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# Planar Laser Imaging of Scattering and Fluorescence of Zooplankton Feeding in Layers of Phytoplankton *in situ*

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Award Number: N00014-06-1-0304
http://spiff.ucsd.edu/

## LONG-TERM GOALS

We intend to quantify the biological, physical, and chemical dynamics that structure marine planktonic ecosystems. Observations of the organisms and their environment on the spatial and temporal scales that characterize their interactions, combined with models of the dominant dynamics, will lead to improved understanding of the dynamics, structure, and function of planktonic ecosystems.

## OBJECTIVES

Our objectives in this work are to 1) visualize and quantify herbivorous copepod feeding in the laboratory, and 2) to apply these methods in the field to observe the dynamics of copepod feeding *in situ*. In particular we intend to test the "feeding sorties" hypothesis vs. the "*in situ* feeding" hypothesis regarding the location and timing of copepod feeding and vertical migration.

## APPROACH

Previous attempts to quantify copepod feeding have either been indirect (measuring the phytoplankton concentration before and after copepods were introduced to a sample), or direct (measuring the gut fluorescence of individual copepods feeding on phytoplankton). The disadvantage of the first method is that we obtain little information about the activities of individual copepods, and how their feeding might change in time. The second method is destructive, and generates only one data point per individual copepod, rendering it ineffective for generating time series of feeding activity. To obviate these problems, we use a planar laser imaging fluorometer (PLIF) system for quantifying copepod gut fluorescence and feeding. A green (532 nm) laser is used to stimulate the fluorescence of chlorophyll *a* ingested by copepods. The fluoresced red (680 nm) light is imaged by a very sensitive CCD camera.

We also designed and built a bi-spectral PLIF system that images both the fluorescence from chlorophyll *a*, and the green light scattered from particles (including copepods) in the imaging plane. This method is non-destructive, allowing time-series measurements to be made on individual copepods.

To image copepods in the field, we constructed a two-camera system, that would take images in synchrony. One camera was equipped with a 530 nm filter to detect the shape of imaged particles; the second camera was equipped with a 685 nm filter to image fluorescent particles; both cameras imaged the same plane. The constraints imposed by the system's geometry limited our image resolution to about 80x80 microns in an imaging plane of 10 x 13 cm. This was adequate for identifying copepods in the images. This bi-spectral imaging system was profiled vertically in the field to identify copepods, determine the depth at which they are located, and determine whether they have recently fed on phytoplankton.

## WORK COMPLETED

*Laboratory work: Quantifying individual copepod gut dynamics*

Our first step in this project was to design a series of experiments to characterize the response of the PLIF to the gut content of feeding copepods. We ran two types of tests on adult females of the calanoid copepod, *Calanus pacificus*; an important grazer in the Southern California Bight and in Puget Sound (WA) where our field work took place. The first experiment had copepods swimming freely in a container with phytoplankton. This was adequate to allow us to determine whether the copepod had phytoplankton in its gut, and could thus be useful to determine *in situ* whether a copepod had fed. However, it was difficult to determine how much phytoplankton had been ingested because the position of the copepod relative to the camera affected the observed fluorescence intensity. Furthermore, free phytoplankton can also affect the fluorescence image when cells are in the imaging plane. In order to quantify the exact gut content, in particular when the goal is to evaluate the change in gut content of a same copepod throughout a feeding bout, we needed to keep the copepod in a consistent orientation relative to the camera. We also had to keep phytoplankton from interfering with the imaging process. This challenge led us to design the second type of experiment.

In the second set of experiments we tethered a copepod, to an acid-washed human hair. The copepod could then be held in position, keeping its side facing the camera. This positioning enables a clear view of the gut tract, and keeps the limbs from affecting the signal. Food was offered to the copepod by injecting a stream of phytoplankton, delivered via a pipette connected to a peristaltic pump, direcly in front of the animal so that it could easily capture the cells by entraining them in its feeding current. We ran two variants of the tethered approach.

The first, the bolus chase experiment, was designed to study the pigment dynamics of a single food bolus passing through the gut.

