A review of information on the seaside organism, *Haplosporidium costale*, with special attention to its presence on the northeastern coast of North America

Hannah Orton[a], Isabelle See[b, c]

[a] Bates College, expected 2024
[b] University of Maine, Quahog Bay Conservancy
[c] Project advisor

Abstract

The protozoan *Haplosporidium costale* is a parasite that causes high mortality rates in the eastern oyster, *Crassostrea virginica*. In 2023, cases of *H. costale* infection were identified in Maine for the first time. Despite its high mortality rates and widespread presence along the eastern coast of North America, research pertaining to this organism is very limited. The objective of this review is to compile a comprehensive body of information from various studies concerning *H. costale* with the intention of using this information to better inform Maine conservationists and oyster farmers about the pathogen and identify potential mitigation efforts. It was found that literature on *H. costale* is outdated and many case studies are inaccessible and lack important information. Future studies were suggested that could potentially resolve these issues.

Introduction

The seaside organism (SSO), or *Haplosporidium costale*, is a protozoan parasitic to the eastern oyster, *Crassostrea virginica*. It was first identified in the Chesapeake Bay in 1962. This discovery was motivated by high rates of eastern oyster mortality within the bay and by the 1957 discovery of another closely related oyster pathogen, *Haplosporidium nelsoni*, which causes a disease commonly known as MSX (Wood & Andrews, 1962). The Haplosporidia family consists of parasitic protists having multinucleate plasmodia and ovoid, walled spores (Burreson & Ford, 2004). In 61 years SSO has spread up the east coast of the United States from Virginia to Maine. The most recent report of a new SSO outbreak on the eastern North American coast that we could find at the time of review was in Long Island Sound (Sunila et al., 2002).

During the summer of 2023 unusually high rates of mortality were observed at two oyster farms in the Quahog Bay of Maine owned by the Quahog Bay Conservancy (Figure 1). The conservancy sent sample oysters from both of these farms to Kennebec River Biosciences in Richmond, Maine for a shellfish health inspection. They were able to detect the presence of SSO

infection in both farms (Merrill, 2023). These cases are some of the first confirmed cases of SSO presence on the Maine coast. Accessible information on SSO, including its life cycle, pathogenesis, and how to reduce its transmission, is scarce and dated. The detection of SSO in the oyster farms in addition to the lack of knowledge known on SSO incentivised a review of case studies on *H. costale*. The purpose of this review is to provide a succinct body of knowledge to further the understanding of this parasite. This understanding can be then used to mitigate its spread both within infected farms and to uninfected areas. Any facets of SSO that are found to not be well researched through this review can then be highlighted and future studies can be developed.

