Spring 2015

The Effect of Temperature on Paralytic Shellfish Toxin Uptake By Blue Mussels (Mytilus Edulis) and Sea Scallops (Placopecten Magellanicus)

Mackenzie Mazur
University of Maine - Main, mackenzie.mazur@maine.edu

THE EFFECT OF TEMPERATURE ON PARALYTIC SHELLFISH TOXIN UPTAKE BY BLUE MUSSELS (MYTILUS EDULIS) AND SEA SCALLOPS (PLACOPECTEN MAGELLANICUS)

by

Mackenzie Mazur

A Thesis Submitted in Partial Fulfillment
of the Requirements for a Degree with Honors
(Marine Science)

The Honors College

University of Maine

May 2015

Advisory Committee:

Laurie B. Connell, Research Professor School of Marine Science, Advisor
Paul Rawson, Associate Professor of Marine Science
Rebecca J. Van Beneden, Professor of Biochemistry and Marine Science
Teresa R. Johnson, Associate Professor of Marine Policy
Robert W. Glover, Assistant Professor of Honors and Political Science

Abstract

Increasing amounts of paralytic shellfish toxins (PSTs) threaten human health, the economy, and marine ecosystems because of paralytic shellfish poisoning (PSP). Therefore, studies about shellfish toxicity can have significant public health and social impact. In this study, the effect of water temperature on PST uptake in blue mussels (*Mytilus edulis*) and sea scallops (*Placopecten magellanicus*) was tested. *Mytilus edulis* and *P. magellanicus* were acclimated to either 10°C or 15°C for two weeks before being fed with the toxic alga, *Alexandrium fundyense,* at a concentration of 100 cells/mL and a non-toxic algae source (Shellfish Diet) at a concentration of 2.4 X 10^3 cells/mL. At different time points, *Alexandrium* cell concentration was determined and individual shellfish were harvested and frozen. Whole mussel tissue, mussel tissue without the digestive gland, scallop tissue without the adductor muscle, and scallop adductor muscles were tested for toxicity using the Jellett Rapid Extraction method. Because the metabolic rates of *M. edulis* and *P. magellanicus* increase with temperature, PST uptake was expected to increase with temperature. The variation in uptake was high in mussels, and the mussels rapidly cleared toxic algae at both temperatures. One set of the scallops used for the experiment had been previously exposed to a harmful algal bloom. Because *P. magellanicus* retains toxin for a long time, the scallops were toxic prior to the experiment. Thus, the relationship between temperature and uptake could not be determined for each species. For future experiments, larger sample size and a new experimental design are recommended.

Acknowledgements

I would like to acknowledge my thesis advisor, Dr. Laurie Connell for all of her time, help, and guidance. I would like to thank Dr. Paul Rawson and Melissa May for providing me with tanks and equipment and for all of their help. I would also like to acknowledge Corey Hirn and Leslie Astbury for all of their help at the Connell laboratory, Tim Miller for finding me a holding tank in the flowing seawater laboratory at the Darling Marine Center, Marissa Giroux for helping me collect blue mussels, and Nate Perry for providing me with the sea scallops. I would also like to acknowledge the School of Marine Sciences for funding for the Shellfish Diet. Also, I would like to thank the Honors College and Charlie Slavin for the Charlie Slavin Research Fund, which provided funding for the Jellett Test Strip shipping. Last but not least, I would like to acknowledge the other members of my thesis committee: Dr. Robert Glover, Dr. Teresa Johnson, and Dr. Rebecca Van Beneden for all of their help, guidance, and advice.

Table of Contents

List of Figures and Tables

Introduction

Paralytic shellfish toxins and shellfish

Harmful algal blooms (HABs) occur when there is a large amount of toxic algae in the water column, and these events cause problems all over the world. In coastal waters, the dinoflagellate species complex *Alexandrium tamarense/ fundyense/ catanella* (hereafter referred to as *Alexandrium*) produces paralytic shellfish toxins (PSTs) naturally. In the Gulf of Maine, this species complex is the main cause of HABs from March through October. *Alexandrium* blooms can reach concentrations of 100 cells/mL (Townsend *et al.* 2001) and extend over wide areas in the Gulf of Maine (McGillicuddy Jr. *et al.* 2014).

Shellfish are filter feeders that can accumulate PSTs by consuming toxic algae from the water column. As a result, PSTs can accumulate in the shellfish tissue, and if the concentration of PST reaches 4.0 X 10^{-4} g STX_{eqv}/kg, in shellfish tissue it can be detected by the Jellett Rapid Extraction method (Cembella *et al.* 2003). The regulation level of PST in shellfish tissue in the United States is 80 µg STX_{eqv}/100 g (FAO 2015).

The toxin in shellfish can be transferred to higher trophic levels, such as other marine organisms and humans. If people consume toxic shellfish from the market or straight from the ocean, then human health is affected by paralytic shellfish poisoning (PSP) in which PST causes muscle paralysis (Setala *et al.* 2013). Marine organisms that consume shellfish, such as sea stars, may also be negatively affected by toxic shellfish. The shellfish themselves may be negatively affected by the accumulation of toxins, and this can affect shellfish feeding, predator avoidance, and other activities (Shumway and

Cucci 1987, Hegaret *et al.* 2012). As a result, shellfish may not be able to modify the marine ecosystem.

Blue mussels (Mytilus edulis) *and sea scallops* (Placopecten magellanicus)

Mytilus edulis and *P. magellanicus* are commercially important shellfish found in the Gulf of Maine. *Mytilus edulis* live in temperate, low and mid intertidal zones along the Atlantic coast (Setala *et al.* 2014). *Mytilus edulis* are edible marine bivalves in the *Mytilidae* family and phylum *Mollusca*, and they grow to eight cm in length. *Mytilus edulis* and *Mytilus trossulus* are morphologically similar. However, Rawson *et al.* (2001) found that the further south blue mussels were collected, the more likely the species distribution was *M. edulis* rather than *M. trossulus*.

