

Wellesley College Wellesley College Digital Scholarship and Archive

Student Library Research Awards

Archives

2017

Investigating the antibacterial properties of mollusks native to the Boston Harbor and Wood Neck Beach: developing a comparative study between *Mytilus edulis* and *Ensis directus*

Nasreen Al-Qadi
nalqadi@wellesley.edu

Follow this and additional works at: http://repository.wellesley.edu/library_awards

Recommended Citation

Al-Qadi, Nasreen, "Investigating the antibacterial properties of mollusks native to the Boston Harbor and Wood Neck Beach: developing a comparative study between *Mytilus edulis* and *Ensis directus*" (2017). *Student Library Research Awards*. 22.
http://repository.wellesley.edu/library_awards/22

This Article is brought to you for free and open access by the Archives at Wellesley College Digital Scholarship and Archive. It has been accepted for inclusion in Student Library Research Awards by an authorized administrator of Wellesley College Digital Scholarship and Archive. For more information, please contact ir@wellesley.edu.

Nasreen Al-Qadi
Marine Biology Final Paper
Dec. 12th 2016

Investigating the antibacterial properties of mollusks native to the Boston Harbor and Wood Neck Beach: developing a comparative study between *Mytilus edulis* and *Ensis directus*

Abstract

The level of biodiversity in the oceans is unmatched by any other ecosystem on earth. Despite this, scientists are only now beginning to explore this untapped center of organismal activity, which offers countless human benefits and gives us insight into the origins of life. A recent discovery has found that many marine mollusks have intrinsic antibacterial properties that allow them to fend against the many pathogens in their environments. This paper aims to compare the antibacterial properties of two New England marine mollusks, *Mytilus edulis* and *Ensis directus* at the Boston Harbor and Wood Neck Beach. This study outlines an experimental procedure that will give us insight into the pathogens that each organism wards against. I also look at sequencing the RNA of each, to identify some previously-characterized genetic markers that indicate antibacterial function. *Ensis directus* lives buried in the sand and is therefore expected to be better equipped to fend against anaerobic bacteria. *Mytilus edulis*, however, will target seawater pathogens as they live in the shallow waters of the intertidal zone. Previous studies indicate that the *Mytilus edulis* should contain MytM peptide precursors, which have been linked to antibacterial activity. Similarly, a close relative of *Ensis directus* has been found to have ILP genes signifying antibacterial ability. Both of the organisms will be found to have high concentrations of the antibacterial lysosomes in their gills, as this structure is constantly exposed to environmental pathogens.

Introduction

The marine environment is rich in organisms, with an unmatched level of biodiversity. Marine species comprise almost half of all the biodiversity around the globe and the oceans correspond to about 99% of the biosphere (Benchley 1995). Despite this, the marine ecosystem has only recently been explored and new, previously uncharacterized bioactive compounds are being discovered, particularly compounds that provide organisms with unique antimicrobial and

antifungal properties. These discoveries are only now happening partly due to past difficulties with collecting marine organisms and culturing marine bacteria (Aneiros and Garateix 2004).

Marine organisms, such as mollusks, fish and algae, have developed antimicrobial capacities due to their harsh environments, which leave them exposed to harmful bacteria, fungus and viruses (Daczkowska-Kozon and Sun Pan 2016). Many of these bioactive products are structurally unique to marine organisms, and not found in terrestrial samples (Arizza 2013).

These antimicrobial properties have been explored mostly by marine chemists working alongside pharmacists to develop new drugs that utilize these natural properties for human benefit. Pioneering studies began in the late 1940s, when scientists demonstrated that marine microorganisms are a viable sources of useful bioactive compounds, as they produce antimicrobial agents (Rosenfeld and Zobell 1947). To overcome the difficulties of isolating marine bacteria in cultures, scientists at the Scripps Institution of Oceanography developed a technique to isolate marine bacteria successfully in the early 1990s (Carte 1996). This work, coupled with the advances in genetic engineering that continues today, meant marine organisms were more thoroughly explored and their potential for human utilization realized more fully.

