# Bacteria Abundance in Response to Increasingly Acidic Conditions

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## Introduction:

Global warming is a worldwide phenomenon that is the result of anthropogenic input of carbon dioxide into the atmosphere. Although the increase in carbon dioxide levels now is 100 times faster than at the end of the last ice age (IPCC 2001, 2007), the ocean is the most important sink of carbon dioxide and have absorbed approximately half of all anthropogenic $CO_2$ emissions to the atmosphere (Sabine et al. 2004). Many consequences are expected of global warming are occurring presently, one of which is ocean acidification.

The average ocean surface water pH has decreased from 8.21 to 8.10 recently (Royal Society 2005). The Intergovernmental Panel on Climate Change predicts $CO_2$ concentrations to reach 800ppmv by the end of the century which is equivalent to a decrease of 0.3-0.4 pH units (Orr et al. 2005). An effect of acidifying seawater is the lowering of calcium carbonate saturation states, a drastic change in environmental condition for shell-forming marine organisms (Doney et al. 2009). The organisms affected directly ranges from plankton to corals, molluscs, and sea urchins (Doney et al. 2009). For example, reduced shell and soft body growth have been reported in mussels (Gazeau et al. 2007) and metabolic depression has been seen in European eels (*Anguila anguila*) at $CO_2$ levels above 2% (Cruz-Neto and Steffensen 1997).

Bacteria are hugely important to all ecosystems, including marine habitats. Marine bacteria are uniquely adapted to saline environments and they need salt for growth (Fenical 1993). Most marine bacteria are unicellular (Fenical 1993) and some such as *Prochlorococcus* and *Synechococcus* are abundant oceanic primary producers (Azam and Worden 2004). Although the distribution of marine bacteria is poorly studied, 90% of marine bacterial flora is of the gram-negative genus *Vibrio* (Fenical 1993).

Sediments, animate and inanimate surfaces, and internal spaces of living organisms are important microhabitats of marine bacteria (Fenical 1993). Internal spaces of marine organisms are often more nutrient rich than seawater (Fenical 1993). In particular, sponges and bacteria can form symbioses (Lee et al. 2009). Sponges can acquire their bacterial partners by filter feeding and selectively retaining bacteria (Taylor et al. 2007). The relationship is often mutualistic; bacteria benefit nutritionally while bacteria help sponges eliminate waste more efficiently (Beer and Ilan 1998), stabilize the skeleton (Rutzler 1985), or defend against pathogens and predators (Bewley et al. 1996). Bacteria are observed on the surface of Oriental shrimp (*Palaeomon macrodactylus)* eggs, American lobster (*Homarus americanus)* eggs, coral reefs, sea jellies, and other bacteria such as cyanobacterium *Microcoleus lyngbyaceus* (Fenical 1993). Bacteria are notably associated with chitinous plankton (Vezzulli et al. 2012). They are also a major food source for invertebrates, which reflects the energy available for higher trophic levels (Hall and Meyer 1998). Hall and Meyer (1998) found that invertebrates could derive <10% to 100% of their carbon requirements from bacteria.

Marine microorganisms such as bacteria have the potential for rapid growth. Thus, marine microorganisms are critical to global nutrient cycles (Arrigo 2005). Nitrogen, an essential nutrient, is used by organisms to form proteins, chlorophyll, and nucleic acids (Murray et al. 2005). In the ocean, it is present as a dissolved gas or as dissolved inorganic ions: nitrate, nitrite, and ammonium (Murray et al. 2005).

Once thought to be restricted to very specific habitats and microbes, now we know that many bacteria are known for their nitrification and denitrification services to the ecosystem (Zehr and Ward 2002). Nitrification is a process of oxidation from ammonia to nitrate with nitrite as an intermediate species (Figure 3). Denitrification is the reverse reaction where nitrate is reduced to nitrite and is converted to nitrogen gas (Figure 3). Bacteria mediate these two processes which makes nutrients available to many organisms, including phytoplankton. Biodiversity of plant and animal communities are dependent on the availability of these nutrients (Bienhold et al. 2012).

The objective of this mesocosm experiment is to determine differences in bacteria abundance in normal (600µatm), medium (1000µatm), and high (1300µatm) $CO_2$ concentrations. Mesocosms serve as an intermediate for lab and field studies; mesocosms provide insight about changes to food webs, communities, and ecosystems.

