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Seasonal Changes of Bioluminescence in Photosynthetic and Heterotrophic Dinoflagellates at San Clemente Island

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1. Introduction

A significant portion of bioluminescence in all oceans is produced by dinoflagellates. Numerous studies have documented the ubiquitous distribution of bioluminescent dinoflagellates in near surface waters (Seliger et al., 1961; Yentsch and Laird 1968; Tett 1971; Tett and Kelly 1973). The number of bioluminescent species and their relative abundance change temporally, with depth, and geographically. Dinoflagellates are most abundant in coastal waters and inland seas and are less abundant in the open ocean (Colebrook and Robinson, 1965; Dodge and Hart-Jones, 1977). Studies have been conducted to determine the species contributing to bioluminescence. In several studies, this involved making plankton collections, isolation and measurement of cells with a laboratory photometer to quantify the light output of several species of bioluminescent dinoflagellates (Lapota and Losee 1984; Batchelder and Swift 1989; Lapota et al., 1992a,b; Swift et al., 1995). These studies were limited to short sampling periods (days-weeks) and to specific locations. There is also evidence that dinoflagellates undergo changes in light output which may be attributable to environmental conditions. For example, cells of *Protoperidinium* spp. produce more bioluminescence when nutritional requirements were optimized in the laboratory (Buskey 1992; Latz 1993). Others have observed that the bioluminescence potential of a dinoflagellate is related to its surface area or cell volume for several species, which might be related to light and nutrient history (Seliger et al. 1969; Seliger and Biggley 1982; Swift et al. 1995; Sullivan and Swift 1995). Bioluminescence may also be a function of light, temperature, and nutrient history (Sweeney, 1981). Other data have suggested that cells of the same species in the same study display differences in bioluminescence. These observations may indicate that cells are exposed to a wide range of environmental conditions affecting light output on a short time scale such as light history, nutrient history, grazing pressure by herbivores and consequent loss of potential bioluminescent capacity (Swift et al., 1981; Sullivan and Swift, 1995).

Despite strong interest in short term process effects on dinoflagellates there have been few investigations on the seasonality of marine bioluminescence (Tett 1971; Bityukov et al. 1967; Lapota et al. 1997). Long term aspects of the development of bioluminescence are unknown for most oceans. The present study was designed to cast light on this question. A station for

measuring bioluminescence was established in August 1993 at San Clemente Island (SCI), 100 km offshore of Southern California. Bioluminescence was measured with a moored bathyphotometer (MOORDEX) hourly through February 1996. Other environmental parameters such as nutrients, chlorophyll, and associated plankton species were measured and collected on a monthly and quarterly basis(Lapota et al. 1997). In the present study, plankton samples were collected and tested for bioluminescence on a quarterly basis to: 1) determine which dinoflagellate species were bioluminescent and 2) observe differences in light output on a seasonal basis. The latter is an important consideration because seasonal changes in bioluminescence from dinoflagellates might possibly indicate a response to regional seasonal environmental changes. These factors include the available nutrients and light for the photosynthetic species (*Ceratium, Gonyaulax*[1], *Pyrocystis*) and the availability of diatoms and smaller algal cells consumed by the heterotrophic *Protoperidinium* dinoflagellates. Seasonal changes in light output will affect the bioluminescence light budget of all species. Published light budgets are limited and specific for limited oceanic areas (Swift et al. 1983, 1985a,b; 1995; Batchelder and Swift 1989; Lapota et al. 1988, 1989, 1992a,b; Buskey 1991) , the number of species tested, or modeled to predict bioluminescence output based on the calculated cell surface area (Seliger and Biggley 1982). This study will complement earlier laboratory work and enlarge these observations by identifying distinct seasonal differences in bioluminescence of open ocean dinoflagellates over a two year period.

2. Methods and materials

2.1 Plankton collections

Plankton samples were collected from the Naval Ordinance Test Station (NOTS) pier at SCI on a quarterly basis from the summer of 1994 through spring 1996. The pier is on the leeward side of SCI and is in a water depth of approximately 15 meters. The island shelf begins to deepen to greater than 100 meters within 150 meters of the pier (Figure 1). A MOORDEX bathyphotometer was suspended under the pier by a 1cm diameter stainless steel cable. Depending on the height of the tide, MOORDEX was usually at a depth of 2-3 meters below the sea surface (Lapota et al. 1994a). A similar MOORDEX bathyphotometer was also deployed in San Diego Bay (SDB) from 1992-1996 for comparative coastal measurements of bioluminescence (Lapota et al., 1997). Plankton samples were always collected in the late afternoon. A 20-µm mesh plankton net and attached collection cup was lowered off the pier to a depth of approximately 10 meters and vertically retrieved. Samples were diluted with fresh filtered (0.45 µm) seawater and transported to a field trailer and kept in a temperature-controlled incubator (Coolatron™) at ambient seawater temperature. Individual cells were viewed microscopically and isolated by pipet and placed in 4-ml spectrophotometric disposable cuvettes with 3-ml of (0.45 µm) filtered seawater. All isolations were completed no later than 1 hour prior to sunset to prevent premature stimulation of bioluminescence. Bioluminescence measurements were always conducted midway into scotophase, about 8-9 hours after collection. Two consecutive nights of isolation and testing were conducted each season (i.e., winter: December 21- March 21; spring: March 22-June 21; summer: June 22-September 21; fall: September 22-December 21).

[1] *Gonyaulax polyedra* has recently been renamed *Lingulodinium polyedrum* Stein (Dodge 1989), but for simplicity we will use the former name because of its wider use in earlier literature.

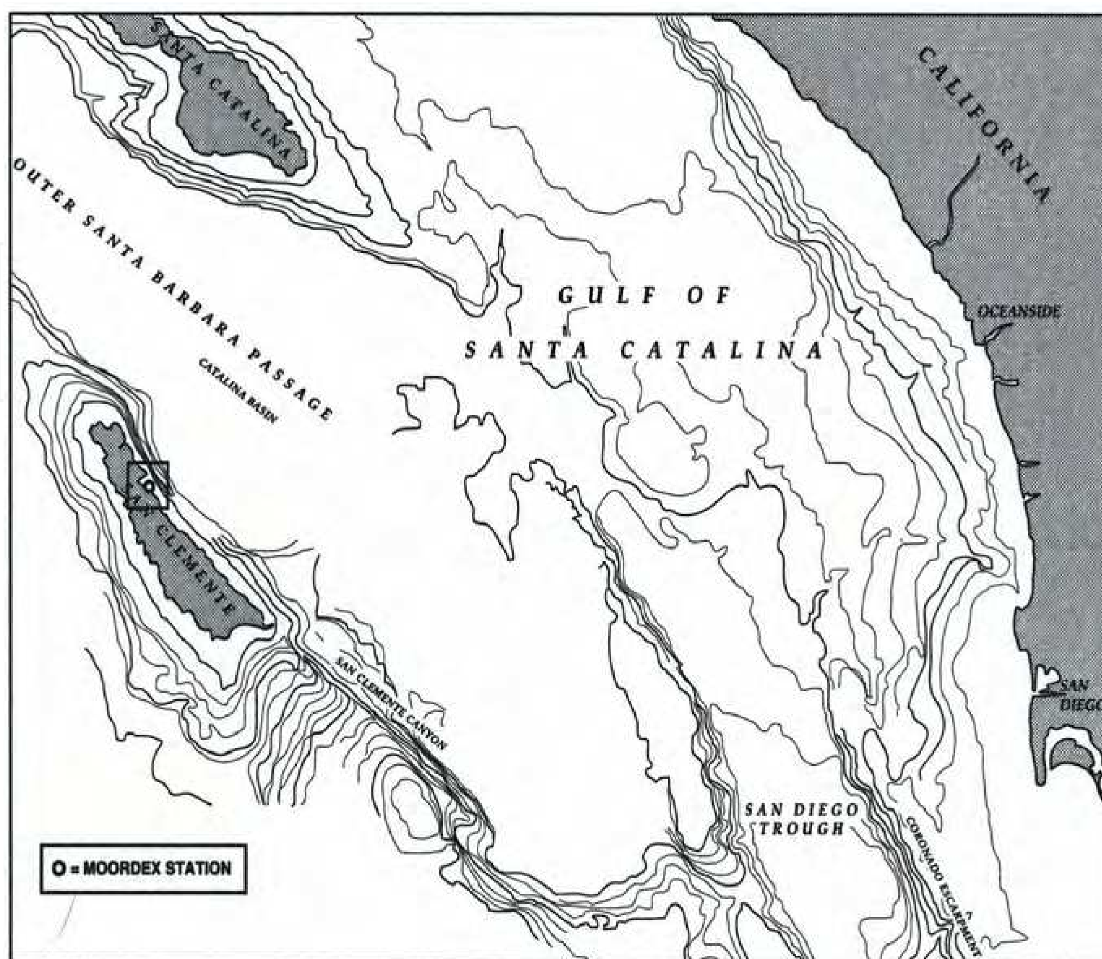

Fig. 1. Bioluminescence study area within the Gulf of Santa Catalina. Boxed area identifies NOTS pier where all plankton collections were made.

