustment for the recent decrease in phytoplankton production. Although these are both large percentages, it is not unreasonable to conclude that quagga mussels are consuming almost all the organic material reaching the lake bottom. Delivery of algae to the benthos is a significant factor in the amount of carbon biomass consumed. A study on Lake Erie found near-bed seston depletion in early to midsummer but little decrease in abundance near the surface (Edwards et al., 2005b). Furthermore, a study on clearance rates and refiltration by zebra mussels concluded that the grazing impact of zebra mussels in thermally stratified lakes depends on vertical distribution of phytoplankton and cannot be predicted simply based on filtration rates and mussel densities (Yu and Culver, 1999).

Estimates of carbon consumption have significant implications for energy flow in lakes. Dreissenid consumption of 15% of annual net phytoplankton production, and the majority—if not all—of settling organic carbon, constitutes a huge loss in energy for other benthic invertebrates, such as the detritivore *Diporeia*, which is completely reliant on settling phytoplankton as a food source (Dermott, 2001; Nalepa et al., 2009). Energy that had been efficiently recycled through *Diporeia* is now contained in dreissenid biomass, creating a benthic energy sink (Hecky et al., 2004; Nalepa et al., 2009) cut off from utilization in upper trophic levels. The consumption of dreissenids by the round goby (*Neogobius melanostomus*) and lake whitefish (*Coregonus*

clupeaformis) indicate dreissenids might not be a complete energetic "dead end." (Madenjian et al., 2010; Kornis et al., 2012).

A recent bioenergetics model of Lake Michigan estimated that total consumption by *Diporeia* declined by 91% between 1995 and 2005, while consumption by dreissenids increased > 25,000 times between 1995 and 2008 (Ryan et al., 2013). This compares to an estimated *Diporeia* assimilation of 61% of deposited spring algal C in Lake Michigan pre-dreissenid colonization, one of the highest rates in the lake at the time (Fitzgerald and Gardner, 1993). It appears that total benthic metabolism, which had been controlled by communities of benthic animals (Fitzgerald and Gardner, 1993), may now be singularly dominated by dreissenids.

In the densely colonized, nutrient poor profundal region, feeding by quagga mussels likely captures the majority of settled organic carbon, restricting benthic organisms' access to an already limited food source and capturing the majority of benthic energy. Coincident with this energetic shift is a decrease in biomass and condition of several fishes in Lake Michigan, attributed to dreissenid colonization and the loss of *Diporeia*, among other factors. (Pothoven et al., 2001; Madenjian et al., 2006; Bunnell et al., 2009). There is evidence that the feces and pseudofeces of dreissenids may provide a nutrient source for benthic invertebrates (Limén et al., 2005), but it is unlikely that the contribution of excreted waste to organic material in the benthos compensates for the loss of settled particulate carbon available to detritivores.

4. Conclusion

Dissolved oxygen plays an important role in assessing the impact of the invasive quagga mussel on energy flow, and changing nearshore oxygen dynamics and can help predict the spatial and temporal frequency of *C. botulinum* vegetative cell growth and toxin production. Areas of hypoxia and anoxia exist in mussel assemblages, sloughed *Cladophora*, and sediment, although the temporal frequency of hypoxic conditions is not consistent for each material. Frequently

observed shallow zones of anoxia in sloughed *Cladophora* and high DOC content in the surface layer of sloughed mats suggests the potential for vegetative cell growth and toxin production in these environments. Mussel beds appear to have changed nearshore benthic oxygen conditions, creating areas of hypoxia within mussed assemblages under conditions of low current velocity and thick algal cover. The frequency of these conditions and the range of bottom velocities and algal cover that may promote hypoxia in assemblages needs further investigation. Although the influence of low DO conditions on benthic nutrient cycling remains unresolved, the potential for hypoxia to form in mussel beds suggests that further studies directly linking DO conditions and nutrient flux may prove useful.

Metabolic adjustments to temperature, food enhancement and deprivation, and decreasing ambient oxygen may explain quagga mussel's suitability for existence within a broad range of environmental conditions. Using oxygen consumption as an estimate of dreissenid carbon consumption in the southern basin of Lake Michigan that accounts for spatio-temporal variations in temperature, food supply, and mussel size, suggests that mussels consume a significant portion of net phytoplankton production and the majority of settled organic carbon. The use of oxygen consumption as an index of seston consumption has broad utility in bioenergetics modeling. The extension of my energy flow estimates to a fully developed Lake Michigan metabolism model would be useful in the continued assessment of the ecosystem scale impacts of dreissenid colonization. Dreissenids appear to exert significant direct and indirect influences on benthic oxygen dynamics, warranting further studies as the impact of mussel colonization continues to be assessed.

