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Encystment, Excystment and Photosynthetic Activity in the Temporary Cysts of *Amphidinium carterae*

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ENCYSTMENT, EXCYSTMENT AND PHOTOSYNTHETIC ACTIVITY IN THE TEMPORARY CYSTS OF *AMPHIDINIUM CARTERAE*

by

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ABSTRACT

ENCYSTMENT, EXCYSTMENT AND PHOTOSYNTHETIC ACTIVITY IN THE TEMPORARY CYSTS OF AMPHIDINIUM CARTERAE.

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Although the formation of temporary cysts has been documented in several papers, the physiology of these stages has remained obscure. Research dealing with dinoflagellate resting stages has primarily targeted sexual cysts. Accordingly, formation, germination and photosynthetic activity in the temporary cysts of *Amphidinium carterae* have been investigated in the present study. The effect of light intensity upon cyst germination was tested by incubating temporary cysts at three different light intensities. In order to assess the potential for photosynthesis in cysts, cellular chlorophyll-*a* concentrations and cellular fluorescence were quantified during encystment. Photosynthetic carbon uptake of $H^{14}CO_3^-$ was also measured in light and dark cultures of temporary cysts. Germination success was found to increase slightly with decreasing light intensity. Chlorophyll $Cell^{-1}$ and Fluorescence $Cell^{-1}$ were each significantly higher in dark-treated cysts than in light-treated cysts. Although temporary cysts were found to possess chlorophyll concentrations only slightly less than those in active cells, no evidence was found to indicate that temporary cysts of *A. carterae* are photosynthetically active.

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1 INTRODUCTION

The formation of resting stages by phytoplankton is an adaptation for survival within an often dynamic environment. Among these stages, dinoflagellate cysts have been the focus of a great deal of research over the last century. The embodiment of this attention has depended greatly upon the motivation and the background of the researchers at hand. Phytoplankton ecologists, for instance, have recognized that the seasonal germination of dinoflagellate cysts can coincide with the onset of favorable growth conditions (e.g., Anderson and Wall 1978). This would likely impart a competitive advantage both in the ability to survive a hostile environment and in the ability to exploit improvement in ambient conditions. Geologists, particularly those associated with the petroleum industry, have long understood the need for careful documentation of fossilized cysts and their distribution in marine sediments. As biostratigraphic markers, dinoflagellate cysts yield important information about the Earth's climatology and may provide clues to the location of oil and gas resources (Loeblich and Loeblich 1984).

The presence of cysts produced by toxic species in vital coastal areas has fueled research concerning the initiation and timing of Harmful Algal Blooms (HABs). It is currently recognized that at least 60 dinoflagellate species produce or exude biologically active and sometimes hazardous compounds (Steidinger and Tangen 1996). Some interesting examples of HABs that have been linked to the presence of dinoflagellate cysts have been given by Anderson and Morel (1979), Burkholder *et al.* (1993), Schwinghammer *et al.* (1994), and Blanco (1995). Since 1978, it has been discovered that cysts may be responsible for both the recurrence of localized blooms and the step-wise spreading of regional blooms through coastal areas (Anderson *et al.* 1982b; Schrey *et al.* 1984; Nehring 1995). The increasing distribution of dinoflagellate HABs has been linked

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The format of this thesis follows that of Limnology and Oceanography.

to human activities, most notably coastal eutrophication and the transport of cysts in ships' ballast water (Hallegraeff and Bolch 1992; Rigby and Hallegraeff 1994; Dale *et al.* 1998).

Considering the attention that has currently been given to understanding the causes and dynamics of Harmful Algal Blooms (HABs), the physiology of dinoflagellate resting stages merits detailed investigation. Among the toxic species mentioned by Steidinger and Tangen (1996), many are planktonic, photosynthetic and produce sexual cysts through gametic fusion. Sexually-derived resting stages, particularly those from high profile toxic species (e.g., *Alexandrium tamarense* Lebour), have drawn much of the research interest in the last 30 years. Several of the species that have recently been associated with toxic blooms were either previously unknown or thought to be non-toxic (Anderson 1989; Hallegraeff 1993; Steidinger *et al.* 1996; Smayda 1997).

An example of a dinoflagellate that has generated a lesser degree of interest is the benthic species *Amphidinium carterae* Hulburt. This organism has been the subject of research involving photosynthesis, pigment composition and culture methods for the last 40 years (e.g., Byerrum and Benson 1975; Galleron 1976; Haxo *et al.* 1976; Gerath and Chisholm 1989). *Amphidinium carterae* is toxic (Thurberg and Sasner 1973; Nakajima *et al.* 1981), cosmopolitan (Steidinger and Tangen 1996), and exhibits some of the highest rates of growth in the Dinophyceae (Smayda 1997). While the formation of sexually-derived cysts has not been confirmed in *A. carterae*, temporary cysts have been observed in laboratory cultures. It is the intention of this thesis to describe the formation of these cysts. The physiological changes that occur during cyst formation will be explored with regard to photosynthesis. The importance of this process and possible ramifications will be addressed. In order to individually examine formation and germination of cysts, as well as their photosynthetic character, this work has been divided into two sections: *Cyst Formation and Germination* and *Cyst Chlorophyll and Photosynthesis.*

2 BACKGROUND

The possibility that resting cysts might play an important role in the development of dinoflagellate blooms was first hypothesized by Prakash (1967) and Wall (1967). This proposal followed an investigation into outbreaks of paralytic shellfish poisoning (PSP) that had plagued the eastern coast of Canada since first recorded in 1793 (Bond 1975). In the United States, more recent interest followed a massive red tide that occurred in the Gulf of Maine in September, 1972. Blooms of the causative organism, *Gonyaulax tamarensis* Lebour, were linked to the germination of benthic resting cysts by Anderson and Wall (1978). Termed the "cyst hypothesis" by Anderson and Wall, this relationship was confirmed the following year by Anderson and Morel (1979). Once this hypothesis was substantiated, awareness that the distribution of cysts could aid in the development of red tides became a focus of research in several regions around the world. Among the first species targeted were: *Alexandrium tamarense* (Anderson *et al.* 1982b; Schrey *et al.* 1984), *Pyrodinium bahamense* (Matsuoka *et al.* 1989), and *Gymnodinium catenatum* (Nehring 1995). It soon became apparent that the ability to understand and predict HAB events might depend upon a knowledge of cyst physiology, ecology, and distribution.

Terminology

Prior to any discussion involving cyst physiology and ecology, it is useful to review some of the terminology associated with dinoflagellate resting cysts as it has evolved in published literature. The extent to which these terms may be interchanged depends upon the environmental conditions during which respective stages were formed and the expertise of the individual researcher. Table 2.1 shows the results of a limited survey of this terminology and the usage thereof. The cyst cycle can be characterized as

Table 2.1. A review of the usage of selected terms pertaining to dinoflagellate cysts.

Terminology	References
Digestive Cyst	Pfiester & Anderson (1987); Kim (1991)
Ecdysal Cyst	Turpin *et al.* (1978); Taylor (1980); Fritz & Triemer (1985); Matsuoka *et al.* (1989); Taylor (1987).
Pellicle Cyst	Dale (1977); Anderson & Wall (1978); Loeblich & Loeblich (1984); Fritz & Triemer (1985); Matsuoka *et al.* (1989); Taylor (1987)
Spherical Cyst	Heiskanen (1993)
Sporangial Cyst	Wall (1975)
Temporary Cyst	Anderson & Wall (1978); Schmitter (1979); Yentsch *et al.* (1980); Walker (1984); Fritz & Triemer (1985); Kita *et al.*(1985); Sampayo (1985); Matsuoka *et al.* (1989); Pfiester & Anderson (1987); Blackburn *et al.* (1989); Marasovic (1985); Heiskanen (1993); Steidinger & Tangen (1996)
Thin-Walled Cyst	Fritsch (1935)
Vegetative Cyst	Taylor (1987); Alldredge *et al.* (1998)

being comprised of four parts; these include the *active cell*, the *resting cyst*, and the appropriate transition points between these stages.

A general scheme for an organism entering into or exiting from a resting stage is given in Figure 2.1. Three of the most commonly utilized terms describing resting stage mechanics are *encystment*, *excystment*, and *dormancy*. The first two terms refer to processes in which active cells enter into and exit from an inactive state, and can be used synonymously with *formation* and *germination*, respectively. Sussman and Halvorson (1966) defined the word *dormancy* as "any resting period or reversible interruption of the phenotypic development of an organism". In addition, the authors have distinguished between two distinct types of dormancy: "Constitutive Dormancy" and "Exogenous Dormancy". Constitutive dormancy describes a mode by which development is prevented by an "innate property" of the dormant stage itself (= *endogenous dormancy*, Pfiester and Anderson 1987). Exogenous dormancy is caused by the presence of unfavorable external conditions which act to prevent normal cell activity. A synonymous term for exogenous dormancy is *quiescence*, which has been utilized to describe fungal spores and copepod

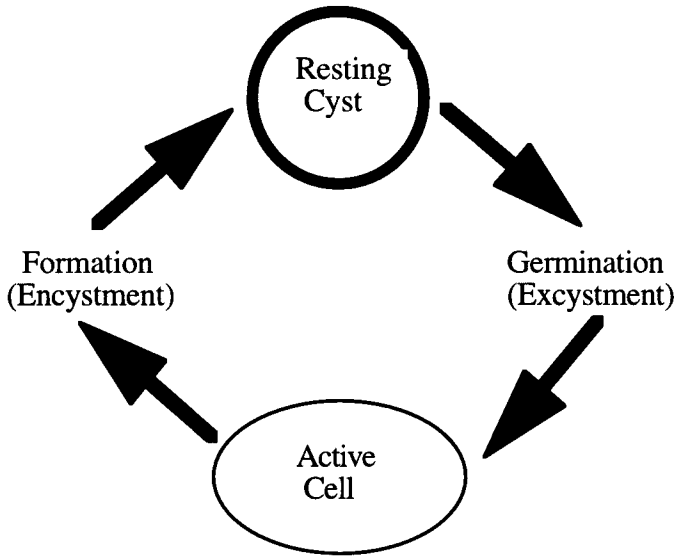


Fig. 2.1. A schematic of resting stage formation and germination by dinoflagellates.

eggs, as well as dinoflagellate cysts (Sussman and Halvorson 1966; Anderson and Wall 1978; Davis 1984).

Function

After reviewing much of the early literature about dinoflagellate cysts, Wall (1975) complied a listing of the probable functions of dinoflagellate resting cysts. These included the following:

1) As cysts are non-motile, they settle to the sediment, thereby ensuring a supply of cells to repopulate the water column in the same area as the "parent" bloom.

2) Cysts have a ripening period that acts to control the timing of repopulation.

3) Cysts seem to be derived sexually and therefore are likely to play a role in genetic recombination.

4) Encystment allows survival of adverse conditions for months and possibly years.

5) Because they are dormant and damage-resistant, transported cysts aid in dispersal and geographic migration of a species.

At the time, much of the list was hypothetical, as little proof existed to authenticate Wall's speculations. Nonetheless, each of these functions has been substantiated by knowledge accumulated over the last 23 years (e.g., Anderson and Wall 1978; Anderson *et al.* 1982a; Keafer *et al.* 1992). Other roles have been assigned to dinoflagellate cysts as well. For example, Ishikawa and Taniguchi (1996) and Alldredge *et al.* (1998) demonstrated that cysts facilitate downward transport of particulate organic carbon (POC) and other organic materials from surface waters. With relatively rapid sinking rates (6 - 11 m d^{-1}), dinoflagellate cysts may contribute up to 45% of the total particulate organic carbon in estuarine sediments (Anderson *et al.* 1985b; Heiskanen 1993). Aside from initiating blooms of toxic species, cysts may also play a role in direct toxicity of shellfish. For example, ingestion of *Alexandrium fundyense* cysts was reported to cause toxicity during non-bloom winter months (Schwinghammer *et al.* 1994).

Species Background: *Amphidinium carterae*

Amphidinium carterae (Dinophyceae) Hulburt, a species common to marine temperate and tropical waters (Thurberg and Sasner 1973; Thomas 1997), was originally described from the salt ponds surrounding Woods Hole, Massachusetts (Hulburt 1957). *Amphidinium carterae* (Fig. 2.2) has been associated with fish kills in Okinawa (Yasumoto 1990), discolored sands in Belize (Faust 1995), and annual blooms in Portuguese aquaculture ponds (Sampayo 1985). As is typical of a species with a global distribution, everal strains are known to exist (Table 2.2). The phylogenetic relationships among these strains have not been adequately explored. Throughout this thesis, each strain will therefore be identified according to the location where it was originally isolated. For example, the strain used in the present study was isolated from the salt ponds

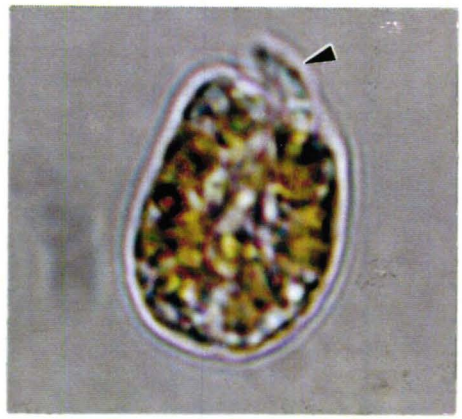


Fig. 2.2. A typical vegetative cell of *Amphidinium carterae* (Length ≈ 17 μm). Arrow indicates the characteristically reduced epicone.

Table 2.2. A list of strains of *Amphidinium carterae*, by location.

Location	Date Isolated	Reference
Woods Hole, MA (USA)	1954	Hulburt (1957); Duff *et al.* (1966); Thurberg & Sasner (1973); Ikawa & Sasner (1975); Sasner & Ikawa (1975); Galleron (1976); Hersey & Swift (1976); Humphrey (1979); Richardson & Fogg (1982); Samuelsson & Richardson (1982); Olson & Chisholm (1986); Gerath & Chisholm (1989); Klut *et al.* (1989).
Tahiti	1978	Yasumoto *et al.* (1980)
Okinawa	1979	Nakajima *et al.* (1981)
Mangladore	-	Nayak *et al.* (1997)
Plymouth, MA (USA)	-	Byerrum & Benson (1975); Haxo *et al.* (1976)
Portugal	-	Galleron (1976); Sampayo (1985)
Kattegat	-	Personal Communication, N. Ekelund (1998)

surrounding Woods Hole, Massachusetts, and will be referred to as the Woods Hole strain. The typical active cell, shown in Figure 2.2 , is characterized by a reduced, crescent-shaped epicone and a large, rounded hypocone. Dorsi-ventrally compressed, the cells average 12-15 μm in length and 8-9 μm in width (Hulburt 1957).

Although it was not originally described as such, *Amphidinium carterae* has been reported to be a benthic species (Nakajima *et al.* 1981; Steidinger and Baden 1984; Taylor and Pollingher 1987). In general, benthic dinoflagellates either reside on the sediment surface (epibenthic), live within interstitial spaces, or attach to a substrate (Steidinger and Baden 1984; Faust 1995). It has been indicated that *A. carterae* is an interstitial species that migrates into the water column each day (Steidinger and Baden 1984). While this behavior is suggestive of positive phototaxis, Eggersdorfer and Haeder (1991) have attributed it to both phototaxis and gravitaxis. Vertical migration is a behavioral pattern common to benthic dinoflagellates in sandy sediments (Steidinger and Baden 1984; Taylor and Pollingher 1987), and has been reported to influence the cell cycle (Gerath and Chisholm 1989) and reduction of nitrate (Hersey and Swift 1976).

Toxicity

Benthic photosynthetic dinoflagellates are often associated with toxicity in marine environments. Faust (1995), for example, reported that up to 60% of the benthic dinoflagellates found in South Water Cay, Belize were toxic. The most notorious of these, *Gambierdiscus toxicus*, has been identified as the causative organism behind Ciguatera poisoning, and reportedly exists in all tropical oceans between approximately 35° N and 35° S (Taylor and Pollingher 1987). Other noteworthy toxic benthic species include *Pfiesteria piscicida*, which has only recently been identified form the Atlantic coast of North America, and *Prorocentrum lima*, which has a global distribution (Yasumoto *et al.* 1980; Carlson and Tindall 1985; Burkholder *et al.* 1993).

Amphidinium carterae (Woods Hole strain) was first reported to produce biologically active compounds by Wangersky and Guillard (1960), who isolated an unidentified organic base from laboratory cultures. They speculated that a fishy odor, common to older cultures, was due to the presence of an acetylcholine analog. This hypothesis was supported by Thurberg and Sasner (1973), who observed choline-like symptoms (muscle paralysis) in two species of fish after exposure to crude extracts from *A. carterae*. The toxin was found to exist within the dinoflagellate cells, as toxicity only developed after cells were burst in some manner. Ikawa and Sasner (1975) concluded that there were at least three active compounds produced in culture: acrylcholine, choline O-sulfate, and an uncharacterized choline ester. More recently, Yasumoto (1990) identified five hemolysins from the Okinawan strain, of which three were considered potent.

As there are little available data regarding temporary cysts by dinoflagellates, it is the objective of this study to document the formation of these stages by *Amphidinium carterae*. Furthermore, the factors that influence formation and germination of temporary cysts (i.e., light, time & nutrients) will be examined using a series of controlled laboratory experiments. It is speculated that temporary cysts, as quiescent life stages, possess much of the photosynthetic machinery that is normally present in the active cells. As such it is hypothesized that both encystment and excystment are light and time dependent. As *A. carterae* has not been widely recognized as a high profile toxic species, it is intended that the information presented herein will be utilized as a focus for future work with more problematic species.

3 CYST FORMATION AND GERMINATION

Introduction

There are two widely recognized types of cysts that can be formed by dinoflagellates; these are *temporary cysts* and *sexual cysts*. The life cycle of a typical cyst-forming dinoflagellate is given in Figure 3.1. A sexual cyst, or *hypnozygote,* is a life stage initiated by fusion of gametes; this is followed by loss of motility and the formation of a rigid outer covering (Walker 1984). The term *temporary cyst* refers to a resting stage that is derived asexually by an individual cell, but has been used as a general descriptive for any nonmotile resting stage other than sexual cysts (Marasovic 1985). Some of the most common synonyms for temporary cysts have included *ecdysal cysts, pellicle cysts, spherical cysts, smooth cysts,* and *vegetative cysts* (Table 2.1). As defined by Taylor (1987), ecdysal cysts are those resulting from *ecdysis*, or "the complete shedding of the theca in response to environmental stress." Pellicle cysts are named for the *pellicle*, which is a flexible, resistant membrane underlying the theca in active cells (Loeblich and Loeblich 1984). Some authors have distinguished between several categories of temporary cysts, each depending upon membrane structure and the manner in which they were derived.

