Mesoscale Structure of Zooplankton In the California Current

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LONG-TERM GOALS

The long range goal of this collaboration is to develop a better understanding of zooplankton population dynamics, especially at the boundaries of distributions, through the use of specifically-designed field efforts utilizing bio-acoustics, biochemical and molecular analyses, and process models.

OBJECTIVES

We are examining how populations of euphausiids maintain high concentrations in coastal regions of the California Current despite the highly advective field. We are testing the hypothesis that mesoscale eddies and coastal counter currents create retention cells, where growth and reproduction are rapid and mortality is reduced. The immediate objective is to produce a data set on the biomass, population genetics, and physiological condition of the targeted euphausiids across the boundary between the eutrophic inshore of the California current and the oligotrophic central gyre waters. This work is supported by ONR Biological Oceanography.

APPROACH

We are using a suite of techniques - including molecular genetics, enzyme biochemistry, and bio-acoustics - to examine the mechanisms that define the boundaries of zooplankton species' distributions in the California Current. We are analyzing the physiological and population genetic characteristics of the target euphausiid species in samples collected along CalCOFI line 83.3. The physiological characteristics are enzyme activities and the genetic characteristics are molecular variants of temperature-sensitive enzymes. The field work is accompanied by the formulation of a set of models of these animals that explicitly considers selection on nuclear genetic traits and the effect of this selection on population structure.

WORK COMPLETED

Molecular genetics: Individual *Nematocelis difficilis* were identified in the alcohol-preserved samples from the 15 MOCNESS tows taken during RR-9610, removed to individual vials, and prepared for molecular analysis. A 450 base-pair region of the mitochondrial cytochrome oxidase I (COI) gene was sequenced for a total of 110 individuals. The sequence data have been analyzed for evidence of population genetic structure by several tests, including a hierarchical analysis of molecular variation (AMOVA; Excoffier et al., 1992) comparing variation within and between samples and regions (i.e., coastal, transition, and offshore).

After some deliberation about technical approaches to identify a trait encoded in the nuclear genome that is likely to be subject to selection, we decided to use differential display, reverse transcriptase PCR (dd-rtPCR) to examine genes that are differentially expressed (i.e., turned on-or-off) in different populations and environmental conditions. We have extracted usable mRNA from individually-frozen *N. difficilis* collected during RR-9610, and are currently adapting established dd-rtPCR protocols to euphausiids. The dd-rt PCR assays require only a small amount of tissue; the biochemical and genetic analyses were thus done using the same individuals (see below).

Biochemical analysis: Enzyme kinetic assays were completed for 100 individuals of *N. difficilis* collected during RR-9610 (see Clark et al., 1992; Clark and Walsh, 1993). The enzymes assayed were: citrate synthetase (CS), lactate dehydrogenase (LDH), phosphoglucose isomerase (PGI), and hexokinase (HK). In addition, the same analyses were done for 300 individuals of several other euphausiid species. Protein analyses were conducted on all samples; all enzyme values were scaled to protein. These data will be analyzed to determine the physiological condition and growth of individual euphausiids across the sampled domain.

High-frequency acoustics: In order to groundtruth the bioacoustics data, the taxonomic composition and size frequency distribution of zooplankton were determined for MOCNESS samples from RR-9610 by silhouette analysis (see Wiebe et al., 1996). Silhouette analysis was completed for four net samples (spanning the top 100 m) from each of four MOCNESS tows. The processing of the bioacoustic backscatter data continue. The acoustic results will provide the biomass context for the single-species molecular and biochemical analyses.

Modeling: A schematic of a model to treat the impact of enzyme expression on a planktonic organism is finished. The process of simplifying the model and testing it for compatibility with the observations has begun. A set of physical models covering upwelling systems was developed based on the MIami Community Ocean Model (MICOM). Three types of plankton models are currently up and running in these simulations. The first two are a simple NPZD model and a multiple foodchain model that is also constructed with nutrients as a conservative currency. These models are used as a background age (stage) and metabolically structured model that runs on particles, and can also simulate genetic characteristics of planktonic populations.

RESULTS

Oceanographic setting: The hydrographic analysis from the domain sampled during RR-9610 indicated the presence of a coastal eddy transected by CalCOFI line 83.3, which was the premise of our proposal. Based on surface hydrography and circulation, our transect of 15 MOCNESS tows sampled from offshore, nutrient-poor waters; a transition region of intermediate properties; and coastal, nutrient-rich waters (see RR-9610 cruise report, T. Hayward, ed.).

