BEHAVIOURAL PATTERNS AND GROWTH STRATEGIES

OF RED TIDE ORGANISMS OF THE SOUTHERN BENGUELA

By

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This thesis is dedicated to my late father,

Albert Horstman, and to my wife Diane.

In the Glass of Sea Water I send with this are some of the Animalcules which cause the Sparkling Light in Sea Water: they may be seen by holding the Phial up against the light, resembling very small Bladders or Air Bubbles, and are in all Places of it from Top to Bottom, but mostly towards the Top, where they assemble when the Water has stood still some Time, unless they have been killed by keeping them too long in the Phial.

Placing one of these Animalcules before a good Microscope; an exceeding minute Worm may be discovered, hanging with its Tail fixed to an opake Spot in a kind of Bladder, which it has certainly a Power of contracting or distending, and thereby of been suspended at the surface, or at any Depth it pleases in the including Water.

H. Baker, 1753

DECLARATION

The studies included in this thesis were initiated and conducted by myself. Presented data and interpretations thereof are my original work. Assistance was received with certain phytoplankton collections and measurements of nutrients.

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ABSTRACT

Red tides are a common feature of the southern Benguela upwelling system and are usually dominated by migratory flagellates and the ciliate *Mesodinium rubrum*. Seasonal blooms of dinoflagellates occur in response to seasonal upwelling and typically succeed diatom blooms. High biomass, multispecies red tides result from concentration by various physical forces and are characteristically found in warm, stratified, nutrient-depleted water overlying cold, nutrient-rich bottom water. The influence of turbulent mixing, light and the availability of nutrients on the migratory behaviour of red tide species was studied by means of both mesocosm and field studies. The mesocosm experiments were conducted in a 3m laboratory column in which a red tide community, collected from the field, was introduced above nutrient-rich bottom water. All the dominant species exhibited directed vertical migration, with ascent and descent starting before sunrise and before sunset respectively. Observations support the hypothesis that red tide organisms can sustain high concentrations in nitrogen depleted surface waters by growing at the expense of nitrate taken up during nocturnal descent. Vertical niche separation of different red tide species was evident both during the night and the day. Observations support the hypothesis that ·pecies are capable of coexisting within a red tide bloom. Division rates were determined from the frequency of paired nuclei and cells. *C. furca* recorded the highest growth rate (μ = 0.24). The relatively low growth rates emphasise the importance of physical processes, as opposed to biological processes, in the formation of red tides within upwelling systems.

CONTENTS

CHAPTER 1

INTRODUCTION

Marine phytoplankton blooms develop over various scales of space and time. The most dramatic and striking examples are the massive, relatively sudden, local or geographically restricted blooms of one or a few species. Such phenomena are called, rather indiscriminately, "red tides", "brown tides", "green tides" or "discoloured waters". All these terms imply that the algal proliferation is so intense as to modify the appearance of the sea surface. This discolouration may vary from green to yellow, orange, red, brown or purple depending on the causative organism and its concentration. Generally red tide patches are small and last only a few days, but periodically the planktonic blooms may discolour the sea for several square kilometres and may last several weeks.

Of the thousands of living phytoplankton species that make up the base of the marine food web, red tide and toxic species amount to about 6% and nearly 2% of the world flora respectively. The class Dinophyceae contains the majority of the toxic species but not more than one half of the red tide species (Sournia 1995). A bloom develops when these single-celled algae photosynthesize and multiply, converting dissolved nutrients and sunlight into plant biomass. The dominant mode of reproduction is simple

asexual fission. Barring a shortage of nutrients or light, or heavy grazing by zooplankton, the population's size can increase rapidly. Given the diverse array of algae that produce toxins or cause problems in a variety of oceanographic systems, attempts to generalize the dynamics of harmful algal blooms have failed. Red tides often occur when heating or fresh water run-off creates a stratified layer above colder, nutrient rich waters. Fast growing phytoplankton, generally diatoms, rapidly deplete nutrients in the upper layer, leaving nitrogen only below the interface of the thermo- or nutricline. Nonmotile phytoplankton cannot easily get to this layer, whereas motile algae, including dinoflagellates can, and are therefore able to thrive. A migrational descent by dinoflagellates during darkness may bring them into contact with deeper, nutrient-rich waters. This will enable the dinoflagellates to meet their growth requirements and maintain the organisms during daylight when they commonly aggregate in the typically nutrient-depleted surface waters. As a result, blooms can suddenly appear in surface waters that are devoid of nutrients and would seem incapable of supporting such prolific growth (Cullen 1985).

Ecologically there are three phases common to the development of all red tide blooms (Steidinger 1975):

a) Firstly a seed population is needed, the initiation phase.

b) This is followed by the support phase - favourable conditions needed for growth of the population. This phase

includes suitable salinity, temperature, nutrients and growth factors.

c) Finally the maintenance and transport of blooms by hydrological and meteorologic forces such as suitable winds, (enrichment of the water column), temperature stratification and the phototactic behaviour of the organisms in order to concentrate the organisms into dense blooms.

Dissipation of the bloom subsequent to unfavourable conditions is often associated with the formation of dormant resting cysts which sink out of the water column and settle in the sediments below. The process of upwelling enables these resting stages to be returned to the nutrient rich euphotic zone where they will germinate under suitable conditions, thereby enabling organisms to bloom repeatedly in the same area (Mulligan 1975). The spectacular growth of marine dinoflagellates and the autotrophic marine ciliate *Mesodinium rubrum* which results in red tides, are generally confined to coastal waters or to those regions of the sea where active upwelling takes place (Blasco 1975, Passow 1991). Red tides do not occur when wind induced turbulence prevents accumulation at the sea surface.

The West Coast of Southern Africa is regarded as one of the four main upwelling regions in the world. Upwelling is most common during spring and summer in the southern Benguela region (Nelson and Hutchings 1983). Red tides along the South African coast line are usually dominated by dinoflagellates and are a common feature

of the southern Benguela upwelling system (Horstman 1981). Red tides occur intermittently throughout the year, but research indicates that red tides are most common in late summer and autumn (Horstman 1981, Pitcher *et al.* 1995), when the decline in the frequency of upwelling - favourable winds result in warm calm conditions (Shannon 1966, Andrews and Hutchings 1980). During these periods of warm dry weather, wind - induced turbulence is weak and stratification of the upper layers is strong. This results in a shallow nutrient-depleted upper mixed layer overlaying a nutrient-rich bottom layer within the range of vertically migrating dinoflagellates. Dense patches of dinoflagellates observed along the coast result from onshore advection combined with positive phototaxis which enables them to accumulate at or near the sea surface (Horstman 1981, Pitcher *et al.* 1995).

During the past 10 to 20 years red tides as well as toxic phytoplankton events have been expanding in geographic extent, in number, frequency, number of toxic species responsible (45 to 57 dinoflagellate species), intensity and resultant ecological damage and economic losses (Anderson 1989; Smayda 1990; Sournia et al. 1991; Hallegraeff 1993; Sournia 1995). Many of these events have been responsible for harmful effects on public health, large scale fish mortalities and destruction of other marine life in these areas (Bodeanu 1993; Dahl and Tangen 1993; Honjo 1993; Ho, K-C. and I.J. Hodkiss. 1993; Yuzao *et al.* 1993). This increase in events are partly attributed to an increase in coastal pollution from nutrient-rich sewage, industrial waste and

fertilizer run off, to the utilization of coastal waters for aquaculture (Smayda 1990; Sournia *et al.* 1991; Chen and Gu 1993; Honjo 1993; Riegman *et al.* 1993;) and to the transport of red tide organisms in ballast water of commercial ships (Hallegraeff and Bolch 1991; Hallegraeff 1993).

Red tides may be harmless and may make a useful contribution to plankton production, but on the other hand they may be harmful to the surrounding fauna, due to physical damage to gills and oxygen depletion as the bloom becomes senescent and decays. They are particularly harmful when the causative organism produces a toxin resulting in devastating mass mortalities of marine organisms. These include the direct poisoning of fish and other marine fauna from toxins produced by certain red tide organisms, and indirect poisoning of upper trophic level predators such as shellfish poisoning in man and sea birds (Oguri *et al.* 1975; Taylor 1990).

Although most dinoflagellates responsible for red tides are non-toxic, more than 20 marine species are known to produce toxins world-wide and the list is constantly growing (Taylor 1990). In South African waters, one of the most potent group of toxins are produced by dinoflagellates of the *Alexandrium* group of which saxitoxin is a major component consisting of at least 18 different compounds. These toxins block the normal entry of sodium ions into nerves which prevents the transmission of normal nerve signals and results in paralysis. *Alexandrium catenella* has been responsible for red tides off the Western Cape coast and has

on occasion destroyed virtually the entire adult mussel population in the Elands Bay region (Horstman 1981; Hutchings *et al.* 1982). The high mortality rates among local mussel populations may be attributed to the exposed nature of the shoreline along the West Coast. Concentration of these poisons by the filter-feeding bivalves followed by human consumption, has led to Paralytic Shellfish Poisoning (PSP) on the South African coast (Popkiss *et al.* 1979; Horstman 1981). Until recently these dinoflagellates were believed to be the only toxic species occurring off the South African coast but it is now known that there are several others.

During the autumn of 1988 a bloom of a previously unrecorded *Gymnodinium* species in False Bay resulted in the mortality of certain fish and intertidal organisms. During the early autumn of 1989 this dinoflagellate again bloomed in False Bay and is thought to have been responsible for the mortality of some 40 tons of abalone, mostly from a marine reserve (Horstman *et al.* 1991). In addition to shellfish mortalities and fish kills, neurotoxic shellfish poisoning (NSP) causes eye, throat and nasal irritation in humans due to its aerosol effect. Examination showed that the cell shape of the causative organism closely resembles *Gymnodinium mikimotoi*, but that it also shares characteristics with *Gyrodinium aureolum* and *Gymnodinium breve*.

In South African waters, diarrhetic shellfish poisoning (DSP) is caused by *Dinophysis acuminata* which produces the toxin okadaic acid, which results in intestinal distress when contaminated

mussels are eaten. The first record of DSP which occurred during the autumn of 1992 (Pitcher *et al.* 1993) on the South African coast, revived interest in toxic dinoflagellate blooms in general and their hazards to public health and the shellfish industry in particular. The toxins produced by *Gymnodinium mikimotoi* (NSP) and *Dinophysis acuminata* (DSP) stimulate the normal entry of sodium ions into nerves. Neither of the toxins is lethal to humans (Anderson 1994).

Deleterious effects are not necessarily confined to blooms of toxic dinoflagellates. Non-toxic dinoflagellate blooms are also important in view of the fact that secondary effects of decomposition and decay of such blooms causes low oxygen conditions, jeopardizing the survival of many coastal marine organisms. Whenever this happens, it results in a drastic change in the ecological balance of the inshore coastal areas and it may take several years before the affected marine communities are able to re-establish themselves.

A decaying bloom may cause oxygen levels to become severely depleted. Animals trapped in such areas soon die, adding to the load of decaying organic matter. In essence, any phytoplankton species which form intense blooms can lead to massive fauna kills. Low oxygen levels following dense phytoplankton blooms have, on several occasions, caused rock lobsters to crawl from the sea as was observed in St Helena Bay in April 1978 following decay of a bloom of the photosynthetic ciliate *Mesodinium rubrum* (Horstman 1981). Entrapment and decay of a massive phytoplankton

bloom dominated by the nontoxic dinoflagellates *Ceratium furca* and *Prorocentrum micans*, was responsible for huge mortalities in St Helena Bay towards the end of February 1994. By March the death of numerous invertebrates and fish and their subsequent decay, was responsible for the most extensive anoxic water mass yet recorded in this area. The shortage of oxygen and poisoning by hydrogen sulphide, generated by anaerobic sulphur-reducing bacteria in the absence of dissolved oxygen was responsible for the largest ever recorded mass mortality of fish and rock lobster on the West Coast. Estimated mortalities were 60 tons of rock lobster, and 1500 tons of fish of 50 different species (Matthews and Pitcher 1996).

Traditionally the word 'plankton' implies organisms which float passively in water. This is however not strictly true. Many organisms, including dinoflagellates and ciliates, posses an effective means of propulsion. This characteristic provides an ability for depth regulation enabling the algae to obtain optimum optical depth, migrate into nutrient-rich layers and avoid detrimental conditions. For autotrophic species motility represents an important adaptation to situations where nutrients are a limiting factor in the euphotic zone. The ability to migrate through temperature gradients of varying magnitude to reach the nutrient-rich bottom layer at night in order to assimilate nutrients and then to return to the euphotic zone during day-time, is clearly advantageous (Cullen 1985; Holligan 1985).

Diel vertical migration (DVM) is best defined as the vertical displacement of an organism, or a group of organisms, which occurs with diel periodicity. In stratified water, the upper water rapidly becomes stripped of nutrients due to their uptake by phytoplankton. Diatoms sink passively but dinoflagellates can regulate their position in the water column over a relatively short time scale by carrying out daily migrations, moving towards the surface during the day and down to the nutricline at night (Heaney and Eppley 1981). In a stratified environment, species with migration capabilities therefore constitute a clear advantage over non-migrating species in the phytoplankton community. Vertical migration further permits these species to accumulate in the surface layers of the sea, resulting in red tides (Crawford and Purdie 1992).

The ability of dinoflagellates and *Mesodinium rubrum* to migrate vertically has been documented in both field studies and laboratory experiments (Passow 1991). A common feature of migrating autotrophs is their tendency to concentrate at a specific depth during daylight and either to disperse or migrate into deeper layers during darkness. At noon two distinct distribution patterns occur: some species maintain maximum numbers at the surface, while others displace their maxima to lower light levels. The extent to which migratory ability is related to environmental factors, has been studied mainly in culture experiments. From such experiments it is known that phototaxis controls the diel migration of autotrophs (Blasco 1978; Heaney and Eppley 1981; Kamykowski 1981(b); Stoecker *et al.*

1984; Prézelin 1992) and that temperature stratification, nutrient concentration and surface irradiance influence migration speed, distance and behaviour (Kamykowski and Zentara 1977; Cullen and Horrigan 1981; Heaney and Eppley 1981; Paasche *et al.* 1984; Kamykowski and McCollum 1986; Olsson and Graneli 1991; Passow 1991). The influence of light intensity is coupled with nutrient concentration in such a way that at limiting nutrient concentrations, organisms either remain at their nighttime depths (Eppley *et al.* 1968; Rasmussen and Richardson 1989; Nielsen *et al.* 1993) or accumulate at depths of comparatively low light intensity (Eppley *et al.* 1968; Heaney and Eppley 1981). Under limiting nutrient conditions, cells have been observed to spend less time in the euphotic zone, beginning upward migration later and leaving the upper layers earlier (Eppley *et al.* 1968, Heaney and Eppley 1981). Some dinoflagellates begin downward migration before it begins to get dark, indicating that migration is correlated with a cellular periodicity which, to some extent, is independent of the light regime (Eppley *et al.* 1968; Olsson and Granéli 1991).

Measurements of population growth rates of individual phytoplankton species *in situ* has been a major challenge for phytoplankton ecologists. It has long been recognised that growth rates of marine dinoflagellates can be determined from the frequency of cell division for the species exhibiting a phased pattern of cell division, whereby division is restricted to a short period of time each day (Blasco 1978; Weiler and Eppley 1979; Weiler and Karl 1979; Weiler 1980; Eppley *et al.* 1984;

Edler and Olsson 1985, Olsson and Granéli 1991; Reguera *et al.* in press - 1996). Several investigators have recognised and explored the suitability of determining the fraction of the cells in some stage of mitosis (usually identified as paired cells or cells with two nuclei), to estimate population growth rates (Olsson and Granéli 1991).

Red tide research is conducted on organisms that live within an environment that is highly variable in scales of time and space, ranging from the macroscale (>100 km, seasons/years) to the mesoscale (0.1-100 km, days/weeks/months) and microscale (<0,1 km, hours/day) (Fig. 1). The present study focuses on processes within the microscale category. Red tides, which occur frequently within the southern Benguela, are considered to result from the interaction between advection and behaviour, with positive phototaxis contributing to the shoreline accumulation of red-tide organisms under gentle onshore wind stress (Pitcher *et al.* 1993).

The study objectives included the following:

* Vertical migration patterns of the dominant red tide species derived from mesocosm and *in situ* measurements; the influence of environmental factors such as temperature gradients and irradiance (mesocosm, *in situ*) and turbulence and advection (*in situ*). The study also examined the active response of nitrate stressed cells to decreasing nitrate concentrations (mesocosm), advection of nitrate into the

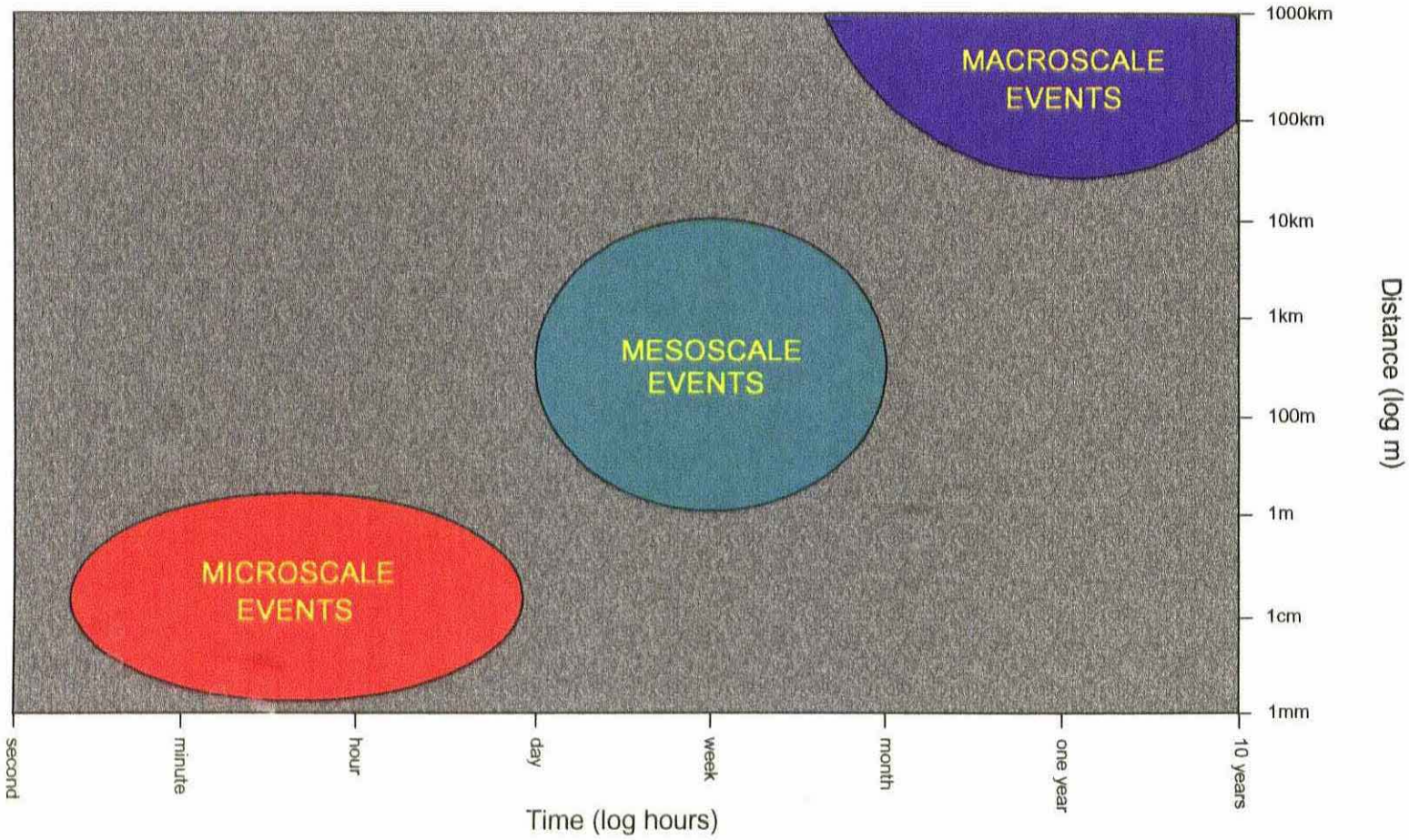

Fig. 1. A visual impression of the scales of time and space relevant to red tide organisms.

study area (*in situ*); and the ability of red tide organisms to take up nitrate during their nocturnal descent.

* Vertical niche separation and species specific migratory behaviour.

* Pattern, growth rates and timing of cell division on a diel and seasonal basis.

Although field observations reveal the complexity of factors influencing migratory organisms, they offer little opportunity for detailed understanding of behavioral cues due to the problem of sampling the same population and isolating the effects of multiple environmental variables. Enclosure experiments although compromising the physical characteristics of the natural water column, provide a more controlled environment for the study of marine diel vertical migration. Experiments in enclosed water columns clearly reveal differences in the behaviour and vertical movement of dinoflagellate species in response to various environmental parameters. Mesocosm experiments provided the opportunity to investigate species-specific responses in multispecies blooms under thermally stratified conditions.

The present study also examined the behaviour of red tide organisms under natural conditions. It focused on vertical niche separation, and the temporal pattern of cell division as well as the estimation of growth rates from the maximum daily frequency of division of red tide organisms occurring in the southern

Benguela. The study also examined how different nitrate regimes affect vertical movement in an attempt to identify some behavioural mechanisms responsible for subsurface chlorophyll maximum layers.

CHAPTER 2

METHODOLOGY

Mesocosm Experiments

The mesocosm was constructed using a 3 m high insulated PVC cylinder with an inside diameter of 0,4 m and a capacity 350l (Fig. 2). Ports to subsample the column were placed at 30 cm intervals, except near the surface where the ports were 10 cm apart. Here the column was sliced into removable 10 cm sections which were removed as the water level declined, thereby allowing the surface of the water to remain directly exposed to the sun. The vertical distribution of the water temperature in the column was controlled by enclosing the lower half of the column with an insulated cement fibre tank, fitted with a cooling coil, filled with fresh water and aerated to ensure a uniform temperature distribution within the cement fibre tank. The temperature of the water jacket was controlled and monitored electronically. A significant temperature difference between the surface and the bottom of the water column was possible ensuring a stable stratified water column.

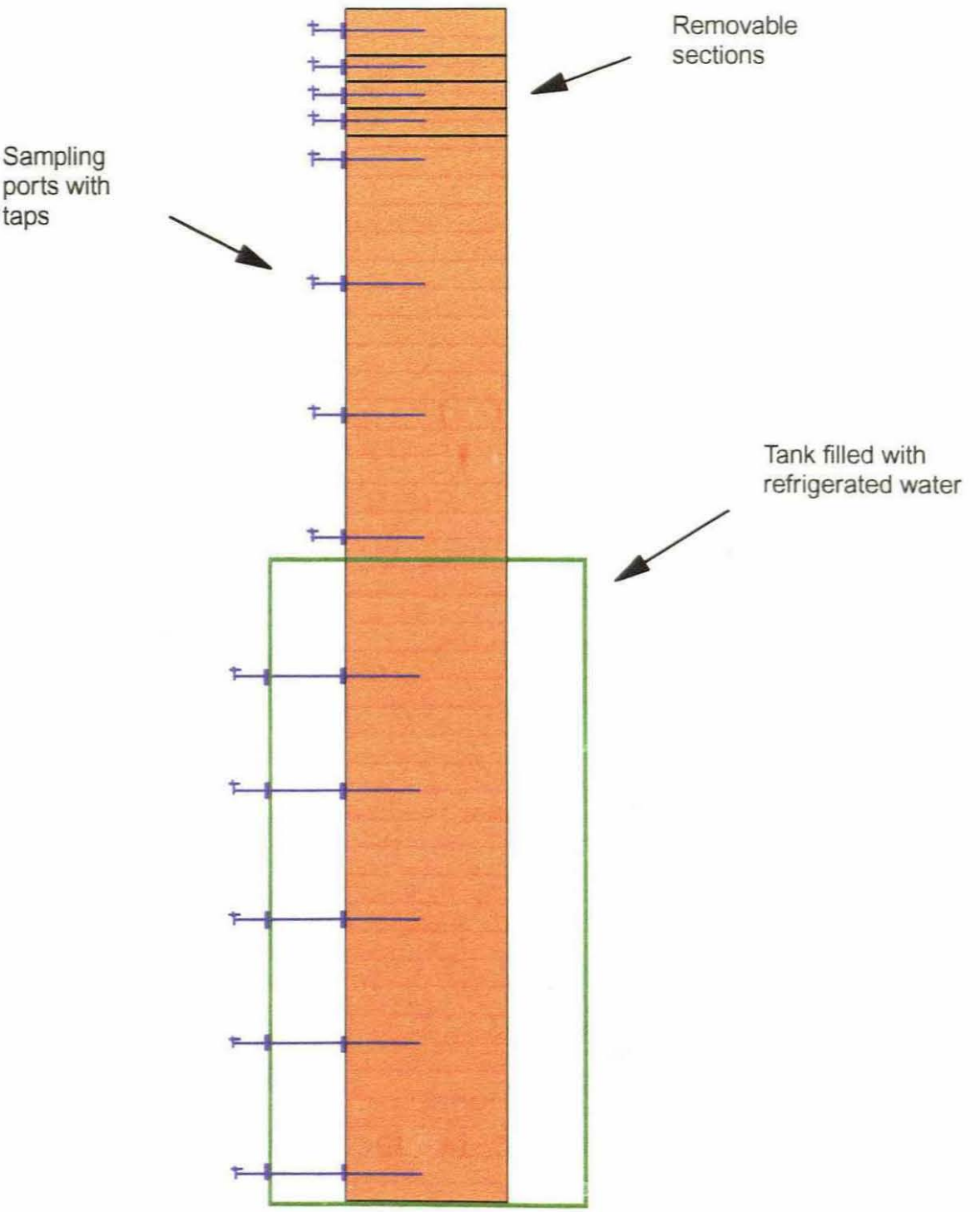

Fig. 2. The mesocosm with the position of the catheters used for sampling.

Newly upwelled water (9 to 11°C) was collected at sea, filtered through a 0,22 µm porosity cellulose acetate membrane cartridge filter and stored in sealed plastic lined, black polyethylene tanks.

At the start of each experiment, 150 l of the stored water was pumped into the column via an in-line filter (0,22 µm) to a level coincident with the thermocline. A neutrally buoyant baffle was carefully lowered and positioned on top of the nutrient-rich water to prevent vertical mixing when the red tide water was introduced into the column. Before adding the red tide sample, it was slowly syphoned through a 300 µm mesh in an attempt to remove any potential grazers. Once the column was filled, the baffle was carefully removed. The following day, stainless steel sampling tubes (catheters), each with a length of tubing and a clamp, were inserted through the membrane sealed flanges at each port. This setup had the advantage that each catheter could be positioned in the centre of the column, and the thermocline was not disturbed during sampling. Sampling volumes and sampling intervals had to be chosen very carefully for fear of running out of water. The mesocosm was left between 12 to 36 hours to stabilise before sampling commenced. The mesocosm was placed outdoors in such a way that the phytoplankton would experience the natural light:dark cycle with light penetrating the mesocosm from the top only.

A total of five mesocosm experiments were carried out. Water samples were collected at the following sites during major red

tides (Fig. 3):

1) Laaiplek: 26 November 1992 - **Mesocosm 1**
2) Doringbaai: 15 January 1993 - **Mesocosm 2**
3) Strandfontein: 4 February 1993 - **Mesocosm 3**
4) Rooiels: 10 March 1993 - **Mesocosm 4**
5) Table Bay: 2 April 1993 - **Mesocosm 5**

Sampling of the mesocosms took place at 14h00, 18h00, 24h00, 06h00, 10h00 and 14h00 during each of the five experiments. The sampling strategy varied. Sampling of Mesocosms 1 and 2 commenced after a 12 hour stabilization period and continued uninterrupted for two days. Sampling of Mesocosm 3 commenced after a stabilization period of 36 h and continued uninterrupted for 4 days. Mesocosms 4 and 5 were also allowed to stabilize for 36 h before sampling. Two 24 h sampling periods were separated by 24h during which no sampling took place.

To minimise sampling error, samples were collected in 1 l bottles which were, in turn, subsampled for the analysis of salinity, chlorophyll *a*, inorganic nutrient concentration (nitrate, phosphate and silicate), CHN and phytoplankton. Water temperature was measured using a submersible thermistor and light penetration was measured using a submersible quantum sensor.

