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Iron And Light Limitation Of Freshwater Chrysophyte Phytoplankton

by

Bradley Hopper

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ABSTRACT

Ambient iron concentrations govern primary productivity in several regions of the world's oceans. The potential for iron limitation in freshwater systems has received little attention. Irradiance levels alter the iron requirements of planktonic algal cells; low light levels elevate iron demands. The goals of this thesis were (i) to determine if the dynamics of freshwater algal populations are regulated by iron, (ii) to examine relationships between irradiance levels and iron requirements of freshwater phytoplankton, and (iii) to compare growth responses of algae using different nutritional modes (i.e. autotrophy vs. mixotrophy) in iron/light stressed environments. Iron-limited growth of the autotroph *Synedra* was induced in the laboratory but not in the field experiments, and low light levels increased this effect. Growth of two mixotrophic algae, *Uroglena* and *Dinobryon*, was unaffected by iron/light manipulation. Results of small-scale laboratory experiments led to the development of successful predictions regarding algal community composition in field experiments.

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1.1 Phytoplankton Growth Limiting Resources and Processes

Phytoplankton communities in freshwater systems are extremely diverse. Algal biomass and species composition vary dramatically from lake to lake, reservoir to reservoir, and stream to stream. Striking patterns of temporal phytoplankton species replacement (succession) also exist within individual systems.

The purpose of this chapter is to review the major principles underlying phytoplankton nutrient limitation and resource competition in aquatic systems. Resource competition, resource ratios, growth limiting factors, essential growth elements (specifically phosphorus, nitrogen, silicon, and iron), and methods of nutrient acquisition will be discussed.

This study takes an unconventional approach and focuses on the roles of iron and light in determining freshwater algal community composition instead of the more traditionally studied nutrients. Phosphorus and nitrogen are generally viewed as the key nutrients limiting primary production in aquatic systems, but they do not necessarily govern all algal growth in all environments. Iron is a nutrient that has recently been shown to limit primary production in several regions of the world's oceans. The results of several marine studies will be examined as well as evidence of the possible existence of iron limitation in freshwater habitats.

1.2 Resource Competition

A frequently encountered hypothesis to account for variation in algal community composition among and within systems over time revolves around resource competition. A component of this hypothesis states that the composition of algal assemblages is determined by the nutrient resources available within an aquatic environment. Nutrients vary in time and space, and their availability to organisms, which depends on several conditions, determines the structure of a phytoplankton communities. Supply rates, relative availability, and relative demands of nutrients along with physical conditions (e.g. light, temperature) will drive primary production.

The total nutrient concentration is partitioned into available and unavailable forms. There are numerous physical and biological processes that alter the partitioning between forms. For a given absolute amount of nutrient, the rate of resource supply would depend on the amount of unavailable resource such that the larger the proportion of the absolute amount that is in the unavailable form, the greater the pool from which available nutrients can be derived (Tilman 1982).

Relative growth rates express growth rates (μ) as a fraction of the maximum potential growth rates(μ_{max}) for given environmental conditions (Falkowski 1980). These variables are key components of the Monod model which is

an equation expressing the requirement by phytoplankton for a specific nutrient (from Sze 1993):

$$\mu = \mu_{max} \frac{S}{K+S} \quad (1)$$

where μ = growth rate based on cell production, μ_{max} = maximum growth rate, S = concentration of nutrient, and K is the value of S at half the maximum rate of growth. The half saturation constant (K) is a measure of the ability of an organism to use a nutrient. These values are determined via laboratory studies and do not always correspond with those observed in natural setting. Species with a low K can grow better at low concentrations of a particular nutrient, while a high K value indicates that high concentrations are required for good growth. Theoretically, K and μ_{max} can be used to predict the outcome of competition between two species, such that individuals that can sustain the greatest positive growth at the lowest concentration of a single limiting nutrient resource will outcompete others for that resource. Coexistence of two species could occur while competing for more than one nutrient if one species is more adept to taking up one nutrient and the other a second nutrient (Tilman 1982).

A focal point of study in the areas of resource competition and nutrient limitation has been to understand how unique dietary requirements of individual organisms, along with differences in ratios of available resources, influence the outcome of competition and lead to observed dynamics of phytoplankton communities in nature (Tilman 1982, Sze 1993). Table 1.1 reports the relationship between the relative supply and

requirements of various elements relative to phosphorus, an element whose ratio of supply to need is often smaller than others in freshwater systems.

Table 1.1: The relative supply of various elements indicated by their abundance relative to phosphorus in the Earth's crust, and the relative requirements of these elements, also relative to phosphorus, by plants and algae. The ratio of supply to need for all elements is greater than that for phosphorus (modified from Moss 1988).

Element	*Ratio of amount of element to that of P in Earth's crust (supply ratio)*	*Ratio of amount of element needed by plants and algae relative to that needed of P (need ratio)*	*Ratio of supply to need*
P	1.0	1.0	1.0
Na	32.5	0.52	62.5
Mg	22.2	1.39	16.0
Si	268.1	0.65	413.0
K	19.9	6.1	3.3
Ca	39.5	7.8	5.1
Mn	0.9	0.27	3.3
Cu	0.05	0.006	8.3
Fe	53.6	0.06	893.0

To introduce the major components of the topics of nutrient limitation and resource competition we briefly review the common nutrients identified as those limiting primary productivity in aquatic environments. We reveal why the particular nutrients are required by organisms, why they are often limiting, and show specific examples of nutrient limited algal growth. Following the 'single' nutrient description we review how differences in nutrient kinetics can promote algal diversity. The subject of resource ratios and corresponding interactions of organisms will also be addressed. When discussing

resource ratios we are referring to the abundance of particular nutrients (i.e. P, N, Si, Fe) or resources (i.e. light) in relation to each other and how those ratios influence phytoplankton community dynamics.

Optimal anabolism demands an abundant supply of available nutrients, sufficient illumination, and an ambient temperature range supportive of biological activity. These three variables (nutrients, light, and temperature) vary spatially and temporally and can simultaneously govern the amount of planktonic primary productivity. However, due to the complexity of their interactions, it is almost impossible to distinguish the individual effects of these variables in a natural setting (Bonin *et al.* 1981). While light and temperature can influence algal growth, the availability of a particular growth-limiting nutrient is frequently described as a major factor determining increases in algal biomass (Sakamoto 1966, Schindler *et al.* 1978, Moss 1988, Wetzel 1975).

Algae are predominantly autotrophic, requiring only light and inorganic nutrients for growth. Yet, some groups utilize organic energy sources (Sze 1993). Mixotrophic algae, for example, have the ability to oxidize organic compounds in the dark to help meet their energy demands (Sze 1993). Most mixotrophs use dissolved organic compounds, but some of these algae are phagotrophic. Phagotrophic algae ingest bacterial cells and detrital particles to supplement their carbon and energy requirements. Phagotrophic species occur in several algal groups including the chrysophytes, prymnesiophytes, euglenoids, and the dinoflagellates. In fact, some algae have come to

depend entirely on heterotrophic metabolism and lack all photosynthetic pigments (Sze 1993).

Irrespective of the method of nutrient acquisition, achieved biomass and growth rates depend upon ambient concentrations and the chemical speciation of the more than 20 essential elements required for metabolism and growth (Moss 1988).

1.3 Major Nutrients (Phosphorus and Nitrogen)

The average algal cell is composed of carbon, nitrogen, and phosphorus in the proportions of 106C:16N:1P by atoms and 42C:7N:1P by weight (Bronmark and Hansson 1998). This stochiometric ratio is referred to as the Redfield ratio. This ratio is indicative of cells having a balanced nutrient supply, and severe departures from this ratio reveal a physiological nutrient limitation. Carbon, nitrogen, and phosphorus are all present in aquatic habitats in concentrations lower than are required by algal cells and active, energy-requiring uptake mechanisms are needed to concentrate them within the cells (Moss 1988). The efficiency at which these mechanisms operate differs between species for each nutrient and can be approximated by the half saturation constant (K) in the Monod model. Phytoplankton N:P generally varies only between 7 and 10 (by weight) and this is very close to the Redfield mass ratio of 7.2 (Downing and McCauley 1992).

Phosphorus and nitrogen are considered to be primary growth limiting nutrients in freshwater and marine environments respectively (Dillon and Rigler 1974, Schindler 1978). The stock of these nutrients in a body of water often sets the upper limit to the

average total algal crop that can exist at any one time (Sakamoto 1966, Shaprio 1980 in Moss 1988, Watson *et al.* 1997). It is important to keep in mind that this upper limit is theoretical which may not always be achieved due to phytoplankton removal processes such as sinking, washout, or grazing by herbivorous zooplankton. A ratio of total nitrogen to total phosphorus (TN:TP) is a nutrient status indicator which relates the concentrations of the two growth limiting nutrients and is a tool frequently utilized to predict the major algal groups that dominate phytoplankton communities (McCauley and Downing 1991, Levine *et al.* 1997). Dissolution, concentration, sedimentation, fixation, and biological transformation result in TN:TP ratios in lakes varying between ~ 200 and <1 (Downing and McCauley 1992). It has been suggested that the relative abundance of N and P in lake water has both quantitative and qualitative effects on phytoplankton communities (Smith 1986, McCauley *et al.* 1989).

Phosphorus (P) is an essential element for all organisms. It is used in such fundamental processes as storage and transfer of genetic information (DNA and RNA), cell metabolism (various enzymes), protein manufacturing, as an energy source for cells (adenosine triphosphate, ATP), and the building of cell membranes (Bronmark and Hansson 1998). Soluble phosphorus is, on average, the scarcest compound in the earth's crust which is absolutely required for algal and higher plant growth (Moss 1988). This fact, along with phosphorus' ability to tightly bind to a variety of colloidal compounds and sediments, and the lack of a gaseous phase in the P-cycle leads to a scarce available supply of phosphorus for aquatic primary production (Goldman and Horne 1983).

Common sources of "new" phosphorus into lakes and reservoirs are from inflowing rivers and precipitation (Wetzel 1975). However, internal loading and recycling of phosphorus also occurs via fish and zooplankton excretions and by the releasing of P from the sediments following fluctuations in redox potentials.

Phytoplankton take up phosphorus as inorganic phosphate (PO_4^{3-}). Most of the total phosphorus (i.e. >80%) is present in the organic form, such as that incorporated into organisms, and is unavailable for direct algal uptake (Bronmark and Hansson 1998). The sum of all organic and inorganic P fractions is collectively called Total Phosphorus (TP). TP is a widely used estimate of lake fertility (Lampert and Sommer 1997) and the nutrient status of an aquatic system may be defined on the basis of internal TP concentrations. Those with low TP (5-10 µg/l) typically exhibit low primary productivity and are categorized as oligotrophic. Systems with TP values in the 10-30 µg/l range are classified as mesotrophic, while those with high TP concentrations (30-100 µg/l) are termed eutrophic and they have the potential to support large amounts of algal biomass (Bronmark and Hansson 1998).

When inorganic phosphorus concentrations are very low, algae may secrete P-specific enzymes (phosphatases) into the water column to break down structurally complex phosphate compounds to simpler inorganic phosphate. Alternatively, when ambient [PO_4^{3-}] is high luxury consumption has been observed where individuals take up amounts greater than required for metabolism and store it for use when external supplies become diminished (Sze 1993).

Increasingly high levels of phosphorus regularly occur in waters receiving waste discharged from urban and rural settlements, and this is a commonly used indicator of eutrophication. Chemical based detergents and fertilizers are familiar sources of anthropogenic P enrichment in aquatic ecosystems that intercept runoff from developed catchments. Since algae are often P-limited, increased growth of many species is stimulated by such discharges.

Nitrogen (N) is primarily utilized by algae to build amino acids, proteins, nucleic acids and chlorophyll molecules. Nitrogen is introduced to the aquatic system via precipitation, diffusion from the atmosphere, surface and groundwater drainage, and fixation of N_2 by specialized prokaryotes. Internal loading/recycling occurs through such processes as the decay of plant and animal material, and the release of urea and ammonium in fish and zooplankton waste products. The most abundant form of nitrogen in lakes is gaseous N_2 (Lampert and Sommer 1997) but only a select few specialist organisms are able to utilize this form. Most algae use ammonium (NH_4^+) and nitrate (NO_3^-) as a nitrogen source. A small number are able to use organic nitrogen, such as amino acids and urea, and the blue-green algae (cyanobacteria) are the only algal class containing representatives that can fix N_2 (Bronmark and Hansson 1998). While the number of chemical species of nitrogen existing in aquatic systems is numerous, their sum represents the total nitrogen (TN) component. Concentrations of TN in lakes typically vary from about 100 µg/l to 6000 µg/l (Bronmark and Hansson 1998). Contrary to popular belief, nitrogen limitation is not restricted to the isolated, deep, open areas of

the oceans. Large segments of the world's oceanic, coastal, and estuarine waters are characterized by nitrogen-limited

primary productivity (Paerl 1997). In deep (>200 m) oceanic regions located away from land masses much of the primary production is dependent on regenerated ammonium derived from the mineralization of organic matter, prokaryotic N_2 fixation, and the introduction of NO_3^- rich water from upwelling regions (Paerl 1997). Nitrogen often limits primary productivity in the world's oceans (Sze 1993), but is less commonly limiting in freshwater. An exception to this occurs in polluted freshwater environments where phosphorus can be available in excess due to increased external loading. Downing and McCauley (1992) demonstrated that TN:TP is high in oligotrophic lakes and very low in eutrophic lakes. They noted that these divergent TN:TP ratios are likely to result from dissimilar nutrient origins such that nutrient sources with a high N:P are dominated by natural runoff from undisturbed infertile terrestrial ecosystems, whereas low N:P sources are typical of runoff from stormwater drainage, agricultural lands, and sewage systems.

Coastal and estuarine environments are heavily influenced by imported nitrogen either supplied naturally (i.e. mineral weathering, lightning) or anthropogenically (i.e. rural wastewater). Increased exogenous N inputs enhance marine primary production, lead to eutrophication, and result in increased frequencies of harmful algal blooms (Paerl 1997).

1.4 Other Growth Limiting Elements (Silicon, Iron, Light)

Silicon (Si) is a third element which has been suggested as a growth-limiting nutrient for both freshwater and marine algae (Boyle 1998). However, its roles as a growth regulator have been analyzed less extensively than those of phosphorus and nitrogen because silicon is apparently only limiting when P and N are abundantly available. Soluble silicon occurs as ortho-silicic acid and is derived from the weathering of silicate materials (Lampert and Sommer 1997). In lakes and oceans silicon plays a vital role since it greatly contributes to the success of the diatoms which dominate many aquatic systems (Goldman and Horne 1983). Most algae have only a minor need for silicic acid (H_2SiO_4) but diatoms utilize relatively large amounts of this form of silica to build a rigid cell wall called a frustule, which may account for as much as 50% of the dry weight of diatoms (Lampert and Sommer 1997). Diminishing effects on the silica cycle by other Si utilizing algae, such as siliceous-scale bearing chrysophytes, is negligible and are usually not considered (Lampert and Sommer 1997). There are several well documented instances of silica-limited phytoplankton growth (see Boyle 1998, Dugdale and Wilkerson 1998, Smetacek 1998) where diatom blooms are limited by ambient silica concentrations. In most instances the dominant diatoms were replaced by algae with much smaller or no silicon demands.

Speculation exists regarding whether nitrogen, phosphorus, and silica are the principle growth-limiting nutrients in all marine and freshwater environments as there are

several other micronutrients that may limit increases in biomass. Many systems fail to indicate the presence of P, N, or Si limited primary production.

Recent investigations (Behrenfeld *et al.* 1996, Coale *et al.* 1996, Boyle 1998) reveal that marine waters containing high levels of nitrogen do not always support greater phytoplankton biomass than those with lower N concentrations. These areas, dubbed 'high-nitrate, low chlorophyll' (HNLC), persist in the surface waters of several large regions of the world's oceans, and factors that limit the uptake of growth-restricting nitrogen by certain marine phytoplankton are currently being intensely examined.

Iron has been suggested as the nutrient restricting primary productivity in these HNLC regions (Menzel and Ryther 1961, Price *et al.* 1994, Sandgren *et al.* 1995). It was not until 1996, however, that a massive *in-situ* experiment by Coale *et al.* (the IronExII) provided unequivocal support for the hypothesis that phytoplankton growth in high-nitrogen, low-chlorophyll regions of the ocean is limited by iron. In this study, fertilization with a low concentration of dissolved iron triggered a massive phytoplankton bloom in surface waters of the equatorial Pacific Ocean. The resulting algal bloom consumed much larger amounts of carbon dioxide and nitrate than the microscopic plants typically utilize under natural conditions (Coale *et al.* 1996). Additional studies have supported the results of Coale *et al.* revealing many other HNLC areas of the oceans exhibit iron-limited primary production (Behrenfeld *et al.* 1996, Frost 1996, Rue and Bruland 1997). The roles of iron as an algal growth limiting nutrient in freshwater habitats are less understood.

As described above, phosphorus is considered to be the nutrient which limits freshwater algal growth. However, this generalization is not true for all classes of algae. Using a compilation of among-lake average summer algal biomass' from temperate lakes, Watson *et al.* (1997) demonstrated that there is a significant positive relationship between increasing phosphorus levels and biomass for many algal groups. A key observation in this study was the lack of a significant positive relationship between chrysophyte (golden-brown algae) biomass and total phosphorus beyond extremely low P values. A high amount of scatter was also observed in the average chrysophyte biomass over a range of phosphorus from oligotrophic to hyper-eutrophic conditions, revealing the potential of this group to display high or low biomass irrespective of P levels. The fact that chrysophytes often dominate low-phosphorus systems (Sze 1993, Sandgren *et al.* 1995) coupled with this groups' weak correlation with phosphorus enrichment provides strong support for the hypothesis that these freshwater algae may be frequently limited by a nutrient other than P. Several researchers (e.g. Sandgren *et al.* 1995, Veen 1991, Van Donk 1983) have suggested that iron is a growth-limiting nutrient for chrysophytes.

Iron is one of the most important trace nutrients for phytoplankton as it is a constituent of many enzymes, cytochromes, other porphyrins, and it is involved in chlorophyll biosynthesis (Van Donk *et al.* 1988, Reynolds 1984). Iron is used by the cells primarily as an electron carrier and /or enzyme activator (Falkowski and Raven 1997).

Although iron is not a physical component of the chlorophyll molecule, it serves many vital roles in photosystems I and II and its absence ultimately leads to the inability of the organism to photosynthesize. Sunda and Huntsman (1997) demonstrated that algae cultured in low light environments (50 $\mu mol/m^2 \cdot sec$) contained increased numbers of photosynthetic units and, because iron is a constituent of these units, had higher internal iron concentrations than algae growing at similar rates in high light environments (500 $\mu mol/m^2 \cdot sec$). Low light levels appear to increase iron requirements to maintain optimal metabolism in phytoplankton cells.

Iron availability is not only a function of absolute concentration, but also chemical speciation. Iron may exist organically or inorganically in either the reduced, divalent ferrous (Fe^{2+}) or the oxidized, trivalent ferric (Fe^{3+}) state and algae are known to take up both forms. Research suggests that algal physiology is analogous to the physiology of higher plants and it is believed that algae utilize the reduced ferrous form for metabolic functions (Hopkins 1995, Veen 1991). Iron is poorly soluble in natural waters. In oxygenated waters of neutral or alkaline pH the ferric state dominates, frequently forming ferric-hydroxide complexes that precipitate. This low solubility is one factor contributing to only a minor portion of the iron in water being dissolved and directly available for the algae (Veen 1991).

There are several ways by which the amount of dissolved iron in aquatic systems can be increased thereby increasing the availability to phytoplankton. Chelators are substances with a strong attraction for trace metals and their presence can be beneficial

for algae because chelation alters both the availability and toxicity of micrometals. The chelating ability of natural waters or the production of specific chelating agents for iron (siderophores) by some algal species, including chrysophytes, has been proposed to be important in determining phytoplankton species composition (Sandgren 1988). Humic and fulvic acids ("Gelbstoff") are examples of natural chelators found in aquatic systems and Ethylenediaminetetraacetic acid (EDTA) is a common artificial chelator that is used in many experimental settings and laboratory cultures. The role of iron and organic acids (natural chelators) with respect to chrysophytes is not well understood (Sandgren *et al.* 1995). Existing experimental evidence for absolute iron limitation for this group is inconclusive and the possibility that growth rates are dependent upon specific reactions involving iron, chelates, and the cells themselves has not been examined (Sandgren *et al.* 1995, Entsch *et al.* 1983). Since chrysophytes use only a soluble cytochrome c in electron transfer between cytochrome b_6-*f* and photosystem I, this group of algae will have an increased iron requirement compared to others (Sandgren *et al.* 1995). Table 1.2 is a summary of the sources, roles, and principle forms of select growth-limiting elements present in aquatic systems.

Light also has a great influence on the amount of dissolved iron present in aquatic environments. Photoreduction is a process by which light reduces colloidal Fe^{3+} into Fe^{2+}, which is then released into the surrounding water column in a dissolved form. The process of photoreduction may help to reduce the severity of iron limitation which can result from such processes as metal-hydroxide copulation (Veen 1991).

Table 1.2: Natural sources, roles, and primary forms of major freshwater and marine growth limiting elements.

ELEMENT	*DOMINANT FRESHWATER FORMS*	*FORMS REQUIRED BY ALGAE*	*EXAMPLE OF BIOLOGICAL ROLES*	*MAJOR INPUT SOURCES TO AQUATIC SYSTEMS*
Phosphorus (P)	Organic/Particulate-P	Inorganic Phosphate (PO_4^{3-})	• Genetic material (DNA, RNA) • Cell membranes • Energy source (ATP)	• Precipitation • Surface and groundwater discharge • Atmospheric dust/fallout
Nitrogen (N)	Nitrogen gas (N_2)	Nitrate (NO_3^-) Ammonium (NH_4^+)	• Amino acids and Proteins • Nucleic Acids • Central structure of chlorophyll molecule	• Diffusion from atmosphere • Precipitation • Surface and groundwater discharge
Silicon (Si)	Silica ($SiO_2 \bullet nH_2O$)	Silicic Acid (H_2SiO_4)	• Frustules of diatoms • Scales of some chrysophytes	• Weathering of silica-containing rocks
Iron (Fe)	Iron-hydroxide ($Fe_2OH_3 \bullet 3H_2O$)	Inorganic and chelated Ferrous (Fe^{2+}) and Ferric (Fe^{3+}) compounds	• Cytochromes • Enzyme activators • Electron carriers in photosystems I and II • Nitrogenase enzyme component	• Atmospheric dust • Surface and groundwater discharge • Weathering of clays, soils, and granite rocks

1.5 Species Interactions

To better understand how available resources can influence algal community composition, it is important to consider how species interact with each other. The following is an introduction to how community dynamics may arise from resource competition and resource ratio partitioning.

A species which appears to be the best competitor for one resource is often the worst competitor for another resource, while the competitive abilities of intermediate species are much less developed. Such tradeoffs in resource requirements are required by theory if the mechanism allowing coexistence of species is the specialization of each species on a different proportion of limiting resources (Tilman 1982).

One must address the aforementioned limiting nutrients and resources both collectively and individually when discussing nutrient competition processes and underlying physiological mechanisms. Let us first consider the concept of resource supply ratios and associated relationships among organisms when several resources are present at once.

If it is assumed that an individual species of algae can only be a superior competitor for a small range of resource supply ratios, such as N:P or Light:Fe, it becomes apparent that spatial variation in resource supply ratios can lead to the stable coexistence of many more species than there are resources (Tilman 1982). The maximum population density of a particular species will occur at the resource ratio at which the species is equally limited by all resources. This state is called the 'optimum resource

ratio' (Tilman 1982). Tilman (1982) suggested that many general predictions about resource competition among species in terrestrial and aquatic systems can be made and are based on the presence of this optimum resource ratio. First, given that all species have at least one stable two-species equilibrium point (a point of coexistence), species which differ in their optimum resource ratio for the limiting resources should separate along a resource gradient becoming dominant at different resource levels. Second, natural communities should show a separation among species presence in relation to the ratio of limiting resources if differences in resource requirements is the mechanism allowing the coexistence of numerous species in a particular habitat. Also, resource enrichment experiments performed on natural communities should show different species becoming dominant depending on the pattern of enrichment with the limiting resources; the species which becomes dominant should be the species with the optimum resource ratio closest to which the resources are being supplied.

Studies on resources ratios are important to understanding competitive interactions among species of organisms. However, the effects of interactions of several resources may be complex enough to render the determination of the effects of individual resources impossible. It is for this reason that it is important to consider each resource in isolation.

Determining the role(s) of a specific nutrient or other resources is dependent upon being able to study an organism's response to the manipulation of one independent variable while holding all other independent variables constant. Such procedures are often

used when assessing the physiological function of a specific nutrient in plants (Hopkins 1995, Bonin *et al.* 1981). Effects of having more than a single variable in such a study would lead to results in which the effects of the specific variable being tested may be modified or masked, rendering them difficult to interpret and misleading.

1.6 Structure of Thesis

In this thesis I focus on freshwater systems falling within a narrow range of phosphorus levels, and asking whether changes in iron availability will stimulate changes in phytoplankton community structure.

This thesis is composed of a series of chapters which address the possibility of iron-limited primary productivity and its link with irradiance in freshwater habitats over a range of ecological scales.

Chapter 2 examines the growth of individual algal populations commonly found in low-phosphorus freshwater environments. It focuses on a series of laboratory experiments in which monocultures of representative algae were reared under a range of iron and light levels. These experiments were used to examine whether iron-limited growth could be induced for each of the chosen species. They also provided an opportunity to determine the effects of reduced light levels on the iron requirements of the phytoplankton. Isolating the individual populations allowed for the growth of autotrophs and heterotrophs to be compared in the absence of any among species interactions. The experimental results provided information from which predictions could

be made about the outcome of competition of these species when individual populations are combined in the same environment. Chapter 2 examines these predictions using experiments with mixed phytoplankton populations subjected to identical iron and irradiance levels as the isolated populations. The outcomes of competition were compared with the previously assembled predictions. These laboratory community competition experiments provided a means to develop yet another set of predictions regarding the outcomes of whole phytoplankton community iron and light manipulation field experiments.

Chapter 3 investigates the possibilities and difficulties of attempting to apply the results of tightly controlled laboratory experiments to a more natural (and therefore more dynamic) field environment. Here the results of a series of field mesocosm experiments performed over an entire growing season are compared with those from the lab cultures. These experiments closely mimic those which successfully identified iron as a nutrient limiting primary productivity in various marine planktonic environments and provide the greatest opportunity to investigate the effects of fluctuating iron and light levels in natural habitats.

Chapter 2: Laboratory Culture Experiments

2.1 Investigation of Iron and Light Limited Growth of a Single Autotrophic and Two Heterotrophic Freshwater Algae

Recent studies (i.e. Coale *et al.* 1996, Behrenfeld *et al.* 1996, Frost 1996, Rue and Bruland 1997) have demonstrated that primary productivity is limited by iron in several regions of the world's oceans and seas. While nitrogen is considered to be the nutrient which most often limits marine phytoplankton growth (Sze 1993), areas of high nitrogen and low chlorophyll (HNLC) persist in marine environments suggesting that algal growth in these regions is limited by a different nutrient. By triggering a massive phytoplankton bloom in a HNLC region of the Pacific Ocean with a large-scale iron fertilization, Coale *et al.* (1996) provided unequivocal evidence that phytoplankton growth in these areas was limited by iron availability.

The potential roles of iron as an algal growth limiting nutrient in freshwater habitats are less understood. Phosphorus is considered to be the nutrient which limits freshwater algal growth (Dillion and Rigler 1974, Sakamoto 1966, Schindler 1978). However, this generalization may not be true for all classes of algae. Using a compilation of among-lake average summer algal biomass' from temperate lakes, Watson *et al.* (1997) demonstrated a significant positive relationship between phosphorus levels and biomass for many algal groups (e.g. blue-green algae). Key observations in this study were the lack of a significant positive relationship between chrysophyte (golden-brown algae) biomass and total phosphorus beyond extremely low P

values, and a high amount of scatter in the average chrysophyte biomass at any given level of P; thus revealing that chrysophytes can display high or low biomass regardless of phosphorus. The fact that chrysophytes often dominate low-phosphorus systems (Sze 1993, Sandgren *et al.* 1995) coupled with this groups' weak correlation with phosphorus enrichment suggests that the observed biomass of these freshwater algae may be influenced by nutrients other than P. Several researchers (e.g. Sandgren *et al.* 1995, Veen 1991, Van Donk 1983) have suggested that iron is a growth-limiting nutrient for chrysophytes and Sandgren *et al.* (1995) proposed that this group of algae may be superior competitors for iron in certain nutrient limited environments.

Iron is one of the most important trace nutrients required by phytoplankton as it is a constituent of many enzymes, cytochromes, other porphyrins, and it is involved in chlorophyll biosynthesis (Van Donk *et al.* 1988, Reynolds 1984). It is primarily used by the cells as an electron carrier and /or enzyme activator (Falkowski and Raven 1997). While iron is not a physical component of the chlorophyll molecule, it serves many vital roles in photosystems I and II and its absence ultimately leads to the inability of the organism to photosynthesize. Sunda and Huntsman (1997) demonstrated that algae cultured in low light environments (50 $\mu mol \cdot m^{-2} \cdot sec^{-1}$) contained larger and/or increased numbers of photosynthetic units and, because iron is a constituent of these units, had higher internal iron concentrations than algae growing at similar rates in high light environments (500 $\mu mol \cdot m^{-2} \cdot sec^{-1}$). Low light levels appear to increase iron requirements to maintain optimal metabolism in phytoplankton cells.

Given that phosphorus may not regulate the growth of all freshwater algae and that iron-limited primary productivity can persist in natural systems, some interesting hypotheses emerge concerning phytoplankton community composition in habitats with similar phosphorus levels.

Several freshwater systems, such as the city of Calgary's Glenmore Reservoir (Canada), have experienced spring and fall blooms of chrysophyte algae despite low relatively stable phosphorus concentrations. This apparent non-phosphorus limited growth begs for an answer to the question of what nutrient is limiting increases in algal populations in such environments.

Uroglena americana and *Dinobryon cylindricum* are two chrysophytes that bloom periodically in Glenmore Reservoir (Dixon *et al.* 1993, Watson *et al.* 1995). Diatoms (e.g. *Synedra*) dominate years when Glenmore Reservoir does not support chrysophyte blooms. *Uroglena* and *Dinobryon* have the ability to utilize organic compounds as energy sources and are able to meet their nutritional demands by ingesting organic compounds and by carrying out photosynthesis. These two chrysophytes are phagotrophs and have the ability to feed on bacteria, providing yet another means of fueling their dietary demands (Sze 1993). In contrast, diatoms are strict autotrophs and depend entirely on light and inorganic nutrients for growth. The ability of mixotrophs to employ alternative methods of nutrient acquisition suggests that they may be superior competitors to autotrophs in low-nutrient environments. If growth of the two chrysophytes (*Uroglena* and *Dinobryon*)

becomes limited by ambient iron concentrations, they should have the ability to supplement their diet by obtaining Fe from organic sources (i.e. bacteria).

The purposes of this study were to (i) examine and compare the growth of *Uroglena*, *Dinobryon*, and *Synedra* in the presence and absence of iron additions under conditions of low and high light, (ii) investigate the effects of reduced light levels on the iron requirements of these three algae, and to (iii) compare the interaction of autotrophs and mixotrophs at three levels of iron in low and high light environments.

We hypothesize that the reduction of iron will lead to decreased growth rates and maximum biomass of all species of algae regardless of light level, and the iron requirements of phytoplankton will be elevated in low light environments. It is predicted that all species of algae will experience reduced growth in the absence of iron. The growth of mixotrophic algae should be less affected than that of the autotrophs because of the ability of mixotrophs to utilize bacteria to supplement their physiological energy demands. Thus, we expect that the growth of the mixotrophs will be negatively affected by the reduction in iron to a lesser degree than for *Synedra*.

Diatoms frequently dominate spring and fall phytoplankton blooms in temperate regions (Sze 1993). The nutrient pools within aquatic environments of such regions increase by elevated runoff and the loss of thermal stratification which recirculates nutrient-rich hypolimnetic waters. The abundance of diatoms during such periods suggests that they are superior competitors when nutrient pools (including silicon, phosphorus, nitrogen, and iron) are elevated, and light and temperatures are relatively

low. Based on this information it is hypothesized that *Synedra* will be a superior competitor in environments containing iron in non-growth limiting quantities. Table 2.1 summarizes these predictions.

To evaluate these hypotheses, populations of each species were reared under a combination of light and iron levels. We used μ of unialgal cultures to derive expectations for competitive outcomes among the three different species. A set of mixed population experiments were used to test these predictions and assess the competitive abilities of these algae in a series of iron additions in low and high light habitats

Table 2.1: Predicted species achieving highest biomass yield under various light and iron environments

	Light	
	Low (50 $\mu mol \bullet m^{-2} \bullet sec^{-1}$)	High (550 $\mu mol \bullet m^{-2} \bullet sec^{-1}$)
	Dinobryon or Uroglena	Dinobryon or Uroglena
	Dinobryon or Uroglena	Synedra
	Synedra	Synedra

2.2 Methodology of Laboratory Culture Experiments

2.2.1 Algal Isolations and Cultures

Cultures of *Uroglena americana, Dinobryon cylindricum,* and *Synedra delicatissima* were established using isolates from the Glenmore Reservoir (Calgary, Canada). The cultures were reared in a 150 nM iron algal growth medium (see table 2.2) within 500 ml Erlenmeyer flasks at 16°C under a 14:10 light:dark cycle with illumination levels of ~ 250 $\mu mol \cdot m^{-2} \cdot sec^{-1}$ using 40 Watt (H768) Sylvania Grolux wide-spectrum fluorescent tubes.

2.2.2 Algal Growth Media

Recipes for three different algal growth media (Table 2.2) differed by their iron concentrations and will be referred to according to the level of iron which they contained. The iron-free, or zero iron, medium did not receive any iron (0 nM Fe). The low iron medium received an iron addition of 15 nM, and the high iron medium was fertilized with 150 nM of iron. Iron was added to the low and the high iron media in a pre-chelated form to reduce the amount of precipitation and leaching of the metal to the inner flask surface.

2.2.3 Individual Species Growth Experiments

Figure 2.1 shows the flask (500 ml) design used for the batch culture experiments. The inner glass surfaces were coated with silicon using Surfasil™ Siliconizing Fluid to reduce the adhering of metals to the inner walls. 250 ml of either 0 nM Fe, 15 nM Fe, or 150 nM Fe medium was then added to the flask.

Algal cells from the primary cultures were concentrated using gravity filtration on Whatman GF/F glass-fiber filters, rinsed 3 times with 0 nM Fe medium, and then separately transferred to a 0 nM Fe medium for 7 days (10 days in second experiment) to deplete internal cell iron reserves and generate a supply of 0 nM Fe acclimated algae to be used in the experiments. At the end of the acclimation period 3 ml samples from each of the 0 nM Fe acclimated (secondary) stock cultures were withdrawn and preserved in Lugol's Iodine solution (45 g/L I_2, 90 g/L KI, 9% glacial acetic acid). 1 ml of these samples was settled in algal counting chambers and ~ 400 cells were enumerated using a Nikon inverted microscope. The biomass (µg carbon/ml) of each stock culture was calculated from the cell counts (using biomass/cell count regressions) and an equivalent biomass of each algal species was separately added to the growth chambers containing either 0 nM Fe (none) medium, 15 nM Fe (low) medium, or 150 nM Fe (high) medium. There were three replicate flasks per treatment.

Experimental cultures of each species were grown at 16°C under a 14:10 light:dark cycle at growth irradiances (Photosynthetically Active Radiation – PAR) of 550 $\mu mol \cdot m^{-2} \cdot sec^{-1}$ (high light, HL) (for experiments 1 and 2), and 55 $\mu mol \cdot m^{-2} \cdot sec^{-1}$ (low light, LL) (experiment 2 only) supplied by 40 Watt (H768) Sylvania Grolux wide-spectrum fluorescent tubes. LL environments were created by shading lights with a neutral-density screening. Cultures (3 ml) were sampled every 2 days. Cells were preserved, enumerated, and the biomass of each population calculated for each sample date. The experiment was continued until populations displayed constant cell densities for

at least 4 days (i.e. equilibrium state). Experiments lasted between 26 and 32 days. Growth curves were plotted for each species and treatment.

Experimental growth rates were measured during the logistic growth phase by calculating the slope of the regression between the natural logarithm population density and time over the appropriate period. Growth rates were compared using an Analysis of Variance (ANOVA).

The maximum biomass achieved over the duration of the study was a second response examined and was also compared using ANOVA . When using maximum biomass as the dependent variable the values were manually read off of the growth plots. For cultures achieving a state of equilibrium , the value used in the ANOVA was the average maximum biomass over the duration of the equilibrium. Else, the greatest biomass achieved by the end of the experiment was the dependent variable (i.e. cultures still in the exponential growth phase at the end of the experiment)

2.2.4 Mixed Populations Growth Competition Experiments

The mixed population competition experiments were performed in identical environments and in the same manner as the individual species growth experiments (i.e. primary and secondary stocks). However, in this experiment the species were not separately added to individual flasks, rather an identical biomass of each species was combined within one vessel. There were 3 replicate flasks per treatment.

