Title: Survival in the dark: strategies adopted by Tetraselmis indica (Chlorodendrophyceae, Chlorophyta)

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Background
Phytoplankton naturally experiences darkness varying from hours to years as a result of diurnal cycles, burial in sediments, deep ocean mixing, and polar winter. Tetraselmis species, a deep diverging lineage of core Chlorophyta, is known to form a bloom in the marine environment. However, the exact cause of the formation and collapse of this bloom is unknown. After a bloom collapses into the aphotic zone, cell death may occur if not upwelled before a species-specific survival time. Hence, the strategies of withstanding darkness can determine the survival and competitive success of species. Even though Tetraselmis species are known to survive in darkness, the physiological processes regulating its adaptation to prolonged darkness has not been attempted. In order to address this problem, a laboratory experiment was carried out with Tetraselmis indica as a model organism.

Methods
Exponentially growing Tetraselmis indica maintained in f/2-Si media was subjected to two different conditions. One set was maintained under 12:12 light:dark condition whereas the other set was subjected to complete darkness for a period of 6 months. The growth potential of dark-adapted cells was tested regularly by transferring it to initial light conditions (12:12 light:dark). The morphological changes were observed using an Olympus BX 53 light microscope at 1000× magnification and analysed by Q-Capture Pro7 cell imaging software. The cell abundance was enumerated using a FACS Verse flow cytometer (BD Bioscience-US). The metabolic activity and neutral lipids were assessed using fluorescein diacetate and Nile Red, respectively. For dissolved organic carbon (DOC) analysis, high-temperature combustion oxidation method (680°C) on a TOC–L carbon analyser (Shimadzu, Japan) was employed. The particulate organic carbon (POC) and particulate organic nitrogen (PON) were analysed using an elemental analyser (Flash EA; Thermo) whereas carbohydrates were determined spectrophotometrically.

Results
In the dark condition, no growth in terms of cell abundance was observed except for day 1. However, in the 12:12 light:dark condition, T. indica followed a typical sigmoid growth curve. The occurrence of thick-walled cells (type 1) and thin-walled cells with the condensed chloroplast (type 2) in light-dark and dark conditions respectively indicated differential resting cell formation. The DOC remained nearly constant throughout the dark period signifying T. indica did not favour heterotrophy. The metabolic activity was reduced in the
darkness whereas in the light:dark condition it increased till the stationary phase. The biochemical composition (neutral lipids, carbohydrates, POC, and PON) also varied significantly in both conditions. Upon re-illumination of dark-adapted cells, the lag phase lengthened from 2 to 28 days when darkness period increased from 8 to 182 days, respectively. However, this negative influence on growth was compensated by an increase in growth rate from 0.35 to 0.95 $d^{-1}$.

**Conclusion**

Our work suggests that *T. indica* can survive a darkness period of 6 months and revive on re-illumination. Possibly, the long dark survival was facilitated by the (i) reduction in catabolic activity and (ii) distinct resting cell formation. These darkness survival strategies of a common green alga, a lineage known to include essentially strict autotrophs, confirms its flexible physiology to adapt to adverse conditions. Thus, the dark adaptability of this ‘hidden flora’ points towards their ecological role in nature. This resurgence capability of *T. indica* after 6 months of dark exposure and distinctive resting cell formation indicate a differential population sustenance mechanism.

**Keywords:** *Tetraselmis indica*; growth; metabolic activity; resting cell