ENVIRONMENTAL REGULATION OF TOXIN PRODUCTION: COMPARISON OF HEMOLYTIC ACTIVITY OF *Amphidinium carterae* AND *Amphidinium klebsii*

Leigh A. Zimmermann

A Thesis Submitted to
University of North Carolina Wilmington in Partial Fulfillment
Of the Requirements for the Degree of
Master of Science

Center for Marine Science

University of North Carolina Wilmington

2006

Approved by

Advisory Committee

_____ _____

_____
Chair

Accepted by

_____
Dean, Graduate School

This thesis was prepared according to the
formatting guidelines of the *Journal of Phycology*.

TABLE OF CONTENTS

ABSTRACT

Many phytoplankton blooms consist of dinoflagellates or diatoms that produce toxins, physical irritants, or noxious effects. While most shellfish that bioaccumulate HAB toxins are not always severely affected, many finfish have adverse reactions to the presence of toxins, particularly neurotoxins, in the water. This can result in massive fish kills. As more becomes known about blooms related to human health, it appears that most algae producing toxins affecting humans also produce ichthyotoxic compounds. *Amphidinium carterae* and *Amphidinium klebsii*, dinoflagellates, have been implicated in fish kills and red tide events and produce compounds known to be hemolytic and antifungal in nature. The aim of this study was to determine what role environmental factors play in the toxicity of two species of dinoflagellates, *A. carterae* and *A. klebsii.* Four environmental factors were considered: nutrient availability, salinity, temperature, and light. Potential toxicity was quantified by measuring the hemolytic activity of these two species grown under different environmental conditions. Growth for both species was highest at the higher salinities, temperatures, and light intensities measured, indicative of the tropical origin of the clones, while nutrient availability did not affect growth. For most methods of normalization, *A. carterae* proved to have more toxic activity than *A. klebsii.* Nutrient availability did affect the toxin production of the species, as did the light, salinity and temperature regimes.

ACKNOWLEDGEMENTS

I give my sincerest thanks to my advisor, Dr. Carmelo Tomas, and to the members of my committee, Dr. Jeffery Wright and Dr. Ronald Sizemore. The technicians and fellow graduate students of the harmful algal bloom lab also deserve a large thank you for their willingness to share knowledge and their encouragement. For her continued support and encouragement of marine science students, I thank Dr. Joan Willey. Travel funds that were used to attend conferences were provided by the UNCW graduate school, as well as Woods Hole Oceanographic Institute. Research funds were provided by the Water Resource Research Institute grant #50337, the North Carolina SeaGrant 2001 leaflet series, the Maryland Coastal Bays Project grant # 2003-0106, and the Center for Disease Control grant #01501-01. Finally, I would like to send a thousand thank yous to my family and friends.

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Harmful algal blooms (HABs) are a problem and concern for the coastal ecosystem and are increasing globally in both intensity and occurrence (Anderson 1989; Hallegraeff 1993; Smayda 2000; Hallegraeff 2003). Many of these phytoplankton blooms consist of dinoflagellates or diatoms that produce toxins, physical irritants, or noxious effects (Baden *et al*. 1998; Anonymous 1999). These blooms and the toxins they produce result in fish kills and some were shown to cause adverse affects on human populations. The aquatic organisms that humans consume such as oysters, clams, mussels, and finfish, temporarily retain or bioaccumulate the toxins produced during blooms. The ingestion of these toxins by humans results in illnesses, such as diarrhetic, amnesic, paralytic, and neurotoxic shellfish poisoning, and ciguatera fish poisoning. Inhalation of some toxins by humans can also result in a wide range of symptoms, including nausea, diarrhea, and bronchoconstriction (Baden & Trainer 1993; Baden *et al*. 1995). The potential threat to human health, loss of resources from commercial fisheries and shellfish industries, and development of strategies for managing these blooms are some of the driving forces of HAB research.

Presently, much is known about HAB toxins and their implications on human health. As mentioned previously, several species produce toxins that result in human syndromes when ingested through seafood vectors. Due to the human health interests and the economic dependence on both the seafood and tourism industries, much research has been focused on these toxin producers. Less understood are the algae that produce ichthyotoxins. They produce the deaths of fish, marine mammals and birds which are easily detected and serve as an indicator of potential human health issues. As more becomes known about blooms and human health, it appears that most algae producing toxins affecting humans also produce ichthyotoxic

compounds. For example, *Karenia brevis*, the causative species of Florida red tide, produces brevetoxins causing human health problems as well as being hemolytic (Kirkpatrick *et al*. 2004). Other species, such as *Chattonella marina* and *Cochlodinium polykrikoides*, were shown to have a toxic cocktail of secondary metabolites in their fish killing mechanism. Neurotoxic compounds working in conjunction with hemolytic compounds, reactive oxygen species (H_2O_2, O_2^-, and OH^-), and hemagglutinating and cytotoxic polyunsaturated fatty acids cause higher ichthyotoxicity than any of the single compounds on their own (Kim *et al*. 2002; Marshall *et al*. 2003). While a hemolytic compound does not always cause death of fish, these compounds are a good measure of potential ichthytoxicity (Eschbach *et al*. 2001).

One of the most studied species of ichthyotoxic algae is *Prymnesium parvum*, known to cause massive fish kills around the world and recently, in Texas and North Carolina (Martin & Padilla 1971; Ralph 1990; Tomas *et al*. 2004; Clouse 2005). This species produces a suite of complex toxins known as prymnesins that are extremely hemolytic in nature (Martin *et al*. 1971; Martin *et al*. 1972; Dafni & Giberman 1972; Doig & Martin 1973; Imai & Inoue 1974; Iragashi *et al*. 1998; Igarashi *et al*. 1999). Research suggests that manipulation of the nutrient environment in which *P. parvum* is grown affects the amount of hemolytic activity produced (Martin *et al*. 1972; Ulitzur 1973; Binford *et al*. 1973; Johansson & Granéli 1999; Granéli & Johansson 2003; Clouse 2005).

Research has shown that many other phytoplankton species produce hemolytic compounds. Rangel *et al*. (1997) showed that extracts from the diatom *Nitzchia* spp. are hemolytic to mouse erythrocytes. When cells were depleted of phosphorous, the freshwater dinoflagellate *Peridinium aciculiferum* was hemolytic to both horse and fish erythrocytes (Rengefors & Legrand 2001). *Heterocapsa circularisquama* displays species specific hemolytic

activity when exposed to a series of mammalian erythrocytes suggesting that its toxins act on erythrocyte membranes through ion channels (Tatsuya *et al*. 2001). *Karlodinium micrum* is a dinoflagellate associated with fish kill events via high hemolytic activity. Cell material of this species is toxic in larval zebrafish hemolytic assays, which suggests a relationship between hemolytic activity and ichthyotoxicity (Kempton *et al*. 2002). Further support for this relationship was shown by Deeds *et al*. (2002), where the LC50 for hemolysis of sonicated *K. micrum* cell material was within cell concentrations known to be toxic to fish. The LC50 for *K. micrum* was substantially higher than the standard saponin compound, used as a positive control.

Members of the genus *Amphidinium* are unarmored dinoflagellates. *Amphidinium klebsii* Kofoid & Swezy has mostly oval cells, with a tongue shaped epicone that deflects to the left and numerous thin chloroplasts. Cells are normally between 20-46 μm long and 14-30 μm wide and dorsoventrally compressed. Studies by Mitchell (1985) and Morton *et al*. (1991) indicated that *A. klebsii* has maximum growth at 27º C. *Amphidinium carterae* Hulburt has a length of 12-18 μm and a width of 8-10 μm. It is also more or less oval and flattened dorso-ventrally with a small epicone deflected to the left. A distinguishing characteristic for *A. carterae* is that it contains one widely branched chloroplast. Both species have a central pyrenoid and are found in temperate and tropical waters worldwide (Steidinger & Tangen 1997; Taylor *et al*. 2003). Species in this genus were shown to spontaneously form cysts under unfavorable environmental conditions, an important role in the sexual cycle of these species (Sampayo 1985; Barlow & Triemer 1988). These two species are photosynthetic, but capable of mixotrophy (Tomas 2003). Members of both species are implicated in historical fish kills and red tide events (Yasumoto 1990).

Growth studies performed by Tomas *et al*. (1987) showed that *Amphidinium carterae* had growth rates of between 0.32 to 0.71 divisions/day. Growth rates varied according to the type of nutrients supplied and, with the exception of the addition of nitrate, the growth rates of all cultures increased with temperature. In another study, nutrient replete cultures of *A. carterae* were shown to have a maximum growth rate of 0.65 divisions/day. The carbon content was 83 pg/cell under nutrient sufficient conditions, but increased when phosphorus was limited (Sakshaug *et al*. 1984).

Both *A. carterae* and *A. klebsii* produce amphidinols (AMs) known to be hemolytic and antifungal in nature. These polyene-polyhydroxy metabolites are characterized by a long carbon chain with multiple hydroxyl groups and polyolefins (Paul *et al*. 1995; Houdai *et al*. 2001). Besides their antifungal nature, AMs are toxic to diatoms (Yasumoto *et al*. 1990). In a recent study, the most toxic amphidinol (AM3) was shown to possess high membrane permeabilizeing activity (Houdai *et al*. 2004). Amphidinolides, another suite of compounds produced by members of the *Amphidinium* genus, are macrolides possessing ether rings, exo-methylene groups, and carbon branches (Kobayashi *et al*. 1996; Tsuda *et al*. 1999; Kubuta *et al*. 2001; Kubuta *et al*. 2001 A and B).

Studies by Yasumoto *et al*. (1980) and Nakajima *et al*. (1981) indicated that both *Amphidinium* species were toxic to mice as well as displaying strong hemolytic activity. When compared to *Prorocentrum lima*, a known producer of okadaic acid, *A. klebsii* was much less toxic, but exceedingly more hemolytic in nature. When the two species were compared to each other, *A. klebsii* displayed much higher hemolytic activity than *A. carterae* (Nakajima *et al*. 1981). Crude extracts of the *Amphidinium* species produced hemolytic activity similar to that demonstrated by *Prymnesium parvum.* Extracted fractions of both were ichthyotoxic during fish

bioassays but not as potent as those from *Prorocentrum concavum* (Nakajima *et al*. 1981). Paul *et al.* (1997) demonstrated that extracts of *Amphidinium* were toxic to diatoms, implying an allelopathic role.

Because these species are widely distributed around the world's oceans, the relationship between environmental influences and the level of toxicity produced is an important issue. A comparison study of these two species that produce similar compounds could serve to provide insights on how environmental factors influence the toxicity of each species and ultimately the potential effects of these species on other organisms, including humans.

The aim of this study was to determine what role environmental factors play in the toxicity of two species of dinoflagellates, *A. carterae* and *A. klebsii.* Four environmental factors were considered: nutrient availability, salinity, temperature, and light. Potential toxicity was quantified by measuring the hemolytic activity of these two species grown under different environmental conditions. The objectives of the study were to determine:

- if *A. carterae* and *A. klebsii* are sensitive to environmental variation, as is seen with *Prymnesium parvum*.
- if the hemolytic activity normalized on a per cell, per carbon, per chlorophyll *a*, and per volume basis was influenced by nutrients, temperature, salinity, and light intensity.
- how the amount of hemolytic activity of one species compares to the other, as well as how the changes in hemolytic activity due to environmental variation compare between species.

METHODS AND MATERIALS

Algal Culture

Clonal cultures of both *A. carterae* and *A. klebsii* (CMS TAC clones 6 and 1, respectively), originally isolated from benthic samples from the Bahamas, were used throughout the course of the study. Reference cultures were grown in an EGC8 environmental growth chamber at 27º C (± 0.5º C) with a 14:10 L:D cycles of cool white fluorescent light and a fluence flux density of ~60 $\mu E \cdot m^{-2} \cdot s^{-1}$. These conditions varied slightly during experiments due to the need to run concurrent experiments in separate environmental chambers or culture rooms with preset L:D cycles and fluence flux densities. The growth media K (Keller & Guillard 1985) was modified by the elimination of Si and trizma and prepared with seawater at a salinity of 39, in order to recreate similar conditions to those from which the cells were originally isolated. Sea water was diluted to make the test salinities, sterilized, and then made into K media by aseptic additions of nutrients. The species tested and the conditions in which they were grown are illustrated in Table 1.

Morphology

General morphological observations and measurements for size and volume were made for each species using a Zeiss Imager Z1 microscope equipped with AxioVision Release 4.4 software, capable of multidimensional depth acquisition such as z-stacks. Length, width, and depth (z-stacks) measurements were obtained for 200 cells of *A. carterae* and *A. klebsii*. These measurements were applied to a biovolume formula developed by Hillebrand *et al*. (1999) to obtain mean cell volume. Members of the *Amphidinium* genus were assigned the geometric shape of ellipsoid and biovolume was calculated using the equation $V= (\pi/6)*a*b*h$, where a

represents the length, b represents the width, and h represents the depth. The mean volume (μm^3) for each species was calculated from the 200 dimensions giving a standard of error <5% of the mean value. Cell densities were measured directly as cell numbers using a Beckman Coulter Multisizer IIE particle counter. Carbon per cell determined from samples collected on precombusted GF/F filters was measured using a CarloErba Elantech NC2100 CHN analyzer according to EPA method 440 (Zimmermann *et al*. 1997). Chlorophyll *a* (Chl *a)* per cell was determined from samples filtered on GF/F glass fiber filters, extracted in 90% acetone (90:10 V:V) and measured using a Turner Designs 10-AU fluorometer equipped with light and filters for Chl *a* without Chl *b* interference (Parsons *et al*. 1984). Carbon and Chl *a* filters were stored in the dark at -80°C until analysis could be performed. The cellular measurements for abundance, volume, Chl *a,* and carbon were used to normalize erythrocyte lysis for both species.

Growth Studies

All *A. carterae* and *A. klebsii* cultures were preconditioned to treatments for at least 7 days prior to their use in experiments. Growth was determined daily from estimates of cell density throughout the course of the experiments. Growth measurements, *in vivo* fluorescence or cell counts, were taken at approximately the same time each day for an entire growth cycle. These measurements were used to determine the growth phase (lag, log, or stationary) of the cultures, so that erythrocyte lysis assays could be performed during each phase. Lag phase was described as the period of growth where the increase in cell numbers or relative fluorescence did not exceed 5% of the initial reading. Log phase is described as the period of growth where greatest increase in cell numbers or relative fluorescence occurred for any consecutive days.

Table 1. Culture conditions (media, temperature, light, and salinity) used for the growth studies of both *A. carterae* and *A. klebsii*.

Treatment	Culture Medium	Temperature (°C)	Light ($\mu E \cdot m^{-2} \cdot s^{-1}$)	Salinity
Nutrient	N-limited K (N:P=4:1)	28	60	39
	P-limited K (N:P=80:1)	28	60	39
	N,P-replete K (N:P=16:1)	28	60	39
	K (N:P=93:1)	28	60	39
Temperature	K	28	60	39
	K	20	60	39
	K	15	60	39
	K	10	60	39
Salinity	K	22	12	39
	K	22	12	30
	K	22	12	20
	K	22	12	15
Light	K	22	21 (100%)	39
	K	22	13 (60%)	39
	K	22	8 (35%)	39
	K	22	5 (20%)	39
	K	22	3 (10%)	39

Stationary phase is described as that period towards the end of the growth period when cell numbers failed to increase consistently and either remained constant or fluctuated around a constant mean. Two replicate experiments were performed for each species growth at different nutrients, temperatures, and salinities, while one experiment for each species was conducted for the light studies. Growth rates, to the base 2, were established using the following equation: $K= [(\log_e N_t * 3.32193) - (\log_e N_0 * 3.32193)]/t$, where N equals either *in vivo* fluorescence or cell numbers and t equals time in days.

Growth Studies: Nutrients, Salinity, and Temperature

Amphidinium klebsii and *A. carterae* were grown in four nutrient conditions. Cultures were grown in nitrogen-limited (N:P = 4:1, 16 μM NO^{3-} + 4 μM PO_4^{3-}), phosphorous-limited (N:P= 80:1, 80 μM NO^{3-} + 1 μM PO_4^{3-}), NP-replete (N:P = 16:1, 58 μM NO^{3-} + 3.63 μM PO_4^{3-}), and modified K media (N:P=93:1). Four salinities (15, 20, 30, and 39) were used to establish a pattern of distribution; salinities were determined using a Fisher hand held salinity refractometer calibrated against seawater of a known salinity. The *Amphidinium* cultures were also grown at four temperatures: 10, 15, 20, and 28° C. Cultures were grown in 60 mL culture tubes with only the treatment variable (nutrient, salinity, or temperature) altered, while all other variables were held constant. Cultures were grown in modified K media, with a constant L:D cycle of cool white fluorescent light. To eliminate potential shock to the cultures, each was preconditioned for at least 7 days to the test treatment. Growth for all temperatures was measured as *in vivo* fluorescence using a Turner Designs 10-AU Fluorometer.

The initial setup of these experiments began with 600 mL of temperature stabilized, sterile media inoculated with enough culture to read ~1.0 on the fluorometer and stored in a 1L

Weaton® bottle. From this homogeneous inoculum, 30 mL aliquots were placed in each of 7 test tubes. Initial fluorescence was measured and recorded as day 0 readings. The remaining inoculum was used to determine initial erythrocyte lysis activity, cellular Chl *a*, cellular carbon, and cell density values. These same analyses were performed at log and stationary phase, as determined indirectly through fluorescence measurements.

Growth Studies: Light

To examine the effect of light on toxin production, five light intensities were used (100, 60, 35, 20 and 10% of ambient light, measured as 21$\mu E \cdot m^{-2} \cdot s^{-1}$). Light was measured with a LI-250A Light Meter equipped with a SR 2π quantum sensor. For these studies a constant L:D cycle (16:8 hour) was maintained. Neutral density screening was attached to plexiglass frames and placed over cultures grown in 2L Corning® aspirator bottles to achieve the desired light range. Daily subsamples were taken after thorough mixing from each culture through a silicon tubing port attached to the aspirator bottles. A 1:10 dilution of this subsample was measured directly for cell number using a Beckman Coulter Multisizer IIE particle counter. Other than light intensity, all other environmental factors were held constant.

A 1.5 L batch of K39 media was placed into 2L aspirator bottles equipped with silicon tubing and pinch clamps. Each bottle was then inoculated with enough preconditioned culture to give a reading of ~1 on the fluorometer. The mesh screening frames were then placed over the aspirator bottles, with the silicon tubing remaining accessible. From the tubing port, approximately 300 mL of homogeneous culture was removed for initial sampling to determine cell densities, erythrocyte lysis activity, Chl *a*, and carbon levels. These same analyses were

performed at log and stationary phase, as determined through cell counts using the Beckman Coulter Multisizer IIE particle counter.

Erythrocyte Lysis Assay

Toxic activity of the two species under various environmental treatments was quantified from dilutions of cell homogenates using the erythrocyte lysis assay (ELA) performed in the 96 well plate format (Eschbach *et al*. 2001). Four methods of normalization were used: per cell, per carbon, per Chl *a*, and per volume. For the purpose of this study hemolytic activity was defined as the lysis of red blood cells (rbc) and the consequent release of hemoglobin. For each treatment/ species combination, an erythrocyte lysis assay was performed during lag phase (day 0 or day 1), log phase (day 8-19) and during stationary phase (day 28-37). Assay plates were prepared, in 96 well plates with V-shaped wells, using a buffer solution as a negative control and a saponin stock solution as a positive control (100% lysis control). Each well contained 125 μL of controls or algal cell dilutions and 125 μL of erythrocyte solution. The 98 well plates were kept on ice during preparation steps. Plates were sealed and stored at 4°C; after a 20-24 hour incubation period, they were then centrifuged at 300 RCF (x g) and 4°C for 10 minutes. The 150 μL of supernatant was transferred to a plate having U-shaped wells. Absorption was read at 414 nm with a Biotek PowerWave$_X$ microtitre plate reader, using KC Junior software. Percent lysis of algal extracts was determined based on the negative (assay buffer and rbc solutions) and positive controls (saponin solution and rbc solution).

Culture samples were centrifuged in 50 mL Fisherbrand® centrifuge tubes at 4°C for 10 minutes at 300 RCF (x g), using a Hermle Labnet Z 383 K Universal Benchtop Centrifuge. The supernatant was removed and 7.5 mL of buffer solution was added to the pellet material. Cell

suspensions were then sonicated for 30 seconds at 50 continuous watts at a frequency of 20 kHz, using a Sonics and Materials Ultrasonic Processor model #GE 130PB. The sonicated cell solution was used to prepare a dilution series at 1:2, 1:5, 1:10, and 1:100 dilutions. All five dilutions, including pure pellet material, were kept on ice during the assay preparation.

The assay buffer used as a negative control and resuspension liquid for all algal extracts contained 150 mM NaCl, 3.2 mM KCl, 1.25 mM $MgSO_4$, 3.75 mM $CaCl_2$, and 12.2 mM TRIS base (all compounds from Sigma and Fisher). The pH was adjusted to 7.4 and the buffer was stored at 4°C (Eschbach *et al*. 2001).

A saponin standard (Sigma-Aldrich, # S4521) of 0.16 mg mL^{-1} in assay buffer solution was made and stored at -80°C as 1.3 mL aliquots. Prior to use in the erythrocyte lysis assay, aliquots were thawed and kept on ice. The saponin stock solution exhibited 100% erythrocyte lysis activity and therefore served as the positive control for each assay. In order to report the findings in a manner similar to those in other studies, the hemolytic activity was converted to saponin equivalents. To do this, a saponin concentration curve was developed and the resulting equation was used to convert all data to saponin equivalency units (Fig. 1).

Human red blood cells were obtained through the American Red Cross. Five to seven mLs of blood was dispensed from the blood bank bags into individual vacutainers and stored at 4°C. Prior to use in the ELA, the desired amount of blood was removed from the vacutainers and placed into a 15 mL Corning® centrifuge tube. The blood was washed at least twice with added assay buffer, gently mixed, and centrifuged at 4°C and 600 RCF (x g) for 5 minutes. After the last washing, the blood cells were mixed with an equivalent amount of buffer solution. From this 1:2 dilution, a 1:50 dilution of blood cells was made. This dilution was kept on ice until used during the assay.

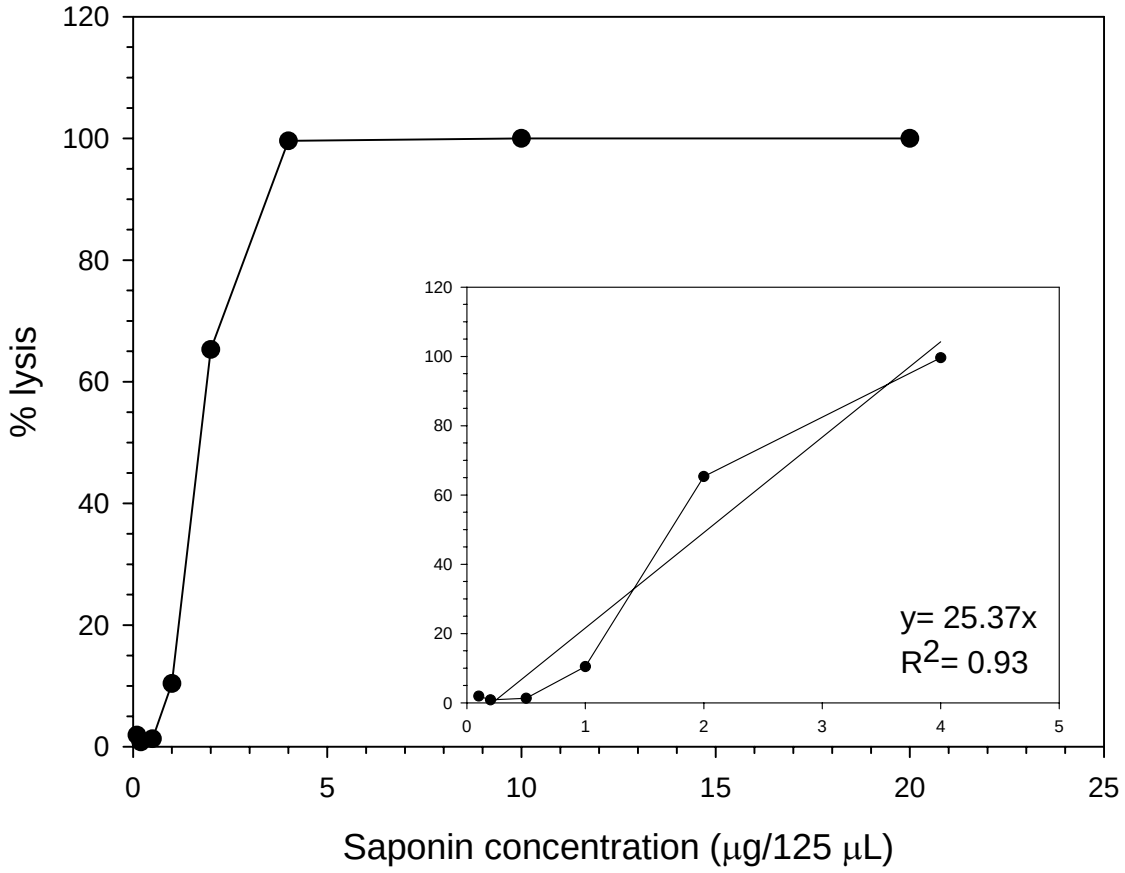

Figure 1. Saponin concentration curve (percent lysis vs. saponin concentration, in μg) used for this study to convert raw erythrocyte lysis data to saponin equivalents.