The second variant of the tethered approach consisted of running experiments, while maitaining food availability constant, at 3 different temperatures. These temperatures were 8, 12, and 16 °C; a range commonly encountered by *C. pacificus* in the Southern California Bight. 12 copepods were experimented upon, resulting in a total of 36 experiments of approx. 5 hours each. The order of experiment temperatures was permuted so that only two copeopds were run through the same sequence of experimental temperatures.

*Field-work: Assessing the foraging sorties hypothesis*
The PLIF was mounted on a free-falling vehicle named the multi-spectral copepod imaging system (MISCIS). The vehicle was deployed *in situ* to allow us to document the short-term (hours) feeding behavior of copepods. Specifically we wanted to explain previously documented lack of co-location between copepods with full guts and the shallow layer of maximum chlorophyll concentrations in Dabob Bay (Dagg *et al. 1997, 1998*). One explanation could be that copepods are able to find sufficient food in the layers below maximum food concentration (the "*in situ* feeding" hypothesis - ISF). Another explanation is that copepods exhibit complex foraging behavior, possibly to limit chances of predation that are higher in the chlorophyll maximum layer: the "foraging sorties" hypothesis (FS). This hypthesis says that the copepod's foraging behavior would consist of short excursions into layers of high food concentration to feed until their gut is temporarily replete. Once the gut is full, the copepod would sink or swim out of that layer while it digests and defecates. This results in a lower predation risk, and the slower gut turnover could imply greater absorption of nutrients from digested food.

To attempt to distinguish between the ISF and FS feeding behaviors *in situ*, we performed constant vertical profiling with a 2-channel MISCIS over periods of hours at a time (approximately 1 profile per 10 min). As explained above, one channel recorded light scatter from the laser sheet to identify copepods. The second channel, focused on the same imaging plane as the first channel, recorded chlorophyll fluorescence. Images were collected during the downcast. The deployments took place in Dabob Bay, a fjord in Puget Sound (WA) that has little physical disturbance and from which the same population of copepods can be sampled repeatedly.

*Individual-based model (IBM) of copepod foraging behavior*
We developed an IBM to explore the dynamics underlying copepod foraging behaviors. An important component of our model was the gut compartment which operated in one of two modes: feeding or non-feeding. A feeding copepod's gut clearance was formulated according to our laboratory results, which included an ambient temperature-dependent response. Ingestion rate was also taken into account, and formulated as a food-dependent saturation response. Our laboratory results were obtained under conditions of food saturation so that ingestion inferred from our data was taken as the maximum ingestion rate possible. A non-feeding copepod gut clearance was taken from previously published temperature-dependent data that were measured in non-feeding copepods.

## RESULTS

*Laboratory work: Quantifying individual copepod gut dynamics*
Initial calibration work in the laboratory showed that the gut fluorescence imaged with PLIF is linearly related to the extracted gut pigment, provided the position of the copepod relative to the imaging system is consistent. This allows a reliable quantification of the gut contents of live copepods.

Rates of gut clearance in non feeding copepods were comparable to previously published values. However significantly more of the fluorescence decay was due to pigment destruction than defecation (Fig. 1). This contradicts the conclusion by Head and Harris (1996) that destruction only occurs early on during feeding. Our results show that pigment destruction occurs throughout the midgut. In some cases an exponential shape gives a good fit to the fluorescence decay, suggesting a constant decay rate. In other cases however, a power law fits the decay better, indicating a decay rate that decreases with time.

Two important observations were made. One is that there were significant differences in feeding characteristics among individual copepods (Fig. 2). This finding is important because most measurements on copepod grazing relate to an average copepod. However whether this average copepod is relevant to ecosystem processes is not clear. It is conceivable for instance that faster feeders would have a disproportionately greater reproductive fitness. On the other hand, in systems where predation pressure is considerable, it is similarly conceivable that slower and thus stealthier feeders have an advantage. The wide variability among individuals could thus offer the population a capacity to adapt to changing conditions.

