enzymes, which play a role in sealing off membranes of cut nerve endings (*14*) and in establishing a path for regeneration, may become activated subsequent to modification. A third possibility is that these reactions may be involved in proteolytic events. The nonlysosomal ubiquitin proteolytic pathway, a system for hydrolyzing damaged proteins, has an absolute requirement for tRNA (*15*). The function of tRNA in the ubiquitin reaction is not known; it may serve as a posttranslational amino acid donor to damaged proteins, thereby targeting those proteins for degradation. If that is the case, then the modification reactions could act to participate in the breakdown of damaged proteins, thus clearing the way for successful regeneration.

In conclusion, both sciatic and optic nerves contain the components necessary for, and are capable of, protein modification by the addition of amino acids. However, the finding that these reactions are dramatically increased in sciatic but not optic nerves after injury suggests a fundamental biochemical difference between them, a difference that may be related to the ability of one and the inability of the other to regenerate.

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9. The connective tissue on the inferior-lateral side of the eyeball was gently teased away with fine forceps to expose the optic nerve. The nerve was then separated from the surrounding connective tissue and crushed for 10 seconds with curved jeweler's forceps at the point where the optic nerve enters the orbital cavity (approximately 4 mm from the back of the eye). Nerves were crushed rather than cut to avoid severing the retinal artery. Sciatic nerves were crushed by the procedure of M. F. Zanakis *et al.* (*7*).
10. Nerves were excised, desheathed, homogenzied in 250 μl of buffer, and extracted with hot and cold 6.5 percent TCA until the wash contained negligible amounts of radioactivity. The pellet was dissolved in Protosol and counted in toluene:Liquifluor. Aliquots of the TCA-soluble fraction were counted in Hydrofluor (National Diagnostics). The calculation for protein synthesis was made by dividing the TCA-insoluble radioactivity by the total radioactivity (TCA-soluble plus -insoluble) in the nerve segment. Thus increases in protein synthesis are indicated by an increase in the percentage of incorporation. (This determination includes radioactive amino acids that may have been incorporated into proteins by modification reactions, but this is likely to be a minor proportion of the amino acids incorporated into proteins by protein synthesis.)
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16. Supported by NIH grant NS19148. We thank D. Richman for valuable contributions.

15 July 1985; accepted 7 November 1985

Herbivores' Direct and Indirect Effects on Algal Populations

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The increase in algal reproductive rates caused by nitrogen regeneration from herbivorous zooplankton approximately equaled the zooplankton-caused mortality. This result demonstrates that nutrient regeneration by herbivores is at least sometimes a strong indirect effect in natural communities.

HERBIVORES AFFECT THE POPULATION dynamics of plants in at least two distinct ways: directly through consumption and indirectly through regeneration of limiting nutrients. Although there has been interest in this dual role of herbivory in both terrestrial (*1*) and aquatic (*2*) plant communities and in indirect effects in general (*3*), until now there have been no studies that have simultaneously assessed the relative importance of these two processes in a natural community. Such experiments were conducted on a natural algal community with a cooccurring crustacean herbivore, *Daphnia pulex*, to separate the direct effect of herbivory from the indirect effect of fertilization. The indirect effect caused by nitrogen regeneration had about as large an impact on the phytoplankton community as the direct grazing effect did.

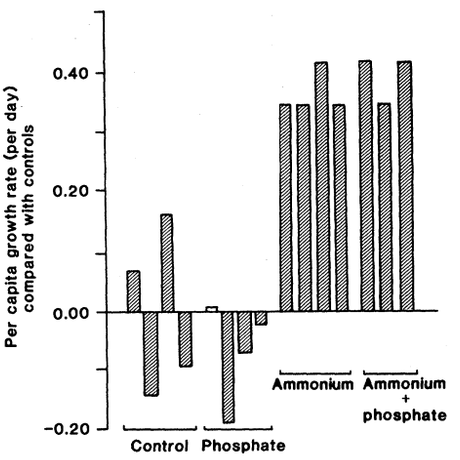

Fig. 1. The difference between growth rates of chlorophyll fluorescence in each vessel and the mean growth rate for controls—as determined from final densities by the equation

$$[\ln F - (\text{mean } \ln F \text{ for controls})]/6$$

where F is fluorescence on day 6—shows a significant nitrogen effect [$F(1, 11) = 86.90$, $P < 0.005$]. The mean for the nitrogen flasks was 0.348 (SE, 0.067), and the mean for the nonnitrogen flasks was −0.035 (SE, 0.068). Nitrogen regeneration could potentially stimulate phytoplankton growth.

