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# The effects of thin layers on the vertical distribution of the rotifer, *Brachionus plicatilis*

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## Abstract

Microscale patches of resources occur in both the horizontal and vertical dimensions, and in the latter case are referred to as thin layers. These layers may affect ecological processes like behavior, predation, growth and reproduction in phytoplankton and zooplankton. The objective of this study was to determine possible effects of physical and biological thin layers on the vertical distribution and diel vertical migration of the rotifer *Brachionus plicatilis*. We used four, 2 m tall, experimental tanks fitted with video cameras which panned the vertical extent of each tank and enabled us to sample on the centimeter scale. The experimental tanks consisted of a thin layer (25 cm thick) of *Nannochloropsis oculata*, whereas control tanks consisted of homogeneously distributed algae. Rotifers aggregated in the thin layers of *N. oculata*, and dispersed (becoming evenly distributed) after depleting the algae within the thin layer (ca. 6 h). In contrast, rotifer aggregation in the physical thin layer of control tanks was shorter in duration (ca. 2.5 h) and rotifers were homogeneously distributed for the remainder of the experiment despite persistent salinity stratification. No signs of diel vertical migration were noted in either experimental or control tanks. A second set of experiments was run to examine the response of rotifers to a choice of food, i.e., thin layers of the diatom, *Skeletonema costatum* versus the eustigmatophyte, *N. oculata*. For the choice experiments, two thin layers were created, one with each food option. Our results suggest that *B. plicatilis* aggregates and feeds preferentially on *N. oculata* over *S. costatum*. In both types of experiments rotifers responded, in terms of distribution, to thin layers of algae within the first half-hour of introduction and remained in the thin layers until the food source was depleted. Our results suggest that rotifers may be important grazers on thin layers because of their ability to quickly locate and take advantage of ephemeral food patches.

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**Keywords:** Vertical distribution; Rotifer; *Brachionus plicatilis*

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## 1. Introduction

In recent years, many studies have been conducted on the spatial variability of phytoplankton and zooplankton in the water column. This spatial variability or patchiness has been documented in various habitats including estuaries (Donaghay et al., 1992), coastal shelves (Cowles and Desiderio, 1993), fjords (Holliday et al., 1998; Deksheniaks et al., 2001), and the open ocean (Bjornsen and Nielsen, 1991). Thin layers, one type of patchiness, have received considerable attention as technology and deployment techniques have improved. Thin layers are generally described as vertical layers ranging in thickness from ~10 cm to ~3.5 m (Donaghay et al., 1992; Cowles and Desiderio, 1993; Cowles et al., 1998; Hanson and Donaghay, 1998) and persist for several days to several weeks (Bjornsen and Nielsen, 1991; Nielsen et al., 1990; Deksheniaks et al., 2001; Rines et al., 2002). They are sites of highly concentrated biological activity and/or areas of distinct chemical or physical attributes (Nielsen et al., 1990; Cowles et al., 1998; Hanson and Donaghay, 1998) which can have important impacts on the structure and dynamics of marine systems.

There are various processes by which thin layers are formed in the ocean. Physical processes that produce thin layers include shear (Eckart, 1948; Franks, 1995), the disintegration of a thicker layer into a thin layer and the horizontal intrusion of water masses (Osborn, 1998). Biological processes that form thin layers include the sinking and accumulation of biological particles at micropycnoclines and the concentration of grazers feeding preferentially just above and below a given area (Franks, 1995). These processes work independently or concurrently to establish thin layers.

The majority of research conducted on thin layers to date has focused on the description of physical and biological thin layers in various habitats using newly developed sampling techniques (Pieper and Holliday, 1984; Desiderio et al., 1993; Cowles et al., 1998; Holliday et al., 1998; Jaffe et al., 1998), but few studies have investigated organism responses to and utilization of thin layers. For instance, Clay et al. (2004) found that the vertical distribution of larval herring (*Clupea pallasii*) was influenced by light and the physical properties of thin layers, while high

concentrations of prey had no effect on distribution. In the controlled laboratory experiments of Bochdansky and Bollens (2004) the copepod, *Acartia hudsonica*, showed a slight response to biological thin layers in terms of vertical distribution, yet this response did not influence egg production. Fecal pellet production was slightly lower in the presence of thin layers. Another laboratory study by Saiz et al. (1993) determined that the copepod, *Acartia tonsa*, remained in food patches but egg production was not affected. Tiselius (1992) studied the effect of thin layers on the behavior and fecal pellet production of *A. tonsa* and found that while the copepods spent more time in thin layers they did not produce more fecal pellets than in homogeneous conditions. Although the last three studies used similar copepod species, they used different species and concentrations of diatoms as the food source (*Skeletonema costatum* vs. *Thalassiosira weissflogii*), and sampled at different spatial scales.

Rotifers are small (130–320  $\mu\text{m}$ ) euryhaline zooplankton (Lubzens, 1987). They are most diverse and abundant in freshwater systems, but may be found in estuarine and coastal habitats (Arndt et al., 1984; Park and Marshall, 2000a; Fradkin, 2001). It has been shown that rotifers can respond to temporally and spatially limited blooms of phytoplankton in terms of growth rate and reproduction rate (Loftus et al., 1972; Heinbokel et al., 1988). In certain areas, for example the Rhode River, a shallow eutrophic estuary, rotifers may equal and/or exceed the abundance of copepods year round (Allan et al., 1976; Dolan and Gallegos, 1992). The majority of field studies conducted on the vertical distribution and diel vertical migration of rotifers suggests seasonal and spatial fluctuations due to several factors, including predation, thermoclines, and oxyclines (Preissler, 1977, 1980; Arndt et al., 1984; Jose de Paggi, 1995).

Rotifers also play an important role in energy and carbon transfer from primary producers to secondary consumers (Stockner, 1988; Park and Marshall, 2000b) as well as within the microbial loop (Fenchel, 1988; Stockner, 1988). Rotifers in both freshwater and estuarine systems can be major grazers of algae and small ciliates (Havens, 1991; Arndt, 1993; Gilbert and Jack, 1993) and prey for invertebrates (e.g. jellyfish, mysids, cladocerans, and copepods) and larval fish (Polgar and Souza, 1981; Setzler-Hamilton et al., 1981; Williamson, 1983; Stoecker and Egloff, 1987;

Conde-Porcuna and Declerck, 1998). The important role of rotifers in aquatic food webs warrants additional investigation into their vertical distribution generally, and responses to thin layers in particular.

In the present study, we investigate the effects of salinity stratification and biological thin layers on the vertical distribution and diel vertical migration of the rotifer, *Brachionus plicatilis*, through a series of laboratory experiments. Previous field studies have been limited in spatial resolution (on the order of meters) due to sampling limitations. In our laboratory investigation, we address rotifer vertical distribution and diel vertical migration using fine scale sampling (cm) to better understand their response to thin layers.