RESULTS

Morphometry

Several analyses were conducted to provide data with which the erythrocyte lysis analyses could be normalized. These proximate analyses included determination of volume, cell numbers, cellular carbon and cellular chlorophyll a content. Much of the raw data for these proximate analyses is found in the appendices.

Volume

Length, width, and depth measurements were taken on two hundred cells of each species. A representative image of each species and two of the dimensions taken appear in Fig.2. The third dimension, depth, was determined optically using the z- stack function of the Axio Imager. For *A. carterae*, the mean length, width, and depth were 13.4, 9.4, and 7.7 μm, respectively, and the mean volume was 511.8 μm^3. *Amphidinium klebsii* had a mean length, width, and depth of 33.5, 22.2, and 15.9 μm, respectively, and the mean volume was 6,257.6 μm^3 (Table 2). This data showed that the volume of *A. klebsii* was 12 times larger than *A. carterae.*

Cell Counts

For log phase *A. carterae* cells in nutrient limited media, there was a slight difference between trial 1 and 2 however, highest cell densities (>4.0 x 10^5 cells/mL) were found in the full treatment of K 39 followed by progressively lower densities at the 80:1 and 4:1 N:P ratios (Fig. 3). Cells in this culture phase were generally less than those observed in stationary phase where 8.0 and 5.0 x 10^5 cells/mL for each trial respectively. The P-limited stationary phase cultures consistently had the highest (>9.0 x 10^5 cells/mL) cell densities. In the trials dealing with the different salinities, no cells grew below a salinity of 15 and higher values in both log and stationary phase were found at 39, 30 and 20. The salinity response contrasted the response

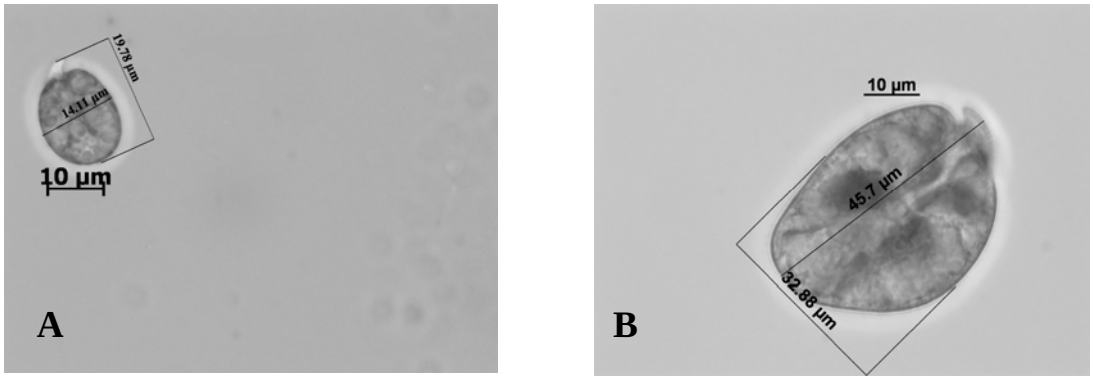

Figure 2. Bright field DIC images at 100x. Cells grown at salinity of 39, 22°C, 16:8 L:D cycle, at 21($\mu E \cdot m^{-2} \cdot s^{-1}$) light intensity for a) *Amphidinium carterae* and b) *Amphidinium klebsii*. These photos represent typical snap photographs used for the measurement of length, width, and cross sections to determine volume of both species.

Table 2. Mean morphometric data for *Amphidinium carterae* and *Amphidinium klebsii.* Two hundred measurements were taken of each to ensure that the standard of error was less than 5% of the mean.

	Amphidinium carterae			*Amphidinium klebsii*		
	mean	n	σ	mean	n	σ
length*	13.4	200	1.6	33.5	200	5.4
width*	9.4	200	1.1	22.2	200	3.8
depth*	7.7	200	1.2	15.9	200	3.8
volume **	511.8	200	143.1	6257.6	200	2352.7

*= μm, **= μm^3

observed in the temperature trials where log phase cells consistently has the highest densities (>4.0 x 10^5 cells/mL) at 28° C and progressively declined with lower temperature. Maximum densities for stationary phase cells occurred at 20° C with no growth occurring at low (10°C) temperatures. The treatment involving different flux densities of light showed a similar response for both log and stationary phases. A progressive decrease from 6.0 x 10^5 cells/mL or less was observed in both the log and stationary phase cells (Fig. 3).

With *A. klebsii* marked differences were observed in the response of cell densities during log and stationary phase (Fig. 4). For the nutrient treatments, log phase of both trials had contrasting results with maximum densities of 4.5 x 10^5 cells/mL with K 39 media in trial 1 with the exact opposite result of maximum densities of >9.0 x 10^5 cells/mL in trail 2. Stationary phase cells were a bit more consistent with values exceeding 6.0 x 10^5 cells/mL for all nutrient treatments in both trials at that stage. The effect of salinity in trial 1 of the *A. klebsii* study was observed most prominently in log phase where densities declined with decline in salinity. Stationary phase cells were slightly variable but always higher than those in log phase. The cells at salinity of 15 or lower showed lowest or no growth. Cell densities in log phase cultures at different temperatures, clearly showed a preference for the highest (28° C) temperature with notable reductions in cell densities at temperatures between 20 and 10° C. A similar pattern was shown in stationary phase cultures with maximum (>3.0 x 10^5 cells/mL) values occurring at 28° C and declining with decreasing temperatures. The light intensity studies with *A. klebsii*, unlike those for A. carterae, showed little response in log phase cells and maximum responses at 100 and 60% ambient light for stationary phase cells (Fig. 4).

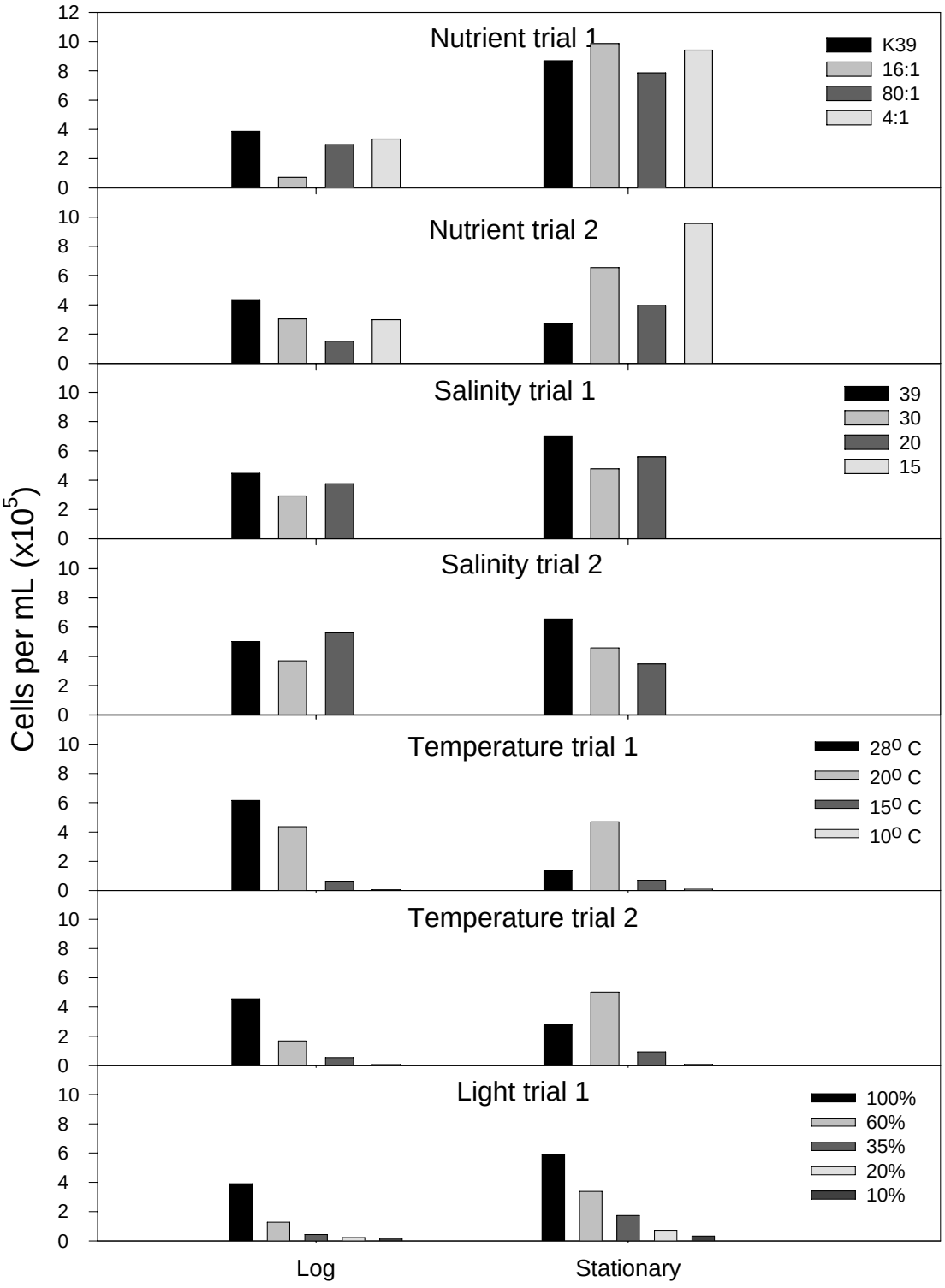

Figure 3. *Amphidinium carterae* cell numbers (cells/mL) sampled during log and stationary growth phases.

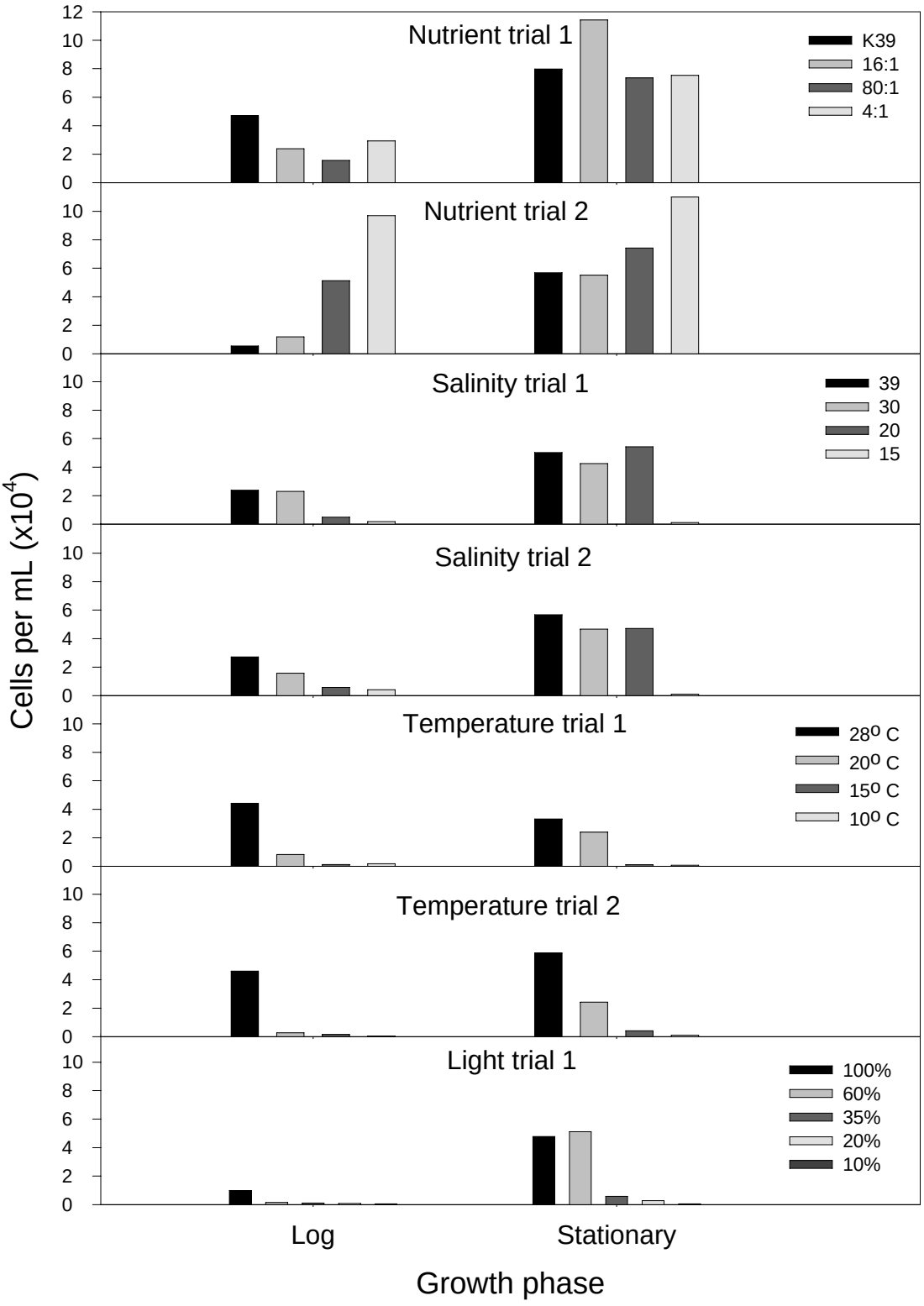

Figure 4. *Amphidinium klebsii* cell numbers (cells/mL) sampled during log and stationary growth phases.

Cellular Carbon

The carbon/cell values of *A. carterae* varied from 36.5 to 1863.6 pg/cell over the entire study (Fig. 5). As to be expected, the carbon values for the log phase cells for all treatments were much lower than those of stationary phase cells. The majority of the values rarely exceeded 100 pg/cell for any nutrient, salinity, temperature and light trial. Values exceeding 100 pg/cell were found in log phase cells grown at salinity of 15 and temperature of 10° C suggesting that this may be a function of a lower growth rate at low salinity/temperature environments. In stationary phase *A. carterae* cells, carbon/cell remained relatively constant (<100 pg/cell) over the nutrient additions. With salinity, only those grown at a salinity of 15 showed values of 10,000 to 150,000 pg/cell. At this salinity *A. carterae* had low cell densities again suggesting that a low growth rate and accumulation of carbon could result in such high cellular carbon values. Unlike salinity, carbon/cell measured at different temperatures for stationary *A. carterae* cells showed a slight increase at the highest (28°C) but consistently lower values at lower temperatures. The light study gave stationary phase cells with between 45 and 80 pg carbon/cell with little effect of intensity (Fig. 5).

Cellular carbon for *A. klebsii* varied from 230.1 to 79,990.0 pg/cell throughout the entire study with this species (Fig. 6). The mean value for carbon/cell for *A. klebsii* was found to be 9 times that of *A. carterae*. As with *A. carterae*, log phase cells showed little differences at the various nutrient treatments with values between 338 and 1087 pg/cell. A similar response was seen with different salinity treatments which were slightly elevated (~ 662 to 1791 pg/cell). The influence of temperature indicated an inverse effect of cellular carbon and temperature. Progressive increases in cellular carbon were seen in *A. klebsii* from ~1000 pg/cell to > 5000 pg/cell with declining temperatures from 28° to 10° C. A definable pattern for cellular

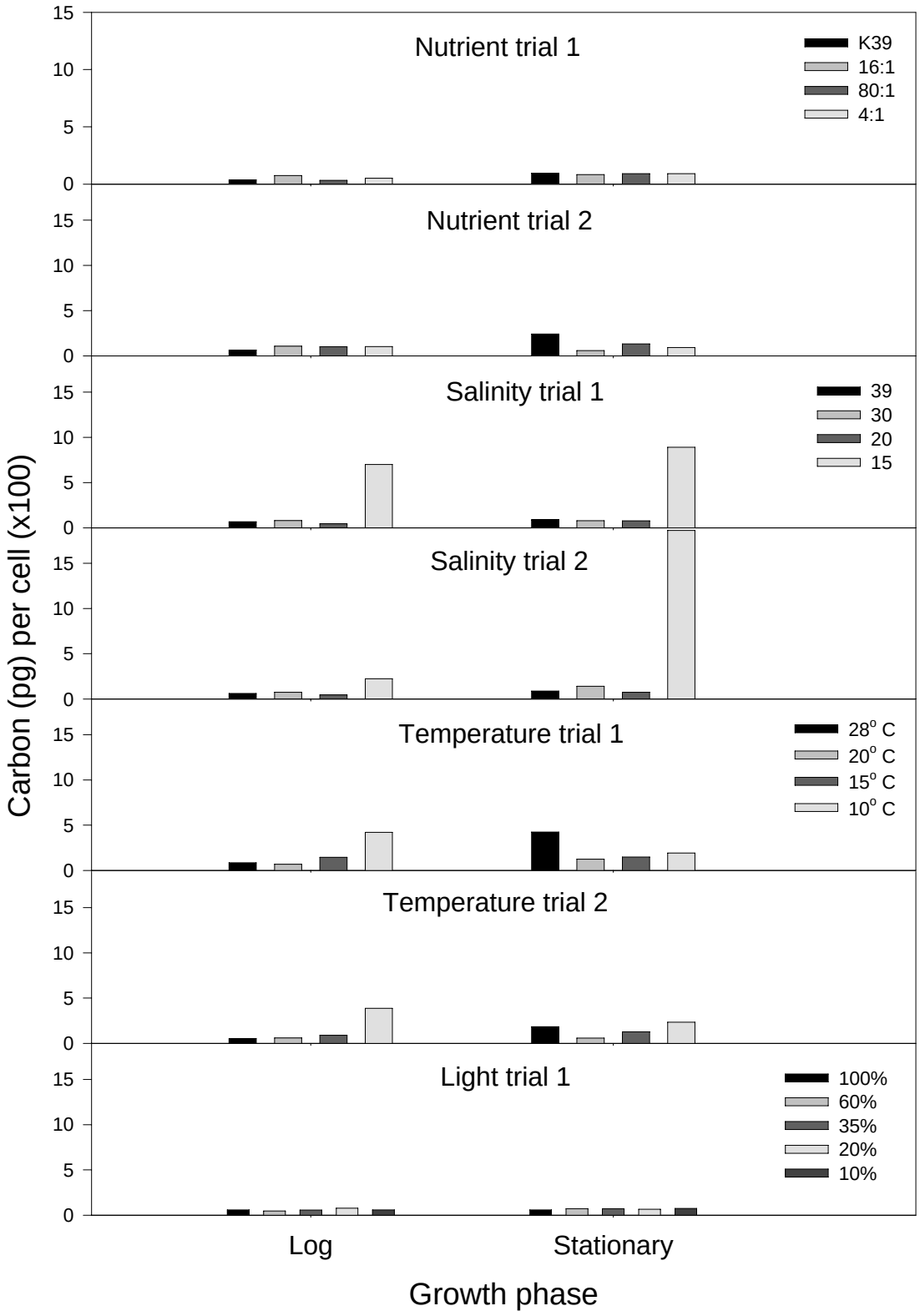

Figure 5. Carbon (pg/cell) for *Amphidinium carterae* at log and stationary growth phases for all treatments.

carbon with varying light was not observed in log phase cells. Stationary phase *A. klebsii* cells had similar carbon/cell values for the two trials of different nutrient ratios. No clear nutrient condition could be identified with increased carbon/cell. Both trials with varying salinities showed no discernable patterns with equivalent (~ 1000 pg/cell) cellular carbon contents for the full range. It was not until the temperature trials that there was any particular response as to carbon/cell. The highest values exceeding 6,000 pg/cell was found only at 15° C during trial 1 and not repeated in trial 2 of stationary phase cells. All other cellular carbon values varied between 1,000 and 2,500 pg/cell at the various temperatures. Cellular carbon in stationary phase was elevated only at the lowest light treatment with a value exceeding 2,000 pg/cell and all other values were near 1,000 pg/cell (Fig. 6).

Cellular chlorophyll *a*

Cellular chlorophyll *a* (Chl *a*)/cell for *Amphidinium carterae* varied from 1.25 to 440 pg/cell throughout this study (Fig. 7). Log phase cells of the nutrient trials rarely had values < 150 pg/cell of Chl *a*. The highest Chl *a*/cell was 253 pg/cell at the balanced 16:1 N:P ratio and for both trials at the phosphorus limited 4:1 treatment. Log phase Chl *a*/cell at different salinities had higher values (~150 to 200 pg/cell) at salinities from 30 to 20 with the lowest cellular Chl *a* occurring at 15. No discernable pattern could be seen in cellular Chl *a* for the different temperature trials and light treatments for log phase *A. carterae* cells. Stationary phase cells generally had higher cellular Chl *a* than log phase cells. Nutrient treatments for stationary *A. carterae* cells were similar to those of log phase cells with no regular variability. Differing salinities, however, had higher cellular Chl *a* at salinity of 15 and roughly equivalent cellular Chl *a* at salinities of 20 to 39. Temperatures between 10 and 28° C appeared to have no affect on lag phase cellular Chl *a* values. The three highest light treatments gave high (between 200 and 250

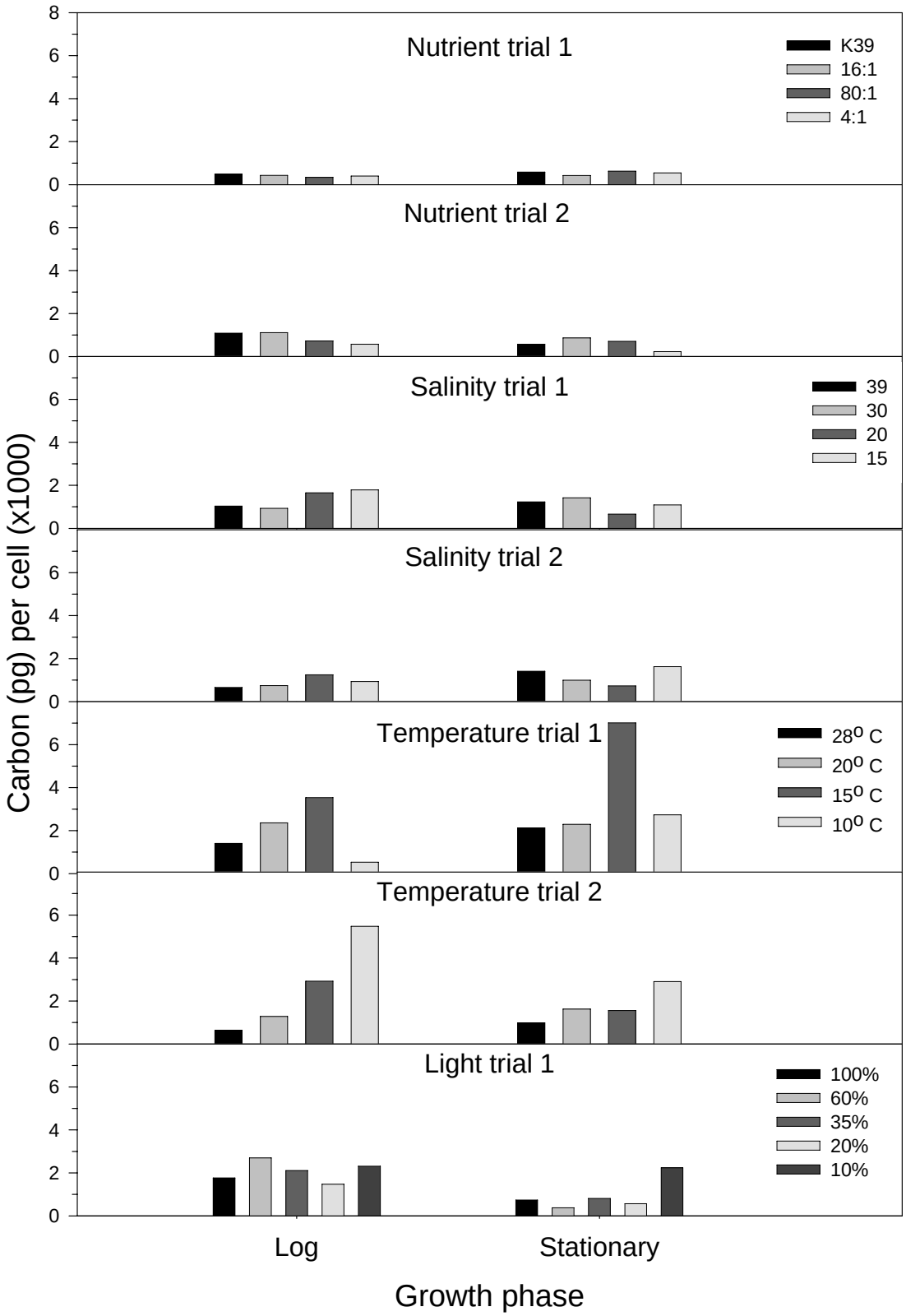

Figure 6. Carbon (pg/cell) for *Amphidinium klebsii* at log and stationary growth phases for all treatments.