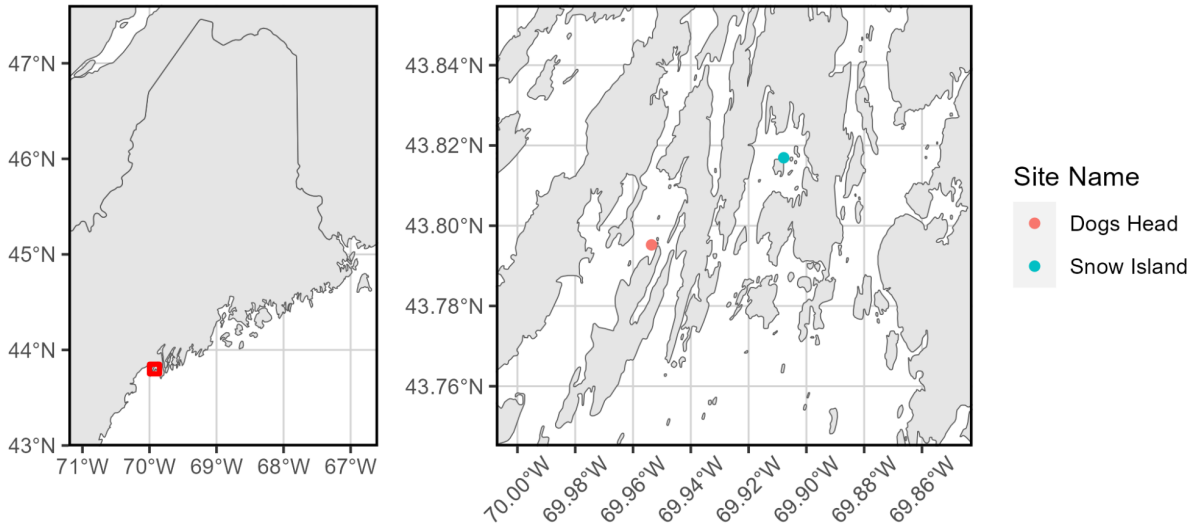

Figure 1: Locations of the Dogs Head and Snow Island oyster farms owned by the Quahog Bay Conservancy in Harpswell, ME.

Literature Review

Life Cycle & Infection

The earliest life cycle stage of *Haplosporidium costale* is the multinucleated plasmodium that enters the oyster during feeding. These cells are typically 6.1 by 7.8 micrometers in size and fix themselves just beneath the epithelium of the digestive tubules (Andrews & Castagna, 1978; Wood & Andrews, 1962). Once inside, the plasmodia develop by successive nuclear and plasmodial division (Fig. 2A) (Ford & Tripp, 1996) and spread throughout the connective tissue of the oyster. After infiltration, the plasmodia remain dormant for about one year. After a year of dormancy, the plasmodia develop systematically into sporocysts for reproduction (Figs. 2A, 2E) (Ford & Tripp, 1996). Sporocysts are typically 7 to 14 micrometers in diameter and contain

20-50 spores each. Sporocyst development is the point at which the infection becomes patent, meaning that it starts to negatively affect the oyster as it degrades the connective tissue for development (Figs. 2B, 2C, 2D, 2F) (Wood & Andrews, 1962). Sporulation occurs in the connective tissue of the oyster rather than the epithelia. The spores that mature into new plasmodia are then transmitted to a different oyster and the cycle begins again (Andrews & Castagna, 1978).

