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**ONR Final Technical Report:
Mesoscale Structure of Zooplankton In the California Current**

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Introduction

Neritic zooplankton species of the California Current exhibit persistent population concentrations along the coast. Some species are associated with active upwelling centers and may be discontinuously distributed along the coast (Brinton, 1962). How are these species' distributions maintained in the highly advective flow field? What are the biological and physical processes that determine the boundaries of their distributions? In order to answer these questions, oceanographers require new tools. This collaborative project was designed to employ several tools that bring new capabilities to a formerly observational science: numerical modeling, molecular population genetic analysis, and bioacoustical characterization of marine planktonic systems. Using these tools, we examined the biological and physical processes that determine the boundaries of species and biomass distributions in the ocean. As hypothesized here, the physical processes are retention cells produced by coastal currents and return flow in deep counter-currents. The biological processes involved include near-shore centers of reproduction, countered by offshore regions of high mortality.

The California Current exhibits persistent meso-scale structure which results in significant spatial structuring of biological properties (see e.g., Palaez and McGowan, 1986; Haury et al., 1986). Persistent physical structures and recirculating current patterns act to retain water parcels in the near shore, mid-Current, and transition zone areas. In the near shore, countercurrents and eddies may generate re-circulation cells. Coastal currents are largely driven by wind events and follow seasonal patterns (Huyer, 1984). During spring and summer, winds and currents are to the south and upwelling is active, while winds are more variable during fall and winter, and currents flow northward. In mid-Current, coastal filaments, cross-shelf transport events, interrupt north-south flow (Strub et al., 1991). In the transition zone between coastal and oceanic regions, there are persistent meso-scale eddies that may retain drifters for months - and presumably biological particles as well (Haury et al., 1986).

Biological processes also determine patterns of zooplankton distribution and abundance. Differential reproduction and mortality, forces of natural selection, may alter the genetic make-up of populations and generate the observed patterns of distribution and abundance. The action of natural selection may be inferred from the genetic character of populations across the species' geographical range. There are a number of methods for the detection of natural selection in wild populations, including: correlation between selected traits and environmental factors; deviation from formal null models; comparisons between unrelated species in similar habitats; non-random allele frequency distribution and number; and changes in allele frequency distributions among life stages (Endler, 1986). These approaches are feasible for zooplankton collected across a

gradient of biological and/or physical characters in the ocean. The traits used to study natural selection in organismal populations must be heritable (i.e., genetic) and should modulate the interaction between the individual organism and its environment. The traits and their method of assay are discussed below.

The factors determining euphausiid distributions have been examined in some detail by Brinton (1979). Some species are exquisitely loyal to particular hydrographic and chemical conditions, while others seem rather more widely tolerant. Our target species (the euphausiids, *Thysanoessa spinifera*, *Nytiphanes simplex*, and *Nematocelis difficilis*, and *Euphausia pacifica*) represent a spectrum. All four species are concentrated in near-shore regions of the California Current, but the species differ in latitudinal range, vertical distribution (including vertical migration behavior), and fidelity to the neritic zone. *Thysanoessa spinifera* and *N. simplex* appear to have narrow tolerances for physical conditions, while both *N. difficilis* and *E. pacifica* have wide distributions and habitat ranges.

The ultimate goal of this study is to understand the parameters and processes that determine the distributional limits of both widely- and narrowly-distributed species in the California Current. Prediction of zooplankton distributions in relation to ocean circulation patterns requires quantitative understanding of both biological and physical processes. The approach outlined here is three-fold: 1) molecular population genetic assessment of field-collected zooplankton; 2) modeling of zooplankton dispersal across a bio-physical gradient in the California Current; and 3) bioacoustic analysis of volume backscattering across a dispersal gradient. Our hypothesis is that the geographical patterns of distribution and abundance of zooplankton species that are largely restricted to coastal regions of the California Current are determined by a combination of passive transport, behavior (including vertical migration) and life history patterns, and natural selection (i.e., differential reproduction and mortality in ocean domains).