Anderson and Wall (1978) described three types of cells reportedly formed by *Gonyaulax excavata*; these included *coccoid, hypnoid,* and *vacuoloid* subtypes. Examples of the coccoid and hypnoid cysts are shown in Figures 3.2b and 3.2c. Coccoid cysts were described as spherical, and were found to be produced when cultures of *G. excavata* were exposed to low temperature or nutrient depleted conditions. Hypnoid cysts were reportedly more ovoid, and were produced when cultures were exposed to copper toxicity. Vacuoloid cysts were found to develop when cultures were exposed to cold, nutrient deficient conditions for more than nine weeks. The morphological similarity between different types of cysts has sometimes led to uncertainty as to how they were derived

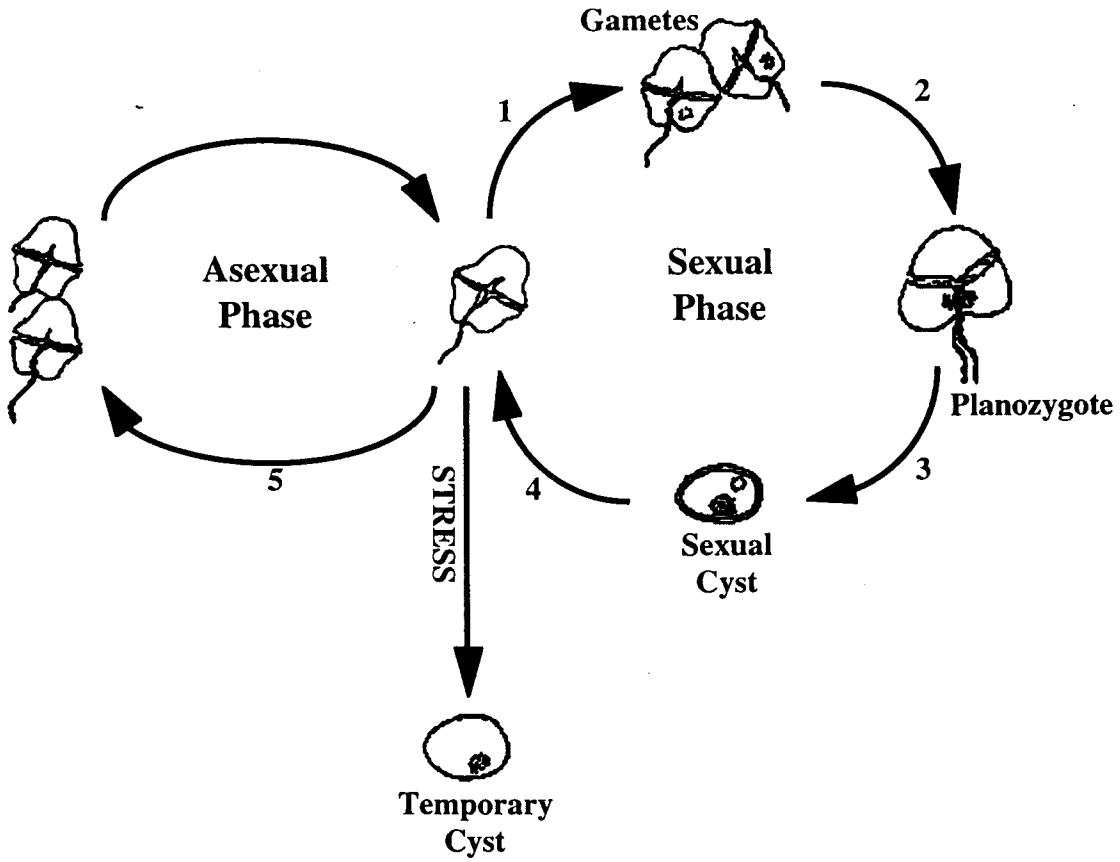
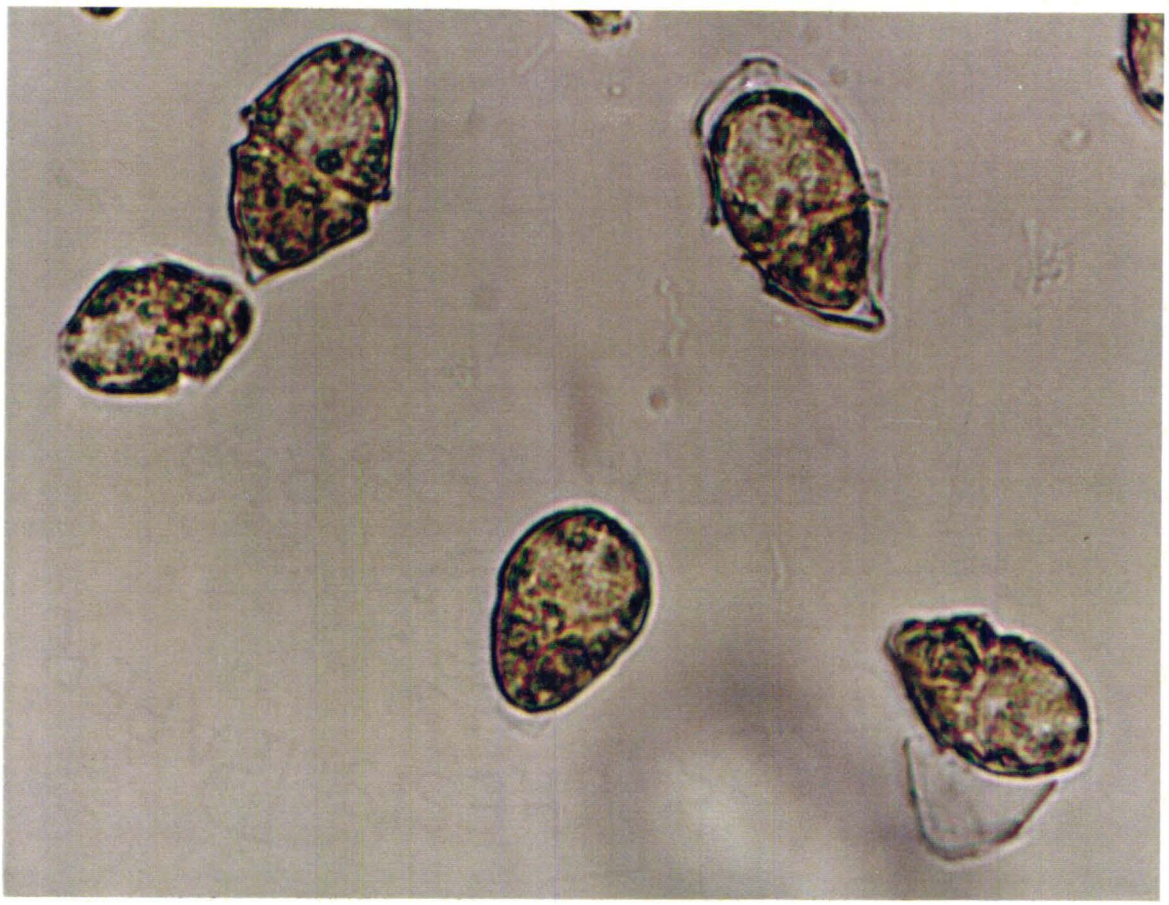


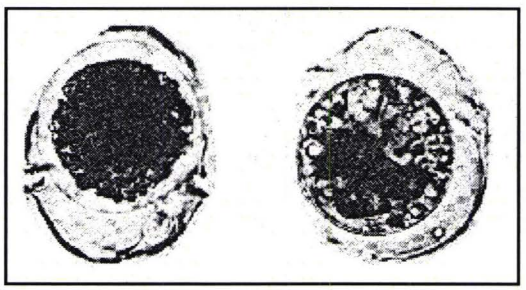
Fig. 3.1. The life cycle of a cyst-forming dinoflagellate, including formation of both temporary and sexual cysts. 1) Release of gametes, 2) Fusion, 3) Encystment, 4) Excystment, 5) Asexual cell division (Recopied from Walker 1984).

(e.g., Sampayo 1985; Heisekanen 1993). The terms *smooth cyst* and *spherical cyst*, as morphologically descriptive names, reflect this uncertainty (Chapman *et al.* 1982; Heisekanen 1993).

Aside from the mode of formation, there are three major differences between temporary cysts and sexual cysts; the first of these concerns the length of dormancy. Sexual cysts, which often undergo more dramatic structural changes than do their counterparts, commonly exhibit a period of endogenous dormancy. This stage has been described by Pfiester and Anderson (1987) as a "mandatory" resting period during which



(a)



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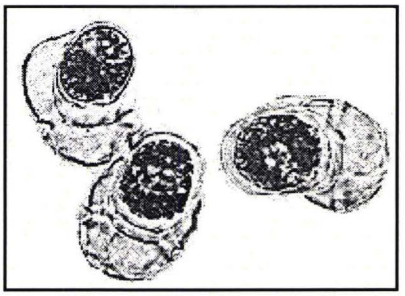


(c)

Fig. 3.2. Examples of dinoflagellate temporary cysts. (a) Cells of *Heterocapsa triquetra* undergoing ecdysis; (b) Immature coccoid cysts of *Gonyaulax excavata* ; (c) Hypnoid cysts of *G. excavata* . (Source: Figs. b - e recopied from Anderson and Wall 1978).

germination cannot proceed. The length of dormancy varies with species and storage conditions, ranging from a few weeks in *Gymnodinium catenatum* to 4 to 5 months in *Alexandrium tamarense* (Anderson *et al.* 1987; Blackburn *et al.* 1989).

Secondly, sexual cysts can remain viable for much longer periods that their counterparts. For example, Huper and Nipkow (1922, 1923) reported that cyst germination in the freshwater species *Peridinium cinctum* was able to proceed after more than 16 years in the deep sediments of Lake Zurich. In contrast, temporary cysts are generally regarded as remaining viable for time periods on the order of days (e.g., Marasovic 1985; Taylor 1987). Accordingly, Anderson and Wall (1978) indicated that the ability to overwinter in *Gonyaulax tamarensis* was a characteristic attributed solely to sexual cysts.

However, Anderson and Wall also demonstrated that 37% of cultured pellicle cysts of this species could remain viable for at least 50 days, provided that they were stored without light, and under reduced temperature. This observation is supported by an earlier report by Pfiester (1975), who indicated that temporary cysts of *P. cinctum* were able to survive for at least 5 months at 4 °C, and were able to overwinter in natural waters. While there is undoubtedly a great degree of variability surrounding survival of both temporary cysts and sexual cysts, the ability to shift rapidly from a resting stage to an active form is what most effectively distinguishes the two types.

Thirdly, temporary cysts generally have been described as having a single membrane. While those with two layers and four layers have been reported (Blackburn *et al.* 1989; Marasovic 1985), most sexual cysts have a three-layered membrane (Pfiester 1975; 1976; Loeblich and Loeblich 1984; Taylor 1987). This covering is often reinforced by cellulose or other materials, and may bear elaborate spines or reticulations (Taylor 1987). It is likely that the ability of sexual cysts to withstand long term dormancy relates in part to the character of these membranes.

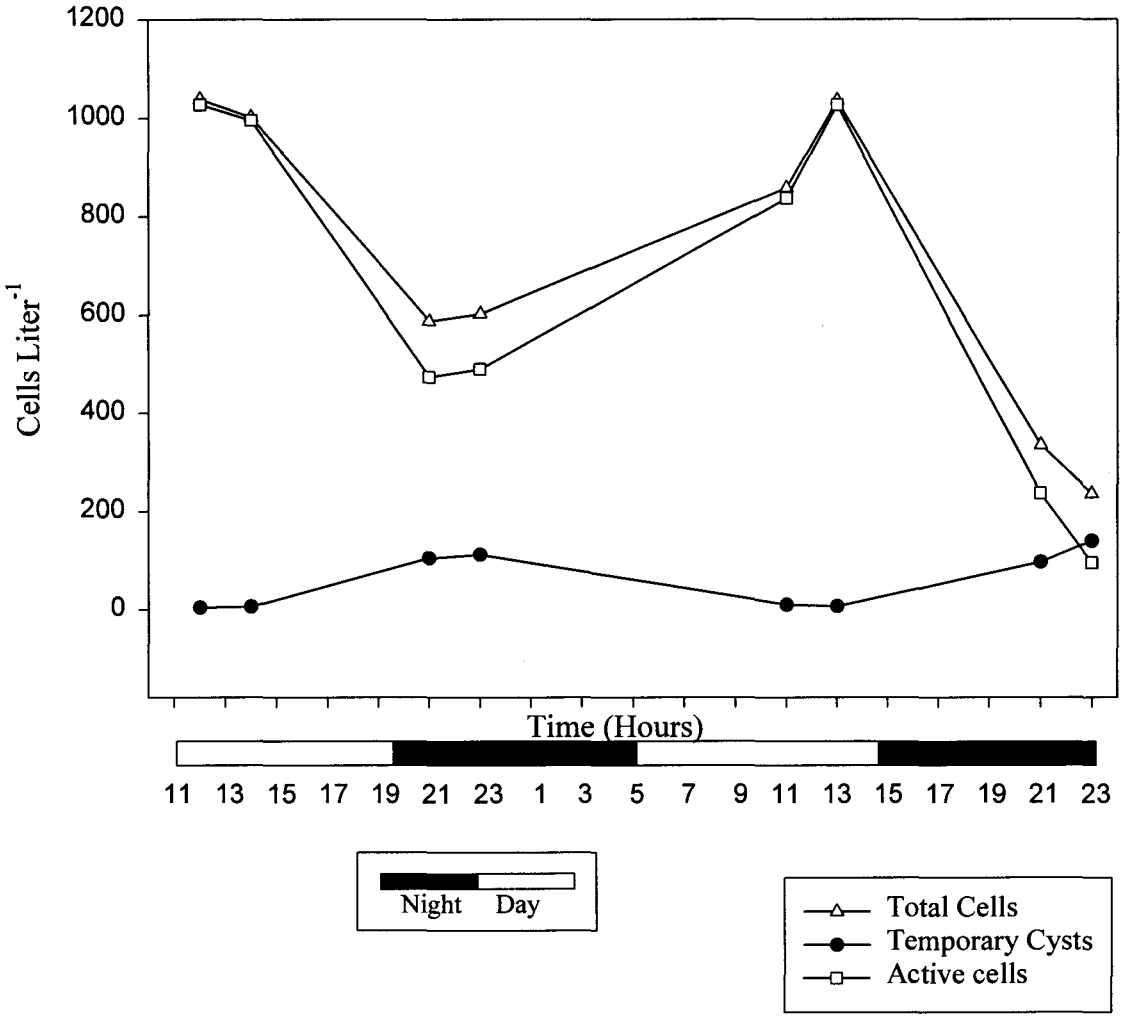


Fig. 3.3. Plots of Temporary Cyst Yield, Vegetative Cell and Temporary Cyst concentrations in a supralittoral tide pool at Jogashima Island (Honshu, Japan) on 6 -7 September 1983. Adapted from Kita *et al.* (1985).

Nearly all of the research concerning the importance of encystment and its role in bloom formation has focused upon sexual cysts, or *hypnozygotes*. Temporary cysts have been described many times under laboratory conditions (Anderson and Wall 1978; Yentsch *et al.* 1980; Fritz and Triemer 1985; Doucette *et al.* 1989), but have been documented in natural samples in only a few instances (Schmitter 1979; Kita *et al.* 1985; Marasovic 1985). Some interesting information concerning the role of temporary cysts in natural populations was reported by Marasovic (1985), following a series of red tides in

Kastela Bay (West Adriatic Sea). When bloom concentrations of *Gonyaulax polyedra* Stein reached a density of 10^5 - 10^6 cells L^{-1}, pH was drastically lowered and the water column became hypoxic. Mass encystment of the dinoflagellate population occurred within 24 hours, presumably in response to toxic H_2S concentrations. Timely sampling detected the presence of significant numbers of temporary cysts in the water column during this event. Within a few days, a second *Gonyaulax polyedra* red tide occurred in the same location. It was concluded that diffusion and flushing quickly eliminated the hypoxic conditions, allowing quiescent temporary cysts to re-establish the bloom.

The occurrence of rapid germination by temporary cysts was also reported by Kita *et al.* (1985), who described cycling between temporary cysts and active cells of *Goniodoma pseudogoniaulax* in a Japanese tide pool. Concentrations of active cells, temporary cysts, and total cells of this species have been plotted over two nightly cycles in Figure 3.3. As is depicted in the graph, temporary cyst concentrations increased at night and declined each day. In contrast, active cell concentrations peaked near mid-day, and decreased as darkness approached. It was speculated that the formation of temporary cysts enabled *G. pseudogoniaulax* to avoid an undefined nightly stress. While the nature of the stress was not known, this cycle reportedly contributed to the re-seeding of planktonic populations each day.

In general, there are three advantages that may be gained by the formation of temporary cysts. These include *a*) avoidance of short term stress, such as hypoxia, *b*) the ability to cycle rapidly between resting and active states, and *c*) the capability for a species to remain within the photic zone while quiescent. As they generally lack a dense cell covering, the relative buoyancy of temporary cysts is likely to be similar to or less than that of the parent cells (Anderson *et al.* 1985b). Decreased buoyancy would raise the probability that temporary cysts remain suspended in the water column, where ample light may allow rapid resumption of activity (Anderson *et al.* 1985b). Furthermore, because there is no known dormancy period in temporary cysts, rapid re-germination of this type

may play a role in the short-term maintenance of blooms. While sexual cysts are certainly more durable, temporary cysts are likely to provide a measure of flexibility within active populations.

As there are little available data regarding temporary cyst physiology, it was the objective of this study to document the formation of these stages by *Amphidinium carterae*. Furthermore, the effect of light intensity upon cyst germination was evaluated using a controlled laboratory experiment. As temporary cysts exhibited signs of high light stress (i.e., bleaching), it was germination success was speculated to vary inversely with light intensity. More specifically, temporary cysts that have been cultured in low intensity light may have a higher rate of germination than cysts cultured in either moderate or high intensity light.

Materials and Methods

Encystment Experiment—A stock culture of *Amphidinium carterae* was prepared in a large boro-silicate glass flask containing 1 Liter of F/2 growth medium. The medium was prepared from filtered,autoclaved Chesapeake Bay water (FACBW), with a salinity of approximately 20. The stock culture was incubated for five days without further enrichment at a temperature of 20 ± 0.2 °C and a light intensity of 175 ± 5 μE m^{-2} s^{-1} (L/D 12:12). A series of 5 ml aliquots was then transferred to 36 glass screw-cap test tubes containing 5 ml of either F/2 medium or FACBW. One half of the tubes were wrapped tightly with aluminum foil such that four groups of culture tubes were created, each with 9 tubes. Treatment groups were referred to as F/2-Light, F/2-Dark, CBW-Light, and CBW-Dark. The tubes were randomly distributed in a modified test tube rack and returned to the incubator.

After 12 days, a 2 ml sample was transferred from each tube to a small vial. The sample was then fixed with 1 drop of Lugol's iodine solution (Throndsen 1978). In

accordance with the method given by Guillard (1978), an Improved Neubauer hemacytometer was utilized to determine the Cyst Yield in each tube. The Cyst Yield is here defined as the ratio of cysts to total cells, expressed as a percentage. A 2-factor crossed ANOVA with fixed effects was applied (SAS, inc.) in order to determine the impact of light and enrichment upon cyst formation. A square root transformation was utilized to ensure that the data met assumptions of the ANOVA (Zar 1996). Tukey's HSD test was then applied as a follow-up to the ANOVA.

Germination Experiment—A series of 10 ml aliquots of *Amphidinium carterae* stock culture was transferred to 60 screw-cap boro-silicate glass test tubes which had been covered with aluminum foil. The tubes were incubated in a water bath for 25 days at a temperature of 20 ± 0.2 °C. On day 23, a 1 ml sample was removed from each of 5 randomly selected tubes and fixed with Lugol's iodine solution. Tubes were sampled in a similar manner every two days, until the average cyst yield was found to surpass 95% (Day 32). The cyst yield was determined using an Improved Neubauer hemacytometer according to methods given by Guillard (1978). As preliminary observations had indicated that *Amphidinium* cysts adhere strongly to the sides bottom of culture vessels, all tubes were vigorously shaken for 3 - 5 seconds prior to removal of the sample. While microscopic examination revealed that shaking had no apparent effect upon cyst integrity, agitation decreased the amount of material retained in each tube. In order to minimize light exposure during sampling, tubes were handled in a darkened room, re-wrapped, and quickly returned to the incubator.

