

Utilization of subsurface food resources for zooplankton reproduction: Implications for diel vertical migration theory

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Abstract

The water columns of lakes and oceans provide a diverse habitat gradient in which light, temperature, food, and predation risk all change with depth. Many planktonic organisms exhibit diel vertical migrations (DVM) in response to daily oscillations in many of these variables. DVM theory often assumes that surface waters are more food-rich than deeper, subsurface layers and proceeds to try to explain why zooplankton migrate out of these beneficial surface layers during the day. Here, we test the assumption that food is best in surface waters by feeding two common crustacean zooplankton with natural epilimnetic and metalimnetic food assemblages from a eutrophic lake and examining their egg production rates. Both *Diaptomus* and *Daphnia* showed greater reproductive rates in the metalimnetic water and significant food limitation in the epilimnetic water. Mass-specific ingestion rates were approximately three times higher in the metalimnion than in the epilimnion. In spite of the poorer food in the surface waters, these two crustaceans migrated into the epilimnion at night. These observations are contrary to the assumption that food is best in the surface water, and a review of the literature suggests that food frequently is not best in surface waters. The upward migrations at night are best explained by the warmer temperatures and reduced predation risk in the surface waters at night.

Diel vertical migration of zooplankton in the world's lakes and oceans is one of the earth's most massive animal migrations. The patterns of these migrations among water bodies and among seasons differ in both their timing and amplitude. These migrations influence the population and community ecology of zooplankton, the trophic dynamics of aquatic food webs, and the vertical transport of nutrients in the water column.

Many hypotheses have been proposed to explain the adaptive significance of vertical migration. Of these, the three that have received the most attention are the metabolic-demographic hypothesis that organisms derive a

physiological and reproductive advantage from spending some time in cooler waters during the day (McLaren 1974; Enright 1977), the photodamage hypothesis that solar radiation may damage plankton (Huntsman 1924; Williamson et al. 1994), and the predation hypothesis that vertical migration reduces predation risk (Zaret and Suffern 1976; Stich and Lampert 1981).

These hypotheses share the assumption that the surface strata of the water column are more food-rich than the deeper layers to which zooplankton migrate. A primary cost of vertical migration is thus a reduction in the availability of food in deeper waters. A fundamental goal of many vertical migration studies thus is to calculate how metabolic or demographic advantages or reduced predation risk or photodamage may balance this cost by conferring an advantage on zooplankton that migrate to deeper waters where food is scarce. The importance of food resources in stimulating vertical migration is evident from experiments in which food resources have been directly manipulated with consequent changes in zooplankton migrations (Johnsen and Jakobsen 1987; Leibold 1990).

This pivotal assumption, that the surface waters of lakes and oceans are more food-rich than deeper strata, has rarely been critically examined. Many workers report vertical profiles of chlorophyll or particulate carbon along with vertical migration data, and highest levels frequently are observed near the surface. However, the nutritional quality of the food at different depths is generally unclear.

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Changes in light and nutrient concentration with depth may influence the biochemical composition of algae and their subsequent nutritional value to zooplankton (Rothhaupt 1991). Sestonic carbon, protein, and carbohydrate may be highest in surface waters of some systems (Stich and Lampert 1981; Pick 1987), but carbon-specific protein and phosphorus may actually increase with depth (Cuhel and Lean 1987; Uehlinger and Bloesch 1987). Furthermore, metalimnetic peaks in microbial communities (e.g. Bennett et al. 1990) combined with the often lower C:P and C:N ratios of bacteria and protozoa compared to algae (Hessen and Andersen 1990) suggest that both the quantity and quality of microbial foods for zooplankton at times may be better in the metalimnion than in the epilimnion. Crustacean zooplankton are known to ingest and utilize various protozoa (Sanders and Porter 1990). In summary, there is reason to suspect that food quantity and quality may not always be best in the surface layers of lakes and oceans.

We tested the hypothesis that food availability for *Diatomus oregonensis* and *Daphnia pulicaria* was better in the epilimnion than in the metalimnion of a eutrophic lake during summer stratification. Our approach was to let the zooplankton define food availability (quantity and quality) through their reproductive response to epilimnetic and metalimnetic foods. Coupled feeding rate experiments provided estimates of the food biovolume ingested in the epilimnion and metalimnion. Although metalimnetic foods supported greater ingestion and reproductive rates in these crustaceans than did epilimnetic foods, both species still migrated up into the surface waters at night. The question then becomes why did these zooplankton migrate up into the food-limiting surface layers?

Methods

Vertical structure of the lake and zooplankton migrations—Oxygen and temperature measurements in Lake Waynewood were taken at 1-m intervals with a Yellow Springs Instrument oxygen-temperature meter on 9 July 1991. Light readings were taken as downwelling irradiance of photosynthetically active radiation (PAR) with two 192SA underwater quantum sensors mounted 1 m apart and a LI-1000 datalogger (LiCor, Inc.).

On 9 July 1991, the depth distribution of phytoplankton was measured as chlorophyll *a* and its photosynthetic activity as $^{14}\text{CO}_2$ uptake (primary productivity). Water samples taken with an all-plastic Kemmerer bottle (Wildco) in late morning were distributed into a 250-ml bottle (for Chl *a* and total inorganic C determination), two clear 45-ml polycarbonate bottles, and one opaque (taped) bottle. ^{14}C was added (74×10^3 Bq per 45-ml bottle) immediately before the bottles were suspended in the lake at the depth of collection. After 4 h of sunny weather, bottles were returned to the laboratory and subsampled for ^{14}C incorporation. An 8-ml subsample of unfiltered water was acidified to 0.1 N H_2SO_4 and allowed to stand uncapped overnight before 10 ml of liquid scintillation

cocktail (Scinti-Verse E, Fisher Scientific Co.) was added. A 5% discrimination factor was incorporated into calculations of C incorporation. Values reported are corrected for dark uptake, which was negligible in the epilimnion.