The scallop *Placopecten magellanicus* lives in the littoral zone from the coast of North Carolina north to Nova Scotia. *Placopecten magellanicus* are edible marine bivalves in the *Pectinidae* family and phylum *Mollusca,* and they grow to 17 cm in length.

In order to feed and obtain oxygen, *M. edulis* and *P. magellanicus* filter water by taking in seawater through the inhalant siphon which passes over the gills before exiting. As this happens, *M. edulis* and *P. magellanicus* consume the sediment, contaminants, and plankton that are in the water column. Sometimes, high production of pseudo-feces occurs because the gill structure of the shellfish will sort through the algae to be digested and sometimes sort out particles they do not want. This is usually based on the size and shape of the particle (Ward *et al.* 2013). Some species of toxic algae are sorted out but not all. The toxic algae then do not enter the digestive gland, and instead exits as pseudo-feces. For example, in a video of *M. edulis* gills with endoscopy, a mucus string of

particles is created within a ciliated groove on the gills (Ward *et al.* 2013). This string is broken down and pieces are dispersed. The particles are then sorted and either ingested or exit as pseudo-feces.

When shellfish consume the *Alexandrium*, PSTs can accumulate in the tissues. The PSTs initially accumulate in the digestive gland and then spread into other tissues; although, as this happens, some of the toxin is degraded or depurated (Setala *et al.* 2014).

Different shellfish species accumulate and depurate toxins at different rates (Setala *et al.* 2014). Individuals of the same species can also have different uptake and/or clearance rates even under the same environmental conditions. Difference in uptake can occur from difference in shell size (Morono *et al.* 2001). Clearance rate is the rate at which seawater is filtered by the mussel, and clearance rate increases with shell size. Clearance rate also varies with sensitivity to PSTs (Twarog *et al.* 1972). Although toxins accumulate quickly in *M. edulis*, the species is relatively unaffected by PSTs (Setala *et al.,* 2014; Twarog *et al.* 1972). In scallops, exposure to PSTs causes increased vulnerability to predators and pathogens (Hegaret *et al.* 2012). When exposed to PSTs, scallops only swam from predators for a short time period, and the tissue and immune system of the scallops were damaged (Hegaret *et al.* 2012). Scallops also accumulate toxins at a slower rate than mussels and can retain PSTs for over a year because of their slow detoxification rate (Haya *et al.,* 2002).

Paralytic shellfish poisoning

Human health can be threatened when toxic shellfish are consumed. This is because of the neurotoxic syndrome known as paralytic shellfish poisoning (PSP), which is major health threat worldwide (Gago Martinez 2003). This syndrome can also occur

from consumption of other types of toxic seafood, such as crabs and fish, and can cause severe illness or death (Gago Martinez 2003). PSTs are a group of related toxins with many congeners. One of the most potent PSTs is saxitoxin (STX) (Thottumkara *et al.* 2014). STX reversibly blocks sodium channel pores, and therefore the flow of sodium ions, thus keeping action potentials from occurring (Thottumkara *et al.* 2014). In an action potential the electrical potential across the cell membrane increases and the sodium channels open up, increasing the electrical potential across the cell membrane even further, because sodium ions are moving into the cell. Eventually, the channels close, and repolarization occurs as the sodium ions are actively transported out of the cell. Next, potassium channels open, and potassium ions move out of the cell. This ion flux then returns the electric potential to the resting state. Ions cannot pass through the sodium channels blocked by STX. As a result, a human victim of PSP will first experience tingling and numbness in the fingers and area around the mouth, and if the toxin level is high enough, the sensation will spread throughout the body. Other symptoms of PSP are headache, dizziness, nausea, and vomiting. In more extreme cases, symptoms include slurred speech, weakness, and paralysis of muscles. A higher toxin level will result in respiratory failure because of muscle paralysis potentially leading to death (Prakash *et al.* 1971). A dose of as low as one mg of STX from shellfish consumption can cause human death (Wiese *et al.* 2010).

Creating policy on PSTs and shellfish can be difficult. The level of PST in shellfish tissue does not determine if PSP will occur, because individual people differ in sensitivity to PSTs. Also, the acidic conditions of the human stomach can make sulphocarbamoyls, from STX, highly toxic (Gago Martinez 2003). If a considerable

amount of sulphocarbamoyls are present in the toxin consumed, then the effects can be disastrous (Gago Martinez 2003).

Reports of PSP cases have increased since the 1970s, and cases are now appearing in areas where PSP had not previously been recorded (Gago Martinez 2003). In Maine, PST have been found in shellfish tissue since the 1960s (Bourne 1965), but PSP cases have been rare because of shellfish monitoring and harvest closures. Only three documented PSP cases have occurred Maine in the past thirty years, and they were due to non-commercial shellfish harvested and consumed from non-regulated areas (Maine DMR 2015). For example, in May through June of 1990 eight fishermen got PSP from eating *M. edulis* from their bycatch at Georges Bank (White *et al.* 1993). Another case occurred in July of 2007, where a fisherman and his family ate mussels from floating barrels off the coast of Jonesport, ME. They cooked the mussels and three out of four of the family members were hospitalized for PSP symptoms (DeGrasse *et al.* 2014).

Impact of Paralytic Shellfish Toxins

If toxic shellfish are put on the market, deaths and hospitalizations may occur from human cases of PSP. Shellfish closures are made when shellfish tissue is toxic (greater than 80 µg STX_{eqv}/100 g) (FAO 2015), and as a result, shellfish fisheries and aquaculture facilities lose profit, which results in economic losses to the state. In the 1990s, the entire shellfish fishery on Georges Bank was closed because monitoring *Alexandrium* blooms was exceedingly difficult because the blooms were occurring frequently and the environmental factors that caused the blooms could not be determined (White *et al.* 1993). Closure of bivalve fisheries can also result in the "halo effect" where consumers change their food preference away from mussels and clams (Bricelj *et*

*al.*1990). Aquaculture facilities, wild harvest fisheries, recreational shellfish harvesters, seafood restaurants, and seafood stores are all affected by these closures. In some areas of the Gulf of Maine, as many as 33 mussel closures have occurred within 17 years (Fig. 1). Therefore, PSTs in shellfish are important to study.

