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# Integrated biochemical, molecular genetic, and bioacoustical analysis of mesoscale variability of the euphausiid *Nematoscelis difficilis* in the California Current

Ann Bucklin[a,*], Peter H. Wiebe[b], Sara B. Smolenack[a], Nancy J. Copley[c], M. Elizabeth Clarke[d]

[a] *Ocean Process Analysis Laboratory, Department of Zoology, University of New Hampshire, Durham, NH 03824, USA*
[b] *Woods Hole Oceanographic Institution, Woods Hole, MA 02834, USA*
[c] *Woods Hole Oceanographic Institution, Woods Hole, MA 02543, USA*
[d] *Rosenstiel School of Marine and Atmospheric Science, University of Miami, Miami, FL, USA*

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**Abstract**

Integrated assessment of the euphausiid *Nematoscelis difficilis* (Crustacea; Euphausiacea) and the zooplankton assemblage of the California Current was designed to investigate individual, population, and community responses to mesoscale variability in biological and physical characters of the ocean. Zooplankton samples and observational data were collected along a cross-shelf transect of the California Current in association with the California Cooperative Fisheries Investigations (CalCOFI) Survey during October 1996. The transect crossed three domains defined by temperature and salinity: nearshore, mid-Current, and offshore. Individual *N. difficilis* differed in physiological condition along the transect, with higher size-corrected concentrations of four central metabolic enzymes (citrate synthetase, hexokinase, lactate dehydrogenase (LDH), and phosphoglucose isomerase (PGI)) for euphausiids collected in nearshore waters than in mid-Current and offshore waters. There was little variation in the DNA sequences of the genes encoding PGI and LDH (all DNA changes were either silent or heterozygous base substitutions), suggesting that differences in enzyme concentration did not result from underlying molecular genetic variation. The population genetic makeup of *N. difficilis* varied from sample to sample based on haplotype frequencies of mitochondrial cytochrome oxidase I (mtCOI; $P = 0.029$). There were significant differences between pooled nearshore and offshore samples, based on allele frequencies at two sites of common substitutions in the mtCOI sequence ($P = 0.020$ and 0.026). Silhouette and bioacoustical backscattering measurements of the zooplankton assemblage of the top 100 m showed marked diel vertical migration of the scattering layer, of which euphausiids were a small but significant fraction. The biochemical and molecular assays are used as indices of complex physiological (i.e., growth and condition) and genetic (i.e., mortality) processes; the bioacoustical observations provide insight into the ecosystem context for the single-species measurements. All data are intended for integration into predictive models of secondary production and biomass concentration in the ocean. 

*Keywords:* Zooplankton; Euphausiids; Population genetics; Bioacoustics; California Current

*Corresponding author. Tel.: +1-603-862-0122; fax: +1-603-862-0243.
*E-mail address:* acb@cisunix.unh.edu (A. Bucklin).

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## 1. Introduction

### 1.1. Mesoscale variability of the California Current

The California Current exhibits persistent mesoscale structure, which results in significant spatial structuring of biological properties (see e.g., Pelaez and McGowan, 1986; Simpson et al., 1986; Haury et al., 1986). Physical structures and recirculating current patterns act to retain water parcels in the nearshore and transition zone areas. Coastal currents are driven largely by wind events and follow seasonal patterns (Huyer, 1984). During spring and summer, winds and currents are to the south and upwelling is active; in contrast, during fall and winter, winds are more variable and coastal counter-currents may create eddies and retention cells. In the transition zone, coastal filaments (i.e., cross-shelf transport events) may interrupt north–south flow and spin off eddies (Strub et al., 1991). Also in this region, mesoscale eddies may persist for months (Haury et al., 1986; Simpson et al., 1986). In the Southern California Bight, a persistent coastal eddy defines a domain of high biological productivity (Wiley, 1966; Jackson, 1986).

The mesoscale features and circulation patterns of the California Current have profound impact on biological processes at all trophic levels. Retention times for water and organisms entrained in the coastal recirculation cell may be as long as months, separating entrained populations from those transported rapidly in the predominant north–south flow of the California Current (Haury et al., 1986). Coastal filaments and other transport events significantly impact patterns of zooplankton biomass and production (Chavez et al., 1991; Mackas et al., 1991; Smith and Lane, 1991) and population genetic structure (Bucklin, 1991).

### 1.2. The target species

The latitudinal range of the euphausiid *Nematoscelis difficilis*, a transition zone species, is 30°–45°N and includes the North Pacific Drift and California Current (Brinton, 1962). Among California Current zooplankton, *N. difficilis* shows a strong preference for the neritic zone (Brinton, 1967a, 1973), although it also has a zonal distribution across the entire N. Pacific Ocean (Brinton, 1962).

The typical center of vertical distribution of *N. difficilis* is between 100 and 200 m; distributions may be shallower where the thermocline breaks the surface and may deepen as the species seeks to remain in waters cooler than 15°C (Brinton, 1967b; Brinton et al., 1999). Temperature may be a primary determinant of the species' depth distribution, based on examination of a sibling species: Wiebe and Boyd (1978) determined that *N. megalops* populations in the N. Atlantic centered around the 10°C isotherm and suggested that individuals may actively maintain their positions in preferred temperature ranges. Despite some conflicting evidence (e.g., Brinton, 1967b; Youngbluth, 1973), it seems likely that *N. difficilis* do not exhibit marked diel vertical migration behavior. Wiebe et al. (1982) suggest that marked differences in day/night collections may result from visual cues in net avoidance.

The factors determining euphausiid distributions have been examined in some detail by Brinton (1979). Some species are loyal to particular hydrographic and biological conditions, while others seem rather more widely tolerant. In light of the diversity of habitat preferences among California Current euphausiids, which include both ubiquitous and endemic species, the parameters and processes that determine their distributional limits must be numerous and complex. Our hypothesis for *N. difficilis* in the California Current is that geographical patterns of distribution and abundance are determined by both biological processes (with higher rates of reproduction and population growth giving rise to nearshore populations and higher rates of mortality depleting offshore populations) and physical processes (with coastal retention cells further concentrating nearshore populations). Our long-term goals are to understand and measure the relative importance of physiological adaptation and selectively driven changes in genetic make-up for euphausiid individuals and populations entrained in different water masses.

In this study, we use a suite of tools available to oceanographers: biochemical analysis of glycolytic

enzymes (indicating physiological condition and growth potential), molecular genetic analysis of mitochondrial genes (indicating dispersal) and genes encoding glycolytic enzymes (indicating differential mortality), and bioacoustical measurement of backscatter (indicating biomass of the zooplankton assemblage). These analyses are intended to provide indices of complex physiological processes for parameterization in biological–physical numerical models (see Olson et al., 1996).

## 2. Materials and methods

### 2.1. Collection and preservation of samples

Zooplankton samples and physical data were collected during the expanded California cooperative fisheries investigations (CalCOFI) Survey on the maiden voyage of the R/V *Roger Revelle* in October 1996 (cruise number RR-9610). Collections for this study were made along CalCOFI survey line 83.3, which intersects the coastline at the Santa Barbara Channel and crosses the Southern California Bight recirculation cell (Wiley, 1966). Zooplankton samples were collected by a 1-$m^2$ multiple opening-closing net and environmental sensing system (MOCNESS) with 335 μm mesh nets (Wiebe et al., 1985). Sampling locations were spaced along the transect, with 13 vertically stratified tows taken at ~20 km intervals (Table 1).

Our sampling was embedded in the CalCOFI survey grid, with temperature, salinity, dissolved oxygen, and chlorophyll measurements at each station on the grid. The hydrographic data were obtained from the RR-9610 cruise report (SIO, 1998) and from the CalCOFI Program website (address: http://www-mlrg.sio.ucsd.edu/CalCOFI.html). Some data analysis, including calculation of dynamic height anomalies (0/500 m), was done by CalCOFI Program investigators and technicians. Dynamic height anomalies (ranging from 0.80 to 1.08 dm over 0 to 500 m; SIO, 1998) and temperature at 10 m were contour mapped over the RR-9610 domain with the MATLAB Toolkit package and EasyKrig, Ver. 2, available from D. Chu (Woods Hole Oceanographic Institution) and the USGLOBEC web site, http://www.globec.whoi.edu.

Table 1
Collection information for MOCNESS samples used for molecular analysis, bioacoustical groundtruthing, enzyme concentrations, and species' abundance from RR-9610 (October 1996) and KIWI-1 (August 1997)

| Tow \# | Date | Time | D/N | CalCOFI Coord. | Latitude | Longitude | Tow depth (m) | Water depth (m) |
|---|---|---|---|---|---|---|---|---|
| *RR-9610* | | | | | | | | |
| MOC-01 | 10/23/96 | 2056 | N | 83.2, 107.7 | 32°00.92N | 124°01.90W | 500 | 4322 |
| MOC-02 | 10/24/96 | 0155 | N | 83.3, 105.0 | 32°05.20N | 123°50.10W | 500 | 4314 |
| MOC-03 | 10/24/96 | 0549 | D | 83.3, 102.3 | 32°9.97N | 123°38.90W | 500 | 4176 |
| MOC-04 | 10/24/96 | 1300 | D | 82.3, 97.4 | 32°20.0N | 123°18.70W | 500 | 4190 |
| MOC-05 | 10/24/96 | 1606 | Dusk | 83.3, 94.8 | 32°25.05N | 123°08.50W | 500 | 4186 |
| MOC-06 | 10/24/96 | 1903 | N | 83.3, 92.4 | 32°29.9N | 122°58.70W | 500 | 4191 |
| MOC-07 | 10/25/96 | 0344 | N | 83.3, 86.7 | 32°41.04N | 122°34.80W | 500 | 4177 |
| MOC-08 | 10/25/96 | 0742 | D | 83.3, 83.2 | 32°48.04N | 122°20.71W | 500 | 4155 |
| MOC-09 | 10/25/96 | 1707 | Dusk | 83.3, 77.4 | 33°00.25N | 121°57.35W | 500 | 4006 |
| MOC-10 | 10/25/96 | 2050 | N | 83.3, 75.1 | 33°05.00N | 121°47.70W | 500 | 3981 |
| MOC-11 | 10/26/96 | 1910 | N | 83.3, 58.8 | 33°37.60N | 120°40.40W | 500 | 1092 |
| MOC-12 | 10/26/96 | 2235 | N | 83.3, 57.5 | 33°40.10N | 120°35.50W | 500 | 1180 |
| MOC-13 | 10/27/96 | 0127 | N | 83.3, 56.2 | 33°42.70N | 120°29.80W | 500 | 1164 |
| *KIWI-1* | | | | | | | | |
| MOC-02 | 8/4/97 | 2347 | N | N/A | 33°55.00N | 119°10.00W | 500 | 850 |
| MOC-03 | 8/5/97 | 1451 | D | N/A | 34°02.38N | 120°48.1W | 500 | 1166 |

Times are given in local ship's time (Pacific Standard Time).

Bioacoustical backscattering measurements, hydrographic data, and samples from two MOCNESS tows made during an August 1997 cruise of the R/V *Revelle* (cruise number KIWI-1) were analyzed as part of this study. One tow (MOC-2) was made inside the Channel Islands, and the other (MOC-3) was on the same transect line (near RR-9610 MOC-13; Table 1).

During RR-9610, live individuals of *N. difficilis* were identified immediately after capture, removed from the samples, placed in cryo-preservation vials, and flash-frozen in liquid nitrogen for biochemical and molecular analysis. The remainder of each MOCNESS sample was 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 of zooplankton species composition (see Wiebe et al., 1997).

### 2.2. Distribution and abundance of N. difficilis

Counts were made of all *N. difficilis* adults and juveniles in quantitative net sub-samples from six MOCNESS tows made along CalCOFI Line 83.3 during RR-9610 (MOC-2, -4, -6, -9, -12, and -13) and one made during KIWI-1 (MOC-3; Table 1). Individual sizes of the *N. difficilis* counted ranged widely and excluded only larval individuals; larger individuals, ranging from 14 to 25 mm in length, were selected for biochemical and molecular analyses. The abundance of *N. difficilis* was plotted as the log of the numbers per $1000\,m^3$ in each net sample for each tow, and total vertically integrated abundances were calculated as numbers per $m^2$. Patterns of vertical distribution and abundance of *N. difficilis* were compared to vertical temperature structure of the water column, based on the MOCNESS temperature data.