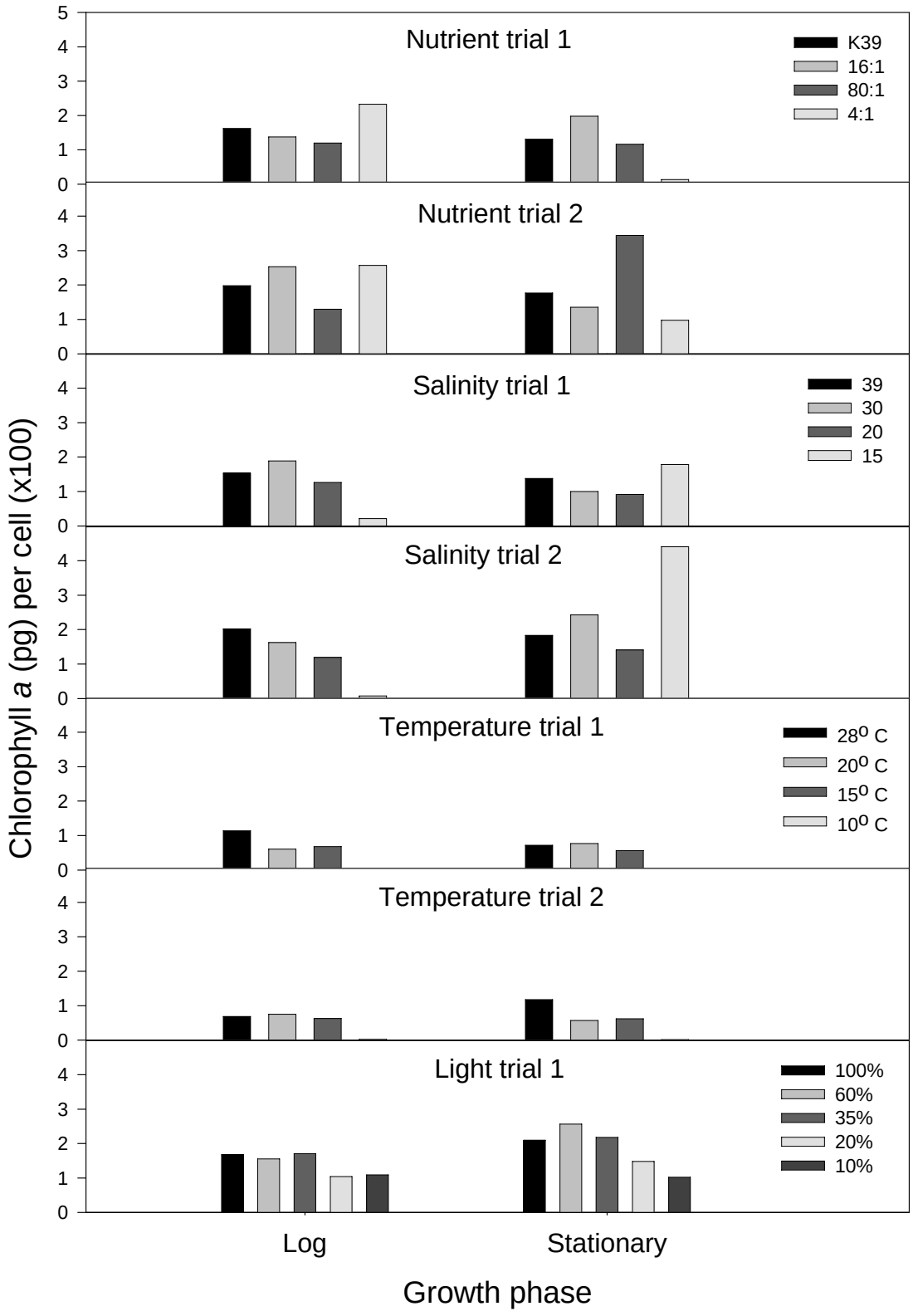

Figure 7. Chlorophyll *a* (pg/cell) for *Amphidinium carterae* at log and stationary growth phases for all treatments.

pg/cell) cellular Chl *a* and values declined thereafter at the lower light treatments.

Cellular chlorophyll *a* for *A. klebsii* varied from 4.01 to 5027.8 pg/cell over the entire study (Fig. 8). Mean Chl *a*/cell values for *A. klebsii* for this study was found to be 9.3 times that of *A. carterae.* The log phase nutrient trials had similar results with slightly varying values from 410 to 1406 pg/cell. No nutrient treatment in log phase gave superior cellular Chl *a* values. Salinity treatments of 20 to 39 gave similar cellular Chl *a* values while temperature treatments gave inconsistent patterns of cellular Chl *a* with some indication of lower Chl *a* values at temperatures below 20° C. Log phase cells at the highest light treatments had higher Chl *a* (between 1600 and 5000 pg/cell). Stationary phase *A. klebsii* cultures showed similar patterns to the log phase cultures. No clear pattern was discernable for the different nutrient treatments although the 16:1 N:P treatment had the highest Chl *a*/cell (1785 pg/cell). Salinities from 20 to 39 gave similar Chl *a*/cell values with very low values (<100 pg/cell) for stationary phase cells at 15 or below. Both temperature and light treatments gave inconsistent patterns for cellular Chl *a*.

Growth

Initial *in vivo* fluorescence readings for all nutrient treatments in trial 1 for *A. carterae* were between 1.0 and 3.0 (Fig. 9a). After a short 5 day lag period for treatment K39, 4:1 and 80:1 N:P, rapid log phase growth was observed progressing throughout a 25 day growth period after which cultures began slower growth signaling the onset of stationary phase. The balanced 16:1 N:P treatment for an unknown reason had a longer lag phase of almost 10 days and an abbreviated log phase and stationary phase beginning around day 24. The second trial, beginning with initial fluorescent values of 0.75 and 3.0 relative unites was similar to the first in having a 5 day lag period and log phase slowly increasing cells again until day 20-25 when stationary phase began for all but the N- limited 80:1 treatment (Fig. 9b). This last treatment had

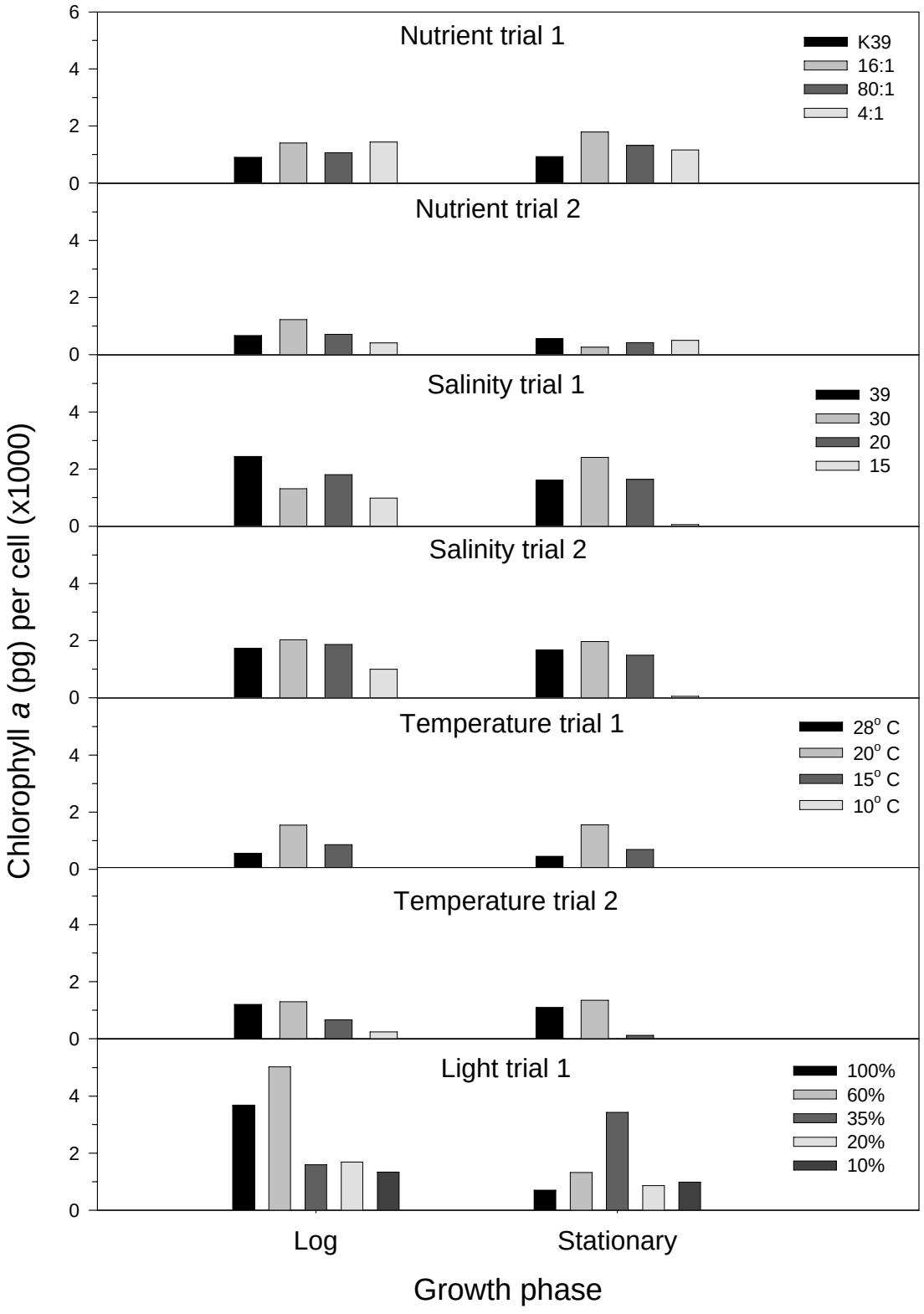

Figure 8. Chlorophyll *a* (pg/cell) for *Amphidinium klebsii* at log and stationary growth phases for all treatments.

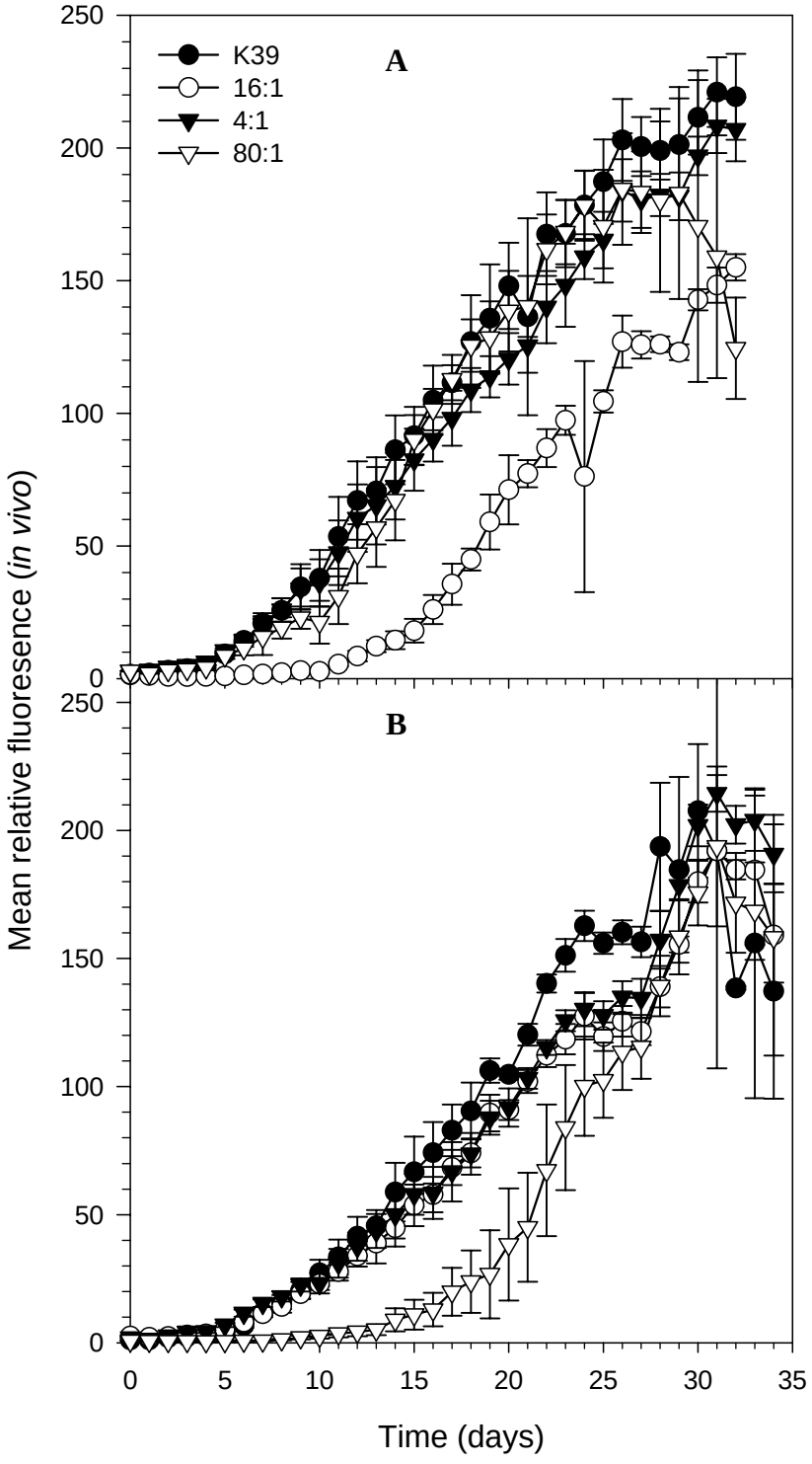

Figure 9. Growth, represented by a mean of 7 replicates, of *A. carterae* at N:P ratios of K39 (93:1), NP-replete (16:1), N-limited (4:1), and P-limited (80:1) over a growth period for a) trial 1 and b) trial 2.

a similar lag and delayed log phase to that of the 16:1 N:P observed in the first trial. Fluorescence based growth rates in both trials varied from 0.12 to 0.33 div/day.

Trial 1 of *A. klebsii* at different nutrient treatments (Fig. 10a) began with relative fluorescence values between 3.0 and 8.0. A similar lag phase of 5 days occurred in all cultures with slow growth following day 5 into day 35. The log phase was much less pronounced in *A. klebsii* than with *A. carterae* presumably due to the larger cell having a slower growth rate. The best growth was observed in the K39 treatment while other treatments were lower but clustered closer together. Fluorescence based growth rates varied from 0.10 to 0.14 div/day during trial 1. Trial 2, had a similar long lag and slowly increasing log phase for the 16:1 and 80:1 treatment reaching maximum readings on days 24 and 25 respectively before declining for the remaining of the growth period (Fig. 10b). The P-limited culture (N:P 4:1) had the highest growth with log phase starting after day 5, continuing until day 13 declining for the next three days and regaining log increases throughout the growth period of 28 days. Trial 2 growth rates varied from 0.11 to 0.36 div/day.

Initial *in vivo* fluorescence values for the first *A. carterae* salinity trial were from 1.0 and 5.5 relative units. Like the previous nutrient study, the lag phase for all treatments lasted approximately 5 days before growth at salinities from 20 to 39 began a rapid increasing have a pronounced log phase lasting until day 22 (Fig. 11a). Cells at the salinity of 15 (Fig. 11a inset) had declining fluorescence values for the first 3 days, showed a slight increase for the next few days before declining to low relative fluorescence values near 0. Fluorescence based growth rates varied from 0.07 to 0.27 div/day during trial 1. Trial 2 showed a similar pattern with relative fluorescence for cultures at salinities of 20 through 39 indistinguishable from one another (Fig. 11b). Again cells at salinity of 15 (Fig. 11b inset) showed an initial decline for the

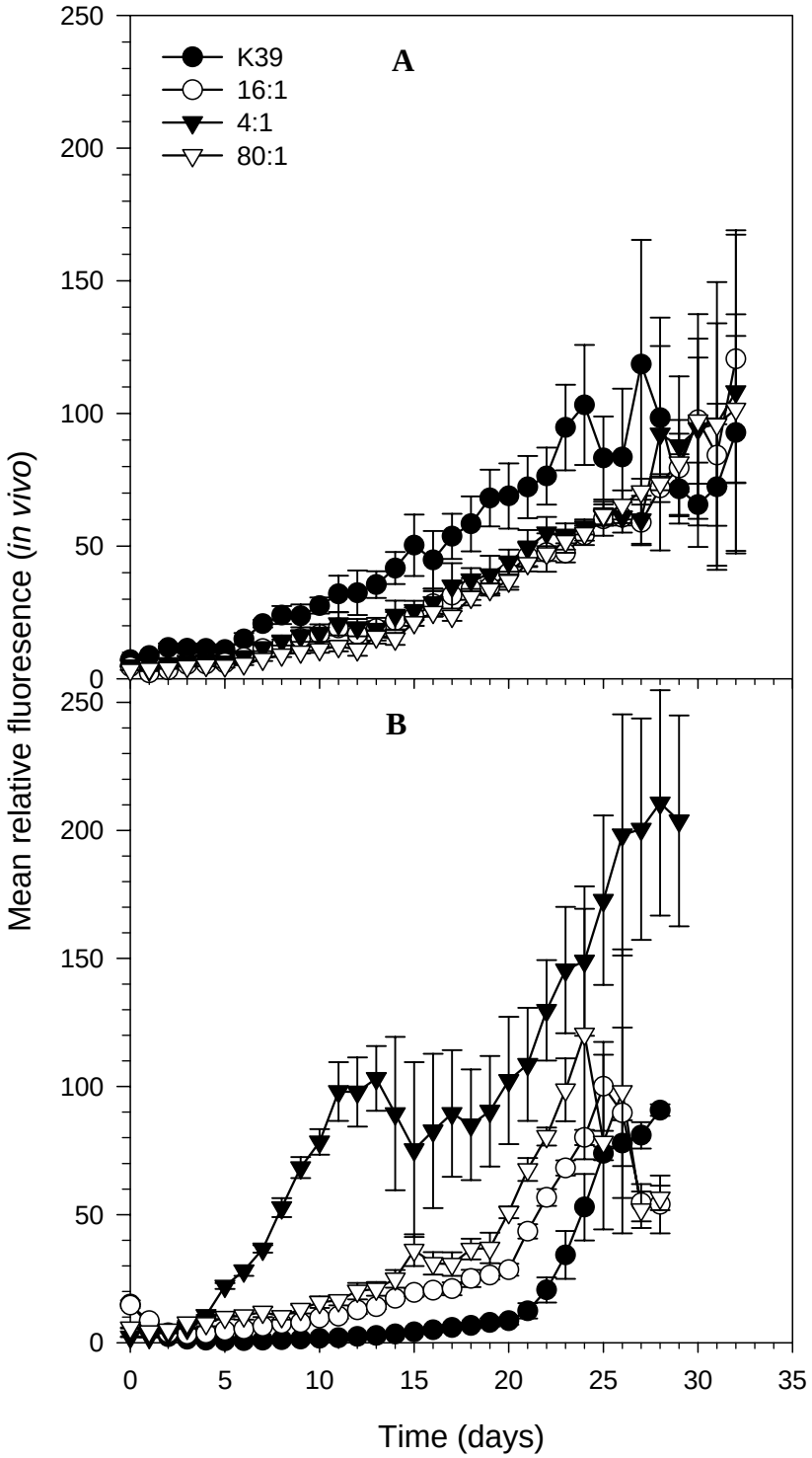

Figure 10. Growth, represented by a mean of 7 replicates, of *A. klebsii* at N:P ratios of K39 (93:1), NP-replete (16:1), N-limited (4:1), and P-limited (80:1) over a growth period for a) trial 1 and b) trial 2.

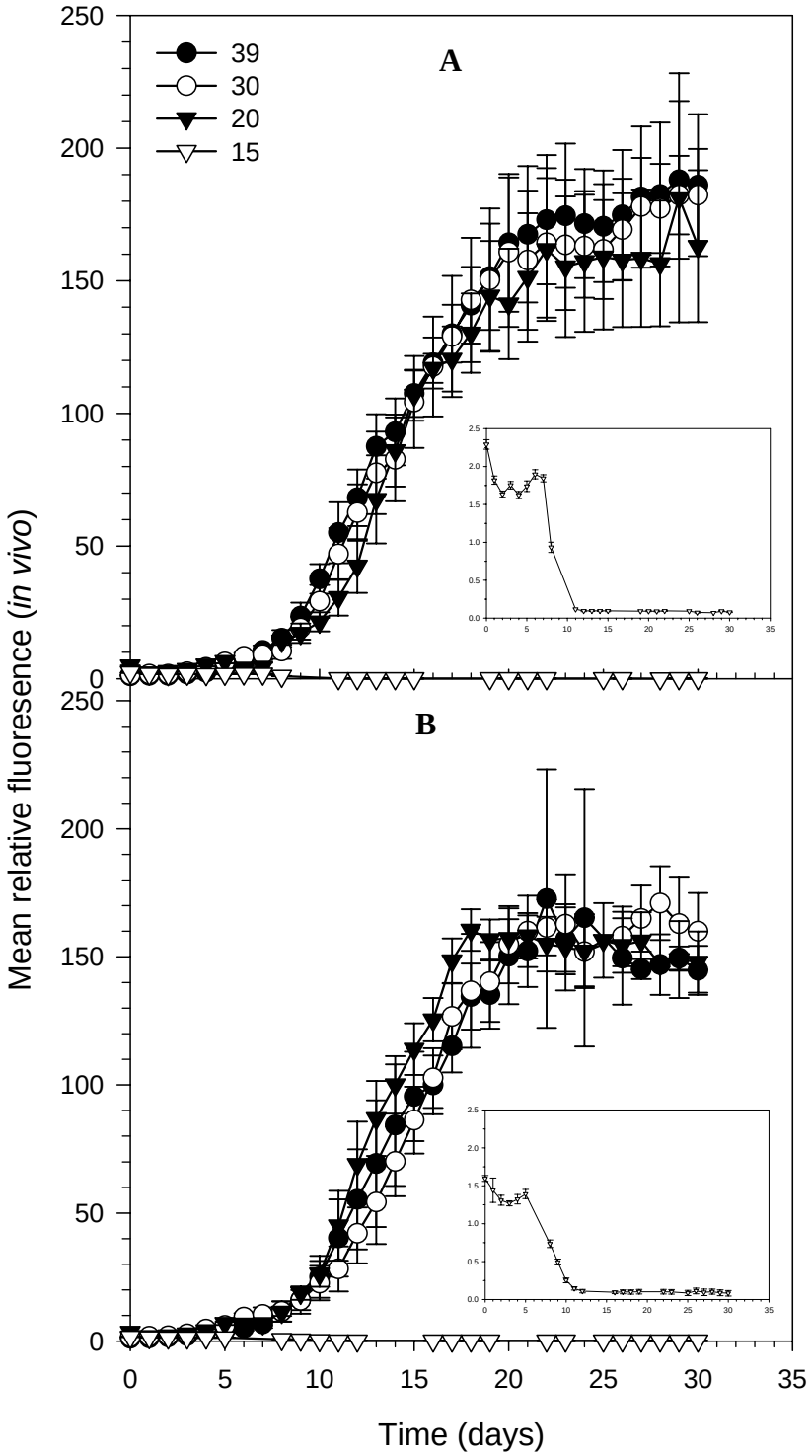

Figure 11. Growth, represented by a mean of 7 replicates, of *A. carterae* at salinities 39, 30, 20, and 15 over a growth period for a) trial 1 and b) trial 2. The mean growth of salinity 15 is inset with a smaller scale for clarity.

first three days, a slight increase and then rapid decline to fluorescence values near 0. Growth rates from trial 2 varied from 0.04 to 0.29 div/day.

Initial relative fluorescence readings for *A. klebsii* was between 2.0 and 4.5 for all salinity treatments. Similar to the nutrient studies, growth at the different salinities (Fig. 12) proceeded after a slow start and lag phase lasting approximately 5 days. Increased in relative *in vivo* fluorescence values followed through day 25 after which fluorescence readings leveled off indicating onset of stationary phase. In the first trial (Fig. 12a) growth at salinities of 30 and 39 were identical. At salinity of 20, growth was slower but showed significant increases during the period from day 10-25. Again, the lowest salinity of 15 failed to support growth and fluorescence values declined after day 3 to values near 0 (Fig12a inset). Fluorescence based growth rates for this trial varied from 0.09 to 0.16 div/day. The second trial (Fig. 12b) had similar results with growth at salinity 30 exceeding that of the other salinities. Stationary phase, denoted by a small increase or similar fluorescent values proceeded after day 25. Again, cells at salinity of 15 showed a decline until values of 0 as in the previous trial (Fig. 12b inset). Growth rates for the second salinity trial varied from 0.11 to 0.21 div/day.

