

## Edge gradients provide evidence of ecological interactions in planktonic thin layers

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### *Abstract*

Physical, optical, and acoustical data from Monterey Bay, California, USA, describing the distributions of phytoplankton, zooplankton, and small fish as well as gradients in the physical habitat were used to examine the interactions between vertically compressed plankton structures (thin layers), their consumers, and the local physical forces they experience. The relationship between upper and lower edge gradients of a layer were used to define layer ‘shape’. The steepness of the vertical gradient on the top vs. the bottom of the plankton layer was correlated to the difference in the relative abundance of consumers above and below the layer. Phytoplankton layer gradients were steeper when more zooplankton were present on one side of the layer vs. the other while zooplankton layers were more diffuse when a greater number of fish were adjacent to one side of the layer than the other. Both layer types showed nearly symmetrical gradients when predators were in low abundance or absent. Predator-associated differences in phytoplankton and zooplankton layer shape were not correlated with vertical gradients in shear or mixing potential surrounding layers. In the absence of strong physical gradients, grazers can play an important role in structuring plankton thin layers. These observations likely represent one end of a continuum of biological and physical forcing responsible for formation and maintenance of thin plankton layers.

The variability of an ecosystem in space and time is usually one of its most important features, influencing both practical problems of sampling and conceptual questions about its structure (Steele 1976). The development and maintenance of spatial and temporal patterns and the consequences of those patterns for the dynamics of populations and ecosystems are fundamental themes in ecology (Levin 1992). The consequences of these patterns of heterogeneity on biota are many, affecting population dynamics, trophic interactions, community organization and stability, and cycling of elements (*see* a review in Levin 1992). In the last two decades, advances in measurement capabilities have led to the discovery of plankton aggregations in a variety of coastal marine habitats with vertical dimensions of tens of centimeters. These ‘thin layers’ can have a horizontal extent of several kilometers and may persist for days (Dekshenieks et al. 2001; Rines et al. 2002; McManus et al. 2003). Sharply distinct from the surrounding water column, the density of phytoplankton and zooplankton in these layers can be orders of magnitude higher than at surrounding depths (Cowles 2003), alluding to their ecological importance.

The formation of thin layers and their subsequent maintenance have been hypothesized to result through a variety of mechanisms. Physical mechanisms for formation include buoyancy forcing (Franks 1992), straining of a patch of passive plankton by near-inertial internal waves (Franks 1995), shear (Birch et al. 2008; Ryan et al. 2008), and the horizontal intrusion of water masses (Osborn 1998). Low mixing levels have been observed in some thin plankton layers, but while this may help layers persist, it has not been viewed as a formation mechanism (McManus et al. 2003). Most studies implicating biological mechanisms for thin layer formation suggest active swimming of both zooplankton (Gallager et al. 2004; McManus et al.

2005) and motile phytoplankton (Klausmeier and Litchman 2001) can create thin plankton layers. A recent model by Stacey et al. (2007) quantifies the relative importance of motility, straining, and buoyancy, integrating biological processes with physical processes. A variety of other biological mechanisms for thin layer formation have been proposed including thinning by preferential grazing at the edges of existing layers, increased reproduction, and increased growth within layers (Donaghay and Osborn 1997). These biological mechanisms have substantial implications for the ecological role of thin layers though they remain untested in field experiments.

Field studies of the ecological consequences of these layers have been limited because of the difficulties in studying the biological processes involved in the formation and persistence of thin layers. Laboratory experiments have focused on predator–prey interactions, showing a variety of responses of predators to thin layers of prey. Bochdansky and Bollens (2004) found very minimal responses of copepods feeding in thin layers of phytoplankton, while two studies found aggregations in microzooplankton predators resulted in areas of thin layers of phytoplankton (Ignoffo et al. 2005; Menden-Deuer and Grunbaum 2006) as a result of behavioral changes by the predators around thin phytoplankton layers (Menden-Deuer and Grunbaum 2006). Thin layers of zooplankton have been shown to directly influence the vertical distribution of larval herring, suggesting an attraction of zooplanktivores to these areas of abundant resources despite their preference for other depths (Clay et al. 2004). Modeling results suggest that a predator’s efficiency inside an even and continuous thin layer is decreased relative to foraging in a patchy thin layer, effectively decreasing the prey’s risk (Leising 2001). Thus, it may be possible for organisms to reduce their predation risk within thin layers by changing their horizontal aggregation, suggesting that predators could have a significant effect on the horizontal distribution of layers. These laboratory and modeling studies

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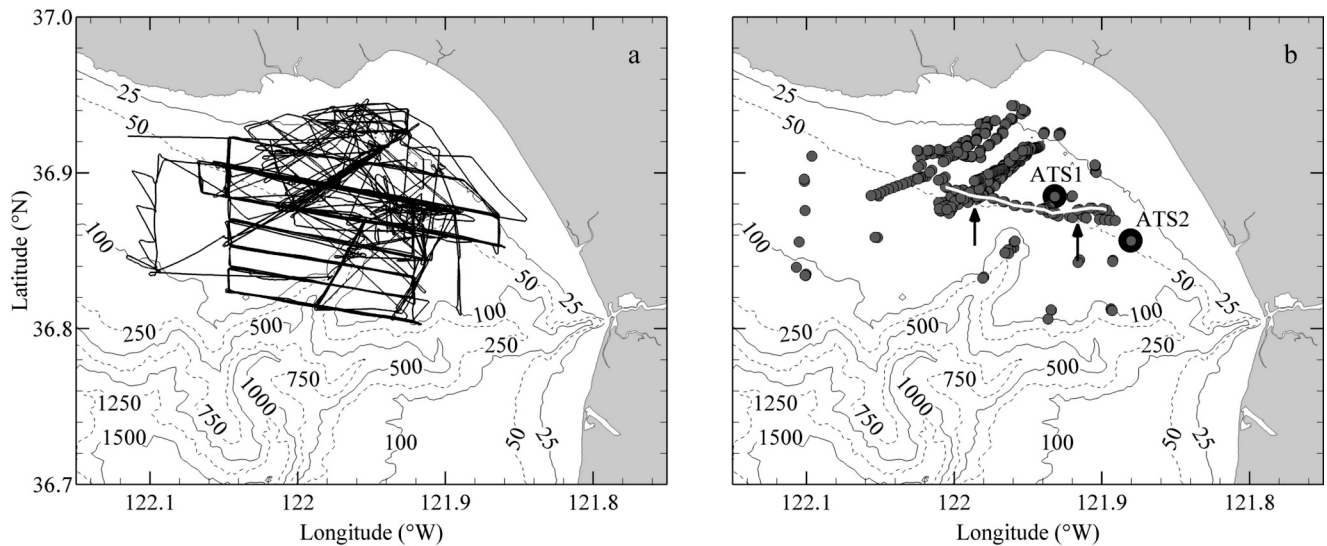


Fig. 1. (a) Shipboard acoustic data were collected nearly continuously over the entire sampling period. The track-lines for this sampling are shown. (b) SlowDROP profiles were collected at numerous stations throughout Monterey Bay, California, USA, indicated by the gray points. Two anchored time-series stations were also sampled and are indicated by the stations outlined in black along with a slow-moving transect with to-yo'd profiling indicated in white.

clearly suggest the role thin layers may play in trophic interactions and thus the behavior, growth, and reproduction of individual organisms and ultimately the flux of carbon and nutrients in ecosystems.