5. Recommendations

Future research should build upon the results presented in this study by (1) enhancing our understanding of the spatial frequency of *Cladophora* depositional areas and the impact of wind

and waves on resuspending sloughed algal mats, (2) investigating the differences in night-dark benthic respiration, (3) isolating the contribution of live *Cladophora* respiration from total assemblage respiration, (4) conducting laboratory DO profiling into constructed mussel beds in a flume that allows for larger assemblage structure and a range of bottom velocities, (5) combining DO profiles with DOC and qPCR data to identify the potential for *C. botulinum* abundance, (6) testing the metabolic response of dreissenid mussels at enhanced food conditions for all experimental temperatures (4, 10, 14, 20°C) used in this study, and (7) developing a full mussel metabolism model using all environmental parameters tested in this study to make ecosystem level assessments of the impacts of dreissenids.

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7. Appendices

Appendix A: Dissolved Oxygen Profiels: *In Situ* Measurements

Benthic mud
Location: ~GH20m
Date: 9.5.12
Collection method: Ponar dredge

#1		#2		#3	
Location	N 44° 58' 27.2" W 85° 48' 47.0"	Location not recorded, SE of #1			N 44° 58' 19.6" W 85° 48' 51.9"
Depth (m): 20.7		Depth (m): 28.5		Depth (m): 29.05	
DOC (ppm) in top cm: 8.96		**DOC (ppm) in top cm: 11.38**		**DOC (ppm) in top cm: 3.05**	
DO (mg/L)	**Depth into sediment (mm)**	**DO (mg/L)**	**Depth into sediment (mm)**	**DO (mg/L)**	**Depth into sediment (mm)**
4.54	1	2.90	1	4.02	1
1.73	2	0.90	2	1.42	2
0.44	3	0.74	3	1.25	3
0.42	4	0.72	4	1.51	4
0.34	5	0.64	5	0.90	5
0.42	7	0.64	6	0.86	6
0.30	9	0.61	7	0.84	7
0.33	11	0.60	8	0.81	8
0.03	13	0.58	9	0.79	9
0.14	15	0.56	10	0.75	11
0.37	18	0.41	13	0.69	14
0.26	20	0.40	14	0.62	17
0.22	22	0.46	15	0.54	21
0.18	25	0.30	16	0.53	23
0.16	28	0.34	18	0.47	33
0.16	31	0.40	22	0.46	63
		0.31	26	0.42	100
		0.21	29		
		0.19	30		

***Cladophora* in PVC tubes**
Location: GH10m
Depth (m): 10
Method: *In situ* profiling

#1 Date: 8.15.12 Temp in mat: 16.9°C No DOC samples collected		#2 Date: 8.29.12 Temp in mat: 20°C No DOC samples collected		#3 Date: 9.19.12 Temp in mat: 17.8°C **DOC (ppm) from triplicate samples: 19.26, 8.65, 16.81**		#4 Date: 9.26.12 Temp in mat: 16.0°C **DOC (ppm) from triplicate samples: 21.18, 18.54, 15.11**	
DO (mg/L)	**Depth into sediment (mm)**	**DO (mg/L)**	**Depth into sediment (mm)**	**DO (mg/L)**	**Depth into sediment (mm)**	**DO (mg/L)**	**Depth into sediment (mm)**
8.36	1	3.53	2	0.17	0	2.00	1
3.91	2	0.63	22	0.12	31	0.42	3
4.17	3	0.36	32	0.04	32	1.61	5
0.15	4	0.35	33	0.07	35	1.31	6
1.51	5	0.34	34	0.12	38	0.87	16
1.76	6	0.26	35	0.12	58	0.79	36
0.00	16	0.22	36	0.15	62	0.78	37
		0.39	37	0.13	66	0.81	38

Ephemeral *Cladophora* mat
Location: GH10m
Depth (m): 10
Collection method: *In situ* profiling
Water temp: 16.9°C

#1
Date: 8.15.12
Ephemeral mat of *Cladophora* only seen on this sampling date.
No DOC sample collected

