

# **Grazing Impacts of Diverse Zooplankton Taxa on Thin Layers**

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## **LONG-TERM GOALS**

The US Navy needs to know how distributions and abundances of light-scattering and sound-scattering organisms in the ocean vary in space and time, particularly in the vertical dimension. Recent field observations have shown that many biological properties may vary substantially over small (e.g. centimeter) scales, commonly referred to as “thin layers” (e.g. Cowles et al. 1998, 1999, Hanson & Donaghay 1998, Holliday et al. 1999, Dekshenieks et al. 2001, Alldredge et al. 2002, Rines et al. 2002). Our previous ONR-funded research has allowed us to begin to understand how zooplankton interact with thin layers and how they can take advantage of biomass of prey concentrated in these small-scale features (Avent et al. 1998, Bollens 2000, Bochdansky & Bollens 2004, Clay et al. 2004, Ignoffo et al., 2005). However, there is almost no information regarding how zooplankton can influence the characteristics and persistence of thin layers.

In this project we proposed to address this issue, with two main long-term goals: First, to determine to what extent zooplankton graze and export carbon from thin layers; and second, to determine whether and how zooplankton influence the physical (e.g. optical and acoustical), chemical, and biological characteristics of thin layers with their presence. These goals require determination of rate processes such as feeding activity and excretion, which are very difficult to assess in the field. Thus our research is focused on detailed experimental studies of biological rate processes and behaviors that contribute to the recycling and export of material in and around thin layers.

## **OBJECTIVES**

The four primary objectives of our proposed research are:

- 1) To understand the spatial (vertical) coherence and temporal persistence of phytoplankton thin layers with and without the impact of zooplankton grazing.
- 2) To understand biological rate processes that influence the carbon budget within and in the immediate vicinity of thin layers.

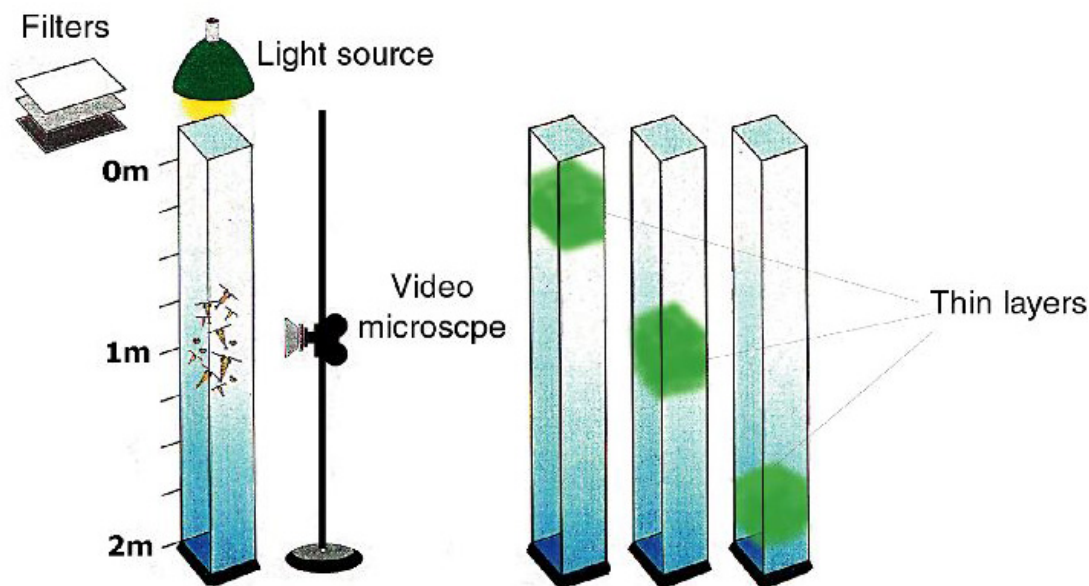
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- 3) To separate local (within thin layers) from non-local (elsewhere in the water column) effects on the thin layers, depending on the type of zooplankton grazers that utilize thin layer organisms as food sources.
- 4) To understand to what extent zooplankton return inorganic nutrients to the autotrophs in the layers and thereby influence the persistence and spatial expanse of thin layers.