As the experiment required tubes to be incubated at three different light intensities, a test tube rack was modified accordingly. The rack was divided into three equal portions, each corresponding to a light intensity of 30 ± 5 $\mu E\ m^{-2}\ s^{-1}$ (Low), 68 ± 2 $\mu E\ m^{-2}\ s^{-1}$ (Medium), or 128 ± 14 $\mu E\ m^{-2}\ s^{-1}$ (High). A Li-Cor Model 189 Radiometer/Photometer

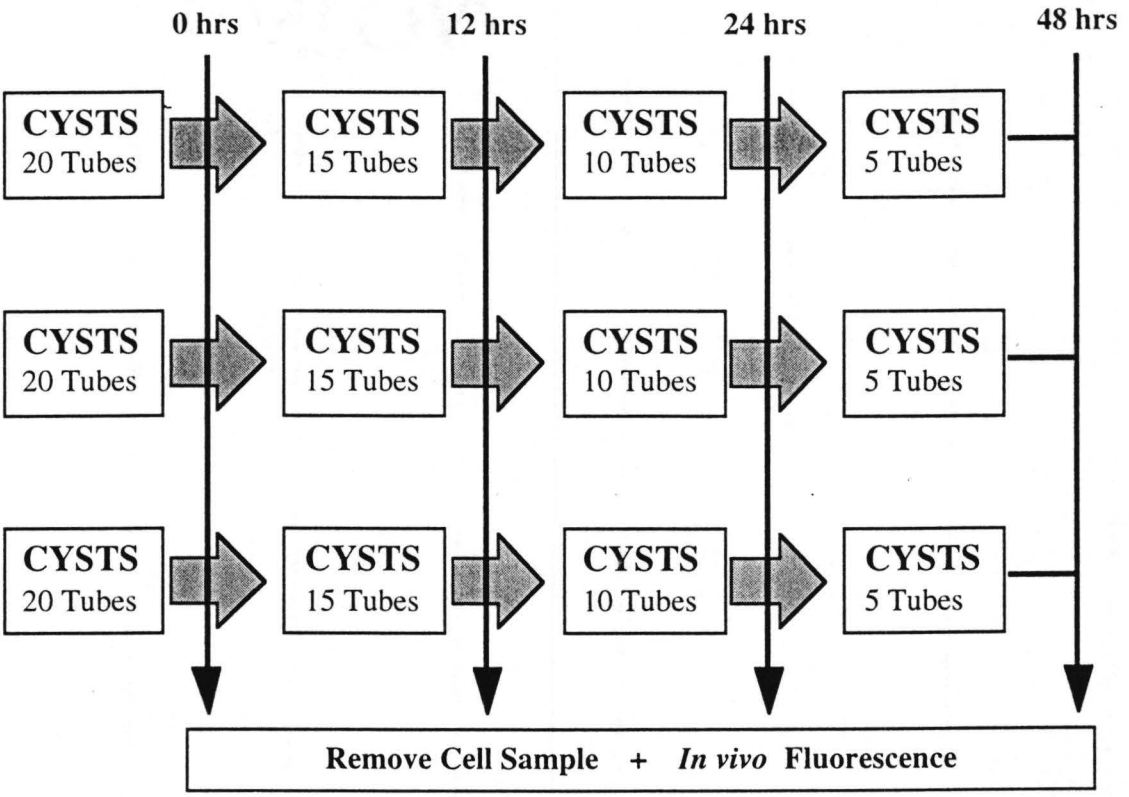


Fig. 3.4. A flow chart demonstrating the sampling scheme utilized during the germination experiment. The small boxes represent the number of replicate culture tubes remaining at each time.

was utilized to measure the light intensity within each portion of the rack. The culture tubes were randomly distributed among the three treatments and incubated at 20 °C with constant irradiance.

Immediately after addition of the culture tubes to the incubator, five randomly selected replicates from each group were removed for sampling (Fig. 3.4). After each tube was shaken, 7 - 9 ml was transferred to a glass cuvette (1 cm pathlength). A 10-AU fluorometer (Turner Designs, Inc.) was then utilized to measure *in vivo* fluorescence in each tube. The measurements were taken in a dimly lit room, and fluorescence was measured within 20 seconds after each tube was unwrapped. A 1 ml sample was removed

from each culture tube and fixed with Lugol's iodine so that the cyst yield could be determined. The 45 remaining tubes were uncovered, shaken, and returned to the incubator. After 12, 24, and 48 hours, tubes were sampled in a similar manner.

The results of a Shapiro-Wilk test indicated that data (i.e., total cell concentration, cyst yield, *in vivo* fluorescence and fluorescence $cell^{-1}$) were not normally distributed ($P > 0.05$, $n = 5$). After a series of unsuccessful transformations, the ANOVA was conducted using ranked values for all variables (Conover and Iman 1981; Zar 1996) .

Results

Encystment Experiment—The results of the encystment experiment indicated that both and enrichment had a significant effect upon cyst yield (Tables 3.1a & 3.1b). The interaction between these variables, however, was found to have no significant effect. The greatest cyst yield was obtained in the enriched medium that had been incubated in the light (Table 3.1a). The lowest yield was obtained from the unenriched, dark-treated cultures (mean = 1.14 ± 0.67). The results of the subsequent Tukey test are given in Table 3.1. As the experiment was designed to identify differences among treatment groups at each time, the Tukey results have been grouped accordingly. With the exception of the dark cultures incubated in unenriched bay water (CBW-Dark), none of the group means were significantly different.

Germination Experiment—Mean cell concentration (Vegetative cells + Cysts) at each light intensity are plotted versus time in Figure 3.5. While the cell concentration in the high and medium light groups declined rapidly over the first 12 hours (63.0% and 49.0%, respectively), that of the Low intensity treatment decreased by only 11.4%. The results of a 2-factor crossed ANOVA (Table 3.2) indicated that light intensity, time, and their inteaction each had a significant impact upon cell concentration. As the emphasis of

Table 3.1. Results of the encystment experiment. The effect of light and nutrient enrichment upon cyst yield in *Amphidinium carterae* were tested. (a) Mean cyst yield and standard deviation (*s*) of each treatment group. (b) Results of a 2-Factor ANOVA. (c) Results of a-Tukey HSD test describing the inter-group relationships between treatment means (CBW = Unenriched Chesapeake Bay Water).

Group	*n*	Mean	*s*
F/2-Light	9	14.622	11.223
F/2-Dark	9	8.8617	8.2206
CBW-Light	9	6.3220	3.5699
CBW-Dark	9	1.1372	0.6719

(a)

Source	df	MS	*F*	*P*	1-β
Light	1	3.364269	10.04	0.0011	N/A
Enrichment	1	4.274755	12.76	0.0034	N/A
Lgt. * Enrich.	1	0.430364	1.280	0.2565	< 0.30
Error	32	0.334922			
Total	35				

(b)

Mean Yield	Mean $(X)^{1/2}$	Treatment Group	Tukey Grouping
14.62	2.247	F/2-Light	A
8.861	1.855	F/2-Dark	A
6.322	1.777	CBW-	A
1.137	0.942	CBW-	B

(c)

Table 3.2. Results of a 2-factor, fixed effects ANOVA concerning the impact of light intensity and incubation time upon total cell concentration and cell/cyst ratio for laboratory cultures of *Amphidinium carterae* ($n_{intensity}$=15; n_{time}=20; α=0.05). (a) Total Cell Concentration, (b) Total Cell:Cyst ratio.

Source	df	MS	*F*	*P*
Intensity	2	787.9630	7.250	0.0018
Time	3	3119.078	28.69	0.0001
Int. * Time	6	1158.209	10.65	0.0001
Error	48	108.7230		
Total	59			

(a)

Source	df	MS	*F*	*P*
Intensity	2	2096.363	12.08	0.0001
Time	3	794.3556	4.580	0.0067
Int. * Time	6	874.8545	5.040	0.0001
Error	48	173.4813		
Total	59			

(b)

this experiment was placed upon identifying differences among groups at each incubation time, the results of the subsequent Tukey HSD tests have been grouped accordingly (Table 3.3). At t = 12 hours, cultures exposed to low intensity light had a significantly greater mean cell concentration than did either the medium or high intensity treatment groups (Table 3.3). By t = 24 hours, however, differences in cell concentration were no longer significant. There were no identifiable trends in total cell concentration other than a general decline over the course of the experiment.

The ratio of total cells to cysts (Cells:Cysts) was calculated and is plotted versus time in Figure 3.5. The graph indicates that the ratio decreased over the first 12 hours in all treatments. The ratio in the high and medium treatments reached a low point at t = 24 hours, before increasing though the second day of incubation. The Cell/Cyst ratio in the low intensity group increased by approximately 13% between hours 12 and 24, before leveling off somewhat through the final 24 hours (Fig. 3.5).

The 2-factor ANOVA results indicated that time, light intensity, and their interaction all had a significant effect upon the Cell/Cyst ratio (Table 3.2). The results of the subsequent Tukey HSD test (Table 3.3) reveal that the mean Cell/Cyst ratios of each treatment were not significantly different at 0, 12, and 48 hours. At t = 24 hours, the ratio in the low intensity group was significantly greater than those of the other two treatments. When grouped according to time, there were no apparent trends in the Total Cell:Cyst ratio.

Plots of mean *in vivo* fluorescence (Fig. 3.6) in each group demonstrate that while fluorescence declined in all treatments, the greatest net decrease (93.0%) was exhibited by the group incubated at the highest intensity. Medium intensity light yielded a decline of 90.7% while fluorescence in the low intensity treatment exhibited a decrease of 81.9%. The results of a 2-factor ANOVA revealed that time, light intensity, and their interaction each had a significant impact upon *in vivo* fluorescence (Table 3.5). The Tukey HSD test results (Table 3.5) indicated that while the high light treatment exhibited a significantly

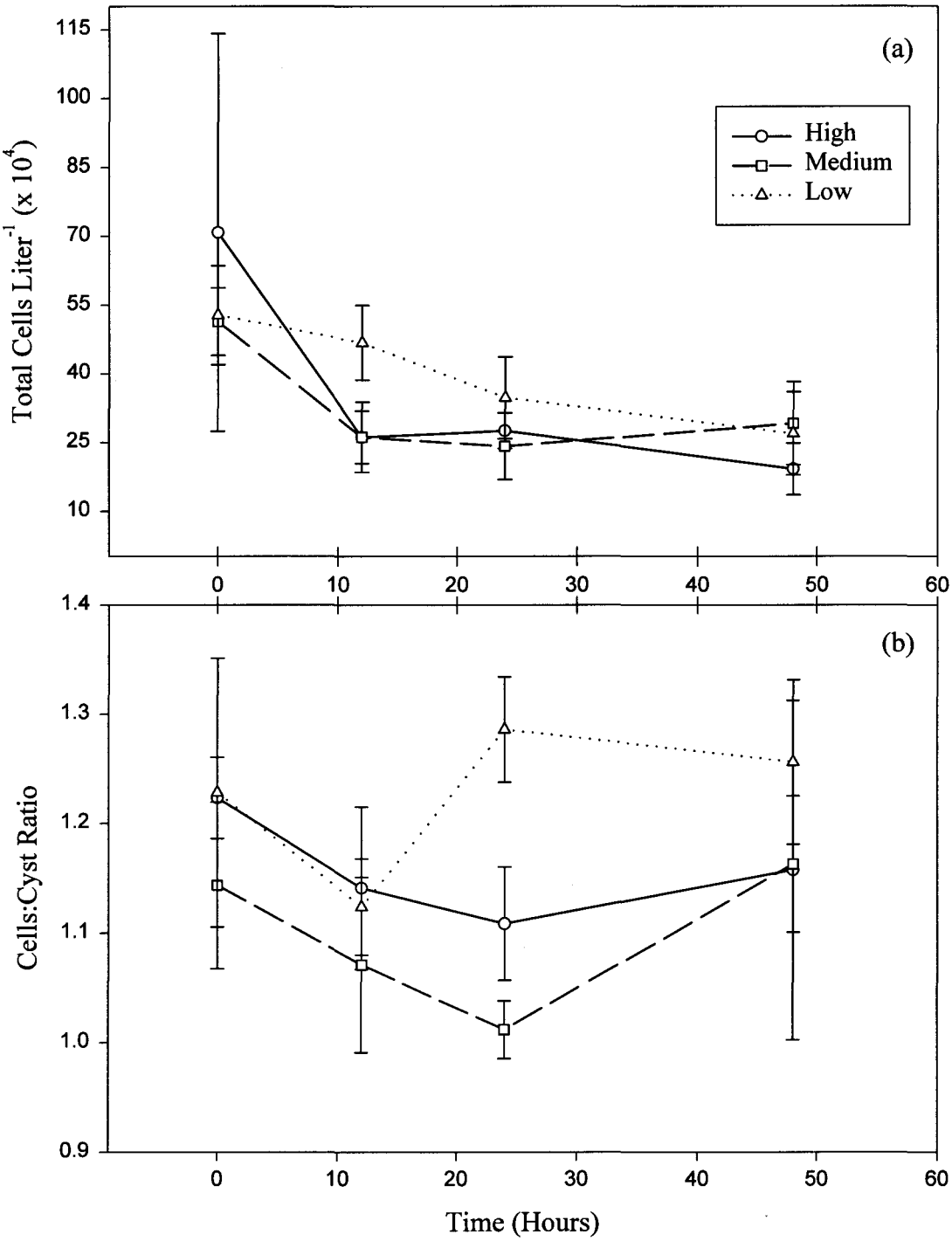


Fig. 3.5. Plots of (a) Total Cell Concentration versus time and (b) Total Cell:Cyst ratio versus time for cyst cultures of *Amphidinium carterae*. Cultures were incubated at High (128± 14 μE $m^{-2}s^{-1}$), Medium (68± 2 μE $m^{-2}s^{-1}$) and Low (30± 5 μE $m^{-2}s^{-1}$) intensity light.

Table 3.3. Results of Tukey test conducted following the ANOVA results in Table 3.2. The inter-group relationships between mean ranks of (a) Total Cell Concentration and the (b) Total Cell/Cyst ratio have been given. In the context of the experiment, results have been grouped according to time in (c) and (d). Columns of capital letters link treatment groups that were not significantly different at the $\alpha = 0.05$ level.

Mean (Cells L^{-1})	Mean Rank	Treatment Group	Tukey Grouping		
70.8×10^4	52.5	H_0		B	
52.8×10^4	51.0	L_0		B	
51.4×10^4	49.8	M_0		B	
46.8×10^4	45.8	L_{12}		B	C
34.8×10^4	32.2	L_{24}	A	B	C
29.2×10^4	23.5	M_{48}	A		C
27.6×10^4	22.7	H_{24}	A		
27.0×10^4	21.7	L_{48}	A		
26.2×10^4	21.4	M_{12}	A		
46.8×10^4	19.6	H_{12}	A		
24.2×10^4	16.5	M_{24}	A		
19.2×10^4	9.30	H_{48}			

(a) Total Cell Concentration

Mean Ratio	Mean Rank	Treatment Group	Tukey Grouping			
1.286	51.8	L_{24}		B		
1.256	46.4	L_{48}		B		D
1.223	42.2	H_0	A	B		D
1.228	41.4	L_0	A	B		D
1.163	31.8	M_{48}	A	B	C	D
1.158	29.9	H_{48}	A	B	C	D
1.143	27.9	M_0	A	B	C	D
1.141	27.4	H_{12}	A	B	C	D
1.124	23.6	L_{12}	A	B	C	D
1.109	22.0	H_{24}	A		C	D
1.071	15.7	M_{12}	A		C	
1.012	5.90	M_{24}			C	

(b) Total Cells : Cysts

t = 0 hrs		t = 12 hrs		t = 24 hrs		t = 48 hrs	
Treatment Group	Tukey Grouping	Treatment Group	Tukey Grouping	Treatment Group	Tukey Grouping	Treatment Group	Tukey Grouping
H	A	H	A	H	A	H	A
M	A	M	A	M	A	M	A
L	A	L	B	L	A	L	A

(c) Total Cell Concentration

t = 0 hrs		t = 12 hrs		t = 24 hrs		t = 48 hrs	
Treatment Group	Tukey Grouping	Treatment Group	Tukey Grouping	Treatment Group	Tukey Grouping	Treatment Group	Tukey Grouping
H	A	H	A	H	A	H	A
M	A	M	A	M	A	M	A
L	A	L	A	L	B	L	A

(d) Total Cells : Cysts

lower mean fluorescence than did the other groups at t = 12 and 24 hours, there was no significant difference among treatment means by t = 48 hours (Table 3.5c). Otherwise, there were no identifiable trends in *in vivo* fluorescence over the course of the experiment.

As cell concentration changed significantly over the course of the experiment (Fig. 3.2), fluorescence was normalized to cell concentration and was plotted versus time in Figure 3.6b. Over the course of the experiment, mean fluorescence cell^{-1} decreased by 62.7% in the low intensity treatment, 82.4% in the medium intensity treatment, and by 74.8% in the high intensity group. The 2-factor ANOVA results (Table 3.4b) indicated that time, light intensity, and their interaction each had a significant effect upon cellular fluorescence. The results of the Tukey HSD test (Table 3.5) indicated no identifiable trends in cellular fluorescence over the course of the experiment. While mean cellular fluorescence in the high intensity group was significantly less than that either of the other groups at t = 24 hours, the means were no longer significant at t = 48 hours. Over the entire 48 hours, the medium intensity treatment exhibited a steady decline in fluorescence cell^{-1} from 9.1 x 10^{-9} to 1.6 x 10^{-9} cell^{-1}. This pattern was not matched by the remaining treatments, as the mean fluorescence in the high and low intensity groups leveled off somewhat by 12 and 24 hours, respectively.

Table 3.4. Results of a 2-factor, fixed effects ANOVA concerning the impact of light intensity and incubation time upon in *vivo* fluorescence and *in vivo* fluorescence cell^{-1} for laboratory cultures of *Amphidinium carterae* ($n_{intensity}$=15; n_{time}=20; α=0.05). (a) *In vivo* Fluorescence, (b) *In vivo* Fluorescence cell^{-1}.

Source	df	MS	*F*	*P*
Intensity	2	1124.64	60.80	0.0001
Time	3	4812.64	260.3	0.0001
Int. * Time	6	1555.08	84.10	0.0001
Error	48	887.600		
Total	59			

(a)

Source	df	MS	*F*	*P*
Intensity	2	904.14	12.65	0.0001
Time	3	3913.8	54.75	0.0001
Int. * Time	6	1323.9	18.52	0.0001
Error	48	71.487		
Total	59			

(b)

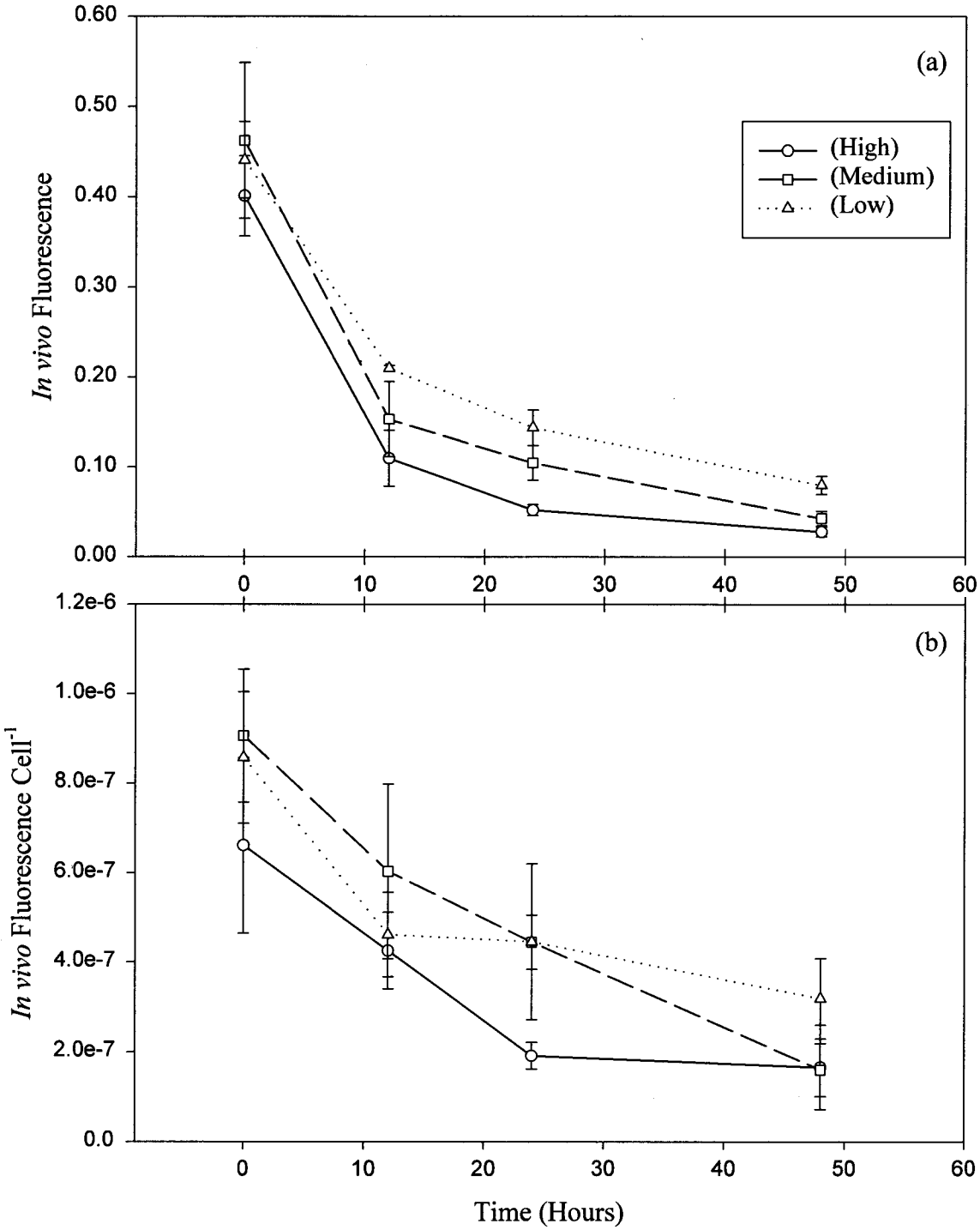


Fig. 3.6. Plots of (a) *In vivo* fluorescence versus time and (b) *In vivo* fluorescence cell^{-1} versus time for cyst cultures of *Amphidinium carterae*. Cultures were incubated at High (128± 14 μE m^{-2}s^{-1}), Medium (68± 2 μE m^{-2}s^{-1}) and Low (30± 5 μE m^{-2}s^{-1}) intensity light.