### 2.3. Kinetic assays of enzyme concentration

The enzyme kinetic assays used fragments of tissue chipped from deep-frozen individual *N. difficilis*. The established techniques of Clark et al. (1992) and Clark and Walsh (1993) were used to assay 56 individuals for the enzymes creatine kinase (CK), lactate dehydrogenase (LDH), phosphoglucose isomerase (PGI), and hexokinase (HK). Enzyme concentrations were measured and reported as rates ($n\,mol\,min^{-1}$) in kinetic assays for each individual; all measurements were scaled to individual size by measuring dry weight of tissue (in μg protein; see Clark and Walsh (1993) for detailed technical description). The mean value ± standard error was determined for each MOCNESS tow; the net samples for each tow were pooled for these analyses. Variation in enzyme concentrations within and between individuals, tows, and regions was statistically analyzed by hierarchical analysis of variance (ANOVA; Sokal and Rohlf, 1981).

### 2.4. DNA sequencing of enzyme-encoding genes

Coding regions of PGI and LDH were sequenced from cDNA synthesized by reverse-transcriptase PCR. Messenger RNA (mRNA) was purified from flash-frozen individual euphausiids, by the protocols and reagents available as part of the simple nucleic acid preparation (SNAP) total RNA isolation kit available from Invitrogen (Carlsbad, CA). The cDNA for PGI and LDH were initially amplified with consensus oligonucleotide primers designed from published sequences for these genes, including degenerate primers designed by Crawford (1995). Once the DNA sequences were known, specific amplification and sequencing primers were designed for *N. difficilis*. The primers designed for *N. difficilis* were:

| | |
|---|---|
| PGI-F | 5′-CCTTCTGACTTCCTTGCT-3′ |
| PGI-DR | 5′-GATGTCCCAGATTA-TACCCTG-3′ |
| LDH-C | 5′-GTCAGCAGGAAGGAGAGT-3′ |
| LDH-GR | 5′-GCTCCAGCGACAACATCTTT-3′ |

The amplification protocol for PGI was: 94°C (1 min); 45°C (2 min); 72°C (3 min) for 40 cycles. For LDH, the protocol was: 94°C (1 min); 50°C (2 min); 72°C (3 min) for 40 cycles. Sequencing was carried out on an American Biotechnology, Inc., Model 373, Automated DNA Sequencer (see detailed description in Bucklin et al., 1997). The

amplified region was sequenced in both directions for PGI; LDH was sequenced with the LDH-C primer. In order to recognize variable bases, assess molecular diversity, and infer genotypic identities, a multiple alignment was done with PileUp (Devereaux et al., 1984) from the genetics computer group (GCG; Madison, WI) software package. For comparison, published PGI and LDH sequences were obtained from GenBank. The DNA sequences for PGI and LDH were translated into the inferred protein sequences, using the reading frames from the published sequences.

### 2.5. MtDNA sequence analysis

Individual *N. difficilis* were identified and removed from the alcohol-preserved portion of each sample and stored in individual vials with fresh ethanol. DNA was purified from portions of these individuals and from flash-frozen individuals by phenol-extraction and ethanol precipitation (for detailed protocols, see Bucklin et al., 1997 and Bucklin, 2000). A ~700 base-pair (bp) region of mtCOI was amplified with conserved primers based on published primer sequences by Folmer et al. (1994):

LCO-1490 5′-GGT CAA CAA ATC ATA AAG ATA TTG G-3′
HCO-2198 5′-TAA ACT TCA GGG TGA CCA AAA AAT CA-3′.

The amplification protocol was: 94°C (1 min), 42°C (2 min), 72°C (3 min) carried out for 40 cycles. An internal, species-specific sequencing primer was designed and used:

LCO-1743 5′-GAT TTT GAT TAC TTC GCC-3′

Sequencing was carried out on an American Biotechnology, Inc., Model 373, Automated DNA Sequencer; sequencing protocols were the same as those described for PGI and LDH. A 350 base-pair region of the mtCOI gene was sequenced for a total of 172 individuals. Multiple sequence alignments and a cluster diagram were generated by PileUp (Devereaux et al., 1984) in order to identify haplotypes and determine haplotype frequencies in each tow. Haplotype diversity, $h$ (Nei, 1987), was calculated by

$$h = 1 - \sum f_i^2, \quad (1)$$

where $f_i$ is the frequency of the $i$th haplotype.

The geographical distributions of mtCOI haplotypes among samples and among the three hydrographically defined regions (i.e., nearshore, mid-Current, and offshore) were statistically evaluated by a chi-square test using a Monte Carlo simulation (Roff and Bentzen, 1989). All unique haplotypes were pooled into a single class to reduce the effects of small sample size. Chi-square tests were also used to evaluate spatial distributions of alleles at two sites of common substitution (bp #9 and #240) in the mtCOI sequence. Each comparison used 1000 simulations and generated a $P$ value $\pm$ the standard deviation. The spatial heterogeneity of *N. difficilis* populations was also evaluated by a hierarchical analysis of variance within and between samples and groups (i.e., nearshore vs. offshore) using analysis of molecular variation (AMOVA; Excoffier et al., 1992).

### 2.6. Silhouette analyses of functional groups

To assess the contributions of the major zooplankton taxonomic groups in terms of their numerical abundance, biomass, and size frequency distribution, silhouette analysis was used. This procedure consists of making a silhouette image of an aliquot of each sample and then counting and making length measurements of each taxonomic group (Davis and Wiebe, 1985). Silhouette analysis were performed on RR-9610 MOCNESS tows collected during the day (MOC-4), at dusk (MOC-9), and during the night (MOC-2 and -6) and for two KIWI-1 tows collected at night (MOC-2) and day (MOC-3). These data were also used to groundtruth the bioacoustical backscattering measurements. Statistical analysis was done on the pooled data for the top four net samples, allowing integrated analysis of zooplankton in the 0–100 m depth interval at each station.

### 2.7. Bioacoustical backscattering

A dual-frequency, dual-beam DT4000 echo sounder, built by BioSonics Inc., was mounted in an ENDECO V-finned towed body (the Greene Bomber) and deployed so the towed body was 2–4 m below the surface (Wiebe and Greene, 1994; Wiebe et al., 1997). The 420 and 120-kHz transducers inside the towed body were down-looking to allow the acoustic sensors to map the fine-scale vertical patterns of acoustic backscatter along the ship's trackline. The 120 kHz transducer was $7^\circ \times 18^\circ$ (narrow beam/wide beam) with a source level of 228.4 dB re 1 μP and a receiver sensitivity of −48.0 dB; the 420 kHz transducer was a $3^\circ \times 9^\circ$ with a source level of 226.3 dB re 1 μP and a receiver sensitivity of −43.5 dB. The ping rate was once per second with a pulse width of 0.3 ms. Volume backscattering strength, $S_v$ (where $S_v = 10 \times \log_{10}(s_v)$ in units of decibels $m^{-1}$ and $s_v$ is the volume backscattering coefficient), is a measure of the efficiency with which scatterers echo sound back to the source in a cubic meter. These data were collected routinely from 6 to 8 m to depths of 120 m in 1-m depth bins for both frequencies, although the effective maximum depth for quantitative work with the 420 kHz system was about 50 m. The data were averaged on ~30 s intervals. The towed body was also equipped with an environmental sensing system including a CTD package, which provided measurements of depth, temperature, conductivity, and fluorescence. Two test runs and five data acquisition runs were carried out during RR-9610. The Greene bomber was towed over the offshore half of the transect (beginning at CalCOFI Station 110). Measurements of backscattering intensity were made through 2 days and two-and-one-half nights, until the acoustic equipment was lost during a storm near the mid-point of the transect (after Station 77.4).

In August 1997, a HTI Model 244 split-beam two-frequency (120 and 200 kHz) echosounder, with transducers towed in a down-looking mode at 3–5 m depth, was used during KIWI-1 to acquire volume backscattering data along the same transect sampled in October 1996. The beam width of the 200 kHz transducer was nominally 6° (3° for the 120 kHz transducer) with a source level of 217.63 dB (223.28 dB for the 120 kHz transducer). The ping rate on both transducers was 11 s and the pulse width was 0.18 ms. The data were collected in 5-m depth bins from ~5 to 200 m; they were averaged on ~30 s intervals.

The backscattering data were groundtruthed with the taxonomic data from the silhouette analyses in order to determine relative contributions of different functional groups to backscattering intensity according to Wiebe et al. (1996, 1997) and Foote (2000). The length measurements for each individual and estimates of the animal's material properties (sound speed contrast and density contrast) were used in equations appropriate for the taxonomic groups developed by Stanton et al. (1994, 1998) to estimate their contribution to the volume backscattering. These estimates were summed to produce an estimate of the contribution of each taxonomic group; the group values were summed to provide an estimate of the total backscattering for the area of the net tow. This assessment was then compared to the measured volume backscattering in the water sampled by the net to determine whether the animals collected by the net could account for the measured backscattering. The volume backscattering values used in the comparison with those computed from the biological data were extracted from the acoustic data files using those values recorded within the time and depths of opening and closing each net on a tow and adjusting for the setback distances between the net and the echo sounder transducers. The volume backscattering coefficients ($s_v$) were averaged before converting the average to the logarithmic form of scattering ($S_v$).

## 3. Results

### 3.1. 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. Contour mapping of dynamic height anomalies (Fig. 1A) and temperature at 10 m (Fig. 1B) shows