Environmental factors affect PST uptake and depuration rate in shellfish. However, there does not seem to be a relationship between average sea surface temperature and shellfish closure frequency (Fig. 1). There have only been two scallop closures off the coast of Maine from 1996-2013. Scallop closures are rare but can last for months and range over the whole coast of Maine because scallops retain toxin for a long time. With scallop closures, there is usually an exception for harvest of the adductor muscle, which does not become toxic as quickly as the rest of the scallop tissue.

Little is known about how environmental factors affect toxin uptake (Morono *et al.* 2001). PST uptake in shellfish can impact marine ecosystems, human health, and the economy. Marine organisms that prey on shellfish, such as sea stars, fish, and seabirds, may be negatively affected by PSTs (Setala *et al.* 2014). The health of fish and other marine organisms is also important to the economy, as humans may consume these marine organisms from the market. Also, PSTs in shellfish may inhibit the ability of the shellfish to filter the seawater and modify the habitat in the marine ecosystem, which may result in a habitat that is not suitable for themselves or other organisms. For example, if shellfish cannot filter feed, then eutrophication may occur. Also, if shellfish cannot avoid predators, then organisms that live in shellfish beds would migrate to find suitable habitat, and as a result, the predators of the organisms that live in shellfish beds would

prey elsewhere. Therefore, knowing how environmental factors affect toxicity in shellfish is important for human health, marine ecosystems, fisheries, and the economy.

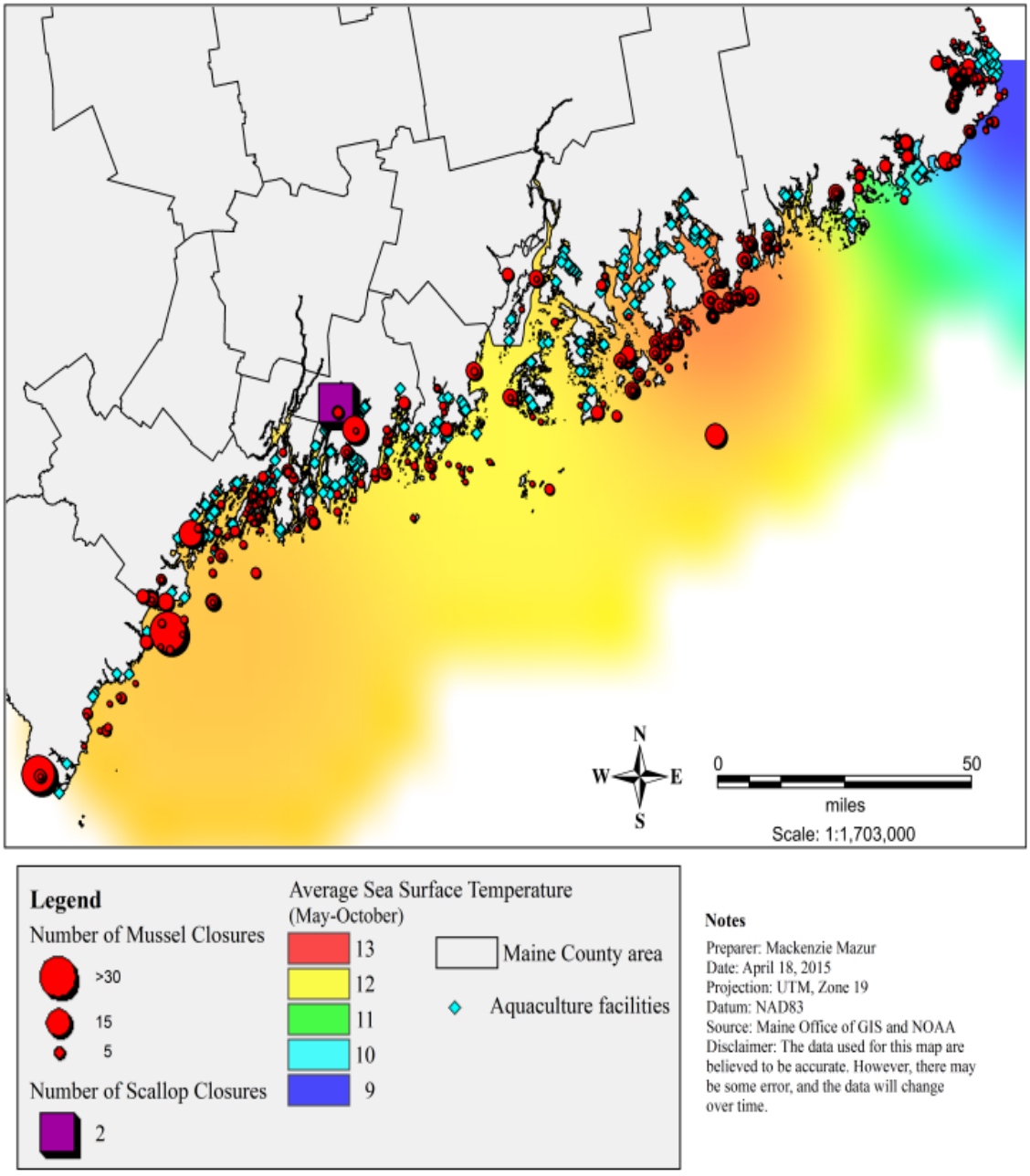

Fig. 1. The number of mussel and scallop closures that have occurred off the coast of Maine from 1996-2013. Shading provides average sea surface temperature from May to October, while the turquoise diamonds indicate the location of aquaculture facilities. Red circles or purple squares represent the center of the closures, and the actual closure covers a wider area.