The second significant finding is that gut clearance occurs at a higher rate during feeding than during refractory periods. Most gut clearance measurements to estimate ingestion have been performed on non-feeding copepods. Our results suggest that copepods feeding under saturation conditions clear their guts in 10 – 60% of the time taken by a non-feeding copepod. This difference increases with increasing temperature. Actual feeding rates in the field are likely to lie between these two extremes (Fig. 3).

*Field-work: Assessing the foraging sorties hypothesis*

The MISCIS package allowed us to map the vertical distribution of fed and unfed copepods in the water column, relative vertical gradients in food contration. A clear result was that copepods with full guts tended to be associated with layers of high food concentration (Fig. 4), a somewhat contradictory result to that found earlier by Dagg *el al.* (1997, 1998) that showed copepods of comparable gut content in and below the layer of maximum food concentration. Our MISCIS package allowed much finer vertical sampling of the copepods, and more accurate estimates of their gut content than the net sampling used in most similar studies.

*Individual-based model (IBM) of copepod foraging behavior*

The copepod vertical and temporal distributions from the IBM agreed well with data collected by the MISCIS in Dabob Bay (Fig 4). Our model was forced with both simplified idealized hydrographic profiles and actual CTD data collected during our field deployment, and during other studies in Dabob bay (Fig 5). Analysis of the various simulation runs showed that the foraging strategy exhibited by a copepod was dependent on the hydrographic environment, specifically on the distribution of food in different layers and, to a lesser extent, on the ambient temperature in the different layers. Thus, the greater the difference in phytoplanton concentration between the shallow and the intermediate layer, the greater the occurrence of foraging incursions into the shallow predator rich layer. This resulted in copepods rapidly filling their gut and letting themselves sink while digesting (FS). On the other hand, the lower the difference in food concentration the greater the occurrence of *in situ* feeding (ISF) with correspondingly less frequent vertical displacement (summarized, Fig 6). Temperature affected the time necessary for gut filling and clearance, demonstrating the necessity of taking this variable into account when studying copepod foraging in the field.

Our simulation results showed that, given the hydrographic settings during our field work, foraging sorties were likely to be common, explaining the greater occurrence of copepods with full guts in the shallow food-rich layer. On the other hand, with the hydrographic settings encounter by Dagg *et al.* (1997, 1998), there was a greater chance of *in situ* feeding being the dominant foraging mechanism. These two strategies are not exclusive of each other in a given copepod, but are emergent behaviors based on the environmental structure and stochastic food encounters experienced by the copepods. Thus both FS and ISF would be expected under different conditions.

## IMPACT/APPLICATIONS

Our bi-spectral PLIF system, MISCIS, gives us an entirely new way to gather data from planktonic organisms in the lab and *in situ*. Combined with appropriate auxiliary data, this system will allow us to investigate the dynamics of the planktonic ecosystem at the level of the individual plankters. The data generated will give us a unique and powerful new view into the dynamics structuring marine planktonic ecosystems.

## RELATED PROJECTS

This work grew from our ONR-sponsored project entitled "Biological and Chemical Microstructure in Coastal Areas" in which we deployed a PLIF system in tandem with an optical nitrate sensor and microstructure sensor. Based on the information gathered in the present work, we will re-analyze the images acquired in our earlier cruises to attempt to identify zooplankton gut fluorescence in the images.

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## PUBLICATIONS

Karaköylü, E., P.J.S. Franks, Y. Tanaka, P.L.D. Roberts and J.S. Jaffe. 2009. Copepod feeding quantified by planar laser imaging of gut fluorescence. Limnology and Oceanography-Methods 7:33-41.