Field Studies

A total of four field studies were conducted at the following

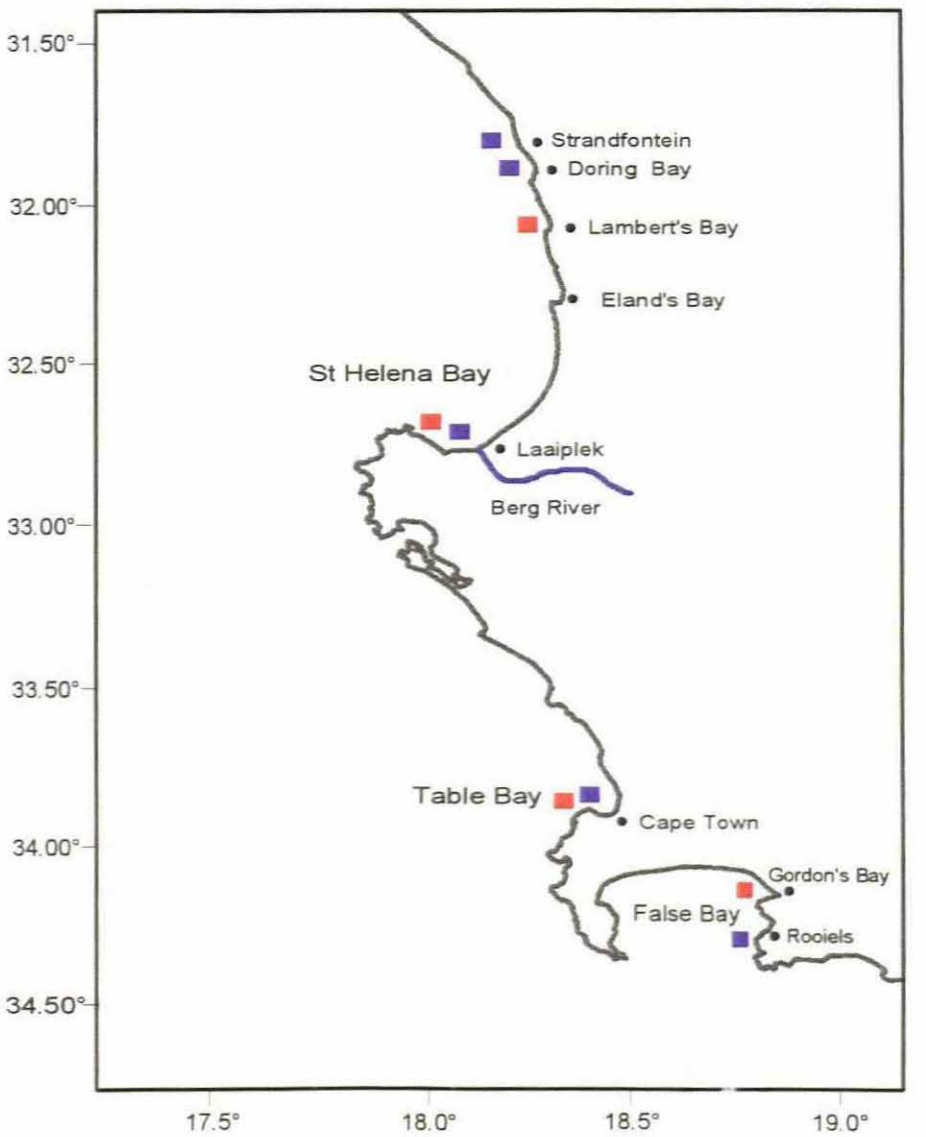

Fig. 3. Map of the west coast of South Africa showing sampling sites for the mesocosm experiments ■, and *in situ* field station positions ■.

locations along the West and South Coasts during the occurrence of extensive red tides (Fig. 3):

1) St Helena Bay: 12 April 1994.
2) Table Bay: 19 April 1994.
3) Gordon's Bay: 22 June 1995.
4) Lambert's Bay: 27 February 1996.

Samples were collected at discrete depths during the St Helena Bay and Table Bay studies at 14h00, 18h00, 24h00, 06h00 and 10h00. Water samples were drawn from the required depths using 5 l National Institute for Oceanography (NIO) bottles. Subsamples were taken for phytoplankton, nutrients, chlorophyll *a* and oxygen analysis. The oxygen samples were immediately fixed until the oxygen content could be measured using the Winkler technique.

A more intensive sampling strategy was followed during the Gordon's Bay and Lambert's Bay studies. Phytoplankton net samples were collected from a fixed station at hourly intervals by means of vertical hauls from the bottom to the surface with a 20 μ mesh net. In addition, the water column was profiled for temperature and *in situ* chlorophyll fluorescence by means of a submersible Chelsea Instruments Aquapack. Every second hour (Gordon's Bay) or third hour (Lambert's Bay), quantitative sampling took place at discreet depths using 5 l water samplers, for nutrients, chlorophyll *a*, phytoplankton and oxygen (Gordon's Bay only).

Sample Analysis

Nutrients

Samples for the analysis of soluble reactive nitrate, silicate and phosphate concentrations were collected from the mesocosm sampling ports or from NOI sampling bottles during field studies in 30 ml polyethylene tubes fitted with screw caps. The tubes were immediately frozen and stored at -20°C. Samples were analysed photometrically using a Technicon AutoAnalyser AA11 as described by Mostert (1983).

Carbon:Hydrogen:Nitrogen

Subsamples (50 ml) for total particulate carbon and nitrogen analysis were vacuum - filtered onto pre-ashed (6 h at 400°C) Whatman GF/F filters. Filters were stored frozen until oven dried (60°C) and analyzed by high temperature oxidation on a LEIO CAN analyser, using cyclohexanone (20.14% N:51.79% C) as a standard (Ehrhardt 1983).

Chlorophyll *a* measurements

Water samples for chlorophyll *a* analysis were vacuum filtered (30 kPa) through GF/F filters and analyzed according to the fluorometric method (Strickland and Parsons 1972; Parsons *et al.* 1984). A 50 ml sample of seawater was filtered. Filters were folded, wrapped in tinfoil and stored frozen until the extraction

process was undertaken. The filter papers were then thawed and placed in polycarbonate centrifuge tubes, prior to maceration in 10 ml of 90 % acetone using a plastic rod. Chlorophyll *a* was extracted in the dark. After 24 hours, the samples were centrifuged for 10 min at 3500 rpm. Fluorescence was read on a Turner Designs fluorometer before and after the addition of 2 drops of 10% HCL. The concentration of chlorophyll *a* was calculated using the formula:

$$\text{chlorophyll } a \ (\text{mg.m}^{-3}) = (R_B - R_A) \times \frac{\text{volume of solvent}}{\text{volume of solvent}} \times F$$

where: R_B - Reading before acidification

R_A - Reading after acidification

t = 2.2 (R_B/R_A for pure chlorophyll *a)*

F = calibration factor (using pure chlorophyll *a)*

Phytoplankton identification and enumeration

Phytoplankton samples were preserved using borax buffered 4% formalin. The preserved phytoplankton samples were counted by means of the Utermöhl method (Hasle 1978). Subsamples of 2 ml were settled in Zeiss settling chambers (diameter = 25 mm) and counted using a Zeiss inverted microscope. Most phytoplankton counts were made using a 16x phase contrast objective. The 40x phase contrast objective was only used when samples had very high cell numbers. Sample counts were expressed as cells per litre.

Red tide species were identified from drawings, photographs and descriptions by Dodge (1982) and Fukuyo *et al.* (1990). Red tide organisms were identified to species level.

Diel vertical migration

Interpretation of diel vertical migration was based on changes in the weighted mean depth (WMD) of the dominant red tide species during the course of each experiment. The WMD was calculated according to Roe *et al.* (1984):

$$WMD = \Sigma(n_i * z_i) \; / \; \Sigma n_i$$

where n_i is concentration (n*cells.l^{-1}) of organisms at depth z_i.

Cell division and growth rates

The method used to determine cell growth rates is based on a time series of the fraction of a population undergoing phased cell division (Weiler and Eppley 1979, Reguera *et al.* 1996). This necessitates estimates of the number of cells in different cell cycle phases. Cells with a single nucleus, cells with two nuclei and recently divided cells were enumerated by means of epifluorescence microscopy after samples were stained with acridine orange (Coats and Heinbokel 1982). Observations were conducted using a Zeiss inverted microscope equipped with a 100 W mercury lamp for epifluorescence (exciting wavelength of

455 to 490 nm, a filter combination: BG12 exciter filter, KP barrier filter), and a 6 V 15 W tungsten lamp for transmitted brightfield/phase contrast illumination.

The relative frequency of division (F) was estimated at fixed times in the cell cycle, using the equation (Weiler 1980):

$$F = (b+c)/(a+b+c)$$

where a = cells with one nucleus

b = cells with two nuclei

c = half no. of recently divided cells

Rates of cell division (μ) were calculated from the above estimates of cell division using the equation (Weiler 1980):

$$\mu = 1/t\ln(F\ max+1).$$

where: t = 1 day

$$F\ max = maximum\ (b+c)/(a+b+c)$$

The doubling time (DT) in days, was estimated from the relationship: $DT = (\ln 2)/\mu$.

CHAPTER 3

DIEL VERTICAL MIGRATION

RESULTS AND DISCUSSION

Mesocosm Experiments

Five mesocosm experiments were conducted in the temperature stratified laboratory column in which natural red tide communities collected from the field were introduced above nutrient-rich water (Fig. 3).

The physical and chemical characteristics of the water column for these experiments are summarised in Table 1. The water for the lower half of the mesocosm was collected subsequent to upwelling favourable winds and was characterised by low temperatures (<12°C) and high nitrates (6.72 - 9.65 $mmol.l^{-1}$). A well stratified water column was established in all instances before the experiments commenced. The surface temperatures which ranged between 20.6 - 31.8°C were allowed to fluctuate in concert with the ambient air temperature, while bottom temperatures were much lower (10.1 - 15.9°C),(Table 1). Significant nutriclines were present at the beginning of four of the experiments. Nitrate concentration were low in the red tide water, ranging between

	Mesocosm 1. (26.11.1992)	Mesocosm 2. (15.1.1993)	Mesocosm 3. (4.2.1993)	Mesocosm 4. (10.3.1993)	Mesocosm 5. (2.4.1993)
Collection Site	Laaiplek	Doringbaai	Strandfontein	Rooiels	Table Bay
Temperature range (noon)	0m = 25.2°C 2.7m = 10.1°C	0m = 31.8°C 2.7m = 12.6°C	0m = 25.7°C 2.7m = 12.5°C	0m = 25.5°C 2.7m = 12.7°C	0m = 20.6°C 2.7m = 15.9°C
Stratification	▲T = 15.1°C	▲T = 19.2°C	▲T = 13.2°C	▲T = 12.8°C	▲T = 4.7°C
Nitrate range (initial)	0m = 0.11μmol.l^{-1} 2.7m = 0.46μmol.l^{-1}	0m = 0.04μmol.l^{-1} 2.7m = 6.72μmol.l^{-1}	0m = 0.46μmol.l^{-1} 2.7m = 9.65μmol.l^{-1}	0m = 3.64μmol.l^{-1} 2.7m = 8.36μmol.l^{-1}	0m = 0.13μmol.l^{-1} 2.7m = 1.92μmol.l^{-1}
Silica range (initial)	0m = 22.8μmol.l^{-1} 2.7m = 27.8μmol.l^{-1}	0m = 14.47μmol.l^{-1} 2.7m = 11.4μmol.l^{-1}	0m = 15.99μmol.l^{-1} 2.7m = 16.04μmol.l^{-1}	0m = 8.25μmol.l^{-1} 2.7m = 9.16μmol.l^{-1}	0m = 2.4μmol.l^{-1} 2.7m = 4μmol.76.l^{-1}
Phosphate range (initial)	0m = 0.93μmol.l^{-1} 2.7m = 1.36μmol.l^{-1}	0m = 9.45μmol.l^{-1} 2.7m = 0.62μmol.l^{-1}	0m = 1.16μmol.l^{-1} 2.7m = 1.47μmol.l^{-1}	0m = 1.23μmol.l^{-1} 2.7m = 1.10μmol.l^{-1}	0m = 0.70μmol.l^{-1} 2.7m = 0.88μmol.l^{-1}
Sunrise	05h34	05h51	06h10	06h41	06h59
Sunset	19h37	20h01	19h49	19h11	18h40
Photoperiod (L:D)	14h03min to 9h59min	14h50 min to 9h10min	13h39min to 10h21min	12h30mim to 11h30min	11h41min to 12h19min

Table 1. Physical and chemical parameters at the start of each mesocosm experiment.

Mesocosm 1.	Mesocosm 2.	Mesocosm 3.	Mesocosm 4.	Mesocosm 5.
Mesodinium rubrum*	Prorocentrum micans*	Prorocentrum micans*	Heterosigma akashiwo*	Ceratium furca*
Scrippsiella trochoideum	Ceratium furca	Ceratium furca	Mesodinium rubrum*	Ceratium lineatum
Dinophysis acuminata	Ceratium lineatum	Ceratium lineatum	Prorocentrum gracile	Prorocentrum micans
	Dinophysis acuminata	Dinophysis acuminata	Prorocentrum rostratum	Dinophysis acuminata
	Peridinium gracilis		Ceratium furca	Mesodinium rubrum
	Peridinium diabolis		Ceratium lineatum	Prorocentrum gracile
			Prorocentrum micans	Peridinium depressum
			Scrippsiella trochoideum	Peridinium diabolus
			Noctiluca miliaris	Gonyaulax polygramma
			Peridinium sp	Scrippsiella trochoideum
			Gyrodinium sp.	Peridinium sp
				Gyrodinium sp.

* dominant

Table 2. Dinoflagellate assemblage at the start of each mesocosm experiment.

0.04 - 3.64 $\mu mol.l^{-1}$, while much higher concentrations of silicate were recorded (Table 1). The low nitrate/silicate ratios pointed to the selective uptake of nitrate over silicate and therefore the dominance of groups such as the dinoflagellates over diatoms. A similar phenomenon was observed by Armstrong *et al.* (1967), Brown *et al.* (1979) and Walker and Pitcher (1991), who found silicate at elevated levels compared to nitrate during red water blooms. The photoperiod changed by a margin of two hours during the course of the experiments.

The red tide assemblages for each mesocosm experiment usually also include taxa other than dinoflagellates, eg. diatoms, ciliates and other small flagellates. These taxa, however, made up only a very small portion of the total cell biomass in each experiment. The dinoflagellate species most abundant during the course of the experiments were *Prorocentrum micans, Ceratium furca and C. lineatum.* The raphidophyte, *Heterosigma akashiwo* and the marine ciliate, *Mesodinium rubrum* also dominated at times. Less dominant dinoflagellates are listed in Table 2.

Experimental results are presented in 15 figures. The figures includes information on temperature, the time course of chlorophyll *a*, the dominant species and the position of the thermocline.

Mesocosm 1

Red tide dominated by *Mesodinium rubrum* was collected in St

Helena Bay, near the Berg river mouth on 25 November 1992, (Fig. 3). As no newly upwelled water was available, water was collected outside the red tide to use in the lower half of the mesocosm (Table 1). The column was intensively sampled for 24 hours.

Temperature data (Table 1) displayed marked stratification with a sharp thermal gradient (Δ15.1°C) between the surface and the bottom of the mesocosm (Fig. 4). Temperatures ranged from 25.2°C on the surface to 10.1°C at 2.7 m. Nutrient data indicated that nitrogen was depleted throughout the water column (Table 1).

A distinct subsurface chlorophyll *a* maximum was initially present at 0.6m at the start of the experiment (Fig. 5). Active downward migration was observed at 14h00, with the formation of a pronounced subsurface maximum at the bottom of the column by 18h00. Upward migration was observed prior to sunrise with the subsurface maximum again present at 0.6 m by 10h00 the following morning, although it was less pronounced.

Examination of the cell counts of *M. rubrum* shows an early pronounced descent, with cell maxima reaching the bottom of the mesocosm by evening (Fig. 6). Upward migration was observed prior to sunrise with a distinct subsurface maximum present in the upper 1 m at 06h00 and 10h00. Maximum observed descending swimming speeds were in the order of 0.3 $m.h^{-1}$. The diel vertical pattern of migration observed during mesocosm 1 was similar to observations by Passow (1991) made both in the field and in the laboratory.

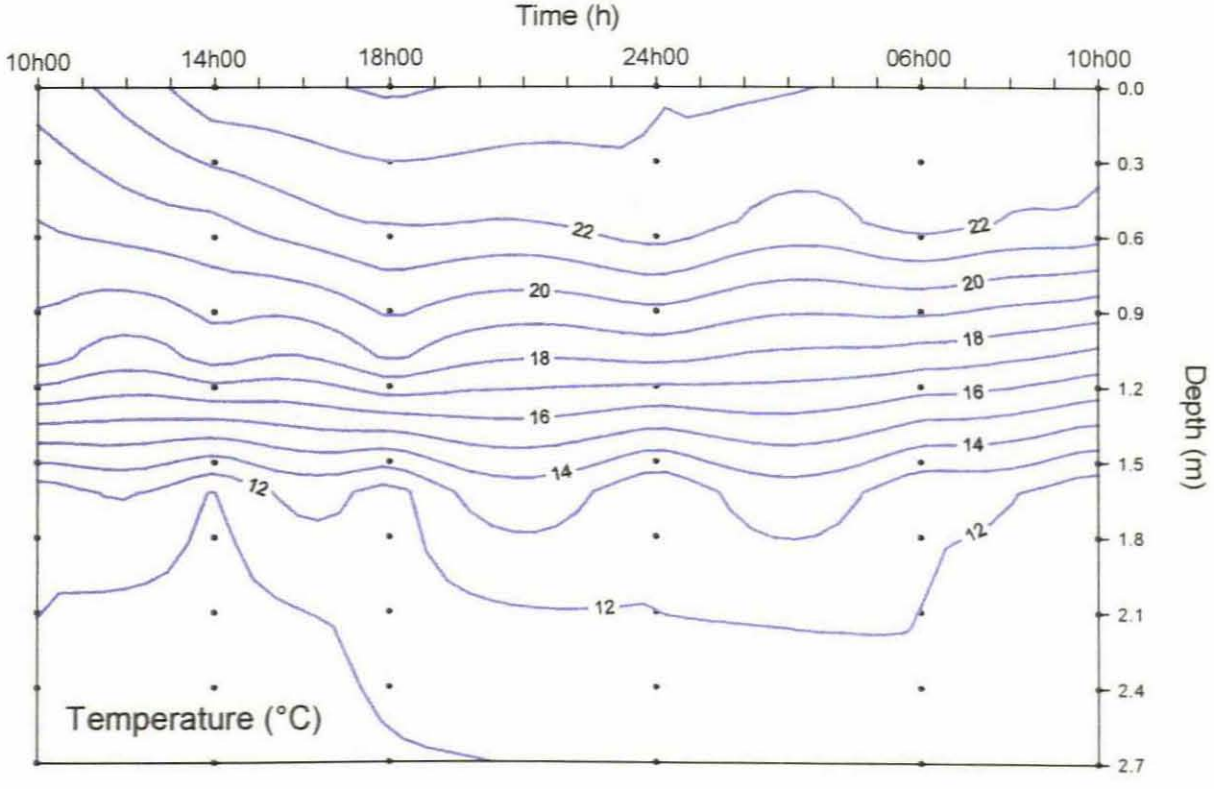

Fig. 4. Vertical changes of temperature (°C) during Mesocosm 1. Sampling was done at indicated intervals of time (h) and depth (m).

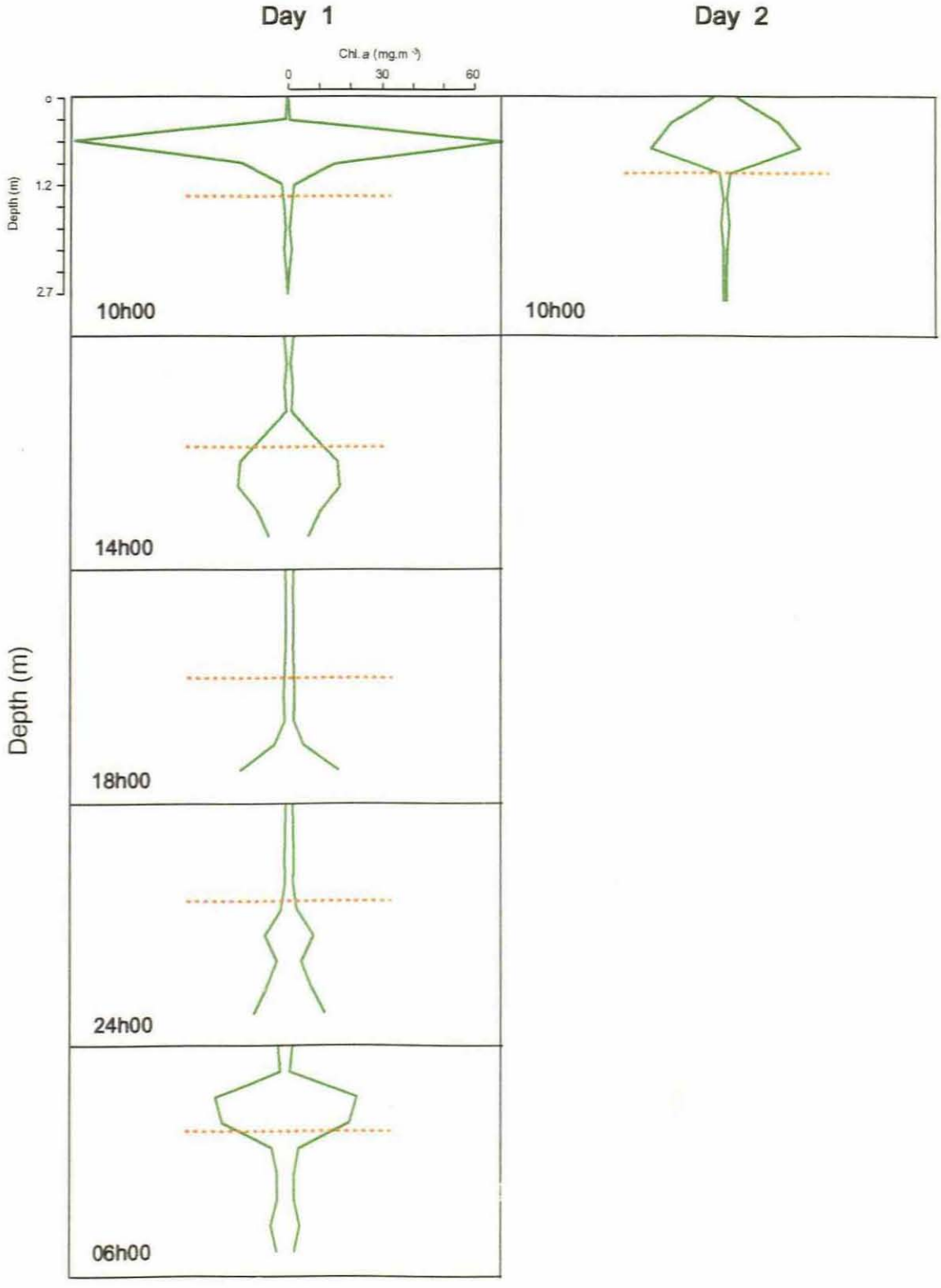

Fig. 5. Chlorophyll *a* profiles relative to the thermocline (·····) during mesocosm 1.

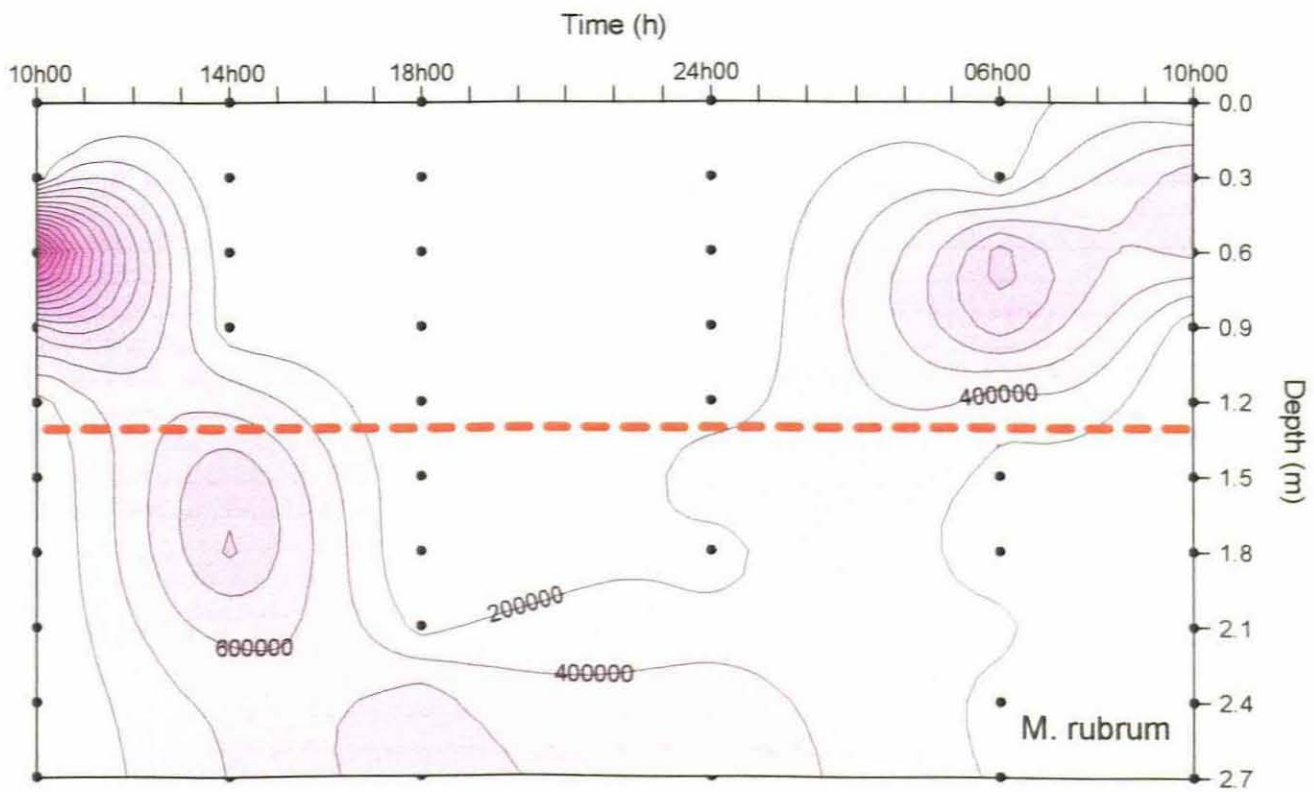

Fig. 6. Time series of cell density (cells.l^{-1}) of *M. rubrum* relative to the thermocline (— -) during mesocosm 1. Sampling was done at indicated hourly intervals of time (h) and depth (m).

Mesocosm 2

Red tide, dominated by *Prorocentrum micans* and *Ceratium furca*, was collected at Doringbaai, north of Lamberts Bay on the 14 January 1993 (Fig. 3). Recently upwelled water was used in the lower half of the column in setting up the experiment the same evening. The column was allowed to stabilise before sampling commenced 12 hours later and continued for 2 days.

During the course of the experiment, extremely high ambient temperatures resulted in surface temperatures reaching a maximum of 31.8°C during the day, before decreasing to 22.3°C at night (Table 1). Temperature profiles displayed an exceptionally sharp thermal gradient (Δ19.2°C) between the surface and bottom waters (Fig. 7). Nitrate concentrations were depleted in the surface layers, ranging between 0.04 - 0.20 μmol.l^{-1}. Below the thermocline, nitrate concentrations were elevated to >6 μmol.l^{-1} (Table 1).

In contrast to the first experiment, the major biomass tended to remain at or near the surface (Fig. 8). Only a small fraction of the population migrated through the thermocline. The migrating population, however, failed to reach the bottom of the column, remaining near the thermocline during the night. The following night the migrating portion of the population increased significantly.

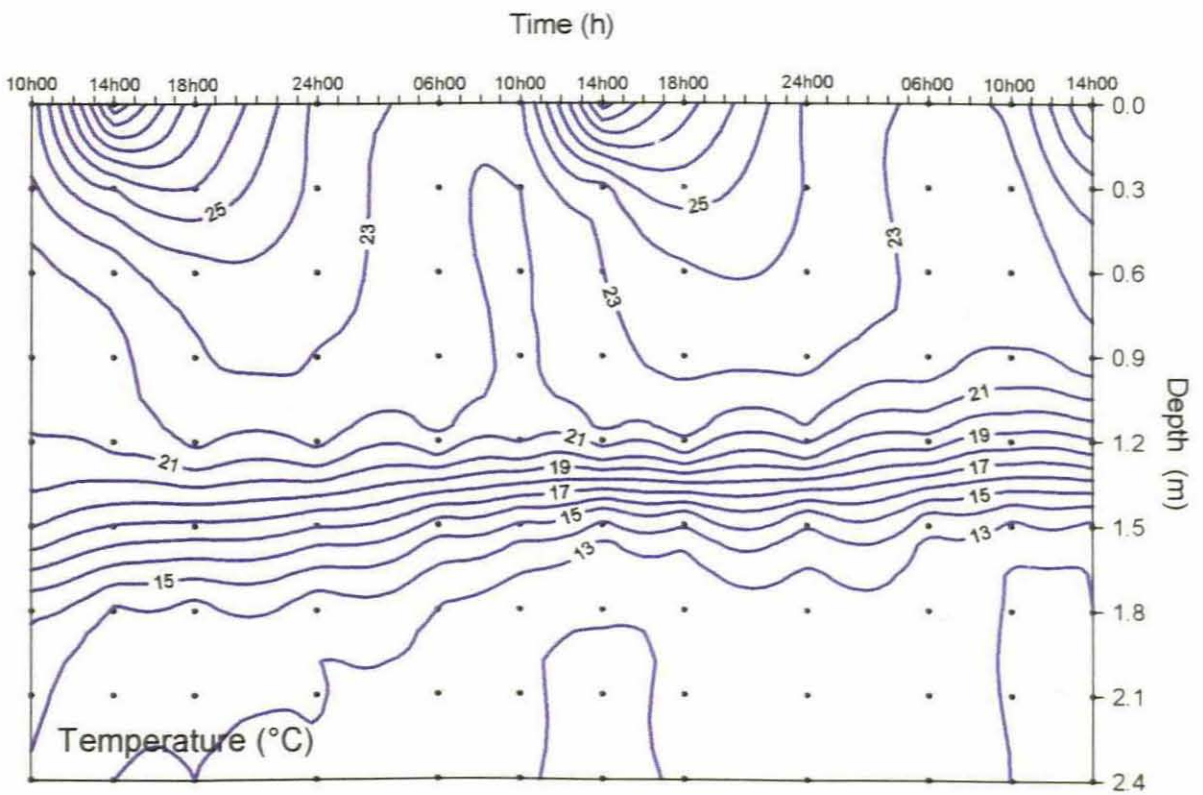

Fig. 7. Vertical changes of temperature (°C) during mesocosm 2. Sampling was done at indicated intervals of time (h) and depth (m).