2.1.1 Laboratory measurements

Single cells in cuvettes were measured in a laboratory photometer system (Lapota et al. 1994b). This system is similar to another photometer system used in previous studies (Lapota and Losee 1984; Lapota et al. 1988, 1989, 1992) and consists of a horizontally mounted 2-inch diameter end window photomultiplier tube (PMT - RCA 8575 with an S-20 response) attached to a darkened chamber with removable opening to receive the cuvettes. Cells were stimulated to luminescence by stirring with an adjustable speed motor driving a plastic stirrer for 30 sec after which the total bioluminescence (PMT counts) was displayed. PMT counts were either logged on a notebook computer operating under Windows™ or hand recorded on data sheets. PMT dark counts were subtracted from all light output values prior to conversion to photons cell^{-1}. The system was calibrated with aliquots of the luminescent bacterium *Vibrio harveyii* measured by the Quantalum 2000 silicon-photodiode detector (Matheson et al., 1984). The detector calibration is traceable to a luminol light standard.

Following testing, the cells were individually placed in borosilicate vials and preserved in a 5% formalin solution for later microscopic identification to species level. Mean light output

values for each species tested for all seasons were calculated. The Student's t test for comparison of two means was used to calculate significant differences between means of cells within species for all seasons. Critical values of the Student's t distribution were calculated using n-2 degrees of freedom.

2.2 Nutrient and chlorophyll data

Nitrate and Chl *a* levels were obtained from archived CalCOFI data bases from 1994 - 1996 for the Southern California Bight and were averaged along CalCOFI lines 90 and 93 which extend offshore west of San Diego to the north and south of SCI (Hayward et al. 1996). Nitrates (µm L^{-1}) and Chl *a* (µg L^{-1}) along each of the CalCOFI transit lines 93.26 to 93.45 and 90.28 to 90.53 were averaged from the surface to a depth of 50 m, seasonally (fall, winter, spring, summer), from spring 1994 through spring 1996. These data were used to calculate correlations with seasonal means of bioluminescence $cell^{-1}$ of the photosynthetic dinoflagellate *Pyrocystis noctiluca* and the heterotrophic dinoflagellate *Protoperidnium pellucidum*. Nutrient data was lagged by one season to calculate correlation coefficients with mean bioluminescence $cell^{-1}$. The Student t-test was used to determine significant differences among seasonal bioluminescence means for *Gonyaulax, Ceratium, Pyrocystis*, and *Protoperidinium* species.

3. Results

3.1 Bioluminescence measured with MOORDEX

A winter maximum and summer minimum in bioluminescence was measured at SCI in contrast to a maximum in the spring and minimum in the fall in San Diego Bay (SDB) (Figure 2). Mean monthly bioluminescence at SCI varied little from August 1993 - February 1996 except during a red tide in January 1995 (maximum of 2 x 10^8 photons s^{-1} ml^{-1} seawater measured in January 1995) which persisted through April (Figure 2). In contrast, seasonal changes in bioluminescence were observed in SDB. Maximum bioluminescence (1 x 10^8 photons s^{-1} ml^{-1} or greater) was measured from March through September for 1993, May through June for 1994, December through May for 1995, and March through April for 1996. Minimum values less than 1 x 10^8 photons sec^{-1} ml^{-1}) were measured in the winter (Figure 2) (Lapota et al., 1997).

3.2 Bioluminescent plankton at San Clemente Island

The numbers of bioluminescent dinoflagellates were typically lower at SCI than at SDB and ranged from 3 - 211 cells L^{-1} of seawater from August 1993 through December 1994 (Figure 3). In contrast, in SDB, maximum numbers of bioluminescent dinoflagellates were collected during the spring-summer months (2430 - 17,216 cells L^{-1}). Minimal numbers of bioluminescent dinoflagellates were collected in the winter in SDB. At SCI, the principal species observed were *G. polyedra* and several species of *Protoperidinium.* A red tide was first observed in January 1995 and persisted through April 1995. Bioluminescence during this event increased approximately 10 times above former levels for both SDB and SCI. Total and bioluminescent dinoflagellates increased to 16,727 cells L^{-1} and 15,939 cells L^{-1}, respectively in January 1995 at SCI (Figure 3). Cell numbers remained high through April 1995. At SCI bioluminescent dinoflagellates comprised a major percentage of total dinoflagellates collected (Figure 4).

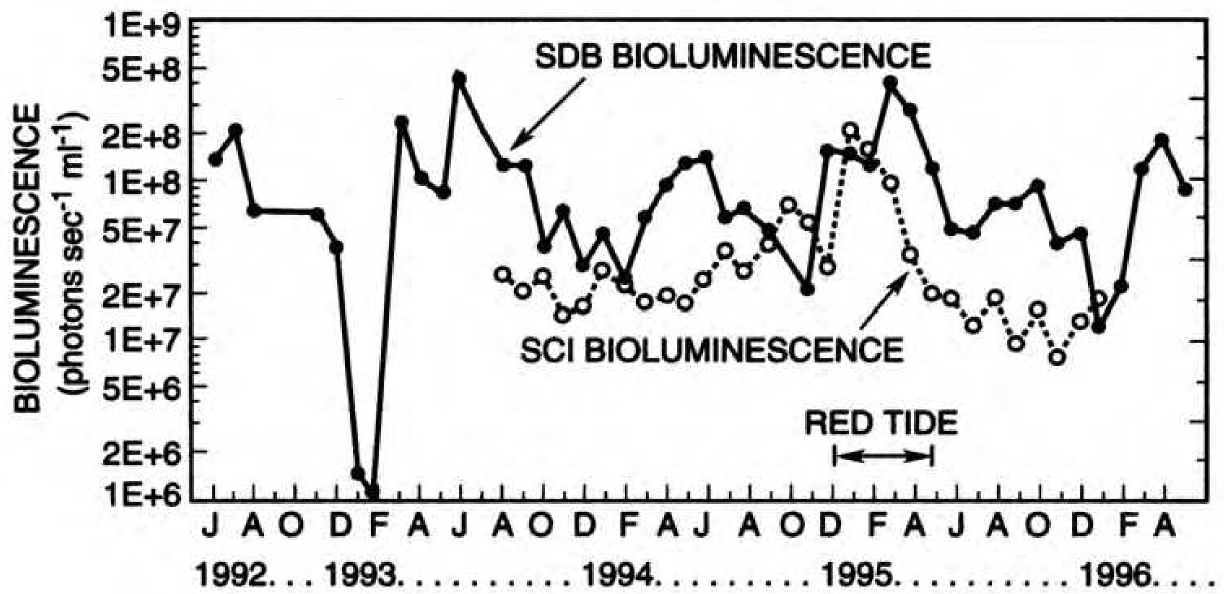

Fig. 2. Mean monthly bioluminescence trends at San Diego Bay and San Clemente Island from 1992-1996.

