A contribution to the limnology of Swartvlei: The effect of physico-chemical factors upon primary and secondary production in the pelagic zone.

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RESUMÉ

The effect of physico-chemical factors on the biology of the upper reaches of Swartvlei has been investigated during 1971 - 1972.

Physico-chemical data have shown that Swartvlei was characterized by an extremely labile ectogenic meromixis. This instability was due to three factors: (1) the inflow of freshwater, (2) the inflow of sea water, and (3) wind stress. The magnitude of the effects of these factors upon the physics, chemistry and biology of the upper reaches was dependent upon whether or not the estuary mouth was open or closed.

The phytoplankton of the pelagic zone of Swartvlei was dominated by nannoplankton. Three major categories were recorded: dinoflagellates, flagellates and diatoms. The major factor regulating their productivity in the upper reaches was light. As a result of humate staining and suspended detrital matter light conditions in Swartvlei were comparable to those in extremely eutrophic northern hemisphere lakes. A maximum integral primary productivity of 39.66 mg C m^{-2} h^{-1} was recorded in November 1972.

The aerobic heterotrophic bacterial population in Swartvlei was usually less than 300 col. ml^{-1} (plate counts). The activity of the total microbial population was measured with ^{14}C techniques. Uptake of acetate was dominated by flagellates and one species of dinoflagellate when they were present. Glucose uptake was due to bacteria as was acetate uptake in the absence of heterotrophic phytoplankton.

Glucose uptake was usually greatest in the anaerobic zone. This, and the presence of H_2S, suggested that a large active photosynthetic and chemosynthetic bacterial population may have been present in the monimolimnion. The possible importance of these bacterial processes in the total productivity of the pelagic zone of Swartvlei was discussed.

Daytime zooplankton population size was statistically correlated with the size of the flagellate population. The zooplankton was dominated by Acartia and Halicyclops. These two animals were found in the anaerobic bottom of Swartvlei after October 1971. This

corresponded to the disappearance of the flagellate population from the water column. The dominance of these animals in the zooplankton population may have been related to their ability to live in anaerobic water where the bacterial population appeared to be considerably more abundant than in the aerobic zone.

Further implications of the results are discussed in reference to phytoplankton cell size and heterotrophy indicating a possible adaptive significance of these factors in Swartvlei.

INTRODUCTION

The study of aquatic microbial ecology began in the latter part of the 19th century. This particular branch of the biological sciences, dealing with the hydrosphere, has been long in its development. Slow progress may have been due, at least in part, to the technical difficulties involved in studying organisms that are normally invisible to the naked eye. It is understandable that the attention of most early aquatic biologists was focused on fish, invertebrates and the large species of phytoplankton.

Although nearly a century has passed, our knowledge of the microbial ecology of natural waters demonstrates a need for further study. The technological advances made in ecological research in recent years, such as the application of radioisotopes, have increased this knowledge considerably but it is still far from complete.

The microorganisms represent only a small portion of the total biomass of a natural water body. However, their function is essential to understand since, as Rodina (1972) pointed out, microorganisms are the first link joining the biotic and abiotic milieu.

An ecosystem was defined by Odum (1971) as, "any unit that includes all of the organisms in a given area interacting with the physical environment so that a flow of energy leads to a clearly defined trophic structure, biotic diversity, and natural cycles within the system". Microbes are known to participate significantly in the turnover and flow of nutrients within trophic levels and from one level to another in ecosystems. They may often break "bottlenecks" which exist in food chains and biogeochemical cycles of macroecosystems (Brock 1966). This fact underscores the need to be aware of the microbial role within an aquatic ecosystem. Brock suggested that the interaction between organisms could be divided into two broad categories: (1) interactions between two microbial populations and (2) interactions between a microbial population and a macroorganism or macropopulation.

Although the present study involved interactions from both these categories it focused mainly on microbe-microbe interactions and their relationship to the physical environment.

Data on South African coastal aquatic ecosystems is extensive but limited to physico-chemical data, aquatic macrophytes, larger phytoplankton, zooplankton and invertebrates and vertebrate groups (Day 1951; Scott et al 1952; Allanson and van Wyk 1969; Boltt 1969; Boltt et al 1969; Hill 1969; Millard and Broekhuysen 1970; etc). The microbial ecology of these ecosystems has never been investigated. In fact, very little work in this regard has been done in Africa (Lawson et al 1969). Furthermore, there is essentially no data available on the ^{14}C primary productivity or the factors controlling it and phytoplankton succession in these coastal aquatic ecosystems. Although enzyme mediated uptake studies of various dissolved organic compounds are relatively common in northern hemisphere aquatic ecosystems they have never been applied to water bodies in Africa. More specifically, physico-chemical and biological data on Swartvlei, the coastal aquatic ecosystem focused on in this study, is virtually nil.

The purpose of this study was to determine the role of the physico-chemical factors of an unstable, meromictic system in regulating the biological limnology. The other major areas of investigation were: (1) the interactions between the aerobic heterotrophic bacterial and phytoplankton populations, (2) the activity of the bacteria and phytoplankton in the removal of dissolved organic compounds and (3) the algal rate of primary productivity and the factors controlling it and phytoplankton succession. In order to try and ascertain the effect of one level of consumers on the planktonic microbial populations the study was extended to include the zooplankton.

Preliminary results indicated that the littoral was distinctly different from the pelagic zone of Swartvlei in terms of its physico-chemical and biological factors. The pelagic also seemed to have a much simpler trophic structure than did the littoral. The recent work

of Allen (1971a and c) and Wetzel and Allen (1972) has shown that the littoral of lakes has a complex trophic structure. The pelagic seemed to be the most suitable area in which to carry out the first study on Swartvlei. Furthermore, an intensive study of the physico-chemical and microbial limnology of the pelagic zone was considered to be a more fruitful proposition than a less critical study of the whole system.

Taken into account in the decision to concentrate the work in the pelagic were the relatively primitive field conditions that would be encountered and which imposed limits on the technical sophistication which could be achieved. Since Swartvlei is 300 miles from the Institute for Freshwater Studies the limit to the amount of sterile equipment that could be taken into the field on each trip restricted the number of stations that could be worked. However, if it became essential to investigate some aspect of the littoral, or some other part of the system, to clarify a point pertaining to the planktonic microbial community the study was expanded to include this aspect.

LITERATURE REVIEW

Although the review will deal mainly with microbe-microbe interactions in aquatic ecosystems the scope of this chapter has been limited to those topics which pertain directly to the project. Other areas of important research dealing with these interactions have been noted during the literature search but were deliberately omitted since they would only mask and confuse the major points related to this study.

The Relationship between Bacteria and Phytoplankton

The relationship between the distribution and occurrence of bacteria and phytoplankton in aquatic ecosystems has received considerable attention, especially by freshwater ecologists. These two groups or organisms appear to be mutually dependent on one another although the relationship apparently differs from one ecosystem to the next. When reviewing the work that has been done, however, it is essential that the method of bacterial enumeration be stated, i.e., direct or total counts or plate counts and if the latter what type of media. The dominant groups of phytoplankton should also be noted. These factors are highly significant in the results obtained. Different culture media select for various groups of bacteria and hence the results may differ from those obtained in other studies.

One of the earliest studies involving the relationship between phytoplankton and heterotrophic bacteria (plate counts, media not specified) was done by Waksman et al (1937). Using mixed and pure cultures of marine diatoms they showed that the functions of these organisms supplemented each other. The diatoms, they believed, synthesized organic matter from the simple chemical substances produced in the decomposition or mineralization of organic matter in the sea by the bacteria.

Henrici (1938) working in lake Alexander, Minnesota found that the number of bacteria, as estimated by plate (glycerol-peptone-mineral salts media) and direct microscopic counts, followed closely the curve for total phytoplankton. The plankton appeared in three

pulses, diatoms first, followed by a heterogenous population of green algae and Protozoa (Volvox, Ceratium, and Dinobryon) which was followed by blue-green algae and finally a reappearance of diatoms. The curves for the aquatic bacteria showed three peaks which lagged behind the plankton peaks. The author concluded that the phytoplankton were providing organic matter which served as a growth substrate for the bacteria.

According to Sverdrup et al (1946) marine bacteria, based on plate counts (media not specified), occupy two main centers in the oceans: (1) a few millemeters below the mud-water interface and (2) in the pelagic zone attached to the floating plants, animals and other particulate matter. Diatoms, copepods, etc., most abundant in the euphotic zone, serve as surfaces for attachment and offer a favourable environment for multiplication of this pelagic bacterial population. The result of such a large bacterial population is prompt decomposition of large quantities of dead organisms before they have sunk to great depths. Therefore, according to Sverdrup et al, a large portion of mineralized plant nutrients is regenerated within or only a little below the euphotic zone.

Probably the most intensive investigations of the relationship between aquatic bacteria and phytoplankton (mainly Cyanophyta) have been done in Germany by Overbeck (1967, 1968a and b). Three major groups of bacteria were usually distinguished by Overbeck (1968b): (1) aerobic bacteria grown at 25°C on Difco Bacto Nutrient agar Bl designated as saprophytes (heterotrophic bacteria), (2) aerobic bacteria grown on a mineral agar without the addition of organic material and designated as oligocarbophiles and (3) bacteria counted on membrane filters or direct counts. All of these groups are reported to follow exactly the vertical distribution of phytoplankton. The correspondingly vertical distribution of aerobic heterotrophic bacteria and phytoplankton in lakes, Overbeck (1968a) wrote, is explained by the organic matter formed by the vegetative phytoplankton

being mineralized in the region of its origin. Mineralization takes place rapidly. The bacteria therefore depend on the liberation of organics either by autolysis or excretion by the phytoplankton and utilize it at the same depth that it is formed. This, Overbeck (1968b) went on to explain, is why there is no substantial accumulation of assimilable organic matter in the water and why it is possible to have narrowly spaced bacterial layers. The bacteria, by rapidly and continuously forming inorganic and organic compounds from waste products, make it possible for a continuous development of phytoplankton. In "healthy" lakes phytoplankton synthesis of organic substances should be counterbalanced by bacterial disintegration (Overbeck 1968a).

Schmidt (1969) investigated the vertical distribution of bacteria and algae (not identified) in Lago do Castanho in the Amazon. He found that the annual fluctuations of bacteria and phytoplankton coincided. Furthermore, there was always a distinct stratification of these organisms with the total number of bacteria attaining their maximum immediately below the algae. Saprophytic bacteria, i.e., those that grow on nutrient agar, appeared to be greatest at the same depth as the phytoplankton maximum. Usually the number of bacteria decreased rapidly with depth after the peak levels. This finding was concluded to support the idea that essential mineralization processes took place at the level of the phytoplankton maximum. However, between October and January, when planktonic populations were decreasing another peak of saprophytic bacteria was noted towards the bottom. At the lowest point in the growth of the phytoplankton the total number of bacteria over the mud was greater than in the euphotic zone. The bottom peak of saprophytes was thought to be due to either specially facultative anaerobic types which found better living conditions, or, saprophyta which were carried to the depths by the frequent circulation of this shallow lake and, at renewed stagnation slowly settled to the bottom. The author concluded that the bacteria, especially saprophyta, depended on a close proximity to the

phytoplankton for their development.

An investigation of the heterotrophic bacteria estimated on YPA 25 media(yeast peptone agar) in lake Malaren by Fonden (1969) yielded a significant positive correlation between bacterial numbers and phytoplankton standing crop (chlorophyll a) in the surface water. He also reported that the vertical distribution of YPA-bacteria in the epilimnion and metalimnion of Ekoln Basin in lake Malaren corresponded to the phytoplankton distribution. Fonden went on to point out, though, that both zooplankton grazing and inflowing water alter the relationship between phytoplankton and bacteria by either lowering or raising the quantity of bacteria present. For example, Boyd and Boyd (1963) in an Arctic coastal lake reported that bacterial maxima (plate counts) corresponded to periods of high inlet discharge. In Ekoln Basin the bacterial vertical peak was found near the metalimnion and was associated with river inflow. The horizontal distribution of bacteria in both lakes Malaren and Hjalmaren showed that bacterial numbers were greatest near towns. In Malaren, sampling stations close to river inflows showed high numbers during spring and autumn. The ideal lake in which to study bacterial growth, Fonden said, is eutrophic, not polluted with domestic wastes and has no major influents.

Olah (1970) reported that under the ice in lake Balaton there was a definite stratification of bacteria with a maximum in the hypolimnion. This peak of total bacteria was higher than any recorded during the rest of the year and was connected to the formation of a large μ-algae (predominantly Chlamydomonas) population. Olah also noted the μ-algae were found in much smaller numbers in reed bed areas and that the total bacterial plankton were also less abundant. A later publication by Olah (1971) on lakes Balaton and Belso stated that the quantity of planktonic bacteria increases only after a definite delay following the phytoplankton maximum. However, in lake Balaton the autumn algal maximum, predominated by diatoms, was accompanied by a total bacterial plankton maximum.

The vertical distribution of bacteria (direct count) in the euphotic zone of the western equatorial Pacific ocean was reported by Vinogradov et al (1970). They found a bacterial and phytoplankton peak between 80 - 100 m. The phytoplankton, they believed, were restricted to this region from below by a lack of light and above by a lack of nutrients. The bacterial development was attributed to the high concentration of seston and to the dissolved organic matter supplied to this region by means of vertical water exchange.

In Titisee a summer maximum of total bacteria was attributed to sewage and a water bloom of Pardonia morum and Anabaena flos-aque by Pokorny (1971) but during the winter the high count of bacteria in the surface layers was thought to be due to flood waters. Seppanen (1971) working in polyhumic lake Hakojarvi, Finland found that the maximum number of heterotrophic bacteria (estimated on TGY media, tryptone-glucose-yeast) occurred in August which corresponded to the summer algal maximum (phytoplankton not identified). Silvey and Wyatt (1971) reported that heterotrophic bacteria, in reservoirs of the Southwestern U.S.A., responded with an abrupt population increase following blue-green algal peaks.

Schegg (1971) noted that high bacterial direct counts were found at the levels with maximum primary production in Rotsee and lake Lucerne. In an attempt to clarify the mutual influence of phytoplankton and bacteria on each other he deivsed a set of in vitro experiments involving aquatic bacterial cultures and Chlamydomonas sp.. The results showed that a nutrient-induced increase in algal growth created a rapid substrate-induced increase in bacterial growth.

The relationship between viable heterotrophic bacteria (ZoBell media 2216) and phytoplankton (not identified) was investigated by Seki (1971) in Saanich Inlet, British Columbia, Canada. He found that small microbial clumps appeared in the seawater, especially before and during a phytoplankton bloom. The largest "clumps", consisting of bacteria and allied microorganisms (Protozoa), were

formed in late summer after the phytoplankton bloom when the viable algae formed approximately one half the total suspended matter. The phytoplankton was probably an important substrate for the formation of the aggregates. The lower number of heterotrophic microorganisms during the bloom was partially ascribed to the antibacterial activity of phytoplankton, whereas before the bloom, the low numbers were attributed to an increase in biochemically stable substances of the particulate matter.

Lange (1970, 1971) suggested that organic matter converted to CO_2 through bacterial respiration enhanced blue-green algal activity, and that the process was a major factor related to algal blooms in natural waters. Keuntzel (1970) presented the hypothesis that bacterial production of CO_2 is the major causative factor in many algal blooms. This was based on two facts: (1) aerobic bacteria require oxygen to degrade organic matter and produce carbon dioxide and (2) algae require CO_2 to photosynthesize organic matter and produce oxygen. Hence, according to Kuentzel, conditions that lead to explosive growths of bacteria might well lead to an accompanying explosive growth of algae. Further, both Kuentzel and Lange maintained that during periods of vigorous algal growth carbon dioxide could become limiting due to the slow diffusion of atmospheric CO_2 and bicarbonate ions dissolved in lake water.

From the preceeding reports it is clear a relationship between aquatic bacteria and phytoplankton exists. Yet, it is not always a simple matter to demonstrate this relationship as the following reports indicate.

As already noted, Olah (1970) found in lake Balaton that usually an increase in phytoplankton corresponded with an increase in the total bacterial number. On the other hand, the saprophytic bacteria (grown on sodium-caseinate agar) showed an inverse relationship. Under the ice, with a large phytoplankton and total bacteria population, the saprophyta were low. In the reed beds, where the algae (diatoms)

and total bacterial numbers were low, the saprophyta were high. Short periodic changes were noted at various times of the year for both the total bacteria and saprophyte populations. These were attributed to temperature variations and heavy winds which stirred up the bottom increasing the organic matter concentration in the water column. Olah (1971) concluded in a following paper that the interrelationship between bacteria and phytoplankton is quite complicated. Although generally being able to report a positive correlation between total bacteria and algae in the surface water of lake Belso, at other times he found no correlation. Instead of the typical increase of bacteria after the proliferation of phytoplankton the bacterial population either decreased or increased depending, it seemed, partially on the type of algae present.

After studying numerous physico-chemical and biological parameters in lake Maggiore and subjecting the data to statistical analysis Goldman et al (1968) were not able to show any relationship between the level of primary productivity and the bacterial population as estimated by direct counts. Bacterial plate counts, however, were positively correlated with the total phytoplankton biomass and with the total zooplankton from June to August. A positive correlation was also found between phyto- and zooplankton during the whole period of the investigation. The authors were of the opinion that the positive correlation between bacteria plate counts and phytoplankton was a result of the positive correlation between phyto- and zooplankton. No explanation was given. Further, they assumed that only zooplankton and bacteria estimated on agar plates were directly related. The basis for this assumption was that bacteria which colonize peptone-yeast extract plates are essentially proteolytic.

Gerletti and Melchiorri-Santolini (1968) also found no evidence of a relationship between bacteria (peptone-yeast extract media and direct counts) and algae in four Italian lakes including Maggiore. This they felt was due to the bateritel count including different types

with various metabolic abilities. Another factor was that the phytoplankton counts did not take into account the different biomass and physiological activities of different blue-green algal species. At the same time the authors noted Overbeck's data for the correlation of bacteria and phytoplankton but felt it was not valid to compare their data to his since their lakes were so different in terms of productivity and other factors.

Jones (1971) studied the factors which influence the freshwater bacterial population and its activity. Estimates of viable bacteria in Esthwaite Water and Windermere lakes were carried out with the spread plate technique on CPS (casein-peptone-starch) media. Statistical analysis of the data indicated no correlation between viable bacteria and the phytoplankton population which was measured by the determination of chlorophyll a levels. However, it was occasionally noted that the bacterial population and enzymatic activity increased soon after phytoplankton maxima indicating some response by the bacteria. In nutrient-rich Esthwaite the major factors which appeared to regulate the bacterial population were temperature, dissolved oxygen and pH. On the other hand, a positive correlation in Windermere existed between the bacterial population and temperature, pH, particulate matter and rainfall.

Obviously many factors are involved in the relationship between aquatic bacteria and phytoplankton. As in so many areas of ecological investigation no set rules can be applied to this relationship. Each aquatic ecosystem with its innumerable internal and external influences should be considered as totally new, in some ways resembling, but in many ways differing from others. Rodina (1972) stressed the fact that every body of water is characterized by its own peculiar bacterial population and the development of microorganisms within this body is determined by the specific conditions of the water mass.

Competition between Bacteria and Phytoplankton for Dissolved Organic Compounds

Another aspect of the interaction between phytoplankton and aquatic bacteria is the competition for dissolved organic substrates. From their studies in lake Erken of the uptake of glucose and acetate by aquatic microbial populations Wright and Hobbie (1965a) postulated two types of uptake. Using laboratory cultures of Chlamydomonas and Gymnodinium inversum (Wright and Hobbie 1966) as well as an aquatic bacterium isolated from lake Erken (Hobbie and Wright 1965b) they were able to show that both an active transport system and a diffusion system were responsible for uptake. Bacteria utilizing a transport system were primarily responsible, they concluded, for the uptake of organic solutes at low concentrations while algae were mainly responsible for uptake by diffusion at concentrations greater than 0.5 mg 1^{-1}. From this information they devised a technique for measuring the uptake of dissolved organic substances by bacteria in aquatic ecosystems (see Materials and Methods). Wright and Hobbie (1966) did not, however, completely rule out the possibility that very small algae, those less than 10 to 15 µm in diameter and with surface/volume ratios close to those of bacteria, may possess transport systems effective for organic substrates at low concentrations.

The outcome of the competition between bacteria and phytoplankton in aquatic systems for organic substances is dependent on substrate concentration (Wright and Hobbie 1966). Since dissolved organic compounds are present in natural water at concentrations of a few µg 1^{-1} (Hobbie and Crawford 1969a) this competition favors the bacteria. Thus, Hobbie and Wright (1965a) concluded the chief obstacle to phytoplankton heterotrophy was the bacteria.

A study by Munro and Brock (1968) showed that when acetate over the range of 10 - 5000 µg 1^{-1} was added to a mixed population of bacteria and diatoms attached to sand grains taken from the sea all the uptake was due to the bacterial population. From these results the

authors concluded that algal heterotrophy is negligible in sea water. They also concluded that bacteria are able to compete successfully for the limited organic matter available in the sea and other aquatic environments.

Indications of algal heterotrophy in the sea comes from data recorded by Fournier (1966). Working in the North Atlantic ocean he found a population of biflagellated, spherical phytoplankton which extended from the euphotic zone downwards with a maximum at 1000 m. These organisms which were 3 to 5 μm in diameter and yellow-green in colour were, he thought, responsible for the uptake of glucose and acetate (no concentrations stated) added to water from this region.

Tezuka (1970) investigated the distribution of heterotrophic bacteria and their possible role in the mineralization of organic matter in lake Yuno-ko, Japan. In addition to finding that planktonic bacteria play a minor role in the mineralization processes in the lake he also found that living algae were present in the lower part of the water column. High photosynthetic activity was considered to be limited in this region due to a lack of light. He decided that algae therefore must have survived at the expense of reserve substances or heterotrophically. As a result, Tezuka concluded, the algae appeared to function in this layer as decomposers rather than primary producers.

Significance of Studies in Ice Covered Lakes

A more likely situation in which to investigate the possibility of phytoplankton heterotrophy being an important process in an aquatic ecosystem would be in waters where light could be considered a limiting factor in phytoplankton production. Such ecosystems are found in regions where lakes have thick ice and snow covers.

A point that needs clarification before continuing this aspect of the review is what is intended by the terms net phytoplankton, nannoplankton and μ-algae. According to Kalff (1967b) net phytoplankton is greater than 65 μm in size. In agreement, Pavoni (1963) noted that nannoplankton have been considered to be algae up to the size of

60 µm. However, for her, nannoplankton average between 1 - 30 µm. At the lower end of the nannoplankton scale are the µ-algae according to Rodhe (Lund 1961). Lund generally defines them as algae less than 15 µm along every axis.

From her study of nannoplankton and net phytoplankton of Swiss lakes, Pavoni (1963) found her data suggested that there was a close relation between extreme environmental conditions and the percentage of nannoplankton in the total phytoplankton population. As the environmental factors became "more extreme" the percentage of nannoplankton increased in relation to the net phytoplankton, i.e., the nannoplankton population is greater, in relation to net phytoplankton, in an oligotrophic lake than in an eutrophic lake. Nannoplankton and µ-algae have been found to form a large percentage of the total phytoplankton in Arctic lakes (Kalff 1970), in alpine and mountain lakes (Nauwerck 1966; Pennak 1968), under the ice in lake Balaton, Hungary (Olah 1970) and in estuaries (Wood 1967). Adding to the possibility of phytoplankton heterotrophy in these ecosystems is the fact that it is the organisms of the nannoplankton and µ-algae size that Wright and Hobbie (1966) cautioned may be capable of effective competition with aquatic bacteria.

Rodhe's 1955 paper, "Can plankton production proceed during winter darkness in subarctic lakes", raised the interesting possibility of effective phytoplankton heterotrophy. The phytoplankton dynamics of an ice covered lake in Massachusetts were investigated by Wright (1964). During a snow free period he found a number of species of flagellated algae. A species-specific preference for light quantity or quality or both was indicated by various species which occupied characteristic levels in the lake. Over 95% of the phytoplankton found in the region where incident light was 0.5 - 20% of above ice readings. A snowfall caused the algae to move to the upper 50 cm of the water column which had been previously avoided because of excess light. A series of snowfalls cut the incident light

penetrating the water column to 0.2% and the phytoplankton population rapidly declined.

The vertical stratification of the species known to occur under the ice indicates, according to Wright, a real spatial separation of species for the purpose of reducing competition for resources such as nutrients and radiant energy. Since the advent of a snow cover drastically reduces light penetration he assumed the phytoplankton must have other adaptations to ensure survival, such as heterotrophy or the development of resting stages. From the algae which migrated to the upper 50 cm of water Wright obtained several species of cryptomonads which he grew in bacteria free cultures. To test his idea of heterotrophy he designed experiments in which three species of cryptomonads were placed in media enriched with different concentrations of glucose (1.35 and 0.135 g l^{-1}) and acetate (1.0 and 0.1 g l^{-1}) and subjected to varying light conditions from total darkness to normal culture light (ca. 1400 lux) at a temperature of 3 - 5°C. Growth was measured in Klett units. The results indicated that two of the species responded with the typical ability of acetate flagellates which are a group of chlorophyllous or colourless flagellates. They grow heterotrophically on acetate and various other fatty acids, Krebs-cycle acids, alcohols, and related compounds, but are typically unable to utilize sugars (Hutner and Provasoli 1951; Danforth 1962). The same two species showed significant autotrophic growth at a light intensity of 65 lux. Uptake of both substrates was greatest in the light. The results, however, only showed a heterotrophic and photoheterotrophic capability and cannot be extended as proof of these processes occurring in natural conditions since the substrate concentrations used were far too high.

Nauwerck (1966) studied the phytoplankton of four Tirolan alpine lakes and four Lapplandic mountain lakes. He found that the most important groups were the Crysomonads, Cryptomonads, dinoflagellates and μ-algae species of the Chlorophyta. Practically all the studied

lakes had the phytoplankton maxima in the greater depths or near the bottom. Smaller peaks were occasionally noted in the upper water zone. The upper maximum was usually composed of Cryptomonads, Chlorophyta and dinoflagellates. The most important factors influencing the plankton were considered to be the physical ones. The number of species was limited by temperature while light controlled the vertical distribution.

Nauwerck went on to point out that all the organisms which produced maxima at greater depths belong to groups where facultative heterotrophy is known and suggested it may be that they survive dark periods by this mode of nutrition. However, he concluded that the presence of well developed chromatophores indicates assimilation was mainly autotrophic.

