

**Fate of *Enteromorpha flexuosa* (Wulfen) J. Agardh and its spores in darkness:  
Implications for ballast water management**

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**Abstract**

Effects of darkness on the filaments and spores of a marine alga, *Enteromorpha flexuosa* (Wulfen) J. Agardh was evaluated through incubation experiments. Sporulation in the algal filaments was observed even after dark exposure of 108 days and their effective period of release ranged from 1 to 7 days. Spores incubated in the dark for 51 days displayed a linear decrease in the survival and germination rate. This is coupled with a linear increase in the effective period of germination for example, 35% of spores survived after exposure to 51 days and these required 21 days for germination. These results indicate the potential of their successful translocation through ballast water of ships, a major vector initiating marine bioinvasion.

*Keywords:* *Enteromorpha flexuosa*; Ballast water; Dark; Light; Spores; Filament

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## 1. Introduction

Ships carry seawater in their ballast tanks for stability and maintenance of trim. This water contains a gamut of organisms and their propagules (Medcof 1975; Carlton and Geller 1993; Drake et al., 2002). In the wild sustenance of these propagules is controlled by natural events. However, once in the dark ballast tanks, their ambient environment is altered. The information on the fate of such organisms during extended incubation in dark and their potential to thrive in new environment is of great interest from the perspective of ballast water management issues (Drake et al., 2002; Gollasch et al., 2000; Lavoie et al., 1999; Williams et al., 1988; Wonham et al., 2001). Generally it is believed that macroalgae disintegrate in a situation where dark conditions prevail and pose less risk of being introduced via ballast waters than hull fouling. If, nonetheless, macroalgae succeed in surviving in ballast tanks, this opens a new potential for dispersal. In this study we evaluated the marine alga *Enteromorpha flexuosa* (Wulfen) J. Agardh an opportunistic species and known to grow on a variety of man-made structures (Evans and Christie 1970; Callow and Evans 1977). It is tolerant to a wide range of environmental conditions and can even grow on antifouling paints containing biocides (Callow 1986). The genus *Enteromorpha* owns importance in ballast water management issues as it has been recommended as one of the surrogate species for evaluating performance of any ballast water treatment technology (IMO, MEPC\ 52\INF-5).

## 2. Materials and methods

*Enteromorpha flexuosa* (Wulfen) J. Agardh, which is abundantly found in Goa (India), was used as a candidate material for the present experiment. The alga was collected from the intertidal rocky area of Vagator, Goa, India and brought to the laboratory in a plastic container in seawater. It was cleaned thoroughly to remove any loosely held material including organisms and used immediately for experimental purpose.

### 2.1. Effect of darkness on the algal filaments (Experiment –1)

A known quantity (50 g wet weight) of the algal filaments were suspended in a opaque tank of 50 liters capacity containing filtered (10  $\mu$  mesh) seawater (35 psu) collected from the same site. The tank was covered with an opaque lid and thick black cloth to avoid entry of light. The water within the tank was unchanged till the end of the experiment.

Sub-sampling of the filaments of known quantity (1 g wet weight) from the tank was done at 35 times during incubation duration of 108 days. Utmost care was taken to avoid any entry of light inside the tank while sub-sampling. The Sub-sampled algal filaments were then suspended in the glass beakers (500 ml) with filtered seawater and exposed to 12h:12h natural light:dark photoperiod (PAR, 900 – 1200  $\mu\text{mol. m}^{-2}. \text{s}^{-1}$ ). Clean glass slides were kept at the bottom of the beaker for collection of spores (Kuroji and Sato, 1967). Slides were also kept at the bottom of the dark tank to observe any release of spores. The slides were removed after every 24h and observed under the microscope for the presence of spores. The water in the beaker was replenished daily to avoid any stagnation effect.

A control experiment was run parallel to the above where *E. flexuosa* was suspended in natural light:dark conditions and monitored for the release of spores as above. It was observed that the spores were released on the ninth day leaving all the mother cells empty. Since the data point was single it is not shown. Water parameters such as temperature, salinity, dissolved oxygen and algal chlorophyll (Chl. *a*) were monitored from the dark tank periodically following standard methods (Parsons et al., 1984)

## 2.2. Effect of darkness on the spores (Experiment – 2)

The experimental set up was almost similar as that of experiment-1 above. Now, the spores of *E. flexuosa* were exposed to darkness for 51 days. Spores were collected on series of slides following procedures of Suto (1952) and Kuroji and Sato (1967). The settled spores on each slide were quantified to record the initial density. All the slides were then suspended together in a dark tank containing filtered seawater. Sub-sampling of these slides was done on predetermined days for examination. The slides were removed in triplicate, labeled and spores were enumerated once again. These slides with spores were then transferred into glass beakers (250 ml) containing filtered seawater (no additional nutrients) and maintained at 12h:12h light:dark photoperiod. The slides were then observed under a compound microscope on a daily basis to study the germination of spores and these values were expressed as percent germination.

