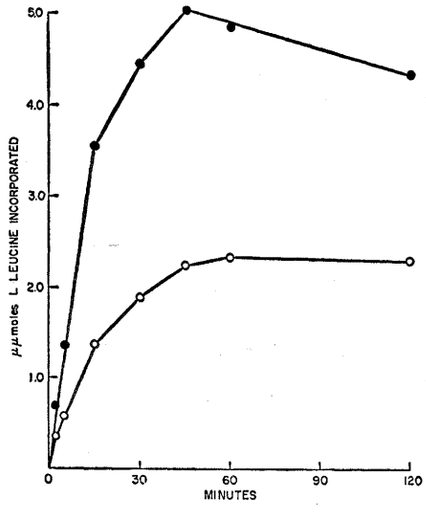


Fig. 1. Amino acid incorporation into protein by microsomes and *p*H-5 fraction from hypertrophied and control muscle. ○, control; ● hypertrophied. Microsomes and *p*H-5 fraction equivalent to 0.2 g of muscle, for both preparations. Assay system same as for Table 2.

average value for four preparations being 73 ± 11 μg of RNA per gram of hypertrophied muscle and 27 ± 4.3 μg of RNA per gram of control muscle. Protein in the microsomal fraction, on the other hand, is only slightly higher (1.8 ± 0.3 mg of protein per gram of hypertrophied muscle versus 1.7 ± 0.4 mg of protein per gram of control muscle). The corresponding values for RNA and protein concentrations in microsomes prepared from the proximal half of the hypertrophied muscle and its control are 41 μg of RNA per gram of hypertrophied muscle and 20 μg of RNA per gram of control muscle; and 1.5 mg of protein per gram of hypertrophied muscle and 1.4 mg of protein per gram of control muscle. These results indicate that the increased rate of protein synthesis in the hypertrophied muscle in vitro is due to an increase in the amount of ribosomes.

The increased activity observed with the system from hypertrophied muscle is probably not due to differences in the concentration of amino acids in the hypertrophied and control muscles. Concentrations of free valine, leucine, and phenylalanine were too low in the microsomes for determination by the method of Stein and Moore (*14*); there was less than 0.3 nmole of these amino acids per 0.34 mg of microsomal protein, which is the amount of the microsomes used in the amino acid-incorporation experiments. In the supernatant fraction obtained after centrifugation at 105,000*g*—the fraction from which the *p*H-5 enzyme is precipitated—the concentration of phenylalanine was identical for control and experimental muscle, whereas the concentrations of valine and leucine were greater in the hypertrophied muscle by about 20 percent.

Our results permit several tentative conclusions concerning the biochemical events leading to skeletal muscle hypertrophy. The higher rate of protein synthesis in vitro with the hypertrophied muscle indicates that the net increase in protein content of the hypertrophied muscle cannot be due solely to a decreased rate of protein degradation. The localization of the higher incorporating activity in vitro in the microsomal fraction is probably related to the higher RNA content of that fraction. It is known, however, that the increased activity in vitro is not due exclusively to increased amounts or availability of messenger RNA since the increase is still apparent, and even enhanced, in the presence of an artificial messenger, polyuridylic acid.

M. Hamosh,* M. Lesch
J. Baron,† S. Kaufman
Laboratory of General and Comparative Biochemistry, National Institute of Mental Health, Bethesda, Maryland 20014