Recent studies continue to show that compounds extracted from mollusks show signs of antimicrobial properties. The origin of these properties is still under investigation. Studies have linked the antimicrobial properties to lysosomes, which are present in virtually all members of the animal kingdom, but are specialized for microbial defense in mollusks and other invertebrates (Michiels and Callewaert 2010). Furthermore, a variety of peptides, including one named mytimycin, have been characterized as being responsible for antifungal and antibacterial properties of marine organisms (Sonthi et al. 2011).

In this study, I investigate the antibacterial properties of mollusks native to the Boston Harbor and Wood Neck Beach, specifically *Mytilus edulis* and *Ensis directus*. I hypothesize that both organisms will exhibit similar levels of antibacterial properties due to their pathogen-filled environments. However, their antibacterial properties will differ in terms of which microbes they defend against depending on their environment. I will investigate this topic through the lens of genetics, as well as take a more practical approach using bacterial assays. *Ensis directus* is expected to better defend against anaerobic bacteria found in its benthic environment, whereas *Mytilus edulis* will defend against water-borne bacteria. Furthermore, *Mytilus edulis* is expected to have two MytM peptide precursors, identical to those found in Mediterranean blue mussels and *Ensis directus* should contain two ILP genes. Both organisms will have heightened gene expression for antibacterial-linked peptides in the gills, as well as higher concentrations of antibacterial lysosomes. Furthermore, organisms from both sampling locations will exhibit similar antibacterial properties, as both locations have maintained similar levels of pollution in recent years.

Methodology (Figure 1)

Sampling and Extraction

Live specimens of *Mytilus edulis* and *Ensis directus* will be collected from Wood Neck Beach in Woods Hole, MA (41.5749° N, 70.6414° W) and Carson Beach at the Boston Harbor (42.3261° N, 71.0475° W). Specimens will be located both under the sediment on the beach and in the salt marsh, as well as adhered-to rocks in the intertidal zone. They will be brought back to the lab and extracts obtained. To obtain the extract, the shells will be opened and bodies

removed. The bodies will then be separated into eight categories so that each group of tissues can be tested on separately:

Sampling Location	Organism	Group	Organs
Carson Beach	<i>Mytilus edulis</i>	A	Gills, foot, muscle, ligament
		B	Mouth, stomach
	<i>Ensis directus</i>	A	Gills, foot, muscle, ligament
		B	Mouth, stomach
Wood Neck Beach	<i>Mytilus edulis</i>	A	Gills, foot, muscle, ligament
		B	Mouth, stomach
	<i>Ensis directus</i>	A	Gills, foot, muscle, ligament
		B	Mouth, stomach

The extracts will be isolated and concentrated using the methods of Karthikeyan et al. (2014).

Anti-bacterial Properties

A bacterial assay of *V. splendidus*, *S. colwelliana*, *C. botulinum*, *E. tarda* and *R. equi* will be tested against Groups A and B of *Mytilus edulis* and *Ensis directus* extracts from each

sampling location. Bacterial samples will be placed on an agar plate and incubated at 30°C for 3 days with each extract. The zone of inhibition will be measured.

GC/MS Analysis

To identify potential bioactive compounds, *Mytilus edulis* and *Ensis directus* extracts will be run through GC/MS analysis. The peaks will allow me to identify the compounds based on molecular weight and fragmentation patterns.

BLAST Search

Previous studies have classified the Phylum Mollusca as containing g-type and i-type lysozymes using a BLAST test, which searches for the amino acid sequence pertaining to those types (Michiels and Callewaert 2010). However, BLAST tests will be conducted in this case to compare our Groups A and B and to survey for i-type and g-type lysozyme homologues.

RNA Extraction

In order to sequence amino acids, RNA must be extracted and purified. RNA will be isolated from both Groups A and B for both *Mytilus edulis* and *Ensis directus* at each sampling location using the techniques described in Cantet et al. (2012).

Results and Discussion

Genetic markers of antimicrobial properties

Mytimycin

Studies have investigated the genetic sequence of the Mediterranean blue mussel, *Mytilus galloprovincialis*, a close relative of *Mytilus edulis*. Sequencing shows evidence of mytimycin (MytM), an antifungal peptide. This amino acid sequence was found in blue mussel samples from three different locations: Venice (Italy), Vigo (Spain) and Palavas (France) (Sonthi et al. 2011) (Table 1).