Effect of temperature on uptake rate

Previous studies have tested the uptake kinetics of PSTs by *M. edulis* (Bricelj *et al.* 1990). *Mytilus edulis* were fed a constant supply of toxic *Alexandrium*, and the mussels reached maximum toxicity levels (0.45 g STX_{eqv} /kg) at twelve to thirteen days, although it took less than an hour for the mussel tissues to reach regulatory levels (8.0 X 10^{-4} g STX_{eqv}/kg).

PST uptake was studied in *Mytilus galloprovincialis,* a species of blue mussel found on the western coast of the United States (Morono *et al.* 2001). They found gonyautoxin (GTX) was the only PST congener that was affected by temperature. The volume-specific toxin concentration of the toxic algae had a significant effect on uptake and thus on the toxicity of the *M. galloprovincialis* tissue. The results may be similar to that of the current study, because PST uptake was studied in another mussel species.

Testing toxin uptake over time at various temperatures is important for monitoring shellfish and marine ecosystems along the coast of Maine. In this experiment, the mussels and scallops were fed *Alexandrium* at 10°C and 15°C. These temperatures represent that of the average Northern and Southern Maine coastal water temperatures in July, which is in the middle of the season for toxic shellfish blooms (March- October) (NOAA 2014). Because metabolic rates, and therefore feeding rates, increase with temperature, PST uptake by *M. edulis* and *P. magellanicus* was expected to increase with temperature.

The hypothesis of my thesis was that PST uptake in *M. edulis* and *P. magellanicus* would increase with temperature. To test the effect of temperature on PST uptake by *M. edulis* and *P. magellanicus*, I conducted an experiment at 10°C and 15°C

with several time points at which shellfish were sampled (n = 9 shellfish/time point). *Alexandrium fundyense* cells and nontoxic algae were fed to the mussels and scallops, and shellfish tissue was tested for toxicity at different time points. I also included control beakers for each temperature in which each species was only fed nontoxic algae.

Methods

Cell Culture

Toxic *Alexandrium fundyense* (CCMP1978) were used in this study. The toxin high-performance liquid chromatography (HPLC) profile had previously been determined by Woods Hole Oceanographic Institute (B. Keefer, personal communication). Algal cultures were grown in one or two L flasks using L-1 medium (Guillard and Hargraves 1993). L-1 medium concentrate (Bigelow Laboratory, Booth Bay Harbor, ME) was mixed with filtered, autoclaved seawater according to manufacturer directions. The seawater was obtained from Lumbol's Hole, Brunswick, ME, the Darling Marine Center (DMC), Walpole, ME, and Kettle Cove (KC), Portland, ME, in September 2014, October 2014, and March 2015 respectively. The flasks were filled half way with medium to provide sufficient airspace for the growing alga and were inoculated with 5-10 mL of stock *A. fundyense* culture at 7.0 X 10^3 cells/mL. The cells were kept in exponential growth by mixing high density (> 4.0 X 10^3 cells/mL) flasks with the same volume of fresh L-1 medium, and then dividing the mixture between two flasks. If fresh L-1 medium was not available, the high density flasks were mixed and divided into two flasks with those of low densities until new L-1 medium was ready. The concentration was determined by fixing an aliquot of the culture in Lugol's solution, placing one mL on a

Sedgewick rafter counter and counting the cells under a light microscope. The cells were counted weekly after initial inoculation.

Collecting blue mussels and sea scallops

In November 2014, 120 *Mytilus edulis* around five centimeters in shell length were collected from the bottom of the dock at the DMC. Rawson *et al.* (2001) found that most of the blue mussels at the DMC were *M. edulis* and not *M. trossulus.* The mussels collected were considered *M. edulis.* In December 2014, 114 *Placopecten magellanicus* of approximately five cm were collected at Pine Point Aquaculture at KC. All of the individual mussels and scallops were selected to minimize size variation and were around five centimeters shell length (Table 1).

Species	**SH (cm)**	**SW (cm)**	**SL (cm)**
Mytilus edulis	1.5 +/- 0.3	3.1 +/- 0.5	5.4 +/- 0.7
Placopecten magellanicus	1.7 +/- 0.3	5.6 +/- 0.7	5.1 +/- 0.6

Table 1. The average shell height, shell width, and shell length and standard deviations in centimeters of *M. edulis* and *P. magellanicus* used in the experiment.

After collection, the shellfish were transported to the DMC where they were held in a flowing seawater tank. Because of the longer distance of transport, *P. magellanicus* was transported on ice with moist paper towels. Half of the shellfish were transported to Orono in January of 2025, and the other half was transported in January of 2015. An

additional set of *P. magellanicus* was transferred from Portland, ME to Orono, ME in March 2015.

Shellfish Acclimation

The shellfish were acclimated so that the metabolic rates of the shellfish could adjust to the new temperature and algal concentration. The temperature of the acclimation tank was recorded from a thermometer each day. *Mytilus edulis* and *P. magellanicus* were acclimated for two weeks in recirculating tanks of 10°C or 15°C at the University of Maine, Orono, ME. When the shellfish were transferred to the 15ºC tank, they were exposed to gradual changes by being placed in buckets of seawater that were replaced daily with water that was approximately 3ºC warmer until acclimation temperature was reached. During this time, the mussels and scallops were fed non-toxic Shellfish Diet (Reed Mariculture) which consists of *Isochrysis, Pavlova, Tetraselmis, Chaetocerous calcitrans, Thalassiosira weissflogii* and *Thalassiosira pseudonana,* at 1.7 X 10^8 cells/shellfish/day.