2.2.5 Iron

Sample preparation; 30 ml samples were taken from each culture and preserved by adding 0.15 ml if 1:1 nitric acid on the final day of the experiment. All samples were stored in the refrigerator prior to analysis.

Sample analysis; Final total iron concentrations were measured using a graphite furnace atomic adsorption (A.A.) spectrophotometer (Perkin-Elmer Model 5000). Repeated standard checks indicated that our minimal level of detection with this instrument was 5 p.p.b.. Operational methods for the graphite furnace A.A. spectrophotometer were derived from the book of Standard Methods For The Examination of Water and Wastewater (A.P.H.A., A.W.W.A., W.P.C.F., 1996). Quality control procedures included frequent iron spikes of samples and calibration of the machine with iron solutions of a known concentration. Initially this procedure was performed using distilled water and 5 iron standards to formulate a standard curve from which the total iron concentrations in the experimental samples could be derived.

Table 2.2: Composition of algal growth medium (pH = 7.5). Note change in iron concentrations depending upon desired iron molarities in medium.

ELEMENT	COMPOUND ELEMENT ADDED AS	FINAL MOLARITY IN MEDIUM
Macronutrients		
Ca	$CaCl_2 \cdot 2H_2O$	2.001×10^{-04}
Mg	$MgSO_4 \cdot 7H_2O$	1.995×10^{-04}
N	$NaNO_3$	5.000×10^{-05}
N	NH_4Cl	4.919×10^{-05}
K	KCl	1.402×10^{-05}
P	KH_2PO_4	5.000×10^{-06}
B	H_3BO_3	1.294×10^{-05}
Si	$Na_2SiO_3 \cdot 9H_2O$	9.993×10^{-06}
Buffer		
HEPES	$C_8H_{17}N_2O_4SNa$	9.604×10^{-04}
EDTA Trace Metals Solution (iron free)		
EDTA	$Na_2EDTA \cdot 2H_2O$	2.501×10^{-07}
Co	$CoCl_2 \cdot 6H_2O$	5.044×10^{-09}
Mn	$MnCl_2 \cdot 4H_2O$	4.649×10^{-08}
Mo	$Na_2MoO_4 \cdot 2H_2O$	1.207×10^{-08}
Zn	$ZnSO_4 \cdot H_2O$	7.947×10^{-09}
V	Na_2VO_4	2.486×10^{-09}
Se	H_2SeO_3	2.016×10^{-08}
Cu	$CuSo_4 \cdot 5H_2O$	4.886×10^{-10}
EDTA Iron Solution		
EDTA	$Na_2EDTA \cdot 2H_2O$	2.501×10^{-07}
Fe	$FeCl_3 \cdot 6H_2O$	(high iron) 1.500×10^{-07} (low iron) 1.500×10^{-08} (no iron) 0.000
Vitamins		Final Concentration ($\mu g \cdot L^{-1}$)
Biotin	$C_{10}H_{16}N_2O_3S$	0.0005
B12	$C_{63}H_{88}CoN_{14}O_{14}P$	0.0005
Thiamin HCl	$C_{12}H_{17}Cl_2N_4OS \cdot HCl$	0.1000

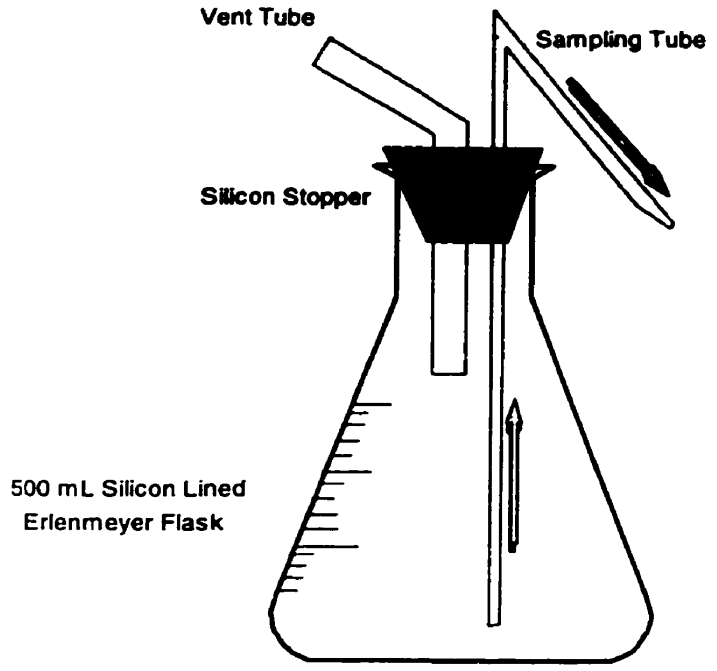

Figure 2.1: Illustration of algal growth chamber used to culture alage in individual species and mixed populations growth competition experiments.

2.3 Results of Individual Populations Laboratory Culture Experiments

The experimental results are presented in a sequence which follows the order which the major hypotheses were tested. The individual species studies focused on the role of iron as a phytoplankton growth limiting nutrient and are addressed in they same order they were performed. The initial batch culture experiment examined the effects of three levels of iron addition on the growth of *Synedra*, *Uroglena*, and *Dinobryon* under a single (high) level of light. The second experiment also tested the effect(s) of three different iron enrichments on the growth of these algal species. However, in the second trial, the impact of light level on the iron requirements and growth of the three phytoplankton species was assessed by introducing a second (low) light environment. The results of the single species studies were used to generate predictions regarding the outcome of competition between *Synedra*, *Uroglena*, and *Dinobryon* in similar experimental conditions (see Table 2.5). These predictions were tested via a set of low and high light mixed population experiments, the results of which are the last to be presented in this section.

2.3.1 Iron

Iron concentrations in all cultures were below the detection limit of the graphite furnace atomic adsorption spectrophotometer for each of the experimental trials.

2.3.2 Individual Species Growth Experiment 1

Synedra average growth rate and average maximum biomass were greater in cultures enriched with iron under high light (Fig. 2.5 a&d). The growth rates of this species increased from 0.267, to 0.360, and to 0.417 day^{-1} upon 0 nM, 15 nM, and 150 nM Fe additions, respectively. Low and high iron fertilization resulted in a similar algal crop yield of 1.67 $\mu g \cdot ml^{-1}$ and were significantly greater than the biomass of 1.24 $\mu g \cdot ml^{-1}$ achieved in an iron free medium (ANOVA, Tukey's test, $P<0.05$).

Under high light, 15 nM and 150 nM iron additions resulted in similar increases of *Dinobryon* biomass (Fig. 2.7 a). Cultures reared in low and high iron media exhibited a greater biomass (8.01 and 7.07 $\mu g \cdot ml^{-1}$ respectively) than those with no iron added (2.71 $\mu g \cdot ml^{-1}$) (ANOVA, Turkey's test, $p<0.05$). Elevated iron levels did not stimulate increased growth rates of *Dinobryon* (Fig. 2.7 b). Growth rates were actually higher in the zero addition treatment than the iron enriched media (ANOVA, Tukey's test, $P<0.05$).

Six of nine *Uroglena* replicates became infected with an unidentified species of amoebae resulting in extinction of algae within these cultures. Non-invaded cultures achieved similar growth rates and biomass' (Fig. 2.6 a&d). The growth patterns of surviving *Uroglena* replicates were similar to *Dinobryon*, the other mixotroph at the same level of iron (Fig. 2.2 d-i). When the growth of *Synedra*, *Dinobryon*, and surviving *Uroglena* replicates under high light was compared (Fig. 2.8 a&d), the mixotrophs achieved a significantly greater biomass than the autotroph at each level of iron enrichment (0 nM Fe; $P<0.01$, 15 nM Fe; $P<0.001$, 150 nM Fe; $P<0.001$) (Fig. 2.8 a). The

magnitude of difference between growth rates of these two algal groups varied depending on the level of iron enrichment (Fig. 2.8 d). Without any iron addition the average mixotroph growth rate (0.522 day^{-1}) was significantly greater than that of the autotroph (0.268 day^{-1}) ($P<0.001$). In the low iron medium the growth rates of the two groups were more similar (autotroph; 0.361 day^{-1}, mixotrophs; 0.471 day^{-1}), yet significantly different ($P<0.05$). With 150 nM iron fertilization there was no significant difference between the average autotroph growth rate (0.417 day^{-1}) and that of the mixotrophs (0.462 day^{-1}). The mixotrophs maintained a similar rate of growth across all level of iron, while the autotroph experienced faster growth with each increase in added iron.

2.3.3 Individual Species Growth Experiment 2

Synedra growth was positively correlated to iron additions in both high and low light environments (Fig. 2.5 b, c, e, f). Under high light, both the average growth rate and average maximum biomass attained were greater in cultures fertilized with iron. (Fig. 2.5 b&e). High light growth rates (Fig. 2.5 e) of 15 nM and 150 nM Fe fertilized cultures were 0.285 and 0.449 day^{-1} respectively, and these were both significantly faster than those replicates not Fe enriched ($P<0.003$). The average maximum biomass of *Synedra* in high light was lower when iron was not added (0.10 $\mu g \cdot ml^{-1}$) compared to enriched cultures (15 nM Fe:1.49 $\mu g \cdot ml^{-1}$; 150 nM Fe: 1.18 $\mu g \cdot ml^{-1}$; $P<0.001$), but the yields in the two enriched treatments did not differ significantly ($P<0.30$).

In contrast, under low light conditions, a low iron addition of 15 nM did not stimulate an increased growth rate or biomass of *Synedra* (Fig. 2.5 c&f). Faster growth

and increased biomass were only observed in the replicates which were fertilized with 150 nM Fe when light levels were reduced. The growth rate and yield of the 0 nM Fe and 15 nM Fe replicates were not different from each other ($P<0.80$, and $P<0.70$, respectively).

Differences between the maximum biomass and growth rate of *Synedra* under high and low light were not significant at iron enrichment levels of 0 nM and 150 nM. In cultures fertilized with 15 nM Fe, the maximum biomass and rate of growth of this diatom were greater under high light.

None of the three iron additions increased *Uroglena* biomass or growth rates under high or low light (Fig. 2.6 b, c, e, f). The biomass of *Uroglena* was similar at all levels of light and iron, and the growth rates of this alga were almost identical across all treatments.

At the same level of iron, *Uroglena* growth rates were not different under high and low light. In 150 nM Fe cultures the biomass achieved under low light was significantly greater ($P<0.03$) than that at high light and the maximum biomass of the replicates fertilized with 0 nM Fe and 15 nM Fe were not different regardless of light level ($P<0.17$ and $P<0.77$ respectively).

In experiment 2, there was a significant effect of iron enrichment on the biomass of *Dinobryon* under low and high light (Fig. 2.7 b&c). An iron fertilization of 15 nM led to increased biomass under low light only, while raising the iron level to 150 nM increased algal yield in both light environments. The addition of iron did not stimulate

faster growth rates under low light (Fig. 2.7 f) and the growth remained at the same rate across all iron levels. There was an increase in the average *Dinobryon* growth rate following the addition of 150 nM of iron under high light. The rate of growth of this alga reached 0.449 day^{-1} in the high iron/high light environment and was significantly greater than those recorded at 15 nM Fe (0.296 day^{-1}, $P<0.01$) and 0 nM Fe (0.333 day^{-1}, $P<0.04$). When the cultures grown in increased iron (15 nM Fe and 150 nM Fe) were combined and compared to those without (0 nM Fe) the effect of iron enrichment was not significant ($P<0.4$), i.e. growth rates were not lower in the absent of iron enrichment.

The average maximum biomass' of *Dinobryon* cultures in experiment 2 were greater under low light at each iron level. Low light conditions also supported faster growth in 0 nM Fe and 15 nM Fe media. Growth rates remained similar in high or low light following the addition of 150 nM Fe.

Combining the responses of the two mixotrophs (*Dinobryon* and *Uroglena*) and comparing them to the autotroph *Synedra* illustrates how differences in methods of energy acquisition (i.e. trophy) affects growth and yield under different environmental conditions. Under high light the average mixotroph biomass (1.89 $\mu g \cdot ml^{-1}$) was greater than the autotroph biomass (0.10 $\mu g \cdot ml^{-1}$) in the iron free medium ($P<0.04$) (Fig. 2.8 b). Iron enrichment at either the 15 nM or 150 nM level led to an increased biomass of the autotroph, and not the mixotrophs. Thus, the difference in biomass the two groups was not significantly different at these iron levels because of increasing autotroph abundance and not decreasing mixotroph biomass. When light was reduced (Fig. 2.8 c) the

mixotroph cultures yielded a significantly greater biomass regardless of iron ($P<0.001$ at all three iron levels). Under high and low light the amounts by which the growth rates differed varied depending on the level of iron enrichment (Fig. 2.8 e-f). 0 nM Fe addition resulted in the mixotrophs achieving significantly higher growth rates regardless of light ($P<0.001$ for both light levels). Under high light only, 15 nM and 150 nM iron fertilization's triggered an increase in autotroph growth rates (but not in the mixotrophs) such that these two trophic groups were able to grow at similar rates in media which had iron added. An iron fertilization of 15 nM Fe did not increase growth rates from those observed at 0 nM Fe under low light and the mixotrophs grew at a significantly faster rate than the autotrophs ($P<0.001$). Under low light and in a 150 nM Fe enriched medium the autotrophs and mixotrophs had similar growth rates.

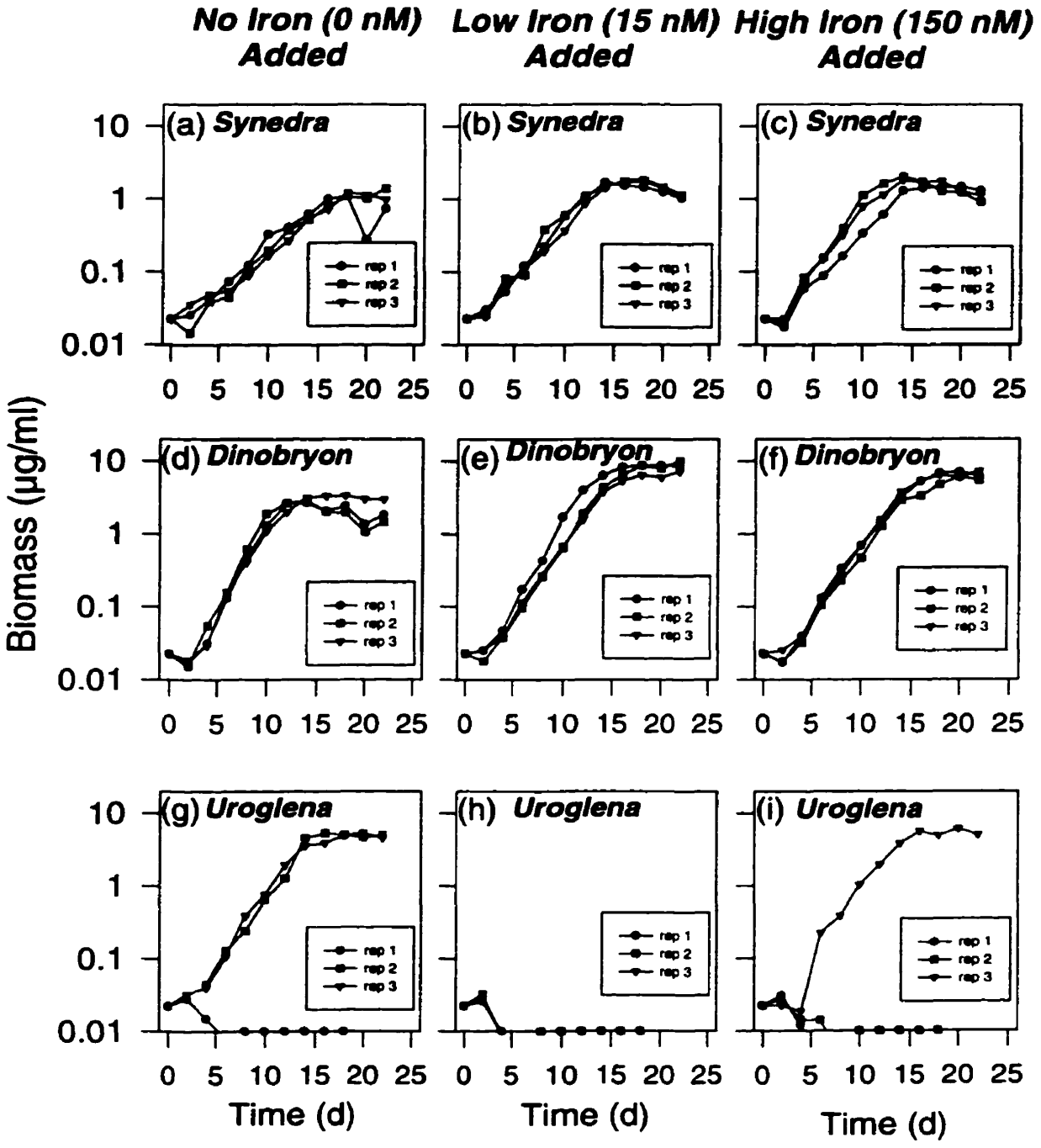

Figure 2.2: Experiment 1 growth plots of *Synedra*, *Dinobryon*, and *Uroglena* reared in three levels of iron addition (0, 15, and 150 nM) under high light

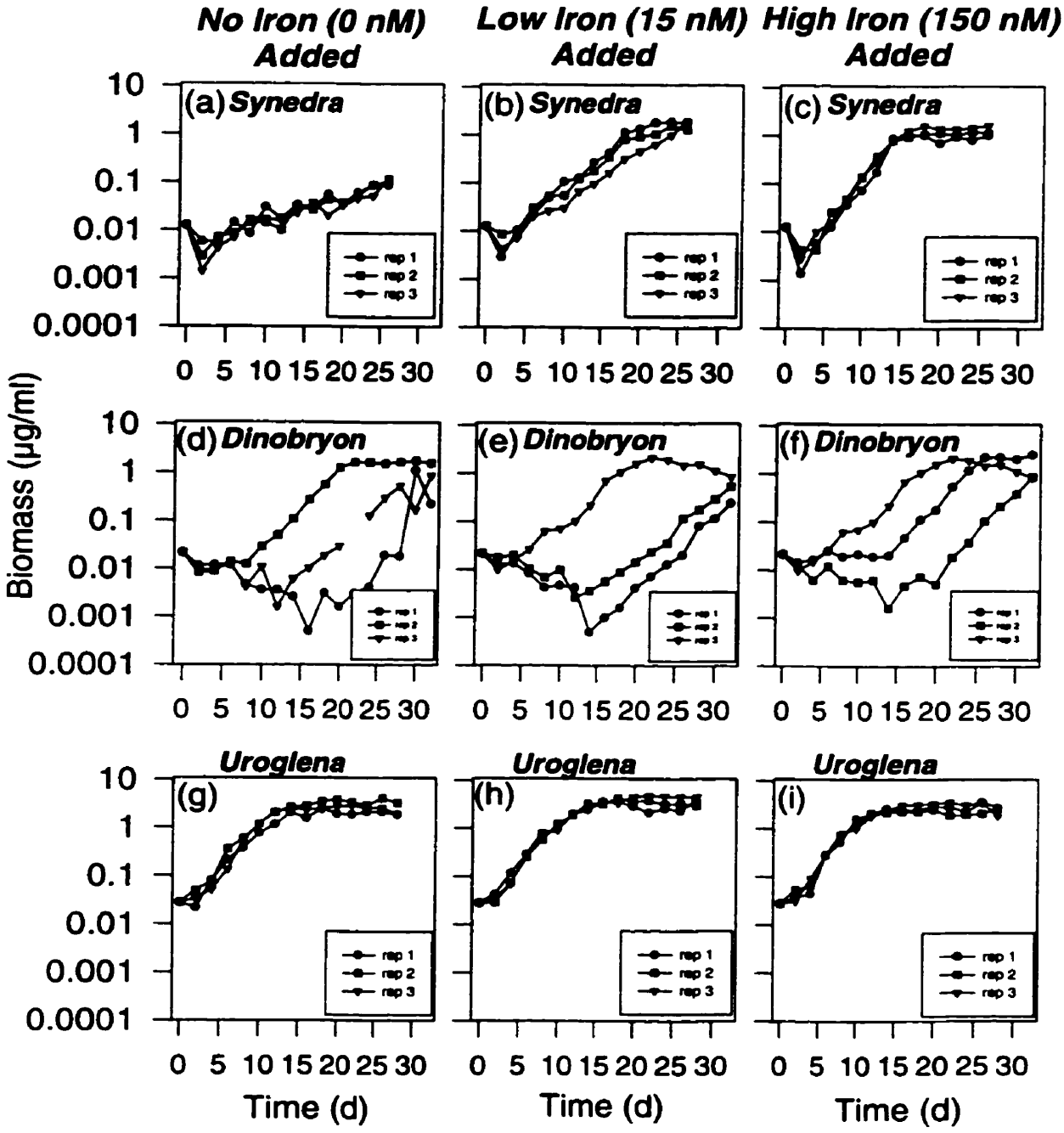

Figure 2.3: Experiment 2 growth plots of *Synedra*, *Dinobryon*, and *Uroglena* reared in three levels of iron addition (0, 15, and 150 nM) under high light

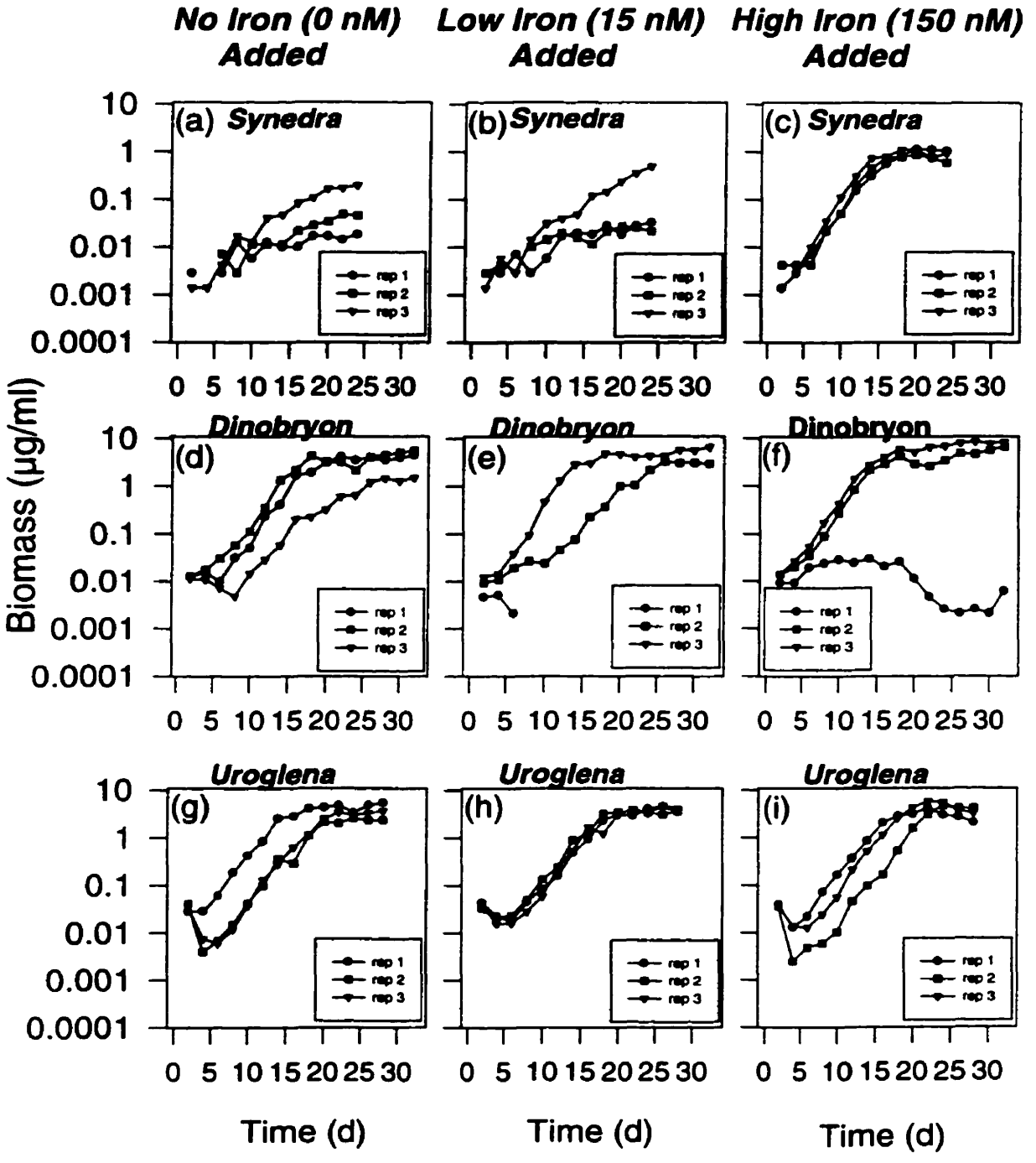

Figure 2.4: Experiment 2 growth plots of *Synedra*, *Dinobryon*, and *Uroglena* reared in three levels of iron addition (0, 15, and 150 nM) under low light

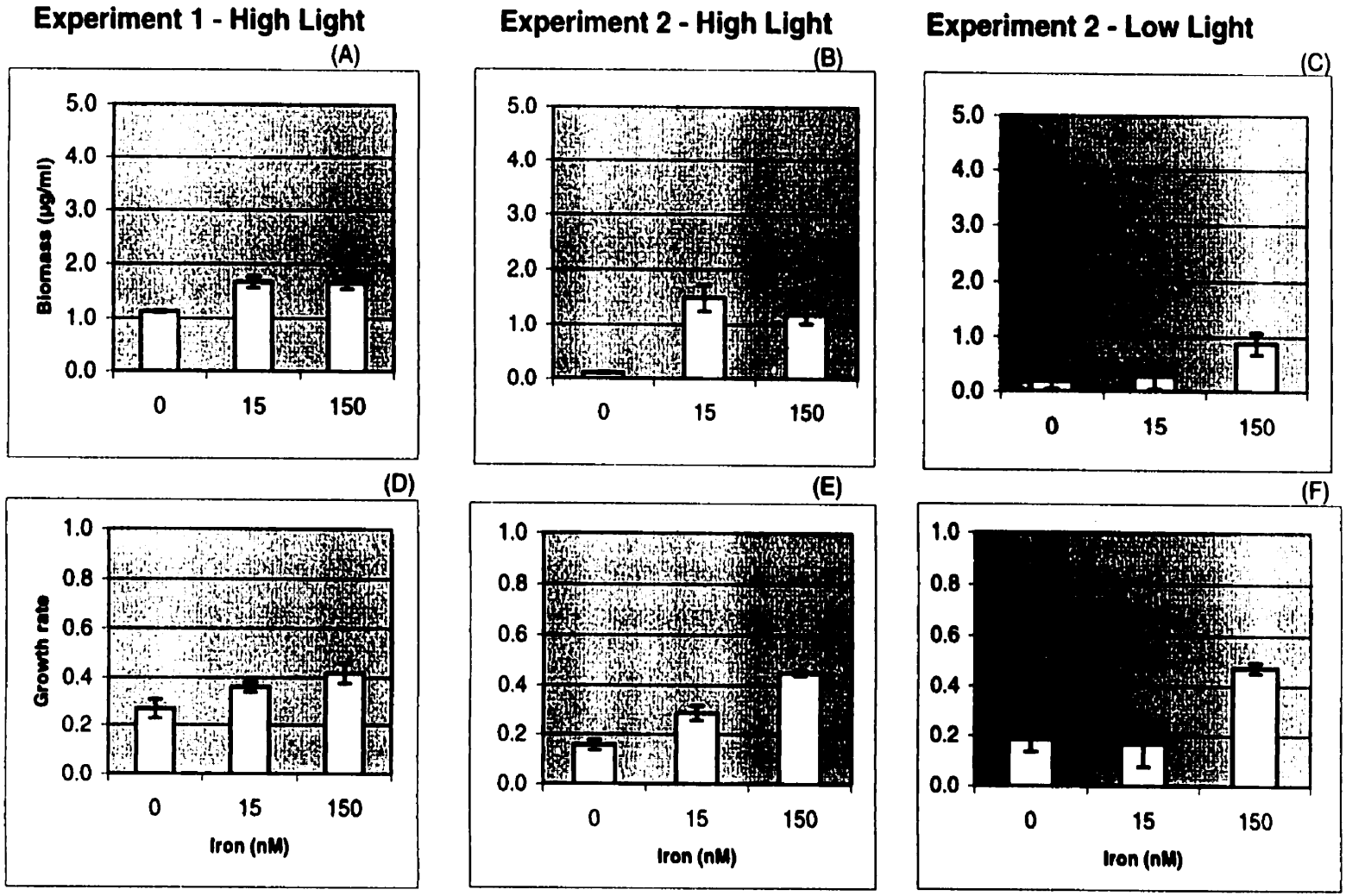

Figure 2.5: Average maximum biomass (+/- 1 SE) and average maximum growth rates (+/- 1 SE) of *Synedra* in three iron additions under two light levels for each experiment

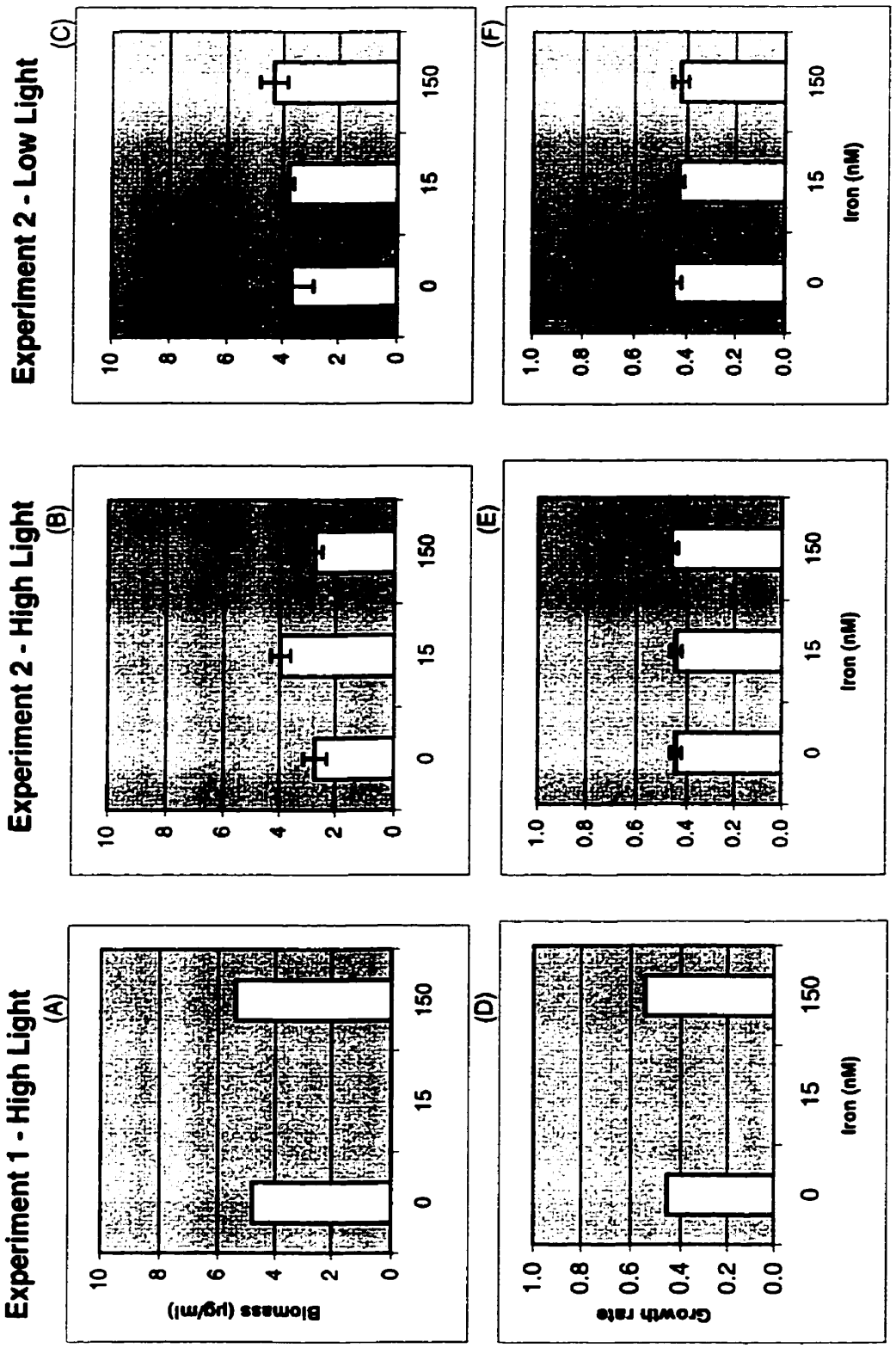

Figure 2.6: Average maximum biomass (+/- 1 SE) and average maximum growth rates (+/- 1 SE) of *Uroglena* in three iron additions under two light levels for each experiment

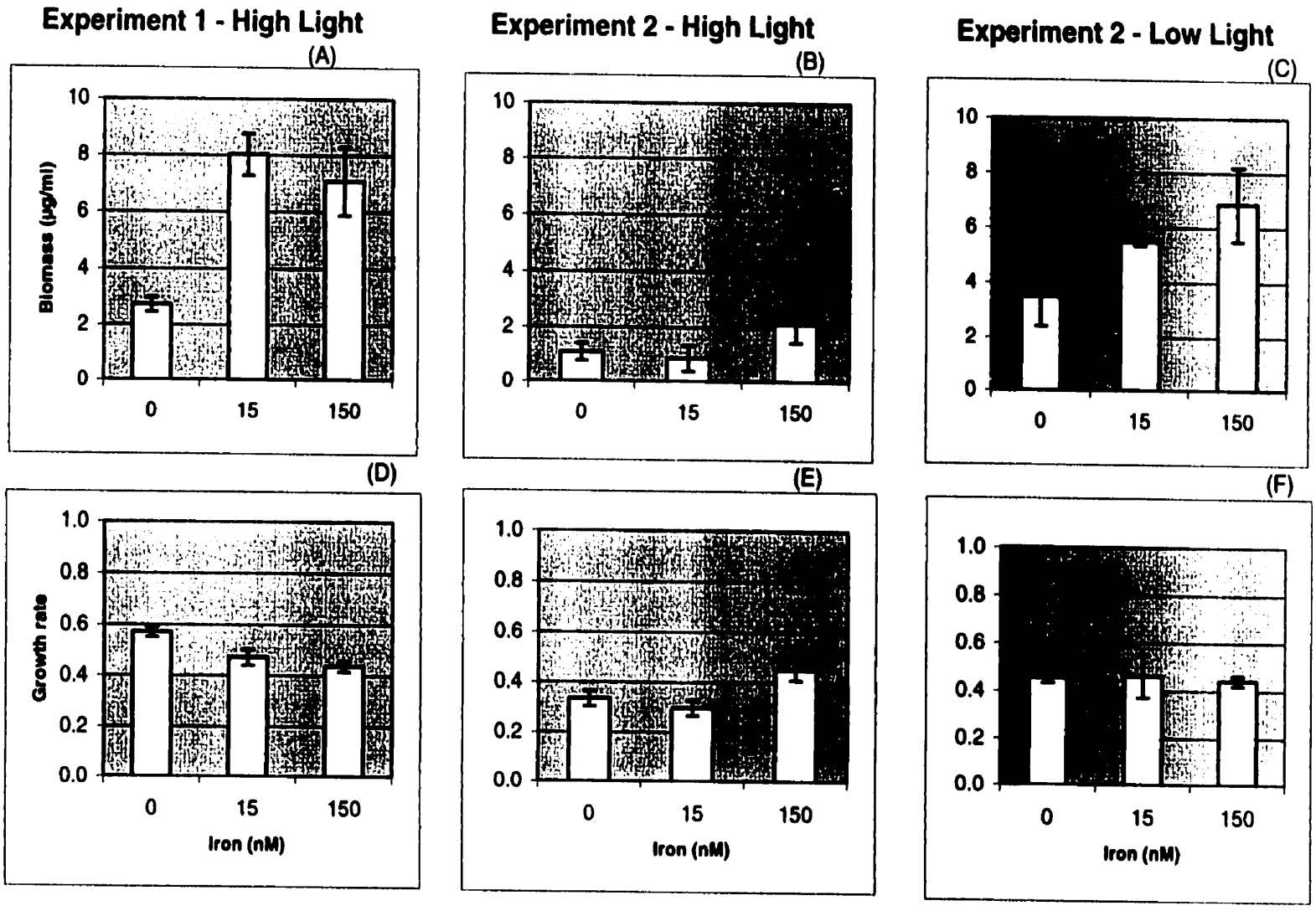

Figure 2.7: Average maximum biomass (+/- 1 SE) and average maximum growth rates (+/- 1 SE) of *Dinobryon* in three iron additions under two light levels for each experiment

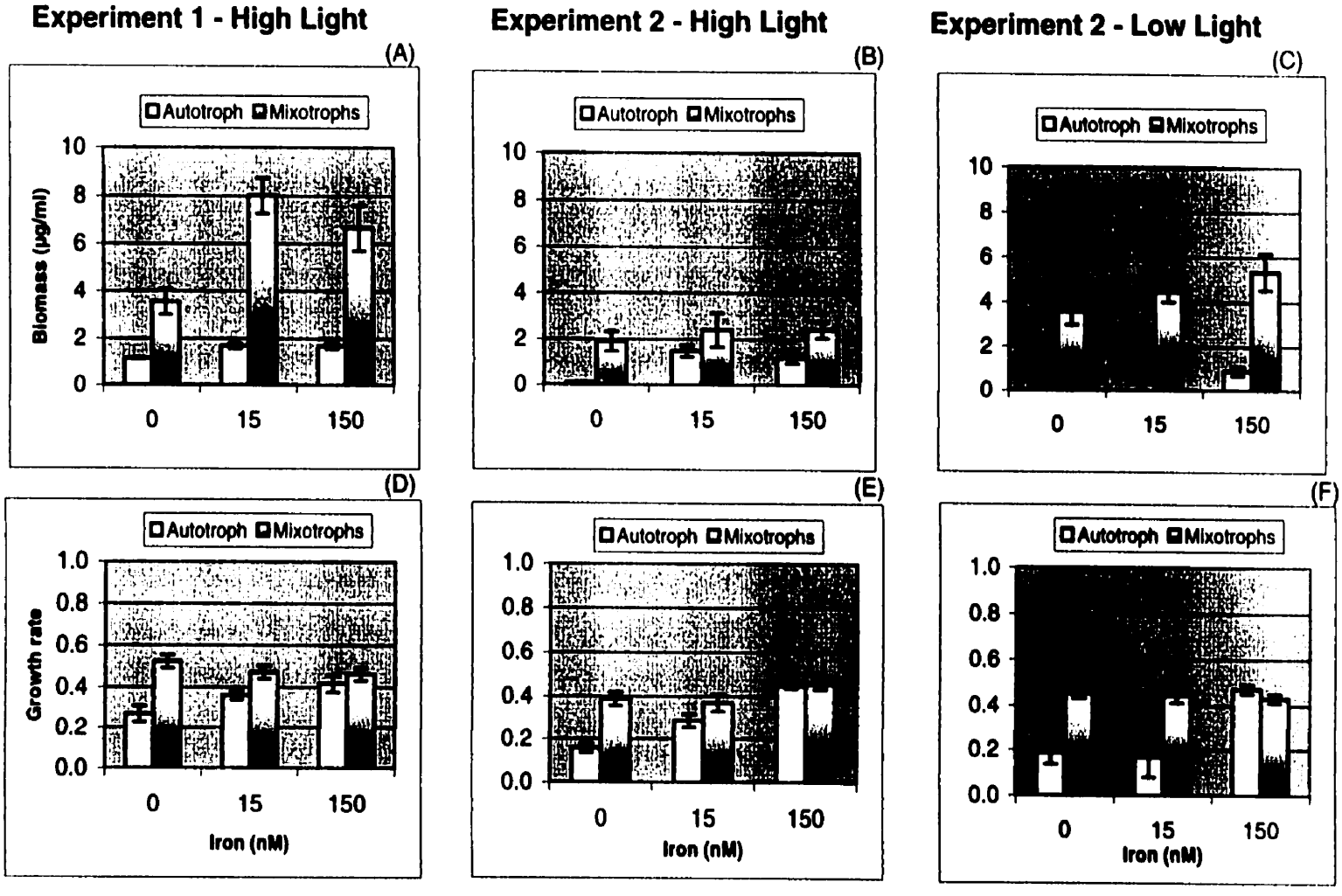

Figure 2.8: Average maximum biomass (+/- 1 SE) and average maximum growth rates (+/- 1 SE) of autotroph and two mixotrophs in three iron additions under two light levels for each experiment

2.4 Results of Laboratory Mixed Populations Competition Experiment

2.4.1 Major Outcomes

The growth curves of each algal species from mixed populations competition experiments reared under high and low light are shown in Figures 2.9 and 2.10.