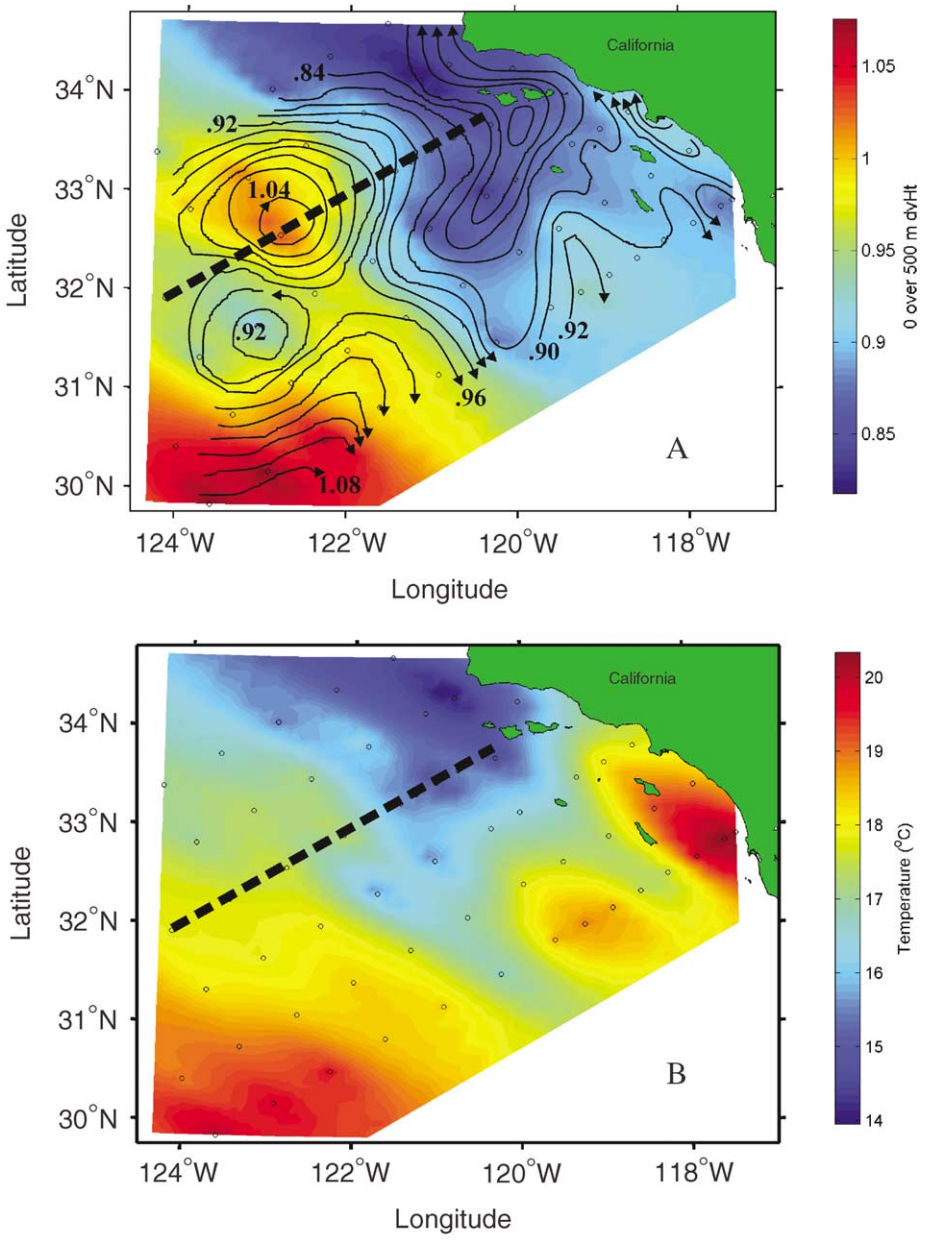


Fig. 1. (A) Dynamic height anomalies (surface relative to 500 m) in the domain sampled by the October 1996 CalCOFI survey on the R/V *Roger Revelle* (cruise RR-9610). Numbers near isopleth lines are dynamic height anomalies, which ranged from 0.80 to 1.08 dm; intervals are 0.02 dm. (B) Temperature at 10 m measured at CalCOFI stations in the sampled domain. Cooler, richer coastal waters were retained within the coastal eddy, while the north–south flow of the California Current in more offshore regions carried warmer, more oligotrophic waters.

that our transect reached from cooler, more saline nearshore waters to warmer, less saline offshore waters. A coastal recirculation cell and two offshore mesoscale eddies were evident in the estimation of current flow from dynamic height anomalies (Fig. 1A).

Hydrographic properties of the water column varied over the length of the transect. Temperature and salinity variation allowed resolution of three distinct oceanographic regions: offshore, mid-Current, and nearshore, with a transition region between the mid-Current and nearshore regions. Nearshore waters between 10°C and 15°C had higher salinity than mid-Current and offshore waters at these temperatures (Fig. 2).

Along-track fluorescence measurements from the Greene bomber and chlorophyll concentration from CalCOFI CTD casts revealed a marked shift from biologically rich nearshore waters to biologically poor offshore waters, with a transition region between (Fig. 3). Along CalCOFI line 83.3, chlorophyll concentrations at 50 m depth ranged from 0.20 ug $l^{-1}$ at the offshore end of the transect (CalCOFI Station 110) to 2.53 µg $l^{-1}$ at the

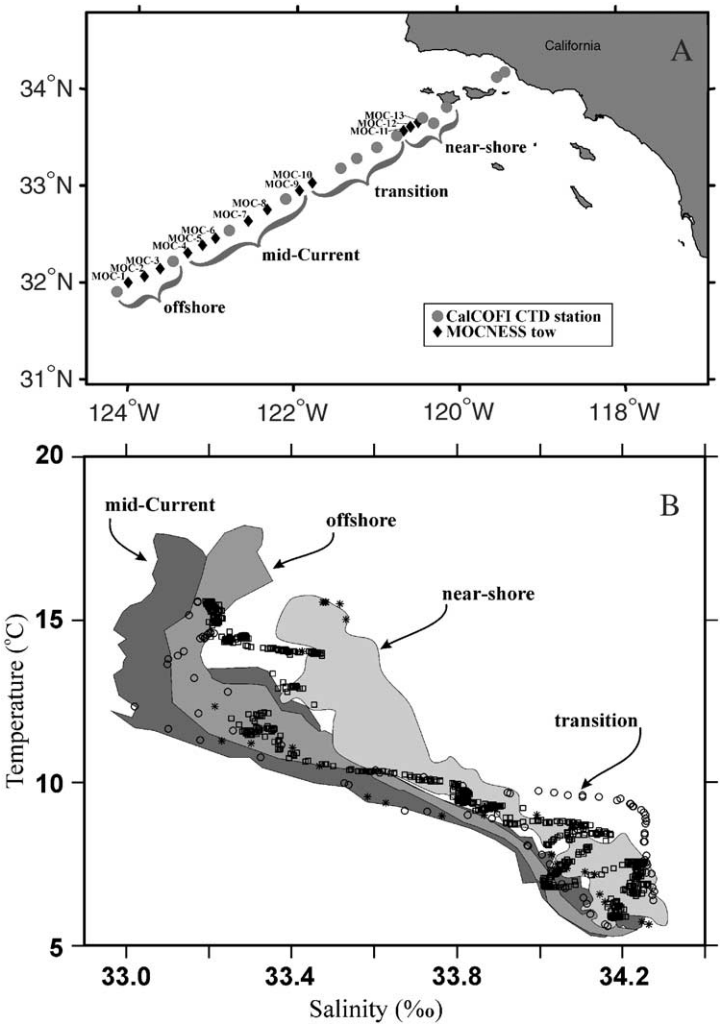


Fig. 2. (A) Positions of CalCOFI CTD profiles and MOCNESS tows during RR-9610 along CalCOFI line 83.3. Hydrographic parameters defined four regions along the transect, indicated by brackets. (B) Temperature and salinity plots for MOCNESS tows and CalCOFI CTD profiles along CalCOFI survey line 83.3. Envelopes are shown for three distinct regions, offshore, mid-Current, and nearshore; data points are shown for a transition area between the mid-Current and nearshore regions.

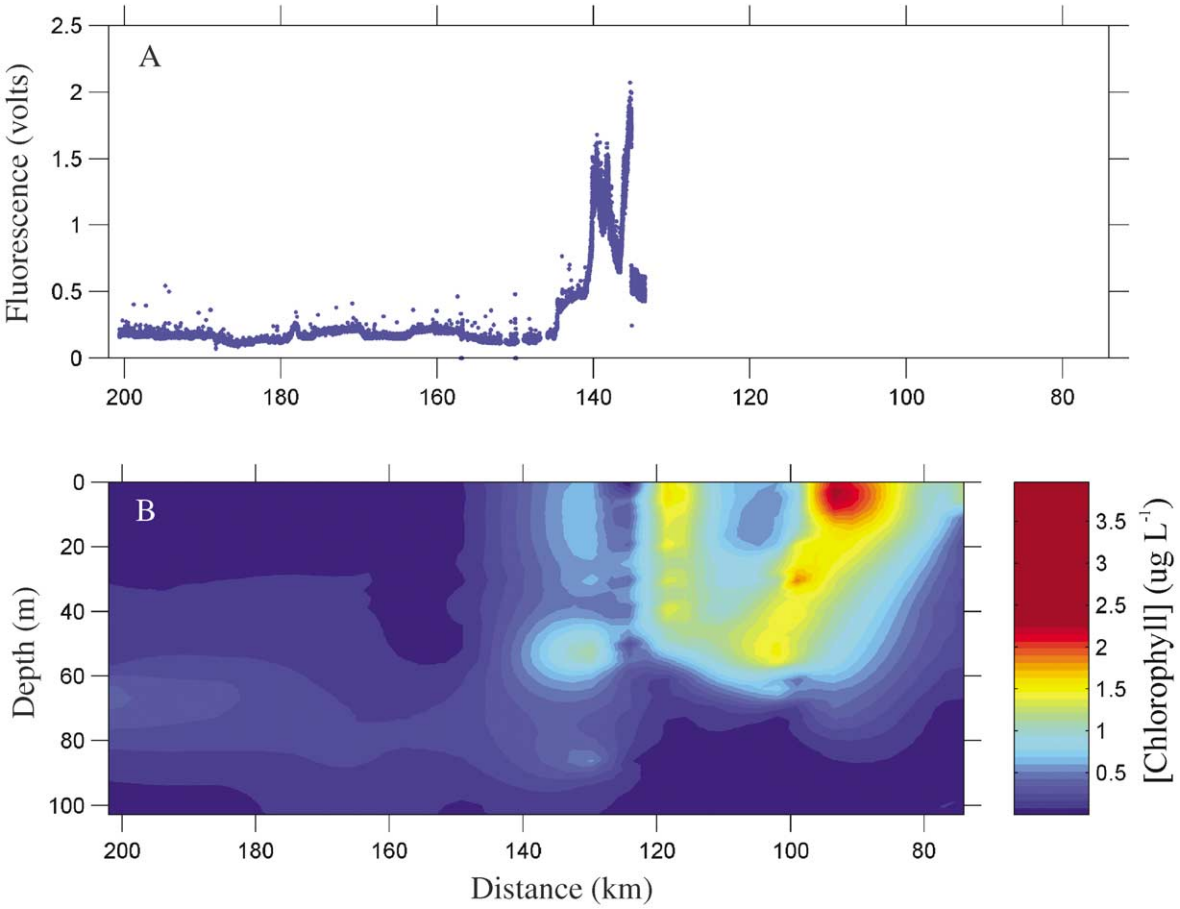


Fig. 3. (A) Surface fluorescence along the offshore portion of CalCOFI line 83.3. (B) Vertical distribution of chlorophyll-*a* concentration (in $\mu g\,l^{-1}$) for the top 100 m along CalCOFI line 83.3, based on data from CalCOFI CTD casts (SIO, 1998). The transect extends from low chlorophyll offshore regions to high chlorophyll onshore waters, with a biologically significant range of chlorophyll concentration from 0.20 to 2.53 $\mu g\,l^{-1}$.

nearshore end of the transect (Station 55; see SIO, 1998).

### 3.2. Distribution and abundance of *N. difficilis*

*N. difficilis* occurred in 13 MOCNESS tows along the transect. The species was counted in quantitative portions of net samples from 6 tows made during RR-9610 and one tow made during KIWI-I. Species abundance ranged from 0.6 to 270.4 $m^{-2}$ for RR-9610 collections, with the highest abundance in the MOC-13 sample collected nearest the coast (Table 2 and Fig. 4). The RR-9610 tow with the lowest abundance was the only daytime tow (MOC-4; Table 2). Abundance in the KIWI-I sample counted was 69.4 $m^{-2}$ for MOC-3 (near MOC-13 from RR-9610; Table 2 and Fig. 4).