Results and Conclusions

Field Collections: The field collections and assessments were done in October, 1996, as part of the expanded California Cooperative Fisheries Investigations (CalCOFI) Survey on the maiden voyage of the *R/V Roger Revelle*. Zooplankton collections were done along CalCOFI Survey Line 83, and included 15 vertically-stratified tows of a Multiple Opening-Closing Net and Environmental Sensing System (MOCNESS; Wiebe et al., 1985); tows were separated by ~ 20 km. The transect line intersected the coastline at Santa Barbara Channel and crossed the Southern California Bight recirculation cell (Wyllie, 1966). In addition to hydrographic data collected during the MOCNESS tows, temperature, salinity, dissolved oxygen, and chlorophyll were determined at the regular CalCOFI Survey grid stations. Dynamic height anomaly (0 / 500 m) and 10 m temperature data were obtained from the RR-9610 cruise report (Hayward et al., 1997) and from the CalCOFI Program website (address: <http://www-mlrg.sio.ucsd.edu/CalCOFI.html>). The data were contour mapped over the Survey domain using a software package, EasyKrig, Ver. 1.0 (D. Chu, Woods Hole Oceanographic Institution; personal communication).

The hydrographic analysis from the domain sampled during RR-9610 indicated the presence of a coastal eddy transected by CalCOFI line 83.3. 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.).

Distribution and abundance of the target species: Immediately after capture, live individuals of *N. difficilis* and other euphausiids were identified and removed from the MOCNESS samples, placed in cryo-preservation vials, and flash-frozen in liquid nitrogen for biochemical and molecular analysis. The remainder of the MOCNESS samples were then split into halves: one-half was preserved in 95% ethyl alcohol for molecular analysis and one-half was preserved in 10% buffered formalin for silhouette analysis to ground-truth the bio-acoustic backscatter measurements. Counts were made of all *N. difficilis* adults and juveniles in net samples from four MOCNESS tows (CalCOFI Stations 77.4, 92.4, 97.4, and 105).

Nematoscelis difficilis were found at all CalCOFI stations sampled by MOCNESS along Line 83, although the transect extended into offshore waters beyond the characteristic distribution of the species. The euphausiid was most abundant in coastal waters, as expected from previous studies of habitat preference and characteristic distribution (Brinton, 1962). The species' vertical

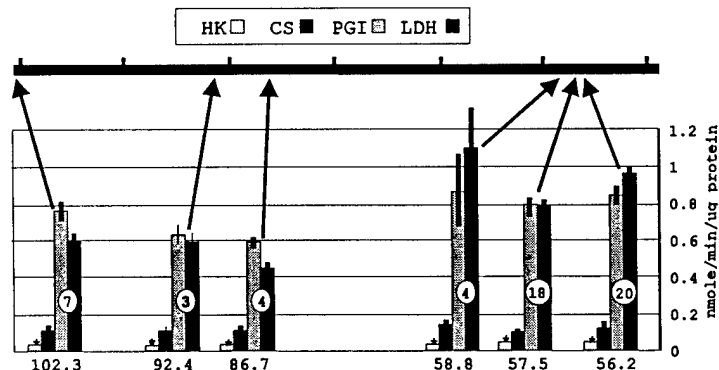


Figure 1. Enzyme concentrations for central metabolic enzymes, LDH, CS, PGI, and HK, from individual *N. difficilis* collected in MOCNESS tows along CalCOFI Line 83. Bars indicate size-corrected mean values; units are nmole/min/μg protein; vertical lines are $\pm$ standard error; numbers in circles are sample sizes; * standard error of HK measurements ranged from 0.001 - 0.005.

distribution was also similar to published reports: *N. difficilis* was most abundant in samples collected in the depth interval 100 - 200 m, with significant numbers between 50 - 100 m and 200 - 300 m. The vertical distribution did not change appreciably from day to night; the species showed no evidence of vertical migration, as expected.

