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research paper

Intensity differences in bioluminescent dinoflagellates impact foraging efficiency in a nocturnal predator

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ABSTRACT.—Bioluminescence in dinoflagellates is thought to function as a "burglar alarm," alerting visual predators to the presence of dinoflagellate grazers. However, many bioluminescent dinoflagellates, particularly those associated with harmful algal blooms (HABs), have a much lower bioluminescence capacity that seems less well-adapted for attracting the attention of distant secondary predators. The present study was motivated by a question regarding the impact of extreme differences in bioluminescence potential among dinoflagellates, particularly those with the capacity to form HABs. This study examined the function of bioluminescence in the bright emitter, *Pyrocystis noctiluca* (Murray, 1876), compared to the much dimmer HAB species, *Lingulodinium polyedrum* F. Stein (Stein 1883). The foraging efficiency of the nocturnal teleost, *Apagon maculatus* (Poey, 1860), was determined at a range of cell concentrations with both dinoflagellate species. At low cell concentrations of *P. noctiluca*, both the foraging efficiency and the orientation distance of the fish to the prey increased, indicating that bioluminescence functions as a burglar alarm. However, neither fish foraging efficiency nor orientation distance increased in the presence of luminescent *L. polyedrum* at low cell concentrations. At higher concentrations, the bioluminescence of *L. polyedrum* improved the foraging efficiency of the fish, but the orientation distance to the prey was no greater than with non-luminescent cells, indicating that at low cell concentrations, bioluminescence does not function as a burglar alarm in *L. polyedrum*. The role of bioluminescence as a possible aposematic signal in *L. polyedrum* is discussed, along with the implications for the role of bioluminescence in HAB dynamics.

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Dinoflagellates are an ecologically important group of microbial eukaryotes within marine systems. Photosynthetic species contribute to global biogeochemical cycling through their role as primary producers, while toxin-producing species possess the capacity to alter food webs and impact both human and ecosystem health via the bioaccumulation and transfer of toxins through the food web. One of the most intriguing biochemical traits found in some species is that of bioluminescence. While they are the only members of the phytoplankton community with the capacity to emit visible light, they are responsible for much of the bioluminescence produced in the

surface waters of the oceans (Marcinko et al. 2013). The reaction is localized to organelles known as scintillons (DeSa and Hastings 1968), which contain the substrate luciferin and the enzyme luciferase, and in some genera, a luciferin-binding protein (LBP) (Schmitter et al. 1976, Johnson et al. 1985, Nicolas et al. 1991). Mechanical stimulation of the cells creates an action potential that opens membrane channels, allowing the rapid entrance of protons from the acidic vacuole and resulting in a rapid and transient drop in pH within the scintillons. This pH decrease results in enzyme activation, and the oxidation of the luciferin results in a flash of light within the wavelength range of 472–475 nm (Hastings 1983, Widder et al. 1983, Wilson and Hastings 1998).

Key features in the circadian rhythm and biochemistry of bioluminescence have been found to differ among species. In *Lingulodinium polyedrum* (Stein) J. D. Dodge, one of the best-studied species in terms of bioluminescence, cellular levels of both luciferase and LBP fluctuate in a circadian manner. However, in others such as *Pyrocystis* sp., the daily levels of luciferase remain constant and there is no LBP. This is in contrast to *L. polyedrum*, in which LBP has been shown to be a necessary component in the biochemical reaction (Knaust et al. 1998). Analysis of the full-length luciferase from multiple species of dinoflagellates has shown that all possess a single polypeptide comprised of an N-terminal region of unknown function followed by three homologous domains, each possessing catalytic activity. Differences exist among gene structure and organization such as a dramatic reduction in synonymous substitution rates in some species, including *L. polyedrum*, while *Pyrocystis* sp. exhibit a higher rate of synonymous substitutions that are uniform along the domains (Liu et al. 2004).

In addition to biochemical and genomic differences among species, there exists within a single species both bioluminescent and non-bioluminescent strains (Marcinko et al. 2013). This suggests a strong selective advantage despite the energetic costs of light production and prompts the question as to the evolutionary role of bioluminescence in dinoflagellates. Overall, bioluminescence is believed to function as a survival strategy in dinoflagellates (Hackett et al. 2004). Early experimental studies showed that nocturnal grazing by copepods was lower in the presence of highly bioluminescent dinoflagellates than in the presence of those with a reduced capacity for luminescence (Esaias and Curl 1972, White 1979). Based on these findings it was suggested that bioluminescence served to startle zooplankton predators, thus allowing the dinoflagellates to escape (Esaias and Curl 1972).

Bioluminescence has also been suggested to function as a "burglar alarm," in which the flashes serve to alert visual predators to the presence of dinoflagellate grazers (Burkenroad 1943). In support of this hypothesis are experiments demonstrating that copepod feeding currents stimulate dinoflagellates to flash, and flashes stimulate swimming bursts in marine copepods, thereby limiting the time available for grazing (Buskey et al. 1983, Buskey and Swift 1983, 1985). By contrast, flash-induced burst swimming does not occur in copepods from freshwater environments, where planktonic bioluminescence is absent, so this behavior cannot be a universal startle response (Buskey et al. 1987). Rather, flash-induced burst swimming is apparently a means of evading visual predators alerted to the presence of the grazers by the dinoflagellate flash. Several laboratory studies have further substantiated the multi-trophic basis for this theory by showing an increased susceptibility

of zooplankton to visual predators in the presence of bioluminescent dinoflagellates (Mensinger and Case 1992, Abrahams and Townsend 1993, Fleisher and Case 1995).

The present study was motivated by a question regarding the possible impact of the extreme differences in bioluminescence potential among dinoflagellates on the foraging behavior of higher order predators. This question is significant because all of the small, armored dim light emitters used in behavioral experiments to date (Table 1) have been identified as harmful algal bloom (HAB) species (Plumley 1997, Smayda 1997). Because experiments have demonstrated that dinoflagellate bioluminescence reduces grazing (Esaias and Curl 1972, White 1979, Buskey et al. 1983), it is important to characterize how this may impact bloom dynamics. While the experimental evidence to date suggests that the burglar alarm defense might help maintain the bloom by causing increased predation on grazers, there is still the question of whether bioluminescence plays any role in initiating the bloom.