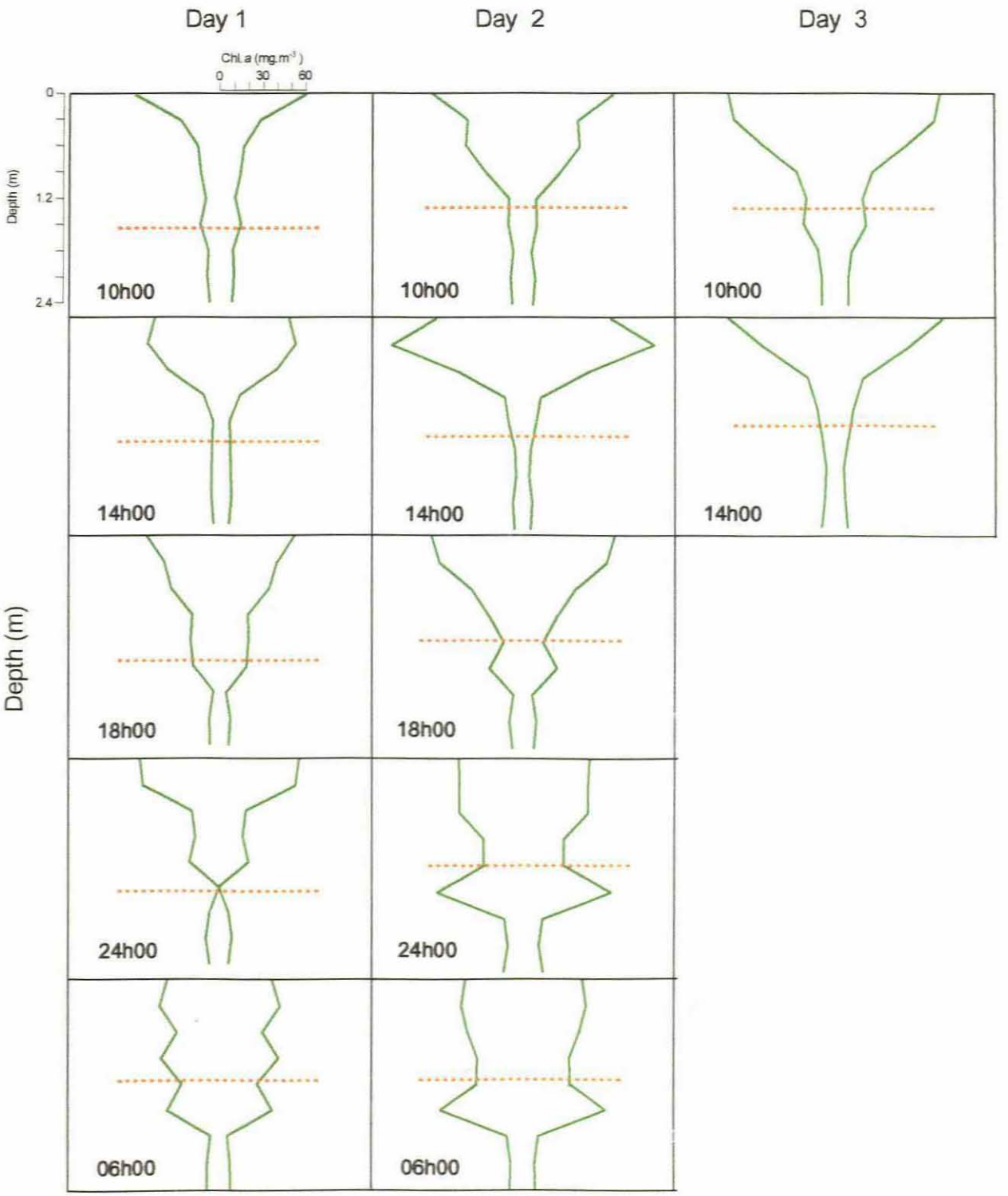

Fig. 8. Chlorophyll *a* profiles relative to the position of the thermocline (·····) during mesocosm 2.

Both the dominant dinoflagellates exhibited DVM. However, their vertical distribution showed a marked difference in migration behaviour (Fig. 9). *P. micans* displayed the most pronounced DVM, but failed to penetrate the thermocline during its descent. *C. furca* remained dispersed throughout the column during the day. At night, *C. furca* formed a dense layer at the thermocline. A small portion of the population ascended to the surface during the early hours of the morning prior to *P. micans* accumulating at the surface. These results are in agreement with the vertical distribution profiles of *P. micans* and *C. furca* described by Edler and Olsson (1985).

Both species started their descent prior to 18h00. *C. furca* had a more pronounced descent while *P. micans* appeared to descend at a much slower rate (Fig. 9). Maximum observed ascending swimming speed of 0.17 $m.h^{-1}$ and descending swimming speed of 0.16 $m.h^{-1}$ were recorded for *P. micans* and *C. furca* respectively.

Mesocosm 3

Red tide dominated by *P. micans*, *C. furca* and *C. lineatum* was collected at Strandfontein on the West Coast on 2 February 1993 (Fig. 3). Sampling was conducted over a four day period after a 12 h stabilization period. Temperature profiles displayed marked stratification with a sharp thermal gradient (Δ13.2°C) between the surface and the bottom of the mesocosm (Fig. 10).

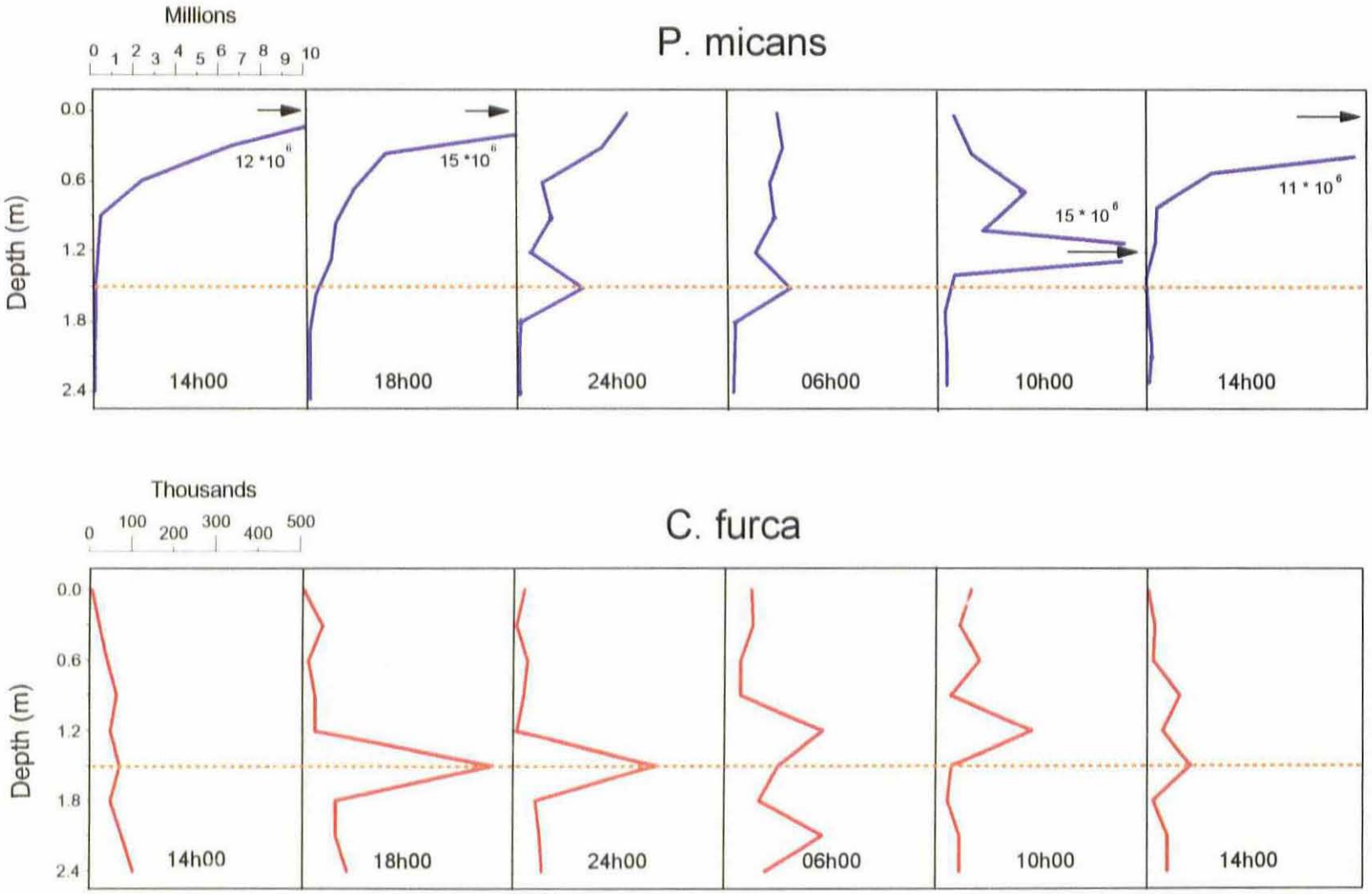

Fig. 9. Time series of cell density (cells.l $^{-1}$) of *P. micans* and *C. furca* relative to the thermocline (·····) during mesocosm 2.

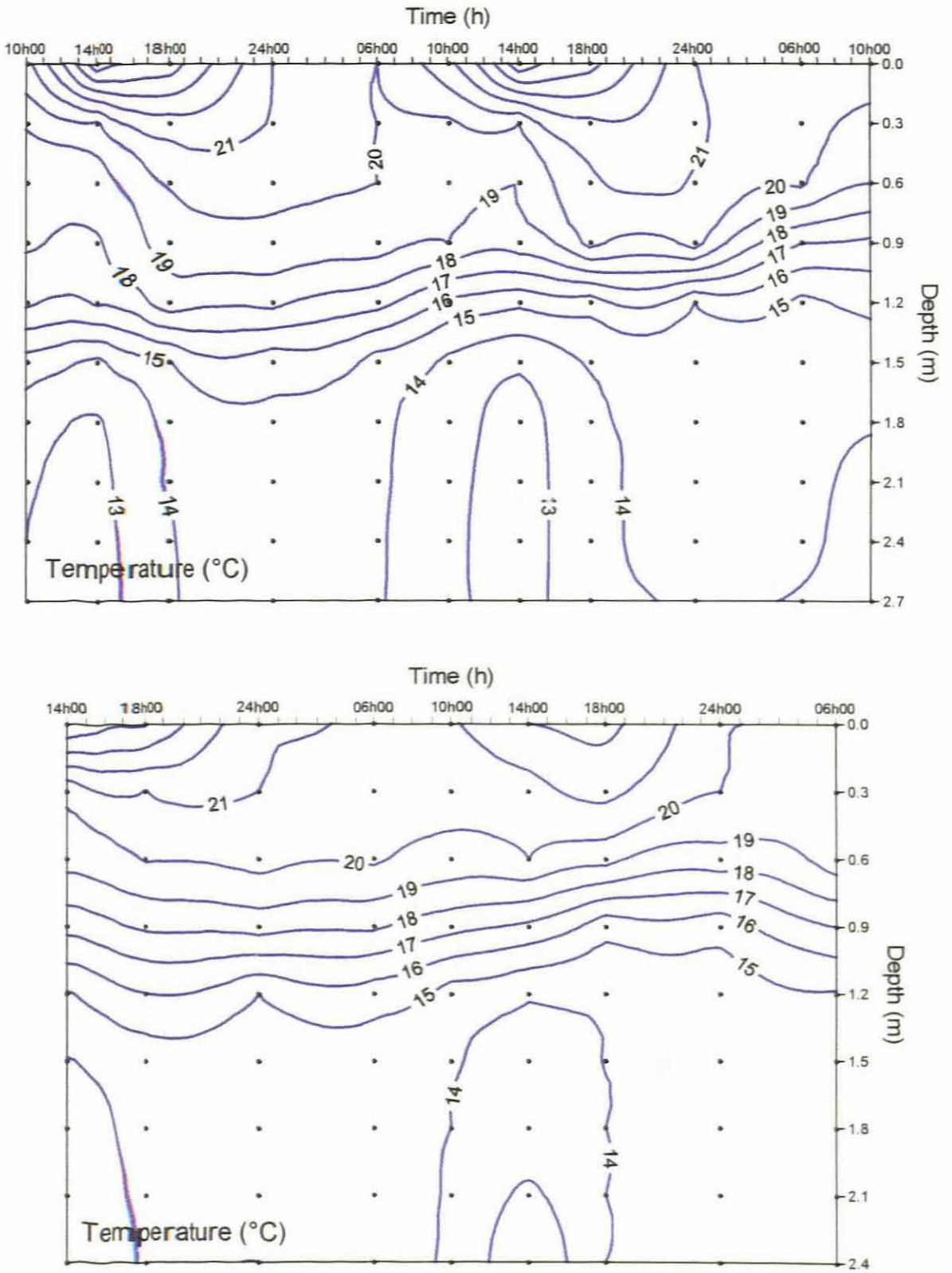

Fig. 10. Vertical changes of temperature (°C) during mesocosm 3. Sampling was done at indicated intervals of time (h) and depth (m).

Temperatures ranged from 25.7°C on the surface to 12.5°C at 2.7 m. After initial sunny weather, cooler cloudy weather set in, resulting in low light intensities (<100 $\mu E\ m^{-2}s^{-1}$ at the surface) and lower surface temperatures. Nitrate concentrations were low in the surface water (< 0.46 $\mu mol.l^{-1}$) but much higher (>9 $\mu mol.l^{-1}$) below the thermocline (Table 1).

The chlorophyll *a* distribution was examined relative to the position of the thermocline (Fig. 11). A dense surface population was evident at 14h00 and was still present at 18h00 on day 1. At night a portion of the population descended to the thermocline. On day 2 a greater portion of the population migrated to below the thermocline. The population was divided on day 3, one portion remained at the surface while the other migrated well below the thermocline at night. During the experiment, the portion of the population that migrated beyond the thermocline not only increased but also remained there for longer periods of time.

There was an order of magnitude difference in the concentration of the three species that dominated the water column (Fig. 12). The diel pattern of migration of *P. micans* was very similar to that of the previous experiment, dominating the surface waters during the day and concentrating at or near the thermocline during the night. In contrast, *C. lineatum* remained subsurface during the day and migrated to the bottom of the column at night. The DVM pattern of *C. furca* was similar to that observed in mesocosm 2 with the population again dividing into two portions.

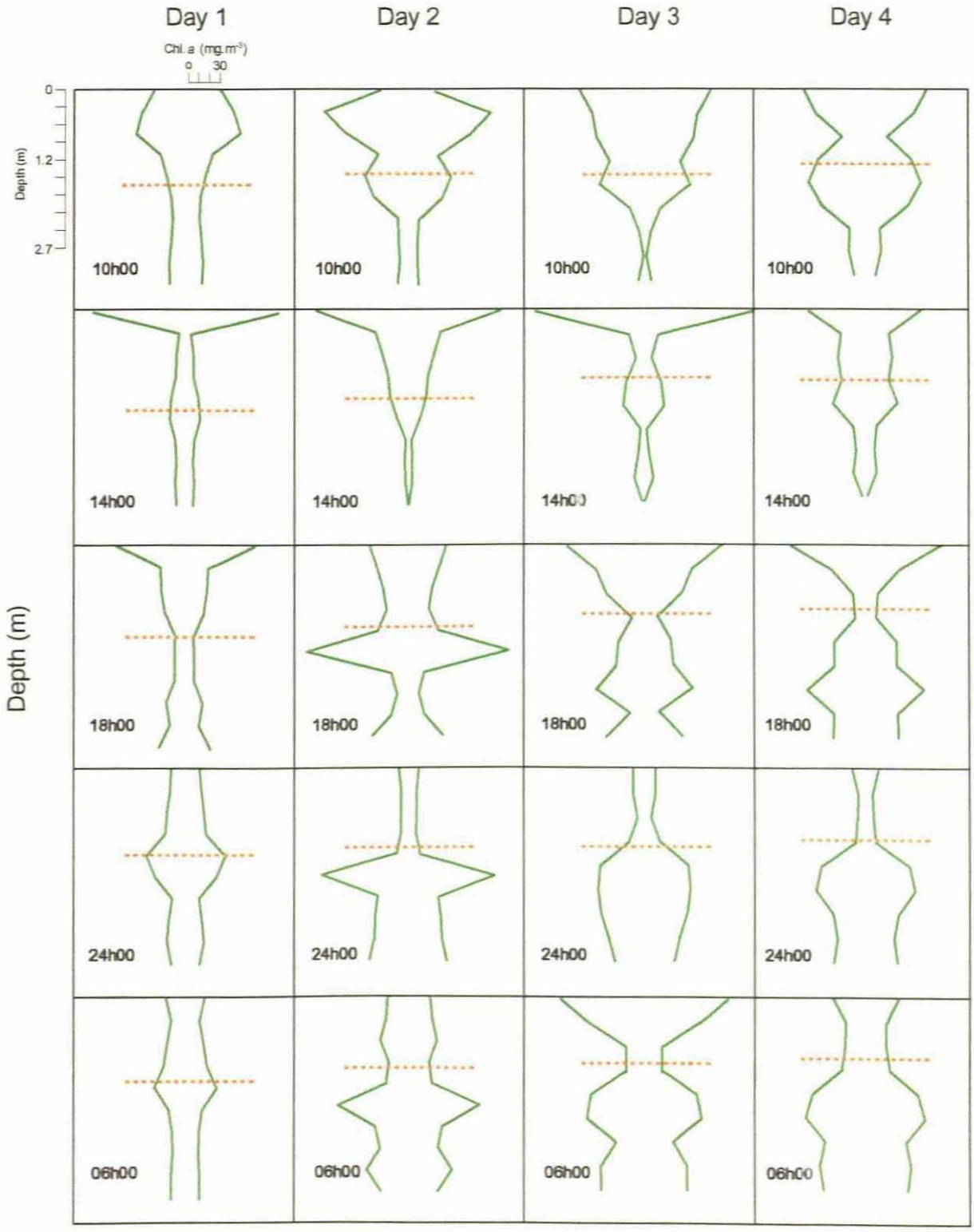

Fig.11. Chlorophyll *a* profiles relative to the thermocline (·····) during mesocosm 3.

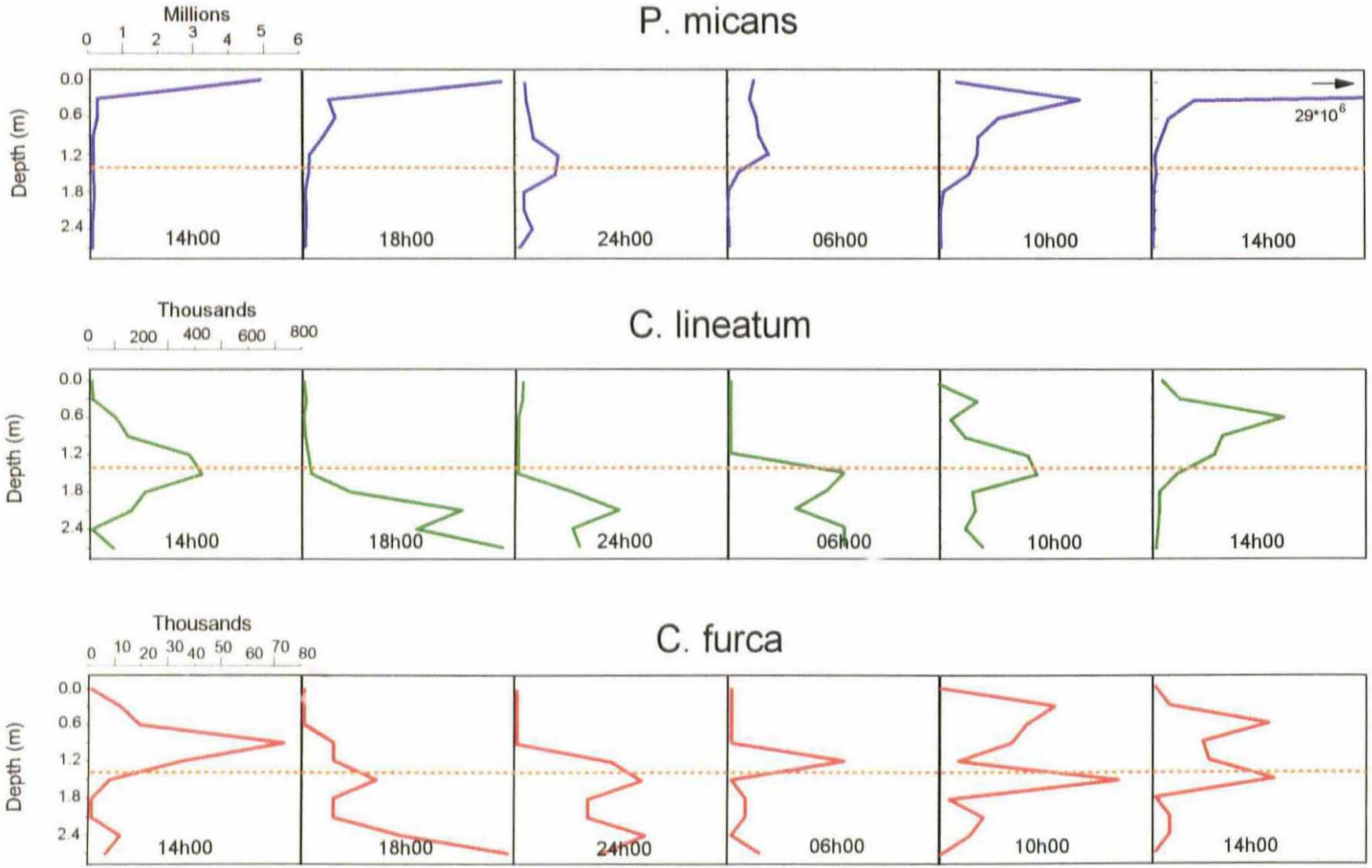

Fig.12. Time series of cell density (cells.l $^{-1}$) of *P. micans*, *C . lineatum* and *C. furca* relative to the thermocline (·····) during mesocosm 3.

As in mesocosm 2, *P. micans* accumulated in the exceptionally warm surface water during the day. This behaviour was also recorded by Lassus *et al.* (1990).

All three species started their descent prior to 18h00. As was the case in Mesocosm 2, the descent of P. micans appeared to be slower than that of the two *Ceratium* species. All species had initiated their ascent by 06h00 (Fig. 12). Maximum observed descending swimming speeds were in the order of 0.23, 0.28 and 0.32 $m.h^{-1}$ for *P. micans, C. furca* and *C. lineatum* respectively.

Mesocosm 4

Red tide dominated by *Heterosigma akashiwo* and *M.rubrum* was collected in False Bay (Fig. 3). After setting up the experiment, the column was left to settle for 36 h before two 24 h sampling periods was undertaken, separated with a 24 h period during which no sampling took place.

Temperature profiles show a distinct stratification with a sharp thermal gradient (Δ12.8°C). Temperatures ranged from 25.5°C on the surface to <13°C at 2.7 m (Fig. 13). Although the temperature conditions were very similar to the previous experiment, the initial irradiance (1550 $\mu E\ m^{-2}s^{-1}$) was much higher at the surface, decreasing to 920 $\mu Em^{-2}s^{-1}$ on the last day of the experiment. Nitrate concentrations were relatively high in the surface water (3.64 $\mu mol.l^{-1}$) while even higher concentrations (8.36 $\mu mol.l^{-1}$) were available below the thermocline (Table 1).

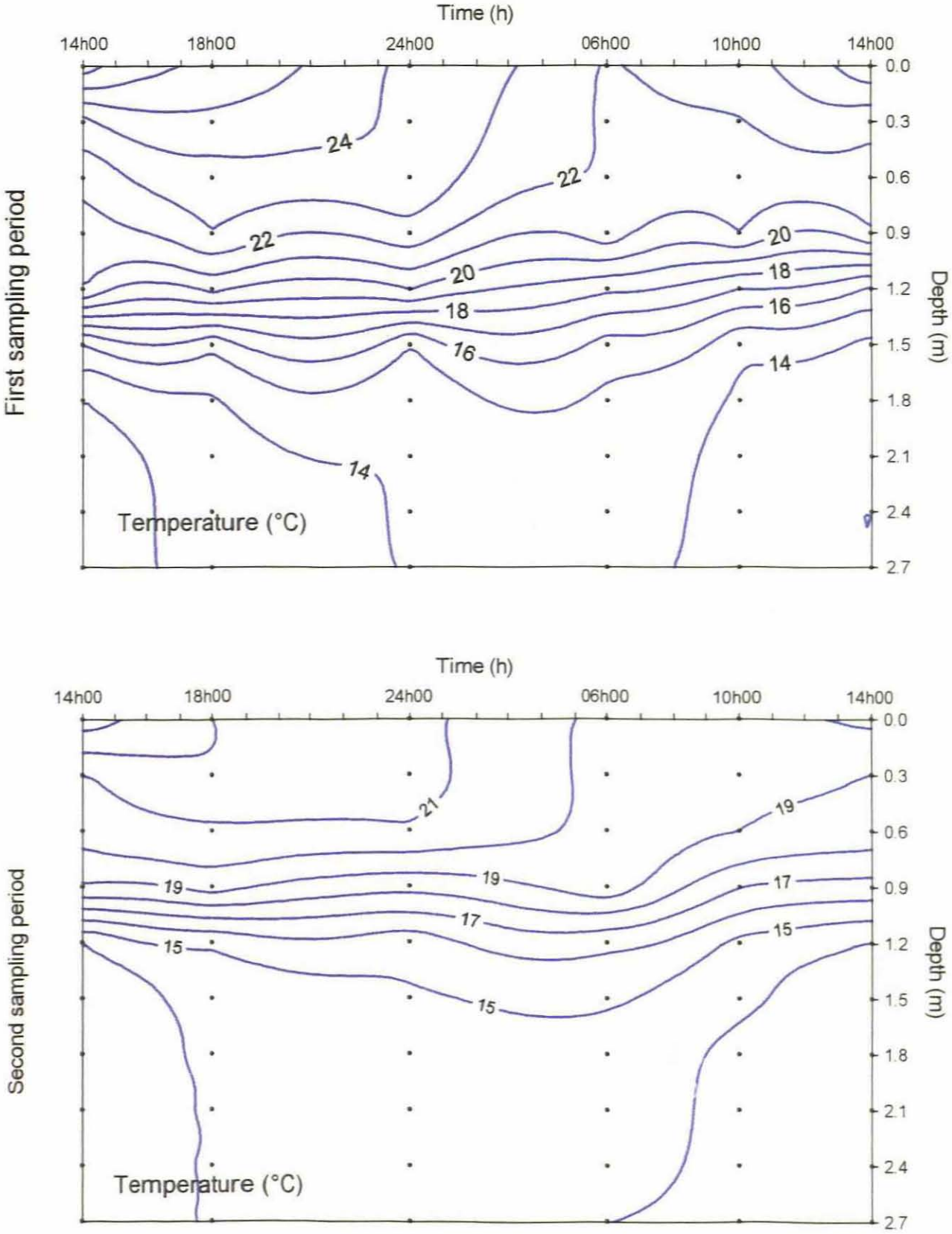

Fig. 13. Vertical distribution of temperature (°C) during mesocosm 4. Sampling was done at indicated intevals of time (h) and depth (m).

The DVM pattern of the red tide population appeared fairly similar during the two periods of sampling (Fig. 14). On day one, the initially subsurface population exhibited pronounced vertical migration, commencing it's descent prior to sunset. The population remained well below the thermocline at night, and accumulated in the region of the thermocline during the following day. During the second period of sampling there was a greater tendency for the population to accumulate near the surface.

This red tide population was initially dominated by *M. rubrum* which undertook vertical migration from below the subsurface during the day to the region of the thermocline at night (Fig. 15). On the last day of sampling, *M. rubrum* accumulated at the surface for the first time under much reduced irradiance. A similar diel pattern was also observed by Cloern *et al.* (1994) and Villarino *et al.* (1995). Passow (1991) hypothesised that *M. rubrum* migrated towards higher light intensities until a certain threshold irradiance was reached, after which migration was directed downwards to avoid light intensities higher than this threshold.

Low numbers of the raphidophycean flagellate, *Heterosigma akashiwo* were recorded at the start of the mesocosm 4 experiment (Fig. 15). This situation was, however, reversed by the early hours of the following morning when a dense concentration of *H. akashiwo* accumulated near the surface. During the second sampling period

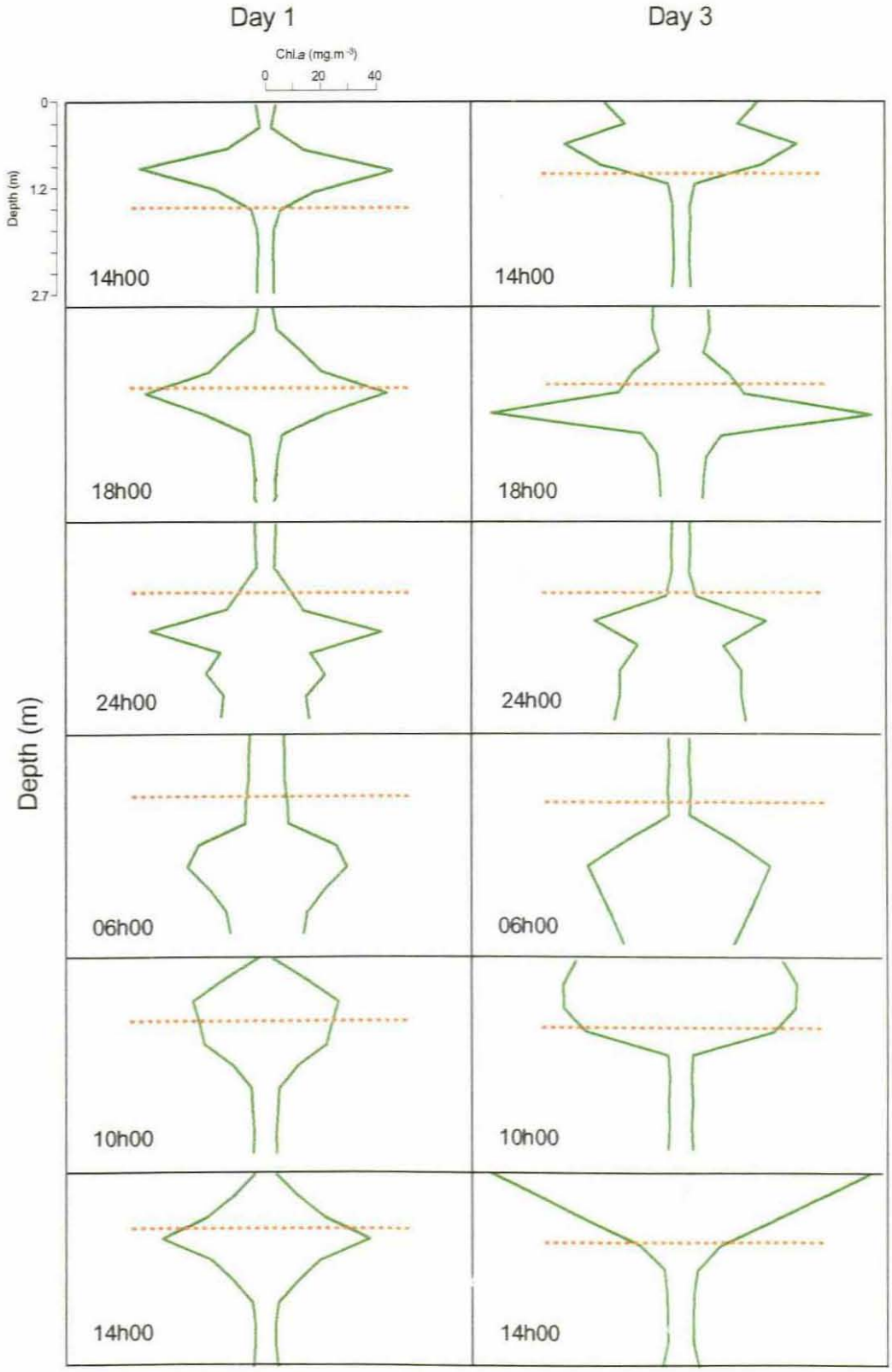

Fig. 14. Chlorophyll *a* profiles relative to thermocline (·····) during mesocosm 4.