Biochemical analyses: 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. Enzyme concentrations were not significantly different among all samples from the MOCNESS tows, but individuals from coastal samples showed higher protein-specific enzyme concentrations for LDH and PGI than those from offshore samples (Fig. 1).

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 190 individuals. The sequence data were 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). We characterized molecular genetic diversity and population structure of *Nematocelis difficilis* across the sampled domain using methods described in Bucklin et al. (1997). There was significant sample-to-sample heterogeneity of mtDNA haplotype frequencies ($P < 0.015$ by Monte Carlo chi-square test). Two groups of samples - coastal and offshore - were differentiated by mtDNA haplotype frequencies ($P < 0.0001$; Fig. 2), suggesting that *N. difficilis* collected in the two regions may have originated from distinct sources.

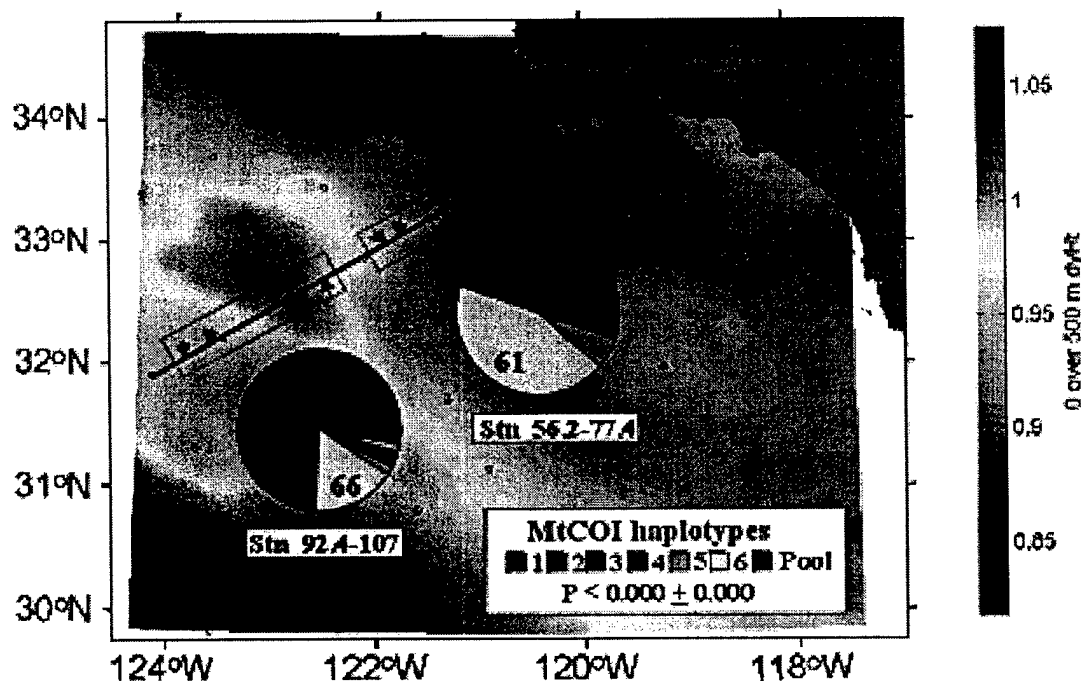


Figure 2. Mitochondrial DNA haplotype frequencies for *N. difficilis* in samples collected along the RR-9610 transect. Samples collected in offshore (Stations 92.4 - 107) and coastal (Stations 56.2 - 77.4) regions differed significantly by Monte Carlo chi-square tests. Background colors represent dynamic height contours (values shown in scale bar at right).