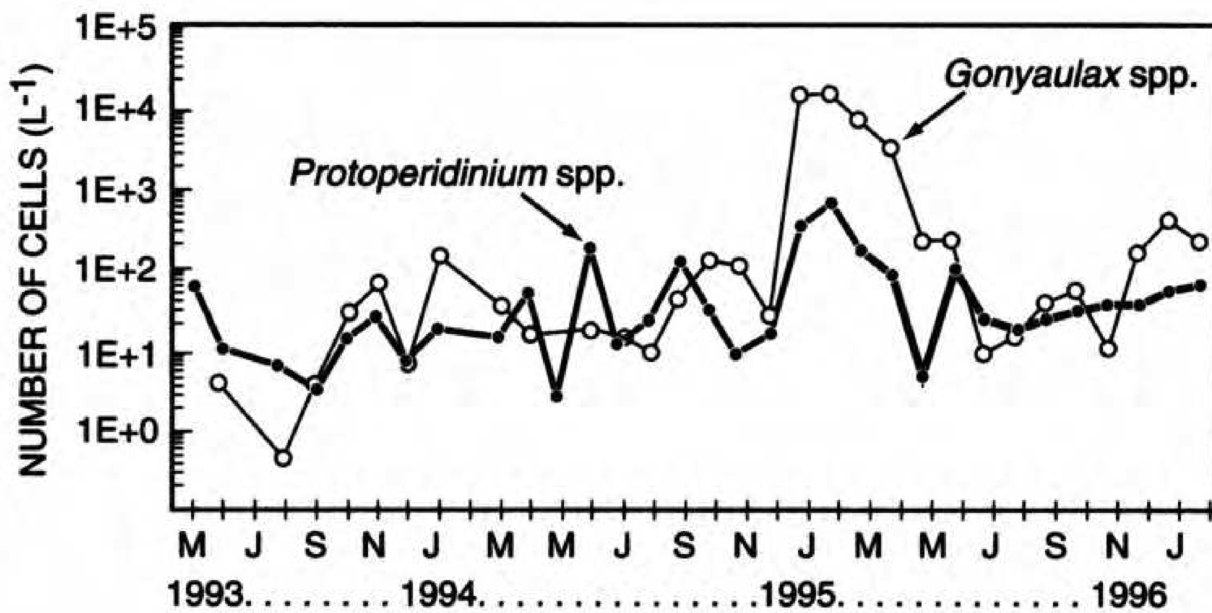

Fig. 3. The abundance of *Protoperidinium* and *Gonyaulax* dinoflagellates at San Clemente Island from 1993-1996.

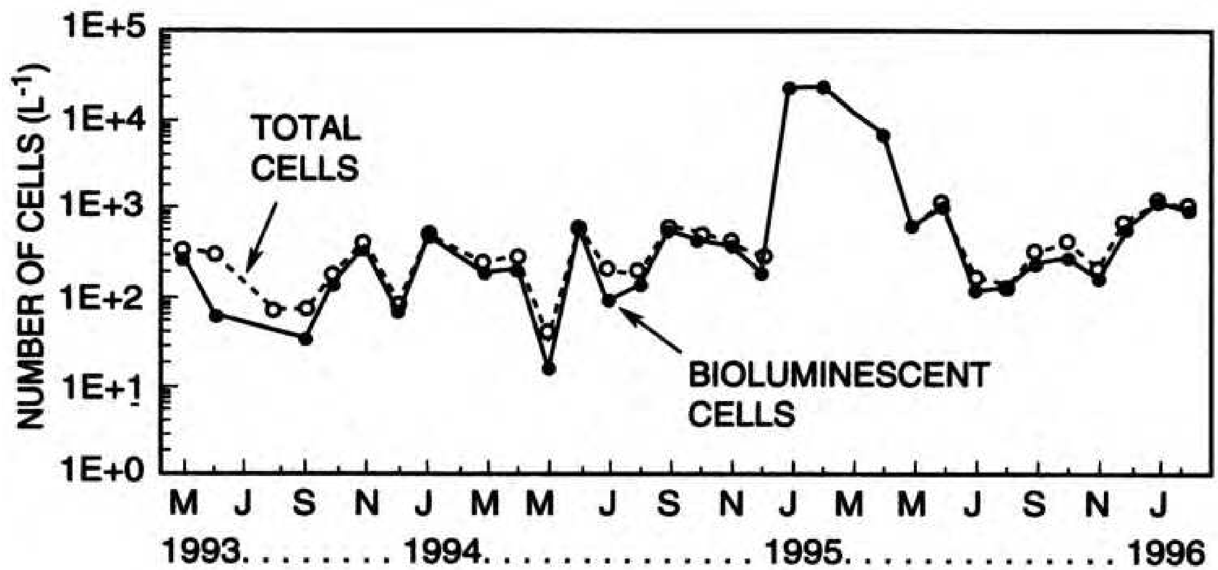

Fig. 4. Total and bioluminescent dinoflagellate cells collected monthly at San Clemente Island from 1993-1996.

3.3 Light budget analysis

The light budget analysis (number of cells L^{-1} of each bioluminescent species multiplied by the mean light output $cell^{-1}$ and then adjusted by dividing the species light contribution by the sum of all bioluminescence from all species) indicated that the *Protoperidinium* dinoflagellates produced most of the bioluminescence (Figure 5).

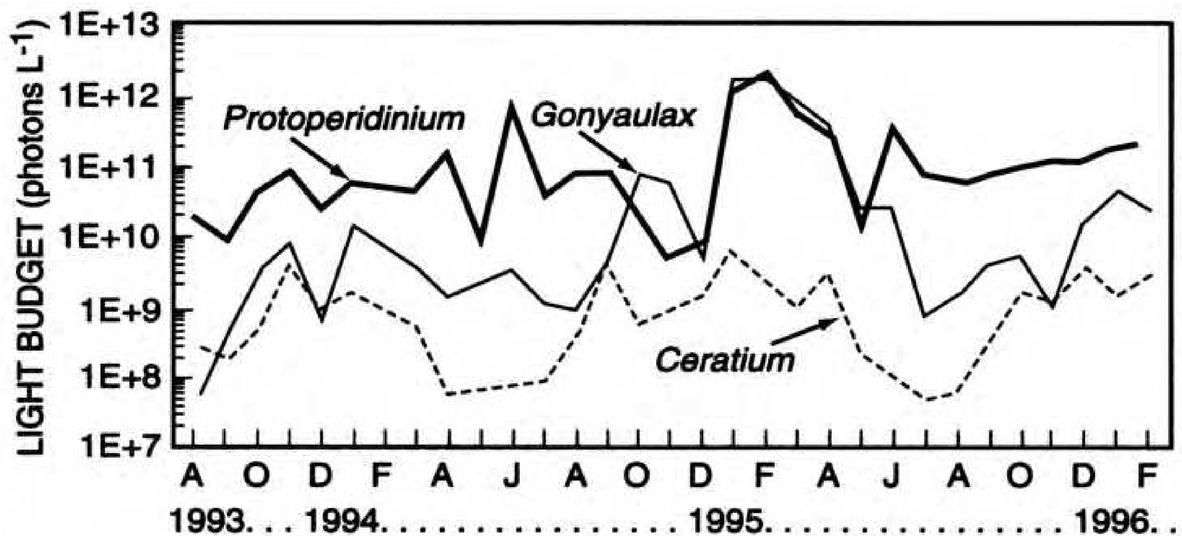

Fig. 5. Bioluminescence produced by each species (photons $liter^{-1}$) monthly at San Clemente Island from 1993-1996.

Gonyaulax spp. and *Ceratium fusus* contributed less. *Protoperidinium* contributed more than 80% of all bioluminescence for 60% of all months (30 months) and more than 50% of all bioluminescence for 77% of all months. In contrast, *Gonyaulax* contributed 80% of all bioluminescence for just 1 month (3.3% of all months) and 50% of all bioluminescence for

only 10% of all months. During the red tide in the winter and spring of 1995, *Gonyaulax* contributed 59%, 42%, 58%, 48%, and 27% of all bioluminescence for the months of January through May 1995, respectively (Figure 6). The open ocean bioluminescent dinoflagellate *Pyrocystis noctiluca* was also found in monthly samples. *Protoperidinium curtipes*, *P. depressum*, *P. divergens*, *P. leonis*, and *P. steinii* were present in the spring and summer months while *Gonyaulax* became more prevalent in the fall and winter months.