A first step toward unraveling the ecological significance of thin layers in field studies may be quantifying the distributions of multiple trophic levels that form or interact with thin plankton layers simultaneously at comparable resolution. Most field studies of thin layers have focused specifically on phytoplankton (Cowles et al. 1998; McManus et al. 2007; Ryan et al. 2008) and to a lesser extent, zooplankton (Holliday et al. 1998; Widder et al. 1999; Cheriton et al. 2007), with only a few studies attempting to integrate measures of multiple trophic levels (McManus et al. 2003; Gallager et al. 2004). These studies have highlighted the different processes driving phytoplankton layers from those influencing zooplankton layers. These forcing factors sometimes resulted in association of layers of various-sized plankters but at other times, displaced distributions were observed (McManus et al. 2003). In this paper, we will present coincident physical, optical, and acoustical data describing the distributions of phytoplankton, zooplankton, and small fish as well as gradients in their physical habitat. Our goal was to use a quantitative framework to assess potential interactions between vertically compressed plankton structures (thin layers), their consumers, and the physical forces they experience. Plankton layers often exhibit asymmetrical boundaries with a noticeably steeper gradient on one edge than the other. We use the relationship between upper and lower gradients of layers to define layer 'shape' and apply this index to evaluate the balance of physical processes and predator-prey interactions in determining layer characteristics. This understanding of the interaction of physical and biological forces is critical for understanding how thin layers are formed, maintained, and dissipated as well as the

potential ecological significance of these intense plankton aggregations.

## Methods

**Data collection**—Data were collected throughout the northern portion of Monterey Bay, California, USA from 20 August to 09 September 2005 from the 52-m R/V *New Horizon* as part of the Layered Organization in the Coastal Ocean (LOCO) program, a large, interdisciplinary project investigating thin plankton layers. The cruise track and station occupation by the vessel was coordinated with the sampling conducted by other LOCO investigators at a well-instrumented mooring array located at the 20-m isobath in the NE corner of Monterey Bay.

Sampling was conducted nearly continuously from a multifrequency echosounder and an acoustic Doppler current profiler (ADCP) mounted 1 m below the surface on a rigid mount on the vessel's port side. This acoustic system was used during transect surveys (at speeds up to  $10 \text{ m s}^{-1}$ ) and while station-keeping during 632 periodic vertical profiles with a slow descent rate optical profiler (SlowDROP) as well as during 69 casts conducted with the ship's CTD (conductivity, temperature, and depth) and rosette system (Fig. 1).

Periodic, vertically integrated plankton tows were conducted using a 0.75-m-diameter 333- $\mu\text{m}$  mesh equipped with a General Oceanics Flowmeter. Tows were conducted from 5 m above the bottom to the surface. Plankton samples were preserved in 5% buffered formalin in seawater and were later identified to genus, measured, and enumerated.

**Resolution of fine-scale vertical structure—velocities:** A 600-kHz ADCP (RD Instruments Workhorse) was used to characterize the fine-scale velocity structure. The transducer was suspended 1 m below the sea surface on a vertical pole

Table 1. Echosounder sampling characteristics.

Frequency (kHz)	Beamwidth (°)	Beam type	Pulse length ( $\mu$ s)	Vertical resolution (cm)	Effective depth range (m)
38	12	Split	256	10	750
70	7	Split	128	5	350
120	7	Split	64	2.5	200
200	7	Split	64	2.5	150
710	7	Single	64	2.5	40

rigidly mounted to the port side of the vessel. The ADCP was configured to resolve 1-m depth bins of horizontal velocity between 3-m and 40-m depth. In addition to determining the horizontal spatial structure of vertical velocities (to be reported in a subsequent publication), we used the ADCP to determine the vertical velocity gradients during each SlowDROP profile. For the analyses in this paper, we used 3-min averages of horizontal velocity, centered on the deployment time of each SlowDROP profile. Estimates of vertical shear were obtained from 1-m vertical gradients in these 3-min averages of horizontal velocity.

Resolution of fine-scale vertical structure—phytoplankton structure: We used a slow descent rate optical profiler (SlowDROP) to obtain centimeter-scale resolution of hydrographic structure as well as O(5 cm) resolution of bio-optical properties. The SlowDROP profiler carried a Sea-Bird 9Plus CTD, an absorption and attenuation meter (AC-9; WET Labs, Inc.), chlorophyll fluorometer (WetStar; WET Labs, Inc.), and an acoustic Doppler velocimeter (Nortek Vector) as well as several other instruments not used in the analyses presented here. SlowDROP was deployed with a slack data cable, allowing resolution of upper ocean properties without contamination of ship motion. The profiler weight in water was compensated with buoyant floats to obtain a free-fall descent rate of 0.20–0.25 m s<sup>-1</sup>. Profiles were obtained throughout the northern portion of Monterey Bay, either closely spaced along specific transect lines or as time series of repeated profiles at specific stations.

Resolution of fine-scale vertical structure—bioacoustics: A five-frequency echosounder system with a range of frequencies from 38 kHz to 710 kHz was used to provide information on a wide size range of meso-zooplankton and fish (Table 1). The centers of each echosounder transducer were no more than 35 cm apart to maximize spatial comparability of the data. Pulse lengths were chosen to allow the highest vertical resolution possible for each instrument while limiting electronic noise from other systems. The echosounder system was calibrated using the settings and physical set up used for data collection just prior to sampling using an indirect procedure incorporating a 38.1-mm-diameter tungsten carbide reference sphere as prescribed by Foote et al. (1987). The echosounder system was mounted at a depth of 1 m below the surface on the same vertical pole as the 600 kHz ADCP.