In support of this conclusion are the data obtained by Rodhe for the lakes studied by Nauwerck (Rodhe et al 1966). He found the most intense zone of photosynthesis, as measured by the ^{14}C-technique, corresponded to the region of the phytoplankton maximum, i.e., in the deepest layers. Although photosynthesis may start at extremely weak light intensities he thought the algae must possess the ability to grow without any photosynthetic activity during the months of complete darkness. This introduces the idea of heterotrophic growth.

The second half of this paper was written by the co-authors, Hobbie and Wright, and deals with the results of heterotrophic experiments in three Swedish lakes of different productivity. One of the lakes, Kuoblatjakkojaure, is one of the series already referred to by Nauwerck and Rodhe. Experimental procedure using ^{14}C glucose and acetate was carried out according to the authors' published technique (see Materials and Methods). From the results it appeared doubtful whether any significant heterotrophic population was accumulating under the ice of the lakes. In fact, heterotrophic activity decreased over the spring months. In Kuoblatjakkojaure the high uptake of

acetate recorded was correlated with a photosynthetic population immediately below the ice rather than with the supposed heterotrophic population at 40 m. From a rough calculation of cell duplication for the phytoplankton at this depth Hobbie and Wright decided the algae would need to be able to utilize a number of substrates and even then they doubted heterotrophy could be their sole energy source. Another possible energy source suggested was phagotrophy. This, however, seemed likely to be restricted to the dinoflagellates and some flagellates and left unexplained how other nannoplankton species such as Chlorella and Scenedesmus survive continuous darkness for three months. A third possibility they considered was the use of stored food reserves at cold temperatures. The very small nannoplankton, it was suggested, may live by a combination of these nutritional processes, i.e., a combination of low level photosynthesis, the use of stored food and a slow uptake of dissolved organic compounds.

The supposition that heterotrophy alone is not the mechanism of winter survival for these phytoplankton populations was based on the ineffective uptake described for Chlamydomonas (Wright and Hobbie 1966). Further Hobbie and Wright pointed out that if there were true heterotrophic algae present then they must have had uptake mechanisms as effective as those of bacteria but such algae have not yet been found in the laboratory. They suggested caution should be exercised before assuming heterotrophy can explain the survival of phytoplankton under extreme conditions.

Pennak (1968) studied the phytoplankton of three Colorado mountain lakes during the winter. Under the ice in Black lake diatoms, green algae, flagellates and ciliates formed only negligible amounts of the algal population. What he did find was a highly variable and sometimes extremely dense population of μ-algae which defied classification. G.W. Prescott said some belonged to the Chlorobacteriaceae or organisms that lie between blue-green algae and bacteria. Various spores and a few of Chroococcus sp. were also

present. In Pass lake diatoms and flagellates were more abundant while the μ-algae population was much smaller. Tea lake had "extreme" (up to 14.5 million cells 1^{-1}) densities of μ-algae with an abundant population of flagellate species. Green algae were present but diatoms were scarce.

Referring to midwinter metabolism, Pennak thought the μ-algae in Black and Pass lakes survived winter by a combination of algal heterotrophy and secondary anaerobic heterotrophy of the sulfur Chlorobacteriaceae. In Tea lake phototrophy was assumed to be the dominant process although heterotrophy may have been functional within a few centimeters of the bottom. The difference in these two situations was related to the fact that a combination of thick ice and snow in Pass and Black lakes was assumed to have completely inhibited all photosynthesis from December to May. Tea lake had small amounts of snow on top of the ice and Pennak regarded the snow thickness as by far the more critical limiting factor for photosynthesis.

Referring to Hobbie and Wright's (1965a) statement that algal heterotrophy is limited by the more efficient uptake systems of the bacteria Pennak wrote that this was not likely to be the case in these mountain lakes because of the large μ-algae populations.

The phytoplankton of the Arctic freshwater lake, Imikpuk, in Alaska, was investigated by Kalff (1967a). Phytoplankton numbers ranged between 2.4 and 24.1 x 10^6 cells 1^{-1}. Small naked unicellular flagellates dominated the total population. Near the bottom there was a greater abundance of nannoplanktonic pennate diatoms, relatively large flagellates and algal cysts. The percentage of the total population represented by organisms of ≤ 10 μm fluctuated between 51 and 100%.

During June the maximum rate of carbon assimilation occurred immediately below the ice although the maximum algal population was located just above the bottom. This suggested that low light levels limited photosynthesis in the water column to just under the ice.

Further, maximum carbon incorporation shifted from the surface in early June to the middle of the water column by June 23 and to near the bottom by June 30. This was attributed to greater light penetration due to a gradual thinning of the ice cover. However, on four sampling periods prior to June 30 carbon fixation occurred at the bottom during the brightest portion of the day. Net carbon fixation was assumed to have been zero just above the bottom during the rest of the 24 hour period and prior to June when even less light penetrated the snow and ice in the deepest part of the lake. Even though light was limiting, this bottom zone contained 24.1×10^6 cells l^{-1} which were mainly flagellates while 50 cm below the ice the population measured only 3.3×10^6 cells l^{-1}. Kalff stated that the only satisfactory explanation for the presence of this large phytoplankton population in a region where little or no light penetrated was a heterotrophic mode of existence. Water colour in this region indicated a high dissolved organic content and reinforced the feasibility of a heterotrophic phytoplankton population during winter and spring. A combination of heterotrophy, phagotrophy and respiration of stored cellular products as suggested by Hobbie and Wright was not ruled out although it was thought that the respiration of stored cell materials was unlikely due to a lack of light.

Continuing his studies of phytoplankton in Arctic aquatic ecosystems Kalff (1967b) found further evidence of phytoplankton heterotrophy. This study involved two small Alaskan tundra ponds. The phytoplankton was composed largely of unicellular flagellates whose numbers ranged between 0.79 and 100×10^6 cells l^{-1}. Cells smaller than 11 μm in size rarely represented less than 75% of the total population. Net phytoplankton remained negligible at all times.

Heterotrophic capability of the phytoplankton in one pond was investigated following the technique of Wright and Hobbie (1966) using glucose as the substrate. Some of the bottles were incubated in the dark while others were incubated in 100, 55 and 27% light

conditions. Labelled substrate concentrations added to the dark bottles ranged from 0.5 to 2.0 mg 1^{-1} while the substrate in all light incubated bottles was 0.5 mg 1^{-1}. The results of the light bottles indicated that photoheterotrophy took place and that more organic carbon was fixed in the presence of some light than in the dark. Dark uptake of glucose increased with concentration and Kalff pointed out that it was much higher than the rate reported by Wright and Hobbie in lake Erken.

In summary Kalff noted, even though a significant correlation between cell number and primary production was obtained, relatively high cell numbers were also found during periods of low solar radiation and inorganic carbon assimilation suggesting the presence of facultative heterotrophs. The fact that on the occasional sunny day during this period of low solar radiation inorganic carbon fixation increased indicated these organisms were capable of shifting to a more autotrophic existence and this idea was supported by experimental data.

Since the substrate concentrations (0.5-2.0 mg 1^{-1}) added by Kalff to pond water samples were greater than 0.5 mg 1^{-1} uptake probably would have been due to a diffusion process (cf. Wright and Hobbie 1966). In most aquatic ecosystems these concentrations would be considered to be far above normal hence proof of a functional phytoplankton heterotrophic uptake at substrate concentrations typical of a natural system would not be obtained. However, the pond water investigated by Kalff had a very high dissolved organic carbon concentration (35.4 mg 0_2 1^{-1} permanganate organic matter). Kalff's results therefore confirmed a phytoplankton heterotrophic capability in a natural system, although no indication of how effective it was, compared to bacterial uptake, was presented.

Goldman, Mason and Hobbie (1967) studied two clear Antarctic desert lakes during the summer. Both lake Vanda and lake Bonney are permanently frozen. Light measurements indicated that light penetrated the entire water column. The phytoplankton in both lakes was dominated

by coccoid blue-green algae and phytoflagellates that were less than 20 μm in size. Total bacteria in lake Bonney reached a peak of 120×10^6 cells l^{-1} at 20 m. Most of the phytoplankton production was considered to occur under favourable light and temperature conditions of the upper 15 m. Heterotrophic activity below this region was probably the dominant process since sinking plankton was subjected to bacterial attack.

The possibility was considered that during the winter months of darkness the unusual planktonic community could survive by fixation of carbon at the expense of an organic energy source. In an attempt to show heterotrophic activity 5 μCi of ^{14}C-acetate and 2.5 μCi of ^{14}C-glucose were added to paired 125 ml light and dark bottles. The glucose concentration was 60 mg l^{-1} and incubation was *in situ*. There was no measurable uptake of acetate recorded. Glucose uptake at 10 m was significant although uptake in the light bottle was higher. Uptake at 3 m was the same as the controls. The bacterial population was assumed to be the agent of the uptake.

The possibility that algae in lake Miers assimilated dissolved organic substances heterotrophically when they could not receive enough light to carry on photosynthesis during the Antarctic winter was suggested by Bell (1967) according to Goldman (1970).

A review of the literature on phytoplankton heterotrophy and its role in aquatic systems has shown that the available data are scarce. Since Rodhe first suggested algal heterotrophy below the ice in subarctic lakes several other authors have used this as a possible explanation for the survival of phytoplankton populations. The few experimental attempts, with the exception of Kalff's (1967b) work, to prove this theory have not produced a positive answer but only shown that phytoplankton heterotrophy may be possible. Pechlaner (1971) concluded, from his study of the factors that control the production rate and biomass of phytoplankton in high-mountain lakes, that the hypothesis of a general heterotrophic growth of phytoplankton during

the winter in these lakes was unnecessary. This conclusion was based on the following points: first, the algae living under ice adapt over a long period of steadily diminishing light conditions with the result that an extremely "shade-type" phytoplankton is present at the end of the period of winter cover, and, on the whole, algae find sufficient radiation for a measurable amount of primary production; second, the substrate concentration is not great enough for osmotrophic heterotrophy of pelagic algae since aerobic heterotrophic bacteria compete successfully for dissolved organics. During the discussion following the presentation of this paper, Rodhe was quoted as saying "I no longer believe in heterotrophy", i.e., algal heterotrophy.

Recent Studies of Phytoplankton Heterotrophy

The theory of heterotrophic phytoplankton in natural systems, however, has not yet been satisfactorily disproved. If there are heterotrophic algae then it needs to be known: (1) how important this process is in an aquatic ecosystem where there is competition from an efficient bacterial transport system and, (2) whether the algae possess an active transport system similar to the bacteria. In an attempt to answer these questions Allen (1971b) studied dissolved organic carbon utilization in size-fractionated algal and bacterial communities.

Allen collected water samples mainly from lake Lotsjon, Sweden while a single phytoplankton sample was taken from Star lake, Vermont, U.S.A. Uptake by diffusion (K_d) and the velocity of active uptake (V_t) by the various microbial components was measured in the lake Lotsjon samples and also in the Star lake samples using ^{14}C-acetate and glucose.

The reduction in algal biomass after filtering the Lotsjon samples through a 20 μm and 58 μm net, corresponded closely to the decrease in the K_d value. Active uptake at low concentrations was only slightly affected by the 58 μm net but was reduced significantly by the 20 μm filter.

The Star lake sample was filtered through 9 membrane filters ranging in porosity from 14.0 μm to 0.22 μm. Organisms ranging in size between 3 and 8 μm were indicated to Allen as being responsible for the majority of the active uptake. Bacterial sized organisms (< 1.2 μm) he believed were responsible for only a minor role in the uptake. Diffusion uptake was considered to be very low and could not be detected in samples which had been filtered through 5.0 μm filters. The planktonic population of the Star lake sample consisted mainly of small flagellates with mean dimensions between 4 and 8 μm and interestingly, few bacteria.

Allen concluded that nannoplanktonic algae with surface/volume ratios similar to those of bacteria may be competitive for organic substrates by utilizing active transport mechanisms. This conclusion supported the hypothesis expressed by Wright and Hobbie (1966).

A recent publication by Monheimer (1972) presented evidence for phytoplankton heterotrophy in an eutrophic, a mesotrophic and an oligotrophic lake. The basic technique involved adding 2 ml of a heterogeneous mixture of dissolved organic compounds labelled with sulphur-35 to 300 ml light and dark bottles. This mixture was prepared in the laboratory by adding $H_2{}^{35}SO_4$ to a 1 litre quantity of eutrophic pond water, incubating it in the light at 25°C for 65 hours and then extracting the labelled organic compounds. At intervals of 1, 2, 4 and 6 hours after inoculation the contents of 4 transparent and 4 opaque bottles were filtered through 0.45 μm membrane filters and the radioactivity counted.

Monheimer interpreted his results by comparing the uptake of the organic compounds to the uptake of inorganic sulphate which was monitored at the same time. The results showed in all three lakes that organic isotope was taken up faster by plankton in the dark and that inorganic isotope uptake was higher by plankton incubated in the light. The greater uptake of inorganic isotope in the light is explained by photosynthesis. Light, however, is not known to affect

bacterial heterotrophy, therefore, Monheimer concluded, if the uptake of organic compounds is by bacteria only, the rates of uptake would be similar in both light and dark incubation conditions. The more rapid uptake of the organic compounds in the dark was explained by the fact that some algae are capable of heterotrophic activities in the dark.

Monheimer pointed out that certain minimum levels of specific organic compounds are required to induce specific microbial active-transport mechanisms into operation. Natural levels of previously used labelled compounds (e.g. glucose and acetate) may not have been sufficient to induce naturally the operation of algal transport mechanisms and therefore, there was no uptake. This point, however, does not seem to be applicable in studies such as reported by Goldman et al (1967) where 2.5 μCi of glucose (60 mg l^{-1}) and 5 μCi of acetate were added to incubation bottles.

Two conclusions were drawn by Monheimer: (1) dark heterotrophic processes are important in the total uptake of inorganic materials by plankton and (2) phytoplankton may conduct up to 50% of the heterotrophic activity that occurs in the limnetic zone of a lake during the dark. Therefore, it seems to Monheimer, that heterotrophy is an important part of the metabolic activities of plankton, especially in oligotrophic systems and more generally, is likely to be an important process in the overall metabolic activities of aquatic ecosystems as a whole.

Two points in criticism can be raised against this work: (1) the total concentration of the organic compounds and the concentration of the various component compounds added to the incubation bottles, were not stated and, (2) depending on these concentrations the algal uptake could have been due either to a diffusion or an active transport system. Since the author would assume a transport system was involved an attempt should have been made to show that this was in fact the case.

Although the work of Allen (1971b) suggested strongly that nannoplanktonic algae are capable of competing successfully with

aquatic bacteria for dissolved organic compounds further studies will be needed before the hypothesis can be accepted. Allen suggested that a more thorough study with independent checks such as micro-autoradiography and organic uptake in axenic cultures was needed. He noted that phytoplankton heterotrophy under varied natural conditions would be difficult to demonstrate. Most algae are probably capable of easily switching from autotrophy to heterotrophy, with a combination of these two types of metabolism often being simultaneously functional.

Competition for Inorganic Nutrients and Phytoplankton Succession

Competition among aquatic microbial organisms has also been noted for inorganic nutrients. A report of this type of activity was given by C.B. van Niel (1955) who referred to work done by H.A. Barker in 1935 on the succession of phytoplanktonic components in marine bays. Barker noted a regular pattern which consisted of the following stages: relative paucity of phytoplankton, fertilization, diatom peak followed by a dinoflagellate peak. This pattern was indicative of a modification of the environment by the diatoms for effective competitive growth by the dinoflagellates. A major factor influencing this succession was thought to be the depletion of nutrients by the diatoms. The results of experiments by Barker with pure cultures indicated that the diatoms were capable of a more rapid growth as long as the nitrogen and phosphorous concentrations exceeded a "certain value". Below these values dinoflagellate growth could exceed diatom development. In fact, these organisms grow exceptionally well in nutrient concentrations some hundred times lower than concentrations needed for optimal diatom reproduction.

Margalef (1960) working in Vigo Bay, Spain also noted a similar phytoplankton succession. Small celled diatoms were followed by a mixed community of diatoms with large cell size and dinoflagellates which, in the third stage of succession, dominated the algal population. In agreement with Barker, Margalef decided several factors were influencing this succession. The most obvious factor was nutrient

depletion which favored the growth of species adjusted to low concentrations and a slower multiplication rate. Similar types of succession involving different groups of phytoplankton have been noted in various freshwater ecosystems (Fogg 1965).

Phytoplankton studies of Long Island Sound have shown that dinoflagellates achieve brief dominance over the diatom population during the summer (Riley 1967). According to Riley, some texts said that this succession is due to the facts that dinoflagellates prefer high temperatures and have fairly low nitrogen requirements. He did not agree. Of the four species of dinoflagellates noted in Riley's study three exhibited wide temperature tolerances and one became a major constituent when the temperature was little above the late winter minimum. Further, the dinoflagellate peak months were June and July and then declined although, in August, the temperature was still rising. Riley believed the nitrogen effect was even more ambiguous. He noted that following a severe post-flowering depletion of nitrogen, there appeared to be a series of small resurgences of ammonia in late spring and early summer, each of which led to phytoplankton growth. In concluding, he said that species succession might well hinge upon such subtle interrelations as the advantage of the dinoflagellates to maintain a relatively stable position in the water column when stability of the water increases the problems of diatom flotation. Also, he noted, we are still very far from a firm understanding of the dynamics of seasonal succession.

Another reference to this type of succession involving inorganic nutrients comes from Martin (1970). He noted that blooms of Gymnodinium breve frequently occur in nutrient depleted waters following diatom blooms. Martin believed the silicate concentration might have an indirect effect since a negative correlation has been found between cell numbers and silicate concentration. Also, Odum (1971) noted that dinoflagellates often follow diatoms in seasonal succession in temperate waters including lakes and the ocean.

The Importance of Bacteria and Phytoplankton in Zooplankton Feeding

Having examined some of the interrelations that exist between aquatic microbial populations it is necessary to briefly place these organisms into their relationship with the consumer organisms or the zooplankton. A great deal of work has been done in this regard by Nauwerck (1963) and Gliwicz (1969a and b). According to Gliwicz the importance of the net and nannoplankton, bacteria and detritus in zooplankton feeding is related to the productivity of the ecosystem. In eutrophic lakes high primary production is due mainly to net phytoplankton and because of the size of these organisms they are not available as food for zooplankton. Organic matter produced from these organisms is available to zooplankton only after it has been partially decomposed by bacteria (Nauwerck 1963; Gliwicz 1969a and b). The type of food available to the zooplankton is then the bacteria. Also influencing this selection is the fact that bacterial production is considerably greater in eutrophic lakes than in oligotrophic lakes (Gliwicz 1969b).

In contrast to this situation is the food availability and selection in oligotrophic ecosystems. The more severe or oligotrophic the environment, the greater will be the proportion of nannoplankton to net phytoplankton (Pavoni 1963). In some lakes it will be the sole phytoplankton type. In these lakes, Gliwicz reported, the macro-filtrators are the dominant zooplankton forms. Since bacterial production is lower here than in a more eutrophic situation the food selectivity is directed towards the nannoplankton. Further, the bacterial population is more important as a food source than detritus.

Kajak (1970) has summarized the work of Gliwicz by presenting the percentages of each of the major components consumed. Thus in an eutrophic lake bacteria dominate as 74% of the total food consumed followed by 14% for detritus and 12% for nannoplankton. In the oligotrophic ecosystem nannoplankton dominate as 44% of the food filtered followed by the bacteria which represent 37.5% and detritus

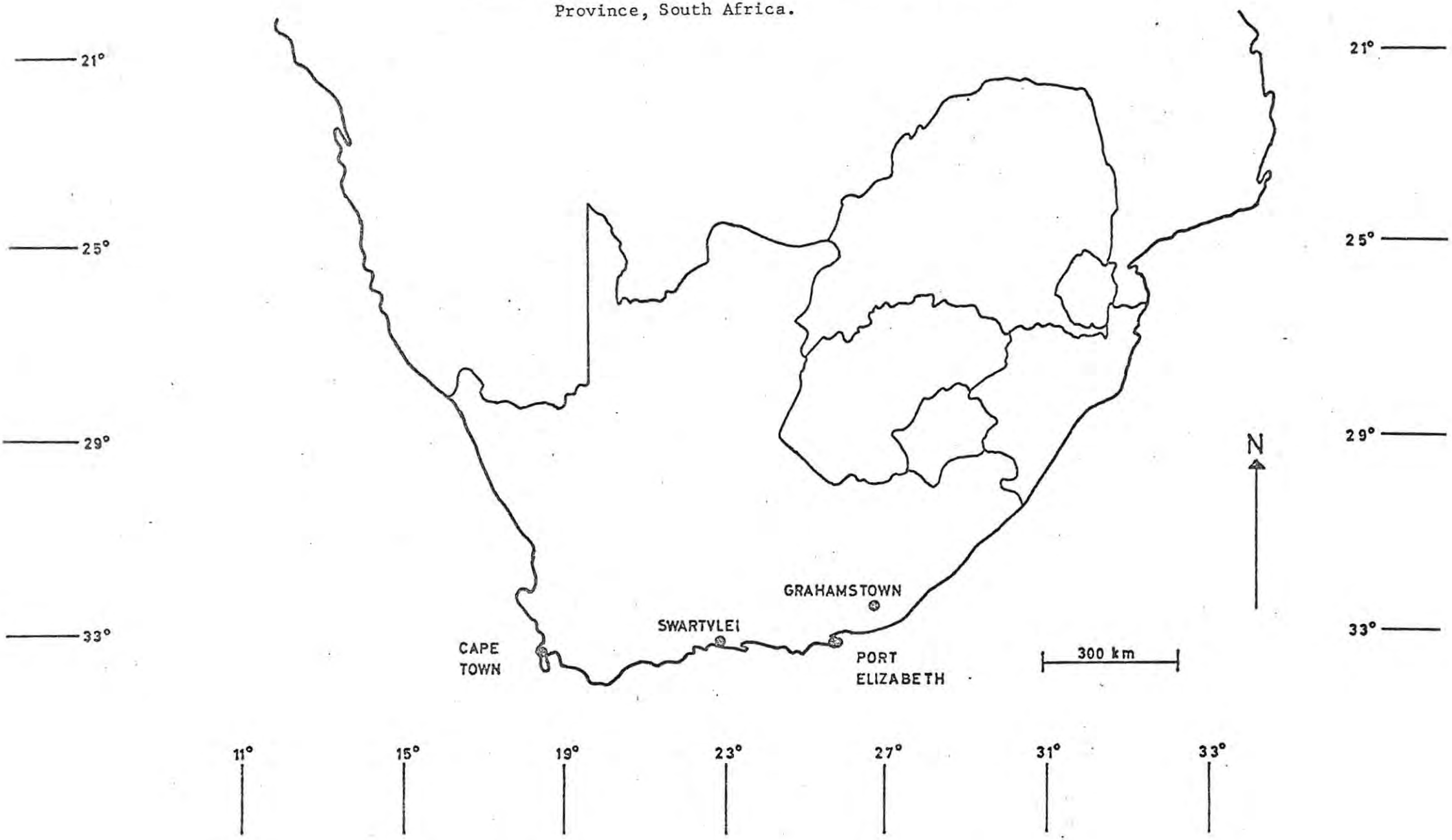
as 18.5%.

Other studies have produced similar conclusions as those of Gliwicz. Straskraba (1966) found that the zooplankton standing crop was in no way directly proportional to the primary productivity of the phytoplankton in two reservoirs. However, he did find that phytoplankton species composition proved to be one of the important factors that influenced the ratio between zooplankton and phytoplankton. In the stagnant lower protion of the water column, and probably in the column as a whole, little effect was noted with an increase of allochthonous matter.

In two Danish lakes studied by Kristiansen (1971) the nannoplankton were considered to be a direct food source for the zooplankton. In support of this idea, Kristiansen noted Nauwerck's (1963) study which concluded that netplankton organisms, because of their size, cannot be ingested by the zooplankton in lakes. As a result, zooplankton feed partly on bacteria living on organic material from the decaying larger algae and partly on nannoplankton. Consequently, zooplankton are of no importance as a limiting factor for the netplankton populations. As Kristiansen pointed out, however, zooplankton do feed on nannoplankton and therefore must be an important factor in determining the nannoplankton population size.

As the literature review has demonstrated, our knowledge of the relationship between bacteria and phytoplankton, phytoplankton succession and phytoplankton heterotrophy is still incomplete. It is hoped that the present study, which encompasses a wide range of physico-chemical and biological factors, will contribute to the understanding of these phenomena.

Fig. 1: Map showing location of Swartvlei in the Cape Province, South Africa.



DESCRIPTION OF THE STUDY AREA

Swartvlei lies approximately midway between Port Elizabeth and Cape Town in the Cape Province of South Africa at latitude 34° S and longitude 22° 46' E (Fig. 1). It is one of the Knysna-Wilderness lakes which occupy east-west valleys parallel to the coast. The lakes were formed during the Pleistocene when this area was inundated by the sea. The exact method of formation is still being disputed by geologists. The theories include such processes as the flooding of interdune depressions and the formation of coastal lagoons behind offshore bars (Martin 1962). According to Martin's outline of the genesis of these lakes there were several periods of low sea level after the initial development when the lakes were probably dry. The Recent lakes were formed from the reflooding and possibly the deepening of their existing depressions. Hutchinson (1957) refers to similar formations as maritime coastal lakes.

If the freshwater inflow into a coastal lake was small the channel which connected the lake to the sea would become permanently closed due to the action of long shore currents and rapidly disappear under sand dunes. The lake would then become land locked. An example is lake Sibaya in Northern Zululand (Hill 1969). If, however, the inflow of freshwater was great enough the lake would remain open due to outflowing water eroding the bar (Hill 1969). This would not prevent the sea channel, during periods of low river discharge, from being closed for a period of time during the year. This is the case with Swartvlei which is usually closed from about April to July.