Initial relative fluorescence measurements for the *A. carterae* were between 1 and 4. For the cultures grown at 28 and 20º C a very short lag phase of around 3 days was followed by a drastic log phase from around day 4 to around day 18 (Fig. 13a). Stationary phase was characterized by a decline in fluorescence. For cells grown at 15º C, log phase was extremely gradual, from around day 4 to 26. Absolutely no growth was seen for the cultures maintained at 10º C (Fig. 13a inset). Growth rates for the first trial varied from no growth at 10º C to 0.41 div/day. Trial two showed a very similar pattern for all four temperature treatments (Fig. 13b). Again, there was no growth observed for cultures maintained at 10º C (Fig. 13b

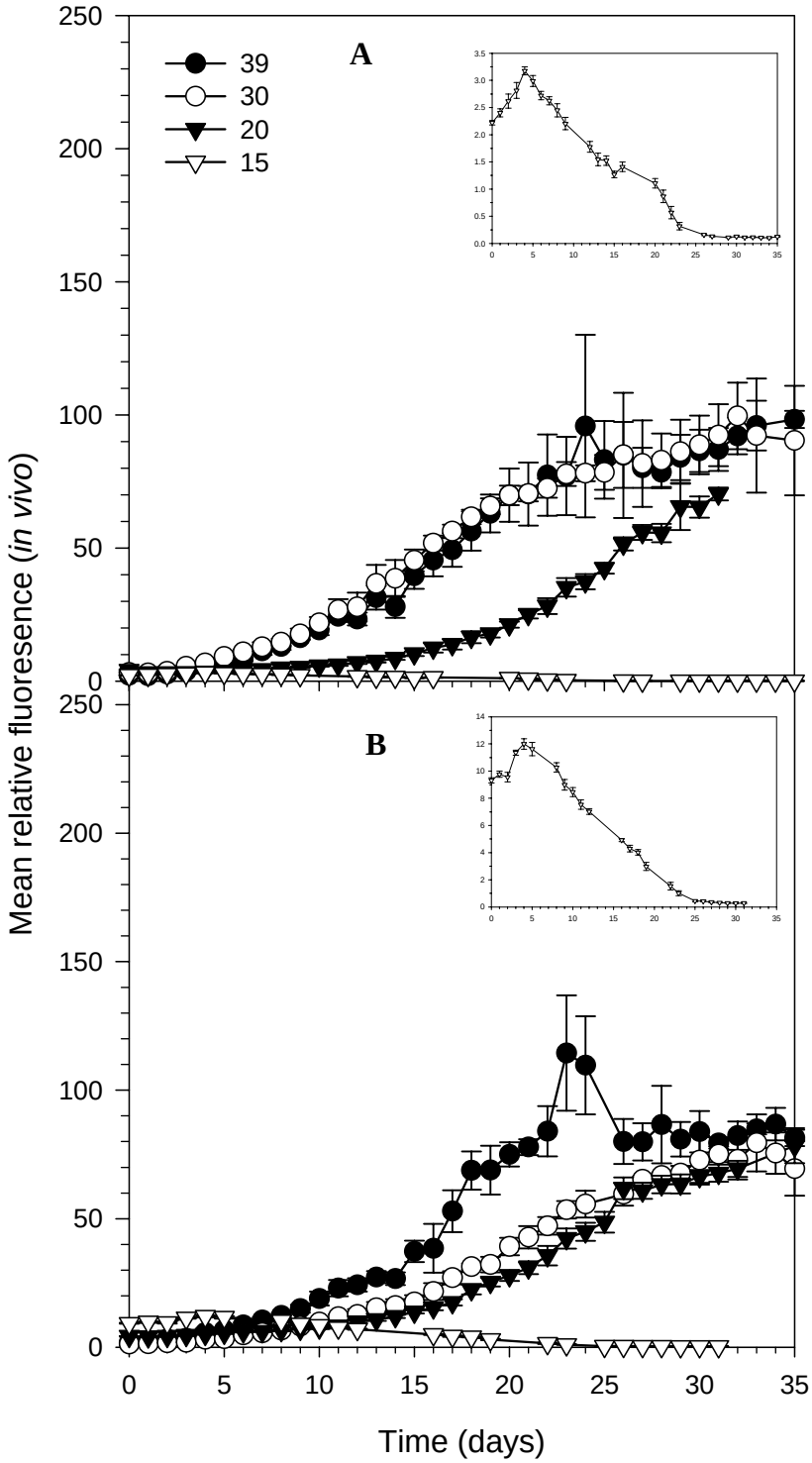

Figure 12. Growth, represented by a mean of 7 replicates, of *A. klebsii* at salinities 39, 30, 20, and 15 over a growth period for a) trial 1 and b) trial 2. The mean growth of salinity 15 is inset with a smaller scale for clarity.

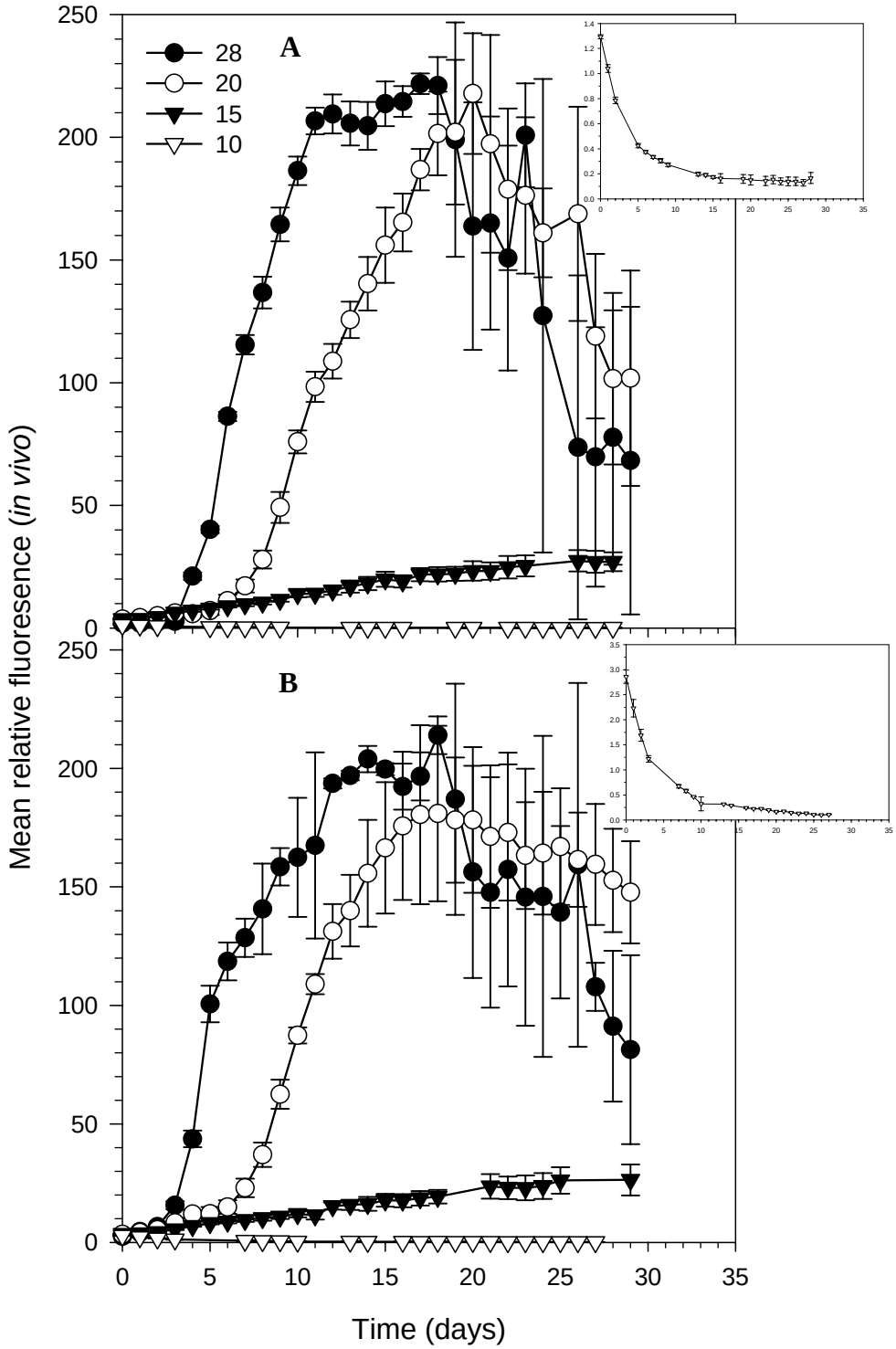

Figure 13. Growth, represented by a mean of 7 replicates, of *A. carterae* at temperatures of 28, 20, 15, and 10°C over a growth period for a) trial 1 and b) trial 2. Mean growth of 10°C is inset with a smaller scale for clarity.

inset). Growth rates for the second temperature trial varied from no growth at the lowest temperature to 0.31 div/day.

Initial fluorescence values for *A. klebsii* varied from 1.4 to 5 for the temperature trials. For cells grown at 28° C, a 3 day lag phase was followed by a short and drastic log phase, of around 6 days, which was then followed by stationary phase marked by a slight decline and a gradual evening out of fluorescence (Fig. 14a). Cells grown at 20° C had a longer lag phase, followed by a more gradual log phase, running from around day 12 to 27. Cells grown at 15° C displayed an initial decline until day 2, followed by a gradual and erratic increase in fluorescence levels. Cells grown at 10° C displayed no growth at all (Fig. 14a inset). Fluorescence based growth rates varied from no growth, seen for cells maintained at 10º C, to 0.45 div/day. Once again, very similar growth patterns were observed during the second trial (Fig. 14b). For the highest temperature, 28º C, there was not as much decline in fluorescence during stationary phase. As seen previously, no growth was observed at 10º C (Fig. 14b inset). Growth rates varied from no growth, at the lowest temperature, to 0.46 div/day.

Initial cell density of the *A. carterae* light experiment varied from ~4000 to 8000 cells/mL (Fig. 15a). All five treatments displayed a lag phase from day 0 to day 5. The log phases were observed from day 6 to around day 25; these varied in the severity of the growth. A typical titration of growth according to light availability is apparent in the growth patterns. The average cell density based growth rates for log phase cells were calculated to be 0.33 for the culture exposed to 100% of available light, 0.11 for 60% of the available light, 0.14 for log phase of culture treated with 35% of available light, 0.09 for cells exposed to only 20% of the available light, and 0.10 for cells exposed to the lowest light treatment, 10%.

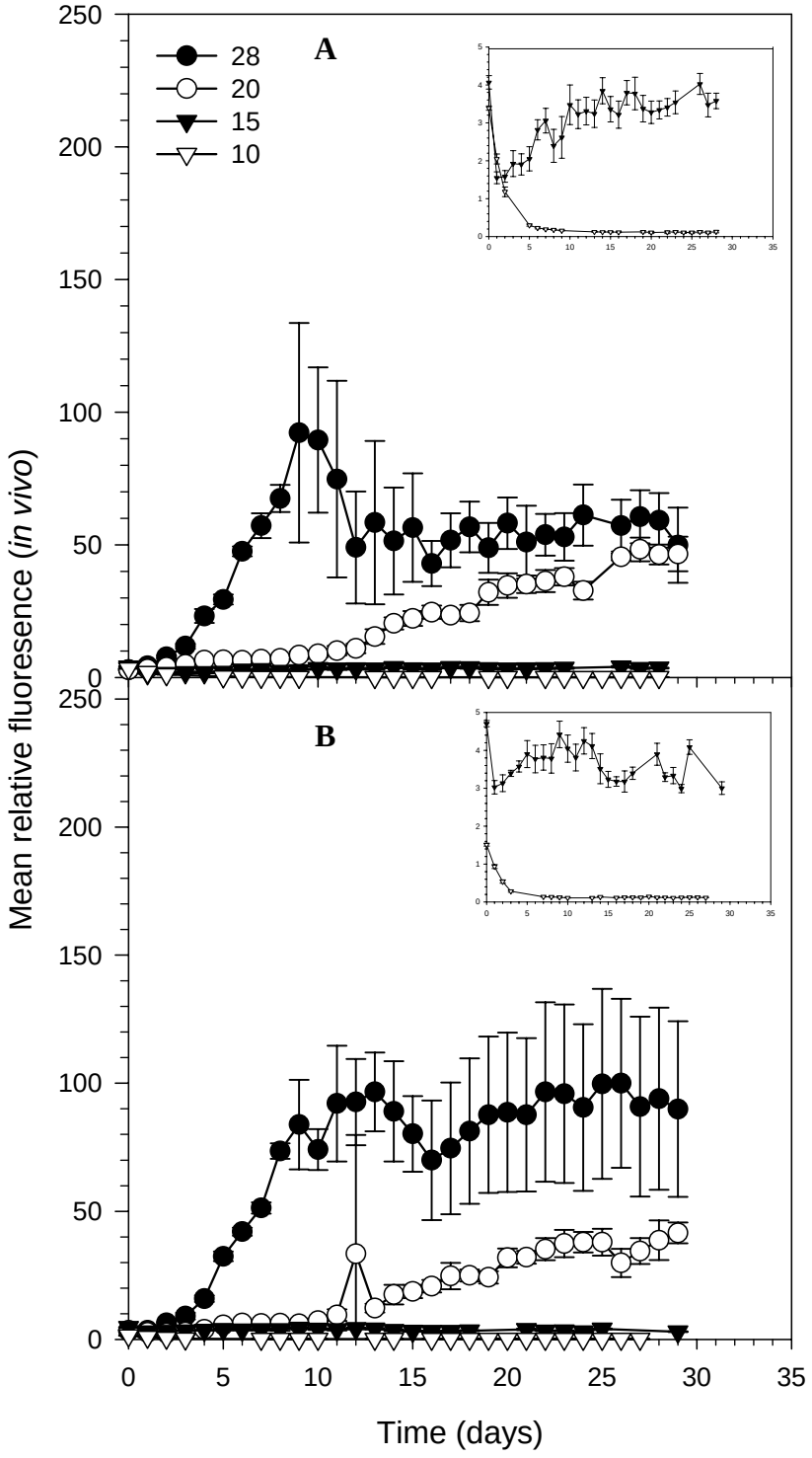

Figure 14. Growth, represented by a mean of 7 replicates, of *A. klebsii* at temperatures of 28, 20, 15, and 10°C over a growth period for a) trial 1 and b) trial 2. Mean growth of 10°C is inset with a smaller scale for clarity.

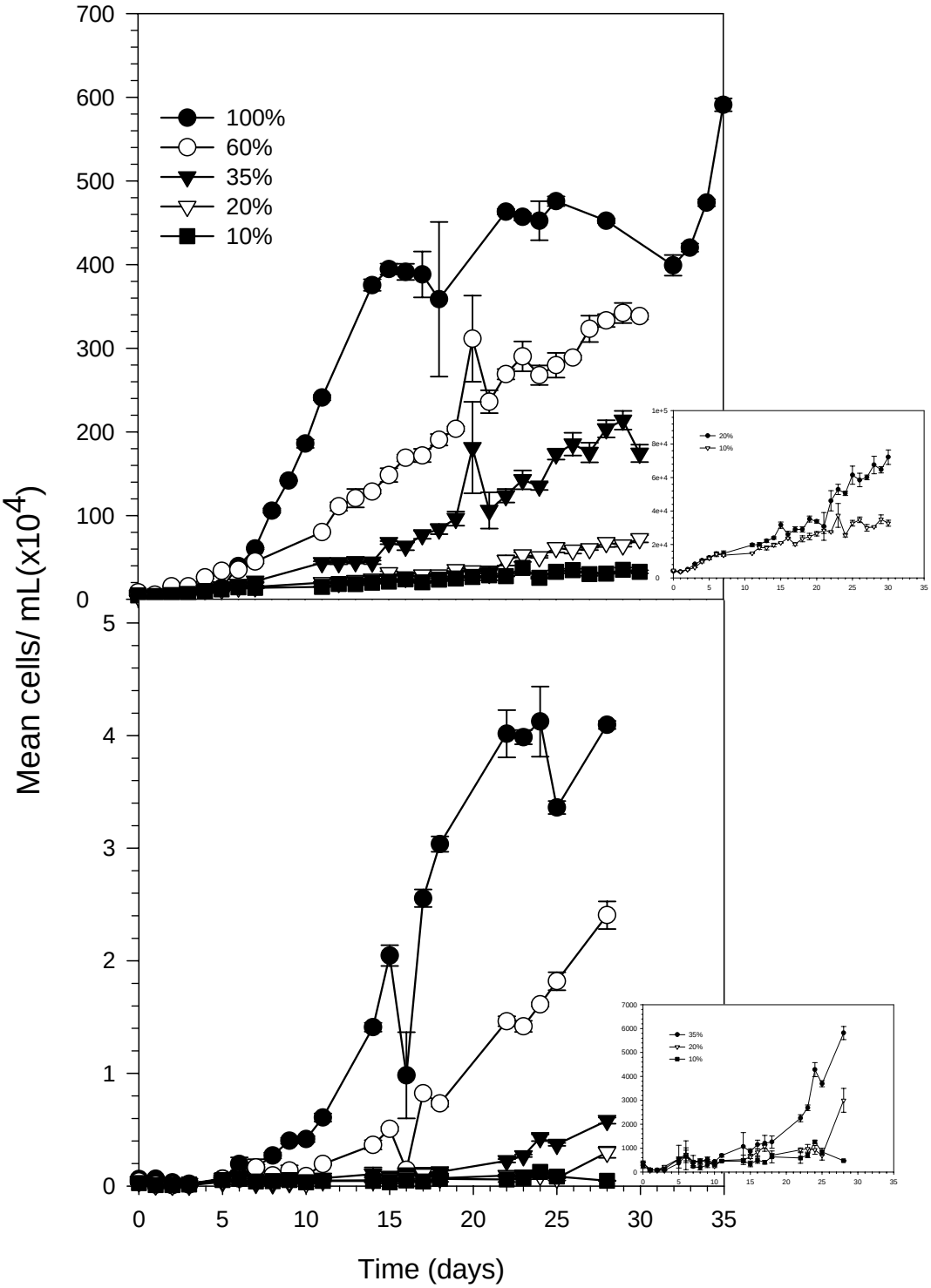

Figure 15. Growth, represented by 3 replicate counts, at light intensities of 100%, 60%, 35%, 20%, and 10% over a growth period for a) *A. carterae* and b) *A. klebsii.* Only one trial was performed for light treatments due to time constraints. Smaller scale graphs of lower light intensities are inset for clarity.

For the *A. klebsii* light experiment, initial cell densities varied from ~250 to 600 cells/mL (Fig. 15b).As seen with *A. carterae*, lag phase for all treatments was around 5 days long, with log phase lasting until around day 25. Once again, a light titration curve is visible in the growth curve patterns. The average growth rate, based on cell densities, during log phase was 0.25 for cells exposed to 100% light, 0.20 for cells exposed to 60% of the available light, 0.17 for those cells with 35% of the available light source, 0.27 cells kept at 20% light, and for those with 10% light the growth rate was 0.09 div/day.

Erythrocyte Lysis Assay

The Erythrocyte Lysis Assay (ELA) was conducted for *A. carterae* exposed to various nutrient treatments, temperatures, salinities and light intensities (Fig. 16). Log phase cells for the two nutrient trials showed some variability in activity however no significant differences was seen in any of the nutrient treatments. Activity as saponin equivalents were between 2 and 4 µg/125µL. Temperature, however did show a marked reduction in activity (>0.5 µg/125µL) in log phase cells for those at 10° C and those at temperatures of 15 to 28° C had identical activity with values between 3.0 and 4.0 µg/125µL. An identical response was found in the salinity trials with log phase cells exposed to salinity of 15 showing little or not activity while those at 20-39 commonly had values near 4.0 µg/125µL. Activity in log phase cells exposed to different light treatments had decreasing activity with decreasing light intensity. The 100 and 60% light treatment had values above 3.0 µg/125µL while all other light levels had values of 0.7 or less. The ELA of *A. carterae* in stationary phase showed identical or similar activity with values nearing 4.0 µg/125µL for all nutrient treatments. With temperature, stationary phase *A. carterae* showed the most drastic reduction at 10° C similar to that seen in log phase cells. While the 28°

C result of trial 1 varied from that of trial 2, all treatments above 10° C were identical. The stationary phase cells at salinities of 15 to 39 showed a similar pattern to the temperature treatments. Only those cells exposed to salinity of 15 had low (<1µg/125µL) or no ELA activity while those above 15 had values above 3.0 and nearing 4.0 µg/125µL. The light treatment showed that for stationary phase cells, ELA activity was not influenced until levels dropped below 20%. The overall pattern was that nutrients did not appear to influence the ELA activity but only those at low temperatures (10° C) and salinity (15 or less) were strongly inhibited in activity while activity in different light levels varied indirectly with the quantity of light given.

When the saponin equivalent activities for *A. carterae* were corrected for difference in cells number the activity patterns seen previously were modified (Fig. 17). In the nutrient trials, log phase cells treated to different nutrient ratios had the greatest ELA values in the N-limited culture with values between 2.0 and >4.0 µg/125µL/cell ($x10^{-5}$) while the P-limited activity was not different from the 16:1 and K39 nutrient treatments. With temperature however, the per cell activity of log phase cells was markedly increased (>6.0 $x10^{-5}$µg/125µL/cell) at 15° C while all other values were similar (~2.0-3.0 $x10^{-5}$µg/125µL/cell). Log phase cells exposed to different salinities did not show significant differences and for light, only those at the 60% level had elevated per cell activity. Cells of *A. carterae* in stationary phase did not show any marked difference when exposed to the different nutrient treatments. As in the log phase cell results, stationary phase cells of *A. carterae* exhibited greatest per cell ELA values at 15° C with values between 4.0 and 7.0 $x10^{-5}$µg/125µL/cell. Consistently discernible patterns were not apparent in the salinity trials. With stationary phase cells at different light treatments, the 35% level was highest at 2.4 $x10^{-5}$µg/125µL/cell with decreasing values towards each extreme in light.

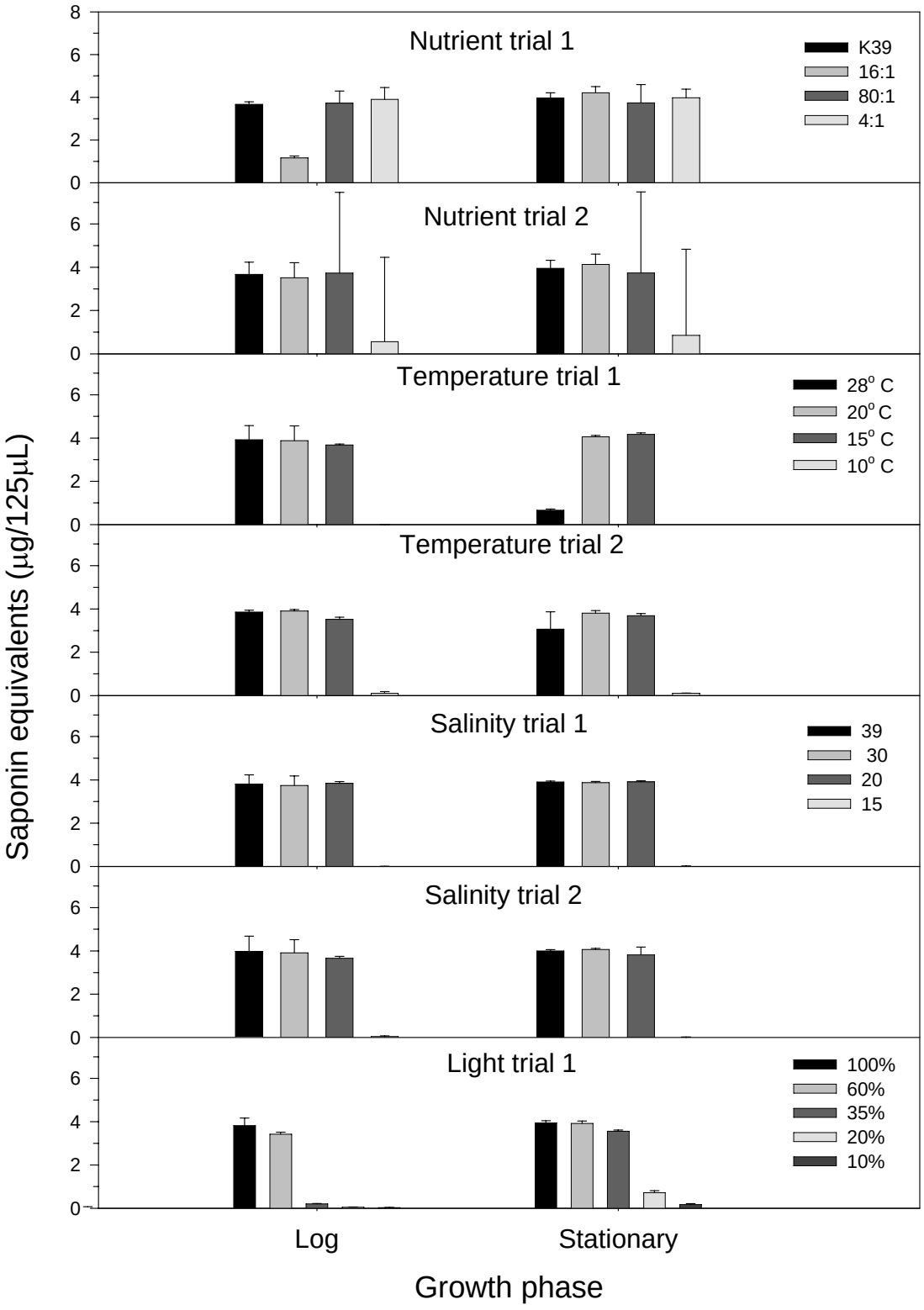

Figure 16. Saponin equivalents(µg/125µL) for all trials and treatments of *Amphidinium carterae* at log and stationary phases.