## Measurement Methods:

All set up and sampling methods can be referenced in Murray et al. 2012.

### Measurements/Calculations:

Nine milliliters of seawater from each mesocosm and off the dock were collected every other day around 09:00; upon collection, one milliliter of formalin or glutaraldehyde was added to stop any cellular processes. Sample water was kept cool until preparation of slides.

DAPI stain was added to three milliliters of fixed seawater for three to five minutes before filtering through a black 0.2µm polycarbonate filter. Slides were prepared using filters and Resolve immersion oil; slides were chilled until counting with an epifluorescence light microscope (Figure 4). Ten fields of view were counted; one hundred percent of the eyepiece grid was counted unless each square (100 squares/grid) exceeded 50 bacterial cells in which case only a subset of squares was counted.

A flow-through method was used where the sample intake is divided into a non-reduced and a reduced split. Nitrite and nitrate were both measured as nitrite on an auto-analyzer; the nitrate was reduced to nitrite with a cadmium column. Sulfanilamide reacted with NEDA to produce a purple solution which was measured with a spectrophotometer. The concentration was measured by peak height against a standard.

Phytoplankton collection and counting methods can be referenced in Peterson 2012; oxygen analysis can be referenced in Schaut 2012 and Tounge 2012.

**Results:**

Low, medium, and high $CO_2$ concentration treatments resulted in similar averages and standard deviations relative to the dock's ambient water (Table 1). Abundance of bacteria in the water column of various sites such as Florida Keys, Philippines, and New Caledonia range between 3E+05 to 9.5E+05 (Garren and Azam 2012). Previous studies such as Watson et al. (1977) showed 1.5E+04 – 6.29E+06 bacteria cells/mL of seawater in the Sargasso Sea. A range of 3.11E+06 to 3.90E+06 using direct counting, epifluorescence, technique was also observed (Watson et al. 1977). Bacteria abundance is relatively low compared to the global bacteria abundance average of 5E+05 cells $ml^{-1}$ (Jackson 2001), for the first six days of the experiment (Figure 1). As oxygen and chlorophyll a were produced in the first six days by primary producers (Figures 5 and 6), nitrate and nitrite were used slowly until they crashed on day six (Figures 2a and 2b). The production of oxygen slowed down after the phytoplankton bloom (Figure 5) due to uptake of oxygen bacterial respiration (Figure 1).

Bacteria became increasingly abundant, higher than previously noted abundances (Garren and Azam 2012), until day 14 (Figure 1). No significant differences in $CO_2$ concentration were observed. Additional nutrients were added on day 12 as shown by a spike in nitrate and nitrite levels (Figures 2a and 2b); at that time, chlorophyll a production started to increase again (Figure 6). Oxygen production only started to increase on day 15 (Figure 5) when bacteria abundance lowered (Figure 1). Ammonia remained low until day 16 when a sudden increase was evident (Figure 2c); nitrite was completely consumed by then (Figure 2b).

Little variation was observed at the dock. Nitrate, nitrite, and ammonia trends for mesocosms are similar compared to a relatively constant level of nutrients at the dock (Figure 2). On the initial day of the experiment nitrate started at the ambient level of 22.5µM. Throughout the whole experiment, nitrate levels at the dock ranged from 20 to 25µM. Ammonia at the dock ranged from 0.5 to 1.3µM and nitrite ranged from 0.14 to 0.32µM, both of which are narrow ranges. Oxygen levels stayed at 270µM throughout the entire experiment (Figure 5); chlorophyll a levels stayed at 1µM (Figure 6).

Based on several simple calculations using Figure 2 and the assumption that the Redfield ratio (106C: 16N: 1P) is accurate, nitrogen uptake occurred at a rate of 4.4µM$L^{-1}$$day^{-1}$ and primary production occurred at a rate of 29µM$L^{-1}$$day^{-1}$. Del Giorgio and Cole (1998) showed that bacterial growth efficiency (BGE) is highest at a range of 5-10 C:N.

