Does dinoflagellate bioluminescence deter shrimp grazing?
An investigation into the Burglar Alarm Hypothesis

Abstract:

Bioluminescence, the emission of light by living organisms, has evolved independently many times. In most simple organisms, including dinoflagellates, the ecological function of light emission is not obvious. Burkenroad's (1943) Burglar Alarm hypothesis suggests that dinoflagellates benefit from bioluminescence because it increases their primary consumers' conspicuousness to secondary predators. Previous work supports the hypothesis; it has been found that grazing is lower on flashing than non-flashing populations of dinoflagellates and predation on dinoflagellates' consumers increases near flashing dinoflagellates. Yet, no study has explored the grazing behavior of those primary consumers whose conspicuousness has been found to increase in the presence of bioluminescent dinoflagellates. In this study, I measured grazing on the dinoflagellate, *Pyrocystis fusiformis,* by the grass shrimp, *Palaemonetes pugio.* I predicted that *P. pugio* would ingest fewer flashing than non-flashing dinoflagellates because the shrimp's swimming movements stimulate the dinoflagellates' bioluminescence, which puts shrimp at risk of predation. I found *P. pugio* consumed a higher percentage of flashing than non-flashing populations of *P. fusiformis*. My result suggests that, in the absence of secondary predators, bioluminescence increases the likelihood *P. fusiformis* is eaten by making the dinoflagellate more conspicuous to its primary consumers. Thus, when flashing, *P. fusiformis* experiences a tradeoff between increasing predation on its consumers and on itself. To evaluate whether *P. fusiformis's* flashing behavior decreases grazing on the dinoflagellate as outlined in the Burglar Alarm hypothesis, future work should determine whether its increased conspicuousness is offset by a sufficient increase in predation on primary consumers when the dinoflagellate is flashing.

Introduction:

Bioluminescence is the emission of light by living organisms. This phenomenon has been documented across a wide range of mostly marine taxa including bacteria, fungi, protists, and animals (Sverdrup et al. 1942, Hastings 1983, Herring 1987, Widder 2001). The light is produced when an enzyme, a luciferase, catalyses a multi-step reaction between oxygen and a substrate, a luciferin (Hastings 1983, Wilson and Hastings 1998). The chemical structures of luciferins and luciferases vary between organisms because bioluminescence appears to have evolved independently at least 30 times (Hastings 1983, Wilson and Hastings 1998).

The multiple evolutions of bioluminescence in a broad range of organisms have prompted biologists to speculate about what benefits an organism might gain from the trait (Harvey 1956). In some organisms, the advantages are obvious. For example, it is well established that fireflies use bioluminescent flashes to find mates (Lloyd 1983, Lewis et al. 2004). Similarly, it is recognized that animals use bioluminescence to both evade predators and to lure prey (Harvey 1929, Sverdrup et al. 1942, Harvey 1956, Lloyd 1983, Lloyd 1984, Johnsen et al. 1999, Johnsen et al. 2004).

In certain organisms, bioluminescence has less obvious ecological functions. Some argue that the evolution of light emission in organisms ranging from bacteria to jellyfish has evolved by chance as a byproduct of chemical reactions (Harvey 1929) or results from normal activities of the organism (Russell 1936). Investigations into some bioluminescent fungi and bacteria support this hypothesis; there is evidence that the organisms benefit from the chemical reactions associated with producing light and not the light itself. For example, in the white rot fungi, *Panellus stypticus*, bioluminescence comes from the decomposition of

lignin (Lingle 1993). Similarly, in bacteria, bioluminescence has been linked to oxidation reactions needed for respiration (Harvey 1956).

In contrast, an optical function for bioluminescence has been discovered in species once thought to emit light for non-optical purposes. Kozakiewicz et al. (2005) found that the light from bioluminescence stimulates DNA repair in several species of bacteria, perhaps by activating DNA repair enzymes. Light emitted by fungi including *Dictyopanus pusillus* and *Mycena sp*, has been found to attract arthropods that might act as spore dispersers, fertilizers, or predators of animals that consume the fungi (Sivinski 1981). Thus, more organisms may benefit from the light they emit than previously thought.