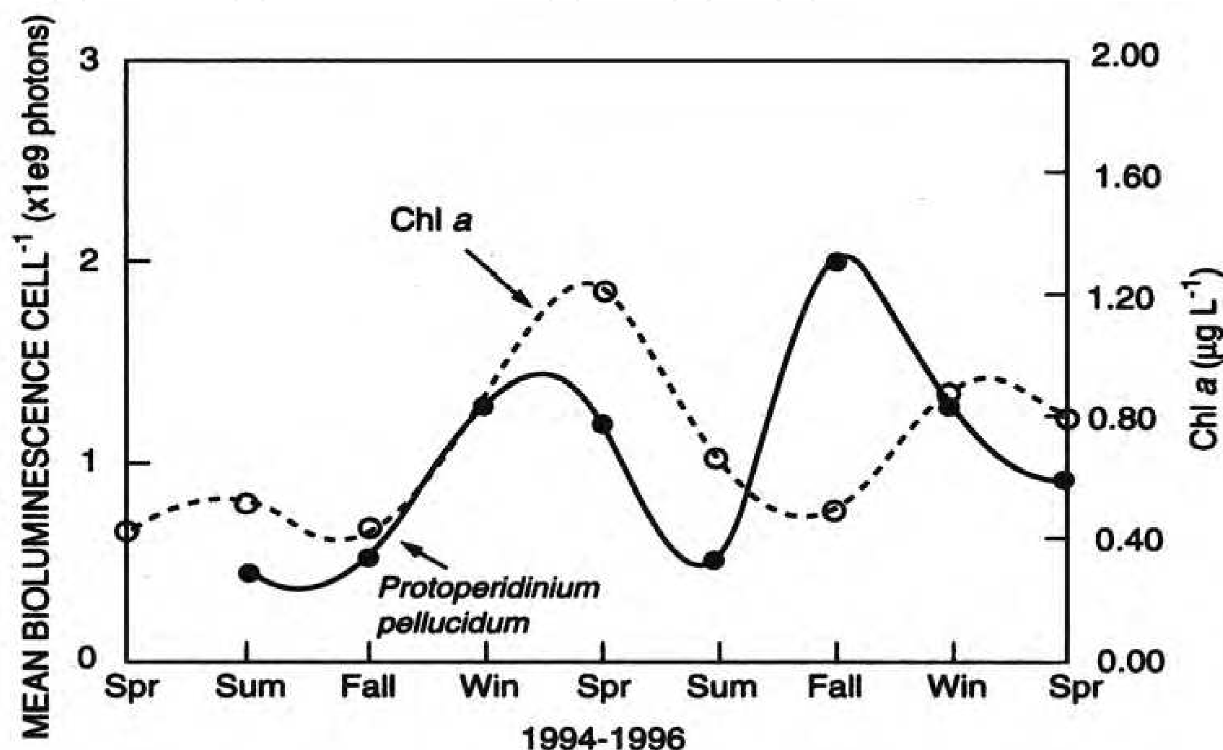

Fig. 6. (a) Seasonal differences in *Protoperidinium pellucidum* mean bioluminescence and mean Chl *a* for waters surrounding San Clemente Island 1994-1996.

3.4 Light output from Dinoflagellates

Three to 6 recurrent species of dinoflagellates were usually found in the collected plankton samples for each season. In 5 of the 9 seasons, more than 45 cells isolated proved to be luminescent and were identified to species level. In all, bioluminescence from 469 identified cells representing 5 genera and 13 species were measured (Table 1). Cells of *Gonyaulax polyedra*, *Ceratium fusus*, *Pyrocystis noctiluca*, and the heterotroph *Protoperidinium pellucidum* were found more frequently in plankton collections than other species, and from these data, seasonal mean bioluminescence cell^{-1} was determined.

Mean light output cell^{-1} for all dinoflagellates is listed in Tables 2,3,4,5 and in Figures 6-9. The mean light output for all quarters for all species is listed in Table 2 and ranges from 1.6e8 photons cell^{-1} for *Ceratium fusus* to 1.8e10 photons cell^{-1} for *Pyrocystis noctiluca*. *G. grindleyi* was the brightest species of *Gonyaulax* tested and was approximately 3 times brighter than *G. polyedra* (Table 2). Cells of species of *Protoperidinium* ranged from 1 to 4e9 photons cell^{-1} (Tables 2, 3; Figure 6a).

Species	Season Tested
G. grindley	Fall'94, Win'95
G. polyedra	Win'95, Spr'95; Fall'95, Win'96
G. polygramma	Spr'94, Fall'95, Win'96
G. spinifera	Sum'95, Fall'95
Ceratium fusus (small cells)	Fall'94, Win'95; Spr'95, Fall'95; Win'96, Spr'96
C. fusus (large cells)	Fall'95, Win'96; Spr'96
Protoperidinium curtipes	Spr'94, Sum'94; Spr'96
P. depressum	Spr'94, Sum'94; Win'96
P. divergens	Spr'94, Sum'94
P. leonis	Spr'94, Spr'95
P. pellucidum	Sum'94, Fall'94; Win'95, Spr'95; Sum'95, Fall'95; Win'96, Spr'96
P. steinii	Fall'94
Noctiluca miliaris	Spr'95
Pyrocystis noctiluca	Spr'94, Sum'94 ; Fall'94, Win'95 ; Sum'95, Fall'95; Win'96, Spr'96

Table 1. Bioluminescent dinoflagellate species surveyed in study.

Species	Mean light output (photons cell^{-1})	Standard Deviation	Number of cells tested
***Ceratium fusus* (small cells)**	1.5e8	1.9e8	52
Gonyaulax polyedra	2.4e8	2.2e8	56
Gonyaulax spinifera	6.1e8	4.5e8	19
Gonyaulax polygramma	6.3e8	4.2e8	36
***Ceratium fusus* (large cells)**	8e8	5.7e8	26
Gonyaulax grindleyi	8.5e8	5.2e8	10
Protoperidinium steinii	1e9	1.1e9	5
Protoperidinium pellucidum	1.2e9	1e9	112
Protoperidinium divergens	2.8e9	3.9e9	19
Protoperidinium depressum	3.1e9	2.8e9	18
Protoperidinium curtipes	4e9	4.7e9	17
Protoperidinium leonis	4.4e9	3e9	7
Pyrocystis noctiluca	1.8e10	9.7e9	105

Table 2. Comparative light output for all species 1994-1996. Listing is by mean light output.

Species	Spr'94	Sum'94	Fall'94	Win'95	Spr'95	Sum'95	Fall'95	Win' 96	Spr'96
P. de.	1.6e9; 7 cells	4.1e9; 9 cells						3.6e9; 2 cells	
P. di.	4.4e9; 8 cells	1.6e9; 11 cells							
P. cur.	5.9e9; 9 cells	7.9e8; 3 cells							2.5e9; 5 cells
P. leo	6.3e9; 4 cells				1.8e9; 3 cells				
P. stei.			4.3e8; 3 cells						1.9e9; 3 cells

Table 3. Light output for other *Protoperidinium* spp. (mean photons cell^{-1}) 1994 – 1996.

Species	Spr'94	Sum'94	Fall'94	Win'95	Spr'95	Sum'95	Fall'95	Win'96	Spr'96
G. poly.	6.2e8;9 cells						5.2e8; 12 cells	7.3e8; 15 cells	
G. spin.						2.2e8; 7 cells	8.4e8; 12 cells		
G. grind.			8.9e8; 8 cells	6.8e8; 2 cells					
G. polyed.				9.8e7 ; 19 cells	1.1e8; 12 cells		4.2e8; 14 cells	4.2e8; 11 cells	

Table 4. Light output for other *Gonyaulax* species (mean photons cell-1) 1994 – 1996.

Cells	Spr'94	Sum'94	Fall'94	Win'95	Spr'95	Sum'95	Fall'95	Win'96	Spr'96
Small cells			3.6e8; 9 cells	5.7e7; 7 cells	2.1e7; 1 cell		8.4e7; 13 cells	1.6e8; 19 cells	2.3e8; 3 cells
Large cells							8.4e8; 6 cells	6.3e8; 15 cells	1.3e9; 5 cells

Table 5. Light output for *Ceratium fusus* (in mean photons cell^{-1})1994-1996.