Experimental Design

Two experiments, one at 10°C and one at 15°C, were conducted for each species to determine the effect of temperature on PST uptake. Both species were fed toxic algae at two temperatures, 10ºC and 15ºC. Individual shellfish were placed into separate beakers, and three sets of three individuals of each species were sampled at different time points (n=9). For each temperature and species, there were six control beakers in which individuals were fed non-toxic algae (2.4 X 10^3 cells/mL of Shellfish Diet). Experimental mussels and scallops were fed *Alexandrium* at a concentration of 100 cells/mL along with

2.4 X 10^3 cells/mL of Shellfish Diet. Water, cooled to the tank temperatures, was changed daily. In a subset of beakers (n=6), *Alexandrium* was counted daily to ensure that shellfish were feeding on *Alexandrium*. *Alexandrium* cells were also counted in beakers holding *M. edulis* during the first three hours of the 15°C experiment. The whole shellfish samples were frozen until toxicity testing.

Toxin Analysis

The toxicity of the shellfish was analyzed following the method of Jellett Rapid Extraction (Jellett Rapid Testing Ltd., Chester Basin, Nova Scotia, Canada). Whole body tissue from three mussels were shucked, rinsed, combined, homogenized, and mixed into the extraction fluid. After filtration, the samples were pipetted onto Jellett PSP testing strips following the manufacture protocol. The Jellett PSP test strips have a limit detection of 2.0 X 10^{-4} – 7.0 X 10^{-4} g STX_{eqv} /kg. To see if toxin concentrated in the digestive gland, the process was repeated with all *M. edulis* except for the digestive gland. Similarly, for scallops, the procedure was applied to *P. magellanicus* without the adductor muscle and *P. magellanicus* adductor muscles alone. The *P. magellanicus* adductor muscles were only tested at zero and 27 hours.

Data Analysis

The results were analyzed and graphed with either scatter plots or bar graphs, and averages and standard deviations were calculated in Excel (Microsoft, Redmond, WA). Error bars were calculated with standard error and the linear trendline was calculated in Excel as well. Statistical significance was determined with a linear regression analysis in Excel. The relationship was considered significant if $P<0.05$.

Results

Temperature over time

Temperature varied over time in the tanks. The temperature was close to the set point during the acclimation period. During acclimation to 10°C, the temperature averaged at 10.5°C (SD +/- 0.90). During acclimation to 15°C, the temperature averaged at 14.5°C (SD +/- 0.25).

Temperature varied more in the 10°C experiment than in the 15°C experiment, and the average temperature during the 10°C and 15°C experiments were 12.2°C (SD +/- 0.88°C) and 14.5°C (SD +/- 0.15°C), respectively (Fig. 2).

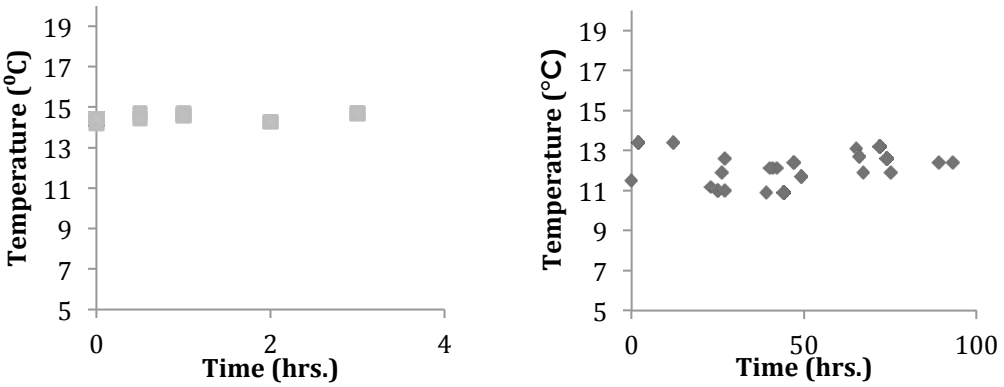

Fig. 2. The temperature in experimental beakers over time. The squares and diamonds represent temperature during the 15^0C and 10^0C respectively.

Alexandrium *consumed over time*

The amount of *Alexandrium* cells consumed over time varied among individual *M. edulis*, especially between one half and one hour. The percent *Alexandrium* consumed increased with time, however, the relationship between percent *Alexandrium* consumed and time was not statistically significant (d.f.=4, P=0.067) (Fig. 3).

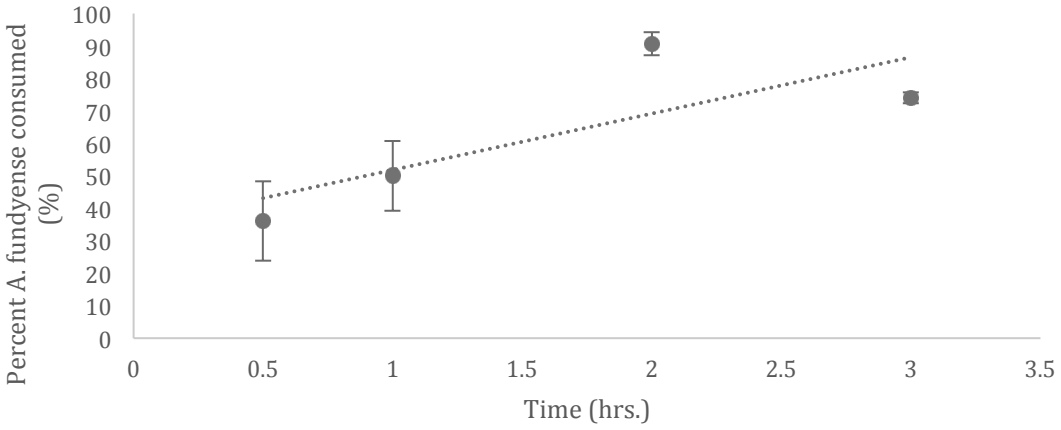

Fig. 3. The percent of *Alexandrium* consumed by *M. edulis* in the experimental beakers at 15°C over time. The points represent the average of the percent consumed, and the error bars were calculated with standard error. The trendline is linear, and the equation is $y = 17.356x + 34.462$, $R^2 = 0.6226$.