The question arises as to whether or not "lake" is the correct description of such a water body as Swartvlei. Since this type of water body is open to the sea it is reasonable to expect that salt water will enter the system during high tides. Swartvlei in this respect may be considered an estuary. According to Day (1951) Swartvlei would be an estuary since it represents "...that part of

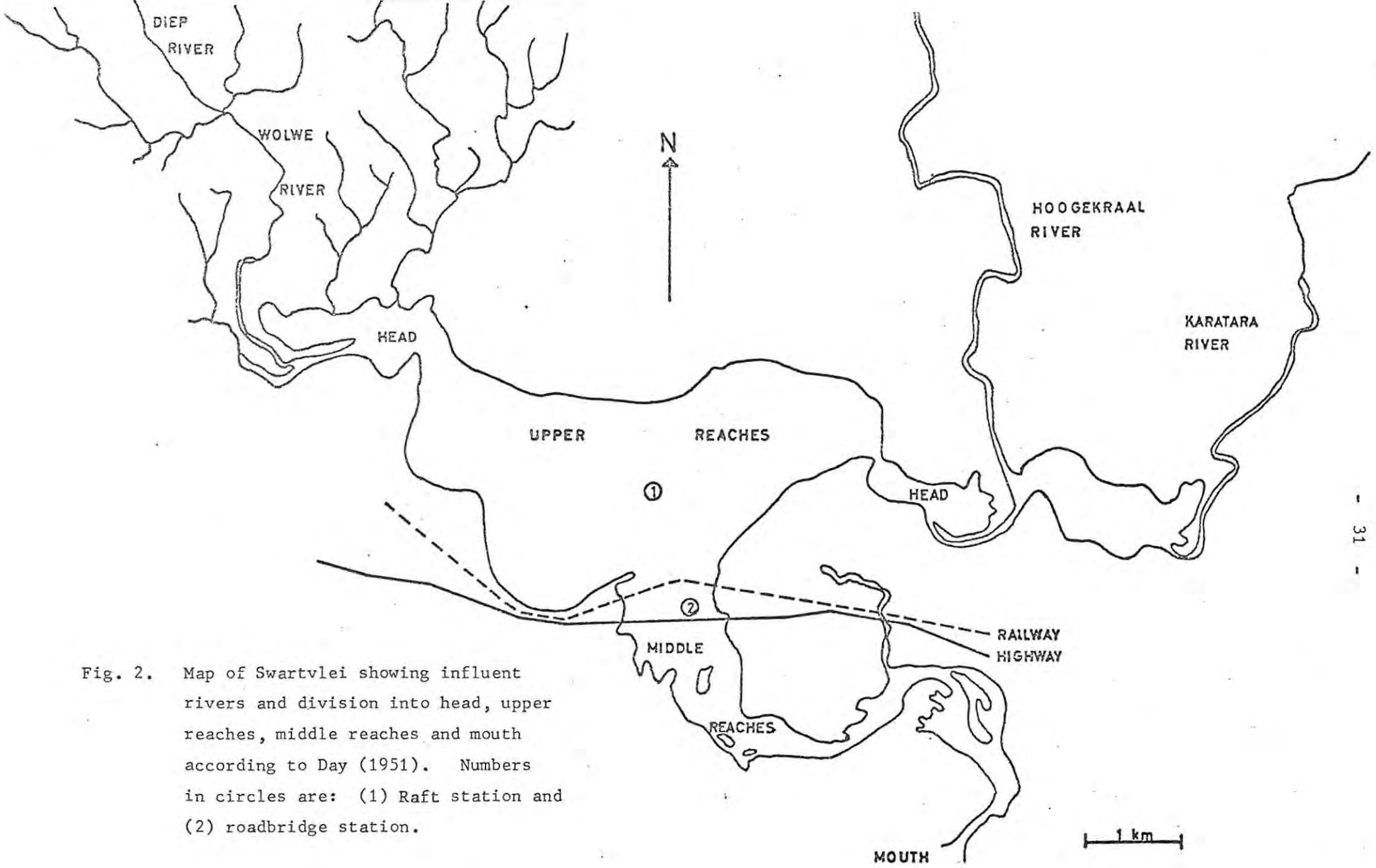


Fig. 2. Map of Swartvlei showing influent rivers and division into head, upper reaches, middle reaches and mouth according to Day (1951). Numbers in circles are: (1) Raft station and (2) roadbridge station.

Fig. 2a: An aerial photograph of the upper reaches of Swartvlei taken by Professor B.R. Allanson, April 1973. Centre, upper left, the western head region can be seen. The white bands in the upper reaches mark the edge of the weed beds. The rail and road bridges, centre right, cross the tidal salt marsh area of the middle reaches.

a river system where there is an appreciable variation of salinity due to the sea". More specifically, Day would classify Swartvlei as a blind estuary, i.e., an estuary which is usually open to the sea but at times is closed by a sand bar. Day's plan of an "ideal" estuary divides an estuary into four regions: (1) head, (2) upper reaches, (3) middle reaches and (4) mouth. Salinities in these regions are <5 °/oo, 5-15 °/oo, 15-25 °/oo and >25 °/oo respectively. Following Day's plan, Swartvlei can be divided, on the basis of salinity, into four major regions: (1) the head, the area where the rivers widen and enter (2) the upper reaches or the main basin, (3) the middle reaches, extending approximately from the railbridge to (4) the mouth, where the estuary enters the sea (Fig. 2). Day points out that his salinity limits are not definite since variations in salinity will occur due to a number of environmental factors such as the variation in the proportion of freshwater inflow to sea water inflow.

Swartvlei presents a rather special ecosystem. As will be seen later both its morphometry and physico-chemical characteristics are lake-like. At the same time, being open to the sea for most of the year, it possesses estuarine characteristics. Since both "lake" and "maritime coastal lake" imply that the system is closed off from the sea they are misleading. For this reason "estuary" more closely describes the physical state of Swartvlei and is used in the following text. Although this situation is not unusual for South African estuaries it differs from most European (Scott et al 1952) and North American estuaries which have been studied in that northern hemisphere estuaries usually remain open to the sea throughout the year.

The upper reaches of Swartvlei have a surface area of 1,085 hect. and a catchment area of 40,240 hect. (Martin 1962). From July or August to March or early April Swartvlei is open to the sea via a shallow channel which is approximately 6.5 km long from the railbridge to the mouth (Fig. 2). Three perennial streams, the Diep-Wolwe,

Fig. 3: Bathymetric map of the upper reaches of Swartvlei (after Hill, 1971). Contours are 3 m.

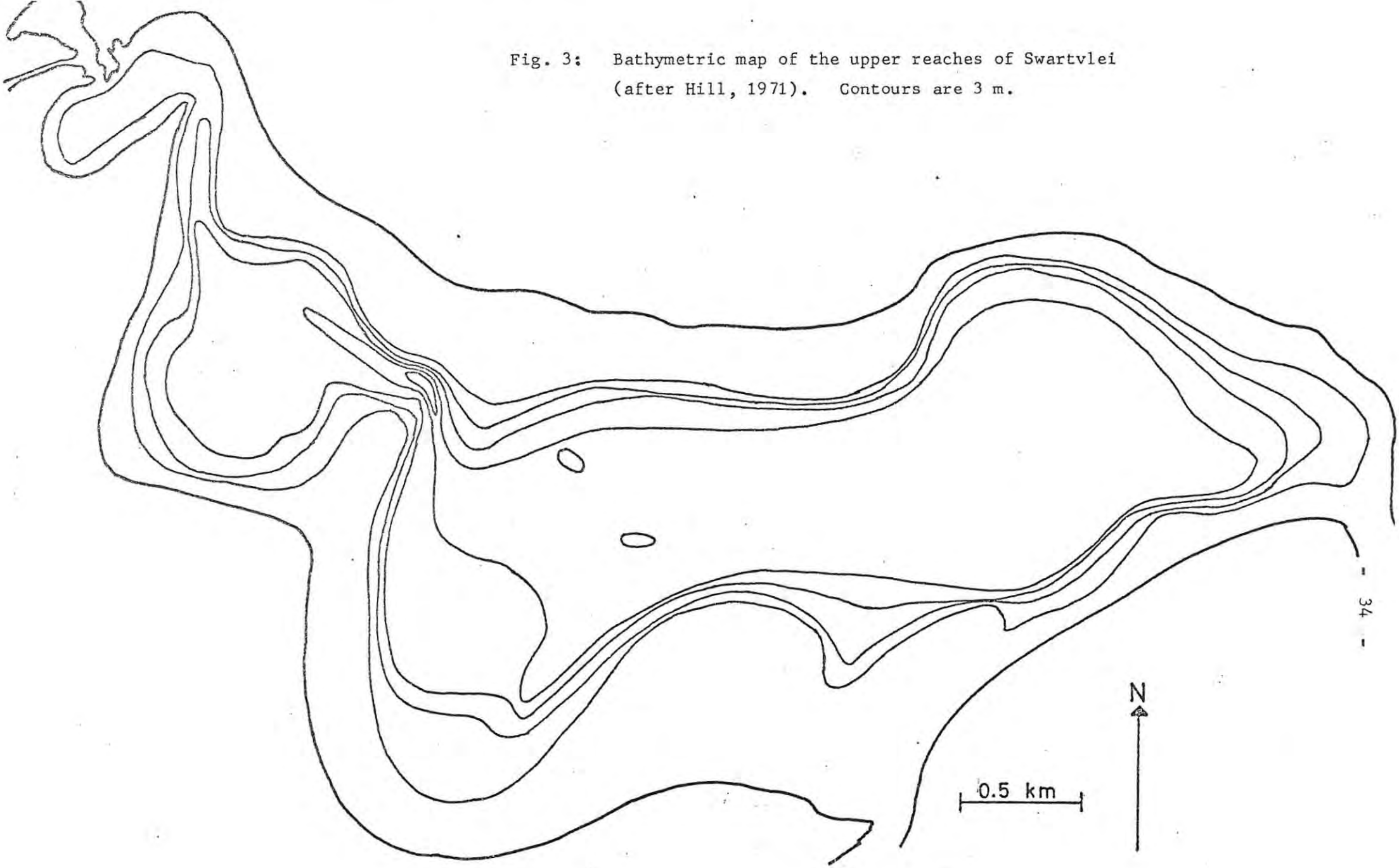
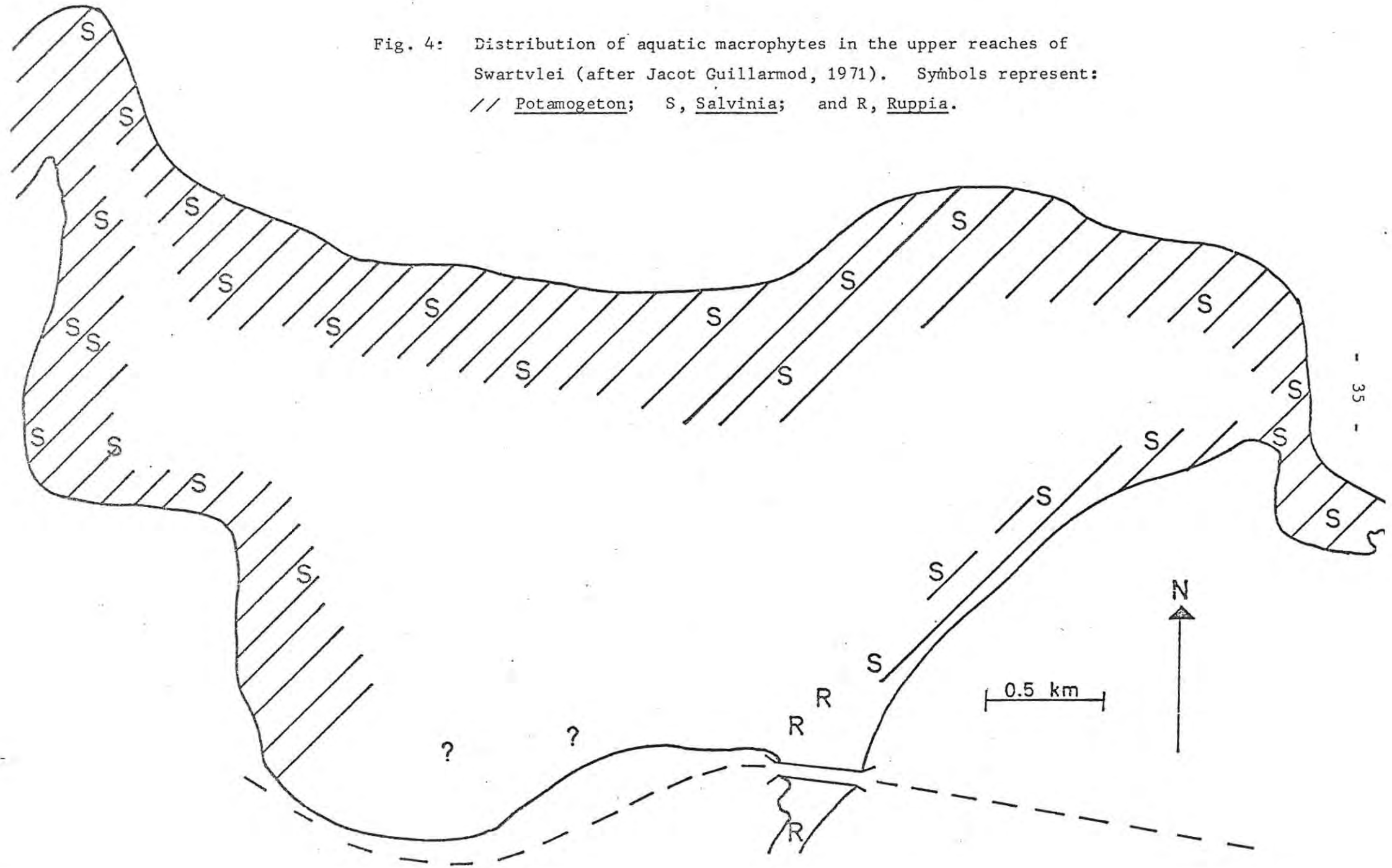


Fig. 4: Distribution of aquatic macrophytes in the upper reaches of Swartvlei (after Jacot Guillarmod, 1971). Symbols represent: // Potamogeton; S, Salvinia; and R, Ruppia.



Hoogekraal and Karatara rivers, flow into the upper reaches. These rivers originate in Table Mountain sandstone. They are characterized by an acidic pH, an unbuffered nature, dissolved solids which are mainly chlorides (Harrison and Agnew 1962) and a dark colour which is due to humates. The humate staining is common to the rivers of this area.

The results of a bathymetric survey of the upper reaches of Swartvlei by Hill (1971) are shown in Fig. 3. Shallow water of 3 m or less extends for a considerable distance from the shore. The upper reaches, in most places, drops rapidly from this 3 m terrace to the main basin. The 3 m terrace and slope to the main basin are mainly sand while the basin itself is soft mud. According to Hill this level plain may represent a silted-in area.

Rainfall in this area tends to be higher during the spring months from September to November and there is no dry season (Martin 1960). According to Martin, rainfall is not usually irregular or seasonal. However, occasional erratic dry spells and periods of torrential rains may occur and this type of climatological behaviour was experienced during this study. As a result of such torrential rains, Swartvlei can be subject to large inflows of humate stained freshwater containing large amounts of particulate matter which lowers the salinity and pH, raises the lake level and increases the normal humate concentration of the upper reaches. The interaction of freshwater and sea water inflow may produce a complex situation according to the season (Scott et al 1952).

The littoral is occupied by aquatic macrophytes which extend in wide bands to the edge of the 3 m terrace (Hill 1971) and border the incoming rivers at their entrances to the upper reaches. These macrophytes are predominantly Potamogeton pectinatus L. with lesser amounts of Salvinia hastata Desv. (Jacot Guillarmod 1971). In contrast to this is the predominance of Ruppia spiralis L. ex Dum. in the tidal salt marsh area of the middle reaches of the estuary (Fig. 4 and 2a) (Jacot Guillarmod 1971).

MATERIALS AND METHODS

General

Samples were taken from the surface and at 1 m intervals to the bottom from a permanently moored raft in the upper reaches of Swartvlei. From the road bridge which passes over the middle reaches samples for chemical analysis only were taken at a depth of 2.5 m (Fig. 2). This gave 13 substations at the raft and 1 station at the road bridge. One station in the upper reaches was considered sufficient as representative of the pelagic zone because of the small surface area and the uniformity of the bottom. As a result of these features the horizontal and vertical distribution of dissolved oxygen, pH, temperature and salinity was uniform throughout the pelagic zone as shown by the data of Allanson et al (1971). Due to the quantity of samples and the resulting data that had to be processed, as well as the preparation of sterile equipment and solutions for the next field trip, the period between field trips was normally 3 weeks. The next sampling period commenced in the fourth week, usually the middle week of each month.

Since it was not possible to complete a sampling program in one day it was necessary to divide it into two sections. All variables, however, were samples within 24 hours of one another in order to minimize the possibility of large changes occurring before the sampling program could be completed. Each section of a field trip involved the following: (1) oxygen, pH, salinity and temperature measurements; the collection of samples for the measurement of the uptake of acetate and glucose as well as for bacteria, phytoplankton and zooplankton enumeration, and (2) samples for primary productivity measurements and for analysis of dissolved carbohydrate, ammonia, nitrate, phosphate and alkalinity. Light transmission through the water column and transparency were also measured. Each month the samples were collected at the same time of day ($\pm$ 20 min).

All glassware, bottles, flasks, pipettes, etc. were washed in a strong HCl acid bath, rinsed with distilled or double distilled water

and autoclaved prior to the next field trip.

Samples labelled with ^{14}C were counted in a Beckman LS 133 liquid scintillation counter using a dioxane based cocktail containing 5 g l^{-1} PPO and 100 g l^{-1} napthalene as primary fluors. 15 ml of this solution were used in each scintillation vial. All counts (cpm) were corrected for the counting efficiency of the machine (94.8%) and for quench using the combined channels external standard method. Samples were counted for 10 minute periods over at least 72 hours to ensure that the data were not affected by chemiluminescence (Kearns 1972). The average of the last three 10 minute counts of each sample was used in the calculations.

Physico-Chemical Limnology

Water for oxygen, pH and salinity determinations was collected in a 1 litre Ruttner bottle. Oxygen was determined using the unmodified Winkler technique. From the 1 litre sample 600 ml were allowed to flow slowly into a 300 ml glass reagent bottle. Fixation took place immediately after collection. Oxygen concentration was determined the next day by titration with 0.025 N sodium thiosulfate (APHA 1965). An aliquot from the remaining portion of this 1 litre sample was used for the determination of pH on board ship with either a Beckman Electromate (Model 1009) or Phillip Harris Ltd (Birmingham, England) portable pH meter. The remainder of the sample was placed in the glass salinity bottle manufactured by the Laboratoire Oceanographique, Denmark. Salinity was determined at the Institute for Freshwater Studies, Rhodes University by means of a calibrated chloride titrator (American Instrument Co., Inc.).

Temperature was recorded with a termistor circuit (Tele-Thermometer, Yellow Springs Instrument Co., Ohio, Model 43TD).

Water samples for the determination of carbohydrate, nitrogen and phosphorous were obtained with the Ruttner bottle from 1, 2, 3, and 8 m at the raft station and from 2.5 m at the middle reaches station. Samples were placed in glass reagent bottles which had been thoroughly

rinsed with double distilled water. It was not possible to complete the analysis at the field station. The samples were brought back the next day to the Institute for Freshwater Studies, a one way trip of 300 miles. Therefore, preservation was essential. The preservatives were: carbohydrate, 2 ml 20 N H_2SO_4 per 500 ml sample; nitrogen, 5 ml 4 N H_2SO_4 per 1000 ml sample and phosphorous 5 ml $CHCl_3$ per 1000 ml sample (Golterman 1969). In the Institute the water samples were filtered through Oxoid sterilizing membrane filters (pore size approx. 0.45 µm) and stored in a refrigerator until the analysis could be done.

Dissolved carbohydrate was determined using anthrone and soluble phosphate phosphorous (PO_4-P) with molybdate-antimony as described by Golterman (1969). Ammonia nitrogen (NH_3-N) was estimated with alkaline thymol, sodium hypobromite, hexanol, and iso-propanol (Mackereth 1963). Nitrate nitrogen (NO_3-N) was measured with sodium salicylate as described by Muller and Widemann (1955) (SACSIR 1969). The spectrophotometer used in this work was a Beckman Model DB.

Samples for alkalinity were placed in 300 ml borosilicate reagent bottles and titrated at the field station within an hour of sampling. Methyl orange was used as the indicator (APHA 1965) and the end point was recognized by comparison to previously prepared buffered solutions.

The level of light extinction was determined by a light meter consisting of a light dependent resistor coupled to a milli-ampmeter which was designed and calibrated by R.E. Boltt (1969). Transparency was measured by a Secchi disc.

Biological Limnology

Uptake of acetate and glucose-kinetic aspects

A series of publications by R.T. Wright and J.E. Hobbie culminated in a paper describing a new method for the study of bacteria in lakes (Hobbie and Wright 1968). Parsons and Strickland (1962) demonstrated that the uptake of organic compounds in the sea could be analysed following

Michaelis-Menten enzyme kinetics. Wright and Hobbie (1965a; 1966, etc) developed this early work and were able to show that there were two different mechanisms of organic substrate removal operating in an aquatic ecosystem. Bacterial uptake operated at low substrate concentrations while algal uptake dominated when the substrate concentration was greater than 0.5 mg l^{-1} (see page 12). Thus, by adding labelled organic substrate to water samples over a range of concentrations at low levels it is possible to analyse the uptake of dissolved organic compounds by bacterial populations. The samples are filtered and the radioactivity counted. The counts obtained are entered into the Lineweaver-Burk modification of the Michaelis-Menten equation for substrate removal (Wright and Hobbie 1965a; 1966):

$$\frac{C\mu t}{c} = \frac{(K+S)}{V} + \frac{A}{V}$$

where C is the cpm of 1 μCi of ^{14}C- labelled substrate, c the cpm of the filter, μ the number of microcuries added, K a transport constant similar to the Michaelis-Menten constant, S the natural substrate concentration (mg l^{-1}), A the mg l^{-1} of substrate added and V the theoretical maximum velocity of uptake attained when all uptake sites are saturated with substrate. After Hamilton and Austin (1967) described the effect of not measuring the loss of substrate taken up due to the production of CO_2 the method was modified (Hobbie and Crawford 1969a). Now c equals the total of the cpm of the filter plus the cpm of the chemically treated filter paper which collects $^{14}CO_2$.

By plotting Cμt/c against A (Fig. 5), the intercept on the negative abscissa is equal to (K+S). The sum (K+S) approximates the natural substrate concentration, S, when K is very small. Since K is unknown in these experiments this figure represents the maximum *in situ* concentration of the organic compound used. Microbial bioassays in freshwater have given quantitative estimations of K as 5 μg l^{-1} for glucose (Hobbie and Wright 1965b). The

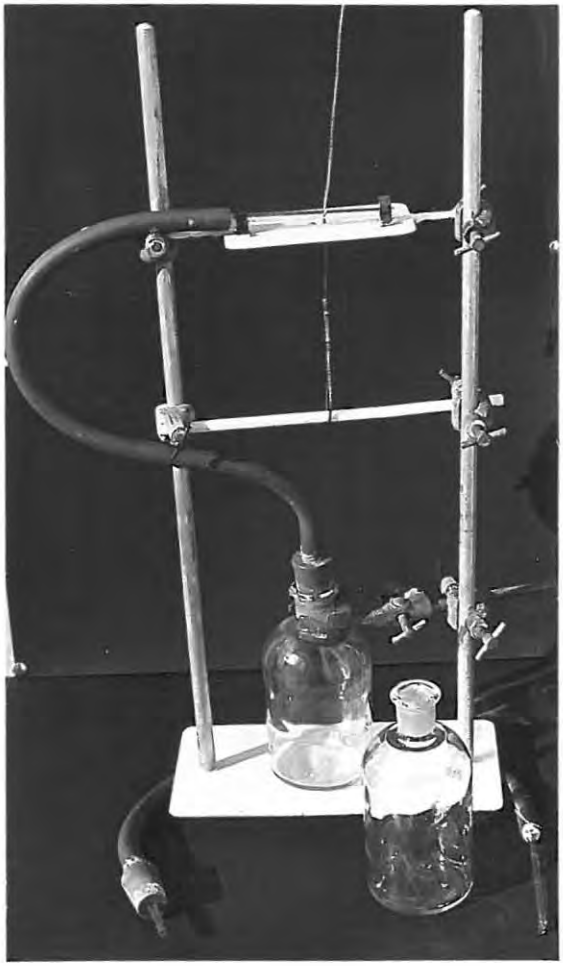


Fig. 6: The 500 ml bacterial sampler and metal harness showing the glass sampling end, which is broken by the messenger, held in position on a metal plate by terry clips.

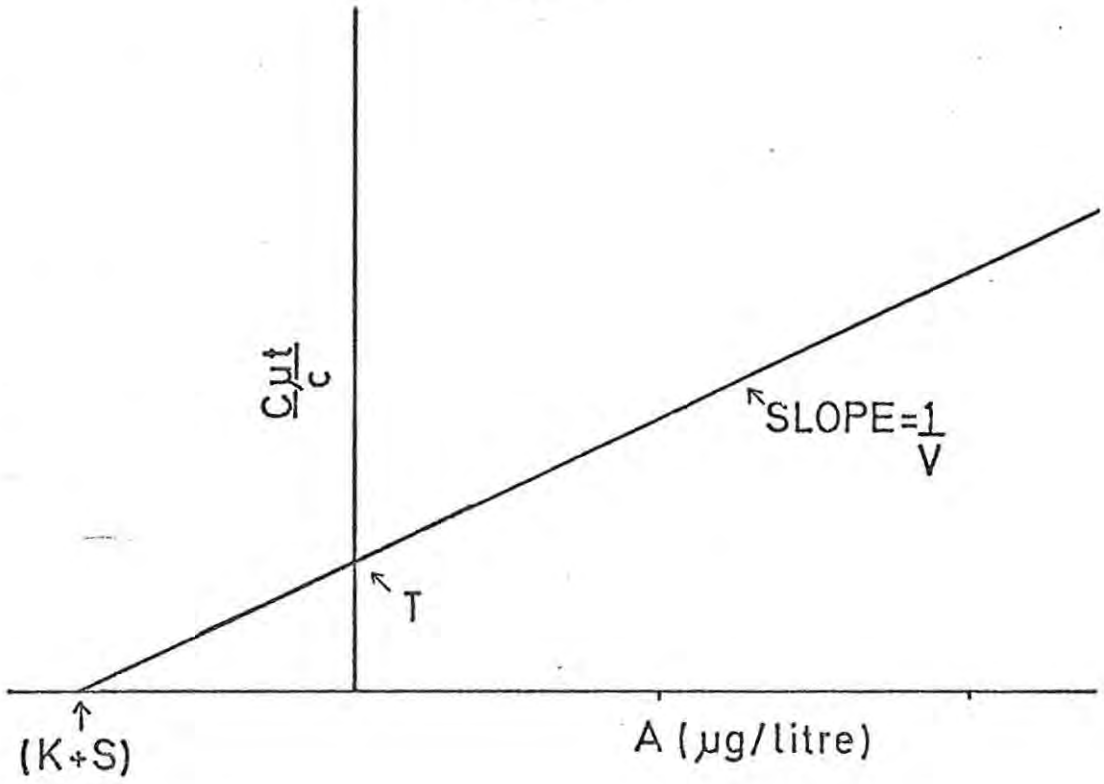


Fig. 5: Theoretical linear plot of data from enzyme mediated uptake studies showing derivation of (K+S), the maximum substrate concentration; T, turnover time and V, the maximum velocity of uptake (after Wright and Hobbie 1965b).

inverse of the slope of this plot is V, the maximum rate of uptake. V gives an estimation of the population size as it is proportional to biomass (Wright and Hobbie 1966; Hobbie and Wright 1968). Further information which can be obtained from the graph is the turnover time, T, which is given by the ordinate intercept. Turnover time represents the time required for complete removal of the natural substrate by the bacterial population (Hobbie and Wright 1968). This technique was applied to the study of heterotrophic uptake in Swartvlei.