Total inorganic C was determined by the gas chromatographic method of Stainton (1973). Chlorophyll *a*, corrected for pheopigments, was determined fluorometrically (Strickland and Parsons 1972) after overnight extractions of filters (Whatman GF/F, stored frozen) in a 5:1 (vol:vol) mixture of alkalized 90% acetone and methanol (cf. Pechar 1987).

On 6 and 12 July, six replicate samples from both epilimnetic and metalimnetic waters, previously passed through an 80- μm sieve, were collected on combusted Whatman GF/F filters for analysis of particulate C and N available in the reproduction experiments. Immediately before sample filtration, filters were rinsed sequentially with dilute HCl and distilled water. Filters were frozen until analyzed with a Carlo Erba model 1106 CHN analyzer.

The diel vertical migration (DVM) patterns of *Diatomus*, *Daphnia*, and the invertebrate predator *Chaoborus* were examined by comparing day (1200–1600 hours) and night (2300–0400 hours) samples on 8–9 and 9–10 July 1991. Samples were collected at 1-m intervals with a transparent 25-liter Schindler trap with a 48- μm net between 1- and 7-m depth and with an integrated tow of a 48- μm -mesh Wisconsin closing net between 7 and 12 m, where the lake was anoxic and organisms likely to be less abundant. This Wisconsin-style net has a narrow opening (15-cm diam) relative to the length of the net (110 cm) and a broad secondary ring where the mesh starts (30-cm diam); prior calibrations with the Schindler trap have shown it to be 95–100% efficient when towed over short vertical distances, as in the current study. Four stations were sampled at each depth on 8–9 July and one station on 9–10 July.

Reproduction experiments—Adult *D. pulicaria* and *D. oregonensis* were collected from Lake Waynewood on 1 July 1991 with a vertical tow of a 202- μm plankton net from a depth of 5.5 m. For *Diatomus*, one clutch-bearing female and two males were placed in each of 16 replicate continuous-flow chambers (Williamson et al. 1985). Each replicate consisted of a separate inner chamber of 13-ml volume with 202- μm mesh on the bottom to retain the adults. Each inner chamber was placed inside a separate outer chamber in which two small holes were cut and covered with 60- μm mesh to retain the nauplii and eggs produced. These two-chamber assemblies were then placed in a large overflow tray to maintain the water level. The continuous flow apparatus provided the copepods with an average flow rate of 105–112 ml chamber $^{-1}$ d $^{-1}$. Each chamber was checked and rinsed daily, the reproductive phase of the adult females was recorded as ovigerous (carrying external egg clutch), gravid (oviducts full of oocytes), both (ovigerous and gravid), or neither (Williamson and Butler 1987), and the offspring were counted and removed. For *Daphnia*, a single adult female was

placed in each of 15 replicate 300-ml BOD bottles for each treatment. The bottles were rotated continuously (1 rpm) on a plankton wheel. Daily, the bottles were checked and rinsed, the food medium was changed, and the offspring were counted and removed.

Four different treatments were established to examine the reproductive response of the two crustaceans to epilimnetic and metalimnetic food supplies in the lake. The food supplies consisted of epilimnetic (1 m) or metalimnetic (5 m) water collected daily with a Kemmerer bottle and strained through an 80- μm mesh to remove the zooplankton. *Cryptomonas reflexa* (culture WCR, Williamson et al. 1985) was added to two treatments to yield a final concentration of 1,000 cells ml^{-1} ($= 0.6 \mu\text{g}$ dry mass ml^{-1}). *Cryptomonas* was grown in modified MBL (Williamson and Butler 1987), concentrated in a light beam, and harvested in exponential growth phase. This cryptomonad is known to be an excellent food source for zooplankton and has supported multiple generations of both crustacean species used here over long periods of time in our laboratory. The four treatments were thus strained epilimnetic water, strained metalimnetic water, strained epilimnetic water plus *Cryptomonas*, and strained metalimnetic water plus *Cryptomonas*. Comparison of the treatments with and without *Cryptomonas* additions provided a test for food limitation in the epilimnion and metalimnion. The entire experiment was run in a walk-in growth chamber with a daily cycle (16 : 8, L/D) of very dim indirect illumination ($0.2 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$) from two 122-cm "cool-white" fluorescent bulbs shaded by black polyethylene film. The temperature (20°C) was intermediate between the epilimnetic (25°C) and metalimnetic (15°C) temperatures at the start of the experiment.

The *Diaptomus* experiment was run for 14 d and the *Daphnia* experiment for 10 d. Total offspring produced per female per day and final clutch sizes were computed from the data after the first (pre-experimental) clutches for both *Diaptomus* and *Daphnia* were eliminated, and treatments were compared with ANOVA (Statpro, Penton Software Inc.). In addition, for *Diaptomus*, interclutch duration was compared among treatments with ANOVA, while the food limitation index (f = percentage of total adult females that have neither egg clutches nor full oviducts, Williamson and Butler 1987) was compared among treatments with the Kruskal-Wallis nonparametric rank test.