The vertical distribution of *N. difficilis* differed somewhat from tow to tow, but was generally delimited by the 8°C and 15°C isotherms. For samples collected during RR-9610, maximum depths of *N. difficilis* varied from 200 to 350 m; the species was absent from the top 50 m in two tows (MOC-6 and MOC-9; Fig. 4). The KIWI-I tow differed, with *N. difficilis* occurring from 50 m through the entire sampled water column (to 500 m) for MOC-3. Maximum concentrations were generally between 75 and 150 m, with

Table 2
Numbers of individuals per 1000 $m^3$ of *N. difficilis* in MOCNESS samples from RR-9610 and KIWI-1

| Net # | Depth interval | MOC-02 | MOC-04 | MOC-06 | MOC-09 | MOC-12 | MOC-13 |
|---|---|---|---|---|---|---|---|
| *RR-9610* | | | | | | | |
| 1 | 500–350 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 350–200 (*350–250) | 0 | *0 | 0 | 0 | 3.78 | 221.24 |
| 3 | 200–150 (*250–150) | 4.13 | *3.80 | 194.65 | 7.74 | 136.29 | NaN (706.10) |
| 4 | 150–100 | 340.91 | 0 | 3479.82 | 1933.65 | 485.42 | 1190.97 |
| 5 | 100–75 | 153.85 | 0 | 581.72 | 328.45 | 1674.51 | 3038.10 |
| 6 | 75–50 | 441.77 | 3.82 | 35.56 | 101.96 | 1395.55 | 2569.76 |
| 7 | 50–25 | 228.35 | 0 | 0 | 0 | 55.031 | 62.81 |
| 8 | 25–0 | 220.10 | 5.90 | 0 | 0 | 39.20 | 22.40 |
| | #/$m^2$ | 43.38 | 0.62 | 199.16 | 107.82 | 110.76 | 270.37 |

| Net # | Depth interval | MOC-03 |
|---|---|---|
| *KIWI-1* | | |
| 1 | 500–350 | 14.120 |
| 2 | 350–200 | 31.78 |
| 3 | 200–150 | 268.37 |
| 4 | 150–75 | 566.18 |
| 5 | 75–50 | 264.29 |
| 6 | 50–25 | 0 |
| 7 | 25–0 | 0 |
| | #/$m^2$ | 69.37 |

Depth intervals for the RR-9610 MOCNESS samples were the same for each tow, except where noted. NaN for net #6 for MOC-13 indicates missing sample; an estimate of the missing value (average of adjoining depth intervals) is given in the parentheses and it was used in computing the #/$m^2$.

significant numbers in the adjacent strata, 50–75 m and 150–200 m (Fig. 4).

### 3.3. Glycolytic enzyme concentrations

The individual, size-corrected (i.e., per unit protein) mean enzyme concentrations for CS, HK, LDH, and PGI differed significantly among the six MOCNESS tow samples, based on an analysis of variance (Table 3, Fig. 5). Comparisons between euphausiids from offshore and mid-Current (samples from MOC-2, -3, -6, and -7 were pooled because of small sample sizes) and near-shore (MOC-11, -12, and -13) regions showed significant differences for all four enzymes (Table 3).

### 3.4. Molecular variation of genes encoding PGI and LDH

DNA sequences were determined for 7 individuals for a 327 bp coding region of PGI (five using cDNA and two using genomic DNA; Fig. 6). Four individuals were identical in DNA sequence; the other three sequences were distinguished by one silent substitution (i.e., a change in the nucleotide sequence that does not alter the amino acid or protein sequence) and five heterozygous sites, of which one was also silent (Fig. 6). Twenty individuals were sequenced for a 349 bp coding region of LDH (12 using cDNA and 8 using genomic DNA; Fig. 7). Seventeen of the individual sequences were identical; one sequence differed at one heterozygous site, and two sequences each had one site of silent substitution (Fig. 7).

### 3.5. Population genetic analysis

Molecular population genetic diversity and structure were characterized. They were based on DNA sequence variation of a 350 bp region of mtCOI for a total of 149 individual *N. difficilis* from 11 net tows (10 MOCNESS and one bongo

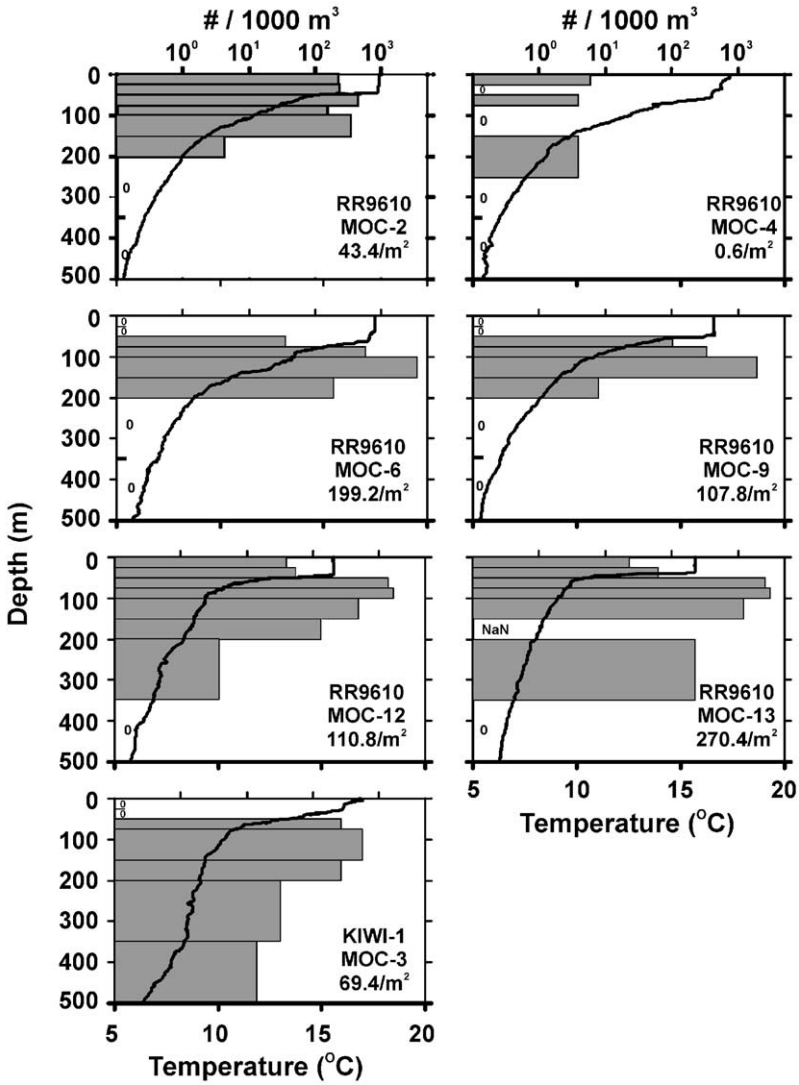


Fig. 4. Vertical distribution of *N. difficilis* in relation to water column temperature. Samples were analyzed from MOCNESS tows at six stations along CalCOFI line 83.3 collected during RR-9610 (in October 1996). MOC-2, -6, -12, and -13 were taken at night; MOC-4 during the day; and MOC-9 at dusk. Also shown is the vertical distribution for one collection during KIWI-1 (in August 1997); MOC-3 was taken during the daytime. Both day- and nighttime tows show maximum concentrations in the depth stratum between 100 and 200 m. (Note: bars show abundances in Log10 numbers per 1000 $m^3$; total depth-integrated abundances are given for each figure in numbers per $m^2$).

net) during RR-9610 (Table 4). There were 46 mtCOI haplotypes among the 149 individuals, of which three were abundant (24%, 26%, and 37% of the individuals) and two were nearly ubiquitous. Thirty-nine haplotypes were unique (i.e., occurred only once in the samples) and were grouped in one haplotype class or "pool" for statistical evaluation (Table 4 and Fig. 8).

MtCOI sequence variation was moderately high; haplotype diversity, *h*, was 0.794. Among the 10 RR-9610 MOCNESS tows from which *N. difficilis* was assayed for mtCOI, there was some

Table 3
Statistical evaluation of glycolytic enzyme concentrations for CS, HK, LDH, and PGI by ANOVA

| Comparison | Enzyme | | | |
|---|---|---|---|---|
| | CS $F$ value ($P$) | HK $F$ value ($P$) | LDH $F$ value ($P$) | PGI $F$ value ($P$) |
| Among all tows | 11.14 ($P<0.0001$) | 23.47 ($P<0.0001$) | 16.59 ($P<0.0001$) | 15.04 ($P<0.0001$) |
| Between regions | 29.62 ($P<0.0001$) | 74.15 ($P<0.0001$) | 56.24 ($P<0.0001$) | 42.54 ($P<0.0001$) |

Variation was evaluated among the six MOCNESS tow samples and between grouped samples for offshore and mid-Current (MOC-2, -3, -6, and -7) vs. nearshore (MOC-11, -12, and -13) regions.

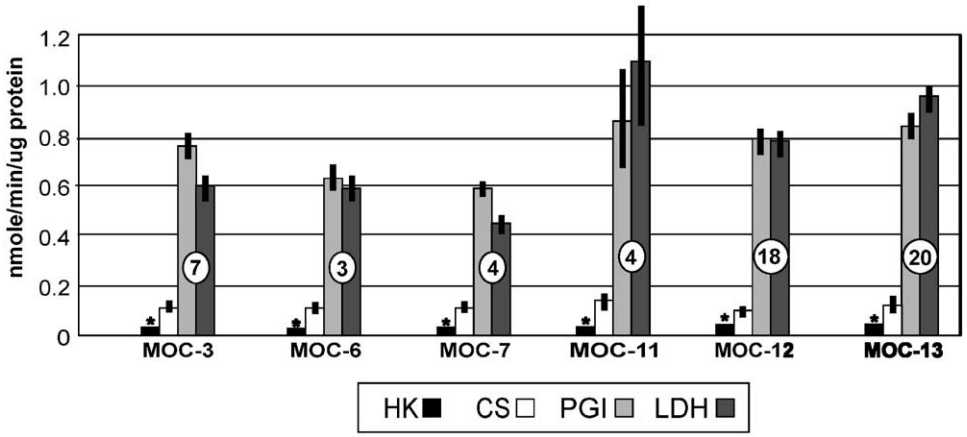


Fig. 5. Enzyme concentrations for central metabolic enzymes, CS, HK, LDH, and PGI, from individual *N. difficilis* collected in MOCNESS tows along CalCOFI line 83.3. Concentrations are given as rates ($n$ mol min$^{-1}$ μg protein) in the kinetic assays; bars indicate mean values; vertical lines are ± standard error; an asterisk (*) indicates the standard error is too small to display; numbers in circles are sample sizes.

tow-to-tow variation in haplotype frequencies ($P = 0.0290 \pm 0.0053$ by Monte Carlo chi-square test; Fig. 9). Variation at two sites of common substitutions (see Fig. 8) revealed significant variation among samples, including allele frequencies at bp #9 ($P = 0.0180 \pm 0.0042$) and at bp #240 ($P = 0.0060 \pm 0.0024$; Table 5).

Samples collected during RR-9610 were grouped according to temperature and salinity domains in order to examine the concordance between population genetic structure and physical structure of the ocean. They were grouped into offshore (B-110, MOC-1, -2, and -3), mid-Current (MOC-6, -7, -9, and -10), and nearshore (MOC-11, 12, and -13) regions based on T/S signatures (Fig. 2). Comparisons among the three groups were not significant for any character. However, pooled samples from the nearshore and offshore domains differed significantly for allele frequencies at bp #9 ($P = 0.0200 \pm 0.0044$) and bp #240 ($P = 0.0260 \pm 0.0050$), but not for mtCOI haplotype frequencies ($P = 0.3690 \pm 0.0153$; Table 5).