A simple sampler was constructed for the purpose of obtaining sterile water samples (Fig. 6). The sampler was autoclaved intact. At the field station, just prior to sampling, a sealing compound was placed in the bottle-stopper joint, the cotton wool was removed from the glass sampling end and the bottle was evacuated with an electric pump. The rubber hose was closed with a G-clamp and the

Fig. 7: The 250 ml incubation flasks used in the uptake of acetate and glucose experiments, showing the folded filter paper for the collection of $^{14}CO_2$, the filter holder and the glass tubing in the rubber bung for the injection of acid through the bottle teat.

glass tube was sealed with a Bunsen burner.

Sampling bottles were lowered to their appropriate depths in the metal frame shown in Fig. 6. A sample was taken by lowering a concave lead messenger. When the glass tube broke the pressure tubing fell away from the frame as the terry clip nearest the rubber hose had a portion of one side removed. This permitted a sample to be taken without contamination from the sampling frame. The sampler was then brought on board where the rubber plug and tubing were replaced by a ground glass stopper. Samples were stored in a shaded area and subdivided within ½ hour of sampling.

The 250 ml incubation flasks, shown in Fig. 7, were indented 33 mm from the top to prevent the large rubber bungs from being forced into the flasks when lowered in the water. The $^{14}CO_2$ trap shown was a modification of the apparatus described by Hobbie and Crawford (1969a and b) and was made large enough to hold an accordian folded piece of 30 x 60 mm Whatman #1 filter paper and 1 ml of 2-phenylethylamine. An open ended glass tube was used for the addition of sterilizing solution at the end of the experiment. Since septum stoppers which would fit over the top of the flasks were not available, rubber baby bottle teats (Nuk, Germany) were used instead. The hole in the nipple was filled with an adhesive sealing compound.

The water samples were subdivided into 50 ml aliquots and dispensed into five incubation flasks with sterile 50 ml volumetric pipettes. Serially increased amounts of uniformly labelled ^{14}C glucose or acetate (Radiochemical Centre, Amersham, England) were added with microsyringes (25, 50, 100, 200 and 100 µl of a 1 µCi ml^{-1} sterile solution). The last flask (100 µl) was a sterilized control which had 2 ml 2 N H_2SO_4 added to it immediately after the addition of labelled substrate. The actual concentration of glucose added normally ranged from 29.64 to 237.12 µg l^{-1} (low specific activity) but sometimes from 2.045 to 16.36 µg l^{-1} (high specific

activity) while the acetate concentration was 0.73 to 5.84 $\mu g\ l^{-1}$ (high specific activity). The time was noted and a rubber teat was stretched over the top of each flask.

The flasks were incubated in situ at their respective depths for 3 hours in the dark using the following procedure. Each flask was placed in 18 x 32 mm light tight canvas bags containing approximately 100 ml of lake water taken from the depth at which the samples were obtained. This ensured the samples would be incubated at ambient temperature. The bags were then folded over twice at the top and placed in rectangular 448 x 223 x 90 mm galvanized tin containers which were subdivided into five compartments and had a securing hinged lid. Enough holes were inserted in the containers so that there was a free flow of water through them. The containers were attached to nylon rope and lowered on the winch to their appropriate depths. The rope was secured to the boat.

To terminate the incubation 2 ml of 2 N H_2SO_4 was added to each flask through the rubber teat with a 2 ml syringe (Hobbie and Crawford 1969 a and b). This killed the sample and drove off the $^{14}CO_2$. Labelled carbon dioxide was collected by adding 1 ml of 2-phenylethylamine to the filter paper in the cup. This was also done with a syringe through the rubber septum. The system thus remained closed. The flasks were taken back to the field station and allowed to stand for at least another hour while the $^{14}CO_2$ was absorbed. At the end of this time the filter paper and phenylethylamine were placed in a scintillation vial containing cocktail for counting. The water was filtered through an Oxoid membrane filter (pore size approx. 0.45 μm). In order to reduce the possibility of rupturing cells, vacuum did not exceed 0.5 atm. (Wetzel 1965b). The filter was washed with distilled water and in order to prevent loss of label associated with drying, was placed wet in another scintillation vial for counting (Ward and Nakanishi 1971).

Relative heterotrophic capability

Due to difficulties with the above method which will be discussed later, a measure of relative heterotrophic uptake (see page 127) was obtained by the addition of a single concentration of labelled substrate at each depth station. A similar technique has been used by Parsons and Strickland (1962) and Goldman et al (1967) and is similar to the measurement of primary productivity.

An estimate of the uptake of glucose and acetate as mg C m^{-3} h^{-1} was obtained from the formula of Parsons and Strickland (1962):

$$\text{mg C m}^{-3}\ \text{h}^{-1} = \frac{\text{c.f. (S+A)}}{\text{C } \mu\text{t}}$$

where μ is the number of microcuries added, C equals 2.22×10^6 or the disintegrations per minute equal to one microcurie, c is the corrected (counting efficiency and quench) counts obtained from the filter, f equals 1.06 and is a constant isotope discrimination factor for ^{14}C, t is the number of hours (3) incubated, S is the natural substrate concentration and A is the added substrate concentration as mg C m^{-3}.

The scope and limitations of this measurement will be discussed later (see page 127).

Samples for this measurement were taken as previously described. However, instead of a series of five incubation flasks for each depth station there were three. Subsamples were 50 ml as before. To one flask was added 100 μl of glucose and to another, 100 μl of acetate. The labelled stock solutions contained high specific activity acetate and glucose at a dilution of 1 μCi ml^{-1}. Actual added substrate concentrations of glucose and acetate were 8.2 μg l^{-1} (3.28 μg C l^{-1}) and 2.3 μg l^{-1} (0.920 μg C l^{-1}) respectively. These values changed slightly when new batches of stock solutions were prepared. The third flask was the control which contained either 100 μl of glucose or acetate plus 2 ml of 2 N H_2SO_4, i.e., there was an acetate or glucose control for alternate depth stations. Incubation and termination of uptake was done as described in the preceeding section.

Bacterial enumeration

The remaining portion of the sterile water sample was kept shaded until taken back to the field station within 6 hours of sampling. Using aseptic techniques, 0.1 ml of this sample was pipetted and spread (Jones 1970) onto duplicate agar plates. The agar used was: E. coli minimally enriched agar (10 g Oxoid iron agar #3, 72.5 ml minimal salts solution, 1 g BDH dextrose and 417.5 ml distilled water); nutrient agar (3 g l^{-1} Bacto-beef extract, 5 g l^{-1} Bacto peptone and 15 g l^{-1} Bacto agar); nutrient agar with salt (same ingredients as for nutrient agar but with the addition of 0.85% NaCl) and YPA 25 media (0.2 g l^{-1} Bacto peptone, 0.12 g l^{-1} yeast extract and 12.0 g l^{-1} Oxoid #3 agar) (Fonden 1969).

The techniques for counting bacteria in water consist of a large variety of procedures which can be condensed to two main methods: (1) indirect counts, the culturing of viable bacteria on or in various solidified agar media and (2) direct counts, the counting of stained colonies on membrane filters (Jannasch 1965). There are advantages and disadvantages to both techniques. The former technique records only viable bacteria but is reported to give significantly lower counts than the direct count procedure. This is due to the fact that different culture media select for different bacterial groups. The difference in the numbers obtained with the two techniques varies with the quality of water (Rodina 1967). According to Rodina bacteria cultured on agar plates from unpolluted water represent only 0.0001 of the entire population while from polluted water 0.01 of the population. Goldman et al (1968) reported that the ratio between cultured and direct counts ranged from 1:10 in highly productive lakes to 1:10,000 in extremely oligotrophic lakes. From general experience Jannasch (1965) found that only 1.0 to 10% of the bacteria present in natural waters could be detected by indirect enumeration and that the percentage varied with the type of water investigated. He went on to point out, however, that similar figures were obtained when applying

the direct count technique. Sources of error in this counting procedure are: (1) the difficulty of differentiating bacterial cells from particulate organic matter and (2) the inability to distinguish viable from non-living cells (Jannasch 1965). Jannasch stressed the point that there is no reason to believe that the percentage of inactive cells is constant or usually low.

In the preliminary stages of this study direct counts were attempted on Swartvlei water. It was found that the large amounts of particulate matter washed into the system severely hindered counting and for this reason, the spread plate technique was used. Membrane filter-fluorescent-antibody techniques and luminescent microscopy are currently being used (Silvey and Wyatt 1971) and may overcome this problem in direct counting but unfortunately such equipment was not available during this study.

Phytoplankton

Phytoplankton numbers were also determined for the water sample taken for the substrate removal study and bacterial enumeration. After the subsamples for bacterial counts were removed a preservative was added to the water. The preservative was a modification of the solution described by Johansen (1940) for Volvox and consisted of 8 g potassium iodide, 4 g iodine, 96 ml of 4% formalin, 16 ml of glacial acetic acid and 400 ml of distilled water. Approximately 20 ml of this solution was added to 500 ml of water sample.

For counting, 200 ml of the sample was filtered through an Oxoid membrane filter (pore size 0.45 μm) and washed with 25 ml of distilled water. If samples had been taken shortly after a period of heavy rain it was necessary to filter 100 ml aliquots otherwise many of the organisms were difficult to see in a relatively thick layer of detritus. After being allowed to air dry the filters were cleared with glycerine. They were then placed on wide microscope slides and a minimum of 20 fields chosen at random were counted at 562.5 X.

Calculation of the number of phytoplankton per litre involved the

following equations:

$$\frac{100X\ (\Pi\ r^2)}{y} = a \qquad (1)$$

where X is the number of fields counted, r is the radius of the objective field, y is the area of the stained portion of the Oxoid filter, 100 converts the figures to a percentage and "a" is the percentage of the stained filter area counted;

$$\frac{100}{a} \cdot b \cdot d = c \qquad (2)$$

where $\frac{100}{a}$ gives a multiplication factor to convert b, the number of organisms counted in X fields, to the total number of organisms on the filter or the number of organisms in the filtered subsample, and d, is a variable dependent on the filtered subsample volume, converting this number to c, organisms per litre.

By counting 100 and 200 ml subsamples from the same sample and by counting 20 random fields across different directions on the filter it was found the counts obtained usually varied by less than $\pm$ 10%.

The phytoplankton of Swartvlei had a very small cell size (see page 88-91). For the counting of very small cells Lund *et al* (1958) recommended the use of haemocytometers or other similar counting chambers. However, Erhan *et al* (1969) have shown that these chambers are not acceptable for counting the cell number in very sparse populations. A haemocytometer, they said, which scans a 1 mm^3 area gives a minimum count of 10^4 cells ml^{-1}. If one organism is seen in the 9 mm^2 area of a Levy Counting Chamber the conclusion drawn is that there is a minimum of 900 cells ml^{-1} in the sample. With such a large minimal cell count, reliable figures for small populations can only be obtained by a large number of replicate counts. Small phytoplankton populations can, however, be enumerated without this problem on membrane filters. The minimum cell count estimated by the technique used in this study was 5,258 cells l^{-1}. With a haemocytometer the small phytoplankton population of Swartvlei would have been significantly overestimated or completely impossible to

calculate without first considerably concentrating the population. The filtration method has the advantage that it is quick. Unlike the Utermohl technique there is no long wait for cells to settle. Further, if the population should be significantly smaller in some months, counting reliability can be improved simply by filtering larger quantities of water.

Zooplankton

Total zooplankton numbers were obtained by filtering 10 litres of water from each metre interval through a canister containing mesh (140 μm pore size). A 2 litre Friedlinger bottle was used for collection. The sample was reduced to about 30 ml and the zooplankton were preserved with a few millilitres of 4% formalin.

Primary productivity

Primary productivity was measured using the ^{14}C light and dark bottle technique. Dark bottles were prepared by painting them with four coats of black school board paint and two coats of epoxy resin to prevent chipping. The carbon source, $NaH^{14}CO_3$, was taken into the field in small reagent bottles as 20 ml portions. This working solution was prepared at a theoretical concentration of 2.5 μCi ml^{-1}. Actual specific activity was verified using the method described by Schindler (1966) in association with an external standard. The working solution was autoclaved and then frozen until needed. After the last incubation bottle had been prepared the $NaH^{14}CO_3$ solution was once again subsampled and its specific activity determined.

Samples were obtained with a Ruttner bottle from the surface and at 1 m intervals to 8 m. The perspex Ruttner bottle was used instead of the metallic Friedlinger bottle since metallic surfaces may be either detrimental or stimulating to the algae (Soeder and Talling 1969). With the transparent Ruttner bottle, however, there was the possibility of light injury to the phytoplankton (Goldman et al 1963). In order to minimize this possibility the sampler was quickly brought on board and a pair of light and dark reagent bottles were filled in the shade.

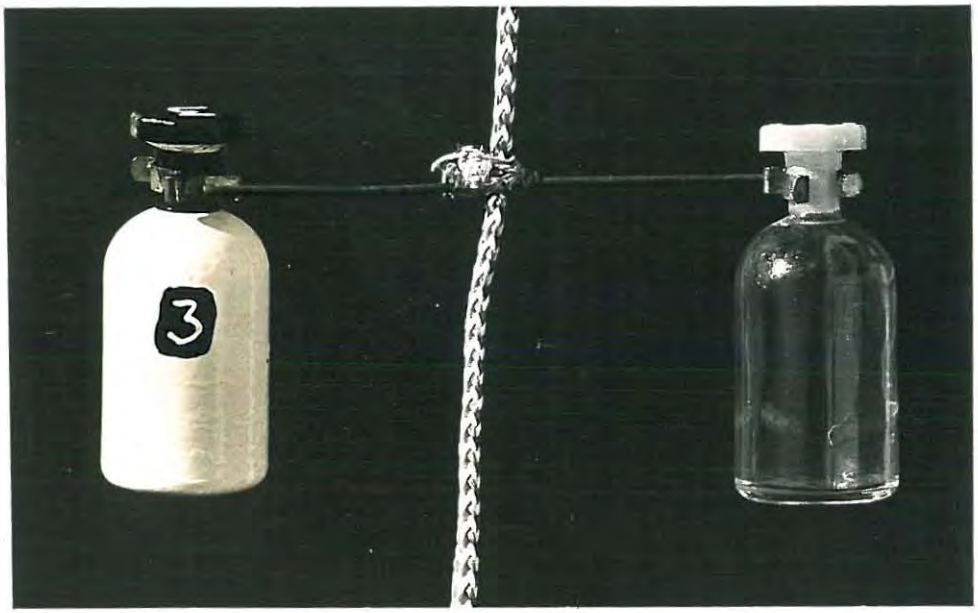


Fig. 8: 125 ml light and dark bottles for primary productivity measurements. Harness is # 10 gauge wire inserted through nylon rope and held in place by soldered cross bars. Bottles are held in # 10 terry clips.

The bottles were then placed immediately into a light tight box until all samples were collected. (The remainder of the water sample was used for total alkalinity determination).

Starting with the 8 m samples, 1 ml portions of the $NaH^{14}CO_3$ solution were quickly injected into each bottle with a calibrated 2 ml syringe. The bottles were shaken vigorously, snapped into the clips and lowered to the correct depth in the harness shown in Fig. 8. This procedure usually took approximately 10 minutes. The rope was then attached to a small buoy and a moored raft. There was approximately 4 m of rope between the buoy and the raft which allowed the suspended bottles to drift away. This was done in order to minimize possible shadowing from the raft.

Incubation time was 4 hours (Vollenweider and Nauwerck 1961), from 1000 h to 1400 h $\pm$ 20 minutes on every field trip. At the end of this time the bottles were quickly brought on board and placed in a light tight box to be taken back to the field station immediately.

At the station 100 ml aliquots were filtered (0.5 atm) through Oxoid membrane filters (pore size approx. 0.45 µm), washed with 30 ml distilled water and exposed in the dark to the fumes of concentrated HCl for 1 minute (Wetzel 1965a; Allen 1971a). The filters, which were still wet, were placed directly into scintillation vials containing the scintillation cocktail (Ward and Nakanishi 1971).

Calculations for the uptake of inorganic carbon as mg C m^{-3} h^{-1} were obtained from a slight modification of an equation given by Wetzel (1965 b):

$$(\dot{x})\ (\mu Ci)\ (2.22 \times 10^6) = \frac{(a)\ (b)\ (c)\ (1.06)\ (1000)\ (d)}{4}$$

where x equals mg C m^{-3} h^{-1}, µCi is the number of microcuries of $NaH^{14}CO_3$ added, 2.22×10^6 equals 1 µCi, "a" is the natural substrate concentration present or the amount of ^{12}C available calculated using the alkalinity value and a pH factor from the table by R.W. Bachman (Saunders et al 1962), b is the bottle volume correction factor

determined volumetrically, 1.06 is a correction factor for ^{14}C isotope discrimination, 1000 is to convert mg l^{-1} to mg m^{-3}, d is a correction factor for the 100 ml subsample filtered and 4 is to convert uptake to an hourly value. This equation differs from Wetzel's: (1) the left hand side of the equation is not divided by a correction factor for the counting efficiency of the scintillation counter as all counts were corrected individually for counting efficiency and quench and, (2) the right hand side of the equation is divided by 4 to give uptake on an hourly basis. Since a pyrheliometer was not available to measure daily solar radiation Wetzel's diurnal correction factor could not be calculated to give uptake for a 24 hour period. Productivity as mg C m^{-2} h^{-1} for the 8 m of water column investigated was estimated by totalling the cubic meter values obtained from the above equation. When this method of obtaining productivity as mg C m^{-2} h^{-1} was compared to Wetzel's planimetry method the values differed by only 0.4% or 0.118 mg C m^{-2} h^{-1} of a total value of 29.114 mg C m^{-2} h^{-1}.

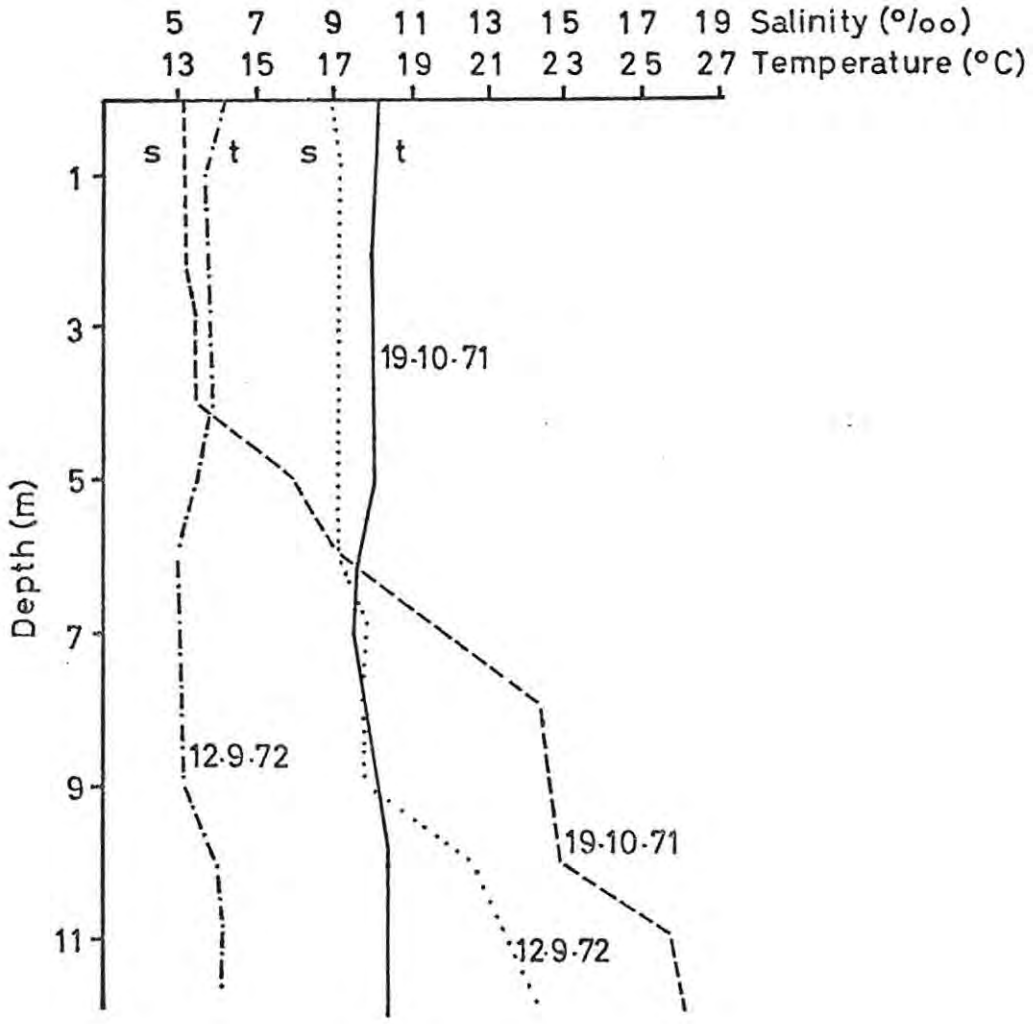


Fig. 9: Showing the stratification produced by the salinity in the upper reaches of Swartvlei.

RESULTS

Physico-Chemical Limnology

Meromixis

While temperature is the factor most commonly responsible for stratification in freshwater lakes this is not so in an estuarine system such as Swartvlei. The limnology of the upper reaches of Swartvlei was dominated by an ectogenic meromixis (Fig. 9). The monimolimnion was a result of the inflow of sea water. A meromictic system in South Africa has only previously been recorded in lake Sifungwe (Allanson and van Wyk 1969). The meromixis in Swartvlei differed from that in lake Sifungwe and from the condition described by Hutchinson (1957) in the Hemmelsdorfersee near Lubeck in that it was of an unstable nature. In this characteristic it matched the conditions described by Yoshimura in 1938 for equally shallow systems along the Japanese coast (Hutchinson 1957).

Figs 10 and 13a illustrate the fluctuations in the volume of the water column occupied by the monimolimnion, halocline and mixolimnion between 1971 and 1972. Temperature and oxygen profiles (Figs 11, 12) for the same months show that this unstable meromictic condition was correlated with the changes in oxygen and temperature. This instability was due to three factors: (1) the inflow of humic freshwater, (2) the inflow of sea water and (3) wind stress. The magnitude of the effect of these factors upon the physico-chemical limnology of the upper reaches was dependent upon whether or not the estuarine mouth was open or closed. Changes in the salinity, temperature and oxygen values for the water column at the raft station were examined for the following time periods: (1) freshwater inflow, August 1971 and March and August 1972, (2) sea water inflow, October and December 1971 and October-December 1972 and (3) abnormal winds, June-September 1972. This analysis will show that these factors controlled not only the physico-chemical limnology but also the biology of the upper reaches of Swartvlei.

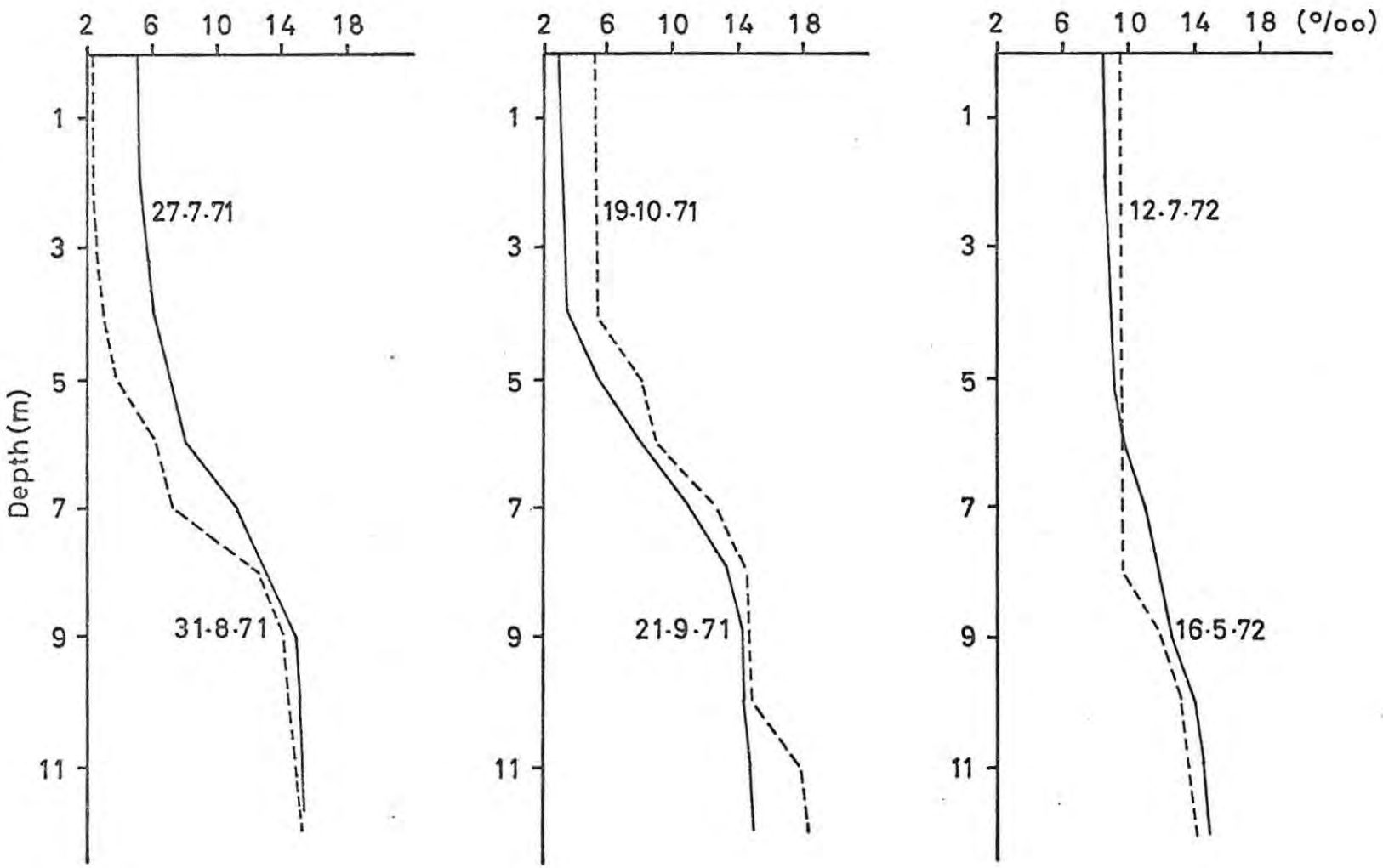


Fig. 10: Salinity depth profiles.