However, Cantet et al. (2012) have also found that the MytM peptide is only expressed in 42% of hemocytes in the Mediterranean blue mussel. Furthermore, there was a lot of variation among the three mussel samples in terms of the amino acid sequence of their MytM gene. The authors hypothesize that due to the locational differences, the mussels face different pathogens, forcing the MytM gene to diversify. Another study, Costa et al. (2009), reports amino acid sequence variation of the MytM gene within any single mussel. However, they did find two MytM sequences that were identical in all samples.

I can extend the results of this study to our organism, *Mytilus edulis*. I can expect to see a great deal of variation in the amino acid sequence of the MytM peptide, but would expect to find the two MytM precursors that have identical sequencing between all mussels surveyed at both Carson Beach and Wood Neck Beach. However, it is also important to investigate the precise contribution of the MytM gene in different tissues, due to the variation amongst hemocytes.

Separating the mussel's organs into two groups, and extracting RNA from each, allows me to sample two different parts of the organism and characterize the MytM precursor for both.

In terms of *Ensis directus*, a different set of genes has been identified and linked to their antimicrobial abilities. Niu et al. (2016) have identified an insulin-like peptide (ILP) in the razor clam, *Sinonovacula constricta*, a close relative to *Ensis directus*. The study identifies two ILP genes that are predominantly expressed in the liver, hemocytes and mantle tissues (Figure 2). These structures are in constant contact with the outside environment, which suggests that the ILPs might contribute to the clam's antimicrobial properties. I should see a reflection of these data when I look at the amino acid sequence of *Ensis directus* - with two ILP genes, expressed more in Group A, which contains the gills, as they are involved in filtering and thus rely more heavily on antimicrobial defense.

Lysosomes

In addition to the MytM and ILP genes, lysosomes have also been identified as contributors to mollusks' antibacterial properties. Michiels and Callewaert (2010) have found that lysosomes defend against both gram-positive and gram-negative bacteria. The study also performed a BLAST test on the lysosomes and characterized them as either g-type and/or i-type (Figure 3). The lysosomes responsible for antibacterial defense were expressed in tissues and body fluids exposed to the environment, mirroring the MytM and ILP gene expression.

These findings can be extended to both *Mytilus edulis* and *Ensis directus*, as the results pertain to the entire Phylum. The study notes that further investigation is needed to determine what tissues are responsible for antibacterial lysosome production. Therefore, I can test our two groups for each species at each sampling location, and determine what lysosomes are expressed

in each of the groups. I would expect to find the highest concentration of antibacterial lysosomes in Group A, which contains the gills, as was found in the scallop *Chlamys farreri* (Michiels and Callewaert 2010).

Bacterial and Fungal Defense

These differences in microbiological properties between *Mytilus edulis* and *Ensis directus* will also translate macroscopically, as they will display different bacterial assays depending on the pathogens they are exposed to in their natural environments.

Mytilus edulis

These mussels are found in the shallow waters of the intertidal zone, adhered to rocks. Studies have documented a variety of bacteria present in the coastal waters around New England.

Marcelino et al. (2006) reported species of *Vibrio* bacteria, notably *V. splendidus* and *V. alginolyticus* (Figure 4). I predict to see large a large Zone of Inhibition (ZOI) in both cases (Table 2). Another study by Romalde and Barja (2010) reported the presence of the probiotic, *Shewanella colwelliana*, which helps adhere larval mollusks to surfaces. I hypothesize that *Mytilus edulis* will not contain a defense mechanism against this strand, as it provides a benefit to the organism at some point during its lifecycle (Table 2).

Ensis directus

These organisms live most of their lives burrowed into beach sand, around 1-4 feet underground. This introduces a host of bacteria that thrive in anaerobic conditions. It has been found that bacteria from the Genus *Clostridium*, such as *C. botulinum*, can be transmitted to

humans via shellfish consumption (Thompson et al. 2005). This bacterium thrives in low-oxygen conditions. I can therefore predict that *Ensis directus* will not form a ZOI around this bacterium, as it freely ingests it, but rather has internal defenses controlled by the genetic mechanisms discussed earlier (Table 2). However, there are other bacteria that are present in seawater, but do not transmit to shellfish, such as *Edwardsiella tarda* (Thompson et al. 2005). I can therefore make the prediction that *Ensis directus* will form a large ZOI when exposed to the bacteria (Table 2).

