Ecology, 74(1), 1993, pp. 258–260

BIOLUMINESCENCE IN DINOFLAGELLATES: A TEST OF THE BURGLAR ALARM HYPOTHESIS

Mark V. Abrahams[1,2] and Linda D. Townsend[1]

Bioluminescence in dinoflagellates, unicellular aquatic organisms, has attracted considerable attention, primarily due to the striking nature of this phenomenon—during blooms, disturbances in the water (e.g., breaking waves, the wakes of boats, etc.) can be intensely phosphorescent. Despite a number of hypotheses regarding the function of bioluminescence in dinoflagellates, it is not clear why dinoflagellates bioluminesce (see Morin [1983] for a review). Dinoflagellates are stimulated to bioluminesce by a deformation of their cell membrane generated by shear forces (Hamman and Seliger 1972). These shear forces are often generated by strong stirring of water, such as breaking waves, or the rapid swimming of fish or invertebrates (Sweeney 1987). Sweeney (1987) noted that the light emitted from dinoflagellates is blue-green in color, with the maximum emission being at 474–476 nm. These wavelengths have a low extinction coefficient in water, allowing the light to be visible over relatively long distances. For this reason many researchers have assumed that bioluminescence serves some communication function.

Schantz (1971) suggested that bioluminescence is a form of aposematic coloration, warning potential grazers of noxious substances contained by the prey. Indeed, many of the species of dinoflagellate that bioluminesce also contain toxins. There are also many nontoxic bioluminescent dinoflagellates, perhaps suggesting the existence of a model/mimic system. However, organisms that consume dinoflagellates are often resistant to their toxins, with toxic effects being realized at trophic levels beyond that of the direct grazer of the dinoflagellate (Schantz 1971).

Bioluminescence has also been hypothesized to serve an antipredator function. Esaias and Curl (1972) demonstrated that grazing rates by copepods on dinoflagellates are increased when the bioluminescent capacity of the dinoflagellate is decreased. They hypothesized that the sudden flash of bright light startles the predator, allowing the prey to escape. Although the startle response appears to benefit the dinoflagellate, it is difficult to explain why copepods would continue to respond to the flashes of light generated by dinoflagellates. Buskey et al. (1986) demonstrated that freshwater copepods do not respond to these flashes of light. Therefore maintenance of this startle response must provide some benefit to the copepod. Buskey et al. (1986, 1987) proposed that copepods respond to rapid decreases in light (e.g., shadows) in order to escape predation by ctenophores (which are not present in freshwater), and concluded that the adaptive value of this response physiologically constrains copepods to respond to light flashes generated by dinoflagellates.

Burkenroad (1943) proposed that bioluminescence in dinoflagellates may serve a different function. He suggested that bioluminescence generated by dinoflagellates serves to attract the predators of the dinoflagellate's grazer. This "burglar alarm" hypothesis argues that dinoflagellates render themselves dangerous as prey upon attack because they generate a signal identifying the location of food to individuals two trophic levels up the food chain. If the risk of predation associated with consuming bioluminescent dinoflagellates results in an additional and significant increase to the cost of foraging, this would reduce the net benefit of consumption to a grazer. A significant reduction in the net benefit may cause these dinoflagellates to be eliminated from the grazers' diet. To date, no experiments have determined whether bioluminescence can exert a multi-trophic layer effect necessary to support the burglar alarm hypothesis. Here, we test one prediction of the burglar alarm hypothesis: that bioluminescence serves

[1] Department of Fisheries and Oceans, Fisheries Research Branch, Pacific Biological Station, Nanaimo, British Columbia, Canada V9R 5K6.

[2] Present address: Department of Zoology, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2.

to increase the mortality rate of copepods grazing upon bioluminescent dinoflagellates.

Materials and Methods

Copepods (*Tigriopus japonicus*) were cultured in brackish water and maintained on a diet of Tetra Min fish food and algae. Wild three-spine sticklebacks (*Gasterosteus aculeatus*), the predator in the experiments, were captured by dip net from the dock of the Pacific Biological Station, Departure Bay, British Columbia, and were maintained in a fiberglass tank providing a continuous flow of sea water and fed live copepods. The sticklebacks and copepods used in these experiments occur together in the coastal waters of British Columbia in the presence of bioluminescing dinoflagellates. *Gonyalaux polyedra* was the species of dinoflagellate used for these experiments and was obtained from the North East Pacific Culture Collection at the University of British Columbia. It is native to the California coast and is known to bioluminesce, and some strains have been demonstrated to be toxic (Schradie and Bliss 1962). Although this species of dinoflagellate does not naturally occur with the other two species used in this experiment, it allowed us to examine interactions between the copepod and stickleback in the presence or absence of a bioluminescing dinoflagellate. The dinoflagellates were cultured in HESNW medium (for details see Harrison et al. 1980) at 17°C. The cultures were grown in an incubator, with half the cultures receiving a 16:8 light : dark photocycle. The other half were grown in a light-proof chamber in the same incubator, with the photoperiod offset by 8 h. Due to the endogenous circadian rhythm of bioluminescence in this species (Sweeney and Hastings 1957), these cultures were not capable of bioluminescing at the same time as the normal culture, and thus served as a control.