Table 3.5. Results of a Tukey test conducted following the ANOVA results in Table 3.4. The inter-group relationships between mean ranks of (a) *In vivo* Fluorescence and (b) *In vivo* Fluorescence Cell^{-1} have been given. In the context of the experiment, results have been grouped according to time in (c) and (d). Columns of capital letters link treatment groups that were not significantly different at the $\alpha = 0.05$ level.

Mean	Mean Rank	Treatment Group	Tukey Grouping	
0.441	55.0	L_0	A	
0.463	54.4	M_0	A	
0.401	49.6	H_0	A	B
0.210	43.0	L_{12}	C	B
0.153	34.2	M_{12}	C	D
0.144	33.2	L_{24}		D
0.105	26.4	M_{24}	E	D
0.110	26.4	H_{12}	E	D
0.080	19.8	L_{48}	E	F
0.052	12.0	H_{24}	G	F
0.043	8.90	M_{48}	G	
0.028	3.10	H_{48}	G	

(a) Fluorescence

Mean	Mean Rank	Treatment Group	Tukey Grouping		
9.06×10^{-7}	54.4	M_0		A	
8.57×10^{-7}	53.2	L_0		A	B
6.61×10^{-7}	42.2	H_0	C	A	B
6.03×10^{-7}	40.6	M_{12}	C	A	B
4.62×10^{-7}	34.9	L_{12}	C	D	B
4.45×10^{-7}	33.9	M_{24}	C	D	
4.26×10^{-7}	30.4	H_{12}	C	D	
4.46×10^{-7}	29.8	L_{24}	C	D	
3.19×10^{-7}	21.2	L_{48}		D	E
1.92×10^{-7}	11.4	H_{24}			E
1.66×10^{-7}	7.00	H_{48}			E
1.60×10^{-7}	7.00	M_{48}			E

(b) Fluorescence Cell^{-1}

t = 0 hrs		t = 12 hrs		t = 24 hrs		t = 48 hrs	
Treatment Group	Tukey Grouping	Treatment Group	Tukey Grouping	Treatment Group	Tukey Grouping	Treatment Group	Tukey Grouping
H	A	H	A	H	B	H	A
M	A	M	B	M	A	M	A
L	A	L	B	L	A	L	B

(c) Fluorescence

t = 0 hrs		t = 12 hrs		t = 24 hrs		t = 48 hrs	
Treatment Group	Tukey Grouping	Treatment Group	Tukey Grouping	Treatment Group	Tukey Grouping	Treatment Group	Tukey Grouping
H	A	H	A	H	B	H	A
M	A	M	A	M	A	M	A
L	A	L	A	L	A	L	A

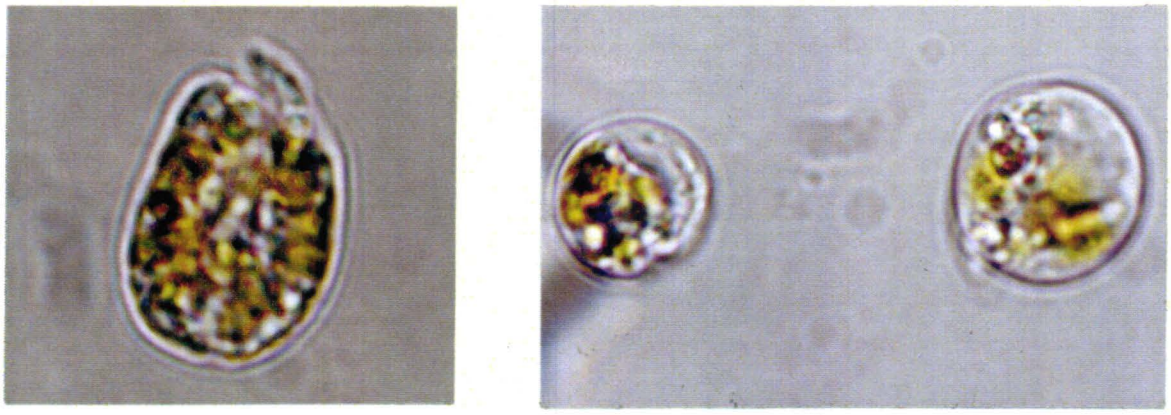
(d) Fluorescence Cell^{-1}

Temporary Cyst Morphology—The formation of temporary cysts occurred spontaneously in cultures of *Amphidinium carterae*. While cysts were routinely observed in both light and dark cultures, vessels that were incubated in the dark typically had greater cyst yields than their counterparts. Prior to encystment, active cells appeared to become sluggish, and the cytoplasm was observed to be withdrawn from the periphery of the cell. While the actual process of encystment was not witnessed, evidence for this process was noted. For example, the remnants of active cells were often found beside newly-formed temporary cysts. Accordingly, it is likely that much of the detrital material that was present at the bottom of culture vessels was comprised of cast-off parental membranes.

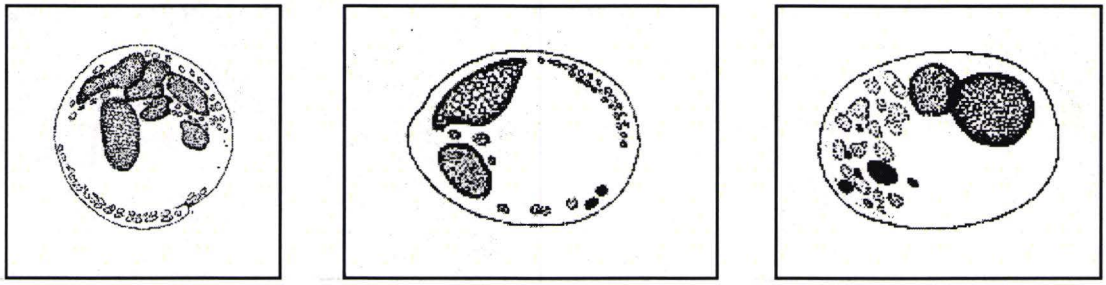
Examples of active cells and temporary cysts of *A. carterae* are shown in Figures3.7a and 3.7 b. While it is not evident from the cells in Figure 3.7, the cytoplasm of respective cells and cysts appeared to be quite similar, with a high level of diffuse pigmentation.

Two distinct types of temporary cysts were found to be formed by *A. carterae*. In accordance with the descriptions given by Anderson and Wall (1978), the first of these (Figure 3.7b) can be termed a coccoid cyst. These cells bore a yellow pigment, and ranged in diameter from 10 to 15 µm. The second type (not shown) was almost exclusively observed in dark treated cultures that were incubated for > 30 days. These cells bore resemblance to the ovoid cysts of Anderson and Wall (1978). Even under high magnification, little pigmentation could be observed in the ovoid cysts. Many of the cells were narrow (< 8 µm x 10 µm), and were apically tapered in a manner similar to those of *G. breve* , as described by Wilson (1967) [Fig. 3.7d] . A large vacuole appeared to account for most of the cytoplasm.

Upon close examination of the cultured cysts, a halo of translucent material was observed to surround many of the temporary cysts. It is likely that this feature represented the mucilaginous envelop that is common to many dinoflagellate cysts (Taylor 1987).

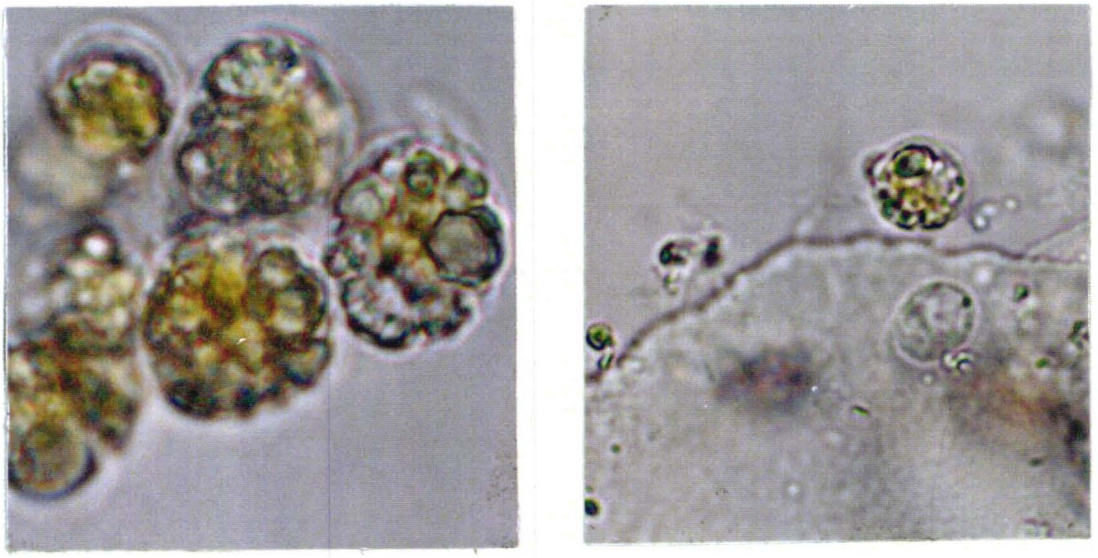


(a) (b)

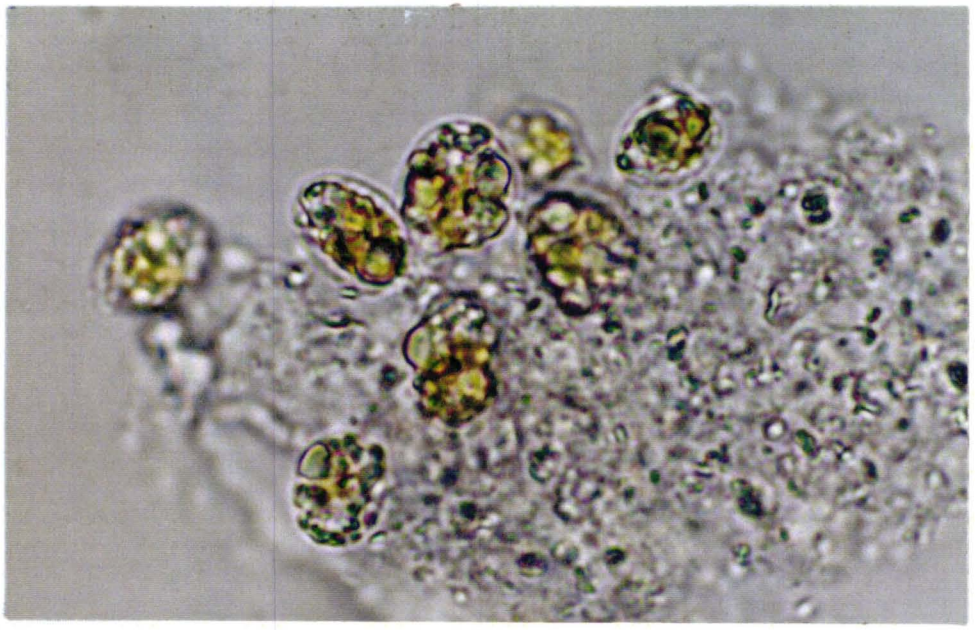


(c) (d) (e)

Fig. 3.7. Examples of temporary cysts formed by *Amphidinium carterae* (a & b) and *Gymnodinium breve* (c - e). (a) An active cell of *A. carterae* that has been fixed with Lugol's iodine. (b) Temporary cysts of *A. carterae*, also fixed with Lugol's. (c - e) Examples of *G. breve* cysts as sketched by Wilson (1967). These cells, while not originally known to be temporary cysts, bear a strong resemblance to those of *Amphidinium carterae*.

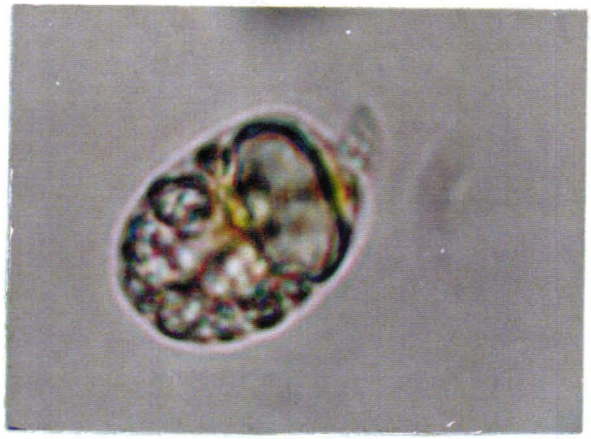


(a) (b)

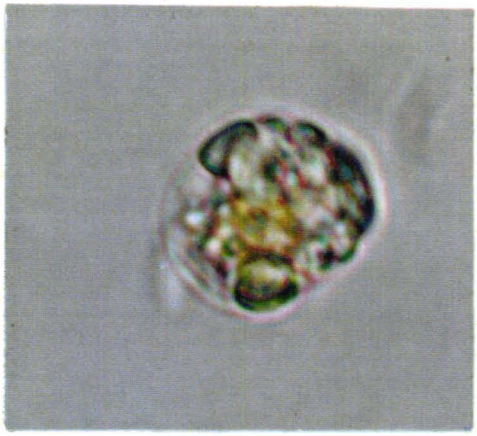


(c)

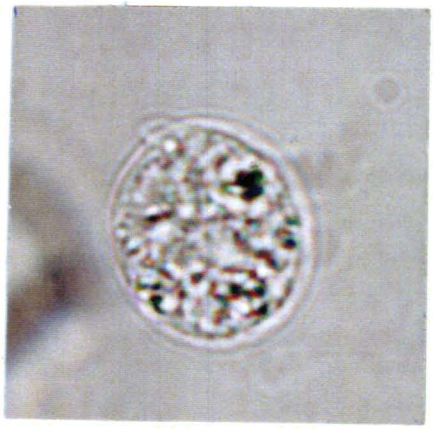
Fig. 3.8. Examples of *A. carterae* temporary cysts showing common patterns of aggregation in culture. (a) A free aggregate of temporary cysts. (b) Mucilaginous sheet that was commonly found at the bottom of culture vessels. As shown, cysts were often embedded in this matrix, and could not be dislodged with violent shaking of the vessel. (c) Another type of accumulation that contained cysts, dead cells, and detritus.



(a)



(b)



(c)

Fig. 3.9. Typical *Amphidinium carterae* cells following incubation for 58 days in the dark. Cells appear colorless except under high magnification. (a & b) Some cells (<5%) were still not encysted after more than eight weeks in the dark. (a) Dorsal view of an non-motile cell. (b) Antapical view of the same cell. (c) Colorless temporary cyst. While the viability of this particular cell was not tested, preliminary experiments have suggested that colorless cysts were still able to germinate.

This envelope may have been responsible for the adhesion that was exhibited. Figure 3.8a depicts a cluster of *A. carterae* cysts, which were commonly found in the water column of culture vessels. These aggregates most often contained four to eight cysts, but clusters comprised of > 15 cells were also observed. Some of the most concentrated aggregates (e.g., Fig. 3.8c) were found at the bottom of culture vessels. These accumulations were comprised of dead cells, live cysts, dead cysts (Fig. 3.9c) and numerous rod-shaped bacteria.

After incubation for more than 30 days, coccoid cysts appeared to undergo a transformation into the ovoid type. The few active cells that survived extended darkness were heavily vacuolated (Figs. 3.9a-b), and swam in slow circles.

Discussion

As no cells were actually observed in the process of encysting, the basis for classifying the observed resting stages as temporary cysts was dependent upon three characteristics. The first of these concerns the structure of the cyst membrane. Light microscopy revealed that the cyst membrane of *Amphidinium carterae* was comprised of a single layer, which contrasts with the heavy cyst wall generally associated with sexual cysts (Walker 1984; Taylor 1987).

Secondly, one of the features indicative of sexual cysts is the presence of a yellow-orange to reddish inclusion in the cytoplasm. This "eye spot", speculated to be comprised of concentrated pigments, was reported to form during encystment and to fade prior to germination (Walker 1984). The diffuse pigmentation of the coccoid cysts signifies that concentration of photosynthetic pigments had not occurred.

The third basis for the classification as temporary cysts concerns the rapidity of germination. Both preliminary studies and the present experiment (Fig. 3.5b) have indicated that a significant degree of germination can occur after 24 hours in a nutrient replete medium. As there have been no reports suggesting that sexual cysts are able to

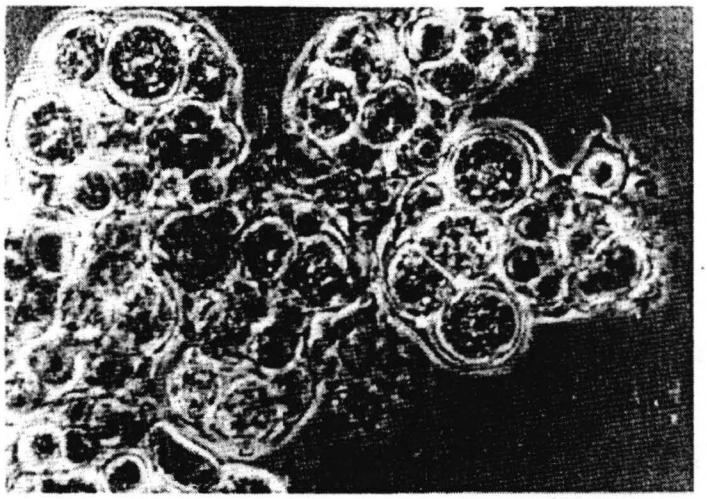


Fig. 3.10. Examples of resting cysts from a Portuguese strain of *Amphidinium carterae* (Sampayo 1985).

germinate within 1-2 days, the cysts in question can be concluded to be temporary cysts. In comparison, Sampayo (1985) expressed a degree of uncertainty as to the origins of cyst produced by a Portuguese strain of *A. carterae*. Figure 3.10 depicts a resting cyst of this strain that appeared to be undergoing meiosis. As meiosis is a process which typically occurs in diploid cells, it would be indicative of sexual cysts. However, the example in Figure 3.10 bears a strong resemblance to the temporary cysts shown in Figures 3.8a and 3.8d. It is possible that the cytoplasmic inclusions evident in Figure 3.8a could be mistaken for the products of meiotic division. Although it was not attempted in the present study, a method such as the Protargol Silver staining technique of Coats *et al.* (1984) would likely have allowed differentiation between sexual and temporary cysts.