Both *Ensis directus* and *Mytilus edulis* are predicted to not form a ZOI around *Rhodococcus equi*. This is because *R. equi* is not present in marine environments, and is rather transmitted through dry sand and soil (Thompson et al. 2005) (Table 2).

Wood Neck Beach versus Boston Harbor

Both Wood Neck Beach and Carson Beach have met Massachusetts bathing beach regulations of fewer than 104 colony forming units (CFU) of Enterococci per 100 ml of water in recent years (Halliday n.d.). Despite this, both have had recent peaks of up to 35 CFU/100 mL after storm events (*Beach Detail Lookup* 2016). We would not expect to see dramatic differences in the bacterial and fungal defenses of the *Mytilus edulis* and *Ensis directus* at either location due to the similarities in environmental conditions.

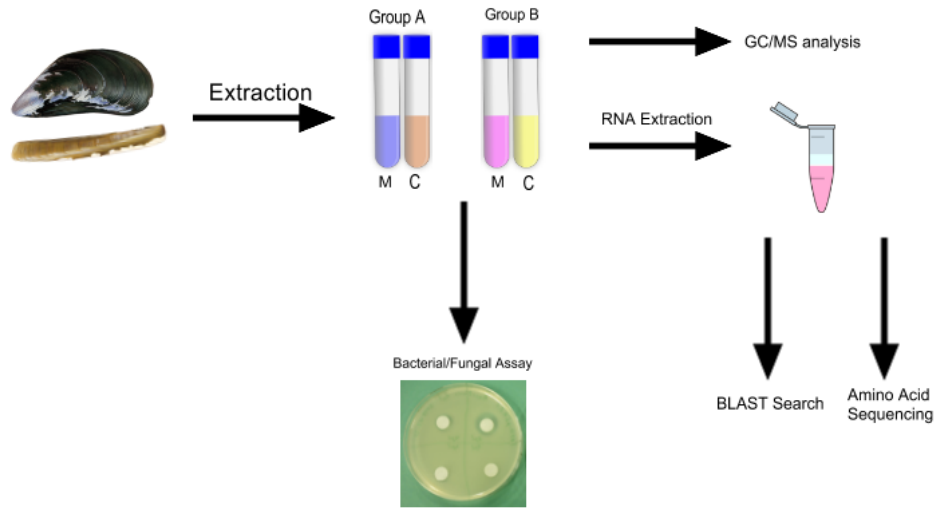


Figure 1: Experimental set-up and methodology, beginning with extraction of organic material from collected specimens and ending with bacterial assay, GC/MS and genetic sequencing.

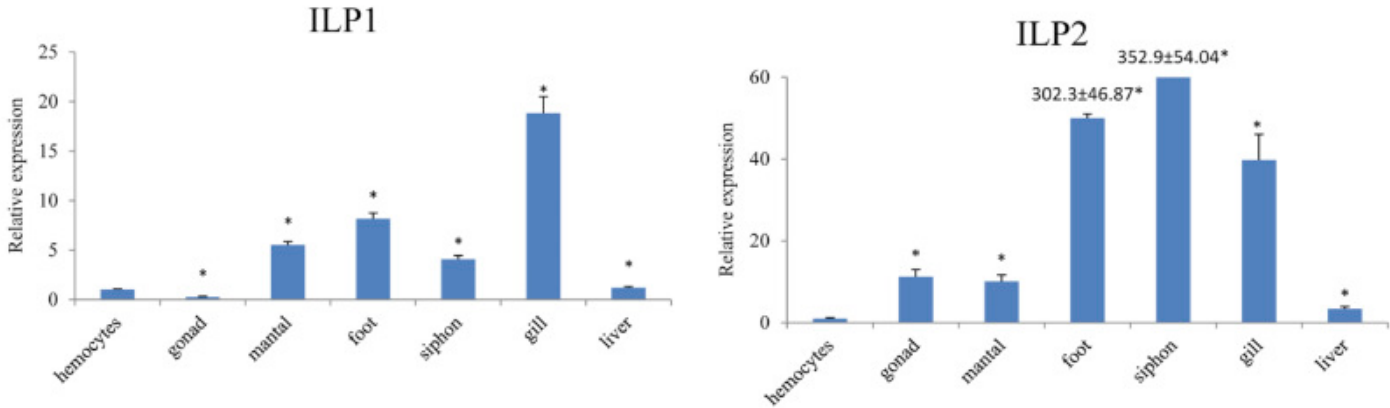


Figure 2: Graph from Niu et al. (2016) showing elevated levels of insulin-like peptide (ILP) expression in the gill, siphon and foot of the razor clam.