The selective advantage of bioluminescence in dinoflagellates is still debated. This group of single-celled protists contains numerous bioluminescent species (Biggley, et al. 1969, Widder 2001) whose flashing behavior was once thought to be of "no possible utility" (Sverdrup et al. 1942). In contrast, Burkenroad (1943) proposes that plankton, including dinoflagellates, may use bioluminescence as a defense mechanism. Burkenroad's (1943) Burglar Alarm Hypothesis states that dinoflagellates' bioluminescence may increase their fitness by increasing their primary consumers' conspicuousness to secondary predators. In the presence of secondary predators, this increased conspicuousness would result in a decreased primary consumer population and decreased grazing on the dinoflagellates. The trait would be maintained by kin selection because dinoflagellate blooms are composed of genetically similar individuals produced asexually (Abrahams and Townsend 1993).

The Burglar Alarm Hypothesis has been tested several times with various combinations of crustacean and dinoflagellate species. Copepods consume fewer flashing dinoflagellates than non-flashing dinoflagellates (Esaias and Curl 1972, White 1979;

Appendix). Also, predation on dinoflagellates' primary consumers increases in the presence of flashing dinoflagellates for a number of consumer-predator combinations including predation on copepods by fish (Abrahams and Townsend 1993), on mysids by fish (Mensinger and Case 1992), and on shrimp by cephalopods (Fleisher and Case 1995; Appendix).

Although each of these studies supported Burkenroad's hypothesis, no test has measured the grazing activity of a dinoflagellate consumer whose susceptibility to predation is known to increase in the presence of flashing dinoflagellates. For example, Fleisher and Case (1995) found that light emitted by the dinoflagellate, *Pyrocystis fusiformis*, makes the grass shrimp, *Palaemonetes pugio*, more conspicuous to predators, but whether grass shrimp alter their grazing behavior in response to dinoflagellates' flashing remains untested. In this study, I investigated whether *P. fusiformis's* bioluminescence directly affects *P. pugio's* grazing by measuring the fraction of *P. fusiformis* consumed by *P. pugio* in flashing and non-flashing populations. I predicted that *P. pugio* would ingest fewer flashing than non-flashing dinoflagellates because the shrimp's swimming movements stimulate *P. fusiformis's* bioluminescence, which puts the shrimp at risk of predation (Fleisher and Case 1995).

Methods:

Study organisms

Stock cultures of *P. fusiformis* (Figure 1A) were obtained from Sunnyside Sea Farms (Goleta, California). Dinoflagellates were incubated under a 12/12 light/dark cycle at 21˚C in 32 ppt artificial seawater with a growth medium (alga-Gro, Carolina Biological Supply, Burlington, NC, USA). I obtained *P. pugio* (Figure 1B) individuals from Gulf Specimen

Marine Lab (Panacea, FL, USA) and kept them in a 40-gallon tank at approximately 21˚C in artificial seawater with a salinity of 32 ppt. Shrimp were fed diced mussels approximately once a week.