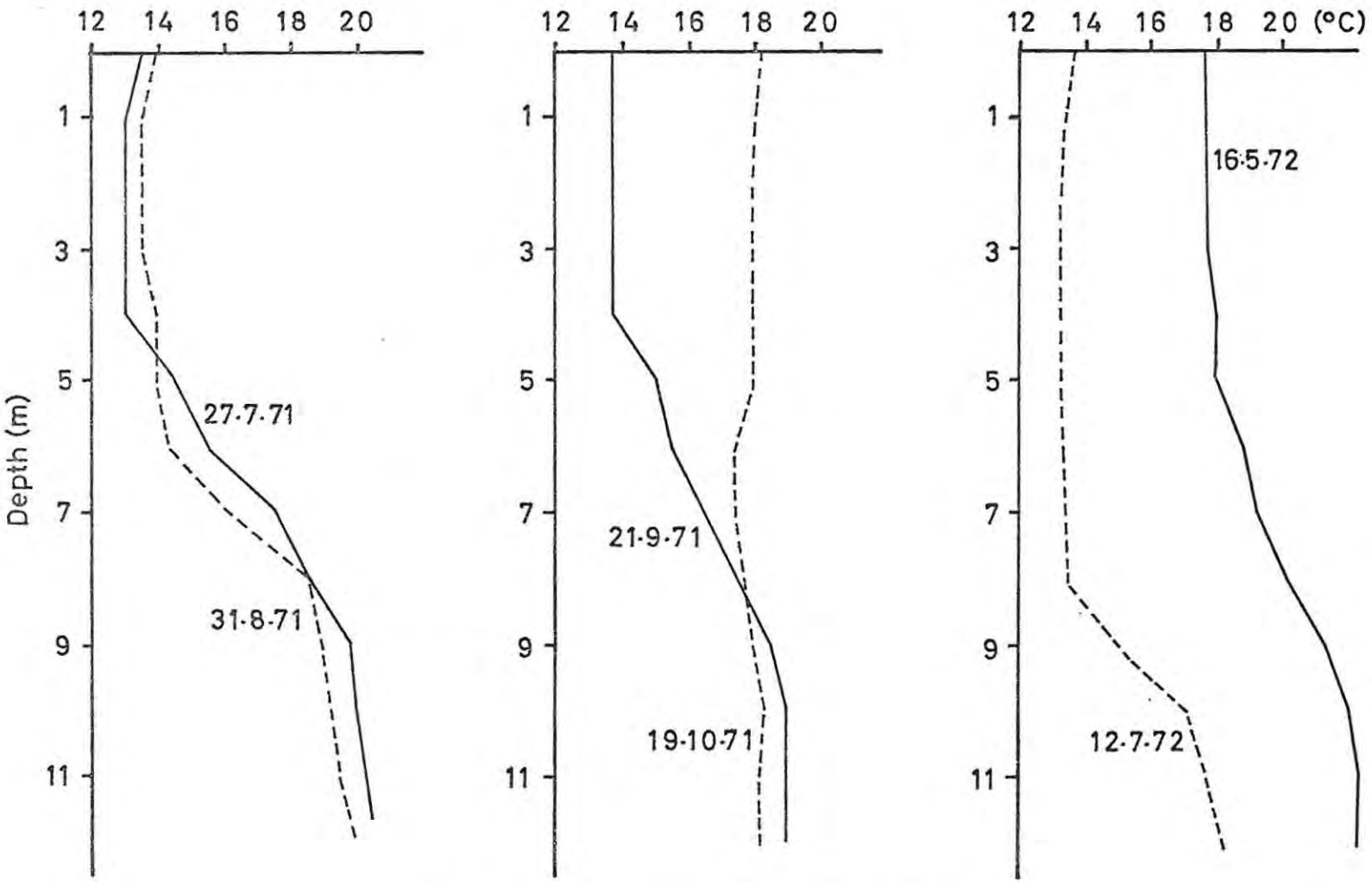


Fig. 11: Temperature depth profiles.

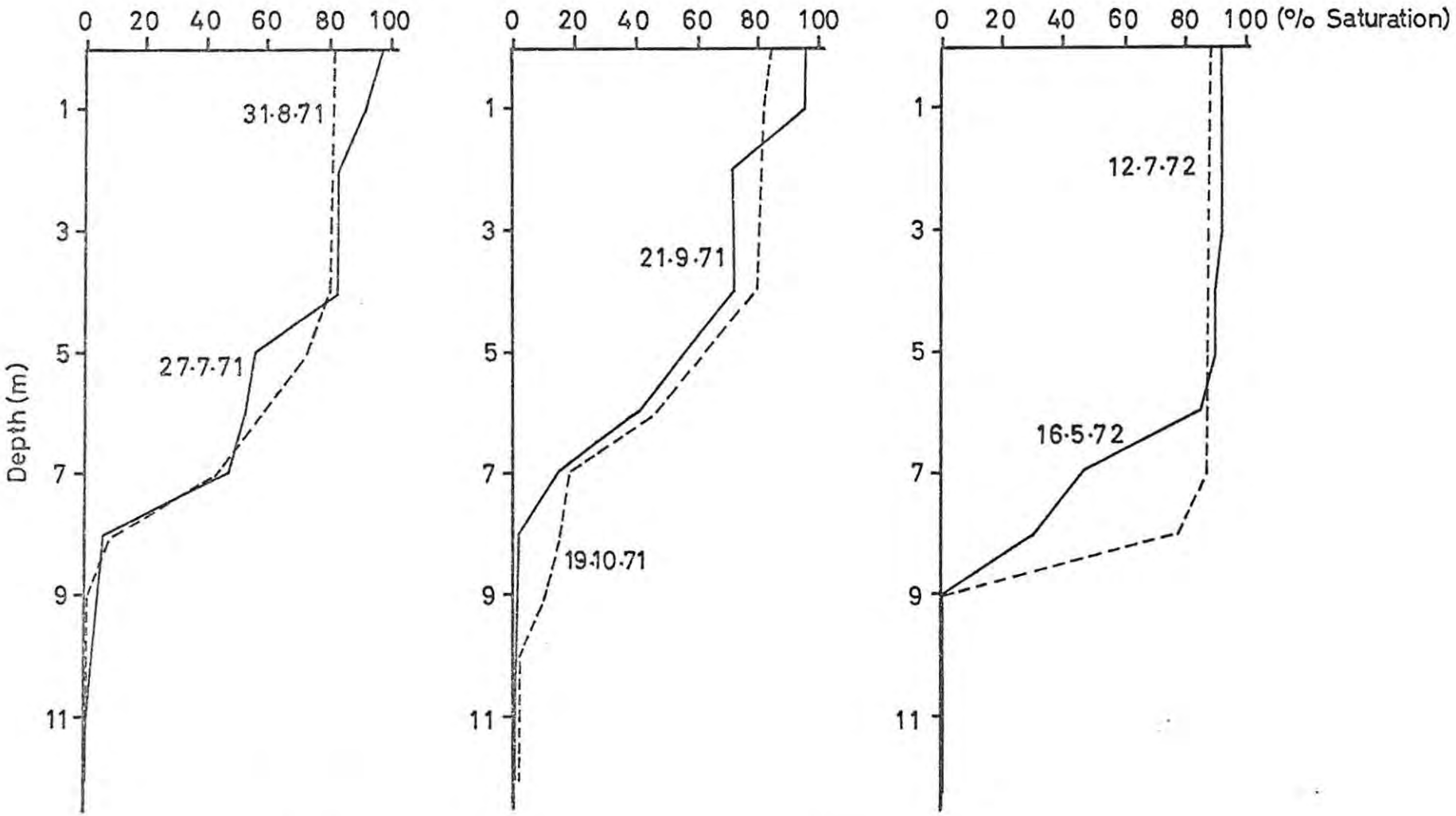


Fig. 12: Percentage saturation of oxygen depth profiles.

Of the three factors which were responsible for the unstable meromixis of Swartvlei wind was the most important as demonstrated in Figs 10 and 13a. The salinity profile for the water column of the upper reaches was multilamina (Figs 9, 10). This layered structure of the mixolimnion and halocline was broken down only by the action of abnormally strong winds from June to September 1972 during which time the mouth of the estuary was closed (Fig. 13a).

During the study the mouth was first opened on July 10, 1971 and closed April 6, 1972 (Figs 13a, 14). It was opened again August 16, 1972 and remained open to the end of the study. The closing of the mouth was a natural process caused by the building of a sand bar through the action of long shore currents. Opening of the mouth, however, was carried out by local residents of the area by digging and blasting. Although the mouth would probably have opened naturally during periods of heavy freshwater inflow this did not happen since large areas of economically important land would have become inundated before this occurred.

The effects of this opening and closing on the water level of Swartvlei are indicated by the broken surface line in Fig. 14. Unfortunately, a permanent water level recorder beacon was not functional during the study. The variation in depth was recorded at the raft station each month by lowering a weight on a winch marked off in 0.1 m to the bottom. On July 2, 1971 the depth was 12.5 m. It dropped to 11.7 m by July 27 after the mouth was opened. A large inflow of freshwater in August produced a 0.3 m rise which persisted into October (Fig. 13a). By November 1971 the water level was back to the more normal depth of 11.7 m. Sampling in April 1972 was done 5 days after the mouth closed. In this time the water level had risen 0.2 m. The water continued to rise until, in August 1972, the depth of water at the raft station reached 12.4 m. The depth of the water column dropped to 11.7 m after the mouth was opened on the 16th.

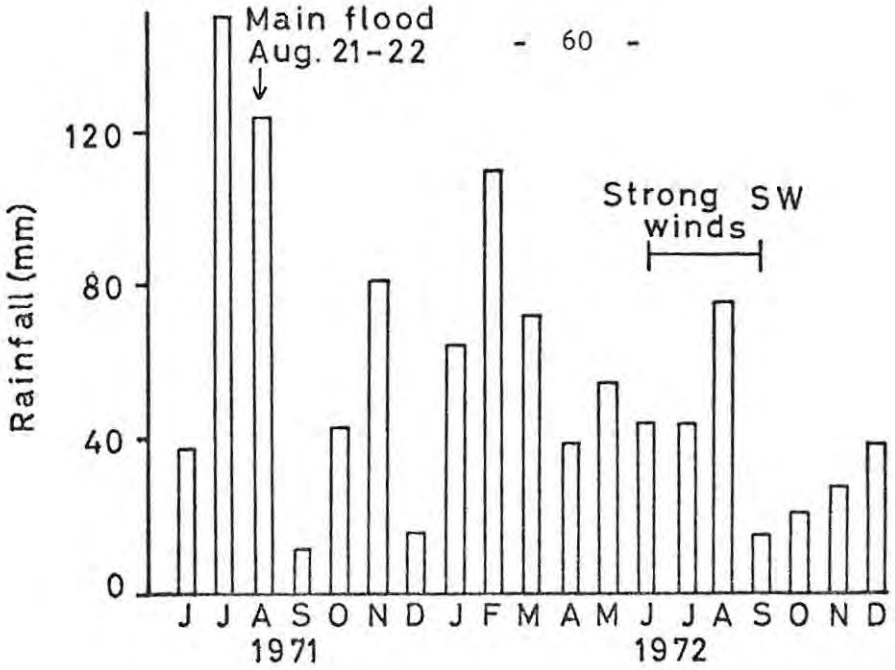


Fig. 13b: Rainfall in Swartvlei Catchment Area (Department of Transport, Weather Bureau, Pretoria).

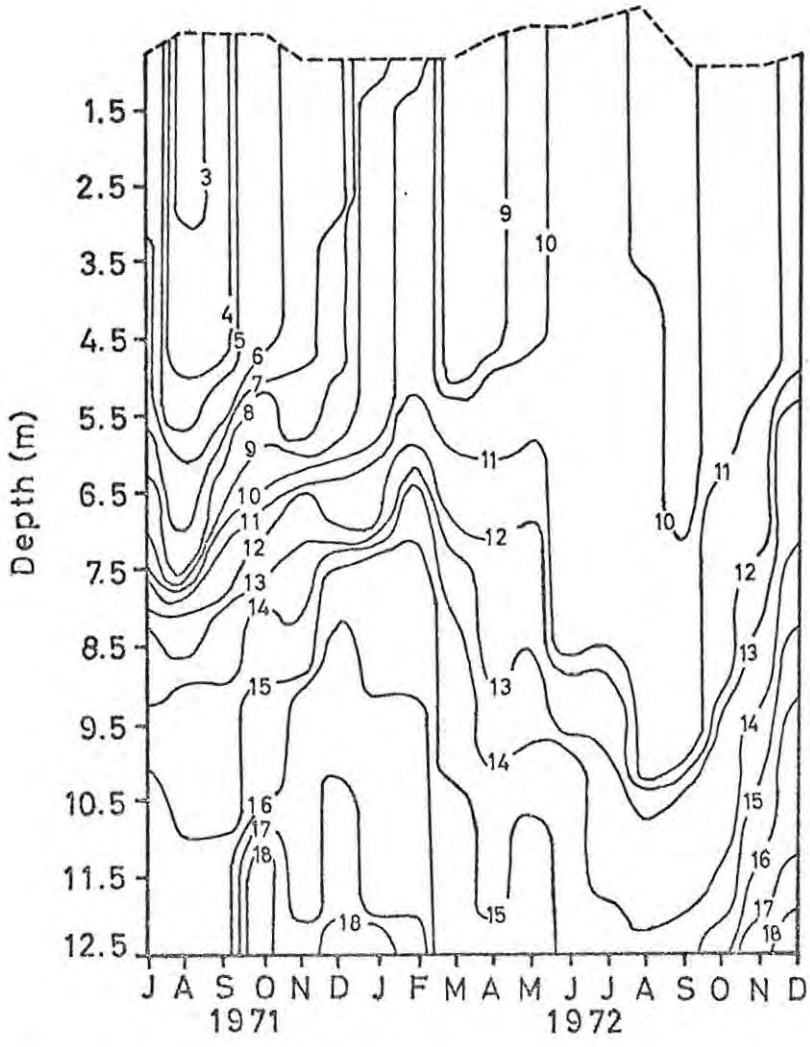


Fig. 13a: Salinity (^{o}/oo) depth-time diagram.

Striped area indicates when mouth of estuary was closed.

Effects of freshwater inflow upon the salinity, temperature and oxygen regimes

When freshwater enters a salt water system the less dense freshwater flows in on top of the denser salt water. In relatively narrow straight systems it is possible for the freshwater to pass over the salt water with little or no mixing. In Swartvlei the wide basin and the fact that the river entrances are not aligned with the exit from the upper reaches (Fig. 2) prevented the freshwater from passing over the salt water. Some portion was always retained in the upper reaches even though the mouth was open. This produced a layer of freshwater on top of the water column. Periods of heavy river inflow and hence rainfall were accompanied by stronger winds. The result of this was a rapid mixing of the freshwater layer with the underlying salt water (Figs 9, 10, 13a).

In August 1971 one of the most severe floods recorded in South Africa occurred between the 21st and 22nd of the month. Fig. 13b indicates that more rain fell in July than in August. The rains in July ended an extended period of drought. A large proportion of the rain falling in July was taken up by the land. Consequently, significant runoff did not occur until August when about 45 mm of rain fell overnight. The resulting flood poured large quantities of acidic humate-stained freshwater into the upper reaches. Although a freshwater layer probably occurred this was rapidly mixed with the salt water as shown by the data in Figs 10 and 13a. The turbulent mixing created by wind stress decreased the salt concentration in the upper 5 m of the water column by 3 $^{o}/_{oo}$ per meter (Figs 10, 13a). The turbulence also mixed freshwater down to 7.5 m resulting in a marked halocline at 7.5-8 m (Fig. 13a). Greater decreases in salinity would probably have occurred if the estuarine mouth had been closed. A much larger quantity of freshwater would have been retained and mixed into the water of the upper reaches. This would have allowed wind stress to increase the volumne of the mixolimnion.

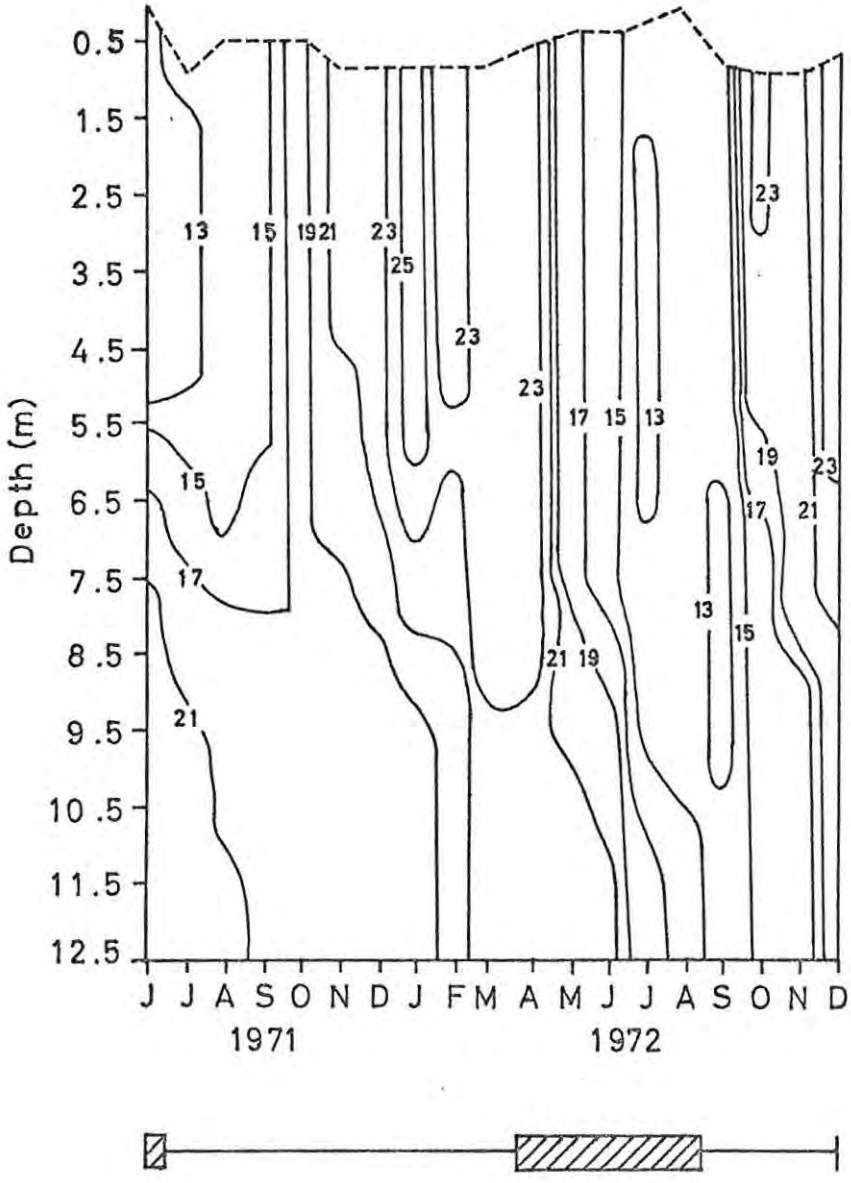


Fig. 14: Temperature (^{o}C) depth-time diagram. Striped areas in lower figure indicate when mouth was closed.

TABLE 1

A comparison of the heat content (K cal. cm^{-2}) in the total water column, mixolimnion and monimolimnion at the raft station in the upper reaches of Swartvlei between June and December 1971 and 1972. Lower table gives heat content as K cal. 100 cm^{-3} so as to take into account the fluctuating volumes of the mixo- and monimolimnions.

MONTH	JUNE 1971	JULY 1971	AUGUST 1971	SEPT. 1971	OCT. 1971	NOV. 1971	DEC. 1971	JUNE 1972	JULY 1972	AUGUST 1972	SEPT. 1972	OCT. 1972	NOV. 1972	DEC. 1972
Mixolimnion	7.83	9.55	11.28	9.93	12.60	12.93	15.55	15.38	12.13	15.15	13.48	17.76	18.36	11.76
Monimolimnion	13.83	9.63	7.63	9.05	8.97	11.32	9.48	6.20	4.96	1.68	2.78	4.98	4.98	15.01
Total	21.66	19.18	18.91	18.98	21.57	24.25	25.03	21.58	17.09	16.83	16.26	22.74	23.34	26.77
Mixolimnion	1.30	1.36	1.41	1.42	1.80	2.15	2.22	1.71	1.21	1.38	1.35	1.97	2.04	2.35
Monimolimnion	1.98	1.93	1.91	1.81	1.79	1.89	1.90	2.07	2.48	1.68	1.39	1.66	1.66	2.14

In March 1972 the salinity of the mixolimnion decreased by 2 °/oo (Fig. 13a). This decrease, and that which occurred throughout the water column, was the result of the rainfall in February (Fig. 13b). A similar process as that in August 1971 occurred. Turbulence due to wind stress mixed the freshwater and salt water and depressed the halocline (Fig. 13a).

A rainfall of 32.2 mm on August 11, 1972 created a flood. The water depth rose 0.2 m at the raft station. The salinity of the mixolimnion decreased by 1 °/oo in the upper 3.5 m (Fig. 13a). Turbulence due to continued wind stress depressed the halocline from 8.5 m in July to 10.5 m in August thus extending the mixolimnion volume. Sampling was done on August 14 and the absence of a freshwater layer emphasized the rapid mixing that occurs after a flood.

Although the inverse thermal stratification of Swartvlei during the winter months conformed closely to the salinity profile (Figs 10, 11) this was not a permanent correlation. As shown in Fig. 9 the water column may become essentially homothermal and during the summer the upper portion of the water column was warmer than the lower (Fig. 14).

In August 1971 and 1972 the temperature of the mixolimnion of the upper reaches rose 1°C over temperatures recorded in July 1971 and 1972 (Fig. 14). During the cold winter months of July and August the "rate of delivery of radiation" (Hutchinson 1957) would have been relatively low even at the latitude of Swartvlei. Consequently, this warming of the mixolimnion can be explained, at least in part, by heat transfer from the distinctly warmer monimolimnion. Table 1 provides evidence to support the validity of this contention. Thus temperature inversion due to meromixis became a real factor in the heat budget of the upper reaches during winter.

Cooling of the water column was also controlled to some degree by the meromixis. In August 1972 the mouth closed and the upper reaches were lake-like. The temperatures of the monimolimnion were lower than for the same period in 1971 (Fig. 14). This was because the rate of

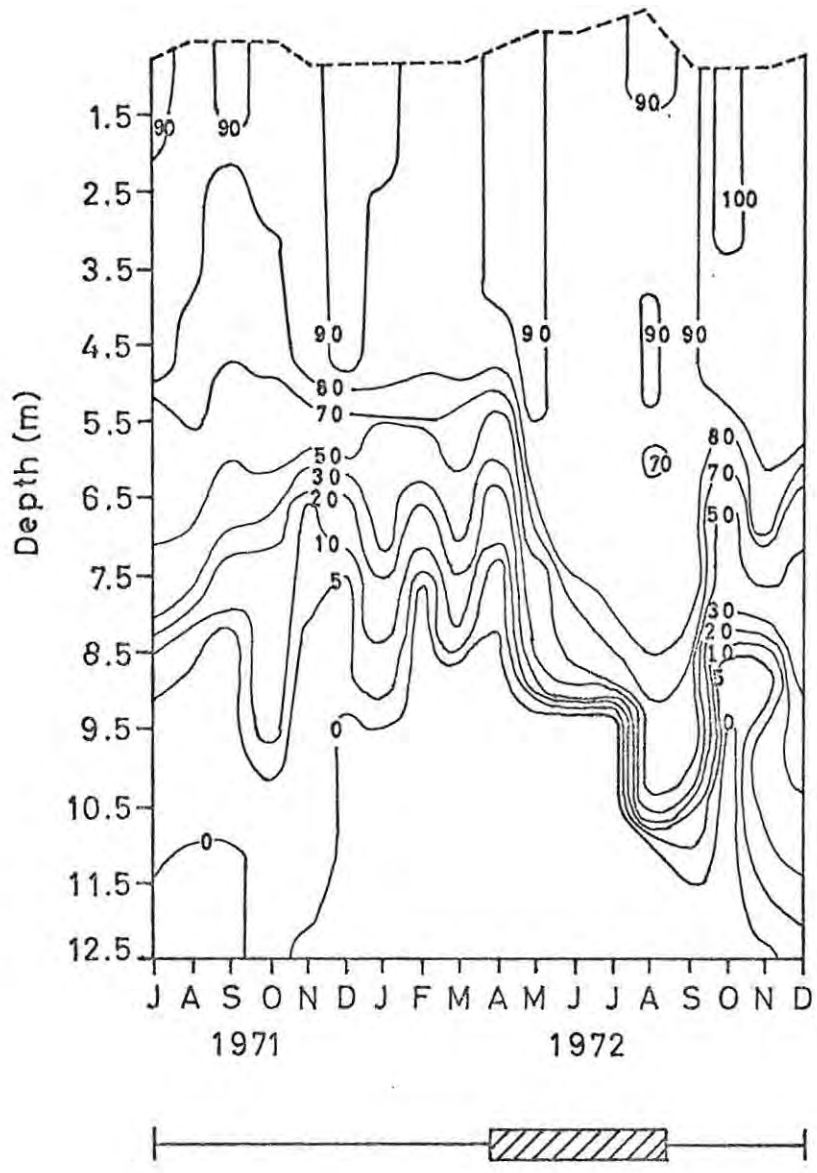


Fig. 15: Oxygen (percentage saturation) depth-time diagram. Striped area in lower figure indicates when mouth was open.

heat loss from this region had been accelerated by the extensive mixing (Fig. 13a; Table 1). The heat contained in the lower levels of the mixolimnion was transported by turbulence to the surface and lost by radiation or evaporative cooling.