DO (mg/L)	**Depth into sediment (cm)**
8.00	1.0
0.80	1.4
0.40	1.5
0.50	1.7
0.00	2.0

***Cladophora* graveyard**
Location: SMI
Depth (m): 10
Method: *In situ* profiling

#1 Date: 8.20.12 Water temp: 18.5°C No DOC samples collected		#2 Date: 8.20.12 No DOC samples collected		#3 Date: 9.25.12 Water temp: 16.1°C **DOC (ppm) from duplicate samples: 123.3, 162.6**		#4 Date: 9.25.12 No DOC samples collected	
DO (mg/L)	**Depth into sediment (mm)**	**DO (mg/L)**	**Depth into sediment (mm)**	**DO (mg/L)**	**Depth into sediment (mm)**	**DO (mg/L)**	**Depth into sediment (mm)**
5.96	1	0.41	1	2.62	1	6.15	1
0.68	2	0.44	2	0.28	2	6.86	2
0.27	3	0.13	3	0.28	4	1.16	4
0.22	4	0.28	4	0.30	5	0.42	5
0.04	5	0.19	5	0.29	6	0.46	6
0.05	6	0.13	6	0.36	7	1.38	47
0.07	7	0.07	7	0.52	17	1.34	77
0.06	8	0.05	8	0.46	19	1.31	97
0.04	9	0.04	9				
0.05	10	0.03	10				
0.00	12	0.05	12				
0.00	13	0.01	15				
0.00	14	0.00	16				

Sediment
Location: GH10m
Depth (m): 10
Method: *In situ* profiling

#1 Date: 9.19.12 Water temp: 16.7°C Sediment under PVC tube **DOC (ppm) from one sample: 9.13**		#2 Date: 9.19.12 Control sediment **DOC (ppm) from one sample: 5.28**		#3 Date: 9.26.12 Water temp: 15.6°C Sediment under PVC tube **DOC (ppm) from one samples: 7.40**		#4 Date: 9.26.12 Control sediment **DOC (ppm) from duplicate samples: 8.35, 8.81**	
DO (mg/L)	**Depth into sediment (mm)**	**DO (mg/L)**	**Depth into sediment (mm)**	**DO (mg/L)**	**Depth into sediment (mm)**	**DO (mg/L)**	**Depth into sediment (mm)**
7.03	1	4.38	1	1.23	0.57	9.67	1
7.19	2	4.25	2	1.17	0.51	6.02	5
6.72	4	2.15	3	1.12	0.45	3.07	8
3.24	5	1.20	4	1.16	0.50	0.94	12
2.23	6	0.92	6	1.19	0.52	1.17	15
1.93	7	0.51	7	1.13	0.47	1.05	18
1.25	8	0.33	8	1.17	0.50	0.97	21
0.86	9	0.42	10			0.71	23
0.80	10	0.36	13			0.88	25
0.76	12	0.49	16			0.68	35
0.55	14	0.49	17			0.64	45
0.53	16	0.23	27			1.00	55
0.55	19						
0.55	21						
0.68	31						

Mussels assemblages with *Cladophora* canopy
Location: GH10m
Depth (m): 10
Method: *In situ* profiling

#1 Date: 9.6.12 Water temp: 20.8°C **DOC (ppm) from duplicate samples: 3.4, 2.7**		#2 Date: 9.6.12 Same location as #1		#3 Date: 9.7.12 Water temp: 20.6°C No DOC samples collected		#4 Date: 9.7.12 No DOC samples collected	
DO (mg/L)	**Depth into sediment (cm)**	**DO (mg/L)**	**Depth into sediment (cm)**	**DO (mg/L)**	**Depth into sediment (cm)**	**DO (mg/L)**	**Depth into sediment (cm)**
10.10	2.0	22.91	0.1	10.84	0.1	9.27	0.1
9.78	3.0	19.98	0.2	15.43	2.1	10.79	1.1
9.36	3.1	20.96	0.3	9.07	4.1	20.21	2.1
9.33	3.2	11.06	1.3	7.52	6.1	9.88	3.1
9.30	3.3	3.34	3.3	4.64	6.2	8.08	3.4
9.26	3.6	2.49	3.6	2.48	6.3	4.46	3.7
2.96	3.9	2.16	3.9	1.11	6.4	2.58	3.9
2.83	4.9	1.67	4.2	0.95	6.5	1.75	4.1
2.64	5.1	1.50	4.5				
2.47	5.2	0.99	4.7				
1.86	5.3	0.61	4.9				
1.71	5.5	0.49	5.1				
1.64	5.8	0.41	5.2				
		0.39	5.4				