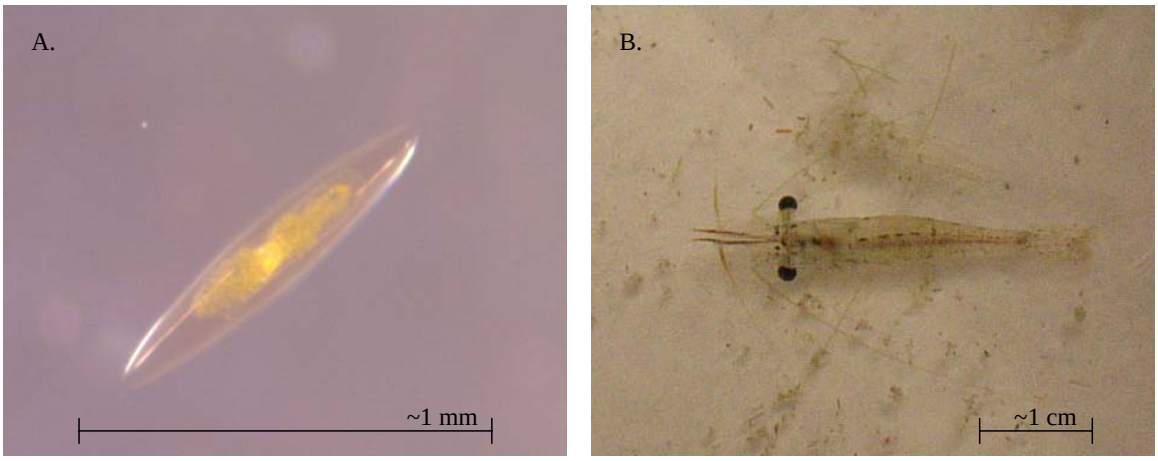

Figure 1: A.) The dinoflagellate, *Pyrocystis fusiformis. B.*) The grass shrimp, *Palaemonetes pugio*.

Determination of palatability of P. fusiformis *to* P. pugio

To determine whether *P. pugio* consumes *P. fusiformis* in darkness, changes in populations of the dinoflagellate with and without the shrimp were compared. Seventy-five ml of seawater was placed in each of ten 250-ml flasks. These flasks were then randomly designated to two treatments: shrimp treatment and shrimp-free control. Two shrimp were placed in each of the five shrimp treatment flasks. No shrimp were placed in control flasks. All flasks were left in the dark for approximately 12 hours to allow shrimp to acclimate to experimental conditions. Fifty ml of seawater containing approximately 140 dinoflagellates/ml were then added to each flask.

After 6 hours under dim light, dinoflagellate density was measured. Each flask was swirled to ensure uniform distribution of dinoflagellates and then a ten milliliter sample was

taken. Dinoflagellates were preserved and stained using a few drops of 1.5% Lugol's iodine solution and sub-sampled into 1 ml aliquots. Using a Sedgwick-Rafter counting cell, the number of dinoflagellates per milliliter was counted for each sub-sample. The mean value of these five sub-samples was calculated to determine the average dinoflagellate concentration.

Manipulation of the circadian cycle of dinoflagellate bioluminescence

P. fusiformis's flashing behavior can be manipulated because its bioluminescence follows a circadian rhythm. When the dinoflagellate is placed in continual darkness, its light production varies cyclically over a 24 hour period with peaks in light production corresponding to night (Sweeney 1982). Using a modified version of Esaias and Curl's (1972) procedure (Figure 2), half of the dinoflagellate cultures had their circadian cycle reversed. First, the entire *P. fusiformis* population was placed under continual light for 5 days to disrupt their circadian rhythm. This arrhythmic population was then diluted to either approximately 120 dinoflagellates/ml, for trial 1, or 150 dinoflagellates/ml, for trial 2. While still in light, 50 ml aliquots of the dilution were placed into beakers and randomly assigned to one of two groups: A or B. Dinoflagellates in group A were then triggered to start a normal circadian cycle by being placed into darkness while dinoflagellates in group B were kept in continual light to maintain their arrhythmic behavior. After 14 hours in darkness, the dinoflagellates in group A nearly ceased flashing due to the circadian rhythm associated with their light production (Sweeney 1982). At this time, group B samples were placed in darkness to prompt flashing so that bioluminescence peaked for group B while group A dinoflagellates barely flashed.

