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Bioluminescent Glows of *Cypridina hilgendorffii* in Kavaratti Lagoon, Lakshadweep Archipelago, India

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Abstract

A temporal, nocturnal sampling along Kavaratti lagoon was conducted in order to find organism(s) exhibiting bioluminescence and factors favoring the proliferation of same. Bioluminescence involves oxidation of luciferin in conjunction with luciferase enzyme. None of the phytoplankton species were found to be bioluminescent. Among the total zooplankton species encountered, only one ostracod crustacean *Cypridina hilgendorffii* was found to be bioluminescent. Low nutrient values ($\text{NO}_2 < 4 \mu\text{mol L}^{-1}$, $\text{NO}_3 < 8.254 \mu\text{mol L}^{-1}$, $\text{NH}_4 < 0.92 \mu\text{mol L}^{-1}$, Inorganic $\text{PO}_4 < 0.25 \mu\text{mol L}^{-1}$, $\text{SiO}_4 < 5.93 \mu\text{mol L}^{-1}$) established oligotrophic condition of Kavaratti lagoon. Most of the abiotic parameters have little or no relationship with *Cypridina hilgendorffii*. However Pearson correlation values showed negative relationship with nitrite (-0.366), ammonia (-0.107) and pH (-0.250). Population of *Cypridina hilgendorffii* reached its zenith during high tide hours (19840 nos m^{-3} , 17764 nos m^{-3} , 18370 nos m^{-3}). Hierarchical cluster analysis showed a close linkage between *Cypridina hilgendorffii* and tide. Analysis of variance diagram represented an inverse relationship of *Cypridina hilgendorffii* with phytoplankton species. This could be explained on the basis of predator prey interaction and active scavenging habit of *Cypridina hilgendorffii* during night. Bioluminescent glows of *Cypridina hilgendorffii* serves a variety of functions like illumination, counter illumination, intra specific communication, and defence mechanism from predators. High tide associated with optimum temperature and salinity favoured growth of *Cypridina hilgendorffii*.

Keywords: *Cypridina hilgendorffii*, Bioluminescence, Tide, Kavaratti

1. Introduction

Water quality monitoring along Kavaratti coast is peculiar in the sense as the island contributes no inlet in to the surrounding lagoon. Kavaratti is one of the coral Islands of Lakshadweep archipelago. In terms of productivity, it is known that coral atolls are highly significant (Dhargalkar and Sheikh, 2000) [13]. Prevalence of oligotrophic condition in Kavaratti Island is cited in most of the literatures. Oligotrophic waters of Kavaratti Island exhibits high level productivity by coral atolls (Raikar and Wafer, 2006) [14]. Phenomenon of bioluminescence in Kavaratti lagoon is attributed to either phytoplankton or zooplankton species. Temporal monitoring of lagoon waters and analysis of phytoplankton and zooplankton groups explains phenomenon of bioluminescence at Kavaratti beach. The bioluminescence could be easily seen at Kavaratti beach during night hours with varying degrees. Hourly sampling programme along lagoon waters describes hydro chemical, physic chemical and marine biological parameters. Primary objective of the study is to find the causative organism for bioluminescent phenomenon. Prime importance was given to the factors responsible for dominance of species exhibiting bioluminescence and their interaction in lagoon waters. Abundance of sea grass beds establishes the sporadic level of productivity in Kavaratti atoll (Nobi *et al*, 2010). Though bioluminescence is a common phenomenon in marine ecosystem, only certain planktons are considered to be bioluminescent (Cussatlegras and Le Gal, 2005) [11]. Mechanical stimulation triggers light emission in bioluminescent planktons (Forey and Patterson, 2006) [8]. The chemistry of bioluminescence involves the oxidation of luciferin by luciferase enzyme (Haddock *et al*, 2010) [10]. Bioluminescence has been found across wide range of organisms from bacteria to fishes and dolphins. Present study also intended to explain temporal variation of plankton species responsible for bioluminescence.