Toxicity and temperature

The time at which *M. edulis* became toxic varied. *Mytilus edulis* became toxic more quickly at 10°C than at 15°C. At 10°C, *M. edulis* was not toxic at zero hours but by two hours all samples (whole body) became toxic. At 15°C, *M. edulis* was not toxic at two but at three hours, all samples were toxic.

Most of the PST did not distribute from the digestive gland in *M. edulis* until 25 hours. Whole shellfish tissue analysis showed that *M. edulis* became toxic after three hours of exposure. When the digestive gland was removed during toxicity testing of *M. edulis*, the rest of the tissue was not toxic until 25 hours (Fig. 4).

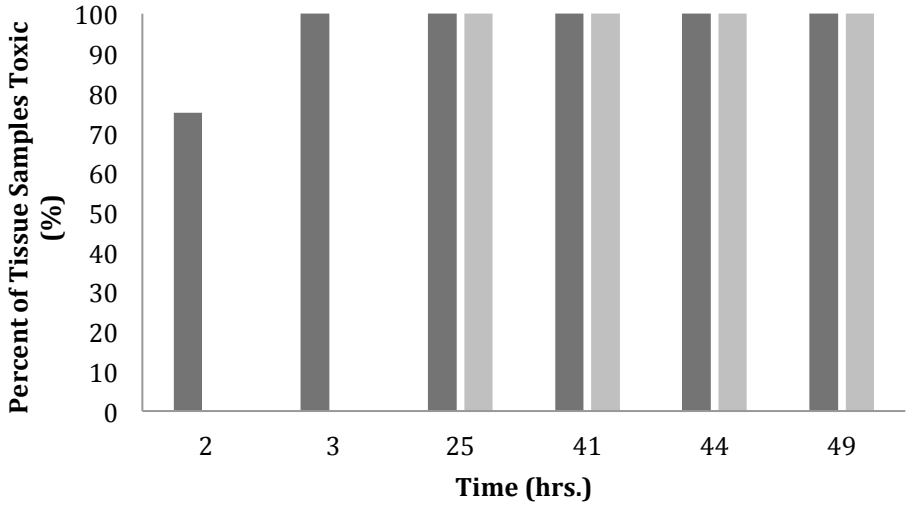

Fig. 4. The percent of *M. edulis* tissue toxic over time. The black bars represent the percent of whole *M. edulis* tissue that was toxic, and the grey bars represent the percent of *M. edulis* tissue, without the digestive gland, that was toxic.

At both temperatures, all but one of the *P. magellanicus* tissue samples, minus the adductor muscle was toxic at all time points including pre-exposure to *Alexandrium.* The *P. magellanicus* adductor muscle alone was not toxic at either zero or 27 hours of exposure to *Alexandrium.* Overall, the mussel and scallop PST uptake relationship with temperature was unclear. However, different types of tissues vary in toxicity, and different mussels vary in cell uptake.

Discussion

Expected results based on the literature of metabolic rates

Because of increased metabolic rate with temperature, clearance rate in shellfish generally increases with temperature. In the Pacific oyster, *Crassostrea gigas,* clearance rate increased with temperature until a critical point of 19°C (Bougrier *et al.* 1995). However, this may not be the case when shellfish are exposed to toxins or contaminants,

because shellfish feeding behavior may change. A previous study on trace metal uptake showed that the concentration of trace metals in *Mytilus galloprovincialis* tissue increased with temperature (Kamel *et al.* 2014). Domoic acid uptake by oysters (*Crassostrea virginica)* increased with temperature (Mafra *et al.* 2010). Based on these results, the relationship between temperature and uptake rate is positive.

Role of temperature in shellfish toxicity

Due to small sample size, previous natural HABs, and difference in cell uptake among individual shellfish, the relationship of PST uptake in *M. edulis* or in *P. magellanicus* with temperature was unclear. In my experiment, *M. edulis* became toxic more quickly at 10°C, but the small sample size, due to the error in the experimental set-up, provided results that were inconclusive. Also, the temperatures during the experiment were not consistent. Therefore, the difference in temperature between the two experiments (10°C and 15°C) was usually less than 5°C. The amount of *Alexandrium* consumed over time was also not significant, which may suggest that multiple factors affect PST uptake. The variance in the amount of *Alexandrium* consumed suggests that individual shellfish differ in cell uptake rate, especially in short time frames. When placed into beakers, individual shellfish took different amounts of time to adjust to their new environment and to start feeding.

The relationship between PST uptake and temperature in *P. magellanicus* could not be determined either, because the *P. magellanicus* were already toxic at the start of the experiment. The *P. magellanicus* must have been toxic from the last HAB that caused a shellfish closure in the KC area in August of 2014. Scallops can retain toxins for

several months to years, whereas mussels depurate in less than a month. Depuration in shellfish consists of two stages: a rapid loss during the first day and a secondary slower loss. More toxins are lost during the first day in scallops than in mussels. However, more toxins are lost in mussels during the secondary slower loss than in scallops, which causes mussels to depurate more rapidly overall (Choi *et al.* 2003).

Toxicity levels

In this study, *M. edulis* became toxic sooner than expected. Several years ago, a similar experiment was performed in SMS 304, an integrative marine science class at the University of Maine. Mussels were fed *Alexandrium* and became toxic by four days. In that study the shellfish were fed only *Alexandrium* cells and no other algae, therefore it was presumed that they may not have fed as vigorously in the shorter time frame because there were no other algal cells to stimulate feeding. *Alexandrium* toxicity depends on the nutritional status, growth phase, and environmental conditions (Anderson *et al.* 1994). In the current study, a specific combination of these factors could have caused highly toxic *Alexandrium* cells. *Mytilus edulis* also consumed the *Alexandrium* cells rapidly. The mussels consumed 90% of the algae within two hours during the 15°C experiment, and if the feeding rate was similar at the 10°C experiment, the short time in which the mussels became toxic is expected.

