Sea star wasting disease transmission in *Pisaster ochraceus*

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Abstract

Sea stars are an important part of marine ecosystems. The sea star *Pisaster ochraceus* serves as a keystone predator and helps to keep mussel populations under control its ecosystem in balance. In the last few years, sea star populations have plummeted along the Pacific Northwest coast of the United States due to sea star wasting disease (SSWD) and the virus that causes it, sea star associated densovirus (SSaDV). This research proposal aims to use a laboratory experiment to determine how quickly *Pisaster ochraceus* sea stars begin to shed their virions after becoming inoculated with the virus as well as how quickly they begin to show morphological symptoms of SSWD after inoculation. The expected results of this study are that SSaDV virions will begin to shed within a few days, and sea stars will begin showing morphological symptoms within one week of innoculation. This research can lead to further exploration of SSaDV as well as the function and transmission of SSWD in an unpredictable environment due to climate change.

Introduction

Disease plays a very important role in the structure and function of an ecosystem. Diseases in aquatic organisms can be have huge effects on individuals as well as populations, but they can also have great ecological importance. For example, in 1983, a bacterial disease of sea urchins in the Caribbean caused 99% mortality of the urchins and an ecosystem shift from a coral dominated ecosystem to an algae dominated ecosystem (Lewin 1988). This epidemic had effects on the community metabolism and herbivores in the ecosystem and overall caused the ecosystem to be much less productive that it was before the disease outbreak (Carpenter 1988).

Another example of disease of great ecological importance is a bacterial disease that caused extreme declines in the sun star, *Crossaster papposus*, in the Gulf of California in 1978. This phenomena caused changes to ecosystem structure as the sea stars usual prey, barnacles, increased and led to a decrease in limpets and encrusting algae in the ecosystem (Dungan et al. 1982). Both of these examples consisted of diseases that created detrimental change in ecosystem structure and caused problems for ecosystem balance. Much is still unknown about diseases in marine environments, so studying these diseases and their effects on ecological systems is important and relevant work.

One such disease that has important ecological effects is sea star wasting disease (SSWD). SSWD is a disease that infects over 20 species of sea stars (Hewson et al. 2014, Eisenlord et al. 2016), but species range in susceptibility to the disease with a few species being especially susceptible (Montecino-Latorre et al. 2016). The disease has been found mostly off the west coast of the United States and has devastated certain species of sea star populations in this region as populations of sea stars have plummeted (Hewson et al. 2014, Eisenlord et al. 2016). The disease is thought to be caused by a densovirus termed sea star associated densovirus

(SSaDV) (Hewson et al. 2014). The disease causes sea stars to waste away through extreme morphological changes. The sea stars begin to form white lesions and their tissues begin to swell (Bates et al. 2009). The sea stars also deteriorate and lose the ability to attach to substrate, and their limbs twist and they begin to melt and disintegrate until they die (Hewson et al. 2014, Menge et al. 2016).

Sea stars are an extremely important part of the ecosystem and in many cases serve as a keystone species (Feder 1970, Petes et al. 2008). *Pisaster ochraceus* is one sea star species that serves as a keystone predator along the northwest coast of the United States and is a huge factor in managing the space taken up by organisms in the rocky intertidal zone. (Paine 1966, Menge et al. 1994, Petes et al. 2008). These sea stars play an important role in keeping mussel populations under control (Feder 1970, Petes et al. 2008), so any disease that threatens them is also threatening to this ecosystem dynamic. Without *Pisaster ochraceus* sea stars, mussels may become too abundant and take up a majority of the area in the rocky intertidal zone and leave much less space for other organisms to populate.

Research is being done on this devastating disease but there is still much that needs to be discovered in order to address the issue of SSWD in *Pisaster ochraceus* sea stars. Although the morphological effects of the disease have been well documented and work has been done on how prevalent the disease is under different ecological conditions, there is still more work to be done on how the disease is transmitted between individuals.

Observational or theoretical motivation for research

Many studies have been done surrounding SSWD that have raised additional questions For example, studies have extensively shown how SSWD affects sea star morphology with the melting features discussed previously (Hewson et al. 2014, Menge et al. 2016), but little has been done on how the SSaDV manifests itself inside the sea star. Previous studies have indicated that SSWD causes apoptosis of cells and proteins are inhibited and genes are expressed that allow for disintegration of the body (Gudenkauf and Hewson 2015), but it is still unknown why exactly this occurs or if there is any way to stop it. Moreover, previous studies have suggested that sea stars which are heterozygous at a certain locus are less likely to have SSWD (Wares and Schiebelhut 2016), but it is not certain whether there are other genetic factors that could also contribute to the likelihood of contracting the disease.