Win'95 bioluminescence is significantly greater than	**Sum'94**	**Fall'94**	**Spr'95**
	p< 0.10	p < 0.001	n.s.
Fall'95 bioluminescence is significantly greater than	**Sum'95**	**Win'96**	**Spr'96**
	p < 0.01	n.s.	p < 0.01

*levels of significance for two-tail student t test

Table 6. Significant differences of light output seasonally in *Protoperidinium pellucidum* from 1994 – 1996*.

Seasons between years

Sum'94 - Sum'95 bioluminescence	**n.s.**
Fall'94 - Fall'95 bioluminescence	Fall'95 > Fall'94; p < 0.001
Win'95 - Win'96 bioluminescence	n.s.
Spr'95 - Spr'96 bioluminescence	n.s.

n.s. = not significant

3.4.1 *Pyrocystis noctiluca*

Significant differences in mean bioluminescence $cell^{-1}$ were observed between spring '94 and summer '94, between spring '94 and fall '94, between spring '94 and winter '95, and between spring '94 and spring '96 (Table 7). Spring '96 cells produced more bioluminescence than spring '94 cells as did winter '96 cells when compared to winter '95 cells (Table 7). *Gonyaulax* spp**.**

	Spr'94	**Sum'94**	**Fall'94**	**Win'95**	**Spr'95**	**Sum'95**	**Fall'95**	**Win'96**	**Spr'96**
Sum'94	p<0.3								
Fall'94	p<0.2	P<0.4*		P<0.6*					
Win'95	p<0.3	P<0.9*							
Spr'95		P<0.6*							
Sum'95		P<0.6*						P<0.8*	P<0.7*
Fall'95			P<0.5*			P<0.8*	P<0.3	P<0.7*	
Win'96				P<0.2					P<0.5*
Spr'96	p<0.1								

Table 7. Significant differences of light output seasonally in *Pyrocystis noctiluca* from 1994 – 1996 (* = not significant).

Seasons between years Fall'94 - Fall'95

Spr'94 - Spr'96	**Spr'96 > Spr'94**	**p < 0.1**
Sum'94 - Sum'95	Sum'95 > Sum'94	p< 0.6*
Fall'94 - Fall'95	Fall'95 > Fall'94	p< 0.5*
Win'95 - Win'96	Win'96 > Win'95	p < 0.2

*= not significant

Significant differences in mean cell light output were measured among *Gonyaulax polyedra, G. spinifera*, and *G. polygramma* (Table 8). Cells of *G. polyedra* of fall '95 produced significantly more light than cells tested previously in the winter and spring '95 ($p < 0.001$; Table 8). Cells tested in the winter '96 were significantly brighter than the previous spring '95. Yearly differences were also observed between both winters of '95 and '96 where '96 cells > '95 cells. The fall '95 cells of *G. spinifera* exhibited more bioluminescence than in the summer while winter '96 *G. polygramma* cells were measurably brighter than earlier in the fall (Table 8).

	G. polyedra	
Win'95 - Spr'95	P<0.8	n.s.
Fall'95 – Win'95	Fall > Win	p < 0.001
Fall '95 – Spr'95	Fall > Spr	p < 0.001
Win'96 – Win'95	Win'96 > Win'95	p < 0.001
Win'96 – Spr'95	Win > Spr	p < 0.001
Win'96 – Fall'95	P < 0.9	n.s.
	G. spinifera	
Fall'95 – Sum'95	Fall > Sum	P < 0.001
	G. polygramma	
Win'96 – Fall'95	Win > Fall	P < 0.2

Table 8. Significant differences of light output seasonally in *Gonyaulax* species from 1995 - 1996.

3.4.2 *Protoperidinium*

The winter '95 *Protoperidinium pellucidum* cells were observed to produce more bioluminescence than cells from summer '94 and fall '94 (Table 6). They were not significantly different than cells tested in spring '95. Cells of *P. pellucidum* in the fall '95 were significantly brighter than cells tested in summer '95, and spring '96, but not winter '96 (Table 6). A significant seasonal difference between years was observed for the fall quarters. These cells were also significantly brighter in Fall '95 than in the fall '94 (Table 6). Significant differences in light output were not found for summer, winter, and spring between 1994 and 1995.

Limited measurements were conducted on *P. curtipes* and *P. divergens*. These cells produced more bioluminescence in the spring ’94 than the following summer while *P. depressum* cells in the summer ’94 were brighter than the previous spring months (Table 9). *P. leonis* cells were observed to produce more bioluminescence in spring ’94 than in spring ’95.

P. curtipes	**Spr’94 - Sum’94**	**Spr > Sum**	**p < 0.2**
P. depressum	Sum’94 - Spr’94	Sum > Spr	p < 0.1
P. divergens	Spr’94 - Sum’94	Spr > Sum	p < 0.2
P. leonis	Spr’94 - Spr’95	Spr’94 > Spr’95	p < 0.05

Table 9. Significant differences of light output seasonally in *Protoperidinium* species from 1994 - 1995.

3.4.3 *Ceratium fusus*

Significant seasonal differences of light output were observed from 1994-1996 in small and larger cells of *C. fusus*. Fall ’94 cells produced more bioluminescence than winter ’95 cells and winter ’96 cells. In contrast, winter ’96 cells produced more bioluminescence than fall ’95 cells (Table 10). Between years, fall ’94 cells were brighter than fall ’95 cells while winter ’96 cells exhibited more bioluminescence than winter ’95 cells. Light output in larger cells of *C. fusus* showed that spring ’96 were brighter than in fall ’95 and winter ’96. Larger cells of *C. fusus* were significantly brighter than smaller *C. fusus* cells for fall ’95, winter ’96 and spring ’96.

	SMALL CELLS	
Fall’94 - Win’95	**Fall > Win**	**p < 0.02**
Fall’94 - Win’96	Fall > Win	**p < 0.05**
Fall’94 - Spr’96	p < 0.5	**n.s.**
Win’96 - Fall’95	Win > Fall	**p < 0.2**
	Seasons between years	
Fall’94 - Fall’95	Fall’94 > Fall’95	**p < 0.01**
Win’96 - Win’95	Win’96 > Win’95	**p < 0.1**
	LARGE CELLS	
Spr’96 - Win’96	Spr > Win	**p < 0.05**
Spr’96 - Fall’95	Spr > Fall	**p < 0.2**
Fall’95 - Win’96	p < 0.5	**n.s.**
	Difference in light output between large and small cells	
Fall’95	Large cells > small cells	**p < 0.001**
Win’96	Large cells > small cells	**p < 0.01**
Spr’96	Large cells > small cells	**p < 0.05**

n.s. = not significant

Table 10. Significant differences of light output seasonally in *Ceratium fusus* from 1994 – 1996.

3.4.4 Seasonal trends of dinoflagellate bioluminescence, nitrates, and Chl *a*

Maximum bioluminescence in *Protoperidinium pellucidum, Pyrocystis noctiluca, Ceratium fusus*, and *Gonyaulax polyedra* was observed in either fall or winter for 1994–1996. Maximum bioluminescence cell^{-1} in *P. pellucidum* occurred in winter 1995 and during the following fall. Bioluminescence in this species was lower in summer ’94 and again in summer ’95 (Figures 6a,b). The percent of peak bioluminescence (the peak refers to maximum bioluminescence measured in winter ’95 and fall ’95) varies with season with values as low as 30% for summer ’94 and 20% for summer ’95 (Figure 6b). Because *Protoperidinium* dinoflagellates are nutritionally dependent on other algal species, any change of algal biomass in waters adjacent to SCI might impact the growth, maintenance and luminescent potential of *Protoperidinium*. Extracted Chl *a* showed a similar periodicity as did mean bioluminescence cell^{-1} in *P. pellucidum* from summer ’94 through summer ’95. Maximum Chl *a* and mean bioluminescence cell^{-1} levels increased in winter 1995 and in spring 1995 (Figure 7a). The seasonal mean bioluminescence cell^{-1} and the seasonal mean Chl *a* in the waters off SCI were positively correlated (Figure 6c). Bioluminescence in *P. pellucidum* was not significantly different between winter and spring quarter 1995, but was significantly brighter than the preceding summer and fall quarters and the ensuing summer 1995 quarter (Table 6).