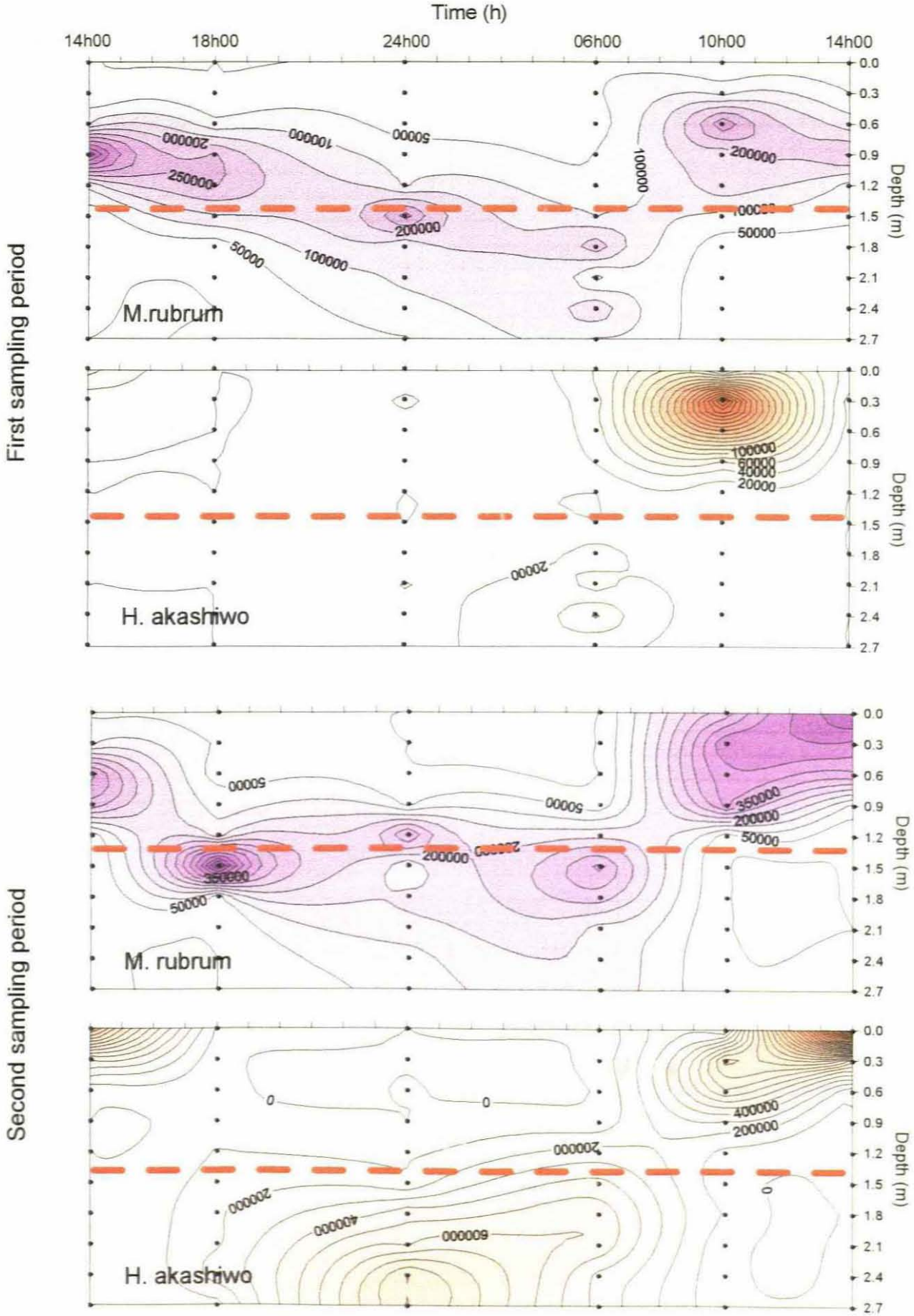

Fig. 15. Time series of cell density (cells.l^{-1}) of *M. rubrum* and *H. akashiwo* relative to the thermocline (— —) during mesocosm 4.

H. akashiwo executed extensive DVM between the surface and the bottom of the column (Fig.15). On the last day, *H. akashiwo* co-inhabited the surface waters with *M. rubrum*. The observations of diel migration confirm similar studies conducted by Yamochi and Abe (1984) on *H. akashiwo* in a dialysis bag.

Both species started their descent prior to sunset, and their ascent prior to sunrise. *H. akashiwo* migrated upwards and downwards at similar speeds, while *M. rubrum* descended very slowly but ascended more rapidly. The maximum observed descending swimming speeds of *H. akashiwo* were in the order of 0.4 $m.h^{-1}$ while the maximum observed ascending swimming speed of *M. rubrum* were in the order of 0.3 $m.h^{-1}$.

Mesocosm 5

Red tide dominated by *Ceratium furca* and *C. lineatum* was collected in Table Bay on 31 March 1993. (Fig. 3). The sampling strategy was similar to the previous experiment whereby the column was stabilised for 36 h before two 24 h sampling periods commenced, separated by a 24 h period during which no sampling took place. Cool, cloudy weather resulted in the irradiance at the surface decreasing from an initial 320 $\mu E\ m^{-2}s^{-1}$ to less than 100 $\mu E\ m^{-2}s^{-1}$. Lower surface temperatures were responsible for a weaker temperature gradient (Δ4.7°C), with the thermocline midway down the column (Fig. 16). Temperatures ranged from 21.7°C on the surface to 13.4°C at 2.7m. Nitrate concentrations were low in the surface water (0.13 $\mu mol.l^{-1}$) and only marginally higher (1.29

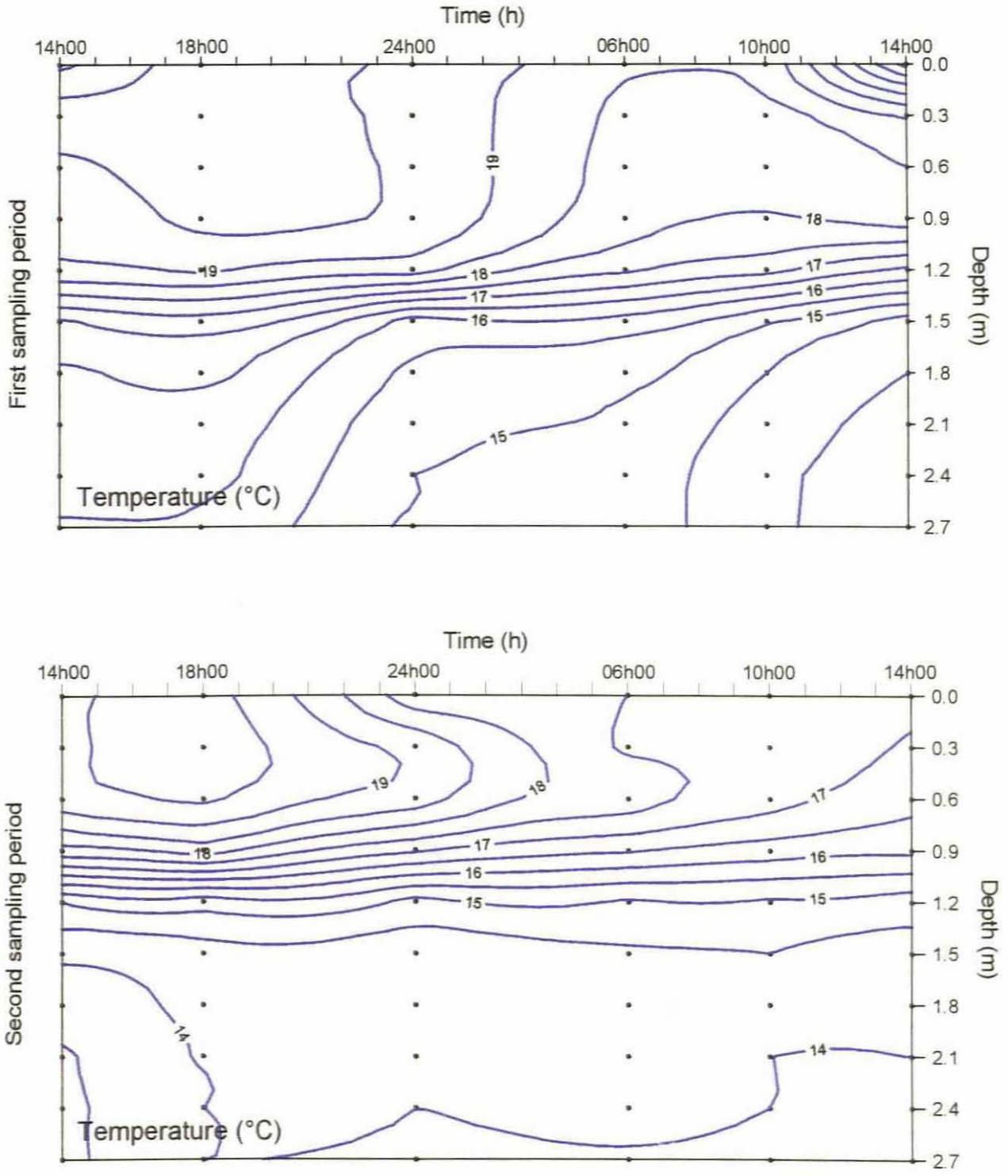

Fig. 16. Vertical changes of temperature (°C) during mesocosm 5. Sampling was done at indicated intervals of time (h) and depth (m).

$\mu mol.l^{-1}$) below the thermocline (Table 1).

The chlorophyll *a* distribution showed that there was a tendency for the red tide to be distributed throughout the column during the first period of sampling (Fig.17). Vertical migration appeared to be poorly defined as only a fraction of the population was seen to perform DVM. A dramatic contrast in behaviour was evident during the second period of sampling. A very intense surface maximum initiated its descent well before sunset and remained below, but close to, the thermocline at night. During the next light cycle a strong ascent through the thermocline commenced prior to 06h00 to form a well-defined surface maximum by 14h00. A secondary subsurface population was present at the beginning and end of the second sampling period. The most significant difference was its position in relation to the thermocline.

The vertical distribution of the two dominant species showed that in the absence of P. *micans, C. furca* displayed a very similar DVM pattern to that previously exhibited by *P. micans* (Fig. 18). From it's position at the surface it descended at night in a thin layer through the weak thermocline. The population again accumulated at the surface by 14h00 the following day. The DVM pattern was essentially the same for the second sampling period (Fig.18). The migrating pattern of *C. lineatum* contrasted substantially from that of *C. furca*. In the first sampling period a significant portion of the population was present below the

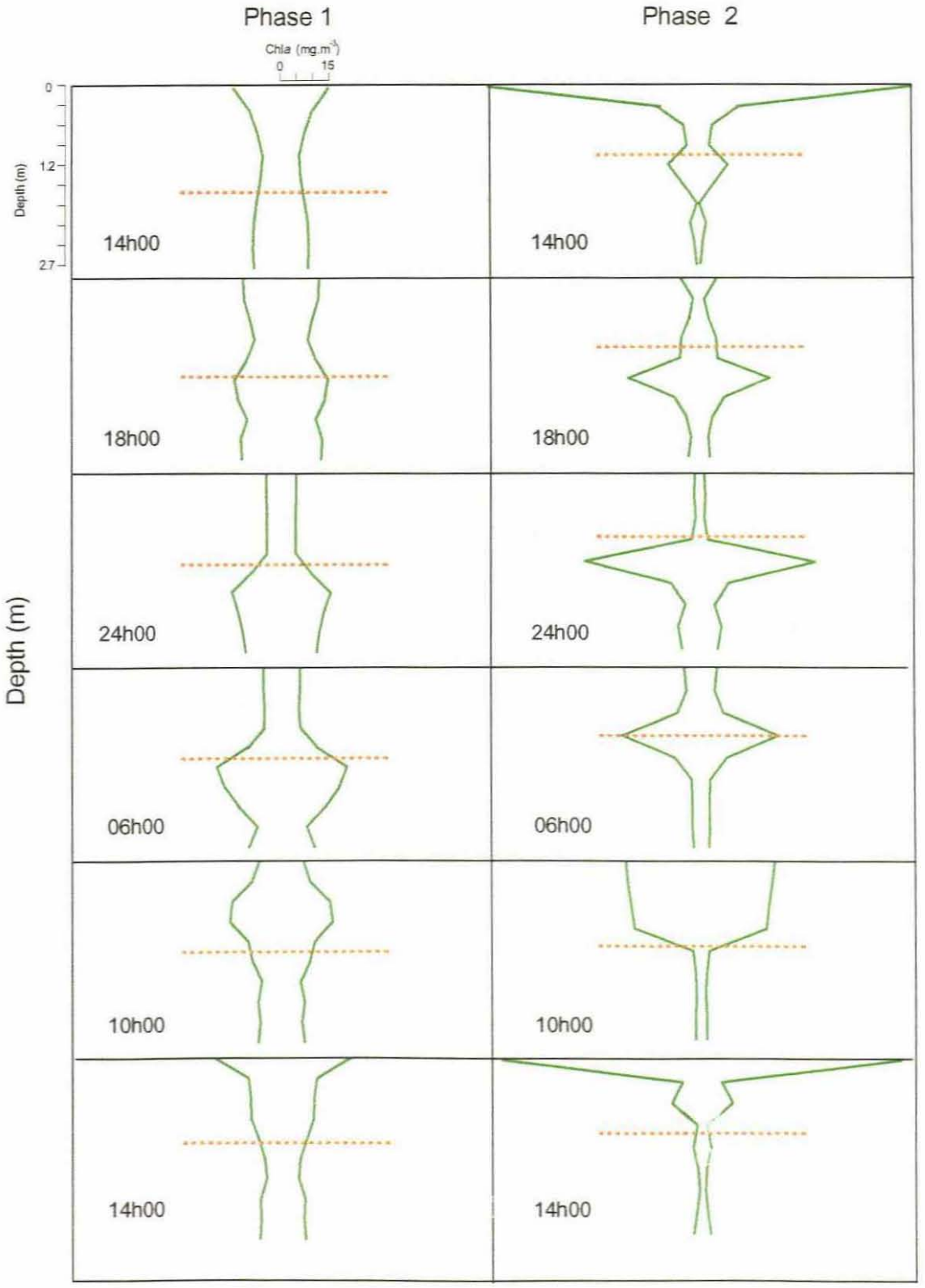

Fig. 17. Chlorophyll *a* profiles relative to the position of the thermocline (·····) during mesocosm 5.

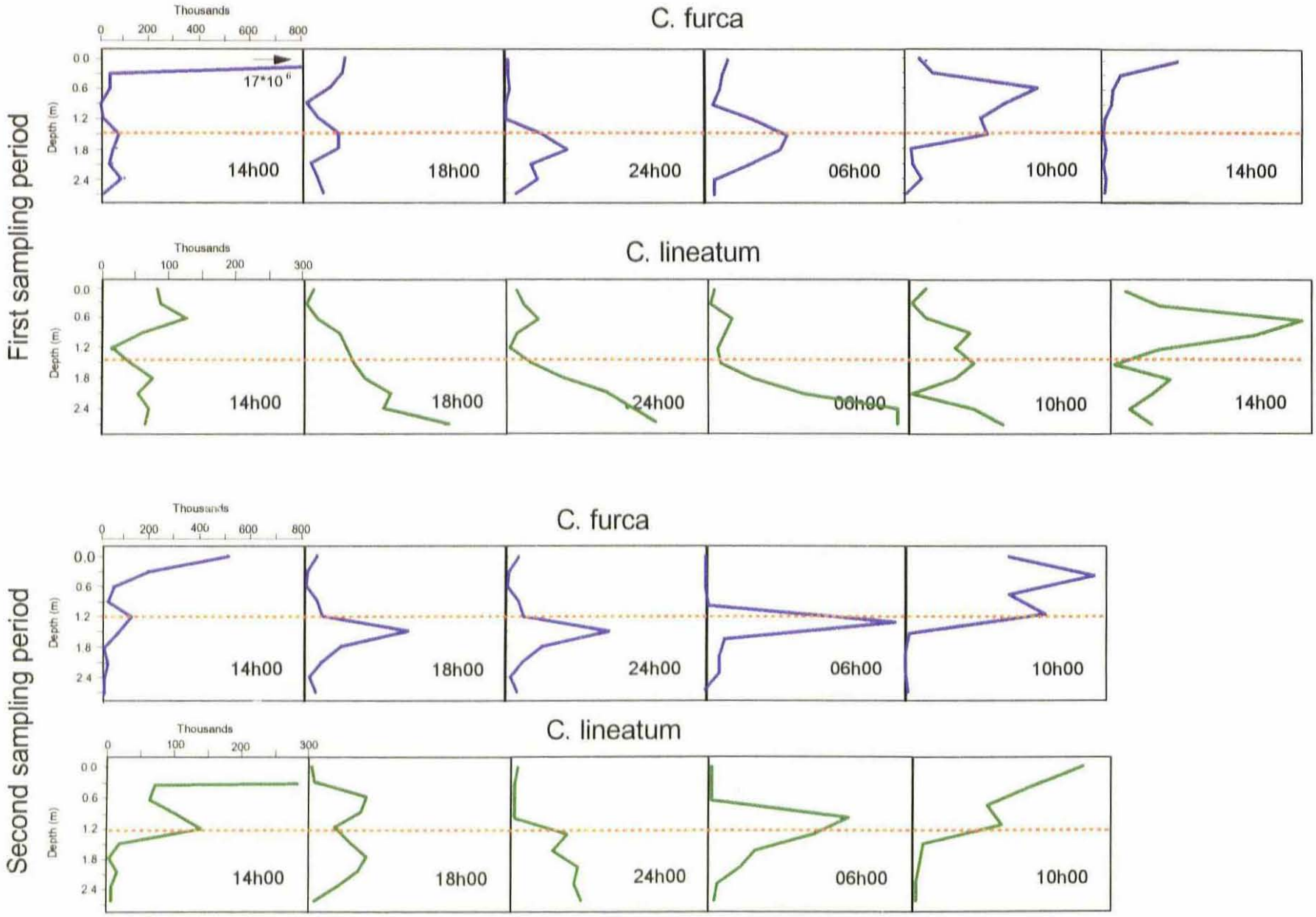

Fig. 18. Time series of cell density (cells.l $^{-1}$) of *C.furca* and *C. lineatum* relative to the thermocline (·····) during mesocosm 5.

thermocline during the initial 24 h. Most of the population exhibited DVM, forming a subsurface maximum during the day. At the start of the second sampling period, *C. lineatum* exhibited a change in behaviour by concentrating at the surface during the day, and performing pronounced DVM, to the bottom of the column at night. On the final day *C. lineatum* appeared to displace *C. furca* at the surface (Fig. 18). Maximum observed descending swimming speeds were in the order of 0.29 and 0.32 $m.h^{-1}$ for *C. furca* and *C. lineatum* respectively.

Field Studies

In addition to the mesocosm experiments, the DVM of red tides under field conditions was studied on four occasions (Table 3). *Prorocentrum micans* was dominant during the first two studies in St Helena Bay and Table Bay, while *Gymnodinium splendens* and *Ceratium furca* dominated the Gordon's Bay and Lambert's Bay studies respectively (Table 4). Only the data from two latter studies will be presented in this chapter.

Gordon's Bay

A red tide which occurred near the Gordon's Bay harbour (Fig. 3), was reported on the 20 June 1995, and our study commenced the following day. The bloom occurred after a few days of calm weather which was preceded by several days of southerly

Survey	St Helena Bay (12.4.1994)	Table Bay (19.4.1994)	Gordon's Bay (21.6.1995)	Lambert's Bay (28.2.1996)
Temperature range (noon)	0m=16.99°C 11m=13.23°C	0m=15.6°C 11m=14.7°C	0m=15.6°C 13m=14.7°C	0m=16.8°C 15m=10.6°C
Stratification	▲T=3°C	▲T=0.9°C	▲T=0.9°C	▲T=6.2°C
Nitrate range (initial)	0m=1.29μmol.l^{-1} 11m=4.02μmol.l^{-1}	0m=2.17μmol.l^{-1} 13m=2.01μmol.l^{-1}	0m=0.34μmol.l^{-1} 7m=11.2μmol.l^{-1}	0m=1.35μmol.l^{-1} 14m=7.82μmol.l^{-1}
Silica range (initial)	0m=17.23μmol.l^{-1} 11m=28.81μmol.l^{-1}	0m=15.27μmol.l^{-1} 13m=18.20μmol.l^{-1}	0m=15.27μmol.l^{-1} 7m=18.20μmol.l^{-1}	0m=10.91μmol.l^{-1} 14m=12.52μmol.l^{-1}
Phosphate range (initial)	0m=7.07μmol.l^{-1} 11m=6.39μmol.l^{-1}	0m=4.9μmol.l^{-1} 13m=1.99μmol.l^{-1}	0m=4.9μmol.l^{-1} 7m=1.99μmol.l^{-1}	0m=2.68μmol.l^{-1} 14m=1.17μmol.l^{-1}
Sunrise	07h06	07h11	07h51	06h34
Sunset	118h31	18h18	17h44	19h27
Photoperiod (L:D)	11h25min to 12h35min	11h07 min to 12h53min	9h53min to 14h07min	12h53mim to 11h07min

Table 3. Physical and chemical parameters at the start of each field study.

St Helena Bay	Table Bay	Gordon's Bay	Lambert's Bay
Prorocentrum micans*	Prorocentrum micans*	Gymnodinium splendens*	Ceratium furca*
Ceratium furca	Ceratium furca	Prorocentrium rostratum	Prorocentrum micans*
Ceratium lineatum	Ceratium lineatum	Prorocentrum gracile	Alexandrium catenella
Dinophysis acuminata	Dinophysis acuminata	Gyrodinium sp.	Gonyaulax gridleyi
Alexandrium catenella	Alexandrium catenella	Ceratium furca	Scrippsiella trochoideum
Gonyaulax grindleyi	Gonyaulax grindleyi	Ceratium lineatum	Dinophysis acuminata
Gyrodinium sp.	Scrippsiella trochoideum	Dinophysis acuminata	Peridinium excentricum
	Gyrodinium sp.		

* dominant

Table 4. Dinoflagellate assemblage at the beginning of each field study.

upwelling wind. During the study the wind changed to the north west and increased in strength during the night resulting in very turbulent seas with rain the following morning (Fig. 19).

The temperature profile shows the initial presence of a highly stratified water column with a shallow upper mixed layer (Fig. 20). During the night the thermocline was gradually eroded as a result of the strengthening wind, resulting in a deepening of the upper mixed layer (Figs 20, 21). The fluorescence time-series reveals the initial presence of a well defined maximum in the upper mixed layer above a well developed thermocline at 15:00 (Fig. 20). Active downward migration of the dinoflagellate population into the high nitrate bottom water was observed in the late afternoon, with the formation of a pronounced subsurface maximum one meter off the seabed before midnight (Figs. 20, 21). At 04h00, the dinoflagellate population was still evident in the bottom nutrient-rich waters although there was an apparent decrease in nitrate concentration in these bottom waters (Fig. 21). Upward migration was observed prior to sunrise with a surface maximum again present at 08h00. By 14h00 the population was dispersed throughout the mixed layer as a result of the deepening of the upper mixed layer in response to increased wind.

The vertical distribution of nitrate shows depletion above the thermocline, whereas deep water concentrations were sufficient to sustain considerable growth (Fig. 21).

Examination of the vertical distribution of the individual

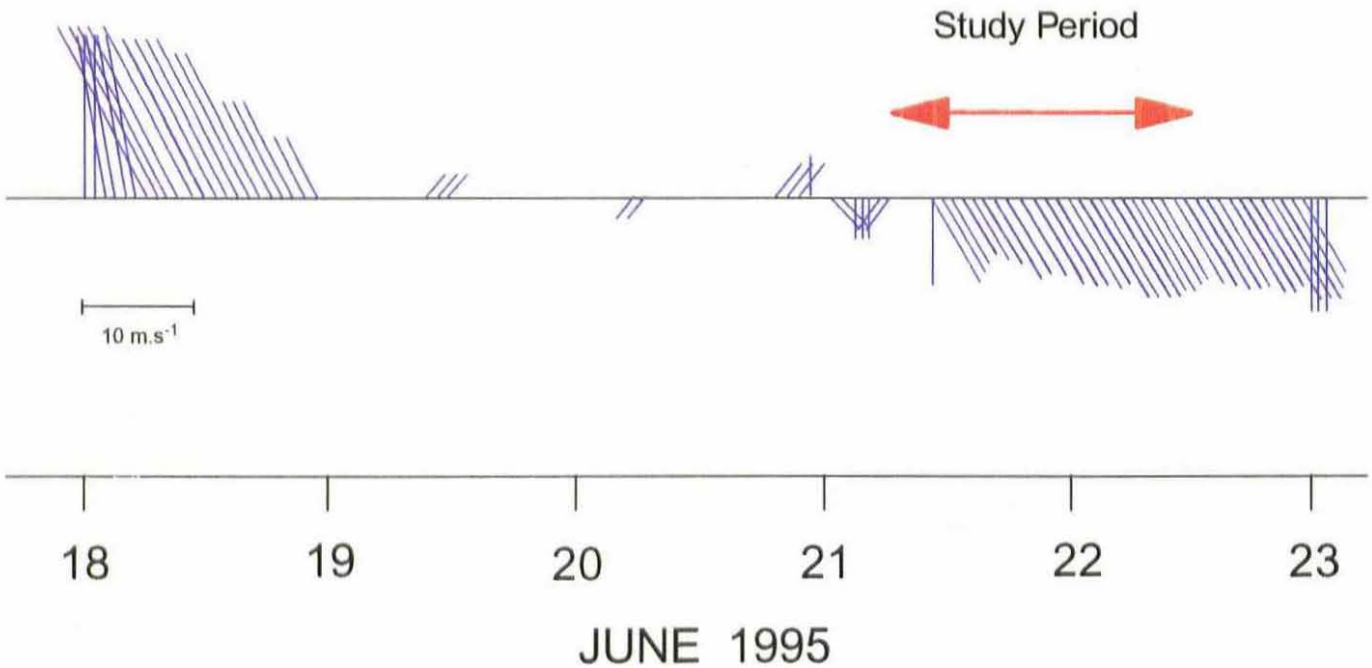

Fig. 19. Hourly stick wind vectors ($m.s^{-1}$) measured at Cape Point lighthouse during June 1995.

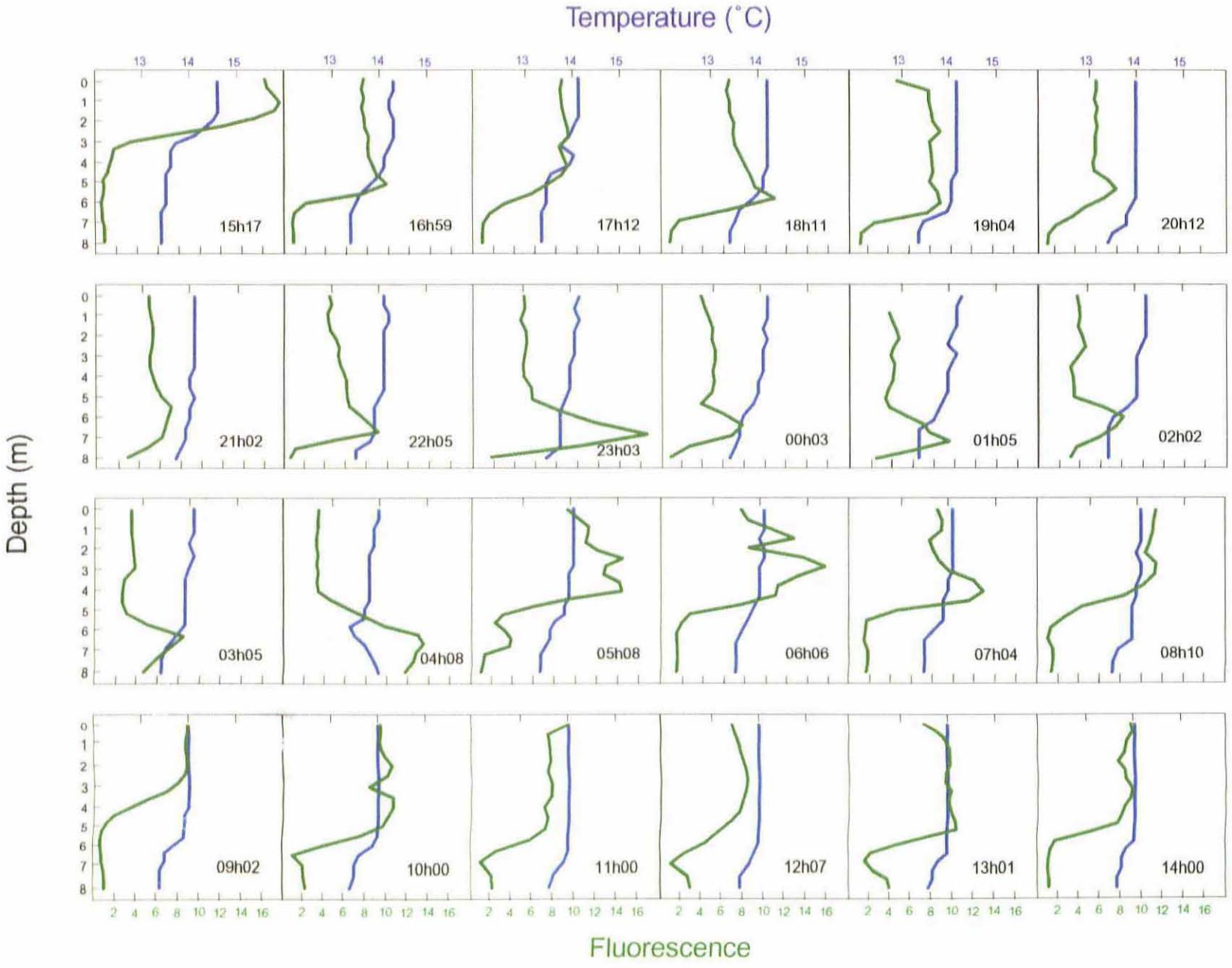

Fig. 20. Vertical profiles of temperature and *in situ*fluorescence from CTD-fluorometer casts during the Gordon's Bay study.