**Discussion:**

The trends seen between bacteria abundance, nutrient, oxygen, and chlorophyll a levels seem to be closely related. Chlorophyll a and oxygen are both produced via primary production and nutrients are used in order to facilitate these cellular processes. Bacteria evolve oxygen through respiration. In aerobic conditions such as the mesocosms where oxygen levels averaged 400µM/L, nitrifying bacteria produce nitrate using ammonia (Figure 3). The second phytoplankton bloom after 12 days presumably used the nitrate and nitrite and produced oxygen as bacteria produced some nitrates using ammonia. A variable slow bloom of phytoplankton towards the end of the experiment resulted in a slow decrease of nitrate (Figure 6 and Figure 2a).

Azam (1998) suggests that patchiness can support high bacterial diversity which might explain why the bacteria bloom occurred following the phytoplankton bloom (Figures 1 and 6).

Table 1 suggests that carbon dioxide concentrations have little influence on bacteria abundance in seawater. Wind strength is a factor in bacteria abundance (Gilbert et al. 2012) but another possible explanation for this idea is that Haro Strait and all the passages around San Juan and neighboring islands are areas of intense mixing (Masson and Pena 2008) implying weak year-round stratification. In addition, the surface layer is consistently deeper than the euphotic zone (Masson and Pena 2008). Therefore, a key factor in consistent low chlorophyll in the Juan de Fuca Strait, or even at the Friday Harbor Laboratories dock, seems to be light limitation.

Growth of plankton is related to bacterial growth. Calbet and Landry (1999) showed that increasing the concentration of mesozooplankton also increased growth of heterotrophic bacteria, which may be seen from day 12 to day 14 of the experiment (Figures 1 and 6). Calbet and Landry also found that the removal of plankton 5-20µm in size resulted in decreased growth rates of bacteria and that removal of organisms smaller than 5µm increased bacterial growth rates to unfiltered control seawater. The exclusion of large grazers, such as copepods, during the initial 500µm filtration to fill the mesocosm bags does not seem to be the reason for continuous low chlorophyll levels. In fact, removal of large grazers seems to decrease chlorophyll levels due to non-existent grazing on meso- and microzooplankton, who are the main grazers of phytoplankton. Dilution experiments show that neither the pump nor polyurethane mesocosm bags were detrimental to the microzooplankton in the mesocosms (Murray et al. 2012).

Bacteria can use both dissolved inorganic nitrogen (nitrate, nitrite, and ammonia; collectively referred to as DIN) and organic matter (Zehr and Ward 2002). Therefore, they might compete for phytoplankton for inorganic nitrogen. Ultimately, much of the primary production in the ocean is processed by planktonic bacteria (Del Giorgio and Cole 1998). Although bacteria grow much faster with ammonium ion as a nitrogen source rather than $N_2$ (Senez 1962), the bacteria in the mesocosms could be using nitrate and nitrate after the nutrient addition on day 12 while the breakdown of cells after the first bloom may have produced some ammonia (Figures 1 and 2). Bacteria have been shown to have strong positive or negative relationships with phytodetritus (Bienhold et al. 2012).

If ocean acidification were to affect the abundance or the chemical metabolites that bacteria produce, organisms that use or wholly depend on bacteria may be adversely affected. The triple bond of atmospheric nitrogen is hard for non-nitrifying organisms to break; therefore, nitrogenous nutrients are made available to organisms by bacteria. The addition of anthropogenic carbon into the major sink of carbon dioxide, the ocean, is suggested to increase fertilization of soils with nitrogen causing a decrease in carbon sequestration (Oren at al. 2001).

Ocean acidification is a pressing concern associated with global warming, but rising sea surface temperatures (SST) is also a concern. Surface temperatures are estimated to increase a few degrees in the next century (Harvell et al. 2002). As SSTs rise due to greenhouse gases trapping heat in the Earth, intense stratification can be expected in the ocean, especially in regions with very little mixing to begin with. Vezzulli et al (2012) showed a major impact on the prokaryotic community over a decadal scale: vibrio bacteria associated with plankton increased in relative abundance in coastal waters. In laboratory studies, growth rate of bacteria declined if temperatures were outside the optimal range for each type of bacteria (Payne and Wiebe 1978). In natural bacterioplankton assemblages though, a strong positive relationship exists between growth rates and temperature (Reitner et al. 1977). One example of the extreme importance of bacteria is in controlling the health and resilience of coral reef ecosystems (Garren and Azam 2012) which are already under stress due to high calcium saturation states and bleaching (Cramer et al. 2012).