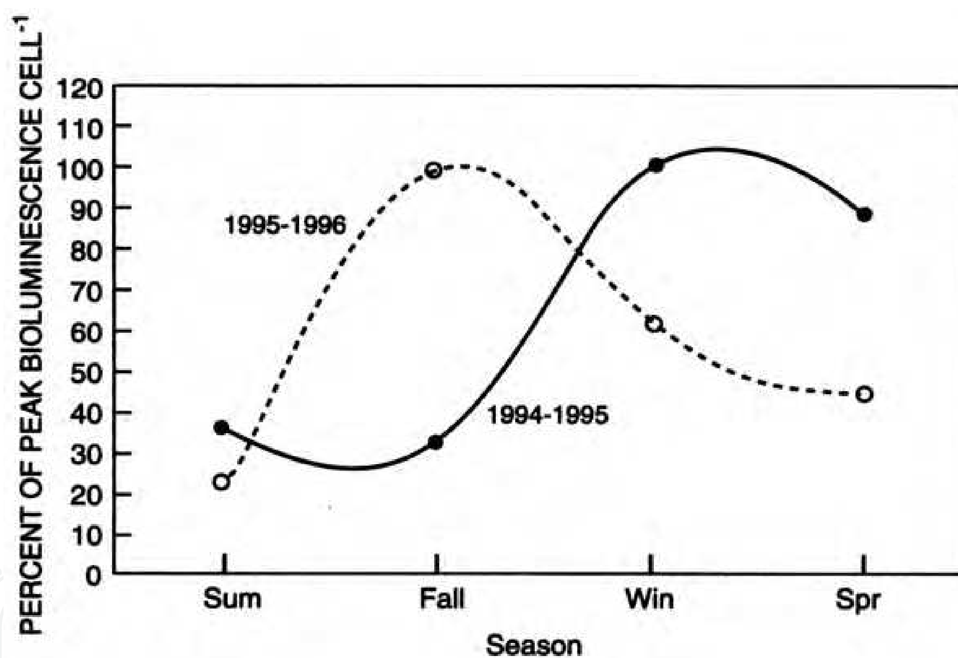

Fig. 6. (b) 1994 to 1996 year comparison of percent of maximum bioluminescence of *Protoperidinium pellucidum* at San Clemente Island.

Maximum bioluminescence in *Pyrocystis noctiluca* was measured in the fall of 1994 and 1995 while minimum bioluminescence was measured in the spring of 1994 and 1996 (Figures 7a-d). Maximum levels of nitrates for both years were in the summer, a time of maximum upwelling in the Southern California Bight. Maximum levels of nitrates preceded maximum bioluminescence for both years (Figure 7c) to possibly explain these seasonal changes in a photosynthetic bioluminescent dinoflagellate. Nitrate levels (μM L^{-1}) were averaged seasonally and compared with bioluminescence (Figure 7b). Both trends exhibit similar amplitudes, i.e., lower levels of nitrates were present in summer and fall of 1994 when

compared to summer and fall nitrate levels in1995. Bioluminescence showed similar trends for the same period (r= 0.859; p < 0.02). Less bioluminescence was measured in cells collected in fall ’94 than in cells measured in fall ’95. Spring bioluminescence was less than fall bioluminescence in *P. noctiluca* for both years (Figure 8d). Spring ’94 bioluminescence was approximately 40% of the maximum of fall ’94 and approximately 65% of maximum for fall ’95.

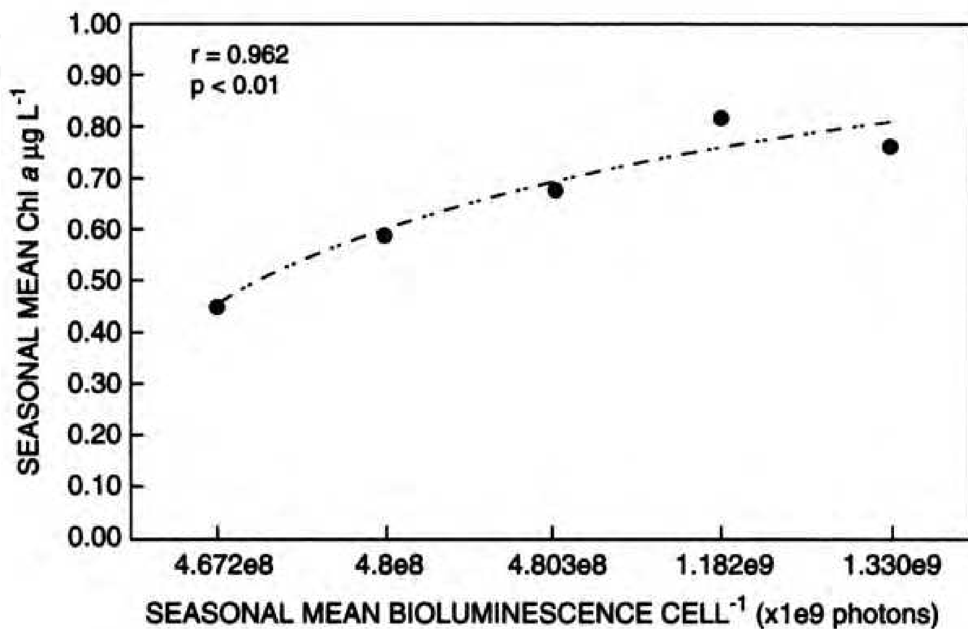

Fig. 6. (c) Correlation of seasonal mean bioluminescence in *Protoperidinium pellucidum* with seasonal mean Chl *a* at san Clemente Island from summer 1994 through sumer 1995.

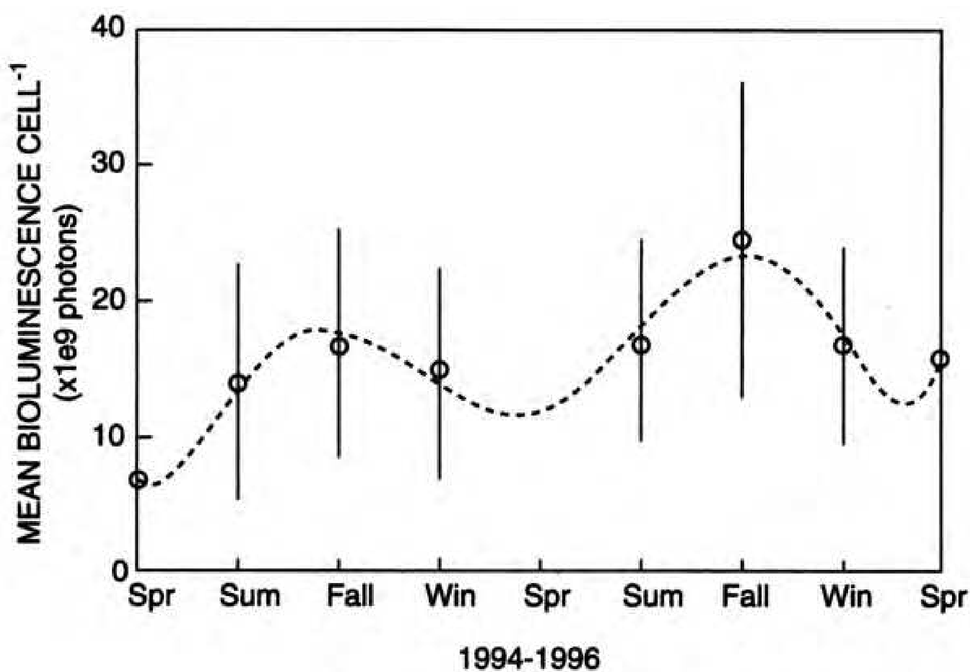

Fig. 7. (a) Seasonal differences in *Pyrocystis noctiluca* bioluminescence at San Clemente Island from 1994-1996. Error bars represent 1 standard deviation of the seasonal means.