## 2. Materials and methods

### 2.1. Culturing

The rotifer *B. plicatilis* was obtained from Aquatic Ecosystems and cultured at 15 psu (InstantOcean<sup>®</sup>, Aquarium Systems) under a light–dark cycle of 12:12 h at 21 °C. Rotifers were fed dissolved bakers yeast and *Nannochloropsis oculata* (Aquatic Ecosystems) in a 1:1 ratio three times a day. Food densities were such that it took approximately 1 h for the rotifers to clear the water. Complete water changes were conducted twice a week by removing the rotifers with a 40- $\mu$ m bag filter and inverting the bag filter into clean water.

The eustigmatophyte *N. oculata* and the diatom *S. costatum* (strain #1281 CCMP Provasoli-Guillard National Center for the Culture of Marine Phytoplankton, Bigelow Laboratories) were cultured at 13, 15, and 17 psu under a 12:12-h light–dark cycle at room temperature. *N. oculata* was cultured with GF/F-filtered San Francisco Bay water while *S. costatum* was cultured with 0.2- $\mu$ m filtered InstantOcean<sup>®</sup> dissolved in deionized (DI) water, supplemented with f/2+silica medium for *S. costatum* and f/2 (without silica) medium for *N. oculata* (Guillard, 1975) once a week.

### 2.2. Experimental design

A total of nine experiments were conducted: five designed to look at diel vertical migration and the

response of rotifers to a thin layer of food (thin layer experiments), and four designed to look at the response of rotifers to thin layers composed of two different types of food (choice experiments). All experiments were run at 21 °C in a temperature- and light-controlled room using four columnar Plexiglas tanks (200 cm tall $\times$ 7.6 cm wide $\times$ 5.1 cm deep). More detailed descriptions of this apparatus and various applications to experimental studies of zooplankton can be found in Speekmann et al. (2000), Lougee et al. (2002), Clay et al. (2004) and Bochsansky and Bollens (2004).

Thin layer experiments were run for 48 h with a 12:12-h light–dark cycle and included two control tanks and two experimental tanks. A homogenous distribution of algae (*N. oculata*) was established in control tanks containing a physical thin layer established through density stratification. Experimental tanks had both biological and physical thin layers. Density stratification was achieved by layering various salinities of water within the tanks, accomplished by slowly filling the tanks through eight evenly spaced valves on the side of the tanks. All tanks were filled simultaneously over 4 h to establish three layers: (1) 18 psu water, from a depth of 200 to 93 cm; (2) 15 psu, water from a depth of 93 to 68 cm, and (3) 12 psu water, from a depth of 68 to 0 cm (Fig. 1). The algae were introduced into the tanks with the water as they were filled. The same total amount of algae ( $1.62 \times 10^8$  cells) was used in all tanks. The concentration of algae in the control tanks was  $2.03 \times 10^4$  cells ml<sup>-1</sup> (evenly distributed throughout the whole water column) and  $3.25 \times 10^5$  cells ml<sup>-1</sup> in experimental tanks (concentrated in the middle layer, 15 psu, 500 ml). The concentration of algae for the experimental (thin layer) tanks was chosen so as to provide food at which *B. plicatilis* would exhibit its maximum ingestion rate (Hirayama and Ogawa, 1972; Lubzens, 1987). The concentration of algae in the control (homogeneous) tanks was one fifth of the experimental tanks. These algal concentrations have been characterized by Walz (1995) as food-saturating and food-limiting conditions, respectively, for a similar species, *B. angularis*. Rotifers (5 ml<sup>-1</sup>) were added to the top of each tank at the start of the experiment (40,000 total rotifers). The treatments were randomly assigned to each of the four tanks at the beginning of each experiment.

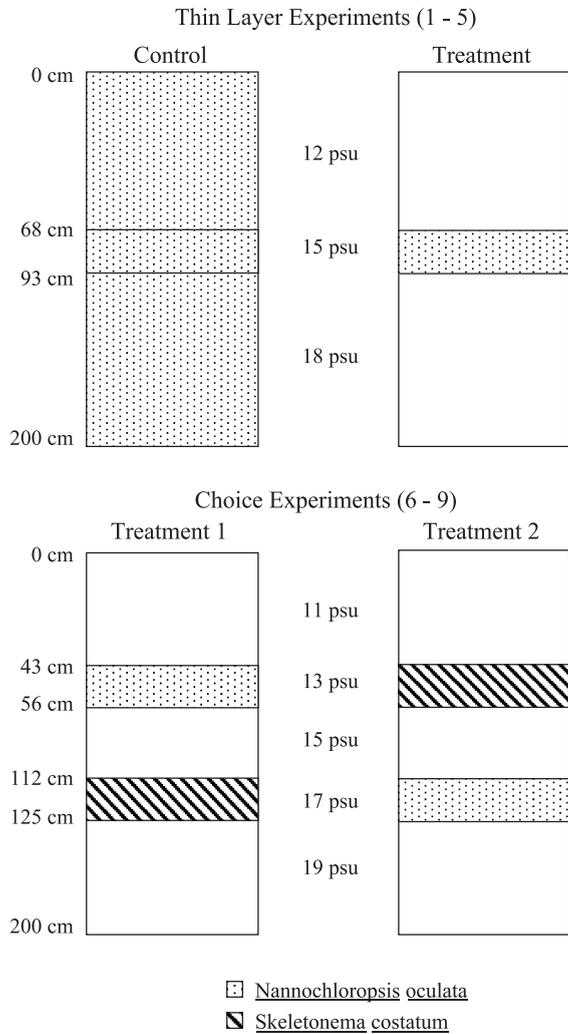


Fig. 1. Experimental design for thin layer (top) and choice (bottom) experiments including salinity and depth profiles.

All thin layer experiments were conducted as described above with the exception of the last (final, Experiment 5) thin layer experiment in which an additional aliquot of algae (30 ml,  $1.62 \times 10^8$  cells) was added to the experimental tanks after 24 h. It was apparent in the previous experiments that the rotifers had depleted the thin layer within the first 12 h and subsequently dispersed throughout the tank. In this final thin layer experiment, we added a secondary algal plume (via a 100-ml syringe through the valve in the middle layer) to determine if the rotifers would redistribute themselves around the thin layer after having dispersed. Control tanks were treated similarly

(receiving the same volume of water) to ensure that any response was due to the injection of a new thin layer of food rather than the increased size of the physical thin layer (ca. 3 cm increase).