The general experimental procedure consisted of establishing an herbivore gradient and monitoring phytoplankton growth rates when influenced by both grazing and nutrient regeneration and when influenced by only nutrient regeneration. In addition, I performed nutrient addition experiments to establish whether regenerated nitrogen or phosphorus or both were potentially limiting (*4*).

In the absence of grazers the algae were limited by nitrogen, but not by phosphorus, as shown by chlorophyll fluorescence (*5*) (Fig. 1) and population densities. Chlorophyll increased at 0.41 per day (SE, 0.09) faster in the nitrogen treatments than in the nonnitrogen ones, providing an estimate of the maximum potential effect of nitrogen regeneration on algal reproductive rates. Population densities of two of four domi-

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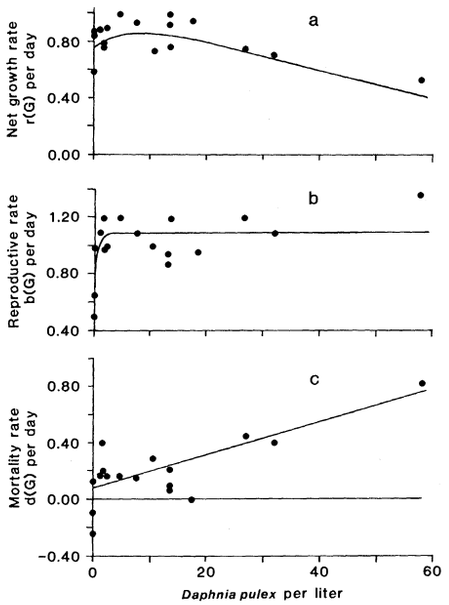

Fig. 2. The direct and indirect effects of herbivory (*20*). (a) Net effect of *D. pulex* on algae, determined by rates of change of chlorophyll outside the grazer exclosures. (b) Indirect effect of *D. pulex* on phytoplankton reproductive rates, determined by rates of change of chlorophyll inside the grazer exclosures. (c) Direct effect of *D. pulex* on phytoplankton mortality rates. The slope of the least squares line fit to these data converts to a filtering rate of 11.7 ml per individual per day (*21, 22*). The regression is significant [slope, 0.012 (SE, 0.003)], and the *y* intercept does not statistically differ from the origin [intercept, 0.071 (SE, 0.050)]. Curves in (a) and (b) are from the fitted equations in (*12*). The curve in (c) is from a linear regression of *d(G)* on *G*.

nant taxa significantly increased with addition of nitrogen [*Scenedesmus* spp., $F(1, 12) = 5.71$, $0.025 < P < 0.010$; pennate diatoms, $F(1, 12) = 34.36$, $P < 0.005$ (*6*)]. Phosphorus and the N by P interaction were always insignificant.

At each grazer density in the herbivore gradient (*7, 8*) 94 percent of the volume of the pond water was in contact with the grazers. These phytoplankton were exposed to both grazing and nutrient regeneration. The remaining 6 percent, separated from the grazers by porous grazer exclosures (*8*), were exposed only to nutrient regeneration. Experiments lasted 6 days.

Both algal reproductive and mortality rates depended on grazer density (Fig. 2). Algal per capita reproductive rates increased with increasing grazer density, saturating at low grazer densities (Fig. 2b). Mortality rates increased as grazer density increased (Fig. 2c). These two simultaneous effects, fertilization and grazing, yielded a net growth function with a shallow hump (Fig. 2a) (*9–11*). Though both the grazing and fertilization effects of herbivores were measurable when separated, they were in opposition, almost canceling when acting simultaneously.

To explore these relationships in more detail, a series of equations (*12*) were fit to the data in Fig. 2. On the basis of that fit, the maximum increase in algal reproductive rates because of nutrient regeneration did not differ significantly from the increase in reproductive rates in the nitrogen additions (*13–15*). This result supports the conclusion that the effect seen in Fig. 2b is due to nitrogen regeneration.

From these data it is possible to estimate the magnitudes of the direct and indirect effects in the units of per day. The density of *D. pulex* in the natural community was estimated to have been 19 individuals per liter (*16*), which was more than the density found to increase phytoplankton reproduction through nitrogen regeneration (Figs. 2b and 3) (*17*). I conclude that the direct and indirect effects were both important to the phytoplankton. In fact, the estimates of those two effects based on the estimated *D. pulex* density were nearly equivalent (*18*).

