Predator avoidance as a possible driver of heterotrophic dinoflagellate diel vertical migration

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Project Summary:

Many dinoflagellates exhibit a diel vertical migration cycle that brings most to the surface water during the day and down at depth during the night (Olsson and Graneli 1991, Olli et al. 1998, Cullen and Horrigan 1981, Suzuki et al. 1997). This migration pattern is opposed to the pattern observed in copepods (Graff 2003, Dagg et al. 1997). The energetic costs of swimming large distances must be balanced by an energetic gain in order for the organisms to continue this cycle. Vertical migration may increase their survival rate because they are rarely colocated with copepods, a potential predator. The proposed study will investigate predator avoidance as a possible driver of vertical migration in heterotrophic dinoflagellates. The study will be carried out March 22-24, 2004 in Dabob Bay of Puget Sound aboard the R/V Barnes. A diel water sampling every 6 meters down to 30 meters will enable microscopic counts of dinoflagellate abundance at discrete depths over a 24 hour period. This will reveal the migration behavior of the population at the time of the study. A concurrent predation experiment will be performed aboard ship in order to measure the predation rates of dinoflagellates on diatoms and copepods on diatoms and dinoflagellates. This will determine whether the copepods are preferentially eating dinoflagellates over diatoms as well as the relative grazing rate of both predators on diatoms. Understanding why dinoflagellates vertically migrate is essential in order to fully understand plankton ecology because such massive movement of biomass through the water column has effects on nutrient cycling, bloom formation, community composition and predator-prey interactions.

Introduction:

Dinoflagellates are an important component of the marine pelagic and coastal ecosystem. Perhaps the most exceptional feature within this protist group is the trophic diversity between different species. There are phototrophic species which absorb nutrients in the water and photosynthesize to fix carbon, heterotrophic species which rely on eating other organism as their energy source, and mixotrophic species that both eat other organisms and photosynthesize. Although still considered planktonic, dinoflagellates are motile organisms. This complicates their role in the ecosystem

because their distribution is influenced by behavioral characteristics and is less dependent on random mixing (Taylor 1987).

Many species of dinoflagellates use their ability to move in order to undergo diel vertical migration. This is an energetically costly behavior of which the benefits are not fully understood. In most dinoflagellates the characteristics of migration are an ascent to the surface during the day and a descent to the depths during the night (Taylor 1987). The deliberate movement of an entire population over such relatively great distances indicates that the behavior must provide a benefit which outweighs the metabolic costs.

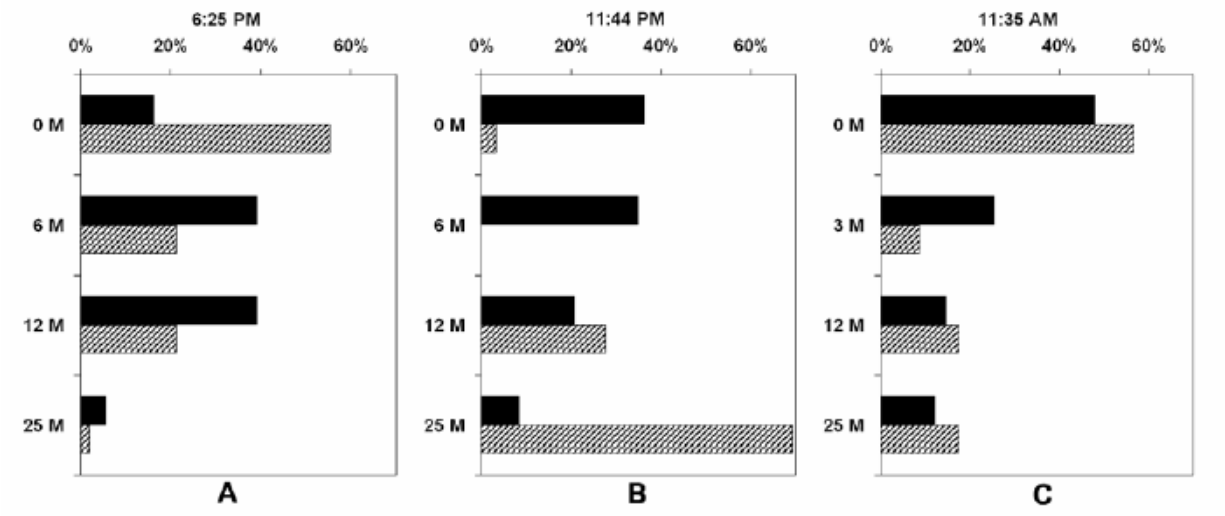
Possible explanations of diel vertical migration and the factors which affect it have been studied extensively in phototrophic dinoflagellates. Because the migration toward the surface occurs during the day, light orientation and intensity has been a factor of interest. In one study, the strength of migration was shown to be proportional to the intensity of light (Staker and Bruno 1997). This supports the idea that light is the driving force behind migration, or at least a cue. However, a study of migration direction under different light orientations and in the absence of light found that movement is independent of light conditions (Cullen and Horrigan 1981). Consequently the importance of the role of light is uncertain, and it is possible that its effects may differ between species. Water temperatures affect the patterns of migration and swimming speed of migrating dinoflagellates when they are at the extreme temperature limits of the species (Kamykowsky 1981). In salt stratified water, cells tend accumulate in thin bands at the halocline (Olsson and Graneli 1991). Although light, temperature, and salinity all affect the patterns of migration, they do not explain the driving force which makes migration beneficial.

