Photosynthesis in Copepods

Abstract. *Zooplankton grazers consume appreciable amounts of algae that are not digested. Indigestibility has been considered of adaptive value to the algae and an unqualified disadvantage to the grazer. In Cartesian diver experiments, freshly killed copepods* (Acanthocyclops vernalis) *produced significant amounts of oxygen when exposed to the light, even after 24 hours of starvation. Also, significant amounts of oxygen were consumed by dead copepods in the dark. These observations suggest that the copepod gut is well suited for photosynthesis by ingested algae.*

While investigating changes in metabolic rate during copepod development (*1*), we found that copepods that were accidentally killed by anesthetic overdose produced substantial oxygen in the light. We concluded that viable organisms, including algae, must be sustaining significant metabolism inside copepods. In this report we describe the magnitude of the photosynthetic and respiratory activity in one copepod species.

Changes in oxygen concentration in *Acanthocyclops vernalis* (Fischer) (*2*) were determined with a Cartesian diver microrespirometer (*3*). There were three groups of experimental animals: group 1 had free access to food, group 2 was starved for 3 hours, and group 3 was starved for 24 hours. All experimental copepods were killed with MS-222 just before we made metabolic rate determinations (*4*). Each experimental group was divided into two treatment categories: illuminated (light) and not illuminated (dark) (*5*). Oxygen change per unit time was the dependent variable in all experiments. The control group consisted of living copepods (*6*).

Differences in oxygen consumption among groups and between treatments were determined by two-way analysis of variance (*7*). The difference between treatments (light and dark) is statistically significant (Table 1). Values of oxygen change for the dead, illuminated animals were always positive, indicating net photosynthesis. Values for the dark treatment were always negative indicating substantial respiration, even though the copepods were dead. Control divers without animals showed no oxygen change, so the changes reported in Table 1 must be associated with the copepods. Microscopic examination showed no external algae on the copepods.

The statistically significant difference among groups (Table 1) indicates that both oxygen production (light) and consumption (dark) change according to the elapsed time since the last food intake before death. This change over time was analyzed by regression analysis in which duration of starvation was the independent variable and oxygen consumption or oxygen production per individual was the dependent variable. Both respiration and net photosynthesis decrease significantly as duration of starvation increases (*8*) (Fig. 1); the slope of the regression line for the dark treatment is significantly steeper ($P < .05$) than that for the light treatment.

We interpret the results as follows. A significant amount of oxygen is produced by algae within the copepod gut. There is also substantial oxygen consumption in the dark by algae, and possibly by other organisms, within the gut. The other organisms might include bacteria living on detritus that has been consumed (*9*). There are measurable changes in oxygen concentration even for copepods starved for 24 hours before being killed, indicating that significant portions of the gut contents are retained during starvation and that some of this material remains alive and metabolically active. Since the rate of oxygen generation decreases with time of starvation at a rate lower than that of the decrease in oxygen consumption, the viable algae must be less damaged than some of the other organisms retained within the gut. The decrease in respiration and photosynthesis with time of starvation also shows that these processes are the result of organisms within the gut, since it is unlikely that other sources of gas change (that is, epizoic organisms) would be affected by starvation.

Table 1. Metabolic activity in dead copepods. Two treatment categories (light, dark) and three feeding regimes are compared. Statistically significant differences exist among groups ($P < .01$) and between treatments ($P < .01$). Values are mean ± the standard error.

Treatment	O_2 change (nl/hour)		
	Group 1	Group 2	Group 3
Light	22 ± 5 ($N = 10$)	27 ± 4 ($N = 6$)	15 ± 3 ($N = 12$)
Dark	−48 ± 12 ($N = 14$)	−66 ± 13 ($N = 6$)	−11 ± 6 ($N = 12$)

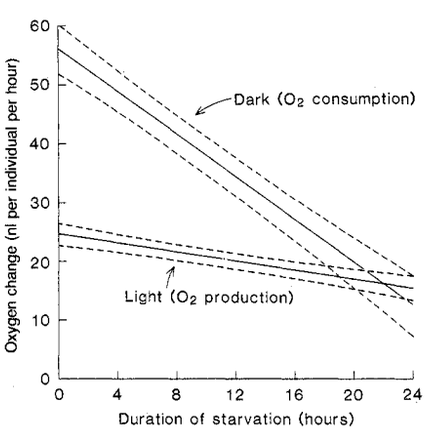

Fig. 1. Regression lines showing oxygen change associated with freshly killed copepods. Standard errors are indicated by the dashed lines. Differences among treatments and between groups are statistically significant ($P < .05$).

