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Field preservation of marine invertebrate tissue for DNA analyses

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Abstract

Successful preservation of tissue samples is a prerequisite for long field studies in remote areas. However, there is little published information concerning field preservation of marine invertebrate tissues for DNA analyses. This omission is significant because marine biodiversity is centered in the Indo-Pacific, where immediate DNA analysis is often impossible. Consequently, we used an assay based on polymerase chain reaction (PCR) to examine the effect of five storage solutions and three temperature regimens on the degradation of DNA from four common classes of marine invertebrates (Anthozoa, Gastropoda, Polychaeta, and Scyphozoa). Control samples were cryopreserved. Storage solution and the type of tissue preserved were the best predictors of preservation success. The length of time in storage and the storage temperature also affected the preservation of DNA. A field test demonstrates that a solution of dimethylsulfoxide and sodium chloride (DMSO-NaCl) preserves a wide range of tissues for DNA analyses and is very simple to use in remote field locations.

Introduction

Plans to investigate scyphozoan phylogenies in the western equatorial Pacific Ocean required the preservation of tissue samples for long periods of time in a hot and humid climate. A survey of the literature revealed that, although many studies have identified methods suitable for preserving plant and animal tissues (Table 1), there were no recommendations on how best to preserve marine invertebrate tissues for DNA analyses. This as a significant omission for several reasons. First, DNA analyses are invaluable in studies of the evolution, systematics, and population genetics of marine invertebrates (e.g., see McMillan et al., 1991; Avise, 1994, p. 154; Burton and Lee, 1994; Palumbi, 1994; France et al., 1996). Second, marine invertebrates are becoming increasingly important to the pharmaceutical industry (Colin and Arneson, 1995). Finally, the marine environment harbors the greatest diversity of invertebrates (Brusca and Brusca, 1990, p. 5), and this diversity is highest in the Indo-Pacific (Colin and Arneson, 1995), where immediate analysis, or cryopreservation, of DNA is often impossible.

DNA is particularly susceptible to degradation by hydrolytic and oxidative endogenous nucleases (Dessauer et al., 1995), which, if not countered, break down highly informative long strands of DNA into small fragments of greatly reduced use for many analyses (Seutin et al., 1991). Enzyme activity, and consequently DNA degradation, may be limited by adjusting the ambient pH, salt concentration, or temperature (Dixon and Webb, 1979). Samples may be successfully preserved by a number of chemical or physical treatments (Table 1). Cryopreservation is the preferred method of DNA protection (Chase and Hills, 1991; Seutin et al., 1991; Rogstad, 1992; Post et al., 1993; Reiss et al., 1995), and may be accomplished by freezing samples over dry ice (−78°C) or in liquid nitrogen (−196°C). However, deep freezing is not always feasible. Both dry ice and liquid nitrogen are difficult to use in the field because they require careful handling and special equipment, and furthermore, strict regulations limit their transport by air (Liston and Rieseberg, 1990; Chase and Hills, 1991; Seutin et al., 1991; Dessauer et al., 1995).

This study was undertaken to identify an alternative to cryopreservation, suitable for the long-term storage of marine invertebrate tissues for DNA analyses, and appropriate for use at remote field sites. After reviewing the published literature, we

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