There is very little published data concerning metabolic activity in dinoflagellate temporary cysts. This necessitates comparison with either vegetative cells or sexual cysts. For example, Binder and Anderson (1986, 1987, 1990) reported that light exposure enhanced germination in the sexual cysts of *Scrippsiella trochoidea.* (Fig. 3.11). This process was believed to involve a pathway in which cysts were "activated" by light

exposure, thereby initiating respiration. This was reportedly followed by protein synthesis, and subsequent reconstruction of photosynthetic systems. The process was concluded by Binder and Anderson (1990) to be a non-photosynthetic response, as similar results were achieved either when irradiance was maintained below 1 $\mu E\ m^{-2}\ s^{-1}$ or when exposure time was limited to less than one second.

While Anderson *et al.* (1987) showed that germination in *Scrippsiella trochoidea* was directly related to light intensity, the current study resulted in evidence to the contrary. Germination in the temporary cysts of *A. carterae* did not appear to be similarly related to light intensity. Instead, Figure 3.5b suggests that cysts incubated at higher light intensities were less likely to germinate than those exposed to lower light levels.

There are three important differences between the present study and that of Anderson *et al.* (1987) that may explain the conflicting results. First, Anderson *et al.* used an incubation time of 7 weeks, as opposed to 12 hours in the present study. Second, cysts of *S. trochoidea* were hypnozygotes that had overwintered at least once in the sediment. Finally, as the cysts used by Anderson *et al.* were collected from natural samples instead of laboratory cultures, little is known about the environmental conditions during which they were formed. It is likely that the process of pigment storage that reportedly accompanies sexual encystment (at least in *S. trochoidea*) somehow insulates chlorophyll from photo-destruction. As temporary cysts in the present study often appeared to bear pigmented chloroplasts, they were probably more susceptible to light exposure than were their sexual counterparts.

While the observed increase in the mean Cell/Cyst ratio in the low intensity group was statistically significant, it does not necessarily reflect cyst germination. As the Cell/Cyst ratio is sensitive to both active cell concentrations and cyst concentrations, it is possible that cell growth may have contributed to the increase. This scenario would involve growth among the small percentage (< 5%) of unencysted cells remaining in each tube. There are two reasons that render this scenario unlikely: *1)* Both cell concentration

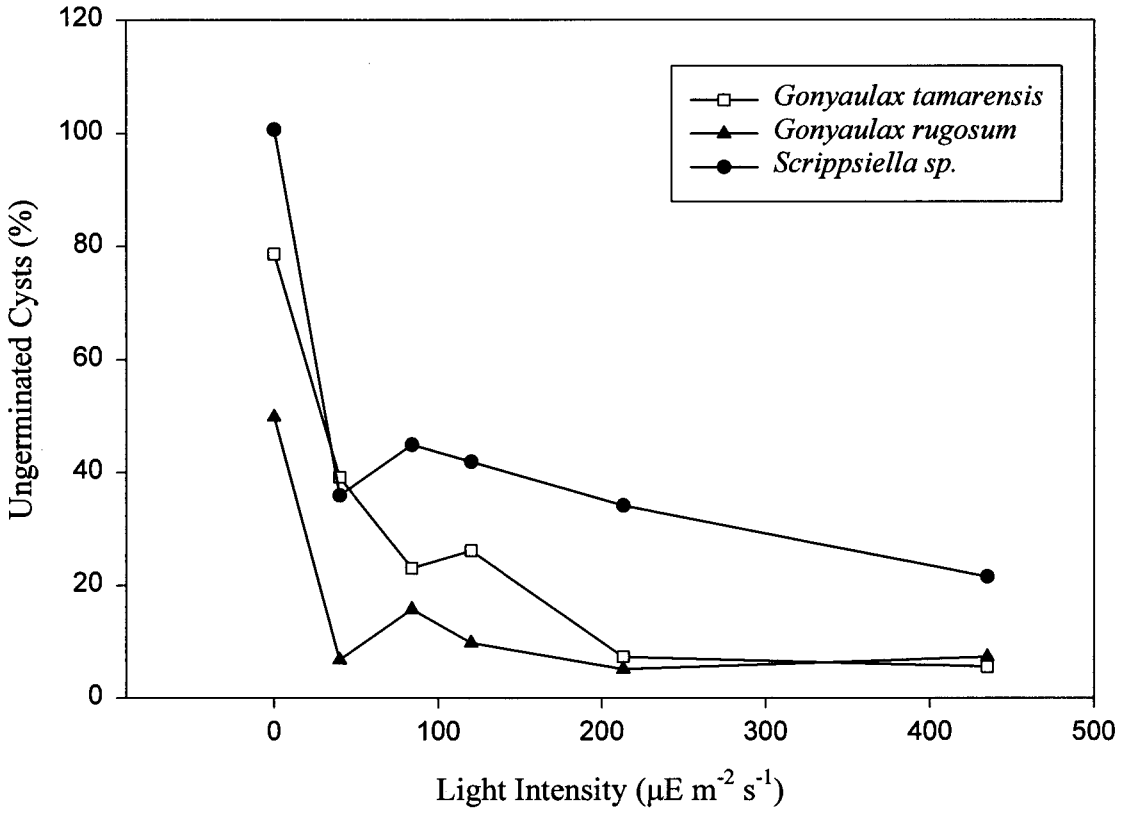


Fig. 3.11. A plot describing germination success in resting cysts of 3 dinoflagellates incubated at a range of light intensities. Values are expresses as the percentage of ungerminated cysts remaining in cultures after an incubation period of 7 weeks (adapted from Anderson *et al.* 1987).

(Fig. 3.5a) and fluorescence (Fig. 3.6a) declined over the course of the experiment, thus suggesting an absence of growth, and *2)* While the experiment was conducted with continuous illumination, *A. carterae* has been reported to undergo cell division exclusively in the dark (Galleron 1976). Therefore, no cell growth would be expected to occur during the course of the experiment.

The loss of cellular fluorescence observed over the course of the germination experiment (Fig. 3.6b) may be attributed to three mechanisms. First, the decline in fluorescence may have been the result of photoimpairment of chlorophyll. This would depend upon either photoinhibition or photodegradation. Photoinhibition constitutes a temporary decline in photosynthetic efficiency due to over-excitation of the chlorophyll-*a*

reaction center (Raymont 1980). In contrast, photodegradation involves the temporary conversion of chlorophyll to phaeopigments, which may have a more substantial impact upon photosynthesis (Raymont 1980). Second, the decline in fluorescence may have been caused by enzymatic breakdown of chlorophyll. However, as the experiment was conducted in a light-incubated, nutrient replete culture medium, with constant temperature, catabolism of chlorophyll seems unlikely. Finally, germination could have been light-related, but independent of photosynthesis. This would suggest the inhibition of a process outside of the photosynthetic mechanisms. Considering the complexity of many cell mechanisms, it is not possible to speculate which of these three pathways was the most likely explanation.

Providing that cyst germination is linked to photosynthesis in some respect, high intensity light would be expected to impact germination in much the same way that fluorescence was effected. Considering that the photo-inhibition in phytoplankton is dependent upon both light intensity and exposure time (Takahashi *et al.* 1971), much the same can be surmised about pigment degradation. Samuelsson and Richardson (1982) reported that *Amphidinium carterae* is photoinhibited at a light intensity between 15 and 80 $\mu E\ m^{-2}\ s^{-1}$. Inhibition was found to occur within 1 to 2 hours after exposure to bright light. Considering the results given herein, it is not possible to identify the lowest light intensity at which cysts were inhibited. As both cell health and light pre-conditioning have a direct impact upon photosynthetic efficiency (Raymont 1980), it is likely that the dark-incubated cultures used for the germination experiment were extremely sensitive to bright light.

4 CYST CHLOROPHYLL AND PHOTOSYNTHESIS

Introduction

Among the factors controlling species succession and bloom formation in natural systems are rate of growth and photosynthetic efficiency (Lalli and Parsons 1993). In a given light field, a photosynthetic organism with the greatest efficiency may be in a position to outstrip its competitors. However, given the degree of spatial and temporal photic variability that phytoplankton must contend with, the ability to adapt to a range of light conditions may be more important. Such adaptability would be particularly important under conditions of either high or low light stress.

Particularly in coastal regions, phytoplankton often encounter drastic changes in the ambient light field, resulting from shifts in intensity, spectral quality, and duration of exposure (Raymont 1980). Prézelin (1987) has described two strategies commonly employed by algal cells to counter changes in intensity. These strategies include either a shift in the number of photosynthetic units or a change in the size of each unit. Each strategy may translate into shifts in cellular chlorophyll concentration. Accordingly, a simple feedback model has been used by Falkowski (1981) to describe the physiological basis for these changes. As depicted in Figure 4.1, the model centers upon the actions of two chloroplastic enzymes. These include glutamine synthetase, which combines ammonium with glutamate (as a precursor to amino acids), and glutamic dehydrogenase (a direct competitor). When a cell is exposed to decreased irradiance, for example, the rate of ATP (Adenosine Triphosphate) production declines markedly. This enables glutamic dehydrogenase to shunt glutamate toward chlorophyll synthesis. In a light field of sufficient intensity, an ample supply of ATP allows the synthetase to monopolize virtually all of the available glutamate. As no more chlorophyll is produced, cellular division will cause a decline in the average amount of chlorophyll per cell (Falkowski 1981).

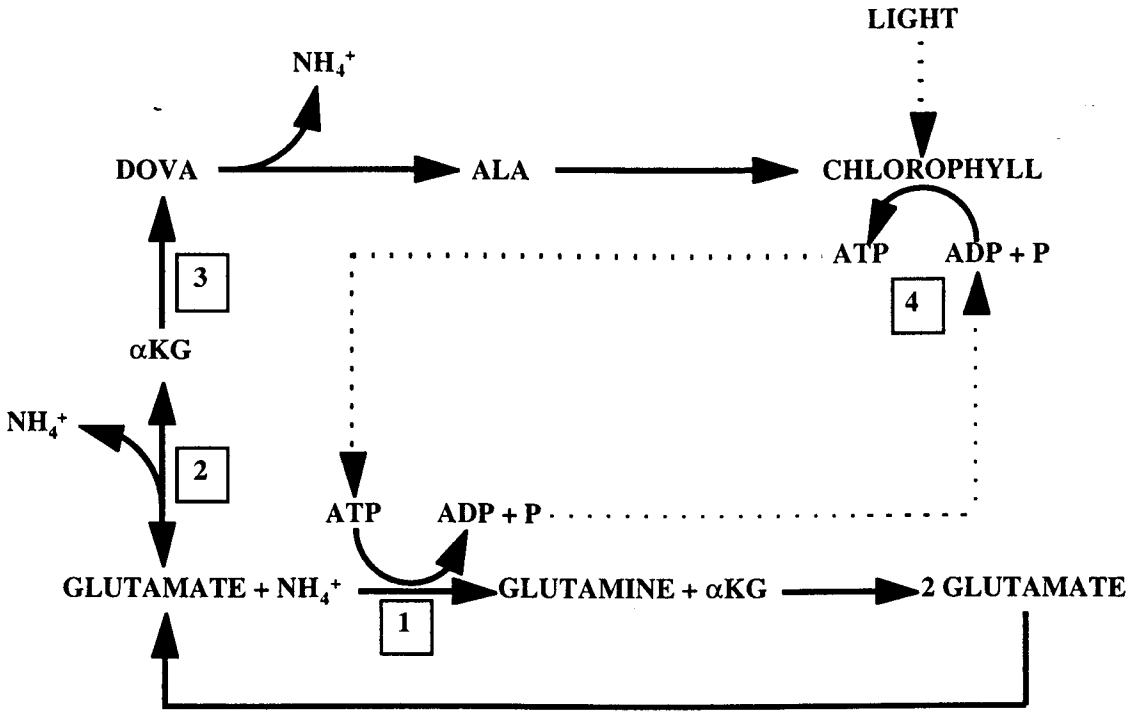


Fig. 4.1. A simple model of chlorophyll regulation during light-shade adaptation. [1] Glutamine Synthetase, which requires ATP for catalysis, incorporates ammonium into amino acids. As light intensity is decreased, there is a decline in available ATP [4], allowing Glutamic Dehydrogenase [2] to compete with the synthetase for glutamate. Glutamate is then used in a chlorophyll synthesis pathway [3], which includes αKG (α-Ketoglutarate), DOVA (4,5-dioxovaleric acid), ALA (δ-aminolevulinic acid), and finally, Chlorophyll-*a*. When light levels increase, αKG is no longer formed, and no new chlorophyll is synthesized. (From Falkowski 1981).

Documentation regarding the impact of dinoflagellate encystment upon photosynthetic systems has been solely concerned with sexual cysts. For example, cellular chlorophyll concentrations, rates of photosynthesis, and respiration during the encystment of *S. trochoidea* have been described by Binder and Anderson (1990). As shown in Figure 4.2, the amount of chlorophyll $cell^{-1}$ declined rapidly during cyst formation. After encystment, cellular chlorophyll-*a* concentrations reached a relatively constant level near 2×10^{-6} μg $cell^{-1}$. It was not known whether this decline in pigment was attributable to decomposition or to a mechanism such as that described by Falkowski (1981). Photosynthetic activity in the sexual cysts of *S. trochoidea* was also quantified by

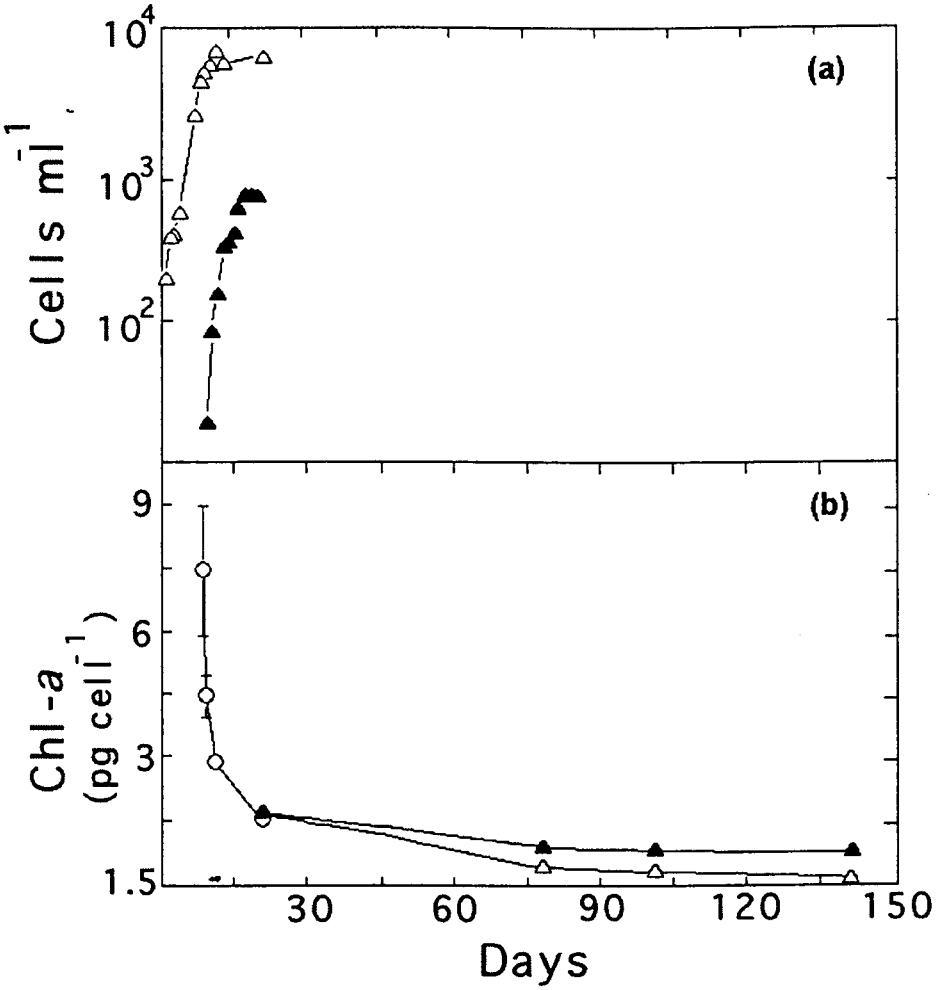


Fig. 4.2. Changes in biochemical composition in vegetative cells of *Scrippsiella trochoidea* during cyst formation, dormancy, and quiescence. (a) Cyst formation in vegetative cells (Δ) and cysts(▲); (b) Chlorophyll-*a*.concentrations in vegetative cells (O); cysts stored at 18 °C (Δ); cysts stored at 3 °C (▲). From Binder and Anderson (1990).

Binder and Anderson (1990), who examined the changes in oxygen production and gross photosynthesis over an eight day period. As shown in Figure 4.3, oxygen production increased with the germination of light-incubated cysts. Because respiration also increased, net photosynthesis became positive only after the third day of incubation. Dark-incubated cysts exhibited a declining rate of respiration, which leveled off after the third day. The authors reported that respiration rates in the cysts of this species were

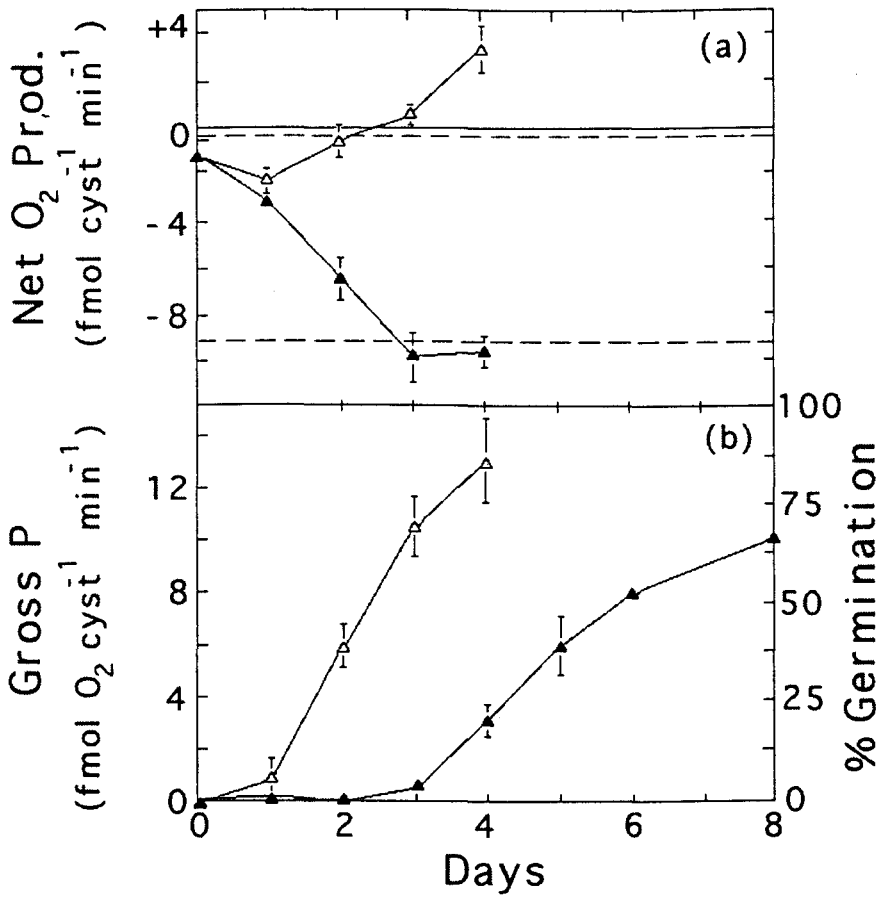


Fig. 4.3. Metabolic activity in cysts of *Scrippsiella trochoidea* during germination. Cysts stored in the dark at 3 °C and transferred to a L/D cycle at 18 °C at time 0. (a) Net oxygen consumption in the dark (▲) and production under saturating light intensity (Δ). Broken lines indicate respiration rates of quiescent and germinating cysts calculated from carbohydrate loss. Means ± SE (n=2). (b) Gross photosynthesis calculated from graph a (Δ); bars indicate SE (df=10); germination frequency (▲), means ± SE (n=3). From Binder and Anderson (1990).

approximately 50% lower than those in corresponding vegetative cells. The rate of gross photosynthesis was reported to be approximately 30% that of active cells, as it was counteracted by respiration. Despite the presence of cellular chlorophyll, the sexual cysts of *Scrippsiella trochoidea* demonstrated little capacity for photosynthesis. It was concluded that the photosynthetic systems of resting cysts were somehow disrupted during encystment, and could only become functional at a point just prior to germination.