Also, it has been determined that warm temperatures cause an increase in the appearance of SSWD and this likely has links to climate change along the Pacific Northwest coast (Staehli et al. 2009, Bates et al. 2009, Menge et al. 2016). This information would explain the more recent explosion of the disease among the Pacific Northwest coast as ocean temperatures are rising. It has also been shown that cooler temperatures may also slow down the infection in sea stars, but does not prevent death (Kohl et al. 2016).

Most importantly, although it is thought that SSWD is caused by SSaDV (Hewson et al. 2014), very little is known about the virus itself. There are gaps in our knowledge about how the virus is spread and at what point a sea star becomes contagious once contracting this virus. The motivation for this research is that although some work has been done, more research needs to be conducted on the mechanisms behind the transmission of the disease in order to better understand the way in which this virus behaves in sea stars and the timeline of this transmission.

Research questions

One of the questions that I aim to answer with this research is: at what stage of infection do *Pisaster ochraceus* sea stars become contagious and start shedding infectious virions? How soon after being inoculated with SSaDV do virions start shedding into the water column? Does temperature have an effect on how quickly virions begin to shed? This question has not been explored much, and there is a lot of research to be done on this topic in order to confirm at what point the sea star becomes contagious and can begin spreading the disease to others in the population.

I also want to answer the question: when do infected *Pisaster ochraceus* sea stars begin showing symptoms after being exposed to the virus? How soon after being inoculated with the virus does the morphology of the sea star begin to change? Does temperature play a role in how quickly the disease manifests itself? Some research has been done on this, but not specifically on *Pisaster ochraceus*, and previous studies have not come to the same conclusions on how long this takes. The morphological consequences of SSWD are well documented, but I aim to discover how the immune system of the sea star is affected and at what point sea stars begin showing symptoms.

Hypotheses

I hypothesize that *Pisaster ochraceus* sea stars will begin to shed their virions within a couple of days of being inoculated with SSaDV. The null hypothesis would be that there is no set timing for these sea stars to begin shedding their virions and that it can happen over any variable amount of time. An alternative hypothesis would be that sea stars do not shed their virions for a very long time and may have SSaDV in their system for a long period of time before they begin to shed the virions.

Additionally, I hypothesize that these sea stars will shed their virions more quickly in warmer temperatures and more slowly in colder temperatures. The null hypothesis would be that temperature has no effect on the speed of virion shedding and that it is equal at every temperature.

I also hypothesize that *Pisaster ochraceus* sea stars will show symptoms of the disease within a week of contracting infectious virions. The null hypothesis would be that sea stars do not have a set time for showing morphological symptoms of the disease after contracting the virus. An alternative hypothesis would be that sea stars do not begin to show their symptoms for a very long period of time after coming in contact with the virus and in some cases may never start showing these morphological symptoms.

Furthermore, I hypothesize that sea stars will begin to show morphological symptoms of the virus more quickly in warmer water and less quickly in colder water. The null hypothesis would be that there is no difference in the time before the sea stars start showing symptoms after being inoculated with the disease at different temperatures.

Experimental design and methods

Specimen collection

To do this study, a laboratory experiment will be conducted on the species *Pisaster ochraceus*. 50 healthy looking sea stars of this species and 10 with signs of SSWD will be collected off the coast of Washington, Oregon, and California by scientific divers. These specimens will be collected from various sights among the coast. These sea stars will be kept separate from each other to prevent disease spread, and will be transported back to the laboratory where they will be put into separate tanks. Healthy sea stars will be split evenly into tanks with either cold water (13 degrees Celsius), average temperature water (23 degrees Celsius), or warm temperature water (33 degrees Celsius), while sea stars that appear diseased will be kept in separate tanks in room temperature water.

Preliminary disease scan

Healthy looking sea stars will be given two weeks to acclimate to their surroundings and their morphology will be examined once daily for lesions, twisting limbs, and other signs of wasting. These sea stars will be checked for SSaDV before the experiment by taking a sample of the sea star's coelomic fluid with a syringe. The coelomic fluid will be run through a quantitative polymerase chain reaction (qPCR) test to detect viral DNA. This test consists of extracting viral DNA from the coelomic fluid and amplifying it in order to detect the amount of viral DNA in the sample. If any viral DNA is detected, this sea star will not be used as test subjects for this experiment.