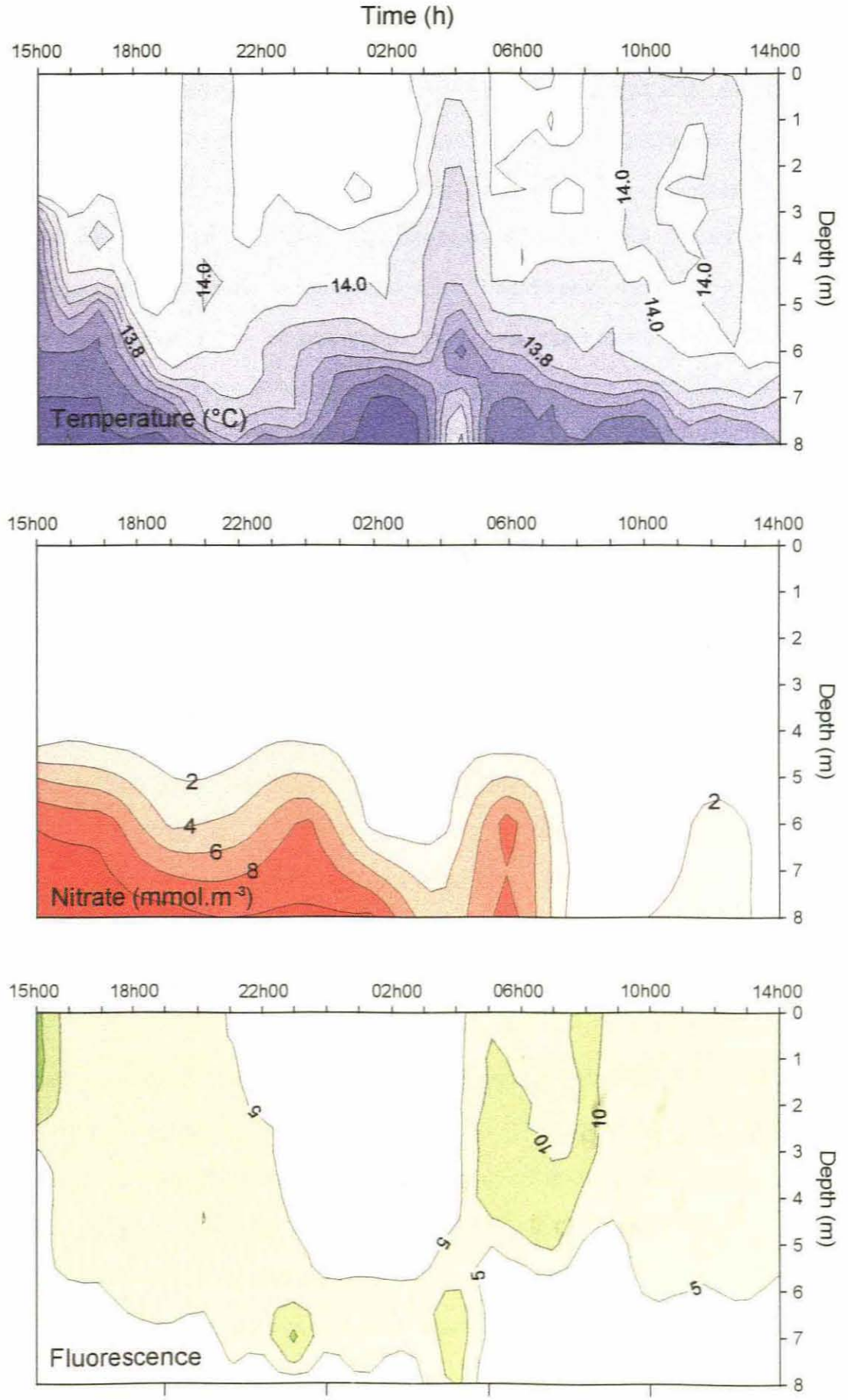

Fig. 21. Time series of temperature, fluorescence and nitrate during the Gordon's Bay study.

species revealed that *Gymnodinium splendens*, the dominant species, displayed a marked diel vertical migration during the period of study (Fig. 22). These results are in general agreement with both the field and laboratory studies of Cullen and Horrigan (1981). Having initially concentrated at the surface, the deteriorating weather prevented the organism from accumulating at the surface the following day, dispersing the organism throughout the mixed layer in response to increased wind. *Gymnodinium sp.*, which initially formed a subsurface maximum, displayed limited DVM descending to only 4 m depth at night. Upward migration of this species was observed at 04h00 and it reached the surface at 06h00, before again descending to form a subsurface maximum. *Prorocentrum rostratum* concentrated below *Gymnodinium sp.* during the day, exhibiting limited DVM during the 24h study (Fig. 22). *P. gracile* formed a distinct subsurface maximum during the day before migrating into high nitrate bottom water in the evening to form a dense layer at 6 m depth. Upward migration was observed prior to sunrise. The maximum estimated swimming speeds were in the order of 0.58, 0.34 and 0.18 $m.h^{-1}$ for *G. splendens*, *Gymnodinium sp.* and *P. gracile* respectively.

Red tides manifest themselves following a decrease in wind stress as the water becomes more stratified. The overriding effect of turbulence on the vertical distribution of dinoflagellate species was clearly seen during the 24 h Gordon's Bay field study. As the wind increased in strength, the increased turbulence was responsible for distributing the population throughout the upper

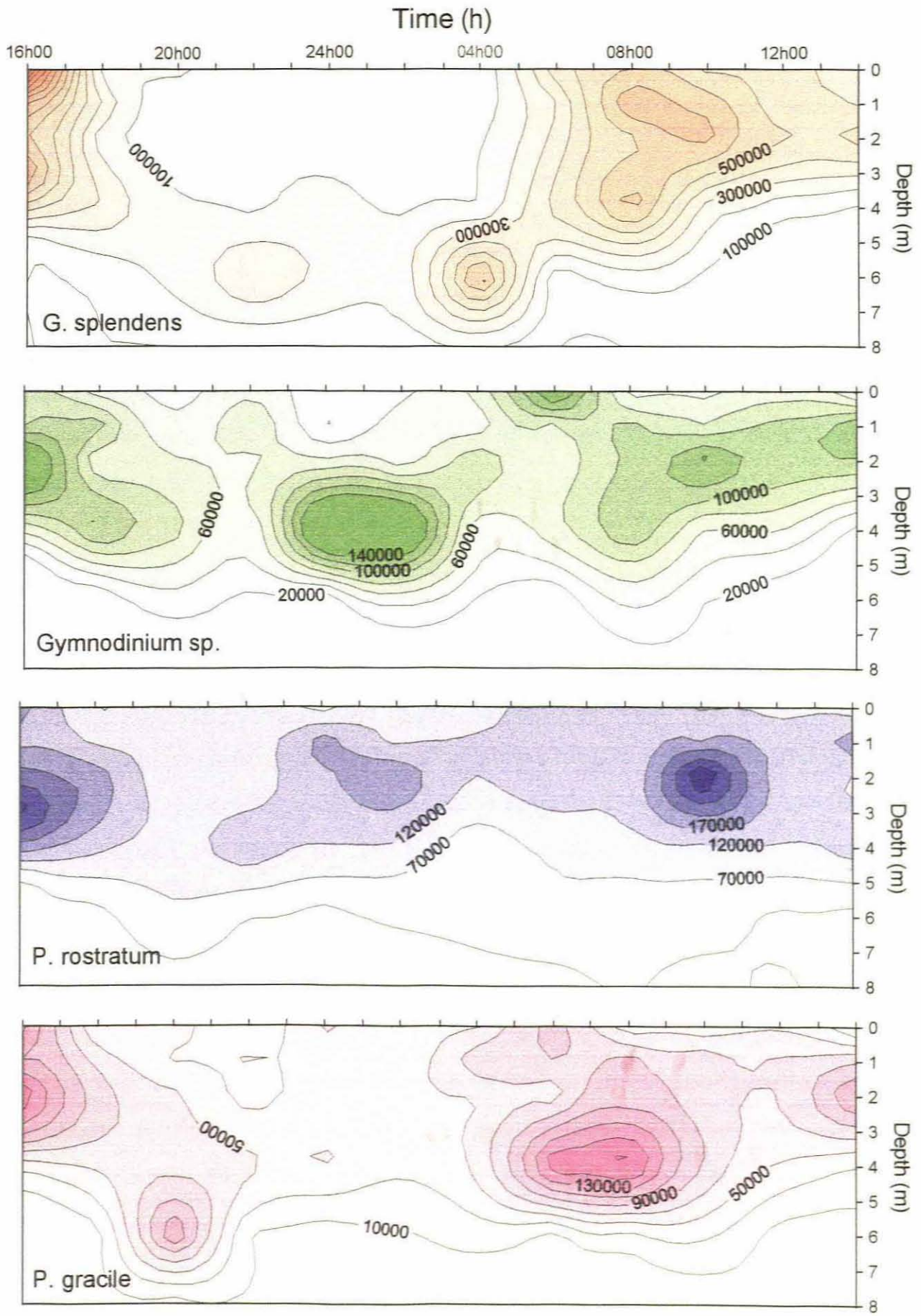

Fig.22. Time series of cell density (cells.l^{-1}) of *G. splendens*, *Gymnodinium sp.*, *P. rostratum* and *P. gracili* during the Gordon's Bay study.

mixed layer. Vertical migration patterns were therefore easily disrupted by mechanical and wind mixing.

Lambert's Bay

An intensive 24 h study in Lambert's Bay formed part of an investigation which was conducted during February 1996 (Fig. 3), where, *inter alia,* the vertical positioning of a red tide population and its interaction with the physical environment was examined.

The red tide occurred during a period of warm, calm weather. The vertical temperature distribution provided evidence of downwelling as a result of relaxation of upwelling (Fig. 23). Nitrate was depleted in the upper layers, whereas the deep water had concentrations sufficient for substantial growth (Fig. 23). A distinct subsurface *in situ* chlorophyll fluorescence maximum was observed at 12h00 at 2m depth (Fig. 23). As the irradiance decreased, the fluorescence peak ascended to the surface where it remained until 17h00. During the course of the study, the red tide displayed marked diel migration. Active downward migration was observed before sunset, while upward migration commenced prior to 06h00 with the formation of a surface fluorescence maximum at 08h00 (Fig. 23). A subsurface maximum was again present at 10h00. The population took approximately 7.5 h to descend, while ascent to the surface took 5 hours.

The population thus appeared to rise towards the surface at dawn,

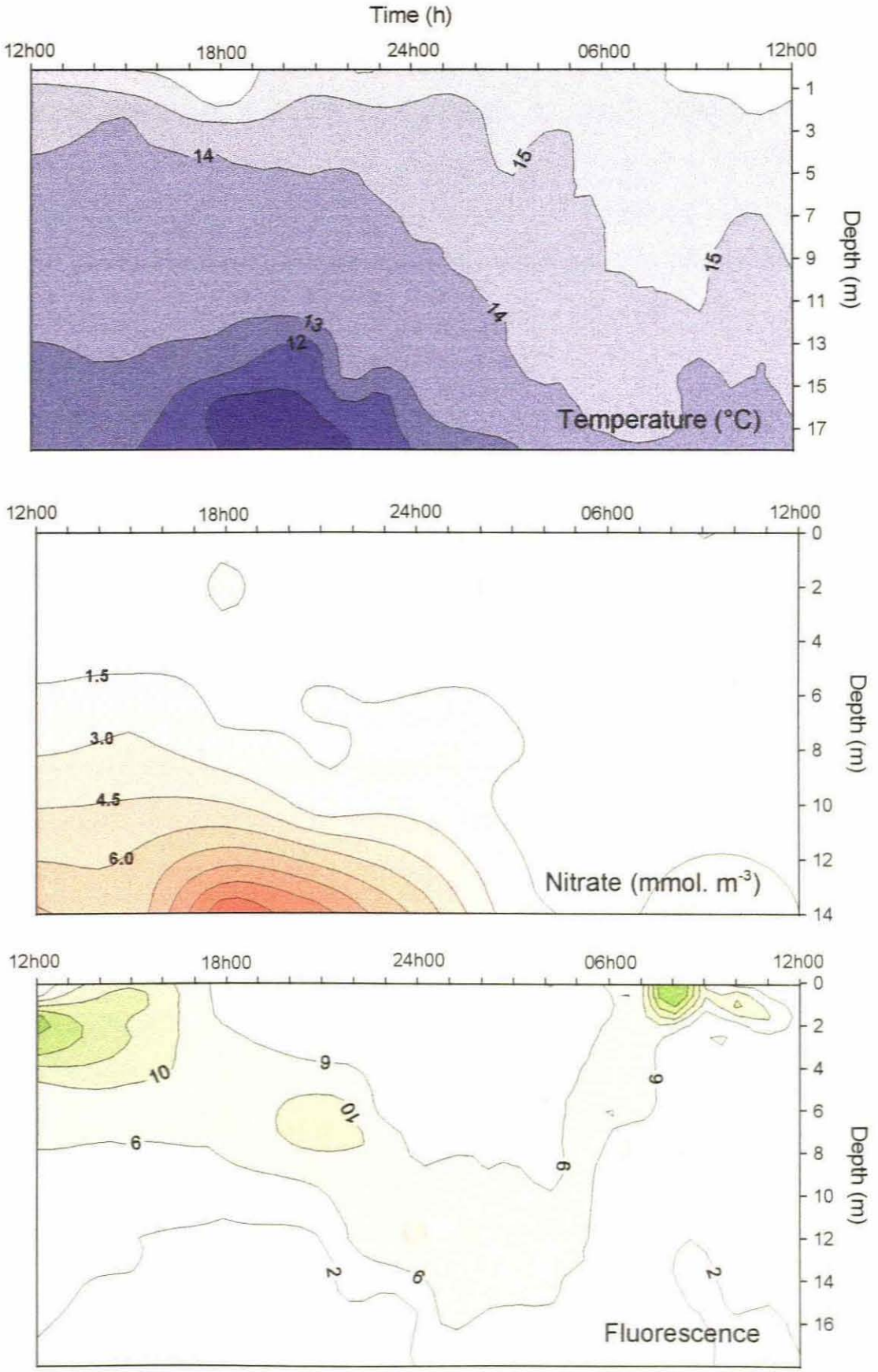

Fig. 23. Time series of temperature, fluorescence and nitrate during the Lambert's Bay survey.

before descending to lower depths at noon because of the inhibiting light intensities, before rising to the surface again by late afternoon. A similar pattern was found by Lassus (1990) when he investigated the role played by light intensities on cell survival.

P. micans accumulated and remained at the surface until after 16h00 before it commenced with it's descent (Fig. 24). It later concentrated in a narrow band between 7 and 9 meters where it remained till 02h00 before ascending to the surface. Although *P. micans* continued to accumulate during the morning, cell concentrations were significantly reduced. *C. furca*, which was initially concentrated at 1 m depth, descended slowly during the afternoon to form a concentrated layer below *P. micans* (Fig. 24). It reached the surface during the early morning where it initially co-occupied the surface water before descending to 1 m depth by 12h00. A small population of *D. acuminata* initially co-inhabited the surface waters with *P. micans*. During the following hours it displayed limited DVM, reaching only 4 m depth before midnight. *D. acuminata* was again present at the surface soon after midnight where it remained before it descended again to 4m by 12h00, suggesting that vertical migration possibly takes place in two phases during each 24 h cycle.

In the case of *P. micans*, the rate of descent appeared to be greater than the ascent. In contrast, the ascending rate of *C. furca* appeared to be considerably quicker than the descending

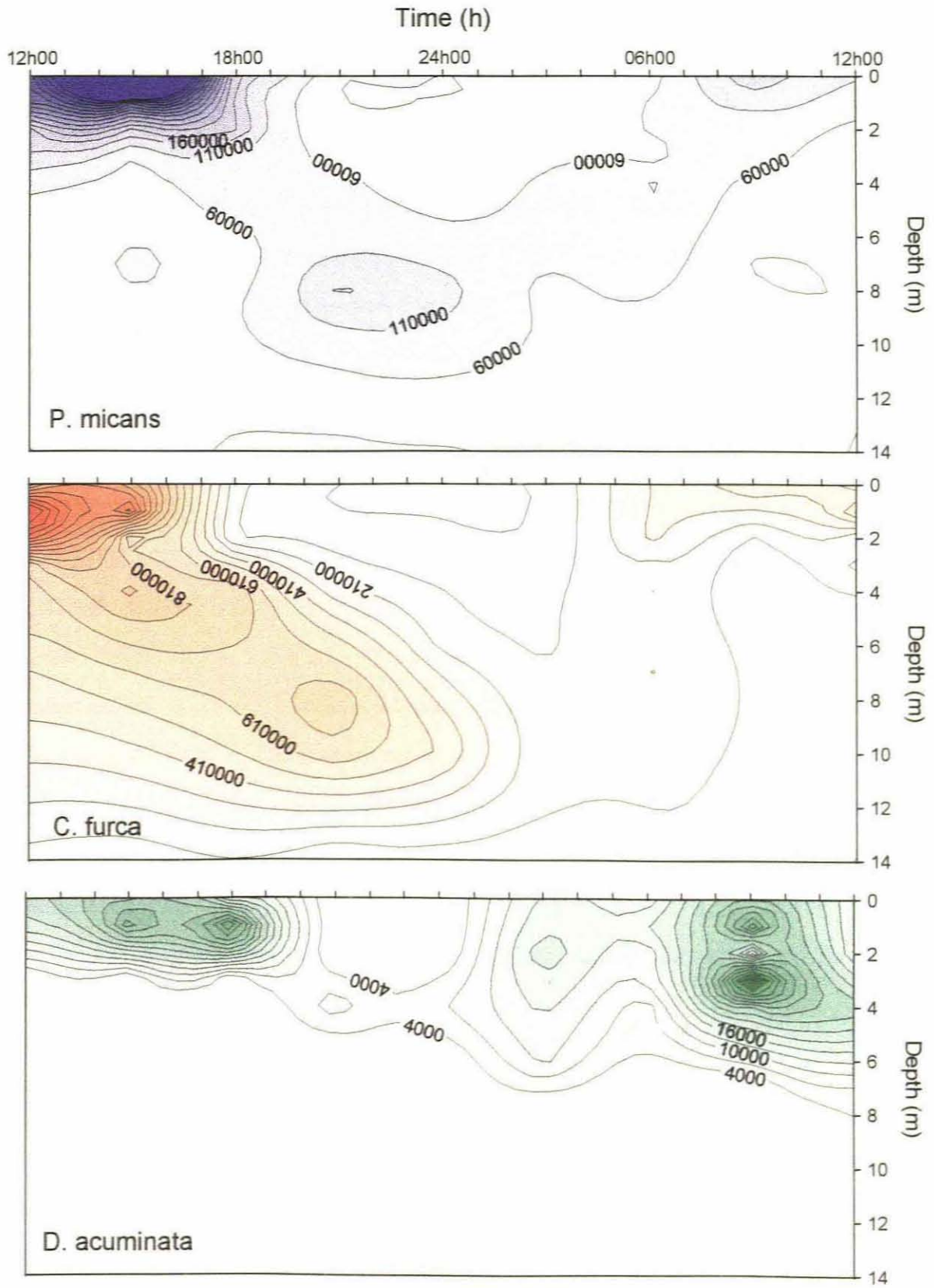

Fig.24. Time series of cell density of *P. micans*, *C. furca* and *D. acuminata* during the Lambert's Bay survey.

rate. Maximum observed descending swimming speeds were 1.64 and 0.87 $m.h^{-1}$ for *P. micans* and *D. acuminata* respectively, while the maximum observed ascending swimming speed for *C. furca* was in the order of 1.35 $m.h^{-1}$.

The chlorophyll *a* distributions presented reveal a marked DVM pattern during both mesocosm and field experiments. Not all aspects of vertical movement could, however, be explained by positive phototaxis as the timing and pattern of migration displayed some interesting features. From chlorophyll *a* and fluorescence data collected during the column experiments and field surveys, red tide organisms would appear to anticipate the end of the light period and begin their descent before dark. In turn they appear to begin their ascent in total darkness. This implies that the cells must have an ability to sense a gravitational field. Eppley *et al.* (1968), Weiler and Karl (1979) and Watanabe (1988) documented DVM in several species of marine flagellates in constant darkness and constant light. Their observations coupled with downward migration at speeds too high to be accounted for by random motion led them to postulate that their motion had to be a geotactic as well as a phototactic response.

All red tide organisms exhibited DVM of varying magnitude in both the mesocosm and field studies. Extensive diel migrations were seen by *P. micans, C. furca, C. lineatum, M. rubrum, H. akashiwo, G. splendens, Gymnodinium sp.* and *P. gracile*. Evidence for DVM by *P. rostratum* and *D. acuminata*, was less convincing. Both

species appeared to be confined to a rather narrow subsurface layer. As observed by Lassus *et al.* (1990), *D. acuminata* appeared to perform two vertical migrations within a 24 h study period. The results of this study are in general agreement with previous studies (Eppley *et al.* 1968; Weiler and Karl 1979; Kamykowski 1981(b); Cullen 1985; Olsson and Granéli 1991; Passow 1991; Villarino *et al.* 1995) and add to the list of dinoflagellate species that appears to involve diel rhythms and geotaxis in addition to phototaxis.

Diel migrations followed during the mesocosm experiments and field surveys confirmed experimental observations of previous authors that thermal stratification and the level of surface irradiance may modify the patterns of diel vertical migration of dinoflagellate populations. *Mesodinium rubrum* reacted directly to changes in light intensity during mesocosm 4. It accumulated at the surface during low light intensities, but evaded the surface when the irradiance exceeded a threshold value. In the mesocosm studies, the exceptionally high surface temperatures experienced during mesocosms experiments 2 and 3 and (25.7-31.8°C) with thermal gradients ranging between 13.2°C-19.2°C prevented *P. micans* from crossing the thermocline during the initial stages of the above mentioned experiments. However, as the cells became nitrate stressed, *P. micans* was able to cross the thermal barrier. The accumulation of cells at thermoclines or their migration through them, in association with water movements can be important in producing temporal and spacial patchiness (Kamykowski, 1974, 1979; Heaney and Talling 1980;

Heaney and Eppley (1981).

Although the study demonstrated the ability of marine dinoflagellates to cross large temperature gradients, it could not be established whether the size of the temperature gradient was more important than the absolute temperatures involved. The study also showed that the extent of migration under stratified conditions varied considerably, with some dinoflagellate species often remaining near the bottom of the euphotic zone during the day. Similar results were obtained by Cullen and Horrigan (1981) who demonstrated that the range of movements of dinoflagellates are frequently variable. Populations of the same species may either undertake appreciable DVM or form relatively stable sub-surface maxima.

In conclusion this study provides evidence of diel vertical migration by most species studied under both laboratory and field conditions. Such active movements provide these species with the ability to migrate between the illuminated and nutrient rich layers on a 24 h cycle, enabling them to grow in an environment where light and nutrient availability are vertically separated.

CHAPTER 4

BEHAVIOURAL PATTERNS

RESULTS AND DISCUSSION

Vertical niche separation

The vertical movement of all the species in both the mesocosm and field studies, demonstrated the ability for populations to concentrate and occupy specific niches in the water column, both during the day and at night.

This vertical niche separation is depicted in mesocosm 3, which illustrates a time series of the weighted mean depth (WMD) of the three dominant red tide species (Fig. 25). The species appeared to be vertical separated with *Prorocentrum micans* situated above *Ceratium furca*, both of which were positioned above *C. lineatum*. The vertical separation was maintained during the day and night with the three species exhibiting DVM in sympathy with each other.

The vertical separation of species was also evident during mesocosm 4, with a tendency for *Heterosigma akashiwo* to be situated above *Mesodinium rubrum* during the day and below *M.*

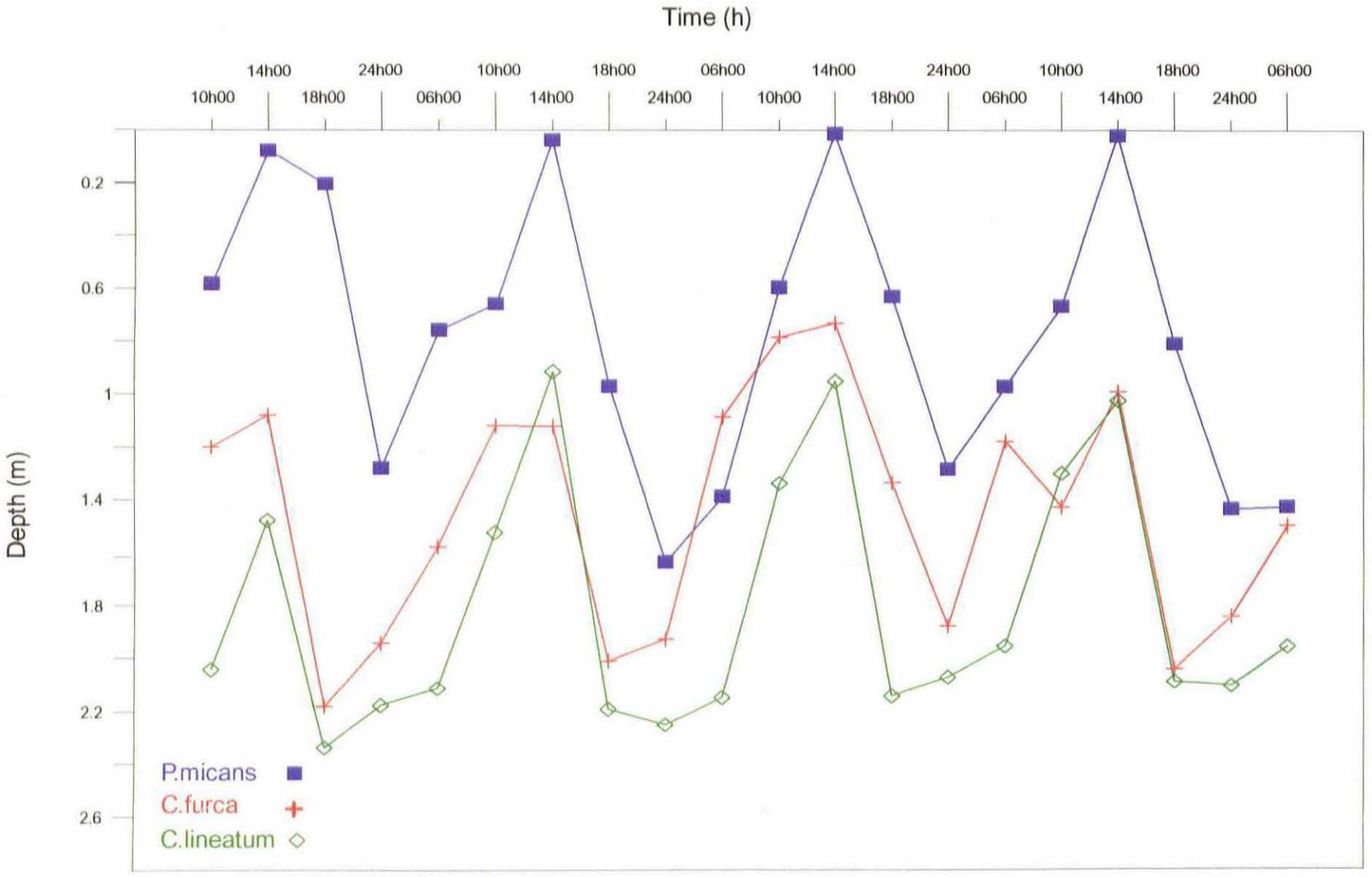

Fig. 25. Weighted mean depth of the dominant red tide species during mesocosm 3.

rubrum during the night. (Fig. 26).

Vertical separation of the two dominant dinoflagellates species was also evident in mesocosm 5 (Fig. 27). *C. furca* was found above *C. lineatum* during the entire first 24 h period of sampling. During the second 24 h period of sampling, *C. lineatum* was on this occasion found above *C. furca* during the day. Both species exhibited DVM in sympathy with another.

Evidence of populations occupying particular niches in the water column was also evident during the Gordon's Bay study. *Gymnodinium splendens* was found above *Gymnodinium sp.*, both of which were positioned above *Prorocentrum rostratum* during the day *(Fig. 28)*. At night *G. splendens* was found below both other species. Their vertical niche separation was maintained both during the day and night despite increased turbulence.

There was again evidence of vertical separation of the various dinoflagellate species during the Lambert's Bay study (Fig. 29). *P. micans* tended to occupy the surface waters and was situated above *Dinophysis acuminata* during the day. Both species were positioned above *C. furca*. At night *P. micans* and *C. furca* underwent a dramatic descent further separating them from *D. acuminata*.