While information concerning the photosynthetic biology of resting cells in other algal groups is limited, there are some similarities between dinoflagellate resting stages and those of diatoms and cyanobacteria. For example, Fay (1968) reported that photosynthetic rates in the resting spores of *Anabaena cylindrica* (Cyanophyceae), while detectable, were 78 to 87% lower than those of active cells. Similarly, Anderson (1975) investigated the changes that occurred during the formation of resting cells in *Amphora coffeaeformis* (Bacillariophycea). Photosynthetic rates were found to decrease dramatically as this process continued. The reduction was not attributed to a decline in photopigments, as chlorophyll-*a* concentrations increased when cells entered the resting phase. Following dark storage, photosynthesis was observed to be negligible. It seems likely that this species underwent a deactivation process functionally similar to that in *Scrippsiella trochoidea*.

The objective of this study was to evaluate photosynthetic capacity in the temporary cysts of *Amphidinium carterae*. Anderson (1975) and Binder and Anderson (1990) reported a decrease in cell chlorophyll concomitant with reduced rates of photosynthesis in the respective resting stages of diatoms and other dinoflagellates. As temporary cysts are short term resting phases, it is likely that cellular photosynthetic systems remain functional even after encystment. On this basis, it is hypothesized that (*1*) a partial reduction in cellular chlorophyll concentration will accompany encystment, and (*2*) cysts will exhibit reduced, yet measurable rates of photosynthetic carbon uptake.

Materials and Methods

Cyst Chlorophyll Experiment—Amphidinium carterae (Woods Hole strain) was obtained from the Provasoli-Guillard Center for the Culture of Marine Phytoplankton (CCMP). A working culture was grown in a large borosilicate glass flask in filtered, autoclaved Sargasso Sea water that had been diluted to 20 ppt. The culture vessel was

covered with a cotton gauze stopper, and incubated above 40-watt Sylvania Cool White fluorescent bulbs at a light intensity of 175 ± 5 μE m^{-2} s^{-1} (LI-COR Model 189 Radiometer/Photometer). Growth temperature was maintained using a flow-through water bath at 22±0.2 °C. After approximately two weeks without further enrichment, a series of 40 ml aliquots were transferred to 48 smaller flasks (125 ml), each with similar stoppers. Half of the smaller flasks were wrapped tightly with aluminum foil to prevent light exposure, and covered with a small piece of foil to further darken the contents. As a precautionary measure to avoid the possibility of metal toxicity, a piece of paraffin film was placed between the cotton stopper and the foil cover.

Each of the small flasks was labeled and placed in a water bath at 20 ± 0.5 °C above 20-watt Sylvania Cool White bulbs with an intensity of 190 ± 5 μE m^{-2} s^{-1}. After approximately 10 minutes, *in vivo* fluorescence was measured in each of six randomly-selected, light treated flasks. This was done by transferring 8 - 9 ml from each flask to a glass cuvette (1 cm pathlength), which was then placed in a model 10-AU digital fluorometer (Turner Designs, Inc.). The contents of each cuvette was then shaken slightly, transferred to 15 ml glass vial, and fixed with one drop of Lugol's iodine solution (Throndsen 1978).

A 10 ml aliquot was withdrawn from the remaining volume in each flask and filtered through a 25 mm Gelman Type A/E™ glass fiber filter at a vacuum pressure of 100 mm Hg. Filters were rinsed three times, folded in half, and stored in plastic vials at -20 °C pending chlorophyll analysis. The contents of six of the foil-wrapped flasks were then processed in the same manner, although the foil covers were quickly replaced after sampling to limit light exposure. The remaining 36 culture flasks were randomly positioned in the gridded incubator. After days 10, 20, and 30, the analysis was repeated on similar groups of light and dark-treated flasks.

$$\frac{\mu g\ Chl\text{-}a}{L} = \frac{(Fa - Fb)\ (0.0148)\ [Vol.\ Acetone\ (ml)]}{[Vol.\ Filtered\ (L)]} \quad (1)$$

Recopied from Parsons *et al.* (1984), where:

F_a = Fluorescence after the addition of HCl.
F_b = Fluorescence before the addition of HCl.
0.0148 = 1.83 x F_D , where FD = the instrument Door Factor.

The chlorophyll extraction method given by Parsons *et al.* (1984) was modified slightly to determine the amount of chlorophyll-*a* ml^{-1} in each aliquot. Fluorescence was read immediately following addition of HCl (0.5 N) to the supernatant, rather than after 5 minutes, as is called for in the original protocol. Equation 1, given by Parsons *et al.* (1984), was utilized to calculate the amount of chlorophyll-*a* in each sample.

The cell concentration in each aliquot was determined using the average of 10 small fields on an Improved Neubauer hemacytometer according to methods given by Guillard (1978). The mean cell concentration in each group was used to calculate the amount of chlorophyll-*a* $cell^{-1}$ and *in vivo* fluorescence $cell^{-1}$. The cyst yield was determined using the ratio of the number of cysts to the total number of cells. In addition, F_b/F_a (Eq. 1) was calculated as a measure of pigment degradation. A 2-factor, fixed-effects ANOVA was applied to assess the impact of irradiance and incubation time upon each variable (SAS Institute, Inc.). As the results of a Shapiro-Wilk test indicated that the data deviated

Table 4.1. Experimental design for the carbon uptake experiment. The <3 µm Fraction was filtered through a 3 µm membrane filter prior to addition of the label.

Treatment	Incubation Time (Hrs)
Cysts (Light)	0, 6, 12
Cysts (Dark)	0, 6, 12
<3µm Fraction (Light)	0, 6, 12
<3µm Fraction (Dark)	0, 6, 12
Fixed Control	0, 6, 12

significantly from normality, a rank transformation was applied prior to the ANOVA. As detailed by Zar (1996), Tukey's HSD test was then applied to identify which groups were significantly different. A Spearman Rank Correlation test was applied in order to test for relationships between variables.

Carbon Uptake Experiment—A series of 60 boro-silicate glass test tubes, each containing 10 ml of *Amphidinium carterae* culture, was prepared from a log-phase stock culture. The tubes were each wrapped in aluminum foil and incubated in the dark, at 20 ± 0.2 °C, for a total of 52 days. The average cyst yield in 10 randomly selected tubes was determined periodically using the method given above. After the average cyst yield was found to reach 95% (day 52), the tubes were randomly assigned to the treatment groups listed in Table 4.1. Approximately 12 hours prior to the experiment, the cysts were removed from groups 3 and 4 by gentle filtration through a 3 μm membrane filter (PC Nucleopore®, 47 mm). The tubes from these two groups were then covered with aluminum foil and returned to the incubator. The control group, which was incubated in the light, consisted of cysts that were fixed with Glutaraldehyde (1% final conc.).

Each culture tube was shaken vigorously prior to the addition of 1 μCi of $Na^{14}CO_3$ and 1 ml of F/2 medium. The cultures were then randomly distributed in a modified test tube rack, and were incubated at 20 ± 0.2 °C at a constant irradiance of 29.8 ± 5 μE m^{-2} s^{-1}. Replicates from the first time interval (t = 0) were filtered immediately after the addition of the label. This procedure was conducted using 25 mm Whatman® GF/F glass fiber filters, with a vacuum pressure of ≤50 mm Hg. After three rinses with filtered and autoclaved seawater (20 ppt), filters were transferred to scintillation vials containing 15 ml of Scintiverse® BD scintillation cocktail (Fisher Scientific). The remaining culture tubes were filtered in a similar manner at designated time intervals. A Packard Model 230TR

$$P = \frac{R_s - R_b \times W}{R} \qquad (2)$$

After Parsons *et al.* (1984), where:

P = Photosynthesis (mg C m^3 hr^{-1})
R = Total Activity (dpm) of bicarbonate added
R_b = Dark bottle sample count (dpm)
N = Incubation time (hrs)
R_S = Sample count per filter (dpm)
W = weight of total CO_2
[As estimated by the method given by Parsons *et al.* (1984)]

scintillation counter was utilized to determine the activity (dpm) on each filter. Equation 2 (Parsons *et al.* 1984) was then applied to determine the amount of carbon fixed, in mg C m^{-3}.

The results of a Shapiro-Wilk test indicated that data (i.e., total cell concentration, cyst yield, *in vivo* fluorescence, and *in vivo* fluorescence $cell^{-1}$) were not normally distributed ($P > 0.05$, $n = 6$). After a series of unsuccessful transformations, the ANOVA was conducted using ranked values for all variables (Conover and Iman 1981; Zar 1996). As detailed by Zar (1996), Tukey's HSD test was applied to identify which groups were significantly different. In the context of the experiment, Tukey test results have been grouped with respect to time.

Results

Cyst Chlorophyll Experiment—The average cell concentration and cyst yield are plotted versus time in Figures 4.4a and 4.4b. Over the duration of the experiment, average cell concentration in the light treated cultures declined by approximately 19.9 %, while those that were not exposed to light declined by 67.4%. A 2-factor analysis of variance with fixed effects was applied to the ranked data to assess the impact of light and time upon cell concentration and cyst yield. The results of the ANOVA indicated that light, time and their interaction each had a significant impact upon both cell concentration

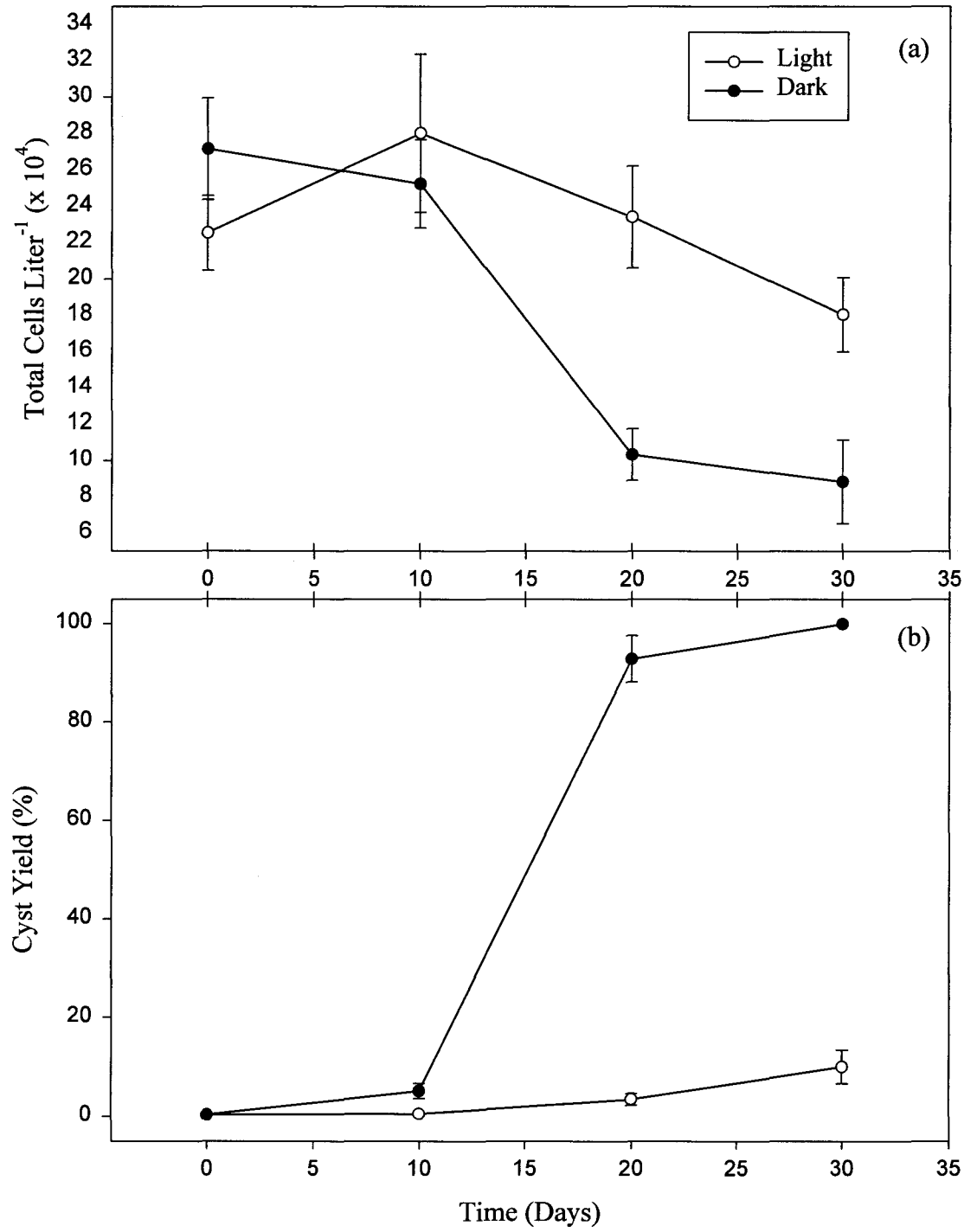


Fig. 4.4. Plots of (a) Total Cell Concentration versus time and (b) Cyst Yield versus time for encysting cultures of *Amphidinium carterae*. Cultures were incubated in either Light (128± 14 μE $m^{-2}s^{-1}$; L/D 12:12) or Dark conditions.

and cyst yield (Table 4.2).

As each of the experiments in this study were primarily designed to identify time-dependent differences among treatment groups, the Tukey HSD test results have been grouped accordingly (Table 4.3). While the Tukey results for cyst yield suggested no identifiable trends with respect to treatment (Table 4.3a),the mean cyst yield in each treatment was significantly different after 20 days (Table 4.3c).

The Cyst yield in the dark treatment group was significantly larger than that in the light treatment group after day 10, reaching a maximum yield of 100 ± 0.0% by day 30. In contrast, the maximum cyst yield in the light-treated cultures reached only 10.0 ± 3.1% by the close of the experiment. When the dark treated culture tubes were visually examined, a mass of sticky, yellow-brown material was found at the bottom of each. A lesser amount of a similar substance was found in the light-treated tubes. Microscopic examination of this material revealed that it was composed of cysts, dead cells, and detritus.

Because average cell concentrations changed significantly over the course of the experiment experiment (Fig. 4.4a), extracted chlorophyll and fluorescence data were normalized accordingly. Figures 4.5a and 4.5b demonstrate the change in fluorescence cell^{-1} and chlorophyll-*a* cell^{-1} over the incubation period. The results of the 2-factor

Table 4.2. Results of a 2-factor, fixed effects ANOVA concerning the impact of light and incubation time upon Total cell concentration and cyst yield for laboratory cultures of *Amphidinium carterae*. ($n_{Light} = 24$; $n_{Time} = 12$, $\alpha = 0.05$). (a) Total Cell Concentration, (b) Cyst Yield.

Source	df	MS	*F*	*P*
Light	1	444.083	10.84	0.0021
Time	3	1773.89	43.31	0.0001
Lgt * Time	3	1081.64	26.41	0.0001
Error	40	40.9625		
Total	47			

(a)

Source	df	MS	*F*	*P*
Light	1	1463.021	99.20	0.0001
Time	3	2174.000	147.4	0.0001
Lgt * Time	3	1227.297	83.22	0.0001
Error	40	14.74790		
Total	47			

(b)

Table 4.3. Results of a Tukey test conducted following the ANOVA results in Table 4.2. The inter-group relationships between mean ranks of (a) Total Cell Concentration and (b) Cyst Yield have been given. In the context of the experiment, results have been grouped according to time in (c) and (d). Columns of capital letters link treatment groups that were not significantly different at the $\alpha = 0.05$ level.

Mean (Cells L^{-1})	Mean Rank	Treatment Group	Tukey Grouping	
28.0×10^4	39.42	L_{10}	A	
27.2×10^4	38.75	D_0	A	
25.3×10^4	34.08	D_{10}	A	C
23.4×10^4	28.83	L_{20}	A	C
22.6×10^4	25.75	L_0	B	C
18.1×10^4	16.17	L_{30}	B	D
10.4×10^4	8.33	D_{20}		D
8.85×10^4	4.67	D_{30}		D

(a) Total Cell Concentration

Mean Yield	Mean Rank	Treatment Group	Tukey Grouping	
0.57	45.50	D_{30}	A	
0.38	39.50	D_{20}	A	D
5.18	33.50	L_{30}	B	D
3.50	26.50	D_{10}	B	E
0.38	22.50	L_{20}		E
10.0	10.50	L_{10}	C	
92.9	9.42	L_0	C	
100	8.58	D_0	C	

(b) Cyst Yield

t = 0 d		t = 10 d		t = 20 d		t = 30 d	
Treatment Group	Tukey Grouping	Treatment Group	Tukey Grouping	Treatment Group	Tukey Grouping	Treatment Group	Tukey Grouping
L	A	L	A	L	A	L	A
D	B	D	A	D	B	D	A

(c) Total Cell Concentration

t = 0 d		t = 10 d		t = 20 d		t = 30 d	
Treatment Group	Tukey Grouping	Treatment Group	Tukey Grouping	Treatment Group	Tukey Grouping	Treatment Group	Tukey Grouping
L	A	L	A	L	A	L	A
D	A	D	B	D	B	D	B

(d) Cyst Yield

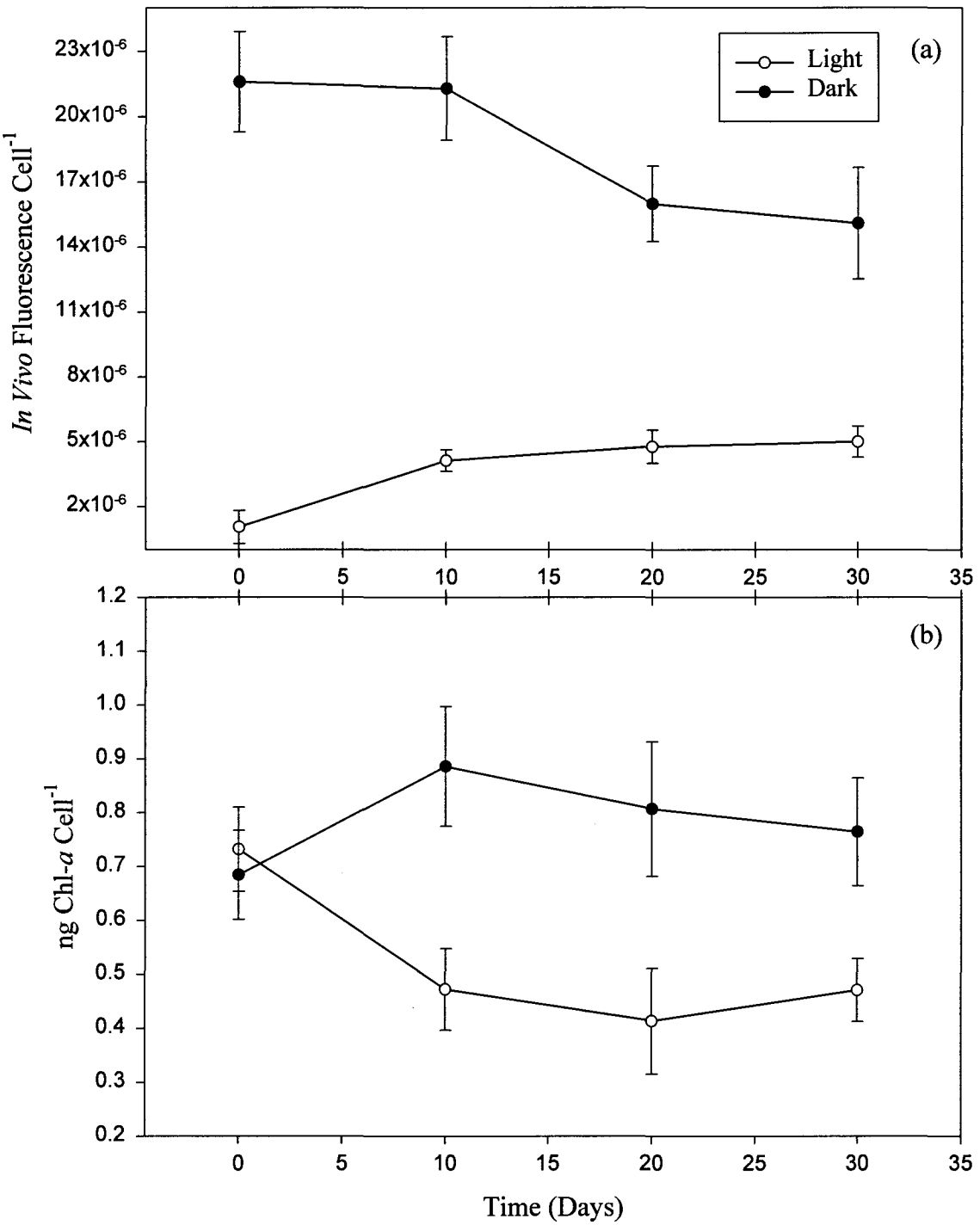


Fig. 4.5. Plots of (a) Cellular Fluorescence versus time and (b) Cellular Chlorophyll-*a* versus time for encysting cultures of *Amphidinium carterae*. Cultures were incubated in either Light (128± 14 μE $m^{-2}s^{-1}$; L/D 12:12) or Dark conditions. Cyst Formation was estimated to have begun after day 10 (see Fig. 4.4).