Feeding experiments—Grazing experiments were run to estimate ingestion rates by *Diaptomus* and *Daphnia*. *D. oregonensis* and *D. pulicaria* were collected and isolated with a 202- μm net on 8 and 9 July 1991. Unstrained, whole-water samples were collected from the epilimnion and metalimnion and placed in 128-ml narrowneck glass bottles with no, low (5 *Diaptomus* or 2 *Daphnia*), or high (10 *Diaptomus* or 4 *Daphnia*) additions of grazers. Other macrozooplankton introduced through the unstrained whole-water samples but not appropriate to the given treatment (e.g. *Daphnia* in the *Diaptomus* treatment, *Chaoborus* and cyclopoids in all treatments) were re-

moved from the bottles at the start of the experiment. Five replicates of each of the six treatments (two grazers at three densities) were incubated for 24 h on a rotating wheel (1 rpm) at 20°C in dim light in the same controlled environment chamber as the reproduction experiments. After 24 h, phytoplankton and nanoflagellate samples were preserved and counted. Phytoplankton samples were preserved in Lugol's acid iodine, and samples for enumeration of nanoflagellates were preserved with equal volumes of 4% glutaraldehyde. Nanoflagellate subsamples were filtered onto black 0.8- μm pore-size Nuclepore filters, stained with the fluorescent dye Primulin (Caron 1983), and 75–150 fields were counted at 1,000 magnifications by epifluorescence microscopy. Samples not counted immediately were stored frozen to preserve algal autofluorescence. Samples for phytoplankton and ciliates were prepared with a freeze transfer technique (Hewes and Holm-Hansen 1983) and enumerated with stratified counts at 200–400 magnifications with a phase-contrast microscope.

Zooplankton grazers were collected with a 153- μm mesh, preserved, counted, and body lengths measured at $30\times$ magnification under a dissecting microscope. Body lengths were converted to biomass with the equations of Rigler and Downing (1984). Weight-specific clearance rates in the grazing experiments were calculated with regression analysis and the standard exponential equations as summarized by Williamson (1991). Instantaneous ingestion rates in the reproduction experiments were estimated by multiplying these clearance rates by the average food concentrations in the reproduction experiments.

Composite samples were counted to assess the food concentrations in the controls (no added grazers), low, and high grazer treatments as an initial screening to permit us to focus counting efforts on treatments where there was evidence of grazing. These analyses indicated that grazing rates were indistinguishable in the low grazer treatments, so only the high grazer treatments and the final controls were counted and used to estimate clearance and ingestion rates.

The only invertebrate predator of any consequence in the lake is the midge larva *Chaoborus*. Population overlap of *Diaptomus* and *Daphnia* with *Chaoborus* was estimated with an overlap index, a predation risk model (Williamson 1993), and field data from the vertical migration study. Overlap of each crustacean with *Chaoborus* was assessed for the whole-water column during the day, the night, and over a 24-h period (day and night data analyzed as one matrix) using the predation risk model and vertical migration data.

Results

Vertical structure of the lake and zooplankton migrations—Lake Waynewood (Wayne County, Pennsylvania $41^\circ23'42''\text{N}$, $75^\circ21'50''\text{W}$) is a dimictic lake with a maximum depth of 12 m. In July 1991, when the experiments were performed, thermal stratification was well-defined, with an epilimnetic layer from 0 to 4 m, a steeply stratified