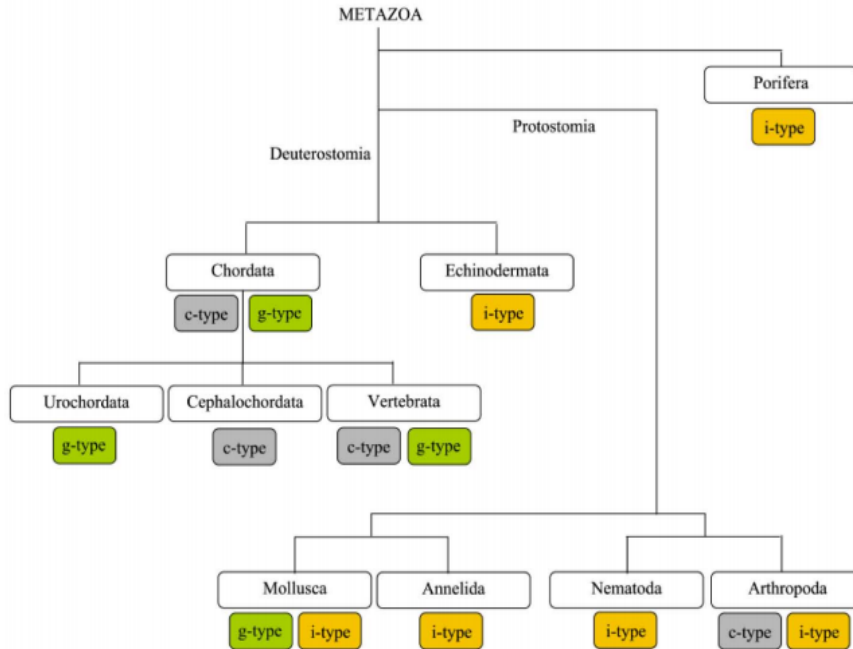


Figure 3: Chart from Michiels and Callewaert (2010) depicting the results of a BLAST test, which categorized the lysosomes of the animal kingdom as either g-type and/or i-type. Mollusca have g-type and i-type lysosomes.

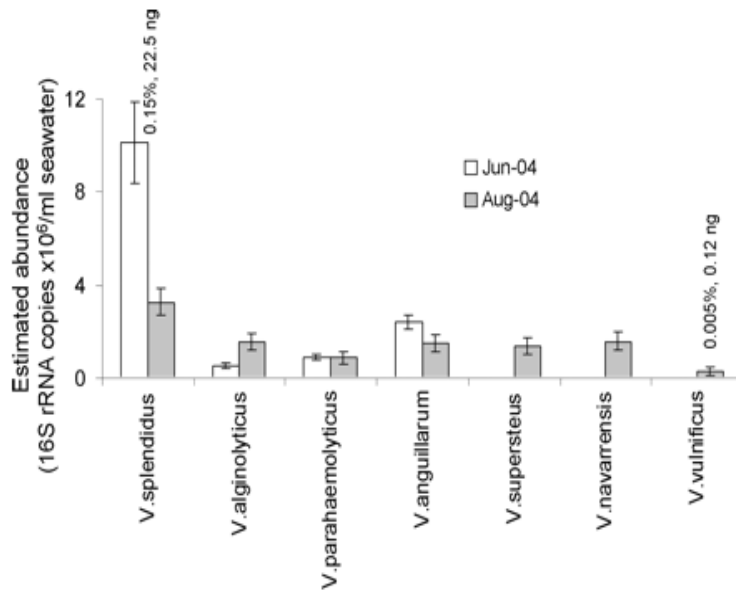


Figure 4: Results from Marcelino et al. (2006) showing common Vibrio bacteria present in the coastal waters around New England.