Future studies should address mixing issues; several mesocosm experiments (Engel et al. 2005, Engel et al. 2008; Kim et al. 2008, Kim et al. 2010) used bubblers and other equipment to maintain constant vertical mixing. Additionally, if organisms larger than 500µm were included in mesocosms, the results could be related to higher trophic levels of the ecosystem. If mixing and filtration remain the same, it would be beneficial and more informative to conduct analyses on bacteria such as the relative abundance of heterotrophic and autotrophic bacteria. Growth rates would be interesting to study as well.

**Appendix:**

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## Tables:

Table 1. Bacterial cell counts over an 18-day experiment at Friday Harbor Laboratories, University of Washington. Mesocosms 1, 4, and 7 had low $CO_2$ concentrations (600 ppmv); mesocosms 2, 5, and 8 had medium $CO_2$ concentrations (1,000 ppmv); mesocosms 3, 6, and 9 had high $CO_2$ concentrations (1,300 ppmv). The dock had an ambient $CO_2$ concentration of about 650 ppmv. Dock average, standard deviation, and range was compiled from one value per sample day; low, medium, and high $CO_2$ concentration averages, standard deviations, and ranges were calculated using three mesocosm values per sample day.

| treatment | average | standard deviation | range |
|---|---|---|---|
| dock | 1.1E+06 | ±3.1E+05 | 7.5E+05 - 1.4E+06 |
| low | 3.3E+06 | ±2.6E+06 | 7.4E+05 - 5.9E+06 |
| medium | 2.7E+06 | ±2.3E+06 | 3.5E+05 - 5.0E+06 |
| high | 2.9E+06 | ±2.4E+06 | 5.2E+05 - 5.3E+06 |