Our primary hypothesis is that trophic processes alter the flux characteristics of organic carbon and inorganic nutrients such as ammonia and phosphorus in the thin layers and in the immediate vicinity of the layers, thereby changing essential properties of the layers (such as persistence, vertical expanse, biological productivity and export flux). Organisms that aggregate and subsequently stay confined within the layers (e.g. microzooplankton such as ciliates, dinoflagellates or rotifers) will have a different effect on recycling and export fluxes than organisms that only transiently visit these layers (e.g. mesozooplankton such as copepods).

## **APPROACH**

Experimental plankton towers: All experiments are being conducted using a plankton tower tank system installed in the Bollens laboratory at Washington State University Vancouver (Fig. 1). This tower tank system has been used successfully in several previous studies (Speckmann et al. 2000, Lougee et al. 2002, Clay et al. 2004, Bochdansky & Bollens 2004, Ignoffo et al., 2005), and was slightly modified for the current project by the addition of valves to allow for 5 cm-spaced subsampling of the tanks in and around the thin layer, installing ethanolamine CO<sub>2</sub> traps to prevent <sup>14</sup>CO<sub>2</sub> release into the atmosphere, and additional high-pressure sodium vapor light sources to increase the range of light intensity for work with autotrophic organisms. In addition to the PI (Bollens) and Co-PI's (Rollwagen-Bollens and Bochdansky), during the past year three research technicians from Washington State University Vancouver (Alejandro Gonzalez, Molli McDonald, and Celia Ross) have been responsible for the set-up and maintenance of the tower tank system. Mr. Gonzalez oversees the operation of the tanks and video recording, and Ms. McDonald and Ms. Ross have been responsible for culturing all experimental organisms as well as monitoring radiation safety, in consultation with the Washington State University Radiation Safety Office.



**Figure 1.** Two-meter high columnar tanks are illuminated by natural light simulators, which incorporate neutral density filters to adjust light intensity. The entire vertical extent of each tank, with a thin layer of phytoplankton, is repeatedly scanned and imaged with an infrared-sensitive video camera to record zooplankton distribution.

The experiments in this research project (2006 – 2008) have overlapped with portions of the ONR field program: Layered Organization in the Coastal Ocean (LOCO) conducted in Monterey Bay, CA. One graduate student (Mr. Joel Quenette from Washington State University Vancouver) is working on the behavioral aspects of the research. A second graduate student (Ms. Joanne Breckinridge of Washington State University Vancouver) is working on a separate, but related, project examining larval decapod distribution and vertical migration in relation to physical structure (i.e. thin layers) in the water column of estuaries.

**Effect of microscale distribution of zooplankton (Year 1):** We determined the fine-scale distribution of autotrophic and heterotrophic protists in the tanks using an external DFL fluorometer (as used in Bochdanský & Bollens 2004), and via direct counts of cells from water obtained through the sampling valves. Mesozooplankton distributions were determined via videomicroscopes that regularly panned the length of the tower tank and recorded onto VHS tapes. Details on the statistical analyses of distributional data resulting from these experiments can be found in our previous thin layer papers (see references above), as well as in Solow et al. (2000) and Beet et al. (2003). In Year 1 (2005-2006) these “behavioral” experiments were conducted simultaneously with radioisotope experiments to measure the redistribution of carbon in and around thin layers (please see Bollens et al. annual report, September 2006; Bochdanský et al. in prep). In Year 2 (2006-2007) the behavioral experiments were conducted with another set of radioisotope experiments described below.

**Redistribution of phosphorus by thin layer organisms (i.e. vertically-migrating phytoplankton) (Year 2):** In our Year 1 experiments, we labeled non-migrating phytoplankton cells with  $^{14}\text{C}$  and observed the redistribution of labeled carbon through the water column in the presence of micro- vs. mesozooplankton grazers. In Year 2, we established a nutricline near the bottom of the 2-m tanks using radiolabeled phosphorus (added as  $\text{NaH}_2^{33}\text{PO}_4$ ) in f/2 medium and introducing cultured

*Akashiwo sanguinea* (= *Gymnodium sanguineum*) to the surface layer containing nutrient-free seawater. The redistribution of phosphorus by *A. sanguinea* was monitored over time (48 hours) at 6-12 hour intervals and over the space of the 2 m tower tanks by taking samples through valves positioned along tanks' walls (please see Bollens et al. annual report September 2007).