Difference in toxicity among species

The scallop tissue without the adductor muscle was toxic even before exposure to *Alexandrium* in this experiment, which means that the tissue was toxic from the last natural HAB at KC. The last PSP HAB event at this location was in August of 2014

(Maine DMR), seven months before the scallops in my study were tested for toxicity. All of the scallops, except one sample, tested positive at the experimental time zero point. This suggests that *P. magellanicus* can retain PST in tissue other than the adductor muscle for at least seven months.

In my study, the adductor muscle in *P. magellanicus* took longer than the rest of the tissue to become toxic. Other studies have also found that the adductor muscle has low toxin content. For example, in 2001, Bauder *et al.* found that diarrhetic shellfish toxin was low in the adductor muscle in the bay scallop (*Argopecten irradians)*. This is one reason why the adductor muscle is usually the only tissue that is put on the market. The rest of the tissue also consists of a texture disliked by most people, and people prefer the taste of the adductor muscle.

The ability of *P. magellanicus* to retain PSTs for extended periods of time raises public health concerns. In many cultures, people consume other tissues along with the adductor muscle in scallops. However, whole scallops should definitely not be served in areas with frequent HABs. If the whole scallop is sold, then toxicity testing should be done before the scallop tissue is put on the market.

Mytilus edulis consumed *Alexandrium* cells rapidly. Although the relationship between percent *Alexandrium* consumed and time was not significant, the mussels ate 90% of the *Alexandrium* cells within two hours. However, *M. edulis* do not retain PSTs for as long as scallops; mussels depurate in less than a month (Choi *et al.* 2003). Diarrhetic shellfish toxins are rapidly released from scallop tissue, because the toxins are not bound tightly to the tissue (Bauder *et al.* 2001). Although diarrhetic shellfish toxins

and paralytic shellfish toxins are different, this finding could give insight into the difference in PST binding to tissues of different shellfish species. Perhaps, PSTs bind tighter to *P. magellanicus* tissue than *M. edulis* tissue.

Various tissues become toxic at different rates (Bricelj *et al.* 1990). In my study, most of the toxin accumulated in the digestive gland in *M. edulis*, and after several hours, the toxins distributed to other tissues. The majority of the PSTs did not leave the digestive gland for 25 hours. *Mytilus edulis* have low absorption rates for PSTs (Shumway *et al.* 1985). This could explain why the PSTs were not detected in other tissues aside from the digestive gland until after 25 hours.

Future Experiments

In this experiment the hypothesis was not confirmed, but modifications in future experiments could sufficiently test the hypothesis. For future experiments, a larger experimental sample size is recommended, in order to achieve accurate results. Several parameters were associated with experimental sample size: 1) number of shellfish, 2) amount of algae, and 3) the experimental tanks. Both a larger number of *M. edulis* and *P. magellanicus* should be collected and a larger amount of *Alexandrium* should be available. The reason that there was not enough *Alexandrium* during the 15°C experiment in November 2014 was because some of the *Alexandrium* cultures unexpectedly died. A holding tank closed off to other animals would also be beneficial. In this study, the shellfish were held in a holding tank at the DMC in between collection and acclimation. When the shellfish were collected for acclimation, seven of the scallops were missing, probably due to green crab predation in the hatchery. Limited use of buckets is suggested

as well. In this experiment, when acclimating the shellfish from 12°C to 15°C, the shellfish were placed in a bucket of intermediate temperature (~13.5°C), so that the temperature change would not be too drastic. An aerator was placed in the buckets to provide dissolved oxygen. However, almost all of the scallops died (n~40), possibly because ammonia accumulated in the holding water. Scallops are sensitive to stress; they should be handled with extra care, and water quality should be monitored at each step. Scallops should always have a supply of dissolved oxygen, and if the scallops need to be held in buckets, the water should be replaced regularly to keep ammonia concentration from becoming too high.

Multiple test runs (at least three) are also recommended. With multiple test runs, the time points will more likely fall in a time range in which the shellfish become toxic. In this study, only one test run was performed. In the test run, *P. magellanicus* was toxic at both four and six days, and *M. edulis* was toxic at four days but not toxic at two days of exposure to *Alexandrium.* Time points were set from two to four days for the shellfish in this experiments based on the one set of tests. However, the results show that the shellfish actually became toxic much sooner than two to four days, and the non-toxic *M. edulis* sample at two days during the test run was an oddity. Therefore, the shellfish, time, and *Alexandrium* used for the long time points did not need to be used. With multiple test runs, a better sense of when the shellfish become toxic would be known beforehand, and shellfish, time, and *Alexandrium* cultures would have been saved.

The experimental set-up of the current study was also somewhat flawed. In future experiments, I would recommend limited use of beakers. The beakers do not represent the natural environment. Also, when *M. edulis* was placed into the beakers, many of the

mussels spawned (n=9). A rise in temperature is a cue for spawning in mussels; however, there was no change in the water temperature between the tank and the beakers. Mussels will also spawn when exposed to environmental stress, in order to release their gametes before their anticipated death. The handling involved when transporting *M. edulis* from the tank to the beakers may have been a source of environmental stress. *Alexandrium* could have been the source of environmental stress because of its toxicity, but this is not likely, because mussels that were not exposed to *Alexandrium* spawned in the beakers as well (n=2). Also, when using the beakers, the *Alexandrium* and Shellfish Diet were placed into the beakers all at once. This method did not replicate the natural environment. Although difficult to set up and maintain, a better design would consist of the shellfish in a tank with a pump providing a more continuous supply algae (MacQuarrie 2002). This experimental set-up seems best for a laboratory setting in that it replicates the natural environment as much as possible.

Varying *Alexandrium* concentration and temperature is recommended to increase the likelihood in determining a difference between PST uptake among different temperatures. With multiple temperatures at greater differences and a lower *Alexandrium* concentration (<100 cell/mL), the results may provide a relationship between PST uptake and temperature. The lower *Alexandrium* concentration will keep shellfish from becoming toxic too quickly, and the temperatures with greater differences will hypothetically provide PST uptakes with greater differences.