The present study utilized the dinoflagellates *Pyrocystis noctiluca* Murray ex Haeckel and *L. polyedrum*. Differences exist between these species in regards to several parameters of bioluminescence. *Pyrocystis noctiluca* is a large (200 to >350 μm) unarmored photosynthetic cell that is one of the brightest of all bioluminescent dinoflagellates, emitting 3.7×10^{10} to 6.5×10^{11} photons cell^{-1} (Swift et al. 1973, Widder et al. 1993). *Lingulodinium polyedrum* is a small (20–30 μm) armored photosynthetic cell that emits light at approximately the same wavelength as *P. noctiluca* [472–474 nm (Widder et al. 1983)], but is >1000 times dimmer, emitting between 3.1×10^{7} and 1.2×10^{8} photons cell^{-1} (Seliger et al. 1969, Swift et al. 1973, Widder et al. 1993). Also noteworthy in comparing the luminescent capacity of these two dinoflagellates is that, under the same conditions, *P. noctiluca* flashes 10 or more times in response to prolonged mechanical stimulation while *L. polyedrum* generally flashes only once (Widder et al. 1993).

One other fundamental difference between *P. noctiluca* and *L. polyedrum* is the potential toxicity of the latter. *Lingulodinium polyedrum* is often referred to as a "red tide" dinoflagellate due to the formation of massive blooms off the coast of California over the past several decades (Allen 1943, Kudela and Cochlan 2000, Omand et al. 2011). It is also a confirmed source of diaherretic shellfish poisoning (DSP) toxins, as both natural populations and clonal isolates have been shown to produce yessotoxin and homoyessotoxin (Draisci et al. 1999, Paz et al. 2004, Armstrong and Kudela 2006, Howard et al. 2009).

Our goal in the present study was to assess whether the burglar alarm defense was effective for dim light emitters at low cell concentrations. To accomplish this, we conducted a series of behavioral experiments that measured the foraging efficiency of the nocturnal reef fish, *Apogon maculatus* (Poey, 1860), on the grass shrimp *Palaemonetes pugio* Holthuis, 1949, in the presence of *P. noctiluca* or *L. polyedrum*. *Palaemonetes pugio*, whose diet includes a range of phytoplankton, typically is found in tidal habitats and thus is likely to encounter bioluminescent dinoflagellates such as *Alexandrium tamarense* (Lebour, 1925) Balech, 1992 and *Lingulodinium polyedrum*, which overlap its geographic range (Kelly 1968, Nixon and Oviatt 1973, Morgan 1980, Hargraves and Maranda 2002). Additionally, *P. pugio* had previously been found to feed on *Pyrocystis* spp. (Fink 2007). Thus, *P. pugio* is a useful model consumer for *Pyrocystis fusiformis* (W. Thomson, 1876) Murray, 1885 because it is exposed to bioluminescent dinoflagellates in nature and is a predator of *P. fusiformis* in laboratory conditions. The natural environment of *A. maculatus* is in the vicinity

of coral reefs, where it can be found in a variety of habitats at depths ranging from approximately 1 to 24 m. Habitats this species is known to occupy include the tidepool/strand area, shallow sand, grass (both shallow and deep), mangrove, shallow forereef, and the patch reef itself (Smith 1997). As *A. maculatus*' feeding schedule coincides with the luminescent phase of dinoflagellates, they are exposed to this phenomenon on a nightly basis.

Methods

Organism Maintenance

Unialgal cultures of *P. noctiluca* were maintained at 20 °C on a 12-hr reverse light:dark cycle. Cells were grown in 2.8-L Erlenmeyer flasks at a volume of 2000 ml in sterilized, filtered seawater enriched with *f/2* medium (Guillard 1975). During day-phase, cells were illuminated from above with fluorescent lights at an irradiance of 25 $\mu E\ m^{-2}\ s^{-1}$. Initial inoculations of *L. polyedrum* were done using an *L1* medium (Guillard and Hargraves 1993) in 250-ml Erlenmeyer flasks at a volume of 200 ml in sterilized, aged seawater. Unialgal cultures were maintained at 15 °C on a 12-hr reverse light:dark cycle. During day-phase, cells were illuminated from above with fluorescent light at an irradiance of 100 $\mu E\ m^{-2}\ s^{-1}$. Once a culture reached a concentration of 300 cells ml^{-1} in a volume of 1000 ml, an *f/2* medium was employed. For all behavioral experiments, two sets of cultures were maintained for each dinoflagellates species: one set on a 12-hr light:dark cycle, and one on a 12-hr reverse light:dark cycle. This allowed for all experiments to be conducted during the night-phase cycle of the fish. Cell counts were performed on all cultures a minimum of once per week using a Sedgewick-Rafter counting cell (volume of 1 ml) to track cell growth. Cell counts were also performed the day before an experiment, and the appropriate cell concentration was transferred from the original culture into an Erlenmeyer beaker for use the following day.

Grass shrimp, *P. pugio* [mean length = 2.52 (SD 0.37) cm] were caught from saltwater retention ponds on the Harbor Branch Oceanographic Institution campus and maintained in a tank containing filtered seawater until needed.

Flame cardinalfish, *A. maculatus* [mean length = 7.10 (SD 0.89) cm], were obtained from Dynasty Marine Associates, Inc. (Marathon, FL). Fish were maintained in filtered seawater with a maximum of three fish per 110-L tank, which was subdivided to provide each fish with its own area. Tanks were maintained at an average temperature of 26 °C and pH of 8.0. Salinity was kept between 35 and 37. Fish were kept on a 14:10 reverse light:dark cycle and fed 3–4 live grass shrimp during the night-phase portion of their cycle. All experiments were conducted during night-phase. Individual fish were transferred from the home tank to the experimental tank via a light-tight 2-L plastic container filled with filtered seawater.