Comparing the importance of nutrient regeneration with the importance of grazing for the total algal community leaves the question of how individual algal species are affected by fertilization and grazing in many environments. Phytoplankton populations inside the grazer exclosures were counted under a microscope to see which algae responded most to nutrient regeneration. Of the four dominant taxa (*Anabaena flos-aquae, Melosira* spp., pennate diatoms, and *Scenedesmus* spp.), the one that seemed to respond most was the same taxon that responded most significantly to nitrogen additions—the pennate diatoms (*6*) (Fig. 3). This result suggests that the indirect fertilization effect was greatest for the most nutrient-limited taxon. Different algal species suffer different mortality losses from grazing (*19*). Also, different herbivore species vary in grazing and nutrient regeneration rates, which also depend on algal density. We can therefore expect these direct and indirect effects in the aquatic food web to be rich in their dynamic possibilities.

To summarize, algal per capita reproductive rates were lower in the absence of the herbivore *D. pulex* than when the grazer was present. The herbivore fertilized the algae by regenerating nitrogen, an indirect effect about as important for phytoplankton population dynamics as the mortality from grazing was. These findings reinforce the concept that nutrient regeneration by herbivores is an important trophic link (*2*). Although in the long term zooplankton grazers generally have an overall negative impact on algal populations, estimates of the effect of zooplankton on phytoplankton communities that consider only grazing may be in error.

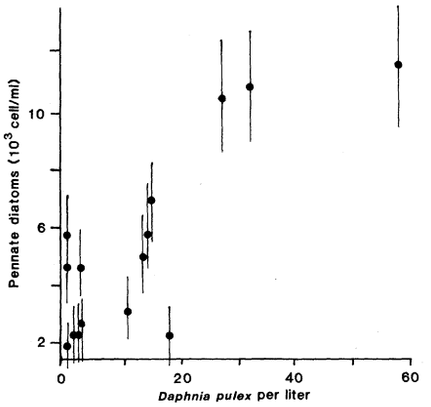

Fig. 3. Pennate diatom (*6*) density [±95 percent confidence intervals (*23*)] inside the grazer exclosures in the herbivore gradient (*7, 8*), showing the indirect effect of fertilization on this taxon.

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4. I collected water from Pleasant Pond, Ramsey County, Minnesota, on 28 June 1984 and removed the macrozooplankton with 80-μm Nitex. I added Na_2HPO_4 to the appropriate treatments to increase the phosphate concentration by 10 μ*M* and $(NH_4)_2SO_4$ to increase the ammonium concentration by 500 μ*M*. These concentrations were chosen to be greater than the concentrations usually found to be limiting to algal growth in laboratory cultures.
5. All fluorescence measurements were determinations of the differences between whole water in vivo fluorescence and fluorescence of the fraction passing through 3-μm-pore Nuclepore filters.
6. *Scenedesmus* spp. included *S. abundans* var. *longicauda, S. dimorphus*, and *S. quadricauda*. Average cell densities (in cells per milliliter) for the treatments were: control, 974; P added, 874; N added, 2461; and P and N added, 1775 (SE, 999). Pennate diatoms included *Fragilaria vaucheriae, F. virescens, Synedra amphicephala, S. faciculata* var. *truncata*, and *S. ulna*. Average cell densities were: control, 7160; P added, 3534; N added, 14983; and P and N added, 15535 (SE, 3381). Decreasing significantly as a result of the additions of N was *Melosira* spp. (including *M. italica* and *M. granulata* var. *angustissima*), with average cell densities of: control, 645; P added, 226; N added, 3.8; and P and N added, 3.8 (SE, 267). *Anabaena flos-aquae* had no significant response to nutrients, with cell densities: control, 305; P added, 181; N added, 41; P and N added, 0.0 (SE, 207).
7. Each of 17 1000-ml beakers was filled with 850 ml of pond water, the predetermined number of freshly collected Pleasant Pond *D. pulex*, and a grazer exclosure chamber (*8*). They and the nutrient addition flasks were incubated at natural light and temperature in a laboratory incubator.
8. Exclosure design followed that described by W. G. Crumpton and R. G. Wetzel [*Ecology* **63**, 1729 (1982)]. Exclosure volume was 55 ml. Porous surfaces were two 90-mm Nuclepore filters of 3-μm pore size. Exclosures were filled with grazer-free pond water. Gentle agitation (10 seconds every 100 seconds), provided mechanically (exclosures were raised and lowered at 0.5 Hz), allowed for an exchange of 80 percent of the chamber volume per hour.