An analysis of molecular variation (AMOVA, Excoffier et al., 1992) partitioned variance between among-sample (4.25% of the variance) and within-sample (95.75% of the variance) components. Nested AMOVAs to assess genetic variance among regional populations revealed no significant patterns: comparison among nearshore, mid-Current, and offshore groupings explained a small-but-significant portion of the variance (0.12%; $P<0.0323$); comparison between near-

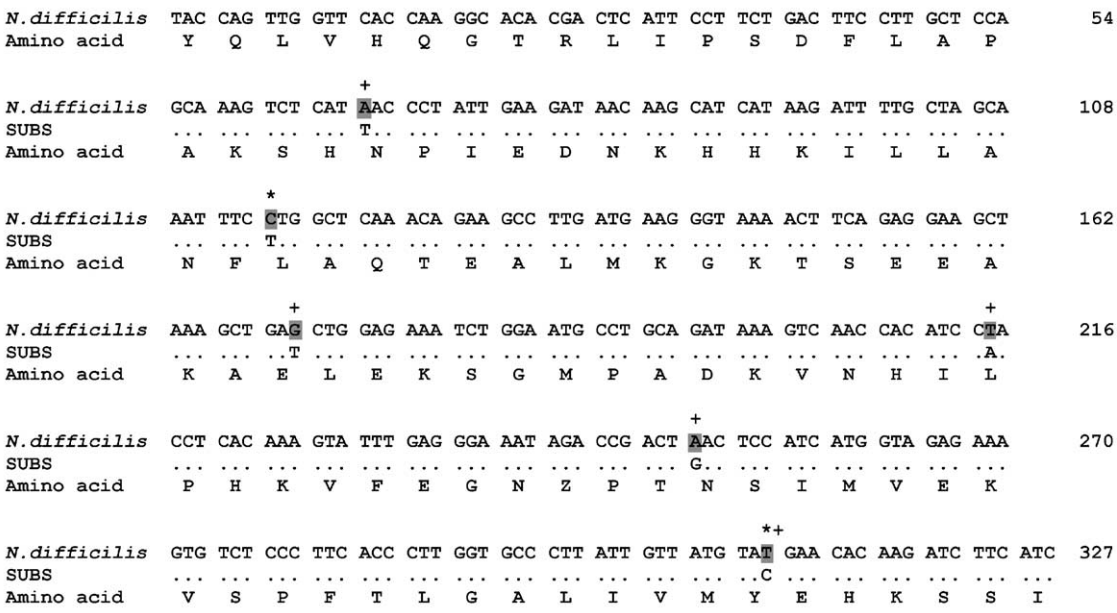


Fig. 6. DNA sequence of the most frequent allele for a 327 bp region of the gene encoding phosphoglucose isomerase (PGI) for *N. difficilis*. Positions of variable bases are shown on the line below (SUBS); all variations occurred only once. Silent substitutions are indicated by an asterisk (*); heterozygous sites are indicated by a plus (+); numbers at end are base positions. A period (.) indicates that the base is identical to the most common sequence.

shore and offshore groupings explained 2.11% of the variance ($P<0.0323$; Table 5).

### 3.6. Silhouette analysis

The relative abundance and estimated biomass of zooplankton functional groups varied markedly among the four RR-9610 and two KIWI-I MOCNESS tows examined (Fig. 10). Analysis of the four samples collected over the top 100 m of the water column showed a numerical predominance of copepods at all stations. Other numerically important groups included spherical bodies (eggs and radiolarians) and, during the day and dusk samples, molluscs (mostly shelled pteropods). More than half of the biomass for the two nighttime and one dusk samples was composed of salps and medusae, which were also abundant in the daytime sample. During nighttime tows, euphausiids were a small but consistent fraction of the abundance (5%) and biomass (10–15%); in the dusk sample, euphausiid abundance was similar but biomass was negligible; euphausiids were rare in daytime tows.

### 3.7. Bioacoustical backscattering measurements

There was a dramatic shift in the backscattering in the upper 100 m between night and day during RR-9610 (Fig. 11). Values at night were approximately −74 dB or about 10 dB higher than during the day (Table 6). The shift in the intensity at dawn and dusk was rapid, requiring only about 30 min. At night, volume backscattering was most intense between depths of 25 and 75 m, in the region of a sharp thermocline (Figs. 4 and 11). Along the offshore portion of the transect, the scattering layer intensified and increased in depth interval closer to shore (near MOC-9; Fig. 11). This trend was evident despite increasing winds and seas that created a surface bubble field, which can absorb some of the outgoing ping energy and reduce scattering at depth. Similar backscattering levels were observed during KIWI-1 in inshore areas of

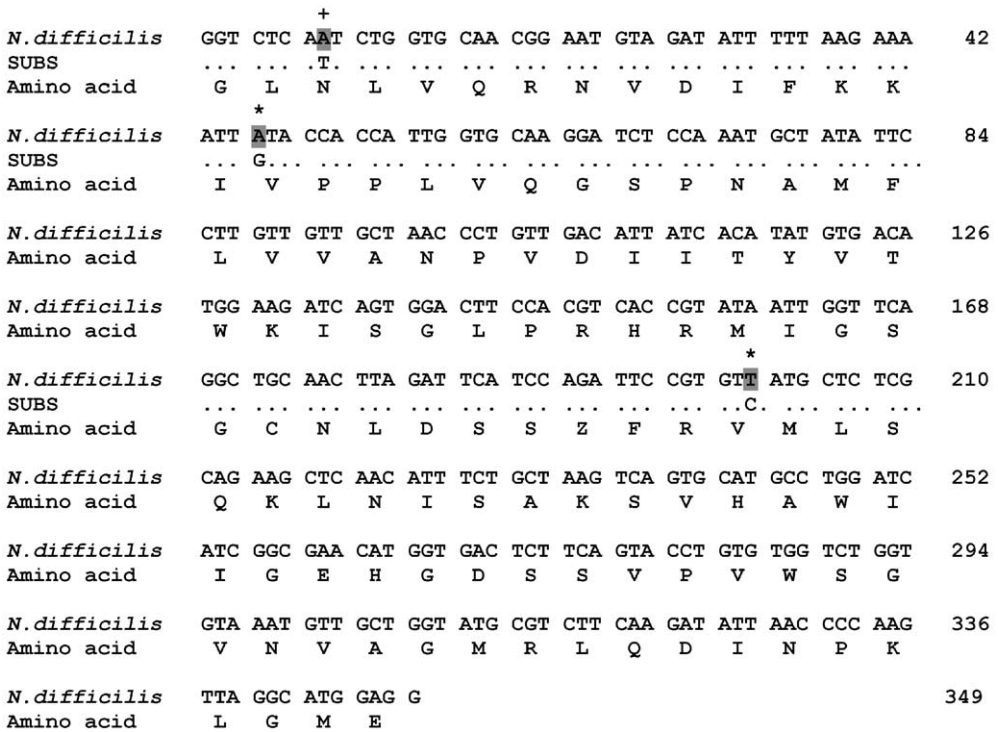


Fig. 7. DNA sequence of a 349 bp region of the gene encoding the enzyme lactate dehydrogenase (LDH) for *N. difficilis*. Variable sites among 20 individuals are shown on the line below (SUBS); all variations occurred only once. Silent substitutions are indicated by an asterisk (*); heterozygous sites are indicated by a plus (+); numbers at end are base positions. A period (.) indicates that the base is identical to the most common sequence.

Table 4
MtCOI haplotype abundances for *N. difficilis* collected in MOCNESS tows and one bongo net tow along CalCOFI line 83.3 during RR-9610

| Sample RR-9610 | Haplotype | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | Pool | Totals |
| Bongo | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 3 |
| MOC-1 | 2 | 0 | 0 | 0 | 2 | 0 | 1 | 5 |
| MOC-2 | 0 | 0 | 4 | 0 | 11 | 0 | 5 | 20 |
| MOC-3 | 0 | 0 | 3 | 0 | 3 | 0 | 3 | 9 |
| MOC-6_ | 0 | 1 | 1 | 0 | 3 | 1 | 8 | 14 |
| MOC-7 | 3 | 0 | 5 | 1 | 4 | 1 | 6 | 20 |
| MOC-9_ | 0 | 1 | 4 | 1 | 8 | 0 | 5 | 19 |
| MOC-10 | 0 | 0 | 3 | 0 | 7 | 0 | 4 | 14 |
| MOC-11 | 2 | 0 | 0 | 0 | 10 | 1 | 2 | 15 |
| MOC-12 | 0 | 0 | 7 | 1 | 5 | 0 | 2 | 15 |
| MOC-13 | 0 | 0 | 8 | 1 | 3 | 0 | 3 | 15 |
| Total | 7 | 2 | 35 | 4 | 55 | 3 | 39 | 149 |

Unique haplotypes were treated as one (Pool), to reduce the statistical bias associated with small sample size.

```
                        *
N.difficilis  TTA TTA TTA GGA AGA GGA CTC GTA GAA AGA GGA GTC GGA ACT GGC TGA ACA    51
SUBS          ... C.. ..G ... ... ... ... ... T.. ... ... ... ... ... ... ... ...
N                       53                    2

N.difficilis  GTT TAT CCT CCT TTA TCA GCT GGA ATT GCT CAT GCA GGA GCT TCA GTA GAT    102
SUBS          ... ... ... ..G ... ... ... ... ..C ... ... ... ... ... ... ... A.C
N                                                                             9

N.difficilis  ATA GGA ATT TTT TCT TTA CAC ATT GCA GGA GCT TCT TCT ATT TTA GGA GCT    153
SUBS          ... ... ... ... ... C.. ..T ... .G. ... ... ... ... ... .C. ... ...
N

N.difficilis  GTA AAT TTT ATT ACT ACT GTA ATT AAT ATA CGA GCA GCA GGT ATA ACA ATA    204
SUBS          ..T ... ..C .CC ... ... ... ... ... ... ... ... ... C.. ... ... ..T ...
N               2          22                                                  3

                                                        *
N.difficilis  GAC CGA ATT CCA CTT TTT GTT TGA TCG GTA TTT ATC ACA GCA ATT TTA CTA     255
SUBS          ... ... ..C .T. ..C ..C ..C ... ..A ... .C. ..T ... .T. ..C CC. ...
N                                     2                   54

N.difficilis  TTA TTA TCT CTT CCA GTT TTA GCT GGA GCT ATC ACA ATA CTA TTA ACA GAT     306
SUBS          .C. .C. ... ..C .T. ..C CC. .G. ... ... ... ... ... T.. .C. ... ...
SUBS          ... .A. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
N                                   2                               2

N.difficilis  CGT AAT TTA AAC ACA TCT TTT TTT GAT CCC GCT GGA GGG GGA                 350
SUBS          ... T.. ... G.T T.T ... CC. C.. ... .T. .T. ... ... ..C
N                       2
```

Fig. 8. DNA sequence of a 350 bp region of mitochondrial cytochrome oxidase I (mtCOI) for *N. difficilis*. The most frequent sequence among 149 individual euphausiids is shown; positions of variable bases (SUBS) and numbers of occurrences of each substitution among the sequenced individuals (N) are also shown. Two sites of common substitutions, at base-pairs #9 and #240, are indicated by asterisks (*); numbers at end are base positions. A period (.) indicates that the base is identical to the most common sequence.

the survey region (Table 6; Fig. 12), although the daytime levels were not reduced.

Although copepods were numerically dominant in the net samples examined from RR-9610 and KIWI-1, they rarely accounted for much of the estimated volume backscattering in the RR-9610 samples (Table 7). The increase in backscattering at night was caused largely by increases in the contributions from euphausiids/decapods, medusae, and siphonophores. Estimated volume backscattering in the nighttime KIWI-1 tow (MOC-2) was accounted for mostly by euphausiids/decapods and siphonophores; in contrast, copepods and pteropods were the dominant contributors for the daytime tow (MOC-3).

The total estimated backscatter was compared to that measured over the same portion of the water column sampled by each MOCNESS net (Fig. 12). For 18 of the 24 samples, estimated volume backscattering values account for most of the backscattering observed. The zooplankton collected in three RR-9610 and three KIWI-1 samples do not begin to account for the measured backscattering. Excluding these six samples, functional regression performed on the remaining samples provided a significant result ($r^2 = 0.67$; $P < 0.05$).