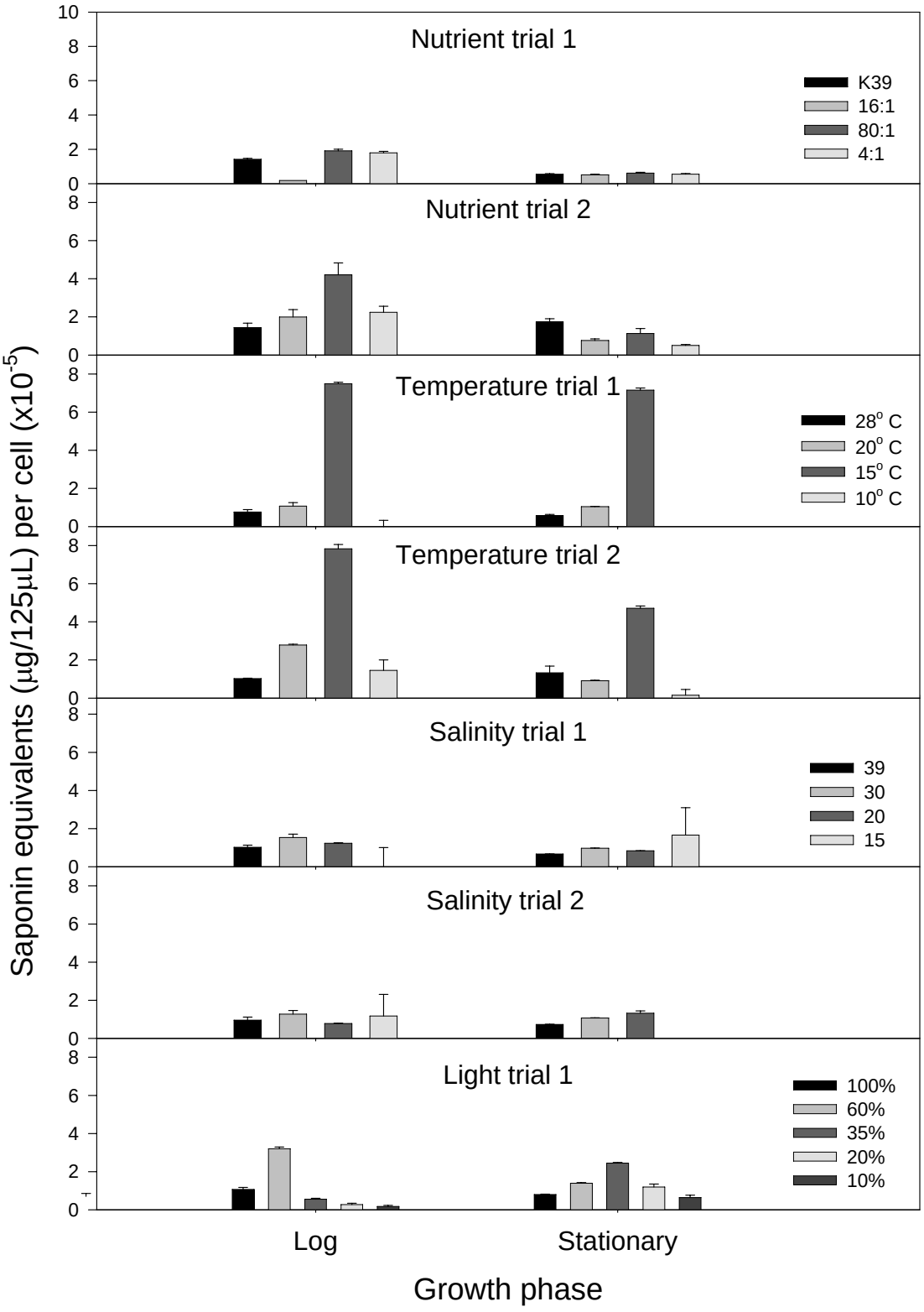

Figure 17. Saponin equivalents (µg/125 µL) per cell for all trials and treatments of *Amphidinium carterae* at log and stationary growth phase.

When the erythrocyte lysis assay values in saponin equivalents were normalized on a per cellular carbon basis (Fig. 18) for *A. carterae,* log phase cells for different nutrients showed variable but distinct patterns. Again the N-limited values were highest with the lowest values occurring at the 16:1 N:P ratio treatment. The temperature response was more distinct with per carbon activity increasing with a decrease in temperature from 28 to 15° C and very low activities (<0.1µg/125µL/µC) at 10° C. The response to salinity was not distinct and except for those per carbon activities at 15° C that were very low (~0.2 µg/125µL/µC) activities at the different salinities were not discernible. With the light treatments, only that at 60% showed any distinct increase over the others. For stationary phase cells of *A. carterae* neither of the nutrient or salinity treatments showed much difference in the pattern of per carbon activity. For temperature, only those at 15° C showed a marked increase and values at 10° C were near the limit of detection. As in the saponin equivalent results, the light treatments gave the highest (~0.4 1µg/125µL/µC) per carbon activities at the 35% treatment while the others decreased from this maximum. The overall pattern was that log phase cells had highest per carbon corrected ELA values with nitrogen limited cells along with those growing at 15° C. Intermediary light levels gave higher carbon corrected ELA values than did other treatments.

When ELA activity as saponin equivalents was normalized to cellular chlorophyll *a* the patterns of activity for log and stationary *A. carterae* cells was highly muted (Fig. 19). For both log and stationary cells, the results from the nutrient, salinity and light treatments basically were not distinguishable. The temperature trials were not consistent and while values at 10° C dominated in trial 2, this was not consistent with those results from trial 1 or previous trials using other normalizations. Trial 2 temperature results can only be considered anomalous and no conclusion can be made of these data. Normalizing the activities on a per cellular chlorophyll *a*

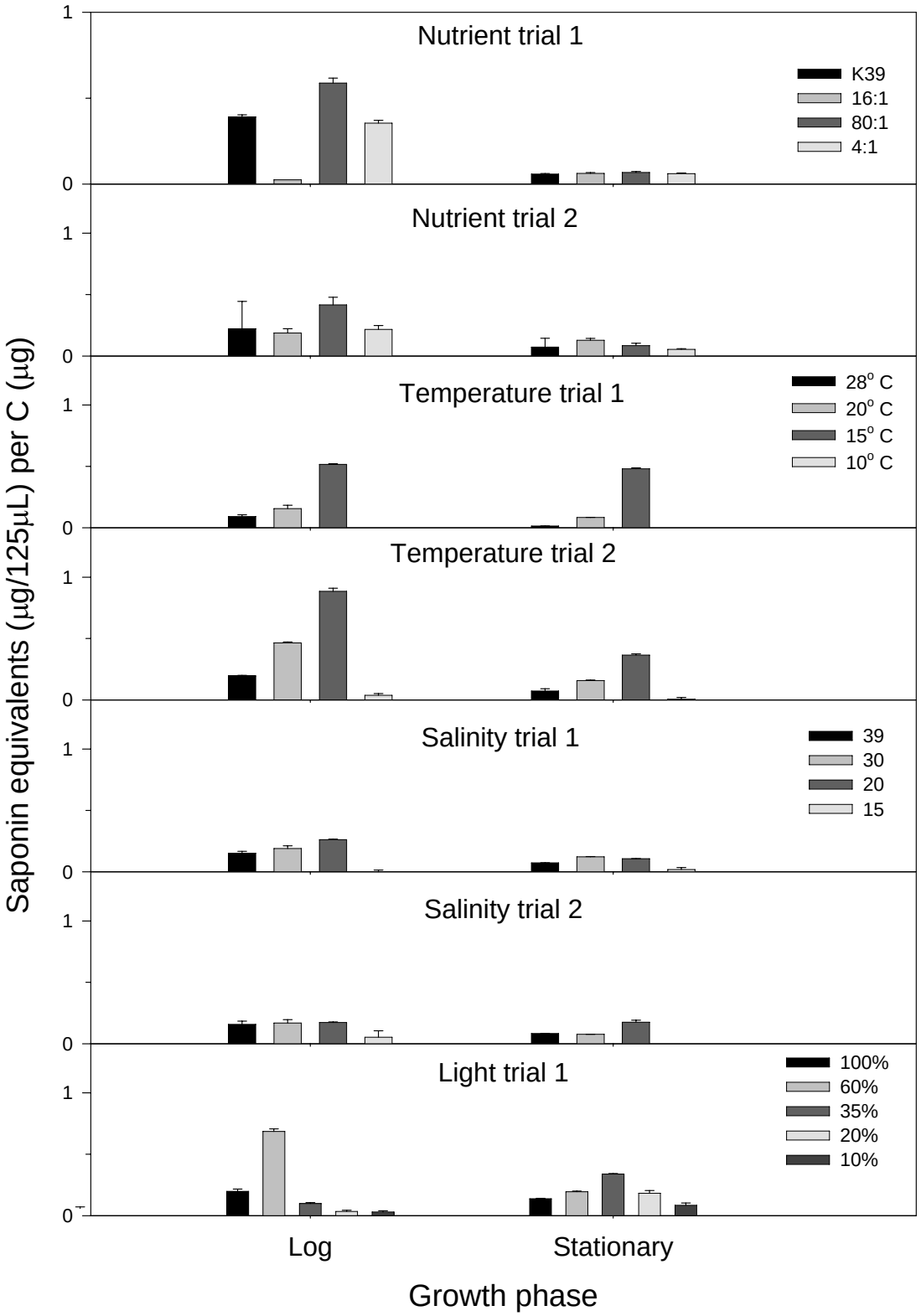

Figure 18. Saponin equivalents (µg/125µL) per carbon (µg) for all trials and treatments of *Amphidinium carterae* during log and stationary growth phases.

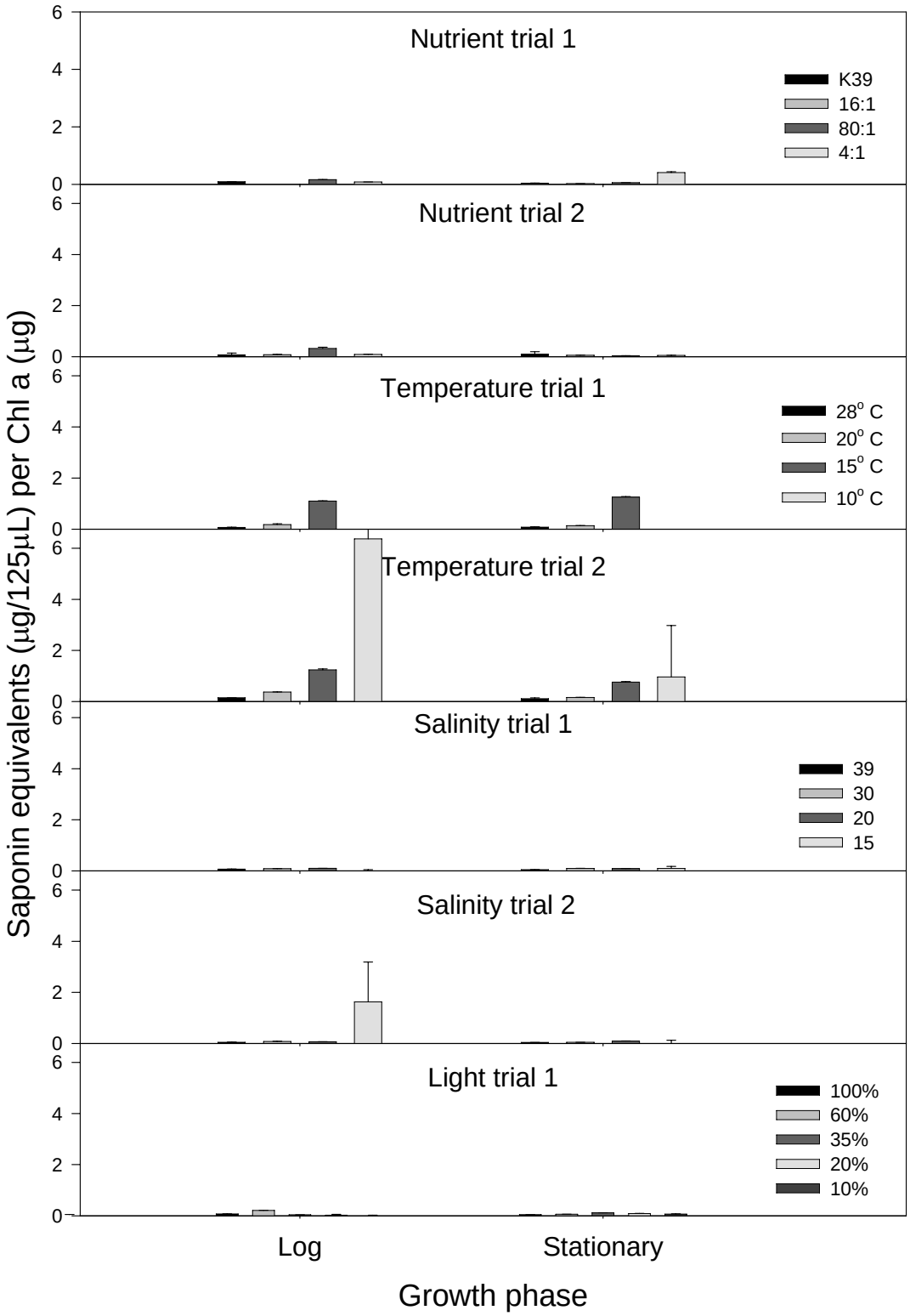

Figure 19. Saponin equivalents (µg/125µL) per chlorophyll a (µg) for all trials and treatments of *Amphidinium carterae* during log and stationary growth phases.

basis was not helpful in differentiating the effects of the experimental treatments and ELA activity.

The ELA analyses for *A. carterae* corrected for cellular volume (Fig. 20) had an identical pattern as that found in values corrected per cell (Fig. 20) was observed. These patterns confirmed that the elevated activities were found at 15° C, N-limited cultures and intermediate (60-35%) light treatments. The other treatments have equivocal results. The patterns were consistent with both log and stationary phase cells.

The ELA activity measured as saponin equivalents for *A. klebsii* showed marked differences between log and stationary phase cells (Fig. 21). Neither nutrients, temperature, salinity nor light treatments showed significant elevated ELA activities for log phase *A. klebsii* cells. For stationary phase cells, nutrient trial 1 showed a direct relationship in ELA as saponin µg/125µL with nutrient stress. The highest values in stationary phase cells exceeding 3.5 µg/125µL equivalents occurred with the nitrogen stressed cells (80:1, N:P). Trial 2 of that series showed a similar pattern with much lower overall values. The stationary phase salinity trials showed some increases with decreasing salinity although most values remained below 2.0 µg/125µL. Only results at 60% light showed elevated ELA values but still remained below 1.0 µg/125µL.

The *A. klebsii* ELA activity normalized per cell (Fig. 22) had log phase cells showing no distinct pattern for nutrient treatments but temperature treatments of 15 and 20° C had values exceeding 5.0×10^5 µg/125µL/cell. At salinities of 20 values were between 5 and 18×10^5 µg/125µL/cell. The light level giving the maxim activity was 60% with activities between 5 and 8.0×10^5 µg/125µL/cell. For stationary phase *A. klebsii*, nutrient trial 1 showed increased activity with cultures having both N-limited (80:1, N:P) and P-limited (4:1, N:P) conditions.

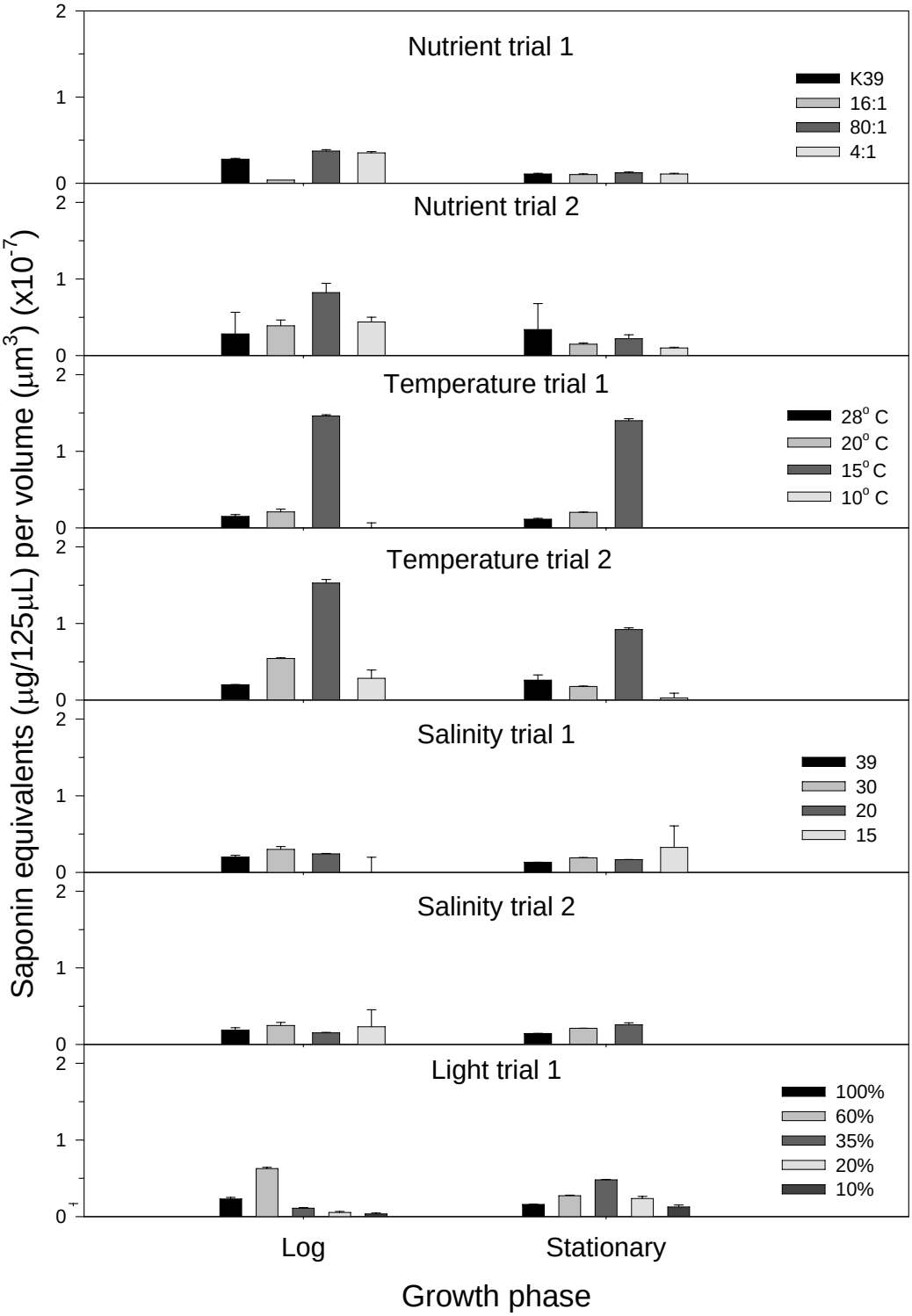

Figure 20. Saponin equivalents (µg/125µL) per volume (μm^3) for all trials and treatments of *Amphidinium carterae* during log and stationary growth phases.

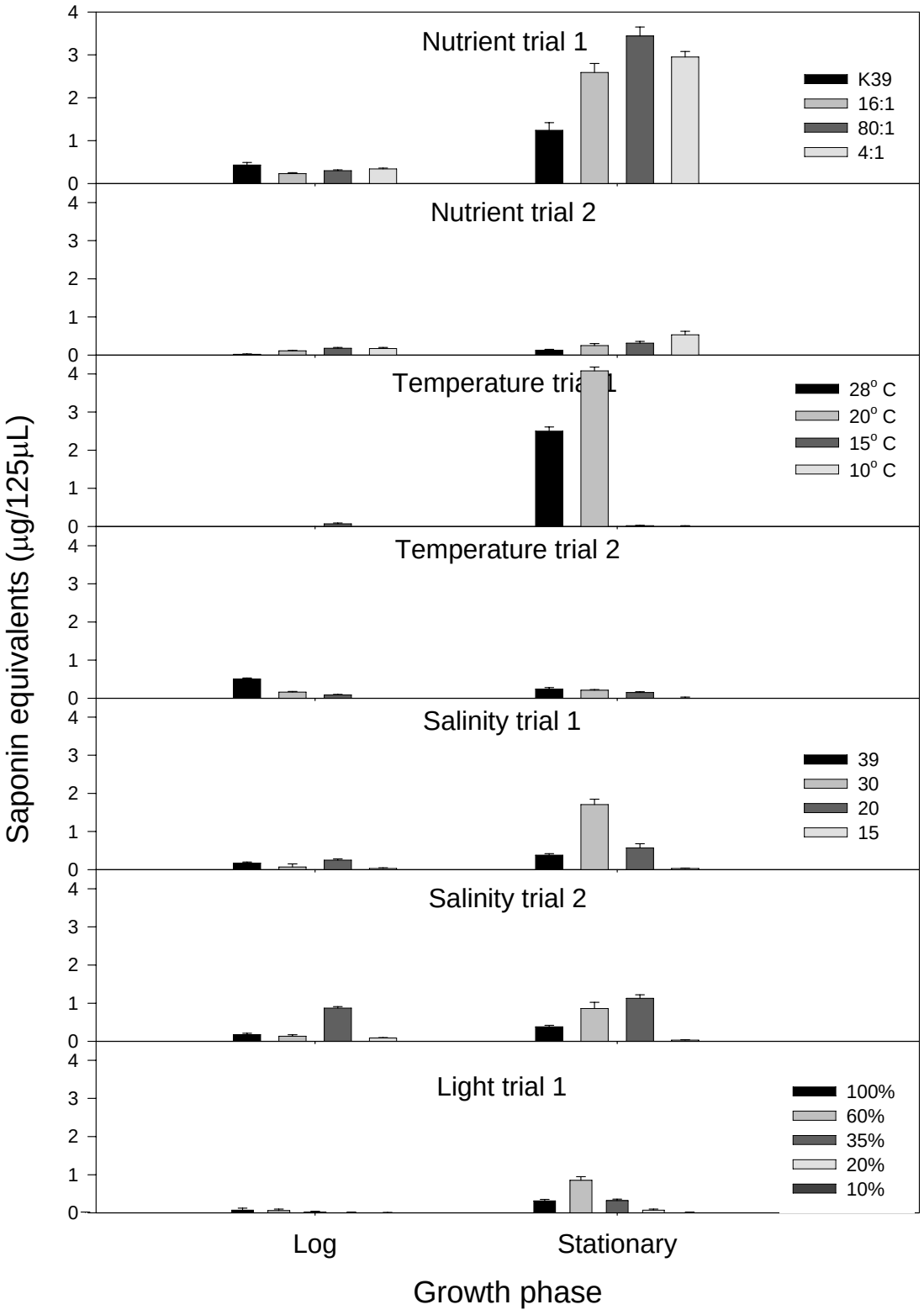

Figure 21. Saponin equivalents (µg/125µL) for all trials and treatments of *Amphidinium klebsii* during log and stationary growth phases.