In the past, the benefits of migration have been rationalized as a way to utilize both the sunlight at the surface and the nutrients at depth (Olsson and Graneli 1991, Olli et al. 1998, Cullen and Horrigan 1981, Suzuki et al. 1997). However, this only applies to phototrophic species and leaves the migration of heterotrophs unaccounted for because they do not require sunlight or nutrient rich water (Taylor 1987). Instead of focusing on resource utilization, perhaps interactions with other organisms in the ecosystem can shed some light onto heterotrophic migration. Both copepods (Bollens and Frost 1992) and artemia (Forward and Hettler 1992) have been shown to vertically migrate as a

mechanism to avoid predation. When they sense the presence of fish they retreat to the darker depths during the day and return to the surface to feed under the cover of night. In addition to making them less visible, this diel pattern opposes that of their predators, which migrate to the surface during the day and to the depths at night.

If predation pressure from fish can drive these zooplankton to migrate, it may be possible for a similar interaction to drive dinoflagellates to migrate. Observations of dinoflagellate and copepod vertical distribution in Puget Sound reveal opposed migration patterns (Graff 2003) (Fig. 1). Perhaps this is not a coincidental phenomenon. If copepods preferentially feed upon dinoflagellates, it would be advantageous for the dinoflagellates to avoid colocating with copepods. The metabolic costs of migrating long distances over 24 hours might be balanced by the increased survival rate due to predation avoidance.

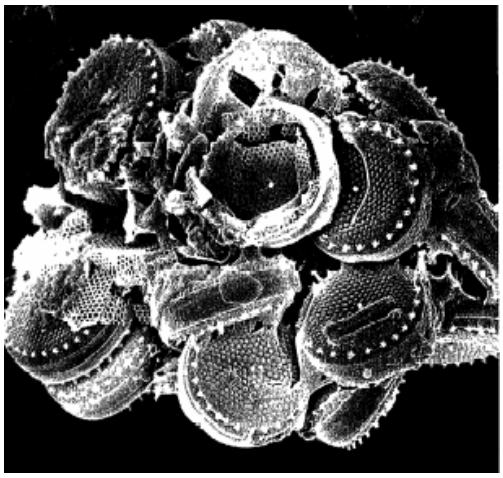
Figure 1. Vertical migration of dinoflagellates (striped) and ciliates (black) in Dabob Bay, Puget Sound. The temporal distribution of dinoflagellates negatively correlated with concurrent measurements of copepod distribution (Graff 2003).