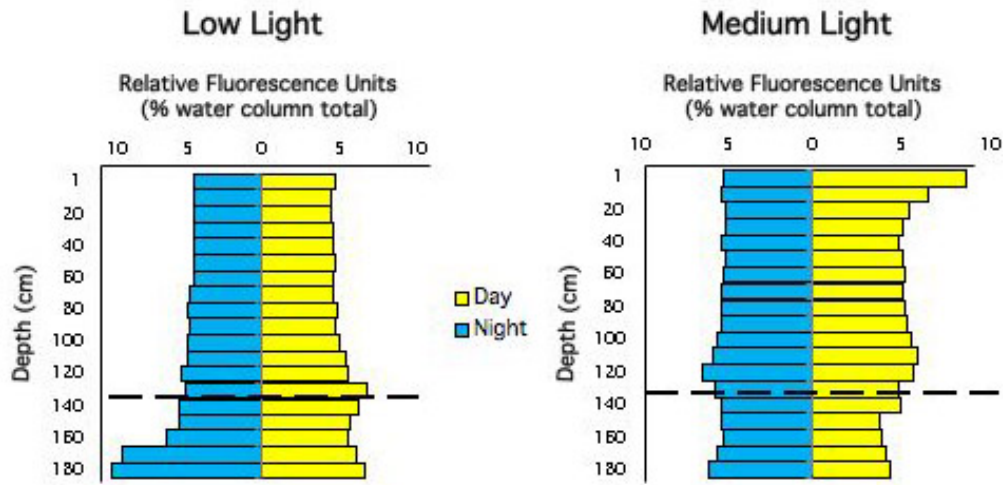
**Vertical migration of dinoflagellate “thin layers” in relation to varying light, nutrients and predators (Year 3):** Based on the results of the  $^{33}\text{P}$ -phosphorus experiments with the vertically migrating dinoflagellate *Akashiwo sanguinea*, in Year 3 we focused specifically on the behavioral aspects of *A. sanguinea* migration and thin layer formation under varying conditions of both physical (light, nutrients) and biological (predators) factors. We examined these phenomena using well-controlled and replicated experiments employing the 2-m high plankton tower system, in which we manipulated both light intensity (0.06 – 140.0  $\mu\text{Einsteins}$ ) and nutrients (0 – 2.0  $\mu\text{M PO}_4$ ), both vertically and between treatments.

## WORK COMPLETED

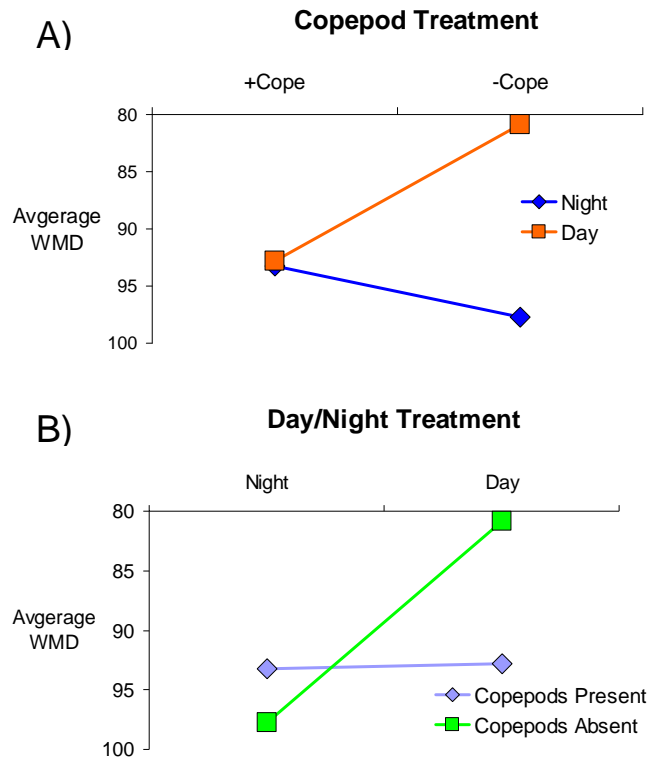
We tested for the effects of light, as well as the presence and absence of copepod predators, on the vertical distribution and diel migration behavior on *A. sanguinea*. A set of 2m high columnar tanks equipped with scanning video microscopes was used to measure the vertical distributions of *A. sanguinea* and the copepod *Acartia tonsa* over 48 hour periods. Relative fluorescence units (RFU) were used as a proxy for *A. sanguinea* abundances. Nutrient concentrations were zero above the pycnocline (140 cm) and 2.0  $\mu\text{M}$  below the pycnocline. Weighted mean depths (WMD) were calculated for each day/night distribution in each tank (treatment) and compared using ANOVA (Bollens et al., 1993). We have run a total of seven experiments, with full data analysis completed on two of these, as presented below.