Shedding of virions

Half of the sea stars will be used in the first part of the experiment. Coelomic fluid will be drawn out from diseased sea stars with a syringe and injected into the healthy sea stars in order to inoculate them with the SSaDV. For the control, sea stars will be injected with autoclaved coelomic fluid in order to inoculate them with a sterile virus. 10 mL water samples will be taken from the individual tanks that the sea stars reside in every 20 minutes to be analyzed for SSaDV, indicating that the virus had been spread. This will be completed using the membrane-absorption technique. This technique consists of passing the coelomic fluid through a membrane that will absorb the virus in a gelatinous substance so that it can be detected (Hill et al. 1971). This experiment will be done on the sea stars in low temperature water, medium temperature water, and high temperature water in order to compare the shedding of virions at the three temperatures.

First signs of symptoms

The other half of the uninfected sea stars will be used in the second half of the experiment. These sea stars will be inoculated with SSaDV taken from the sea stars that were collected with SSaDV virions in their coelomic fluid just as in the previous part of the experiment. For the control, sea stars will be inoculated with coelomic fluid that has been autoclaved to sterilize the SSaDV. Sea stars will be monitored once every 4 hours for visible morphological signs of disease such as lesions and wasting. Results will be recorded in high temperature water, medium temperature water, and low temperature water in order to test for differences between the three temperatures.

Anticipated results

Through this lab experiment, it is expected that that virions will begin to shed within the first 48 hours. Few virions will likely be shed at first, but more and more will be shed over time until shedding reaches a plateau (Figure 1). Also, as mentioned previously, virions are likely to shed more quickly in warmer waters due to the fact that a higher amount of diseased stars have been found in warmer temperatures in previous studies (Staehli et al. 2009, Bates et al. 2009, Menge et al. 2016).

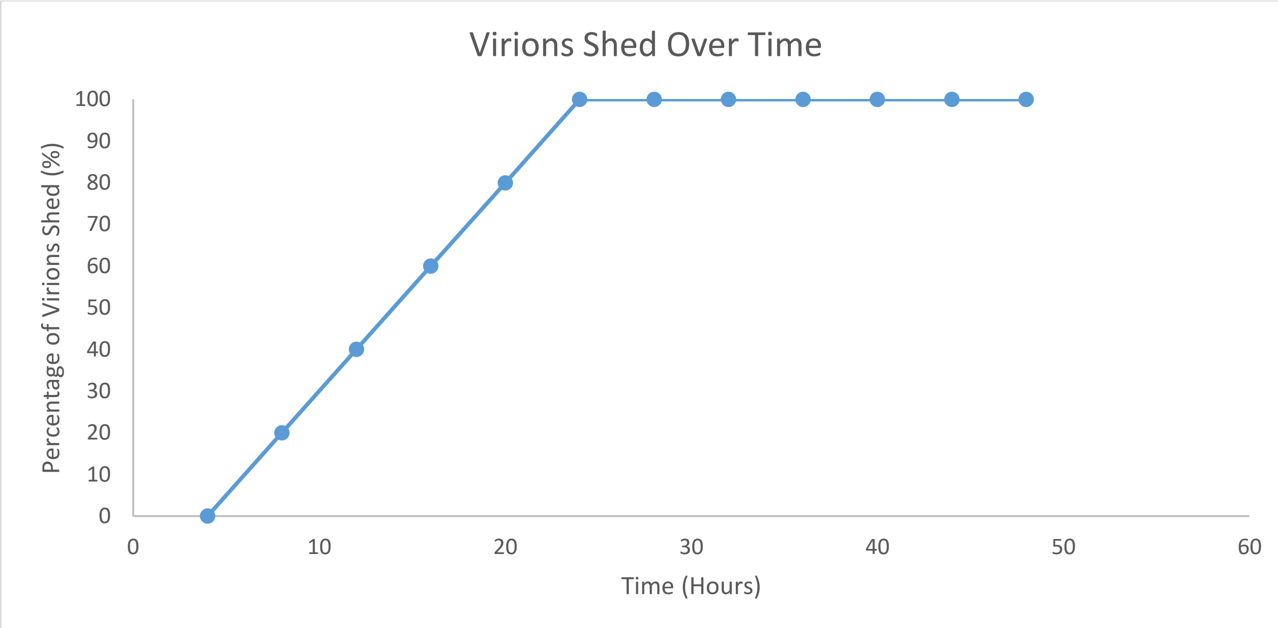

Figure 1. Expected amount of virions to be shed into the water column over time.

Additionally, sea stars will likely begin to show signs of wasting within 7 days of being inoculated with SSaDV (Figure 2) as this is similar to what has been seen in previous studies in alternate species (Bates et al. 2009, Fuess et al. 2015). I expect *Pisaster ochraceus* will begin to show signs more quickly than other species because it is a species that has been reported to be largely affected by SSWD. Also, these sea stars are more likely to begin showing morphological signs of SSWD quicker in warmer water rather than colder water due to warmer temperatures leading to more reports of sea stars with SSWD (Staehli et al. 2009, Bates et al. 2009, Menge et al. 2016).