References and Notes

1. S. Gudbjarnason, M. Telerman, C. Chiba, P. L. Wolf, R. J. Bing, *J. Lab. Clin. Med.* **63** 245 (1964); F. Z. Meerson and T. L. Zayats, *Bull. Exp. Biol. Med. Transl.* **50**, 678 (1960).
2. S. S. Schreiber, M. Oratz, M. A. Rothschild, *Amer. J. Physiol.* **211**, 314 (1966).
3. J. Kendrick-Jones, S. V. Perry, *Nature* **213**, 407 (1967).
4. A. L. Goldberg, *Physiologist* **8**, 175 (1965).
5. W. C. Schneider, in *Methods Enzymol.*, S. P. Colowick and N. O. Kaplan, Eds. (Academic Press, New York, 1957), vol. 3, p. 680.
6. E. J. Masoro, B. R. Loving, R. M. McDonald, *Biochim. Biophys. Acta* **84**, 493 (1964).
7. H. Drury, *Arch. Biochem. Biophys.* **19**, 455 (1948).
8. J. R. Florini, *Biochemistry* **3**, 209 (1964).
9. P. Siekevitz, *J. Biol. Chem.* **195**, 549 (1952).
10. T. C. Hall and E. C. Cocking, *Biochem. J.* **96**, 626 (1965).
11. O. H. Lowry, N. Y. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
12. A. Fleck and D. Begg, *Biochim. Biophys. Acta* **108**, 333 (1964).
13. E. W. Yemm and E. C. Cocking, *Analyst* **80**, 209 (1955).
14. W. H. Stein and S. Moore, *J. Biol. Chem.* **211**, 915, 927 (1954).
15. E. Helander, *Acta Phys. Scand.* **41**, suppl. 141 (1957).
16. E. Helander, *Biochem. J.* **78**, 478 (1961).
17. F. Z. Meerson, T. D. Beloshapkina, E. F. Lushnikov, E. M. Leikina, G. N. Markovskaya, G. V. Chernyshova, *Fed. Proc.* **18**, transl. suppl. T667 (1964).
18. V. D. Arutyunov, *ibid.* **25**, transl. suppl. T353 (1966).
19. E. E. Gordon, K. Kazimierz, M. Fritts, *Amer. J. Physiol.* **210**, 1033 (1966).
20. W. Sandritter and G. Scomazzoni, *Nature* **202**, 100 (1964).
21. We thank Dr. L. Guth for demonstrating the tenotomy technique, Dr. L. Laster and W. Edwards for the amino acid analysis, R. Funk, A. Ferguson, and F. Gold for technical assistance.
* Permanent address: Department of Biochemistry, Hebrew University-Hadassah Medical School, Jerusalem, Israel.
† Present address: University of Chicago Medical School, Chicago, Illinois.

12 April 1967; revised 12 July 1967

Vertical Diurnal Migration and Endogenous Rhythmicity

Abstract. *Experimental studies of mixed populations of marine zooplankton have demonstrated that internal rhythms, synchronized by a light-dark cycle, are of dominant importance for the vertical migration of several species of crustaceans. For certain other organisms, the vertical migrations observed in the experiments can be accounted for as direct responses to light intensity only. Performances intermediate between these extremes were also observed, as well as behavior based on biological timing mechanisms that are not rhythms in the usual sense.*

The vertical diurnal migration of zooplankton represents one of the most conspicuous and widespread natural manifestations of daily rhythmicity that can be observed in animal activities. It is now well established for terrestrial organisms that internal biological rhythms are the primary mechanisms responsible for cyclic activity under field conditions, although concurrent environmental stimuli also play important roles in modifying that activity (*1*). The hypothesis that endogenous rhythmicity contributes to the phenomenon of vertical migration of plankton, however, has been largely neglected. The current viewpoint, as reflected in reviews and summaries of the subject (*2*), is that the animals are responding primarily, if not exclusively, to concurrent environmental stimuli, that is, that the rhythmic behavior is exogenous only. While this conclusion derives support from several lines of evidence, it has by no means been rigorously established.

Extensive laboratory studies leave no doubt that light intensity as well as other environmental factors such as temperature, hydrostatic pressure, and

*p*H can affect the direction and intensity of swimming of zooplankters. Furthermore, field studies have often demonstrated strong correlations between light intensity and vertical position of zooplankton, but these observations cannot distinguish between a primary role of the stimulus, as a direct cause of vertical distribution, and a secondary role, as a modifier of an internal rhythmicity. Backus, Clark, and Wing (*3*) have recognized, for example, that the rise of the deep scattering layer toward the surface during a solar eclipse confirms that light influences behavior, but does not rule out internal rhythmicity as the underlying cause for normal migration.