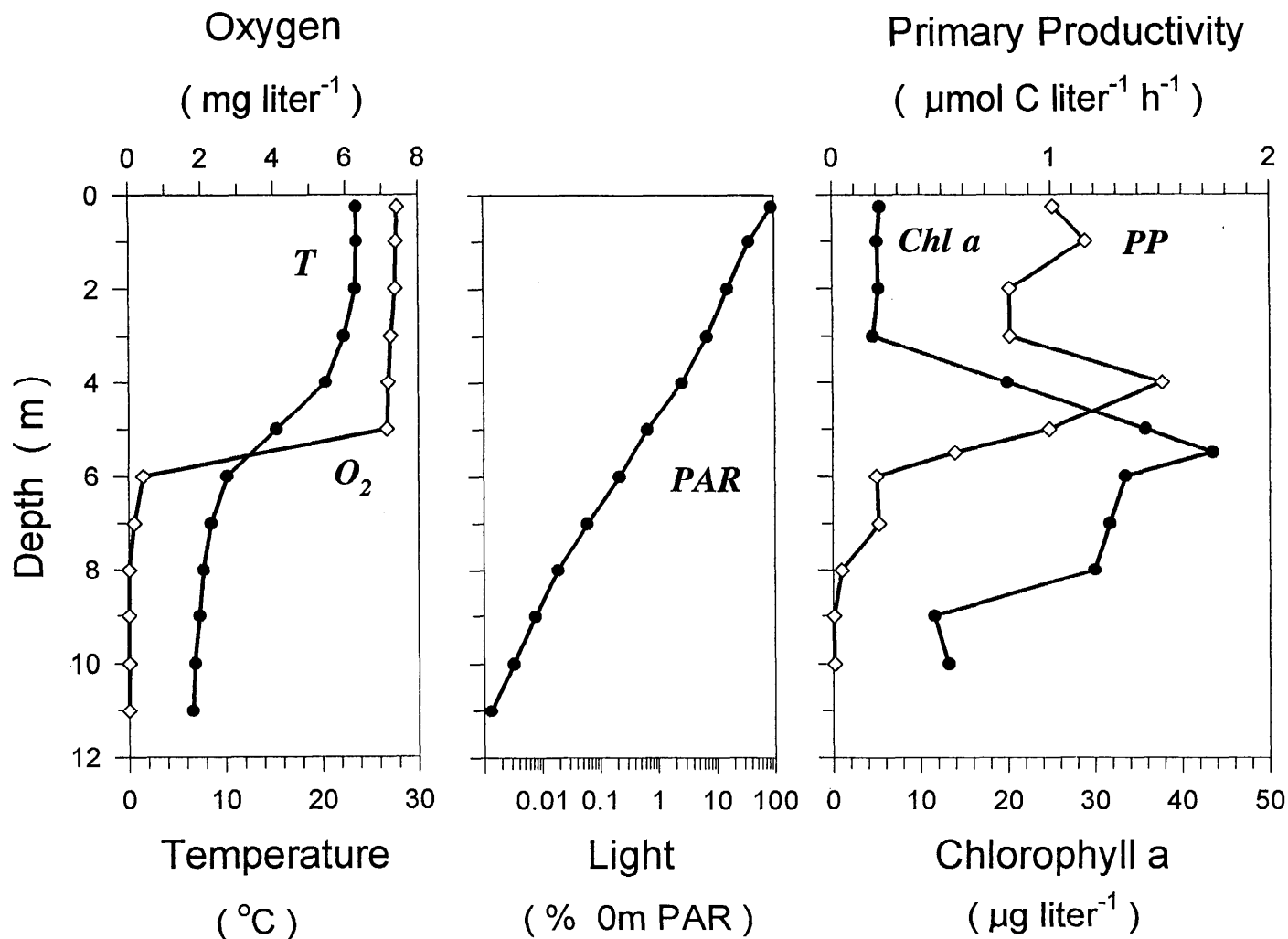


Fig. 1. Profiles of the stratified water column in Lake Waynewood, 9 July 1991: dissolved oxygen, temperature, relative light intensity (as photosynthetically active radiation, PAR), primary productivity (from 4-h ¹⁴C uptake), and chlorophyll *a* (corrected for pheopigments).

metalimnion from 4 to 6 m, and a largely anoxic hypolimnion from 6 to 12 m (Fig. 1). Chlorophyll *a* concentrations were lowest in the epilimnion (4 $\mu\text{g liter}^{-1}$) and highest in the metalimnion and upper hypolimnion (35–40 $\mu\text{g liter}^{-1}$), although primary productivity per unit chlorophyll was higher in the epilimnion than in the metalimnion (Fig. 1). C and N concentrations and C:N ratios of the <80- μm particulate matter were similar in the epilimnion (C = 0.73 mg liter⁻¹, N = 0.14 mg liter⁻¹, C:N = 5.2) and metalimnion (C = 0.84 mg liter⁻¹, N = 0.16 mg liter⁻¹, C:N = 5.2) on the two days sampled.

The vertical migrations of *Diaptomus* and *Daphnia* consisted of migrations of portions of the populations from the metalimnion into the epilimnion at night and back into the metalimnion during the day. Few or no individuals remained at 3 m or above during the day (Fig. 2). Peak population densities were at a depth of 5 m during both the day and the night for both species. Both *Diap-*

tomus and *Daphnia* were far more abundant (300+ and 200+ times) at 5 m than at 1 m during the day, while at night they were only 2–4 times as abundant at 5 m as at 1 m due to DVM.

Population densities of the invertebrate predator *Chaoborus* peaked in the metalimnion at night and in the lower metalimnion and upper hypolimnion during the day (Fig. 2). The lower densities of *Chaoborus* during the day compared to the night are probably due to a diurnal migration into the anoxic sediments (Woodmansee and Grantham 1961).

Reproduction experiments.—The rate of offspring production (eggs female⁻¹d⁻¹) and final clutch size were significantly greater in the metalimnetic treatment than they were in the epilimnetic treatment for both crustaceans (Table 1). Egg production by *Diaptomus* and *Daphnia* fed metalimnetic food was 59 and 40% greater, respectively,