Prior to experiments the copepods were sieved through a 300-μm mesh screen, which retained only the adults. These copepods were resuspended in filtered sea water and starved for 24 h prior to beginning these experiments. Cell densities of the control and bioluminescent dinoflagellate cultures were measured with a Coulter Counter and equalized by diluting the culture containing the highest density of cells with filtered sea water.

Experiments were conducted in 20 8-L, round, sterile, glass jars. To each jar was added 400 mL of dinoflagellate culture (10 normal and 10 control jars), 1500 mL of filtered sea water, 150 mL of starved copepod culture at a density of 3.33 copepods/mL, and one juvenile stickleback (average mass: 0.27 g). In order to provide a uniform background for all experiments, each jar was surrounded by a neutral gray cardboard cylinder.

The jars were placed on a bench in a random order. Sticklebacks were the last organism to be added to the jars and were all added in a random order within 5 min. After the last stickleback was placed in the jar, the room was darkened. The experiment began at 1300 and was terminated at 1630. At that time, the sticklebacks were immediately removed from all jars, in the same order in which they had been added. After all sticklebacks were removed, the water in each jar was passed through a 100-μm mesh screen, which retained only the copepods. The number of surviving copepods was then counted.

Results and Discussion

Samples from two control jars were lost due to spillage and were eliminated from statistical analysis. The mortality rate of copepods in the presence of bioluminescing dinoflagellates was 33.4 $\pm$ 10.3 copepod deaths per hours (mean $\pm$ 1 SE), compared to a mortality rate of 19.6 $\pm$ 5.4 deaths per hour in the absence of bioluminescence. When predator size was considered as a covariate, the ability of dinoflagellates to bioluminesce resulted in a significant increase in copepod mortality rates (ANCOVA, $F_{2,15} = 3.95$, $P = .042$). Thus, bioluminescing dinoflagellates significantly increased the foraging efficiency of sticklebacks preying on copepods. This increased mortality rate of the copepods should increase the survival of dinoflagellates.

The copepods had two alternatives in this experiment. In the presence of bioluminescing dinoflagellates, they could continue to feed, or they could cease feeding. If the copepods chose not to feed in the presence of the bioluminescing dinoflagellates, the final density of the bioluminescing culture should have been greater than that of the control culture. Furthermore, even if the copepods did feed during these experiments, dinoflagellate densities should be higher in the bioluminescent cultures since higher mortality rates of copepods should result in a reduced rate of grazing. Unfortunately, the bacterial contamination of the dinoflagellate culture resulting from the addition of the fish prevented us from obtaining accurate measures of the final dinoflagellate density.

The sticklebacks used in this experiment had never been exposed to the experimental conditions. That the sticklebacks were able to use the light emission of the dinoflagellates suggests that they use this cue to increase foraging efficiency in nature (it is unlikely they learned to use this cue given the short duration of these experiments). Furthermore, all sticklebacks survived the experiment, indicating that the strain of *Gonyaulax polyedra* used for these experiments was unlikely to be toxic.

These data demonstrate that bioluminescent dinoflagellates increase the mortality rate of copepods graz-

ing on them, a result consistent with the burglar alarm hypothesis. It should be noted, however, that the burglar alarm is not a mechanism that must operate independently of other proposed functions. Bioluminescence may still provide other benefits to dinoflagellates. Nevertheless, these data do suggest that reduction in feeding efficiency of grazers, as predicted by the burglar alarm hypothesis, may be adaptively significant in maintaining bioluminescence in dinoflagellates.

Bioluminescence emitted by dinoflagellates may not necessarily prevent an individual cell from being consumed. Blooms of dinoflagellates occur during an asexual reproductive phase, so that most cells in a local area should be clones. Even though individuals may perish, reduced grazing (either avoidance by grazers or a reduction in grazers by predators) will allow genetically similar individuals to survive. Thus, bioluminescence in dinoflagellates is probably maintained by kin selection.