The usual techniques involved in laboratory studies of vertical migration leave much to be desired. The animals are usually collected in a net, which, at the very least, disturbs them. Then they are placed in a narrow column of water, which greatly restricts their movements. Changes in vertical distribution are followed over time, but this has generally been of short duration, owing to rapid death of the animals. Observing these tiny organisms has required that either continuous light be used, or that the counts be quickly effected during a brief period of illumination. But bright light often masks rhythmic responses, and even brief light flashes can cause marked phase shifting of an internal rhythm. The existing reports of endogenous rhythmicity in zooplankters are not particularly convincing, probably as a direct result of these several experimental difficulties.

The early studies of Esterey (*4*) led him to conclude that three species of copepods (*Acartia tonsa, A. clausi,* and *Calanus finmarchicus*) can, under some experimental conditions, show internal timing in their swimming behavior, but Esterley's experimental animals were usually kept in darkness in the laboratory for only a few hours. On the average, a larger fraction of the copepods tended to be in the top portion of a cylinder of seawater between 6 and 8 p.m. than were present during the midday hours. After 8 p.m. the animals again returned to lower levels, and on two occasions when enough specimens survived, a similar rise, of short duration, was observed on the second night of captivity. These results have received relatively little attention, perhaps because such a short-duration rise does not correspond with expectations based on field observations of vertical migration. Furthermore, Schallek (*5*), studying one of these same species, *A. tonsa,* found no evidence for persistent rhythmicity.

In a more recent study, Harris (*6*) suggested again that the vertical migration of *C. finmarchicus* may involve internal timing, but in this case the experimental observations were presented in the form of a single graph based on "daily averages," a method of data summary which does not permit a decision about whether rhythmicity in the usual sense was actually involved (*7*). Somewhat more convincing data were presented for the freshwater cladoceran, *Daphnia magna.*

The experiments described below were performed to reassess the possible contribution of internal rhythmicity to the vertical diurnal migration of nearshore zooplankters. We have used experimental techniques that eliminate or minimize several of the possible difficulties inherent in previous methods.

The studies involved repetitive sampling of a mixed population of zooplankton contained in a large concrete tank, 11 by 5 by 2½ m deep. The building which enclosed the tank was light tight; access to the tank enclosure was provided by a door fitted with two sets of opaque curtains attached in the form of a light-lock. The imposed light-dark cycle included a maximum light intensity of about 100 lux at the water surface, and an intensity of about 0.02 lux during the dark phase. Artificial twilight transitions of 30 minutes' duration were produced by a motor-driven rheostat.

The experimental animals were obtained from the Scripps Institution of Oceanography seawater system, which pumps unfiltered seawater continuously from a depth of about 6 m about 300 m offshore. Following the technique of Fleminger and Clutter (*8*), the tank enclosure was brightly illuminated while a continuous stream of seawater, with contained plankters, was poured into one side of the tank; drainage was through surface overflow ports on the opposite side. This procedure avoided direct handling of the animals. In addition, plankton were concentrated within the tank, because most species swam downward in response to the bright overhead light and thereby avoided the outflow ports. Such a concentration procedure presumably selected for dark-active animals (that is, those that are normally present at the ocean surface only during the night), although light-active animals were also observed (see below).

Sampling of the organisms was accomplished with a set of three nets, 25 cm in diameter and having 0.24-mm apertures. The three nets were mounted in a horizontal row beneath the front end of a floating wooden

Table 1. Vertical migration (first experiment).

Animal	Vertical migration*		Pattern†
	During light cycle	Persistence during constant conditions	
Dark-active animals‡			
Nototropis sp. (amphipod)	Yes	Yes	A
Exosphaeroma sp. (isopod)	Yes	Yes	A
Peltidiad copepods	Yes	Yes	B
Cyclaspis sp. (cumacean)	Yes	Yes	B
Tiron sp. (amphipod)	Yes	No	C
Metamysidopsis elongata (mysid)	Yes	No	D
Epicarid isopods (larval stages)	Yes	No	D
Light-active animals‡			
Lacophontid copepods	Yes	No	E
Euterpina sp. (copepod)	Yes	No	E
No migration observed			
Labidocera spp. (copepod)	No	No	
"*Acartia*" spp. (copepod)	No	No	
Chaetognaths	No	No	
Medusae	No	No	