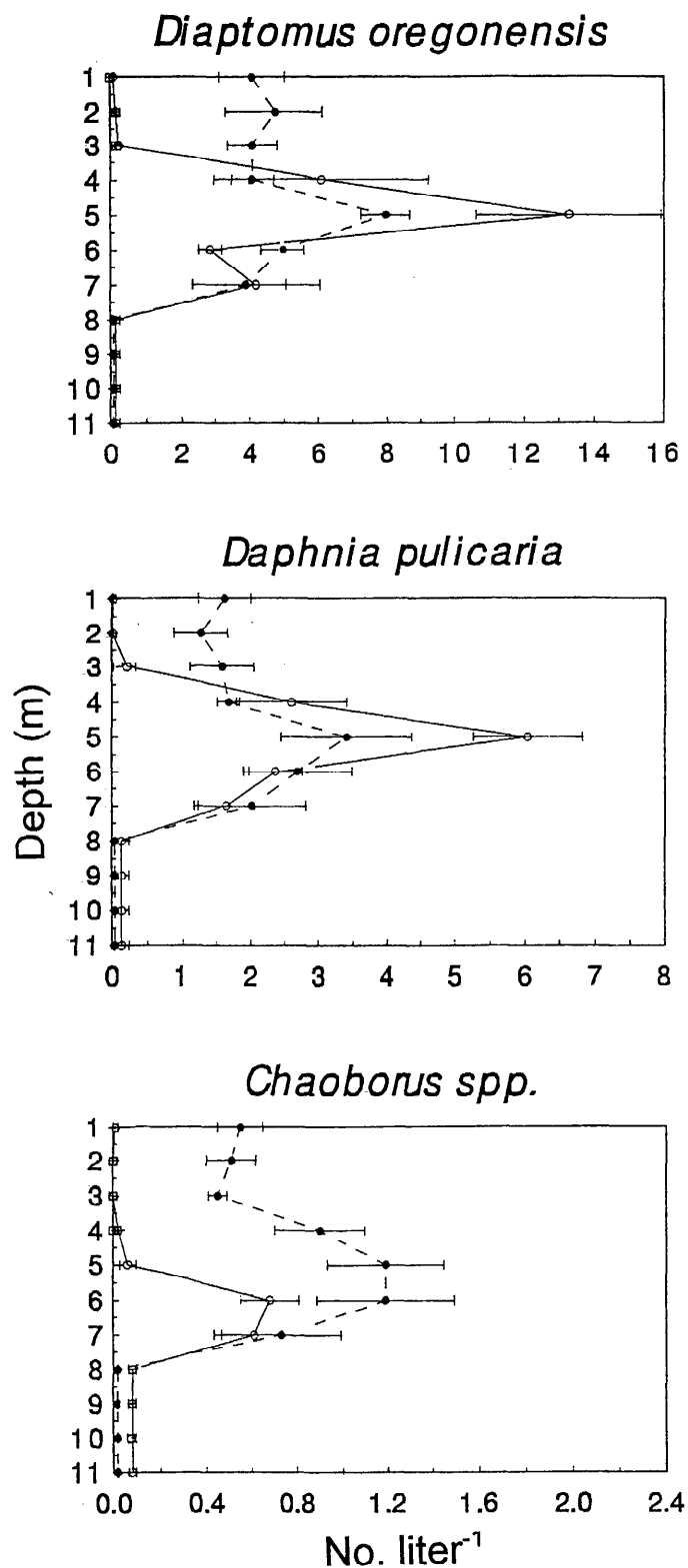


Fig. 2. Diel vertical migration pattern of *Diaptomus oregonensis*, *Daphnia pulicaria*, and *Chaoborus* predators in Lake Waynewood, 8–9 July 1991. Daytime (○) and nighttime (●) values are means and SE ($N = 4$). Vertical migration patterns were similar on 9–10 July.

than it was in those offered epilimnetic food. Furthermore, *Diaptomus* had a lower food limitation index in the metalimnetic treatment than it did in the epilimnetic treatment (Table 1).

Food limitation in the epilimnion was indicated by an increased rate of offspring production and larger final clutch sizes in both crustaceans when the epilimnetic treatments were supplemented with *Cryptomonas* (Table 1). Further evidence for food limitation in *Diaptomus* was the significantly shorter interclutch duration and the lower food limitation index in the epilimnetic treatments with *Cryptomonas* additions vs. those without (Table 1). Egg production rates were high and similar for all diaptomus treatments in the first 3 d, when the initial (pre-experimental) clutches were being dropped. After 4 d, the food-limiting conditions in the epilimnetic treatment became apparent. The *Daphnia*, only some of which were initially clutched, showed a divergence in the treatments after 3–4 d.

Differences in the rate of offspring production and final clutch size indicated that food was limiting for *Daphnia* in the metalimnion (Table 1). In contrast, and despite similar patterns of offspring production for both crustaceans, there was no indication of food limitation for *Diaptomus* in the metalimnion. There were no significant differences in the rate of offspring production, final clutch size, interclutch duration, or in the food limitation index for *Diaptomus* in the metalimnetic treatments with and without the addition of algae (Table 1).

Feeding experiments—Of the broad range of algae and protozoans, food species were defined as those on which statistically significant grazing rates were obtained for at least one of the crustaceans in the grazing experiment. The one exception to this was that statistically significant ($P < 0.05$) “clearance” rates on *Ceratium hirudinella* were observed in the grazing experiments in the epilimnetic treatments with both *Diaptomus* ($0.87 \text{ ml } \mu\text{g}^{-1} \text{ d}^{-1}$, $r^2 = 53\%$) and *Daphnia* ($0.69 \text{ ml } \mu\text{g}^{-1} \text{ d}^{-1}$, $r^2 = 68\%$). However, the large size ($260 \mu\text{m}$ long) and armored nature of these cells suggest that although these grazers induced mortality in *Ceratium*, ingestion of these cells was not likely. We therefore excluded *Ceratium* from the ingestion rate analyses.

The species identified as available food and their average contribution to food biovolume throughout the reproduction experiment are given in Table 2. Ingestion rates for both crustaceans were 2–3 times higher in the metalimnion than they were in the epilimnion. In the epilimnion, *Gonyostemum* made up 44% of the diet of *Diaptomus*, while *Cryptomonas erosa* and large ciliates ($> 30 \mu\text{m}$) each made up 19% of the diet. Several food types contributed to *Daphnia*'s diet in the epilimnion, including *Gonyostemum* (27%), *C. erosa* (19%), *Rhodomonas* (15%), and ciliates $> 30 \mu\text{m}$ (22%). Heterotrophic foods contributed 26 and 29% of the diets of *Diaptomus* and *Daphnia* in the epilimnion, but 0 and 6% of the diets of these two species in the metalimnion. Although ingestion rates were not statistically detectable for the relatively low densities of *Cryptomonas* in some of the treat-

Table 1. Comparison of epilimnetic (EPI) and metalimnetic (MET) treatments with (+) and without added food to test for food limitation in the two layers. Asterisks—significance levels that provide for rejection of the following three null hypotheses: *— $P < 0.05$; **— $P < 0.01$; ***— $P < 0.001$ (NS—not significant). Null hypothesis 1 (SIM): Food resources are similar in the two strata. Null hypothesis 2 (EPI): Food is not limiting in the epilimnion. Null hypothesis 3 (MET): Food is not limiting in the metalimnion.