A summary of the vertical positions of the dominant red tide species at 14h00 (Fig. 30), revealed that:

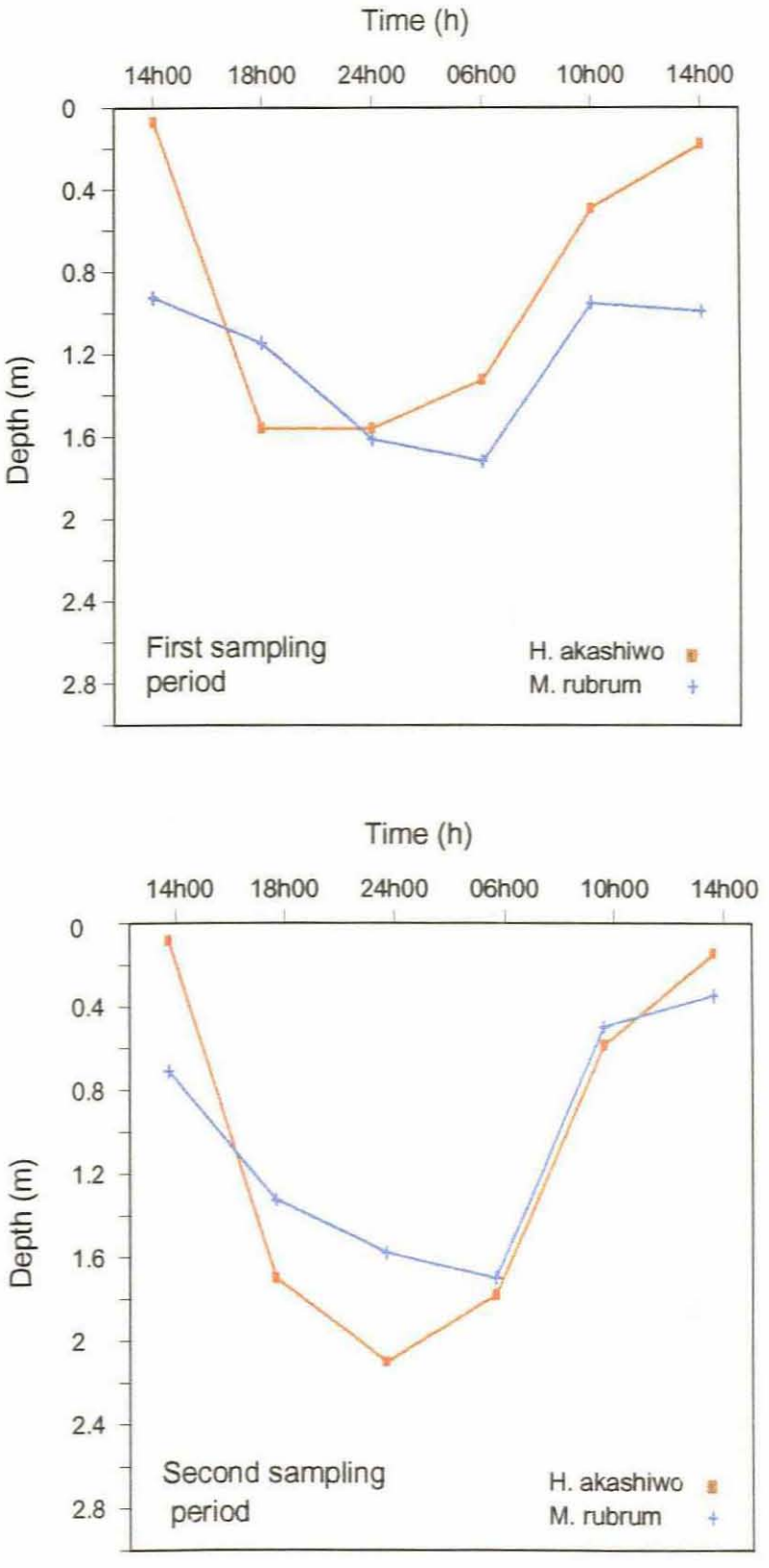

Fig. 26. Weighted mean depth of the dominant species during mesososm 4.

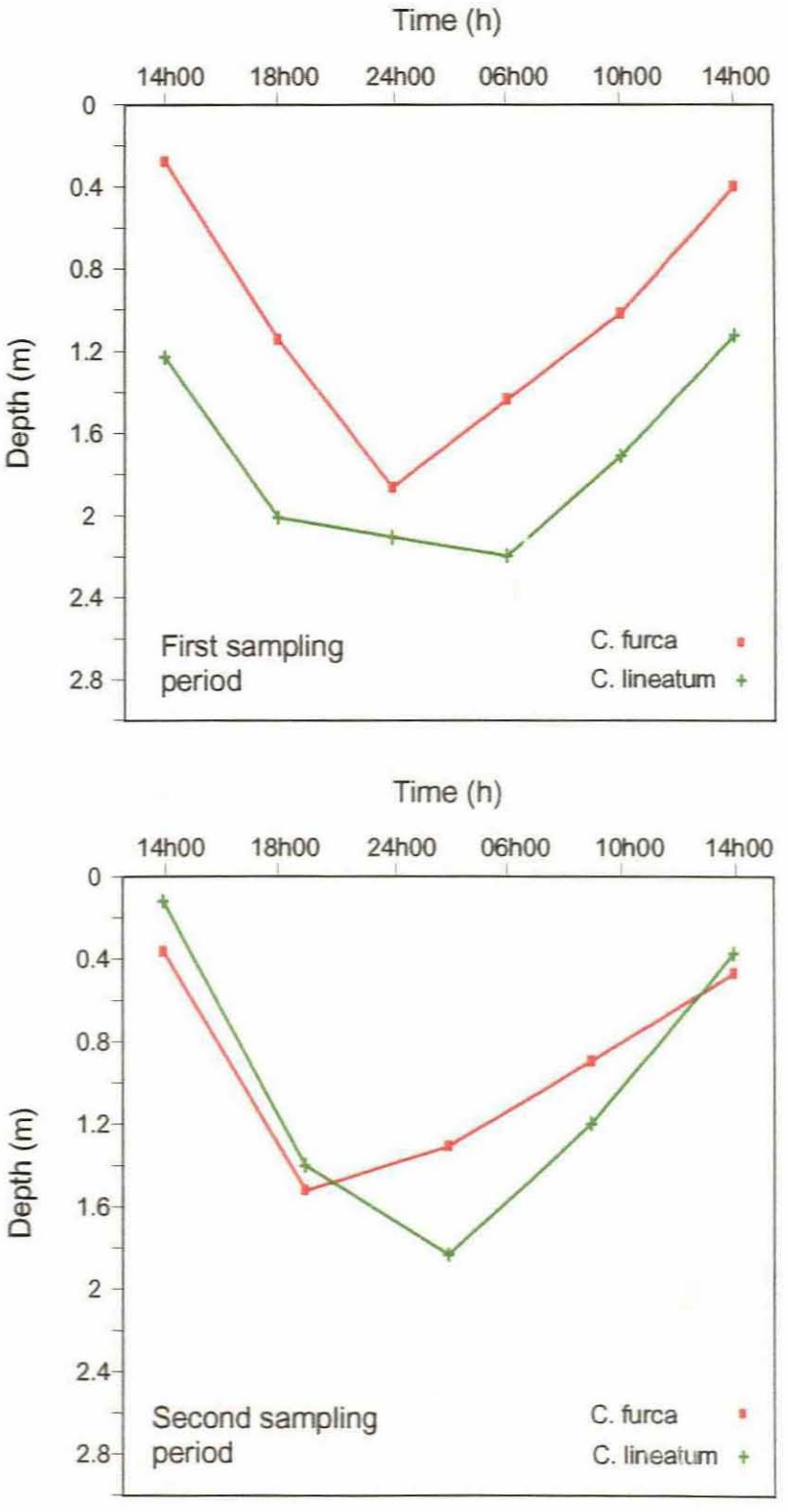

Fig. 27. Weighted mean depth of the dominant species during mesocosm 5.

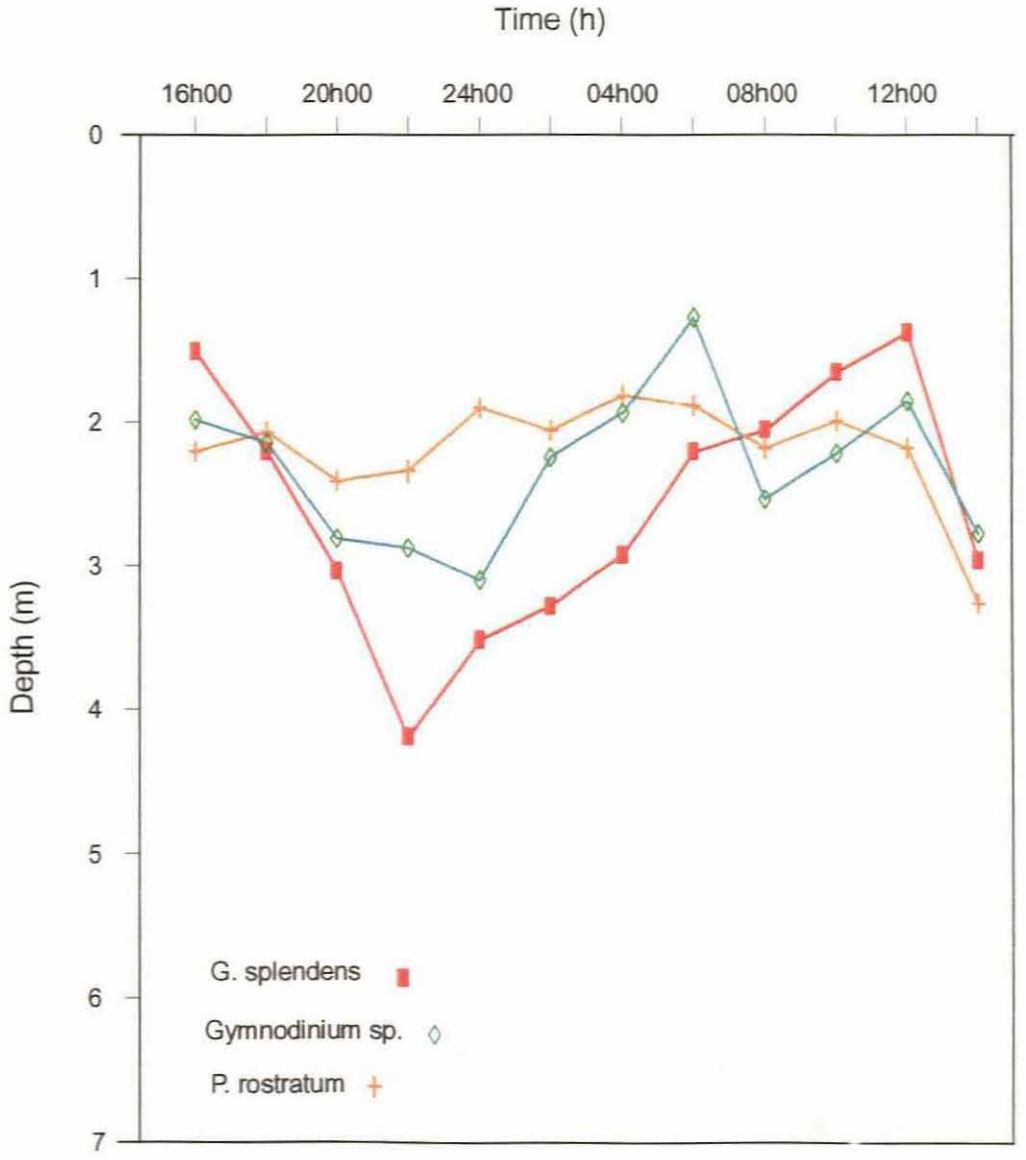

Fig 28. Weighted mean depth of the dominant species during during the Gordon's Bay study.

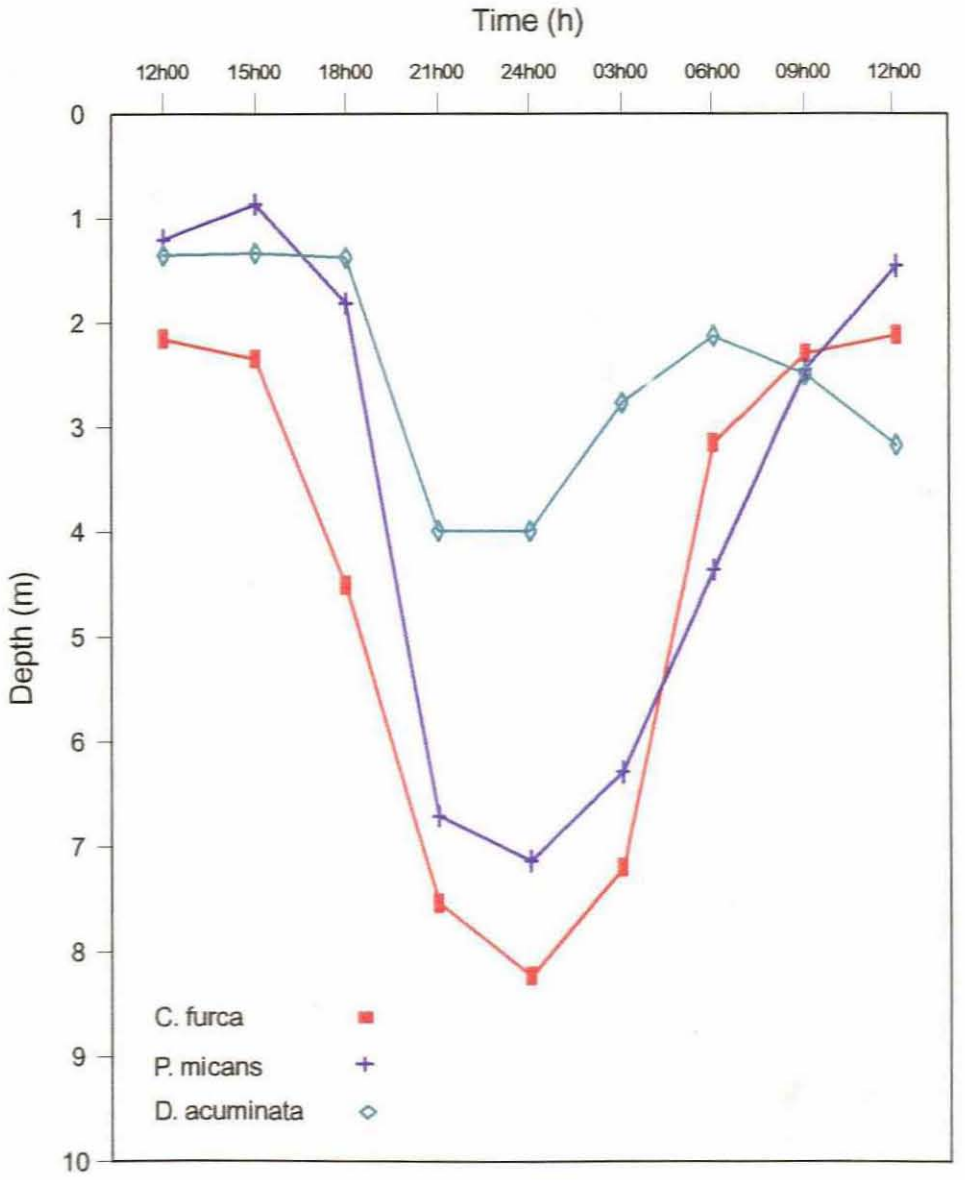

Fig. 29. Weighted mean depth of the dominant species during the Lambert's Bay study.

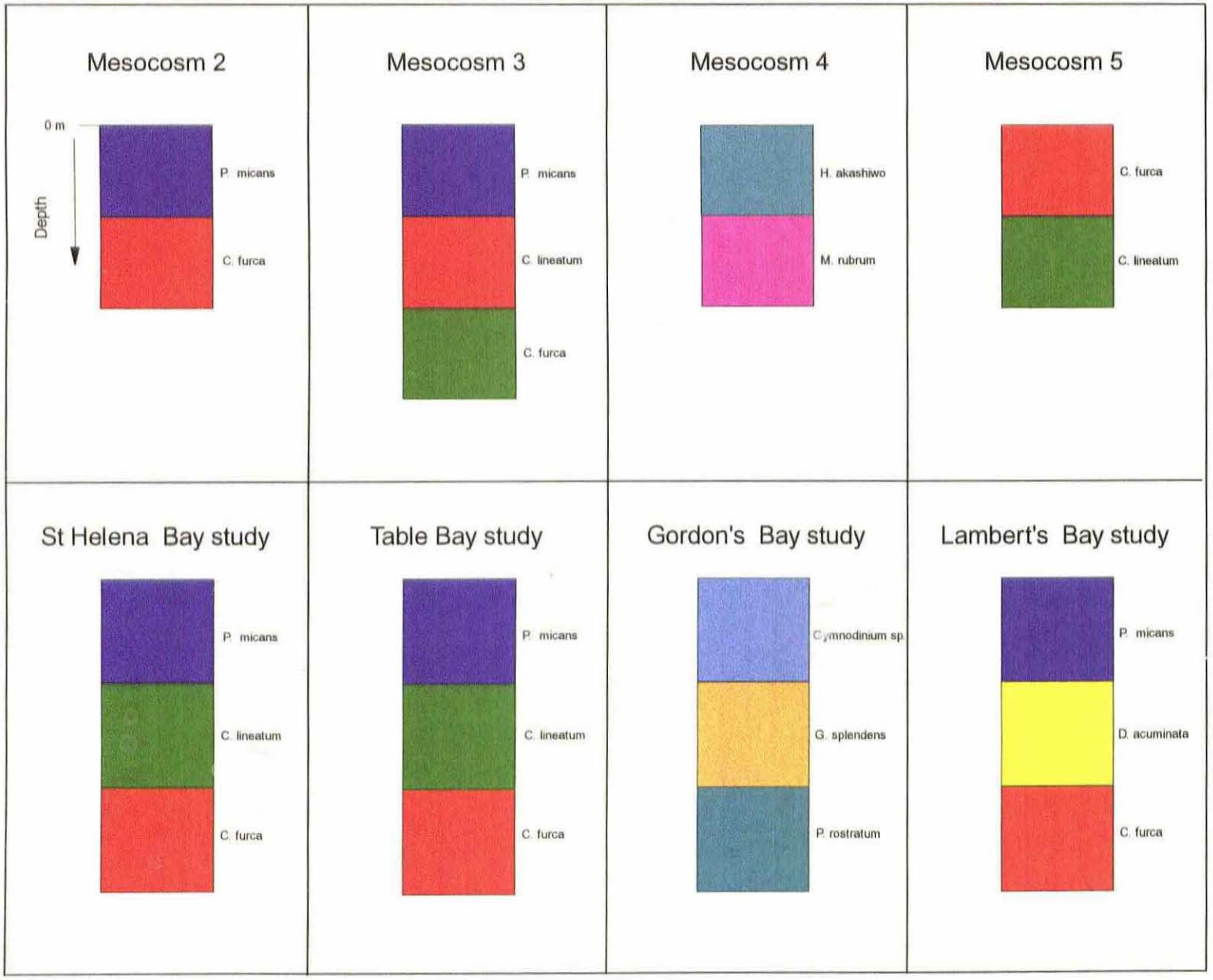

Fig. 30. A schematic presentation of the vertical positioning of the dominant red tide species at 14h00.

1) *P. micans* when present was always at the surface.

2) *C. furca* always occurred below *P. micans*.

Planktonic flagellates are mainly phototactic and regulate their vertical position during the day by concentrating at specific depths in response to underwater lightfield (Smith 1990). At night they position themselves in response to the availability of nutrients and the position of the nutricline. When different species of the same community seek a resource in short supply, they compete with one another. Interspecific competition may be exploitive when the individuals utilising the same resource, reduce the availability for another. A particular form of interference competition among plants is the production and release of chemical substances by one species that inhibit the growth of other species, (allelopathy), thereby reducing competition for nutrients, space and light (Branch 1984).

Light and nutrients are the two most important resources, each of which is characterised by opposing vertical gradients. These opposing gradients cause a significant variability the continuously changing physical environment. Two or more species can, however, compete for the same resource without being in direct competition by occupying and exploiting different parts of the mesocosm. This situation occurred during mesocosm 3 (Fig. 25) when layered peaks were evident for all the species for the duration of the experiment. Differential resource utilization (resource partitioning) is based on the premise that species

compete simultaneously for several resources, such as light and nutrients, but differ in their requirements for the two limiting resources (Smith 1990).

Closely associated with the concept of interspecific competition is the concept of the niche. In the absence of any competition, an organism occupies its fundamental niche, but for this to happen is very rare. In the presence of interspecific competition, the fundamental niche is reduced to a realised niche (Smith 1990), which relates to the conditions under which an organism actually exists. Similar conditions existed during eg. mesocosm 2 and mesocosm 5, when *C. furca* moved into the "vacant" position on the surface normally occupied by *P. micans* (Fig. 30). Dinoflagellates are thus capable of modifying their behaviour depending on the presence of other species. The niche therefore provides an explanation for the population's structural, physical, and behavioral adaptations (Begon and Mortimer 1986). Niche differentiation is also a means of allowing competitors to co-exist.

When two or more organisms use a portion of the same resource, these niches can overlap. Most populations in a natural ecosystem show varying degrees of niche overlap. All species require certain resources, and one can expect a certain amount of overlap for them. The most likely way for competitors to co-exist both during the day and during the night, is to partition their physical habitat. The theoretical model of the niche also assumes that when competition is intense, only one species can occupy a

niche space, and that competitive exclusion takes place in areas of overlap. Evidence of competitive exclusion took place during mesocosm 2 when a small portion of *C. furca* concentrated at the surface during the early hours of the morning, before being displaced by *P. micans* (Fig. 9). Also during the Gordon's Bay study, *Gymnodinium sp.* reached the surface at 06h00 prior to the dominant *G. splendens*. By 08h00 *C. splendens* had formed a very distinct maxima at the surface while a subsurface maxima was formed by *Gymnodinium sp.* (Fig. 23).

This data clearly demonstrates interspecific variability of diel vertical migration patterns of dinoflagellates. There is also evidence that populations display the tendency to concentrate at specific depths. Edler and Olsson (1985) used the depth of maximum cell concentration to calculate migration rates and to determine optimum light intensity for *P.micans* and *C. furca*. The data further illustrates the view that interspecific competition for resources may be important in structuring natural communities. These observations, therefore, support the hypothesis that certain flagellate species are capable of co-dominating red tide blooms by discrete resource partitioning. This is achieved by species differing in migratory behaviour which minimizes niche overlap and therefore reduces interspecific competition for the essential resources of light and nutrients.

This phenomenon has not been investigated in detail previously. The possibility of niche separation in dinoflagellates based on variability of vertical nutrient profiles and differing migration

patterns was suggested by Dugdale (1979). This study provides the first data in support of vertical niche separation.

Nutrients and vertical migration

Diel vertical migrations were also found to vary under different nutrient conditions. During three of the experiments conducted during this study, the same red tide assemblage (*P. micans, C. furca and C. lineatum*) displayed vastly different behavioural patterns.

During the first example, a 24 h study in Table Bay, low nitrates were present throughout the column (Fig. 31). This resulted in the major portion of the population remaining in the surface waters both during the day and at night. Only a small portion of the population underwent DVM, descending towards the sea bed during the early evening (Fig. 31). The migrating portion remained close to the sea bed for approximately six hours before it returned to the surface waters.

During the second example, a 24 h study undertaken in St Helena Bay, a similar population was found to behave very differently. The relatively low bottom nitrate levels at the beginning of the experiment changed dramatically when cold nutrient-rich bottom water was advected into the study area during the night (Fig. 32). The red tide population which had accumulated at the surface during the day, descended into the high nitrate region during the

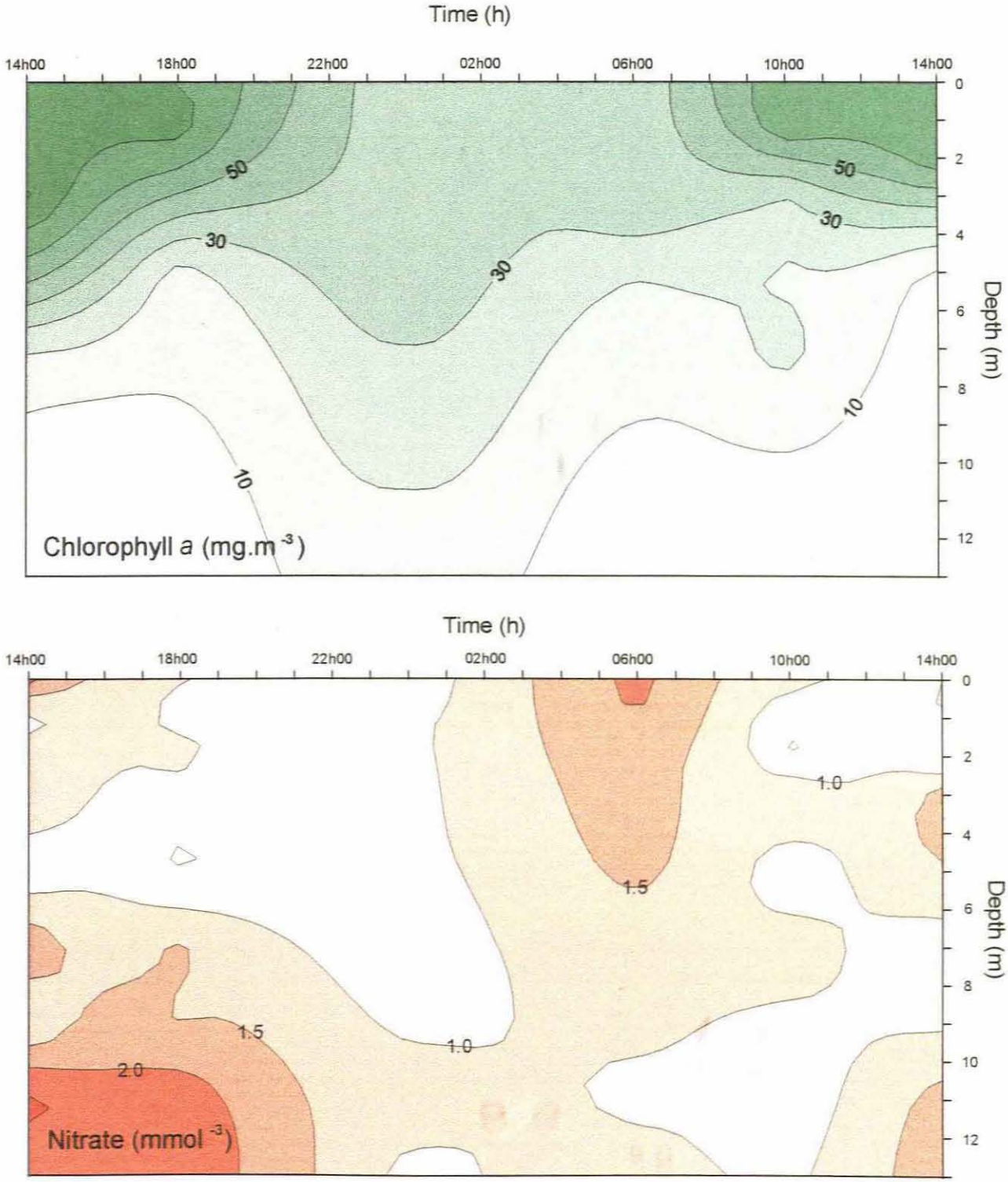

Fig. 31. Time series of chlorophyll *a* and nitrate during the Table Bay study.

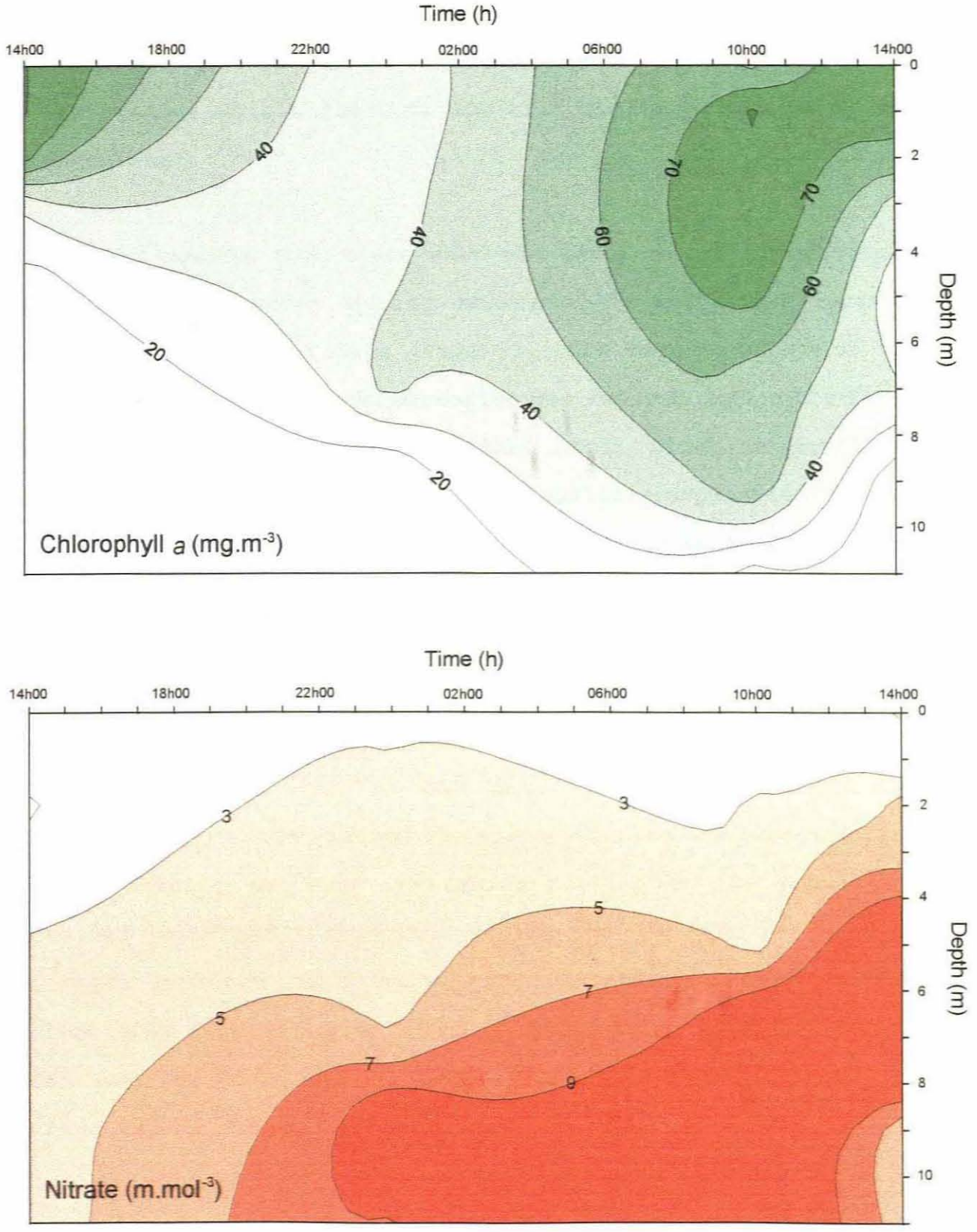

Fig. 32. Time series of chlorophyll *a* and nitrate during the St Helena Bay study.

early evening. Although the weather remained calm and sunny, the population apparently modified its behaviour by delaying its ascent. By 10h00 a significant portion of the population was still present within the nutrient-rich bottom waters below the thermocline.