Light Measurements

Two bioluminescence parameters were measured for both dinoflagellate species: the peak photon flux per cell and the average total stimulated light (TSL). Cell counts were conducted and samples prepared 1 d prior to light measurements. A sample consisted of 5 ml of culture and 10 ml of seawater loaded into a 20-ml vial. The vial was placed in an integrating sphere (Labsphere) fitted with a photomultiplier tube (Hamamtsu HC124-06) and calibrated with an NIST referenced standard (Optronic

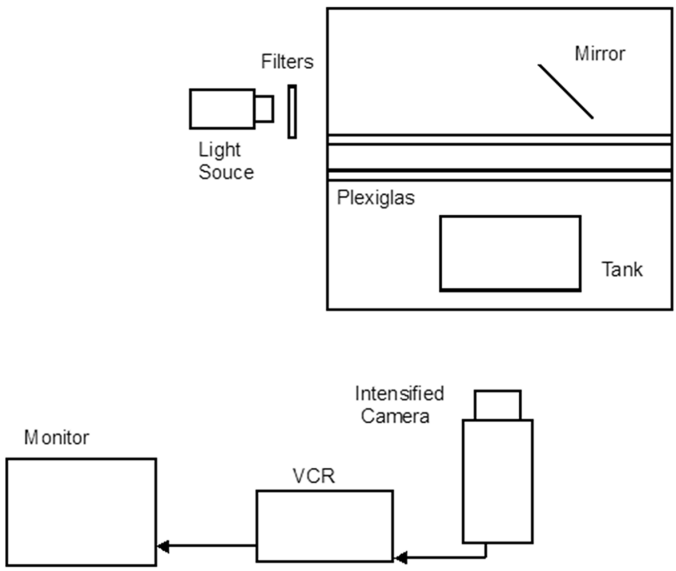

Figure 1. Overhead schematic of darkroom design and video set-up used to record fish behavior and bioluminescence in feeding experiments. Experimental tank illuminated from behind with infrared light. Events of trials captured with intensified-intensified charge-coupled device (I^2CCD) camera and recorded on Hi-8 video recorder. Monitor located outside darkroom for simultaneous viewing of trial events.

Laboratories 310 multi-filter calibration source). Bioluminescence was stimulated to exhaustion with a DC motor-driven stirring rod.

Video Setup

All experiments were conducted in a darkroom and recorded under infrared illumination. Two types of video systems were employed, one for each type of experiment, which allowed for real-time monitoring of fish behavior and dinoflagellate bioluminescence.

In the video setup used to record capture success experiments, the experimental tank was illuminated from behind using a slide projector (500 W incandescent bulb) with 2 Kodak IR filters (Wratten No. 89B) and a neutral density filter (Tiffen 0.6). The light was projected onto a mirror situated at a diagonal behind the tank and further diffused through white Plexiglas. The events of the trials were monitored with an intensified camera (I^2CCD) (Intevac GenIII Nitemate 1305/1306 with a CCTV intensifier) (Fig. 1).

In the video setup used to record distance experiments, the experimental tank was illuminated from behind using a 7.5 W incandescent light source filtered through two Kodak IR filters (Wratten 89B) and diffused through white Plexiglas. A mirror (14.6 × 22.2 cm) was situated at a 45° angle from the top rear rim of the tank to give a three-dimensional view of the position of the fish relative to the shrimp. The tank and mirror were also illuminated from below using a slide projector with a 500 W incandescent bulb filtered through a Kodak IR filter (Wratten 89B) and white Plexiglas. The video system in these trials was based on the design of Widder (1992). Briefly, an intensified camera (Dage-MTI ISIT 66) that recorded bioluminescence was synced to an infrared sensitive camera (Dage-MTI SC-68 with an IR Ultricon

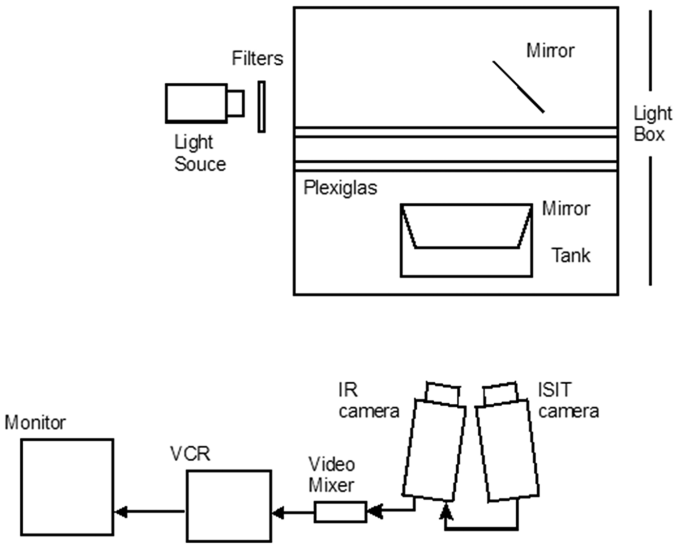

Figure 2. Overhead schematic of darkroom design and video set-up used in distance experiments. Experimental tank illuminated from behind and below with infrared light. Mirror positioned above tank used to measure coordinates (length, height, width) of distance formula in determining fish orientation distance. Video system consisted of an intensified camera synced to an infrared camera with the two signals fed through a video mixer. Events of trials stored on Hi-8 video recorder. Monitor located outside of darkroom for simultaneous viewing of trial events.

tube) that simultaneously recorded the actions of the organisms in the chamber and the two signals fed through a video mixer (Primebridge MicroSeries PVM-1) (Fig. 2). For both video setups, the events of the experiments were projected onto a Panasonic monitor outside the darkroom while simultaneously recorded on a Sony Hi-8 video recorder for later viewing.

Experimental Protocol

Two types of experiments were conducted: (1) those that examined the foraging efficiency of the fish ("capture success" experiments), and (2) those that measured the orientation distance of the fish to the prey prior to the first capture attempt ("distance" experiments). For each type of experiment, five to seven trials each were conducted with each species in both night-phase (luminescent) and day-phase (non-luminescent) at each cell concentration (5, 10, 20, 40, and for *L. polyedrum* 500 cells ml^{-1}). For example, five capture success trials were conducted with *L. polyedrum* at a concentration of 5 cells ml^{-1} with cells in night-phase, and five trials at a concentration of 5 cells ml^{-1} with day-phase cells. One fish participated in a maximum of four trials, only two of which contained cells in night-phase. Conditioning trials, described in the Online Appendices, were conducted prior to the beginning of the study to examine the possibility of the fish becoming conditioned to the bioluminescence of the cells and the experimental conditions.

Capture Success Experiment.—Trials were conducted with *P. noctiluca* and *L. polyedrum* at concentrations of 5, 10, 20, and 40 cells ml^{-1}, with cells in night-phase or day-phase. As *L. polyedrum* is a red-tide dinoflagellate and at times occurs in bloom numbers, trials were also conducted with this species at a concentration of

500 cells ml^{-1}. Two trials were also conducted in which the cells were filtered from the culture and only the media (equivalent to a concentration of 500 cells ml^{-1}) was added to the experimental tank. Control trials were also conducted with no dinoflagellate cells in the experimental tank.