* Because of the short duration of this experiment, no statistical test for rhythmicity can be applied to the data which will make adequate allowances for possible monotonic time trends, such as those due to mortality. The interpretations of the data in these columns, therefore, represent subjective but deliberately conservative decisions. † Pattern refers to a categorization of the observations, in terms of the component graphs of Fig. 1; for example, *Exosphaeroma* sp. (pattern A) showed a behavior most comparable with that of *Nototropis* sp. (Fig. 1A). ‡ Dark-active animals are defined as those that showed significant maxima of surface abundance during the dark phase of the light-dark cycle; light-active animals showed opposite behavior.

frame, with net centers at a depth of 30 cm from the water surface. The entire assemblage could be towed the length of the tank by means of an above-water bridle and a tow line which was reeled by hand onto a large drum. Average towing speed was about 25 cm/sec. The sampling method was designed to minimize net-avoidance by the animals (*8*).

The samples thus obtained provided information only on the changes in abundance of the various species at the surface, but such changes adequately reflected temporal patterns of vertical migration. Since samples were not taken at the bottom and intermediate depths, the technique did not distinguish between the possibility that the organisms moved en masse from a position near the bottom to a position near the top, and the possibility that the organisms alternated from high densities near the bottom to a more uniform distribution throughout the water column.

The first experiment was conducted between 31 March 1966 and 2 April 1966. Animals were concentrated for 4 days within the tank, and thereafter were treated with a light-dark cycle with light from noon until midnight for 3 days before sampling was begun. Samples were taken at 4-hour intervals for 2 days. The light cycle was discontinued at midnight on 31 March, permitting 1½ days of sampling under constant dim-light conditions (0.02 lux). The second experiment was conducted in July 1966. Animals were concentrated for 5 days, and then treated with a light-dark cycle with light from midnight to noon (reverse from the first experiment) for 6 days. Sampling was begun on the 4th day of the light cycle (24 July) and continued at 3-hour intervals for 6 days, of which 4 days were under constant dim-light conditions. Water temperatures were not measured in the first experiment; during the second experiment, regular surface temperature measurements after each sampling ranged between extreme values of 19.7° and 20.2°C, with no indication of a daily cycle.

Observations from the two experiments are summarized in Figs. 1 and 2 for selected species and species groups. Tables 1 and 2 provide additional information on these as well as other organisms. Those organisms which are listed in only one of the two tables were either absent in the other experiment or were present in such low abundance that no conclusions seem warranted. A comparison of the results of the two experiments for various taxonomic groups indicates that the kind of behavior shown is a species characteristic which was generally reproducible.

Most of the organisms showed significant vertical migratory behavior in the presence of the light-dark cycle. In addition, several species (see Fig. 2E) showed inverse vertical migration, with greater abundance at the surface during the hours of light, a kind of behavior seldom reported from field studies. Data obtained during these normal light-dark cycles did not provide any indication of the behavioral mechanisms involved, but do suggest that the tank approximated a natural environment for many of the species; it seems to us unlikely that factors such as the shallowness of the tank could induce vertical migration in a species which does not normally show such behavior. It is conceivable, however, that the environment provided may have interfered with normal behavior in other species, and therefore negative evidence from these experiments should not necessarily be interpreted to mean that the species does not migrate vertically in the field.