Mussel assemblages without *Cladophora* canopy
Location: GH10m
Depth (m): 10
Method: *In situ* profiling

#1 Date: 8.15.12 Water temp: 16.9°C *Cladophora* removed by hand 30 min. before profiling No DOC sample collected		#2 Date: 9.19.12 Water temp: 16.7°C *Cladophora* cover sparse following sloughing event No DOC sample collected	
DO (mg/L)	**Depth into sediment (cm)**	**DO (mg/L)**	**Depth into sediment (cm)**
12.87	0	9.60	0.1
10.09	1	9.64	0.2
11.94	2	9.79	0.3
		10.18	0.4
		10.68	1.4
		13.94	2.4

B. Dissolved Oxygen Profiles: Aquarium Experiment

Current in front of mussel assemblage
Location: Aquarium experiment

Height from bottom of bed (cm)	Pump on Velocity (cm/sec)	Pump off Velocity (cm/sec)
13.0	0.64	0.29
11.5	1.12	0.73
9.0	1.26	0.29
7.5	1.17	0.16
6.0	1.54	0.34
4.5	3.28	0.18
3.0	1.66	0.22
1.2	1.58	0.83

Profiles into aquarium
Trial 1: pump off
Experiment run time: 16 hours

Pre-trial profile		#1 Post-trial profile		#2 Post-trial profile		#3 Post-trial profile	
DO (mg/L)	Height from bottom of bed (cm)	DO (mg/L)	Height from bottom of bed (cm)	DO (mg/L)	Height from bottom of bed (cm)	DO (mg/L)	Height from bottom of bed (cm)
10.71	17.00	7.59	15.00	7.98	17.00	6.80	15.00
10.81	0.50	8.15	8.20	7.46	15.00	7.46	10.00
		5.92	5.20	7.71	9.68	8.33	8.15
		5.36	2.20	8.06	7.68	6.46	5.15
		4.17	2.10	8.00	6.68	6.28	2.15
		3.38	1.80	8.04	5.68	6.14	1.65
		3.10	1.50	7.97	4.68	5.02	1.15
		2.98	1.00	8.01	3.68	4.98	1.05
		2.79	0.95	8.30	2.68	4.95	0.95
		2.60	0.85	7.62	1.68	3.33	0.85
		2.51	0.75	3.84	0.68	2.21	0.60
		2.41	0.60	2.11	0.18	0.72	0.55
		2.56	0.50	2.08	0.08	0.00	0.50
		2.60	0.30	1.95	0.03	0.00	0.45
		2.55	0.10	0.00	0.01	0.00	0.30
						0.00	0.25
						0.00	0.20
						0.00	0.10

Profiles into aquarium
Trial 2: pump off
Experiment run time: 6 hours

Pre-trial profile		#1 Post-trial profile		#2 Post-trial profile	
DO (mg/L)	**Height from bottom of bed (cm)**	**DO (mg/L)**	**Height from bottom of bed (cm)**	**DO (mg/L)**	**Height from bottom of bed (cm)**
11.38	17	10.41	15.00	10.69	15.00
11.29	15	10.51	8.55	10.33	8.90
11.25	13	10.58	5.55	10.05	3.90
11.25	12	10.49	4.55	9.83	1.90
11.34	10	10.46	2.55	9.57	1.50
11.31	6	9.36	2.15	9.51	0.85
11.28	5	9.34	1.85	8.91	0.55
11.19	3	9.10	1.55	8.72	0.45
11.19	1	8.83	1.05	8.96	0.35
11.19	0	8.23	0.45	7.73	0.25
		7.45	0.25	7.05	0.15
				6.71	0.08
				4.72	0.05

Profiles into aquarium
Trial 3: pump on
Experiment run time: 5 hours

Pre-trial profile		#1 Post-trial profile	
DO (mg/L)	**Height from bottom of bed (cm)**	**DO (mg/L)**	**Height from bottom of bed (cm)**
11.33	15.00	10.89	17
11.43	4.65	10.91	10
11.44	6.65	9.80	8
11.38	5.65	9.76	6
11.33	4.65	11.21	4
11.14	3.65	10.05	2
11.19	2.65	11.14	0
11.30	1.65		
11.39	0.65		
11.14	0.05		
11.04	0.00		