The experimental chamber consisted of a 5-L tank. Upon placement into the experimental tank from the transfer container, fish were given an acclimation period of 1 hr. The times of cell addition to the experimental tank differed based on cell species and cell phase to assure maximum bioluminescence potential in night phase cells and minimum bioluminescence potential in day phase cells. For night-phase trials using *P. noctiluca*, the cell culture was added 15 min before the start of the trial. Due to the fact that *L. polyedrum* needs a longer recovery time after stimulation, *L. polyedrum* night-phase cell cultures were added 30 min before the start of the trial. In day-phase trials, both *P. noctiluca* and *L. polyedrum* were added 5 min prior to trial initiation to reduce the possibility of the cells beginning to luminesce. Both cell species were introduced gently into the tank, with care taken to minimize mechanical stimulation of night-phase cells. Ten grass shrimp were gently introduced to the experimental tank following the 1-hr fish acclimation period and the trials proceeded for 30 min. For each trial, the number of shrimp consumed and the capture success of *A. maculatus* were recorded. Capture success was calculated based on the number of shrimp consumed by the fish divided by the number of strike attempts recorded as a percentage.

Distance Experiments.—The primary goal of the distance trials was to measure the initial orientation distance of the fish to the prey in the presence and absence of bioluminescent dinoflagellates. Trials were conducted at a concentration of 40 cells ml^{-1}, with fish and dinoflagellate cells introduced to the experimental tank as described above. Following fish acclimation, a single shrimp was added to the chamber, and the trial proceeded for 8 min or until the fish consumed the shrimp. Two behavioral parameters were recorded from each distance trial: orientation distance and capture success. Orientation distance was defined as the final alignment of the fish's body before a strike at the prey was initiated. This parameter was calculated using the distance formula $\sqrt{(x^2 + y^2 + z^2)}$, where x = length, y = height, and z = width of the distance between the fish and the prey. As the input of the individual sensory systems of the fish were not known, the value obtained for each coordinate was the shortest distance between the two organisms—i.e., there was not a specific body part that served as the constant point of reference. Distances were obtained by freezing the display screen and measuring the three variables. When recording capture success, only the first strike of *A. maculatus* was counted, as subsequent strikes toward the single prey may have involved some degree of learning or may have been influenced by sensory systems other than vision, such as lateral line or olfaction. This produced a capture success of either 0% or 100%.

Statistical Analyses.—For all experiments, data were analyzed for statistical significance between day- and night-phase experiments at each cell concentration with the two-sample *t*-test or paired two-sample *t*-test if data passed the tests for normality (Kolmogorov-Smirnov) and homogeneity of variance (F_{max}) (Zar 1996). A single-factor ANOVA was applied to data comparing day-phase cells and no cells.

Results

Light Measurements

The mean peak photon flux per cell for *P. noctiluca* was 3.01×10^{12} photons s^{-1} (SE 9.0×10^{10}), and the mean total stimulated light (TSL) was 1.48×10^{12} photons (SE 5.73×10^{11}). For *L. polyedrum,* the mean peak photon flux was 8.30×10^{10} photons s^{-1} (SE 2.40×10^{10}) and the mean TSL per cell was 1.17×10^{7} photons (SE 3.38×10^{6}).

Capture Success Experiments

There was no significant difference in the number of prey consumed by *A. maculatus* at any cell concentration between trials conducted with day- and night-phase *P. noctiluca* (two-sample *t*-test assuming equal variance). The mean number of shrimp consumed at each concentration, regardless of cell phase, ranged between 3 and 5. There also was no significant difference in the number of shrimp consumed by the fish between day-and night-phase trials utilizing *L. polyedrum* at any cell concentration (paired two-sample *t*-test for means; two-sample *t*-test assuming equal variances at 40 and 500 cells ml^{-1}) ranging between approximately 2 and 3 shrimp per treatment type (data not shown). Additionally, there was no significant difference in shrimp consumption between either day-phase *P. noctiluca* trials and trials conducted without cells (single-factor ANOVA: $F_{(4,22)} = 2.69$, $P = 0.06$) or *L. polyedrum* day-phase trials and trials conducted without cells ($F_{(4,25)} = 0.40$, $P = 0.80$).

Capture success, defined as the percentage of successful prey captures relative to the number of attempts, was significantly higher at all concentrations when *A. maculatus* was in the presence of night-phase *P. noctiluca* cells (two-sample *t*-test assuming equal variances at each concentration; 5 cells ml^{-1}, $P < 0.001$; 10 cells ml^{-1}, $P < 0.05$; 20 cells ml^{-1}, $P < 0.05$; 40 cells ml^{-1}, $P < 0.01$). The greatest capture success occurred at a concentration of 5 cells ml^{-1}, with an average of 93% of strike attempts resulting in prey capture. Capture success in night-phase trials ranged between 83% and 93%, whereas day-phase values ranged between 42% and 52% (Fig. 3A). There was no significant difference in the capture success between day-phase *P. noctiluca* trials and trials that did not contain cells (single-factor ANOVA: $F_{(4,22)} = 2.69$, $P = 0.22$) (Fig. 3A).

The capture success of *A. maculatus* did not differ significantly between day- and night-phase trials conducted with *L. polyedrum* at concentrations of 5, 10, and 20 cells ml^{-1} (paired two-sample *t*-test for means). Capture success was significantly higher in the presence of night-phase *L. polyedrum* at concentrations of 40 cells ml^{-1} (two-sample *t*-test assuming equal variances: $P = 0.001$) and 500 cells ml^{-1} ($P < 0.05$) in comparison to day-phase *L. polyedrum*. Capture success increased to approximately 87% at a concentration of 500 cells ml^{-1} (night-phase), compared to approximately 62% at all other concentrations of night-phase *L .polyedrum* (Fig. 3B). There was also no significant difference in capture success between trials conducted with day-phase *L. polyedrum* and trials that did not contain cells (single-factor ANOVA: $F_{(4,25)} = 0.40$, $P = 0.80$) (Fig. 3B). Additionally, in the two trials conducted with the media from *L. polyedrum* cultures, the results of both prey consumption (two to three shrimp) and capture success (about 33%) were comparable to trials conducted with day-phase cells and no cells (data not shown).