Molecular genetics: We have characterized the molecular genetic diversity and population structure of *Nematoscelis difficilis* across the sampled domain using DNA sequence variation of the mitochondrial gene, cytochrome oxidase I (COI; see Bucklin et al., 1997). Interestingly, the species population is heterogeneous across the California Current (Fig. 1). The sample-to-sample heterogeneity ($P < 0.015$ based on a chi-square test of haplotype frequencies) suggests a complex response to the environmental gradient along the transect. Based on AMOVA, 8% ($P < 0.032$) of the variation was explained by variation between samples; there was no evidence of regional groupings (i.e., onshore vs offshore) in the spatial patterns of mtDNA diversity.

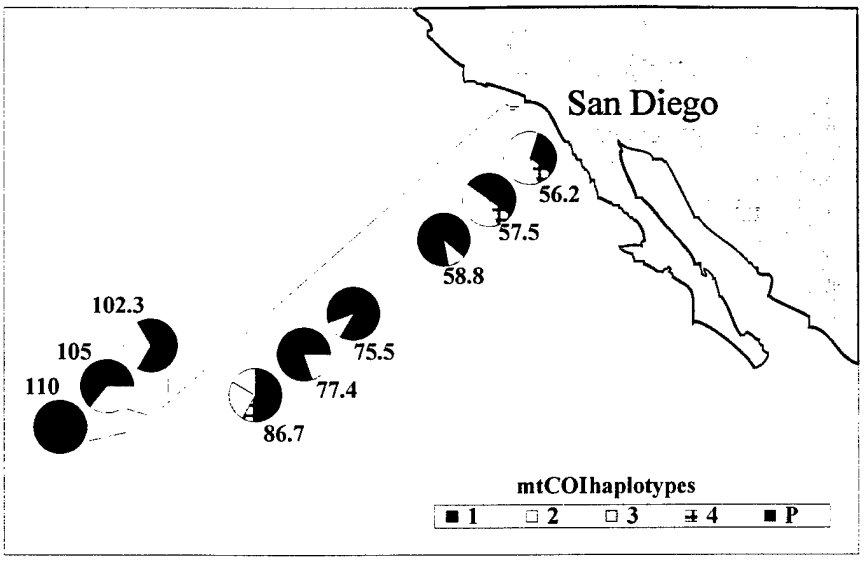

Figure 1. MtCOI haplotype frequencies of the euphausiid *Nematoscelis difficilis* in samples collected along CalCOFI line 83.3; numbers are CalCOFI station designations. Haplotype frequencies differed significantly among the samples ($P<0.015$), but the spatial heterogeneity of the populations was not resolved into regional patterns.

We have modified protocols for assay of gene expression differences by differential display-reverse transcriptase PCR (dd-rtPCR) for euphausiids, and have obtained high-quality mRNA from individual *N. difficilis*. We are now screening for gene expression differences between individuals collected from the coastal eddy vs. those collected offshore using

differential display. In addition, we are working to obtain cDNA sequences for glycolytic enzymes, including phosphoglucose isomerase (PGI) and others assayed biochemically. Comparison of the DNA sequence of the encoding gene with the assays of enzyme activities *for the same individuals* will allow us to determine whether any observed differences in individuals collected along the environmental gradient result from molecular genetic diversity (i.e., DNA sequence variation) or physiological adaptation (i.e., differences in gene expression and enzyme concentrations).

Biochemical analysis: Frozen samples of *N. difficilis* yielded high-quality data on the physiological condition of individual euphausiids, based on the kinetic activities of citrate synthetase (CS), hexokinase (HK), lactate dehydrogenase (LDH), and phosphoglucose isomerase (PGI). Activities of all enzymes were measurable, high, and highly variable. Although enzyme concentrations were not significantly different among individuals collected in different MOCNESS tows (by analysis of variance: CS, $P<0.54$; HK, $P<0.14$; LDH, $P<0.25$; PGI, $P<0.76$), it is possible that patterns of enzyme concentration may be related to genetic characters. We will expand our statistical analyses of the molecular and biochemical data to include multivariate analysis of DNA sequence variation of mtCOI and enzyme-encoding genes and differential gene expression, once all the analyses are complete.

High-frequency acoustics: Silhouette analysis of taxon-specific biomass revealed considerable variation in the taxonomic composition of samples along the transect (Fig. 2), suggestive of mesoscale patchiness structure. The silhouette analysis data will provide the biomass context for our single-species modeling and will be used to ground-truth the bioacoustical observations.

Modeling: The numerical model incorporating population genetic data has been developed (Olson et al., 1998) and is being modified for the California Current coastal region. The mtDNA haplotypes are used a tags of dispersal in the numerical model; previous efforts have demonstrated the model's ability to explain and reproduce complex geographic patterns of mtDNA haplotype frequencies (Olson et al., 1996, 1998).