*Data analysis*—Acoustic separation of zooplankton and fish: Our assessment of ecological interactions between trophic levels requires the partitioning of acoustic volume-scattering into zooplankton and fish components. We split the echosounder data into echoes that were consistent with

swim-bladdered fish and those attributable to zooplankton. First, using the split-beam capabilities of the four lower frequencies, all echoes from solitary individual targets (i.e., targets at densities lower than one per sampling volume) were separated and identified as individual fish (MacLennan and Simmonds 1992). This was accomplished using Myriax's Echoview software using a target strength threshold of -80 dB, consistent with a small swim-bladder-bearing fish. A 'pulse length determination level', the value in dB below peak value considered when determining the pulse length, or envelope, of a single-target detection, of 12 dB was used. 'Normalized pulse lengths', the measured pulse length divided by transmitted pulse length, were required to be between 0.8 and 2.0. The 'maximum beam compensation' for correcting for transducer directivity was set to 12 dB. To confirm all sources of scattering within the measured pulse length were from a single target, all samples within this pulse envelope must have had a standard deviation in angular position of <3° in both the along- and athwart-ship directions of the beam. The single targets identified at 38 kHz were used for further analysis while those at other frequencies were simply removed from the scattering measurements.

The next step in signal separation was to remove targets likely to be aggregations of fish. Over the frequency range used in this study, the echoes from plankton are more dependent on frequency than the echoes from fish. Targets likely to be aggregated fish, those that were at densities >1 per sampling volume, were separated from zooplankton using the observed frequency response in volume backscatter at 38 kHz and 120 kHz (Korneliusson 2000; Kang et al. 2002). All echosounder data were averaged into 0.5-m vertical by 10-ping horizontal cells and then the frequency difference in each cell was computed. Cells with a mean volume backscatter difference ( $s_{v120} - s_{v38}$ ) of between -3 dB and 3 dB were categorized as fish while those with a volume backscatter difference >4 dB were categorized as zooplankton. These threshold values were selected based on the 95% confidence interval of the mean difference between the scattering at 38 kHz and 120 kHz by single targets converted to  $s_v$  units. The areas with a high likelihood of aggregations of fish were excluded from zooplankton layer analysis. The shape of these distributions of likely fish were most often discrete and relatively ovoid, consistent with schools of fish rather than layer formations, reducing the likelihood of misclassification.

Layer identification: Plankton layers were identified as local maxima (fluorescence, zooplankton acoustic volume backscatter) that exceeded nearby (within 2 m) background

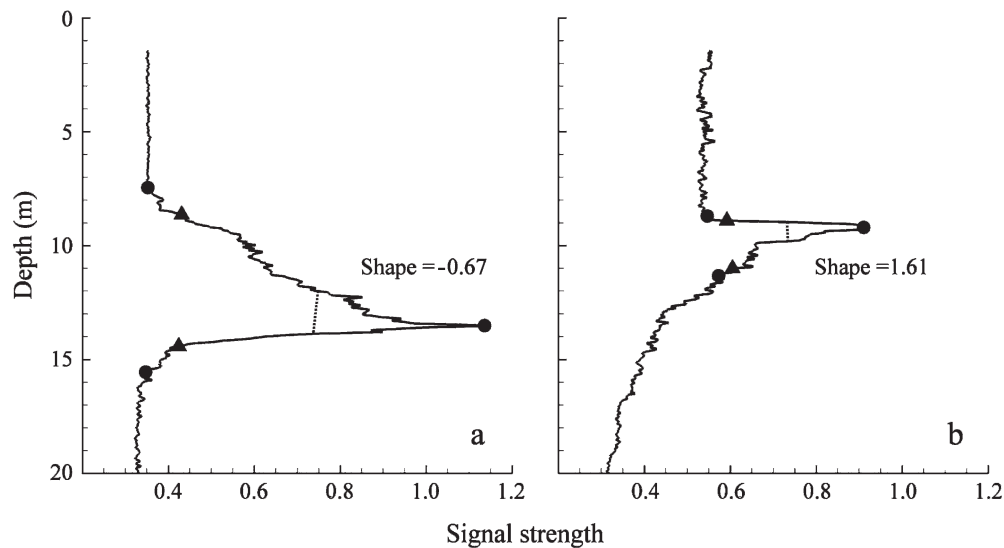


Fig. 2. Two sample profiles of thin layers are shown along with the features measured from each profile. The peak of each layer and its intersections with the baseline are indicated by filled circles. The 10% of peak values assuming that the baseline = 0 and the peak = 1 are shown as solid triangles. Note that because of differences in the baseline above and below (b), these 10% of peak values are different for the upper and lower gradients. These values and their depths are combined in the layer shape parameter. (a) has a negative shape value because it is steeper on its lower edge, while (b) has a positive shape index value because it shows the opposite pattern in edge gradient. Also shown on each plot are the 50% of maximum points of the layer, connected by a dotted line. The difference in depth between these points defines the thickness of the each layer. The example profiles shown here are phytoplankton layers. However, profiles of the vertical distribution of acoustic backscatter would be similar.

levels by a factor of 1.5 or more (Fig. 2). This criterion is somewhat different from the commonly cited definition of thin layers, which uses a factor of three times higher than the global rather than local background values (Deksheniaks et al. 2001) but is robust when layers are found in a variable background (e.g., there are other patches in the plankton), a situation common in Monterey Bay. In order to avoid any potential bias by manually selecting the intersection points of each thin layer with the local baselines, we used an objective criterion based on the local maxima in the second derivative of the fluorescence or volume-scattering depth profile above and below the depth of the layer peak. Before calculating the first derivative, each vertical profile was smoothed with a median filter. The second derivative then was calculated, following smoothing of the first derivative with a 'loess' filter. The depth of the local maxima in the second derivative above and below the layer peak was assigned to the fluorescence or volume-scattering profile as the upper and lower baseline intersection points, respectively. The depth of a layer was defined by the depth of the maxima. We calculated layer thickness at the location of the full width, half-maximum (FWHM) value (Deksheniaks et al. 2001).

We used chlorophyll fluorescence as a proxy for phytoplankton concentration in thin layers because more of our SlowDROP profiles have high-quality fluorescence data than have beam attenuation (650 nm from the unfiltered ac-9). The majority of identified layers occurred at night, minimizing the degree of fluorescence quenching in the upper euphotic zone. We also compared the vertical structure of

chlorophyll fluorescence in >50 profiles with simultaneously obtained profiles of beam attenuation (650 nm) and found no evidence of fluorescence-quenching influencing our resolution of the vertical gradients of fluorescence.