The ability to manipulate predator–prey interactions at higher trophic levels as a means of deterring or escaping predation may not be unique to dinoflagellates. Curio (1976) proposed a similar mechanism for the function of fear screams in mammals and birds. He hypothesized that screams are intended to attract other predators (of the screamer or the predator). The ensuing dispute will allow the otherwise doomed prey animal some finite chance of escape. Both Högstedt (1983) and Koenig et al. (1991) have experimentally demonstrated that fear screams in birds induce the rapid approach of secondary predators. Furthermore, Koenig et al. (1991), demonstrated that fear screams in acorn woodpeckers (*Melanerpes formicivorus*) do not attract conspecifics, a result contrary to Rohwer's (1975) "calling for help" hypothesis. In a recent review Dicke et al. (1990) described a similar system involving interactions among plants, herbivorous mites, and predatory mites. In this system, plants that are infested by the herbivorous mite release a chemical that attracts predatory mites. Consequently, plants that release these chemicals are avoided by the herbivorous mites.

Our understanding of predator–prey interactions has advanced dramatically in the last ten years. No longer is it assumed that the only effect of predators on prey populations is consumption. The mere presence of predators can cause substantial modification in the behavior of the prey. Here, we have presented evidence that prey species may be able to exploit predator–prey interactions at higher trophic levels and turn them to their own advantage.

Acknowledgments: We thank M. Healey for providing lab space for these experiments and the staff of the scallop lab at the Pacific Biological Station for assisting with the culture of the dinoflagellates. Helpful comments and criticisms were provided by L. Dill, M. Hay, and two anonymous referees. M.V. Abrahams was supported by a Canada Department of Fisheries and Oceans Visiting Fellowship. Data analysis and preparation of this manuscript were supported by research grants from the University of Manitoba and NSERC to M.V. Abrahams.

Literature Cited

Burkenroad, M. D. 1943. A possible function of bioluminescence. Journal of Marine Research **5**:161–164.

Buskey, E. J., C. G. Mann, and E. Swift. 1986. The shadow response of the estuarine copepod *Acartia tonsa* (Dana). Journal of Experimental Marine Biology and Ecology **103**: 65–75.

Buskey, E. J., C. G. Mann, and E. Swift. 1987. Photophobic responses of calanoid copepods: possible adaptive value. Journal of Plankton Research **9**:857–870.

Curio, E. 1976. The ethology of predation. Springer-Verlag, Berlin, Germany.

Dicke, M., M. W. Sabelis, J. Takabayashi, J. Bruin, and M. A. Posthumus. 1990. Plant strategies of manipulating predator–prey interactions through allelochemicals: prospect for application in pest control. Journal of Chemical Ecology **16**:3091–3118.

Esaias, W. E., and H. C. Curl, Jr. 1972. Effect of dinoflagellate bioluminescence on copepod ingestion rates. Limnology and Oceanography **17**:901–906.

Hamman, J. P., and H. H. Seliger. 1972. The mechanical triggering of bioluminescence in marine dinoflagellates: chemical basis. Journal of Cellular Physiology **80**:397–408.

Harrison, P. J., R. E. Waters, and F. J. R. Taylor. 1980. A broad spectrum artificial medium for coastal and open ocean phytoplankton. Journal of Phycology **16**:28–35.

Högstedt, G. 1983. Adaptation unto death: function of fear screams. American Naturalist **121**:562–570.

Koenig, W. D., M. T. Stanback, P. N. Hooge, and R. L. Mumme. 1991. Distress calls in the Acorn Woodpecker. Condor **93**:637–643.

Morin, J. G. 1983. Coastal bioluminescence, patterns and functions. Bulletin of Marine Science **33**:787–817.

Rohwer, S. 1975. The social significance of avian winter plumage variability. Evolution **29**:593–610.

Schantz, E. J. 1971. The dinoflagellate poisons. Pages 3–25 *in* S. Kadis, A. Ciegler, and S. Ajl, editors. Microbial toxins. Volume 7. Academic Press, New York, New York, USA.

Schradie, J., and C. A. Bliss. 1962. The cultivation and toxicity of *Gonyaulax polyedra*. Lloydia **25**:212–221.

Sweeney, B. M. 1987. Bioluminescence and circadian rhythms. Pages 269–281 *in* F. J. R. Taylor, editor. The biology of dinoflagellates. Blackwell, Oxford, England.

Sweeney, B. M., and J. W. Hastings. 1957. Characteristics of the diurnal rhythm of luminescence in *Gonyaulax polyedra*. Journal of Cellular and Comparative Physiology **49**: 115–128.

Manuscript received 10 January 1992; revised and accepted 7 May 1992.