## 4. Discussion

Zooplankton, like other organisms, are capable of complex responses to environmental variability, including both physical (e.g., temperature, salinity,

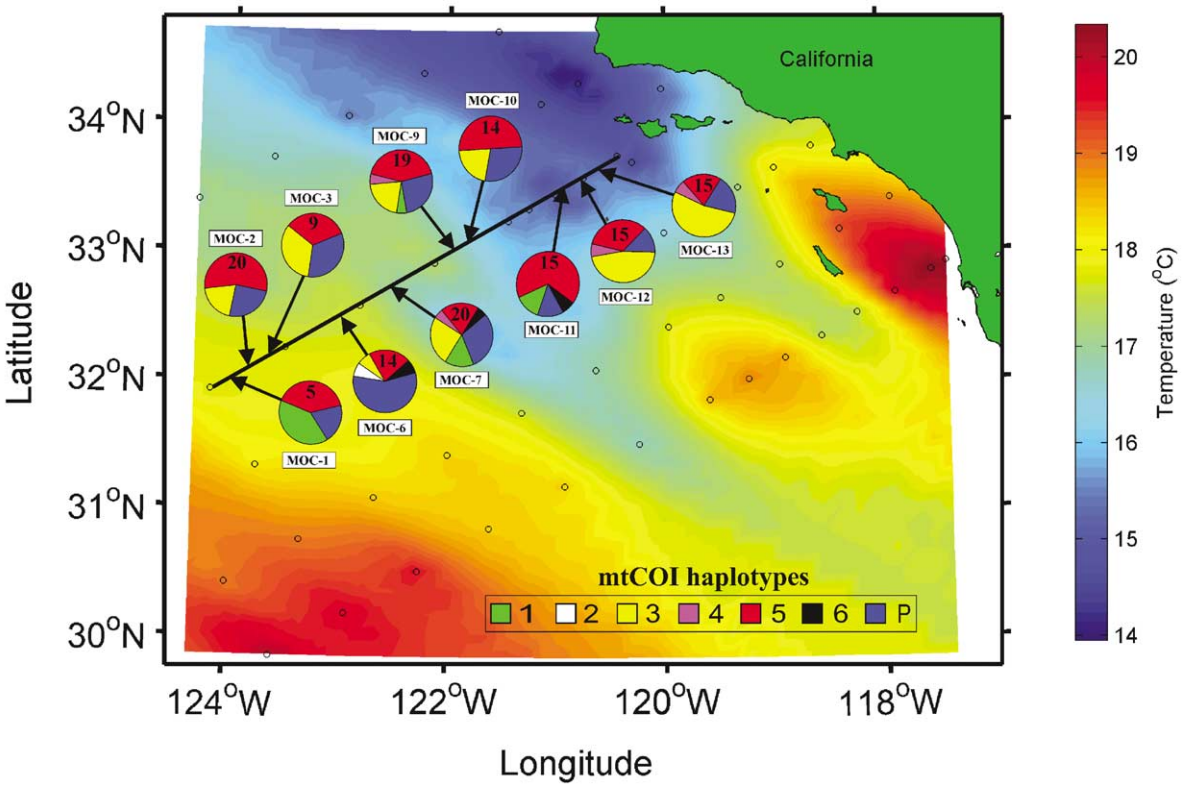


Fig. 9. MtCOI haplotype frequencies of the euphausiid *N. difficilis* in samples collected along CalCOFI line 83.3. Numbers in boxes are the MOCNESS tows (see Table 1 for detailed collection information); numbers on the pies are sample sizes.

Table 5
Statistical analysis of population genetic structure based on mtCOI sequence variation of *N. difficilis* collected during RR-9610

| | All RR-9610 samples | Three-region | Nearshore vs. offshore |
|---|---|---|---|
| (A) *Chi-square test* | | | |
| MtCOI haplotypes | $P = 0.029 \pm 0.005$* | $P = 0.256 \pm 0.014$ | $P = 0.369 \pm 0.015$ |
| base-pair \#9 | $P = 0.018 \pm 0.004$* | $P = 0.068 \pm 0.008$ | $P = 0.020 \pm 0.004$* |
| base-pair \#240 | $P = 0.006 \pm 0.002$** | $P = 0.124 \pm 0.010$ | $P = 0.026 \pm 0.005$* |
| (B) *Nested analysis of molecular variation* | | | |
| Variance among groups | | $V_A = 0.12\%$, $P < 0.0323$* | $V_A = 2.11\%$, $P < 0.0323$* |
| Variance among populations within groups | | $V_B = 4.16\%$, $P < 0.0323$* | $V_B = 8.62\%$, $P < 0.0323$* |
| Variance within populations | | $V_C = 95.72\%$, $P < 0.677$ | $V_C = 89.28\%$, $P < 0.807$ |

*$P < 0.05$ and **$P < 0.01$.

(A) Results of Monte Carlo chi-square evaluation of geographic patterns of frequencies of mtCOI haplotypes and common substitutions (bp \#9 and \#240) using the restriction enzyme analysis program (REAP; Roff and Bentzen, 1989). Values indicate statistical significance, $P \pm$standard deviation. (B) Nested analysis of variance using the analysis of molecular variation (AMOVA; Excoffier et al., 1992). Values indicate % of the variance and statistical significance ($P$). Three-region (i.e., nearshore, mid-Current, and offshore) comparisons were done by pooling or grouping RR-9610 MOCNESS tow samples as follows: nearshore samples (MOC-11, -12, and -13), mid-Current (MOC-6, -7, -9, and -10), and offshore samples (B-110, MOC-1, -2, and -3).

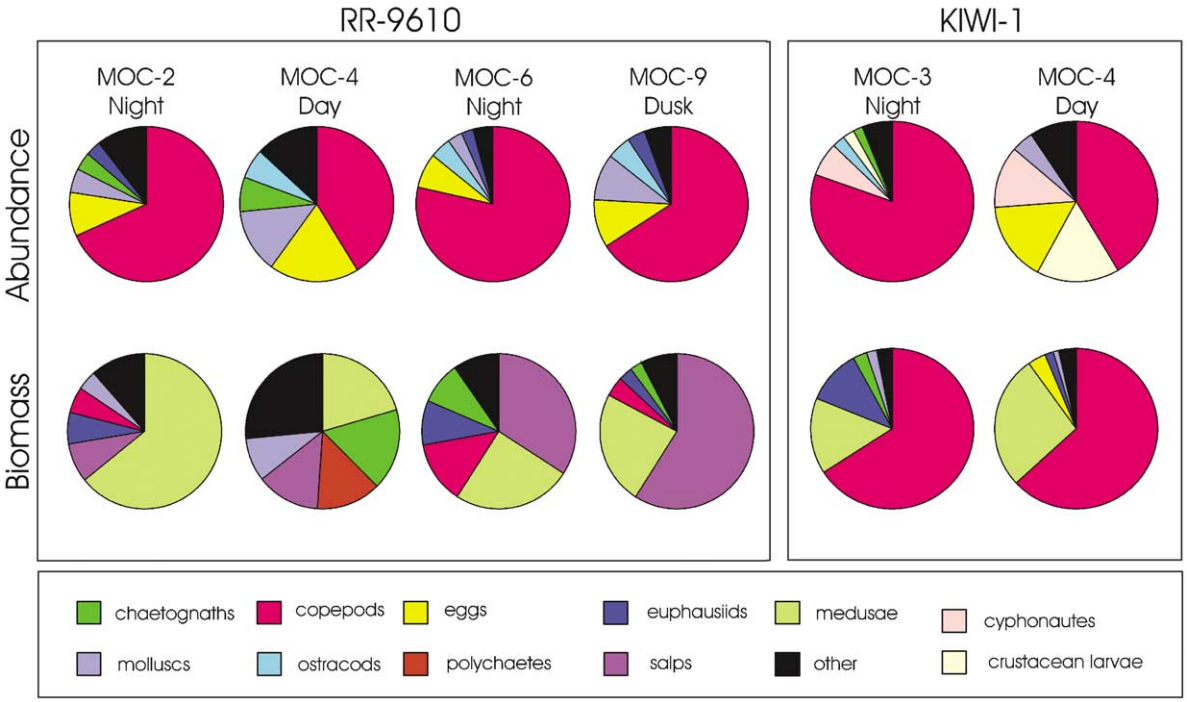


Fig. 10. Biomass fractions of diverse taxa determined from silhouette analysis from depth stratified samples in four MOCNESS tows made during RR-9610 and two tows during KIWI-I. Each pie diagram represents pooled samples from the top four MOCNESS nets, which together sampled the 0–100 m depth range. The data are useful to determine the absolute and relative abundance and biomass of euphausiids and other groups in the zooplankton assemblage. These data are used to evaluate the importance of each group for the bioacoustical backscatter observations.

current flow, illumination, turbulence) and biological (e.g., nutritional richness, predator and prey abundances, population density) properties. Possible responses by zooplankton include changes in physiological condition of individuals, changes in the genetic make-up of populations, and changes in relative species' abundance of the zooplankton assemblage. Our goal was to examine the range of possible responses for *N. difficilis* along an environmental gradient ranging from the biologically productive, cool coastal waters entrained in the Southern California Bight eddy to the biologically poor, warmer offshore waters. Our hypothesis is that the recirculation cell of the Southern California Bight will retain euphausiid populations in nearshore waters and result in a spatial pattern of individual (biochemical and molecular), population (distribution, abundance, and genetic structure), and biomass (species abundance) characteristics that are concordant with mesoscale variability. We recognize a number of shortcomings in our multi-faceted data set, including: small sample sizes, some technical difficulties, limited spatial resolution of the field sampling, and restricted overall range of the study area. However, we continue to be optimistic about the prospects for integrated individual, population, and ecosystem analysis in order to understand and predict the distribution, abundance, and dynamics of zooplankton.

For the October 1996 CalCOFI Survey on the R/V *Revelle* (Cruise RR-9610), the dynamic height topography confirmed the presence of a coastal recirculation pattern, consistent with the long-term average flow fields calculated from decades of CalCOFI hydrographic data (see Lynn et al., 1982). Contour mapping of the dynamic height anomaly also showed two mesoscale eddies that

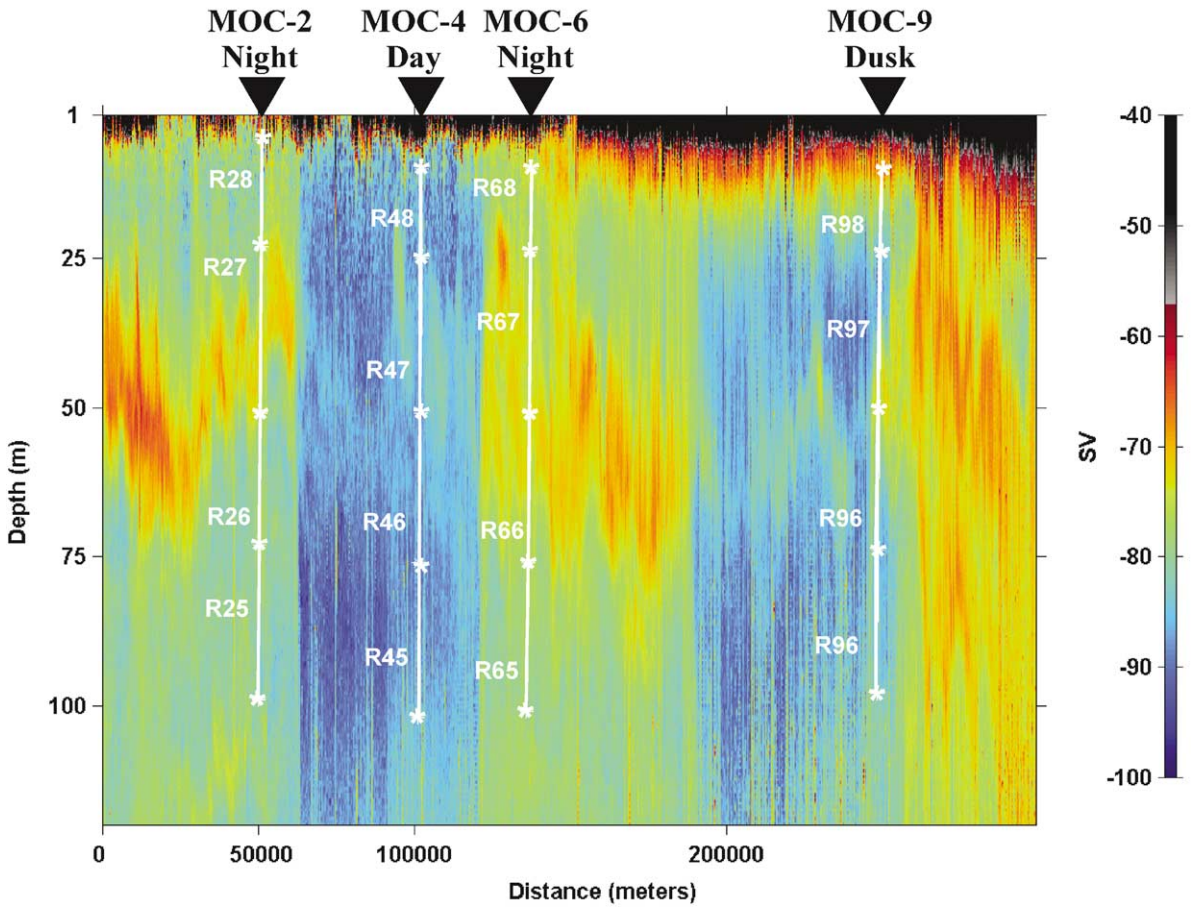


Fig. 11. Volume backscattering at 120 kHz along the section running between MOC-1 and MOC-9 (see Fig. 2A). The vertical lines marked by asterisks represent the positions of the MOCNESS tows used to compare the zooplankton taxonomic data with the backscattering data. Each net is designated with a letter and number (i.e. R25: R = RR-9610, 2 = MOC-2, 5 = Net 5).

were farther offshore and embedded in a southward-flowing current (Fig. 1A). Temperature, salinity, density, and oxygen all showed characteristic gradients in conditions along the transect (see SIO, 1998). In October 1996, there were marked along- and cross-shelf gradients in temperature (Fig. 1B). The cross-shelf temperature gradient appeared to result from influxes of warm water from the south into the sampled domain. The oceanographic conditions of the sampled domain were thus suitable for testing our hypothesis.