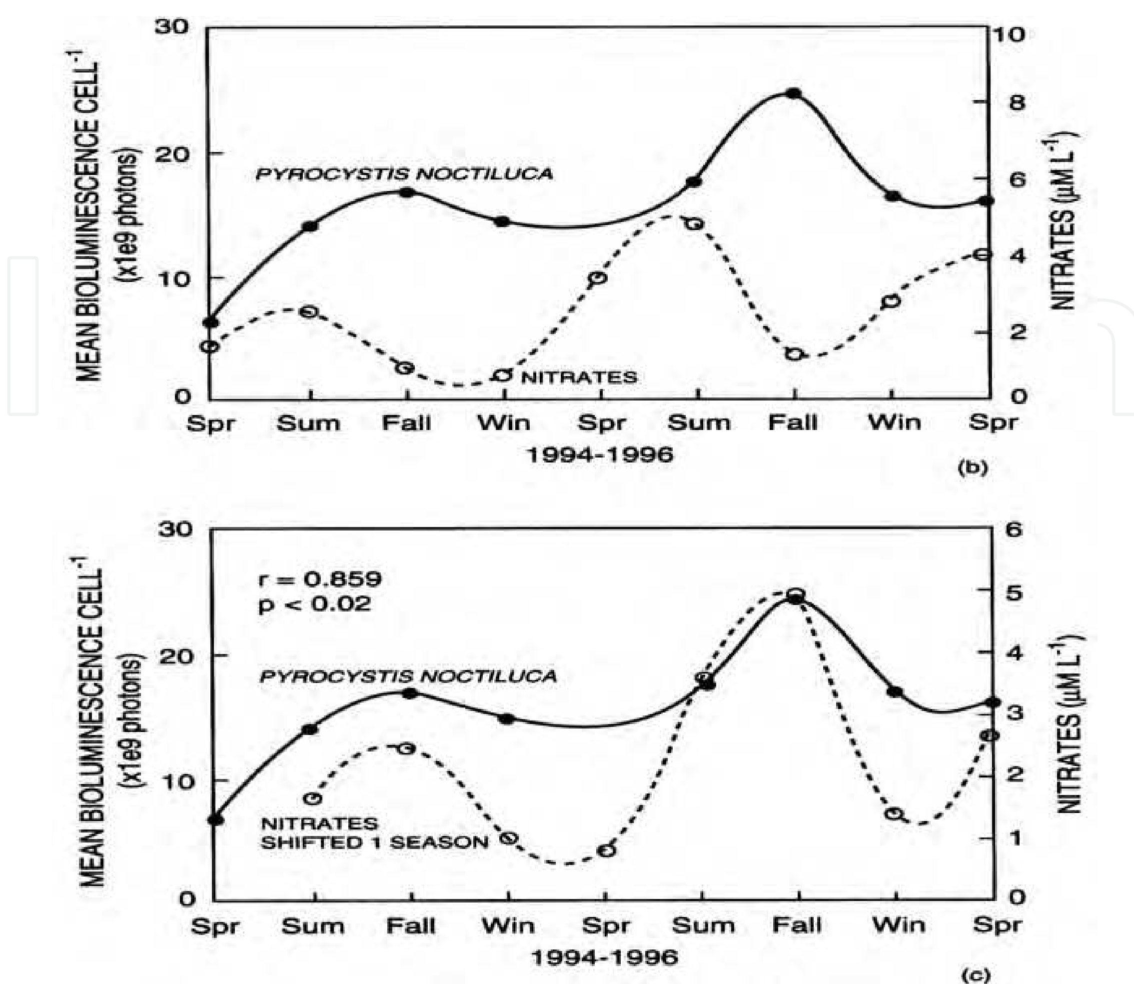

Fig. 7. (b) Seasonal differences in mean bioluminescence of *Pyrocystis noctiluca* and mean nitrates in waters surrounding San Clemente Island from 1994-1996. (c) Correlation of seasonal means of *Pyrocystis noctiluca* bioluminescence and mean nitrates. Mean nitrates were shifted to the right by 1 season to illustrate temporal and magnitude similarities in both trends for both years.

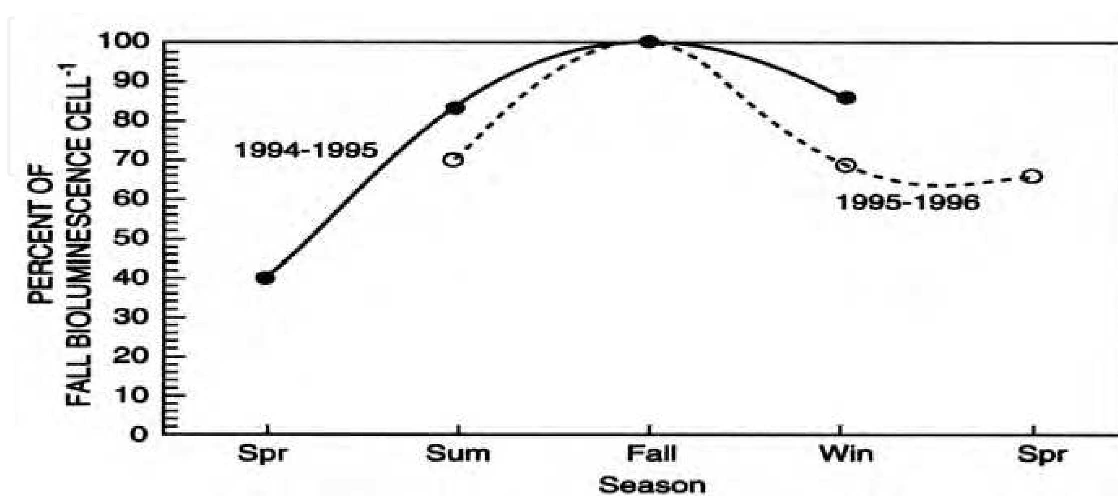

Fig. 7. (d) 1994 to 1996 comparison of percent of maximum bioluminescence of *Pyrocystis noctiluca* at San Clemente Island.

Seasonal and yearly differences of bioluminescence in *Gonyaulax polyedra* and *Ceratium fusus* were also observed (Table 8, 10). While both data sets are incomplete with respect to a continuous record, the data do show a maximum bioluminescence in fall '94 for *C. fusus* and differences in bioluminescence in *G. polyedra* between winter '95 and winter '96.

4. Discussion

The data agree that dinoflagellate bioluminescence has a marked seasonality in the open ocean which is affected by regional environmental events such as upwelling and rainstorms resulting in enhanced terrestrial runoff. Other laboratory observations support the view that nutritional requirements are important in determining bioluminescent capacity (Sweeney 1971). For example, *Protoperidinium* dinoflagellates underwent increases in bioluminescence potential when fed to excess with diatoms (Buskey et al., 1992; Latz 1993) In an earlier study, when the heterotroph *Noctiluca miliaris* was fed with the flagellate *Dunaliella* more light was emitted than from unfed *Noctiluca* (Sweeney 1981). It was also reported that a strain of this dinoflagellate carrying a photosynthetic algal symbiont produced bioluminescence which was proportional to the light intensity at which the symbiont was grown, suggesting a nutritive contribution by the algal symbiont (Sweeney 1981; Sullivan and Swift 1995). Certainly other observations support the view that nutritional requirements are important in determining bioluminescent capacity (Sweeney 1971). Laboratory investigations have also shown that increased irradiance elevates photosynthesis with consequent increased bioluminescence (Sweeney et al., 1959; Sweeney 1981; Swift et al., 1981; Sullivan and Swift 1995).

When cultures of *Gonyaulax polyedra* were maintained in artificial seawater media for periods of up to 37 days, mean bioluminescence decreased by almost a factor of 10 when compared to cells after 5-13 days in culture (Sweeney 1981). Because cell numbers increased throughout the study (from 6,300 cells ml^{-1} to 21,830 cells ml^{-1}), auto-toxicity is not a likely explanation of this significant decrease in bioluminescence, leaving nutrient limitation a possibility. This was tested by Sweeney in the same report with the finding that nitrates and phosphates appeared to enhance cell growth, but not bioluminescence capacity, while only trace levels of iron sequestrine supported maximum bioluminescence and cell growth. At higher concentrations, iron sequestrine appeared to reduce the bioluminescent capacity and cell division in *G. polyedra* (Sweeney 1981). The bioavailability of nutrients and trace metals are often not addressed with respect to impact on different physiological mechanisms (cell division *vs* bioluminescent capacity) within the same cell. Particularly in coastal waters environmental contaminants might complicate interpretation of nutrient effects. Thus bioluminescence enhancement has been observed in toxicity studies using *G. polyedra* incubated for up to 4 days with bay sediment pore waters. Ammonia is commonly found in sediment pore waters and levels of 200-400 μg L^{-1} have been observed to increase light output 3-4 times above controls. The data suggest that the organism may be responding to a readily available increased source of nitrogen, possibly an example of hormesis (Unpublished data, Lapota and Liu 1997).