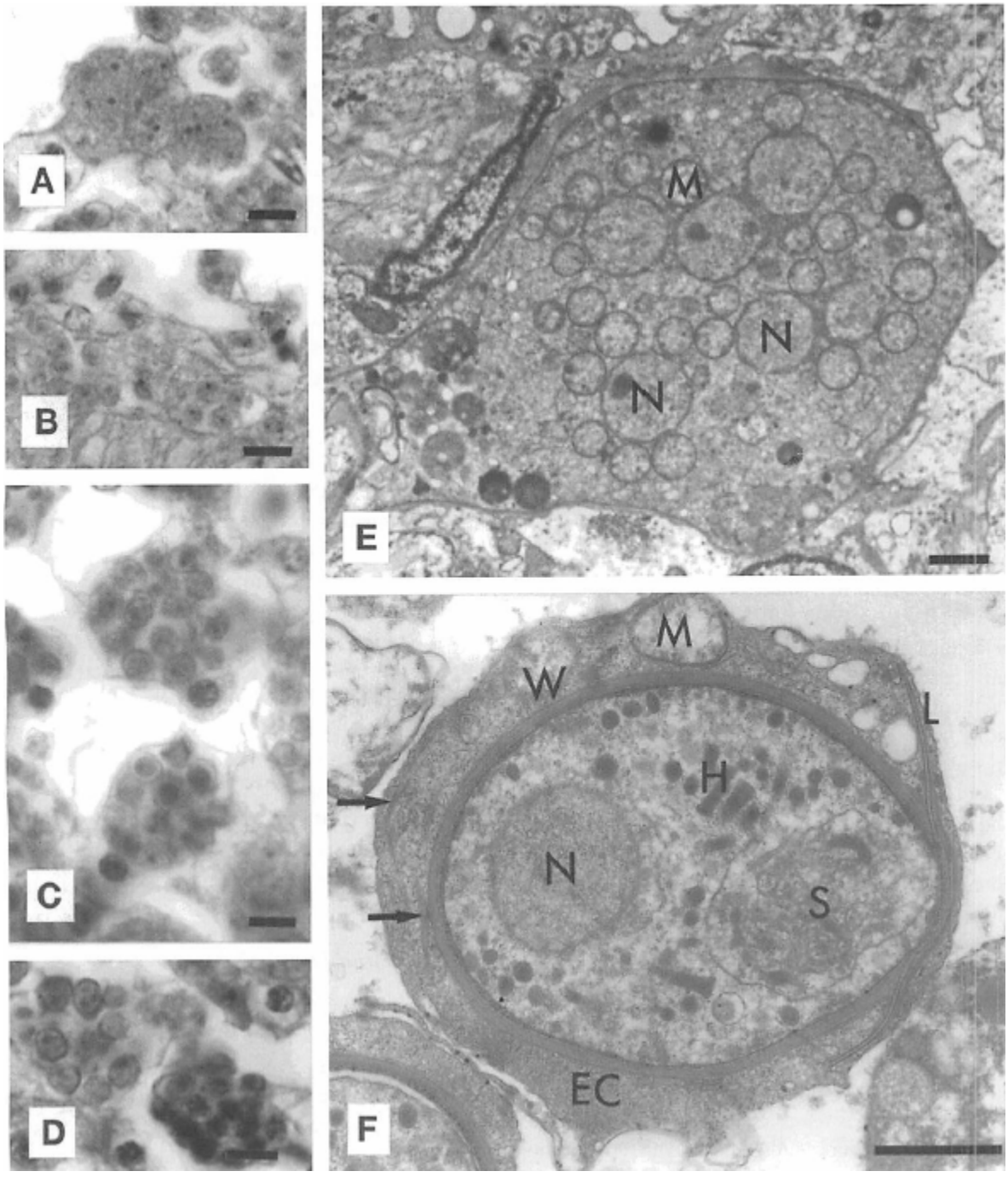

Figure 2: Stages of *Haplosporidium costale* in the eastern oyster *Crassostrea virginica*. (A) plasmodia; (B) early sporonts; (C) sporonts; (D) sporocysts; (E) plasmodium showing nucleus; (F) immature spore. Scale bars = 5 micrometers in A to D and 1 micrometer in E and F (Ford & Tripp, 1996).

The complete infection cycle of SSO takes about a year and two months to occur. Infiltration occurs in May and June concurrent with mature spore production and subsequent mortality from infections that developed over the past year. Typically 20% to 60% of an oyster population exposed to SSO will become infected. The level of exposure to *H. costale* cells needed to produce an infection is unknown. The plasmodia lie dormant until March and April of the following year when they rapidly develop into sporocysts. Sporulation occurs during the subsequent May and June and the infection becomes patent. Heightened oyster mortality linked to SSO infection occurs almost instantaneously after sporocyst development in May and lasts until the end of July, with mortality rates ranging from 20% to 60% (Ford & Tripp, 1996). This timeline indicates that mortality occurs synchronously with new infiltrations and occurs relatively quickly, and that advancement through stages of the *H. costale* life cycle are likely temperature-dependent.

Some differences in pathogeny have been found between infection sites at different latitudes. A case study of SSO infection from Long Island Sound reported that infected oysters did not experience high mortality during May and June like infected oysters in the south do. All oysters in this study infected with SSO were also infected with MSX (Sunila et al., 2002). Sporulation was found to occur in October to December, a time when SSO in Virginia is subpatent. There are a few cases of patency developing in the fall in Virginia, but it is rare. Sporulation also occurs more rarely in the north. Although there are differences in pathogeny, there were not any differences found in morphology between SSO in the south and the north.