*N. difficilis* occurred in some nets of all MOCNESS tows taken along line 83.3 and was most abundant in nearshore waters, as expected from previous studies of habitat preference and characteristic distribution (Brinton, 1962). The species' vertical distribution in the RR-9610 tows and the KIWI-I tow did not change appreciably, with highest concentrations in the 100–200 m depth interval (Fig. 4). Consistent with published reports (e.g., Brinton, 1962), the species seemed to exhibit little diel vertical migration.

Temperature has profound effects on organisms, including wide-spread impact on the enzymatic machinery driving metabolism, growth, and reproduction. Enzymatic activities, reaction rates, and reaction equilibria may vary with temperature. In particular, changes in temperature may

Table 6
Mean volume backscattering values for the RR-9610 and Kiwi-1 acoustic sections

| Time of day | Number of observations | Mean $S_v$ |
|---|---|---|
| *RR-9610* | | |
| Night 1 | 66560 | −74.42 |
| Day 1 | 52240 | −85.50 |
| Night 2 | 68800 | −74.38 |
| Day 2 | 41600 | −83.73 |
| Night 3 | 19040 | −70.07 |
| *KIWI-1* | | |
| Night—section 1 | 5648 | −74.48 |
| Day—section 1 | 3248 | −77.37 |
| Day—section 2 | 3248 | −70.86 |

Mean values were computed by summing the $s_v$ values in the appropriate time period, dividing by the number of observations, and then converting the value to $S_v$. The RR-9610 acoustic data were recorded at ~30 s intervals and had 1-m depth bins; the Kiwi-1 data were recorded at ~30 s intervals and had 5-m depth bins.

affect the Michaelis–Menten constant $K_m$ (the concentration of substrate at which velocity = $\frac{1}{2}$maximum velocity) of important metabolic enzymes, with higher temperatures resulting in a higher $K_m$ (see Hochachka and Somero, 1984). However, observed variation in enzyme concentration may be the result of many different processes, including biochemical (i.e., increased rates of protein synthesis) and genetic (i.e., DNA sequence variation of the encoding genes). Thus, an individual organism may undergo physiological changes to adapt to a new temperature by producing more enzyme or by switching to a different allelic form (i.e., allozyme) of the enzyme (see Gillespie, 1991). Both responses have been observed: a large body of work on the killifish *Fundulus heteroclitus* has elegantly demonstrated a direct tie between LDH allozymes and temperature-dependent development time (e.g., Powers and Place, 1978; Place and Powers, 1984). On the other hand, physiological condition, growth, and survivorship have been correlated with concentrations of selected enzymes—CS, HK, LDH, and PGI—that control fundamental metabolic processes for individual zooplankton (Clarke et al., 1992; Clarke and Walsh, 1993).

There is justification for hypothesizing that PGI and LDH concentrations may result from genetic differences (i.e., allozymic or DNA sequence variation) of the encoding genes. Frequencies of PGI allozymes have been shown to co-vary significantly with temperature for the butterfly *Colias* (Watt et al., 1983), the sea anemone *Metridium* (Hoffmann, 1983), and many other organisms (see Gillespie, 1991). For euphausiids in particular, considerable allozymic variation of enzymes has been observed in the Antarctic krill *Euphausia superba* (Kuehl and Schneppenheim; 1986; Marquez, 1987; Fevolden and Schneppenheim, 1989), but not in other species (Sundt and Fevolden, 1996).

Concentrations of all four enzymes—CS, HK, LDH, and PGI—showed significant sample-to-sample and regional variation for *N. difficilis* collected along the RR-9610 transect: size-corrected enzyme concentrations were higher in individuals collected from nearshore waters and lower in offshore waters (Table 2, Fig. 5). The low levels of DNA sequence variation—as well as the predominance of silent and heterozygous substitutions—for the genes encoding PGI and LDH suggest that the observed differences may have resulted from physiological response by individuals (e.g., differences in rates of protein synthesis), not from genetic differences between populations (i.e., DNA sequence variation of the encoding genes).

Our results must be interpreted cautiously, since we almost certainly underestimated levels of molecular variation of the enzyme-encoding genes. Our sample sizes were small (seven individuals for PGI and 20 individuals for LDH) and our assay success rate was low: DNA sequencing was attempted for 37 individuals for PGI and 62 individuals for LDH. It is likely that our PCR and sequencing primers were designed from too few individuals, resulting in the successful amplification of only those individuals sharing the same priming site sequence (see Bucklin, 2000). It also should be noted that nuclear genes, in general, can present difficulties in DNA sequence analysis. Since the two homologous strands are sequenced together, heterozygous sites are identified by "double peaks" in the DNA sequence

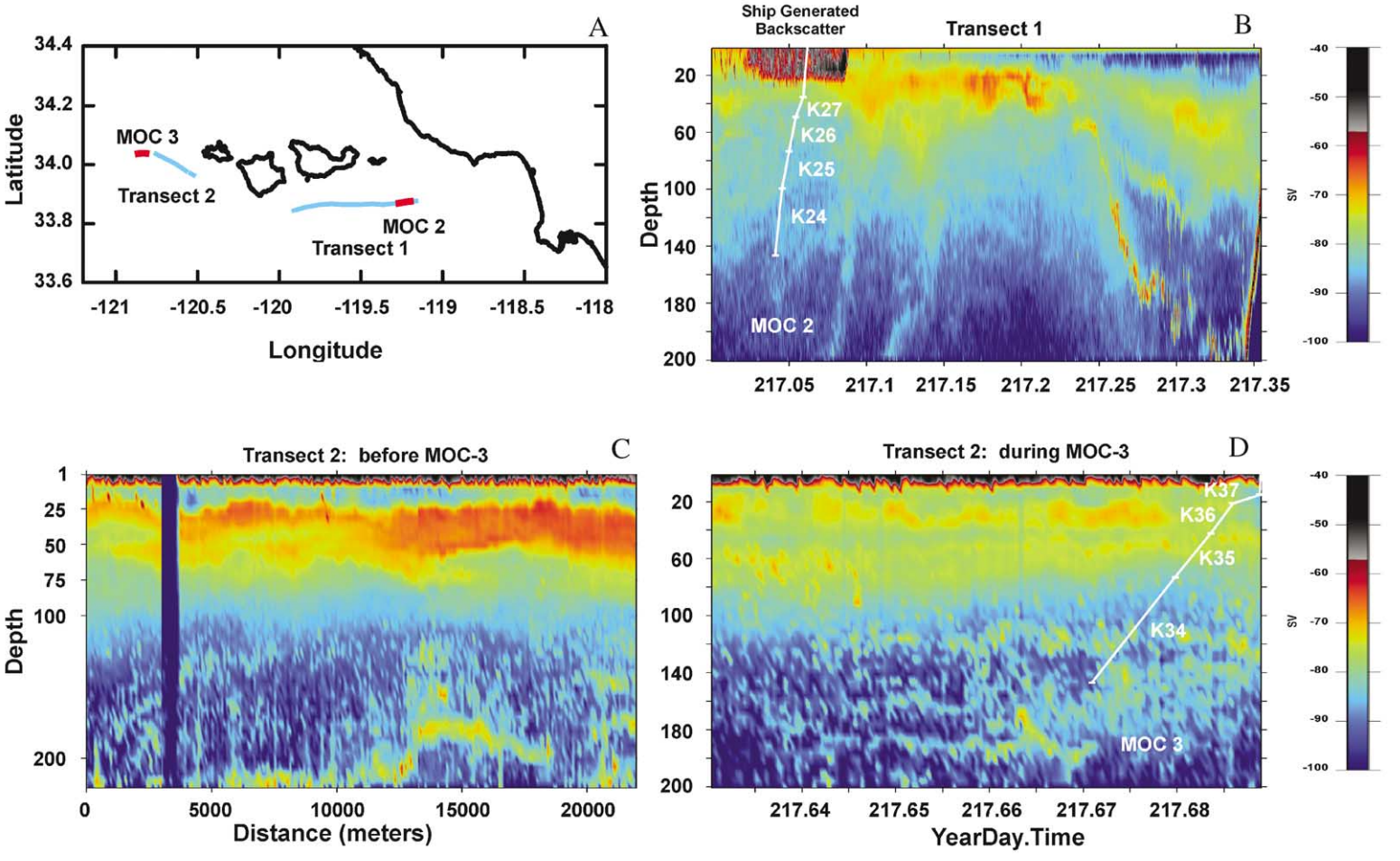


Fig. 12. (A) Position of the bioacoustical transects and MOCNESS tows during KIWI-1 (August 1997). (B) Backscattering at 120 kHz along transect 1, with total length of 70 km.The vertical line marked by asterisks represents the position of MOC-2. Each net is designated with a letter and number (see caption of Fig. 11). (C) and (D) Backscattering at 120 kHz along transect 2, with total length of 20 km, including position of MOC-3 (shown in (D)).