The third example took place when the water became increasingly depleted of nitrogen during mesocosm 3, which was sampled continuously for four days (Fig. 33). The major portion of the initially high nitrate concentration was removed during the first night leaving a diminished nitrate pool, which dramatically influenced the behaviour of the dinoflagellate population. During the first 24 h, 42% of the population migrated below the thermocline into the nutrient-rich water at night (Fig. 33). The following 24 hours, this migrating portion increased dramatically to 80% as the population became nutrient stressed, concentrating near or at the bottom of the mesocosm where very low concentrations of nitrates were still available. Although the ascending population reached the surface during the expected time of the morning, an ever increasing portion of the population remained below the thermocline during the day. The presence of nitrogen depleted cells below the thermocline during the day potentially enabled a greater migration depth into deeper layers than when the cells were nitrogen sufficient and at the surface during the day (Fig. 11).

This behavioural pattern is also reflected in the analysis of the vertical distribution of *P. micans*, the dominant dinoflagellate

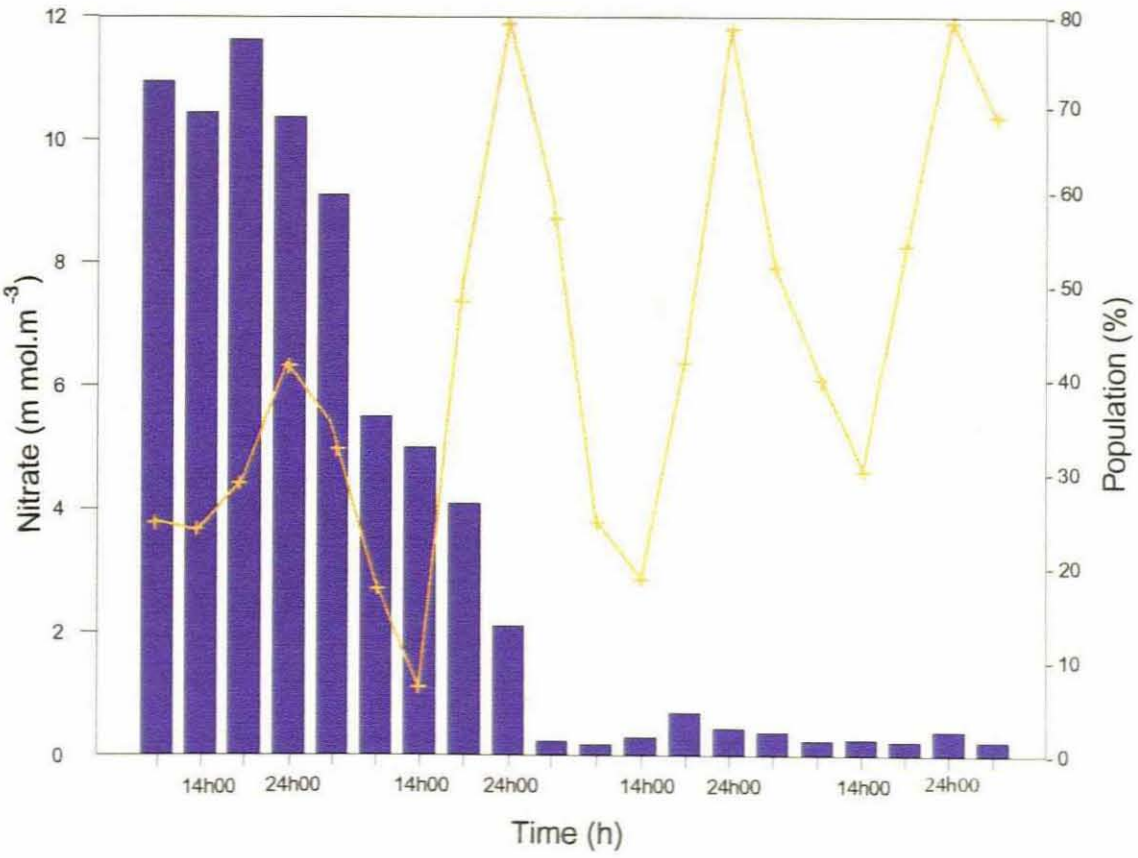

Fig.33. Percentage of the population below the thermocline and intergrated nitrate of the water column during mesocosm 3 .
Nitrate: ■ Chlorophyll: —

species in mesocosm 3 (Fig. 34). The progressive nitrate depletion was associated with a significant increase in the concentration of cells below the thermocline during the course of the experiment. It would also appear that the cells spent more time below the thermocline before returning to the surface.

The behavioural patterns of dinoflagellates appear therefore to be closely linked to physiological responses and to nutrition. The progressively earlier descent of cells with increasing nitrogen depletion was a persistent feature during the mesocosm studies. Cells that swim downwards earlier in the day are more likely to reach the nitracline while light is still available than cells that leave the surface layers later in the day. If the range of upward movement of nutrient-starved cells is decreased because of altered light sensitivity such that they remain near the nitracline in the day, then the time required for downward migration into the nitracline is reduced. The Table Bay field study showed that when nutrients were limiting throughout the water column, vertical migration is such that the maximum time is spent near the surface within the constraints of completing vertical migration. When nitrate is depleted in the surface layer, behaviour is modified to provide more exposure to nutrients at depth while still maintaining a high rate of photosynthesis during the day. Heaney and Eppley (1981) showed that Gony*aulax polyedra* increases its exposure to nutrients by beginning its nocturnal descent early, whereas *Gymnodinium splendens* restricts its diel ascent to a subsurface layer, often close to the nutricline, in which light intensity is saturating

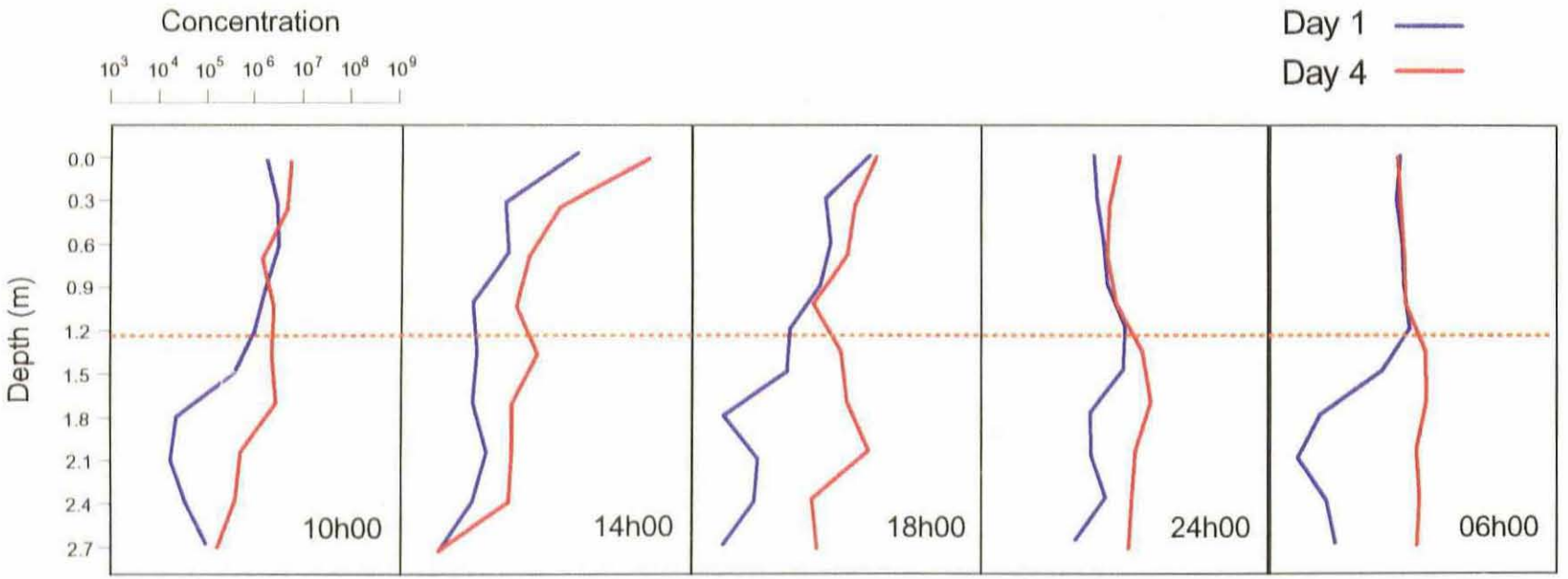

Fig. 34. Cell concentration of *P. micans* relative to the thermocline (·····) at the beginning and end of mesocosm 3.

for photosynthesis (Cullen and Horrigan 1981). When nutrients are no longer available within the range of vertical migration, the pattern of migration of *G. polyedra* was modified further such that it no longer migrated to the surface during the day. In a study on the effect of density and nutrient gradients on the behaviour of dinoflagellates, evidence was produced that illustrated the inability of *Gymnodinium aureolum* to penetrate a density barrier of 10 ppt salinity in a nutrient sufficient environment. When introduced into a nutrient deficient environment, the ability of the organism to penetrate a greater density gradient was significantly enhanced (Nielsen *et al.* 1993). It would therefore appear that modifications to the behaviour of red tide organisms is of prime importance for the survival of the cells in the sea. The significance of vertical migration by dinoflagellates in terms of resource exploitation under varying environmental conditions is thus established.

During the course of each mesocosm study, nitrate concentration decreased substantially in the bottom layer indicating nocturnal uptake of nutrients by the migrating red tide species (Table 5).

Depth	Mesocosm 1.	Mesocosm 2.	Mesocosm 3.	Mesocosm 4.	Mesocosm 5.
Bottom	0.5 to 0.3	6.7 to 0.6	8.7 to 0	8.4 to 2.7	1.9 to 0.2

Table 5. Nitrate (mmol.m^{-3}) reduction at the bottom of the column during the 5 mesocosm experiments.

The largest decrease in nitrate concentration occurred during mesocosm 3, when the integrated nitrate concentration decreased from 10.37 mmol.m^{-3} to 5.50 mmol.m^{-3} after 10h and to 0.19 mmol.m^{-3} after 48 h (Fig. 34). Of particular importance was the substantial decrease in nitrate between 06h00 and 10h00 during the morning of the second day (9.10 mmol.m^{-3} to 5.50 mmol.m^{-3}), a direct result of a large proportion of the population remaining below the thermocline before 10h00. The C:N ratio of the red tide populations in both the second and third experiments showed significant increases between day one and day two. The C:N ratio's increased from 5.85 to 6.45 (Mesocosm 2) and from 4.15 to 5.55 (Mesocosm 3). Using a clonal culture of *Chattonella antiqua*, Watanabe *et al.* (1988) similarly reported on the removal of nitrates at night. Olsson and Granéli (1991) concluded that a decrease in the nitrate concentration in the bottom water of a cylinder provided evidence for the assimilation of nitrate at night.

The motility of dinoflagellates enables them to exploit the dissolved nutrients over a relatively large part of the water column, attaining in the process higher densities at specific depths than non-motile diatoms under conditions of stratification and nutrient depletion. Blooms of dinoflagellates are often found in surface waters depleted of nutrients. Their ability to migrate into nutrient-rich zones, and their ability for dark uptake and assimilation of nutrients, are of great importance to dinoflagellates. The hypothesis that nitrate uptake by dinoflagellates in the dark is an important factor in competing

with diatoms has been put forward in several papers (Eppley *et al.* 1968; Eppley and Harrison 1975; Harrison 1976). The decrease in nitrate concentration in the bottom waters of the mesocosm studies provided the necessary proof that dinoflagellates are capable of dark nitrogen assimilation. These observations support the hypothesis that certain dinoflagellates can sustain high concentrations in nitrogen depleted surface waters by growing at the expense of nitrate taken up during nocturnal descent of their diel vertical migration. Species specific migratory behaviour appears, therefore, to regulate adequate light exposure during the day and access to nutrients at night.

CHAPTER 5

CELL DIVISION

RESULTS AND DISCUSSION

The measurement of *in situ* rates of growth is essential for understanding the dynamics of dinoflagellate blooms. Many techniques are available to assess the growth rate of planktonic communities, but there are few methods suitable for determining growth of an individual species when it occurs in a mixed population.

Recent studies have determined the growth rates of dinoflagellate species by establishing the frequency of cell division. The method requires a time series of the fraction of a population undergoing phased cell division. The temporal pattern of division in natural populations was determined by calculating the percentage of dividing cells in different cell cycle phases. The following stages of division, applied only to *Ceratium furca* and *C. lineatum* (Fig. 35), were counted (Weiler and Eppley 1979).

Stage A - Parent cells, no nuclear division visible.

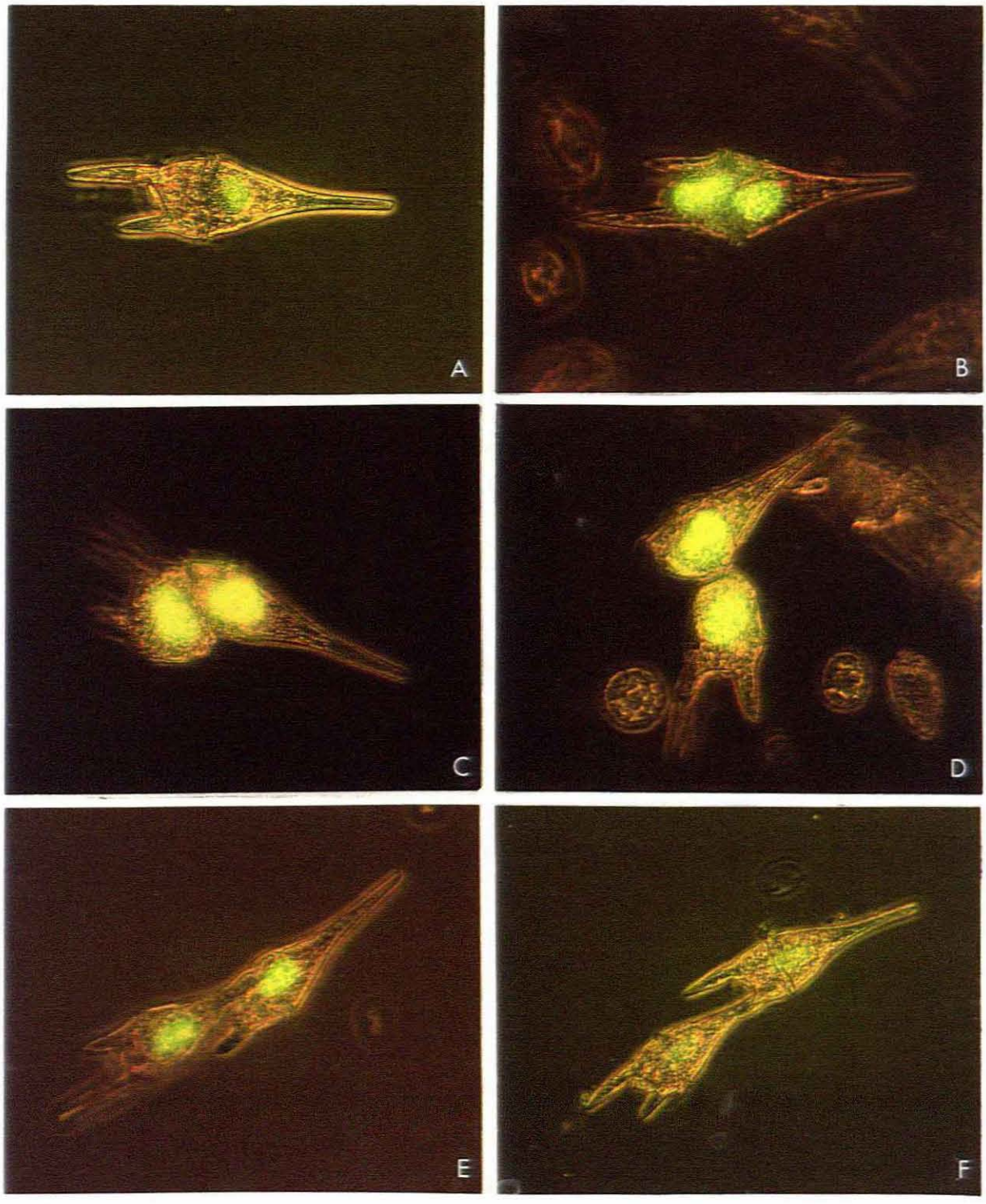

Fig. 35. ***Ceratium furca:*** Delineation of division stages as defined in the present study. A: non-dividing cell, B: cell undergoing nuclear division, this stage lasted from the time two distinct nuclei were observed until a break occurred in the theca; C-F: recently divided cells, this stage lasted from when a break was observed in the theca until horn generation could no longer be detected.

Stage B - Nuclear division, prior to cell division

Stage C - Recently divided cells. This stage lasted from the time when a break was first observed in the theca until horn generation could no longer be detected.

Only nuclear and cell division were observed in *Gymnodinium sp.* and *G. splendens* (Fig. 36), whilst only paired cells could be distinguished in *Prorocentrum micans* and *P. rostratum* (Fig. 37). In *D. acuminata* paired nuclei, paired cells and recently divided cells that is before regeneration of the sulcal list, were recognized (Fig. 38). Cells with paired nuclei were identified by epifluorescent microscopy after staining with acridine orange.

Field Studies

Cell division was examined during two field studies, one in Gordon's Bay in early winter and the other in Lambert's Bay in late summer.

Gordon's Bay

This study took place in early winter when day-length was 10h. Surface temperatures ranged between 14.0 - 14.6°C, and bottom temperatures between 13.4 - 13.7°C. *G. splendens, Gymnodinium sp, C. furca, C. lineatum* and *P. rostratum* were the only species which were both abundant and dividing frequently enough for

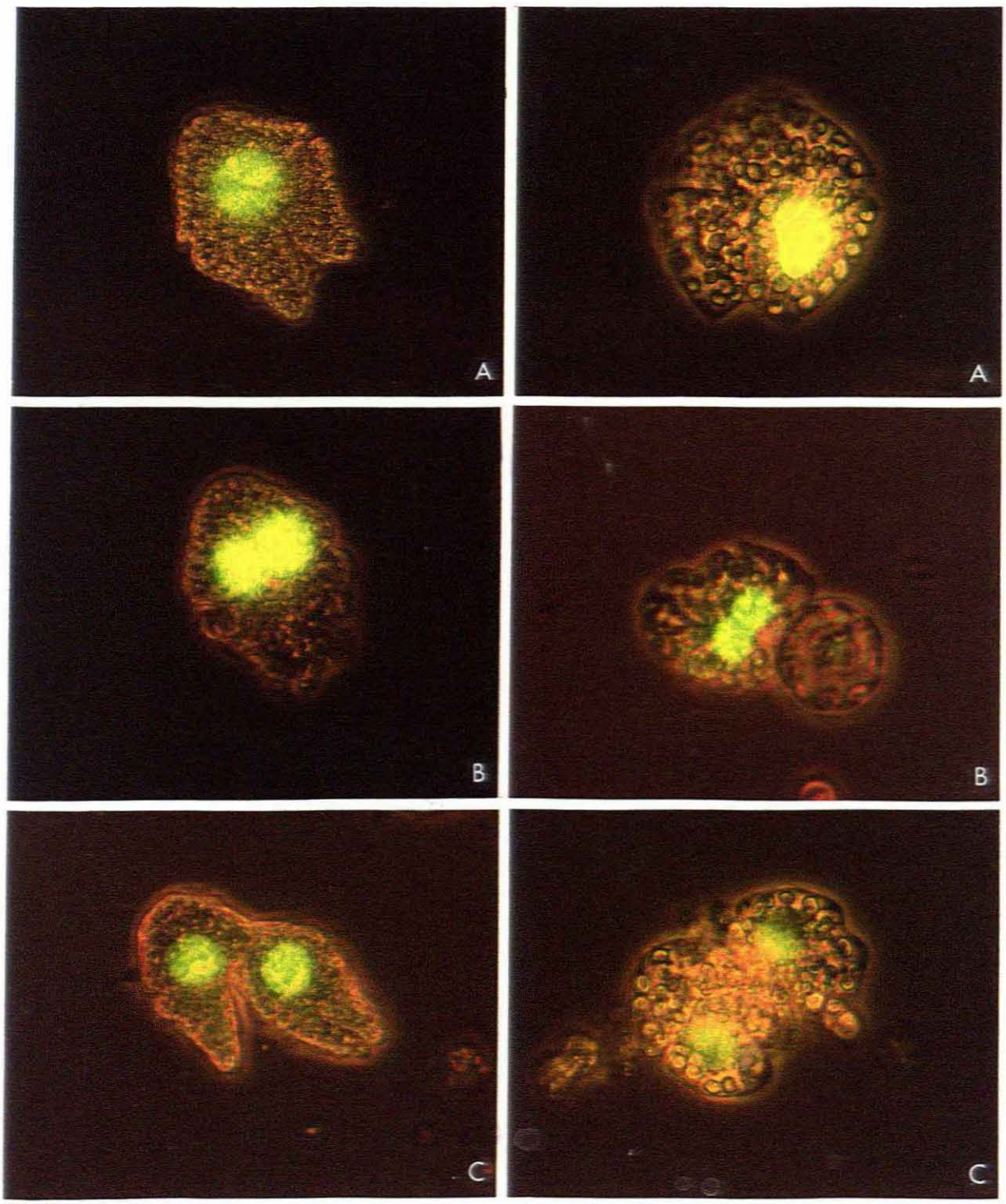

Fig. 36. ***Gymnodinium splendens (L) and Gymnodinium sp (R) :*** Delineation of division stages as defined in the present study. A: non dividing cell; B: cell with paired nuclei, C: cell division in progress.

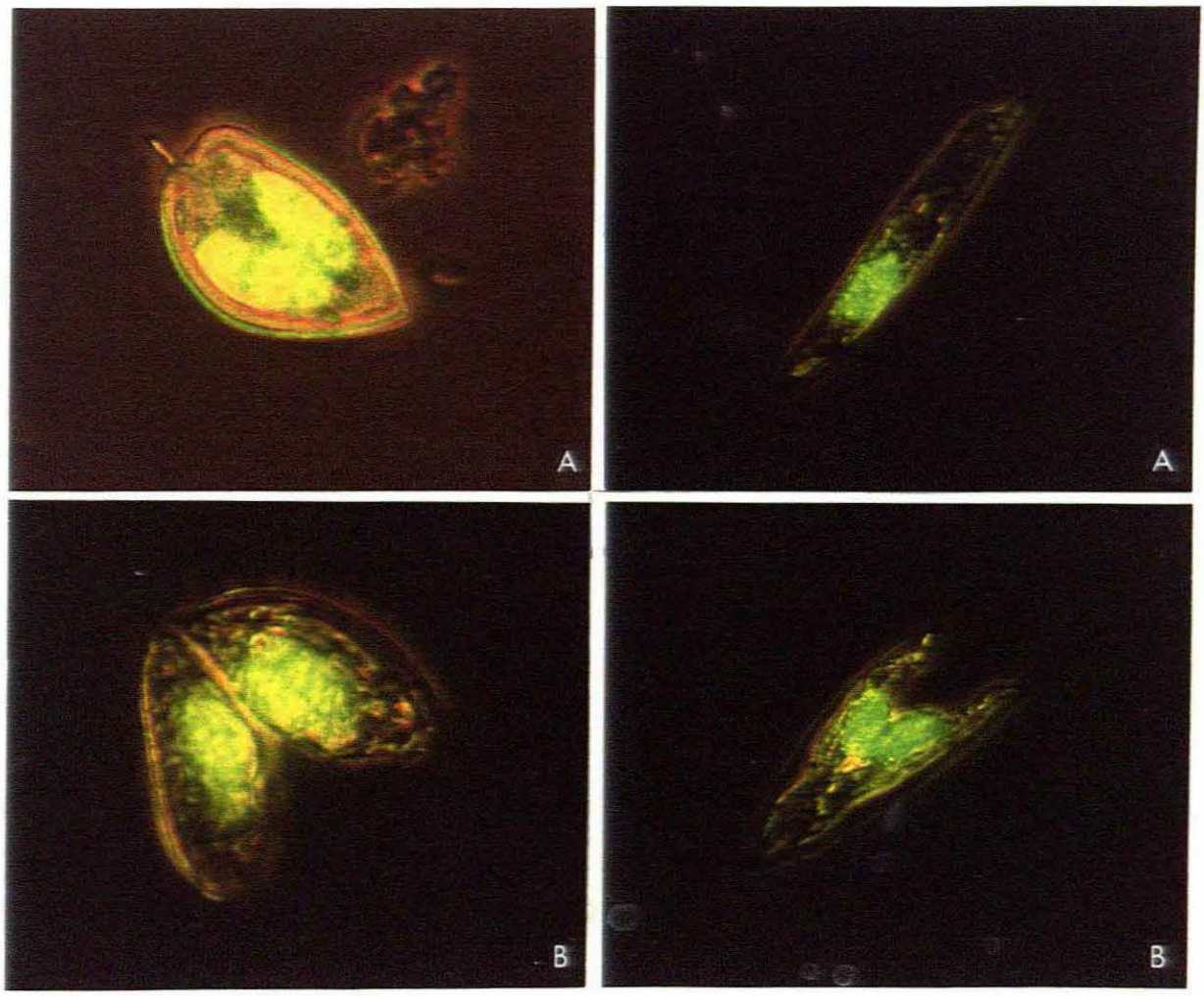

Fig. 37. *Prorocentrum micans (L) and P. rostratum (R) :* Delineation of division stages as defined in the present study. A: non dividing cell; B: paired cells.

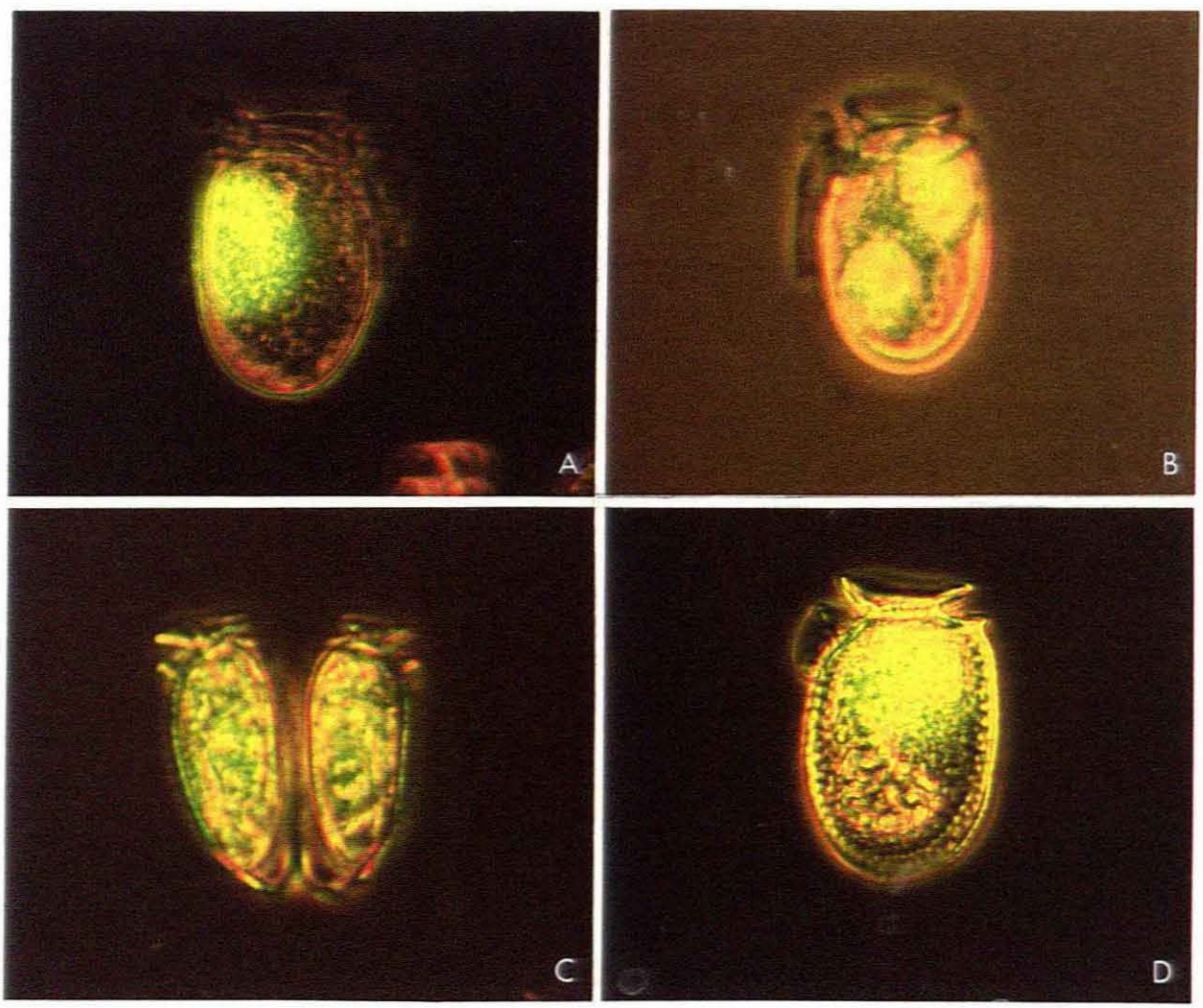

Fig. 38. ***Dinophysis acuminata :*** Delineation of division stages as defined in the present study. A: non dividing cell; B: cell with paired nuclei; C: paired cells; D: recently divided cell before regeneration of the sulcal list.

reliable measurements. Neither *G.splendens* nor *Gymnodinium* sp. displayed distinct phased growth patterns. Cell division in these species occurred over an extended period of time, commencing at 17h00 and 22h00 respectively with maximum rates before midnight (Fig. 39). Growth rates were not determined for these two species as cell division was not sufficiently phased.