Sample Type	Site	Depth (m)	Date Sampled	Date Analyzed	Replicate	Sample Vol. (ml)	DI Vol. (ml)	Conc. (ppm)	Corresponding DO profile?
Sediment under ephemeral sloughed *Cladophora* mat	GH10m	10	8/29/2012	9/11/2012	1	4.80	1.20	3.75	
Sediment under PVC tubes	GH10m	10	8/29/2012	9/11/2012	1	3.60	2.40	5.20	
	GH10m	10	9/19/2012	10/4/2012	1	2.20	3.80	9.13	yes
	GH10m	10	9/26/2012	10/4/2012	1	6.00		7.40	yes
	GH10m	10	10/16/2012	10/23/2012	1	6.00		4.00	
Sediment not under PVC - control sediment	GH10m	10	9/19/2012	10/4/2012	1	2.00	4.00	5.28	yes
	GH10m	10	9/26/2012	10/4/2012	2	6.00		8.35	yes
	GH10m	10	9/26/2012	10/4/2012	1	6.00		8.81	yes
Cladophora from PVC tubes	GH10m	10	9/19/2012	10/4/2012	2	6.00		19.26	yes
	GH10m	10	9/19/2012	10/4/2012	1	6.00		8.65	yes
	GH10m	10	9/19/2012	10/4/2012	3	6.00		16.81	yes
	GH10m	10	9/26/2012	10/4/2012	1	6.00		21.18	yes
	GH10m	10	9/26/2012	10/4/2012	3	6.00		18.54	yes
	GH10m	10	9/26/2012	10/4/2012	2	6.00		15.11	yes
Sediment/biodepositis at base of mussel assemblages	GH10m	10	9/6/2012	9/11/2012	1	6.00		3.40	yes
	GH10m	10	9/6/2012	9/11/2012	2	6.00		2.70	yes
	Atwater 10m	10	10/2/2012	10/4/2012	3	6.00		1.59	
	Atwater 10m	10	10/2/2012	10/4/2012	1	6.00		3.10	
	Atwater 10m	10	10/2/2012	10/4/2012	2	6.00		3.88	
	GH10m	10	10/16/2012	10/23/2012	1	6.00		2.40	
	GH10m	10	10/16/2012	10/23/2012	2	6.00		2.60	
	GH10m	10	10/16/2012	10/23/2012	3	6.00		3.20	
Benthic mud from Ponar grabs	GH20m	21	9/5/2012	9/11/2012	1	4.40	1.60	6.53	
	GH20m	20.5	9/5/2012	9/11/2012	2	2.20	3.80	8.96	yes
	GH20m	28.5	9/5/2012	9/11/2012	3	1.40	4.60	11.38	yes
	GH20m	29	9/5/2012	9/11/2012	4	1.80	4.20	3.05	yes
Sediment under sloughed *Cladophora* mat	SMI10m-SMI Graveyard	8	9/25/2012	10/4/2012	1	3.30	2.70	55.19	
	SMI10m-SMI Graveyard	8	9/25/2012	10/4/2012	2	6.00		64.76	
	SMI10m-SMI Graveyard	8	9/25/2012	10/4/2012	3	2.00	4.00	151.26	
Sloughed *Cladophora* mat	SMI10m-SMI Graveyard	8	9/25/2012	10/4/2012	1	0.60	5.40	123.30	yes
	SMI10m-SMI Graveyard	8	9/25/2012	10/4/2012	2	0.60	5.40	162.60	yes
Sloughed *Cladophora* D=dead *Cladophora*/detritus F=Fresh, fluffy. Decomposing material beneath S=Sand. Decomposing material beneath	GH20m-GH Graveyard	23	10/17/2012	10/23/2012	1	6.00		6.60	
	GH20m-GH Graveyard	23	10/17/2012	10/23/2012	2	6.00		6.10	
	GH20m-GH Graveyard	23	10/17/2012	10/23/2012	1	6.00		7.80	
	GH20m-GH Graveyard	23	10/17/2012	10/23/2012	2	6.00		6.70	
	GH20m-GH Graveyard	23	10/17/2012	10/23/2012	1	6.00		3.00	
	GH20m-GH Graveyard	23	10/17/2012	10/23/2012	2	6.00		4.40	