

STUDY TITLE: How does produced water cause a reduction in the genetic diversity of harpacticoid copepods?

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CONTRACT NUMBER: 14-35-001-30660-19949

SPONSORING OCS REGION: Gulf of Mexico OCS Region

APPLICABLE PLANNING AREA: Central Gulf of Mexico

FISCAL YEARS OF PROJECT FUNDING: 1998-2001

COMPLETION DATE OF REPORT: September 2001

COSTS: FY 1998 \$88,250; FY 1999 \$63,369; FY 2000 \$34,704; CUMULATIVE PROJECT COST: \$186,323

PROJECT MANAGERS: John W. Fleeger and David W. Foltz

AFFILIATION: Louisiana State University

ADDRESS: Department of Biological Sciences, 508 Life Science Building, Baton Rouge, LA 70803

PRINCIPAL INVESTIGATORS*: John W. Fleeger and David W. Foltz

KEYWORDS: cryptic species, meiofauna, harpacticoid copepods, genetic diversity, toxicity tests

BACKGROUND: Research associated with the GOOMEX project found a reduction in genetic diversity in meiobenthic copepods found in close proximity to production platforms. Specifically, the 16S rRNA gene of the mitochondrial genome in several meiobenthic harpacticoid copepod species was analyzed by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analysis. Such a loss of genetic diversity may have several causes. A reduction in genetic diversity may be the direct result of exposure to contamination via the process of genotypic selection. Alternatively, it is possible that some of the mitochondrial DNA genotypes found in the GOOMEX study represent interspecific variation in the form of cryptic species rather than within-species variants. If cryptic species with differing levels of tolerance to sediment contaminants occur among harpacticoids, the apparent reduction in genetic diversity near platforms could represent a loss of species diversity rather than genotype selection.

OBJECTIVES: Using a multilocus genetic approach and limited morphological observations, we analyzed *Cletocamptus deitersi* (a common harpacticoid copepod frequently found at contaminated sites with known morphological variability and highly cosmopolitan in distribution) from four localities in North America to determine if separate populations are represented by morphologically and/or genetically differentiated species. Two sites were intensively sampled to determine if cryptic species live in sympatry. We also obtained genetic data from *C. helobius*, a readily identifiable and morphologically differentiated congeneric species, to establish a level of interspecific genetic differentiation. An undescribed harpacticoid and a planktonic calanoid were used as outgroups in copepod phylogenetic reconstructions. In addition, tests for tolerance to PAH and metal exposure were conducted on *C. deitersi* from two locations.

DESCRIPTION: *C. deitersi* was collected at an inland brine seep near Jackson, Alabama and at two locations on the coast of Louisiana. *C. deitersi* was also obtained from laboratory cultures established from collections made in the Salton Sea, California and at the mouth of the "Estero del Yugo" estuary in Mazatlán, Sinaloa, Mexico. All copepods were subject to an initial PCR amplification of the mitochondrial DNA (mtDNA) cytochrome oxidase subunit I (COX-I) gene. We sequenced two additional gene regions in copepods representative of the major lineages identified on the basis of COX-I sequences (1) part of the mitochondrial large subunit ribosomal DNA (LSU rDNA 16S) and (2) part of the nuclear rDNA, comprising the entire ITS1, ITS2 and the intervening 5.8S rDNA. Limited morphological observations were made on the third swimming leg of specimens from all locations. Toxicity bioassays, over 96 hours, were conducted on specimens from Alabama and Louisiana for tolerance to metals and phenanthrene.

SIGNIFICANT CONCLUSIONS: Our genetic analyses and limited morphological comparisons strongly suggest that *C. deitersi* in North America is not mono-specific. Our analysis identified four putative species among specimens obtained from four widely separated locations. Based on our molecular data, two species (referred to as type I and II below) within *C. deitersi* co-occurred at each of two intensively studied field sites. Overall, 11 (7.7%) of the specimens we examined from Louisiana harbored type I and 132 (92.3%) type II COX-I sequences. Conversely, 90 (92.8%) *C. deitersi* from the Alabama brine seep were of type I whereas seven (7.2%) were of type II. Furthermore, our research suggests that type I and type II *C. deitersi* from two locations have similar tolerances to poly-cyclic aromatic hydrocarbons but different tolerances to heavy metals. The results of 96-h bioassays reveal that both type I and type II are very resistant to phenanthrene. Type I and type II *C. deitersi* are also both relatively tolerant to a mix of Zn, Pb and Cd (at 6249 $\mu\text{g Zn/l}$, 685 $\mu\text{g Pb/l}$ and 62 $\mu\text{g Cd/l}$, based on ratios present in produced water). Mortality in exposed groups was significantly greater than in controls for both species. However, significant differences between type I and type II were identified; type I (from Alabama) was found to be more tolerant than type II (from Louisiana).

STUDY RESULTS: For cryptic species to contribute to losses in genetic diversity associated with contaminated sites, three observations must be true. Firstly, some taxa among the groups studied must form a cryptic-species complex. The harpacticoid copepod *Cletocamptus deitersi* is not mono-specific in North America but exists as a complex of at least four species. Secondly, cryptic species must co-occur at uncontaminated sites. Our research found that cryptic species of *C. deitersi* are sympatric at two locations. Thirdly, cryptic species must exhibit different responses to contaminants such that differential mortality occurs at contaminated sites. Toxicity bioassays with *C. deitersi* from two locations found a difference in tolerance to metals. It is possible that losses in genetic diversity at contaminated sites may be associated with losses in species diversity due to a reduction in the number of cryptic species at contaminated sites. Investigators employing harpacticoids for genetic analysis in the future may wish to consider taking the extra precaution of dissecting and archiving a single swimming leg (as was done in this study) to allow limited morphological comparisons among individuals used in genetic analysis. In addition, DNA sequence data offer many advantages over RFLP primarily by facilitating the identification of cryptic species by examining all of the nucleotide diversity of gene region. A loss of genetic diversity associated with a reduction in species diversity at contaminated sites is fundamentally different than adaptation-related changes in genetic diversity within species and has an ecological rather than an evolutionary basis. If genetic diversity is used as a marker for contamination effects in the future, the possible contributions of cryptic species should be considered.

STUDY PRODUCTS: Fleeger, J.W., D.W. Foltz and A. Rocha-Olivares. How does produced water cause a reduction in the genetic diversity of harpacticoid copepods? Final report. OCS Study MMS 2001-078. U.S. Dept. of the Interior, Minerals Management Service, Gulf of Mexico OCS Region, New Orleans, Louisiana, 48 pP.