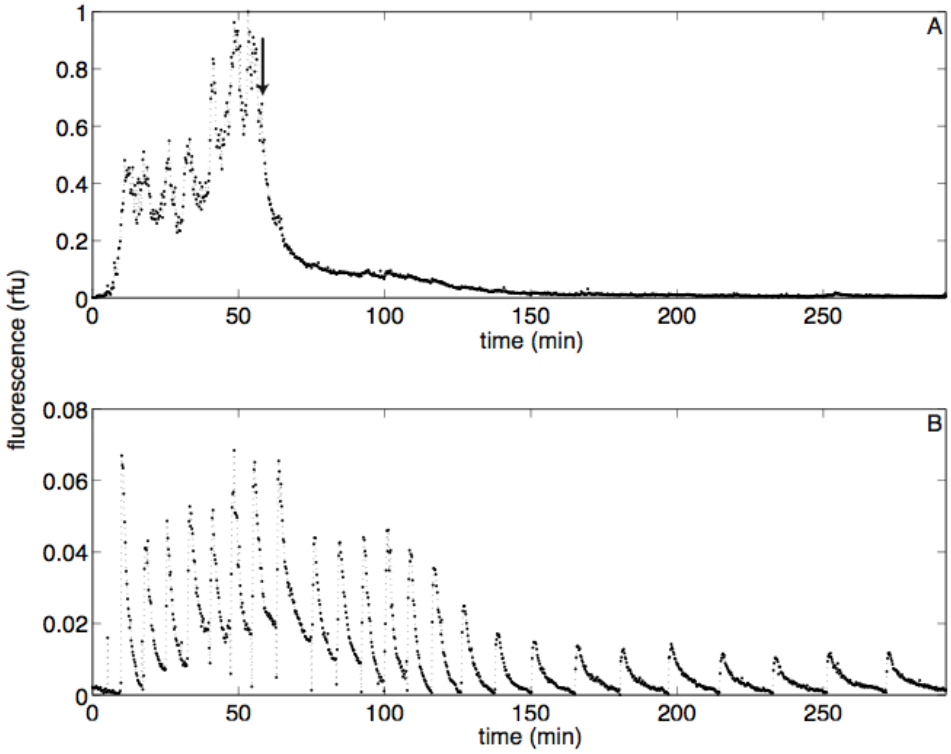


***Figure 1. Gut fluorescence decay in an individual non-feeding copepod. Panel A shows total midgut content, the arrow shows the time at which food was interrupted. Panel B shows posterior midgut (PMG) content. Total midgut and PMG data show that defecation is not sufficient to account for the observed decay in total midgut content, indicating that pigment destruction is present throughout the midgut.***

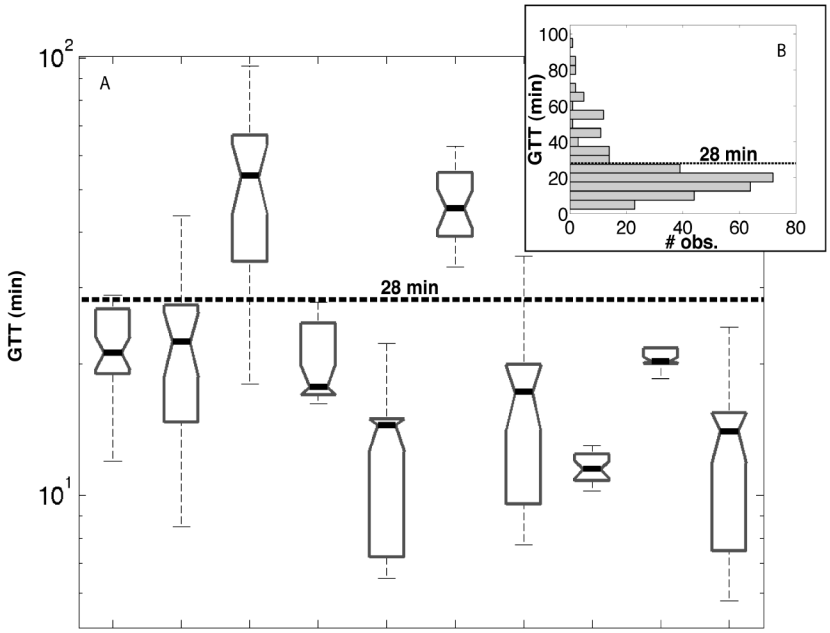


***Figure 2. Statistics of individual copepod's gut clearance. Panel A shows individual gut clearance, expressed as gut throughput time (GTT). Top and bottom of the boxes are the upper and lower quartiles of an individual's GTT, respectively. The thick black line is the median; side notches mark 95% confidence intervals. The horizontal dashed line references the expected GTT, given the experimental temperature (12 °C, 28 min, Dam and Peterson 1988). Panel B combines all copepods.***