Under high light, cultures with either no iron added or low levels of iron fertilization were dominated by mixotrophic algae (Fig. 2.12 a-f), and *Uroglena* achieved the greatest biomass in these communities by the time the experiment was terminated. Under conditions of high light and high iron enrichment the algal assemblages were co-dominated by the autotroph (*Synedra*) and one of the two mixotrophs (*Uroglena*). On average, the autotroph established a greater representation in the low-light mixed population cultures than those in high light.

2.4.2 Species Trends

The average maximum biomass of *Synedra* was positively correlated with iron enrichment levels (Fig. 2.11 a-c), and the proportional abundance of this diatom increased with increasing iron additions under high light. The equilibrium biomass of *Synedra* (Fig. 2.12 a-c) represented approximately 10% of the total community biomass in those flasks not fertilized with iron. Mixed communities enriched with 150 nM Fe consisted of approximately 50% *Synedra* at the end of the logistic growth phase (Fig. 2.12 g-i).

The average maximum biomass of *Dinobryon* remained relatively low across all iron levels and did not increase with elevated iron concentrations (Fig. 2.11 a-c). The proportion of *Dinobryon* present decreased with increasing iron additions (Fig. 2.12).

This alga was eliminated from all 150 nM replicates prior to the completion of the experiment.

Uroglena achieved a similar maximum biomass irrespective of iron addition (Fig. 2.11 a-c), and it was the dominant or co-dominant species in all the high light communities (Fig. 2.12). No trends relating total community algal biomass to iron levels are apparent in Figure 2.12.

Observed trends in low light (Fig. 2.15) were the same as those in high light (Fig. 2.12) such that cultures enriched with low iron or no iron were dominated by mixotrophs and those cultures with high levels of iron enrichment yielded algal communities with a greater representation by the autotroph. An increase in *Synedra* biomass was stimulated upon addition of 150 nM Fe only (Fig. 2.14 a-c). Under low light and high iron *Synedra* was the dominant species in two of three replicates (Fig. 2.15 g-i). The high iron/low light flask not dominated by *Synedra* contained similar amounts of the autotroph as the other two replicates. However, this vessel supported a large *Uroglena* population not seen in the other two replicates and this greatly reduced the proportional representation of *Synedra* (Fig. 2.15g).

Mixotroph biomass' were highly variable at each level of iron addition under low light (Fig. 2.14 a-c). No relationship between *Dinobryon* and *Uroglena* biomass and iron enrichment under low light was evident.

The total algal biomass in all replicates under low light was independent of iron concentration. No trends relating total algal community biomass to iron enrichment levels are apparent in Figure 2.16.

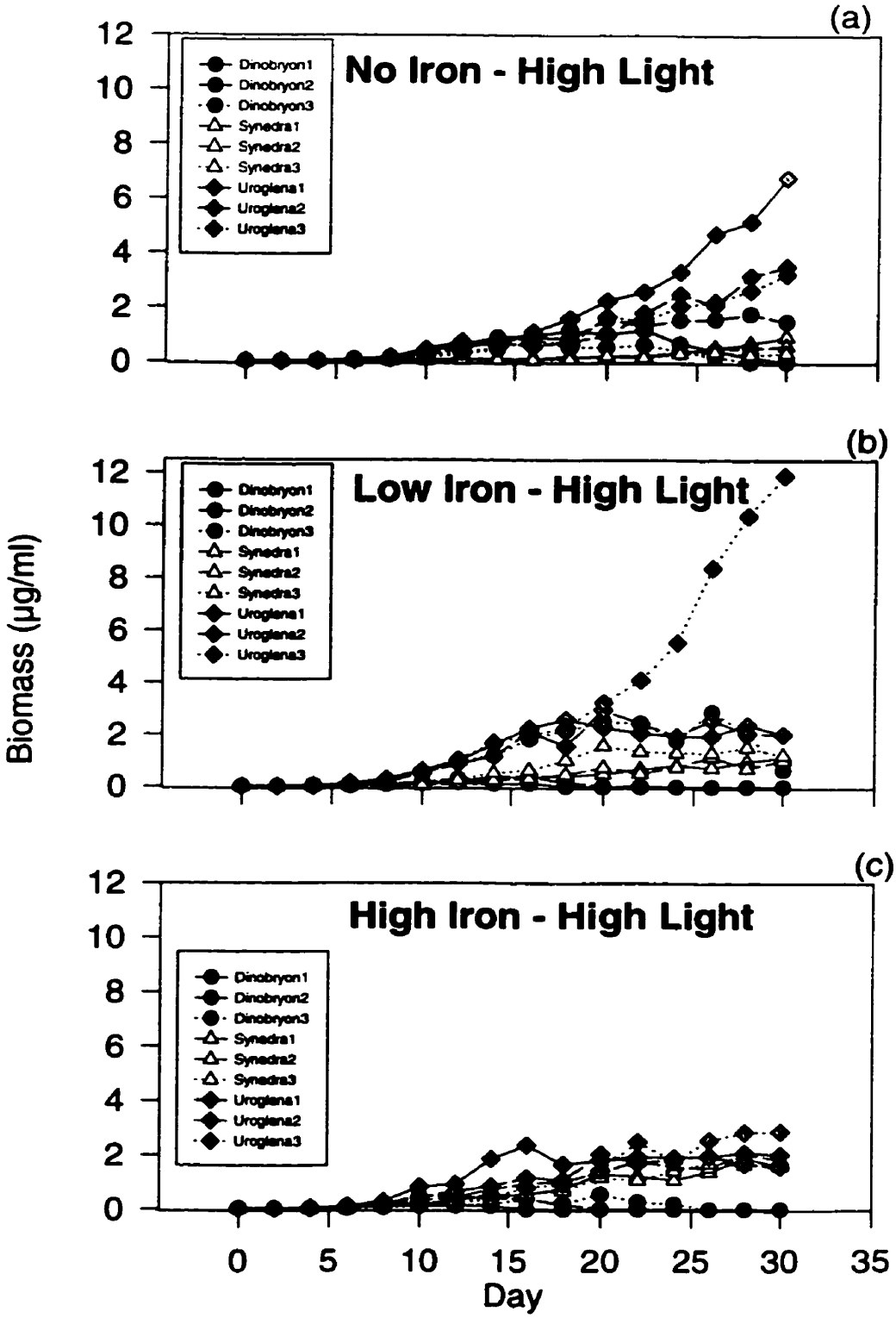

Figure 2.9: Biomass of each algal species in each replicate in mixed competition experiments performed under high light

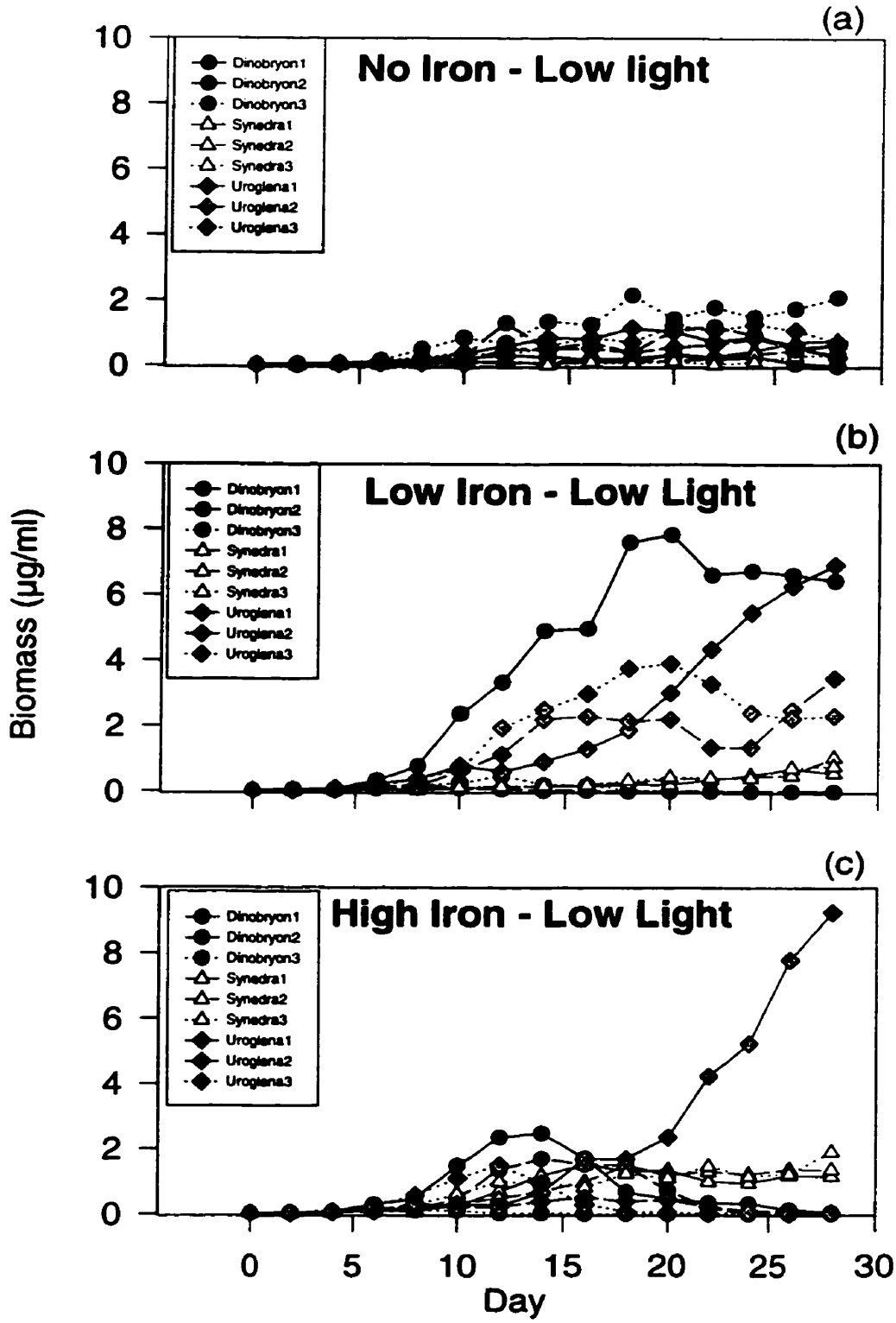

Figure 2.10: Biomass of each algal species in each replicate in mixed competition experiments performed under low light

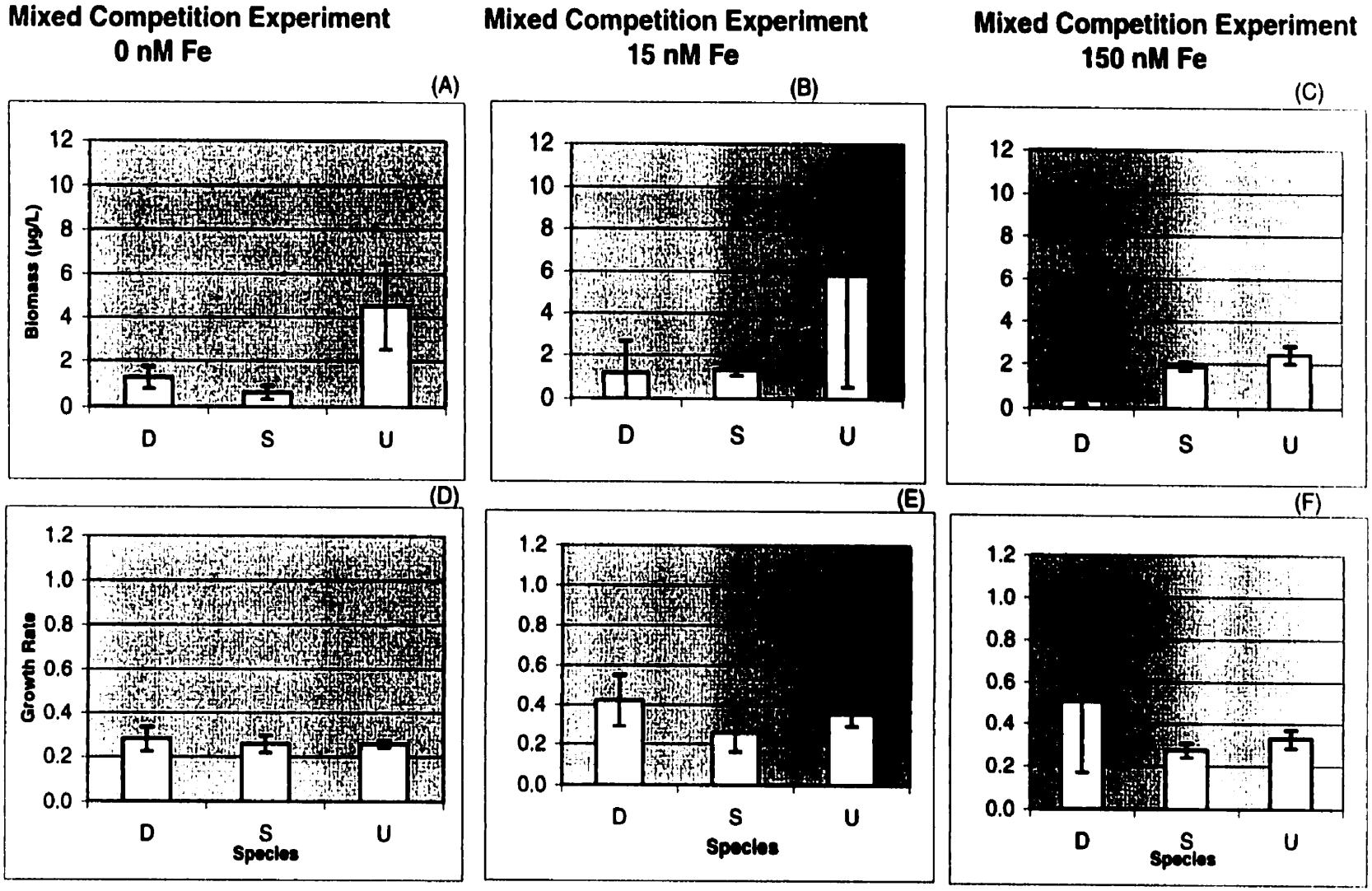

Figure 2.11: Average maximum biomass (+/- 1 SE) and average maximum growth rates (+/- 1 SE) of *Dinobryon* (D), *Synedra* (S), and *Uroglena* (U) from mixed competition experiments in three iron additions under high light

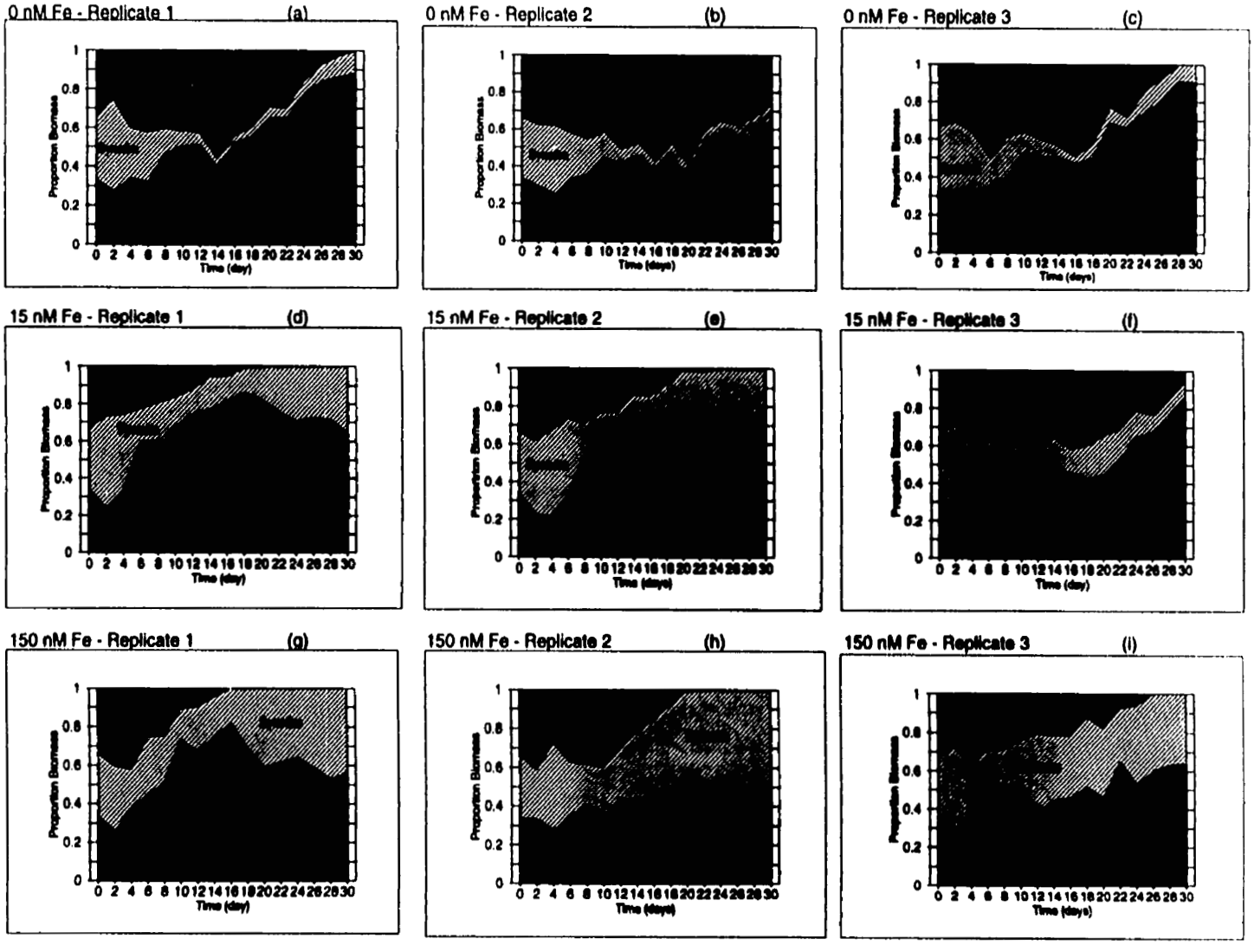

Figure 2.12: Proportion biomass of each species of algae in each replicate of the mixed cultures in three iron additions reared under high light

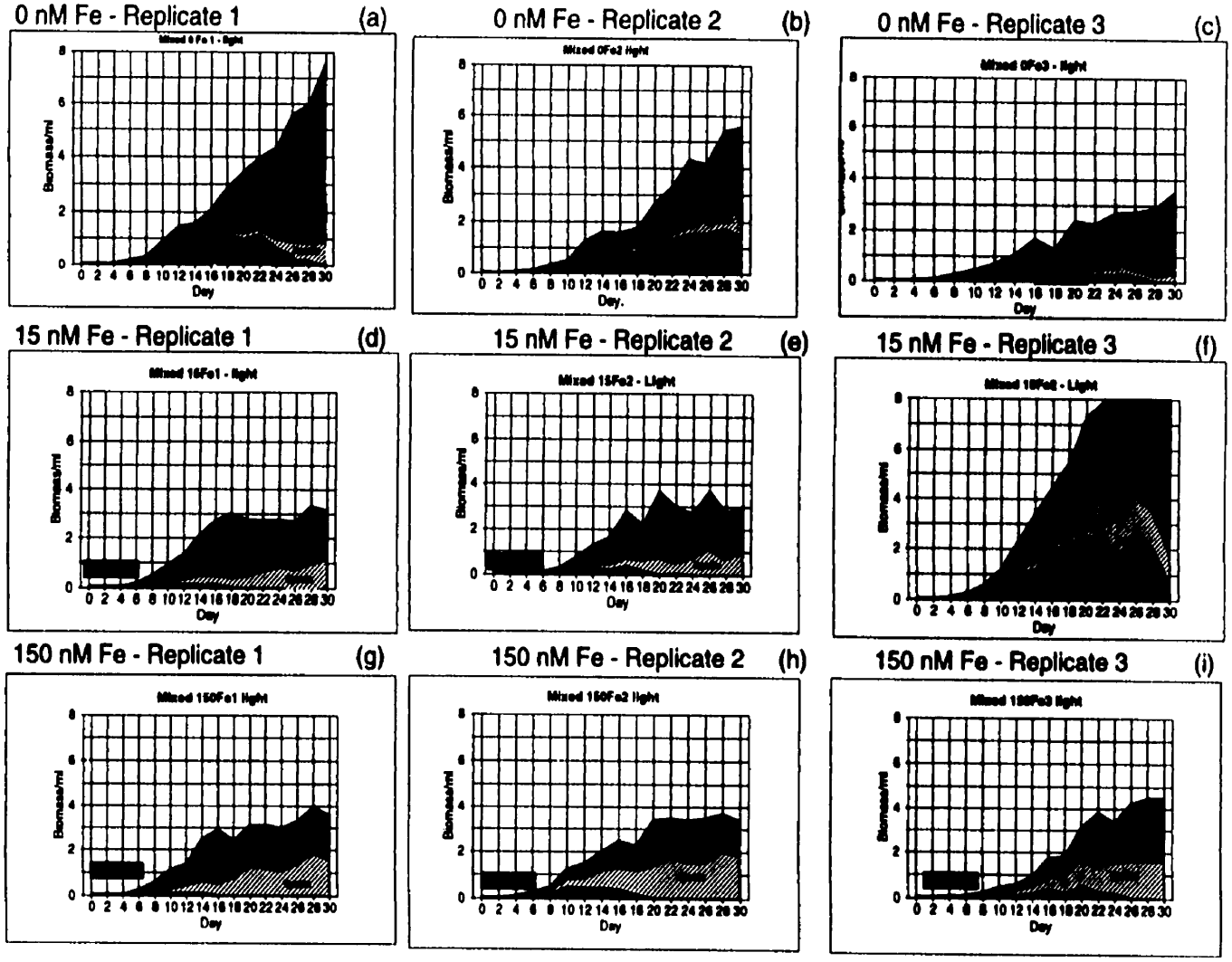

Figure 2.13: Area plots illustrating biomass of algae in each mixed culture replicate at 3 iron additions reared under high light

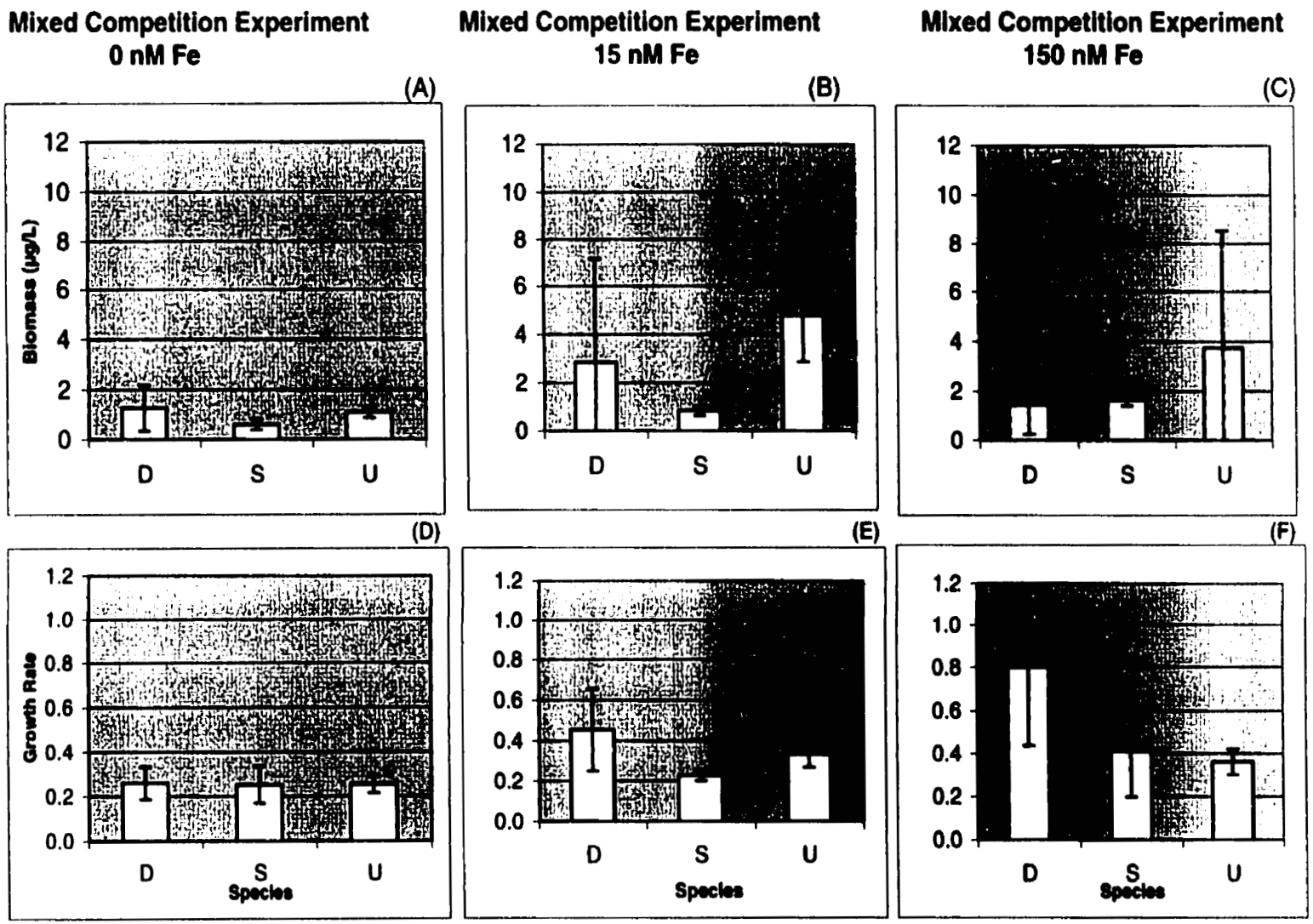

Figure 2.14: Average maximum biomass (+/- 1 SE) and average maximum growth rates (+/- 1 SE) of *Dinobryon* (D), *Synedra* (S), and *Uroglena* (U) from mixed competition experiments in three iron additions under low light

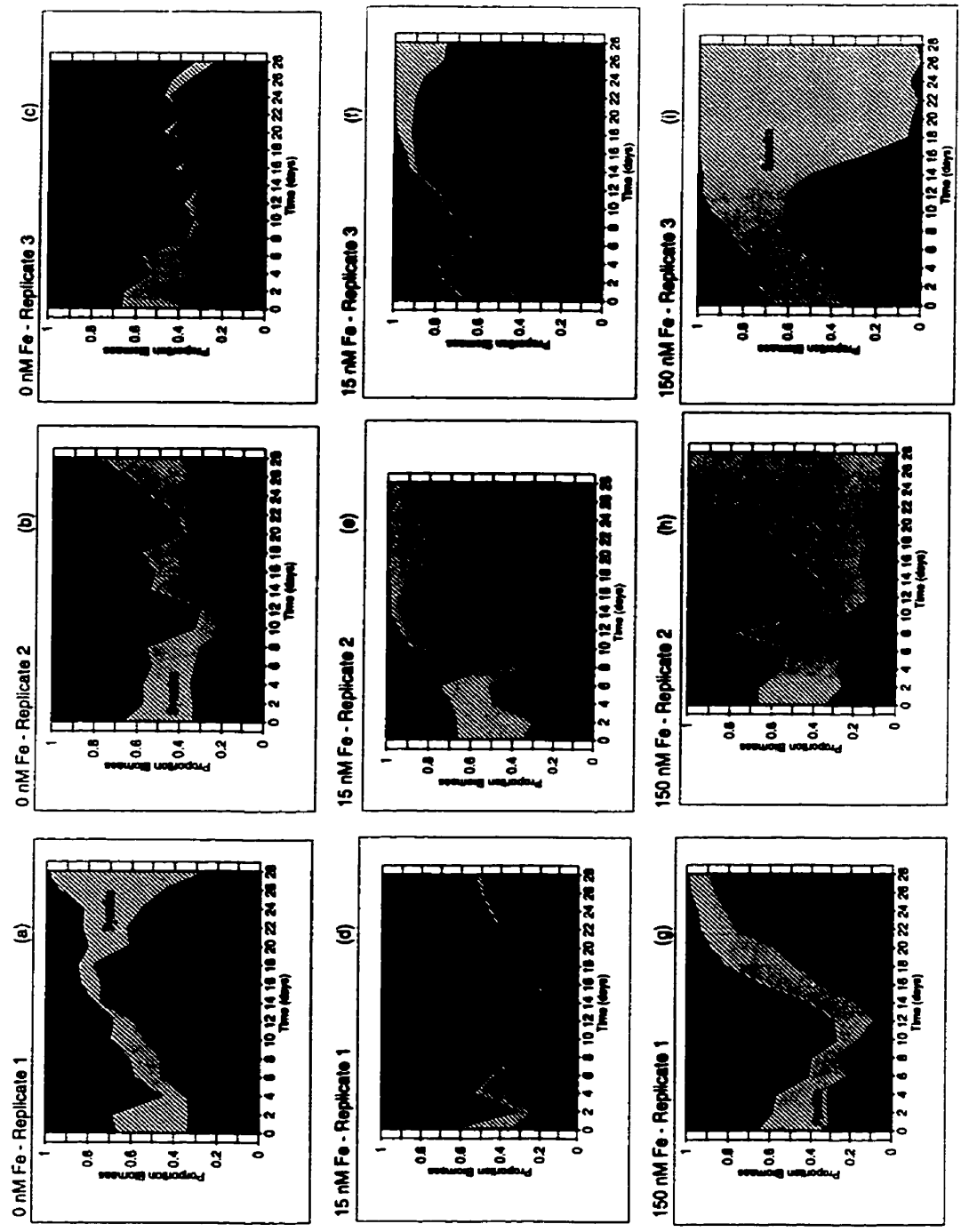

Figure 2.15: Proportion biomass of each species of algae in each replicate of the mixed cultures in three iron additions reared under low light

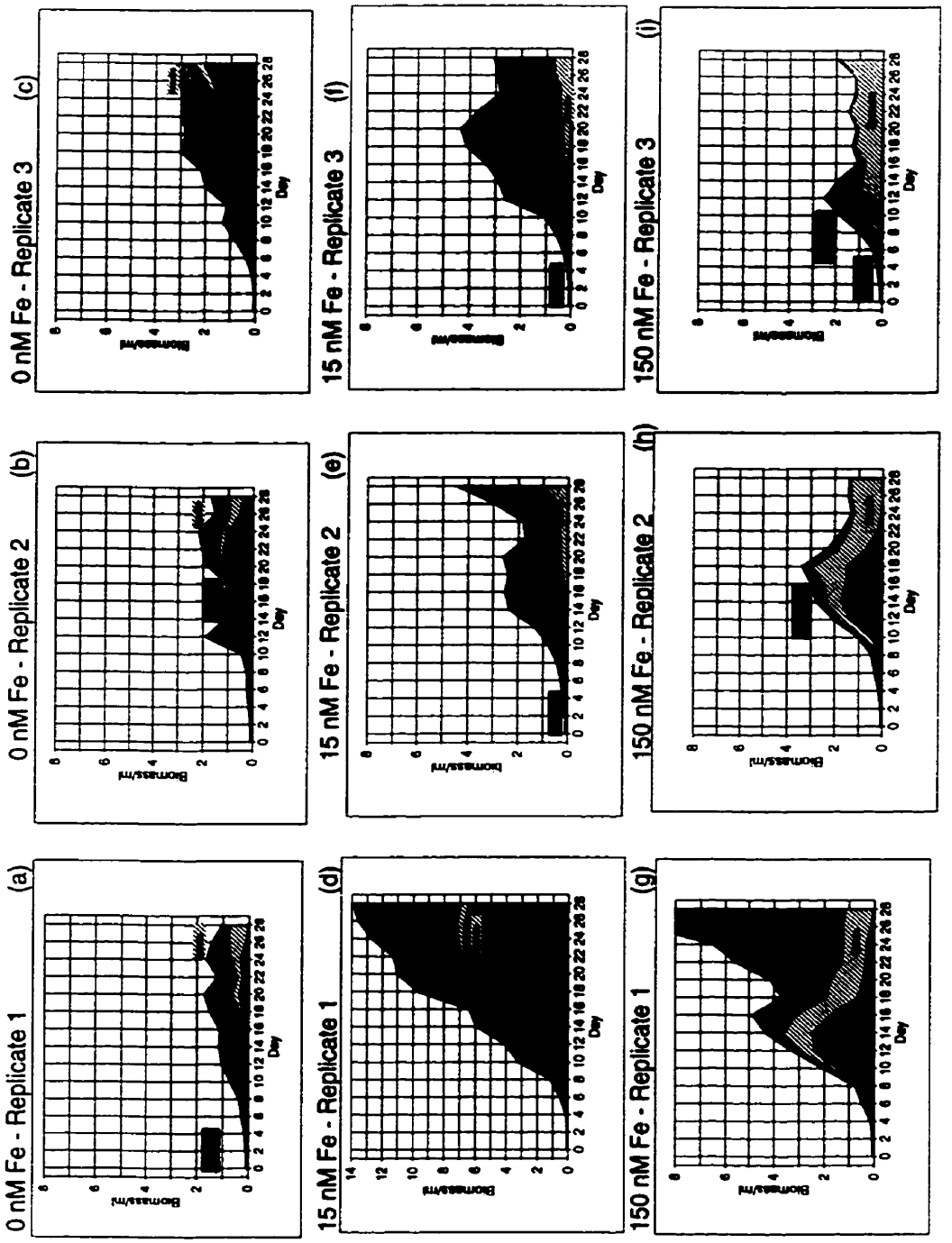

Figure 2.16: Area plots illustrating biomass of algae in each mixed culture replicate at 3 iron additions reared under low light (note difference in ordinate scale of fig. d)

Table 2.3: Results of nine ANOVA's on average growth rates and average maximum biomass of *Synedra*, *Dinobryon*, *Uroglena*, and all species combined from individual species experiments 1 and 2

EXPERIMENT	EFFECT	SPECIES	DF	GROWTH RATE MS	GROWTH RATE F	GROWTH RATE P>F	MAXIMUM BIOMASS MS	MAXIMUM BIOMASS F	MAXIMUM BIOMASS P>F
1	Iron (I)	**Synedra**	2	0.017	4.54 ♣	0.060	0.301	13.22♣♣	0.006
	Error (I)		6	0.004			0.023		
1	Iron (I)	**Dinobryon**	2	0.015	8.21♣♣	0.020	23.994	11.70♣♣	0.009
	Error (I)		6	0.002			2.050		
1	Iron (I)	**Uroglena**	2	0.005	2.12	0.380	0.221	4.80	0.273
	Error (I)		6	0.002			0.05		
1	Iron (I)	**All Species**	2	0.001	0.29	0.754	11.641	8.79♣♣	0.003
	Trophy (T)		1	0.102	25.09♣♣♣♣	0.0002	105.881	79.98♣♣♣♣	< 0.0001
	I x T		2	0.021	5.09♣♣	0.021	6.767	5.11♣	0.020
	Error (I x T)		5	0.004			1.324		
2	Light (L)	**Synedra**	1	0.002	0.33	0.577	1.007	9.89♣♣	0.009
	Iron (I)		2	0.142	22.74♣♣♣♣	< 0.0001	1.345	13.21♣♣♣	0.001
	L x I		2	0.010	1.67	0.229	0.667	6.55♣♣	0.012
	Error (L x I)		12	0.006			0.102		
2	Light (L)	**Dinobryon**	1	0.033	8.94♣♣	0.014	58.961	38.47♣♣♣♣	< 0.0001
	Iron (I)		2	0.006	1.64	0.241	6.461	4.22♣♣	0.047
	L x I		2	0.009	2.64	0.122	2.387	1.56	0.258
	Error (L x I)		10	0.004					
2	Light (L)	**Uroglena**	1	0.001	0.51	0.491	2.848	4.36♣	0.059
	Iron (I)		2	0.0001	0.08	0.927	0.684	1.05	0.381
	L x I		2	0.001	0.42	0.664	1.282	1.96	0.183
	Error (L x I)		12	0.002			0.653		
2	Light (L)	**All Species**	1	0.001	0.17	0.679	8.873	6.53♣♣	0.015
	Iron (I)		2	0.112	24.51♣♣♣♣	< 0.0001	4.278	3.15♣	0.054
	L x I		2	0.005	1.09	0.347	0.783	0.58	0.567
	Trophy (T)		1	0.219	47.82♣♣♣♣	< 0.0001	82.95	61.04♣♣♣♣	< 0.0001
	L x T		1	0.010	2.24	0.142	21.156	15.57♣♣♣	0.0003
	I x T		2	0.075	16.29♣♣♣♣	< 0.0001	0.108	0.08	0.923
	L x I x T		2	0.014	3.06♣	0.058	0.891	0.66	0.525
	Error (L x I x T)		40						

Note: ♣ P < 0.1, ♣♣ P < 0.05, ♣♣♣ P < 0.001, ♣♣♣♣ P< 0.0001

Table 2.4: Average growth rates and average maximum biomass' with associated standard errors at three levels of iron and two levels of light for each species/group of algae studied in the individual species growth experiments.