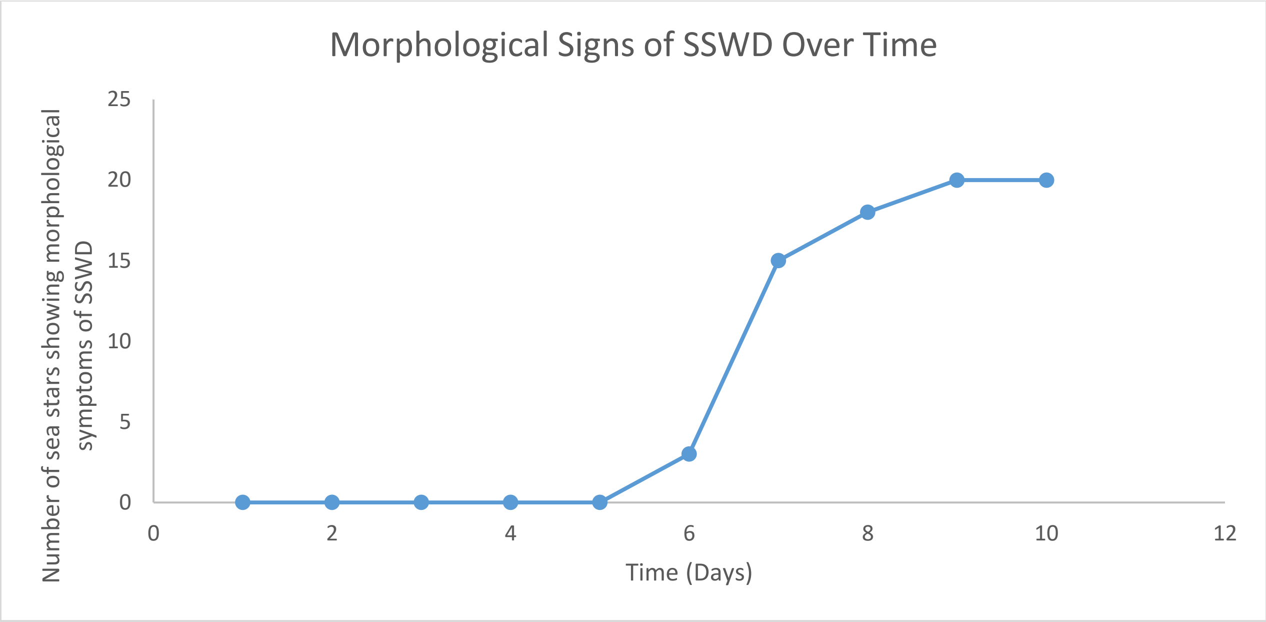

Figure 2. Expected number of sea stars to show morphological signs of SSWD over days.

Discussion

From these possible results, *Pisaster ochraceus* sea stars are likely able to transmit SSWD relatively quickly. If shedding of virions happens over just a few days and morphological signs begin to show within a week of contracting the virus, SSWD is likely a very contagious disease that can be spread rapidly amongst sea stars. This would also explain the massive die offs and decreases in population abundances of this sea star.

This information on transmission of SSWD would be very important for future scientific work. Scientists could have a much better idea of how SSaDV manifests in sea stars and how it is transmitted amongst individuals and even populations. It is known that sea stars have a simple immune system with antibody-like responses and it is possible that through the use of antimicrobial peptides and transcriptional changes sea stars may have some level of protection against sea star wasting disease (Brillouet et al. 1984, Li et al. 2015, Fuess et al. 2015). However, much more information is still lacking on how SSaDV seems to evade most sea star immune responses and this study could be a starting point for figuring out the function of the virus.

Furthermore, figuring out exactly how long before sea stars can begin to infect one another is vital information and could lead to further studies on determining how long the SSaDV can persist inside the sea star. Previous studies have mentioned that SSaDV has been found in sea star specimens from the 1940’s and has been found in plankton and sediment samples as well (Hewson et al. 2014). Information from this research proposal could potentially be used to discover how the virus behaves in other organisms and the environment and for how long this virus can persist within a population.

Additionally, information on SSWD transmission can be an important step in the management and conservation of these species. Transmission rates and signs of infection are important to know when creating mathematical models of disease, and this information can be used to map the spread of SSWD and diseases like it in the future. As climate change continues to create warmer temperatures and more uncertainty along the Pacific Northwest coast and other locations, the likelihood of disease outbreak is likely increasing. Climate change may also make disease outbreaks even more unpredictable than before, so it is important to have this kind of information available for when this disease or diseases like it appear. With this information we can continue researching in order to protect sea star populations, ecosystems, and our marine environment.

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