Zooplankton thin layers were defined using volume backscatter at 120 kHz as an index of zooplankton abundance. Volume backscatter data at 120 kHz from all times and positions that were consistent with zooplankton were averaged into by 0.1-m vertical by 10-ping horizontal cells. Note these cells are smaller vertically than the cells used to initially categorize data because the differences in the sampling resolution between frequencies necessitated larger cells for the frequency differencing analysis. Each 5-min interval within the 120-kHz data was analyzed for the presence of thin layers. All 5-min intervals when the vessel was moving were used to characterize acoustic thin layer overall presence. To characterize features of these layers without oversampling, only the data at the midpoint of 5-min periods during moving surveys were used. Acoustic layer characteristics during these 5-min midpoints were compared to the abundance of fish  $\pm 2$  m of layer peak depth  $\pm 30$  s of the 5-min midpoint. Fish included both counts of single targets and area scattering from schools. The moving surveys were chosen for this comparison to reduce the effects of fish responses to the vessel's lights, which were noticeable at night from stationary observations.

Zooplankton scattering was also analyzed with respect to SlowDROP profiles. For all SlowDROP profiles with identified fluorescence thin layers, 120-kHz volume scattering consistent with zooplankton at depths 2 m above

and 2 m below the fluorescence peak depth were averaged for the 30 s surrounding the midpoint of the cast. The scattering below the layer was subtracted from the scattering above the layer to provide an estimate of the zooplankton gradient surrounding the thin phytoplankton layer. In addition, all 120-kHz volume-scattering data at the times of SlowDROP profiles were analyzed for the presence of zooplankton thin layers, resulting in 94 profiles for comparison of zooplankton thin layers with local physics.

Layer ‘shape’: Plankton layers frequently exhibit asymmetrical boundaries, with a steeper vertical gradient on one edge of the layer relative to the other edge. This asymmetry could be a result of differential physical forcing (e.g., shear, mixing) as well as differential growth or loss across the depth range of the layer. We have used layer asymmetry as an index of shape in which relatively steeper upper gradients yield positive values, and relatively steeper lower gradients yield negative values. The upper and lower gradients were quantified as

$$\text{Upper gradient (value m}^{-1}\text{)} = \frac{|\text{Value}_{\text{peak}} - \text{Value}_{10\% \text{ of peak re upper baseline}}|}{|\text{Depth}_{\text{peak}} - \text{Depth}_{10\% \text{ of peak re upper baseline}}|} \quad (1)$$

$$\text{Lower gradient (value m}^{-1}\text{)} = \frac{|\text{Value}_{\text{peak}} - \text{Value}_{10\% \text{ of peak re lower baseline}}|}{|\text{Depth}_{\text{peak}} - \text{Depth}_{10\% \text{ of peak re lower baseline}}|} \quad (2)$$

where ‘Value’ is fluorescence (volts) in the case of phytoplankton and volume-scattering cross-section ( $\sigma \text{ m}^{-3}$ ), a linear form of volume backscatter, in the case of zooplankton. For the upper and lower gradients of each layer (Fig. 2), we determined (1) the intersection point of that gradient with the local baseline, (2) the depth of the maximum value, or peak, of the layer, and (3) the depths of 10% maximum and 50% maximum value, assuming baseline = 0 and maximum = 1.0. Thus, the value at 10% of the peak can be different for the upper and lower edges of a layer.

These gradient values were combined to provide a shape index:

$$\text{Layer shape (value m}^{-1}\text{)} = \frac{\text{Upper gradient}}{\text{Value}_{\text{peak}}} - \frac{\text{Lower gradient}}{\text{Value}_{\text{peak}}} \quad (3)$$

The upper and lower edge components of the shape parameter were scaled by the intensity of the layer to remove any bias in the shape index created by the magnitude of the peak value. The resulting shape indices, for both phytoplankton and zooplankton layers, were uncorrelated with layer magnitude and were approximately normally distributed. In contrast, shape values calculated from unnormalized gradients displayed a weak but noticeable correlation with layer magnitude.

The most challenging aspect of these estimates was the determination of the intersection point of the upper or lower gradient with the local baseline of fluorescence or

volume scattering. In order to avoid any potential bias by manually selecting the intersection points of each thin layer with the local baselines, we used an objective layer selection criterion based on local maxima in the second derivative. An estimate of layer shape that is based on the vertical gradient displayed at the top and bottom of a thin layer will be sensitive to the choice of the baseline intersection point. We therefore chose to calculate the gradients between the maximum value and the 10% of peak value (see Fig. 2), thus minimizing bias caused by local discontinuities in the profiles. This approach yields a more conservative estimate of the upper and lower gradients of each layer than would be obtained by using the baseline intersection point in the gradient calculation. For those profiles that had unusual fluctuations in the increase or decrease of fluorescence or volume scattering near the baseline intersection points, we selected specific bounding points for the upper and lower gradients before proceeding with the first and second derivative calculations. These profiles accounted for <5% of all SlowDROP profiles and 1% of all acoustic profiles.

## Results

Of 632 vertical profiles with the SlowDROP, 94 contained thin phytoplankton layers. Thin phytoplankton layers were detected during 2% of casts conducted during daylight hours and 29% of casts conducted during nighttime hours, consistent with observations during the same time period in the northeast corner of Monterey Bay that layers consisted of migrating dinoflagellates (J. Sullivan and J. Rines pers. comm.). These layers ranged in thickness from 0.25 m to 2.0 m in thickness with a mode thickness of 0.5 m (Fig. 3a.). Of 4903 5-min intervals in the acoustic data set, 913 contained thin acoustic scattering features consistent with zooplankton. Thin zooplankton layers were detected during 12% of profiles conducted during daylight hours and 25% of profiles conducted during nighttime hours. Zooplankton layers ranged in thickness from 0.25 m to 2.5 m with a mode thickness of 1 m (Fig. 3b).

No net sampling focused directly on thin layers was possible during this study; however, water-column-integrated zooplankton tows showed that zooplankton were dominated by herbivorous copepods numerically and by biomass. The most abundant genera of copepods were *Calanus* (mean body length = 1.35 mm, standard deviation (SD) = 0.27), *Ctenocalanus* (mean length = 0.88 mm, SD = 0.09), and *Acartia* (mean length = 0.95 mm, SD = 0.07). Together, these three groups made up >90% of the zooplankton both numerically and by biomass. The relatively limited diversity of body types and the lack of any extremely strong scatterers such as gastropods or those with air inclusions suggests that we can reasonably use scattering as an estimate of relative abundance.