## 2.3 Statistics

Influence of darkness on the algal filaments, survival of spores and germination of spores were evaluated by power or linear regression analysis (Sokal and Rohlf, 1981).

## 3. Results

### 3.1. Environmental conditions within the dark tank

The water temperature inside the dark tank ranged between 30 to 34 °C. The salinity of the water within the tank exhibited a marginal increase during the period of observations (34.5 to 35.0 psu). The dissolved oxygen concentration at the beginning of the incubation was 4.59 mg l<sup>-1</sup> and decreased to 2.41 mg l<sup>-1</sup> at the end of day 51. The Chl. *a* concentration of *Enteromorpha* decreased from 861 µg g WW<sup>-1</sup> (STD=±96; n=3) on initial day to 53 µg g WW<sup>-1</sup> (STD=±4; n=3) on final day.

### *3.2 Effects of total darkness on the algal filaments*

The filaments exposed to total darkness were able to bear spores upto 108 days and their release was evident only when transferred to light:dark condition. The effective period for spore release increased significantly with the period of incubation ( $r = 0.78$ ,  $p \leq 0.001$ ; Fig. 1). It was on the first day for 4 days incubation, whereas after 108 days incubation release took place on the 5<sup>th</sup> day. In contrast, the filaments in the control tank released all their spores during beginning of the 2<sup>nd</sup> week, leaving all the mother cells empty. The released spores were found to germinate in seawater when exposed to light:dark conditions.

### *3.3 Effects of total darkness on the spores*

The density of spores decreased significantly with prolonged exposure to darkness ( $r = 0.94$ ,  $p \leq 0.001$ , Fig 2a), survival decreased from 92% after day one to 35% after day 51 and the corresponding rate of germination was 53% and 18%, respectively (Fig.2a).

The spores exposed to 51 days of darkness exhibited germination after 21 days, whereas, at the beginning of the incubation germination has occurred within a week (Fig. 2b).

## **4. Discussion**

Firstly, disintegration of filaments in the dark took a longer time as compared to the control (light:dark exposure) instead of nine days, dark exposure delayed spore release to even over 100 d in a part of the thalli. This prolonged perseverance of the algal filaments can possibly be attributed to a reduced light-induced fracture of the outer wall of the mother cell, which should result in release of the spore. Strasser et al., (1970) reported a similar pattern in *Chlorella*.

In total darkness the spores were kept in the mother cells for 108 days with no evident release. However, when exposed to a light:dark cycle, subsequent to dark incubation, release of spores was observed. Furthermore, the effective period of germination was prolonged, suggesting an overall dormant survival in the dark. The spore germination experiment (Exp.2) did indicate that about 6% of the original inoculum was capable to survive for 51 days. We therefore conclude that algal thallus may well survive over 100 d and that spores can do so for some 50 d. Effectively, modern travel from continent-to-continent is in the order of a few weeks (Carlton and Geller 1993). Thus *E. flexuosa* (Wulfen) J. Agardh must be considered, to have the capacity of inter continental passage in ballast water.

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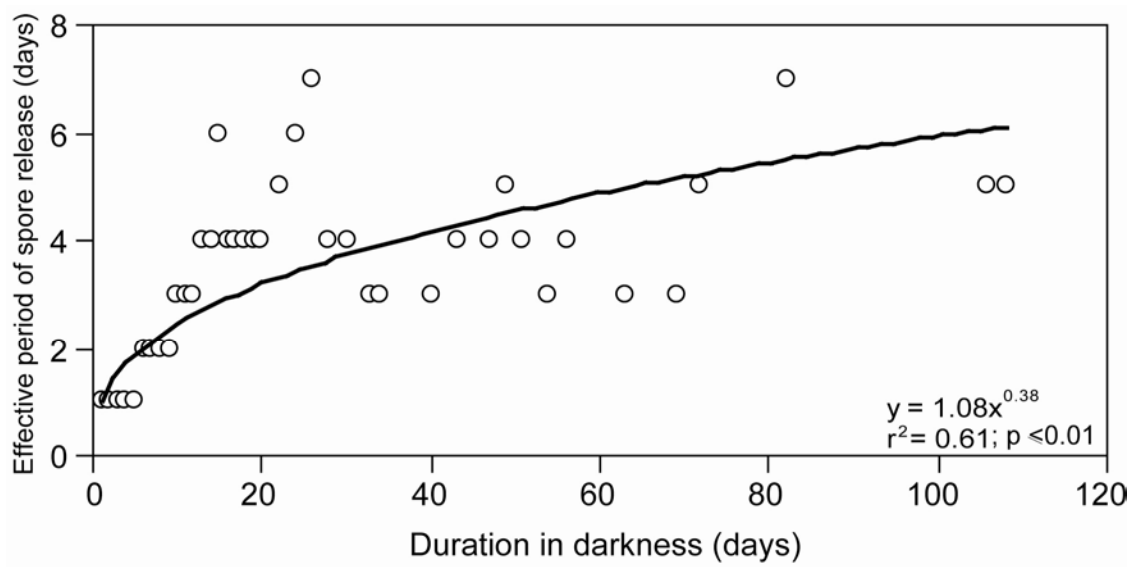