Metabolic activity in the gut has important implications both from a technical and from an ecological-evolutionary standpoint. From a strictly technical perspective, photosynthesis and respiration of the magnitude we have observed in the gut can create seriously misleading information concerning metabolic rates. In our experiments, metabolism of living *Acanthocyclops vernalis* would have been overestimated by 40 percent if measured in the dark and underestimated by 20 percent if measured in the light because of metabolic activities of organisms within the gut (*10*). Since we made no attempt to maximize photosynthesis either by increasing the amount of algae in the diet or by optimizing light intensity, maximum photosynthesis and associated technical problems in determining metabolism could be much greater. These problems can be especially important in experiments involving systematic changes in light regime, as in diel studies.

The possible adaptive significance of metabolically active algae within the gut is considerable. Certain phytoplankton are known to pass through the guts of planktonic grazers without undergoing any appreciable digestion (*11, 12*). Porter (*13*) has referred to the relation between grazer and algae as nonobligatory mutualistic symbiosis, thereby implying that both members profit from the association. Although Porter has shown several advantages to the algae associated with the phenomenon, hypotheses concerning the possible adaptive advantages of the relation to the grazer have not been convincing. Porter (*13*) suggests that the nutritive value gained from partial digestion of the gelatinous sheath may be a significant source of energy for the grazer during resource scarcity. Although the sheath may be nutritious, most of the potential energy is not used because the algal cell itself is not digested. Furthermore, many indigestible species lack a gelatinous sheath and therefore would be of no nutritional value (*12*). Without

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some better argument for the advantage to the herbivore, its failure to digest many algae appears to be a major adaptive inadequacy of the herbivore. This is possible, but it seems unlikely that the herbivore is truly blocked from selection of digestible foods or from development of mechanisms for digesting refractory foods.

Research (*13*) on the significance of undigested algae to the grazer has not dealt with the potential for photosynthetic activity during passage through the gut, probably because it would not be obvious that photosynthesis would proceed so effectively. Since active photosynthesis can occur in the gut, even for long periods, advantages may accrue to the herbivore by this route. Gases, photosynthate, or both, may be involved. Algae may be trading photosynthate to copepods in return for viable gut passage; this would imply coevolved mechanisms, including extraordinary release of photosynthate or important growth factors such as vitamins (*14*) by the cells and enzymatic selectivity by the herbivore to protect the algae. Gases are a second line of interaction. An internal oxygen pump of this magnitude opens possibilities for improved efficiency in food gathering and locomotion. Removal of CO_2 is also affected to the benefit of both herbivore and algae. Behavioral alteration of gut photosynthesis by adjustment of light climate during vertical migration is a potentially important by-product of algal-copepod coevolution.

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References and Notes

1. R. W. Epp and W. M. Lewis, Jr., *Ecology* **61**, 259 (1980).
2. Animals were obtained from University Pond on the campus of the University of Colorado, Boulder. All animals were acclimated to a temperature of 22.5°C in raw pond water for 24 hours before the experiments. Animals were fed on the natural assemblage of organisms in the pond, including an abundance of mixed phytoplankton. Copepodids were drawn at random from the last six developmental stages in the population.
3. R. W. Epp and W. M. Lewis, Jr., *Oecologia (Berlin)* **42**, 123 (1979).
4. The animals were observed under a microscope after anesthetization. Peristalsis of the gut continued after heavy anesthetization. Death was coincident with cessation of peristalsis. The test animals were all placed in fresh culture medium (medium without MS-222) after the experiments, and none showed vital signs.
5. Treatment was performed in total darkness or at a light intensity of 150 $\mu E\ m^{-2}\ sec^{-1}$ measured at the divers. This is approximately 7 percent of midday sunlight [R. J. List, Ed., *Smithsonian Meteorological Tables 1963* (Smithsonian Institution, Washington, D.C., ed. 6, 1963)]. All oxygen change experiments were conducted between 1200 and 1600 hours.
6. Metabolism of the control group was determined under normal laboratory illumination (< 50 $\mu E\ m^{-2}\ sec^{-1}$) on copepods starved for 24 hours before the experiment.
7. Metabolic rate per unit weight decreases with increasing body size in copepods (*1*) and most animals generally [C. L. Prosser, *Comparative Animal Physiology* (Saunders, Philadelphia, 1973)]. We first tested for the effect of size on metabolism. Body length was determined with an ocular micrometer. Length was then converted to weight by the equation of H. J. Dumont [*Oecologia (Berlin)* **19**, 75 (1975)]. Weights ranged from 1 to 13 μg (dry). We did not find a statistically significant effect of copepod size on metabolism of organisms in the gut. The typical effect of size on metabolism was significant in the control group, however. In our study, we need not consider size for any animals except the living controls; hence we can compare O_2 change per individual directly among experimental animals.
8. The normal retention time for food in the gut is only a few minutes during feeding periods [J. D. Green, *Oecologia (Berlin)* **21**, 345 (1975)]. A continual filtration is necessary for movement of material through the digestive system, however. Food appears to be retained in the gut for an extended time during periods of starvation (*13*).
9. R. J. Conover, in *Zoogeography and Diversity in Plankton*, S. van der Spoel and A. C. Pierrot-Bults, Eds. (Halsted, New York, 1979), p. 66.
10. The basal metabolic rate for the smallest copepodid that was used in this study (1.0 μg, dry weight) was 142 nl per individual per hour.
11. T. S. Ho and M. Alexander, *J. Phycol.* **10**, 95 (1974); C. M. Nadin-Hurley and A. Duncan, *Freshwater Biol.* **6**, 109 (1976).
12. G. S. Fryer, *J. Anim. Ecol.* **26**, 263 (1957).
13. K. G. Porter, *Am. Sci.* **65**, 159 (1977); *Science* **192**, 1332 (1976); *Verh. Int. Ver. Limnol.* **19**, 2840 (1975).
14. Copepods consume a significant amount of detritus [R. P. Gerber and N. Marshal, *Limnol. Oceanogr.* **19**, 815 (1974); S. A. Poulet, *Mar. Biol.* **34**, 117 (1976)], and they are apparently capable of digesting most particulate organic matter [P. Mayzaud and R. J. Conover, in *Tenth European Symposium on Marine Biology* (Ostend, Belgium, 1975), vol. 2, p. 415; J. Boucher, A. Laurec, J. F. Samain, S. L. Smith, in *ibid.*, p. 65]. Nevertheless, living organic matter is a dietary requirement. Conover (*9*) speculates that living organic matter may be a source of vitamins or other trace nutritional requirements. If this is the case, copepods may obtain an important nutritional supplement such as a vitamin while sequestering only a small fraction of the organic matter that is represented by the oxygen production.
15. We thank J. D. Allan and T. M. Frost for reviewing the manuscript and J. F. Saunders for identifying copepods. This work was supported by NSF grant DEB 8003883.