9. Since nutrient regeneration is coupled to feeding, it seems impossible for the net effect of grazers on the entire phytoplankton assemblage ever to be an increasing function of grazer density. But grazing on colloidal or bacterial material may supply nutrients to algae. Also, zooplankton may excrete previously assimilated nutrients (*10*). Finally, chemical compounds released by zooplankton such as phosphatases (*11*) or chelators may supply otherwise unavailable nutrients. In six other experiments performed in this and another field site, fertilization effects were weaker than grazing effects; none were stronger.
10. For example, J. H. Martin, *Limnol. Oceanogr.* **13**, 63 (1968).
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12. The three equations were:

$$r(G) = \mu_1 + \mu_2\left[\frac{G}{K_1 + G}\right] + fG \quad (1)$$

$$b(G) = \mu_3 + \mu_4\left[\frac{G}{K_2 + G}\right] \quad (2)$$

$$d(G) = \mu_5 + fG \quad (3)$$

where: $r(G)$ is per capita rate of change of algal density as a function of grazer density (per day), $b(G)$ is algal per capita reproductive rate as a function of grazer density (per day), $d(G)$ is algal per capita mortality rate as a function of grazer density (per day), μ_i are growth rates (per day), K_i are half-saturation constants (individuals per liter), G is grazer density in individuals per liter, and f is proportional to filtering rate (per individual per day). These three equations were chosen because they provided an adequate fit to the data, not because they are expected for mechanistic, physiological reasons. However, analysis of a series of first-order nonlinear differential equations derived from first principles suggests they are approximately correct.
13. When Eq. 1 is fit to the data in Fig. 2a, a value of 0.281 (SE, 0.177) per day is obtained for μ_2. When Eq. 2 is fit to the data in Fig. 2b, a value of 0.387 (SE, 0.108) per day is obtained for μ_4. By a multivariate nonlinear regression analysis, these two estimates are not significantly different ($-2\ln\Lambda = 0.09$, 1 d.f., $0.75 < P < 0.90$) (*14, 15*). These estimates do not significantly differ from the observed effect of nitrogen additions (0.41 per day).
14. The value Λ is the maximum likelihood ratio (*15*).
15. D. R. Cox and D. V. Hinkley, *Theoretical Statistics* (Chapman & Hall, London, 1974).
16. Density was determined from ten vertical hauls of an 80-μm mesh size Wisconsin zooplankton net. I assumed the capture efficiency was 100 percent; in the likely event that the efficiency was less than 100 percent, 19 individuals per liter is an underestimate.
17. The *D. pulex* density that half-saturates the fertilization effect is K. The data in Fig. 2a and Eq. 1 estimate K_1 as 4.02 (SE, 7.28) *D. pulex* per liter, and the data in Fig. 2b and Eq. 2 estimate K_2 as 0.162 (SE, 0.610) *D. pulex* per liter. Multivariate analysis finds these two estimates to be statistically different ($-2\ln\Lambda = 6.11$, d.f. = 1, $0.01 < P < 0.025$) (*14, 15*), casting doubt on the validity of the grazer exclosures for estimating K. The data in Fig. 2a do not depend on the growth of algae inside the grazer exclosures, so the larger of the two estimates may be more accurate.
18. Solving Eq. 1 at a density of 19 *D. pulex* per liter with the fitted parameter values reveals that this density of grazers increased phytoplankton reproductive rates 0.232 per day. Algae reproduced 30 percent faster than in the absence of grazers. With the slope of the line in Fig. 2c used to estimate the grazing effect, 19 *D. pulex* per liter increased phytoplankton mortality rate by 0.228 per day. The estimated fertilization effect (0.232 per day) is approximately equal to the estimated grazing effect (0.228 per day).
19. J. T. Lehman and C. D. Sandgren, *Limnol. Oceanogr.* **30**, 34 (1985).
20. Each datum in (a) and (b) represents the slope of a linear regression of ln (fluorescence) versus day for days 2 to 6, allowing for a lag in algal growth. Because removing a fraction of the volume inside the exclosure dilutes the remaining algae, a dilution factor of ln (a), where a is the fraction of the population remaining after dilution each time t, was added to the oberved rates of change inside the grazer exclosures. Mortality rates in (c) were obtained from the differences of the corrected reproductive rates and the net rates of change.
21. It follows directly from the derivation of filtering rate (*22*) that the slope of $d(G)$ (or f in Eqs. 1 and 3) multiplied by the volume of fluid indicated on the horizontal axis (in this case 1000 ml) yields a filtering rate in the conventional units of milliliters per individual per day.
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24. I thank K. Larntz for his help with the statistical analyses. D. Tilman and J. P. Grover provided advice during all phases of this work, and they and D. McNaught, J. Shapiro, and D. Wright read and improved this report. Financial support was provided by the National Science Foundation Predoctoral Fellowship Program and the Dayton-Wilke Fund (to R.W.S.), NSF grant NSF/BSR 8114302, and Sea Grant NA82AA-D-00039, Project R/F-9 (to D. Tilman). This is Minnesota Sea Grant contribution 174.