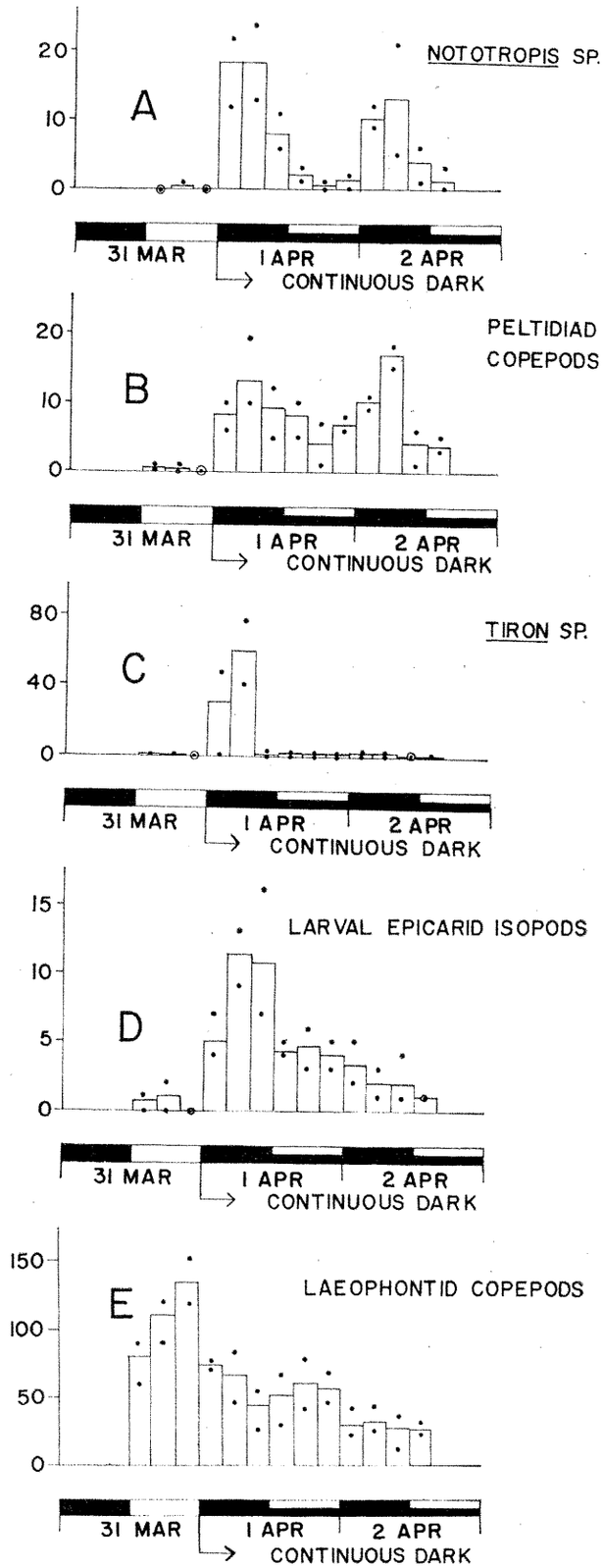
The observations under constant conditions, following the light cycle, provide evidence for the existence of endogenous timing in several species. For *Nototropis* sp. (Figs. 1A and 2A), the internal rhythmicity was so strongly expressed that the vertical migration under constant conditions differs only in amplitude from the migration during the light cycle. The data also clearly imply that the endogenous circadian rhythm of this species can be rapidly and completely entrained by a lighting cycle, since the timing of migration under constant conditions corresponded closely in both experiments with the prior imposed lighting cycle, and showed no consistent relationship with the outdoor daytime. The inverse timing produced in the two experiments also shows that the rhythmic behavior was not a response to some cyclic environmental factor that was uncontrolled in the experiment (for example, atmospheric pressure, cosmic radiation and so forth).

The data for *Nototropis*, as well as for several other organisms shown in Figs. 1 and 2, suggest a general trend toward decreasing abundances as the experiment progressed. In some cases, these trends may represent behavioral

Table 2. Vertical migration (second experiment).