EXPERIMENT	LIGHT ($\mu mol \cdot m^{-2} \cdot sec^{-1}$)	IRON ADDITION (nM)	SPECIES	AVERAGE GROWTH RATE ($cells \cdot day^{-1}$)	STD. ERROR OF AVERAGE GROWTH RATE	AVERAGE MAXIMUM BIOMASS ($\mu g \cdot ml^{-1}$)	STD. ERROR OF AVERAGE MAXIMUM BIOMASS
1	550	0	Synedra	0.268	0.04	1.124	0.02
1	550	15	Synedra	0.361	0.02	1.676	0.10
1	550	150	Synedra	0.417	0.04	1.667	0.02
1	550	0	Dinobryon	0.570	0.02	2.714	0.26
1	550	15	Dinobryon	0.471	0.03	8.014	0.74
1	550	150	Dinobryon	0.436	0.02	7.074	1.20
1	550	0	Uroglena	0.450	0.04	4.975	0.15
1	550	15	Uroglena	------	-----	------	-----
1	550	150	Uroglena	0.540	-----	5.370	-----
2	550	0	Synedra	0.156	0.02	0.103	0.01
2	550	15	Synedra	0.286	0.03	1.492	0.24
2	550	150	Synedra	0.449	0.01	1.181	0.16
2	550	0	Dinobryon	0.333	0.03	1.092	0.32
2	550	15	Dinobryon	0.296	0.03	0.858	0.47
2	550	150	Dinobryon	0.449	0.04	2.125	0.67
2	550	0	Uroglena	0.442	0.02	2.702	0.42
2	550	15	Uroglena	0.443	0.02	3.959	0.37
2	550	150	Uroglena	0.460	0.02	2.711	0.25
2	55	0	Synedra	0.186	0.05	0.194	0.15
2	55	15	Synedra	0.168	0.09	0.283	0.247
2	55	150	Synedra	0.472	0.02	0.880	0.20
2	55	0	Dinobryon	0.454	0.02	3.487	1.09
2	55	15	Dinobryon	0.461	0.09	5.468	0.13
2	55	150	Dinobryon	0.442	0.02	6.850	1.35
2	55	0	Uroglena	0.450	0.03	3.666	0.81
2	55	15	Uroglena	0.431	0.02	3.758	0.18

2	55	150	Uroglena	0.424	0.03	4.335	0.50
1	550	0	Autotroph	0.268	0.04	1.124	0.02
1	550	15	Autotroph	0.361	0.02	1.676	0.10
1	550	150	Autotroph	0.417	0.04	1.667	0.02
1	550	0	Mixotroph	0.522	0.03	3.546	0.53
1	550	15	Mixotroph	0.471	0.03	8.014	0.74
1	550	150	Mixotroph	0.462	0.03	6.64	0.95
2	550	0	Autotroph	0.156	0.02	0.103	0.01
2	550	15	Autotroph	0.286	0.03	1.492	0.24
2	550	150	Autotroph	0.449	0.01	1.181	0.16
2	550	0	Mixotroph	0.388	0.03	1.897	0.43
2	550	15	Mixotroph	0.370	0.04	1.492	0.24
2	550	150	Mixotroph	0.455	0.02	2.418	0.34
2	55	0	Autotroph	0.186	0.05	0.194	0.15
2	55	15	Autotroph	0.168	0.09	0.283	0.247
2	55	150	Autotroph	0.472	0.02	0.880	0.20
2	55	0	Heterotroph	0.452	0.02	3.576	0.61
2	55	15	Heterotroph	0.442	0.03	4.442	0.43
2	55	150	Heterotroph	0.431	0.02	5.341	0.80

Table 2.5: Summary of predicted (based on yields of individual population experiments) and observed results of mixed competition experiment at three levels of iron addition under high and low light environments.

Treatment	Predicted Competition Outcome (based on growth rates) (in order of expected of abundance)	Predicted Competition Outcome (based on maximum biomass) (in order of expected of abundance)	Observed Competition Outcome (based on growth rates)	Observed Competition Outcome (based on maximum biomass)
0 nM Fe High Light	**Dinobryon** **Uroglena** Synedra	**Uroglena** Dinobryon Synedra	**2/3 Uroglena** **1/3 Dinobryon**	3/3 **Uroglena**
15 nM Fe High Light	**Uroglena** **Dinobryon** Synedra	**Uroglena** Synedra Dinobryon	**2/3 Dinobryon** 1/3 Synedra	3/3 **Uroglena**
150 nM Fe High Light	**Synedra** Dinobryon Uroglena	**Uroglena** Dinobryon Synedra	2/3 Dinobryon 1/3 Uroglena	2/3 **Uroglena** 1/3 Synedra
0 nM Fe Low Light	**Uroglena** **Dinobryon** Synedra	**Uroglena** Dinobryon Synedra	2/3 Synedra **1/3 Dinobryon**	2/3 **Uroglena** & Synedra 1/3 Dinobryon
15 nM Fe Low Light	**Dinobryon** **Uroglena** Synedra	**Uroglena** Dinobryon Synedra	**2/3 Dinobryon** 1/3 Uroglena	2/3 **Uroglena** 1/3 **Uroglena** & Dinobryon
150nM Fe Low Light	**Uroglena** **Synedra** Dinobryon	**Dinobryon** Uroglena Synedra	2/3 Dinobryon **1/3 Uroglena**	2/3 Synedra 1/3 Uroglena

2.5 Discussion of Results of Laboratory Experiments

2.5.1 Individual Populations

As iron is an essential element for phytoplankton growth, cell populations reared without iron additions should be expected to have slower growth rates and smaller biomass yields than those cultured with iron. The results suggest that the trophic modes employed by phytoplankton and light environments significantly influence the expression of iron limitation.

Autotrophic *Synedra* populations fertilized with a high (150 nM) amount of iron achieved faster growth rates and greater maximum biomass' than those in a 0 nM Fe medium regardless of light. As bacteria and other organic matter represent a viable nutrient supply for mixotrophic organisms, algae employing mixotrophy should be less affected by reductions in the ambient concentrations of an essential element than strict autotrophs. This prediction was supported because the effects of iron additions under the same conditions as the autotroph were less substantial (although somewhat variable) for the mixotrophs. The growth rate of the mixotrophs did not increase with iron fertilization. In fact, as observed in experiment 1, *Dinobryon* was capable of growing more quickly without any iron enrichment. Increasing iron levels did have a positive effect on total *Dinobryon* population biomass. In the first experiment those cultures subjected to low and high iron fertilization's reached a significantly greater biomass than those without. Under high light in the second experiment, only those cultures in 150 nM Fe enriched medium experienced an increased biomass compared to those in the 0 nM Fe medium.

The difference between the results of the first and second experiment was likely due to the difference in the 0 nM Fe acclimation periods before each experiment. In experiment 1 all algae were acclimated for a period of 7 days and in the second experiment this duration was extended to 10 days. Those three extra days cells were in the 0 nM iron medium in experiment 2 might have resulted in the internal iron reserves of the cells being depleted to an even greater extent. Thus, the *Dinobryon* cells in experiment 2 likely required a greater amount of iron to replace the internal stores and to maintain metabolism, therefore less iron could be utilized for growth. Additions of 150 nM Fe are believed to provide iron in excess amounts (Watson, pers. comm.), thereby reducing the effect(s) of a longer acclimation period in experiment 2. Under low light, each addition of iron resulted in increased yields, and this indicates that this mixotroph was iron limited in this light environment. The maximum biomass *Dinobryon* achieved during the second experiment at each iron level was greater under low light than high light. This result suggests that under irradiance levels of 550 $\mu mol \cdot m^{-2} \cdot sec^{-1}$ *Dinobryon* growth was actually photoinhibited. Results for experiment 1 and 2 were consistent for *Uroglena* and closely follow the predictions made about mixotrophic organisms. In both of the experiments, *Uroglena* biomass yields and growth rates were independent of iron concentrations in high and low light environments.

There was no obvious correlation between rate of growth and biomass for those species which experienced increased growth rates as a result of iron fertilization under

high light (*Synedra*, *Dinobryon*). In the first experiment, *Dinobryon* had greater yields and slower growth rates when either high or low iron concentrations were added. *Synedra* growth was faster under high light following each iron enrichment, but this did not translate into greater biomass. Thus, under high light, 15 nM of iron allowed *Synedra* to achieve a similar biomass as 150 nM Fe, but at a slower rate.

Cells need higher Fe:C for growth under low light (Sunda and Huntsman 1997). Because of this it was expected that these three species of algae would need more iron in low light to maintain growth at levels similar to those in high light. This prediction is supported by the *Synedra* results which illustrate that increasing iron concentrations by 15 nM only elevated growth rates and biomass' beyond those which had no iron added at irradiance levels of 550 $\mu mol \cdot m^{-2} \cdot sec^{-1}$. Under irradiances of 55 $\mu mol \cdot m^{-2} \cdot sec^{-1}$ a 150 nM increase in iron was needed to elevate growth rates and crop size. Results of mixotrophs, however, failed to reveal an increased iron demand under reduced light environments.

Predictions (Table 2.1) over estimated the yields of the autotroph in the individual populations experiments. It was expected that the autotroph would achieve the greatest biomass when low and high iron concentrations were added under high light and upon a high iron fertilization in low light. *Synedra* was only observed to reach a level of biomass similar to *Uroglena* under high light/low iron conditions. Low light levels seem to favor the growth of the mixotrophs regardless of iron enrichment when species are reared individually, a result which changes when the populations are mixed within a single vessel.

2.5.2 Mixed Competition Experiments

Predictions, based on the results of the individual populations experiments, were made about the outcome of competition between *Synedra*, *Dinobryon*, and *Uroglena* reared under different iron and light environments (Table 2.5).

When grown in isolation the average *Uroglena* biomass was greater than the biomass of *Synedra* and *Dinobryon* under high light regardless of iron. We therefore predicted that *Uroglena* would achieve the greatest biomass and dominate the mixed populations under high irradiance at all levels of iron enrichment. Our predictions were correct when no or low amounts of iron were added to the high light flasks. By the time the experiments were terminated *Uroglena* represented the largest proportion of the total algal biomass within the vessels enriched with 0 nM and 15 nM Fe. The abundance of *Dinobryon* was not correlated with iron and this species was observed to be the inferior competitor of the three algae at each iron level under high light. *Synedra* biomass was, as in the individual species experiments, positively correlated with increasing iron concentrations. The proportion of the autotroph in each community increased with iron enrichment to the extent that at 150 nM Fe *Synedra* became a co-dominant species along with *Uroglena* under high light. It is evident from these results that *Uroglena* is the superior competitor of the two mixotrophs at all levels of iron used in this study at light levels of 550 $\mu mol \cdot m^{-2} \cdot sec^{-1}$, and the superior competitor of all three algae when no iron or 15 nM of iron was added. The ability of *Uroglena* to dominate the mixed assemblages was greatly reduced upon iron enrichment of 150 nM. Increasing iron levels by this

amount allowed *Synedra* populations to achieve biomass' similar in size to those of *Uroglena*. Under high light, the competitive advantage(s) held by *Uroglena*, possibly due to its ability to acquire iron from organic sources (i.e. bacteria), appear to be greatly reduced in environments abundant in light and nutrients in which the autotroph can increase its numbers and achieve similar biomass levels.

Based on the observed population yields in the low light individual species experiments, it was predicted that mixotrophs would dominate the low light mixed community algal assemblages at each level of iron addition. We expected that *Uroglena* would dominate communities not Fe enriched as well as those fertilized with a low concentration of iron. Individual *Dinobryon* populations achieved the greatest biomass under high iron and low light. Thus, it was expected that this species would dominate mixed populations in a similar environment.

There was a greater autotroph component in mixed populations reared under low light compared to high light. On the basis of the individual populations experiments we did not expect to see a significant *Synedra* component under any level of iron enrichment at irradiance levels of 55 $\mu mol \cdot m^{-2} \cdot sec^{-1}$. However, *Synedra* was a co-dominant species in a majority of the 0 nM Fe replicates and represented nearly 100% of the final algal community in two thirds of the 150 nM iron fertilized vessels. In fact, *Synedra* yields were nearly the same in all the high iron fertilized cultures. This species did not dominate

one of the replicates because, compared to the others, the *Uroglena* population grew to a much greater level in one of the flasks and in that replicate the mixotroph represented nearly 90% of the final biomass (Fig. 2.16 g).

High proportions of *Uroglena* persisted in the 0 nM Fe and 15 nM Fe cultures and it was either the dominant or co-dominant species in these vessels. Low level iron addition did not encourage increased autotroph representation under low light. The mixotrophs, specifically *Uroglena* which dominated two 15 nM Fe enriched communities and co-dominated the third with *Dinobryon*, achieved greater biomass levels than the autotroph in all three replicates.

The predictions generated from the yields of the individually raised populations were less accurate in low light than those made in high light conditions. Trends suggest that the dominant species in communities reared under reduced light levels in the absence of iron enrichment the dominant species is not easily predicted. When all the algae are competing for the same low concentration of iron resource the outcome of competition appears to be dependent upon the trophic mode an organism utilizes to meet energy demands. The dominance of the mixotrophs in low light communities fertilized with 15 nM Fe reveals that the small pulse of iron provided the mixotrophs with enough of the nutrient that they were able to increase their biomass to levels greater than *Synedra*. Even if low light levels did increase the iron demands of these algae, a 150 nM addition was often enough to allow the autotroph to overcome this and *Synedra* could increase its biomass as, or more, successfully than the mixotrophs.

Low light levels yielded higher variability among replicates and this could have been caused by mixotrophic species switching modes of nutrient acquisition under light/nutrient limited environments.

The results of the single species culture experiments generally led to the development of a successful set of predictions regarding the outcome of competition when *Synedra*, *Uroglena*, and *Dinobryon* populations were mixed in various iron environments under high light. The predictions were less accurate when applied to the competition experiments performed under reduced irradiance levels.

Uroglena was accurately predicted to dominate phytoplankton communities in all high light and half of the low light replicates receiving no or low iron enrichment. This species also represented a major component of the biomass in all high iron high light replicates.

Dinobryon was able to co-dominate/dominate a few (3 of 6) no or low iron/low light replicates. These outcomes were not anticipated because of *Dinobryon's* inferior growth compared to *Uroglena* under the same conditions in the individual population experiments.

The representation of *Synedra* was unexpectedly high in 150 nM Fe enriched vessels under both high and low light. The autotroph co-dominated three of three high light replicates and dominated two of the three low light/high iron flasks.

One of the low light/150 nM Fe replicates supported an extremely large *Uroglena* population and this resulted in this species representing the greatest proportion of these three algal species. This raises the question of how this occurred in one vessel and not the others. A potential source of variation may have been the bacterial community within the flasks. Variation in the species and the abundance of bacteria may have (although this should have been minimal due to identical experimental procedures among replicates and experiments) influenced the abundance of this mixotroph.

Chapter 3: Field Mesocosm Experiments

3.1 Iron and Light Manipulation of Whole Phytoplankton Communities

The laboratory culture experiments featured in chapter 2 provided a means to test the iron and light-limited growth hypotheses for individual and mixed populations of three algal species. These experiments were performed under tightly controlled environmental conditions (i.e. light levels, water temperature, nutrient concentrations were all specified by the experimenter), and were effectively utilized to determine if iron/light-limited growth could be induced for *Synedra*, *Uroglena*, and *Dinobryon*. By studying the algal populations in isolation we were able to eliminate the effects of among-species interactions and focus on physiological responses underlying phytoplankton growth. Mixing the three algal populations in a single vessel in the lab provided a method to observe the effects of among-species interactions on the growth of the test species, while maintaining strict control of ambient environmental conditions.

The laboratory studies led to the development of a set of predictions regarding the response of natural (i.e. not isolated) phytoplankton communities to iron and light manipulations. This chapter presents the results of a series of field based mesocosm experiments used to test these predictions.

Concern exists in ecology regarding the validity of extending the results of experiments performed at one scale (i.e. algal cultures in flasks) to explain mechanisms driving processes at another scale (i.e. phytoplankton community dynamics in field mesocosms or whole systems). The roles and effectiveness of meso/microcosms is a

commonly debated subject of aquatic ecologists (Carpenter 1996, Drenner and Mazumder 1999, Carpenter 1999, Huston 1999). Criticism often centers around the belief that the scale of microcosm experiments limits the extent to which they can be used to represent ecological phenomena. Some feel that without the addition of appropriately scaled field studies, microcosm experiments become irrelevant and diversionary (Carpenter 1996). The purpose of utilizing mesocosms in this study was to test the extent to which predictions generated in the laboratory can be applied to a larger whole community scale. Besides some of the economic incentives associated with the use of mesocosms, such as minimal expense, they can greatly contribute to the replicabililty and statistical power of one's research (Carpenter 1996). In this study, a particular advantage of utilizing mesocosms in the University of Calgary's campus pond was that they served as a tool used to isolate and manipulate whole phytoplankton communities containing the same blooming algal species found in Glenmore Reservoir.

Based on the results of the laboratory experiments, it was predicted that diatom populations, specifically *Synedra* populations, would increase in mesocosms enriched with iron. It was also expected that the abundance of the two mixotrophic algae, *Uroglena* and *Dinobryon*, would be unaffected by iron fertilization in the field tanks. In the lab, reduced light levels and high iron levels generally resulted in a decreased mixotroph representation and an increased abundance of *Synedra*. Therefore, we

predicted that natural phytoplankton communities reared under low irradiances would favour the growth of *Synedra* when natural iron levels were increased.

High light conditions in the lab favoured the growth of the mixotrophs when no or low amounts of iron were added. Supplementing cultures with a high concentration of iron (150 nM) in high irradiance environments resulted in a co-dominance of algal communities by *Uroglena* and *Synedra*. Thus, mesocosms not enriched with iron were expected to contain a greater proportion of *Uroglena* and *Dinobryon*. Following iron addition, it was anticipated that *Uroglena* and *Synedra* would be equally abundant in tanks not subjected to a reduction in light.

3.2 Methodology of Whole Phytoplankton Community Iron and Light Manipulation Experiments

These experiments were performed in the western most of 2 ponds located at the south west corner of the University of Calgary campus (Calgary, Canada). This pond is a meso-oligotrophic system whose water is supplied from the Bow River (Calgary, Alberta). It contains algal, macrophyte, and invertebrate communities, but does not contain fish. Typical planktonic algal assemblages consist primarily of populations of chrysophytes, diatoms, chlorophytes, dinoflagellates, and some cyanophytes.

A series of 200 liter mesocosms were established within this pond. 12 polyethylene tanks were filled with 35μm filtered pond water to ensure the presence of natural phytoplankton communities and the exclusion of crustacean zooplankton grazers.

In an attempt to mimic natural light and temperatures, these tanks were placed directly in the pond to a minimum depth of 2/3 the height of the tank. Half of the mesocosms (the low light treatments - LL's) were covered with a neutral density filter to lower ambient light levels and prevent contamination, the other mesocosms (the natural light treatments – NL's) were covered with transparent polyethylene sheeting to permit natural illumination levels while ensuring contamination of the enclosures was kept to a minimum. Iron levels of half of the NL and LL mesocosms were increased by 370 µg/L via the addition of a pre-chelated EDTA-Ferric chloride complex ($Na_2EDTA \cdot 2H_20$ + $FeCl_3 \cdot 6H_20$) at day 0 of the experiment. The remaining tanks were left unfertilized to serve as controls.

One liter samples were collected weekly. Parameters measured included algal biomass, algal species, chlorophyll a, silica, zooplankton, light, pH, temperature, and phosphorus (total and soluble reactive). A summary of analytical methods is provided below. Tanks were scrubbed and stirred three times per week to reduce the growth of nuisance epiphytic algae and to prevent the settling of planktonic algae and nutrients.

To avoid problems associated with repeated measures, a fixed census date of 28 days was used to describe a time series similar to the laboratory experiments. It is important to note that even if the experiments been terminated after 21 days, the major results would be qualitatively identical.

Table 3.1: List of variables, frequency of measurement, and analytical methods utilized in whole phytoplankton community iron and light manipulation experiments

Variable	Frequency of Measure	Method
Algal species	Weekly	Inverted Microscope
Algal biomass	Weekly	Inverted Microscope
Chlorophyll a & Phaeophytin	Weekly	Acetone Extraction / Spectrophotometric analysis
Zooplankton	Day 0 and Day 35	Filtration through 35μm screening
Silica	Weekly	Silicomolybdate / Spectrophotometric analysis
Phosphorus (Total and Soluble reactive)	Weekly	Phosphomolybdate / Technicon© Autoanalyzer
Total Iron in Water	Day 35	Graphite Furnace Atomic Adsorption Spectrophotometer
pH	Weekly	pH meter
Light	3 Times Per Week	Light meter (Li-Cor LI-192SA)
Air Temperature	Daily	Daily weather reports
Water Temperature	Daily	Submersible Thermal Recorder

3.2.1 Algal Speciation and Biomass Determination

Sample preparation; 125 ml samples were collected from the mesocosms. All samples were preserved with Lugol's iodine solution (45 g/L I_2, 90 g/L KI, 9% glacial acetic acid). *Sample analysis*; Preserved samples were well shaken and a 1-5 ml aliquot was settled over night and then analyzed with a Nikon inverted microscope. A minimum of 400 individuals from the samples were counted and classified to the genus level (and to species when possible). The level of identification was often limited by the resolution of the inverted light microscope.

3.2.2 Chlorophyll and Phaeophtyin

Sample preparation; 100 ml of sample was filtered through a Whatman GF-F glass-fiber filter at a pressure no greater than 20 mm Hg. The filters were then kept dark and frozen until analysis.

Sample analysis; The frozen filter was placed in test tube and 6 ml of 90% acetone was added. The filter was then ground into a slurry using a tissue grinder, after which an additional 6 ml of 90% acetone was added. The tube containing the ground filter/acetone solution was sealed and stored in the refrigerator in complete darkness for 24 hours to allow for chlorophyll extraction. Upon completion of the 24 hour extraction process the test tube was centrifuged at 1500 rpm for 3 minutes. The fluorescence of the supernatant was measured using a fluorometer (Sequoia-Turner Model 450). 2 drops of 1:1 HCl were then added and a second fluorescence reading was recorded. Sample readings were compared with a previously calculated standard curve, the first fluorescence reading

corresponded to chlorophyll a concentrations and the second to phaeophytin concentrations.

3.2.3 Zooplankton

Sample preparation; Zooplankton were removed by filtering 2 liters of a stirred sample through a 35µm screen. Samples were then preserved and stored in a 3% Formalin solution.

Sample analysis; Samples were visually scanned to ensure that there were no replicates that had been invaded by herbivorous zooplankton populations.

3.2.4 Silica

Sample Preparation; 125 ml of sample was collected in polyethylene containers and stored in a dark and cool refrigerator until analysis.

Sample analysis; Silica was measured colorimetrically using the silicomolybdate - spectrophotometric analysis technique. Molybdate reagent was added to the water sample and was allowed to bind with the silica, forming a yellow colour in the process. Phosphorus can interfere with this method (Strickland and Parsons 1968). To control for this interference citric acid was added to the samples to remove any yellow colour which may have resulted from the presence of phosphorus-molybdate complexes. The samples were allowed to sit for 10 minutes to allow for full silica-molybdate coupling and colour development to occur. After 10 minutes extinction values were measured at a wavelength of 8100 Å using a spectrophotometer. This procedure was repeated using distilled water

and 3 silica standards to formulate a standard curve which was used to determine the silica concentrations in the samples.

3.2.5 Total Phosphorus (TP)

Sample preparation; 75ml samples were collected, filtered through a 250 µm screening, and stored in 250 ml Pyrex tubes which had been washed for 24 hours in a 25% HCl bath. An excess amount (~ 0.5 gram) of Potassium persulfate ($K_2S_2O_8$) was added to each sample and the bottles were capped with polypropylene lids. Samples were digested by autoclaving at ~ 120°C for 20 minutes and were analyzed within 5 days after collection and preparation.

Sample analysis; Total phosphorus was measured colorimetrically (@ 8850 Å) using the phosphomolybdate method. This method is dependent upon the formation of a blue colour which results from the coupling of phosphorus and molybdenum in the sample. Reagents and samples were mixed and the intensity of blue colour developed using a Technicon© AutoanalyzerII (Pulse Instrumentation Ltd.). Details of this analysis are lengthy and can be found in any version of Standard Methods For The Examination of Water and Wastewater (A.P.H.A., A.W.W.A., W.P.C.F., 1996). This procedure was repeated using distilled water and 3 phosphorus standards to formulate a standard curve which was used to determine the phosphorus concentrations in the samples.

3.2.6 Soluble Reactive Phosphorus (SRP)

Sample preparation; 20 ml of sample was filtered through a 0.45µm Gelman membrane

filter and stored in 25 ml Pyrex tubes which were capped with polypropylene lids. The samples were then analyzed within 24 hours of collection and preparation.

Sample analysis; Prepared samples were analyzed using the same phosphomolybdate/Technicon© autoanalyzer method described for total phosphorus.

3.2.7 Total Iron

Sample preparation; 50 ml of water was collected from each tank and stored in polyethylene containers on the last day of each field experiment. Samples were preserved via the addition of 0.25 ml of 1:1 nitric acid:H_2O. Samples were stored in the refrigerator prior to analysis.

Sample analysis; Final total iron concentrations were measured using a graphite furnace atomic adsorption (A.A.) spectrophotometer (Perkin-Elmer Model 5000). Repeated standard checks indicated that our minimal level of detection with this instrument was 5 p.p.b. Operational methods for the graphite furnace A.A. spectrophotometer were derived from the book of Standard Methods For The Examination of Water and Wastewater (A.P.H.A., A.W.W.A., W.P.C.F., 1996). Quality control procedures included frequent iron spikes of samples and calibration of the machine with iron standards. This procedure was repeated using distilled water and 5 iron standards to formulate a standard curve which was used to determine the total iron concentrations in the samples.

Chemical conditions within the tanks were measured when possible. The Hach© spectrophotometer and Technicon© autoanalyzer were out of service on several occasions

throughout the experimental study period. Missing silica and phosphorus values indicate times when measurements were not made.

3.3 Results of Whole Phytoplankton Community Iron and Light Manipulation Experiments

3.3.1 Three Experiments, One Ice-Free Season

The three field experiments were performed at separate times of the 1998 ice-free growing season. Experiment 1 began in May and continued through mid-June. It should be noted that the weather during this experiment was much more cloudy and rainy (i.e. ~ 80% days experiencing precipitation) than the other two experiments (each with < 40% precipitation days). Experiment 2 began in July and ran until mid-August. The third experiment quickly followed experiment 2 and was terminated in late September. Table 3.2 summarizes the initial biological, chemical, and physical conditions within the tanks for each trial.

3.3.2 Initial Conditions

The initial phytoplankton community crop size was higher in experiment 1 (~ 2368μg/L) than in experiment 2 (~ 1159 μg/L) or experiment 3 (~ 533 μg/L).

Following this trend, the biomass on day 0 of total, chrysophytes, *Synedra*, *Uroglena*, and *Dinobryon* were all greater in the first experiment. With the exception of *Synedra* (whose day 0 biomass was similar in the second and third experiment), the initial biomass of these species was smaller in each successive experimental trial.

Variation in initial chlorophyll a concentrations mirrored differences in biomass.

As a result of filtering pond water through 35 μm mesh, the populations of all zooplankton were greatly reduced. Their numbers and effects on phytoplankton dynamics were considered negligible in each of the experiments.

Initial silica, total phosphorus (TP), and soluble reactive phosphorus (SRP) concentrations were similar among experiments. Day 0 silica concentrations were > 3 mg/L in the first and third experiment. TP was initially > 25 μg/L in experiment 1 and 2, and day 0 SRP concentrations in the first and second trial were > 4 μg/L.

The pH of the tanks averaged ~ 8 at the beginning, and throughout, each experiment.

Due to seasonal heating of the campus pond, the day 0 water temperature of the mesocosms increased each time the experiment was repeated. When the first experiment began in May the tank water temperature was 14.2°C, this increased to 17.3°C for the second experiment, and further increased to 18.0°C at the onset of experiment 3.

The air temperature reported in table 3.2 is the daily maximum air temperature on the first day of each experiment. Day 0 air temperature varied only slightly among experiments, averaging 20.5°C.

Appendix I contains figures illustrating air/water temperatures, chlorophyll a concentrations, total and soluble reactive phosphorus concentrations, and irradiance levels (subsurface, mid-depth, and bottom of mesocosms) for the duration of the whole phytoplankton community field experiments.

Statistical and summary statistics are provided in tables 3.3 and 3.4.

The results of field mesocosm experiments are presented in the same order as they were performed. The first experiment took place early in the ice-free growing season (May/June), the second experiment was performed in mid-season (July/August), and the third trial took place late in the season (August/September).

3.3.3 Field Experiment 1

Total algal biomass was significantly lower in tanks subjected to reduced light levels than those exposed to natural irradiances ($P < 0.001$) (Fig. 3.4 a). Iron fertilization failed to stimulate an increase in total algal yield under low light (no Fe added, average biomass ~ 481 µg/L; iron added, average biomass ~ 481 µg/L), or under natural light (no Fe added, average biomass ~ 2277 µg/L; with iron added, average biomass ~ 2382 µg/L).

The amount of chrysophytes in the mesocosm communities was also greatly influenced by light levels. Chrysophyte biomass was significantly higher in those communities reared under natural light levels ($P < 0.0001$) (Fig. 3.5 a). The effect of iron enrichment was only marginally insignificant ($P < 0.06$). Under low light this group's yield averaged ~ 70 µg/L without iron addition and ~ 252 µg/L with iron enrichment, and when light was not manipulated (i.e. natural levels) the average chrysophyte biomass increased from ~ 1239 µg/L to 1720 µg/L upon iron fertilization.

Shifting focus to some individual species in the communities, *Synedra* populations were influenced by light. This autotrophic diatom achieved significantly higher biomass yields in the natural light treatments (with Fe added, ~ 307 µg/L; without

Fe added, ~ 282 µg/L) than in the low light ones (with Fe added, ~ 55 µg/L; without Fe added, ~ 120 µg/L) ($P < 0.02$), but did not respond to iron fertilization , regardless of light (Fig. 3.6 a).

The biomass of the mixotrophic *Uroglena* and *Dinobryon* populations reached higher levels under natural light compared to when light levels were decreased (*Uroglena*; $P < 0.0001$, *Dinobryon*; $P < 0.02$) (Figs. 3.7 a and 3.8 a respectively). Fertilization with iron led to increased yields of *Uroglena* in treatments of natural light (average biomass with Fe added, ~ 208 µg/L; without Fe added, ~ 23 µg/L), and low light (average biomass with Fe added, ~ 1270 µg/L; without Fe added, ~ 916 µg/L), the differences were marginally insignificant ($P < 0.07$).

Populations of *Dinobryon* achieved similar maxima under each iron regime.

Comparing populations of *Synedra*, *Uroglena*, and *Dinobryon* with each other allows for the proportional representation of each in respect to each other to be analyzed. In relation to *Uroglena* and *Dinobryon*, the proportion of *Synedra* was significantly higher under low light levels (Fig. 3.15 a) ($P < 0.02$). Populations of the autotroph accounted for, on average, 48% and 83% (with and without iron enrichment, respectively) of the total biomass achieved by these species under low light. In natural light environments, *Synedra* represented 18% (with Fe added) and 22% (without Fe added) of the combined biomass. In each light environment, iron enrichment did not significantly alter the abundance of the autotroph in comparison to the two mixotrophs.

Opposite to the *Synedra* results, the proportion of *Uroglena* was significantly higher in natural light communities ($P < 0.02$). Representing an average of 77% (with Fe added) and 76% (without Fe added) of the combined biomass, *Uroglena* had the highest yield of the three species under natural irradiance levels. Iron fertilization did not stimulate significant changes in the representation of this alga under natural or reduced light environments (Fig. 3.15 a).

A significant iron effect on the proportion of *Dinobryon* was observed in this study, such that iron fertilization resulted in an increased abundance of this mixotroph ($P < 0.01$). Light levels did not alter the proportion of *Dinobryon* in relation to the other two species. *Dinobryon* populations only accounted for a small component (maximum of 4%) of the combined biomass regardless of environmental conditions (Fig. 3.15 a).

3.3.4 Field Experiment 2

Community biomass yields seem to be lower under low light (no Fe added; ~ 908 µg/L, Fe added; ~ 530 µg/L) compared to natural light levels (No Fe; ~ 1132 µg/L, Fe added; ~ 1255 µg/L). However, differences in total algal biomass were not significantly affected by either the light or iron treatments (Fig. 3.4 b).

Low light levels had a negative effect on the abundance of chrysophytes. The average biomass of this group was marginally lower under reduced irradiances (no Fe added; ~ 215 µg/L, Fe added; ~ 94 µg/L) compared to natural light levels (no Fe added;

~ 571 µg/L, Fe added; ~ 312 µg/L) ($P < 0.045$) (Fig. 3.5 b). Iron enrichment did not stimulate significant changes in the total chrysophyte biomass yield regardless of light level.

Synedra populations achieved an average biomass of 261 µg/L and 467 µg/L (without and with iron respectively) under natural light levels, and 307 µg/L (no iron added) and 290 µg/L (iron added) under low light conditions. Neither the light ($P < 0.68$) nor iron ($P < 0.56$) treatments resulted in significant changes in the biomass of this diatom.

The biomass of *Uroglena* was significantly higher in the natural light treatments ($P < 0.03$). Without iron addition, the average yield of *Uroglena* decreased from ~ 547 µg/L to ~ 2 µg/L when irradiance levels were lowered, and reduced from an average of ~ 66 µg/L to ~ 11 µg/L when light was decreased with iron enrichment. Iron fertilization affected *Uroglena* biomass differently depending on the light environment. Enriching the communities with Fe resulted in a decreased average biomass of this species under natural light, whereas in low light conditions, increasing iron levels stimulated increased population sizes (Fig. 3.7 b).

Dinobryon crop size was unaffected by the treatments applied in this trial. The significance of an elevated biomass in the natural light/+Fe tanks (Fig. 3.8 b) was offset by the large amount of variability among replicates.