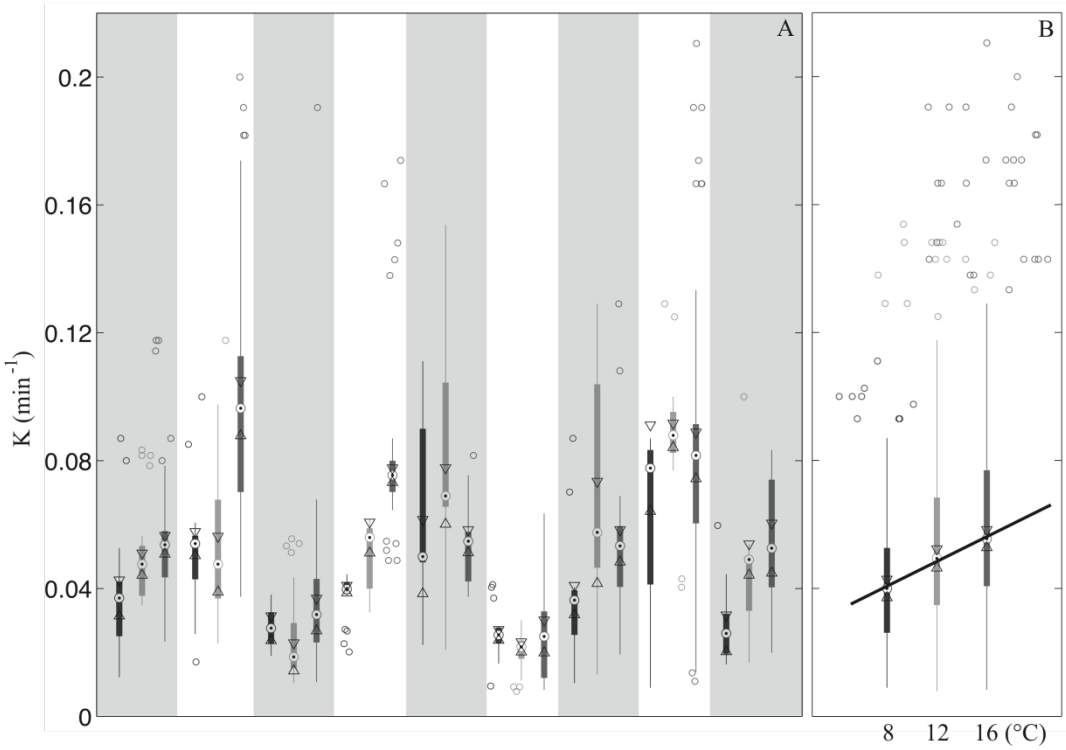


***Figure 3. Gut clearance rate K (min$^{-1}$). Panel A shows individual response, by temperature. Each vertical bar (gray, white) represents data from an individual copepod at three different temperatures (8 °C, 12 °C, 16 °C from left to right). Panel B shows group response to temperature. Black line shows the fit to the group median.***