The oxygen concentration of the water, as percentage saturation corrected for temperature and salinity, shows that a marked decrease occurred after the flood in August 1971 (Fig. 15). This decreased oxygen saturation was probably due to two factors: (1) increased biochemical oxidation of the visibly increased detritus concentration and (2) a reduction of photosynthesis due to decreased transparency. The marked oxygen depeltion below the halocline at 8-8.5 m was due to increased oxygen consumption and reduction in turbulent mixing. The increased oxygen depletion between 8.5-11.5 m, as compared to July 1971, suggested that sinking detritus increased microbial activity and utilization of oxygen in this region (Fig. 15).

In March 1972 oxygen saturation increased following the large amount of rain in February (Figs 13b, 15). It was possible that any oxygen deficit that may have occurred due to increased freshwater inflow had been compensated for prior to sampling in late March. In August 1972 the oxygen saturation from the surface to 1.5 m increased as a result of increased primary production (See Fig. 32). At deeper levels effected by the flood (to 3.5 m, Fig. 13a) oxygen decreased (Fig. 15).

The effect of sea water inflow on the salinity, temperature and oxygen regimes

The first inflow of sea water into the upper reaches during the study period occurred between September-October 1971. The resulting salinity increases are shown in Fig. 13a. This isopleth diagram shows that denser, more saline water flowed into the upper reaches following the bottom contour. This caused a 3 $^{o}/oo$ increase in bottom salinities. The bottom wedge of saline water increased the depth of the water column. The less saline surface water left the upper reaches on the next low tide. A 2 $^{o}/oo$ increase was noted in the mixolimnion (Fig. 13a).

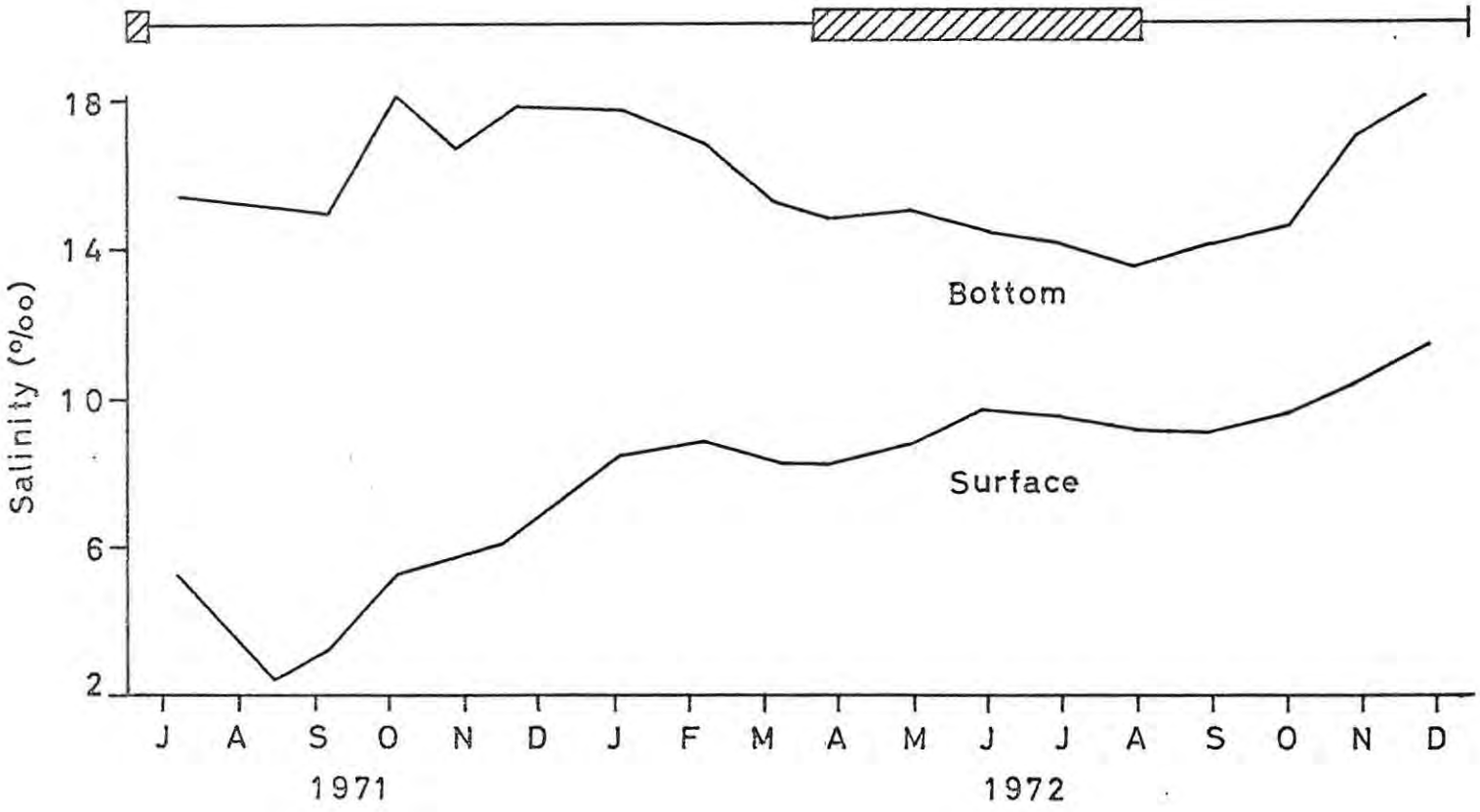


Fig. 16: Variation in surface and bottom salinity of Swartvlei. Striped area in upper graph indicates when the mouth of the estuary was closed.

As sea water entered the upper reaches varying quantities were mixed into all levels of the water column. The overall effect of this sea water inflow on the salinity profile was to further subdivide the water column into a number of salinity layers (Figs 10, 13a).

Sea water entered the upper reaches again between November-December 1971. Sea water was mixed into the water column below 4 m with the greatest increase in salinity (2 °/oo) noted between 7.5-8.5 m (Fig. 13a). The magnitude of the salinity increases indicated that a much smaller quantity of sea water entered in December 1971 than in October 1971.

In January 1972 salinity values above 6 m increased 2-3 °/oo per meter from those in December (Fig. 13a). Sea water may have entered the upper reaches and mixed into the water column above 6 m.

Fig. 16 indicated that further inflows of sea water into the upper reaches occurred from October to December 1972. Water from the middle reaches entered the upper reaches in a similar manner as in October and December 1971 (Fig. 13a). In October 1972 turbulence mixed sea water into all levels of the water column. Further salinity increases in the water column occurred below 6 m in November. Another inflow of sea water, between November and December 1972, resulted in a 1 °/oo salinity increase in the mixolimnion and a 2 °/oo increase at all levels below the halocline (Fig. 13a). The overall result of these sea water inflows between October-December 1972 was to re-establish the multilamina salinity profile which had been broken down during June-September 1972.

The high tides (South African Tide Tables 1971-1972) were recorded in a histogram and compared to the salinity data in Fig. 16. No correlation was found between tide height and salinity increases. Many factors, such as wind direction and velocity, may influence the degree of movement of sea water in the middle reaches. A combined south-westerly wind and high tide produced a strong current in the channel thereby forcing water into the upper reaches (H. Watts 1972, pers.comm.). Such a combination was noted between October and November 1972 and would

explain the salinity increases. In April 1973 the salinity values in the upper 4.5 m of the water column had risen to 13 °/oo (Allanson 1973, pers.comm.). This was due to the mouth remaining open since the last field trip in December 1972.

The thermal profile of the upper reaches in October and December 1971 appeared not to have been effected to any great extent by the inflow of sea water (Fig. 14). The transfer of heat from the monimolimnion (Table 1) combined with radiation and mixing in the upper water column between September and October 1971 resulted in a uniform temperature profile in October (Fig. 14). By November 1971 the temperature in the upper water column was warmer than in the lower. As Fig. 14 and Table 1 indicate heat was transferred to the lower water column from the mixolimnion. It is not known if the inflow of sea water between September-December 1971 influenced the heat gain of the water column.

Total heat content of the water column in October and November 1972 was lower than for the same months in 1971 (Table 1). Marked temperature increases and heat gains by the water column occurred between September-October 1972 (Fig. 14; Table 1). This heat gain continued into December and was due mainly to radiation. However, the large heat gain of the monimolimnion in December indicated that the inflow of sea water may have been a contributing factor.

Other than salinity the chemical characteristic of Swartvlei most altered by these inflows of sea water was oxygen. After the inflows of sea water between September-October 1971 and October-December 1972 oxygen was detected at the bottom of the water column (Fig. 15). The halocline in a meromictic lake reduces the degree of mixing occurring below it and hence the transport of oxygen into the monimolimnion. If oxygen in the monimolimnion becomes totally depleted due to decreased eddy diffusion and biochemical processes then anaerobic microbial reduction of sulphate in the sea water produces H_2S (Yoshimura 1932; Jannasch 1970). H_2S is also produced by the decomposition of

phytoplankton and detritus but Yoshimura has shown that in systems open to the sea the dominant source of hydrogen sulphide is the sulphate of sea water. Sulphide and oxygen quickly eliminate each other producing a definite aerobic-anaerobic interface (Jannasch 1970). Large changes in the oxygen and hydrogen sulphide concentrations may occur at this interface during the day due to microbial activity (Sorokin 1965). The variation in microbial activity at the interface may account for the changes in volume of the anaerobic zone in Swartvlei (with the exception of October 1971 and December 1972) (Fig. 15).

In October 1972 an increase in the anaerobic zone was noted (Fig. 15) although sea water had entered at the bottome (Fig. 13a). In October 1971 and December 1972 the bottom became aerobic after a sea water inflow. Although it is not clear why the anaerobic zone increased in October 1972 it may have been related to the smaller quantity of sea water (based on salinity values) that entered the upper reaches between September-October 1972 than between September-October 1971 and October-December 1972 (Fig. 13a).

In the upper part of the water column oxygen saturation increased between October-December 1972 (Fig. 15). The oxygen saturation increased from July 1972 and was probably the result of the increasing primary productivity in these months (See Fig. 34).

Effects of abnormal wind stress on the salinity, temperature and oxygen regimes

The meromixis in Swartvlei conformed to the classical definition of this term only between June and September 1972 (Fig. 13a). This was the result of two interacting factors: (1) wind stress and (2) closure of the mouth. The uniform salinity region down to 4.5 m in May 1972 was extended to 8.5 m in June and July (Fig. 13a). This occurred due to stress from extremely strong south-westerly winds overcoming the salinity gradient which was not immediately re-established as sea water could not enter. Fig. 16 shows that the salinity increase in the mixolimnion in June was related to the salinity decrease of the

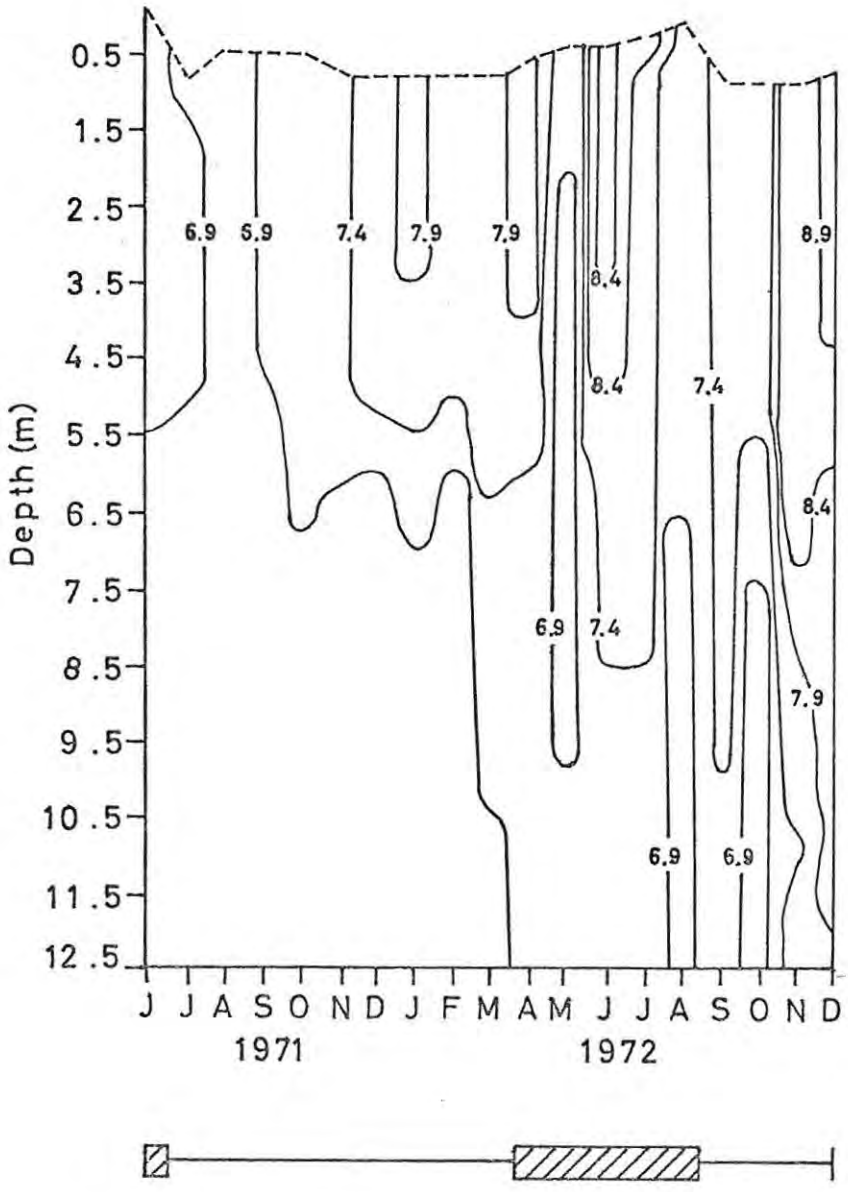


Fig. 17: pH depth-time diagram. Striped area in lower figure indicates when mouth was open.

monimolimnion. The halocline was depressed to 8.5-10 m between June and September due to the large volume of circulating water (Fig. 13a). In September as the salinity gradient was being reformed in the upper levels of the mixolimnion by the inflow of freshwater the halocline started to rise (Fig. 13a). The sea water inflows in the following months produced the characteristic multilamina salinity profile.

The temperature profile (Fig. 14) of the mixolimnion for each month from June to September 1972 was essentially uniform. By September both surface and bottom temperatures were 14°C. Cooling of the mixolimnion until August, and of the monimolimnion into September, was the result of turbulent mixing and evaporation which would have increased markedly with high winds. Compared to the same period in 1971 the heat content and temperatures of the monimolimnion from August to November 1972 were low (Fig. 14; Table 1).

The oxygen profiles for June to September 1972 reflect the extended volume of turbulent mixing which carried oxygen to greater depths (Fig. 15). The zone of rapid oxygen depletion at 9-9.5 m in June-July and 10.5-11 m in August-September overlapped the halocline (Fig. 13a). Below these levels the monimolimnion was anaerobic and H_2S was present.

pH and the bicarbonate budget

The pH value of the upper water column, with the exception of August 1971, fluctuated between 7.0 and 7.3 up to December 1971 (Fig. 17). After December, the pH became more variable, fluctuating between 7.0 and 9.1. The reading of 9.1 recorded in December 1972 was not only the highest value recorded during the study but was the only time that the pH approached the value of 9.2 recorded for Swartvlei, April 1951 (Martin 1962). The small deviation in pH for 1971 indicated a well buffered system which is characteristic of estuaries generally (Wood 1965). This buffering capacity is acquired by the presence of the excess basic radicals of carbonate and bicarbonate which are common to the sea (Day 1951). A further possible source of this buffering capacity is the humolimnic acids. Shapiro (1957) suggested a reasonable

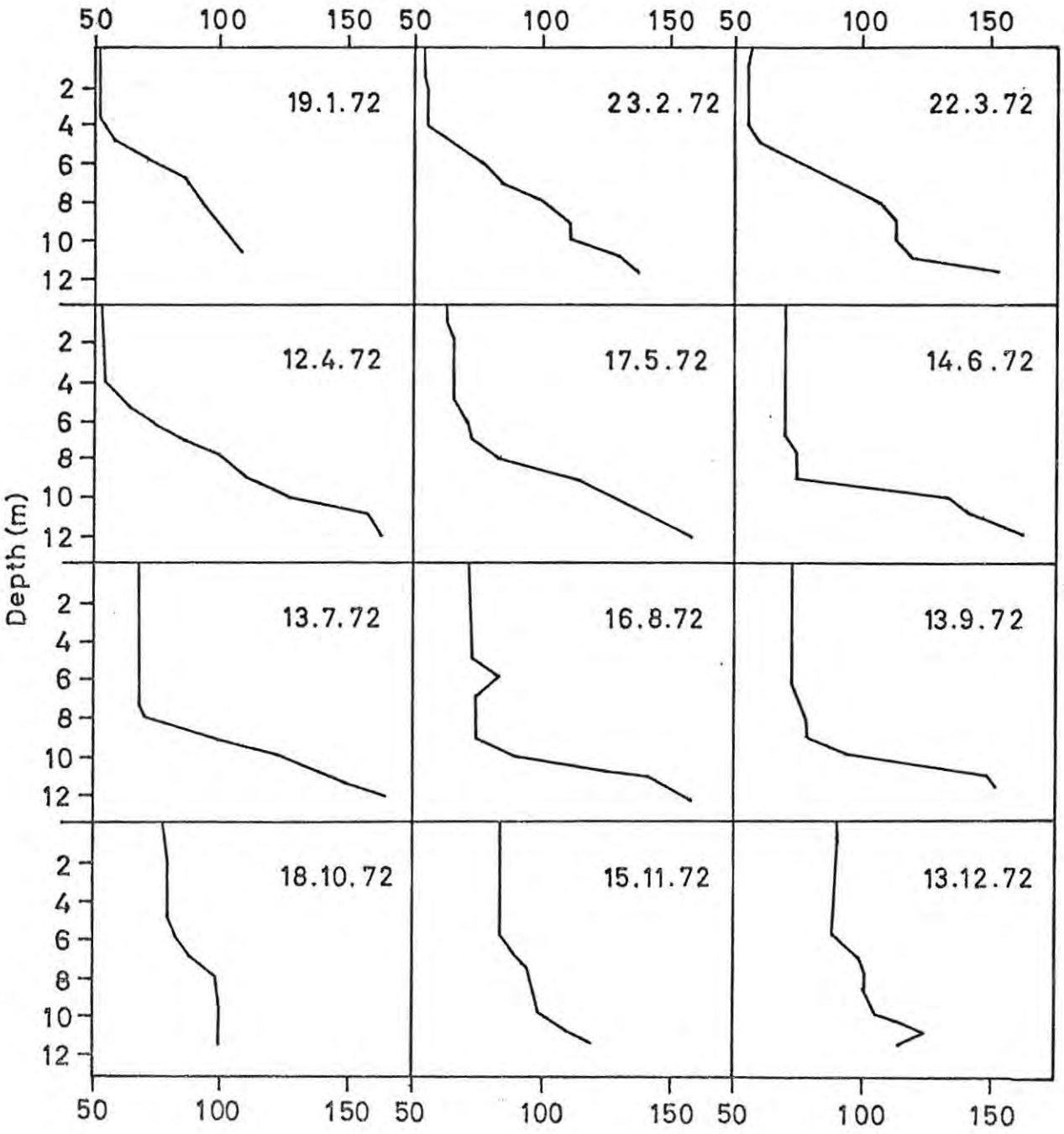


Fig. 18: Depth profiles of Methyl Orange Alkalinity (mg l^{-1} Ca CO_3).
1972

amount of colouring matter would tend to keep a basic lake basic. The importance of these compounds in Swartvlei needs further research.

Fig. 17 shows that pH decreased with depth. The pH value below the halocline (Fig. 13a) was normally less than 7.0. This pH decrease was due to the presence of carbon dioxide, hydrogen sulphide and other compounds.

The inflow of acidic, unbuffered river water in August 1971 decreased the pH by 0.3 units in the upper 4 m of the water column (Fig. 17). This decrease became smaller with depth so that between 6.5 and 7.5 m only a 0.1 unit difference was noted between these levels in July and August. No pH change was noted below 7.5 m. These pH decreases were due to the acidic, unbuffered nature of the river water but perhaps more importantly, to a reduction of transparency due to increased colouring and suspended matter which increased markedly after a flood. This decrease in transparency would decrease photosynthesis hence the CO_2 concentration would rise. In conjunction with this, bacterial production of CO_2 would increase with the added suspended matter. The generally lower (average 0.9 units) pH values in 1971, as compared to the same period in 1972, were therefore correlated with the greater rainfall in 1971 (Figs 13b, 17).

The distribution of bicarbonate, the dominant basic radicle, in the water column governed by the meromixis is shown in Fig. 18. Below the area of circulating water the bicarbonate concentration started to increase rapidly (Figs 13a, 18). According to Hutchinson (1957), Findenegg found in the meromictic lakes of Carinthia that carbon dioxide and bicarbonate increased towards the bottom concomitant with a very considerable oxygen deficit. A comparison of Fig. 12 to Fig. 18 shows that the oxygen and bicarbonate profiles in May and July 1972 were inversely related. The accumulation of aggressive CO_2 in the anaerobic monimolimnion releases carbonate from the sediments (Mortimer 1941/42; Boltt 1969) which would form predominantly bicarbonate at the pH values recorded in Swartvlei. Although no measurements of

carbon dioxide are available it seems reasonable to assume that aggressive CO_2 was responsible for the increase in bicarbonate with depth in Swartvlei. Any increase in the release of bicarbonate from the anaerobic monimolimnion would therefore add to the buffering capacity of the upper reaches.

The bicarbonate concentration in the upper half of the water column from January to September 1972 increased as the degree of mixing increased. From May to June, with the increased depth of the mixolimnion, the bicarbonate concentration increased 4-6 mg l^{-1} per meter above 5 m (Figs 13a, 18). Continuation of this mixing caused further increases in this region. From October to December 1972 the bicarbonate concentration in the lower water column was much lower than in previous months (Fig. 18). This indicates that turbulence and, as a result, eddy diffusion in these months transferred the bicarbonate to the upper water column at a rate faster than it could be dissolved from the bottom sediments. Consequently, in the upper water column the bicarbonate concentration increased between October and December 1972 (Fig. 18).

Therefore, the variation in the pH of the upper water column was a result of the complex interactions of carbon dioxide, bicarbonate and humic acids. Changes in the concentration of these compounds resulted from fluctuations in river inflow, sea water inflow, wind stress, photosynthesis and loss of CO_2 to the atmosphere. Further data will be needed to explain fully the variations in the pH of the upper reaches of Swartvlei since numerous biologically mediated reactions such as, nitrification, denitrification, sulphide oxidation and sulphate reduction affect the pH in natural water systems (Goldman et al 1972).