The choice experiments were designed to determine how rotifers respond to thin layers of two different types of food: the eustigmatophyte, *N. oculata* and the diatom, *S. costatum*. Two thin layers, each containing a different food option, were established (one at a depth of 125 cm and the other at 56 cm) and the experiment was run for 24 h on a 12:12-h light–dark cycle at 21 °C. To account for any bias in the position of food in the water column (i.e. top of tank versus bottom of tank), two treatments were created. Treatment 1 consisted of *N. oculata* in the top position and *S. costatum* in the bottom position, while in Treatment 2 the food options were reversed (Fig. 1). As in the thin layer experiments, the treatments were randomly assigned to each of the four tanks at the start of each experiment. The placement of the thin layers was accomplished by arranging algae and diatom layers equidistant from the center valve. The tanks were filled simultaneously to produce five layers: (1) 19 psu water from a depth of 200 to 125 cm; (2) 17 psu water, from a depth of 125 to 112 cm; (3) 15 psu water, from a depth of 112 to 56 cm; (4) 13 psu water, from a depth of 56 to 43 cm; and (5) 11 psu water, from a depth of 43 to 0 cm (Fig. 1). *N. oculata* ( $1.62 \times 10^8$  cells) and *S. costatum* ( $2.16 \times 10^8$  cells) were introduced with the water as the tanks were being filled (Fig. 1). Rotifers were introduced to the tanks by injection through the center valve using a 100-ml syringe. This method ensured that the rotifers would initially have an equal chance of encountering either food option. Equivalent amounts of *N. oculata* and *S. costatum* were determined by calculating the carbon/volume ratio for both. Menden-Deuer and Lessard's (2000) carbon/volume equation for calculating carbon content was used for *N. oculata* (20 pg C cell<sup>-1</sup>) and the literature value from Strathmann (1967) was used for *S. costatum* (15 pg C cell<sup>-1</sup>). The rotifers used in the choice experiments were maintained and fed only baker's yeast to ensure that there was no prior predisposition to either experimental food item.

In all experiments, the salinities were adjusted using 5- $\mu$ m filtered mixtures of InstantOcean® and DI water. Prior to each experiment, the tanks were rinsed

and soaked in DI water for at least 12 h. The salinity and temperature distributions in each tank were measured immediately prior and immediately following each experiment using a hand held probe (YSI 85, YSI). The concentrations and distributions of *N. oculata* and *S. costatum* were measured (every 15 cm for thin layer experiments, every 10 cm for choice experiments) using a hand held digital chlorophyll fluorometer (DFLB, WET Labs). Prior to the first experiment, light intensities were measured on all tanks and averaged ca.  $9 \mu\text{mol s}^{-1} \text{m}^{-2}$  at the top of the tanks, and ca.  $1.5 \mu\text{mol s}^{-1} \text{m}^{-2}$  on the bottom of the tanks.

### 2.3. Sample collection and analysis

To monitor the vertical position of the rotifers, we used infrared video cameras, mounted along the vertical axis of each tank, to pan the depth of each tank once every half hour for the first 3 h and each hour for the remainder of the experiment. Each tank was fitted with a monochrome video camera (Cohu) with a macro/zoom lens. An infrared light-emitting diode (LED) placed in combination with a plano-convex lens was used to collimate light from the LED into the camera. The camera and light system was mounted on a motorized linear bearing/rail system with the lens focused at maximum magnification to observe the vertical distribution of the rotifers. Video was recorded on videocassette time-lapse recorders (VCR, Panasonic, model AG-6124) with a date/time recorder. Specialized computer software (Home Control Assistant<sup>®</sup>) controlled the timed synchrony of the motor, camera, lights, and VCR equipment. Rotifer distributions were analyzed by counting the number of rotifers  $\text{screen}^{-1}$  at 10 cm depth increments. For Experiment 5, rotifers  $\text{screen}^{-1}$  were counted at higher resolution in 2 cm increments between 80 and 100 cm to determine how the rotifers re-distributed themselves around the additional plume of algae.

### 2.4. Data analysis

In order to quantify the number of rotifers counted at each 10-cm stratum, a conversion factor was calculated for each tank. The conversion factors were calculated by taking the inverse of the volume of

water sampled  $\text{screen}^{-1}$ . Rotifers  $\text{screen}^{-1}$  counts were then converted to rotifers  $\text{ml}^{-1}$  by multiplying rotifers  $\text{screen}^{-1}$  count by the conversion factor for each tank. Only data from homogeneously distributed tanks (control) were used for the conversion factor calculations and included four time periods ( $t=12, 16, 20$  and  $24$  h) to ensure homogeneity of rotifer distributions. Since the conversion factors were significantly different among tanks (ANOVA,  $df=3$ ,  $F=33.62$ ,  $p=0.000$ ), a specific factor was used for each tank (i.e. Tank 2=0.225, Tank 3=0.191, Tank 4=0.177, and Tank 5=0.156).

Due to the natural aggregation of moving rotifers at the two boundaries (bottom of tank and air–water interface), the top two and bottom-most two depths were not included in the statistical analyses. Regressions were run between rotifer abundance data and chlorophyll data for the thin layer experiments at  $t=4$  and  $8$  h. Because the chlorophyll data were not collected on the same spatial scale as the rotifer data, we averaged the data into 30-cm depth bins and used the arcsine square root transformation, because the data were proportions (Zar, 1999), for the regression analysis.

Patchy distributions of prey have been shown to adversely affect traditional statistical methods (Venrick, 1986). Various researchers have suggested modifications to traditional statistical methods to address the issue of comparing vertical distributions (Smith et al., 1998; Solow et al., 2000; Beet et al., 2003). We used the Beet et al. (2003) approach, which was based on a modification of the Kolmogorov–Smirnov test, and is insensitive to patchiness, to analyze the vertical distributions of rotifers in all of our experiments.

We also ran two-way ANOVAs at each time period for each of the two types of experiments, combining the data for all thin layer (Experiments 1–5) and all choice experiments (Experiments 6–9). The ANOVAs were run using SPSS<sup>®</sup> version 11.5 using a type III sums of squares and an alpha level of 0.05. We used a completely within subjects design designating rotifer abundance as the independent variable and depth and treatment as factors.

Lloyd's (1967) index of patchiness was calculated for the thin layer experiments to determine the degree of rotifer aggregation. The patchiness index ( $P$ ) is based on Lloyd's index of mean crowding ( $m^*$ ),

which reflects the number of rotifers in the near vicinity of each other rotifer. This is a measure based on number of individuals and not on area.

$$m^* = m + \left( \frac{\sigma^2}{m} - 1 \right) \quad (1)$$

where  $m$  is the mean and  $\sigma^2$  the variance of the number of rotifers in an average quadrat. Therefore, Lloyd's index of patchiness ( $P$ ) is calculated by

$$P = \frac{m^*}{m} \quad (2)$$

Paired-sample  $t$ -tests were run in SPSS® to determine any difference in patchiness values between control and experimental tanks. Patchiness values (means and variances) were determined by combining data for all five thin layer experiments.

All figures and tables presented below depict only a representative subset of the results.

### 3. Results

Consistent salinity and temperature distributions were maintained throughout all experiments (Figs. 2, 3, 4 and 5). *N. oculata* layers were maintained within the salinity gradient in both thin layer and choice experiments (Figs. 2, 3, 4 and 5). *S. costatum* layers remained suspended within the salinity gradient with minimal sinking over time (Fig. 5).