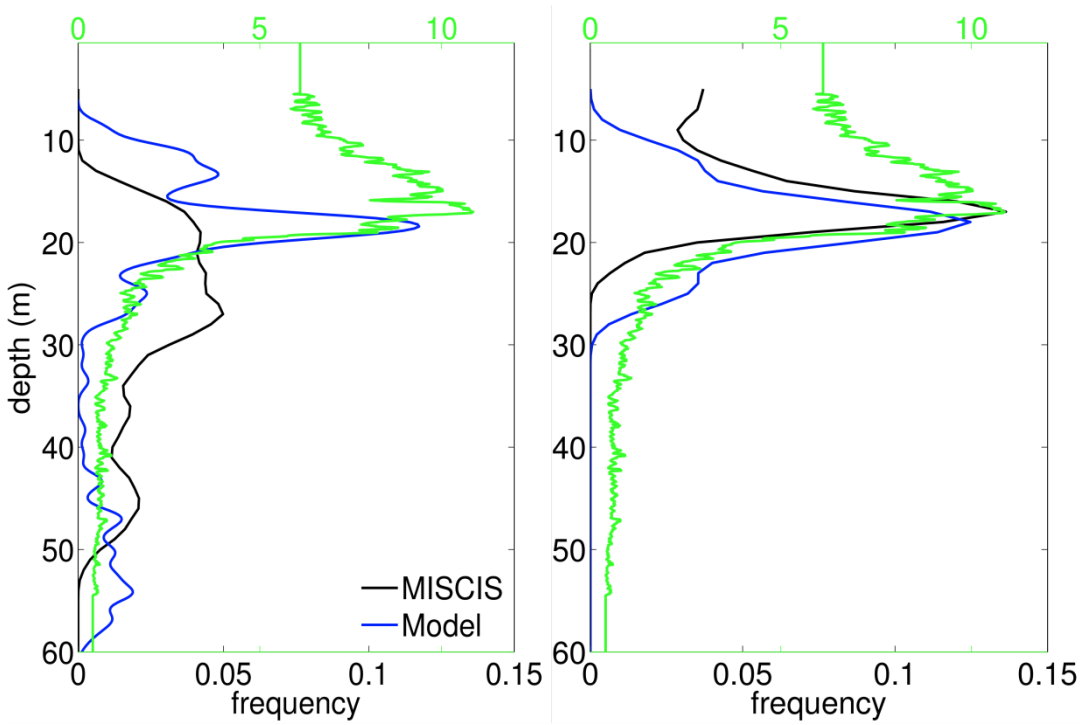


***Figure 4. Vertical distribution of full and empty copepods from data and from the model at dusk. Copepods with empty guts shown left; copepods with full guts shown right. —: MISCIS field data. —: Model data, —: chlorophyll (µg $L^{-1}$) from CTD.***