Conclusion

This study has suggested that *Mytilus edulis* and *Ensis directus* have adapted to a life full of pathogens in very distinguished ways. In terms of genetic sequencing, blue mussels rely on MytM peptides, although the sequence is variable and dependent on the mussel's particular environment. I would still expect to at least see two MytM precursors after sequencing the extracted amino acids in our *Mytilus edulis*.

Similarly, razor clams also have been found to have particular genes responsible for antibacterial defense, or ILPs. These genes are found throughout the organism and have been tied to their antimicrobial abilities. I would expect to find evidence of these ILPs in our amino acid sequence for *Ensis directus*.

Despite these differences, there are some similarities between the two species. Firstly, the gills are most exposed to the outside environment, and therefore I would expect to see heightened gene expression for MytM and ILP, as well as the highest concentration of antibacterial lysosomes in Group A, which contains the gills.

In terms of antibacterial defenses, both *Ensis directus* and *Mytilus edulis* are predicted to have defensive properties against bacteria that are present in both marine waters and sands. However, they will also have unique adaptations against bacteria specific to their environments. *Ensis directus* will show antibacterial properties towards anaerobic bacteria in particular, such as *E. tarda*. On the other hand, *Mytilus edulis* will defend against bacteria such as those from the genus *Vibrio*. It also must be noted that a ZOI is not the best indicator of whether or not a

mollusk has a defense mechanism against a particular bacterium. This is because the mollusk may not actively ward off the bacteria by forming a ZOI and still not be affected by the bacteria after ingestion; such is the case with *C. botulinum*, which is transmitted to humans through shellfish.

In terms of the differences between the specimens of our two sampling sites, we expect to see little variation, as there are no specific differences in bacteria populations, specifically in Enterococci, between Carson Beach at the Boston Harbor and Wood Neck Beach.

The broader implications of this study include both human influence and climate change. Humans are affecting the habitats of mollusks by introducing invasive bacterial species, for which the organism has no natural defenses. Ballast water is one cause associated with this unnatural spreading. This includes the spreading of bacteria that causes cholera in Chesapeake Bay, where an estimated ten billion liters of foreign ballast water is dumped each year, contaminated with around 8.3×10^8 bacteria per liter of ballast water (Ruiz et al. 2000). Current studies are working towards classifying the pathogens present in ballast water in order to further understand the implications it will have on the ecosystem, as previous research has mainly focused on larger invasive organisms. However, microorganisms may have an even larger impact than their macroscopic counterparts as they have high rates of asexual reproduction, can tolerate wide environmental conditions, and can rest in dormancy if conditions are not ideal before invasion. Furthermore, as mollusks are filter feeders, they are heavily exposed to such microorganisms. I predict that with such a volume of pathogens being introduced, there will be large economic, social and environmental impacts, including those on human health, the productivity of fisheries and the health of bivalves. Studies have shown an increase in disease

among many marine taxa, including mollusks, which have a hypothesized association with ballast water (Pagenkopp Lohan et al. 2016). Furthermore, it has been shown that this process of infection is accelerated by climate change. In a recent study, *Perna viridis* mussels showed increased loads of pathogens and higher mortality rates when exposed to warming and hypo salinity compared to control groups, which indicates direct negative effects on the mussels' defense mechanisms (Turner et al. 2016).

Current work that is being done to limit the distribution of invasive pathogens includes a regulation instituted in 2013 by Congress that requires all ships with ballast water capacities of 1,500 to 5,000 cubic meters to treat their water before releasing it back into the ocean (Begich 2013). However, much more research and work must be done to not only regulate the spreading, but also to investigate the implications the large scale introduction of pathogens will have on our coastal ecology and beyond.