22 April 1985; accepted 15 October 1985

Stress-Induced Inhibition of Reproductive Functions: Role of Endogenous Corticotropin-Releasing Factor

CATHERINE RIVIER, JEAN RIVIER, WYLIE VALE

In the adult castrated male rat, exposure to inescapable, intermittent electroshocks inhibited the pulsatile pattern of luteinizing hormone release and markedly lowered its plasma concentrations. The central administration of the corticotropin-releasing factor (CRF) antagonist α-helical ovine CRF residues 9 to 41 reversed the inhibitory action of stress. Neither its peripheral injection, nor the intraventricular injection of the inactive CRF analog des-Glu[17] to Arg[35] ovine CRF was effective. These results suggest that endogenous CRF may mediate some deleterious effects of noxious stimuli on reproduction.

EXPOSURE TO STRESS IS ACCOMPANIED by disruption of reproductive functions in many species (*1–10*) including human beings (*11–17*), but the exact mechanisms that mediate these effects are not fully elucidated. Possible sites involved include (i) direct gonadal effects of the hormones secreted during stress [such as adrenocorticotropin (ACTH), steroids, catecholamines, and vasopressin] with subsequent alterations in sex steroid output (*8, 18–20*); (ii) a corticosteroid-mediated decrease in pituitary responsiveness to gonadotropin-releasing hormone (GnRH), resulting in decreased luteinizing hormone (LH) secretion (*21–23*); and (iii) a centrally mediated inhibition of GnRH release (*24*). While there is evidence that each of these mechanisms can indeed operate during stress and could interfere with normal pituitary and gonadal function, recent studies have additionally indicated that corticotropin-releasing factor (CRF), which is secreted by the brain during stress (*25*), will inhibit GnRH secretion into the hypophyseal portal circulation (*26*). These observations prompted us to investigate a possible central role of endogenous CRF in mediating stress-induced alterations in LH output in the rat.

We have studied castrated male rats because their pattern of pulsatile release is inhibited by stress (*5*). In the castrated rat, forced immobilization or a broken leg abol-

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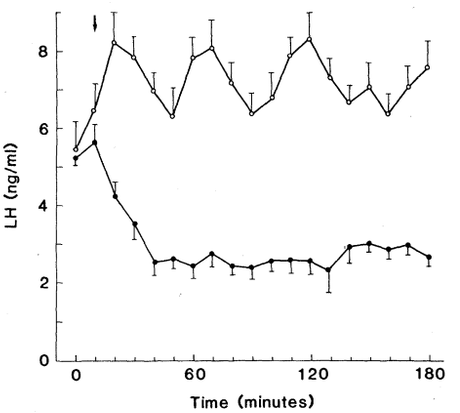

Fig. 1. Effect of electroshocks on basal plasma LH in castrated male rats. The rats were placed in individual shockers and exposed to footshocks for 3 hours. The arrow indicates the onset of the electroshocks. The shocks (2 mA, 2-second duration) were delivered randomly on an average of four per minute to the grid floors of plexiglass chambers (30 by 126 by 30 cm) by a Colbourn shocker. Blood samples (0.3 ml) were obtained every 10 minutes and replaced with an equal volume of lactated Ringer or Plasmanate (plasma protein fraction, 5 percent; Cuttler Biological, Berkeley, CA). Open circles, control rats; closed circles, shocked rats. Each point represents the mean ± SEM of six to eight rats. Plasma LH was measured by radioimmunoassay (intra- and inter-coefficients of variation, 5.2 and 9.8 percent, respectively). Data were analyzed by one- and two-way analyses of variance. For reasons of clarity, levels of statistical significance are not indicated on figures. In this experiment, plasma LH concentrations of stressed rats were significantly ($P \leq 0.01$) different from control animals at all times.