**Figures:**

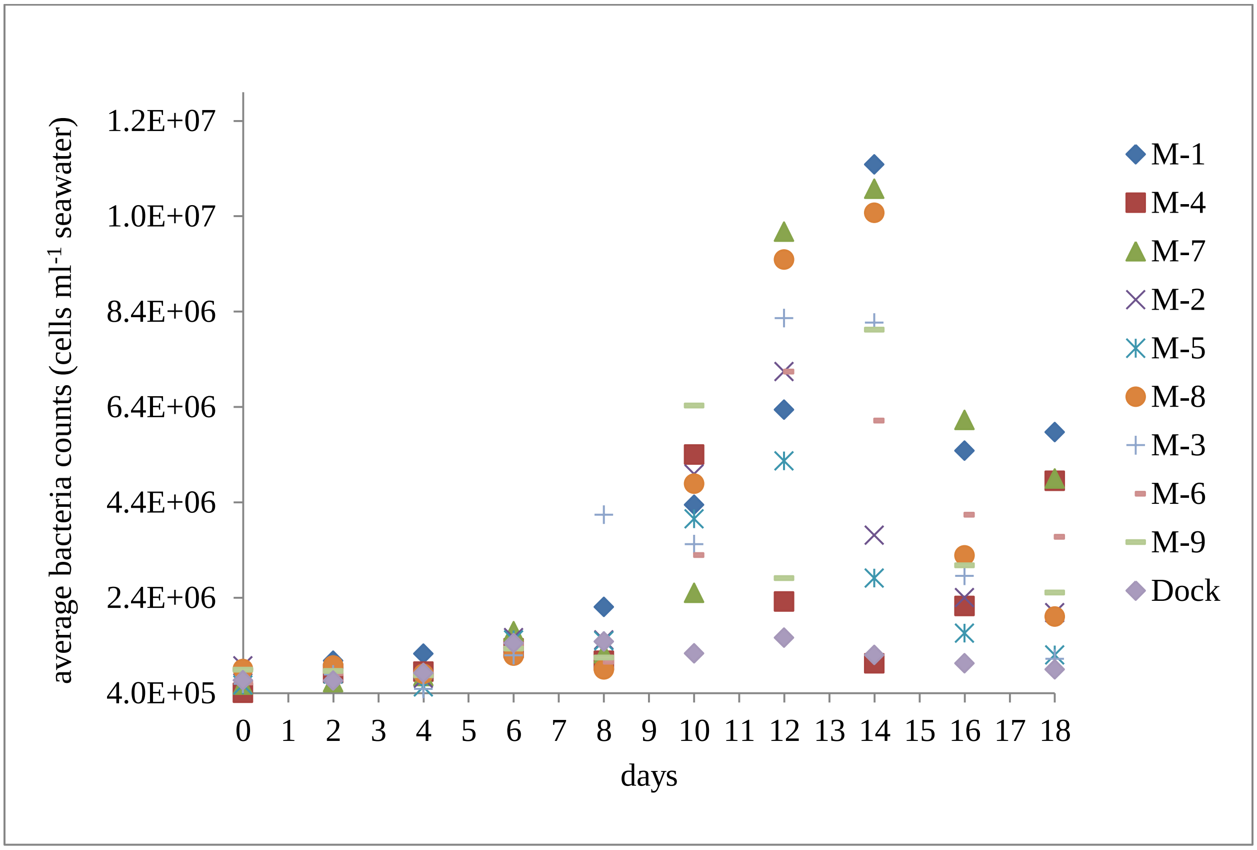

Figure 1. Bacterial cell counts in a mesocosm experiment at Friday Harbor Laboratories, University of Washington. Averages per mesocosm and dock were calculated with 10 fields of view; samples were taken every two days. Mesocosms 1, 4, and 7 had low $CO_2$ concentrations (600 ppmv); mesocosms 2, 5, and 8 had medium $CO_2$ concentrations (1,000 ppmv); mesocosms 3, 6, and 9 had high $CO_2$ concentrations (1,300 ppmv). The dock had an ambient $CO_2$ concentration of about 650 ppmv.