Soluble phosphate phosphorous (PO_4-P)

The PO_4-P data for March to December 1972 for the upper reaches of Swartvlei are shown in Fig. 19. The most striking feature of this figure is the almost total depletion of this anion in June followed by an increase in concentration which exceeded the previous high recorded

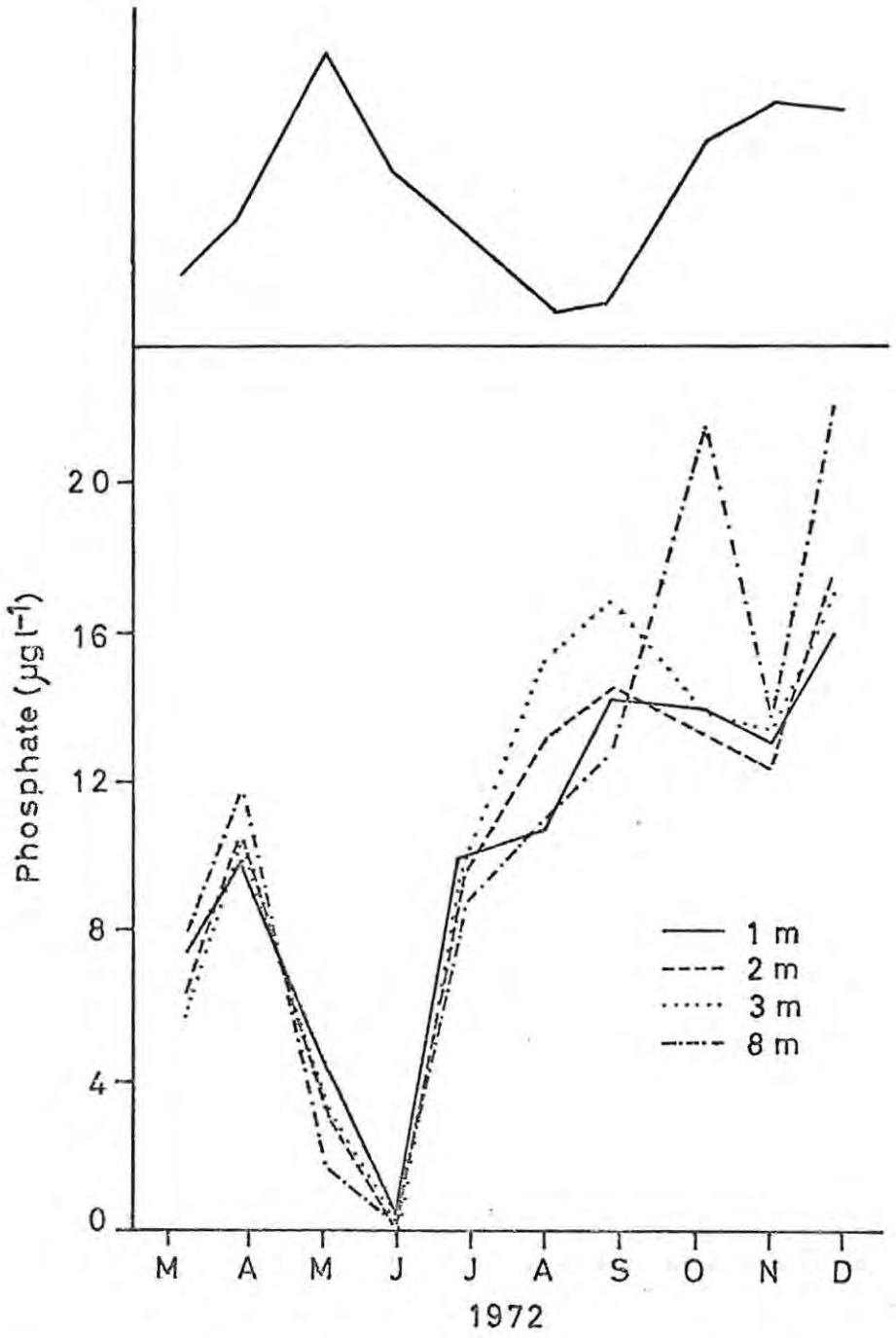


Fig. 19: Soluble Phosphate Phosphorous (PO_4-P) in the upper reaches of Swartvlei. Upper figure shows the monthly changes in the total phytoplankton population.

in April. The depletion of phosphate was related to the increasing phytoplankton population (Fig. 19) combined with a lack of replenishment of this nutrient in the upper water column from the monimolimnion. Soluble phosphorous is produced in the decomposition of detritus. This process occurs mainly in the hypolimnion of thermally stratified lakes or in the monimolimnion of chemically stratified lakes and is unavailable for primary productivity until the lake overturns. As this did not occur in Swartvlei (see August-September 1972; Fig. 13a) PO_4-P may well become limiting with a large increase in the phytoplankton population. Other possible sources of phosphate in the upper reaches were river and sea water. However, the greatest increase in phosphate in the water column occurred in July 1972 (Fig. 19) when the mouth was closed and river inflow was low (Figs 13a, 13b). These two sources of phosphate therefore seemed to be of little account in Swartvlei. An exchange between the monimolimnion and mixolimnion may occur and its efficiency depends on the energy of the water movements that cause turbulence and hence eddy diffusion in the sense of Findenegg (1965 a). This fact adequately explains the PO_4-P increase between June and December 1972 (Fig. 19). As already noted, there was a large degree of mixing, turbulence and consequently eddy diffusion from June to September. With the re-establishment of the salinity gradient at higher levels in the water column the exchange of phosphate between mixolimnion and monimolimnion decreased slightly from September to November (Figs 13a, 19). The PO_4-P concentration at 8 m in October 1972, however, increased (Fig. 19). This was probably due to the sample being taken from water with a very low oxygen concentration (Fig. 15) in which phosphate would be more soluble. This increased solubility was related to two factors: (1) ferrous iron and (2) hydrogen sulphide. H_2S was detectable in this region. When there is a lack of oxygen in the water ferric iron is reduced to the ferrous form and the precipitation of ferric phosphate is retarded (Mortimer 1941/42; Hutchinson 1957; Ruttner 1963). In addition, if H_2S is present,

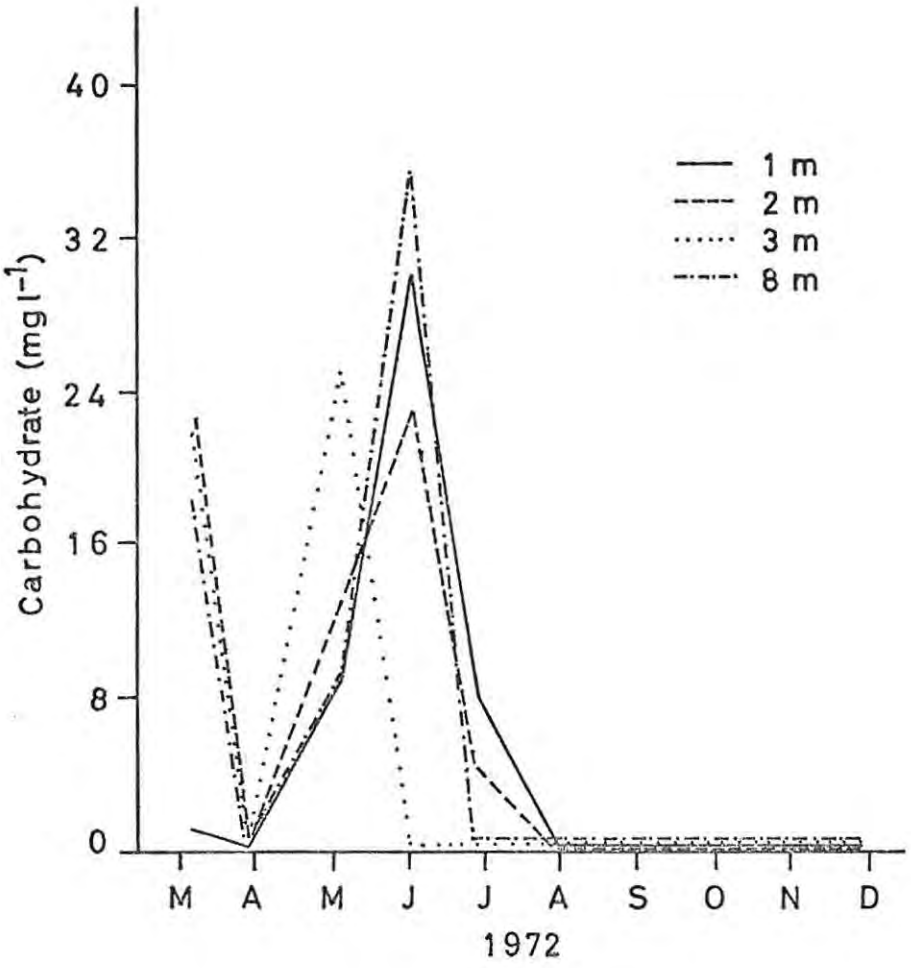


Fig. 20: Dissolved Carbohydrate in the upper reaches of Swartvlei.

especially under alkaline conditions, insoluble FeS is formed thus releasing phosphate (Hutchinson 1957; Ruttner 1963; Brock 1966). From November to December 1972 a further increase of PO_4-P occurred at all depths sampled due to turbulent diffusion transferring phosphate from the sediments to higher water levels.

Dissolved carbohydrate

The results of the carbohydrate determinations are shown in Fig. 20. With the exception of March and May to July 1972 carbohydrate could not be measured with the anthrone technique indicating the concentration was less than 0.4 mg l^{-1}. With the series of zero concentration results from August to December the technique was suspected of being faulty. This was not the case, however, as the method was checked on several occasions by adding known amounts of glucose (0, 10, 25, 50, 100 mg l^{-1}) to both distilled and Swartvlei water and these concentrations were recovered.

Carbohydrate is well documented as an extracellular product of both freshwater and marine phytoplankton (Guillard and Wangersky 1958; Fogg 1962; Hellebust 1965; Marker 1965; Vaccaro et al 1968; Aaronson 1971; Fogg 1971; Myklestad et al 1972) and also, as an aerobic decomposition product of detritus (Biggs and Wetzel 1968) and algae (Otsuki and Hanya 1972a). A comparison of Fig. 20 with the phosphate values in Fig. 19 shows that when the phosphate concentration was high the carbohydrate concentration was low. This would seem to be related to two facts: (1) as the phytoplankton population increases the phosphate concentration decreases and (2) carbohydrate release increases with the age of the cell population (Guillard and Wangersky 1958; Fogg 1962; Marker 1965; Aaronson 1971). The contribution of carbohydrate by a total phytoplankton population to the total concentration in a natural aquatic system is not known. Guillard and Wangersky (1958) working with the estuarine phytoplankton data of other workers estimated that for a total population of 10^5 cells ml^{-1}, assuming that the organisms behaved like stationary cultures, the amount

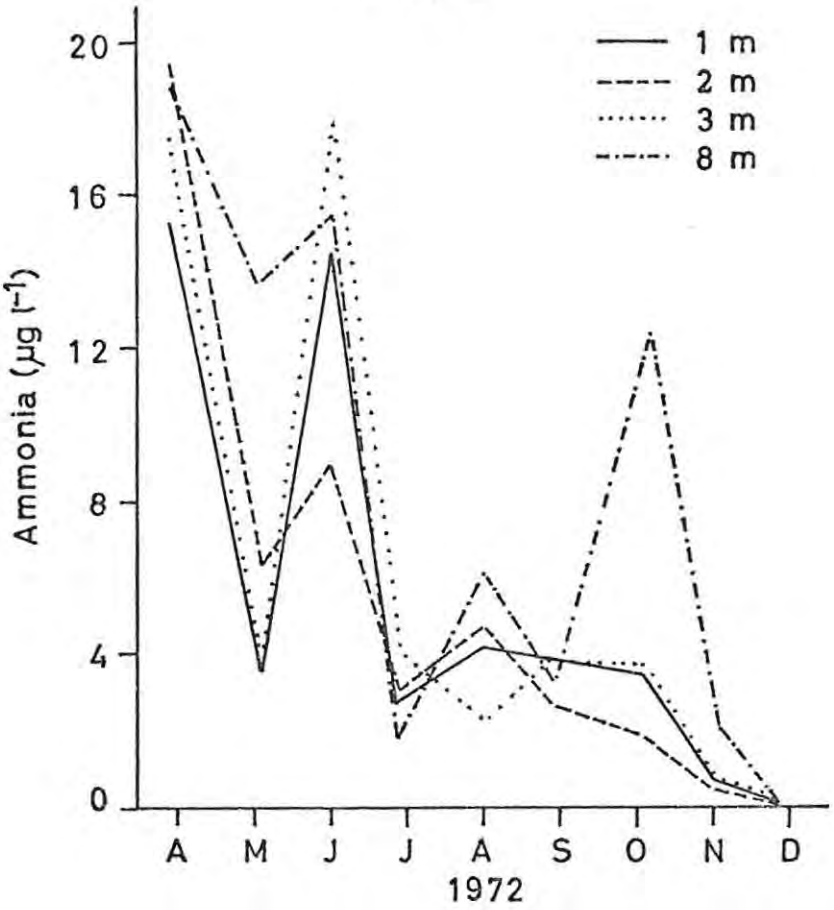


Fig. 21: Ammonia nitrogen (NH_3-N) in the upper reaches of Swartvlei.

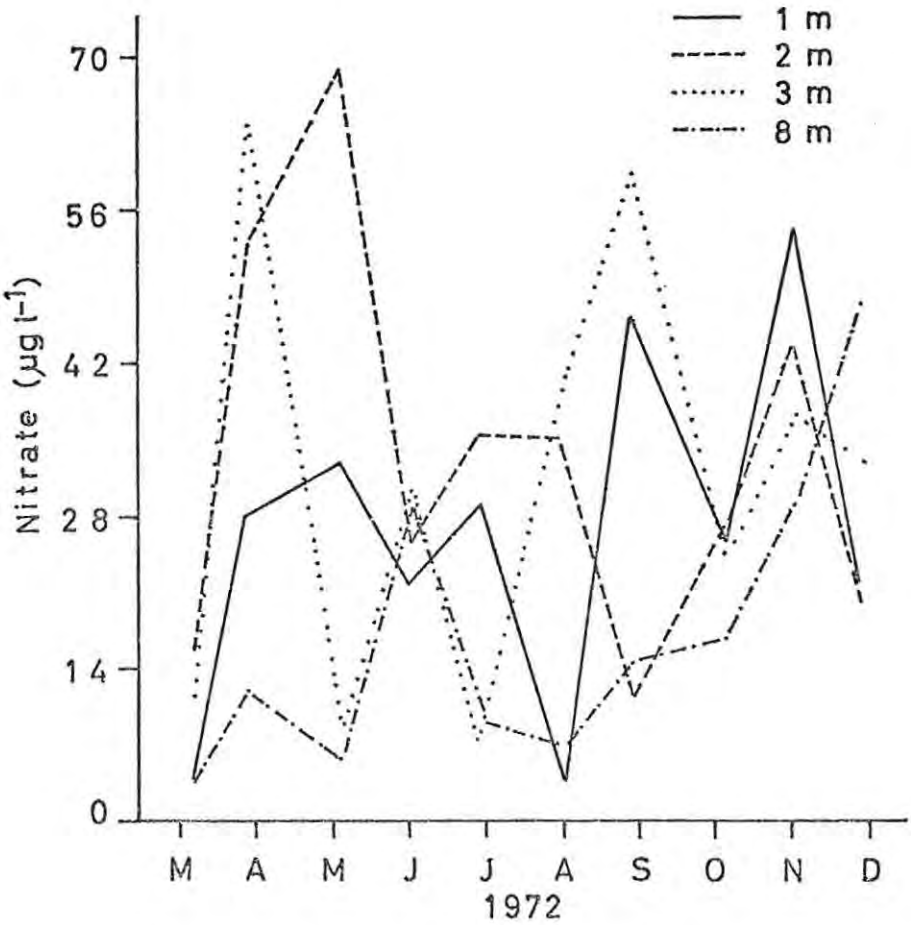


Fig. 22: Nitrate nitrogen (NO_3-N) in the upper reaches of Swartvlei.

of carbohydrate liberated would not exceed 1 mg l^{-1}. This fact combined with the theory that natural aquatic bacterial populations keep dissolved organic compounds at low concentrations (Hobbie and Wright 1965a) indicates that the high carbohydrate values obtained in March and from May to July may be the result of some allochthonous source. Therefore, if this source of carbohydrate was removed the soluble carbohydrate concentration could very well remain below the minimum value of 0.4 mg l^{-1} which could be detected with the anthrone method. Furthermore, the maximum total phytoplankton population at any depth in Swartvlei was always at least 3 orders of magnitude lower than the population used in the carbohydrate estimate of Guillard and Wangersky (See Figs 28, 30, 31). The exact source of the few high carbohydrate values recorded and their relationship to the PO_4-P concentration remains unknown at this time. However, the recent work of Allen (1971a and c) and Wetzel and Allen (1972) showing the formation of a dissolved organic matter pool in the littoral and its release to the pelagic is a possible avenue for further investigation.

Ammonia nitrogen (NH_3-N) and nitrate nitrogen (NO_3-N)

Ammonia is produced by practically all heterotrophic bacteria in the decomposition of plant and animal proteins (Hutchinson 1957; Ruttner 1963). In the presence of oxygen this compound is immediately transformed by nitrifying bacteria into nitrate. Consequently, in the mixolimnion of a meromictic lake nitrate is present in greater concentrations than ammonia while in the anaerobic monimolimnion nitrate is not present and ammonia is found to accumulate. Fig. 21 shows that the NH_3-N concentration in the aerobic upper reaches of Swartvlei never rose above 19.5 $\mu g\ l^{-1}$ while NO_3-N approached 70 $\mu g\ l^{-1}$ (Fig. 22). The ammonia concentration at the depths monitored was usually highest at 8 m. This reflects the lower oxygen concentration found at this level. Conversely, the NO_3-N values obtained at 8 m were usually the lowest recorded (Fig. 22).

In June 1972 the ammonia concentration was high (Fig. 21). This

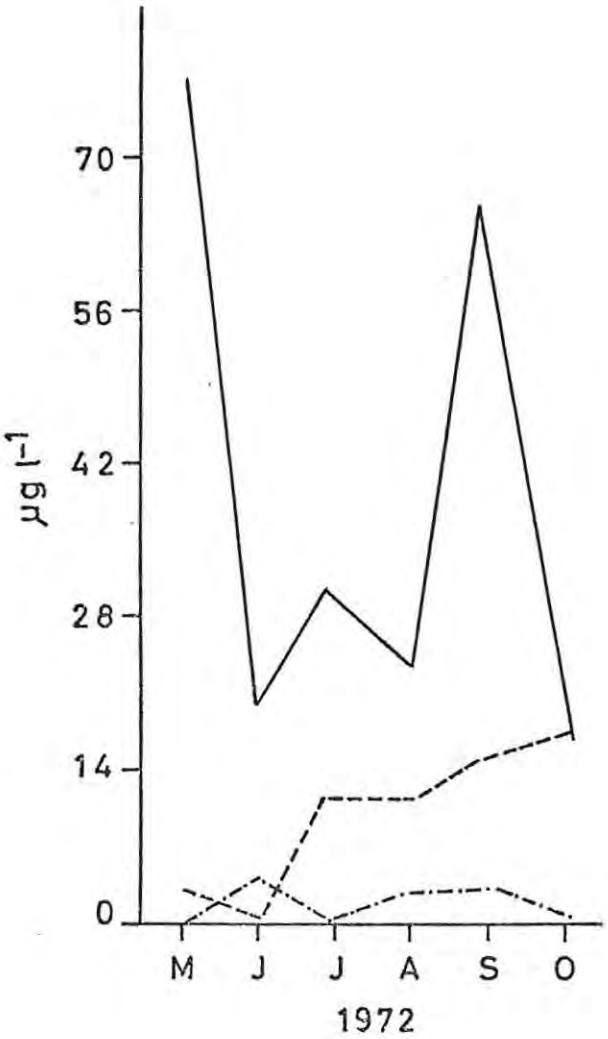


Fig. 23: Chemical analysis of the middle reaches (roadbridge station). Broken line, PO_4-P; broken line and dots, NH_3-N; solid line, NO_3-N.

increase may have been related to increased phytoplankton decomposition at the end of a long growth phase (Fig. 19). After July ammonia decreased, with the exception of August and October at 8 m, to zero by December 1972 (Fig. 21). This decrease was related to the increased oxygen saturation in the water column (Fig. 15) which was the result of turbulent mixing and increasing primary productivity (Fig. 34). The ammonia concentration increase in August 1972 was related to the microbial decomposition of detritus brought in by the flood. The high ammonia value at 8 m in October 1972 was the result of the sample being taken from a level of very low oxygen saturation (Fig. 15).

Nitrate (Fig. 22) generally showed an increase from July to December 1972. This may have been due to such factors as increased transfer of ammonia from the monimolimnion into the mixolimnion and its biochemical oxidation to nitrate and the rate of phytoplankton and detritus decomposition. Different rates of utilization may partially explain the variation in nitrate concentration with depth. The decreased nitrate concentration at 1-3 m in December possibly was the result of the sustained high rate of primary production (See Fig. 34).

Chemical analysis of the middle reaches

The results of the chemical analysis of the middle reaches in 1972 are shown in Fig. 23. The PO_4-P values were similar to those recorded in the upper reaches and showed the same type of monthly pattern (Fig. 19). The NH_3-N concentrations, however, were lower than those found in the upper reaches, probably a result of high oxygen concentrations in this shallow (5 m) area, and had a range of 0 to 5.75 $\mu g\ l^{-1}$. Monthly variation was similar to that of the upper reaches. NO_3-N concentrations were also similar to those recorded in the upper reaches. However, in May a concentration of 77.2 $\mu g\ l^{-1}$ was recorded and in September, 65.6 $\mu g\ l^{-1}$. In the salt marsh area of the middle reaches horses and cattle are allowed to graze. These very high nitrate values are probably related to urine and faeces from these animals. The middle reaches may have provided a source of nitrogen for

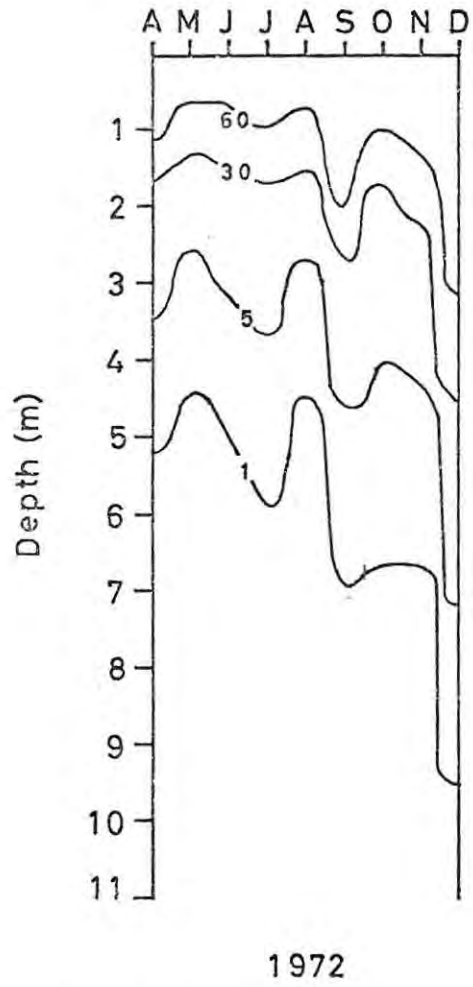


Fig. 25: Percentage transmission of light.

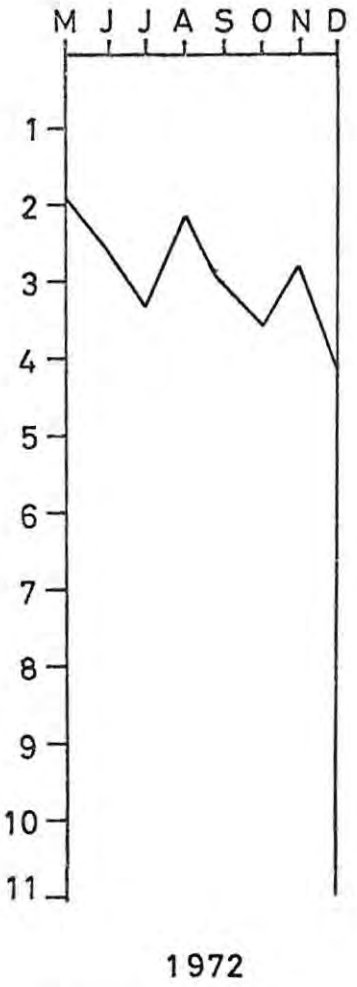


Fig. 24: Secchi disc.

the upper reaches as indicated by the increase of NO_3-N at 1 and 2 m in May and at 1 and 3 m in September (Fig. 22).

The carbohydrate values (not shown in Fig. 23) obtained for the middle reaches ranged between 0 and 2.7 mg l^{-1}. Except for May when the reading was 2.7 mg l^{-1} and July when it was 2.46 mg l^{-1} carbohydrate was not detected by the anthrone method.

Light penetration and transparency

Hutchinson (1957) points out that Secchi disc transparency roughly corresponds to a light intensity of 5-15% of that at the surface. This measurement depends on the ratio of the intensity of light scattered from around the disc to light reflected off the disc and scattered upward above the disc. The value obtained, however, is affected by such factors as surface reflections, clearness of the sky and water colour (Tyler 1968).

The Secchi disc values (Fig. 24) and percentage transmission of white light (Fig. 25) were plotted from the surface downwards, i.e. in the opposite manner in which the other isopleth graphs were done. This was because the variations in the depth of the water column did not affect penetration and transparency since they were a function of depth from the surface. The other physico-chemical variables, however, change with changes in overall depth.

The minimum Secchi disc reading between May-December 1972 in the upper reaches of Swartvlei was 2.0 m in May and the maximum reading 4.1 m in December (Fig. 24). In August 1972, after a rainfall of 32.2 mm, Secchi disc transparency decreased to 2.1 m. This indicated that transparency and hence light penetration was extremely sensitive to changes in humic freshwater inflow.

A comparison of Fig. 25 with Fig. 24 shows that the Secchi disc value, with the exception of September, November and December, corresponded to 8-14% of surface illumination. In September 1972 the Secchi disc reading equalled 27% of the surface light intensity, in November, 19% and December, 42%. The reason for these large percentage

transmission values of the Secchi disc readings is not known at this time. Hutchinson (1957) noted that it is possible for two lakes to exist, one more transmissive and less transparent than a second which is less transmissive and more transparent. The difference between these two lakes was that the first was more turbid and less coloured than the second. Shapiro (1957) in his study of the humolimnic acids of lake water pointed out that the yellow materials must play a very important role in determining the quantity and quality of light reaching various depths. The role of these acids in Swartvlei and their effect on light penetration and transparency, and indeed on the system as a whole, needs to be examined in detail.

The 1% level of white light penetration, which usually marks the lower limit of the euphotic zone Talling (1962), varied between 4.4 m and 9.6 m from April to December 1972 (Fig. 25). In August the 1% level was raised to 4.4 m after the flood.

The general increase through 1972 of light penetration and Secchi disc value may be related to the increasing salinity of Swartvlei (Fig. 13a). and decreased inflow of freshwater due to low rainfall (Fig. 13b). The highest Secchi disc value recorded in Swartvlei was 5 m recorded July 1952 when the salinity was 13.74 $^{o}/oo$ (Martin 1962). In 1972 the highest Secchi disc value was 4.1 m (Fig. 24) when the salinity was the highest value recorded (11 $^{o}/oo$ Fig. 13a). Laboratory experiments have shown that suspended matter in rivers is flocculated and deposited where it reaches salt water (Meade 1968). Meade noted that although the suspended matter may be flocculated, as indicated by the laboratory results, it may not be deposited in estuaries where velocities and internal shears are strong enough to inhibit settling or to disrupt the flocculated aggregates. In the estuaries studied by Meade the frequently observed seaward decrease in suspended matter could be attributed to progressive downstream dilution by sea water rather than salt flocculation. In Swartvlei most suspended matter was probably deposited on the bottom as was indicated by the large amount of

flocculated bottom malm or sediment. The extent of light penetration in the upper reaches, therefore, may be the result of five interacting factors: (1) humate staining, (2) the influx of freshwater, (3) the inflow of sea water, (4) water turbulence and (5) the loss of suspended matter to the middle reaches.

Biological Limnology

Energy entering an aquatic ecosystem arrives mainly in the form of sunlight and is converted into organic matter by the photosynthetic organisms (Brock 1966). Some of this energy is consumed by the primary producers themselves, during respiration; the rest is passed on to other components of the system. There are two main channels of energy flow, one to the herbivores (zooplankton) and the other to the decomposers (bacteria). In order to analyse the biological data from this study it seems logical to follow the flow of energy in the ecosystem. The obvious starting point in the analysis is the phytoplankton and primary productivity since data on photosynthetic bacteria were not collected. The aerobic heterotrophic bacterial population and its role in the uptake of acetate and glucose will then be examined followed by a discussion on the daytime pelagic zooplankton population. The purpose of this section is to relate the different biological components to one another while at the same time examining the governing role of the physico-chemical limnology on the biology of the upper reaches of Swartvlei.

Phytoplankton- classification and seasonal cycles

Three major categories of phytoplankton were recorded: diatoms, dinoflagellates and flagellates. The diatoms were identified by Professor M.H. Giffen, Department of Botany, University of Fort Hare, Alice, South Africa. The centric diatom, Coscinodiscus lineatus Ehrenberg was the dominant organism. This species of Coscinodiscus is found in estuarine and marine habitats in South Africa (Giffen 1970). Other diatoms found in varying abundance during the study are listed in Table 2.