## RESULTS

*Akashiwo sanguinea* exhibited diel vertical migration (up during the day, down at night) under the medium light treatment, but not the low light treatment (Figure 2). Notably, *A. sanguinea* did not migrate in the presence of copepod predators. More specifically copepods were distributed in the upper water column during the day, resulting in a deeper distribution of *A. sanguinea* (Figure 3, Table 1). The degree to which this is due to individual *A. sanguinea* behavior versus population level effects of copepod grazing remains to be determined.



**Figure 2.** Vertical distributions of *Akashiwo sanguinea*. (A) Low light intensities ( $1.5 - 0.1 \mu\text{Em}^{-2}\text{s}^{-1}$ ). (B) Medium light intensities ( $74.5 - 5.0 \mu\text{Em}^{-2}\text{s}^{-1}$ ). Area below dashed line represents stratum of high nutrients ( $2.0 \mu\text{M PO}_4$ ). Under low light conditions there was no significant difference in weighted mean depth of *A. sanguinea* between day and night. Under medium light conditions, *A. sanguinea* were evenly distributed during the night, but migrated toward the surface during the day.



**Figure 3.** Mean weighted mean depth (WMD) of *Akashiwo sanguinea* during the day and night in the presence or absence of copepod predators. There was a significant interaction between the copepod and day/night treatments, such that in the absence of copepods *A. sanguinea* were migratory but in the presence of copepods *A. sanguinea* descended during the day, thereby becoming non-migratory.

ANOVA: WMD versus Treatment, Day Night					
	Factor	Type	Levels	Values	
Copepod	fixed	2	- C, + C		
Day Night	fixed	2	d, n		
Analysis of Variance for WMD					
Source	DF	SS	MS	F	P
Copepod	1	55.27	55.27	3.66	0.080
Day Night	1	300.96	300.96	19.94	0.001
Cope*Day Night	1	271.74	271.74	18.01	0.001
Error	12	181.10	15.09		
Total	15	809.08			
S = 3.88478		R-Sq = 77.62%	R-Sq(adj) = 72.02%		

**Table 1. Two-way ANOVA results of effects of day/night and presence/absence of copepods on vertical distributions (WMD) of *Akashiwo sanguinea*. There was a significant ( $p < 0.001$ ) interaction effect of predator presence and time of day on vertical distributions of *A. sanguinea*.**

## IMPACT/APPLICATIONS

This research will be an important contribution to the Thin Layers program, as it directly addresses the influence of zooplankton on rate processes in thin layers, and moreover addresses the influence of migrating thin layer phytoplankton on the distribution of major nutrients within and around thin layers. While field studies have to rely primarily on inference from distributions, our controlled laboratory experiments provide flux patterns of important inorganic and organic nutrients in and around thin layers. In this experimental setting we are able to manipulate predator – prey ratios and available nutrients. We will therefore be able to understand potential effects of zooplankton on the persistence and internal dynamics of thin layers, as well as the effect of thin layer phytoplankton themselves. Our experimental work will provide sufficient data to allow us to make predictions about the contribution of biological processes to thin layer dynamics given the presence and abundance of various plankton species found in the field.

## RELATED PROJECTS

This research is relevant to virtually all of the many field studies previously and currently being undertaken within the “Thin Layers” program.

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