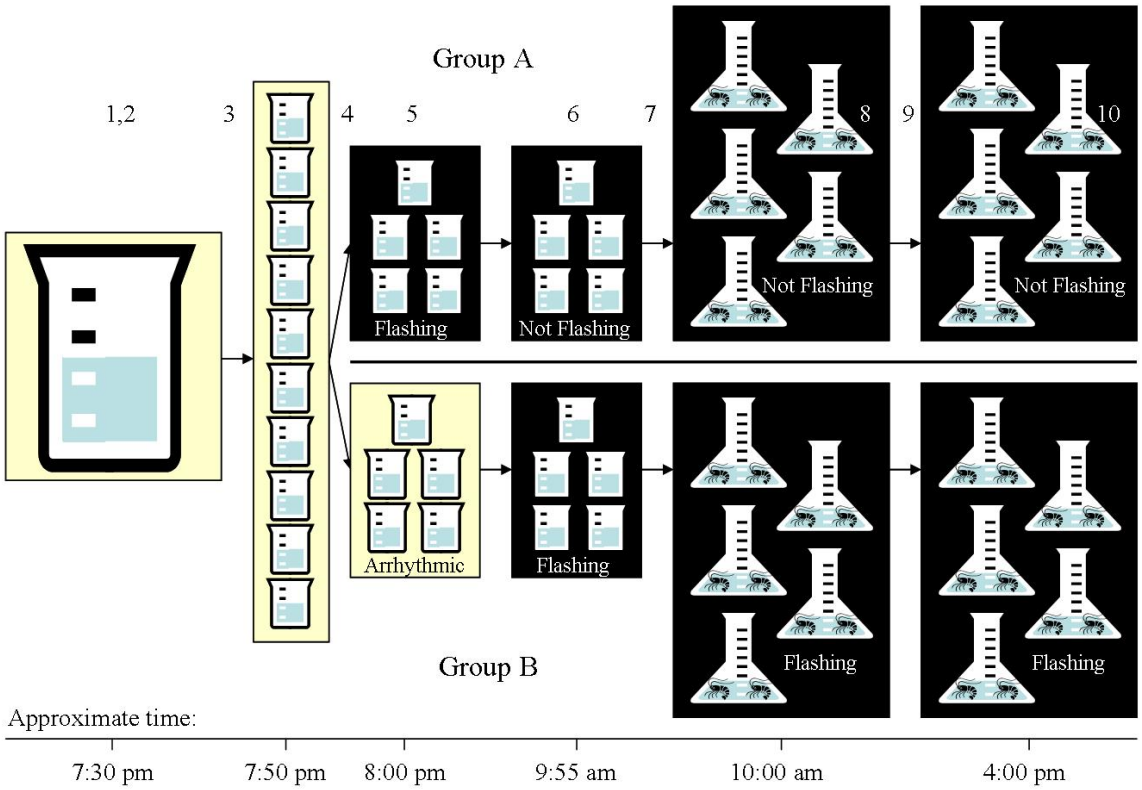

Figure 2: Manipulation of dinoflagellate flashing behavior and shrimp grazing trials. 1.) Maintained dinoflagellates in light for several days. 2.) Diluted to set cell concentration. 3.) Separated aliquots. 4.) Randomly assigned aliquots to groups. 5.) Placed Group A in dark for 14 hours. 6.) Placed Group B in dark. 7.) Added aliquots to flasks with shrimp. 8.) Measured initial dinoflagellate population. 9.) Allowed shrimp to feed for six hours. 10.) Measured final dinoflagellate concentration.

Grazing trials

Within approximately 5 minutes of placing group B in darkness, each aliquot of dinoflagellates was added to a flask containing seawater and two randomly assigned *P. pugio* individuals. Flasks contained 100 ml seawater in trial one and 75 ml seawater in trial 2. This resulted in each flask containing two shrimp, and either 150 ml seawater and 40

dinoflagellates/ml for trial 1 or 125 ml seawater and 60 dinoflagellates/ml for trial 2. These cell densities correspond to *P. fusiformis* population levels equal to and slightly higher than those found to increase *P. pugio's* susceptibility to predation (Fleisher and Case 1995). For the remainder of the trial, dinoflagellates flashing was maximal for group B and minimal for group A. To ensure that the shrimp would eat dinoflagellates, they were starved for at least 2 days and allowed to acclimate to the flasks in dim light for 12 hours before addition of dinoflagellates. Following addition of dinoflagellates, shrimp were allowed to graze for six hours. During trials, each flask was enclosed in an opaque cylinder so that the activity within each flask would not influence another.