If dinoflagellates are migrating in order to avoid copepods, it must be shown that copepods will preferentially feed upon them over other sources of food. One way to

measure copepod prey selection has been through the use of predation experiments (Leising et al. unpublished). Conducting both a traditional dilution experiment and a copepod incubation experiment enables calculation of the copepod and microzooplankton grazing rates on phytoplankton, as well as grazing rates of copepods on microzooplankton. In this study, copepods were found to be highly selective feeders. It also suggested that selective feeding upon microzooplankton could release predation pressure on phytoplankton. Thus the presence of copepods may enable greater phytoplankton growth through the mechanism of a trophic cascade. Because phytoplankton encompass a wide range of sizes, the cascade effect may only be detected within one of these size classes depending on what the dinoflagellates are consuming. A study of zooplankton fecal pellets found that dinoflagellate pellets in Dabob Bay of Puget Sound were composed entirely of diatoms (Fig. 2) and indicated that they supply almost one third of the total fecal pellet carbon flux (Buck and Newton 1995). This suggests that the cascade effect will be detected within the phytoplankton of the large diatom size class.

Figure 2. Scanning electron micrograph of a dinoflagellate fecal pellet in Dabob Bay. It is composed entirely of *Thalassiosira* cells. (Buck and Newton 1995)



Much is already known about the environmental factors which affect the migration behavior in dinoflagellates, but little research has focused on the effects of predator-prey interactions. The proposed study will test the hypothesis that the diel

vertical migration is driven by predator avoidance. This will be accomplished by two experimental methods. Diel water sampling and microscopic counts of dinoflagellate cells will reveal migration patterns of the dinoflagellate *Gyrodinium.* Predation experiments will show whether copepods preferentially feed upon dinoflagellates and how the grazing rates of dinoflagellates and copepods on diatoms compare. If the dinoflagellates migrate in order to avoid predation, then diel sampling will reconfirm that their migration pattern opposes the copepods. In addition, the copepods will preferentially feed upon dinoflagellates in the predation experiment. It is important to understand the migration of dinoflagellates because their behavior has effects on the rest of the ecosystem. The results of the proposed study will help understand the motivation for the daily migration of an entire population which affects nutrient cycling, predator prey interactions, biodiversity, and plankton abundance.

Proposed Research:

Location- The proposed study will take place in Dabob Bay of Puget Sound aboard the R/V Barnes on March 22-24, 2004. All sampling and shipboard experiments will take place at the buoy station located at 47 46.12.N 122 50.09W.

Migration Behavior- Diel water samples will be taken for 24 hours, beginning on the evening of the 22nd. Water samples will be collected approximately every 4 hours at 6 meter depth intervals down to 30 meters in 10 L Niskin bottles attached to a CTD system. A 200 mL subsample from each Niskin bottle will be preserved with Lugol's fixative and stored for microscopic analysis on shore. One density profile of the water column will be taken from the CTD sensor in order to determine how well mixed the profile is. Large dinoflagellates within the samples will be counted using a light microscope. The volume analyzed will be varied depending on the abundance of dinoflagellate cells present in order to obtain statistical significance using a Poisson distribution (Larson and Farber 2003). One triplicate sample will be counted in order to determine the variability caused by the sampling method. The resulting abundances will reveal depth distribution of dinoflagellates over a 24 hour period.

Microzooplankton dilution experiment- An onboard dilution experiment will begin after the first water sampling on the 22nd. Filtering the seawater through a small

mesh will remove large copepod predators so the measurements will only apply to microzooplankton and phytoplankton. The seawater will be incubated in 1 L jars for 24 hours in an onboard incubator in one of four dilutions, 100%, 50%, 25% or 10% seawater. Each dilution condition will be done in triplicate in order to determine experimental variation. After 24 hours all samples will be filtered using a size fractionated vacuum pump through 0.7 μm Whatman GF/F filters and 10 μm filters. The filters will be stored in acetone and stored in the freezer until they are centrifuged and read with a fluorometer on shore. The fluorescence of each sample will be converted to chlorophyll using the Lorenzen equations (1966). When chlorophyll concentration is plotted versus dilution, the slope of the line created is equal to the grazing rate. The y-intercept is equal to the phytoplankton growth rate in the absence of predators (Landry and Hassett 1982). Measuring size fractionated chlorophyll will enable calculation of growth rates of small phytoplankton and large diatoms.