Literature Cited

- Aneiros, A., and A. Garateix. 2004. Bioactive peptides from marine sources: Pharmacological properties and isolation procedures. *Journal of Chromatography B* 803:41–53.
- Arizza, V. 2013. Marine biodiversity as source of new drugs. *Italian Journal of Zoology* 80:317–318.
- Begich, S. M. 2013. A bill to provide for the establishment of nationally uniform and environmentally sound standards governing discharges incidental to the normal operation of a vessel. Pages 113-304.
- Beach Detail Lookup. 2016. . Lab Data, Commonwealth of Massachusetts Department of Public Health.
- Benchley, P. 1995. *Ocean Planet: Writings and Images of the Sea*. (J. Gradwohl, Ed.). H.N. Abrams, Incorporated and the Times Mirror Magazines, Incorporated.
- Cantet, F., M. Toubiana, M.-G. Parisi, M. Sonthi, M. Cammarata, and P. Roch. 2012. Individual variability of mytimycin gene expression in mussel. *Fish & Shellfish Immunology* 33:641–644.
- Carte, B. K. 1996. Biomedical potential of marine natural products. *Bioscience* 46:271–286.
- Costa, M. ., M. Prado-Alvarez, C. Gestal, H. Li, P. Roch, B. Novoa, and A. Figueras. 2009. Functional and molecular immune response of Mediterranean mussel (*Mytilus galloprovincialis*) haemocytes against pathogen-associated molecular patterns and bacteria. *Fish & Shellfish Immunology* 26:515–523.

- Daczowska-Kozon, E. G., and B. Sun Pan, editors. 2016. *Bacteria on Seafood: Friend or Foe*. Page 401 *Environmental Effects on Seafood Availability, Safety, and Quality*. CRC Press, New York.
- Halliday, E. (n.d.). *Testing the Waters and Closing Beaches*.
- Karthikeyan, C., S. Velmurugan, S. Donio, M. Michaelbabu, and T. Citarasu. 2014. Studies on the antimicrobial potential and structural characterization of fatty acids extracted from Sydney rock oyster *Saccostrea glomerata*. *Annals of Clinical Microbiology and Antimicrobials* 332.
- Marcelino, L., V. Backman, A. Donaldson, C. Steadman, J. Thompson, S. Preheim, C. Lein, E. Lim, D. Veneziano, and M. Polz. 2006. Accurately quantifying low-abundant targets amid similar sequences by revealing hidden correlations in oligonucleotide microarray data. *Proceedings of the National Academy of Sciences of the United States of America* 103:13629–13634.
- Michiels, C. W., and L. Callewaert. 2010. Lysosomes in the animal kingdom. *Bioscience* 35:127–160.
- Niu, D., F. Wang, H. Zhao, Z. Wang, S. Xie, and J. Li. 2016. Identification, expression, and innate immune responses of two insulin-like peptide genes in the razor clam *Sinonovacula constricta*. *Fish & Shellfish Immunology* 51:401–404.
- Pagenkopp Lohan, K. M., R. C. Fleischer, K. J. Carney, K. K. Holzer, and G. M. Ruiz. 2016. Amplicon-based pyrosequencing reveals high diversity of protistan parasites in ships' ballast water: Implications for biogeography and infectious diseases. *Microbial Ecology* 71:530–542.

- Romalde, J. L., and J. L. Barja. 2010. Bacteria in molluscs: good and bad guys. *Technology and Education Topics in Applied Microbiology and Microbial Biotechnology*:136–147.
- Rosenfeld, W., and C. Zobell. 1947. Antibiotic Production by Marine Microorganisms. *Bacteriol* 54:393–398.
- Ruiz, G., T. Rawlings, F. Dobbs, L. Drake, T. Mullady, A. Huq, and R. Colwell. 2000. Global spread of microorganisms by ships. *Nature* 408:49–50.
- Sonthi, M., M. Toubiana, A. Pallavicini, P. Venier, and P. Roch. 2011. Diversity of coding sequences and gene structures of the antifungal peptide Mytimycin (MytM) from the Mediterranean Mussel, *Mytilus galloprovincialis*. *Marine Biotechnology* 13:857–867.
- Thompson, J. R., L. A. Marcelino, and M. F. Polz. 2005. Diversity, Sources, and Detection of Human Bacterial Pathogens in the Marine Environment. Pages 29–68 *in* S. Belkin and R. R. Colwell, editors. *Oceans and Health: Pathogens in the Marine Environment*. Springer US, Boston, MA.
- Turner, L. M., C. Alsterberg, A. D. Turner, S. K. Girisha, A. Rai, J. N. Havenhand, M. N. Venugopal, I. Karunasagar, and A. Godhe. 2016. Pathogenic marine microbes influence the effects of climate change on a commercially important tropical bivalve. *Scientific Reports* 6.