Determination of percent dinoflagellates consumed

To control for variation in initial dinoflagellate populations amongst flasks, percent dinoflagellates consumed was used to compare shrimp grazing in each flask. This percentage was calculated by taking the difference between initial and final dinoflagellate densities and then dividing by the initial dinoflagellate density. Initial and final dinoflagellate densities were measured and calculated as describe above.

Statistical analyses

To evaluate whether *P. pugio* eats *P. fusiformis*, Wilcoxon tests were used to determine if average initial dinoflagellate densities differed from average final dinoflagellate densities for each treatment. To determine whether shrimp grazing differed between flashing and non-flashing treatments, Wilcoxon tests were again used to determine if percent dinoflagellates consumed differed between treatments for each trial. All analyses were

conducted using JMP 6.0.0 (SAS Institute Inc. 2005). Throughout mean ± standard deviation is presented and $p < 0.05$ is considered significant.

Results:

In trials to determine whether *P. pugio* eats *P. fusiformis*, dinoflagellate density was initially near 55 dinoflagellate/ml ($\bar{x} = 56.5 \pm 4.8$). In the presence of shrimp, dinoflagellate populations significantly dropped from 56.5 ± 5.9 dinoflagellates/ml to 29.1 ± 5.5 dinoflagellates/ml (Wilcoxon $X^2 = 5.33$, $DF = 1$, $p = 0.02$; Figure 3). In shrimp-free controls, dinoflagellate populations barely decreased from 56.5 ± 4.5 dinoflagellates/ml to 53.3 ± 7.8 dinoflagellates/ml (Wilcoxon test, $X^2 = 0.53$, $DF = 1$, $p = 0.46$; Figure 3).

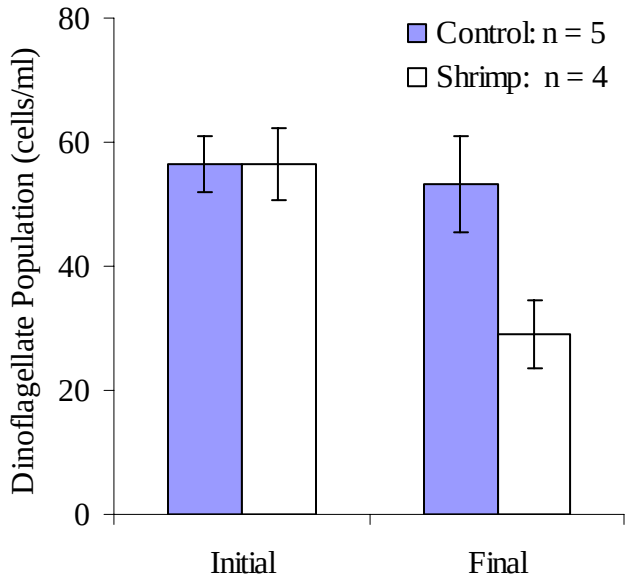

Figure 3. Effects of shrimp (*P. pugio*) and non-shrimp treatments on *P. fusiformis* populations. Mean ± SD population initially and at the end of six-hour trials by treatment.

Trials to determine whether *P. pugio's* grazing differs between flashing and non-flashing *P. fusiformis* populations began with either 40 ($\bar{x}$ = 41.1 $\pm$ 4.9) or 60 ($\bar{x}$ = 58.9 $\pm$ 8.7) dinoflagellate/ml. For trials conducted with initial dinoflagellate density near 40 cells per milliliter, shrimp similar percentages of flashing (54.1 $\pm$ 16.5%) and non-flashing (53.7 $\pm$ 13.5 %) dinoflagellates (Wilcoxon test, X^2 = 0.01, DF = 1, p = 0.92, Figure 4A). For trials conducted with initial dinoflagellate density near 60 cells per milliliter, shrimp consumed significantly more flashing (67.9 $\pm$ 13.5 %) than non-flashing (50.9 $\pm$ 16.0 %) dinoflagellates (Wilcoxon test, X^2 = 6.4, DF = 1, p = 0.01, Figure 4B). The change in initial dinoflagellate density is confounded by the change in light level between trials.