Table 4.4. Results of a 2-factor, fixed effects ANOVA concerning the impact of light and incubation time upon cellular fuorescence and cellular chlorophyll-*a* concentration for laboratory cultures of *Amphidinium carterae*. (n_{Light} = 24; n_{Time} = 12, α = 0.05). (a) Cellular Fluorescence, (b) Cellular Chlorophyll-*a* concentration.

Source	df	MS	*F*	*P*
Light	1	6768.75	341.0	0.0001
Time	3	342.500	17.25	0.0001
Lgt * Time	3	1202.57	60.58	0.0001
Error	40	19.8500		
Total	47			

(a)

Source	df	MS	*F*	*P*
Light	1	4524.08	89.38	0.0001
Time	3	155.500	3.070	0.0385
Lgt * Time	3	1026.76	20.29	0.0001
Error	40	50.6167		
Total	47			

(b)

ANOVA (Table 4.4) indicate that light, time and their interaction each had a significant impact upon both cellular chlorophyll-*a* and cellular fluorescence.

The Tukey test results indicate that *in* vivo fluorescence in each group was significantly different at all times during the experiment (Table 4.5c). Cellular fluorescence in the light treatment decreased by 52.3% over the first ten days of incubation but did not significantly change over the next 20 days (Fig. 4.4a). In the dark treatment, normalized fluorescence declined by 25.0% between days 20 and 30, but showed no other significant changes (i.e., $D_0 \approx D_{10}$; $D_{20} \approx D_{30}$).

While there were no significant differences in mean chlorophyll $cell^{-1}$ at t = 0 days, mean concentrations of cellular chlorophyll in each treatment diverged by day 10. At t = 10 days, mean cellular chlorophyll concentration in the dark-treated cultures had increased by 22.3%, while that from the light-treated cultures exhibited a decrease of 35.5%. Neither group exhibited significant changes in cell chlorophyll concentrations after day 10.

Figure 4.6 describes the change the degradation ratio (F_b/F_a) over time. The graph demonstrates a continual increase in chlorophyll degradation in the dark-treated cultures. The results of the 2-factor ANOVA indicate that light and the interaction between light and time each had a significant effect upon the degradation ratio (Table 4.6a). Time had no significant impact upon the Degradation ratio. After 30 days, the mean ratio in the light

Table 4.5. Results of a Tukey test conducted following the ANOVA results in Table 4.4. The inter-group relationships between mean ranks of (a) Fluorescence Cell^{-1} and (b) Chlorophyll-*a* Cell^{-1} for laboratory cultures of *Amphidinium carterae* have been given. In the context of the experiment, results have been grouped according to time in (c) and (d). Columns of capital letters link treatment groups that were not significantly different at the α= 0.05 level.

Mean	Mean Rank	Treatment Group	Tukey Grouping	
5.82	42.50	D_0	A	
5.34	41.83	D_{10}	A	
1.64	31.33	D_{20}	B	
1.29	29.83	D_{30}	B	D
2.39	22.00	L_0		D
0.89	12.33	L_{30}	C	
1.11	10.33	L_{20}	C	
1.14	5.830	L_{10}	C	

(a) Fluorescence Cell^{-1}

Mean	Mean Rank	Treatment Group	Tukey Grouping	
1.06×10^{-5}	41.17	D_{10}	A	
4.13×10^{-5}	35.17	D_{20}	A	C
4.78×10^{-5}	33.33	D_{30}	A	C
5.01×10^{-5}	30.67	L_0	A	C
2.16×10^{-5}	27.17	D_0		C
2.13×10^{-5}	10.67	L_{10}	B	
1.60×10^{-5}	10.67	L_{30}	B	
1.51×10^{-5}	7.170	L_{20}	B	

(b) Chlorophyll-*a* Cell^{-1}

t = 0 d		t = 10 d		t = 20 d		t = 30 d	
Treatment Group	Tukey Grouping	Treatment Group	Tukey Grouping	Treatment Group	Tukey Grouping	Treatment Group	Tukey Grouping
L	A	L	A	L	A	L	A
D	B	D	B	D	B	D	B

(c) *In vivo* Fluorescence Cell^{-1}

t = 0 d		t = 10 d		t = 20 d		t = 30 d	
Treatment Group	Tukey Grouping	Treatment Group	Tukey Grouping	Treatment Group	Tukey Grouping	Treatment Group	Tukey Grouping
L	A	L	A	L	A	L	A
D	A A	D	B	D	B	D	B

(d) Chlorophyll-*a* Cell^{-1}

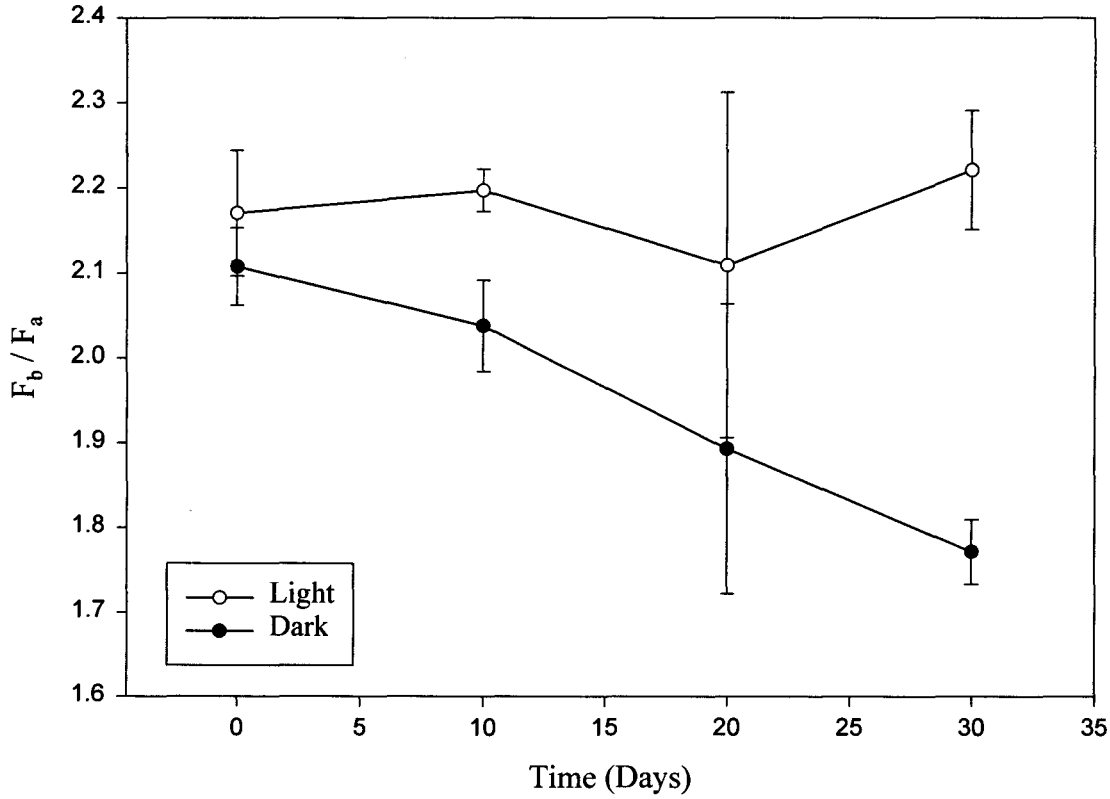


Fig. 4.6. A plot demonstrating changes in the Degradation Ratio (F_b/F_a) with incubation time for encysting cultures of *Amphidinium carterae*. Cultures were incubated in either Light (128± 14 μE $m^{-2}s^{-1}$; L/D 12:12) or Dark conditions.

treatment (2.221) was significantly greater than that of the dark treatment (1.771). The Tukey test results indicated that the degradation ratios in the light and dark treatment groups were significantly different only on days 10 and 30 (Table 4.5 b & c). Therefore, there appeared to be no significant trend in inter-group differences over the duration of the experiment.

In order to gain some insight into the relationships between variables, the degree to which chlorophyll-a $cell^{-1}$, fluorescence $cell^{-1}$, chlorophyll degradation, and cyst yield were related was explored by linear correlation. The results of a Spearman rank correlation (Table 4.7) indicate that, in the light treatments, there was a significant positive correlation between cellular fluorescence (LIVC) and cellular chlorophyll (LCC). There was no significant correlation in the dark treatments. The correlation results also indicate a

Table 4.6. Results of a 2-factor, fixed effects ANOVA and subsequent Tukey test concerning the impact of light and incubation time upon the Degradation Ratio (F_b/F_a) for laboratory cultures of *Amphidinium carterae* (n_{Light} = 24; n_{Time} = 12, α = 0.05). Columns of capital letters link treatment groups that were not significantly different at the α= 0.05 level. (a) ANOVA results, (b) Tukey Test results, (c) Time-grouped Tukey results.

Source	df	MS	*F*	*P*
Light	1	4466.021	50.07	0.0021
Time	3	85.73610	0.960	0.4204
Lgt * Time	3	806.0952	9.040	0.0001
Error	40	89.19580		
Total	47			

(a)

Mean Ratio	Mean Rank	Treatment Group	Tukey Grouping		
2.221	38.42	L_{30}		B	
2.198	36.92	L_{10}		B	
2.170	31.08	L_0		B	C
2.109	30.17	L_{20}		B	C
2.108	22.83	D_0	A	B	C
2.038	16.42	D_{10}	A		C
1.893	14.17	D_{20}	A		C
1.771	6.000	D_{30}	A		

(b)

t = 0 d		t = 10 d		t = 20 d		t = 30 d	
Treatment Group	Tukey Grouping	Treatment Group	Tukey Grouping	Treatment Group	Tukey Grouping	Treatmen t Group	Tukey Grouping
L	A	L	A	L	A	L	A
	A				A		
D	A	D	B	D	A	D	B

(c)

Table 4.7. Results of a linear correlation between select variables from the cyst chlorophyll experiment (n = 24).

Correlation	Spearman Coefficient (r_s)	P(α=0.05)	Power (1-β)	Key to Abbreviations
LIVC * LCC	0.822	0.0001	N/A	LCC = Chl-*a* $Cell^{-1}$ (Light)
DIVC * DCC	0.293	0.1648	0.5438	DCC = Chl-*a* $Cell^{-1}$ (Dark)
LCC * LYD	- 0.353	0.0909	0.6064	LIVC = Fluorescence $Cell^{-1}$ (Light)
DCC * DYD	0.192	0.3688	0.8577	DIVC = Fluorescence $Cell^{-1}$ (Dark)
LIVC * LYD	- 0.073	0.7340	0.9484	LFF = F_a/F_b (Light)
DIVC * DYD	- 0.752	0.0001	N/A	DFF = F_a/F_b (Dark)
LFF * LYD	0.124	0.5633	0.9177	LYD = Cyst Yield (Light)
DFF * DYD	- 0.823	0.0001	N/A	DYD = Cyst Yield (Dark)

significant negative correlation between both *in vivo* fluorescence $cell^{-1}$ and F_a/F_b with cyst yield. This negative relationship occurred exclusively in the dark treatments (Table 4.7). No significant correlation was found to exist between these variables in the light-treated cultures.

Carbon Uptake Experiment—Carbon uptake by temporary cysts of *Amphidinium carterae* has been plotted versus time in Figure 4.7. The graph demonstrates a steady increase in mean activity in the dark-incubated cysts, the light-incubated cysts, as well as the fixed control group. The impact of each treatment, as well as that of time, has been assessed with a 2-factor crossed ANOVA. The results of the ANOVA indicate that light, time and their interaction each had a significant impact upon ^{14}C activity (Table 4.8a). The Tukey test results indicate that, at no time during the experiment, were there any significant differences in activity between light treated cysts, dark treated cysts and the dark treated bacterial fraction. The light-treated < 3 μm fraction, which had an initial mean activity that was approximately three times greater than the remaining groups, exhibited a decline in activity over the entire 12 hours. With the exception of the control group, there were no significant differences among any of the treatments by t = 12 hours (Table 4.8b).

Discussion

There are a number of factors that cause dinoflagellates to form cysts; among these, nutrient stress and dark storage have been widely utilized by researchers to prompt encystment in laboratory experiments (e.g., Anderson and Wall 1978; Anderson *et al.* 1982a). While preliminary data suggested that *Amphidinium carterae* forms temporary cysts after dark-incubation, the results of the present study have indicated that light, time, and their interaction each effect encystment. Many photosynthetic processes are both light and time dependent (Takahashi *et al.* 1971); therefore, the same may be true for cyst

Table 4.8. Results of a 2-factor, fixed effects ANOVA and subsequent Tukey test concerning the effect of light and incubation time upon Carbon Uptake for laboratory cultures of *Amphidinium carterae* (n=60, α =0.05). Cultures in respective treatment groups were incubated for (i) 0 hours, (ii) 6 hours, and (iii) 12 hours. Capital letters link group means that were not significantly different at the α = 0.05 level. (a) ANOVA results, (b) Tukey Test results.

Source	df	MS	*F*	*P*
Time	2	1250.06	40.43	0.0001
Treatment	4	2817.47	91.12	0.0001
Time * Trt.	8	1124.97	36.38	0.0001
Error	44	30.9200		
Total	58			

(a)

t = 0 hrs				t = 6 hrs				t = 12 hrs			
Mean (dpm)	Mean Rank ($X^{1/2}$)	Trt. Group	Tukey Grouping	Mean (dpm)	Mean Rank ($X^{1/2}$)	Trt. Group	Tukey Grouping	Mean (dpm)	Mean Rank ($X^{1/2}$)	Trt. Group	Tukey Grouping
1069.8	6.9640	LC	A A	1733.8	7.4576	LC	A A	2098.7	7.6464	LC	A A
1026.3	6.9298	DC	A A	1688.3	7.4215	DC	A A	2279.9	7.7275	DC	A A
1436.5	7.2659	DB	A	1780.7	7.4736	DB	A	2372.2	7.7654	DB	A A
793.57	6.5757	LCF	B	670.60	6.4964	LCF	B	2648.1	7.8757	LB	A
3463.2	8.1364	LB	C	2897.1	8.0656	LB	C	1039.2	6.9268	LCF	B

LC = Light Treated Cysts LB = Light Treated < 20 µm Fraction

DC = Dark Treated Cysts DB = Dark Treated < 20 µm Fraction

LCF = Light Treated Cysts Fixed with 1% (v/v) Glutaraldehyde

(b)

formation. The effect of nutrient limitation was not of direct interest in the present study,but cannot be ruled out entirely. The results of a related experiment (Table 3.1) have suggested that the interaction between nutrient limitation and darkness had a significant impact upon encystment in *A. carterae*.

Binder and Anderson (1990) have reported that the formation of sexual cysts by *Scrippsiella trochoidea* occurred during approximately 25 days of dark incubation.

Temporary encystment has been described as a means by which dinoflagellates avoid sudden, short term stress (Wall 1975; Marasovic 1989). Conversely, hypnozygote formation generally includes gametogenesis, fusion, settling, and encystment (Taylor 1987), which is likely to require more time than simple ecdysis. Fritz and Triemer (1985), for example, have demonstrated that encystment occurred within ten minutes following the introduction of *Gonyaulax tamarensis* cells into distilled water. In the present study, *Amphidinium carterae* required approximately 30 days of dark incubation before cultures approached 100% encystment.

Although the encystment process may have been complicated by other factors, the difference may be due to the nature of the stress. Rapid shifts in physiochemical factors such as salinity and temperature are more likely to be lethal to a cell than the same degree of light stress. Therefore, selective pressure would dictate that encystment must proceed rapidly if the cell is to survive the stress. Light deprivation, which first affects photosynthetic systems, may allow a greater time lag before the stress elicits a similar response. Had nutrient depletion been combined with light deprivation in the current experiment, it is possible that the amount of time required for encystment would have been reduced. It seems likely that dark-dependent encystment induces a gradual disassembly of photosynthetic mechanisms.

It is possible that the experimental design may have introduced a nuisance factor into the encystment process. In particular, oxygen stress may have been induced due to restricted gas exchange within the cyst cultures. The dark-treated flasks were stoppered with cotton gauze, paraffin film, and aluminum foil in order to prevent exposure to light. The light-treated flasks were covered only with cotton gauze. Due to the presence of organic detritus and dead cells within cyst cultures, the biological oxygen demand within the darkened flasks may have caused hypoxia. As oxygen was not monitored during this experiment, the impact of this factor cannot be assessed.

Based upon the results given in Figure 4.5b, the average concentration of chlorophyll-*a* per *Amphidinium* cyst was 760 pg chl-*a* cell^{-1}. This concentration is more than two orders of magnitude greater than that reported for *S. trochoidea* (ca. 1.5 pg chl-*a* cell^{-1}) by Binder and Anderson (1990). While the average size of *A. carterae* cysts was 10 to 15 μm, that of *S. trochoidea* is approximately 25-45 μm (Hallegraeff *et al.* 1995). Assuming a roughly spherical cyst for *A. carterae* (radius = 12.5 μm), cyst volume would approximate 8181 μm^3 cyst^{-1} ($V_{sphere} = 4/3\ \pi\ r^3$). A rough approximation of the amount of chlorophyll-a per μm^3 in a temporary cyst of *A. carterae* would be 9.3 x 10^{-2} pg chl-*a* μm^{-3}. Utilizing the volume given by Anderson *et al.* (1985b) for a spineless cyst (radius = 12.9 μm), *S. trochoidea* would have an approximate chlorophyll-*a* concentration of 1.7 x 10^{-4} pg chl-*a* μm^{-3}. Therefore, although the sexual cysts (*S. trochoidea*) and temporary cysts (*A. carterae*) had similar diameters, the amount of chlorophyll per μm^3 in the temporary cysts exceeded that of the sexual cysts by more than 500 times.

The formation of sexual cysts in *Scrippsiella* was found to prompt a decline in cellular chlorophyll of nearly 80%. This pattern was not paralleled in *Amphidinium carterae*, which exhibited an average chlorophyll gain of almost 31% during encystment. As Binder and Anderson demonstrated some degree of photosynthetic activity in *Scrippsiella* cysts, a respectively greater rate of photosynthesis could be expected in Amphidinium. As this was not the case, photosynthetic capacity does not appear to be a direct function of cellular chlorophyll concentration in temporary cysts.