The most striking feature of this study was the pronounced and staggered phased cell division exhibited by *Ceratium furca, C. lineatum* and *P. rostratum* (Fig. 39). The percentage of dividing cells within these species ranged from 0 to 40 percent during the 24 h period. A 40 percent division rate corresponds to a generation time of 2.5 days. The maximum frequency of division of *Ceratium furca, C. lineatum* and *P. rostratum* was staggered at 04h00, 06h00 and 07h00, 10, 12 and 13 hours after sunset respectively. The duration of each phased division was in the order of 3 hours. Of the three species, *C. furca* had the highest specific growth rate (μ = 0.32.d^{-1}). The values of μ for *C. lineatum* and *P. rostratum* were 0.18 and 0.08.d^{-1} respectively. This corresponded to generation times of 2.6, 5.6 and 12 days respectively (Table 6).

Lambert's Bay

This 24 h study took place during late summer (February 1996), when the day length was 13 h and the water temperature ranged between 15.2 - 16.5°C at the surface and 10.6 - 13.7°C at the bottom.

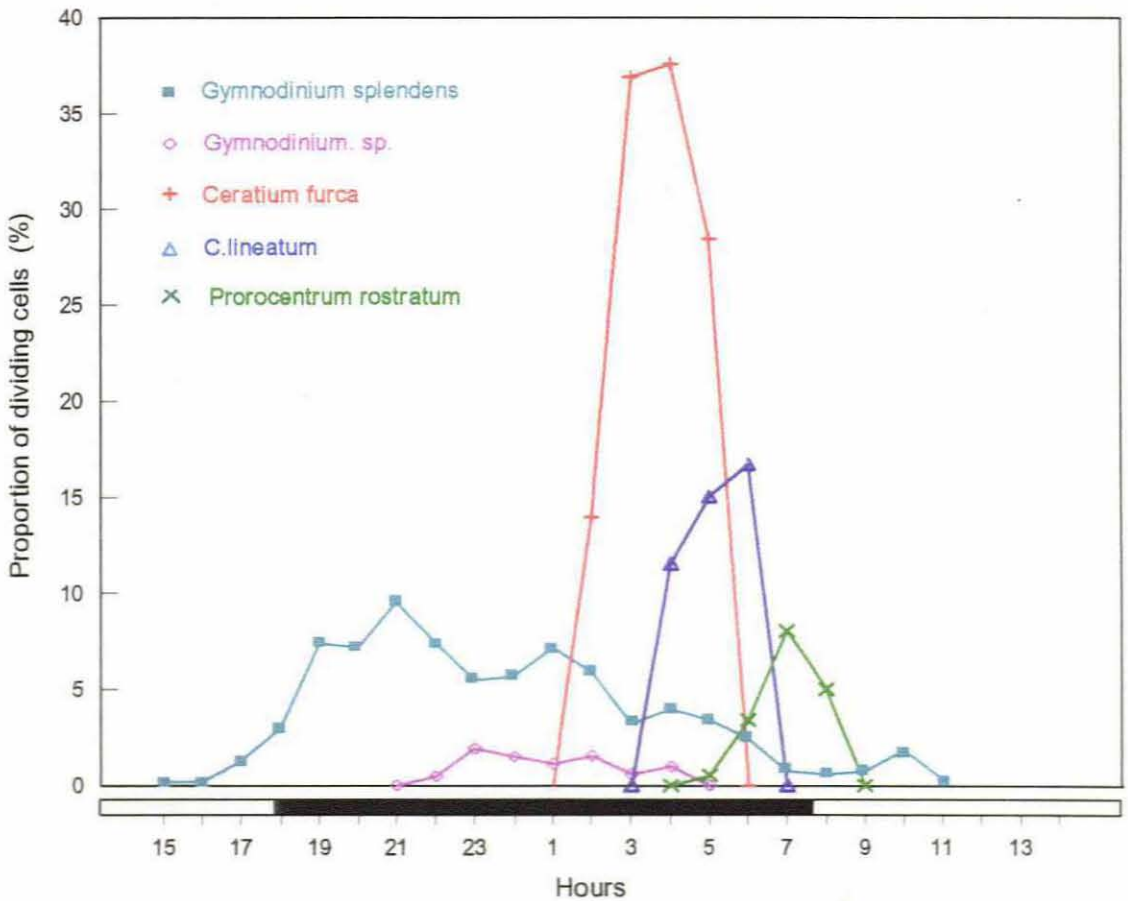

Fig. 39. A 24h time series of the fraction of dinoflagellate cells undergoing cell division during a red tide in Gordon's Bay during June 1995.

Species	Initiation of division (hours after dark)	Time of maximum cell division	Growth rates (μ)	Doubling times (days)
Gordon's Bay				
C. furca	8(h)	03h00 - 04h00	μ = 0.32	2.6 d
C. lineatum	10(h)	05h00 - 06h00	μ = 0.18	5.6 d
P. rostratum	12(h)	07h00	μ = 0.18	12.0 d
Lambert's Bay				
C. furca	5(h)	03h00 - 04h00	μ = 0.16	5.7 d
C. lineatum	9(h)	05h00 - 06h00	μ = 0.21	4.2 d
D. acuminata	10(h)	08h00	μ = 0.14	6.8 d

Table 6. Initiation of nuclear division, growth rates (μ) and doubling times

Phased cell division was observed during this study period by *C. furca, C. lineatum* and *D. acuminata* (Fig. 40). Phased cell division of *C. furca, C. lineatum and D. acuminata* commenced 5 h, 10 h and 11 h respectively after sunset.

The maximum frequency of division the two *Ceratium* species (hours after sunset in brackets), occurred at 03h00 (7 h) and 05h00 (9 h) respectively. Specific growth rates of 0.16 and 0.21 div.day^{-1} were calculated for *C. furca* and *C. lineatum*. This corresponds to generation times of 5.7 and 4.2 days respectively (Table 6).

In the case of *D. acuminata*, the frequency of the sum of paired cells and half the number of recently divided cells (only those missing the lower sulcal list) were followed with time. Cytokinesis and wing generation lasted from 06h00 to 10h00 (Fig. 40). *D. acuminata* showed synchronised phased cell division with maximum frequency (19%) at 08h00. The specific growth rate was 0.18 div.d.$^{-1}$ which corresponds to a generation time of 6.8 days (Table 6).

Only *C. furca* was present in sufficient numbers in the bottle samples and divided frequently enough to give an indication of the depth-differentiation in the proportion of dividing cells (Fig. 41A). Cell division occurred only among cells undergoing downward vertical migration during the night.

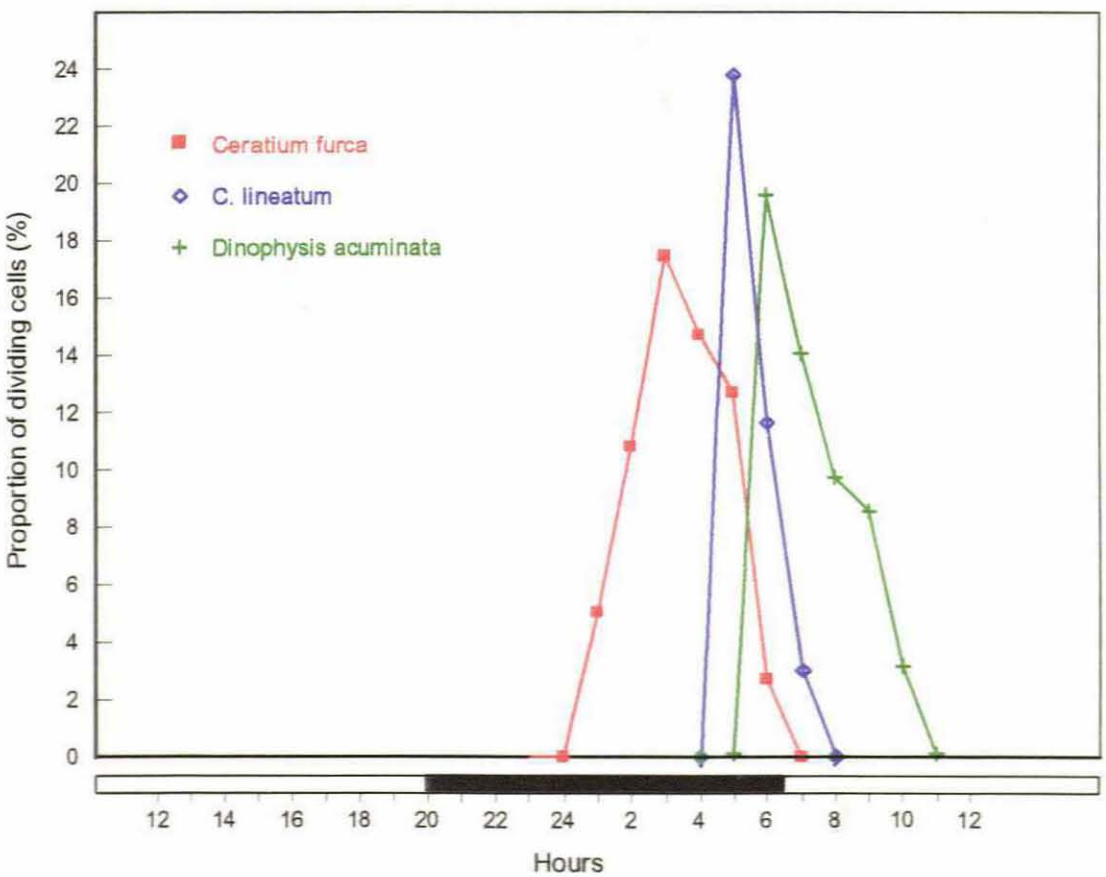

Fig.40. A 24h time series of the fraction of dinoflagellate cells undergoing cell division during a red tide in Lambert's Bay during February 1996.

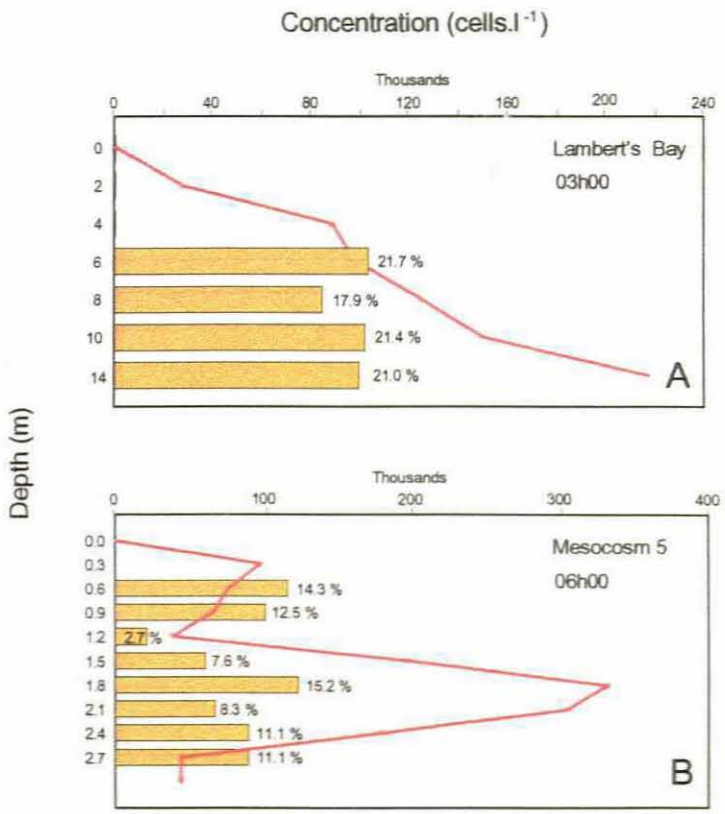

Fig. 41. Percentage of dividing cells ( ) , and cell concentration (cells.l^{-1}, ——) of *C. furca* during mesocosm 5 and Lambert's Bay study.

Mesocosm Studies

The vertical distribution of dividing cells was examined in selected mesocosm experiments. Recently divided cells of *P. micans* were present mainly at or above the thermocline (0.9 to 1.2 m) during mesocosm 3 (Fig. 42). This was initially attributed to the fact that vertical migration to the surface had commenced prior to 06h00. However, during the course of the experiment, the fraction of the population below the thermocline increased dramatically, with no corresponding increase in cell division below the thermocline. As *P. micans* became nitrate stressed, the percentage of the population dividing decreased, suggesting a deterioration of the conditions necessary for continued growth of this species.

During the same experiment, *C. lineatum* was found below the thermocline at 06h00 (Fig. 43). The maximum frequency of division thus occurred at or below the thermocline with division rates decreasing towards the bottom of the mesocosm in all cases. In contrast to *P. micans*, the percentage of cells dividing tended to show a small increase during the course of the experiment.

Although *C. furca* was present in the majority of the experiments, the sporadic occurrence of cell division could be attributed to the sampling times. Division frequencies were, however, high when cell division took place (Fig. 41B). No pattern as regards depth-differentiation was observed.

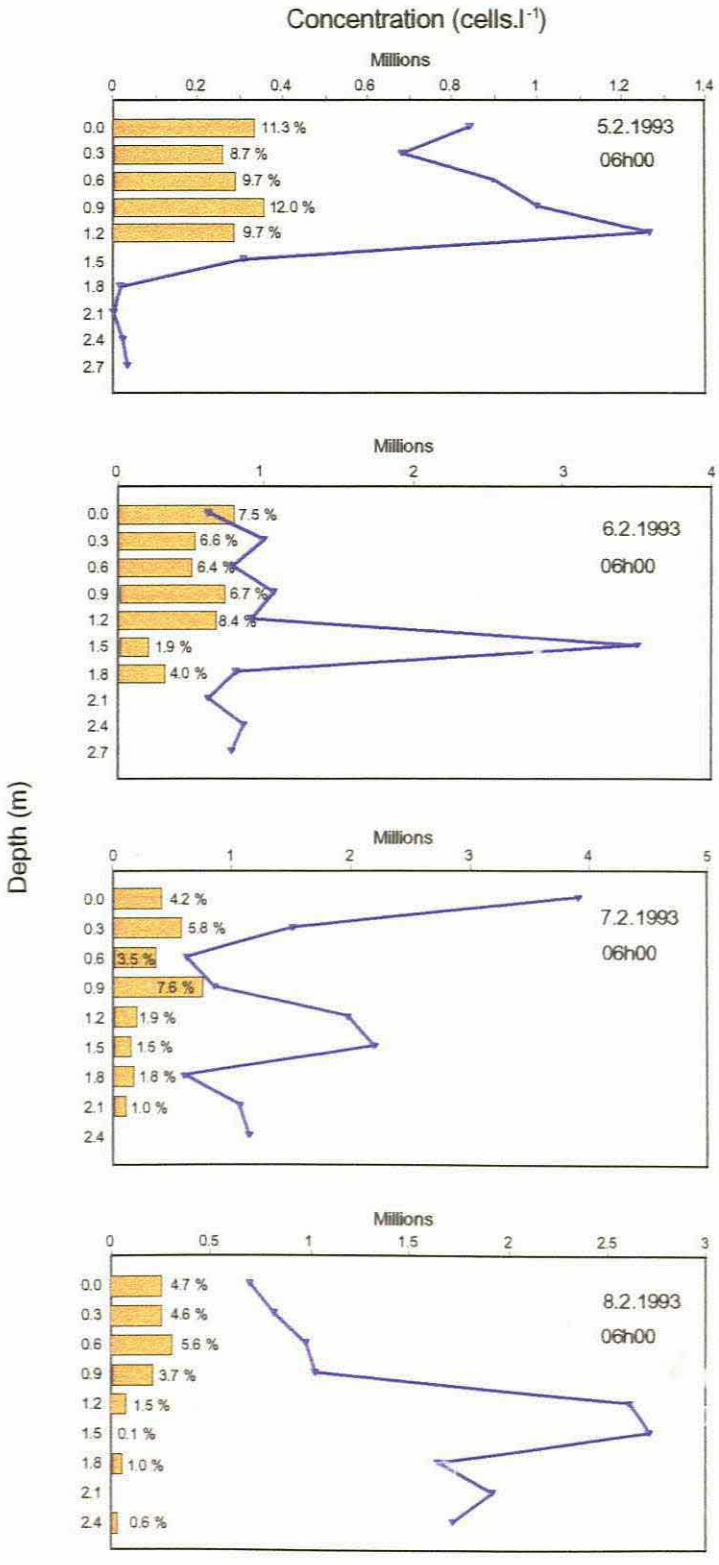

Fig. 42. Percentage of dividing cells (▭) , and cell concentration (cells.l^{-1} , ——) of *P. micans* during mesocosm 3.

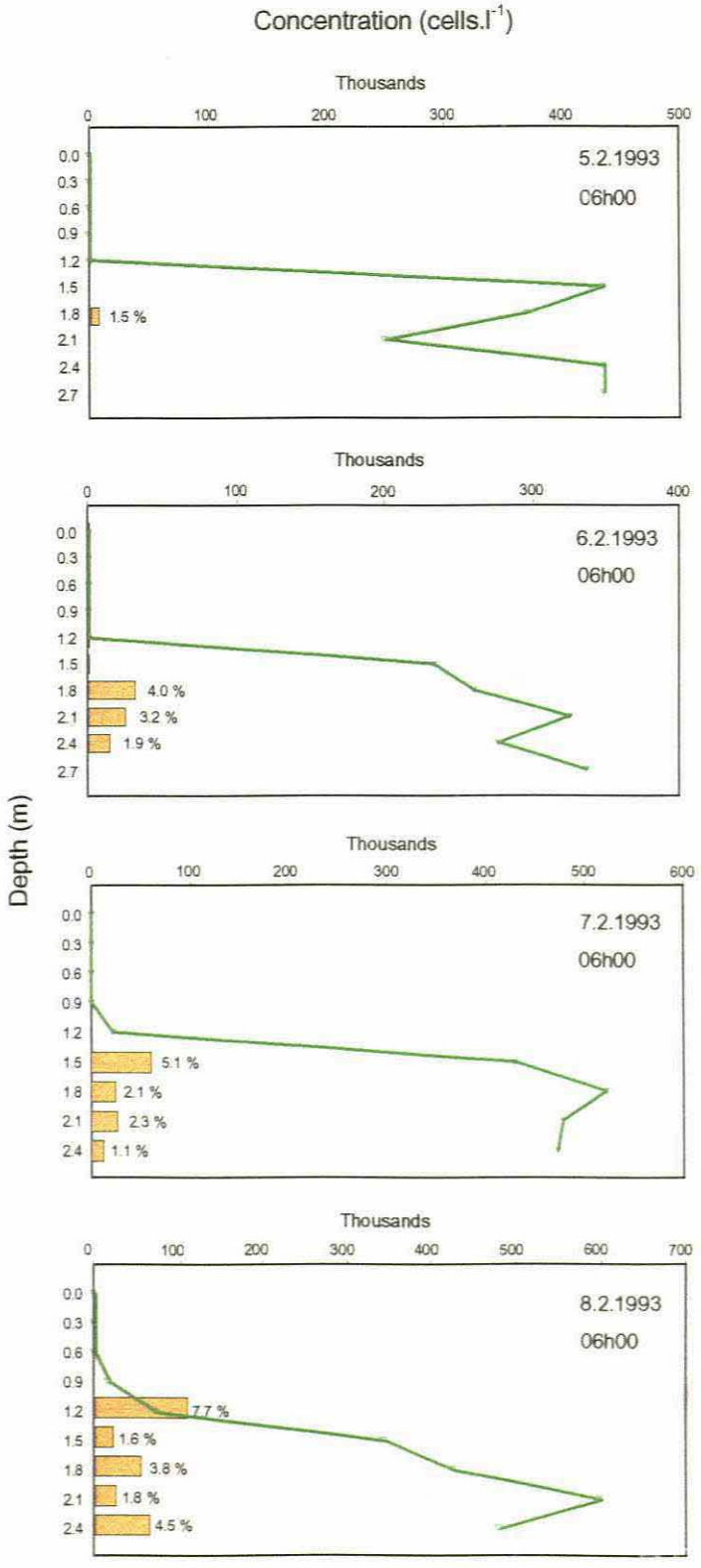

Fig. 43. Percentage of dividing cells ( ), and cell concentration (cells.l^{-1}, ——) of *C. lineatum* during mesocosm 3.

Three significant results emerged from the mesocosm studies:

1) The marked depth-differentiation in the proportion of dividing cells.

2) The depth of the maximum frequency of division did not correspond with the depth of the maximum population. This implies that the portion of the population that is destined to divide is not necessary part of the maximum concentration of that population.

3) The dividing fraction of the population does not migrate vertically or has reduced vertical migration.

The above behavioural patterns can be attributed to the harmful effects of allelopathy, to the deleterious effects of too many cells packed in a very narrow layer competing for the available nutrients, or to the conservation of energy for cell division as an appreciable amount of energy is needed for motility in dinoflagellates (Ronkin 1959).

Several species were fairly abundant in the experiments but were rarely or never observed to divide. These included *Alexandrium catenella, Protoperidinium trochoideum, Peridinium diabolus, P. exentricum, Gonyaulax polygramma, G. grindleyi, Prorocentrum gracile, P. balticum, Mesodinium rubrum* and *Noctiluca miliaris*.

Patterns of cell division in unicellular algae have long been of

interest and there have been numerous observations on periodicity of division of phytoplankton. Most *in situ* observations deal with various *Ceratium* species (Elbrächter 1973, Weiler and Chrisholm 1976 and Weiler and Eppley 1979). Patterns of cell division in other flagellates have not been well studied.

The temporal pattern of cell division was examined at two extremes in photoperiod: one during early winter (Gordon's Bay June 1995) and the other during late summer (Lambert's Bay February 1996). In both instances cellular division was strongly phased in *C. furca, C. lineatum, P. rostratum* and *D. acuminata*. Phased division in *C. furca* and *C. lineatum* peaked between 03h00 and 04h00, and 05h00 and 06h00 respectively during both winter and summer conditions. It would appear, therefore, that division in coastal populations of *Ceratium* species does not maintain a constant phase relationship with dusk under different photoperiod and temperature conditions. These observations suggest that light or photoperiod was not responsible for synchronizing cell division of *Ceratia*. It would appear, therefore, that phased cell division follows a circadian rhythm for these species. Little information exists in the literature about the effects of environmental conditions on the timing of cell division.

Division in photosynthetic marine dinoflagellates is generally late at night or early in the morning under laboratory (Weiler 1978) and field (Weiler and Chisholm 1976) conditions. There appear to be at least some exceptions to this rule. *G. splendens* divides near the light-dark border on LD12/12 cycles (Sweeney,

1959). Videau and Partensky (1990) found that *Gymnodinium cf nagasakiense* in culture reached its F_{max} towards the middle the night and that the period of division was very variable (3-12h). During the present study *G. splendens* under field conditions, was observed to divide over an extended period (18h) and F_{max} was reached at 21h00 (Fig. 39).

The ecological significance of diel timing of biological activities has been recognised for higher trophic levels in the marine environment. The temporal pattern of cell division in algal species is a subject of speculation. Unlike the 5 species (*Ornithocerus magnificus, Ceratium tripos, Peridinium sp., Ceratocorys horrida, Ceratium trichoceros)* observed by Doyle and Poore (1974), the dinoflagellates observed during the 24 h *in situ* studies divided at different times of the night. The existence of phased cell division in natural phytoplankton populations is of significance because of ecological implications involving both competition (light and nutrients) and predation (Doyle and Poore 1974). The timing of cell division could also be an adaptation to minimize competitive interactions and grazing pressure (Chrisholm *et al.* 1978).

Many authors have used the maximum frequency of division in natural populations of dinoflagellates for the assessment of population growth rate. It is possible that the measured growth response of the species being studied could reflect the previous environmental history of the cells rather than the growth response to the immediate environment.

The observed growth rates and doubling times indicate that conditions were favourable for the growth of the species present at Gordon's Bay and Lambert's Bay at the time of the experiments. The large variability in growth rates observed between the two studies could be due to the different hydrographic conditions (temperature, nutrient availability, etc) and the population age. These results are, however, consistent with other studies involving *C. furca* both with respect to the timing of cell division and the position in the water column (Weiler and Eppley 1979; Weiler and Karl 1979; Heaney and Eppley 1981; Kamykowski 1981a,b and Olsson and Granéli 1991) and of *D. acuminata* (Reguera *et al.* 1996).

The present studies have shown that dinoflagellates have different growth strategies and that resource partitioning occurs both in time and space. The relatively low specific growth rates determined during these studies emphasise the importance of physical processes as opposed to biological processes in the development of red tides within upwelling systems. The observations indicate that red tides do not result from rapid cell growth but rather represent localised accumulations of widespread, slow-growing, seasonal blooms.

CHAPTER 6

CONCLUSIONS

There is general agreement that the scale and complexity of red tides are expanding; the frequency of toxic blooms, the diversity of toxic species, the resources affected, the kinds of toxins and their economic impact have all increased (Riegman *et al.* 1993; Hallegraeff 1993; Sournia 1995). To address these issues, an understanding of the ecological, physiological and toxicological mechanisms underlying the growth of red tide algae is essential.

Red tides dominated by migratory dinoflagellates are a common feature of the southern Benguela upwelling system. Seasonal blooms of dinoflagellates occur in response to seasonal upwelling and typically succeed diatom blooms. Red tides usually occur following concentration by various physical forces and are characteristically found in warm, stratified, nutrient-depleted water overlying cold, nutrient-rich water.

This study is amongst the first to contribute to our understanding of the behavioural and physiological mechanisms and strategies of red tide species from the southern Benguela. Behavioural patterns and growth measurements of natural red tide populations under thermally stratified conditions were collected.

During this study, the influence of turbulent mixing (field study), light and the availability of nutrients on migratory behaviour were also examined.

The design of the mesocosm experiments was important in maintaining the red tide population in a system simulating the natural environment. A mesocosm was used to overcome two of the principal difficulties of plankton research in the open sea. viz.,

a) The isolation of the red tide organisms from influences of the turbulent motions associated with wind, waves and advective processes.

b) Repetitive sampling of the same group of organisms in the same body of water.

It was encouraging to find that the behaviour and growth of red tide organisms in the mesocosm studies was in most respects similar to naturally occurring red tides in the southern Benguela. The main drawback of the mesocosm studies were the restricted sample volume per sampling interval.

With the above considerations incorporated into the design of the mesocosm experiments, it was assumed that the mesocosm could be used with some confidence to investigate and explain behavioural patterns and growth strategies observed under natural conditions.

Red tides investigated during this study were dominated by the dinoflagellates *Prorocentrum micans, Ceratium furca, C. lineatum* and *Dinophysis acuminata*, a marine ciliate *Mesodinium rubrum* and a raphidophyte *Heterosigma akashiwo*. They all exhibited directed vertical migration, with ascent and descent starting before sunrise and before sunset respectively. Some interspecific differences were found in the timing and rate of migration, both during ascent and descent. Species also differed in their diel vertical migration patterns. Some species aggregated at the surface during the day and at the bottom at night, while others exhibited less extensive vertical movements between the surface and immediately below the thermocline. All dominant species penetrated strong temperature gradients in the mesocosm experiments. *D. acuminata* exhibited very limited diel vertical migration during the field study at Lambert's Bay. Vertical migration patterns were found to be strongly influenced by mechanical wind mixing during the Gordon's Bay study.

A major finding of this study was the clear vertical niche separation of different red tide species, both during the day and at night. Observations support the hypothesis that flagellate species are capable of coexisting within a red tide bloom. This is achieved by species occupying different depths at all times thus minimizing niche overlap and therefore reducing interspecific competition for the essential resources of light and nutrients. The coexistence of multiple species during a red tide event is thus a consequence of resource partitioning. From the study, it is clear that red tide species in well stratified

water will be found at their behaviourably determined preferred depths.

By photosynthesising near the surface during the day, and utilising nitrogen at depth during the night, dinoflagellates have a competitive advantage over non-motile phytoplankton such as diatoms, especially in nutrient-depleted surface waters. This is corroborated by the ability of dinoflagellates to sustain high concentrations in nitrogen-depleted surface water, presumably growing from nitrogen taken up during nocturnal diel vertical migration. It is noteworthy that the behavioural patterns of some vertically migrating species seem to be closely linked to their nutritional physiology. When nutrients are depleted throughout the water column, vertical migration is such that the maximum time is spent near the surface, within the constraints of completing the nocturnal descent. When nitrate is introduced in the bottom layer, behaviour is modified to increase exposure to nutrients at depth while still maintaining a high rate of photosynthesis during the day. Migratory behaviour therefore optimises both exposure to light conditions during the day and access to nutrients at night.

Measurements of *in situ* growth rates provided insight into the population dynamics of red tides. In this study the growth rates of dinoflagellate species were determined by estimating the frequency of dividing cells. During two 24-hour time series, *C. furca*, *C. lineatum*, *Prorocentrum rostratum* and *Dinophysis acuminata* exhibited pronounced phased division during the early

hours of the morning. Most significant were the temporal separation between the dividing species on a diel and seasonal basis. As the timing of cell division for the individual species was similar for both summer and winter, it is suggested that the duration of sunlight is not responsible for synchronizing cell division. *C. furca* recorded the highest specific growth rate of $0.32.day^{-1}$. Growth rates could not be determined for the *Gymnodinium* species as phased cell division was not evident. The relatively low specific growth rates ($0.08-0.32.day^{-1}$) determined during these studies emphasise the importance of physical processes as opposed to biological processes in the formation of red tide within upwelling systems. These findings indicate that red tides do not result from rapid cell growth but rather represent localised accumulations of widespread, slow-growing seasonal blooms.

Although dinoflagellates are regarded as having a lower productivity than diatoms, their migratory ability and their different physiological requirements enable them to out-compete diatoms in mature upwelled water. Previously this dinoflagellate production has been largely overlooked, with diatom production in the early phase of upwelling being the focus. As a result of a suite of behavioural and physiological adaptations, dinoflagellates are able to exploit mature, nutrient-depleted waters, thus providing the potential to make an important contribution to phytoplankton production in a fluctuating system such as an upwelling area, especially in the stratified stage of the upwelling cycle.

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