In the present study, we have observed seasonal trends in nitrates, Chl *a*, and bioluminescence in numerous species of bioluminescent dinoflagellates. Maximum bioluminescence in *Protoperidinium pellucidum* was observed in winter '95 and in fall '96

which might be explained by the availability in the diet of diatoms and *Gonyaulax polyedra* (Figure 7a).

Increased levels of Chl *a* were measured in the winter and spring ’95 and were strongly correlated with increased *P. pellucidum* bioluminescence. Species of *Protoperidinium* are known to graze on *G. polyedra* in laboratory studies (Buskey et al., 1992; Latz and Jeong 1993; Jeong and Latz 1994). Latz (1993) demonstrated the maintenance of *Protoperidinium divergens* growth, survival, and bioluminescence capacity when grazing on a variety of dinoflagellates, but found maximum bioluminescence when the diet was solely *G. polyedra*. The winter ’95 period within the Southern California Bight was characterized by an extensive red tide and extended from Santa Barbara south to San Diego and west to San Clemente Island. *G. polyedra* was the principal dinoflagellate present, reaching concentrations of approximately 16,000 cells l^{-1} in January 1995, although increases in *Protoperidinium* spp. were also observed (Lapota et al., 1997). Heavy rainfall was recorded during this winter period (17-18 inches, as compared with the norm of 5-10 inches) and consequently extensive runoff was observed along the entire southern California coast. Total bioluminescence (photons ml^{-1} $year^{-1}$) was positively correlated with rainfall (inches $year^{-1}$) for a 4 year period in San Diego Bay (1992-1996) (Lapota et al., 1997). Nitrates and trace metals are carried off from land with the runoff into surface waters (Dugdale and Goering 1967). Thus, the *G. polyedra* red tide was probably triggered by extensive runoff including nutrients such as nitrates from this “wet” year which in turn stimulated growth of phytoplankton grazed by *Protoperidinium pellucidum* and other *Protoperidinium* species. Increases in bioluminescence were observed in more than 60% of all rain events from 1992-1996 in San Diego Bay (Lapota et al., 1997). Others have observed these sudden blooms and they often occur in spring or summer following heavy rains that produce nutrient-rich land runoff (Eppley, 1986). The reason for a fall ’95 peak in *P. pellucidum* bioluminescence is unknown but may be due to grazing by *P. pellucidum* on lower numbers of *G. polyedra* and other algal cells. Upwelling and nitrate levels (Figure 8b) were greatest during the summer months and could result in a later increase in photosynthetic biomass in the fall. However, Chl *a* levels were actually low during the period when bioluminescence was high and may indicate previous grazing by *P. pellucidum*. Seasonal mean Chl *a* and mean bioluminescence $cell^{-1}$ were strongly correlated ($r = 0.962$; $p < 0.02$) for 1994-1995 which may suggest that as Chl *a* levels increased, so did bioluminescence $cell^{-1}$ (Figure 7c). These field measurements support previous laboratory studies (Buskey et al., 1992; Latz and Jeong 1993).

Both nitrates and mean bioluminescence $cell^{-1}$ in *Pyrocystis noctiluca* show similar trends temporally and in magnitude (Figures 7b, 7c). Peak levels of nitrates were found in the summer months followed by increases in bioluminescence during the fall months. Nitrate levels were greater in summer ’95 than in summer ’94. Peak bioluminescence was also greater in fall ’95 than in fall ’94. Lagging data comparisons + 1 season for nitrates produced a strong correlation with bioluminescence for the entire 2 year period ($r = 0.859$; $p < 0.02$). That is, lower nitrate levels measured in 1994 correlated with lower bioluminescence in 1994 while greater nitrate levels correlated with an increased bioluminescence (Figure 7c). Peak bioluminescence was also observed to occur in the fall for both years (Figure 7d). The effect of nitrate on bioluminescent capacity within photosynthetic dinoflagellates is unclear, but perhaps may be related to the overall health of the cell and how *Pyrocystis*, *Gonyaulax* spp. and *Ceratium* partition their metabolic resources when nitrate satiated. Others have

observed the photosynthesis-irradiance relation to bioluminescence capacity. That is, cells grown at higher irradiance levels produce more photosynthetic products which may be diverted to the bioluminescence system (Sweeney et al., 1959; Sweeney 1981; Swift et al., 1981; Sullivan and Swift 1995). It is very possible that increased levels of nutrients from upwelling and storm runoff events may override diminished irradiance levels found during the fall and winter months to explain maximum bioluminescence observed in these species.

5. Conclusion

A significant portion of bioluminescence in all oceans is produced by dinoflagellates. The number of bioluminescent species and their relative abundance changes temporally and spatially. There is evidence that dinoflagellates exhibit changes in per cell bioluminescence magnitude which may be attributable to environmental conditions such as light, temperature, and nutrient history. In the present study, photosynthetic and heterotrophic dinoflagellates were collected and tested for bioluminescence on a quarterly basis from 1994-1996 at San Clemente Island, located 100 km off the Southern California coast. Per cell bioluminescence was measured for the phototrophs *Ceratium fusus, Pyrocystis noctiluca, Gonyaulax polyedra* as well as 3 other species of *Gonyaulax*, and in 6 species of the heterotroph *Protoperidinium*. Our data strongly suggests that dinoflagellates have a marked seasonality in the open ocean which may be attributable to regional environmental events such as upwelling and associated winter storm land runoff. We observed correlations between surface (0-50m) nitrates and Chl *a* with bioluminescence in *Pyrocystis noctiluca* and *Protoperidinium pellucidum*. Increased levels of Chl *a* measured in the winter and spring '95 correlated with increased *P. pellucidum* bioluminescence. Both nitrates and mean bioluminescence cell^{-1} in *P. noctiluca* showed similar trends temporally and in magnitude. Peak levels of nitrates were found in the Southern California Bight in the summer months followed by increases in bioluminescence during the fall months. Peak bioluminescence was observed to occur in the fall for both years in *P. noctiluca* whereas peak bioluminescence in *P. pellucidum* was measured in winter '95 and later in fall '95.

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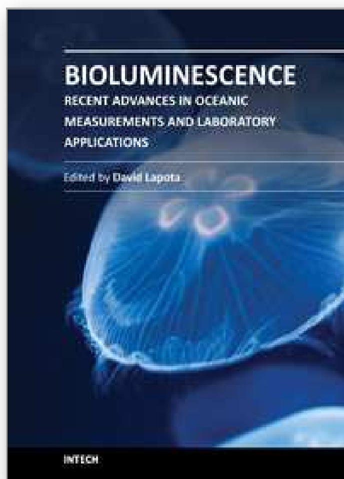

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We now find ourselves utilizing luciferase - luciferin proteins, ATP, genes and the whole complex of these interactions to observe and follow the progress or inhibition of tumors in animal models by measuring bioluminescence intensity, spatially and temporally using highly sophisticated camera systems. This book describes applications in preclinical oncology research by bioluminescence imaging (BLI) with a variety of applications. Chapters describe current methodologies for rapid detection of contaminants using the Milliflex system, and the use of bioluminescence resonance energy transfer (BRET) technology for monitoring physical interactions between proteins in living cells. Others are using bioluminescent proteins for high sensitive optical reporters imaging in living animals, developing pH-tolerant luciferase for brighter in vivo imaging, and oscillation characteristics in bacterial bioluminescence. The book also contains descriptions of the long-term seasonal characteristics of oceanic bioluminescence and the responsible planktonic species producing bioluminescence. Such studies are few and rare.

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