#### 3.1. Thin layer experiments

In the thin layer experiments, there was an immediate response of the rotifers to the physical thin layer (Figs. 2 and 3). By the first sampling period (0.5 h), there was an accumulation of rotifers in the middle salinity layer (15 psu) regardless of treatment. However, the rotifers formed a more distinct peak in

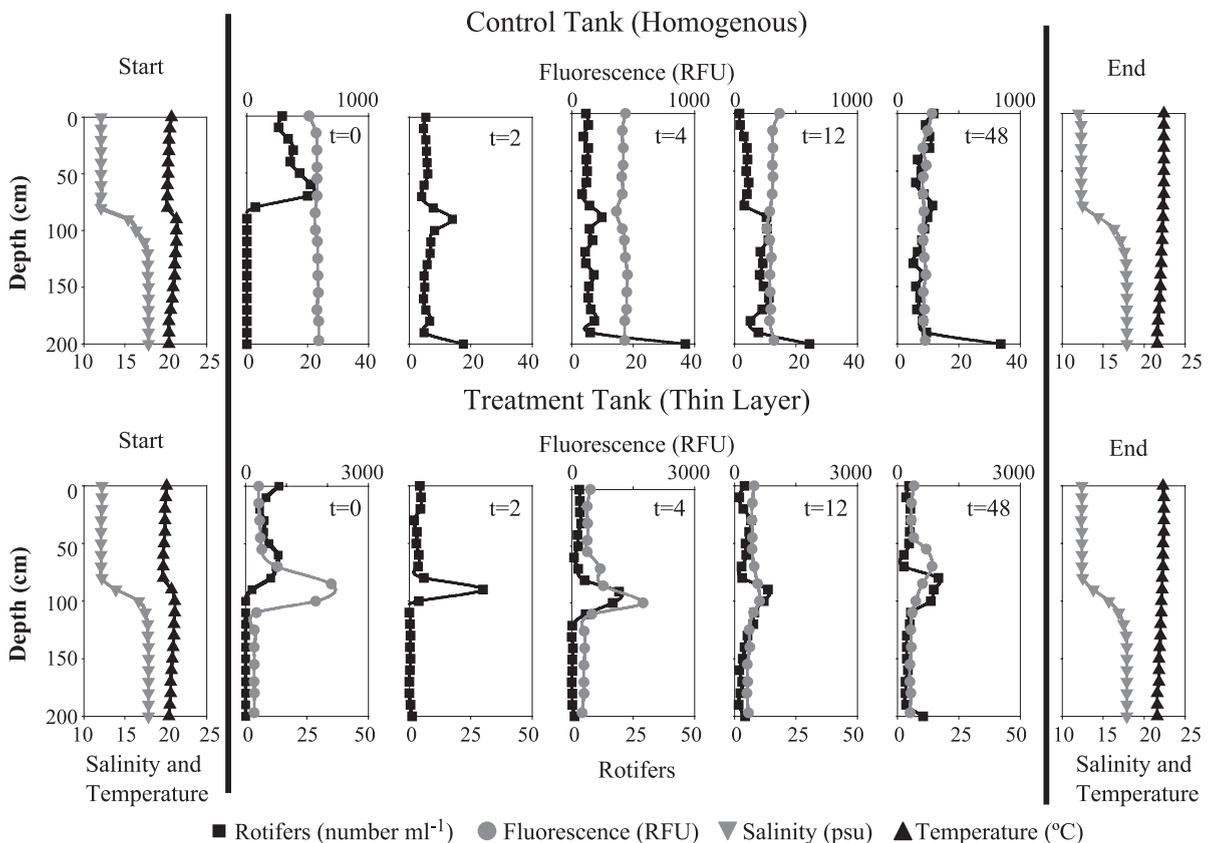


Fig. 2. A representative time series of the vertical distribution of salinity, temperature, rotifer abundance, and fluorescence data from thin layer experiment #4. Fluorometry data are expressed on the top x-axis while salinity, temperature, and rotifer abundance are shown on the bottom x-axis.

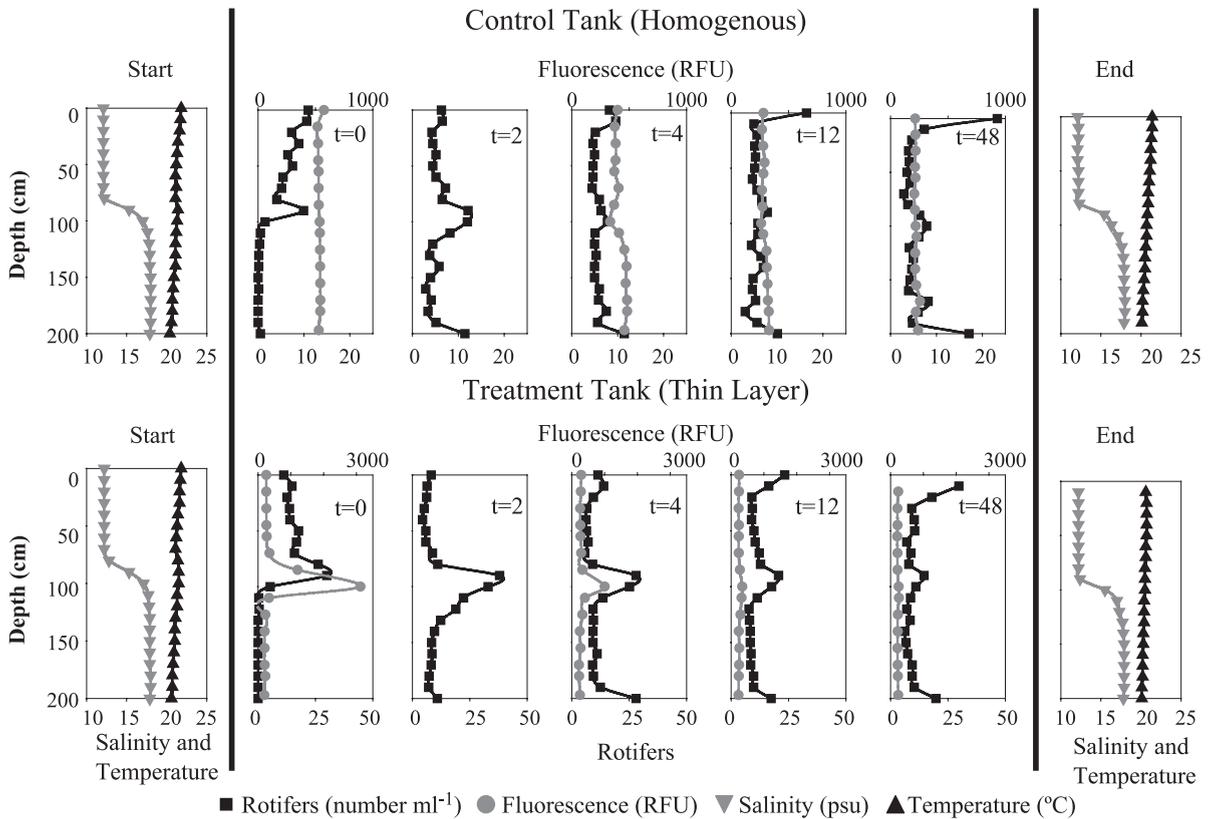


Fig. 3. A representative time series of the vertical distribution of salinity, temperature, rotifer abundance, and fluorescence data from thin layer experiment #14. Fluorometry data are expressed on the top x-axis while salinity, temperature, and rotifer abundance are shown on the bottom x-axis.

the thin layer treatments than in the homogenous treatments (Figs. 2 and 3). Rotifers in the control tanks accumulated in the salinity gradient for about 2 h and then began to disperse throughout the tanks for the remainder of each experiment (Figs. 2 and 3). Rotifers in the experimental tanks remained in the thin layer for about 6 h, during which they depleted the food supply and subsequently dispersed throughout the tanks for the remainder of each experiment (Figs. 2 and 3). These trends were consistent for all five thin layer experiments with slight variation seen in the duration of the response to biological thin layers. There was no indication of diel vertical migration, yet rotifers accumulated at the surface and bottom of all tanks throughout all experiments. Regression analysis between rotifer abundance and chlorophyll a abundance (binned in 30 cm depth strata) showed that rotifers collocate with their food (Fig. 6).