Animal	Vertical migration*		Pattern†
	During light cycle	Persistence during constant conditions	
Dark-active animals‡			
Nototropis sp. (amphipod)	Yes	Yes	A
Peltidiad copepods	Yes	Yes	B
Tiron sp. (amphipod)	Yes	No	C
Megaluropus sp. (amphipod)	Yes	No	C
Cyclaspis sp. (cumacean)	Yes	No	C
Epicarid isopods (larval stages)	Yes	No	D
Metamysidopsis elongata (mysid)	Yes	No	D
Labidocera spp. (copepod)	Yes	No	D
Emerita analoga larvae (decapod)	Yes	No	D
Light-active animals‡			
Laeophontid copepods	Yes	No	E
Euterpina sp. (copepod)	±	No	E
Acanthomysis macropsis (mysid)	±	No	E
Cyprid larva (cirripedia)	Yes	No	E
No migration observed			
"*Acartia*" spp. (copepod)	No	No	F
Archaeomysis sp. (mysid)	No	No	
Chaetognaths	No	No	
Medusae	No	No	
Mollusc larvae	No	No	

* Significance of the day-night differences was evaluated by the Mann-Whitney U test. For the "light-cycle" decision, data from the hours of illumination on 25 and 26 July were compared with data from the dark hours of these same dates (24 values in each class); similarly, for the "persistence" decision, data from midnight to noon on 27 and 28 July were compared with data from noon to midnight. A "Yes" decision indicates significance at the .001 level; ± indicates $.01 > P > .001$; "No" indicates a probability greater than .01. † Pattern refers to a categorization of the observations, in terms of the component graphs of Fig. 2. Thus, *Megaluropus* sp. (pattern C) showed behavior most comparable with that of *Tiron* sp. (Fig. 2C). ‡ Dark-active animals are defined as those that showed significant maxima of surface abundance during the dark phase of the light-dark cycle; light-active animals showed opposite behavior.