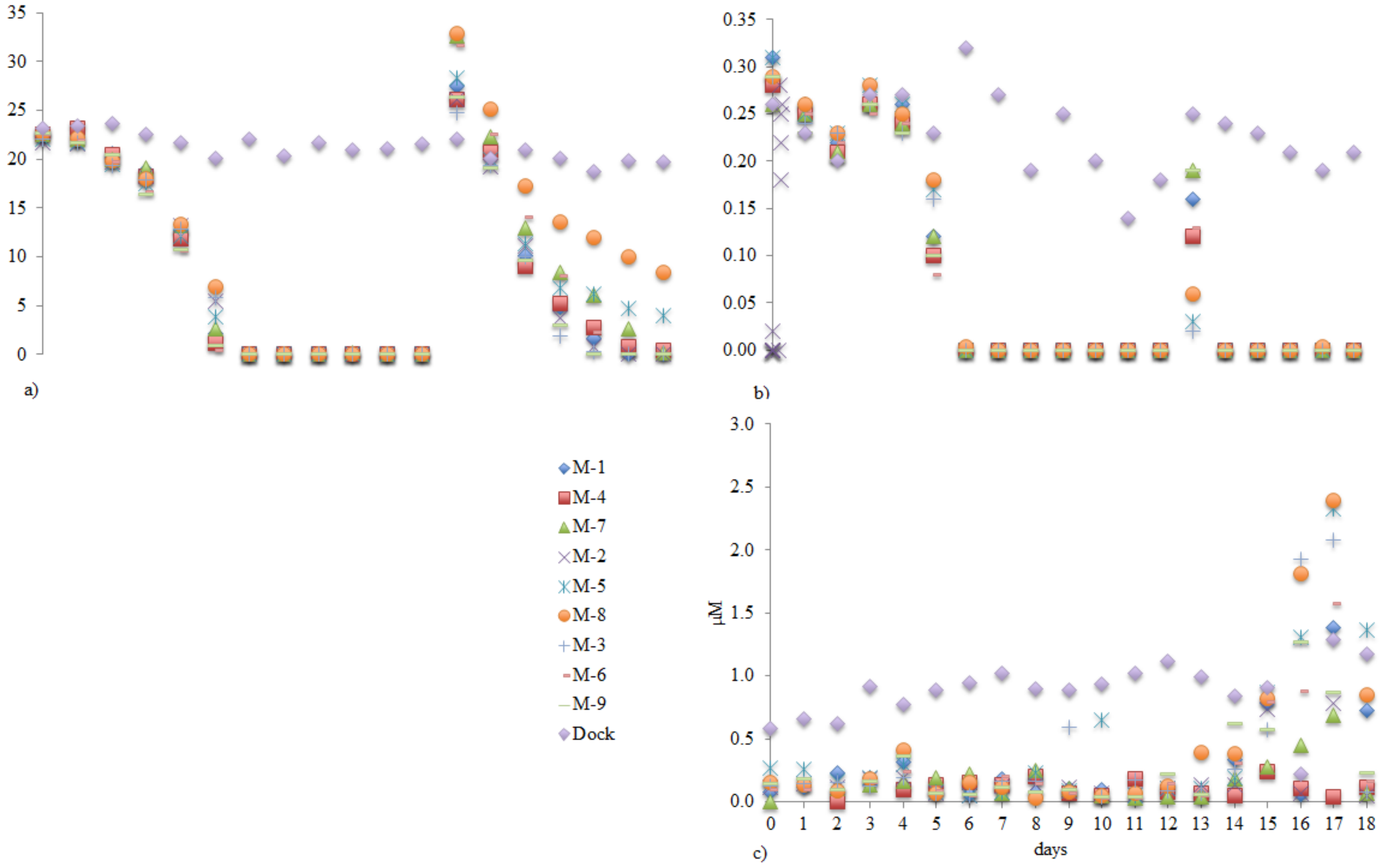

Figure 2. Daily a) nitrate, b) nitrite, and c) ammonia values in a mesocosm experiment at Friday Harbor Laboratories, University of Washington. Mesocosms 1, 4, and 7 had low $CO_2$ concentrations (600 ppmv); mesocosms 2, 5, and 8 had medium $CO_2$ concentrations (1,000 ppmv); mesocosms 3, 6, and 9 had high $CO_2$ concentrations (1,300 ppmv). The dock had an ambient $CO_2$ concentration of about 650 ppmv.

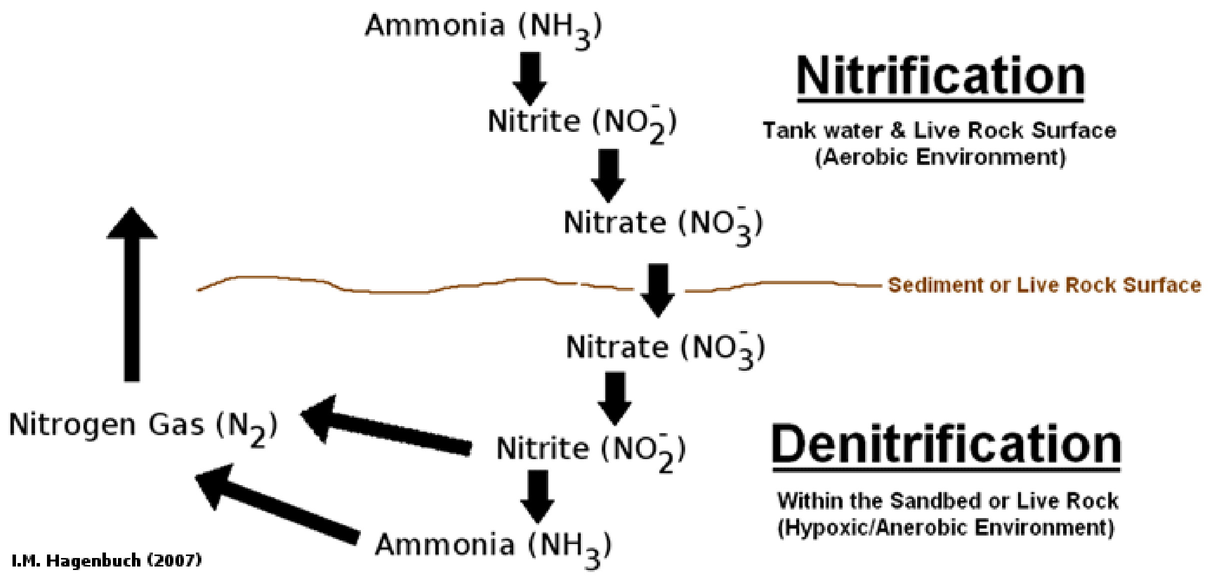

Figure 3. Visual representation of two processes mediated by bacteria: nitrification and denitrification (Hagenbuch 2007, unpublished figure).

