Developing Molecular Methods to Identify and Quantify Ballast Water Organisms: A Test Case with Cnidarians

Compliance
CP-1251

Background:

To comply with Federal and International regulations, the Department of Defense (DoD) needs to quantify the abundance and diversity of organisms in the ballast water of DoD vessels. Traditional identification methods, such as morphology, are difficult and time-consuming. Full identification of certain taxa is not always possible because many of the larval forms do not have unique morphology, which can lead to an underestimate of the diversity of organisms present. New and novel approaches are needed to overcome many of the obstacles encountered in using traditional identification methods for ballast water organisms. Deoxyribonucleic acid (DNA) identification techniques have been successful in estimating mixed bacterial communities in soil and water; however, this technique has not been explored fully for ballast water organisms.

Objective:

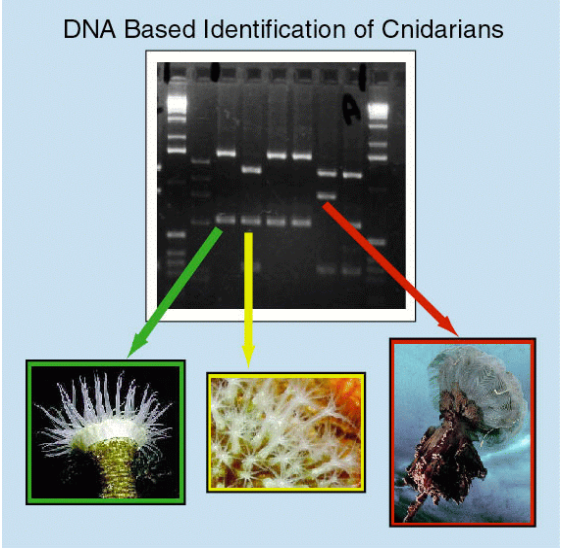
The objective of this exploratory research is to describe molecular markers and refine the methods necessary to identify a specific cnidarian taxa in ballast water samples by developing protocols that apply molecular methods to detect, identify, and quantify ballast water organisms.

Summary of Process/Technology:

A four-step protocol for the identification of cnidarian taxa using molecular methods will be developed. Bulk DNA from all the organisms present is extracted from a sample. Polymerase chain reaction (PCR) is used to amplify a specific target gene from a specific group of organisms (cnidarians in this case). The PCR products are then cloned as a means of isolating the contributions of each species present. Identifications are made by screening these cloned PCR products using a variety of techniques including size variation, restriction fragment length polymorphisms (RFLPs), and selective amplification by PCR. To develop the molecular protocol, markers that identify various cnidarian species will be characterized by determining which genes in the genome will provide the appropriate level of taxonomic resolution. Lab tests will be conducted to determine the marker's ability to detect the presence of specific cnidarian taxa from a variety of concentrations in mixed samples. The technique that is developed will be tested on actual ballast water samples as a final evaluation.

Benefit:

If successful, this project will provide a powerful and versatile protocol for determining cnidarian taxa in ballast water that can be applied by any worker with basic skills in molecular biology and adapted to any taxonomic group.



Accomplishments:

This project began in FY 2002. Accomplishments will be noted upon completion of the project.

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