While no data were provided by Binder and Anderson (1990) regarding the fate of the lost pigment, in the present study, a portion of it was degraded to phaeopigment within the cell. The correlation between the degradation ratio and the cyst yield was negative in the dark treatments, suggesting that degradation of chlorophyll was at least indirectly related to cyst formation. Whether the degradative end products remained within the cell or were released during ecdysis is not known.

With respect to culture volume, the mean degradation ratio in the dark-treated cultures declined from 2.11 to 1.77 over the course of the experiment. Following an incubation experiment with *Phaeodactylum tricornutum* (Bacillariophyceae), Yenstch (1965) reported that rapid degradation of chlorophyll began after 70 hours of dark incubation. After 270 hours of light deprivation, all chlorophyll was converted to phaeopigments (i.e., $F_b/F_a \approx 0$). In the present study, *A. carterae* cultures reached a ratio of no less than 1.77 after 720 hours. Therefore, pigment degradation was by no means complete.

As there were no significant differences in uptake of ^{14}C-bicarbonate between light-treated cysts, dark-treated cysts, and the dark-treated < 3 μm fraction (Fig. 4.7), the measured uptake can not be termed photosynthetic. The lack of a significant increase in activity in the control group suggests that simple diffusion cannot explain the pattern of uptake. The results of the uptake experiment are consistent with those of Binder and Anderson (1990), who found no evidence of significant photosynthetic activity in *S. trochoidea*. This evidence would require that photosynthetic uptake of labeled CO_2 occurred, and more importantly, that it was enough to compensate for respiration.

There are three alternatives that can be used to explain the lack of activity in *Amphidinium* cysts: (*1*) High light stress may have caused photoinhibition, thereby negating any gain in carbon uptake, (*2*) photorespiration my have lowered net photosynthesis through the oxidation of glycolate, and (*3*) bacterial transformation to CO_2 gas would result in a loss of label.

With regard to photoinhibition, it is interesting to note that Binder and Anderson (1990) incubated *Scrippsiella* cysts at a saturation intensity for active cells. Some questionmay be raised as to whether saturating light intensity would be expected to remain stable while many of the normal photosynthetic functions were interrupted. As chlorophyll concentration decreased rapidly during encystment, it is likely that the saturating intensity also declined. The effects of photoinhibition have been described by

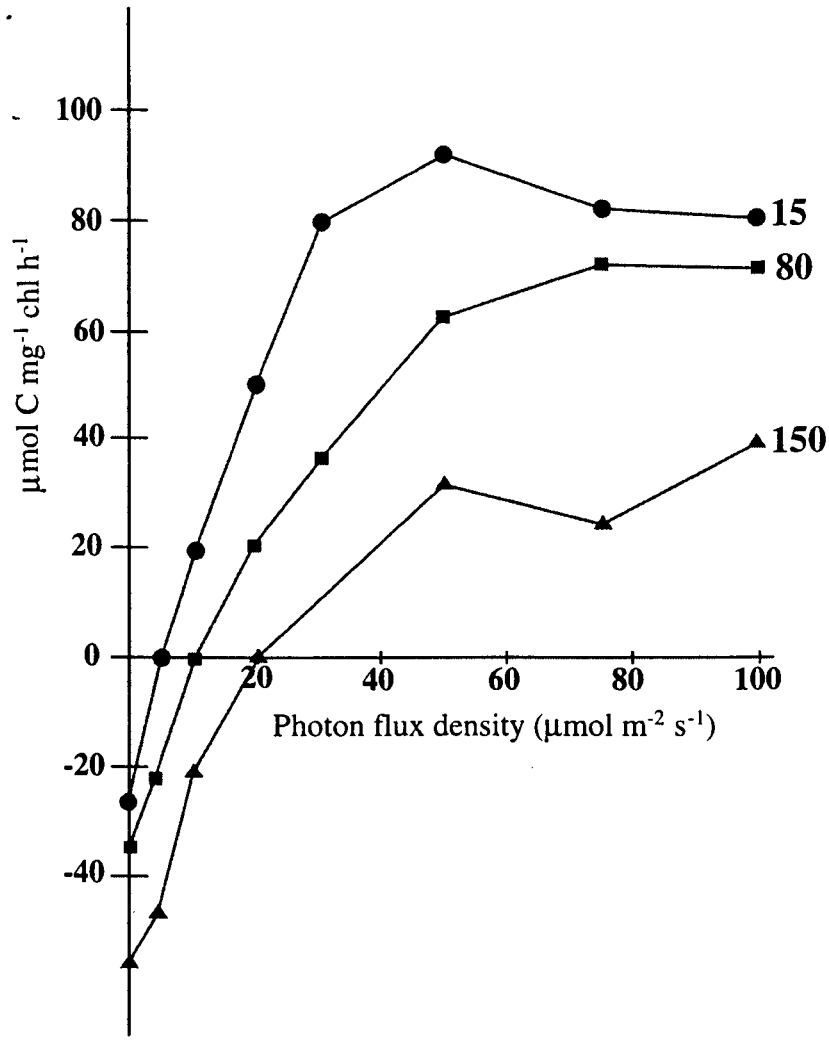


Fig. 4.8. Photosynthetic light saturation curves for *Amphidinium carterae* cultures that were preconditioned at three different light intensities. Rates of carbon fixation are expressed per µg Chl-*a*. (Recopied from Samuelsson and Richardson 1982.)

Peterson (1980) as resulting in an increase in respiration as well as a reduction in the rate of photosynthesis. Not only is there an inhibition of light harvesting, but carbon may be respired as quickly as it is fixed. The result is a decrease in both gross and net photosynthesis.

The impact of high-light photostress upon photosynthesis in *Amphidinium carterae* was examined by Samuelsson and Richardson (1982). As is depicted in Figure 4.8, low-

light conditioned (15 μE m^{-2} s^{-1}) cultures were found to be photoinhibited when exposed to light intensities over 50 μE m^{-2} s^{-1}. The evidence of photoinhibition was reinforced by the observation that cells exhibited signs of photo-bleaching. Specifically, when *Amphidinium* cultures were grown at 15 μE m^{-2} s^{-1}, cells appeared to have a brownish-red color. In cultures grown at 80 and 150 μE m^{-2} s^{-1}, cells were observed by the authors to be greenish-yellow in color. In the current study, microscopic examination of cysts and recently-germinated cysts indicated that cells appeared bleached when incubated at an intensity of approximately 175 μE m^{-2} s^{-1}.

In addition to reducing photosynthesis, photoinhibition may also prompt a decline in chlorophyll fluorescence (Peterson 1980). This decline can likely be attributed to quenching, which would cause a further reduction in photosynthetic efficiency. The correlation data given in Table 4.7 indicates that there was a relatively strong negative linear relationship between dark encystment (cyst yield) and both fluorescence $cell^{-1}$ and F_b/F_a. However, there was a significant linear relationship between cellular chlorophyll and cellular fluorescence only in the light treatment. Therefore, although both fluorescence and the degradation ratio were negatively correlated with cyst formation, the two variables were not directly related. This can be explained by the fact that measurements of *in vivo* fluorescence cannot distinguish between degraded and undegraded forms of chlorophyll.

Both Anderson *et al.* (1987) and Yentsch *et al.* (1980) have utilized *in vivo* fluorescence as an indicator of cyst activation. The initiation of chlorophyll activity was therefore an indication that cysts were nearing germination. In the current study, cellular fluorescence was not linearly related to the amount of chlorophyll in a given cyst (Table 4.7). The findings in the present study agree with those of Anderson *et al.* (1987) and Yentsch *et al.* (1980), as dark-incubated cysts would not be expected to exhibit fluorescence. It is therefore likely that while temporary cysts of *A. carterae* bear chlorophyll, it is present in a state of reduced fluorescence. This would explain the

observed lack of photosynthetic activity.

The likelihood that photorespiration may have lowered rates of net photosynthesis such that it was undetectable must also be addressed. Photorespiration refers to the oxidation of glycolate, which is a short-term product of photosynthesis (Voet and Voet 1995). Tolbert (1974) has reported that up to 50% of recently fixed CO_2 can be photorespired in some plants. Therefore, photorespiration is another process by which fixed carbon can be released into the medium without being detected. According to Peterson (1980) and Voet & Voet (1995), photorespiration usually occurs under CO_2 limiting, oxygen replete conditions. As the uptake experiment was conducted in sealed serum bottles, there is some question as to the degree of gas exchange with the atmosphere inside each bottle. However, as the maximum uptake of $H^{14}CO_3$ (3463 dpm) represented only 0.16% of the available activity per bottle, it is unlikely that CO_2 was limiting. Furthermore, as *Amphidinium* cyst cultures were routinely observed to contain significant amounts of detritus and miscellaneous organic material, the potential for oxygen saturation seems remote as well. Therefore, the impact of photorespiration is expected to have been minimal.

The third explanation for the lack of photosynthetic uptake concerns the bacterial component, that is, the < 3 μm fraction. Even in short term experiments, the bacterial component can have a significant effect upon dissolved carbon pools (Peterson 1980). For example, Larsson and Hagström (1979) reported that bacteria could release labeled DOC into the medium as fast as $^{14}CO_2$ was produced by phytoplankton. This was based upon the existence of a relatively constant rate of bacterial assimilation coupled with a declining rate of phytoplankton uptake. Indeed, phytoplankton commonly exhibit a rapid initial rate of carbon uptake, which generally declines after the first three hours of incubation (Peterson 1980). In the current study, the anomalously high activity exhibited by the light-treated < 3 μm fraction (Fig. 4.7) is suggestive of a similar pattern.

McAllister *et al.* (1964) was unable to utilize cultures of *Amphidinium carterae* for carbon uptake experiments due to excess organic material present in cultures. This material, which was described as a gelatinous matrix of "non-cellular" material was routinely observed in cultures during the present study (Figs. 3.8b-c). The gelatinous material may have influenced carbon uptake in two ways. First, due to its adhesive nature, the mucilage may have removed cysts from the water column of each culture vessel. A similar problem was encountered during the cyst chlorophyll experiment, in which a significant number of cysts were lost due to adhesion to the culture flask. The result of this removal would be a reduction in measurable label, yielding underestimates of carbon uptake.

Examination of a sample of mucilage using differential interference contrast microscopy revealed the presence of many rod-shaped bacteria. While the majority of those observed were attached to the surface of cysts and detritus, others appeared to be suspended within the matrix itself. Hobbie *et al.* (1972) reported that clumps of algal detritus, which often form in senescent cultures, harbored significant populations of associated bacteria. Accordingly, it seems likely that the bacteria observed in the present study were heterotrophic bacteria which utilized the ample supply of organic material in each flask.

While the source of this contamination is not known, bacterial contaminants appear to be common in *Amphidinium carterae* cultures. Both Galleron (1976) and Nayak *et al.* (1997) have reported an inability to obtain axenic cultures of this species. This difficulty stemmed from the presence of bacteria intimately associated with the algal cells. These bacteria repeatedly escaped antibiotic treatments. Particularly in older cultures, such as those used for encystment experiments, heterotrophic bacterial populations may be well developed. The presence of these bacteria further complicates carbon activity experiments, as they may assimilate and release labeled carbon into solution. Again, this would act to reduce the amount of detectable fixed carbon.

5 SUMMARY AND CONCLUSIONS

Amphidinium carterae has been found to produce temporary cysts in laboratory cultures. Cells, which were examined by light microscopy, were classified as temporary cysts based upon (*1*) the presence of a single membrane around each cell, (*2*) the absence of a yellow-orange eyespot, and (*3*) the ability of cysts to germinate within 1 - 2 days of formation. As there was no evidence of cell division prior to or closely following excystment, the possibility that the cysts were sexually derived seems remote. In accordance with the descriptions provided by Anderson and Wall (1978), both coccoid and ovoid types of cysts were identified in culture vessels. The former were spherical, contained a yellow pigment, and ranged between 10 and 15 μm in diameter (Fig. 3.7b). Ovoid cysts were smaller (ca. 8 X 10 μm), apically tapered, and virtually colorless. After 30 days of dark incubation, most coccoid cysts were replaced by the ovoid type.

Based upon preliminary observations, it was hypothesized that germination of the temporary cysts of *Amphidinium carterae* was dependent upon light intensity. The results given in Tables 3.1 and 4.2b suggest that encystment is significantly impacted by light, time, and nutrient enrichment. The results of the excystment experiment indicated that cyst germination is influenced by incubation time, light intensity, and their interaction (Table 3.2b). However, any interaction among time, light and nutrients is beyond the scope of this work. When the ratio of Total Cells:Cysts was used as a measure of germination success (Fig. 3.5), incubation at light intensities greater than 68 μE m^{-2} s^{-1} yielded lower ratios than incubation at 30 μE m^{-2} s^{-1}. As *A. carterae* can be expected to divide only in the dark (Galleron 1976), such a decrease in the number of cysts is indicative of germination. This pattern was paralleled by a decline in fluorescence at similar light intensities (Figs. 3.6a-b). Cysts incubated at 30 μE m^{-2} s^{-1} exhibited a smaller decline in fluorescence $cell^{-1}$ than did the remaining treatment groups. Observation suggests that cysts were bleached

by light exposure at all three light intensities. As cellular chlorophyll was not quantified in this experiment, declining fluorescence could be interpreted either as photodestruction of chlorophyll or as quenching.

The results of the germination experiment contrast with those of Anderson *et al.* (1987), who reported increased germination success in sexual cysts of three dinoflagellates when light intensities were maintained above 400 $\mu E\ m^{-2}\ s^{-1}$. It is likely that physiological differences between sexual and temporary cysts may partially explain the apparent contradiction in results obtained in each study. Further work is required before variability in light-response between different types cysts and/or different species may be adequately addressed.

It was additionally hypothesized that individual cells of *A. carterae* retain relatively low concentrations of chlorophyll during cyst formation. The effect of encystment upon chlorophyll concentrations and *in vivo* fluorescence has been depicted in Figures 4.5. As total cell concentration did not remain constant with time (Fig. 4.), chlorophyll concentration and *in vivo* fluorescence were first normalized to the number of cells. The cyst yield in dark-treated cultures increased dramatically after the first 10 days of incubation (Fig. 4.4b), reaching a maximum of 100% by day 30. As depicted in Figure 4.4b, the greatest rate of encystment (days 10-20) was concomitant with the maximum (nearly 60%) of decline in the total cell concentration (Fig. 4.3b). Therefore, there was a lag of approximately 10 days between initial light deprivation and the majority of cyst formation. It is possible that short term light deprivation (<10 days) fails to provide sufficient metabolic stress to elicit encystment in *Amphidinium carterae*.

In contrast to the data given by Binder and Anderson (1990), cellular chlorophyll concentrations in *A. carterae* did not decline greatly during cyst formation (Fig. 4.4c). Instead, there was a net increase in cellular chlorophyll of approximately 31%. As the two studies targeted dinoflagellates from different dinoflagellate species (as well as different

families), the variation in results is not suprising. It is likely that the physiological differences between sexual and temporary cysts may, in part, explain the difference between each species.

Cyst formation was also paralleled by degradation of chlorophyll (Fig. 4.6). As this process was exclusive to the dark-treated cultures, photodegradation can be ruled out. The presence of significant quantities of degraded chlorophyll within temporary cysts may be viewed as a reservoir of stored photosynthetic pigment. This reservoir would likely provide a metabolically inexpensive, readily accessible supply of chlorophyll to each cell to be used upon improvement of the ambient light field.

Light incubated cultures exhibited cellular chlorophyll concentrations that were consistently less than those of the dark treatments. Cellular chlorophyll in both treatments remained approximately constant after ten days of incubation (Fig. 4.5b). Therefore, different processes appear to have been at work in light and dark groups. As total cell concentration decreased through the incubation period (Fig. 4.4a), reduced cellular chlorophyll concentrations cannot be attributed to simple cell division. A light-dependent feedback mechanism, such as was suggested by Falkowski (1980), may have provided an internal control upon chlorophyll production (Fig. 4.1).

Aside from chlorophyll concentration and *in vivo* fluorescence, carbon uptake was also evaluated in the temporary cysts of *A. carterae*. It was hypothesized that cells retained photosynthetic activity after encystment. As depicted in Figure 4.7, the activity in light-treated cysts was not significantly different from those incubated in the dark. Thus, no evidence of photosynthetic activity was observed in temporary cysts during 12 hours of light-incubation. Samuelsson and Richardson (1982) reported that *Amphidinium carterae* appears to be a species adapted for low light intensities (Fig. 4.8). While either photorespiration or photoinhibition may have been involved in the apparent lack of photosynthetic activity, observations of germinating cysts have suggested the latter. Namely, germination of temporary cysts was observed to be followed soon after by cell

bleaching and mortality. Given the results given in Figures 3.6, it is likely that photoinhibition occurred. Because CO_2 must be limiting before photorespiration will occur (Peterson, 1980), the introduction of further HCO_3^- into each bottle would render this scenario unlikely. Interestingly, stock cultures of *A. carterea* were routinely incubated at light intensities >100 μE m^{-2} s^{-1}. Although self-shading may have prevented some degree of photoinhibition from occurring, temporary cysts appear to be less tolerant of light exposure than are active cells.

Considering the conclusions presented here regarding the impact of high light intensity upon cyst germination, *in vivo* fluorescence and photosynthesis, *Amphidinium carterae* appears to be adapted for low light intensities. This conclusion is in agreement with that of Samuelsson and Richardson (1982), who reported that the Woods Hole strain was photoinhibited at light intensities above approximately 50 μE m^{-2} s^{-1}. Research conducted in tropical and equatorial regions has typically indicated that *A. carterae* is a benthic species (e.g., Faust 1995; Yasumoto 1990; Nakajima *et al.* 1981). Conversely, similar studies dealing with exclusively temperate strains have made no such indication (e.g., Sampayo 1985; Byerrum and Benson 1981; Hulburt 1957). Given that the depth of the euphotic zone in equatorial waters is often much greater than in temperate latitudes (Lalli and Parsons 1993), surface light intensities would tend to be higher in the former. It is possible that benthic habitats in lower latitudes offer protection from light stress. Vertical migration in and out of interstitial microhabitats may allow such a shade-adapted species to survive in an otherwise unfavorable environment.

The presence of bacterial contaminants or excess organic material may have had an impact upon the results of carbon uptake experiment as well. As bacteria may rapidly take up labeled CO_2 during short term experiments (Peterson 1980), the anomalously high activity observed in the light-incubated, < 3 μm group may reflect this process. In such a manner, the bacterial component would function as a sink for labeled carbon. Particulate

organic material may also act as a sink for labeled carbon. Cultures of *Amphidinium carterae* have been reported to produce relatively large quantities of detritus (Galleron 1976). This characteristic has been confirmed in the present study. In particular, cellular detritus and cysts were observed to accumulate at the bottom of culture vessels. Any label associated with these materials would be effectively removed from solution. McAllister *et al.* (1964) suggested that *A. carterae* was not suitable for labeling experiments due to uptake of label by detritus. Therefore, the results obtained in the present experiment contain a degree of uncertainty.

In conclusion, the results of this study have suggested the following: (*1*) The observed resting stages were actually temporary cysts, (*2*) Cyst formation was prompted by light deprivation, incubation time, and their interaction, (*3*) Germination success increased with decreasing light intensity, (*4*) Temporary cysts of *A. carterae* retained most of the chlorophyll that was present in the parental cell, although it may be stored in a degraded form, and (*5*) Temporary cysts exhibited no evidence of photosynthetic activity, even at a relatively low light intensity. Experimental results, when coupled with direct observations, indicated that temporary cysts of *Amphidinium carterae* are sensitive to light. The effect of light exposure may manifest as photoinhibition, quenching, photo-destruction of chlorophyll, decreased germination success and/or mortality. As stock cultures were routinely incubated at light intensities greater than 100 $\mu E\ m^{-2}\ s^{-1}$, temporary cysts appear to be more sensitive to light stress than corresponding active cells.

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