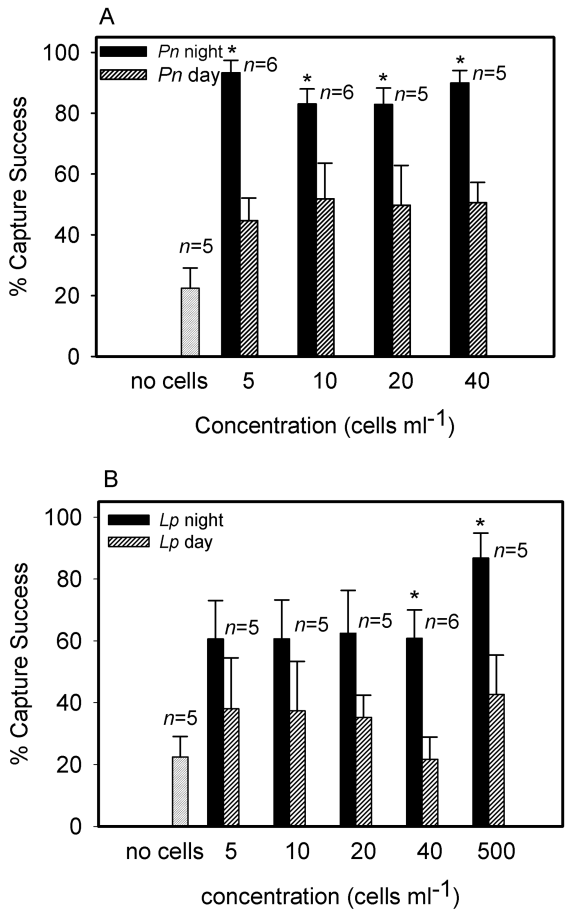

Figure 3. Average percent capture success (number of shrimp consumed by fish divided by the number of capture attempts) of *A. maculatus* during feeding trials conducted with day- and night-phase *P. noctiluca* (*Pn*, A) or *L. polyedrum* (*Lp*, B). Trials were conducted with cell concentrations of 5, 10, 20, and 40 cells ml^{-1} for both species, and an additional concentration of 500 cells ml^{-1} with *L. polyedrum*. For each cell concentration, an equal number of trials were conducted using cells in day-phase and cells in night-phase, as denoted by *n* above the bars of each concentration. * denotes significant difference between pairs (two-sample *t*-test assuming equal variances: $P < 0.05$). Error bars represent standard error of the mean.

Distance Experiment

All trials that examined the distance at which fish oriented to the prey were conducted at a concentration of 40 cells ml^{-1} for both dinoflagellate species. The average distance at which fish oriented toward the prey before initiating the first attempt at prey capture was significantly greater in trials conducted with night-phase *P. noctiluca* (5.64 cm) than when cells were in day-phase (0.98 cm) (two-sample *t*-test: $P < 0.05$) (Fig. 4A). The orientation distance of *A. maculatus* did not differ significantly between day- and night-phase trials with *L. polyedrum*, with a mean distance of 1.50 cm in night-phase trails and 1.02 cm in day-phase trials (two-sample *t*-test assuming unequal variances) (Fig. 4A). Additionally, the orientation distance of *A. maculatus* was significantly greater in trials conducted with night-phase *P. noctiluca* than in trials conducted with night-phase *L. polyedrum* (two-sample *t*-test: $P < 0.05$).

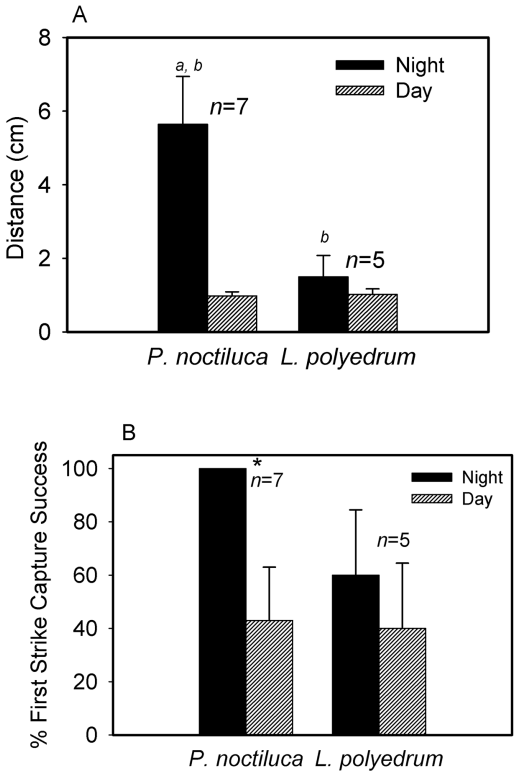

Figure 4. (A) Average orientation distance of *A. maculatus* in distance trials conducted with day- and night-phase *P. noctiluca* or *L. polyedrum* at a concentration of 40 cells ml^{-1}. Orientation distance was calculated using the distance formula, where length = x, height = y, and width = z of the distance between the fish and the shrimp. n denotes individual number of trials conducted with cells in day-phase and cells in night-phase for each dinoflagellate species. ^{a} indicates significant difference (two-sample t-test assuming unequal variances, $P < 0.05$) between day- and night-phase trials of *P. noctiluca*, ^{b} indicates significant difference between night-phase trials of *P. noctiluca* and *L. polyedrum* (two-sample t-test assuming equal variances: $P < 0.05$). (B) Average percent capture success based on first strike of *A. maculatus* in distance trials conducted with day- and night-phase *P. noctiluca* or *L. polyedrum* at a concentration of 40 cells ml^{-1}. n denotes individual number of trials conducted with cells in day-phase and cells in night-phase for each dinoflagellate species. * indicates significant difference (two-sample t-test assuming unequal variance: $P < 0.05$).

The capture success of *A. maculatus* based on the first strike was also examined at a concentration of 40 cells ml^{-1}. Capture success was significantly greater in the presence of night-phase *P. noctiluca* (two-sample t-test assuming unequal variance: $P < 0.05$) in comparison to day-phase, 100% to approximately 43%, respectively (Fig. 4B). There was no significant difference in capture success between day- and night-phase *L. polyedrum* trials based on the first strike of *A. maculatus* (paired two-sample t-test for means). The average capture success in trials conducted with cells in day-phase was 40%, comparable to the results of day-phase trials utilizing *P. noctiluca*, while the average capture success of the fish when in the presence of night-phase *L. polyedrum* was 60% (Fig. 4B).