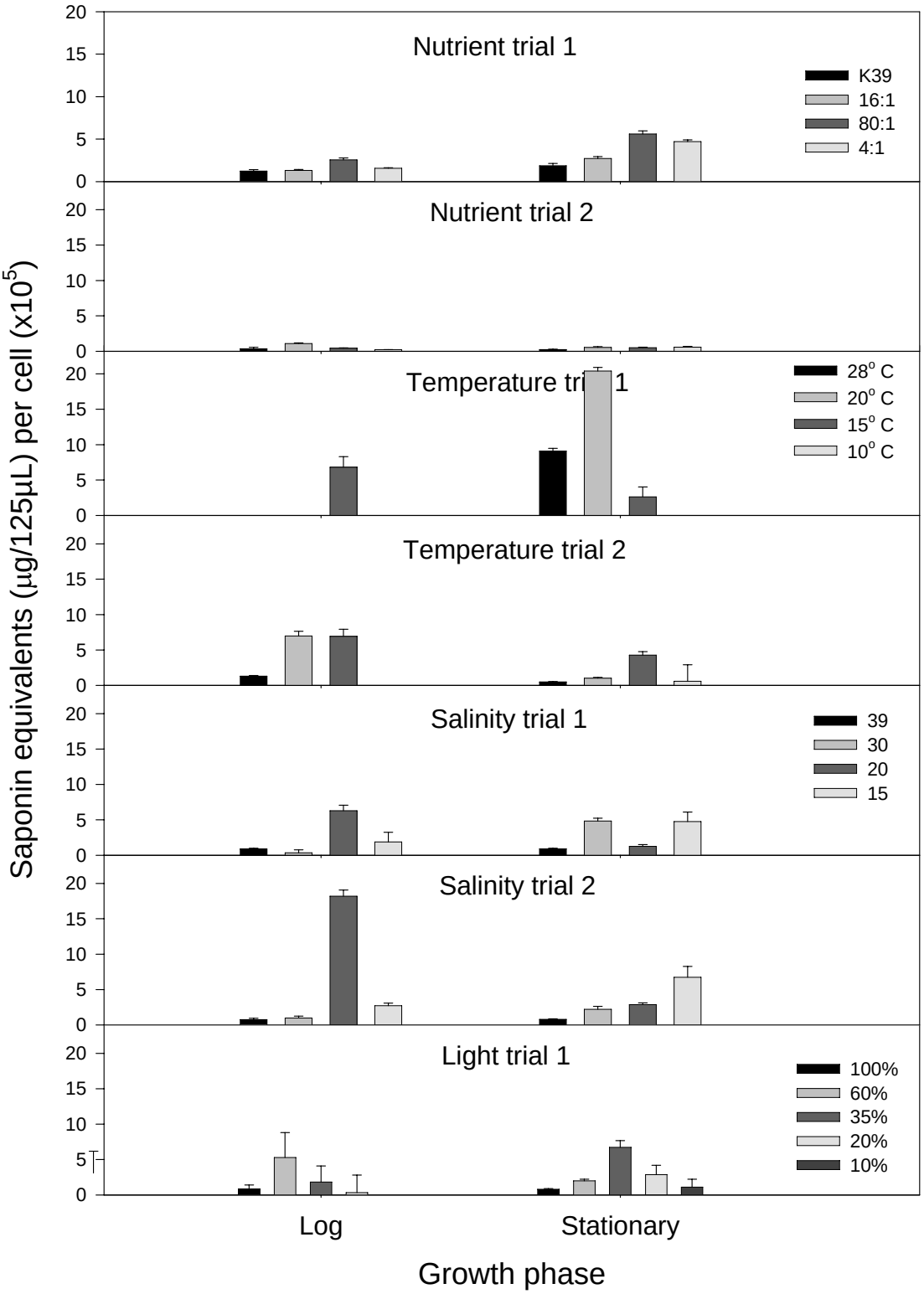

Figure 22. Saponin equivalents (µg/125µL) per cell for all trials and treatments of *Amphidinium klebsii* during log and stationary growth phases.

The activity values rarely exceeded 5.0 x 10^5 µg/125µL/cell for any nutrient treatment. At different temperature treatments, stationary phase *A. kelbsii* cells showed elevated activities (>5.0 x 10^5 µg/125µL/cell) at 28 and 20° C in trial 1 but was not reproduced in trial 2. When salinity was varied, those stationary cells at 15 through 30 had decreasing or variable per cell activity. Lower light intensities (35%) appeared to favor higher activity than either increased or decreased light.

The ELA values for *A. klebsii* normalized for cellular carbon (e.g., µg/mL/µg C) in nutrient trial 1 for log phase cells showed a distinct increase from 4.0 to 8.5 x 10^{-2} µg/125µL/µg C in activity with the N and P limited cultures (Fig. 23). Trial 2 values were not as informative presumably due to differences in culture condition between trial 1 and 2. During the temperature treatments of log phase cells trial 2 showed increased at 20 and to a lesser extend at 28 and 15° C. No activity was recorded for cultures at 10° C. A salinity of 20 clearly favored increased activity and maximum values exceeded 14 x 10^{-2} µg/125µL/µg C. The light treatments showed little or no effect on log phase cells. For stationary phase cells of *A. kelbsii*, again increased nutrient stress in the N-limited 80:1 and P-limited 4:1 treatments gave the greatest values above 8.0 x 10^{-2} µg/125µL/µg C. Elevated activities were also found during trial 1 at 20 and 28° C respectively while activity at other treatments was not detected. For salinity treatments, activities at 15 through 30 were elevated while those at 39 were depressed. No activity was observed below a salinity of 15. The light treatments indicated that the highest per cell activity (~ 8.0 x 10^{-2} µg/125µL/µg C) occurred at the 35% level with progressively lower values at either higher or lower percent intensities.

When values for the ELA were normalized to cellular chlorophyll *a*, activity values were detectable only in the temperature and salinity trials for stationary phase *A. klebsii* cells (Fig. 24).

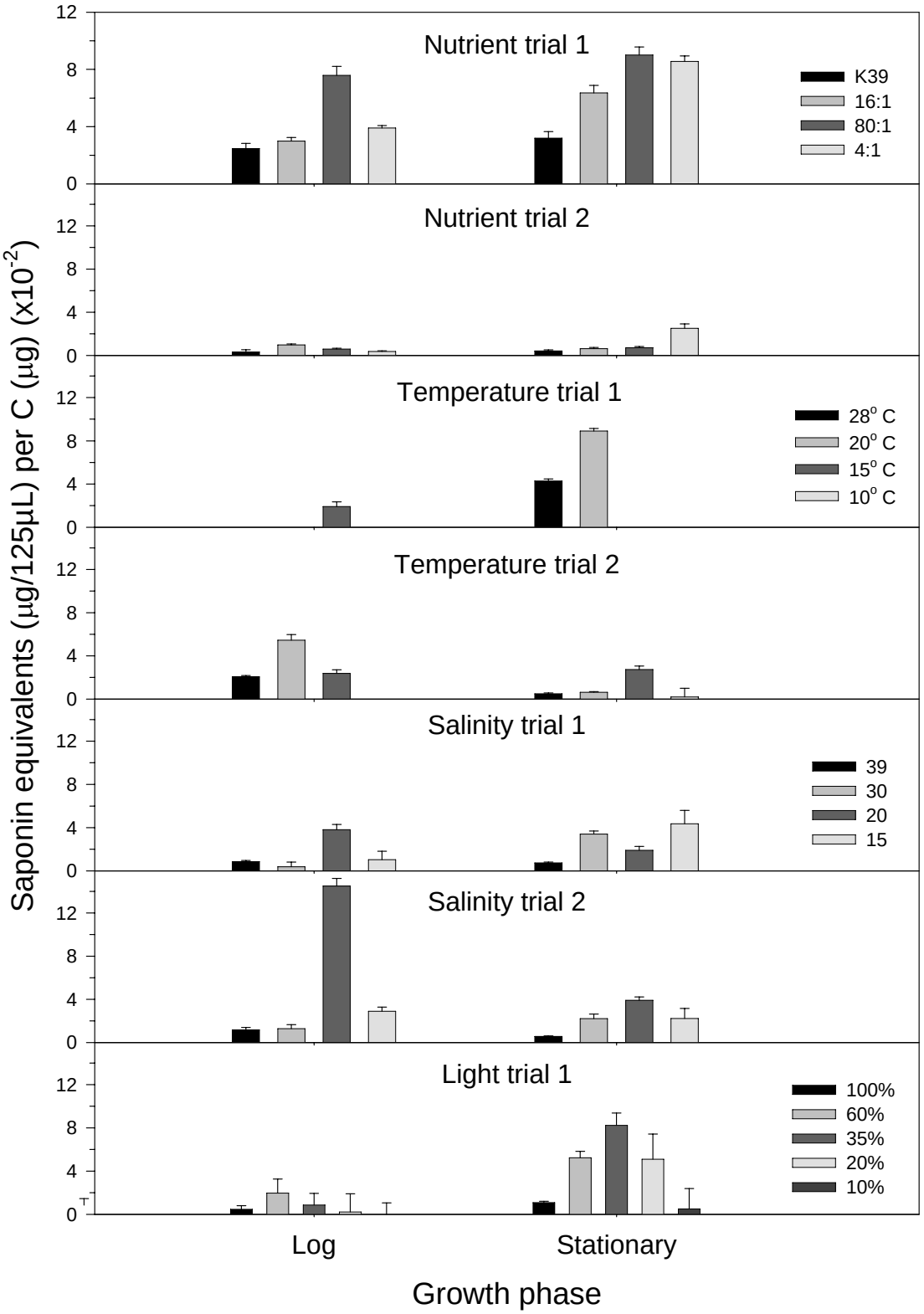

Figure 23. Saponin equivalents (µg/125µL) per carbon (µg) for all trials and treatments of *Amphidinium klebsii* during log and stationary growth phases.

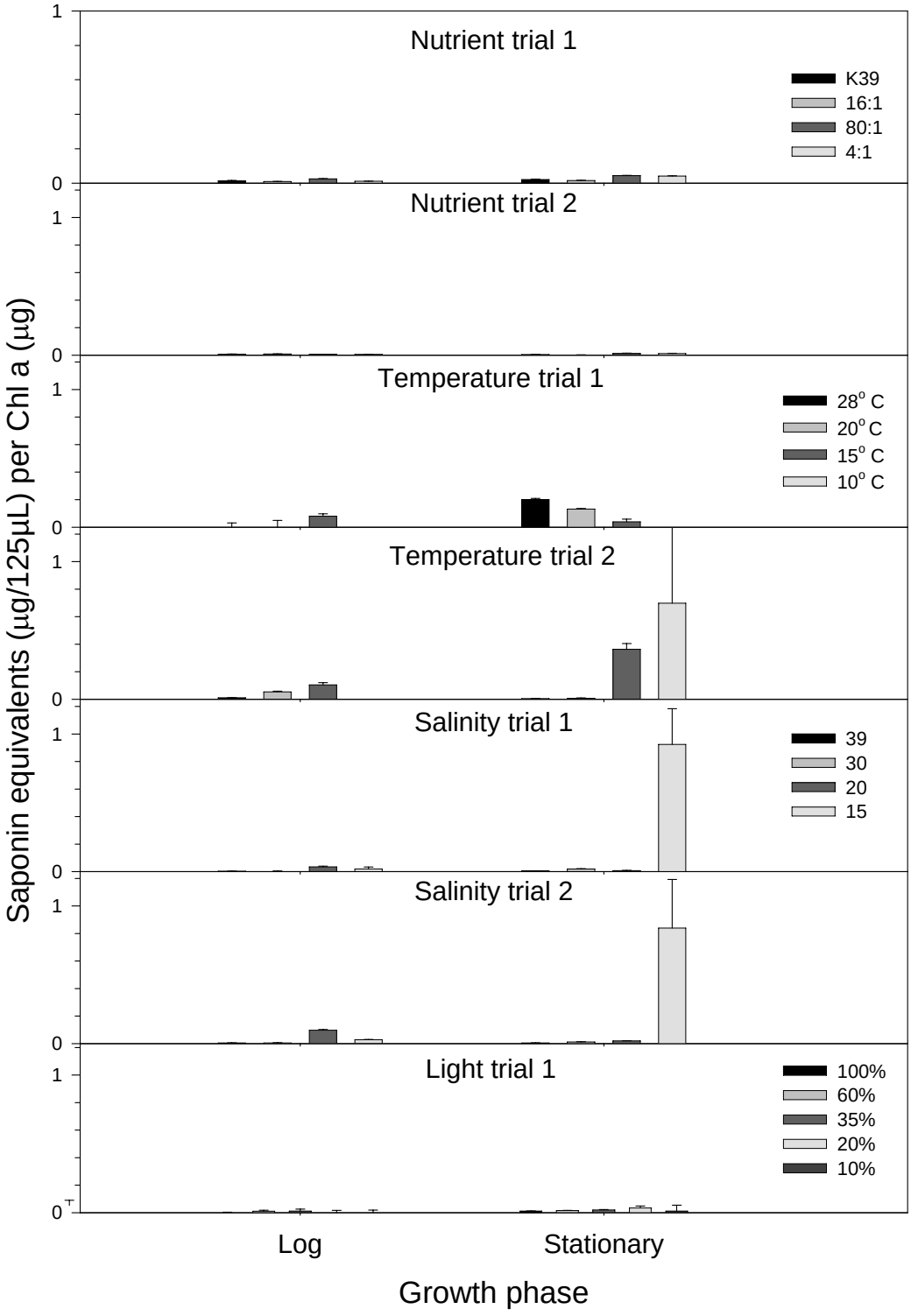

Figure 24. Saponin equivalents (µg/125µL) per chlorophyll a (µg) for all trials and treatments of *Amphidinium klebsii* during log and stationary growth phases.

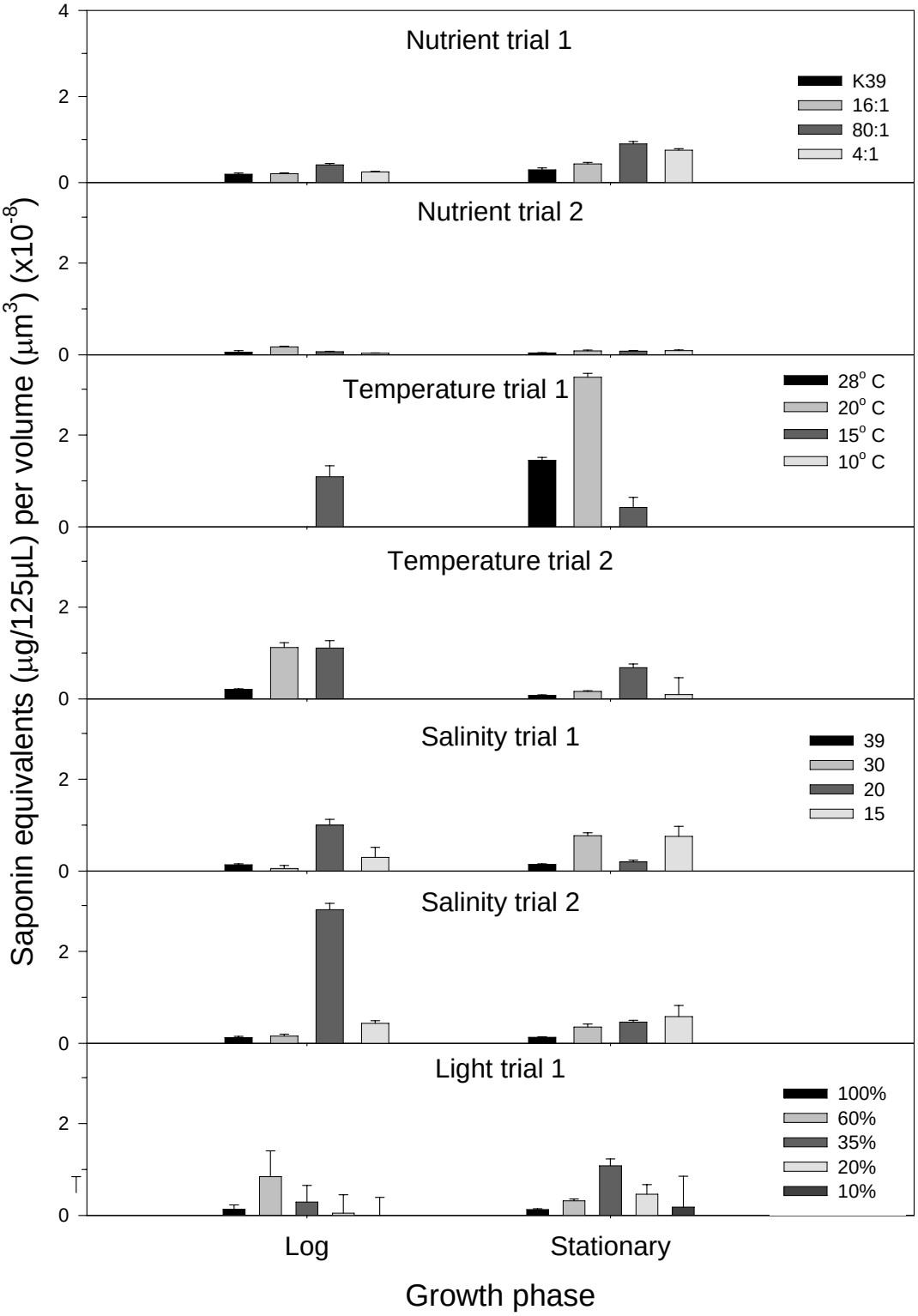

Figure 25. Saponin equivalents (µg/125µL) per volume (μm^3) for all trials and treatments of *Amphidinium klebsii* for log and stationary growth phases.

For log phase cells, the only detectible activities were in the 15° C and15 and 30 salinity treatments. All other values were very low. Stationary phase cells exhibited measurable activities at temperatures of 15 to 28° C and salinities of 15. All other values were very low to non detectable. Normalizing to cellular Chl *a* was not very helpful in revealing further details of the activity.

The ELA activity of *A. klebsii* corrected for cellular volume had ELA values at >2.0 x 10^{-8} μg/125μL/μm^3 (Fig. 25). Light at 60% gave elevated values relative to all other treatments. For stationary phase cells, nutrients conditions of N-limited and P-limited cultures showed slightly higher ELA values in trial 1 but not in trial 2. With temperature treatments, 20, 28 and 15°C gave elevated ELA values in trial 1 but this was not repeated in trial 2. Salinity responses of stationary cells showed slightly elevated activities at all treatments of 15 or higher with the highest values at salinities of 15 and 30. Light treatments of stationary cells gave highest values at 35% with lower values at treatments below or above that intensity.

DISCUSSION

Morphometry and Growth

According to previous studies, nutrient type played a role on growth rate. Although this study did not vary type of nutrients, only the availability of them, the growth rates of this study did vary for *A. carterae* under different nutrient ratios. Nitrogen-limited cultures has the lowest growth rates for both trials; growth rates varied from 0.12 to 0.33 div/day. These growth rates correspond with the previously discussed growth studies by Tomas *et al* (1987). The growth rates for cells grown at the various nutrient treatments varied among trials and species, much more than the growth rates of the temperature, salinity, and light intensity treatments. The

temperature, salinity, or light intensity at which cultures were maintained had more distinct boundaries for growth than nutrient availability, meaning that growth did not occur below certain thresholds. There was not an obvious threshold for growth of either species in terms of nutrient availability.

Log phase growth rates were consistently higher for *A. carterae* than *A. klebsii* throughout the study for all treatments. This was expected given the size difference between the two species. Growth rates for *A. carterae* were higher at higher salinities and temperatures. The tropical origin of this clone presumably plays a role in this.

Low cell numbers of *A. carterae* were found at the lowest salinity of 15, where high cellular carbon and relatively high cellular Chl *a* levels were observed. This could be attributed to few cells that had all but shut down physiologically due to the environmental stress. There was almost no growth at the lowest salinity; the cells were presumably storing their reserves of carbon and Chl *a*, as described by Cembella *et al*. (1984). When placed in another unfavorable environment, 10º C, *A. carterae* cells stored carbon, but not chlorophyll. The loss of color for phytoplankton (chlorosis) can be indicative of physical stress. Visual observation of these cultures recorded lightly colored cells, not the normal golden brown of a healthy cell. Carbon per cell levels were higher at 10º C than other temperature treatments, indicating that the cells reserved carbon at a higher rate at the lowest temperature. Nutrient availability did not seem to affect this species as far as cell density, cellular carbon or chlorophyll a.

While *A. carterae* is found worldwide at a range of temperatures and salinities, this particular clone was isolated from the Bahamas. Because this species did not gradually acclimate to lower temperatures and salinities while in culture, this could suggest a genetic

component regulating its ability to thrive at certain temperatures and salinities rather than an environmental adaptation of the particular clone.

Amphidinium klebsii had lower growth rates than *A. carterae*. Its growth was also affected by the amount of nutrients available and did not show healthy growth at the lowest salinity of 15 and the lowest temperatures of 15 and 10º C. Tropical origin of the species most likely predisposes it to thrive in warmer, more saline waters. The inhibition of growth at salinity of 15, 15º, and 10º C agrees with Morton *et al*. (1991). The growth rates for *A. klebsii* in the light experiments followed a similar trend similar to those seen for *A. carterae*. They generally decreased according to the amount of light available. This indicates that since these species are benthic in nature they may require less light than pelagic species, but growth is still a function of the amount of light available to the cells.

Amphidinium klebsii is considered a tropical species, so high cell numbers correlating with high temperatures and salinities is no surprise. Cells for this species did not reach high densities for the lowest salinity (15) nor the lowest temperatures (15 and 10º C) and lowest light intensities of 35, 20, and 10%. As described by Cembella *et al*. (1984), storage of carbon was definitely seen at the lowest temperatures, indicating that the cells were stressed, had stopped dividing and were reserving carbon. Chlorosis took place within *A. klebsii* cells at the lowest salinity, 15, and the lowest temperatures. Under these conditions, almost no cellular Chl *a* was detected, even though cells were still present in the cultures.

Hemolytic Activity

When measured by volume, cellular carbon, or cellular chlorophyll, *A. klebsii* was around 10 times larger than *A. carterae*. Since larger phytoplankton cells do no grow as quickly as

smaller ones, it was expected that *A. carterae* would have greater growth rates and reach higher cell densities. However, because *A. klebsii* is that much larger it could be argued that it should be that much more toxic. Also, previous reports indicated that *A. klebsii* was more hemolytic than *A. carterae.*

This study found quite the opposite; for almost all treatments, no matter how the data was normalized, *A. carterae* showed higher activity levels than *A. klebsii.* Many treatments for *A. carterae* displayed activity at or around 4 µg/125µL of saponin, which was close to the upper limit of the ELA. Normalization by cell number indicated that *A. klebsii* was more toxic for each of the nutrient treatments during stationary phase of the first nutrient trial. However, the size of the two species must be taken into consideration. When normalized on a per carbon basis to account for the size variation, the saponin equivalences were comparable between species for all the nutrient treatments. Comparison based on Chl *a* normalization, showed hardly any activity for *A. klebsii* when compared to *A. carterae*, indicating that Chl *a* normalization is quite variable. This suggests that Chl *a* pigments within these species vary on a different cycle than the hemolytic compounds. When the volume normalization data was compared between species, it was again observed that *A. carterae* produced more toxic activity on a per volume basis for most of the treatments. It is most useful to normalize on a per volume basis when comparing activity of cells with distinctly different volumes, such as *A. carterae* and *A. klebsii*.

Other species have been shown to react to environmental factors, namely nutrient limitation. Toxin production increased when nutrient availability was limited, specifically phosphorus. This study also found an interesting difference in hemolytic activity between nutrient treatments for one species, *A. klebsii*. Both nutrient limited media cultures, N- and P-limited, produced higher hemolytic activity than the nutrient replete cultures. This trend was

visible in all normalization methods, exclusive of Chl *a* normalization. The availability did not affect the amount of hemolytic activity produced by *A. carterae,* regardless of the type of normalization.

Other environmental variables tested did not have a clear pattern related to hemolytic activity. There were several unexpected peaks of hemolytic activity, found in most methods of normalization, at various temperatures for both species. The origin of these higher hemolytic activities is unclear.

The theory exists that toxin production in dinoflagellates is designed to offset their low nutrient affinity uptake (Smayda 1997). Other theories for this phenomenon include defense against non-native bacteria (Nayak *et al*. 1997) and an overwintering strategy (Rengefors & Legrand 2001). The production of toxic compounds, hemolytic in this case, by *Amphidinium* species may be an adaptation designed to offset one of the previously mentioned or some other ecological shortcoming.

CONCLUSION

The clones used in this study were originally from a tropical origin and at no point over the course of this study did they not respond like tropical species. They grew faster and healthier at the highest temperatures and salinities tested. While temperature, salinity, and light did have a significant impact on the growth of both of these species, nutrient availability did not.

The two species used were very different in size, with *A. klebsii* being around 12 times larger than *A. carterae* according to volume. The amount of cellular carbon and chlorophyll a within *A. klebsii* is around 9 times that of *A. carterae.* Based solely on these analyses, the assumption could be made that *A. klebsii* was that much more hemolytic. However, the

normalized data from the erythrocyte lysis assay proved otherwise. For most treatments, all methods of normalization indicate that *A. carterae* is more hemolytic than *A. klebsii.*

Throughout the course of this study, normalization by carbon or Chl *a* seemed to give an unclear picture as to what was actually happening with the hemolytic activity of the species. These normalization methods are very susceptible to being misleading due to processes like chlorosis, the loss of chlorophyll pigments, and the storage of carbon reserves.

While hopefully shedding some light on certain aspects of these two species, this study also highlights some areas in which further research would be appropriate. A genetic study of clones from different global waters would help resolve the origin of some of the growth barriers that were seen in this study. Fish bioassays would solidify if the hemolytic compounds found in these species are causing toxic effects. Chemical analyses of the active compounds are essential to determine if the hemolytic activity seen in the study was the action of a lone compound or of several working together as a toxic cocktail. Another interesting study would be to try to link bacterial communities, nutrient availability, and hemolytic activity.

LITERATURE CITED

Anderson, D.M. 1989. Toxic algal blooms and red tides: a global perspective. In: Okaichi, T., Anderson, D.M., and Nemoto, T. (Eds.), Red Tides: Biology, Environmental Science, and Toxicology. Elsevier Science Publishing Company, Inc., New York, pp. 11-16.

Anonymous. 1999. From Monsoons to Microbes: Understanding the Ocean's Role in Human Health. A National Research Council Publication. National Academy Press, Washington, D.C., pp. 59-70.

Baden, D.G., Fleming, L.E., and Bean, J.A. 1995. Marine toxins, intoxications of the nervous system part II. In: Vinken, P.J. and Bruyn, G.W. (Eds.), Handbook of Clinical Neurology, Elsevier Science, the Netherlands, pp. 141-175.

Baden, D.G., and Trainer, V.L. 1993. Mode of actions of toxins of seafood poisoning. In: Falconer, I.R. (Ed.) Algal Toxins in Seafood and Drinking Water. Academic Press, London, pp. 49-74.