Comparing the *Synedra*, *Uroglena*, and *Dinobryon* populations, treatment effects on the abundance of these species in regards to each other can be assessed.

The proportion of *Synedra* was much greater under low light conditions ($P < 0.001$). Regardless of the amount of iron added, *Synedra* represented ~ 96% of the combined biomass of these three algae when light levels were reduced. Under natural irradiance, the abundance of this diatom decreased to an average of 65% and 25% with and without iron enrichment, respectively (Fig. 3.15 b).

Opposite to the response of the diatom, the proportion of *Uroglena* was significantly higher in natural light communities ($P < 0.003$). Representing an average of 73% (without Fe added) and 27% (with Fe added) of the combined biomass, *Uroglena* had the highest yield of the three species under natural iron and irradiance levels (Fig. 3.15 b). The abundance of this alga was greatly reduced when light levels were lowered. Under this light regime, *Uroglena* contributed a mere 3% and 1% to the combined total biomass of these species in tanks with and without iron added. Iron fertilization did not stimulate significant changes in representation of this mixotroph under either light environment.

Neither iron ($P < 0.44$) nor light ($P < 0.38$) treatments altered the proportion of *Dinobryon* in relation to the other two species. *Dinobryon* populations accounted for a very small component (ranging from and average of 0% to 8%) of the day 28 combined biomass regardless of experimental environments.

3.3.5 Field Experiment 3

Total community biomass, chrysophyte biomass, and the biomass of *Synedra*, *Uroglena*, and *Dinobryon* were not significantly affected by the treatments applied in this trial. The total biomass yields of the natural light communities in the third experiment were lower than either of the first two experiments (Fig. 3.4). The natural light communities supported total, chrysophyte, and *Synedra* biomass amounts similar to, or lower than, those reared under low light regardless of iron fertilization. Trends of *Uroglena* (Fig. 3.7 c) were consistent with the first two trials. However, low population densities of this species in each treatment and variability among replicates rendered differences not statistically significant. *Uroglena* biomass was lower when light levels were reduced (natural light with iron added, ~ 248 µg/L; without iron added ~ 162 µg/L, compared to low light, with iron added ~ 19 µg/L; without iron added ~ 66 µg/L). Iron additions did not significantly increase/decrease the crop size of this mixotroph.

In comparison to *Synedra* and *Uroglena*, *Dinobryon* biomass yields were low (maximum of 51 µg/L) and highly variable (Fig. 3.8 c). Although no significant effects were observed when the total biomass was the response variable of interest, significant changes in the proportion of *Synedra*, *Uroglena*, and *Dinobryon* (with respect to each other) occurred as a result of the treatments applied.

The proportion of *Synedra* , in relation to the other two species, was significantly higher under low light levels ($P < 0.01$) (Fig. 3.15 c). Populations of this autotroph accounted

for, on average, 65% and 29% (with and without iron enrichment, respectively) of the total biomass of these three algae under low light. In natural light environments, *Synedra* represented 14% (with Fe added) and 3% (without Fe added) of the combined biomass. Under natural and reduced irradiance levels, iron addition led to increased yields of *Synedra*. These differences were marginally insignificant ($P < 0.09$).

In contrast to the *Synedra* results, the proportion of *Uroglena* was significantly higher in the natural light communities ($P < 0.01$). Comprising an average of 73% (with Fe added) and 93% (without Fe added) of the combined biomass, *Uroglena* populations achieved the greatest day 28 yield of the three species under natural light. The addition of iron resulted in decreased representation of this alga in both light regimes. The difference in proportions resulting from Fe enrichment were marginally insignificant ($P < 0.08$).

Neither iron ($P < 0.88$) nor light ($P < 0.43$) treatments altered the proportion of *Dinobryon* in relation to *Synedra* and *Uroglena*. Representing anywhere from 4% to 19%, *Dinobryon* populations accounted for a small component of the day 28 combined biomass in each experimental environment.

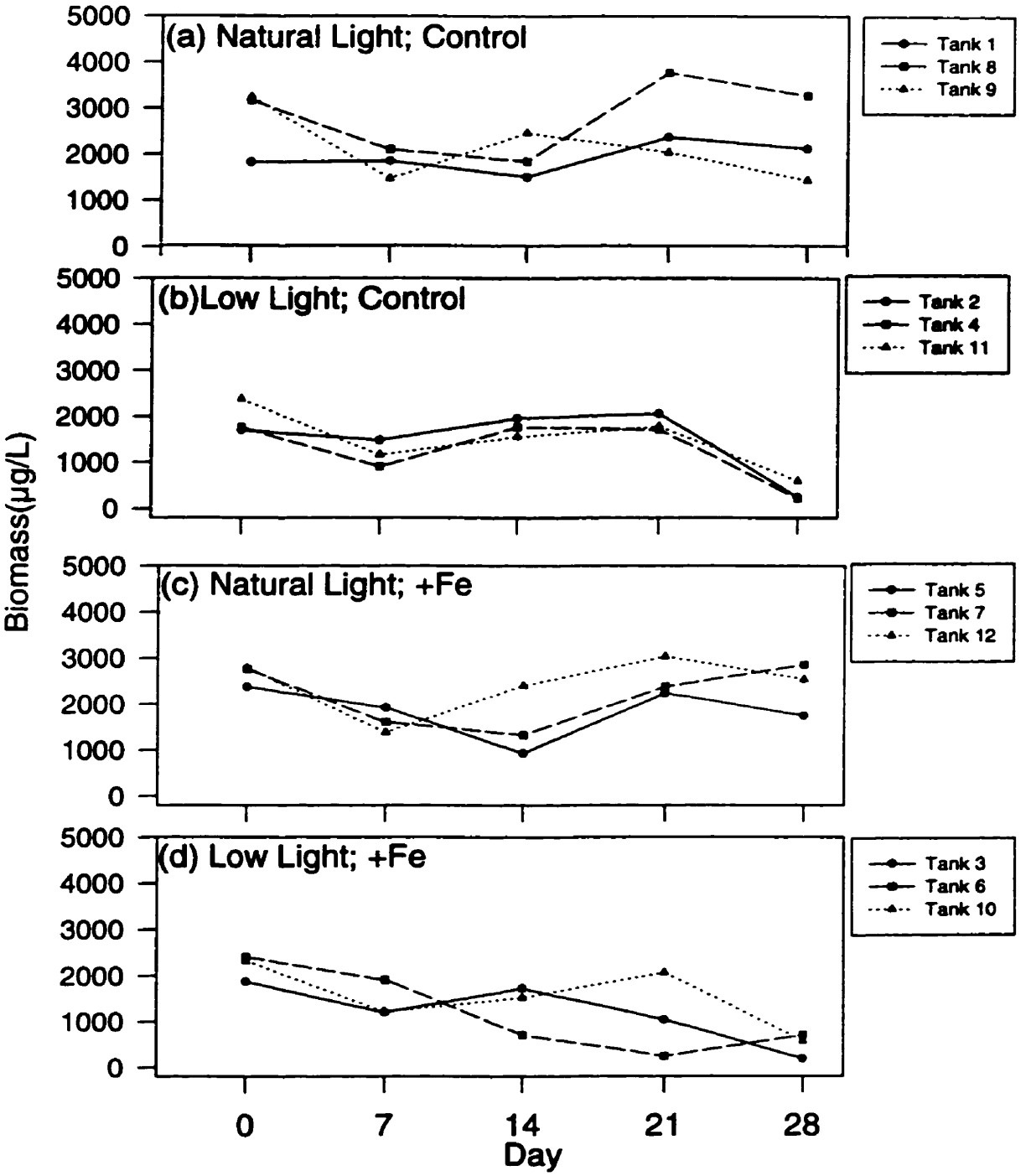

Figure 3.1: Phytoplankton biomass of each replicate at each sampling date for all treatments of whole community field experiment 1

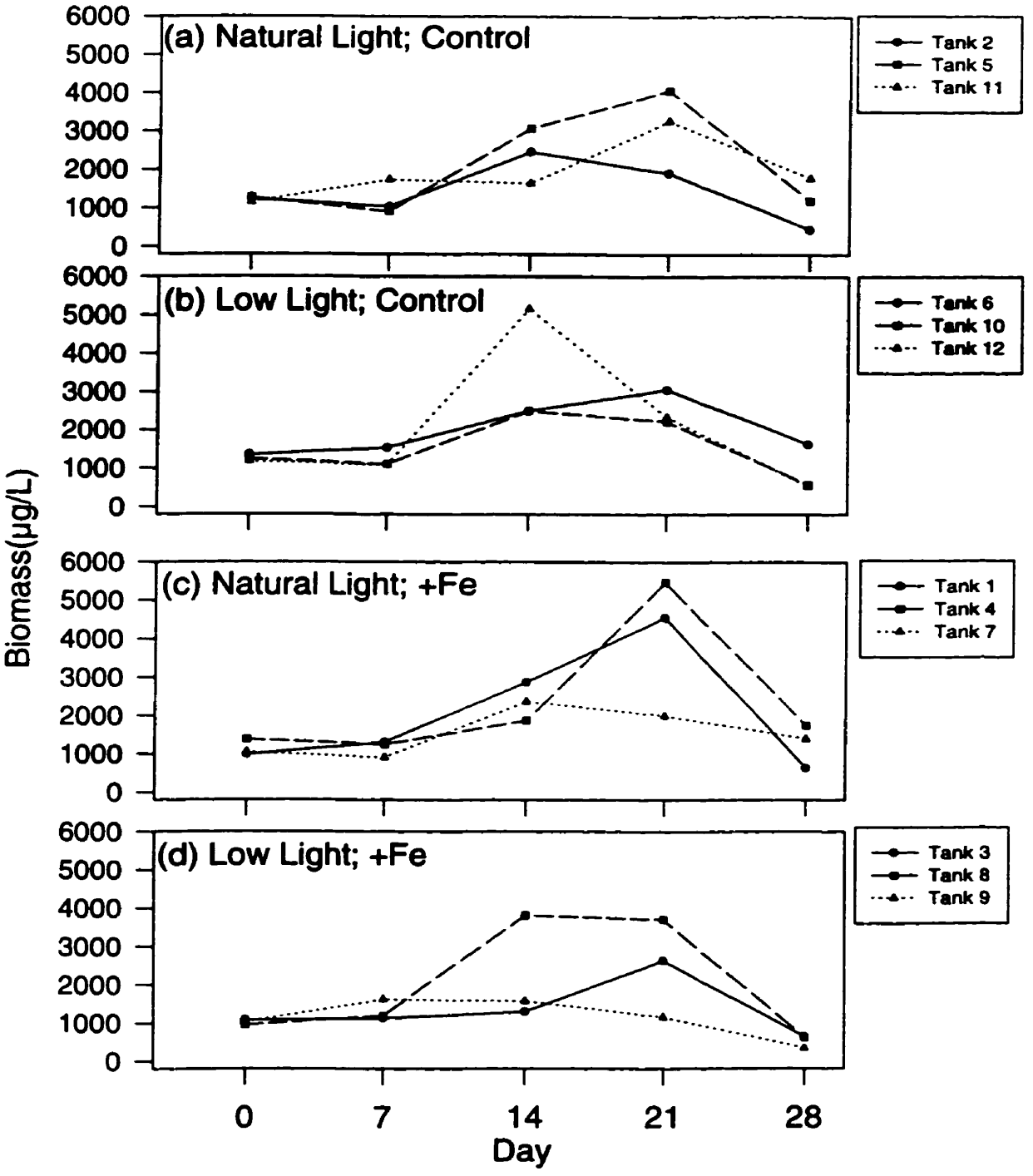

Figure 3.2: Phytoplankton biomass of each replicate at each sampling date for all treatments of whole community field experiment 2

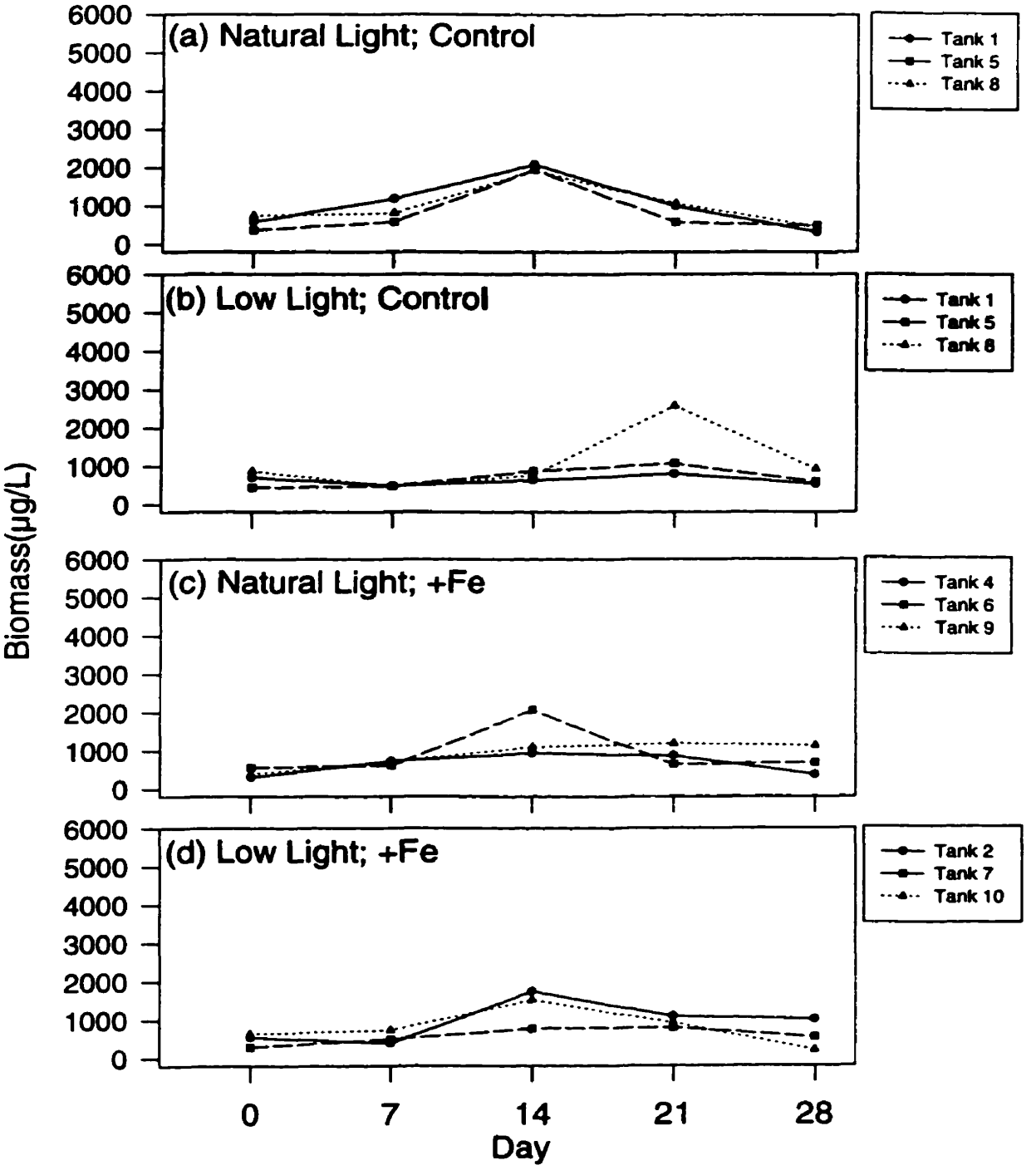

Figure 3.3: Phytoplankton biomass of each replicate at each sampling date for all treatments of whole community field experiment 3

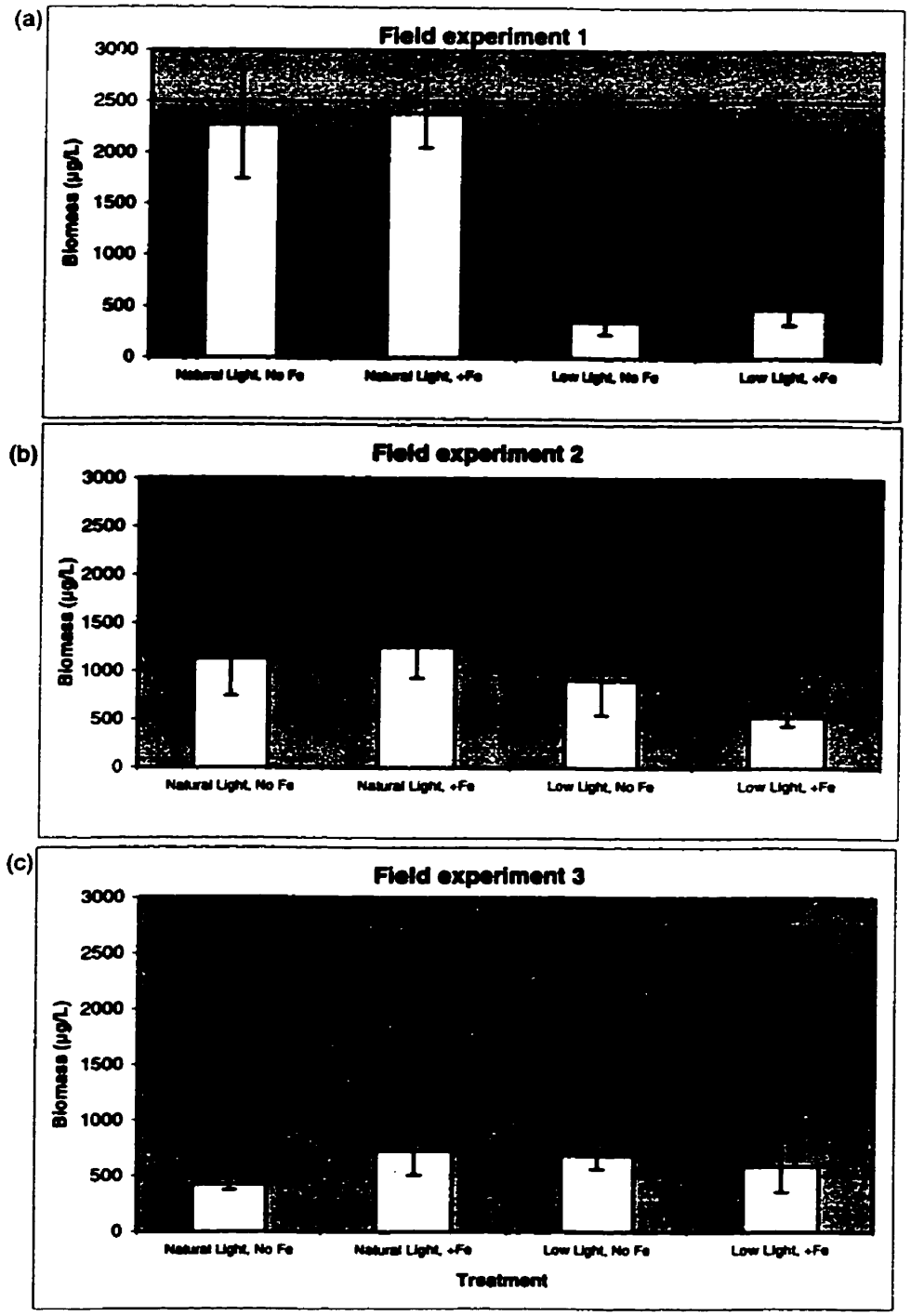

Figure 3.4: Average total phytoplankton day 28 biomass (+/- 1 SE) from whole community field experiments 1, 2, and 3

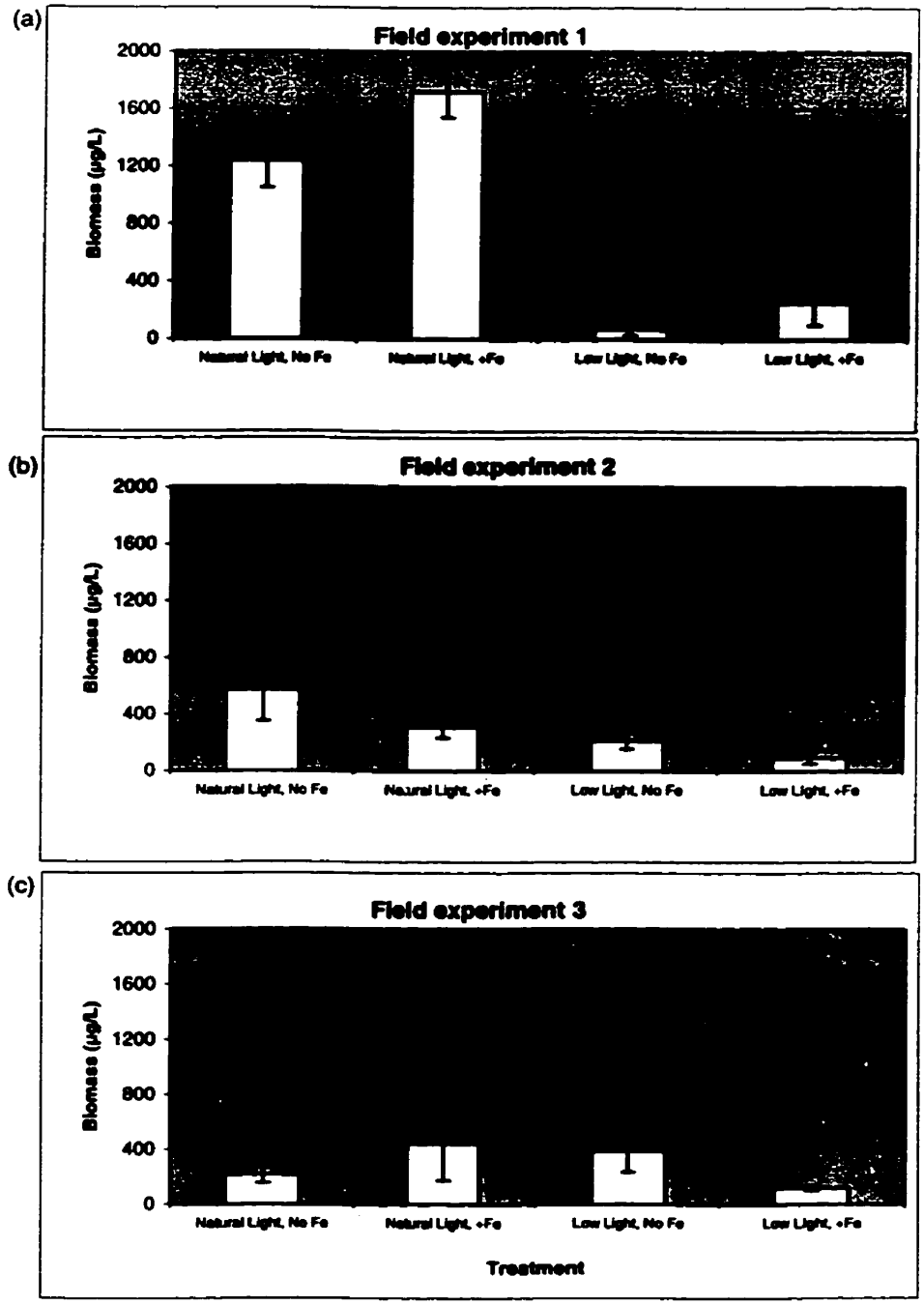

Figure 3.5: Average Chrysophyte day 28 biomass (+/- 1 SE) from whole community field experiments 1, 2, and 3

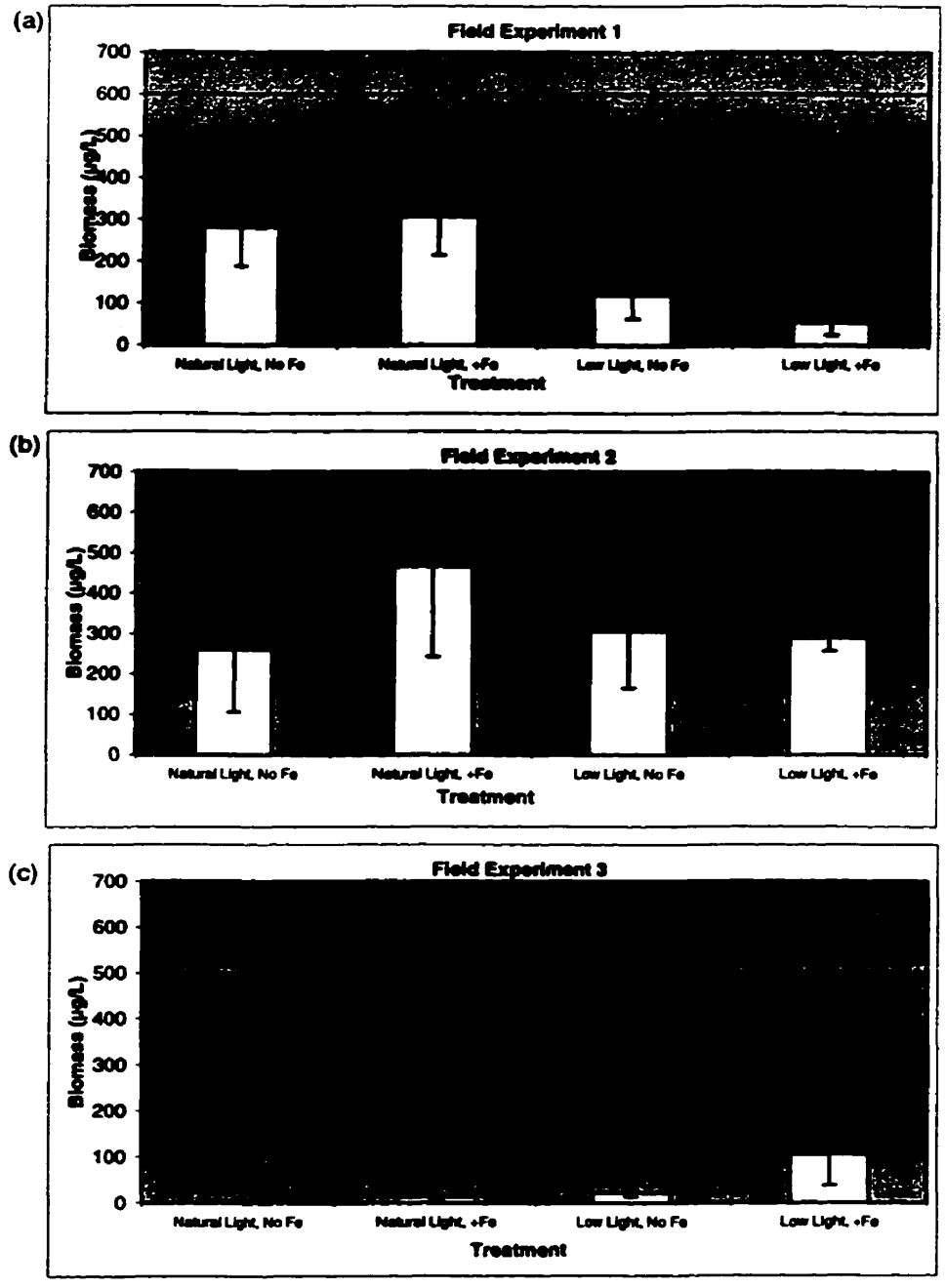

Figure 3.6: Average *Synedra* day 28 biomass (+/- 1 SE) from whole community field experiments 1, 2, and 3

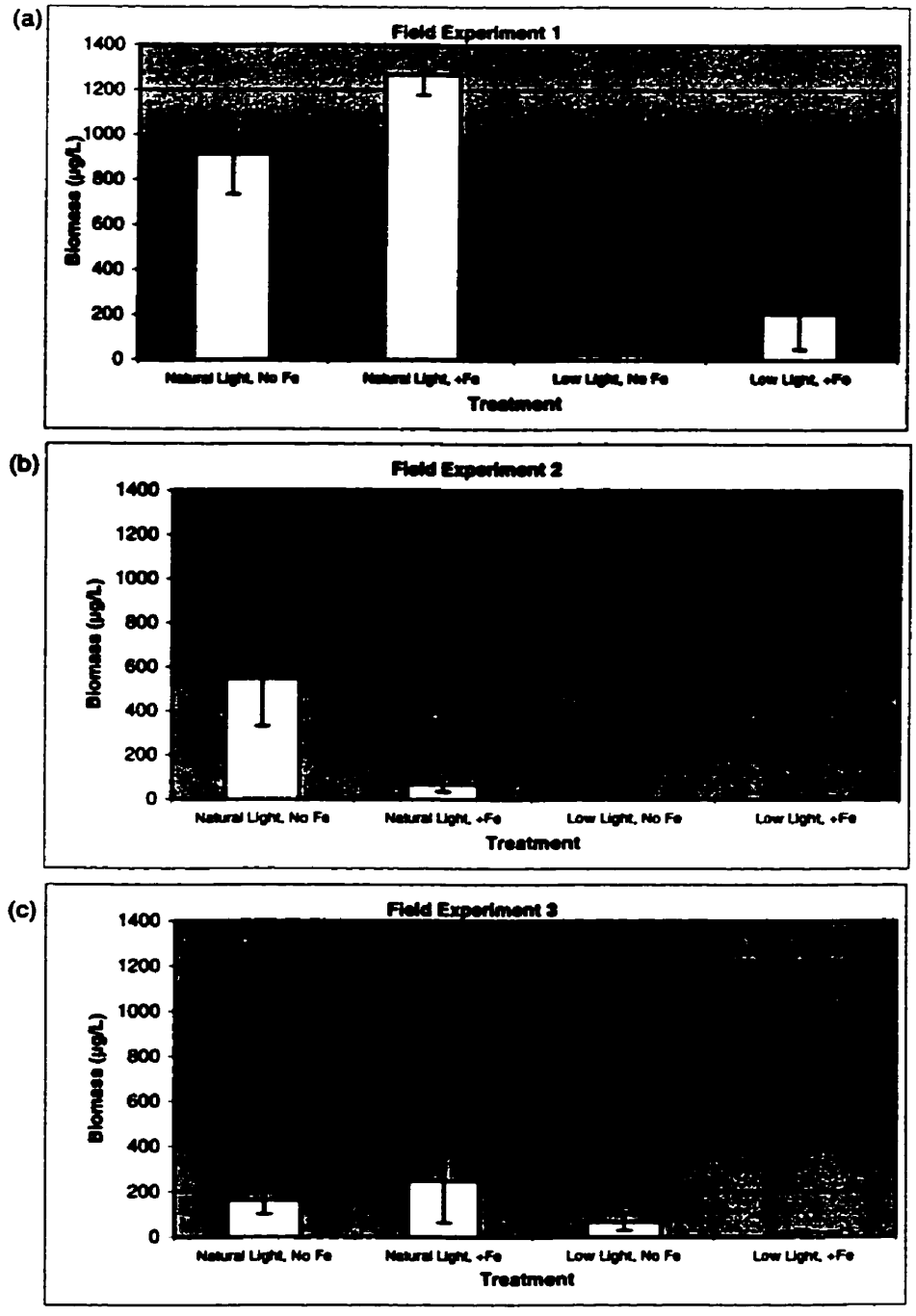

Figure 3.7: Average *Uroglena* day 28 biomass (+/- 1 SE) from whole community field experiments 1, 2, and 3

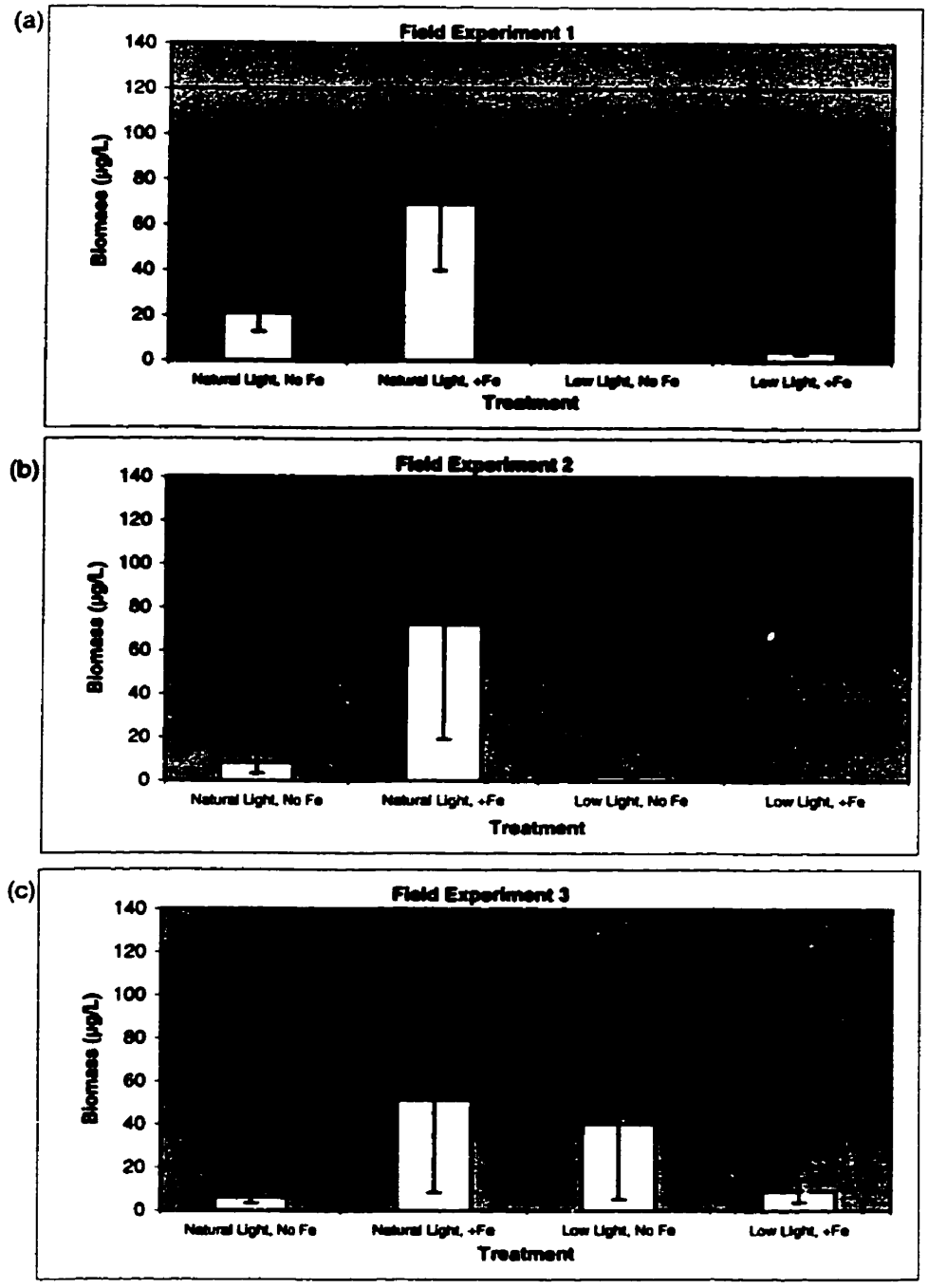

Figure 3.8: Average *Dinobryon* day 28 biomass (+/- 1 SE) from whole community field experiments 1, 2, and 3

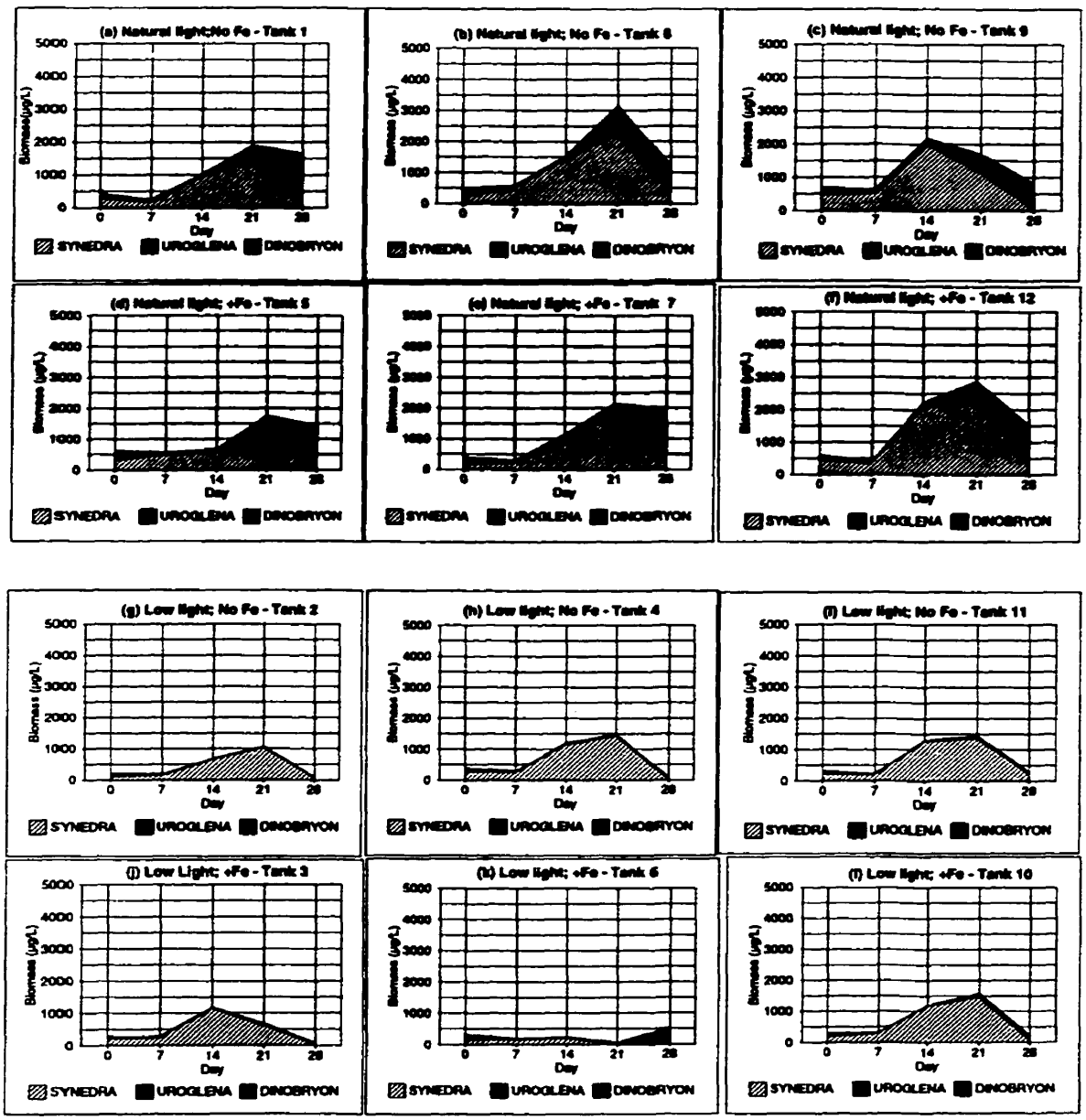

Figure 3.9: Area plots illustrating biomass of Synedra, Uroglena, and Dinobryon in each replicate of all light and iron treatments of whole community field experiment 1