Transmission

While the life cycle and infection process of *Haplosporidium costale* has been researched and reviewed extensively, the details of the transmission of SSO are still unknown. In early studies of SSO infection, there was uncertainty as to whether SSO is transmitted from oyster to oyster or through a different host. It was noted that exposure to dying oysters infected with SSO is necessary for new infections and that a majority of oysters that were killed by SSO contained a low abundance of mature *H. costale* spores (Andrews & Castagna, 1978; Ford & Tripp, 1996). It is therefore presumed that transmission occurs through a non-oyster host, rather than from oyster to oyster.

Resistance

The ability of the eastern oyster to resist SSO is also widely unknown. Oysters do have an immune response to infection; as sporulation occurs, hemocytes enter the affected connective tissue. Hemocytes are responsible for the phagocytosis of food and foreign organisms, including SSO. They are also capable of repairing the shell, protecting wounds for repair, and blood clotting (Fisher, 1986). However, the hemocytes "seem ineffective in phagocytosing or killing pathogen cells" (Andrews, 1988). It has been noted that oysters that develop in enzootic waters experience a lower mortality rate than oysters that have been transported to live in waters containing SSO later in life. However, a study of the mortality of both native and imported eastern oysters in Virginia found that the mortalities of imported infected oysters were only slightly higher than that of native oysters (Andrews & Castagna, 1978). More studies would need to be done to determine if this was an isolated or recurring event.

Despite high mortality and infection rates, eastern oysters are still able to reproduce and avoid extinction. This could be related to the relatively long incubation period. Infected oysters have up to a year for reproduction before the infection degrades their tissue (Arzul & Carnegie, 2015). Research has revealed that some oysters are able to recover after mild SSO infection, as evidenced by plasmodial regression observed in early spring (Andrews & Castagna, 1978). More research is needed to determine why or how this occurs.

Environmental Influences

Several environmental factors influence the spread and infection severity of *H. costale*. The protozoan thrives in warmer waters with a high salinity (>25 ppt). Some cases have been detected in waters with salinities of 20-23 ppt, though "infections regress when oysters are moved to water below 20 ppt" (Andrews, 1979). Recent studies of SSO in Maine suggest that the temperature and salinity of Maine's coastal waters might resemble those of Virginia coastal waters in 1962 when SSO was discovered (Sunila et al., 2002). As global oceans warm and northern environments become better-suited for *H. costale* (Pershing et al., 2021), more research is needed to confirm exactly how SSO spreads.

Detection Methodologies

At the time of its discovery, SSO was detected in oysters histologically and this method continued to be used for many years. To identify SSO, oysters were fixed in alcohol and viewed under a microscope. Any histological sections that contained an unrecognizable parasite were

stained and further examined (Wood & Andrews, 1962). This histological method was used for many years, but it posed inherent challenges. The primary issues are time, expense, and histomorphological similarities between MSX and SSO (Andrews & Castagna, 1978). These issues motivated researchers to find different methodologies to identify SSO.

In 1995, researchers motivated by the struggles of histological identification developed a detection for *H. nelsoni* and *H. costale* using polymerase chain reaction (PCR) amplification (Stokes et al., 1995). They noted that "PCR diagnostic protocols will be especially valuable in elucidating complex parasite life cycles," (Stokes et al., 1995) as DNA can be acquired and amplified from any life stage of MSX and SSO. They identified small subunit (SSU) rRNA *H. nelsoni* primers that successfully amplify its DNA and determined that this method is more successful and sensitive than the histological method. When they tested SSU rDNA from *H. costale* as a primer for the PCR amplification of its DNA, they found that it was unsuccessful (Stokes et al., 1995). In that same year, *H. costale* primers were successfully identified and the pathogen was able to be identified by PCR amplification (Ko et al., 1995), allowing for a more rapid and non-lethal detection of SSO.

Since the development of the PCR detection method, many improvements to this technique have been implemented. In 2001, multiplex PCR (MPCR) technology was developed to detect the presence of multiple pathogens in an eastern oyster at once, including *H. costale*, *H. nelsoni*, and *Perkinsus marinus* (Penna et al., 2001). *P. marinus* is the pathogen which causes dermo, another common oyster disease. This technology was then expanded upon in 2004 by adding an internal standard for DNA quality control. This internal standard allows for fewer false positive and negative results (Russell et al., 2004).