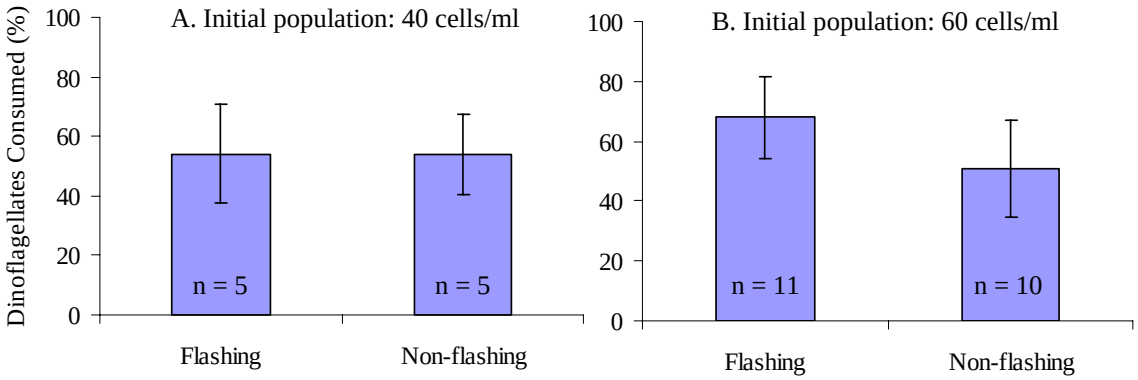

Figure 4: Consumption of flashing and non-flashing *P. fusiformis* populations by *P. pugio*. *P. fusiformis* populations were initially A). approximately 40 cells per milliliter and B). approximately 60 cells per milliliter. Mean percentage consumed $\pm$ SD.

Discussion:

My results confirm that if placed together, *P. pugio* consumes *P. fusiformis* even though the two species are unlikely to encounter each other in nature; *P. fusiformis* is found in the deep-sea (Swift et al. 1976) while *P. pugio*, is found in tidal marshes (Williams 1983, Jayachandran 2001). Dinoflagellate populations decreased significantly in the presence of

shrimp but did not decrease in shrimp-free controls. This result is not surprising; *P. pugio* readily consumes a broad range of foods including phytoplankton (Nixon and Oviatt 1973, Morgan 1980) and is likely to encounter other bioluminescent dinoflagellates, such as *Alexandrium tamarense* and *Lingulodinium polyedrum*, which are found in shallow water and overlap its geographic range (Kelly 1968, Hargraves and Maranda 2002). Thus, *P. pugio* is a useful model consumer for *P. fusiformis* because it is exposed to bioluminescent dinoflagellates in nature and is a predator of *P. fusiformis* in laboratory conditions.

My results did not match my prediction that shrimp would decrease grazing activity in the presence of bioluminescent dinoflagellates to decrease their susceptibility to predation. *P. pugio's* grazing did not differ between flashing and non-flashing *P. fusiformis* populations at 40 cells per milliliter and was significantly higher for flashing than non-flashing *P. fusiformis* populations at 60 cells per milliliter. These results differ from those of two studies that found that copepod grazing decreases in the presence of flashing dinoflagellates in the absence of a secondary predator (Esaias and Curl 1972, White 1979). Although I used methods similar to the two copepod grazing experiments, I used a different primary consumer and far lower dinoflagellate concentrations (the two copepod experiments used initial cell concentrations ranging between 200 and 3500 cells per milliliter). Thus, my results may differ from other studies' because I used more realistic initial cell concentrations that were only moderately above natural populations (Fleisher and Case 1995) which range from 2 to 500 cells per liter (Esaias and Curl 1972, Mensinger and Case 1992).