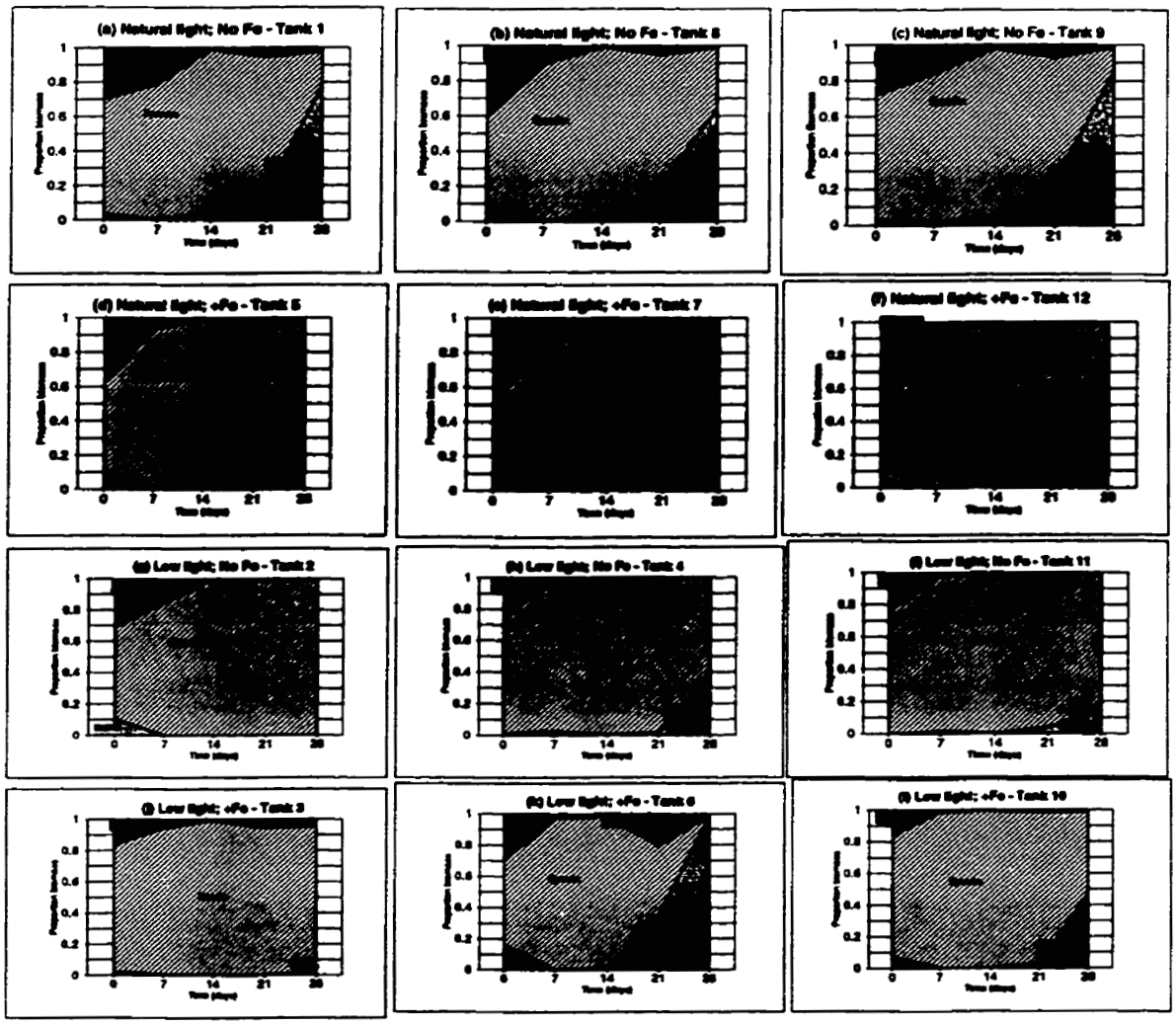

Figure 3.10: Areas plots illustrating proportional biomass of Synedra, Uroglena, and Dinobryon (in relation to each other) in each replicate of all light and iron treatments of whole community field experiment 1

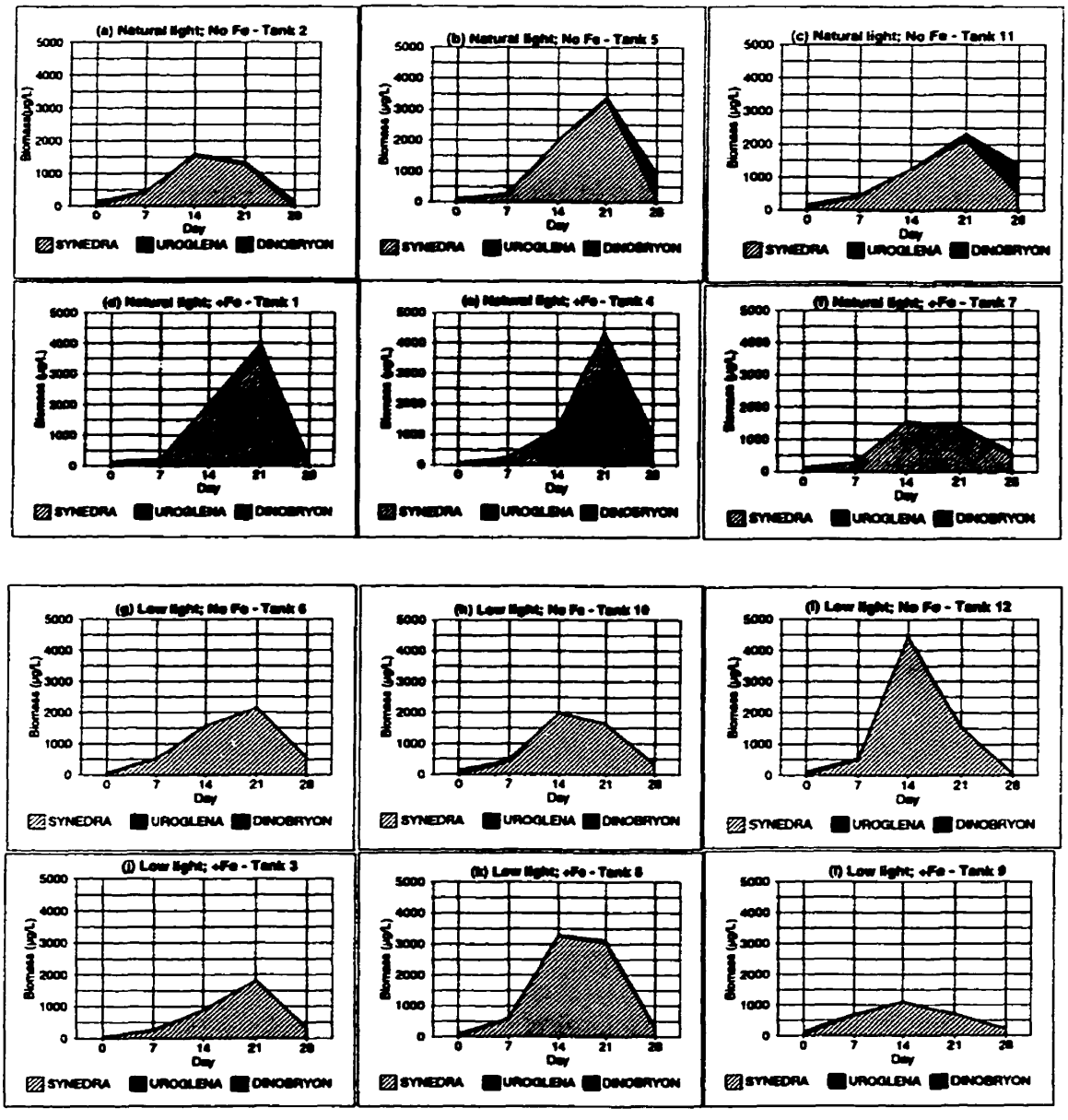

Figure 3.11: Area plots illustrating biomass of Synedra, Uroglena, and Dinobryon in each replicate of all light and iron treatments of whole community field experiment 2

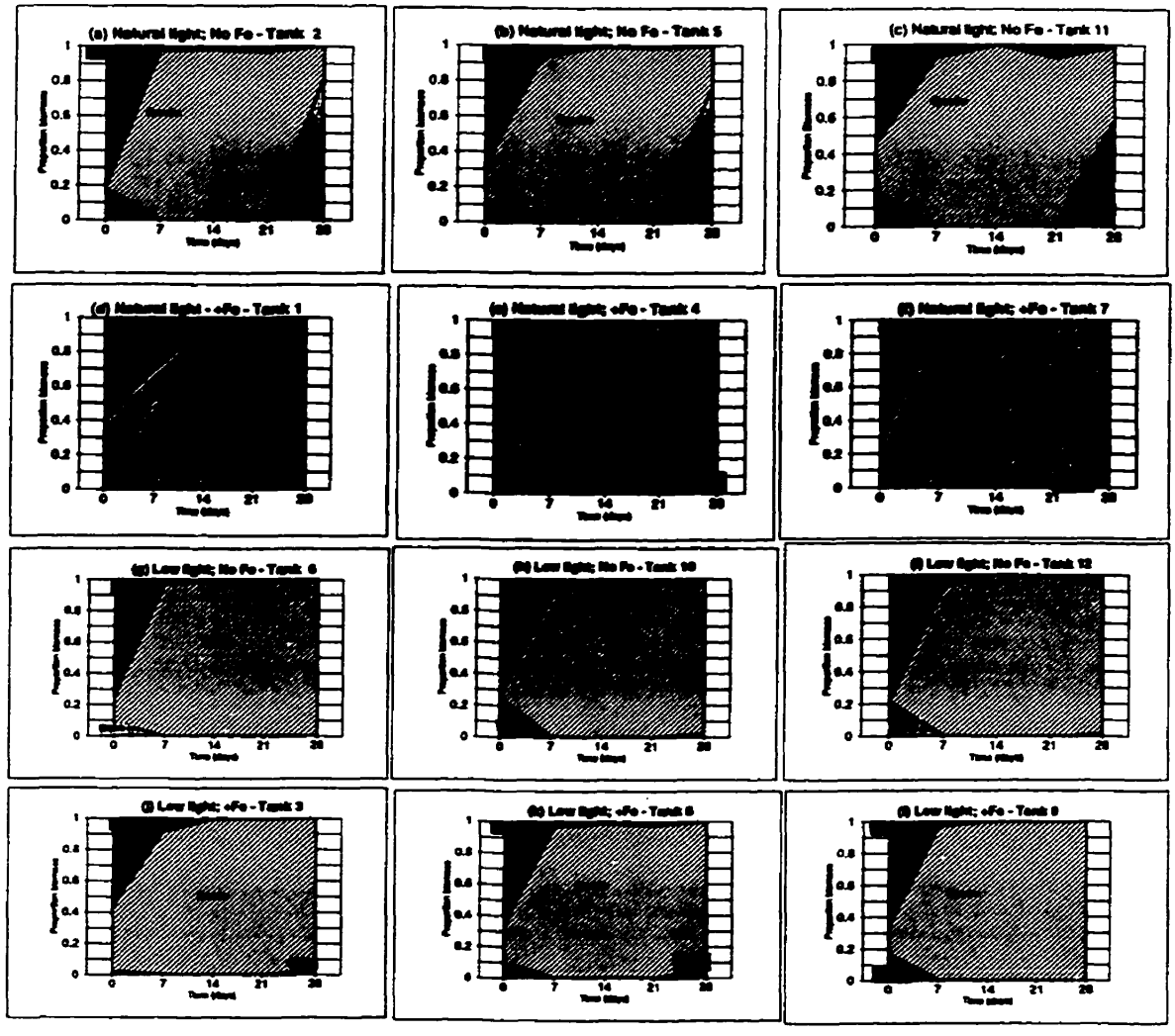

Figure 3.12: Areas plots illustrating proportional biomass of Synedra, Uroglena, and Dinobryon (in relation to each other) in each replicate of all light and iron treatments of whole community field experiment 2

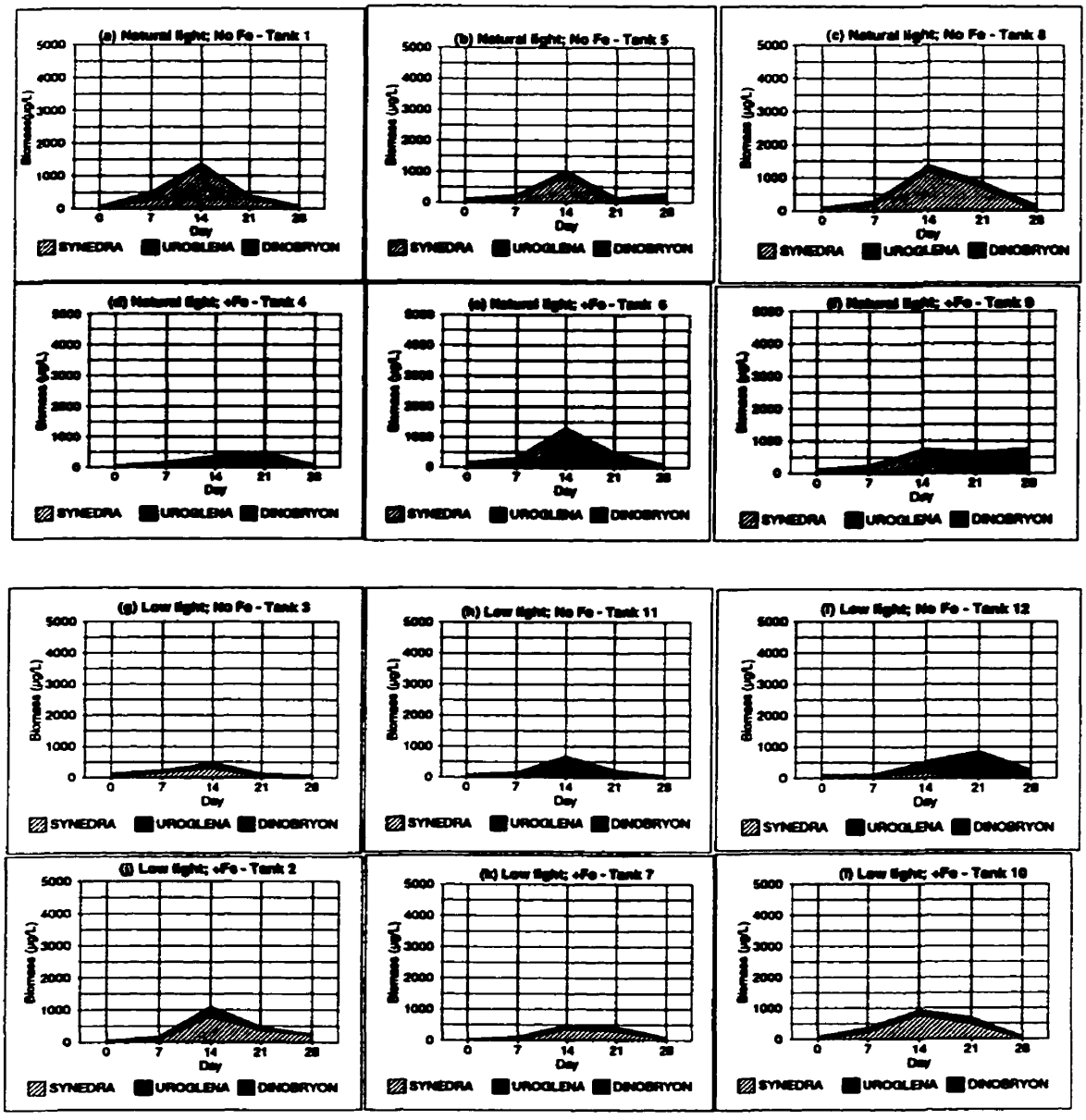

Figure 3.13: Area plots illustrating biomass of Synedra, Uroglena, and Dinobryon in each replicate of all light and iron treatments of whole community field experiment 3

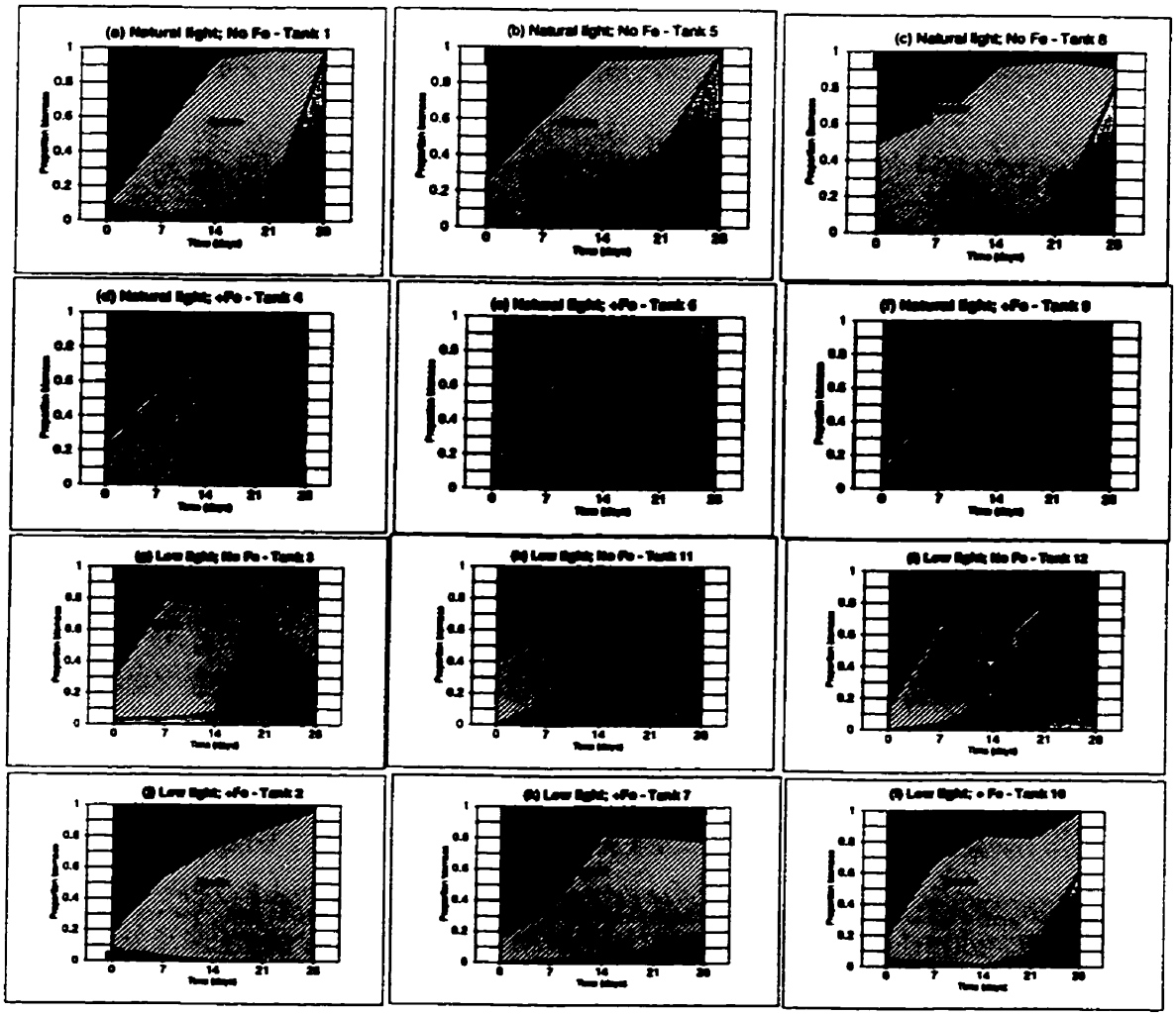

Figure 3.14: Areas plots illustrating proportional biomass of Synedra, Uroglena, and Dinobryon (in relation to each other) in each replicate of all light and iron treatments of whole community field experiment 3

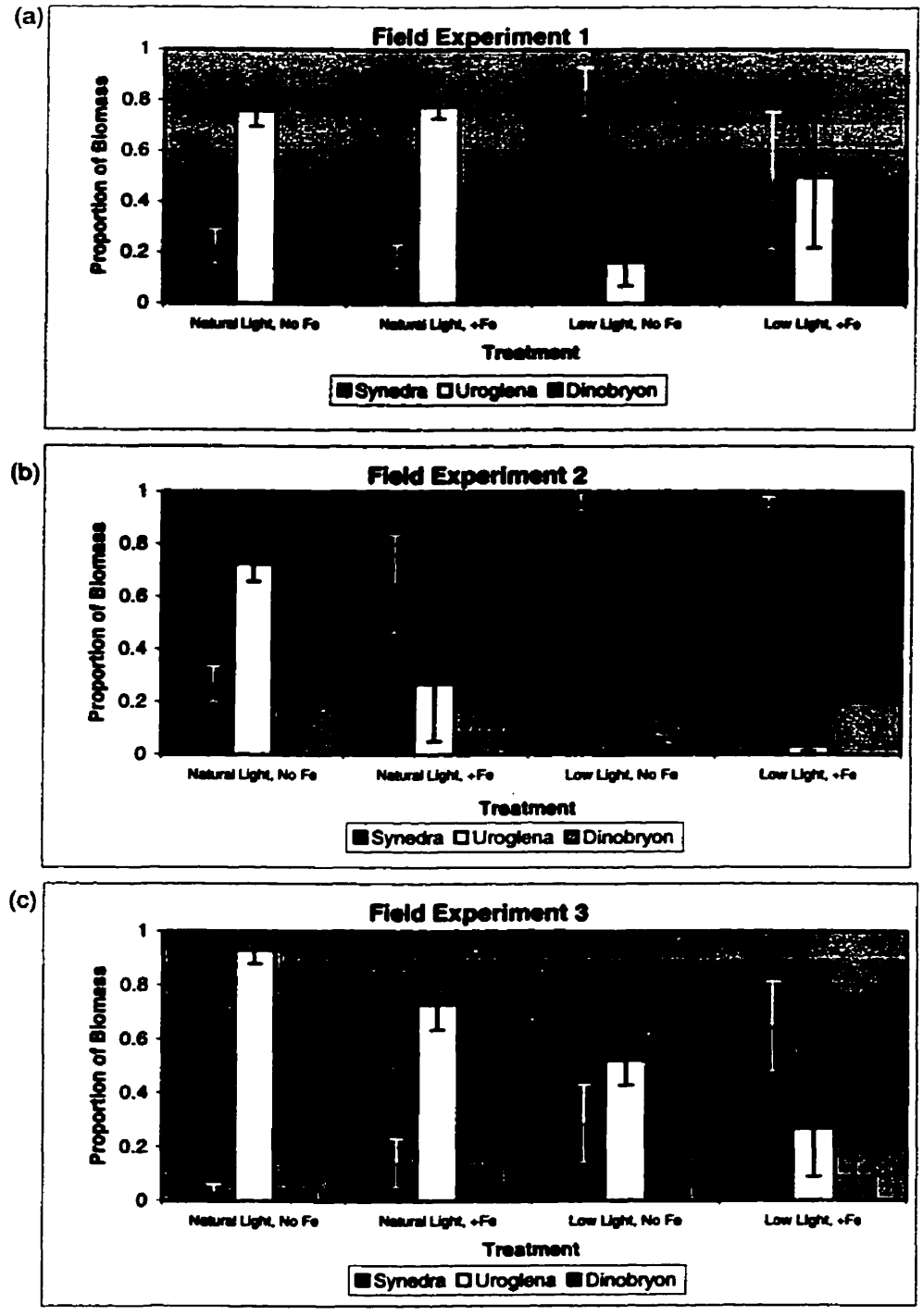

Figure 3.15: Average day 28 proportional biomass (+/- 1 SE) of *Synedra*, *Uroglena*, and *Dinobryon* (in relation to each other)

Table 3.2: Initial (day 0) biological, chemical, and physical conditions for field experiments 1, 2, and 3. Numbers reported as an average of all twelve mesocosms.

VARIABLE	Units	EXPERIMENT 1 (19-May-1998)	2 (02-July-1998)	3 (17-August-1998)
BIOLOGICAL				
Total algal biomass	µg/L	2368	1159	533
Chrysophyte biomass	µg/L	652	585	274
Synedra biomass	µg/L	268	15	17
Uroglena biomass	µg/L	24	20	4
Dinobryon biomass	µg/L	128	82	72
Chlorophyll a	µg/L	4.45	2.18	1.89
Zooplankton biomass	µg/L	Negligible	Negligible	Negligible
CHEMICAL				
Silica Concentration	mg/L	3.12	—	3.79
Total Phosphorus concentration	µg/L	28.7	26.5	—
Soluble Reactive Phosphorus Concentration	µg/L	5.5	4.1	—
PHYSICAL				
pH		7.8	8.0	7.9
Water Temperature	°C	14.2	17.3	18.0
Air Temperature	°C	20.0	20.0	21.0

* Denotes start date of experiment

Table 3.3: Results of ANOVA's on total biomass and proportional biomass (among S, D, and U) of *Synedra*, *Uroglena*, and *Dinobryon* from whole phytoplankton community experiments 1, 2, and 3.

EXPERIMENT	EFFECT	DEPENDENT VARIABLE	df	MS	F	P>F
1	Light (L)	**Total**	1	10 949 839	33.72	0.0004♣♣♣
	Iron (I)		1	39 710	0.12	0.7356
	L x I		1	285	0.00	0.9771
	Error (L x I)		8	324 718		
2	Light (L)	**Total**	1	676 633	2.30	0.1680
	Iron (I)		1	48 121	0.16	0.6966
	L x I		1	187 775	0.64	0.4476
	Error (L x I)		8	294 486		
3	Light (L)	**Total**	1	10 698	0.12	0.7392
	Iron (I)		1	31 345	0.35	0.5715
	L x I		1	118 385	1.31	0.2847
	Error (L x I)		8	90 050		
1	Light (L)	**Chrysophytes**	1	5 212 558	75.52	<0.0001♣♣♣♣
	Iron (I)		1	330 904	4.79	0.0600♣
	L x I		1	66 976	0.97	0.3534
	Error (L x I)		8	69 020		
2	Light (L)	**Chrysophytes**	1	246 763	5.61	0.0453♣♣
	Iron (I)		1	108 186	2.46	0.1554
	L x I		1	14 077	0.32	0.5871
	Error (L x I)		8	43 978		
3	Light (L)	**Chrysophytes**	1	17 626	0.24	0.6395
	Iron (I)		1	1 325	0.02	0.8971
	L x I		1	184 140	2.48	0.1543
	Error (L x I)		8	74 398		
1	Light (L)	**Synedra**	1	129 065	7.99	0.0223♣♣
	Iron (I)		1	1 174	0.07	0.7943
	L x I		1	6 089	0.38	0.5564
	Error (L x I)		8	16 158		
2	Light (L)	**Synedra**	1	12 766	0.18	0.6848
	Iron (I)		1	26 885	0.37	0.5581
	L x I		1	37 297	0.52	0.4922
	Error (L x I)		8	72 008		
3	Light (L)	**Synedra**	1	8 976	2.53	0.1502
	Iron (I)		1	6 422	1.81	0.2152
	L x I		1	4 301	1.21	0.3027
	Error (L x I)		8	3 545		
1	Light (L)	**Uroglena**	1	2 865 248	57.53	<0.0001♣♣♣♣
	Iron (I)		1	218 349	4.38	0.0696♣
	L x I		1	21 463	0.43	0.5299
	Error (L x I)		8	49 801		
2	Light (L)	**Uroglena**	1	269 280	7.49	0.0256♣♣
	Iron (I)		1	167 324	4.65	0.0631♣
	L x I		1	179 781	5.00	0.0558♣
	Error (L x I)		8	35 956		
3	Light (L)	**Uroglena**	1	79 462	2.70	0.1390

	Iron (I)		1	1 112	0.04	0.8508
	L x I		1	13 501	0.46	0.5174
	Error (L x I)		8	29 437		
1	Light (L)	**Dinobryon**	1	5 517	7.75	0.0238♣♣
	Iron (I)		1	2 087	2.92	0.1258
	L x I		1	1 472	2.07	0.1883
	Error (L x I)		8	711		
2	Light (L)	**Dinobryon**	1	4 578	2.13	0.1827
	Iron (I)		1	2 889	1.34	0.2799
	L x I		1	3 300	1.53	0.2506
	Error (L x I)		8	2 151		
3	Light (L)	**Dinobryon**	1	51	0.02	0.8858
	Iron (I)		1	151	0.06	0.8054
	L x I		1	4 362	1.87	0.2085
	Error (L x I)		8	2 331		
1	Light (L)	**Proportion**	1	0.621	9.24	0.0161♣♣
	Iron (I)	**Synedra**	1	0.114	1.70	0.2290
	L x I		1	0.072	1.07	0.3308
	Error (L x I)		8	0.067		
2	Light (L)	**Proportion**	1	0.765	25.15	0.0010♣♣♣
	Iron (I)	**Synedra**	1	0.114	3.75	0.0888♣
	L x I		1	0.106	3.50	0.0983♣
	Error (L x I)		8	0.030		
3	Light (L)	**Proportion**	1	0.433	9.97	0.0134♣♣
	Iron (I)	**Synedra**	1	0.168	3.87	0.0848♣
	L x I		1	0.045	1.05	0.3354
	Error (L x I)		8	0.043		
1	Light (L)	**Proportion**	1	0.576	8.18	0.0211♣♣
	Iron (I)	**Uroglena**	1	0.091	1.3	0.2864
	L x I		1	0.075	1.07	0.3316
	Error (L x I)		8	0.070		
2	Light (L)	**Proportion**	1	0.682	16.88	0.0034♣♣
	Iron (I)	**Uroglena**	1	0.141	3.49	0.0988♣
	L x I		1	0.173	4.28	0.0724♣
	Error (L x I)		8	0.040		
3	Light (L)	**Proportion**	1	0.572	14.49	0.0052♣♣
	Iron (I)	**Uroglena**	1	0.154	3.90	0.0836♣
	L x I		1	0.002	0.05	0.8220
	Error (L x I)		8	0.039		
1	Light (L)	**Proportion**	1	0.00068	3.12	0.1156
	Iron (I)	**Dinobryon**	1	0.00188	8.65	0.0187♣♣
	L x I		1	0.000008	0.04	0.8494
	Error (L x I)		8	0.0002		
2	Light (L)	**Proportion**	1	0.0021	0.85	0.3834
	Iron (I)	**Dinobryon**	1	0.0016	0.65	0.4430
	L x I		1	0.0090	3.40	0.1023
	Error (L x I)		8	0.0030		
3	Light (L)	**Proportion**	1	0.0096	0.70	0.4269
	Iron (I)	**Dinobryon**	1	0.0003	0.02	0.8862
	L x I		1	0.0280	2.04	0.1912
	Error (L x I)		8	0.0138		

Note: ♣ $P < 0.1$, ♣♣ $P < 0.05$, ♣♣♣ $P < 0.001$, ♣♣♣♣ $P < 0.0001$

Table 3.4: Average day 28 biomass with associated standard errors with and without iron addition under natural and low light levels for each species/group of algae studied in the whole phytoplankton community experiments.

EXPERIMENT	LIGHT LEVEL	IRON			
1	Natural	No	Total biomass	2276.97	533.76
1	Natural	Yes	Total biomass	2382.27	330.30
1	Low	No	Total biomass	356.73	121.95
1	Low	Yes	Total biomass	481.53	155.20
2	Natural	No	Total biomass	1132.30	385.09
2	Natural	Yes	Total biomass	1255.83	327.90
2	Low	No	Total biomass	907.53	357.38
2	Low	Yes	Total biomass	530.70	95.74
3	Natural	No	Total biomass	424.83	51.90
3	Natural	Yes	Total biomass	725.70	220.62
3	Low	No	Total biomass	683.20	121.89
3	Low	Yes	Total biomass	586.77	232.04
1	Natural	No	Chrysophyte biomass	1238.50	188.72
1	Natural	Yes	Chrysophyte biomass	1720.03	176.75
1	Low	No	Chrysophyte biomass	69.77	42.31
1	Low	Yes	Chrysophyte biomass	252.47	152.91
2	Natural	No	Chrysophyte biomass	570.60	217.98
2	Natural	Yes	Chrysophyte biomass	312.20	79.23
2	Low	No	Chrysophyte biomass	215.30	
2	Low	Yes	Chrysophyte biomass	93.90	38.56

3	Natural	No	Chrysophyte biomass	213.40	58.61
3	Natural	Yes	Chrysophyte biomass	440.13	267.97
3	Low	No	Chrysophyte biomass	384.50	153.73
3	Low	Yes	Chrysophyte biomass	115.73	17.90
1	Natural	No	Synedra biomass	281.90	93.97
1	Natural	Yes	Synedra biomass	307.17	92.30
1	Low	No	Synedra biomass	119.53	57.06
1	Low	Yes	Synedra biomass	54.70	30.65
2	Natural	No	Synedra biomass	260.87	155.86
2	Natural	Yes	Synedra biomass	467.03	224.51
2	Low	No	Synedra biomass	307.13	142.13
2	Low	Yes	Synedra biomass	290.30	33.93
3	Natural	No	Synedra biomass	4.20	4.20
3	Natural	Yes	Synedra biomass	12.60	7.27
3	Low	No	Synedra biomass	21.03	8.43
3	Low	Yes	Synedra biomass	105.17	67.71
1	Natural	No	Uroglena biomass	915.73	178.65
1	Natural	Yes	Uroglena biomass	1270.10	92.50
1	Low	No	Uroglena biomass	23.03	13.22
1	Low	Yes	Uroglena biomass	208.23	160.47
2	Natural	No	Uroglena biomass	546.73	216.64
2	Natural	Yes	Uroglena biomass	65.77	31.13
2	Low	No	Uroglena biomass	2.33	1.86
2	Low	Yes	Uroglena biomass	10.97	6.05
3	Natural	No	Uroglena biomass	162.10	61.15
3	Natural	Yes	Uroglena biomass	248.43	184.08
3	Low	No	Uroglena biomass	66.43	38.60
3	Low	Yes	Uroglena biomass	18.60	11.60

1	Natural	No	Dinobryon biomass	21.10	8.31
1	Natural	Yes	Dinobryon biomass	69.57	29.62
1	Low	No	Dinobryon biomass	0.36	0.36
1	Low	Yes	Dinobryon biomass	4.53	1.33
2	Natural	No	Dinobryon biomass	8.30	5.14
2	Natural	Yes	Dinobryon biomass	72.50	53.27
2	Low	No	Dinobryon biomass	2.40	2.01
2	Low	Yes	Dinobryon biomass	0.27	0.27
3	Natural	No	Dinobryon biomass	6.13	2.78
3	Natural	Yes	Dinobryon biomass	51.37	43.02
3	Low	No	Dinobryon biomass	40.13	34.95
3	Low	Yes	Dinobryon biomass	9.10	5.34
				AVERAGE DAY 28 PROPORTION	STD. ERROR
1	Natural	No	Proportion Synedra	0.22	0.07
1	Natural	Yes	Proportion Synedra	0.18	0.05
1	Low	No	Proportion Synedra	0.83	0.10
1	Low	Yes	Proportion Synedra	0.48	0.27
2	Natural	No	Proportion Synedra	0.27	0.07
2	Natural	Yes	Proportion Synedra	0.65	0.19
2	Low	No	Proportion Synedra	0.96	0.03
2	Low	Yes	Proportion Synedra	0.97	0.02
3	Natural	No	Proportion Synedra	0.03	0.03
3	Natural	Yes	Proportion Synedra	0.14	0.09

3	Low	No	Proportion Synedra	0.29	0.14
3	Low	Yes	Proportion Synedra	0.65	0.17
1	Natural	No	Proportion Uroglena	0.76	0.06
1	Natural	Yes	Proportion Uroglena	0.78	0.05
1	Low	No	Proportion Uroglena	0.16	0.10
1	Low	Yes	Proportion Uroglena	0.50	0.28
2	Natural	No	Proportion Uroglena	0.73	0.07
2	Natural	Yes	Proportion Uroglena	0.27	0.22
2	Low	No	Proportion Uroglena	0.01	0.005
2	Low	Yes	Proportion Uroglena	0.03	0.02
3	Natural	No	Proportion Uroglena	0.93	0.05
3	Natural	Yes	Proportion Uroglena	0.73	0.10
3	Low	No	Proportion Uroglena	0.52	0.09
3	Low	Yes	Proportion Uroglena	0.27	0.18
1	Natural	No	Proportion Dinobryon	0.02	0.003
1	Natural	Yes	Proportion Dinobryon	0.04	0.01
1	Low	No	Proportion Dinobryon	0	0
1	Low	Yes	Proportion Dinobryon	0.03	0.01
2	Natural	No	Proportion Dinobryon	0.003	0.003
2	Natural	Yes	Proportion Dinobryon	0.08	0.05
2	Low	No	Proportion Dinobryon	0.03	0.03
2	Low	Yes	Proportion Dinobryon	0	0
3	Natural	No	Proportion Dinobryon	0.04	0.02

3	Natural	Yes	Proportion Dinobryon	0.13	0.04
3	Low	No	Proportion Dinobryon	0.19	0.11
3	Low	Yes	Proportion Dinobryon	0.09	0.07

3.4 Discussion of Results of Field Experiments

Despite the fact that the three field experiments were performed during three separate time intervals of a single ice-free growing season, and each began with unique initial conditions (see Table 3.2), results were generally consistent among trials.