Previous work has indicated the importance of shear and small-scale mixing in layer processes. The relationship between the shape of phytoplankton and zooplankton layers was examined in response to the differential shear between the 2 m above and 2 m below each phytoplankton and zooplankton layer (Fig. 4a,b). No significant relationship was found between differential shear and the shape of

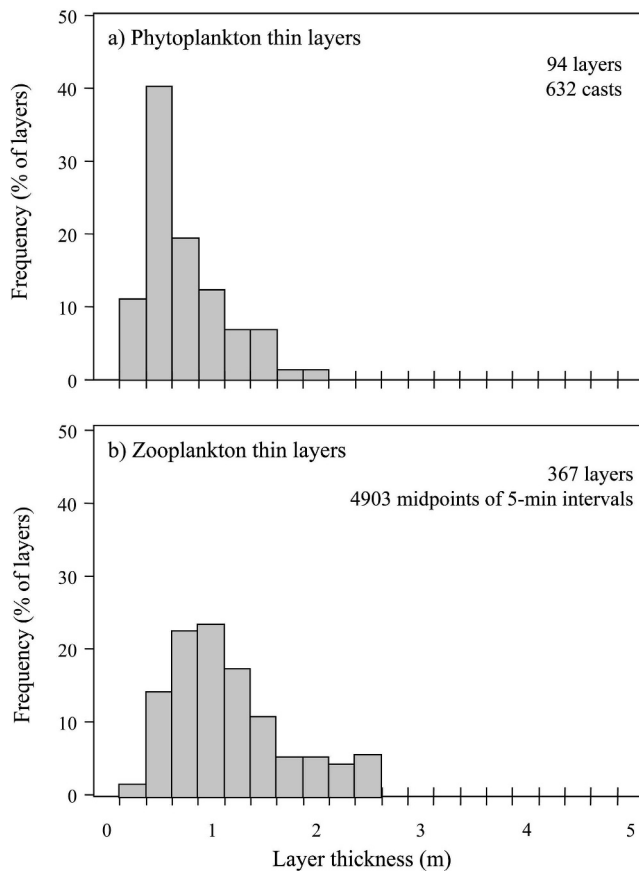


Fig. 3. Distribution of (a) phytoplankton, and (b) zooplankton thin layer thickness, defined as the width of the layer at half peak intensity (width, half maximum [FWHM]). For phytoplankton layers, thickness was calculated for all casts containing layers. For zooplankton layers, layer thickness was calculated only at the midpoint of each 5-min interval in order to minimize oversampling, resulting in fewer samples than used for layer presence calculations.

either phytoplankton or zooplankton layers. Note that several phytoplankton and zooplankton layers show large shape values but very little differential shear. Similarly, the difference in Richardson number in the 2 m above and below each layer was found to have no significant relationship with phytoplankton or zooplankton layer shape. These results are not plotted.

We found that layer shape for each trophic interaction (phyto-zoo, zoo-fish) was correlated with the abundance of potential consumers around the layer. In the case of phytoplankton layers (Fig. 5), relatively more zooplankton scattering in the 2-m interval above the layer was associated with steeper upper gradients in phytoplankton concentration (positive layer shape), while more zooplankton scattering in the 2-m interval below the layer correlated with steeper lower gradients (negative layer shape). In other words, the side of the phytoplankton layer with the steepest gradient also had relatively more zooplankton scattering, and the side of the layer with the weaker gradient had relatively less acoustic scattering from zooplankton. In contrast, we found that zooplankton layer shape responded to the local vertical gradient in fish abundance (Fig. 6) by having weaker vertical gradients on the side of the zooplankton layer with more fish within 2 m.

We also observed that low grazer abundance around thin layers was correlated with symmetrical upper and lower gradients. As noted in Fig. 5, phytoplankton shape values ranged from  $-4.4$  to  $6.1$  for all 94 phytoplankton thin layers. When we evaluated only those phytoplankton thin layers that had nearby zooplankton concentrations below a threshold  $-60$  dB re  $1 \text{ m}^{-1}$  (23 out of 94 phytoplankton layers), those 23 layers had a reduced distribution of layer shape ( $-0.74$  to  $0.51$ ) with a mean near zero. Similarly, the shape parameter for all zooplankton thin layers ranged from  $-4.1$  to  $4.8$ . This range was reduced to  $-0.6$  to  $0.6$  when only those 142 zooplankton thin layers that had no fish within 2 m of their peak were examined. The mean

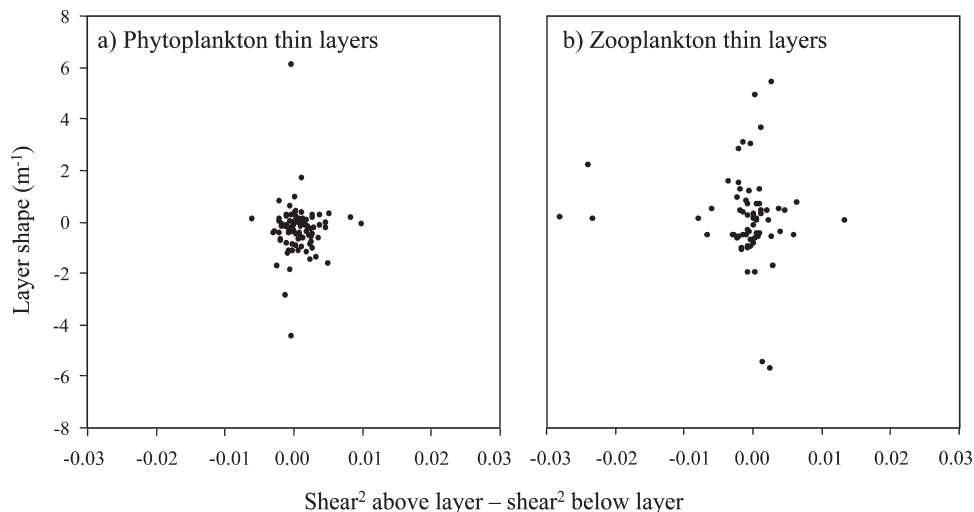


Fig. 4. (a) Phytoplankton, and (b) zooplankton layer shape as a function of the difference in shear 2 m above and 2 m below the thin layer. Differential shear was relatively weak during this study and showed no clear pattern with layer shape.