Fig. 1. Influence of darkness on the effective period of spore release from *Enteromorpha flexuosa* algal filaments.

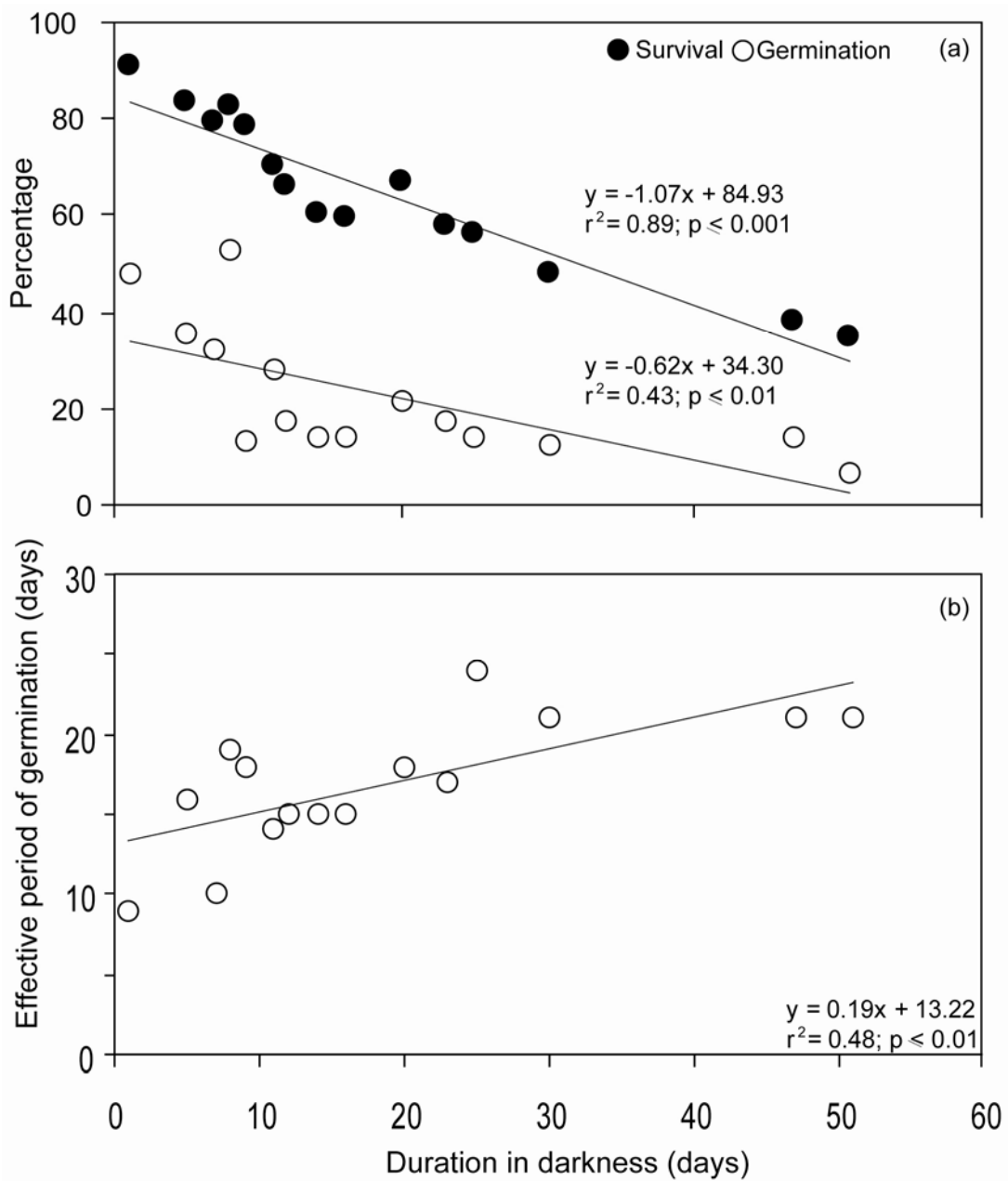


Fig. 2. Effect of dark incubation on the (a) survival and germination and (b) effective period of germination of spores.