Metric	EPI	MET	EPI+	MET+	Significance levels		
	Mean \pm SE (N)				SIM	EPI	MET
<i>Diaptomus</i>							
Offspring female ⁻¹ d ⁻¹	1.51 \pm 0.27 (16)	2.40 \pm 0.32 (12)	3.24 \pm 0.39 (13)	3.27 \pm 0.41 (16)	*	***	NS
Final clutch size	10.87 \pm 0.85 (8)	14.45 \pm 0.97 (11)	16.27 \pm 1.26 (11)	16.25 \pm 1.14 (12)	*	**	NS
Interclutch duration (d)	8.19 \pm 0.83 (16)	6.07 \pm 0.96 (12)	4.70 \pm 0.34 (13)	4.87 \pm 0.50 (16)	NS	**	NS
Food limitation index	45.49 (16)	17.67 (12)	16.88 (13)	23.54 (16)	**	***	NS
<i>Daphnia</i>							
Offspring female ⁻¹ d ⁻¹	2.97 \pm 0.15 (13)	4.16 \pm 0.29 (14)	5.31 \pm 0.26 (14)	6.00 \pm 0.18 (14)	**	***	***
Final clutch size	14.15 \pm 1.06 (13)	17.50 \pm 1.12 (14)	19.64 \pm 1.50 (14)	22.28 \pm 0.69 (14)	*	**	**

Table 2. Available food (mean \pm SE) and percent of biovolume for 4, 6, 8, and 10 July 1991 and ingestion rates ($10^3 \mu\text{m}^3 \text{d}^{-1}$) of food types in the epilimnion (EPI) and metalimnion (MET) of Lake Waynewood during the experiments. Instantaneous ingestion rates were estimated from clearance rate estimates in the grazing experiment and food concentrations in the reproduction experiment. Metalimnetic clearance rates were used to estimate epilimnetic ingestion rates of *Gonyostemum*.

Food type		Available food		Ingestion rates		
		(cells ml ⁻¹)	(%)	<i>Diap-</i> <i>tomus</i>	<i>Daphnia</i>	
Autotrophs						
<i>Gonyostemum semen</i>	EPI	4.93 \pm 5.20	32	180	120	
	MET	45.9 \pm 24.0	81	1,700	1,200	
<i>Cryptomonas rostratiformis</i>	EPI	1.91 \pm 0.84	5	15	20	
	MET	0.37 \pm 0.36	<1	—	—	
<i>Cryptomonas erosa</i>	EPI	42.53 \pm 18.62	15	78	90	
	MET	8.76 \pm 8.44	1	—	—	
<i>Tabellaria fenestrata</i>	EPI	35.2 \pm 23.4	5	6	17	
	MET	18.2 \pm 27.9	1	—	—	
<i>Cryptomonas marssonii</i>	EPI	42.36 \pm 18.54	2	—	12	
	MET	12.69 \pm 12.22	<1	—	—	
<i>Rhodomonas lacustris</i>	EPI	343 \pm 99.0	7	27	70	
	MET	190 \pm 256	1	—	—	
<i>Ankyra judayi</i>	EPI	9.53 \pm 7.40	<1	<1	<1	
	MET	19.30 \pm 8.80	<1	—	—	
Heterotrophs						
ciliates >30 μm	EPI	2.55 \pm 1.40	17	78	104	
	MET	5.95 \pm 5.00	10	—	—	
20–30 μm	EPI	3.70 \pm 0.87	6	20	14	
	MET	7.15 \pm 2.70	3	—	—	
10–20 μm	EPI	7.20 \pm 4.00	2	9	18	
	MET	10.0 \pm 7.00	1	—	29	
<10 μm	EPI	0.21 \pm 0.10	<1	<1	<1	
	MET	0.15 \pm 0.13	<1	—	<1	
nanoflagellates <5 μm	EPI	3,410 \pm 784	8	—	—	
	MET	3,161 \pm 1,550	2	—	50	
Ingestion rate totals						
				EPI	415	467
				MET	1,700	1,280

Table 3. Chlorophyll *a* concentrations and position of maximum in three Pennsylvania lakes of different trophic status in June, July, and August 1988 (nine dates), 1989 (seven dates), 1990 (six dates), 1991 (seven dates), and 1992 (seven dates). The percentage of dates on which the chlorophyll maximum was located in a given stratum and the mean \pm SD of chlorophyll *a* concentrations ($\mu\text{g liter}^{-1}$) in that stratum over the 5-yr period are given.

Stratum	L. Giles (oligotrophic)	L. Lacawac (mesotrophic)	L. Wayneood (eutrophic)
Epilimnion			
% dates	0	6	22
mean Chl	0.7 ± 0.61	2.8 ± 1.46	9.5 ± 11.5
Metalimnion			
% dates	22	61	69
mean Chl	1.5 ± 1.6	4.9 ± 3.9	22.0 ± 25.9
Hypolimnion			
% dates	75	11	3
mean Chl	2.2 ± 2.0	1.5 ± 1.78	4.2 ± 4.78
No clear maximum			
% dates	3	22	6

ments, these desirable food algae were most likely ingested.

The impact of vertical migration by both prey and predators on invertebrate predation risk was estimated by calculating overlap coefficients (O_i , unitless) and density risk (DR, predators liter $^{-1}$, =overlap times mean predator density, Williamson 1993). These calculations showed that the vertical overlap was very similar during the day and at night for both crustaceans (1.70–1.96) but that DR was about four times higher at night (0.94–0.98) than it was during the day (0.24–0.26) due to migration of *Chaoborus* into the water column at night.

Discussion

Food availability is notoriously difficult to quantify in plant-herbivore interactions due to the complex nutritional biochemistry of these interactions, as well as the variety of physical and chemical defenses that plants have against herbivores. Planktonic systems are no exception, and like many other grazers, zooplankton consume microbial and detrital particles as well as plants. We used the reproductive rates of the two vertically migrating crustacean zooplankton *Daphnia* and *Diaptomus* to examine food availability and found that the food was better in the metalimnion than in the epilimnion of Lake Wayneood for both crustaceans.