Table 1. Bioluminescence capacity and cell concentrations used in previous studies with dinoflagellates.

Dinoflagellate	Photons per cell	Cells cm^{-3}	Reference
Alexandrium tamarense	2×10^4 to 4×10^6	500–3,500	White 1979
Alexandrium tamarense	2×10^5 to 2×10^7	330–7,870	Buskey et al. 1983
Lingulodinium polyedrum	4×10^7	600	Esaias and Curl 1972
Alexandrium catenella	7×10^7	200–2,300	Esaias and Curl 1972
Alexandrium acatenella	3×10^7	200–1,800	Esaias and Curl 1972
Pyrocystis fusiformis	$2–6 \times 10^{10}$ [a]	1–30 [b]	Swift et al. 1973, Mensinger and Case 1992
Pyrocystis fusiformis	10^{10}	1–20	Fleisher and Case 1995

[a] Swift et al. 1973
[b] Mensinger and Case 1992

Discussion

The present study was motivated by a question regarding the impact of the extreme differences in bioluminescence potential among dinoflagellates as to the ecological function of this trait among the bioluminescent species. Previous studies investigating the use of bioluminescence as a burglar alarm have used both "bright" (i.e., *P. fusiformis*) and "dim" (i.e., *Alexandrium* and *Lingulodinium* spp.) light emitters (Table 1 and references therein). However, cell concentrations of the dim light emitters were 100–1000 times greater than those used in experiments with bright emitters. As *P. fusiformis* produces at least 1000 times more light per cell than *Alexandrium* and *Lingulodinium* spp., these differences in cell concentrations have made direct comparisons based on bioluminescence potential impossible. Therefore, we compared the foraging efficiency of a teleost predator using the same range of cell concentrations with both a bright (*P. noctiluca*) and dim (*L. polyedrum*) emitter. These comparisons dictated an experimental design that utilized combinations of species that do not all typically occur together in natural plankton communities: based on its tidal habitat range, the grass shrimp *P. pugio* is more likely to occur with *L. polyedrum*, while *A. maculatus* most likely occurs with *Pyrocystis* spp. However, *A. maculatus* was shown to consume grass shrimp, and previous studies have established the predation of *P. pugio* on *Pyrocystis* (Frank 2007). The results of our study demonstrate that the bioluminescence produced by *P. noctiluca* aided in the foraging efficiency of *A. maculatus* at both low and high cell concentrations. However, the dimmer flash of *L. polyedrum* did not enhance the foraging capabilities of *A. maculatus* at low cell concentrations, and only became effective at higher cell concentrations. Additionally, the flashes produced by *P. noctiluca*, but not *L. polyedrum*, allowed *A. maculatus* to detect the prey at a greater distance, as evidenced by the significant increase in orientation distance.

The results of the present study indicate that bioluminescence functions as a burglar alarm in bright emitting dinoflagellates throughout a range of cell concentrations, but only above a certain threshold in dim emitters. The question our study raises is whether or not dim emitters like *L. polyedrum* have any effect on grazers at low cell concentrations—and if so, by what mechanism. Since so many of the dim light emitters among the dinoflagellates, including *L. polyedrum*, are toxic, the possibility arises that bioluminescence in these species functions as an aposematic signal. These different functions for bioluminescence are further supported when one considers the variations in molecular biology and cell physiology between dim and bright emitters. Multiple *Alexandrium* spp, *L. polyedrum*, and *Protoceratium reticulatum* (Claparède & Lachmann) Butschli, 1885, which can be considered dim emitters, are all toxic and possess the luciferin binding protein (LBP), which through its

sequestration of the luciferin substrate controls the flash (Liu et al. 2004). *Pyrocystis noctiluca*, *P. fusiformis*, and *Pyrocystis lunula* (J. Schütt) J. Schütt, 1896, all of which can be classified as bright emitters, are not toxic and do not possess a LBP (Liu et al. 2004). Additionally, cellular levels of both luciferase and LBP fluctuate in a circadian manner in *L. polyedrum*, while in *Pyrocystis* spp., the daily levels of luciferase remain constant and there is no LBP (Knaust et al. 1998).

No systematic phylogenetic analysis currently exists between bioluminescence and toxicity in dinoflagellates. At present, 68 species are classified as bioluminescent (Marcinko et al. 2013). Of these 68 species, a literature search revealed information on toxin (or lack of) production in 45 of them. Of these 45 species, 12 are toxic. *Alexandrium* spp. and the closely-related *Pyrodinium bahamense* Plate, 1906 are the primary genera that are both toxic and bioluminescent, along with several *Gonyaulax* and a *Protoceratium* species (Online Table S1). In general, most of the dim emitters are small cells (*L. polyedrum*, approximately 20–25 μm, *Alexandrium*, approximately 25–50 μm, *P. bahamense*, approximately 40–50 μm), while the bright emitters are large (greater than 80 microns). Therefore, the difference in flash intensity may be explained by "Seliger's rule," which proposes a general relationship between bioluminescence potential and surface area of dinoflagellate cells based on a constant ratio of TMSL to cell surface area of about 10^{11} photons mm^{-2} (Buskey et al. 1992). Ultimately, while bioluminescence potential may be attributable to cell size, its function among dinoflagellates likely differs when taking into account the differences in molecular biology (i.e., LBP) and biochemistry (i.e., toxin production) between dim and bright emitters.

Based on the inverse square law and the peak flash intensity that we recorded in *P. noctiluca* (3.01×10^{12} photons s^{-1}) compared to *L. polyedrum* (8.30×10^{10} photons s^{-1}), one would predict that the peak of a single flash from *P. noctiluca* would be visible at six times the distance of a single flash from *L. polyedrum*. Examining the habitats and physiologies of these two species used in this study suggests that *P. noctiluca*, a non-toxic species typically found at low cell concentrations in the open ocean, evolved a more intense, longer duration flash that allows for detection over greater distances. Thus, bioluminescence is likely to function as a burglar alarm in this species. Although the flash of *L. polyedrum* and other toxic dinoflagellates does not appear to be bright enough to function as a burglar alarm at low cell concentrations, it may signal unpalatability to grazers, leading to rejection of the luminescent cells, thereby conferring a direct selective advantage on individual cells. There are several well-known instances where bioluminescence appears to function as an aposematic warning of unpalatability, as in firefly larvae (Underwood et al. 1997), railroad worms (Sivinski 1981), nocturnal millipedes (Marek et al. 2011), and echinoderms (Grober 1988). Bioluminescence in dinoflagellates may therefore function in conjunction with toxin production at low cell concentrations, to deter predation by primary predators and thereby aid in the initiation of a bloom. In this type of situation, it would not be beneficial to the HAB species as a population to remove the zooplankton predators, thereby relieving predation pressure on not only the bioluminescent species, but other phytoplankton competitors as well. The results of numerous behavioral studies conducted with toxic dinoflagellates and their zooplankton predators have led to the theory that the toxins function as a grazing deterrent (Cusick and Sayler 2013). Because of the proximity of the grazer to its prey, there is no need for the warning signal to be broadcast over any distance, as is required for a burglar alarm; therefore,