Results from the modified Kolmogorov–Smirnov test described in Beet et al. (2003) (see also Solow et al., 2000) showed significant differences between the vertical distribution of rotifers in control and experimental tanks at all times between  $t=1$  to  $t=8$  and  $t=16$  (Table 1).

Results from the two-way ANOVAs were generally consistent with the results from the modified Kolmogorov–Smirnov test. The depth factor was significant throughout the whole experiment (minimum  $p<0.001$ ,  $df=16$ ,  $F=3.33$ ), the treatment factor was only significant at  $t=33$  ( $p<0.01$ ,  $df=1$ ,  $F=8.19$ ) and the interaction term (depth  $\times$  treatment) was significant from  $t=0.5$  to  $t=8$  (minimum  $p<0.05$ ,  $df=1$ ,  $F=1.679$ ) (results not shown). The absence of a consistent treatment effect in the ANOVA is not surprising, since the ANOVA tests for differences in abundances, which were purposefully manipulated to

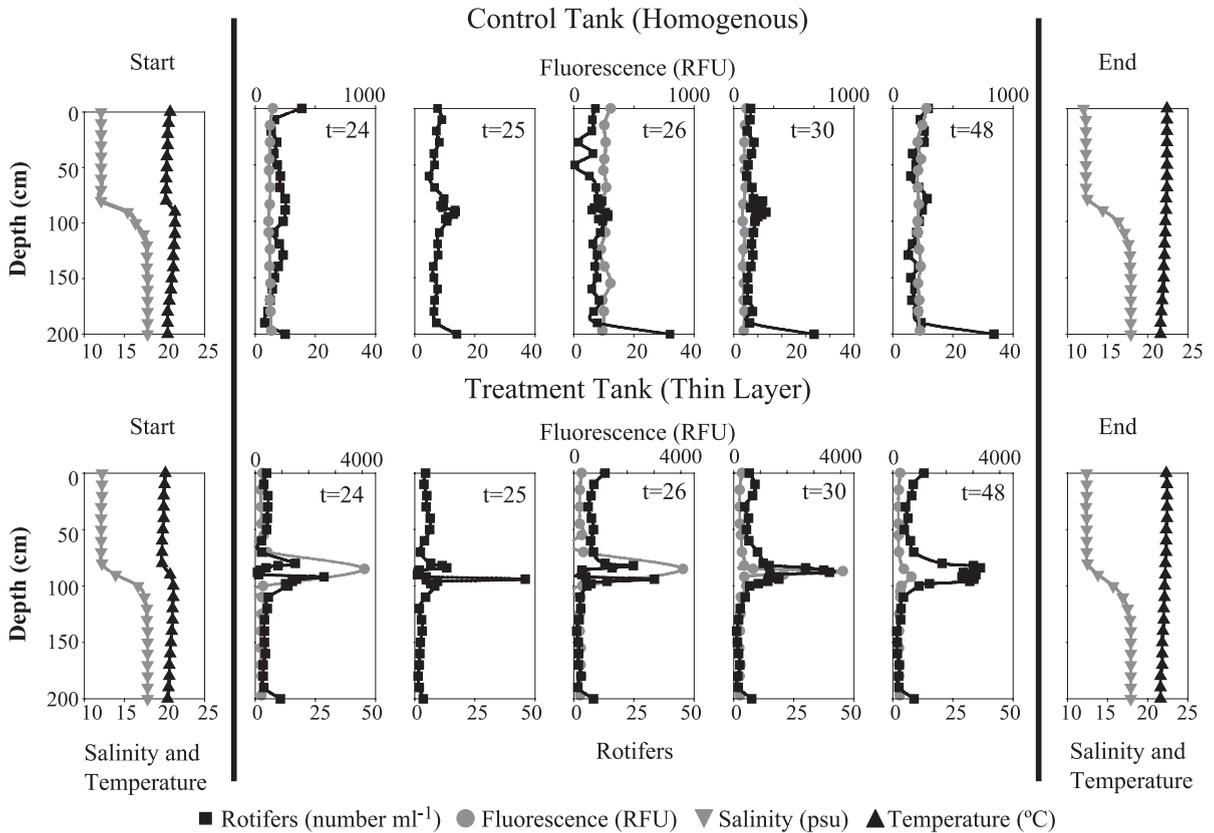


Fig. 4. Time course of vertical distribution of salinity, temperature, rotifer abundance and fluorescence from experiment 5 after injection of an additional plume of *N. oculata* at  $t=24$ . Fluorescence samples were not taken at  $t=25$  and  $t=27$ . Fluorescence data are expressed on the top x-axis while salinity, temperature, and rotifer abundance are shown on the bottom x-axis.

be equal. The effect of thin layers on vertical distribution can be seen in the consistently significant interaction term between depth and treatment.

In the thin layer experiment where we added an additional aliquot of algae to the experimental tanks after 24 h (Experiment 5), the rotifers re-aggregated at the biological thin layer (Fig. 4). The number of algal cells added to the tanks was the same as at the beginning of the experiment ( $t=0$ ), but was concentrated into 30 ml, resulting in a denser and narrower (ca. 12 cm) layer. Within the first half hour, the rotifers began to accumulate at the top and bottom of this patch, forming two peaks during the next several hours ( $t=24, 25$ , and  $26$ ; Fig. 4). The rotifers remained, and presumably fed, at the top and bottom of the layer until a single peak of rotifers was seen at the depth of highest alga concentration ( $t=30$ ; Fig. 4). The rotifers accumulated in the biological thin layer for the remainder of the experi-

ment. There was no accumulation of rotifers at the physical thin layer in the control tanks (Fig. 4).

Lloyd's index of patchiness was calculated at all time periods for all thin layer experiments combined. We hypothesized that the rotifers in the experimental tanks would be more aggregated than rotifers in the control tanks, with a null hypothesis of equal patchiness indices for experimental and control. The patchiness indices for the experimental tanks were significantly higher than the control tanks from time periods  $t=0.5$  to  $t=12$  (Fig. 7).