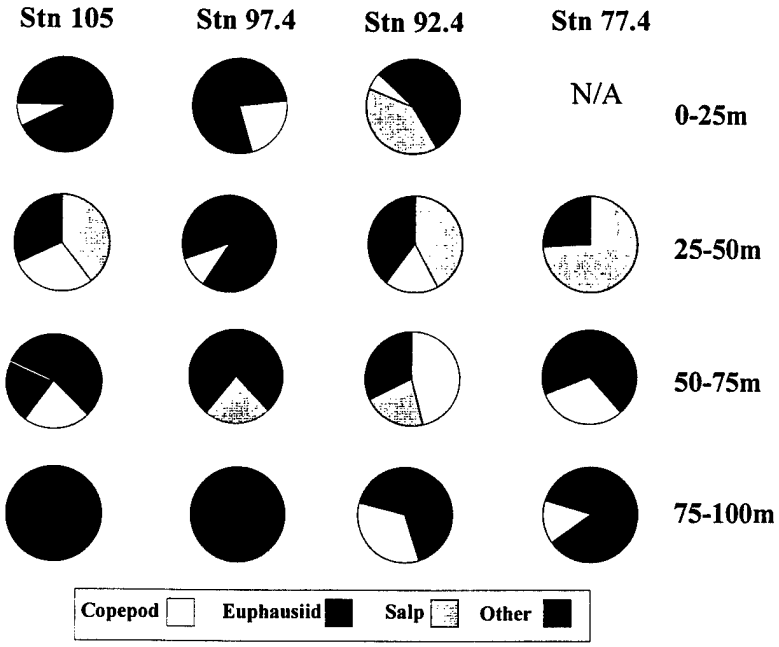

Figure 2. Biomass fractions of copepods, euphausiids, and salps determined from silhouette analysis for depth-stratified samples from four MOCNESS tows at standard stations (Stn) along CalCOFI line 83.3. All other taxa are grouped as "other". These data are important to understand the biomass contribution of euphausiids and to ground-truth the bioacoustical analysis. Data for the 0-25m sample at Stn 77.4 are not available (N/A).

A preliminary study, based on transport of a planktonic copepod around the N. Atlantic gyre, revealed significant spatial pattern in the population genetic character of zooplankton as they are advected through varying environmental carrying capacities. The model results were consistent with the observations in the Gulf Stream system, based on a published study of the copepoda, *Nannocalanus minor* (Bucklin et al., 1996). A manuscript submitted to the Journal of Marine Research is now being revised for resubmission.

Synthesis: We are working toward synthesis of our diverse data sets, and full assimilation into the numerical population dynamic models. We continue to refine our integrated tools (genetics, bioacoustics, and numerical modeling) in order to evaluate both model results and field observations. We are also experimenting with predictive capabilities in various field regions, including the coastal boundary region of the California Current.

IMPACT

Our fundamental approach, which combines experimental data and modeling, should provide new insights into interpretation of population dynamics in the ocean. In addition, the integrated analysis of biochemical, molecular, and high-frequency acoustic data will provide new information for biological oceanographers who seek to understand complex processes and will also provide new indices for rapid assessment of the planktonic assemblage. The impact of this work will be to improve biological models of ocean assessment and prediction.

TRANSITIONS

The model codes will be made widely available after they have been further tested and reviewed for publication. Olson is discussing possible transitions of the Lagrangian code to Naval Research Lab (NRL) via collaboration with Dr. John Kindle at NRL.

RELATED PROJECTS

Using samples collected during RR-9610, A.B. has examined population genetic diversity and structure of the copepoda, *Calanus pacificus*, in coastal regions of the California Current. These data are being analyzed now, to determine whether the nearshore regions provide opportunities for retention and persistence for one of the most abundant and ecologically important members of the California Current zooplankton assemblage. This analysis will help us interpret our results for *Nematoscelis difficilis*, which has different behavior patterns and preferred habitats, but may also be retained in coastal cells and counter currents.

REFERENCES

Bucklin, A., T.C. LaJeunesse, E. Curry, J. Wallinga, and K. Garrison. 1996. Molecular genetic diversity of the copepoda, *Nannocalanus minor:* genetic evidence of species and population structure in the N. Atlantic Ocean. J. Marine Res. 54:285-310.

Bucklin, A., S.B. Smolenack, A.M. Bentley, and P.H. Wiebe. 1997. Gene flow patterns of the euphausiid, *Meganyctiphanes norvegica*, in the N. Atlantic based on DNA sequences for mitochondrial cytochrome oxidase I and cytochrome b. J. Plank. Res. 19:1763-1781.