Control Measures

It has been suggested that oysters should be planted in September after the summer mortality, maintained through the following summer and subsequent fall and winter, and then harvested right before the second summer. This is based on the observation that exposure to two summer's worth of infections results in significantly greater mortality (Couch & Rosenfield, 1968). However, I was not able to find any record of this method being implemented so its success is unknown. Other than that, I was not able to find other suggestions that would help mitigate the infection of any protozoan pathogen in oysters other than a general suggestion to modify planting and harvesting schedules.

Discussion

There was a range of information provided on each facet of *Haplosporidium costale* that was reviewed. There was a sufficient amount of information available on the life cycle, infection process, and detection methodologies, though only one study on SSO has been done in the northeast and there are no SSO studies from the Gulf of Maine. This becomes a larger issue when newly-discovered latitudinal differences in pathogeny are considered. These differences suggest that the pathogenesis and possibly other facets of SSO differ between sites of different latitudes. Without more research on SSO in the northeast, the extent, cause, and impact of these differences are unknown. Thus, more research on SSO needs to be done in the Gulf of Maine. More research in the north will also allow us to develop effective mitigation strategies for SSO infection in Quahog Bay.

There is little information available on SSO transmission, resistance, and control methods. All that is known about the transmission of SSO is that the pathogen is not transmitted from oyster to oyster due to the lack of mature spores found in infected oysters. It has not been determined if there is another host involved in the transmission, let alone what that host may be or if it is transmitted through an abiotic factor such as the water column. It is also not known how SSO has spread from Virginia to Maine. Similarly, the ability of an eastern oyster to resist or recover from SSO infection is virtually unknown. What is known is that some oysters that experience light infection, which is not quantified, are able to recover and that oysters that are raised in enzootic waters experience lower infection rates. It is also known that an oyster's hemocytes are ineffective in killing the pathogen. However, it is unknown how some oysters are able to recover or avoid infection altogether. It is essential to understand how SSO is transmitted and how some oysters can resist and recover from infection if oyster farmers want to determine a method for SSO mitigation and spread reduction.

These gaps in the information available about *H. costale* are caused by a few issues. The greatest issue is the lack of relevant, available literature on SSO. All but two of the literature cited in this review were published more than 15 years ago, and many of the sources that this literature cited were inaccessible online. This limited the amount and quality of information that I could provide. There was also no information available on the presence of SSO in the northeastern coast of North America. This, in part, is due to the fact that we have identified the first case of SSO in Maine, so it's understandable that there are not any published studies on the

presence of SSO in Maine. However, SSO has been in Long Island Sound since 2004, so it is surprising that I was not able to find a case study that identified the presence of SSO on the coasts of Rhode Island or Massachusetts.

These issues could be resolved with future studies. I suggest a study that investigates the mode of transmission of SSO involving a controlled transmission study. A couple of the sources that I reviewed expressed interest in a study of this nature as well. I also suggest a study that involves testing an eastern oyster's resilience to SSO. This could involve SSO exposure to oysters for different amounts of time and under different conditions. Finally, I suggest that once more information on SSO is known, that researchers and oyster farmers develop control methods to help its mitigation. Many people rely on oyster farming for income and many organisms, including us, rely on oysters for food and water filtration, so it is vital that we gain more knowledge on SSO so we can reduce oyster mortality.

Acknowledgements

I thank Kennebec River Biosciences for identifying the presence of *Haplosporidium costale* in our oyster farms, Snow Island Oysters for providing sample oysters from both their Dogs Head and Snow Island oyster leases to be sampled for pathogens, and the Quahog Bay Conservancy for assisting me in the literature searching process and providing connections to people that have knowledge on oyster disease.

References

Andrews, J. D. (1979). Oyster Diseases In Chesapeake Bay. *Marine Fisheries Review*, *21*(2), 45–53.

Andrews, J. D. (1988). Haplosporidium costale disease. In *Disease Diagnosis and Control in North American Marine Aquaculture* (2nd ed., pp. 296–299). Elsevier Science.