We also characterized molecular genetic variation of the genes encoding two enzymes, PGI and LDH, that showed significant concentration differences across our transect. DNA sequences for ~300 base-pair regions of the genes encoding PGI and LDH were determined for individuals of *N. difficilis* collected in coastal and offshore samples during RR-9610. mRNA was extracted from individually-frozen euphausiids; cDNA was synthesized by reverse-transcriptase PCR (RT-PCR); and the cDNA was sequenced. Oligonucleotide primers were designed from the cDNA sequences to allow direct amplification and sequencing of genomic DNA for these two genes. Levels of variation of the ambiguous diploid sequence were analyzed; putative heterozygous individuals were cloned to identify genotype and allele frequencies. These data are now being analyzed. 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).

High-frequency acoustics: Bio-acoustical backscatter showed dramatic day-night differences along the offshore portion of the transect as well as clear vertical stratification. 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, 1997). Silhouette analysis was completed for four net samples (spanning the top 100 m) from each of four MOCNESS tows. Silhouette analysis of taxon-specific biomass revealed considerable variation in the taxonomic composition of samples along the transect. In general, euphausiids were a small component of the total zooplankton biomass. The bioacoustical analyses will provide the biomass context for the single-species molecular and biochemical analyses.

Numerical modeling: The numerical model incorporating population genetic data was developed (Olson et al., 1998) and modified for the California Current coastal region. The mtDNA haplotypes are used as 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). 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 copepod, *Nannocalanus minor* (Bucklin et al., 1996). A manuscript is in preparation for resubmission to the Journal of Marine Research.

A schematic of a model to treat the impact of enzyme expression on a planktonic organism was completed. The process of simplifying the model and testing it for compatibility with the observations is still underway. 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 food chain 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.

A new model formulation explicitly took into account starvation and its metabolic effects; the model accurately treats the edge of a population distribution. This model formulation produces rapid mortality (based on the fundamental metabolic processes of the Krebs cycle) and makes explicit predictions of the activities of the enzymes assayed for biochemical and molecular genetic variation.

Conclusions

During the October, 1996 CalCOFI Survey of coastal regions of the Southern California Bight, the euphausiid *N. difficilis* was concentrated in cool, biologically rich near-shore waters of the California Current. The species may be retained in the persistent Southern California eddy, and thus respond to the mesoscale variability in environmental conditions on time scales of days to weeks. *Nematoscelis difficilis* also occurred in the warmer, biologically poorer waters farther offshore. Integrated biochemical and molecular genetic analysis of individual euphausiids collected along a transect crossing these two domains revealed a complex array of differences that may reflect the consequences of both physiological adaptation and natural selection.

Our analyses revealed differences between individual euphausiids collected from near-shore and offshore regions in the size-corrected concentrations of PGI and LDH, central metabolic enzymes associated with growth and condition in zooplankton and larval fish (Clarke et al., 1992; Clarke and Walsh, 1993), which were higher for individuals collected in near-shore waters. The variation in enzyme concentration was found not to result from allelic variation of the encoding genes (which did not differ in cDNA sequence based on small sample sizes), suggesting that the observed spatial variation was a consequence of physiological adaptation (increased rates of protein synthesis) rather than natural selection acting through differential mortality of individuals based on their PGI or LDH genotypes. The long-term goal of this and related studies is the assimilation of biochemical and molecular genetic information into numerical population dynamic models. Using biological-physical models that explicitly model life history and the complex processes that determine zooplankton distribution and abundance, we are working toward developing accurate predictive capabilities for community dynamics and biomass patterns in the coastal ocean.

Significance of the research

Our fundamental approach, which combined 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.

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

1 - Using the N. Atlantic gyre formulation of the model, we are determining the model's ability to explain and reproduce geographic patterns of mtDNA haplotype frequencies for the euphausiid, *Stylocheiron elongatum*, in the Gulf Stream (based on a study funded by ONR Biological Oceanography during 1992 - 1994). A manuscript describing the population genetic results is in preparation. We will test the sensitivity of the model to the genetic data and life history parameters by direct comparison with similar studies of the copepod, *Nannocalanus minor* in the Gulf Stream (Bucklin et al., 1996; Olson et al., 2000).