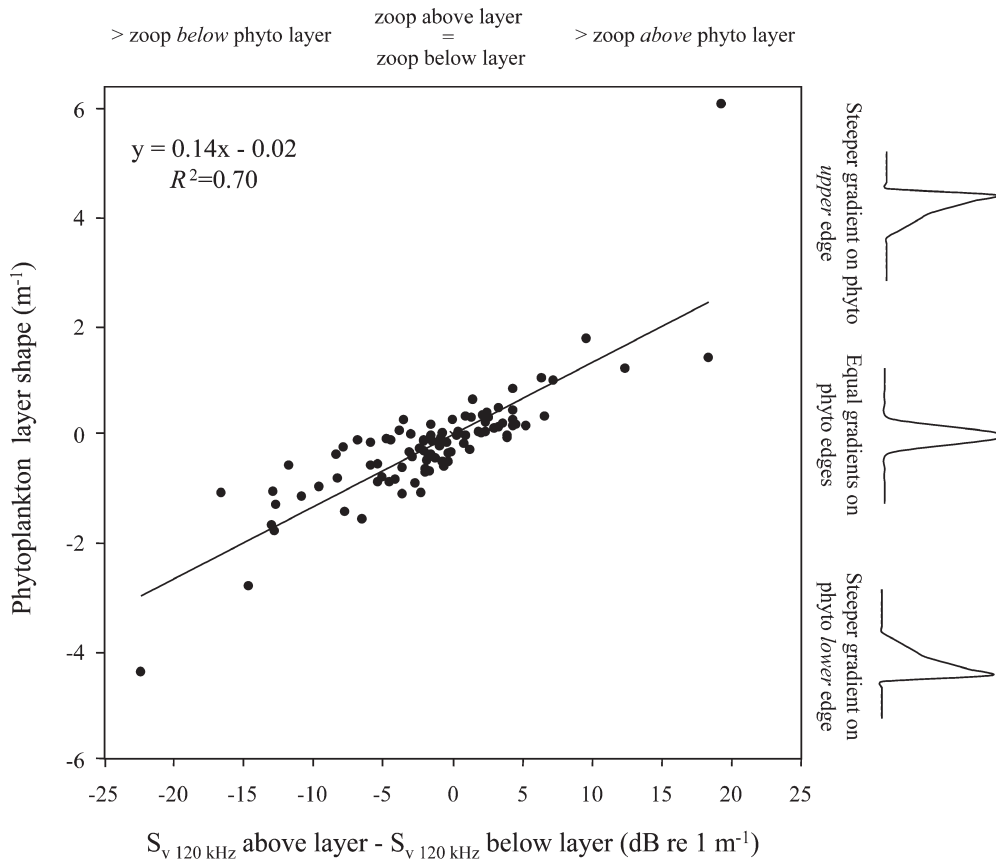


Fig. 5. Phytoplankton thin layer shape as a function of the local zooplankton gradient. A positive shape value means that the top edge of the phytoplankton has a steeper gradient than the lower edge while a negative value, indicates that the lower has the steeper gradient. Volume scattering at 120 kHz was used as a metric of zooplankton abundance. A positive value on the x-axis means that more zooplankton was found in the 2 m above the phytoplankton layer's peak than below it, and a negative value, the reverse. Note that the volume-scattering data are in dB, a log unit, to permit compression of the scale. Thus, a value of 3 indicates twice as much zooplankton above the layer than below it while a value of 20 represents a two-orders-of-magnitude difference.

shape value of these zooplankton thin layers in the absence of fish grazers was also 0, representing layers with symmetrical upper and lower gradients (Fig. 7).

We present two final examples that demonstrate the potential for insight into ecological interactions through the application of these simultaneous high-resolution measurements of multiple trophic levels. In the first example, we used a slow 10-km transect within Monterey Bay to illustrate spatial patterns in layer structure. During the transect we obtained centimeter-scale vertical resolution of fluorescence and other optical parameters from Slow-DROP profiles every 0.25–0.5 km and continuously measured the vertical structure in acoustic volume-scattering. We encountered a horizontally coherent thin layer of phytoplankton (Fig. 8a) ~2 km offshore of the 20-m isobath and repeatedly detected it until we were 8 km offshore of the 20-m isobath. The shape value of the phytoplankton layer was between 0 and –0.5 until a zooplankton layer ascended to within a few meters of the peak of the phytoplankton layer between kms 4 and 5, at which point the phytoplankton shape value dropped below

–2.0. In the second example, we conducted a 24-h time series of observations at a fixed point in Monterey Bay and examined the temporal change in layer shape as a zooplankton layer appeared above the phytoplankton layer (Fig. 8b). Between 03:00 h and 05:00 h local time, the zooplankton thin layer was observed ~2 m above the persistent phytoplankton layer. By 05:00 h the phytoplankton layer shape had increased from –0.5 to 1.0. In both examples, significant changes in the shape parameter of the phytoplankton layer were observed after a zooplankton layer came within 2 m of the phytoplankton thin layer. These changes were consistent with the patterns shown in Fig. 5. Note that fish were not quantified in this portion of the analysis because of the potential for the lights from the stationary vessel to affect their behavior.

## Discussion

Thin layers of phytoplankton were found in 15% of the SlowDROP casts and zooplankton were found in ~19% of 5-min acoustic sampling intervals during the study period.

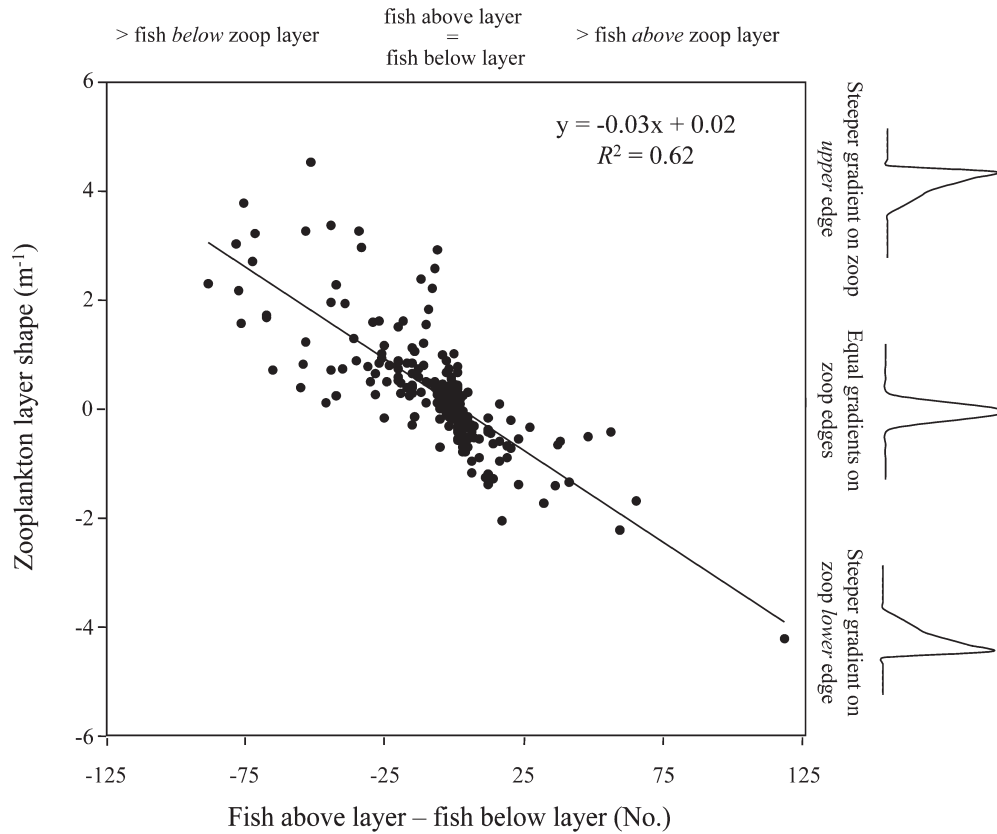


Fig. 6. Zooplankton thin layer shape as a function of the local gradient in fish abundance. Zooplankton shape was calculated from the 120-kHz data while fish abundance was calculated from the individual target detections from the 38-kHz echosounder  $\pm 2$  m of the zooplankton layer peak. Schools and other dense aggregations of fish were rarely found at the depth of thin layers and are not included.