Baden, D.G., Rein, K.S., and Gawley, R.E. 1998. Marine toxins: how they are studied and what they can tell us. In: Cooksey, K.E. (Ed.) Molecular Approaches to the Study of the Ocean. Chapman and Hall, London, pp. 487-514.

Barlow, S.B., and Triemer, R.E. 1988. Altermate life history stages in *Amphidinium klebsii* (Dinophyceae, Pyrrophyta). *Phycologia* 27(3): 41-420.

Binford Jr., J.S., Martin, D.F., and Padilla, G.M. 1973. Hemolysis induced by *Prymnesium parvum*: toxin calorimetric studies. *Biochimica et Biophysica Acta (BBA)-Biomembranes* 291(1): 156-164.

Cembella, A.D., Antia, N.J., and Harrison, P.J. 1984. The utilization of inorganic and organic phosphorous compound as nutrients by eukaryotic microalgae; a multidisciplinary perspective. *Critical Review of Microbiology* 2: 13-81.

Clouse, M. 2005. Comparison of four clones of the ichthyotoxic flagellate *Prymnesium*. MS Thesis, University of North Carolina Wilmington, 75 pp.

Dafni, Z., and Giberman, E. 1972. Nature of initial damage to Ehrlich ascites cells caused by *Prymnesium parvum* toxin. *Biochimica et Biophysica Acta (BBA)-Biomembranes* 255(1): 380-385.

Deeds, J.R., Terlizzi, D.E., Adolf, J.E., Stoecker, D.K., and Place, A.R. 2002. Toxic activity from cultures of *Karlodinium micrum* (=*Gyrodinium galatheanum*) (Dinophyceae)- a dinoflagellate associated with fish mortalities in an estuarine aquaculture facility. *Harmful Algae* 1(2): 169-189.

Doig III, M.T., and Martin, D.F. 1973. Anticoagulant properties of a red tide toxin. *Toxicon* 11(4): 351-355.

Eschbach, E., Scharsack, J.P., John, U., and Medlin, L.K. 2001. Improved erythrocyte lysis assay in microtitre plates for sensitive detection and efficient measurement of haemolytic compounds from ichthyotoxic algae. *Journal of Applied Toxicology* 21:513-519.

Granéli, E., and Johansson, N. 2003. Increase in the production of allelopathic substances by *Prymnesium parvum* cells grown under N- or P-deficient conditions. *Harmful Algae* 2(2): 135-145.

Hallegraeff, G.M. 1993. A review of harmful algal blooms and their apparent global increase. *Phycologia* 32(2): 79-99.

Hallegraeff, G.M. 2003. Harmful algal blooms: a global overview. In: Hallegraeff, G.M., Anderson, D.M., and Cembella, A.D. (Eds.) Manual on Harmful Marine Microalgae. UNESCO, Paris, pp. 25-49.

Hillebrand, H., Dürselen, C., Kirschtel, D., Pollingher, U., and Zohary, T. 1999. Biovolume calculation for pelagic and benthic miroalgae. *Journal of Phycology* 35: 403-424.

Houdai, T., Matsuoka, S., Murata, M., Satake, M., Ota, S., Oshima, Y., and Rhodes, L.L. 2001. Acetate labeling patterns of dinoflagellate polyketides, amphidinols 2, 3, and 4. *Tetrahedron* 57: 5551-5555.

Houdia, T., Matsuoka, S., Matsumori, N., and Murata, M. 2004. Membrane-permeabilizing activities of amphidinol 3, polyene-polyhydroxy antifungal from a marine dinoflagellate. *Biochimica et Biophycica Acta* 1667: 91-100.

Igarashi, T., Aritake, S., and Yasumoto, T. 1998. Biological activities of prymnesin-2 isolated from a red tide alga *Prymnesium parvum*. *Natural Toxins* 6(1): 35-41.

Igarashi, T., Satake, M., and Yasumoto, T. 1999. Structures and partial sterochemical assignments for prymnesin-1 and prymnesin-2: potent hemolytic and ichthyotoxic glycosides isolated from the red tide alga *Prymnesium parvum*. *Journal of the American Chemical Society* 121(37): 8499-8511.

Imai, M., and Inoue, K. 1974. The mechanism of the action of prymnesium toxin on membranes. *Biochimica et Biophysica Acta(BBA)- Biomembranes* 352(3): 344- 348.

Johansson, N., and Granéli, E. 1999. Influence of different nutrient condition on cell density, chemical composition and toxicity of *Prymnesium parvum* (Haptophyta) in semi-continuous cultures. *Journal of Experimental Marine Biology and Ecology* 239:243-258.

Keller, M.D. and Guillard, R.R.L. 1985. Factors significant to marine dinoflagellate culture. In: Anderson, D.M., White, A.W., and Baden, D.G. (Eds.), Toxic Dinoflagellates. Elsevier, New York, pp. 113-116.

Kempton, J.W., Lewitus, A.J., Deeds, J.R., Law, J.M., and Place, A.R. 2002. Toxicity of *Karlodinium micrum* (Dinophyceae) associated with a fish kill in a South Carolina brackish retention pond. *Harmful Algae* 1(2): 233-241.

Kim, D., Oda, T., Muramatsu, T., Kim, D., Matsuyama, Y., and Honjo, T. 2002. Possible factors responsible for the toxicity of *Cochlodinium polykrikoides*, a red tide phytoplankton. *Comparative Biochemistry and Physiology Part C* 132: 415-423.

Kirkpatrick, B., Fleming, L.E., Squicciariani, D., Backer, L.C., Clark, R., Abraham, W., Benson, J., Cheng, Y.S., Johnson, D., Pierce, R., Zaias, J., Bossart, G.D., and Baden, D.G. 2004. Literature review of Florida red tide: implications for human health effects. *Harmful Algae* 3:99-115.

Kobayashi, J., Takahashi, M., and Ishibashi, M. 1996. Amphidinolide Q, a novel 12-membered macrolide from the cultured marine dinoflagellate *Amphidinium* sp. *Tetrahedron Letters* 37(9): 1449-1450.

Kubota, T., Endo, T., Tsuda, M., Shiro, M., and Kobayashi, J. 2001. Amphidinolide T5, a new 19-membered macrolide from a dinoflagellate and X-ray structure of amphidinolide T1. *Tetrahedron* 57: 6175-6179.

Kubota, T., Tsuda, M., and Kobayashi, J. 2001A. Biosynthetic study of amphidinolide C. *Tetrahedron* 57: 5975-5977.

Kubota, T., Tsuda, M., and Kobayashi, J. 2001B. Amphidinolide V, novel 14-membered macrolide from marine dinoflagellate *Amphidinium* sp. *Tetrahedron Letters* 41: 713-716.

Marshall, J., Nichols, P.D., Hamilton, B., Lewis, R.J., and Hallegraeff, G.M. 2003. Ichthyotoxicity of *Chattonella marina* (Raphidophyceae) to damselfish (*Acanthochromis polycanthus*): the synergistic role of reactive oxygen species and free fatty acids. *Harmful Algae* 2(4): 273-281.

Martin, D.F. and Padilla, G.M. 1971. Hemolysis induced by *Prymnesium parvum* toxin: kinetics and binding. *Biochimica et Biophysica Acta* 241:213-225.

Martin, D.F., Padilla, G.M., and Brown, P.A. 1971. Hemolysis induced by *Prymnesium parvum*: toxin effect on primaquine treatment. *Biochimica et Biophysica Acta (BBA)-Biomembranes* 249(1): 69-80.

Martin, D.F., Padilla, G.M., Heyl, M.G., and Brown, P.A. 1972. Effect of *Gymnodinium breve* toxin on hemolysis induced by *Prymnesium parvum* toxin. *Toxicon* 10(3): 285-290.

Mitchell, L.E. 1985. Ecological studies of benthic dinoflagellates associated with ciguatera in the Florida Keys; the 0-38 micrometer size fraction. MS thesis, Florida Institute of Technology, 46 pp.

Morton, S.L., Norris, D.R., and Bomber, J.W. 1991. Effect of temperature, salinity, and light intensity on the growth and seasonality of toxic dinoflagellates associated with ciguatera. *Journal of Experimental Marine Biology and Ecology* 157: 102-113.

Nakajima, I., Oshima, Y., and Yasumoto, T. 1981. Toxicity of benthic dinoflagellates in Okinawa. *Bulletin of the Japanese Society of Scientific Fisheries* 47(8): 1029-1033.

Nayak, B.B., Karunasagar, I., and Karunasagar, I. 1997. Influence of bacteria on growth and hemolysis production by the marine dinoflagellate *Amphidinium carterae*. *Marine Biology* 130: 35-39.

Parsons, T.R., Maita, Y., and Lalli, C.M. 1984. Fluorometric determination of chlorophylls. In: A Manual of Chemical and Biological Methods for Seawater Analysis. Pergamon Press, New York, pp. 107-109.

Paul, G.K., Matsumori, K., Konoki, K., Murata, M., and Tachibana, K. 1997. Chemical structures of amphidiniols 5 and 6 isolated from marine dinoflagellate *Amphidinium klebsii* and their cholesterol-dependant membrane disruption. *Journal of Marine Biotechnology* 5: 124-128.

Paul, G.K., Matsumori, N., Murata, M, and Tachibana, K. 1995. Isolation and chemical structure of amphidinol 2, a potent hemolytic compound from marine dinoflagellate *Amphidinium klebsii*. *Tetrahedron Letters* 36(35): 6279-6282.

Ralph, J. 1990. Association of *Prymnesium parvum* with fish kills in Texas. 44th Annual Southeastern Association of Fish and Wildlife Agencies Conference. Richmond, VA (USA), 21-24 Oct. 1990. World Meeting # 904 5006.

Rangel, M., Malpezzi, E.L.A., Susini, S.M.M., and De Freitas, J.C. 1997. Hemolytic activity in extracts of the diatom *Nitzschia*. *Toxicon* 35(2): 305-309.

Rengefors, K. and Legrand, C. 2001. Toxicity in *Peridinium aciculiferum*- an adaptive strategy to outcompete other winter phytoplankton? *Limnology and Oceanology* 46(8): 1990-1997.

Sakshaug, E., Graneli, E., Elbrachter, M., and Kayser, H. 1984. Chemical composition and alkaline phosphatase activity of nutrient-saturated and P-deficient cells of four marine dinoflagellates. JEM 263

Sampayo, M.A., 1985. Encystment and excystment of a Portuguese isolate culture of *Amphidinium carterae* in culture. In: Anderson, M.A., White, M. and Baden, L. (Eds.),

Toxic Dinoflagellates. Elsevier Science Publishing Company, Inc., New York, pp.125-130.

Smayda, T.J. 1997. Harmful algal blooms: their ecophysiology and general relevance to phytoplankton blooms in the sea. *Limnol. Oceanogr.* 42(5): 1137-1153.

Smayda, T.J. 2000. Novel and nuisance phytoplankton blooms in the sea: Evidence for a global epidemic. In: Graneli, E., Sundström, B., Edler, L., and Anderson, D.M. (Eds.) Toxic Marine Phytoplankton. Elsevier Science Publishing Company, Inc., New York, pp. 29-40.

Steidinger, K.A. and Tangen, K. 1997. Chapter 3 Dinoflagellates. In: Tomas, C.R. (Ed.), Identifying Marine Phytoplankton. Academic Press, New York, pp. 443- 445.

Tatsuya, O., Sato, Y., Kim, D., Muramutsu, T., Matsuyama, Y., and Honjo, T. 2001. Hemolytic activity of *Heterocapsa circularisquama* (Dinophyceae) and its possible involvement in shellfish toxicity. *Journal of Phycology* 37: 509-516.

Taylor, F.J.R., Fukuyu, Y., Larsen, J., and Hallegraeff, G.M. 2003. Chapter 15 Taxonomy of harmful dinoflagellates. In: Hallegraeff, G.M., Anderson, D.M., and Cembella, A.D. (Eds.), Manual on Harmful Marine Microalgae. UNESCO Publishing, Paris, pp. 393.

Tomas, C.R. 2003. Observations on mixotrophic feeding by the two autotrophic HAB species *Amphidinium carterae* and *Prymnesium parvum* in estuarine waters. Second Symposium on Harmful Marine Algae in the U.S. Symposium Agenda, Abstracts, and Participants. Woods Hole, MA (USA), December 9-13, 2003.

Tomas, C., Glass, J., Ralph, J., and Lewitus, A. 2004. Blooms of the ichthyotoxic flagellate *Prymnesium parvum* in U.S. waters: an emerging or a perennial problem? In: Steidinger, K.A., Landsberg, J.H., Tomas, C.R., and Vargo, G.A. (Eds.) Harmful Algae 2002. Intergovernmental Oceanographic Commission of UNESCO, pp. 369-370.

Tomas, C.R., Montresor, M. and Tosti, E. 1987. Nutrient and temperature effects on growth and sexual phases of four marine dinoflagellates. In: Okaichi, T., Anderson, D.M., and Nemoto, T. (Eds.) Red Tides: Biology, Environmental Science, and Toxicology. Elsevier Science Publication Co, Inc., New York, pp. 293-294.

Tsuda, M., Endo, T., and Kobayashi, J. 1999. Amphidinolide U, novel 20-membered macrolide from marine dinoflagellate *Amphidinium* sp. *Tetrahedron* 55: 14565-14570.

Ulitzer, S. 1973. The amphiphatic nature of *Prymnesium parvum* hemolysin. *Biochimica et Biophysica Acta (BBA)- Biomembranes* 298(3): 673-679.

Yasumoto, T. 1990. Marine microorganism toxins- An overview. In: Graneli, E., Sundstrom, B., Edler, L., and Anderson, D.M. (Eds.) Toxic Marine Phytoplankton. Elsevier Science Publications, New York, pp. 3-8.

Yasumoto, T., Oshima, Y., Murakami, Y., Nakajima, I., Bagnis, R., and Fukuyo, Y. 1980. Toxicity of benthic dinoflagellates found in coral reef. *Bulletin of the Japanese Society of Scientific Fisheries* 46(3): 327-331.

Yasumoto, T., Underdal, B., Aune, T., Hormazabal, V., Skulberg, O.M., and Oshima, Y. 1990. Screening for hemolytic and ichthyotoxic components of *Chrysochromulina polylepis* and *Gyrodinium aureolum* from Norwegian coastal waters. In: Graneli, E., Sundstrom, B., Edler, L., and Anderson, D.M. (Eds.) Toxic Marine Phytoplankton. Elsevier Science Publications, New York, pp. 436-442.

Zimmermann, C.F., Keefe, C.W., and Bashe, J. 1997. Determination of carbon and nitrogen in sediments and particulates in estuarine/ coastal waters using elemental analysis. In: Methods for the Determination of Chemical Substances in Marine and Estuarine Environmental Matrices- 2cd Edition. National Exposure Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati Ohio 45268.

APPENDICES

Appendix A. Cell density data.

Table 3. Cells densities (cell/mL) for *Amphidinium carterae* during lag, log, and stationary phases of all trials and treatments.

Trial	Treatment	Lag phase	Log phase	Stationary phase
Nutrient 1	K39	7580	387447	869180
	16:1	7153	71773	986453
	80:1	7747	295373	786267
	4:1	7707	333393	942713
Nutrient 2	K39	4387	435753	271993
	16:1	5653	304020	654420
	80:1	1320	152073	395747
	4:1	6047	298720	956887
Salinity 1	39	1900	447093	702013
	30	1667	292447	477673
	20	4947	375567	559567
	15	2180	1653	1527
Salinity 2	39	1800	501633	655427
	30	2107	369867	457133
	20	5580	560740	347620
	15	4827	3667	440
Temperature 1	28°	2860	616167	136400
	20°	7140	436773	470080
	15°	10240	59040	69880
	10°	7540	5500	8927
Temperature 2	28°	7960	455640	277087
	20°	8113	168500	501747
	15°	14947	54033	93947
	10°	12020	6753	8180
Light 1	100%	5807	391153	590867
	60%	5300	128493	338227
	35%	3133	44033	174313
	20%	3660	23833	72140
	10%	3640	19613	32680

Table 4. Cell densities (cells/mL) for *Amphidinium klebsii* during lag, log, and stationary phases of all trials and treatments.

Trial	Treatment	Lag phase	Log phase	Stationary phase
Nutrient 1	K39	6047	47053	79840
	16:1	4387	23820	114233
	80:1	6833	15613	73553
	4:1	12080	29333	75353
Nutrient 2	K39	1560	5427	56860
	16:1	2147	11893	55160
	80:1	4327	51347	74253
	4:1	1993	96940	110047
Salinity 1	39	787	23853	50247
	30	1460	22973	42440
	20	933	4833	54247
	15	1080	1820	1120
Salinity 2	39	980	27160	56767
	30	427	15673	46660
	20	1527	5731	47173
	15	1713	4187	1007
Temperature 1	28°	2633	44207	33120
	20°	940	8310	23993
	15°	427	1233	1087
	10°	1967	1747	760
Temperature 2	28°	1660	46067	58740
	20°	427	2733	24227
	15°	1007	1533	4220
	10°	820	431	1027
Light 1	100%	647	9833	47740
	60%	127	1440	51180
	35%	93	1147	5760
	20%	100	827	2767
	10%	80	493	527

Appendix B. Cellular carbon data.

Table 5. Carbon (pg/cell) for *Amphidinium carterae* during lag, log, and stationary phases of all trials and treatments.

Trial	Treatment	Lag phase	Log phase	Stationary phase
Nutrient 1	K39	124.93	36.51	94.58
	16:1	148.73	75.54	82.09
	80:1	153.43	32.44	90.47
	4:1	133.13	50.58	90.34
Nutrient 2	K39	256.57	65.07	241.58
	16:1	188.56	107.18	59.15
	80:1	807.58	100.65	133.52
	4:1	180.76	103.16	93.32
Salinity 1	39	389.47	67.59	90.68
	30	342.00	80.68	79.01
	20	208.22	46.97	78.31
	15	509.17	701.61	890.83
Salinity 2	39	441.67	60.80	87.82
	30	417.72	75.22	140.40
	20	225.81	45.33	75.43
	15	111.88	223.64	1863.64
Temperature 1	28°	360.14	83.61	424.93
	20°	204.48	68.59	125.09
	15°	168.95	144.99	148.54
	10°	401.86	421.82	192.68
Temperature 2	28°	229.90	51.97	183.99
	20°	195.97	59.94	57.96
	15°	137.15	88.46	128.58
	10°	440.10	387.96	234.72
Light 1	100%	134.33	59.21	58.39
	60%	173.58	46.62	71.62
	35%	169.15	56.96	72.17
	20%	128.42	78.04	66.20
	10%	120.88	58.94	74.97

Table 6. Carbon (pg/cell) for *Amphidinium klebsii* during lag, log, and stationary phases of all trials and treatments.

Trial	Treatment	Lag phase	Log phase	Stationary phase
Nutrient 1	K39	855.08	495.25	581.86
	16:1	435.48	434.48	427.52
	80:1	347.11	338.05	623.49
	4:1	250.25	400.47	549.15
Nutrient 2	K39	903.85	1087.22	573.69
	16:1	1080.73	1108.19	867.30
	80:1	919.87	725.66	709.19
	4:1	1791.27	571.38	230.07
Salinity 1	39	2122.88	1033.82	1232.32
	30	1513.70	928.03	1416.12
	20	3225.00	1654.76	659.95
	15	1388.89	1791.21	1089.29
Salinity 2	39	1040.82	661.93	1407.87
	30	3796.88	746.49	997.86
	20	1283.84	1249.42	735.16
	15	2214.98	936.31	1629.14
Temperature 1	28°	546.84	1407.48	2120.17
	20°	2414.89	2355.86	2293.14
	15°	6773.44	3535.14	7012.27
	10°	1194.92	526.72	2736.84
Temperature 2	28°	1439.76	631.69	979.23
	20°	4007.81	1280.49	1623.83
	15°	4440.40	2921.74	1559.24
	10°	3170.73	5475.64	2902.60
Light 1	100%	634.02	1771.53	736.49
	60%	6473.68	2694.44	381.40
	35%	4821.43	2110.47	815.97
	20%	7990.00	1475.81	563.86
	10%	3625.00	2310.81	2240.51

Appendix C. Cellular chlorophyll *a* data.

Table 7. Chlorophyll *a* (pg/cell) for *Amphidinium carterae* during lag, log, and stationary phases of all trials and treatments.

Trial	Treatment	Lag phase	Log phase	Stationary phase
Nutrient 1	K39	81.66	162.34	130.93
	16:1	73.11	137.52	197.68
	80:1	124.96	119.85	116.25
	4:1	167.39	232.76	13.54
Nutrient 2	K39	131.08	197.82	176.47
	16:1	196.35	253.27	135.69
	80:1	303.03	129.54	344.16
	4:1	170.34	256.76	97.61
Salinity 1	39	130.53	152.99	137.89
	30	256.80	188.75	100.07
	20	125.94	126.21	91.50
	15	234.86	21.29	178.17
Salinity 2	39	217.78	202.14	183.39
	30	263.92	162.76	242.82
	20	134.77	119.13	140.96
	15	55.32	7.25	440.91
Temperature 1	28°	151.40	113.93	71.70
	20°	110.50	60.44	77.01
	15°	69.53	68.09	56.38
	10°	4.75	2.04	1.25
Temperature 2	28°	131.91	68.48	117.65
	20°	86.15	75.25	57.00
	15°	50.91	62.92	61.95
	10°	4.04	2.28	1.52
Light 1	100%	49.77	167.71	209.86
	60%	220.75	155.34	256.63
	35%	158.94	171.23	218.00
	20%	155.19	104.06	148.32
	10%	178.30	109.11	102.20

Table 8. Chlorophyll *a* (pg/cell) values for *Amphidinium klebsii* during lag, log, and stationary phases of all trials and treatments.

Trial	Treatment	Lag phase	Log phase	Stationary phase
Nutrient 1	K39	947.62	898.98	921.84
	16:1	508.35	1406.38	1785.82
	80:1	339.51	1063.20	1318.77
	4:1	209.44	1435.23	1157.22
Nutrient 2	K39	1006.41	665.23	562.79
	16:1	1355.57	1227.58	257.80
	80:1	1451.45	708.91	420.18
	4:1	416.46	410.56	497.97
Salinity 1	39	2059.32	2439.91	1616.03
	30	1226.03	1305.86	2408.11
	20	2132.14	1804.14	1640.65
	15	962.96	980.22	51.43
Salinity 2	39	2255.10	1730.49	1673.52
	30	4406.25	2028.92	1967.42
	20	1906.11	1863.66	1488.13
	15	2626.46	993.63	43.31
Temperature 1	28°	778.48	561.00	454.11
	20°	1148.94	1542.66	1550.43
	15°	1971.09	859.46	688.34
	10°	84.92	4.01	12.37
Temperature 2	28°	698.80	1202.60	1096.36
	20°	2165.63	1295.12	1345.62
	15°	1013.25	658.70	118.48
	10°	23.54	236.66	8.38
Light 1	100%	218.04	3681.36	703.81
	60%	955.26	5027.78	1320.83
	35%	2517.86	1597.67	3427.08
	20%	1350.00	1686.29	860.24
	10%	588.75	1337.84	987.34

Appendix D. Growth rate data.

Table 9. Average growth (K) for log phase of *Amphidinium carterae* and *Amphidinium klebsii* for nutrient, salinity, temperature, and light treatments.

		Amphidinium carterae		*Amphidinium klebsii*	
Trial	Treatment	Log length*	K**	Log length*	K
Nutrient 1	K39	19	0.33	13	0.13
	16:1	10	0.28	13	0.10
	4:1	17	0.14	13	0.10
	80:1	15	0.20	13	0.14
Nutrient 2	K39	15	0.17	8	0.36
	16:1	19	0.12	6	0.30
	4:1	19	0.12	14	0.11
	80:1	13	0.22	6	0.28
Salinity 1	39	15	0.23	21	0.09
	30	15	0.27	21	0.09
	20	15	0.23	12	0.16
	15	3	0.07	5	0.10
Salinity 2	39	16	0.22	10	0.21
	30	11	0.16	20	0.11
	20	11	0.29	10	0.18
	15	3	0.04	3	0.11
Temperature 1	28°	8	0.41	8	0.45
	20°	12	0.30	16	0.13
	15°	18	0.07	9	0.13
	10°	n/a	n/a	n/a	n/a
Temperature 2	28°	12	0.31	7	0.46
	20°	10	0.29	13	0.13
	15°	18	0.07	5	0.07
	10°	n/a	n/a	n/a	n/a
Light 1	100%	10	0.33	13	0.25
	60%	19	0.11	18	0.20
	35%	16	0.14	18	0.17
	20%	20	0.09	8	0.27
	10%	13	0.10	18	0.09

*= log phase in days
**= divisions per day

Appendix E. Growth rate comparison data.