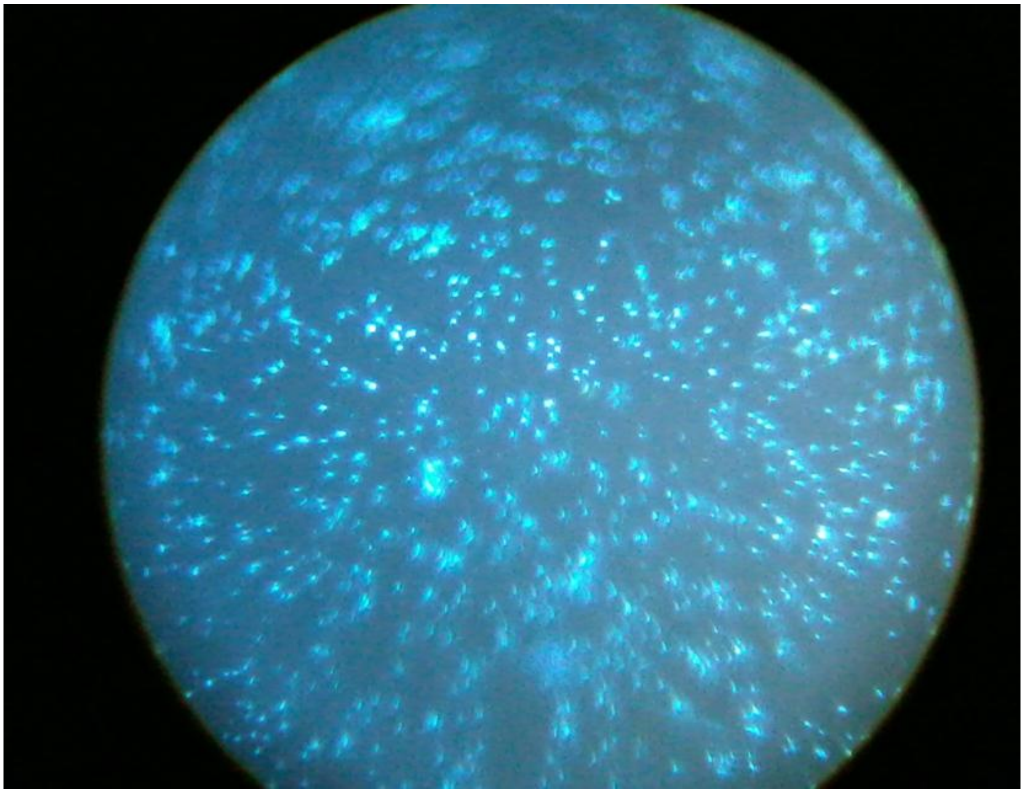

Figure 4. Photograph of one field of view of DAPI-stained bacteria in an epifluorescence light microscope. Photograph taken by Anne Wang.

