

## METHODOLOGY:

### **Culturing of *Noctiluca scintillans*:**

*N. scintillans*, a mixotrophic, large dinoflagellate (~600–1000 µm in diameter), harboring thousands of photosynthesizing endosymbionts, which swim freely within its large central vacuole, was isolated from the open waters of Arabian Sea.

It is maintained in the laboratory in f/20 medium without silicate (f/20 – Si) following the recipe prescribed in <https://ncma.bigelow.org/cms/index/index/> at 26°C and exposed to a light intensity of approx. 200 m E m<sup>-1</sup> sec<sup>-1</sup> for a photoperiod of 11.5 h light:12.5 h dark. This culture is maintained without extraneous food.

**Culturing of symbiotic flagellate of *Noctiluca*:** The endosymbionts of *N. scintillans* which were previously classified as *Pedinomonas noctilucae* (~10 µm in diameter), have been reclassified by us as *Protoeuglena noctilucae* using molecular methods (Wang *et al.*, 2016). *P. noctilucae* is being maintained in the laboratory under the same conditions listed above for *N. scintillans*.

**Cell Counts:** As the cells of *N. scintillans* are large, counts are performed visually as follows. 5 ml of the media is drawn from a culture bottle containing *N. scintillans*, using a sterile plastic transfer pipette and the number of individual cells in the pipette is counted. This process is repeated 5 times to ascertain accuracy and the average of 5 counts determines the cell count of *N. scintillans*. Counts of *P. noctilucae* are undertaken using an inverted light microscope (generally 20x magnification) and with a standard hemacytometer as described in Andersen (2005).

Cell counts were also undertaken using FlowCAM<sup>®</sup>, which is an imaging particle analyzer that combines imaging and laser light to detect particles and capture their images which are then identified from pre-built libraries (Poulton, 2016).

Growth Rates (µ) of *N. scintillans* and *P. noctilucae*, measured as the change in cell number over time, are computed using the formula:

$$\mu = [\ln(Nt1/Nt0)]t^{-1}$$

where Nt0 = concentration of cells at time 0 (cells ml<sup>-1</sup>),

Nt1 = concentration of cells at time 1 (cells ml<sup>-1</sup>), and

t = duration of each experiment in days.

### **Chlorophyll *a* (Chl *a*):**

Chl *a*, an indicator of phytoplankton biomass was measured fluorometrically (Holm-Hansen and Riemann., 1978) using a Turner Designs Trilogy Laboratory Fluorometer<sup>®</sup> to estimate endosymbiont biomass. The pigment was extracted in acetone after the *N. scintillans* cells or the endosymbiont culture solution was filtered through 0.7 µm Whatman<sup>®</sup> GF/F filters.

### **Photophysiology of endosymbionts using Variable Fluorescence:**

A comprehensive suite of photosynthetic and physiological characteristics of photosynthetic endosymbionts in *N. scintillans* was measured using a Fluorescence Inductance and relaxation (FiRE) technique in a mini-FiRe<sup>®</sup> benchtop instrument (Gorbunov and Falkowski, 2004; Park *et al.*, 2013). This active fluorescence method is a noninvasive technique to rapidly assess the photosynthetic physiology of phytoplankton and provides parameters that characterize photosynthetic light-harvesting processes, photochemistry in Photosystem II (PSII), and the photosynthetic electron transport rates (ETR) down to

carbon fixation. It also provides  $\sigma_{PSII}$ , the cross section of PSII, which is a measure of the size light-harvesting antenna system associated with PSII. Using these parameters, the photosynthetic ETR per PSII Reaction Center (RC), can then be calculated (Park et al., 2013) which is related to the rate of primary production or carbon fixation (Gorbunov and Falkowski, 2004). FIRE, also provides ETR measurements over a range of light levels using an Actinic Light Source (ALS). Curves thus obtained, with ETR rates as a function of irradiance provide parameters such as maximum ETR (ETR<sub>max</sub>), initial light-limited ETR and saturation irradiance  $E_k$  (Platt et al., 1980).

Usually, about 10 cells *N. scintillans* are carefully picked with transfer pipettes, washed in sterile Filtered Seawater (FSW) and suspended in glass tubes containing sterile FSW. The glass cuvette is inserted into the compartment of FIRE and the ALS is placed on top of the holder. The length of the multi-turnover flash (MTF) is set to 100 ms, the number of pulses in the relaxation sequence (MTRP) is set to 40, while the maximum PAR for the ALS is set to 1200  $\mu\text{mole}/\text{m}^2/\text{s}$ . The number of PAR steps is set to 10, and the time delay in which the ALS is on prior to data acquisition is set to 5 s. To acquire the parameters described above the data is further processed with a program supplied with the instrument.

Depending on the presence or absence of photoinhibition two different models of (Platt et al., 1980) are fitted to the ETR data. All curve fitting is done using MATLAB 2016b routines.

#### **Intracellular Ammonium Concentrations:**

$\text{NH}_4^+$  accumulation in *N. scintillans* cells is measured by placing generally 5-10 FSW washed cells into 15 mL plastic centrifuge tubes containing 10 mL Milli-Q distilled water. Several batches which serve as replicates are vortexed, and frozen at  $-80^\circ\text{C}$  prior to analysis. Ammonium is measured colorimetrically with the Turner Designs Trilogy laboratory fluorometer according to Holmes et al. (1999).

#### ***N. scintillans* Grazing and Ingestion rates:**

A known number of *N. scintillans* cells (approx. 50) are placed in cleaned 250 mL polycarbonate bottles filled with 200 mL of sterile f/20 medium. Different prey suspensions of phytoplankton isolated from the Arabian Sea are prepared by growing them in ideal conditions prescribed by the Provasoli-Guillard National Center for Marine Algae and Microbiota (NCMA) <https://ncma.bigelow.org/> to obtain healthy cells in exponential growth. Small volumes of the phytoplankton cultures are added as prey to *N. scintillans*. Some bottles where no food is provided serve as controls. All bottles with prey and predator as well as controls with only predator are grown in incubators at  $26^\circ\text{C}$  with a photoperiod of 11.5 h light: 12.5 h dark for 15-25 days on dimensional gyratory rockers to gently agitate the cells and encourage natural interaction between predator and prey. After a period of acclimatization, samples are drawn every 3-4 days for *N. scintillans* counts, endosymbiont Chl  $a$ , prey counts, and variable fluorescence based photophysiological measurements. Growth rates are measured as described in the Cell Counts section and Ingestion rates are estimated according to (Hansen et al., 2004).

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