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Phase Locking, Period-Doubling Bifurcations, and Irregular Dynamics in Periodically Stimulated Cardiac Cells

Abstract. *The spontaneous rhythmic activity of aggregates of embryonic chick heart cells was perturbed by the injection of single current pulses and periodic trains of current pulses. The regular and irregular dynamics produced by periodic stimulation were predicted theoretically from a mathematical analysis of the response to single pulses. Period-doubling bifurcations, in which the period of a regular oscillation doubles, were predicted theoretically and observed experimentally.*

The phase of neural and cardiac oscillators can be reset by a single brief depolarizing or hyperpolarizing stimulus (*1–3*). Experimental determination of the dependence of the phase shift on the phase of the autonomous cycle at which the stimulus was delivered allows computation of a mathematical function called the Poincaré map (*4*). Analysis of the Poincaré map is carried out to predict the response to periodic stimulation (*1, 2, 4*). This work provides experimental confirmation of a recent theoretical prediction (*4*) that period-doubling bifurcations and irregular dynamics (*5*) should be observable in periodically stimulated oscillators.

The preparation has been described in detail (*6*). Briefly, apical portions of heart ventricles of 7-day-old embryonic chicks were dissociated into their component cells in 0.05 percent trypsin. The cells were transferred to a flask containing tissue culture medium (818A with a potassium concentration of 1.3 m*M*), which was placed on a gyratory shaker. Spheroidal aggregates (100 to 200 μm in diameter) of electrically coupled cells that beat spontaneously with a period between 0.4 and 1.3 seconds form after 48 to 72 hours of gyration. Experiments were performed on aggregates in the same culture medium at 35°C under a gas mixture of 5 percent CO_2, 10 percent O_2, and 85 percent N_2. Intracellular electrical recordings were made with glass microelectrodes filled with 3*M* KCl (resistance, 20 to 60 megohms). Current pulses were delivered through the same electrode and measured with a virtual ground circuit. Impalements were maintained for 2 to 5 hours. This report presents results for two aggregates out of ten studied.

Consider the response of an aggregate to a single current pulse delivered δ msec after the upstroke of the action potential (Fig. 1A). The length of the cycle immediately preceding the perturbation is called τ, and the phase ϕ of the cycle at which the stimulus was delivered is $\phi = \delta/\tau$, $0 \leq \phi < 1$. Control cycles with the phase labeled are shown in Fig. 1B. The cycle time of the perturbed cycle (the time from the upstroke immediately preceding the stimulus to the next upstroke) is called *T*. A stimulus was delivered after every ten beats, with δ increased by 10 msec each time. In Fig. 1C the normalized perturbed cycle length T/τ is plotted for two different preparations. In a single preparation, an increase

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