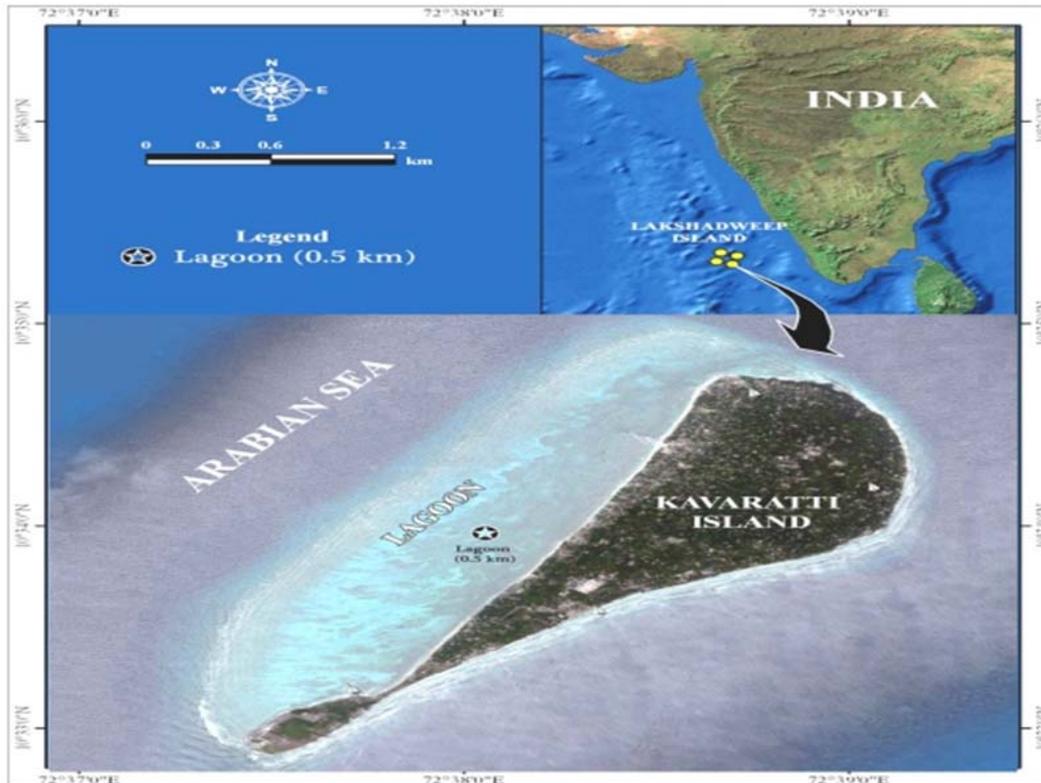


Fig. 1: Study Area



Fig. 2: Microscopic picture of *Cypridina hilgendorffii*

2. Materials and Methods

The sampling location was selected at a distance of 0.5 km from the beach (10°34'21" N, 72°38'09" E) (Fig.1). A post monsoon cruise (October, 2015) on Ocean Research Vessel Sagar Manjusha confirmed bioluminescence phenomenon in Kavaratti lagoon. Five hour sampling programme for abiotic parameters, phytoplankton and zooplankton were conducted at an interval of three hours viz. 18:00 hrs, 21:00 hrs, 00:00 hrs, 03:00 hrs and 6:00 hrs. Atmospheric and water temperatures were recorded using standard mercuric thermometer. Secchi disc was used for measuring transparency. Salinity was measured by Mohr's method. Water samples for nutrient analysis, Dissolved Oxygen were collected by Niskin sampler. Dissolved Oxygen values were measured using water quality Analyzer (Eutech probe PCD 650). Nutrient analysis were Carried out spectrophotometrically (Varian 50 UV – Visible

spectrophotometer) (APHA, 2005) [2].

Phytoplankton samples were collected by filtering 100 liters of water through 20 μ m plankton cup. The crude filtrate was preserved with 4% neutralized formalin solution (Tomas, 1997) [3]. For zooplankton collection, vertical and horizontal hauling was performed using a bongo net (General Oceanic). Quantitative analysis of phytoplankton and zooplankton were made under a phase contrast binocular microscope (Olympus CX 41) using Sedgwick Rafter counting chamber

3. Result and Discussion

3.1 Abiotic components

Nocturnal sampling data of Kavaratti lagoon represented two tidal periods. Low tide at 18:00 hours and morning 6:00 hours. While rest of the sampling was taken during high tide. Transparency was considerably low ranging from 0.9 to 1.7 m. Atmospheric and water temperature showed very low variation. Atmospheric temperature ranged from 26.5 to 27.0 °C. While lagoon waters recorded a narrow range of temperature from 26.1 to 26.9 °C. pH was found to be slightly alkaline (<8). Salinity values showed an obvious fluctuation during morning 6:00 hours, where the value was considerably higher (29.43 psu) as compared to other sampling periods. Dissolved oxygen showed a decreasing trend from evening 18.00 hours to morning 6.00 hours (5.25 to 3.85 mg L⁻¹). Assessment of major nutrients depicted a primary picture of oligotrophic nature of lagoon waters. Nitrite (3.06 – 3.91 μ mol litre⁻¹), nitrate (5.19 – 7.7 μ mol L⁻¹), ammonia (0.20 – 0.92 μ mol L⁻¹), inorganic phosphate (0.15 – 0.25 μ mol L⁻¹) and silicate (2.73 to 5.93 μ mol L⁻¹) values establishes lack of entrainment of nutrients to lagoon as the Island doesn't possess any dynamic bodies (Table 1).