a single dim flash would be more energetically efficient than a bright one. Evidence that the bioluminescence of dim emitters at low cell concentrations functions aposematically will require a demonstration that grazers reject such cells in response to their flash.

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Literature Cited

Abrahams MV, Townsend LD. 1993. Bioluminescence in dinoflagellates: a test of the burglar alarm hypothesis. Ecology. 74:258–260. http://dx.doi.org/10.2307/1939521

Allen WE. 1943. Red Water in La Jolla Bay in 1942. Trans Amer Microsc Soc. 62:262–264. http://dx.doi.org/10.2307/3223029

Armstrong M, Kudela R. 2006. Evaluation of California isolates of *Lingulodinium polyedrum* for the production of yessotoxin. Afr J Mar Sci. 28:399–401. http://dx.doi.org/10.2989/18142320609504186

Burkenroad MD. 1943. A possible function of bioluminescence. J Mar Res. 5:161–164.

Buskey E, Mills L, Swift E. 1983. The effects of dinoflagellate bioluminescence on the swimming behavior of a marine copepod. Limnol Oceanogr. 28:575–579. http://dx.doi.org/10.4319/lo.1983.28.3.0575

Buskey E, Mann CG, Swift E. 1987. Photophobic responses of calanoid copepods: possible adaptive value. J Plank Res. 9:857–870. http://dx.doi.org/10.1093/plankt/9.5.857

Buskey EJ, Swift E. 1983. Behavioral responses of the coastal copepod *Acartia hudsonica* (Pinhey) to simulated dinoflagellate bioluminescence. J Exp Mar Biol Ecol. 72:43–58. http://dx.doi.org/10.1016/0022-0981(83)90018-7

Buskey EJ, Swift E. 1985. Behavorial responses of oceanic zooplankton to simulated bioluminescence. Biol Bull. 168:263–275. http://dx.doi.org/10.2307/1541239

Buskey EJ, Strom S, Coulter C. 1992. Bioluminescence of heterotrophic dinoflagellates from Texas coastal waters. J Exp Mar Biol Ecol. 159:37–49. http://dx.doi.org/10.1016/0022-0981(92)90256-A

Cusick KD, Sayler GS. 2013. An overview on the marine neurotoxin saxitoxin: genetics, molecular targets, methods of detection, and ecological functions. Mar Drugs. 11:991–1018. http://dx.doi.org/10.3390/md11040991

DeSa R, Hastings JW. 1968. The characterization of scintillons. Bioluminescent particles from the marine dinoflagellate, *Gonyaulax polyedra*. J Gen Physiol. 51:105–122. http://dx.doi.org/10.1085/jgp.51.1.105

Draisci R, Ferretti E, Palleschi L, Marchiafava C, Poletti R, Milandri A, Ceredi A, Pompei M, et al. 1999. High levels of yessotoxin in mussels and presence of yessotoxin and homoyessotoxin in dinoflagellates of the Adriatic Sea. Toxicon. 37:1187–1193. http://dx.doi.org/10.1016/S0041-0101(98)00254-2

Esaias WE, Curl HC. 1972. Effect of dinoflagellate bioluminescence on copepod ingestion rates. Limnol Oceanogr. 17:901–905. http://dx.doi.org/10.4319/lo.1972.17.6.0901

Fink R. 2007. Does dinoflagellate bioluminescence deter shrimp grazing? An investigation into the Burglar Alarm Hypothesis. Thesis, Duke University.

Fleisher KJ, Case JF. 1995. Cephalopod predation facilitated by dinoflagellate luminescence. Biol Bull. 189:263–271. http://dx.doi.org/10.2307/1542143

Grober MS. 1988. Brittle-star bioluminescence functions as an aposematic signal to deter crustacean predators. Anim Behav. 36:493–501. http://dx.doi.org/10.1016/S0003-3472(88)80020-4

Guillard RRL. 1975. Culture of phytoplankton for feeding marine invertebrates. *In:* Smith WL, Chanley MH, editors. Culture of marine invertebrate animals. Plenum Press. New York. http://dx.doi.org/10.1007/978-1-4615-8714-9_3

Guillard RRL, Hargraves PE. 1993. *Stichochrysis immobilis* is a diatom, not a chrysophyte. Phycologia. 32:234–236. http://dx.doi.org/10.2216/i0031-8884-32-3-234.1

Hackett JD, Anderson DM, Erdner DL, Bhattacharya D. 2004. Dinoflagellates: a remarkable evolutionary experiment. Am J Bot. 91:1523–1534. http://dx.doi.org/10.3732/ajb.91.10.1523

Hargraves PE, Maranda L. 2002. Potentially toxic harmful microalgae from the northeast coast. Northeastern Nat. 9:81–120. http://dx.doi.org/10.1656/1092-6194(2002)009[0081:PTOHMF]2.0.CO;2

Hastings JW. 1983. Biological diversity, chemical mechanisms, and the evolutionary origins of bioluminescent systems. J Mol Evol. 19:309–321. http://dx.doi.org/10.1007/BF02101634

Howard MDA, Smith GJ, Kudela RM. 2009. Phylogenetic relationships of yessotoxin-producing dinoflagellates, based on the large subunit and internal transcribed spacer ribosomal DNA domains. Appl Environ Microbiol. 75:54–63. http://dx.doi.org/10.1128/AEM.00818-08

Johnson CH, Inoue S, Flint A, Hastings JW. 1985. Compartmentalization of algal bioluminescence—autofluorescence of bioluminescent particles in the dinoflagellate *Gonyaulax* as studied with image-intensified video microscopy and flow cytometry. J Cell Biol. 100:1435–1446. http://dx.doi.org/10.1083/jcb.100.5.1435