### 3.2. Choice experiments

There was an immediate response in terms of rotifer distribution within the first sampling interval (0.5 h) regardless of treatment (Fig. 5). The rotifers accumulated within food layers, but were more highly

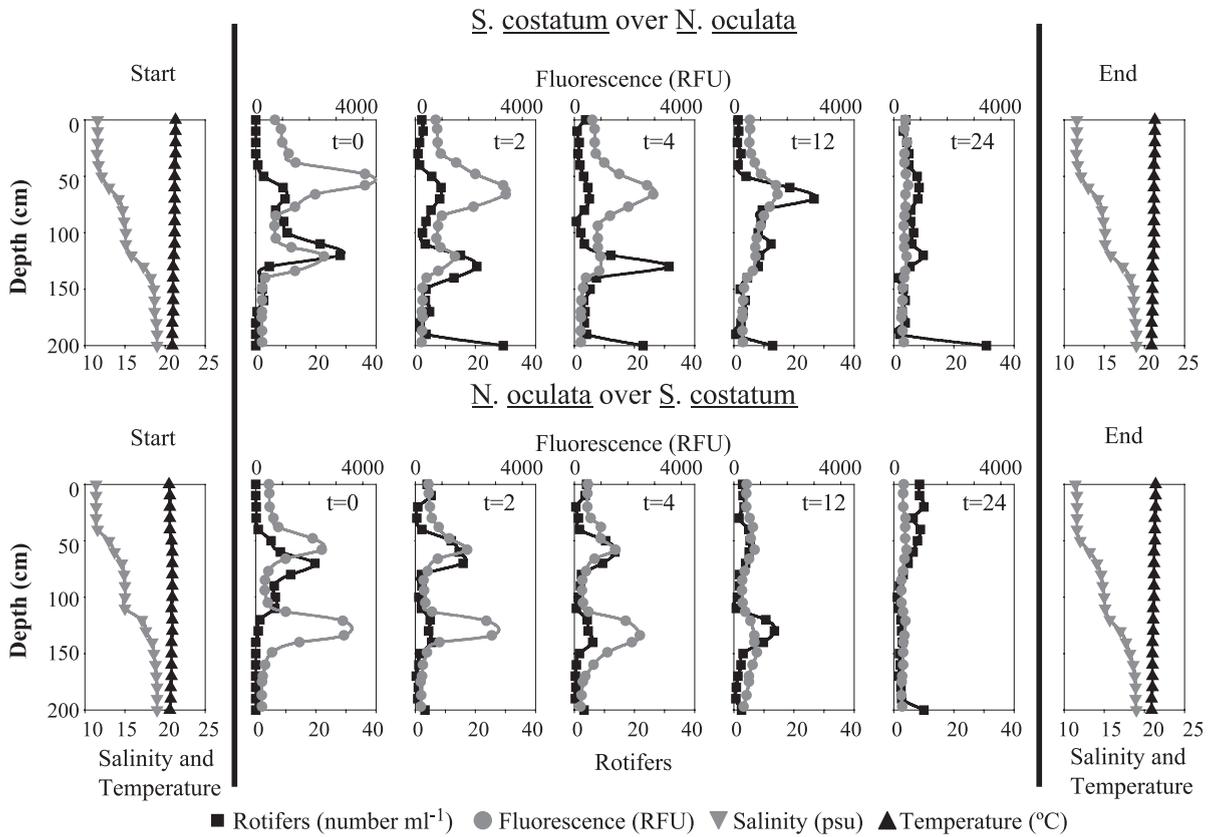


Fig. 5. A representative time series of the vertical distribution of salinity, temperature, rotifer abundance and fluorescence data for choice experiment #6. Fluorescence data are expressed on the top x-axis while salinity, temperature, and rotifer abundance are shown on the bottom x-axis.

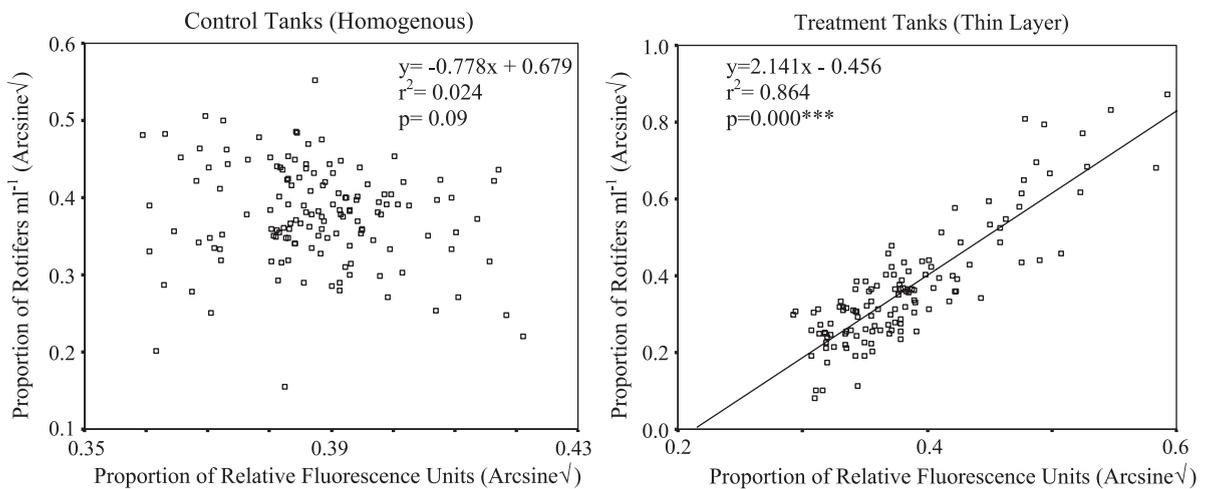


Fig. 6. Regression analysis between the proportion of water column total abundance of rotifer counts (30 cm depth stratum) and relative fluorescence units (30 cm depth stratum) at time=4 h and t=8 h over all five thin layer experiments for control tanks and treatment tanks.

Table 1  
Results from the modified Kolmogorov–Smirnov statistical approach (Beet et al., 2003) for the first 24 h of the experiments

	Time (h)												
	0	0.5	1	1.5	2	2.5	3	4	8	12	16	20	24
<i>Thin layer experiments</i>													
Test statistic	16.25	25.02	41.49	46.02	36.09	42.42	42.91	41.89	51.58	24.37	28.10	9.87	3.96
<i>p</i> value	0.4360	0.0694	***	***	**	***	***	***	***	0.0817	*	0.8734	0.9990
<i>Choice experiments</i>													
Test statistic	45.95	21.68	38.29	35.63	39.92	39.47	40.16	20.91	27.08	48.25	64.37	65.15	25.36
<i>p</i> value	***	0.1540	**	**	***	***	***	**	0.0406	***	***	***	0.0638

\*  $p < 0.05$ .

\*\*  $p < 0.01$ .