In future studies, other methods of toxicity testing are recommended. In my study, the Jellett test strips only provided a qualitative result: a positive or negative value for toxicity. Jellett test strips are a less expensive and easier option to use. With more

funding, different types of toxicity testing can be used. In many studies and monitoring programs, mouse bioassays are used to provide quantitative results. In the mouse bioassay method, a shellfish tissue extract is injected into a live mouse. The amount of PST in the sample is calculated using the time it takes for the mouse to die. However, this method is expensive, time-consuming, and requires a considerable amount of live mice. Using mice also raises ethical concerns. A method that provides more quantitative data is HPLC. In HPLC, the shellfish tissue extract is injected into a solvent reservoir. Then, a detector will provide different light wave lengths or the absorption of the shellfish extract. Based on the absorption values, one can find how much PST is in the shellfish tissue (Gago Martinez 2003). If a quantitative method were used to test *P. magellanicus* for toxicity, PST uptake could be determined, because a PST concentration would be provided. Quantitative results would also provide more of an insight into how and at what rate PSTs distribute throughout shellfish tissue.

This study also raised other interesting topics of study for future experiments. Testing the toxicity of the *P. magellanicus* adductor muscle and the *M. edulis* tissue without the digestive gland provided interesting results. Testing more tissue types for toxicity is recommended, as it will provide more of an insight into how PSTs are distributed throughout shellfish tissue and which tissues should be put on the market for human consumption. Observation of shell valves and measuring pseudo-feces and clearance rate is also recommended to see how PST affects shellfish behavior. With increased PST concentrations, bivalves have a high production of pseudo-feces, close their shell valves, and have lower clearance rates (Shumway and Cucci 1987). Shell valves may be closed more often when shellfish are exposed to *Alexandrium*, because the

shellfish may feed less often. Pseudo-feces production may increase when exposed to *Alexandrium,* because the shellfish may be trying to rid their bodies of *Alexandrium* cells. To measure pseudo-feces, the protocol in Shumway and Cucci's experiment in 1987 could be followed. Individual shellfish could be placed in bell jars in the tanks with the algal mixture and an aerator. After an hour, the pseudo-feces could be collected with a pipette (Shumway and Cucci 1987). Also, clearance rate may decrease, because shellfish may be trying to avoid *Alexandrium* consumption. In order to provide a comparison, these measurements and observations should be collected for shellfish both exposed and not exposed to *Alexandrium.*

Climate Change

Seasons are not the only cause of changing temperatures. The sea surface temperature in the Gulf of Maine is increasing around 1°C every one hundred years because of climate change (Shearman and Lentz 2010). Therefore, if PST uptake is affected by temperature, then climate change may affect the PST uptake in shellfish. Climate change seems to affect the frequency, duration, and range of HABs, but scientists are unsure as to how. Temperature does affect plankton growth and ocean stratification, and therefore the nutrient availability. Higher sea surface temperatures may also result in wider geographical ranges of toxic algae species, as some species, such as *Alexandrium catanella* grow faster in higher water temperatures (Moore *et al.* 2008). Other studies suggest that PST outbreaks will occur in earlier months during the year because of climate change (Joo *et al.* 2015). These studies suggest that climate change will have an effect on PSTs in shellfish, so more research on temperature and PSTs in shellfish is recommended.

Policy Implications

Currently, human health is well protected by resources managers through shellfish closures. In the last thirty years, there have only been three cases of PSP from shellfish off the coast of Maine. None of the cases were from shellfish that were sold on the market. In all three cases, fishermen consumed shellfish that they found themselves (Maine DMR).

The next step is to make the shellfish closures more efficient without putting human health in danger. This way, the fishery could reduce economic losses during the primary HAB season. To make the shellfish closures more efficient, more research needs to be done on the depuration rate of shellfish. With more research, the amount of time that the shellfish depurates after a HAB will be better estimated, resulting in more efficient shellfish closures. Efficient shellfish closures will not only help fisheries and aquaculture facilities, but it will also help grocery stores and seafood restaurants maximize profit, because they would be able to serve shellfish sooner. Efficient shellfish closures will also lessen the "Halo" effect, where the public is hesitant to buy any shellfish, because they heard about a shellfish closure on the news. With shorter closures, this effect will be diminished.

Conclusion

The relationship between temperature and PST uptake in *P. magellanicus* and *M.edulis* could not be determined due to small sample size, previous natural HABs, and differing cell uptake among individual shellfish. *Mytilus edulis* probably became toxic within two to three hours because of the nutritional status, growth phase, and the

environmental conditions of the *Alexandrium. Placopecten magellanicus* retains toxin for a longer time than *M. edulis,* probably because PSTs bind tighter to scallop tissue than to mussel tissue. In *M. edulis,* the digestive gland retained most of the PSTs when first feeding on toxic algae, probably because *M. edulis* was experiencing low absorption rate. Also, the adductor muscle in *P. magellanicus* takes longer to become toxic than the rest of the scallop tissue. Future experiments with a new experimental design and a larger sample size are recommended, because climate change may have an effect of PSTs and shellfish. Experiments on shellfish depuration rate are also recommended, because efficient shellfish closures are important to the economy.

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Author's Biography

Mackenzie Mazur was born in Halifax, Nova Scotia, Canada, and grew up in Northbridge, Massachusetts. She graduated from Nipmuc High School in Upton, Massachusetts in 2011. She is a member of the International Golden Key and Phi Kappa Phi Honor Societies. She attends college at the University of Maine and majors in marine science with a concentration in marine biology and with fisheries minor. She plans to graduate in May of 2015. She enjoys tutoring, teaching, and SCUBA diving in her spare time. After graduation in 2015, Mackenzie is attending graduate school at the University of Maine for a dual masters in marine science and policy.