Table 7
Estimated volume backscattering ($S_v$ dB) contributions of the taxa collected on a MOCNESS tows off Southern California during RR-9610 (in October 1996) and KIWI-1 (in August 1997)

| Moc# /Net\# | Meaured $S_v$ | Estimated $S_v$ | Amphipod | Chaetognath | Copepod >2.5 mm | Copepod <2.5 mm | Crustacean larvae | Euphausiids /decapods | Fish | Pteropods | Medusa | Ostracods | Salps | Sipho bracts | Sipho necto-phores | Spheres egg<1 mm |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Percent of total $S_v$ contributed by taxa in Revelle 9610 MOCNESS samples* | | | | | | | | | | | | | | | | |
| 2.5 | −80.73 | −82.95 | 0.55 | 0.67 | 2.30 | 0.67 | 0 | *21.77* | 1.78 | 0.04 | 64.30 | 0.03 | 0 | 7.77 | 0 | 0.12 |
| 2.6 | −78.00 | −81.95 | 0.25 | *8.54* | 0.82 | 3.93 | 0 | *73.97* | 6.05 | 0 | 0 | 0.01 | 0 | 0 | 0 | *6.43* |
| 2.7 | −74.47 | −85.14 | 2.85 | 0.96 | 1.88 | 4.01 | <0.01 | 4.75 | *26.53* | 0.34 | 0 | <0.01 | 0.37 | *57.49* | 0.59 | 0.20 |
| 2.8 | −76.14 | −76.42 | 4.11 | 0.35 | 0.91 | 0.66 | <0.001 | 4.99 | 0 | *14.94* | *22.12* | <0.01 | 0.74 | *50.87* | 0.28 | <0.01 |
| 4.5 | −83.66 | −85.01 | *18.23* | *5.54* | 0.32 | 0.05 | <0.01 | 0.96 | 0.32 | *12.64* | *7.95* | 0.03 | 0 | *53.93* | 0 | 0.03 |
| 4.6 | −85.87 | −83.23 | *9.51* | 1.75 | 1.06 | 0.13 | <0.01 | 3.08 | 0.85 | *23.47* | *25.27* | 0.07 | 0.18 | *34.59* | 0 | 0.03 |
| 4.7 | −85.92 | −84.38 | *15.60* | 0.89 | 0.30 | 0.27 | <0.01 | 1.70 | 1.36 | 0.54 | *5.92* | 0.02 | <0.001 | *72.26* | 0 | 1.13 |
| 4.8 | −82.76 | −84.87 | *18.59* | 4.67 | 0.53 | 1.97 | <0.01 | 0.30 | 2.56 | 8.08 | 0.15 | <0.01 | 0.12 | *60.47* | 2.53 | 0.01 |
| 6.5 | −78.19 | −83.98 | 0.02 | 1.55 | 3.58 | 3.46 | 0 | *57.09* | *20.67* | 0 | 1.27 | 0.21 | 0.04 | *12.10* | 0 | 0.01 |
| 6.6 | −74.63 | −85.70 | 6.82 | *6.66* | 4.62 | *8.55* | <0.01 | *14.22* | *12.45* | <0.01 | 4.15 | 0.10 | 0.44 | *39.16* | 2.81 | 0.02 |
| 6.7 | −75.07 | −80.34 | 2.36 | *9.87* | 0.49 | 1.14 | <0.001 | 4.47 | 1.55 | 5.10 | *6.10* | <0.01 | 0.27 | *59.79* | *8.85* | <0.01 |
| 6.8 | −76.63 | −79.54 | *18.89* | *9.51* | 0.53 | 0.34 | 0 | 3.90 | 3.49 | 1.67 | *38.83* | <0.01 | 0.40 | *20.62* | 1.80 | <0.01 |
| 9.5 | −83.93 | −84.22 | *5.83* | 0.57 | 1.65 | 1.42 | <0.001 | *16.00* | *19.02* | 0 | *51.65* | 0.04 | <0.01 | 2.81 | 0.99 | 0.02 |
| 9.6 | −78.07 | −85.68 | 2.10 | *6.19* | 1.64 | *12.78* | 0 | *6.24* | *16.65* | 0.12 | *43.91* | 0.12 | <0.001 | *8.48* | 1.21 | 0.57 |
| 9.7 | −77.65 | −79.86 | 0.96 | *6.31* | 0.09 | 1.15 | <0.001 | 0.68 | 3.62 | *8.14* | *40.60* | <0.01 | 2.85 | *35.59* | 0 | 0.02 |
| 9.8 | −74.02 | −75.22 | *5.24* | 1.93 | 0.07 | 0.14 | <0.001 | 3.07 | 0 | *72.32* | 4.26 | <0.01 | 0.10 | *11.55* | 0.07 | 1.27 |

| Moc# /net\# | Measured $S_v$ | Estimated $S_v$ | Amphipod | Chaetognath | Copepod >2.5 mm | Copepod <2.5 mm | Crustacean larvae | Euphausiids /decapods | Fish | Pteropods | Ostra-cods | Sipho bracts | Sipho necto-phores | Spheres |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Percent of total $S_v$ contributed by taxa in Revelle Kiwi leg 1 MOCNESS samples* | | | | | | | | | | | | | | |
| 2.4 | −85.11 | −84.88 | 2.44 | *8.84* | *8.38* | 0.65 | <0.001 | *17.14* | 0 | 0.01 | 0.07 | *57.93* | 4.52 | 0.0477 |
| 2.5 | −83.82 | −83.10 | 1.53 | 0.32 | 1.87 | 0.35 | <0.001 | *19.32* | 2.82 | 0.00 | 0.03 | *70.62* | 3.13 | 0.0135 |
| 2.6 | −81.70 | −83.72 | *5.87* | 0.54 | *18.64* | 2.88 | <0.001 | *34.69* | 0 | 0.02 | 0.06 | *34.53* | 2.75 | <0.01 |
| 2.7 | −78.43 | −81.10 | *9.64* | 0.99 | *19.36* | *5.00* | 1.19 | *55.24* | *8.09* | 0.05 | 0.03 | 0 | 0 | 0.4043 |
| 3.4 | −82.05 | −84.84 | 1.64 | 0.30 | *93.52* | 1.68 | 0 | 1.20 | 0 | 0.46 | <0.001 | 1.11 | 0 | 0.0751 |
| 3.5 | −76.30 | −93.73 | 3.94 | 0.10 | *59.73* | *28.35* | 1.01 | 2.51 | 0 | 0.48 | <0.01 | 2.65 | 0 | 1.2201 |
| 3.6 | −75.87 | −100.35 | 0.21 | 0.50 | *59.65* | *8.92* | *10.62* | 0.38 | 0.24 | 0.86 | 0.03 | *18.10* | 0 | 0.4958 |
| 3.7 | −80.35 | −104.31 | 0 | 0 | *22.47* | *12.14* | 2.11 | 0.66 | 0.15 | *61.95* | 0.04 | 0 | 0 | 0.4817 |

Italic entries account for 5% or greater of the percent of total $S_v$.

chromatogram (Hare and Palumbi, 1999; Bucklin et al., 2000). It is possible that this practice contributed to underestimating DNA sequence variability.

Population genetic diversity of *N. difficilis*, measured as haplotype diversity ($h = 0.738$), was similar to that determined for a different region of mtCOI for the euphausiid, *Meganyctiphanes norvegica* ($h = 0.6847$; Bucklin et al., 1997). In contrast to *N. difficilis*, *M. norvegica* showed no significant population genetic structuring on mesoscales, although populations on either side of the N. Atlantic Ocean were genetically distinct. The most likely explanation for the population genetic distinctiveness of onshore and offshore populations of *N. difficilis* is the ocean circulation and water mass structure of the California Current. Nearshore populations may be retained in the Southern California Bight eddy, where abundant food enhances individual growth and reproduction and circulation patterns retain populations in persistent eddies. Similarly, genetic differentiation in allozymic frequencies was observed between populations of the copepod, *Metridia pacifica*, entrained in a persistent, coastal mesoscale eddy and those in the surrounding California Current waters (Bucklin, 1991). However, it is not possible to identify the sources of the populations nor to determine the time since divergence.

The bioacoustical observations were intended to provide a synoptic view of biomass fields in the study area. Existing technology does not allow direct identification and measurement of single species using bioacoustical backscatter. Thus, the mismatch in vertical distribution of the target species and the depth stratum for which backscattering intensity was observed is unfortunate, but not fatal to this aspect of the study. The bioacoustical backscattering observations and silhouette analysis indicated that the zooplankton assemblage of the top 100 m varied significantly, in terms of relative abundance and biomass of functional groups, among samples collected in the offshore portion of the transect. Diel vertical migration may have caused some of the variation, since maximum backscattering intensities were observed between 25 and 75 m during the night for both RR-9610 and KIWI-1 but were negligible during daytime hours (during RR-9610 but not KIWI-1). It is likely that zooplankton migrated below the 100 m lower depth limit of our acoustical observations, although additional day–night comparisons will be needed to confirm this.

The KIWI-1 transect was designed to add nearshore bioacoustical observations that were impossible after the loss of instrumentation during RR-9610. Comparisons between the offshore RR-9610 and nearshore KIWI-1 observations revealed strong similarity in the backscattering environment of the two transects (Figs. 11 and 12). However, daytime backscattering levels did not change dramatically during KIWI-1 (Fig. 12). The six samples for which estimated backscattering was markedly lower than that observed (i.e., three KIWI-1 daytime samples from 75 m to the surface and three RR-9610 samples from 75 to 25 m) were quite low in numbers and biomass of zooplankton (Fig. 13). Interestingly, all of these samples were collected from regions where the mixed layer bottomed and a strong thermocline began. It seems most likely that microstructure contributed significantly to the observed backscattering in these depth intervals.

A consistent feature across all samples analyzed was the numerical predominance of copepods, with significant contributions from spherical bodies (eggs and radiolarians) and molluscs (shelled pteropods). Salps and medusae were the biomass dominants at three of the four RR-9610 stations (Fig. 10). Euphausiids made a small but significant contribution to bioacoustical backscatter measurements in many samples. It is likely that many euphausiids either persisted, as *N. difficilis* was observed to do, or vertically migrated below the depth range of the bioacoustical system employed. The marked sample-to-sample variation in the zooplankton assemblage made it difficult to evaluate the relative importance and role of euphausiids, and of *N. difficilis* in particular, in the California Current zooplankton assemblage.

Although *N. difficilis* was one of the most numerous euphausiids in the RR-9610 samples, we did not attempt to correlate *N. difficilis* abundance patterns with the bioacoustical backscattering measurements, since the target species

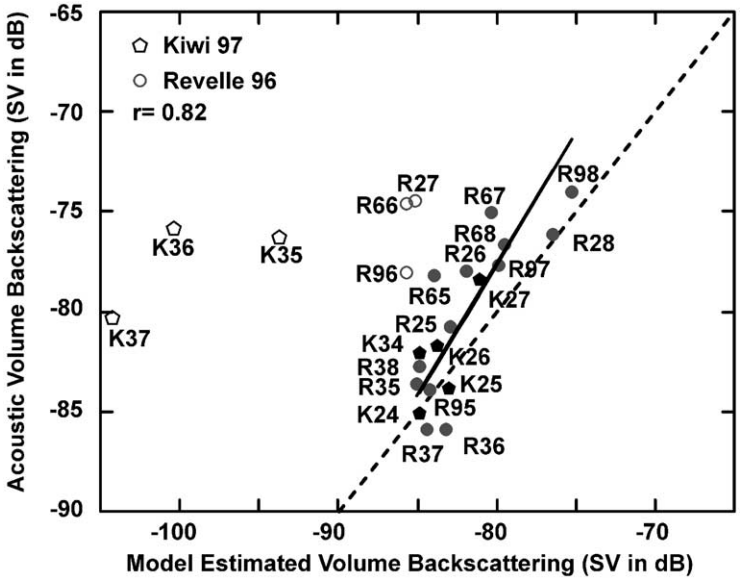


Fig. 13. Comparison of the estimated volume backscattering determined from the size and abundance of individual taxa in RR-9610 and KIWI-1 MOCNESS samples and the measured volume backscattering in the immediate vicinity of each net. The diagonal line is the expected, when the estimated and measured values agree. Only filled markers were used in the regression analyses. (See Figs. 11 and 12 for explanation of data labels).

typically occurred below the depth range of the bioacoustical instrumentation. A tow-yoed deployment (i.e., periodic oscillation of an instrument between two depths while being towed through the water, creating a sawtoothed pattern; see Wiebe et al., 1985) would have allowed us to achieve our goal of directly relating the target species distribution and abundance to the zooplankton assemblage, by taxonomic and silhouette analysis of MOCNESS samples and bioacoustical backscattering measurements (see Wiebe et al., 1996).

## 5. Conclusion

The goal of this study was to conduct an integrated examination of the physiological condition of individuals, molecular population genetic diversity and structure of a species, and bioacoustical backscattering of the zooplankton assemblage in hydrographically distinct regions of the California Current. At the individual level, *N. difficilis* exhibited significant variation in concentrations of important metabolic enzymes between nearshore and offshore regions, which we hypothesized to result from physiological rather than genetic variation. However, nearshore and offshore populations of *N. difficilis* differed in their genetic make-up, based on mtDNA "tags" of population differentiation, suggesting distinct sources in upstream regions. Future goals for this research include parameterization of the biochemical, molecular, and bioacoustical backscattering measurements for use in biological/physical numerical models (e.g., Olson et al., 1996). Integration across individual-, species-, and assemblage-levels of organization will help us understand and quantify the processes that determine the spatial and temporal patterns of distribution and abundance of zooplankton in the ocean.

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