Andrews, J. D., & Castagna, M. (1978). Epizootiology of Minchinia costalis in susceptible oysters in Seaside Bays of Virginia's Eastern Shore, 1959–1976. *Journal of Invertebrate Pathology*, *32*(2), 124–138. https://doi.org/10.1016/0022-2011(78)90022-8

Arzul, I., & Carnegie, R. B. (2015). New perspective on the haplosporidian parasites of molluscs. *Journal of Invertebrate Pathology*, *131*, 32–42. https://doi.org/10.1016/j.jip.2015.07.014

Burreson, E. M., & Ford, S. E. (2004). A review of recent information on the Haplosporidia, with special reference to *Haplosporidium nelsoni* (MSX disease). *Aquatic Living Resources*, *17*(4), 499–517. https://doi.org/10.1051/alr:2004056

Couch, J. A., & Rosenfield, A. (1968). Epizootiology of Minchinia costalis and Minchinia nelsoni in oysters introduced into Chincoteague Bay, Virginia. *Proceedings of the National Shellfisheries Association*, *58*, 51–59.

Fisher, W. S. (1986). Structure and Functions of Oyster Hemocytes. In M. Brehélin (Ed.), *Immunity in Invertebrates* (pp. 25–35). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-642-70768-1_3

Ford, S., & Tripp, M. R. (1996). Diseases and Defense Mechanisms. In *The eastern oyster: Crassostrea virginica* (2nd ed., pp. 626–641). University of Maryland Sea Grant Publications.

Ko, Y. T., Ford, S. E., & Fong, D. (1995). Characterization of the small subunit ribosomal RNA gene of the oyster parasite Haplosporidium costale. *Molecular Marine Biology and Biotechnology*, *4*(3), 236–240.

Merrill, P. (2023). *Shellfish Health Inspection* [Lab Report]. Kennebec River Biosciences.

Penna, M. S., Khan, M., & French, R. A. (2001). Development of a multiplex PCR for the detection of Haplosporidium nelsoni, Haplosporidium costale and Perkinsus marinus in the eastern oyster (Crassostrea virginica, Gmelin, 1971). *Molecular and Cellular Probes*, *15*(6), 385–390. https://doi.org/10.1006/mcpr.2001.0386

Pershing, A. J., Alexander, M. A., Brady, D. C., Brickman, D., Curchitser, E. N., Diamond, A. W., McClenachan, L., Mills, K. E., Nichols, O. C., Pendleton, D. E., Record, N. R., Scott, J. D., Staudinger, M. D., & Wang, Y. (2021). Climate impacts on the Gulf of Maine ecosystem: A review of observed and expected changes in 2050 from rising temperatures. *Elementa: Science of the Anthropocene*, *9*(1), 00076. https://doi.org/10.1525/elementa.2020.00076

Russell, S., Frasca, S., Sunila, I., & French, R. (2004). Application of a multiplex PCR for the detection of protozoan pathogens of the eastern oyster Crassostrea virginica in field samples. *Diseases of Aquatic Organisms*, *59*(1), 85–91. https://doi.org/10.3354/dao059085

Stokes, N., Siddall, M., & Burreson, E. (1995). Detection of Haplosporidium nelsoni (Haplosporidia:Haplosporidiidae) in oysters by PCR amplification. *Diseases of Aquatic Organisms*, *23*, 145–152. https://doi.org/10.3354/dao023145

Sunila, I., Stokes, N., Smolowitz, R., Karney, R., & Burreson, E. M. (2002). Haplosporidium

Costale (Seaside Organism), A Parasite Of The Eastern Oyster, Is Present In Long Island Sound. *Journal of Shellfish Research*, *21*(1), 113–118.

Wood, J., & Andrews, J. (1962). Haplosporidium costale (Sporozoa) Associated with a Disease of Virginia Oysters. *Science*, *136*, 710–711. https://doi.org/10.1126/science.136.3517.710.b