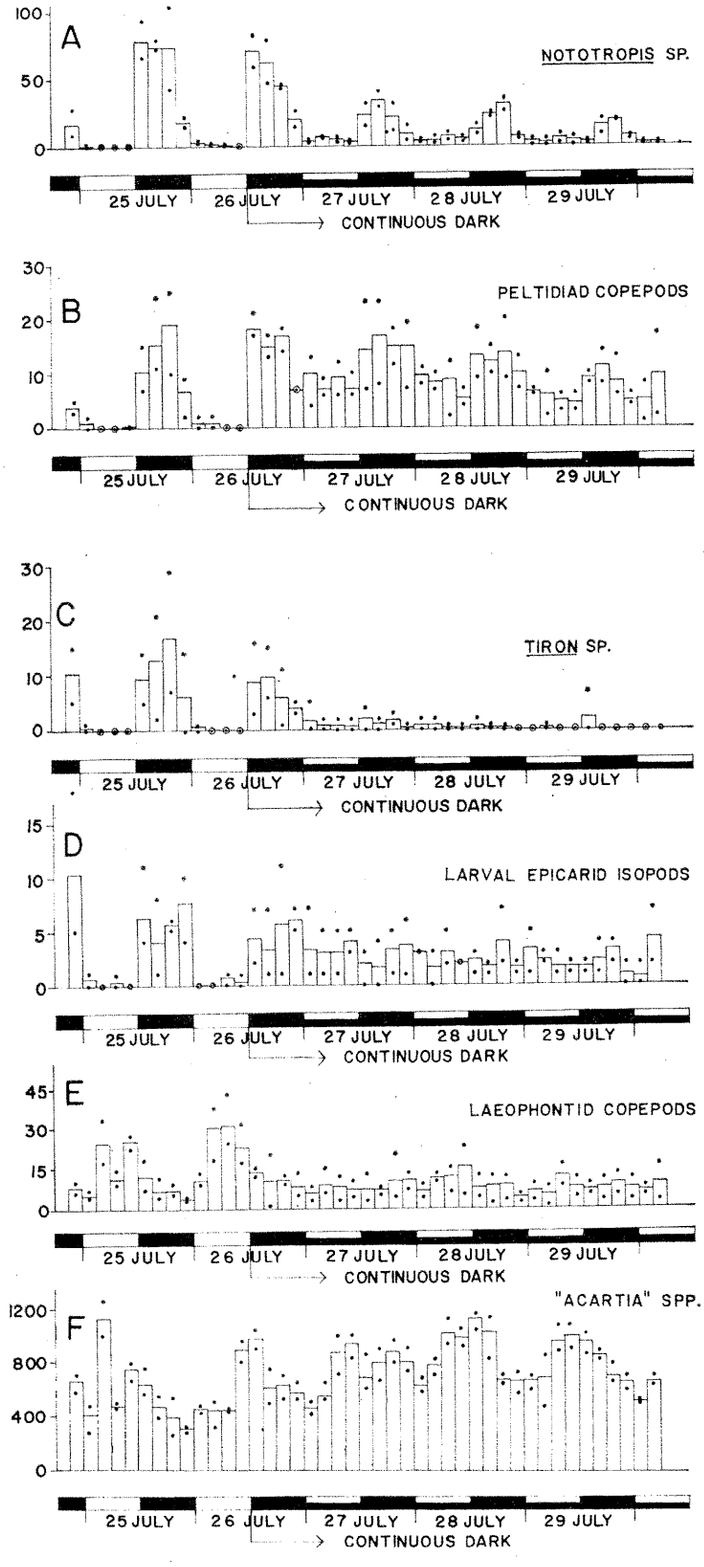


Fig. 1 (left). Summary from first experiment of changes with time in abundances of selected organisms. The heights of the vertical bars represent average abundance from three simultaneous samples; the dots present minimum and maximum observed values. The horizontal bars beneath the graphs indicate the initial light cycle, and, during continuous dark, also include an extrapolation of the preceding light cycle. See text for further details. Fig. 2 (right). Summary from second experiment of changes with time in abundance of selected organisms. Other details are as in Fig. 1.

adaptations of the organisms to the environment provided, but true decreases in population size are also likely. "Grazing" by the net would contribute to this phenomenon. Each net-tow filtered slightly more than 1 percent of the total volume of water present, and a larger fraction of a given species could have been removed during those times at which the organisms may have been concentrated in a relatively thin layer near the surface. Gut contents of predators, particulary chaetognaths and medusae, suggest that appreciable predation also occurred, probably intensified by the heavy concentration of organisms present. In addition, abnormally high mortality may have been induced in certain species by the lack of circulation in the tank, perhaps due to reduced oxygen tension or to the accumulation of metabolic by-products.

The data for the peltidiad copepods (Figs. 1B and 2B) indicate that both internal rhythmicity and concurrent light intensity play important roles in their normal vertical migration. Under constant dim light, there was clear evidence for rhythmic changes in surface abundance in phase with the prior light cycle. Large numbers of the animals, however, remained at the surface during the hours corresponding to the previously imposed "daytime," a behavior which is in marked contrast to the virtual absence of these animals at the surface during the previous light phase of the actual light cycle. The results imply, then, that the vertical migration of these species is based upon an internal rhythm that is entrained by a light-dark cycle, but that bright light also acts directly as a stimulus, superimposed on the endogenous rhythmicity, to suppress swimming near the water surface.

In the design of this study, we had anticipated two probable outcomes: internal rhythmicity, or dependence of the organism primarily on prevailing light conditions. Behavior such as that shown by *Tiron* sp. (Figs. 1C and 2C), therefore, came as a surprise to us, in that it indicates the presence of a nonrhythmic timing mechanism. Following the last 12-hour light treatment, the animals rose to the surface, and then returned spontaneously to the bottom several hours later in the absence of any light stimulus. Thereafter they never reentered the surface in any appreciable numbers. This suggests the existence of a timing mechanism related in principle to an hourglass, which requires stimulation by light in each cycle, rather than the oscillatory timing mechanism implied by persistent rhythmicity. Presumably such internal timing would be sufficient under field conditions to induce animals to leave the surface waters during the very early morning hours, long before any perceptible change in the light intensity served to "drive" them from the surface. This sort of interaction with the environment, incorporating a time lag, would (like persistent rhythmicity) result in behavior completely unrelated to concurrent environmental conditions. These results suggest, then, that considerable caution is necessary in searching for environmental correlates of vertical distributions of zooplankters.