Table 1: Table showing abiotic parameters

	18.00 hrs	21.00 hrs	00.00 hrs	03.00 hrs	06.00 hrs
Tide	Low (0.56 m)	High (1.21 m)	High (1.34 m)	High (1.41 m)	Low (0.72 m)
Transparency (m)	0.9	1.7	1.7	0.9	0.9
Atmospheric Temperature ($^{\circ}\text{C}$)	26.60	26.50	27.00	26.50	27.00
Water Temperature ($^{\circ}\text{C}$)	26.70	26.20	26.90	26.90	26.10
pH	8.02	7.81	7.80	7.96	7.68
Salinity (psu)	26.08	27.07	26.45	26.42	29.43
Dissolved Oxygen (mg L^{-1})	5.25	4.90	4.90	4.90	3.85
Nitrite (NO_2) ($\mu\text{mol L}^{-1}$)	3.12	3.03	3.06	3.76	3.91
Nitrate (NO_3) ($\mu\text{mol L}^{-1}$)	6.17	8.25	4.49	7.70	5.19
Ammonia (NH_4) ($\mu\text{mol L}^{-1}$)	0.92	0.52	0.20	0.83	0.34
Total Nitrogen (TN) ($\mu\text{mol L}^{-1}$)	28.90	26.88	28.83	26.42	23.60
Inorganic Phosphorous (IP) ($\mu\text{mol L}^{-1}$)	0.15	0.25	0.13	0.17	0.21
Silicate (SiO_4) ($\mu\text{mol L}^{-1}$)	3.46	4.64	5.93	2.73	3.38

3.2 Phytoplankton and Zooplankton Analysis

In order to detect the organism(s) causing phenomenon of bioluminescence, both phytoplankton and zooplankton samples were analyzed as both class might include species causing bioluminescence. A total of twenty five phytoplankton species have been encountered in lagoon waters. They were classified under five classes. Dinophyceae contributed maximum number of species, while ulvophyceae and cyanophyceae contains minimum number of species. Bacillariophyceae comprises *Bellerophon malleus*, *Biddulphia reticulata*, *Cosinodiscus gigas*, *Eucampia zoodiacus*, *Rhizosolenia alata*, *Skeletonema costatum* and *Triceratium favus*. Dinophyceae recorded eight number of species viz. *Ceratium furca*, *Ceratium tripos*, *Ceratium macroceros*, *Ceratium pentagonium*, *Ceratium trichoceros*, *Peridinium steinii*, *Prorocentrum micans* and *Dinophysis caudata*. Chlorophyceae included green algae such as *Actinastrum sp.*, *Chlamydomonas sp.*, *Volvox sp.*, *Paediastrum sp* and *Oedogonium sp.* Ulvophyceae contributed only two species viz. *Cladophora sp.* and *Ulothrix sp.* Class cyanophyceae include two species *Oscillatoria sp.* and *Trichodesmium erythreum*. *Trichodesmium erythraeum* was found to be dominating species all over the sampling hours followed by *Bellerophon malleus*. Total phytoplankton counts were found to be decreasing from evening 18.00 hours ($22660 \text{ cells L}^{-1}$) to morning 6.00 hours ($6300 \text{ cells L}^{-1}$). Among encountered phytoplankton species none of them was found to be bioluminescent. Zooplankton analysis recorded an abundance of species numbering up to fifty. Copepodans dominated in

number of species. Other classes include foraminiferans, malacostraca, ciliata, chaetognaths, ostracoda, ichthyoplankton and larvae. Among these an ostracodan crustacean *Cypridina hilgendorfi* was found to be dominating throughout the sampling hours (Fig. 2). The species is well known for its bioluminescence phenomenon (Fig. 2). Numerical density of *Cypridina hilgendorfi* was low at evening 6:0 hours (5028 nos m^{-3}) and high at night 21:00 hours (19840 nos m^{-3}). During night hours of sampling i.e. 21:00 hours, 0:00 hours and 3:00 hours, *Cypridina hilgendorfi* outnumbered rest of the other zooplankton species. It is important to note that, during these hours high tide condition prevailed. Pearson correlation data describes low significant correlation among *Cypridina hilgendorfi* and abiotic components including nutrients. Further, pH, ammonia (NH_4) and nitrite (NO_2) shows negative correlation (values = -0.250, -0.107, -0.366 respectively). Hierarchical cluster analysis reports a significant linkage between *Cypridina hilgendorfi* and tide (Fig.3). High tide favours abundance of *Cypridina hilgendorfi* at lagoon near shore, provided optimum atmospheric temperature and silicate concentration was present. As the season of sampling is post monsoon, the temperature remained humid and optimum. Other abiotic parameters showed more or less negative relation with *Cypridina hilgendorfi*. ANOVA (Analysis of Variance) diagram demonstrates how phytoplankton population varied according to abundance of *Cypridina hilgendorfi*. Inverse relationship of phytoplankton groups to *Cypridina hilgendorfi* explains the it's feeding habit (Fig.4).