Kelly MG. 1968. The occurrence of dinoflagellate luminscence at Woods Hole. Biol Bull. 135:279–295. http://dx.doi.org/10.2307/1539782

Knaust R, Urbig T, Li L, Taylor W, Hastings JW. 1998. The circadian rhythm of bioluimnescence in *Pyrocystis* is not due to differences in the amount of luciferase: a comparative study of three bioluminescent marine dinoflagellates. J Phycol. 34:167–172. http://dx.doi.org/10.1046/j.1529-8817.1998.340167.x

Kudela RM, Cochlan WP. 2000. Nitrogen and carbon uptake kinetics and the influence of irradiance for a red tide bloom off southern California. Aquat Microbial Ecol. 21:31–47. http://dx.doi.org/10.3354/ame021031

Liu L, Wilson T, Hastings JW. 2004. Molecular evolution of dinoflagellate luciferases, enzymes with three catalytic domains in a single polypeptide. Proc Natl Acad Sci USA. 101:16,555–16,560. http://dx.doi.org/10.1073/pnas.0407597101

Marcinko CLJ, Painter SC, Martin AP, Allen JT. 2013. A review of the measurement and modelling of dinoflagellate bioluminescence. Prog Oceanogr. 109:117–129. http://dx.doi.org/10.1016/j.pocean.2012.10.008

Marek P, Papaj D, Yeager J, Molina S, Moore W. 2011. Bioluminescent aposematism in millipedes. Curr Biol. 21:R680–R681. http://dx.doi.org/10.1016/j.cub.2011.08.012

Mensinger AF, Case JF. 1992. Dinoflagellate luminescence increases susceptibility of zooplankton to teleost predation. Mar Biol. 112:207–210. http://dx.doi.org/10.1007/BF00702463

Morgan MD. 1980. Grazing and predation of the grass shrimp *Palaemonetes pugio*. Limnol Oceanogr. 25:892–902. http://dx.doi.org/10.4319/lo.1980.25.5.0896

Nicolas MT, Morse D, Bassot JM, Hastings JW. 1991. Colocalization of luciferin binding-protein and luciferase to the scintillons of *Gonyaulax polyedra* revealed by double immunolabeling after fast-freeze fixation. Protoplasma. 160:159–166. http://dx.doi.org/10.1007/BF01539967

Nixon SW, Oviatt CA. 1973. Ecology of a New England salt marsh. Ecol Monogrphs. 43:463–498. http://dx.doi.org/10.2307/1942303

Omand MM, Leichter JJ, Franks PJS, Guza RT, Lucas AJ, Feddersen F. 2011. Physical and biological processes underlying the sudden surface appearance of a red tide in the nearshore. Limnol Oceanogr. 56:787–801. http://dx.doi.org/10.4319/lo.2011.56.3.0787

Paz B, Riobo P, Fernandez AL, Fraga S, Franco JM. 2004. Production and release of yessotoxins by the dinoflagellates *Protoceratium reticulatum* and *Lingulodinium polyedrum* in culture. Toxicon. 44:251–258. http://dx.doi.org/10.1016/j.toxicon.2004.05.021

Plumley FG. 1997. Marine algal toxins: biochemistry, genetics, and molecular biology. Limnol Oceanogr. 42:1252–1264. http://dx.doi.org/10.4319/lo.1997.42.5_part_2.1252

Schmitter R, Njus D, Siulzman FM, Gooch V, Hastings JW. 1976. Dinoflagellate bioluminescence: a comparative study of in vitro components. J Cell Physiol. 87:123–134. http://dx.doi.org/10.1002/jcp.1040870115

Seliger HH, Biggley WH, Swift E. 1969. Absolute values of photon emission from the marine dinoflagellate *Pyrodinium bahamense, Gonyaulax polyedra,* and *Pyrocystis lunula*. Photobiol Photochem. 10:227–232. http://dx.doi.org/10.1111/j.1751-1097.1969.tb05685.x

Sivinski J. 1981. The nature and possible functions of luminescence in coleoptera larvae. Coleopts Bull. 35:167–179.

Smayda TJ. 1997. Harmful algal blooms: their ecophysiology and general relevance to phytoplankton blooms in the sea. Limnol Oceanogr. 42:1137–1153. http://dx.doi.org/10.4319/lo.1997.42.5_part_2.1137

Smith CL. 1997. National Audubon Society field guide to tropical marine fishes of the Caribbean, the Gulf of Mexico, Florida, The Bahamas, and Bermuda. Alfred A Knopf, Inc., New York.

Swift E, Biggley WH, Seliger HH. 1973. Species of oceanic dinoflagellates in the genera *Dissodinium* and *Pyrocystis:* interclonal and interspecific comparisons of the color and photon yield of bioluminescence. J Phycol. 9:420–426.

Underwood TJ, Tallamy DW, Pesek JD. 1997. Bioluminescence in firefly larvae: a test of the aposematic display hypothesis (Coleoptera: Lampyridae). J Ins Behav. 10:365–370. http://dx.doi.org/10.1007/BF02765604

White HH. 1979. Effects of dinoflagellate bioluminescence on the ingestion rates of herbivorous zooplankton. J Exp Mar Biol Ecol. 36:217–224. http://dx.doi.org/10.1016/0022-0981(79)90117-5

Widder EA. 1992. Mixed light imaging system for recording bioluminescence behaviors. J Mar Biol Assoc UK. 72:131–138. http://dx.doi.org/10.1017/S0025315400048839

Widder EA, Latz MI, Case JF. 1983. Marine bioluminescence spectra measured with an optical multichannel detection system. Biol Bull. 165:791–810. http://dx.doi.org/10.2307/1541479

Widder EA, Case JF, Bernstein SA, MacIntyre S, Lowenstine MR, Bowlby MR, Cook DP. 1993. A new large volume bioluminescence bathyphotometer with defined turbulence excitation. Deep-Sea Res I. 40:607–627. http://dx.doi.org/10.1016/0967-0637(93)90148-V

Wilson T, Hastings JW. 1998. Bioluminescence. Ann Rev Cell Dev Biol. 14:197–230. http://dx.doi.org/10.1146/annurev.cellbio.14.1.197

Zar JH. 1996. Biostatistical Analysis. Prentice Hall. Upper Saddle River, NJ.