\*\*\*  $p < 0.001$ .

aggregated within the layer of *N. oculata* (in most cases rotifer densities associated with *N. oculata* were twice those associated with the diatom layer, *S. costatum*). This trend was consistent for at least 3.5 h, at which point the *N. oculata* layer was depleted and the rotifers moved toward areas containing *S. costatum* (Fig. 5). An exception to this general trend was in Experiment 8 (Choice Experiment 3 of 4) in which the rotifers accumulated at the food layer near the bottom of the tank regardless of the cell type (food) (results not shown).

The results from the modified Kolmogorov–Smirnov test described by Beet et al. (2003) revealed significant differences between treatments during most time periods ( $t=0$ ,  $t=1$  to  $t=3$ ,  $t=8$  to  $t=20$ ; Table 1).

As with the thin layer experiments, the results from the two-way ANOVAs for the choice experiments generally supported the results from the modified

Kolmogorov–Smirnov test. The depth factor was significant for the whole experiment (minimum  $p < 0.001$ ,  $df=16$   $F=2.90$ ), the treatment factor was significant at  $t=1$  ( $p < 0.05$ ,  $df=1$ ,  $F=5.92$ ) and  $t=2$  ( $p < 0.01$ ,  $df=1$ ,  $F=8.82$ ) and from  $t=16$  to  $t=24$  (minimum  $p < 0.05$ ,  $df=1$ ,  $F=6.47$ ), and the interaction term (depth  $\times$  treatment) was significant from  $t=0$  to  $t=3$  (minimum  $p < 0.05$ ,  $df=16$ ,  $F=1.72$ ) and  $t=8$  to  $t=20$  (minimum  $p < 0.01$ ,  $df=16$ ,  $F=2.34$ ) (results not shown).

#### 4. Discussion

Our study is the first of its kind as it clearly separates physical from biological effects on the distribution of rotifers. This was not possible in previous field studies because of confounding biological and physical variables. Consequently, the

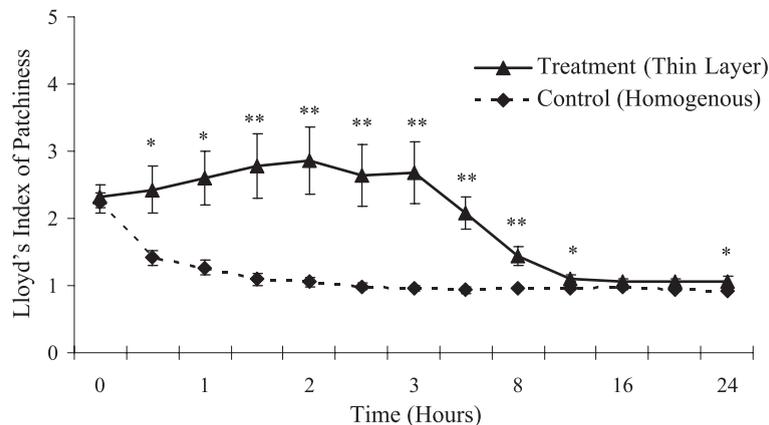


Fig. 7. Lloyd's index of patchiness (mean  $\pm$  S.E.) for all thin layer experiments combined. \* $p < 0.05$ , \*\* $p < 0.01$ , and \*\*\* $p < 0.001$ .

numerous field studies that have investigated the vertical distribution of rotifers in a variety of systems reported varying results as to which environmental factors (e.g., temperature, oxygen content, food resources, or the presence of predators) are most important (Preissler, 1977; Miracle and Vicente, 1983; Mikschi, 1989; Armengol-Diaz et al., 1993; Miracle and Alfonso, 1993; Jose de Paggi, 1995; Baiao and Boavida, 2000). These variable results may be attributed to the fact that each study looked at a specific species in specific systems with varying physical and biological conditions. For example, Armengol-Diaz et al. (1993) found that with the development of stratification some rotifer populations showed a downward migration following the thermocline, while others migrated upward following the oxycline. Conversely, Jose de Paggi (1995) studied the vertical distribution of rotifers in a floodplain lake and found that there was no direct effect of low oxygen content on the rotifers and that, depending on sample location (limnetic versus littoral), rotifer vertical distribution was influenced by phytoplankton and the presence of predators. Baiao and Boavida (2000) found that food availability was crucial in shaping the vertical distribution and species composition of rotifers in a reservoir. These field studies suggest that physical characteristics have a strong influence on rotifer vertical distribution, yet many attribute food as a major factor. We, therefore, chose to specifically study the affects of vertical heterogeneity of food on rotifer distribution. Moreover, we used well replicated and controlled experiments to specifically address the role of vertical heterogeneity, or microscale thin layers of food, on the vertical distribution of rotifers.

The vertical distribution of rotifers, in our study was strongly affected by the vertical distributions of available food. Rotifers collocated with their food source resulting in increased patchiness index when the food was offered in a thin layer (Fig. 7). Conversely, when food was provided in a biologically homogenous distribution, rotifers were homogeneously distributed and a lower patchiness index of rotifers was observed (Fig. 7). The accumulation of rotifers in the physical thin layer in homogenous tanks at the beginning of the experiments was attributed to rotifers seeking similar salinities to those in which they were cultured (acclimated). It was only in the experimental

tanks, where a thin layer of food was provided, that the rotifers remained aggregated in the middle layer (Figs. 2 and 3). Significant differences in distribution were found between experimental and control tanks using the modified Kolmogorov–Smirnov test described by Beet et al. (2003), confirming that biological thin layers affected rotifer distribution.

Furthermore, the re-establishment of biological thin layers (subsequent addition of algae 24 h into the experiment, Experiment 5) after rotifers had dispersed was used to confirm that rotifers actively seek out patches of food. The additional aliquot of algae was more highly concentrated than the original thin layer, yet contained the same original amount of algae. Rotifers initially re-aggregated at the top and bottom of this re-established layer, essentially forming two peaks of rotifer abundance. After ca. 12 h, the rotifers were concentrated in one layer (95 cm) associated with the area of highest concentration of algae. (Sampling valve location precluded us from taking water samples within the thin layer itself.) The higher rotifer density at the top and bottom edges of the thin layer, rather than throughout the thin layer, may be explained in several ways. One possibility is that since the additional aliquot of algae was introduced during the evening, a time during which the algae would be respiring and depleting dissolved oxygen, the conditions in the area of highest algal concentration may not have been suitable for the rotifers. Alternatively, or additionally, the concentration of algae in this area may have been above the optimal density for rotifer feeding. While we do not know of any studies that specifically address or test the effect of food concentration found in thin layers on rotifer feeding success, Hotos (2002) observed greatly reduced filtration and ingestion rates when algal densities were above the optimum level. Alldredge et al. (2002) did not directly measure the effect of particle concentration on rotifer feeding, but inferred that high concentrations of marine snow found in thin layers in East Sound, Washington, may inhibit feeding by planktonic herbivores.