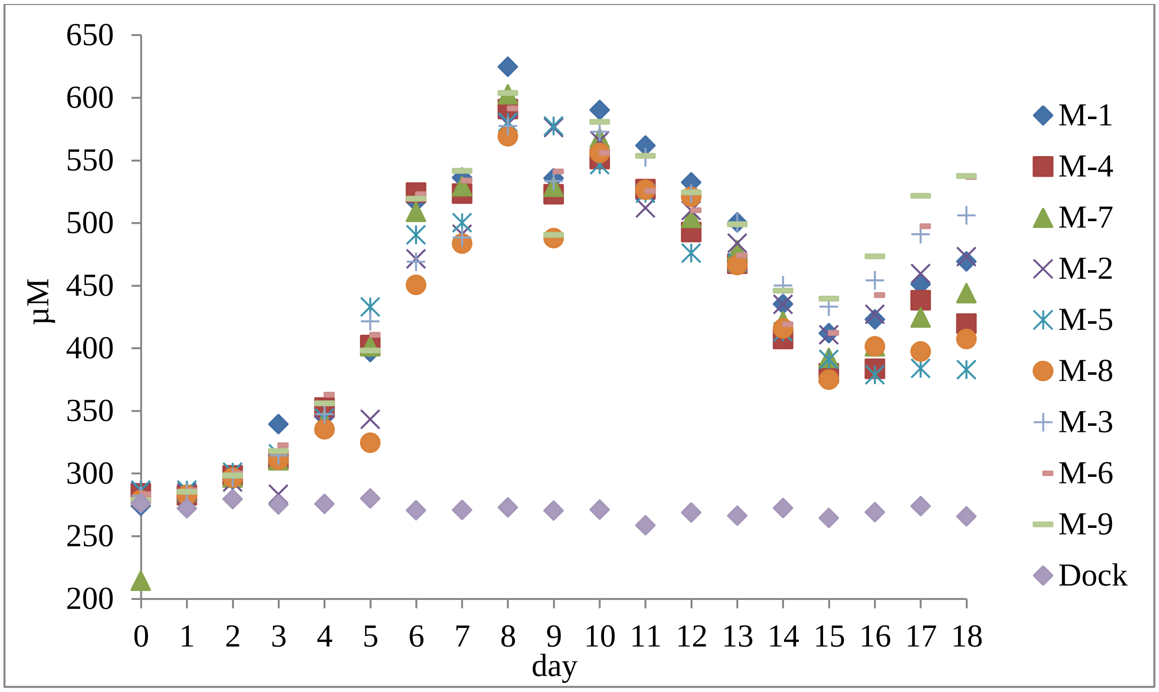

Figure 5. Daily $O_2$ levels in mesocosms at Friday Harbor Laboratories, University of Washington. Mesocosms 1, 4, and 7 had low $CO_2$ concentrations (600 ppmv); mesocosms 2, 5, and 8 had medium $CO_2$ concentrations (1,000 ppmv); mesocosms 3, 6, and 9 had high $CO_2$ concentrations (1,300 ppmv). The dock had an ambient $CO_2$ concentration of about 650 ppmv.

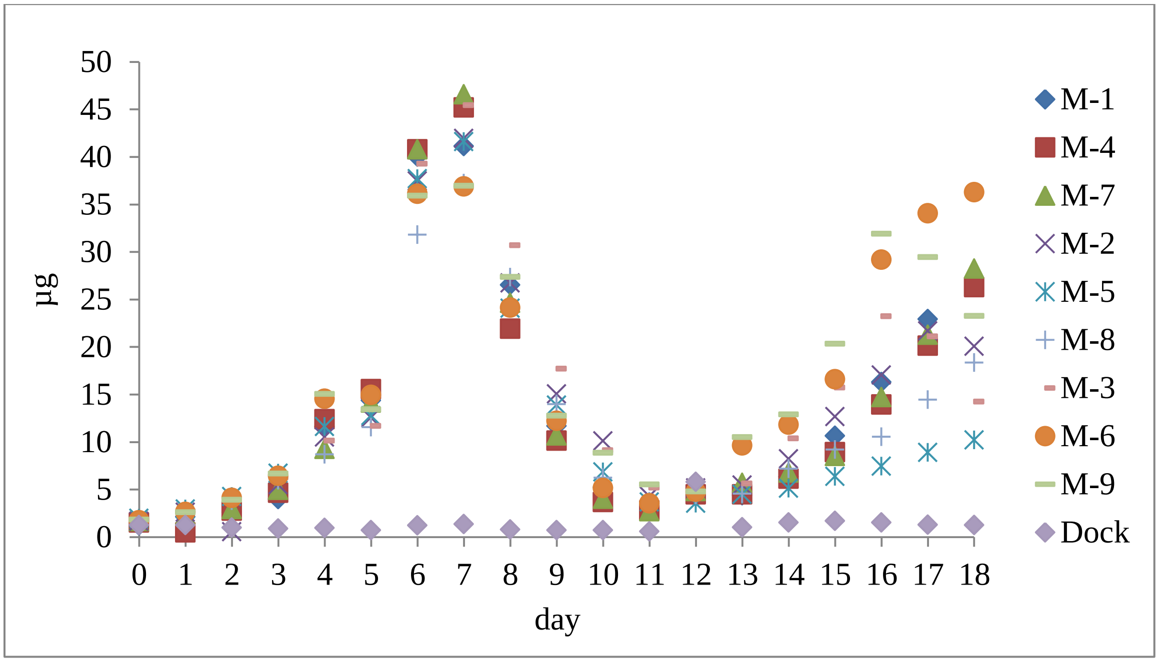

Figure 6. Daily chlorophyll a levels in mesocosms at Friday Harbor Laboratories, University of Washington. Mesocosms 1, 4, and 7 had low $CO_2$ concentrations (600 ppmv); mesocosms 2, 5, and 8 had medium $CO_2$ concentrations (1,000 ppmv); mesocosms 3, 6, and 9 had high $CO_2$ concentrations (1,300 ppmv). The dock had an ambient $CO_2$ concentration of about 650 ppmv.