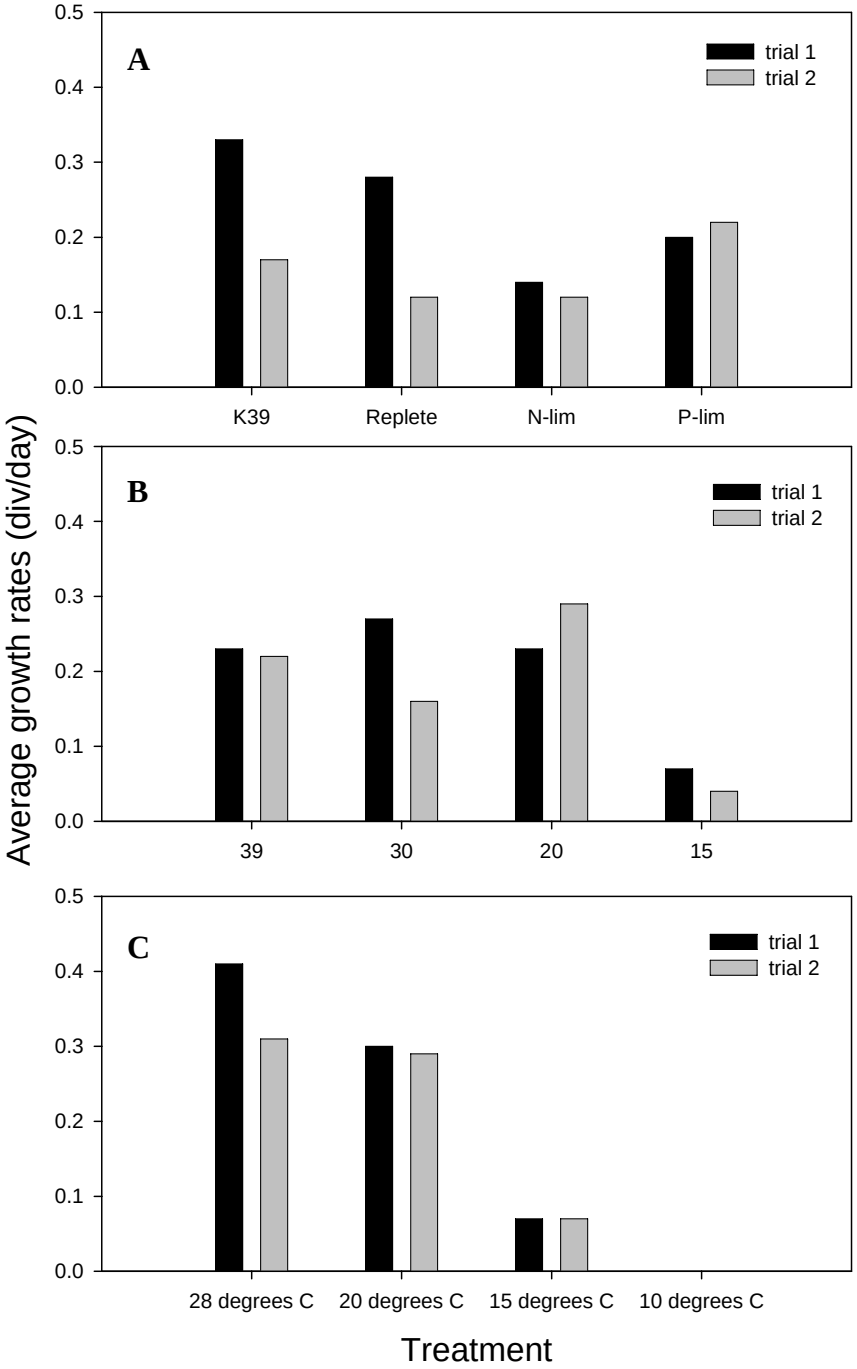

Figure 26. Growth (K) of *Amphidinium carterae* for a) nutrient, b) salinity and c) temperature treatments and both trials.

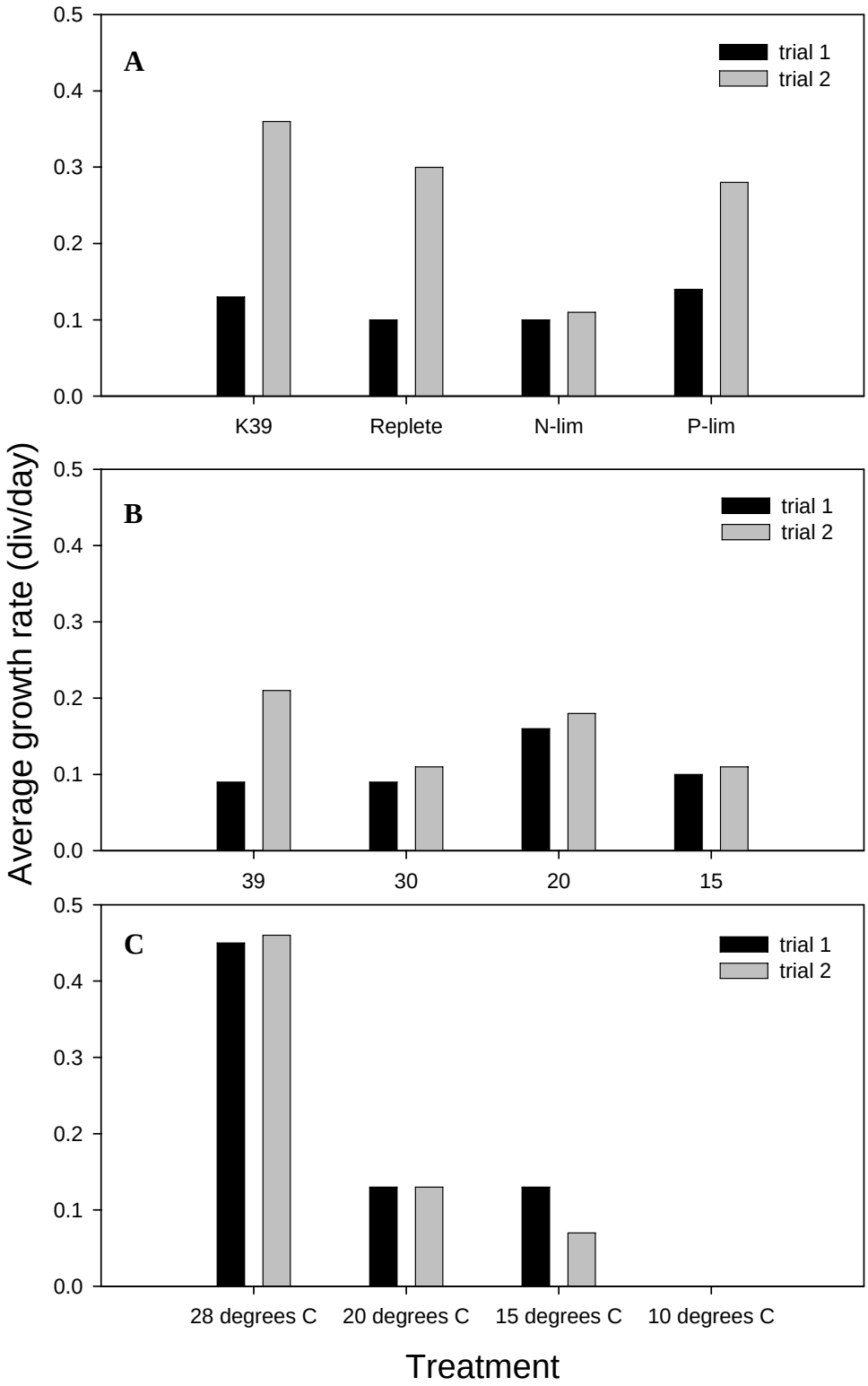

Figure 27. Growth (K) of *Amphidinium klebsii* for a) nutrient, b) salinity and c) temperature treatments for both trials.

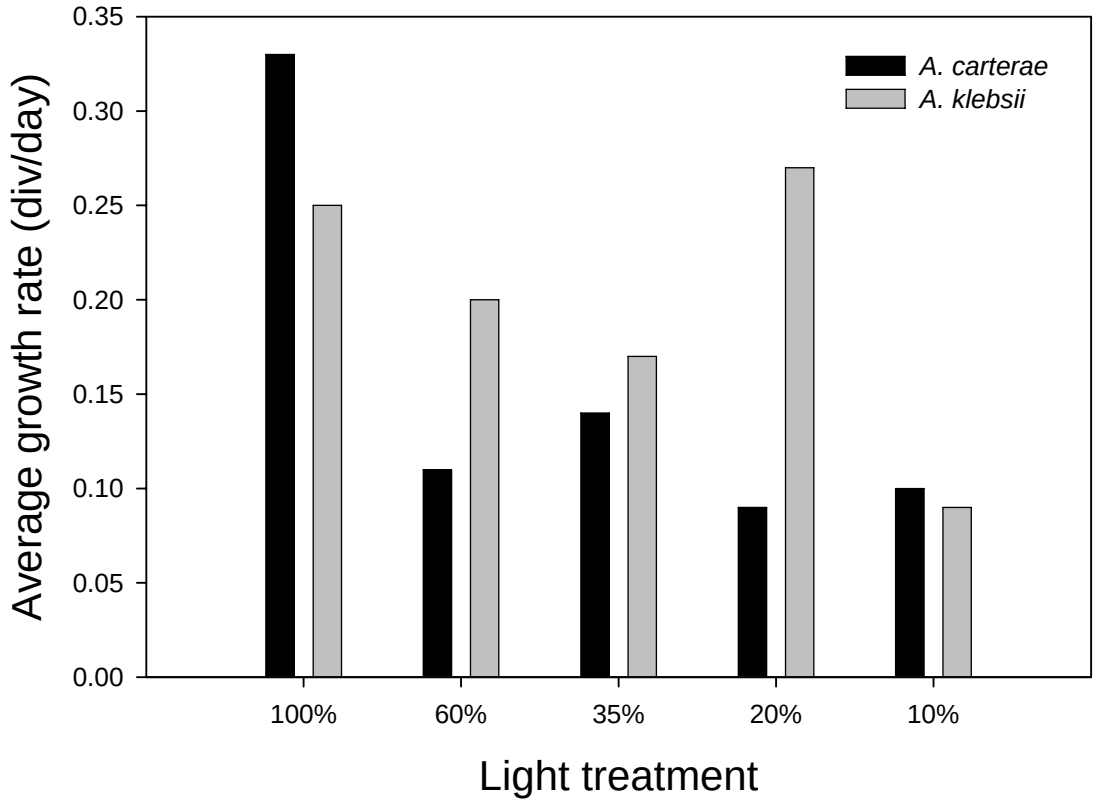

Figure 28. Growth (K) of *Amphidinium carterae* and *Amphidinium klebsii* for light treatments.

Appendix F. Erythrocyte lysis data for *A. carterae* and *A. klebsii.*

Table 10. Mean saponin equivalents (µg) for *Amphidinium carterae* during lag, log, and stationary phases for all trials and treatments.

Trial	Treatment	Lag phase	Log phase	Stationary phase
Nutrient 1	K39	3.64	3.67	3.96
	16:1	0.57	1.16	4.21
	80:1	0.00	3.73	3.74
	4:1	2.35	3.90	3.98
Nutrient 2	K39	0.02	3.67	3.95
	16:1	2.07	3.52	4.13
	80:1	0.00	3.73	3.74
	4:1	2.35	3.90	3.98
Temperature 1	28°	0.47	3.92	0.66
	20°	3.48	3.88	4.06
	15°	3.66	3.68	4.17
	10°	0.17	0.00	0.00
Temperature 2	28°	3.94	3.86	3.07
	20°	3.61	3.90	3.81
	15°	4.06	3.52	3.69
	10°	2.56	0.08	0.01
Salinity 1	39	1.24	3.81	3.90
	30	0.24	3.74	3.87
	20	3.65	3.84	3.91
	15	3.93	0.00	0.01
Salinity 2	39	0.06	3.98	4.00
	30	1.84	3.91	4.07
	20	1.33	3.66	3.82
	15	4.17	0.04	-0.01
Light 1	100%	0.00	3.82	3.95
	60%	0.09	3.42	3.92
	35%	0.00	0.20	3.56
	20%	0.03	0.05	0.72
	10%	0.06	0.03	0.17

Table 11. Mean saponin equivalents (µg) for *Amphidinium klebsii* during lag, log, and stationary phases for all trials and treatments.

Trial	Treatment	Lag phase	Log phase	Stationary phase
Nutrient 1	K39	0.17	0.43	1.24
	16:1	0.32	0.23	2.59
	80:1	0.36	0.30	3.44
	4:1	0.40	0.34	2.95
Nutrient 2	K39	0.01	0.02	0.12
	16:1	0.43	0.11	0.25
	80:1	0.17	0.18	0.31
	4:1	0.13	0.17	0.53
Temperature 1	28°	0.02	0.00	2.50
	20°	1.14	0.00	4.08
	15°	0.87	0.07	0.02
	10°	0.00	0.00	0.00
Temperature 2	28°	0.17	0.50	0.24
	20°	0.15	0.16	0.21
	15°	0.10	0.09	0.15
	10°	0.00	0.00	0.01
Salinity 1	39	0.06	0.17	0.38
	30	0.04	0.07	1.71
	20	0.06	0.25	0.57
	15	0.00	0.03	0.03
Salinity 2	39	0.09	0.17	0.38
	30	0.01	0.13	0.86
	20	0.20	0.87	1.13
	15	0.12	0.09	0.03
Light 1	100%	0.00	0.07	0.31
	60%	0.06	0.06	0.85
	35%	0.02	0.02	0.32
	20%	0.00	0.00	0.07
	10%	0.01	0.00	0.00

Table 12. Mean saponin equivalents (μg) per cell for *Amphidinium carterae* during lag, log, and stationary phases for all trials and treatments.

Trial	Treatment	Lag phase ($x10^{-5}$)	Log phase ($x10^{-5}$)	Stationary phase ($x10^{-5}$)
Nutrient 1	K39	8.34	1.42	0.55
	16:1	1.39	0.18	0.51
	80:1	7.19	1.91	0.62
	4:1	7.19	1.80	0.55
Nutrient 2	K39	0.10	1.44	1.74
	16:1	6.34	1.99	0.76
	80:1	0.00	4.20	1.13
	4:1	6.73	2.24	0.50
Temperature 1	28°	3.31	0.76	0.58
	20°	9.75	1.07	1.04
	15°	6.22	7.49	7.15
	10°	0.45	0.00	0.00
Temperature 2	28°	9.89	1.02	1.33
	20°	8.90	2.78	0.91
	15°	5.43	7.83	4.71
	10°	4.25	1.45	0.15
Salinity 1	39	130.00	1.02	0.67
	30	2.86	1.53	0.97
	20	148.00	1.23	0.84
	15	361.00	0.00	1.66
Salinity 2	39	0.70	0.95	0.73
	30	174.00	1.27	1.07
	20	4.76	0.78	1.32
	15	173.00	1.18	0.00
Light 1	100%	0.00	1.07	0.80
	60%	0.72	3.20	1.39
	35%	0.00	0.56	2.45
	20%	0.35	0.27	1.20
	10%	0.70	0.18	0.64

Table 13. Mean saponin equivalents (µg) per cell for *Amphidinium klebsii* during lag, log, and stationary phases for all trials and treatments.

Trial	Treatment	Lag phase (x10-6)	Log phase (x10-6)	Stationary phase (x10-6)
Nutrient 1	K39	4.77	12.20	18.60
	16:1	12.60	13.00	27.20
	80:1	9.14	25.60	56.10
	4:1	5.75	15.60	47.00
Nutrient 2	K39	1.02	3.56	2.45
	16:1	34.80	10.80	5.42
	80:1	6.80	4.28	5.07
	4:1	13.00	2.15	5.78
Temperature 1	28°	1.85	0.00	90.70
	20°	242.00	0.00	204.00
	15°	354.00	68.10	26.30
	10°	0.00	0.00	0.00
Temperature 2	28°	20.90	13.00	4.84
	20°	69.30	69.80	10.20
	15°	20.80	69.30	42.80
	10°	0.00	0.00	5.85
Salinity 1	39	15.60	8.66	8.98
	30	5.79	3.42	48.30
	20	13.20	62.80	12.60
	15	0.00	18.70	47.50
Salinity 2	39	19.10	7.68	7.96
	30	3.26	9.61	22.10
	20	26.00	182.00	28.80
	15	14.00	27.10	67.30
Light 1	100%	0.16	8.26	7.88
	60%	150.00	52.90	19.90
	35%	55.60	18.10	67.30
	20%	0.00	3.11	28.80
	10%	30.90	0.00	11.10

Table 14. Mean saponin equivalents (µg) per carbon (µg) for *Amphidinium carterae* during lag, log, and stationary phases for all trials and treatments.

Trial	Treatment	Lag phase	Log phase	Stationary phase
Nutrient 1	K39	0.67	0.39	0.06
	16:1	0.09	0.02	0.06
	80:1	0.47	0.59	0.06
	4:1	0.54	0.36	0.06
Nutrient 2	K39	0.00	0.22	0.07
	16:1	0.34	0.19	0.13
	80:1	0.00	0.42	0.08
	4:1	0.37	0.22	0.05
Temperature 1	28°	0.09	0.09	0.01
	20°	0.48	0.16	0.08
	15°	0.37	0.52	0.48
	10°	0.01	0.00	0.00
Temperature 2	28°	0.43	0.20	0.07
	20°	0.45	0.46	0.16
	15°	0.40	0.88	0.37
	10°	0.10	0.04	0.00
Salinity 1	39	0.34	0.15	0.07
	30	0.08	0.19	0.12
	20	0.71	0.26	0.11
	15	0.71	0.00	0.02
Salinity 2	39	0.02	0.16	0.08
	30	0.42	0.17	0.08
	20	0.21	0.17	0.18
	15	1.54	0.05	0.00
Light 1	100%	0.00	0.20	0.14
	60%	0.04	0.69	0.19
	35%	0.00	0.10	0.34
	20%	0.03	0.03	0.18
	10%	0.06	0.03	0.09

Table 15. Mean saponin equivalents (µg) per carbon (µg) for *Amphidinium klebsii* during lag, log, and stationary phases for all trials and treatments.

Trial	Treatment	Lag phase ($x10^{-3}$)	Log phase ($x10^{-3}$)	Stationary phase ($x10^{-3}$)
Nutrient 1	K39	5.58	24.60	31.90
	16:1	28.90	29.90	63.60
	80:1	26.30	75.80	90.10
	4:1	23.00	39.00	85.60
Nutrient 2	K39	1.13	3.27	4.26
	16:1	32.30	9.71	6.25
	80:1	7.39	5.89	7.15
	4:1	7.29	3.76	25.10
Temperature 1	28°	3.39	0.00	42.80
	20°	101.00	0.00	89.10
	15°	52.30	19.20	3.75
	10°	0.00	0.00	0.00
Temperature 2	28°	14.50	20.50	4.94
	20°	17.30	54.50	6.32
	15°	4.67	23.70	27.40
	10°	0.00	0.00	2.02
Salinity 1	39	7.38	8.40	7.30
	30	3.83	3.69	34.00
	20	4.10	38.10	19.00
	15	0.00	10.40	43.60
Salinity 2	39	18.40	11.60	5.64
	30	0.86	12.90	22.20
	20	20.30	145.00	39.10
	15	6.34	29.00	22.30
Light 1	100%	0.26	4.67	10.70
	60%	23.20	19.60	52.30
	35%	11.50	8.58	82.40
	20%	0.00	2.10	51.00
	10%	8.51	0.00	4.97

Table 16. Mean saponin equivalents (µg) per chlorophyll a (µg) for *Amphidinium carterae* during lag, log, and stationary phases for all trials and treatments.

Trial	Treatment	Lag phase	Log phase	Stationary phase
Nutrient 1	K39	1.02	0.09	0.04
	16:1	0.19	0.01	0.03
	80:1	0.58	0.16	0.05
	4:1	0.43	0.08	0.41
Nutrient 2	K39	0.00	0.07	0.10
	16:1	0.32	0.08	0.06
	80:1	0.00	0.32	0.03
	4:1	0.40	0.09	0.06
Temperature 1	28°	0.22	0.07	0.08
	20°	0.88	0.18	0.14
	15°	0.90	1.10	1.25
	10°	0.95	0.00	0.00
Temperature 2	28°	0.75	0.15	0.11
	20°	1.03	0.37	0.16
	15°	1.07	1.24	0.76
	10°	10.50	6.37	0.97
Salinity 1	39	1.00	0.07	0.05
	30	0.11	0.08	0.10
	20	1.17	0.10	0.09
	15	1.53	0.00	0.09
Salinity 2	39	0.03	0.05	0.04
	30	0.66	0.08	0.04
	20	0.35	0.07	0.09
	15	3.12	1.63	0.00
Light 1	100%	0.00	0.07	0.04
	60%	0.03	0.21	0.05
	35%	0.00	0.03	0.11
	20%	0.02	0.03	0.08
	10%	0.04	0.02	0.06

Table 17. Mean saponin equivalents (µg) per chlorophyll a (µg) for *Amphidinium klebsii* during lag, log, and stationary phases for all trials and treatments.

Trial	Treatment	Lag phase ($x10^{-3}$)	Log phase ($x10^{-3}$)	Stationary phase ($x10^{-3}$)
Nutrient 1	K39	5.03	13.50	20.20
	16:1	24.70	9.20	15.20
	80:1	2.69	24.20	42.50
	4:1	27.50	10.80	40.50
Nutrient 2	K39	1.01	5.35	4.34
	16:1	25.60	8.76	2.10
	80:1	4.69	6.03	12.10
	4:1	31.40	5.22	11.60
Temperature 1	28°	2.38	0.00	200.00
	20°	211.00	0.00	132.00
	15°	180.00	79.20	38.30
	10°	0.00	0.00	0.00
Temperature 2	28°	29.90	10.80	4.40
	20°	31.90	53.70	6.68
	15°	20.50	105.00	363.00
	10°	0.00	0.00	698.00
Salinity 1	39	7.59	3.55	5.54
	30	4.71	2.61	20.00
	20	6.21	34.90	7.66
	15	0.00	19.10	924.00
Salinity 2	39	8.45	4.44	4.77
	30	0.74	4.73	11.20
	20	13.60	97.80	19.30
	15	5.32	27.30	839.00
Light 1	100%	0.74	2.24	11.20
	60%	157.00	10.50	15.10
	35%	22.10	11.30	19.60
	20%	0.00	1.84	33.50
	10%	52.40	0.00	11.30

Table 18. Mean saponin equivalents (µg) per volume (μm^3) for *Amphidinium carterae* during lag, log, and stationary phases for all trials and treatments.

Trial	Treatment	Lag phase ($x10^{-8}$)	Log phase ($x10^{-8}$)	Stationary phase ($x10^{-8}$)
Nutrient 1	K39	16.30	2.78	1.07
	16:1	2.71	0.36	1.00
	80:1	14.00	3.72	1.20
	4:1	14.10	3.51	1.07
Nutrient 2	K39	0.19	2.82	3.40
	16:1	12.40	3.88	1.48
	80:1	0.00	8.21	2.21
	4:1	13.10	4.38	0.98
Temperature 1	28°	6.47	1.49	1.13
	20°	19.10	2.08	2.03
	15°	12.10	14.60	14.00
	10°	0.88	0.00	0.00
Temperature 2	28°	19.30	1.99	2.60
	20°	17.40	5.43	1.78
	15°	10.60	15.30	9.20
	10°	8.31	2.84	0.29
Salinity 1	39	25.50	2.00	1.30
	30	5.58	3.00	1.90
	20	28.90	2.40	1.64
	15	70.40	0.00	3.25
Salinity 2	39	1.38	1.86	1.43
	30	34.10	2.48	2.09
	20	9.29	1.53	2.57
	15	33.80	2.31	0.00
Light 1	100%	0.00	2.29	1.57
	60%	1.40	6.25	2.72
	35%	0.00	1.09	4.79
	20%	0.69	0.53	2.35
	10%	1.37	0.34	1.25

Table 19. Mean saponin equivalents (µg) per volume (µm^3) for *Amphidinium klebsii* during lag, log, and stationary phases for all trials and treatments.

Trial	Treatment	Lag phase ($x10^{-10}$)	Log phase ($x10^{-10}$)	Stationary phase ($x10^{-10}$)
Nutrient 1	K39	7.62	19.50	29.70
	16:1	20.10	20.70	43.50
	80:1	14.60	41.00	89.70
	4:1	9.18	24.90	75.10
Nutrient 2	K39	1.63	5.69	3.91
	16:1	55.70	17.20	8.66
	80:1	10.90	6.84	8.10
	4:1	20.90	3.43	9.23
Temperature 1	28°	2.96	0.00	145.00
	20°	387.00	0.00	326.00
	15°	566.00	109.00	42.10
	10°	0.00	0.00	0.00
Temperature 2	28°	33.40	20.70	7.73
	20°	111.00	112.00	16.40
	15°	33.20	111.00	68.40
	10°	0.00	0.00	9.35
Salinity 1	39	25.00	13.80	14.30
	30	9.25	5.47	77.20
	20	21.20	100.00	20.10
	15	0.00	29.80	75.90
Salinity 2	39	30.50	12.30	12.70
	30	5.21	15.40	35.30
	20	41.50	291.00	46.00
	15	22.40	43.30	58.10
Light 1	100%	0.26	13.20	12.60
	60%	240.00	84.50	31.80
	35%	88.90	28.90	108.00
	20%	0.00	4.97	46.00
	10%	49.90	0.00	17.80