Our data support previous experimental evidence that food may be limiting to cladocerans in the epilimnion. When incubated in epilimnetic water with and without added *Chlamydomonas*, *Daphnia* exhibited higher intrinsic rates of increase in the food addition treatments ($r = 0.313$) than it did in the treatments without added food ($r = 0.094$, Dini and Carpenter 1992). Hoenicke and

Goldman (1987) performed short-term reciprocal transfer experiments in which cladocerans were incubated in the epilimnion, metalimnion, and hypolimnion with seston from the same and the other two strata. Changes in lipid-ovary-egg indices indicates that food resources were best in the epilimnion early in summer but were better in the deeper strata by late August and September.

The occurrence of better food in deeper water is counter to the central assumption of DVM theory that the surface waters of lakes and oceans are more food-rich than deeper strata. The importance of this assumption to DVM theory is evident from its repeated presence in review papers (Haney 1988; Lampert 1989), in textbooks, and even its citation in ecological studies of trophic interactions with other organisms, such as stream insects (Flecker 1992). The balance between the advantages of a food-rich epilimnion and the reduced predation pressures in deeper, food-poor waters is perhaps summarized most cogently in the conflicting selective pressures model of Gliwicz and Pijanowska (1988). This model proposes that two conditions are necessary for normal (nocturnal upward) migration to occur: food resources must be greatest in the surface waters to encourage ascent to the epilimnion, and visual predators must be present to force descent out of this food-rich epilimnion during the day. Although most investigators who examine DVM in plankton communities are undoubtedly aware of the possibility that food availability can be better in deeper waters, the conventional DVM paradigm and the assumption of better food in surface waters seem to persist.

One possibility is that this assumption persists because subsurface food maxima are not widespread in lakes and oceans. However, vertical profile data in lakes and oceans suggest that subsurface food maxima are common. The most often used metric of phytoplankton biomass in planktonic systems is chlorophyll. Although chlorophyll concentrations may often peak in the surface waters of lakes and oceans, subsurface chlorophyll maxima are frequently observed in the oceans (Cullen 1982) as well as in a wide variety of lakes (Fee 1976). Often, the depth of zooplankton population and grazing maxima may coincide with these chlorophyll maxima (Kettle et al. 1987), suggesting that zooplankton can exploit subsurface food maxima. In marine systems, the zooplankton abundance may peak just above the subsurface chlorophyll maximum, where phytoplankton growth rates and perhaps food quality are better (Napp et al. 1988). In reviewing our own data collected on three lakes of different trophic status over the past 5 yr, we found that during summer stratification, chlorophyll levels were maximal in the surface waters 22% of the time in the eutrophic lake, 6% of the time in the mesotrophic lake, and never in the oligotrophic lake (Table 3). Our data support other published data in suggesting that food quantity, in terms of algal biomass, frequently is not best in the surface waters of lakes and oceans.

In addition, chlorophyll has clear limitations as an indicator of food quality due to variability in algal biochemistry and grazer nutritional needs. Chlorophyll may exaggerate subsurface phytoplankton biomass due to in-

creases in chlorophyll concentration per unit cell volume at greater depths (Cullen 1982). However, vertical profiles of algal cell numbers, total algal volume, and particulate organic C also provide evidence that subsurface food maxima are widespread in both marine and freshwater systems (Napp et al. 1988). In addition, increases in the nutritional quality (protein content, nutrient ratios) of the algae are likely to correlate with increases in chlorophyll per cell with increasing depth (Cuhel and Lean 1987). Thus, decreases in quantity of food per unit chlorophyll may be offset by increases in food quality with increasing depth. In our experiments, there was no evidence of differences in C:N ratios in the epilimnion vs. the metalimnion.

Chlorophyll does not provide an accurate estimate of microbial biomass, which may also be an important food resource for zooplankton (Bennett et al. 1990; Hessen and Andersen 1990). We compared heterotrophic microbial food resources and the phytoplankton by biovolume. The absolute abundance of heterotrophic food was greater in the metalimnion than it was in the epilimnion. However, due to the high ingestion rates of *Diaptomus* and *Daphnia* on the abundant large chloromonad *Gonyostemum* in the metalimnion, heterotrophic foods made up a larger percentage of the diet in the epilimnion than they did in the metalimnion (Table 2). *Gonyostemum* did, in fact, account for most of the food biomass in the metalimnion of Lake Wayneood. It is not uncommon for such subsurface chlorophyll maxima to be due to a single species of phytoplankton at light levels of only 0.3–3.5% of surface irradiance (Fee 1976; Pick et al. 1984).

Clearance and ingestion rate estimates on bacteria were not made in the grazing experiments in this study. However, our previous laboratory experiments with these same two crustacean species have demonstrated that bacteria can contribute to the reproductive potential of *Daphnia* but not *Diaptomus* (Sanders et al. in prep.) We used the clearance rate estimates for *Daphnia* from these previous experiments ($0.24 \text{ ml } \mu\text{g}^{-1} \text{ Daphnia d}^{-1}$), an estimated biovolume of $0.075 \mu\text{m}^3$ per bacterium (Porter et al. 1983), and epifluorescence bacteria counts made in the current experiments to estimate the potential contribution of bacteria to the diet of *Daphnia* in the current experiments. Bacterial concentrations (mean \pm SE, $N =$ four dates) were $5.1 \pm 0.26 \times 10^6$ cells ml^{-1} in the epilimnion and $7.0 \pm 1.03 \times 10^6$ in the metalimnion. From these numbers, we estimated that the bacteria contributed 16 and 9% of the food volume ingested by *Daphnia* in the epilimnion and metalimnion, respectively. These estimates are very similar to those of Wylie and Currie (1991), who found that in systems where cladocerans dominate, bacteria and picoplankton can contribute 16–21% of carbon uptake by crustaceans, while in copepod-dominated systems, uptake rates of these foods are much lower. Our estimates of ingestion for natural bacteria may be high, however, because of the relatively large size of bacteria used in the laboratory experiments ($1 \times 3 \mu\text{m}$). Furthermore, the actual contribution of bacterial biomass to reproduction may be less than implied by ingestion rates since not all bacteria are digested by daphnids (King et al. 1991).