Clarke, M.E., C. Calvi, M. Domeier, M. Edmonds, and P.J. Walsh (1992) Effects of nutrition and temperature on enzyme activities in larval and juvenile red drum, *Sciaenops acellatus*, and lane smapper, *Lutjanes synagris*. Mar. Biol. 116:31-36.

Clarke, M.E. and P.J. Walsh (1993) Effect of nutritional status on citrate synthetase activity in the copepods *Acartia tonsa* and *Temora longicornis*. Limnol. Oceanogr. 38:414-418.

Excoffier, L., P.E. Smouse, and J.M. Quattro (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479-491.

Olson, D.B., C. Davis, M. Pascual, P. Wiebe, and A. Bucklin. 1996. Model studies of copepod demography. EOS 76:44.

Olson, D.B., A. Bucklin, and Peter H. Wiebe. 1999. Distributions of genetic traits in copepods: a model study. J. Mar. Res. (in revision)

Wiebe, P.H., D. Mountain, T.K. Stanton, C. Greene, G. Lough, S. Kaartvedt, J. Manning, J. Dawson, L. Martin, and N. Copley (1996) Acoustical study of the spatial distribution of plankton on Georges Bank and the relation of volume backscattering strength to the taxonomic composition of the plankton. Deep-Sea Research 43: 1971-2001.

PUBLICATIONS

Bucklin, A., C.C. Caudill, and M. Guarnieri. 1998. Population genetics and phylogeny of marine planktonic copepods. Chapter 14 in: *Molecular Approaches to the Study of the Ocean*, K.C. Cooksey (ed.). Chapman & Hall, London, UK. Pp. 303-317.

Bucklin, A. 1998. Molecular analysis of biogeographic patterns and processes: the copepod *Nannocalanus minor* in the N. Atlantic Ocean. In: *Proceedings of the Second International Conference on Pelagic Biogeography*, 9-14 July 1995. Intergovernmental Oceanographic Comm. Workshop Rpt. No. 142:78-85.

Bucklin, A. and P.H. Wiebe. 1998. Low mitochondrial diversity and small effective population sizes of the copepods Calanus finmarchicus and Nannocalanus minor: possible impact of climatic variation during recent glaciation. American Genetics Assoc. Symposium Issue: Conservation and Genetics of Marine Organisms. J. Heredity 89: 383-392.

Bucklin, A. 1999. Methods for Population Genetic Analysis of Zooplankton. Chapter 11 in: *The ICES Zooplankton Methodology Manual*, R.P Harris and H.-R. Skjoldal, Eds. Academic Press (in press)

Bucklin, A., R.S. Hill, and M. Guarnieri. 1999. Taxonomic and systematic assessment of planktonic copepods using mitochondrial COI sequence variation and competitive, species-specific PCR. Special Issue on Molecular Ecology of Aquatic Communities, J.P. Zehr and M. Voytek (eds.) Hydrobiol. (in press)

Cosner, C., D.L. DeAngelis, J.S. Ault and D.B. Olson. 1999. Mass action, spatial heterogeneity, and the functional response of predators. J. Theor. Bio. (In review)

Guarnieri, M., E. Maly, and A. Bucklin. 1999. Population genetic structure of the freshwater copepod, *Diaptomus leptopus:* evidence for dispersal in post-glacial drainage patterns. Can. J. Fish. Aquat. Sci. (in review)

Flierl, G., D. Grunbaum, S. Levin and D.B. Olson. 1999. Individual-based perspectives on grouping. J. Theor. Bio. (In review)

Hood, R.R. and D.B. Olson. 1998. Multispecies modeling in the North Atlantic Ocean. *Proceedings of the 2nd International Conference on Pelagic Biogeography*, Noordwijkerhout, The Netherlands, July 9 - 14, 1995. Intergovernmental Oceanographic Comm. Workshop Report No. 142: 176 - 181.

Olson, D., A. Bucklin and P. Wiebe. 1999. Distributions of genetic traits in copepods: A model study. J. Mar. Res. (In review)

Olson, D. 1999. Biophysical interactions and the Kuroshio/Oyashio transition zone. Fish. Oceanogr. (In review)

(III) STATISTICAL INFORMATION

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<pi2>Donald B. Olson
<pi3>Peter H. Wiebe

[Note: For P.H. Wiebe, see statistical information provided in the Annual Report by T.K. Stanton and P.H. Wiebe entitled "Acoustic Scattering Models of Zooplankton and Micronekton"]

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<bestaccomplishment>Use of multi-disciplinary observational and analytical tools (molecular genetics, biochemistry, bio-acoustics, numerical modeling) to improve our understanding and prediction of secondary production in the coastal ocean.