3.4.1 Light Manipulation

Underwater light climate is an important factor in determining phytoplankton community structure (Smith 1986, Tilman 1985). Many concepts in aquatic ecology are based on irradiance levels and light gradients (i.e. it is possible to identify the euphotic depth where most photosynthesis takes place), yet resource competition theory primarily centers around competition for nutrients (see Tilman 1977, Van Donk and Kilham 1990). Light has received far less attention as a selective factor of algal dominance, probably because the presence of a vertical gradient makes light competition conceptually and experimentally more complex than nutrient competition (Huisman *et al.* 1999).

The critical light intensity, the light intensity supporting a steady-state algal population, is species specific and plays a critical role when phytoplankton species compete for light (Huisman 1999). A limited light supply per unit area can sustain only a limited number of phytoplankton cells per unit area. The algal species with the lowest critical light intensity should be the superior light competitor (Huisman and Weissing 1994). Recent light competition experiments have confirmed this prediction (Huisman *et al.* 1999).

In this study, similar to the results of the mixed populations laboratory experiments, light levels had relatively little effect on the total community biomass, and altering light levels most greatly affected the composition of species within a community. One exception to this generalization occurred in experiment one where low light treatments significantly reduced the total community biomass (Fig. 3.4). This experiment was performed in the latter part of May and the first half of June 1998, a time period in which the weather was frequently cloudy and rainy. Such weather naturally reduces irradiance levels and thereby intensifies the effects of the low-light treatments on the ambient light levels in the mesocosms. Thus, the low-light treatment combined with naturally lower irradiance levels likely resulted in the differences in total algal yield between light treatments to be significant in this trial.

Light manipulation most greatly affected the algal community composition in the whole phytoplankton community experiments. Similar to the laboratory experiments, the proportion of *Synedra* and *Uroglena* was correlated with light level. In general, the proportional representation of *Uroglena* decreased and that of *Synedra* increased as irradiance levels were lowered (Fig. 3.15).

The processes of light and shade adaptation in aquatic environments are also important processes in the determination of algal species selection/succession (Falkowski 1980). An organism may become more suited to its environmental light regime as a result of its physiological plasticity (i.e. changes in intracellular pigment content, chemical composition, cell volume, and changes in numbers and sizes of photosynthetic units

(PSU's)) (Falkowski *et al.* 1981). Some species of algae appear to be genotypically shade adapted and thrive at subsaturating light intensities, while other species demand high irradiance levels (Falkowski 1980). *Uroglena* biomass yields were greater than both *Synedra* and *Dinobryon* under natural light levels. Based on this result, *Uroglena* appears to be the most light adapted of these species as it was often the superior competitor under natural light environments regardless of iron fertilization

Opposite to the trends of *Uroglena*, *Synedra* dominated a majority of the low-light algal communities regardless of iron enrichment. This suggests that this diatom is more of a shade tolerant species than the other two mixotrophs and may have the lowest critical light intensity of the three species. These results support other published studies where diatoms have been found to be superior low light competitors (see Marks and Lowe 1993).

As seen in the mixed populations laboratory experiments, *Dinobryon* populations failed to achieve biomass levels greater than *Synedra* or *Uroglena* in any of the field experiment replicates.

The second treatment applied to the field mesocosms was an iron fertilization. The following section discusses the results of this manipulation.

3.4.2 Iron Fertilization

There are numerous documented examples of iron limited primary productivity in marine ecosystems (see Coale *et al.* 1996, Behrenfeld *et al.* 1996, Frost 1996, Rue and

Bruland 1997) and comparatively fewer examples of iron limitation in freshwater systems (see Van Donk 1983, Ishida *et al.* 1982).

In contrast to the work of Van Donk (1983), Ishida *et al.* (1982), and in contrast to initial expectations at the onset of this study, it was predicted, on the basis of the laboratory flask experiments, that chrysophyte growth would remain unaltered by the addition of iron. The lab studies also suggested that growth of the autotrophic diatom would be positively correlated with iron enrichment, and it was expected that populations of this alga would increase with iron enrichment.

Iron enrichment failed to consistently stimulate significant changes in the community biomass or species representation within the tanks (Fig. 3.15). The addition of iron did trigger significant changes (positive and negative) in algal abundance in each of the three experiments, but the results were not repeatable. For example, the proportional abundance of *Dinobryon* increased upon iron addition in the first experiment, but was not affected by the same treatment in either of the following trials.

In contrast to the predictions, the total biomass yield and proportional representation of *Synedra* was not significantly altered by the addition of chelated iron. One possible reason for the lack of consistency between the results of the laboratory and field work is the iron levels naturally present in the source water of the field studies.

The iron levels in the tanks prior to fertilization with chelated Fe were much greater (i.e. concentrations of total Fe > 10 μg/L) than the high iron treatment fabricated in the laboratory. It is possible that the algae in the field tanks were not limited by iron at

any point during the experiments. The amount of particulate and dissolved organic material in the ponds may have also contributed to the lack of an iron effect in the field studies. Munch (1972), Van Donk (1983), and Sandgren (1988) suggested that the chelating ability of lake water may have an impact on algal seasonality through its effect on iron availability for phytoplankton growth. The stained appearance and relatively high carbon levels (DOC ~ 25 mg/L) of the University of Calgary pond indicates that iron limitation may have been minimal, or non-existent, due to a large pool of chelating substances which would limit the amount of essential nutrients leaving this system.

3.4.3 Low Light Induced Iron Limitation

Based on the results presented by Sunda and Huntsman (1997) and the physiological mechanisms associated with the processes of photosynthesis (see Hopkins 1995, Sunda 1989), it was predicted that the iron requirements of freshwater algae will be greater under low light environments. This prediction was modified following the laboratory culture experiments (Chapter 2) such that only autotrophic phytoplankton were expected to express greater iron demands in low light environments. It was anticipated that low light tanks fertilized with Fe would have a greater autotroph component than non-fertilized low light tanks. Compared to communities exposed to natural light levels, the growth/abundance of autotrophic organisms was expected to exhibit a stronger positive correlation with iron addition under low light.

In contrast to the laboratory experiments, iron fertilization did not stimulate increased crop yields of the autotroph *Synedra* under low light, and no evidence that

growth of this alga is more correlated with iron addition under low light compared to high light was found.

The lack of an effect of iron addition under low light and the differences in the laboratory and field results may be attributed to the difference in ambient light levels in the low light treatments (lab and field), as well as the pre-existing iron levels in the pond water (see previous section).

In the laboratory, light levels were tightly controlled and were reduced to a uniform level of ~ 55 $\mu mol \cdot m^{-2} \cdot sec^{-1}$. Irradiance levels in the low light tanks (reaching subsurface levels of ~ 300 $\mu mol \cdot m^{-2} \cdot sec^{-1}$) were often higher than in the low light flasks in the lab and varied along a spatial gradient within the mesocosm (light levels could be 250 $\mu mol \cdot m^{-2} \cdot sec^{-1}$ lower at the bottom of the tank compared to the top). Because tanks were stirred daily and because some of the algae present in the tank community were mobile, it is unlikely that the algal cells were exposed to the low light levels at the bottom of the tank for a period of time greater than 24 hours. This point is supported by the fact that, as previously discussed, total community biomass did not differ between the low and natural light tanks in two of the three trials.

3.4.4 Conclusions and Future Considerations

The results of the field experiments strongly suggest that the total yields of phytoplankton populations within the experimental communities were not reduced by iron and light manipulations.

As seen in figures 3.1, 3.2, and 3.3, the total algal biomass reached an asymptotic maximum in each trial and phytoplankton populations failed to maintain exponential growth for the duration of the studies. The obvious, yet not simple, question now becomes what factor(s) govern the growth of these algal populations?. Inspection of the measured physical and chemical parameters (independent variables) reveals that no single answer can be derived for this question. The lack of a single explanation is both curious and intriguing.

As phosphorus is considered the nutrient which most frequently limits freshwater primary productivity (Schindler 1978, Sakamoto 1966), this nutrient emerges as the primary suspect in this ecological mystery. The possibility of phosphorus limitation, however, is quickly dismissed when the experimental levels of this nutrient are noted. As seen in figures A5 and A6, total phosphorus (TP) concentrations and, more importantly, soluble reactive phosphorus concentrations (SRP) levels remained within detectable limits (SRP was no less than ~ 2µg/L) in these experiments. There is no indication from these values that phosphorus was a growth-limiting resource.

Because diatoms represented a substantial component of the experimental phytoplankton communities, silica is another resource which may have played a vital role in controlling primary productivity. Silica concentrations remained high in the experiments in which it was monitored (experiment 1: Si > 1 p.p.m., experiment 2: Si > 2 p.p.m.) (Figs. A7, A8). As with phosphorus, these values fail to provide evidence of Si limitation in these trials.

Water temperature fluctuated on a daily cycle, but did not exhibit any significant increasing/decreasing trends during the experiments. These results fail to provide evidence of a mechanism which is driving the temporal phytoplankton dynamics.

Total organic and dissolved organic carbon (TOC and DOC, respectively) levels were randomly monitored and both were always found to be present in amounts well above levels that would render them growth-limiting nutrients.

Daily maintenance activities performed throughout each experiment included stirring the tanks and scrubbing the interior walls. Because the tanks were stirred each day, it is unlikely that algal cells were settling out of the phytoplankton communities. Reductions in algal biomass resulting from cells settling out of the water column should not have been a significant factor in these experiments. Epiphytic algal growth was kept to a minimum as a result of scrubbing the mesocosm walls on a daily basis. Nutrient uptake and/or other competitive/interfering mechanisms commonly introduced by the presence of epiphytes were unlikely to have limited the growth of the planktonic algal community.

The presence of zooplankton grazers can significantly decrease the crop size of a phytoplankton community (McCauley and Murdoch 1987, McCauley and Briand 1979). However, because these experiments were performed in an environment which was virtually zooplankton-free, grazing effects on phytoplankton yields were assumed to be minimal.

The measured physical and chemical parameters do not provide any conclusive evidence about the mechanisms limiting primary productivity in the experimental systems. It is, therefore, important to consider some factors which were not analyzed in these studies.

Nitrogen, as is phosphorus, is an essential macronutrient of algal growth which can limit aquatic primary productivity (McCauley and Downing 1991, Smith 1986). Nitrogen levels were not measured in these experiments, and the possibility that the assemblages were N-limited should not be excluded. However, it should be noted that current nitrogen manipulation experiments on similar aquatic systems by Vandermeulen, Watson, Hopper, and McCauley (University of Calgary) have failed to reveal that these phytoplankton communities are limited by nitrogen (pers. comm.). It is recommended that any future studies carefully incorporate regular monitoring of nitrogen levels into their design.

Bacterial community assemblage and biomass were not measured for the field experiments. Due to the fact that algal communities contained populations of mixotrophic and heterotrophic algae (phagotrophs), it is possible that bacterial abundance may have decreased during the experiments, thereby reducing the nutrient pool for the phagotrophs. This possibility, however, does not account for the declining total biomass as it is unlikely that autotrophic organisms were affected (they may even benefit) from such a change in prokaryote abundance.

Alternatively, bacterial communities may have increased in size throughout the experiments. As bacteria compete with phytoplankton for nutrients, and recognizing that changes in bacterial abundance can trigger changes in algal abundance (Maranger *et al.* 1998), it is possible that the bacteria may have been more effective nutrient scavengers than the algae, and increases in their numbers may have resulted in a decline in phytoplankton cells. It is highly recommended that bacterial communities be monitored in any future studies.

The results presented provide strong evidence that growth of these phytoplankton communities was not limited by any of the variables measured/manipulated during this study. The field experiments also raised suspicions about what factor, or set of factors, regulate algal growth in such systems. Any similar future experiments should be designed in such a way as to ensure all possible growth-limiting factors are carefully monitored.

Chapter 4: Concluding Statements

The following statements describe the major questions asked and conclusions achieved during this thesis.

(I) Does iron limit the growth of freshwater phytoplankton populations which display growth patterns that are independent of phosphorus concentrations?

Although phosphorus is considered the nutrient which most often limits freshwater primary productivity, this generalization does not hold true for all classes of algae. Growth of chrysophyte (golden-brown) algae is only weakly correlated to phosphorus levels, and this group of planktonic algae frequently dominate aquatic systems containing low levels of phosphorus. It was hypothesized that freshwater chrysophyte growth is limited by iron. While several researchers have proposed that iron-limited primary productivity can exist in freshwater systems, relatively few studies have attempted to address this hypothesis. Many more examples of iron-limited primary productivity exist for marine systems.

It is apparent from the results of the laboratory and field experiments that growth of *Uroglena* and *Dinobryon* is not limited by iron.

In the laboratory, *Uroglena* and *Dinobryon* populations were unaffected by the lack of an iron fertilization to the growth medium. The levels of iron addition that resulted in greatly increased biomass yield of the diatom (15 nM and 150 nM) failed to stimulate higher biomass of the chrysophyte populations.

It was initially anticipated that the abundance of chrysophytes in the whole phytoplankton communities would increase following fertilization with iron. This expected result was not observed in any of the field trials. In fact, iron enrichment of the mesocosm assemblages failed to stimulate significant changes in the algal proportional abundance and total biomass of the communities.

It is important to remember that the phytoplankton populations isolated in the lab and mesocosm communities were established from natural systems (Glenmore Reservoir and University of Calgary campus pond) which are subject to sporadic blooms of *Uroglena* and *Dinobryon*.

(II) Do freshwater phytoplankton exhibit higher iron requirements in reduced light environments?

Support for the hypothesis that iron requirements of freshwater phytoplankton increase as irradiance levels decrease was provided by *Synedra* reared in flasks in the laboratory. Under conditions of high light, biomass yields of cultures reared in 15 nM Fe medium increased to levels significantly greater than those reared in the 0 nM Fe medium. Under low light, however, enriching cultures with 15 nM of iron failed to elevate *Synedra* populations to sizes significantly greater than observed in cultures excluded from iron enrichment. Only the 150 nM Fe treatment resulted in *Synedra* biomass yields greater than that observed at 0 nM Fe under low irradiance.

In contrast to the results of the autotroph, there was no evidence of a low-light-induced elevated iron requirement in the mixotroph cultures. *Uroglena* and *Dinobryon*

populations achieved a similar maximum biomass with and without iron addition in low light environments.

Whether differences in low light growth responses of the autotroph and two mixotrophs occurred as a result of differences in the abilities of the algae to acquire nutrients (discussed in greater detail in the following section), or as a result of differences in the individual critical light intensities, or as a result of a combination of mechanisms, lower irradiances appear to elevate the iron requirements of *Synedra* more so than of *Uroglena* and *Dinobryon* in these experiments.

There was no evidence of elevated iron requirements of the experimental phytoplankton species as a result of decreased light levels in the field studies. The total algal biomass' within the mesocosms were not significantly different in assemblages reared under low light regardless of iron fertilization. More specifically, the individual populations of *Synedra*, *Uroglena*, and *Dinobryon* did not consistently increase in size following iron enrichment of the field tanks. It is possible that iron concentrations naturally present in the pond water were sufficient to overcome the physiological affects imposed by reducing light levels.

(III) Are mixotrophs like *Uroglena* and *Dinobryon* more robust to the effects of iron limitation than obligate autotrophs (i.e. *Synedra*) in freshwater systems?

As iron is an essential element required by algae to perform metabolic and physiological processes, it was expected that the growth of all algae would be negatively affected by reductions in ambient iron concentrations (see (I)). However, it was also

predicted that growth *Uroglena* and *Dinobryon* would be less affected by such reductions in non-axenic environments due to their abilities to acquire essential nutrients from bacteria.

The results of the laboratory culture experiments strongly supported these predictions. Populations of *Synedra* (an autotrophic diatom) achieved smaller biomass yields in flasks not fertilized with iron regardless of light level. While it is unlikely that omitting Fe from the medium resulted in an iron-free environment (as indicated by the ability of the autotroph to maintain positive growth in the 0 nM Fe treatments), the lack of iron addition consistently dampened *Synedra* yields.

Unlike *Synedra*, populations of *Uroglena* and *Dinobryon* reared in the 0 nM Fe medium achieved biomass yields similar to, and sometimes greater than, those replicates to which iron was added. These results indicate that the mixotrophic species have a competitive advantage over the autotroph when growing in iron-deficient environments. Differences between the growth responses of mixotrophic and autotrophic phytoplankton may be the result of differences in their method(s) of nutrient acquisition.

The results of the field mesocosm experiments failed to support the prediction that iron addition would stimulate an increase in *Synedra* biomass. Iron fertilization did not increase the proportional abundance of *Synedra* in the tanks, and *Uroglena* and *Dinobryon* proportions were, in general, not correlated with the absence/presence of iron enrichment. Unlike the lab culture experiments, the Fe treatments of the field mesocosm

experiments simply elevated naturally existing iron levels of the pond water. It is possible that natural iron levels (organic and inorganic) were sufficient to support populations of all algae such that iron competition was not a factor driving the dynamics of these phytoplankton communities.

(IV) (a) Can results obtained from laboratory experiments that examined the effect(s) of iron/light limitation on the growth of isolated algal populations be used to predict the outcome of competition between the same species in the laboratory? (b) Can the results of iron/light manipulation experiments between a fixed number of algal populations in the laboratory be used to predict the outcome of competition between the same species in whole phytoplankton community iron/light manipulation field experiments?

Much debate exists in ecology about the roles and effectiveness of using microcosms as tools to explain processes driving ecological phenomena at larger scales (Carpenter 1996).

Predictions derived from the results of the individual population experiments were, for the most part, successful when used to estimate the outcome of competition between three algal species in culture.

It was accurately predicted that when individual populations of *Synedra*, *Uroglena*, and *Dinobryon* were mixed within a single vessel the species that would become dominant was dependent upon iron addition and the light environment.

Under high light, the proportional abundance of *Synedra* increased with each increase in iron level to the extent that this species co-dominated (i.e. ~50% of total biomass) algal assemblages reared in the 150 nM Fe medium. It was also successfully predicted that high light assemblages fertilized with 0 nM and 15 nM of iron would be dominated by mixotrophs.

Under low light, the results of the individual population experiments also allowed for generally accurate predictions about the outcome of competition. *Synedra* only increased significantly following high iron additions. Two of three replicates became dominated (i.e. > 90% of total biomass) by this diatom, and the third supported similar autotroph yields. It was only due to a large, and unexpected, increase of *Uroglena* that *Synedra* did not dominate.

The 0 nM and 15 nM Fe high light replicates became dominated by mixotrophs.

A series of expectations were generated regarding the day 28 algal assemblage in the whole phytoplankton communities within the field mesocosms based on the results of laboratory competition experiments.

The derived expectations were generally successful despite the number of independent variables introduced by performing the experiments in a field setting (i.e. time of year, weather, initial conditions).

It was accurately predicted that light levels would have a significant effect on algal community composition. *Synedra* dominated low light mesocosms, whereas

Uroglena , and to a lesser extent *Dinobryon*, consistently dominated natural light assemblages.

The results of the field experiments indicate that algal abundance in natural systems is not solely regulated by ambient iron concentrations. The species composition of algal communities within the tanks did not change significantly as a result of iron enrichment. This discrepancy between the results of the laboratory and field experiments was likely a function of the natural iron levels in the campus pond. Unlike the lab cultures, in which an iron-limited growth environment was established (as indicated by trends of *Synedra* growth), it is improbable that similar conditions existed within the field mesocosms (natural iron levels in the field (controls) were greater than high iron treatments in the lab). It is, in hind sight, not reasonable to expect similar growth responses to iron enrichment in the lab and field studies.

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APPENDIX I

Additional Detailed Results of Field Mesocosm Experiments

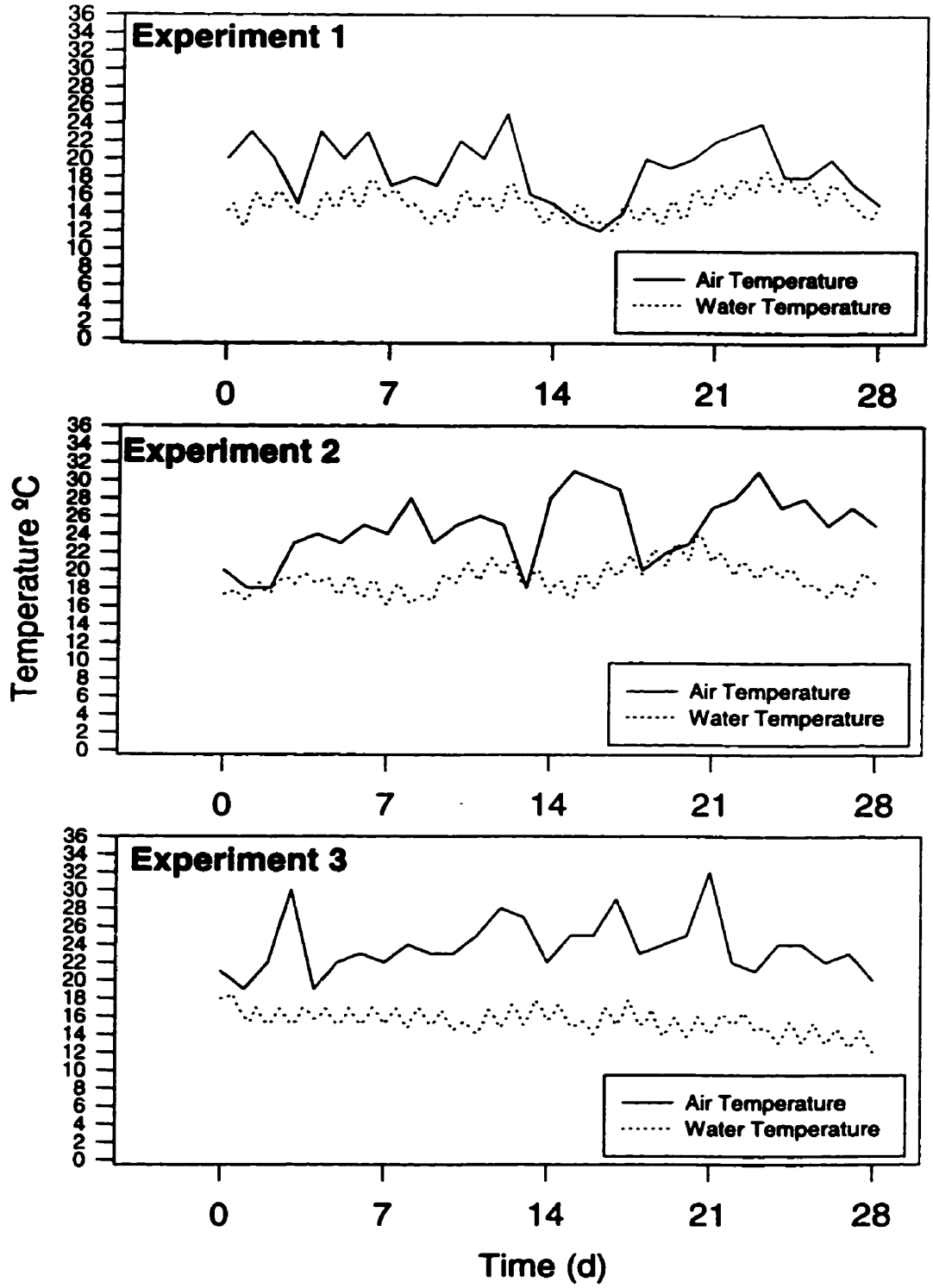

Figure A1: Water temperature of representative mesocosm and air temperature for field experiments 1, 2, and 3.

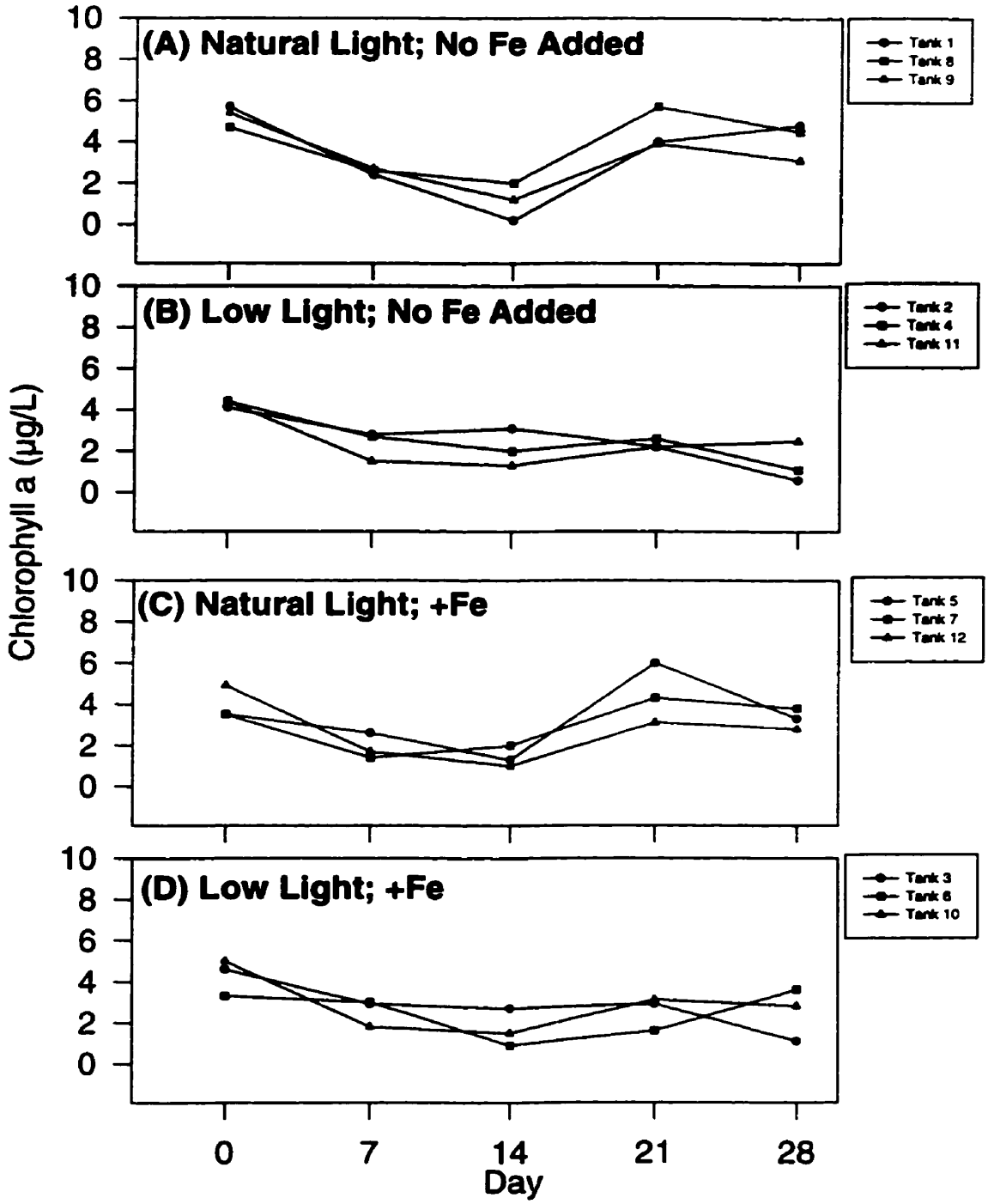

Figure A2: Chlorophyll a concentrations of each mesocosm of field experiment 1

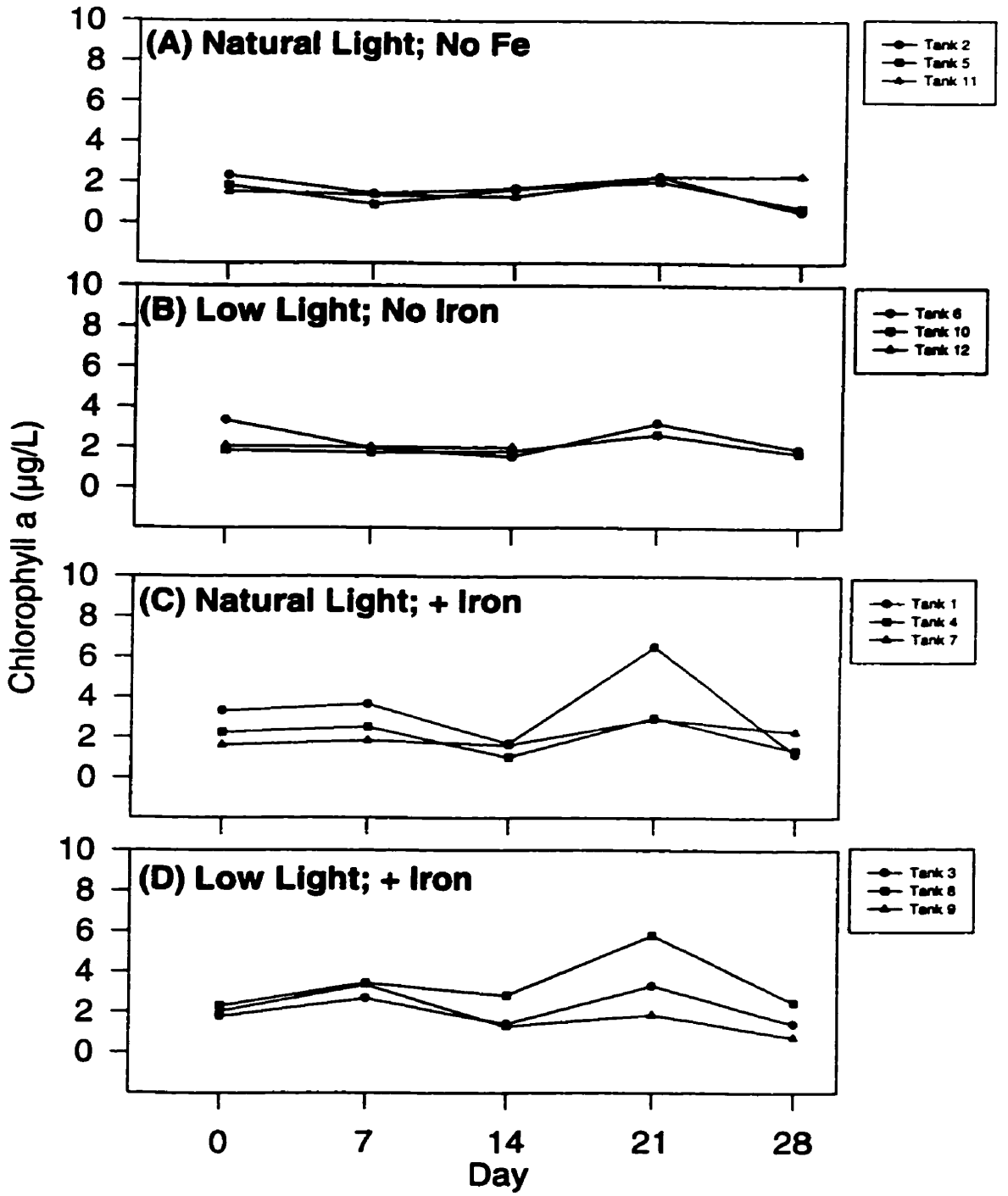

Figure A3: Chlorophyll a concentrations of each mesocosm of field experiment 2

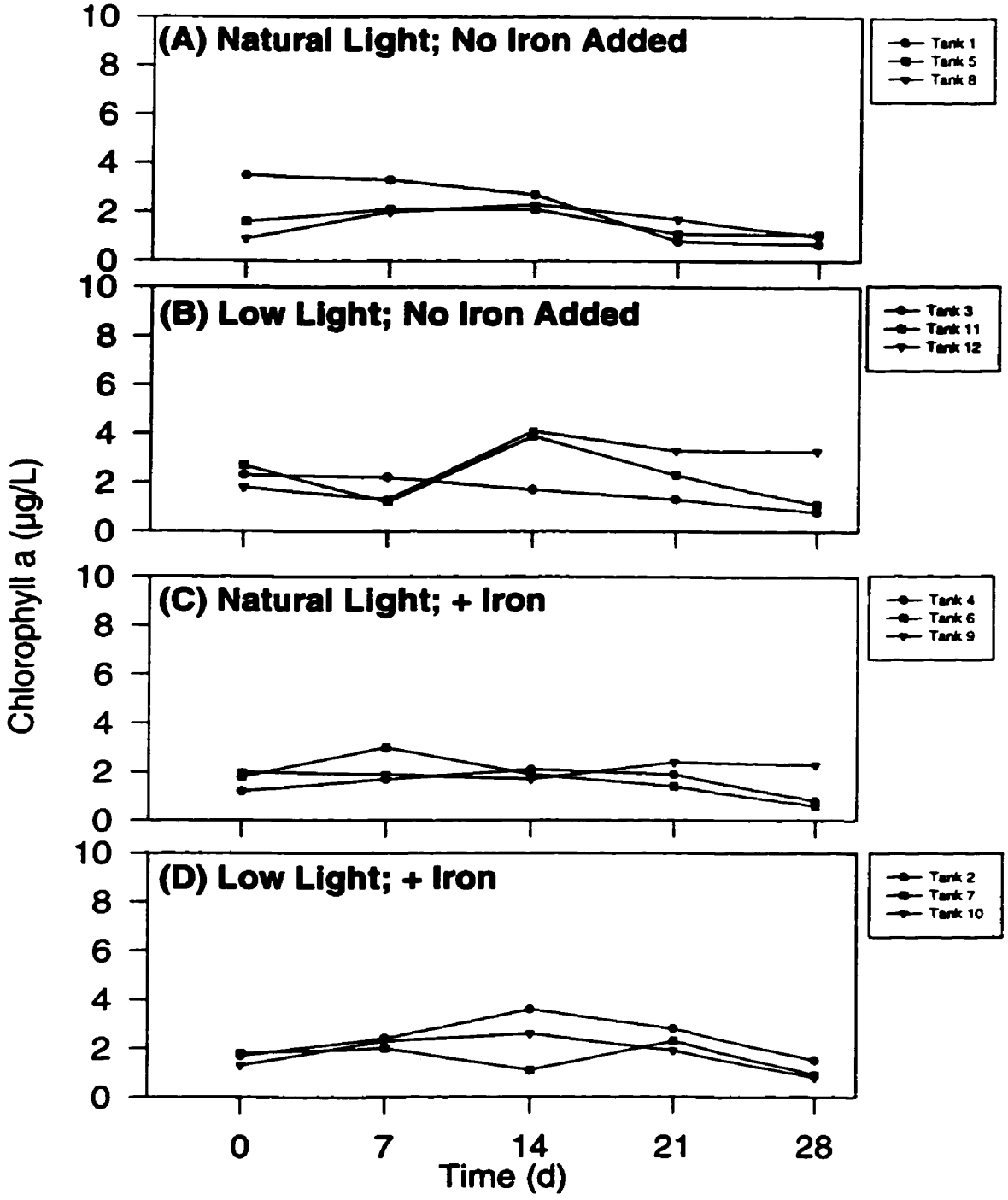

Figure A4: Chlorophyll a concentrations of each mesocosm of field experiment 3

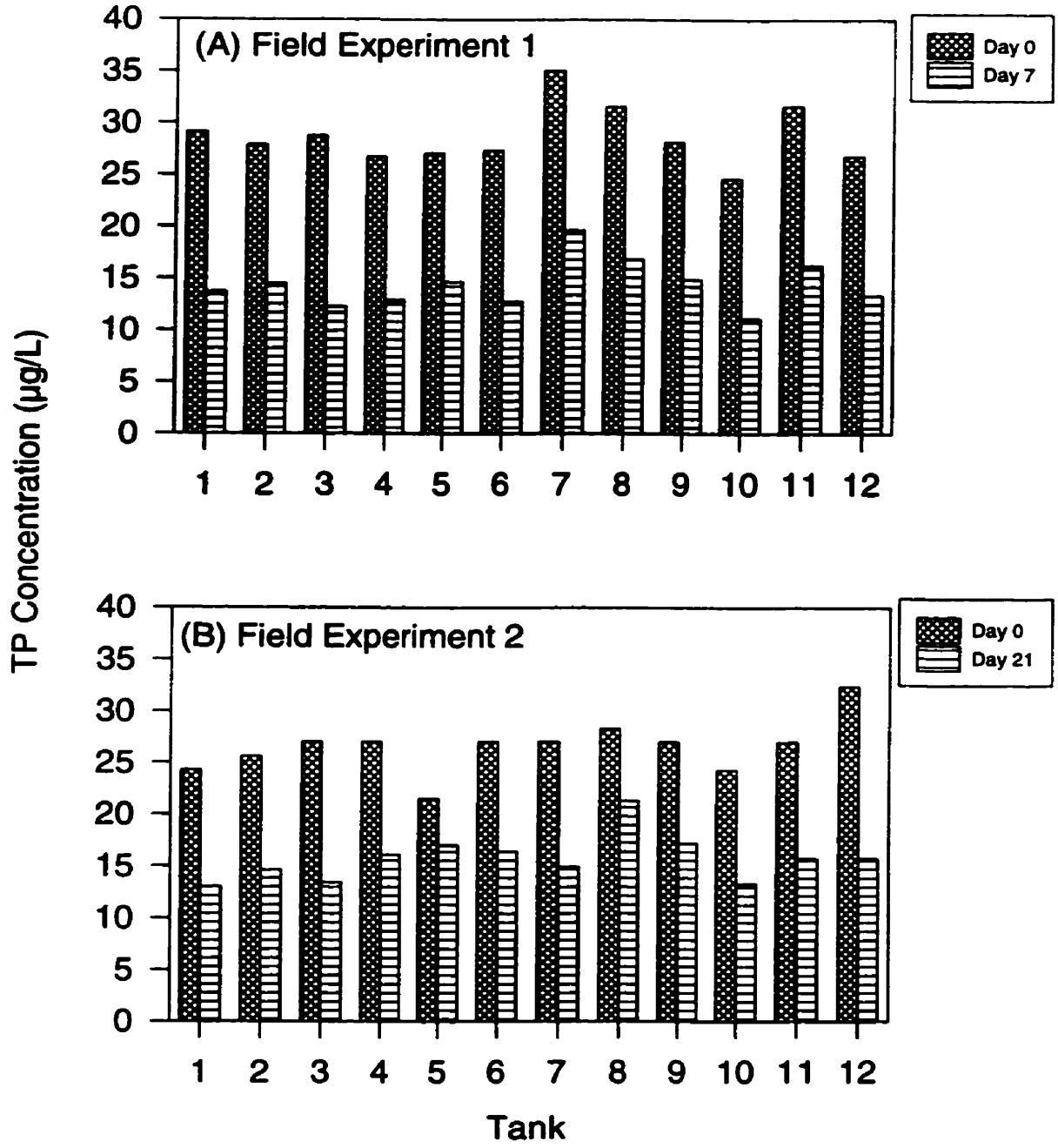

Figure A5: Total phosphorus concentrations for each tank at selected dates of field experiments 1 and 2