Phytoplankton layers had a mode thickness of  $\sim 0.5$  m while zooplankton layers had a mode thickness of  $\sim 1.0$  m. However, all layers were thinner than 3 m, showing a discrete separation from other, larger vertical-scale aggregations.

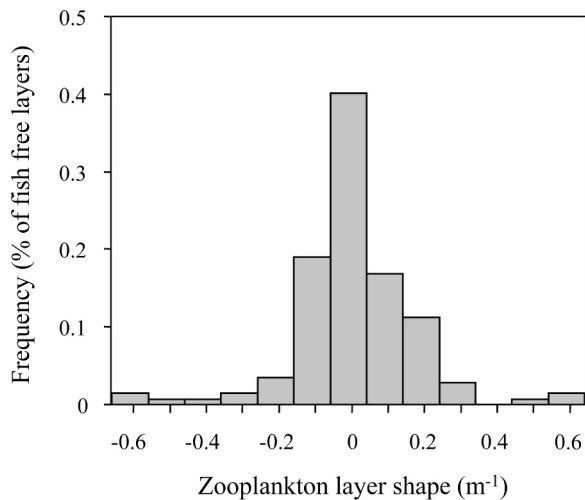


Fig. 7. Zooplankton thin layer shape for all layers where no fish were detected around the layer. The histogram shows that most zooplankton thin layers were nearly symmetrical in the absence of fish.

Thin layers of both phytoplankton and zooplankton were most commonly found at night during our study. This is consistent with observations in the Northeast corner of Monterey Bay during the same time period that showed that nighttime phytoplankton layers resulted from a reverse diel vertical migration consisting of dinoflagellates (J. Sullivan and J. Rines pers. comm.). The formation of zooplankton layers likely also resulted at least partly from behavior; however, the mechanisms of zooplankton layer formation were not investigated during this study.

Plankton thin layers frequently exhibit a steeper vertical gradient on one edge of the layer relative to the other edge. This asymmetry could result from differential physical forcing as suggested by Stacey et al. (2007) as well as from differential growth, loss, or behavior across the depth range of the layer. Our application of a layer shape index in conjunction with estimates of consumer biomass, shear, and mixing potential on the same spatial scale as the layer provides an approach for assessing the balance between physical processes and predator-prey interactions. In our observations, differences in the vertical edge gradients (layer shape) of both phytoplankton and zooplankton thin layers were not correlated with the physical processes of shear and small-scale mixing. For most of the layers we measured, vertical gradients in shear and Richardson

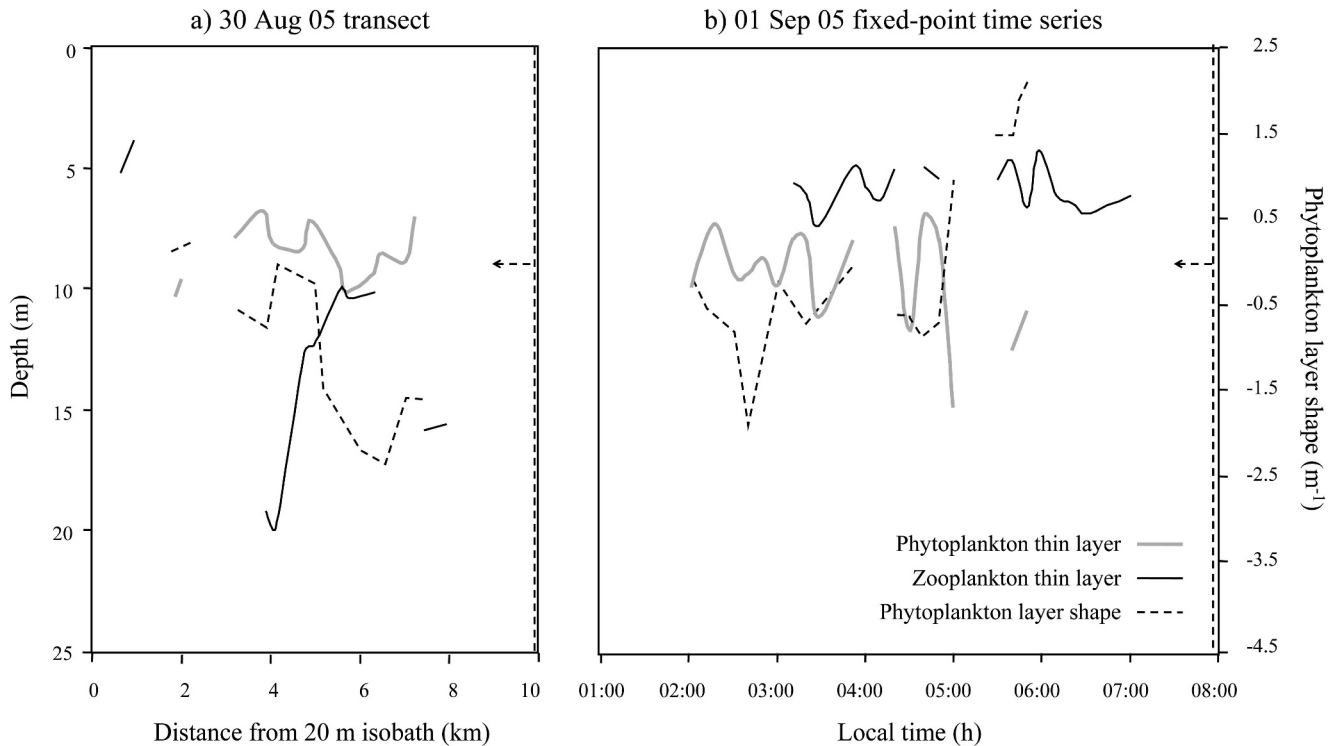


Fig. 8. (a) Data from a 10-km-long toyo'd transect running offshore from the 20-m isobath. This slowly moving transect began at 20:00 h and took  $\sim 5$  h to complete. This transect is highlighted in white in Fig. 1b with arrows indicating the first and last detections of the phytoplankton thin layer. (b) A section of a 24-h fixed-point time series, ATS2 in Fig. 1b, near the same location on the 20-m isobath. In each panel, the depth of the peak of the phytoplankton thin layer is shown in grey and the zooplankton thin layer is shown as a solid black line (left y-axis). Gaps in each line represent times that these features did not fit the definition of a thin layer as described in the methods. Shown as black dashed lines is the shape of the identified phytoplankton thin layer (right y-axis). The position of a symmetrical layer, as in the center example of Fig. 5, is indicated by the arrow near the right axis.

number were weak within 2 m of the layer, suggesting that physical forces were not dominating layer processes.