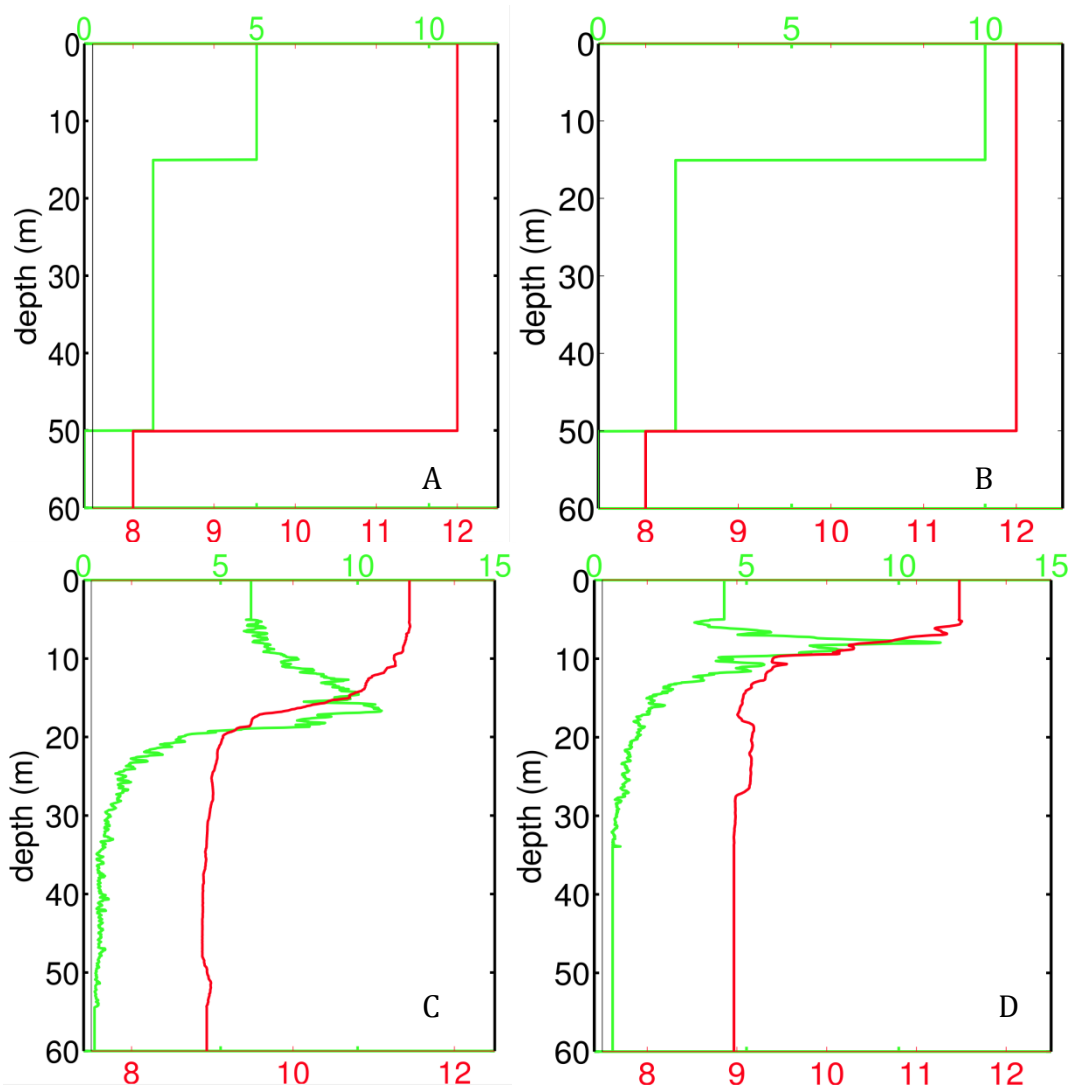


***Figure 5. Hydrographic conditions – Examples of food distributions used to drive the copepod feeding model. Chlorophyll distribution shown in green, temperature in red. A and B: Idealized scenarios with slab-shaped layers. C and D: real hydrographic data acquired during a cruise in May 2007 in Dabob Bay, WA. — temperature (ºC) — chl (µg L$^{-1}$)***