A number of species, for which the epicarid isopods serve as an example (Figs. 1D and 2D), showed no clear evidence of internal timing mechanisms in their behavior. Following clear vertical migration during the light cycle, the animals tended to remain at the surface during constant dim light, with no significant cyclic time trends in surface abundance. It should be emphasized, of course, that such evidence does not indicate the absence of endogenous rhythmicity in the species, but only the probable noninvolvement of an endogeous rhythm (if such is present) in the phenomenon of vertical migration. The vertical migratory performance can be most easily interpreted by the hypothesis that the animals were responding exclusively to concurrent light intensity.

Of the few organisms which showed an inverse vertical migration during the lighting cycle (Figs. 1E and 2E), none showed statistically significant evidence for the persistence of the behavior during constant conditions. The data suggest that these organisms, also, were primarily dependent on concurrent lighting conditions as a determinant of vertical position.

The data for the most abundant group of organisms, "*Acartia*" spp. (*9*), are open to alternative interpretations. In the first experiment (data not illustrated) these animals showed no evidence of consistent time trends. In the second experiment (Fig. 2F), statistical tests for day-night differences gave no indication of significant changes between day and night, either during or after the light cycle. The raw data, however, suggest a weak tendency toward greater abundance in those samples taken immediately before and after evening twilight transition than at other times, and there is some suggestion in the data that this trend continued in a rhythmic manner after the end of the light cycle. Such a migratory pattern, if real, would not be detected by a statistical test based solely on day-night differences. It seems best to us to withhold judgment in interpreting the suggestion of rhythmicity in these data pending further studies, and we have therefore grouped these organisms with those that showed no significant vertical migration in the tank environment. It should not be overlooked, however, that the apparent peaks in abundance for "*Acartia*" spp. coincided with evening twilight, a timing which seems anomalous on ecological grounds but which corresponds with the observations of Esterley (*4*) for both *Acartia tonsa* and *A. clausi*.

A surprisingly wide variety of behavioral responses involved in vertical migration were obtained in these experiments, ranging from persistent endogenous rhythms synchronized by environmental light cycles, to direct responses to concurrent light intensity. Responses intermediate between these extremes were also observed. Still other organisms possess internal timing mechanisms which do not appear to be self-sustained rhythms. Although the vertical migration of zooplankters may appear to be a single phenomenon in an ecological context, conceivably evolved on the basis of a common selective pressure, the physiological mechanisms underlying the field behavior are by no means uniform.

J. T. ENRIGHT
Scripps Institution of Oceanography, University of California, La Jolla
W. M. HAMNER
Department of Zoology, University of California, Davis

References and Notes

1. *Cold Spring Harbor Symp. Quant. Biol.* **25** (1960); *Circadian Clocks*, J. Aschoff, Ed. (North-Holland, Amsterdam, 1965).
2. D. H. Cushing, *Biol. Rev. Cambridge Phil. Soc.* **26**, 153 (1951); J. E. Raymont, *Plankton and Productivity in the Oceans* (Macmillan, New York, 1963), p. 418; K. Banse, *Progr. Oceanog.* **2**, 53 (1964).
3. R. H. Backus, R. C. Clark, A. S. Wing, *Nature* **205**, 989 (1965).
4. C. O. Esterley, *Univ. Calif. Berkeley Publ. Zool.* **16**, 393 (1917); *ibid.* **19**, 1 (1919).
5. W. Schallek, *Biol. Bull.* **82**, 112 (1942).
6. J. E. Harris, *J. Marine Biol. Assoc. U.K.* **42**, 153 (1963).
7. J. T. Enright, *J. Theoret. Biol.* **8**, 426 (1965).
8. A. Fleminger and R. I. Clutter, *Limnol. Oceanog.* **10**, 96 (1965).
9. The organisms classified as "*Acartia*" spp. consisted predominantly of *Acartia tonsa* and *Acartia clausi*, but because of difficulty in identification, some few individuals of several other species, including the genera *Calanus* and *Pseudocalanus*, were counted together with *Acartia*.
10. Supported by grants GB 2469 and GB 5471 from NSF. We thank Catherine A. Brown for assistance throughout the research.

21 June 1967