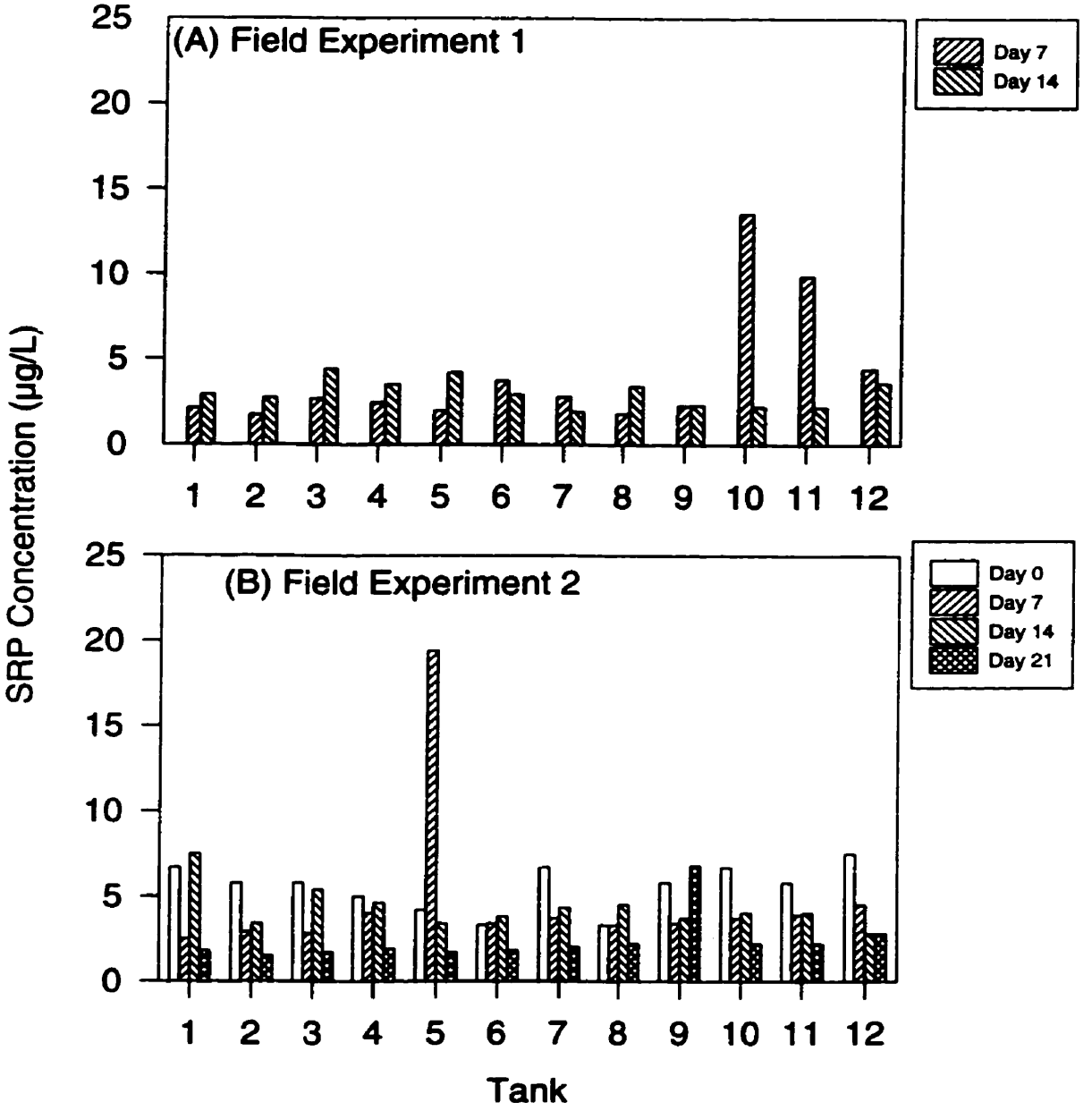

Figure A6: Soluble reactive phosphorus concentrations for each tank at selected dates of field experiments 1 and 2

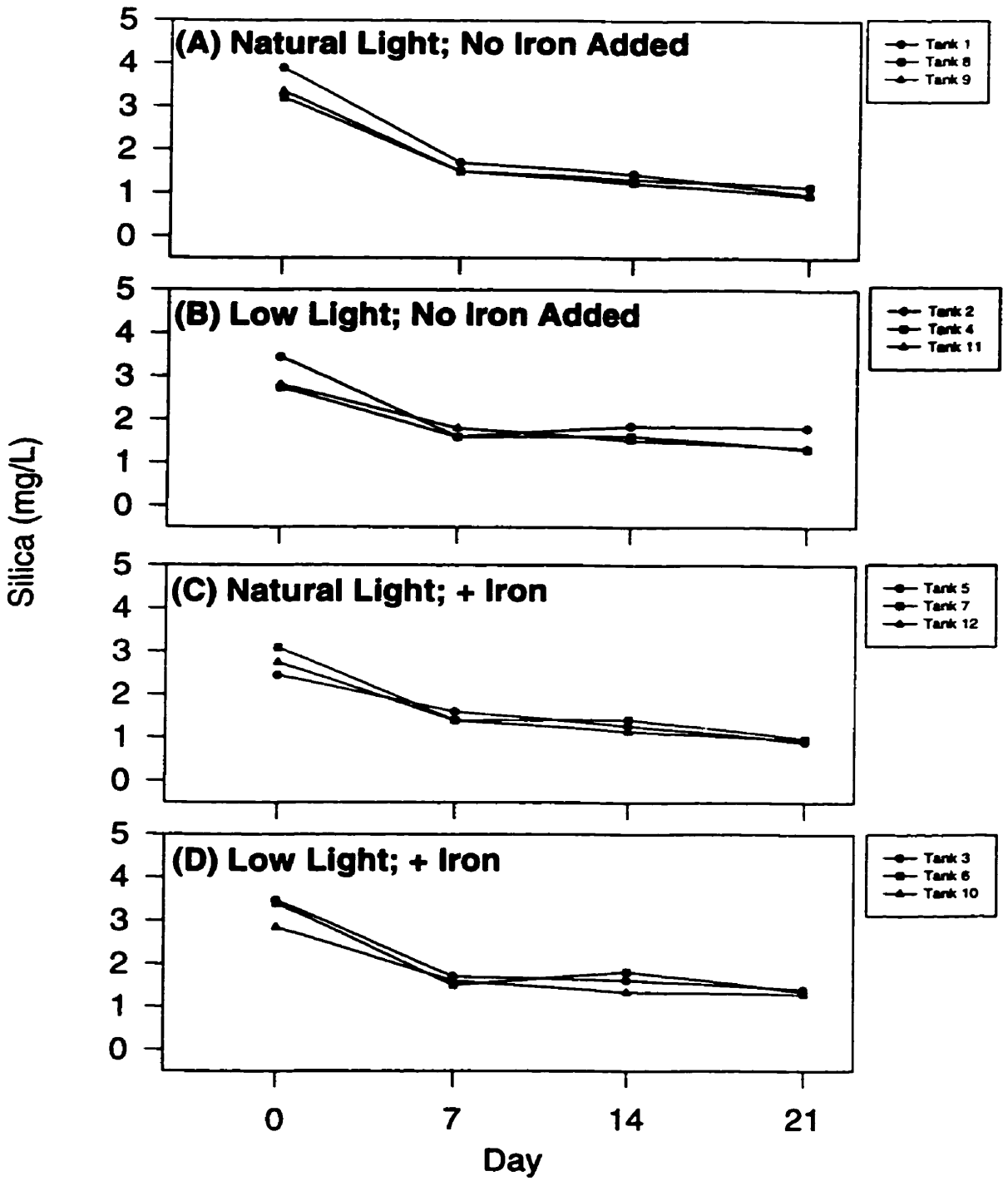

Figure A7: Mesocosm silica concentrations at first four sampling dates of field experiment 1

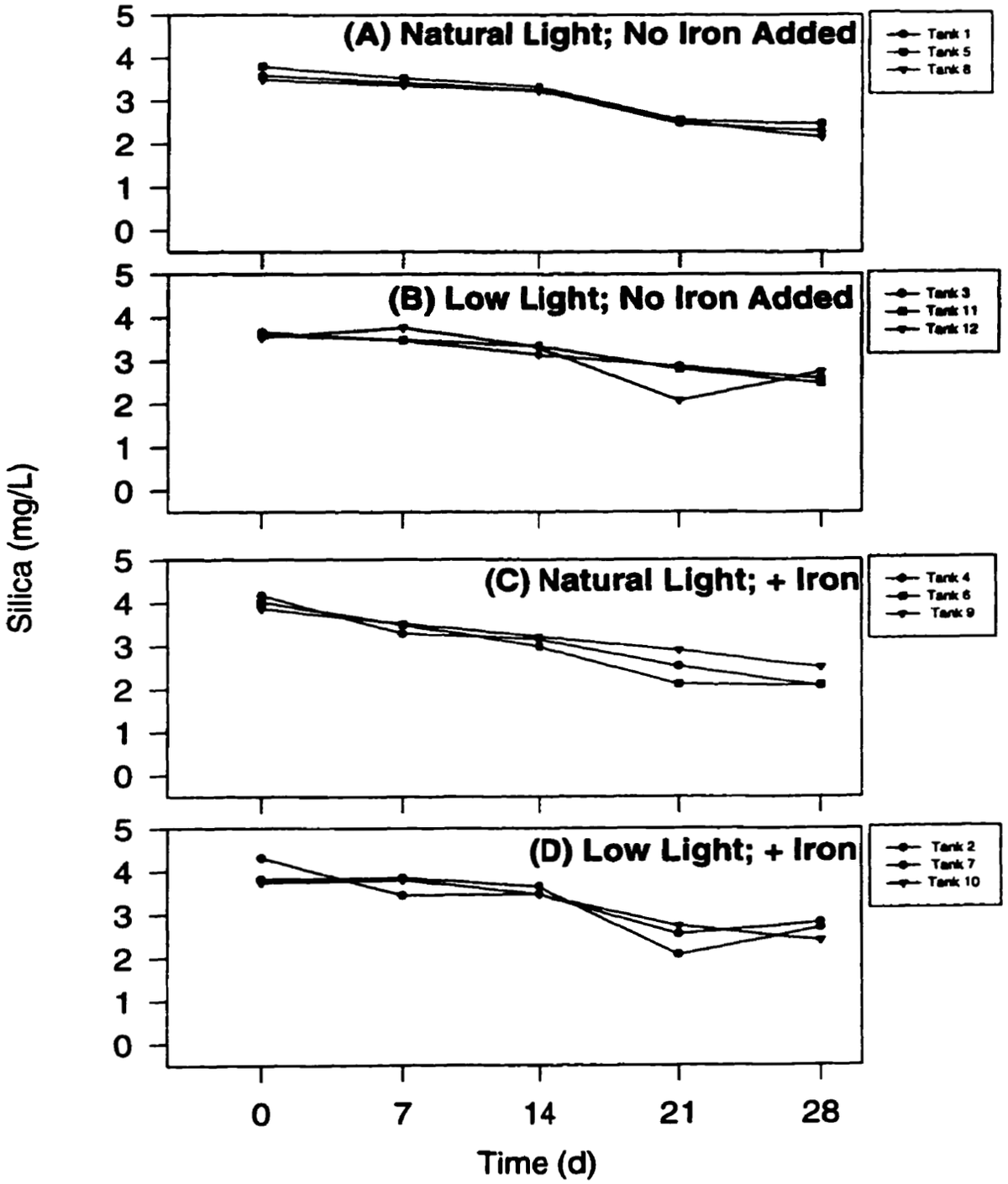

Figure A8: Mesocosm silica concentrations for field experiment 3

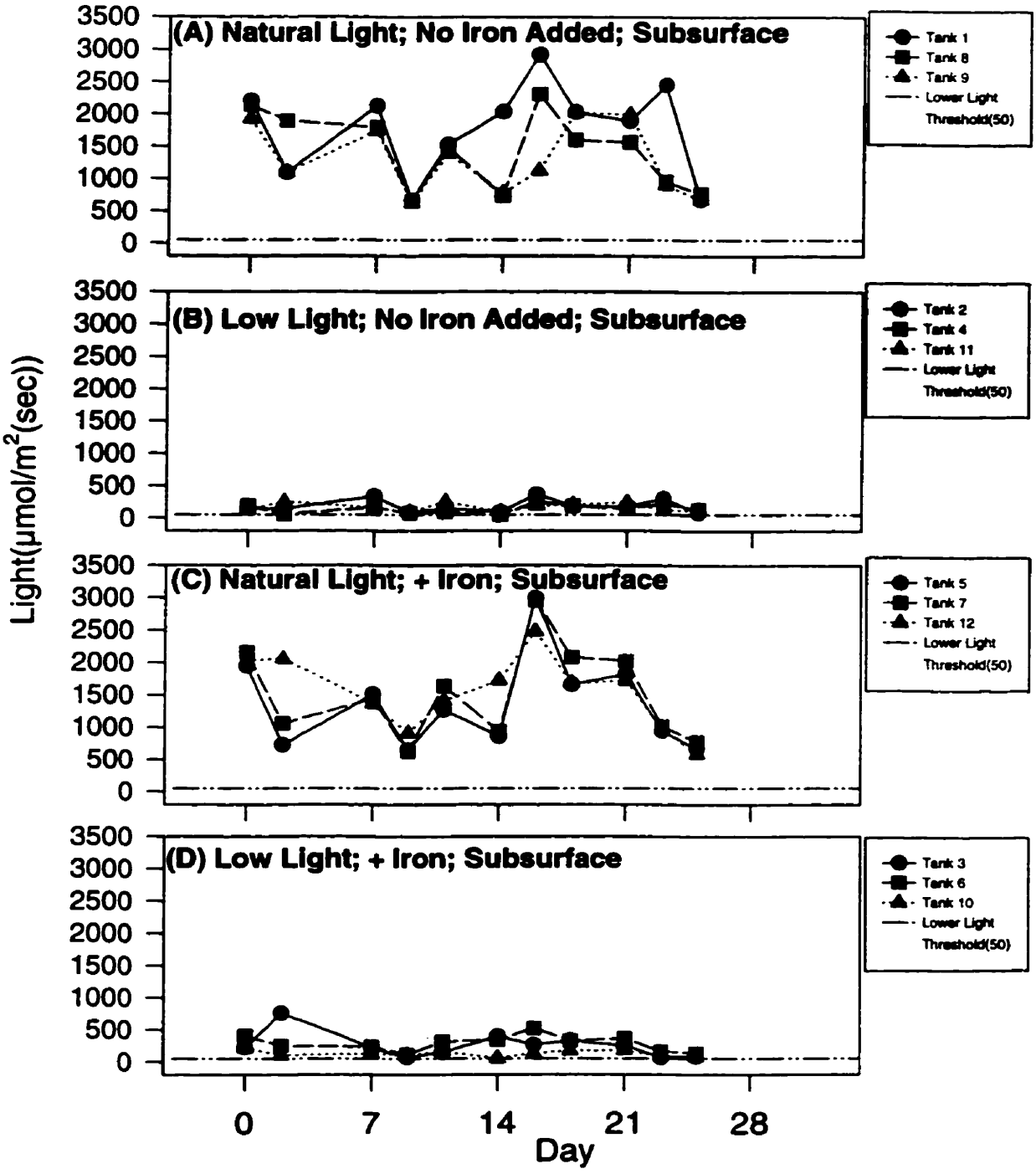

Figure A9: Subsurface mesocosm light levels for field experiment 1

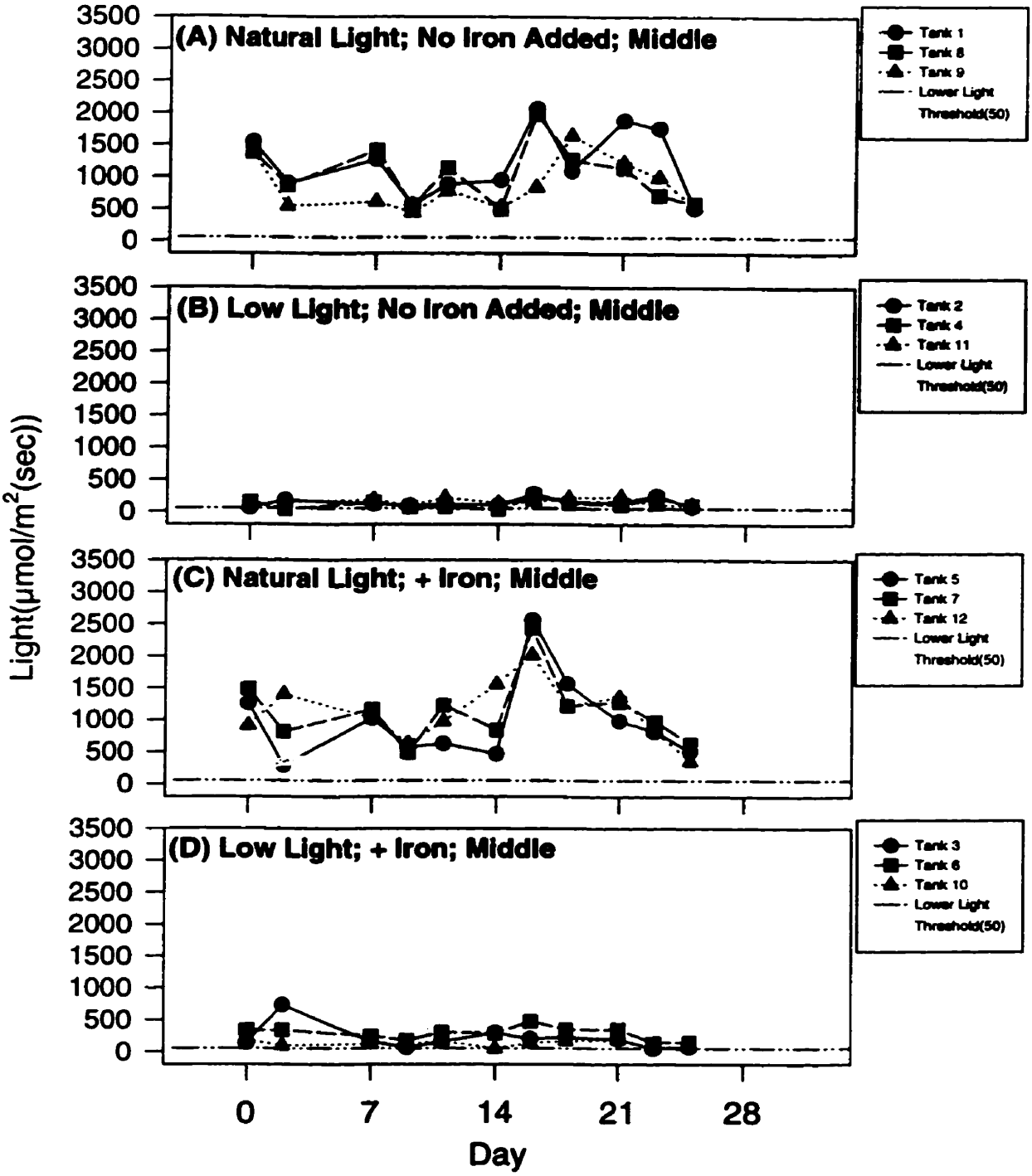

Figure A10: Middle mesocosm light levels for field experiment 1

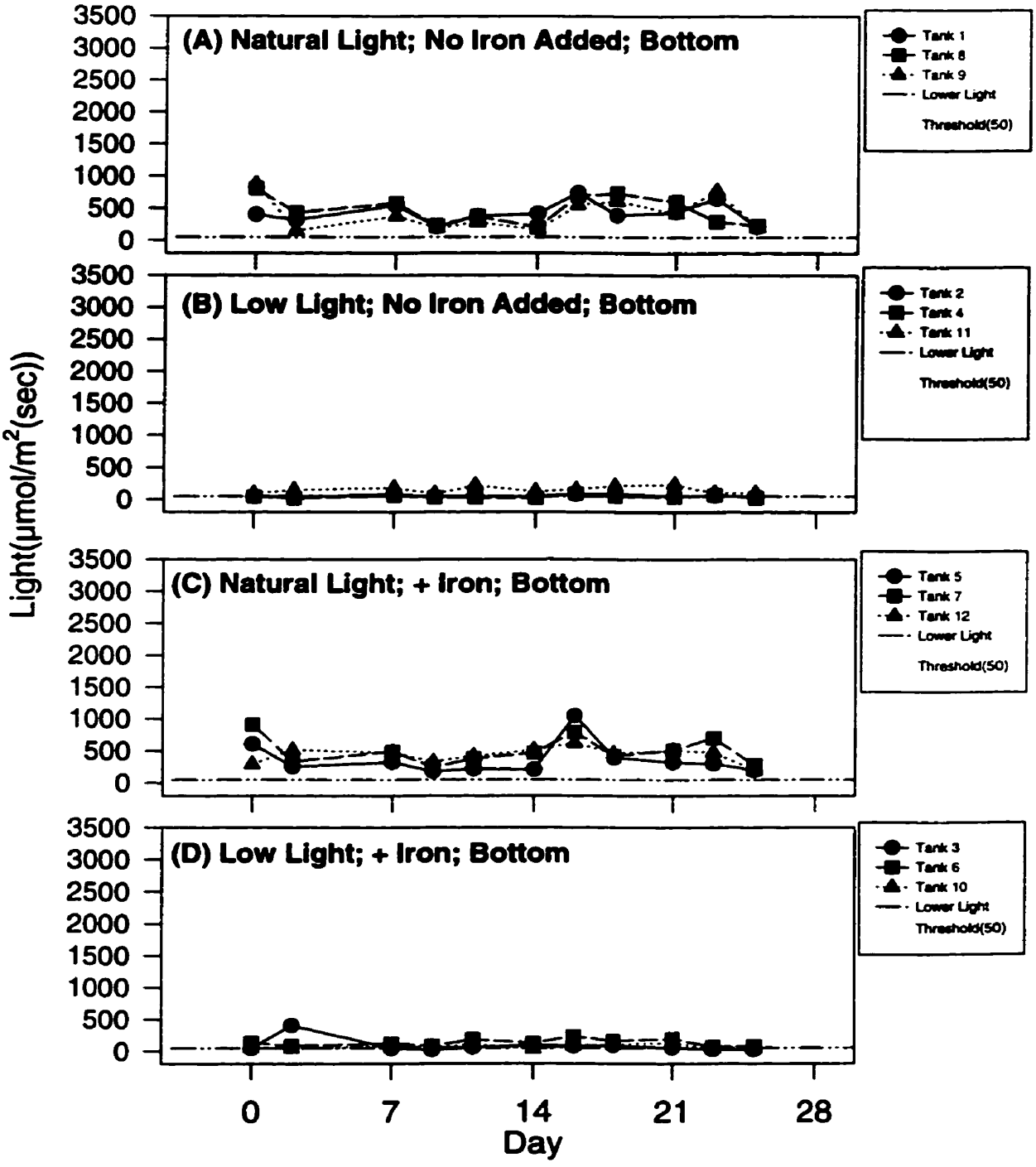

Figure A11: Bottom mesocosm light levels for field experiment 1

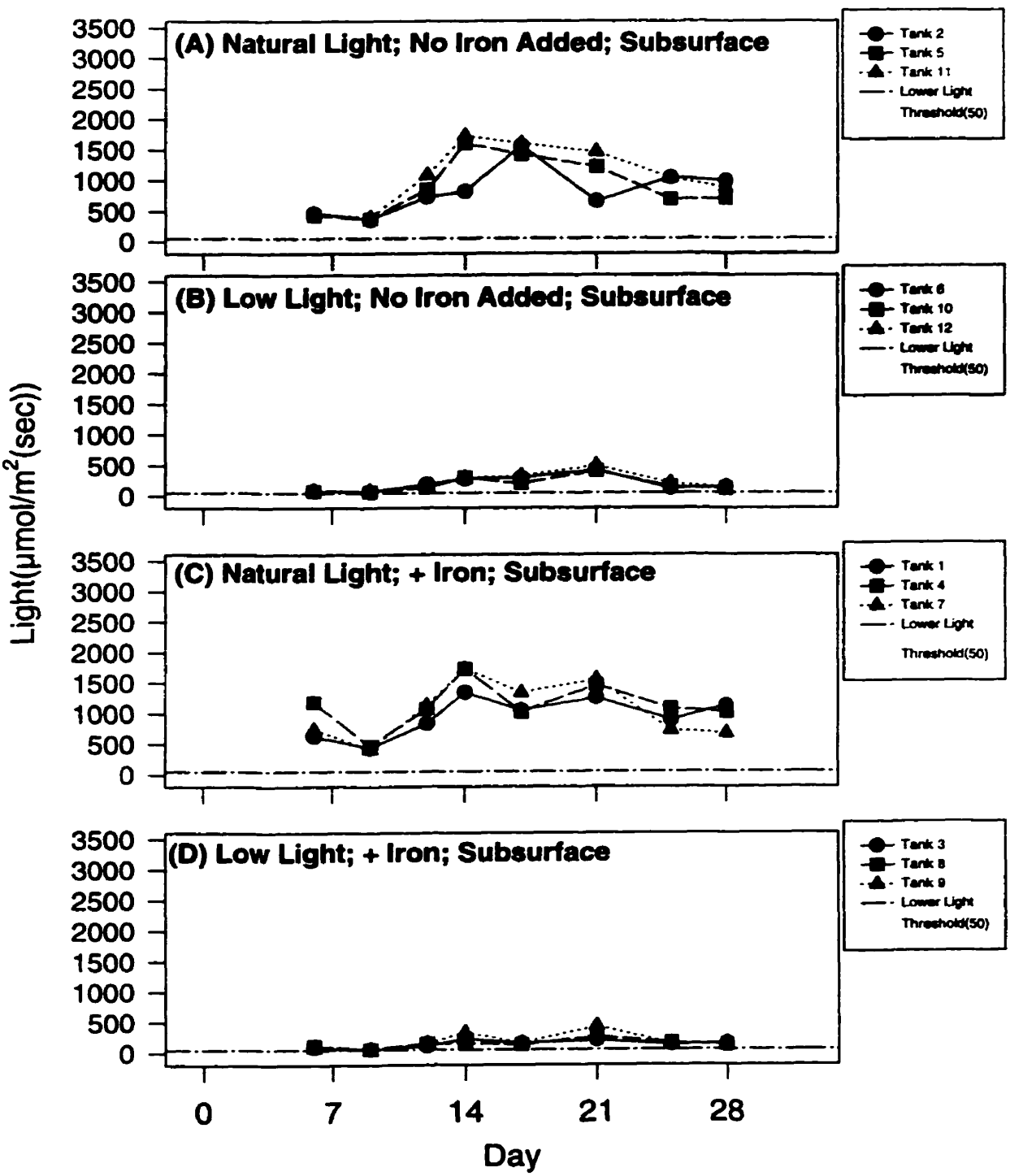

Figure A12: Subsurface mesocosm light levels for field experiment 2

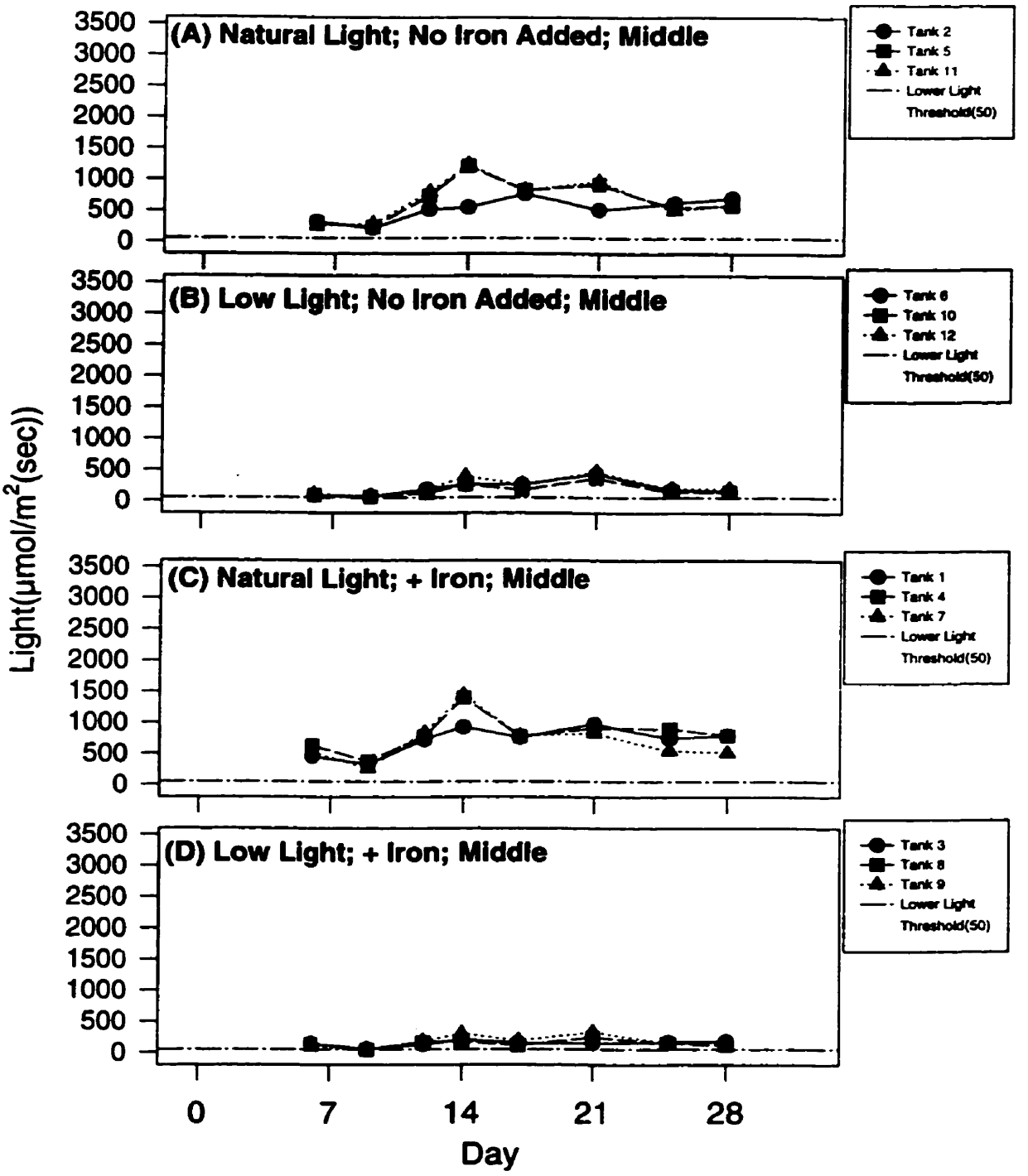

Figure A13: Middle mesocosm light levels for field experiment 2

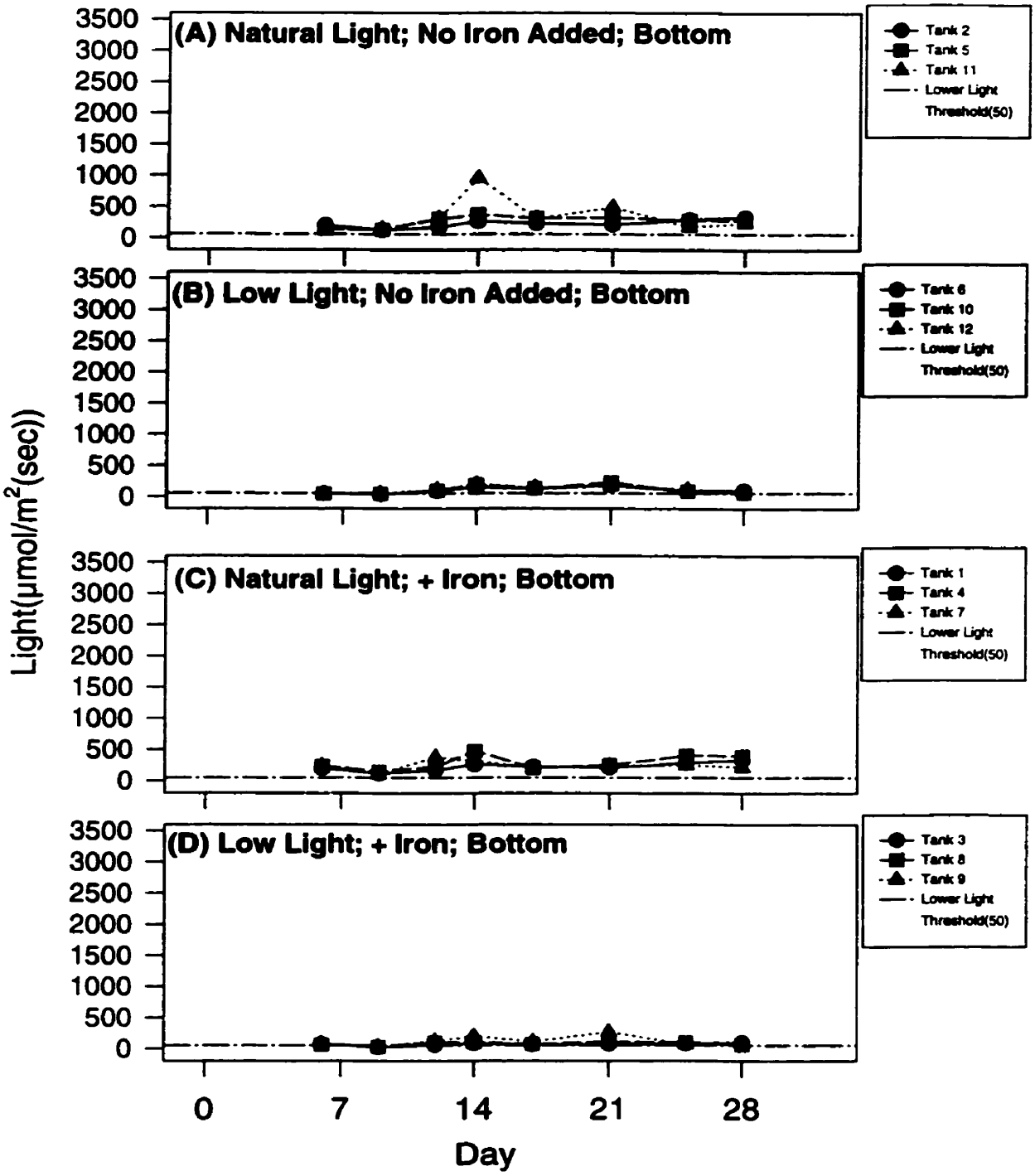

Figure A14: Bottom mesocosm light levels for experiment 2