2 - A.B. examined population genetic diversity and structure of the copepod, *Calanus pacificus*, along a latitudinal transect in coastal regions of the California Current using samples collected during RR-9610. There was no evidence of population genetic structure among the samples, perhaps suggesting that populations are not retained in nearshore regions for long enough to become genetically differentiated. This result will help us interpret the genetic data for *N. difficilis*, which showed genetic differentiation between coastal and offshore regions.

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, *Meganctiphanes 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 snapper, *Lutjanus 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.

Wiebe, P.H., T.K. Stanton, M.C. Benfield, D.G. Mountain, and C.H. Greene. 1997. High-frequency acoustic volume backscattering in the Georges Bank coastal region and its interpretation using scattering models. IEEE J. Ocean. Engin. 22: 445-464,.

Publications resulting from the project (to date)

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. J. Heredity 89:383-392.

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. 401:239-254.

Bucklin, A. 2000. 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. Pp. 533 - 570.

Bucklin, A., U. Goswami, M. Guarnieri, and S. Kaartvedt. 2000. Population genetic analysis of drifting (*Calanus finmarchicus*) and resident (*Acartia clausi*) plankton in Norwegian fjords. J. Plankton Res. (In press)

Cosner, C., D.L. DeAngelis, J.S. Ault and D.B. Olson. 1999. Effects of spatial grouping on the functional response of predators. Theor. Pop. Bio. 56: 65 - 75.

Cowen, R.K., K.M.M. Lwiza, S. Sponaugle, C.B. Limouzy-Paris and D.B. Olson. 2000. Connectivity of marine populations: open or closed? Science (In review)

Flierl, G., D. Grunbaum, S. Levin and D.B. Olson. 1999. From individuals to aggregation: the interplay between behavior and physics. J. Theor. Bio. 196: 397 - 454.

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.

Humston, R., J. Ault, M. Lutcavage and D. Olson. 2000. Schooling and migration of large pelagics relative to environmental clues. Fish. Oceanogr. (In press)

Olson, D., A. Bucklin and P. Wiebe. 2000. Distributions of genetic traits in copepods: A model study. J. Mar. Res. (In revision)

Olson, D. 2000. Biophysical dynamics of western transition zones. Fish. Oceanogr. (In review)

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13. ABSTRACT (Maximum 200 words) This collaborative project was designed to improve our quantitative understanding and predictive abilities for 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. <i>Nematoscelis difficilis</i> (Crustacea, Euphausiacea) were collected in a persistent, eutrophic coastal eddy and in oligotrophic offshore waters of the California Current. The euphausiids were assayed for biochemical and molecular genetic traits. Kinetic assays of enzyme concentrations for phosphoglucose isomerase (PGI), lactate dehydrogenase (LDH), citrate synthase (CS), and hexokinase (HK) were used to infer physiological adaptation. DNA sequence variation of the genes encoding PGI and LDH were used to estimate allele and genotype frequencies, and to infer selective mortality. DNA sequence variation of a 350 base-pair region of mitochondrial cytochrome oxidase I (mtCOI) revealed significant sample-to-sample heterogeneity; two groups of samples - coastal and offshore - were differentiated by mtCOI haplotype frequencies, suggesting that <i>N. difficilis</i> collected in the two regions may have originated from distinct sources. Concentrations of PGI and LDH were higher in individuals from the cool, rich near-shore waters. Genotype frequencies of the genes encoding these enzymes did not differ between samples or domains, suggesting that the patterns were not the result of direct selection on the traits assayed. This study provided evidence that <i>N. difficilis</i> individuals responded to mesoscale variability by physiological adaptation, not by differential mortality and selection. Bio-acoustical backscatter showed dramatic day-night differences along the offshore portion of the transect as well as clear vertical stratification. Silhouette analysis of taxon-specific biomass revealed considerable variation in the taxonomic composition of samples along the transect, with euphausiids constituting a small component of total biomass.				
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