In contrast, our results show that the steepness of the vertical gradient on the top vs. the bottom of the plankton layer is correlated with the relative abundance of consumers above or below the layer. These results suggest at least two hypotheses: that zooplankton and fish have a preference for the gradient of their prey (steep in phytoplankton gradients, diffuse in zooplankton gradients, respectively), or that the presence of consumers causes plankton layer edges to have distinct gradients. In the absence of nearby grazers, both phytoplankton and zooplankton layers were approximately symmetrical with equal upper and lower edge gradients. This observation, combined with the observations of changes in phytoplankton layer shapes as zooplankton layers moved (or were advected) closer to the phytoplankton layers (Fig. 8), support the conclusion that predator-prey interactions were responsible for the observed asymmetry in layer gradients.

It is particularly interesting that our layer shape analysis revealed distinctly different relationships between phytoplankton and zooplankton layers and their respective consumers. A greater abundance of zooplankton on one side of a phytoplankton layer coincided with a steeper phytoplankton gradient at that edge of the layer. However,

the edges of zooplankton layers with a greater number of fish displayed weaker gradients on that side of the layer. These differences in observed shape effects between phytoplankton and zooplankton layers may be the consequence of recent grazing activity (predation losses), of mixing caused by predator swimming (Farmer et al. 1987), or of prey dispersion as a predation-avoidance response. There is little information available to discriminate between these hypotheses, or to assess the relative contribution of each process to the observed layer shape. Grazers respond to thin layers of food in experimental conditions by increasing ingestion rates and altering swimming behavior (Clay et al. 2004; Ignoffo et al. 2005; Menden-Deuer and Grunbaum 2006). Although these studies measured the depletion of the food in layers, they did not examine changes in the distribution of the prey. Particularly in zooplankton layers, there is also potential for the observed pattern to result from the behavioral response of the zooplankton to nearby fish predators (Bollens and Frost 1989), or an interaction of behavior with other factors. Although there have been efforts to study the escape responses of zooplankton in laboratory studies and to quantify predator correlations with zooplankton spatial distributions, relatively few field studies outside the schooling fish literature have examined the response of marine pelagic prey distributions to predators (Orr 1981;

De Robertis et al. 2003; Benoit-Bird and Au 2009). The limited field observations of changes in the distribution of pelagic prey in response to predator presence have shown conflicting results, with dramatic changes in distribution documented in ichthyoplankton (Axelsen et al. 2001) while euphausiid distributions showed no response to fish predators at small scales (De Robertis et al. 2003). However, zooplankton distributions have been observed to change in response to deployed oceanographic equipment, suggesting a behavioral avoidance response to the hydrodynamic disturbance created by these potential 'predators' (Graves 1975; Orr 1981; Farmer et al. 1987). The differences in layer shape that we observed between phytoplankton and zooplankton layers suggest that there are differences in the mechanisms affecting the edge gradients of these layers. These differences could be a consequence of the particular combination of species making up the layers, the physical scales at which they interact with their predators, or species-specific behavior of the predators themselves. One likely explanation lies in the relative differences in motility of the plankton in the layers and their predators. This difference in predator and prey motility is substantially greater for phytoplankton layers than zooplankton layers. In addition, phytoplankton have not been observed to show strong escape responses or cuing of neighboring individuals as has been observed in many copepod species (Fields and Yen 1997) and, thus, would be less likely to be able to effectively avoid their predators. These differences might result in diffuse layer edges as zooplankton avoid predators but distinct layer edges in phytoplankton layers as individual cells are unable to avoid predators and are consumed. Laboratory studies similar to those used to examine predator responses to plankton layers (Bochdansky and Bollens 2004; Clay et al. 2004; Menden-Deuer and Grunbaum 2006), but instead focused on the response of prey in layers to predators, could provide insight into the processes driving layer shape. Although the mechanisms affecting layer shape remain to be tested, our data indicate that layer edge gradients can be used to detect and assess the consequences of trophic interactions within plankton aggregations.

Numerous studies have demonstrated regulation of plankton by physical oceanographic processes (*see reviews in Denman and Powell 1984; Legendre and Demers 1984*). However, there are few studies on the biological causes of patchiness in the ocean as described by Hutchinson (1961): reproductive, interactions between parents and offspring; social intraspecific signaling between individuals; and coactive, intraspecific actions such as competition, predation, and parasitism. The importance of biological processes in causing and structuring plankton patchiness have become increasingly apparent, particularly when physical processes alone cannot account for the observed patterns (*see a review in Folt and Burns 1999*). Our results suggest some first steps in discriminating between physical and biological processes from in situ studies, and show that it is possible to assess their relative effect. Plankton thin layers provide a unique in situ experimental laboratory for studying plankton aggregation in the ocean because of the inherent compression of biomass

and the potential intensification of ecological processes in and around the layers.

This work provides indirect evidence of interactions of three trophic levels (phytoplankton, zooplankton, and fish) associated with small vertical-scale structures of plankton. Although planktonic thin layers have been documented in several coastal habitats around the world, in situ evidence of ecological effects has been difficult to obtain. The correlation of layer parameters with the presence of higher trophic levels, but not with physical forcing, highlights the importance of biological processes, specifically trophic interactions, in structuring thin layers. Our observations likely represent one end of a continuum of biological and physical forcing mechanisms responsible for formation and maintenance of thin plankton layers. We have presented evidence showing thin layers dominated by biological forcing, while the measurements presented in Ryan et al. (2008) suggest an environment dominated by physical forcing. These results support the need for coincident multiparameter measurements within and around plankton aggregations that include data on potential biological forces to assess the ecological significance of aggregations. Our results also reinforce the need for investigating predation effects, because these interactions provide a valuable window into layer dynamics (formation, maintenance, dissipation) as well as insights into the ecological significance of these intense plankton aggregations.

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