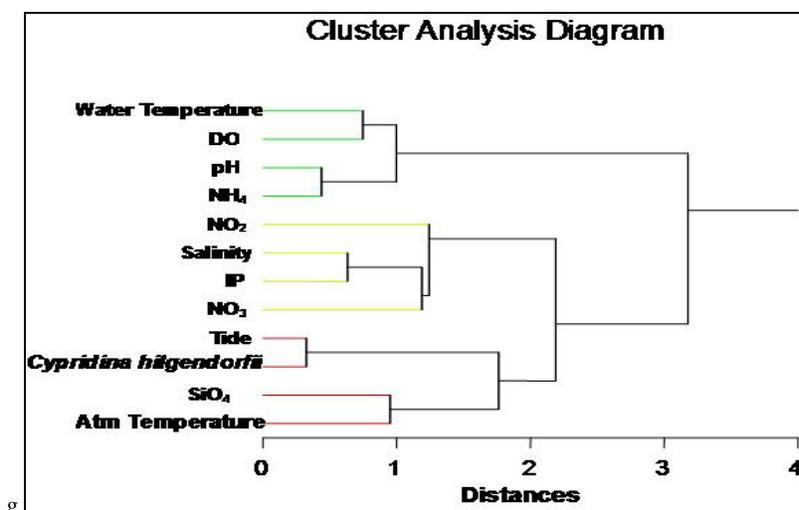


Fig. 3: Cluster showing linkage of *Cypridina hilgendorfi* with abiotic parameters

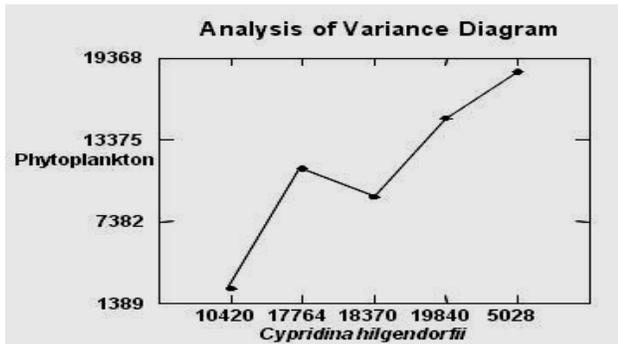


Fig. 4: ANOVA diagram showing variation of phytoplankton against *Cypridina hilgendorffii*

Inverse relation of *Cypridina hilgendorffii* to phytoplankton groups could be attributed to predator prey mechanism in lagoon waters (Haddock *et al.*, 2010) ^[10]. The overabundance of *Cypridina hilgendorffii* could be easily seen during night as the beach is bedded with blue sparkling light. Ostracod crustaceans like *Cypridina hilgendorffii* generate light in response to mechanical stimuli (Rohr *et al.*, 1998) ^[6]. During high tide hours, the number of *Cypridina hilgendorffii* exceeded other zooplankton groups. This establishes the fact that, a mechanical stimulus during high tide brings *Cypridina hilgendorffii* to the surf waters of lagoon. Oxidation of light emitting molecule luciferin by luciferase enzyme occurs during bioluminescence by *Cypridina hilgendorffii*. Though a little known about it, it was established that ostracod crustaceans like *Cypridina hilgendorffii* synthesize luciferin from arginine, isoleucine and tryptophan (Kato *et al.*, 2007) ^[11]. The illumination in lagoon waters by *Cypridina hilgendorffii* explains many phenomenon such as intra specific communication (Morin and Cohen 2010) ^[5], illumination and counter illumination (Harper and Case, 1999) ^[9]. Mating of crustaceans like *Cypridina* occurs during post monsoon season where medium and high saline conditions are available (Pillay and Nair, 1971) ^[7]. Our study supported this finding as the number of *Cypridina hilgendorffii* was enormous during sampling period (post monsoon) where bioluminescence by these organism was crucial for mating. Present study suggest the little role of nutrients for the proliferation of *Cypridina hilgendorffii*, rather tidal impact and optimum salinity proved to be the major factors affecting the dominance of *Cypridina hilgendorffii*. Proliferation of *Cypridina hilgendorffii* resulted in reduction of phytoplankton cell density from 18.00 hours to 6.00 hours evening. This was due to voracious feeding of *Cypridina hilgendorffii* during night hours. *Cypridina hilgendorffii* became active scavenger during night and they escapes from predators by throwing blue luminescent puff (Wilson and Hastings, 2013) ^[12]. In nutshell, proliferation of *Cypridina hilgendorffii* with its bioluminescence mechanism explains their mode of survival in oligotrophic lagoon waters.

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