The question that remains for Lake Wayneood is why these two crustaceans migrate into the surface strata of the lake at night if food availability is poorer there than it is in the metalimnion. Food availability is not the only factor influencing vertical migration, and most investigators recognize a multiplicity of factors (Haney 1988; Lampert 1989). In addition to food, temperature and predation are frequently implicated. We can only speculate on the possible contribution of these two variables based on limited data.

In our study, the increased density risk in the metalimnion at night due to nocturnal migration of *Chaoborus* may contribute to the vertical migration of *Diaptomus* and *Daphnia* out of this layer and into the epilimnion (Fig. 2). However, these estimates of DR include all instars of *Chaoborus*, and the younger instars would not be able to consume the large *D. pulicaria*. In addition, we do not have reliable predation rate estimates to convert these density risk values into predation risk. Experimental manipulation of *Chaoborus* has been shown to induce migration of *D. pulicaria* into the epilimnion (Leibold 1990). Several previous studies have demonstrated migrations induced by invertebrate predators, although these migrations were most commonly reverse migrations upward during the day (Ohman 1990; Neill 1990). We assume that vertebrate predation will exclude these large crustaceans from the epilimnion during the day because Lake Wayneood has a rich population of planktivorous fish, including several species of sunfish (*Lepomis macrochirus*, *Lepomis gibbosus*, *Lepomis auritus*), yellow perch (*Perca flavescens*), and golden shiners (*Notemigonus crysoleucas*). Because of the anoxic hypolimnion in the lake, *Daphnia* and *Diaptomus* do not have the option of migrating into deeper layers at night when *Chaoborus* migrates upward. The result is that this invertebrate predator may contribute to the nocturnal ascent of these grazers: a migration pattern that is more commonly attributed to a combination of visually feeding vertebrate predators and the presence of better or more food in surface waters.

Temperature is also likely to be important in the upward migration of zooplankton (Kerfoot 1985; Dawidowicz and Loose 1992). If planktivorous fish exclude zooplankton from surface waters during daylight, vertical migration into the warmer waters at night may offer a demographic advantage by increasing egg production rates even if food resources are better in deeper strata. Our experiments were performed at a temperature that was intermediate to that in the epilimnion and metalimnion in order to eliminate temperature effects and focus on food availability. Data from the literature for similar species of *Diaptomus* (*Diaptomus pallidus*, Williamson and Butler 1987) and *Daphnia* (*Daphnia parvula*, Orcutt and Porter 1983) show that the instantaneous birth rates differ between the temperatures of 15 and 25°C by a factor of 1.6 for *Diaptomus* and 2.0 for *Daphnia*. These increases in birth rate with temperature suggest that the demographic advantages of temperature alone could account for the nocturnal upward migration of *Diaptomus* and *Daphnia* in Lake Wayneood. Previous experiments in which *D. pulicaria* was incubated in the epilimnion (20–

27°C) and hypolimnion (14–18°C) of Wintergreen Lake and fed identical epilimnetic food resources similarly demonstrated greater population growth rates for *Daphnia* in the warmer epilimnion (Threlkeld 1980). One must be cautious in interpreting our extrapolations based on temperature, however, because food and temperature have interactive effects on *Daphnia* and *Diaptomus* reproductive rates (Orcutt and Porter 1984; Williamson and Butler 1987). Lower oxygen concentrations in the metalimnion may also be important (Hanazato 1992).

In addition to temperature and predation, other factors may also have contributed to the persistent migration of a portion of the zooplankton into the food-poor epilimnion in Lake Waynewood. For example, vertical migration patterns can vary among clonal subpopulations of *Daphnia* (King and Miracle 1995). Although we have no data on this, the migration patterns we observed may represent a persistent spring clone that migrated into the surface during a period when food resources were better in the epilimnion. Interestingly, *Daphnia middendorffiana* has been shown to migrate upward out of deeper food-rich layers in the dark in laboratory column experiments under isothermal conditions in the absence of predators (Haney 1993). This observation supports the idea that upward migrations at night are not driven primarily by the benefits of food-rich surface strata and may occur in the absence of alternative selective forces from either predators or temperature.

We suggest that food quality may not always be best in the surface waters of lakes and oceans and that the classic vertical migration paradigm, which focuses on why zooplankton migrate down out of food-rich surface waters, should be abandoned. Our results are limited to one time period in one lake. Although they cannot be simply extrapolated to other lakes or oceans, a critical look at the literature suggests that food may not always be best in surface waters. A more extensive and systematic survey of lakes and oceans will be necessary to confirm the general applicability of these observations. Our data reinforce the suggestion of Dini and Carpenter (1992) that future DVM studies will benefit from an increased emphasis on documenting the vertical distribution of food resources. The spectrum of food in deeper strata may result in an equally good or higher quality diet for herbivorous and omnivorous zooplankton than the algal food in surface waters. In addition, temperature may play a more consistent role than is played by food in offering a demographic advantage in the stratified surface waters (Kerfoot 1985). Models that use a common currency that takes into consideration the relative intensity of the various positive and negative selective forces at different depths in the water column will provide greater insight into the adaptive significance of diel vertical migration in lakes and oceans.

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