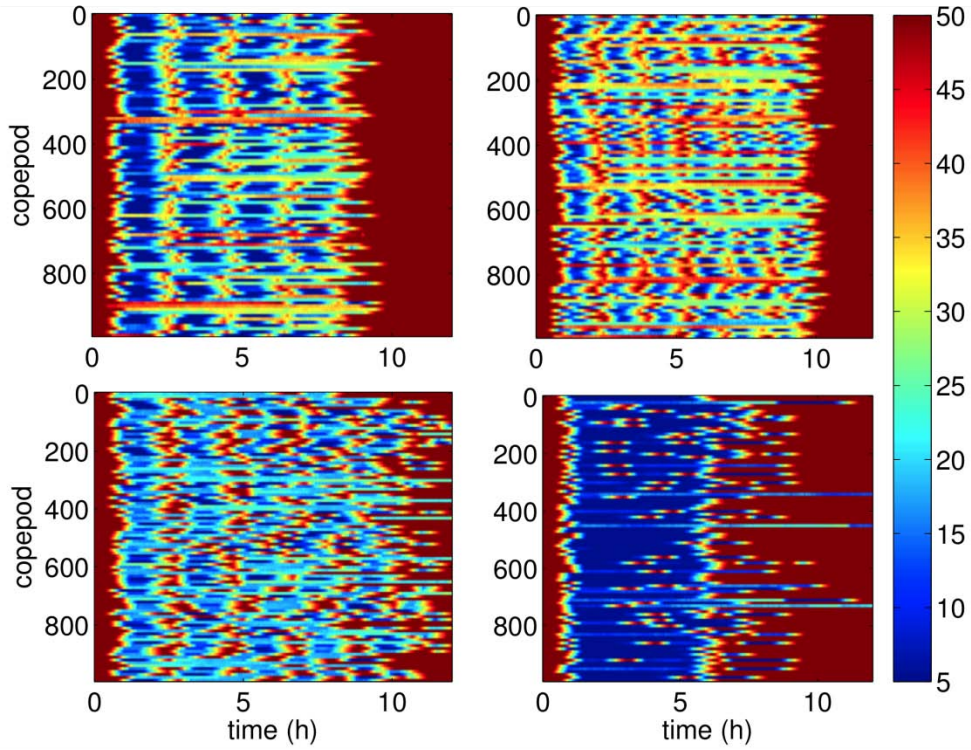


***Figure 6. Composite of copepod trajectories in the water column. Rows report individual copepod vertical location through time. Panels correspond to scenario panels A-D in Fig 5. Warmer colors indicate deeper location; colder colors imply a shallow station (color bar at right gives depth of copepod in meters). Red implies relative safety, blue implies relative danger due to visual predators. Depth range includes the shallow and intermediate layer. Alternating bands of warmer and colder colors are diagnostic of foraging sorties. Width of bands indicates time spent at either surface or depth.***

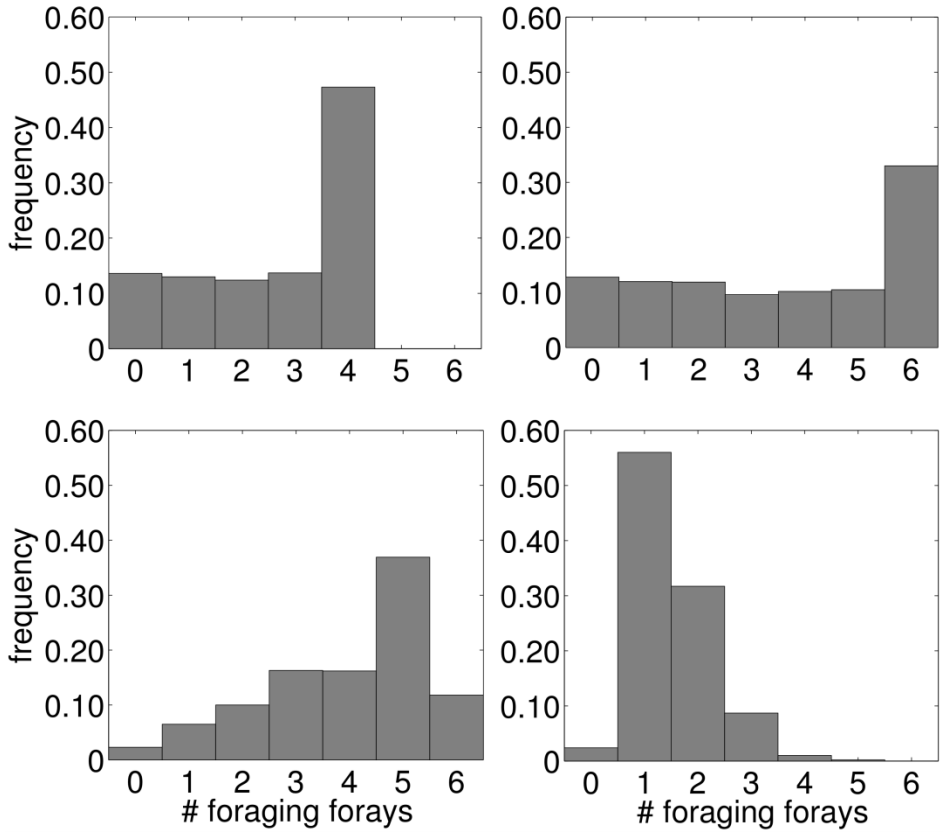


***Figure 7. Foraging sorties distribution frequency. Panels A-D correspond to panels in Figs. 5 and 6. A and B: idealized hydrography; C and D: real hydrography. A-C: increasing shift toward foraging sorties; D: in situ feeding is dominant.***