Copepod grazing experiment- The second on board experiment will include the copepod predators. Copepods will be collected with a 571 micron 1 meter diameter plankton net (a Puget Sound net) and picked out of the sample using a microscope and pipette. Water samples will be filtered through a small mesh in order to remove all copepods and placed in 1 L jars. A triplicate sample will be fixed with Lugol's fixative before the incubation begins in order to record the initial diatom and dinoflagellate abundance. The size fractionated chlorophyll of these water samples will also be measured before the incubation begins in order to determine initial chlorophyll concentrations among the different size classes before being incubated with copepods. The copepod *Calanus pacificus* will be spiked into triplicate water samples, incubated in the onboard incubator for 24 hours, and then both fixed with Lugol's fixative and filtered for chlorophyll concentration. For statistical significance of grazing rate, the copepods must clear between 25 and 75 percent of the water sample. If they filter 5-10 mL of water per copepod per hour, 3 copepods must be added to the samples. If they filter 15-25 mL of water per copepod per hour, only 1 copepod is needed. To ensure a statistically significant clearance of the water sample, water samples with 1 copepod and with 3 copepods will be incubated. After 24 hours, the fixed samples will be stored until they can be analyzed on shore with a light microscope. After determining which copepod

treatment had the most ideal clearance rate from an initial count, the abundance of diatoms and dinoflagellates will be counted in each triplicate sample. The change in dinoflagellate and diatom population from the initial sample will reflect predation by copepods. The grazing rates will be determined by the Frost equations (Frost 1972) and can be compared to the microzooplankton grazing rate and phytoplankton growth rate calculated from the dilution experiment. The change in growth rate of the different size classes of phytoplankton will help to detect a trophic cascade and indicate what each grazer is eating.

Fecal pellet observations- As a qualitative analysis of zooplankton grazing, the composition of fecal pellets within the microscopically analyzed water sample of the predation experiment will be noted. If the contents of the pellet can be determined visually, it will provide further insight into diet composition of the zooplankton.

Expected results- I expect that the diel water sampling will reveal dinoflagellate abundance at depth during the night and at the surface during the day, as reported by Graff (2003). This sampling will be of higher resolution than that study and will confirm dinoflagellate behavior at the time of experimentation. I expect the grazing experiments to show a copepod prey preference of dinoflagellates over diatoms, which would provide motivation for dinoflagellates to avoid copepods. This will be detected by the growth and grazing rates calculated by the microzooplankton dilution and copepod grazing experiments. If copepods preferentially feed on dinoflagellates, the dinoflagellate counts will decrease after incubation, the diatom growth rate will increase because they are not under as much predation pressure, and the smaller phytoplankton will increase because there are less dinoflagellates feeding on them.

Budget:

		Actual Cost	My Budget
Platform cost			
R/V Clifford A. Barnes	$1700 x 3 =	$5100	$0
Shipboard equipment			
CTD system	$135 x 3 =	$405	$0
Water sample incubator	$3 x 3 =	$9	$9
Zooplankton townet	$6 x 3 =	$18	$0
Lab equipment			
Dissecting microscope			$0
Filtration Rack and Pump	$6 x 3 =	$18	$0
Shipboard supplies and expendables			
Pipet for plankton capture		$0.30	$0
Glass incubation bottles	$6 x 3 x 1 =	$18	$0
Lugol's fixative		$20	$20
Filters			
• 0.7 μm GF/F		$17.50	$17.50
• 10 μm		$16.85	$16.85
• 0.7 μm GF/F		$6.80	$6.80
Acetone		$24.25	$24.25
25 ml Falcon tubes		$17.76	$17.76
Water sample bottles	$6 x 3 x 2 =	$36	$0
Graduated cylinders			$0
Post-cruise equipment			
HCl		$1	$1
Fluorometer cuvettes			$0
Fluorometer			$0
Centrifuge	$6 x 3 =	$18	$0
Dissecting microscope			$0
Total amount:		**$5726.46**	**$113.16**

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