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13. ABSTRACT ***(Maximum 200 words)***

The research funded by this award, sponsored investigations on novel marine viruses that were isolated in British Columbia coastal waters and the Gulf of Mexico. It was a continuation of Grant N00014-92-J-1676 awarded at The University of Texas. The results included the isolation of a viral pathogen that infects a eukaryotic toxin-producing phytoplankton. The phytoplankton that is infected has been responsible for extensive fish kills in North American and Asia. The ultimate goal is to use these and other viral isolates as vectors for transforming and studying genetics in eukaryotic microalgae. As part of the research an unknown viral pathogen that infects microzooplankton has begun to be characterized. Ultimately, this research may lead to the development of *in situ* PCR-based methods to detect the presence of viral genes within infected cells, and for detection of novel and rare virus types in seawater. This will allow us to more effectively screen for the presence of potential lysogens, as well as allow us to determine the presence of infected cells in natural populations. We have also continued work on the development of fluorescently labeled virus technology as a tool to localize and isolate viral receptors on cell surfaces.

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FINAL REPORT

Grant#: N000149610793

PRINCIPAL INVESTIGATOR: Curtis Suttle

GRANT TITLE: Marine Viral Pathogens

INSTITUTION: University of British Columbia

AWARD PERIOD: 1 May 1996 - 30 September 1997

OBJECTIVE: To isolate, characterize and develop probes for marine viruses.

APPROACH: Natural marine virus communities were concentrated from a wide range of geographical and oceanographic locations, including the Gulf of Mexico, a transect along the west coast of South America from the southern tip of Chile to Panama, and from coastal waters of British Columbia. These were added to our library of natural marine virus communities; the library is being used to screen for novel viruses that infect marine phytoplankton. As a result of the screening experiments pathogens were isolated that infect the toxic bloom forming microalga, *Heterosigma akashiwo*, and the coccolithophorid *Emiliania huxleyi*. Experiments are continuing to determine whether the pathogens are viral. We have continued the development of PCR primers that are specific for viruses that infect microalgae. We are also continuing work on fluorescently labeled viruses that can be used as probes to identify specific cell types and localize viral receptors on cell surfaces.

ACCOMPLISHMENTS: A major accomplishment was the establishment a new research laboratory at The University of British Columbia. We have significantly expanded our library of natural viral communities by adding samples collected along a transect from the Southern tip of Chile to Panama, from fjords and other coastal waters of British Columbia, and from the Gulf of Mexico. We have begun screening these samples for novel viral pathogens. Evidence of the importance of these recent additions our library is documented by the fact that we have used them to isolate a pathogen that infects *Heterosigma ashikawa*, a representative of an unusual group of bloom-forming and toxin producing microalgae (Raphidophyceae). Although we have not definitively shown that the pathogen is viral, it has many characteristics that are consistent with it being a virus. The pathogen can be filtered through a 0.2-µm filter, it can be propagated indefinitely and is resistant to antibiotics. We were also able to obtain DNA from an unknown virus that infects a microflagellate (*Bodo* sp.). This involved amplifying the virus in several 200-liter cultures, and then purifying the virus by filtration, ultrafiltration and ultracentrifugation. We have isolated DNA from the virus and have been working on sequencing a DNA polymerase from the virus. A DNA polymerase sequence will help determine if the virus belongs to a known group or represents an undescribed group of viruses. As well, the sequence should permit us to design PCR primers that are specific for these viruses, and thereby allow us to screen potential host cells for the presence of lysogens. Recently, we have isolated a pathogen that causes lysis of *Emiliania huxleyi*; further work will be required to determine if the pathogen is viral.

We have also continued methodological work to improve our ability to study viruses in aquatic environments. This includes developing methods for quantifying the abundance of free viruses in seawater samples and cultures, and examining the genetic relationships among viral isolates and within natural viral communities. In addition, we have continued work optimizing our phage-labeling protocol. This method allows us to identify cells that specific viruses bind to, as well as localize and quantify viral receptors on cell surfaces. Ultimately, fluorescently labeled virus probes may be used to examine the regulation of receptors on cell surfaces. Finally, we have been examining factors responsible for the destruction of infectious viruses in surface waters. This research is necessary so that we can

understand when and where infectious viruses will occur in the greatest abundance. This aspect of the research has focussed on the effects of UV radiation on the destruction of viruses, and the role of photoreactivation in restoring infectivity to viruses damaged by solar radiation.

SIGNIFICANCE: The research has led to a number of significant advances. 1) The virus communities that we have concentrated have greatly expanded the range of environments represented in our library of natural viral communities. This is important as many of these communities were collected from very different habitats than were previously represented in the library; therefore, the virus communities should also be different. 2) It is also significant that we have isolated a pathogen that infects the Rhaphidophyte, *Heterosigma ashikawa*. This is not only a toxic bloom former, but rhaphidophytes have a number of unusual ultrastructural features including trichcysts, which are small projectiles that can be shot out from the cell surface. Ultimately, viruses may provide a mechanism to understand the genetic basis controlling such architecture. Developing PCR primers for this virus, as well as the unknown virus that infects *Bodo* sp. will provide tools for identifying cells and populations that contain viral genes. 3) We have been able to use fluorescently labeled viruses to identify a number of specific cell types, including bacteria that are pathogens of humans. 4) We have been able to follow iron stress in bacterial populations by using fluorescently labeled viruses that bind to an iron transport protein. 5. We have convincingly shown that viruses can be quantified accurately in aquatic samples by epifluorescence microscopy. 6. We have demonstrated in natural viral communities the importance of photoreactivation in restoring infectivity to viruses damaged by sunlight.

This research has led to a number of publications and abstracts that have appeared and are still in preparation. As this award was completion of work funded under grant N00014-92-J-1676, publications arising under both awards are included in this final report.

PUBLICATIONS IN REFEREED JOURNALS:

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