A decrease in chlorophyll concentration over time was observed in both types of treatments, i.e. those containing homogeneously distributed algae and thin layers of algae. These results, and those described previously (Experiment 5) suggest that rotifers actively seek out and graze in food-rich areas. We

conclude that this decrease in chlorophyll concentration was a result of rotifer grazing, and not sinking of algae, because no subsequent increase in chlorophyll concentration was observed at the bottom of the tanks. Further, the change in vertical distribution of rotifers in experimental tanks (i.e., moving from a patchy distribution to a homogenous distribution) coincided with the decrease in chlorophyll in the previously food-rich thin layer (Figs. 2 and 3). The concurrent decrease in chlorophyll concentration and increased vertical homogeneity of rotifers therefore suggest that rotifers consumed the phytoplankton and migrated away from the grazed areas (Fig. 7).

It is important to note that we did not observe any indication of diel vertical migration of rotifers during any of our experiments. Many field studies have looked at rotifer diel vertical migration in freshwater systems and have reported varied results depending on the species studied, the physical characteristics of the system, the season and the occurrence of predators (Preissler, 1980; Armengol-Diaz et al., 1993; Jose de Paggi, 1995; Baiao and Boavida, 2000; Kuczynska-Kippen, 2001; Holst et al., 2002). Two of these studies reported that the occurrence of predators induced diel vertical migration. The cultured rotifers used in this study, however, might not have exhibited this behavior because natural cues (e.g., predator exudates) needed to induce diel vertical migrations were absent.

In addition to abundance of food, food quality can have a significant affect on rotifer distribution. It has been shown that *B. plicatilis* is capable of a certain degree of food selectivity (Chotiyaputta and Hirayama, 1978; Hotos, 2002). When offered a mixture of algae of various sizes and types (e.g., flagellated, spiny, or motile) the rotifers adjusted both filtration rate and ingestion rate depending on food quality (Hotos, 2002). Hotos (2002) used a mixture of *Chlorella* sp. and *Chaetoceros* sp. and found a decrease in filtration and ingestion rates with increasing proportions of a spiny species of *Chaetoceros* sp. We offered *B. plicatilis* a similar but different mixture of algae (*N. oculata* and *S. costatum*). *Chlorella* sp. and *N. oculata* are both green, circular cells. However, the unidentified *Chaetoceros* species used by Hotos (2002) was brown, rectangular, with four long spines extending from each corner (personal communication), whereas the diatom used in our study, *S.*

*costatum*, was brown, chain forming and spineless. Nevertheless, our findings are consistent with Hotos (2002) in that *B. plicatilis* preferred *N. oculata* over *S. costatum*. In our experiments, the rotifers not only showed a feeding preference for *N. oculata* over *S. costatum*, but were also capable of actively moving into a thin layer of their preferred prey.

Three previous laboratory studies that employed the same laboratory apparatus as this study reported species-specific differences in the utilization of physical and biological thin layers. Clay et al. (2004) found the key factors affecting vertical distribution of larval Pacific herring to be physical (light and salinity). Clay et al. (2004) suggested that since many biological thin layers found in oceans and estuaries are associated with physical characteristics (salinity), larval herring may rely on the physical cues of the water column to influence their vertical distribution in order to locate prey patches more easily and to help decrease advective losses out of estuaries. Lougee et al. (2002) studied the effects of haloclines on the vertical distribution and migration of four species of copepods (*Acartiura* spp., *Acanthacartia* spp., *Tortanus dextrilobatus*, *Oithona davisae*), and larval Pacific herring. Lougee et al. (2002) found that smaller copepod taxa (*Acartiura* spp. and *Acanthacartia* spp.) accumulated below the halocline while the response of larger taxa was more subtle. A study conducted by Bochdanský and Bollens (2004) looked at the affect of thin layers of algae on vertical distribution, egg production and fecal pellet production of the copepod *Acartia hudsonica*. Bochdanský and Bollens (2004) found a short-term, ephemeral response in terms of vertical distribution, but found no difference in egg production when the same number of phytoplankton cells was provided in homogenous versus heterogeneous distributions, and speculated that it may be more advantageous for copepods to respond to persistent physical cues rather than ephemeral food patches. These three studies indicate that vertical distribution and migration of zooplankton can vary between species depending on what physical and/or biological factors are present.

Two additional laboratory studies are worth noting, although they used different laboratory apparatus systems and sampled on smaller scales (20 cm) than our study. Tiselius (1992) investigated the behavior and fecal pellet production of *A. tonsa*

in response to thin layers of the diatom *T. weissflogii*, while Saiz et al. (1993) looked at the effect of thin layers of the same diatom on egg production in the presence and absence of a predator (*Labidocera aestiva*). Tiselius (1992) found a change in swimming behavior in the presence of biological thin layers, which allowed the copepods to remain inside the patch, but he did not see a difference in fecal pellet production. Saiz et al. (1993) determined that egg production of *A. tonsa* was higher in homogenous tanks and that predation had a positive effect on egg production. It is not clear whether discrepancies in the interpretation of copepod responses to thin layers were a consequence of the differences in experimental design or actual species-specific differences between the two copepod species. However, using very similar experimental designs and the same apparatus in a series of laboratory experiments allowed us to directly compare representatives of three very different groups: fish larvae (micronekton), copepods (mesozooplankton), and rotifers (microzooplankton). While the vertical distribution of herring larvae was almost entirely driven by physical cues (Clay et al., 2004), copepods showed a small but ephemeral response to food layers (Bochdansky and Bollens, 2004). In contrast, rotifers (this study) responded most strongly to thin layers of food, actively moving towards preferred food patches and exploiting the resource until depletion. This suggests that there exist at least three different strategies in the way zooplankton interact with thin layers.

Despite a clear effect of thin layers on the vertical distribution and apparent grazing of *B. plicatilis*, the question remains as to whether this is of any ecological consequence. One such potential ecological consequence is the population (numerical) response of rotifers to thin layers. For instance, while abundances of *B. plicatilis* increased by approximately 30% during our 2-day-long thin layer experiments ( $r=0.16 \text{ day}^{-1}$ ), there were no consistent differences in abundances between treatments (Table 1). Had the concentration of food used in the homogenous treatment been more severely food limiting, or the concentration of food in the thin layer treatment been completely saturating, a significant difference in population growth rate may have emerged. Future studies of the effects of thin layers on zooplankton

should look for demographic and biogeochemical consequences, in addition to behavioral and physiological effects.

In summary, we found that the vertical distribution of *B. plicatilis* is strongly influenced by the vertical distribution and quality of food (algae). Our experimental results generally support previous field studies in that although physical factors have an effect on rotifer distribution, food also plays a key role. Our findings also show that rotifers are capable of locating and quickly taking advantage of food patches, which is important because rotifers play a significant role in linking the microbial food web to higher trophic levels (e.g. copepods and larval fishes). Our results, combined with those of previous investigators, indicate that responses of zooplankton to thin layers are variable, with *B. plicatilis* responding more strongly than copepods of the genus *Acartia* (Tiselius, 1992; Saiz et al., 1993; Bochdansky and Bollens, 2004) or larval herring (Clay et al., 2004). We recommend that future studies of the effects of thin layers should go beyond evaluation of distribution and abundance and focus on ecological consequences (e.g. rate processes such as feeding, growth and mortality).

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