My results do not confirm that *P. fusiformis's* bioluminescence decreases grazing on the dinoflagellate. Instead, for trials in dim light with initial concentrations of 60 dinoflagellates per milliliter, bioluminescence substantially increased the likelihood that *P.*

fusiformis was consumed by *P. pugio*. Thus, if *P. pugio* and *P. fusiformis* were found together without a secondary predator in nature, bioluminescent behavior would increase the likelihood the dinoflagellate is eaten. Previous work has suggested *P. fusiformis's* bioluminescent behavior may indirectly decrease grazing on the dinoflagellate by making *P. pugio* more susceptible to predation (Fleisher and Case 1995). These contrasting consequences of bioluminescence indicate that, in the presence of *P. pugio* and a secondary predator, flashing *P. fusiformis* populations may experience a tradeoff between exposing themselves and exposing their consumers to increased predation.

This tradeoff does not refute the Burglar Alarm Hypothesis (Burkenroad 1943); it highlights the importance of secondary predators. Without a secondary predator, bioluminescence in *P. fusiformis* should be selected against because it increases the likelihood that the dinoflagellate will be eaten. However, with a secondary predator, bioluminescence may be advantageous to *P. fusiformis* if the increase in its conspicuousness is balanced by a sufficient decrease in primary consumer population. This necessity of a secondary predator to increase *P. fusiformis's* fitness is central to Burkenroad's (1943) hypothesis.

Although my results suggest that a secondary predator is needed to decrease *P. pugio's* grazing on *P. fusiformis*, this study does not provide sufficient evidence to declare that the dinoflagellate's bioluminescence increases its fitness as outlined in the Burglar Alarm Hypothesis (Burkenroad 1943). Instead, my results show the combination of *P. pugio* and *P. fusiformis* would be useful for future investigation. I did find that *P. pugio* ingests *P. fusiformis*. Thus, it is possible to directly measure the effects of *P. fusiformis* ' flashing behaviors in the presence of *P. pugio* and a secondary predator by quantifying grazing rate on

P. fusiformis by *P. pugio*. Future work should determine if increased grazing on *P. pugio* by secondary predators counterbalances *P. fusiformis's* increased conspicuousness and results in an overall decreases in grazing on populations of *P. fusiformis*. Results finding total grazing on *P. fusiformis* to be lower for flashing than non-flashing populations in the presence of *P. pugio* and a secondary predator would confirm bioluminescence is advantageous to the dinoflagellate as outlined in the Burglar Alarm Hypothesis (Burkenroad 1943) and would imply the dinoflagellates' bioluminescence has evolved in response to ecological pressures.

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Appendix:

Table 1. Study organism used in experiments which found copepods graze less on groups of flashing than non-flashing dinoflagellates.

Study	Dinoflagellate	Copepod
Esaias and Curl 1972	*Gonyaulax polyedra*	*Calanus pacificus*
Esaias and Curl 1972	*Gonyaulax acatenella*	*Acartia clausi*
Esaias and Curl 1972	*Gonyaulax acatenella*	*Acartia longiremis*
White 1979	*Gonyaulax excavata*	*Acartia tonsa*

Table 2. Study organisms used in experiments which found dinoflagellates' bioluminescence increases the susceptibility of their primary consumers to secondary predators.

Study	Dinoflagellate	Primary Consumer	Secondary Predator
Fleisher and Case 1995	*Pyrocystis fusiformis*	*Holmesimysis costata*	*Sepia officinalis*
Fleisher and Case 1995	*Pyrocystis fusiformis*	*Palaemonetes pugio*	*Euprymna scolopes*
Abrahams and Townsend 1993	*Gonyalaux polyedra*	*Tigriopus japonicus*	*Gasterosteus aculeatus*
Mensinger and Case 1992	*Pyrocystis fusiformis*	*Holmesumysis costata*	*Porichthys notatus*