TABLE 2

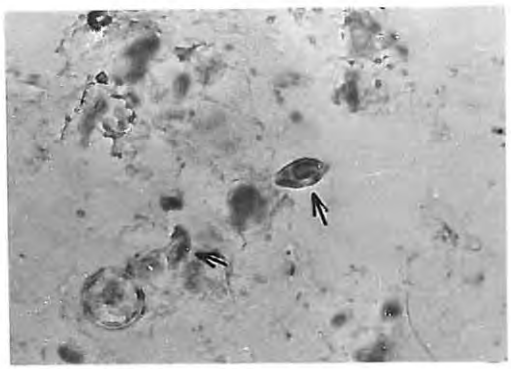
Some of the diatoms occurring in the upper reaches of Swartvlei between 1971-73. Identification by Professor M.H. Giffen, University of Fort Hare.

Diatom	Habitat
Chaetoceras (Sp.)	estuarine
Chaetoceras wighamii Brightwell	estuarine
Cocconeis scutellum Ehrenberg	estuarine
Coscinodiscus lineatus Ehrenberg	estuarine-marine
Grammatophora oceanica (Ehr.) Grun. var. Macilenta	marine
Grammatophora sepentina (Ralfs) Ehr.	marine
Navicula pseudony Hust.	littoral
Raphoneis mirabunda Giffen	estuarine
Raphoneis superba (Janisch) Grunow	estuarine
Synedra tabulata (Aq.) Kutzing	indifferent

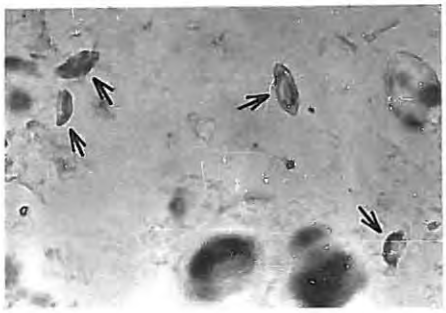
The chlorophyllous flagellates and dinoflagellates were not identified. Included in the flagellate counts were Volvox and a few silicoflagellates. These organisms formed only a minor part of the plankton and were infrequently seen and never reached a concentration greater than 1×10^4 cells 1^{-1}. No ciliates were found at any time.

A dinoflagellate species shift was believed to have occurred between December 1971 and January 1972. In December the dominant dinoflagellates had an irregular diamond shape whereas those present from January to the end of the study had an ovoid shape typical of estuarine dinoflagellates (Lackey 1967). The population shift and consequently the absence of the irregular diamond-shaped dinoflagellate species in 1972 may have been due to the 3 °/oo salinity increase of the upper water column between December 1971 and January 1972 and the maintenance of the salt concentration between 9-11 °/oo in 1972 (Fig. 13a).

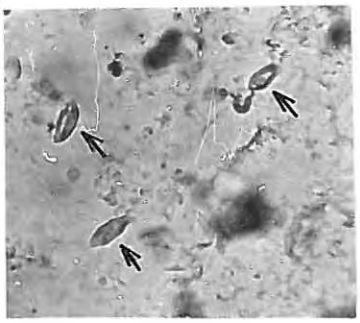
The average size of the flagellates was 10 to 12 µm (Fig. 26) while the dinoflagellates (after December 1971) measured 14 to 18 µm



Oct. 19/71
2 m



Oct. 20/71
8 m - night



Oct. 20/71
8 m - night

Fig. 26: Flagellates, indicated by arrows, on counting filters. Magnification 12 x 40.

Scale: ⊢⊣
10 µm

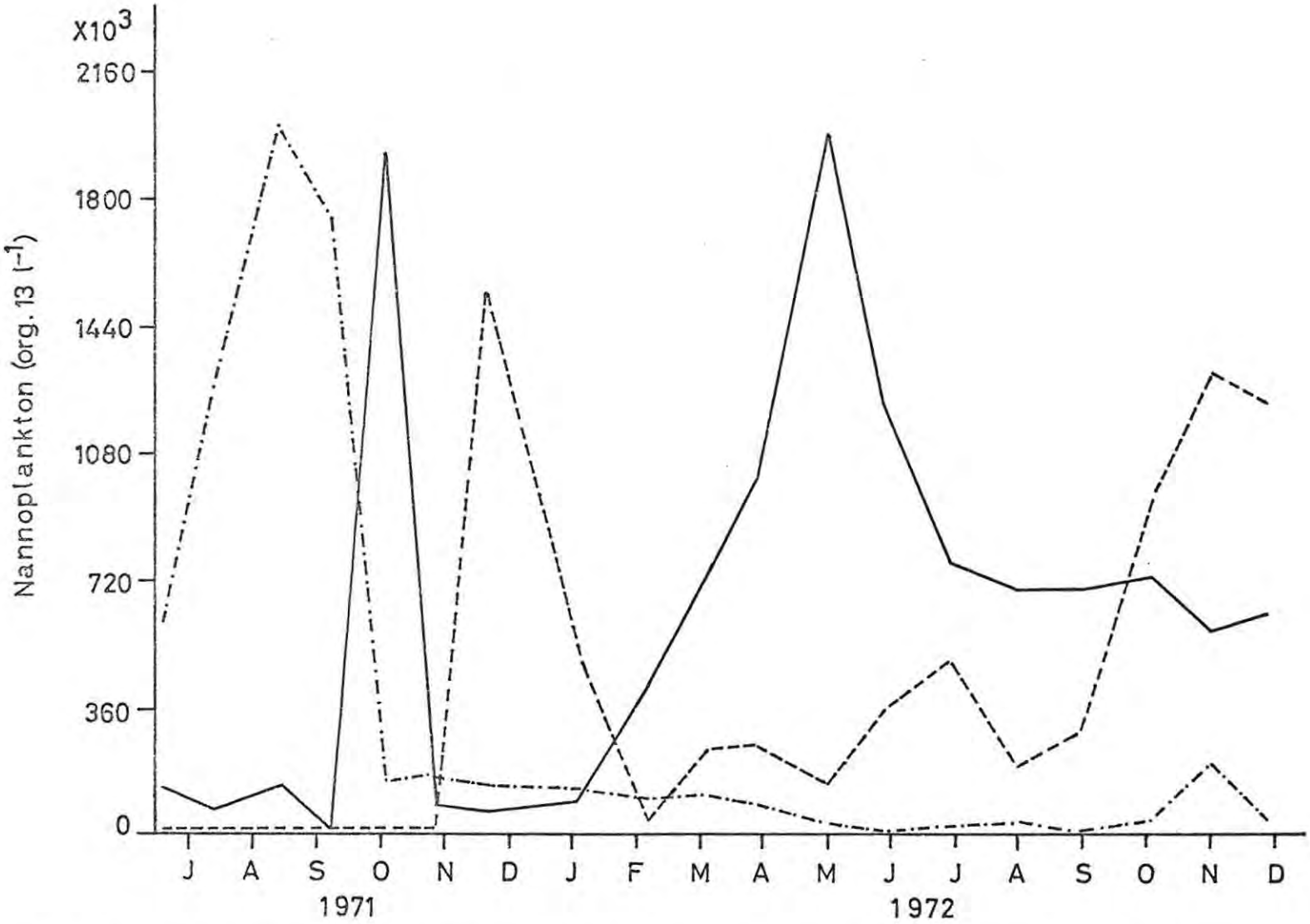


Fig. 27: Diatom-dinoflagellate-flagellate total population seasonal cycles in the upper reaches of Swartvlei. Solid line represents diatoms; broken line, dinoflagellates; and broken line and dots, flagellates.

along their longest axis. The diatoms varied most in size, having a diameter range of 18 to 60 μm, while the majority of cells measured 30-40 μm. All the phytoplankton could be classified as nannoplankton, i.e., organisms less than 60 μm.

In the text, phytoplankton densities at each level in the water column are expressed as organisms per litre. As 13 samples were taken through the water column the integral expression, or total population, is recorded as organisms per 13 litres. The seasonal changes in phytoplankton number and type, taken over the entire water column sampled, are given in Fig. 27. The details of the successions which have been observed are more clearly seen by examination of the density-depth-time diagrams, Figs 28, 30, 31.

From July to September 1971 the flagellates were definitely the dominant organisms, reaching a maximum of 2×10^6 cells 13 1^{-1} in August. In October there was a sharp decrease in flagellate numbers followed by an increase in the diatom population. November 1971 the smallest total phytoplankton population during the study was recorded. By November the diatom population had decreased drastically and the flagellate population was marginally the dominant group. This was the last time during the study, with the exception of November 1972, that the flagellates showed signs of increasing.

The major phytoplankton changes occurred between the dinoflagellates and diatoms from December 1971 to the end of the study (Fig. 27). Until December 1971 the dinoflagellates remained at less than 5×10^3 cells 1^{-1}. In December they rose to 154×10^4 cells 13 1^{-1} and although by January they had decreased in number, they still remained the dominant organisms. Between December and January a change in the dinoflagellate species occurred. The second species of dinoflagellates showed a small increase in March-April and June-July before finally dominating the phytoplankton population in October 1972. The diatoms, meanwhile, had increased steadily from February 1972 to a maximum total population of 199×10^4 cells 13 1^{-1} in May. From

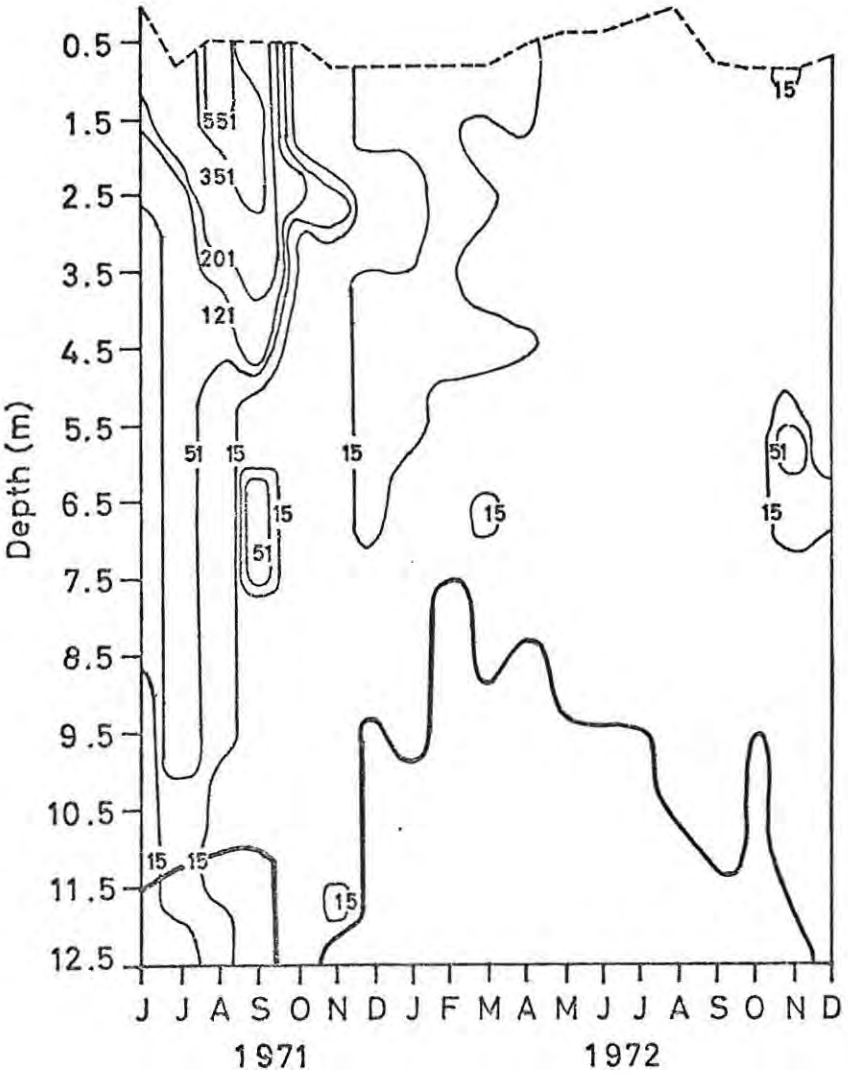


Fig. 28: Density-depth-time diagram of the flagellates in the upper reaches of Swartvlei. (Org. $1^{-1} \times 10^{3}$). Thick line represents aerobic-anaerobic interface.

this peak they declined to August after which the population remained fairly constant.

Flagellate-diatom succession

Fig. 28 the vertical distribution of flagellates shows that in July 1971 the organisms reached a maximum at about 1 m. This peak shifted to the surface in August, after the flood, and increased to 810 x 10^3 cells l^{-1}. The flagellate population decreased steadily with depth. This upward vertical migration was a response to decreased light penetration. In the months following August 1971 moderately large numbers of flagellates could be found at increasing depths. Although the reason for this was unclear it may have been partially attributable to increasing light penetration. In October 1971 the flagellate population peak was located at 2.5 m, moving down to 3 m in November (Fig. 28). During the rest of the study flagellate population numbers remained insignificant except at 5.5 m in November 1972 when the population increased to 84 x 10^3 cells l^{-1} and light penetration was only 2.7% of the surface value (Fig. 25).

In October 1971 sea water entered the upper reaches (Fig. 13a). In December 1972 a similar inflow of sea water occurred and one of the changes in the water noted was a significant increase in the percentage transmission of light (Fig. 25). A similar effect may have occurred in October 1971 and as a result of the increased light penetration the flagellates may have moved to the mud-water interface or into the upper layers of the bottom sediments. The oxygen concentration was very low in this area (Fig. 15). However, certain species of phytoflagellates such as Cryptomonas are commonly encountered in proximity to black muds (Hutner and Provasoli 1951).

Night sampling of the nannoplankton population in October 1971 showed a population change from the daytime distribution. Fig. 29 shows that the flagellate population increased in the water column at night. The most striking increase, however, was the population peak of 584 x 10^3 cells l^{-1} at 8 m. This population increase could have

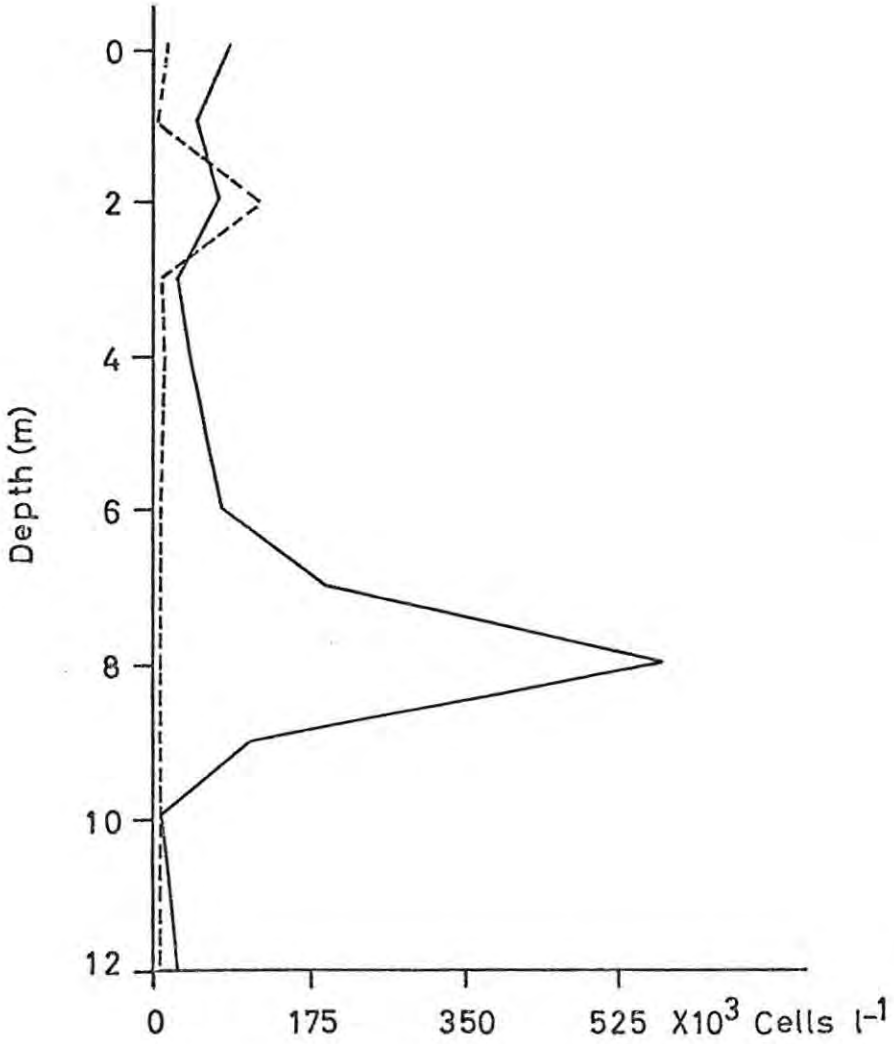


Fig. 29: Flagellates - daytime and night-time populations, October 19, 1971. Broken line, 10:00 a.m.; solid line, midnight.

only come from the bottom sediments which further suggests that the flagellates left the water column because of increased light penetration. Hutner and Provasoli (1951) were uncertain whether facultative anaerobic phytoflagellates left the anaerobic bottom water at night and migrated to the oxygen rich surface. The answer would seem to be positive with regards to the flagellates in Swartvlei if, in fact, they were living anaerobically. A large flagellate population was found living in the anaerobic mud of Swartvlei. Whether these flagellates were the same as the planktonic organisms was not known. In February 1972 another night collection of nannoplankton samples indicated no vertical migration. It was significant that in October 1971 the water was homothermal at 18°C (Fig. 14) and that oxygen was present to the bottom (Fig. 15). In February there was a temperature gradient with a bottom temperature of 20°C and the anaerobic zone extended up to 7.5 m. The facultative anaerobic capabilities of the pelagic flagellates in Swartvlei are questionable. The role of oxygen and temperature in regulating the migration of flagellates in the upper reaches requires further research.

In November 1972 Professor I. Manton, FRS, of the University of Leeds visited South Africa to collect planktonic flagellates in the waters off Cape Town. After several sampling trips she was distressed to find almost no flagellates suggesting that perhaps some error had occurred in her technique for extracting these organisms from the water. Large numbers of diatoms were present. When I joined Professor Manton (Nov. 7-10) I suggested, on the basis of results obtained in Swartvlei, that light was possibly the major factor controlling the seasonal cycles and vertical distribution of flagellates in the water column. I suggested that rather than sample from the surface, as she had been doing, she should take samples from deeper levels or from water in which there was a large quantity of suspended matter. This suggestion was taken up. However, samples taken by divers at 30 m produced no increase in the flagellate

population. While waiting for the ship to take us out on the collecting trip it was noted that the water where a dredger was in operation contained a high concentration of suspended matter. A bucket was lowered into the water and the sample was taken back to the lab. When Professor Manton examined this sample it was found to have a very large planktonic flagellate population. This seemed to support the theory that light was a major controlling factor in the succession and vertical distribution of flagellates in Swartvlei.

The flagellate population decreased in October 1971 and the total diatom population increased markedly (Fig. 27). Javornicky (1966) has shown that light was the main factor limiting the development of diatoms in the Slapy Reservoir. The passively floating diatoms with a specific gravity greater than that of the brown-coloured water were unable to maintain themselves within the narrow (3 m) euphotic zone of the Slapy Reservoir as the actively migrating flagellates were able to do. The replacement of a flagellate population with a diatom population in Swartvlei further reinforces the theory that light conditions in the upper reaches had significantly improved by October 1971 and that light was a major factor controlling the absence of flagellates in the water column after September 1971. Other factors such as organic constituents (vitamins, etc) dissolved in the water may also have been involved. These substances play an important role in regulating phytoplankton species distribution, succession and blooms, and they exert their influence at extremely low concentrations (Gold 1967).

Diatom-dinoflagellate succession

The factors regulating the succession of dinoflagellates after a diatom maximum generally are not clearly understood. Van Niel (1955) stated that Barker's work in 1935 showed dinoflatellates follow a diatom peak due to a decrease in the phosphorous and nitrogen concentrations. Margalef (1960) would agree that this would be a major factor controlling this type of succession although Martin

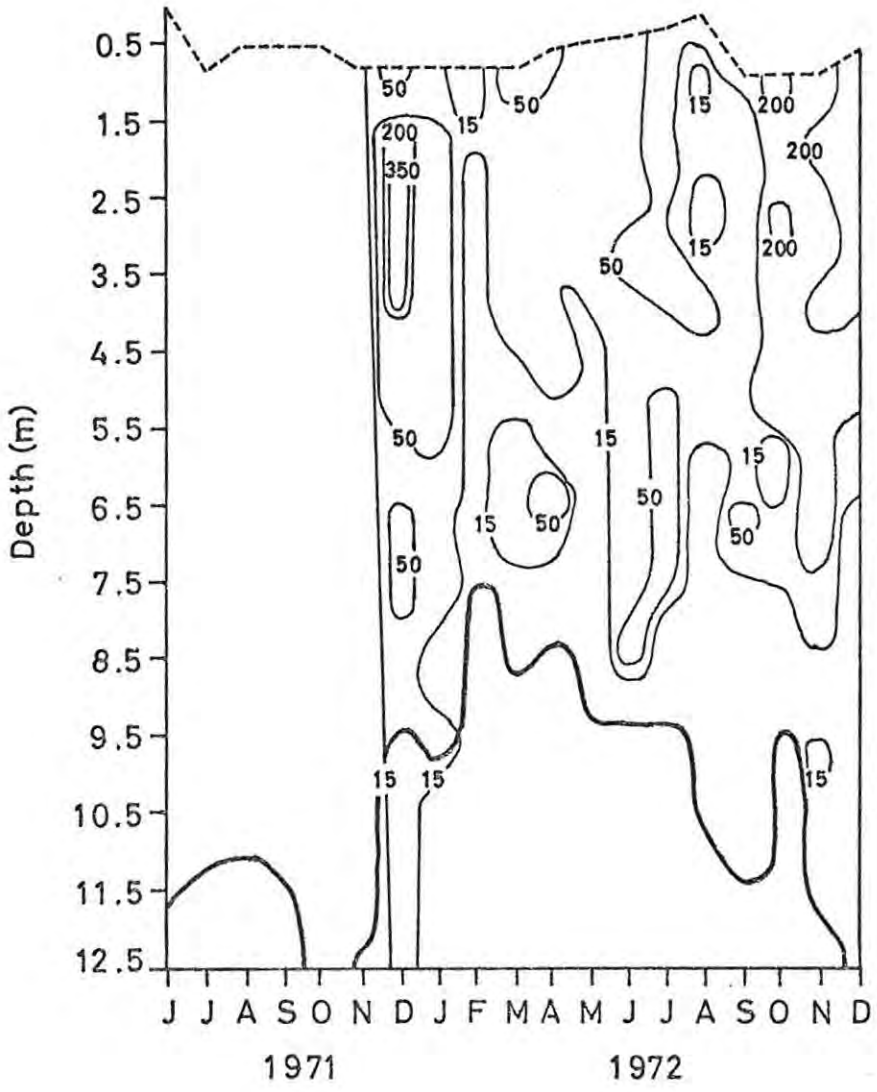


Fig. 30: Density-depth-time diagram of dinoflagellates in the upper reaches of Swartvlei (Org. $1^{-1} \times 10^{3}$). Thick line represents aerobic-anaerobic interface.

(1970) might place more emphasis on the silicate concentration. If a low phosphate concentration was responsible for the dinoflagellate increase in Swartvlei a peak would be expected to have occurred in June 1972 when the PO_4-P concentration was at its lowest due to the large diatom population in May (Figs 19, 27). A small dinoflagellate increase did occur between June and July but the population never dominated the total algal population. From October to December 1972, however, the dinoflagellates achieved dominance but the phosphate concentration had reached its highest levels of the year. The nitrate concentration (Fig. 22) remained at relatively high levels throughout the year. It would seem as Riley (1967) has noted for studies of other systems, that neither phosphate or nitrate were the sole controlling factors of diatom-dinoflagellate succession in Swartvlei.

It was noted that both major dinoflagellate maxima in Swartvlei occurred when the water temperature rose above 21°C (Figs 14, 27). Pomeroy et al (1956) found that the series of dinoflagellate blooms in Delaware Bay occurred only when the surface water temperature was between 25 and 29°C. When samples from the blooms were brought into the laboratory they showed marked positive phototaxis. Barker (1935) found that cultures of dinoflagellates reached their maximal division rates at relatively high light intensity (the intensity of a 60 w lamp at a distance of 2 cm). Rhyther (1956) reported the optimum radiation for growth in diatoms was 0.07 ly min^{-1} and for dinoflagellates 0.16 ly min^{-1} (Qasim et al 1972).

The dinoflagellates in Swartvlei generally maintained maximum numbers within the upper 4 m of the water column (Fig. 30). Pomeroy et al (1956) found the dinoflagellate blooms in Delaware Bay were always limited to the surface layer of the bay. Although the total dinoflagellate population in Swartvlei increased with increasing transparency, as in July and October 1972 (Figs 24, 30) this would seem more coincidence than significant correlation. That other factors were involved in the production of large dinoflagellate populations

was suggested by the dinoflagellate decrease in December 1972 (Fig. 27) when the water temperature was still increasing and transparency and light penetration were at a peak (Figs 14, 24, 25). In January 1972 when the temperatures in the upper water column were the warmest recorded the total dinoflagellate population decreased.

Riley (1967) concluded in his paper on the plankton of estuaries that we are still very far from fully understanding the dynamics of seasonal succession. The present study must also conclude on the same note as regards the shift from diatoms to dinoflagellates in Swartvlei. Smayda (1963) would refer to Swartvlei as a holocoenotic environment, i.e., an environment in which the observed phytoplankton dynamics are the resultants of the collective, simultaneous and interdependent action of many parameters. The combined action of these variables is expressed in the recurrent succession and cyclic abundance of phytoplankton.

Nannoplankton vertical distribution

In Swartvlei, dinoflagellates and flagellates (see Fig. 29) may migrate to optimum light and nutrient conditions. The ability of dinoflagellates to perform active vertical migration is generally well known in the marine environment but less documented in lacustrine systems (Talling 1971). In Windermere, Talling has shown that the vertical movements of Ceratium allowed the cells to pass through the metalimnion below the boundary of the euphotic zone. These organisms constantly avoided the deoxygenated hypolimnion.

In December 1971 the dinoflagellates (Fig. 30) in Swartvlei were found in the anaerobic monimolimnion where a peak of 47×10^3 cells l^{-1} occurred at 10 m. Numbers decreased below this but at the mud-water interface 26×10^3 cells l^{-1} were still present. These were probably dying cells which had fallen through the halocline. At all other times the dinoflagellates were located above the anaerobic zone (Fig. 30). The flagellates were also found in significant numbers below the halocline where oxygen was greatly reduced (Figs 28, 31).

The approximate upper and lower limits of the halocline.

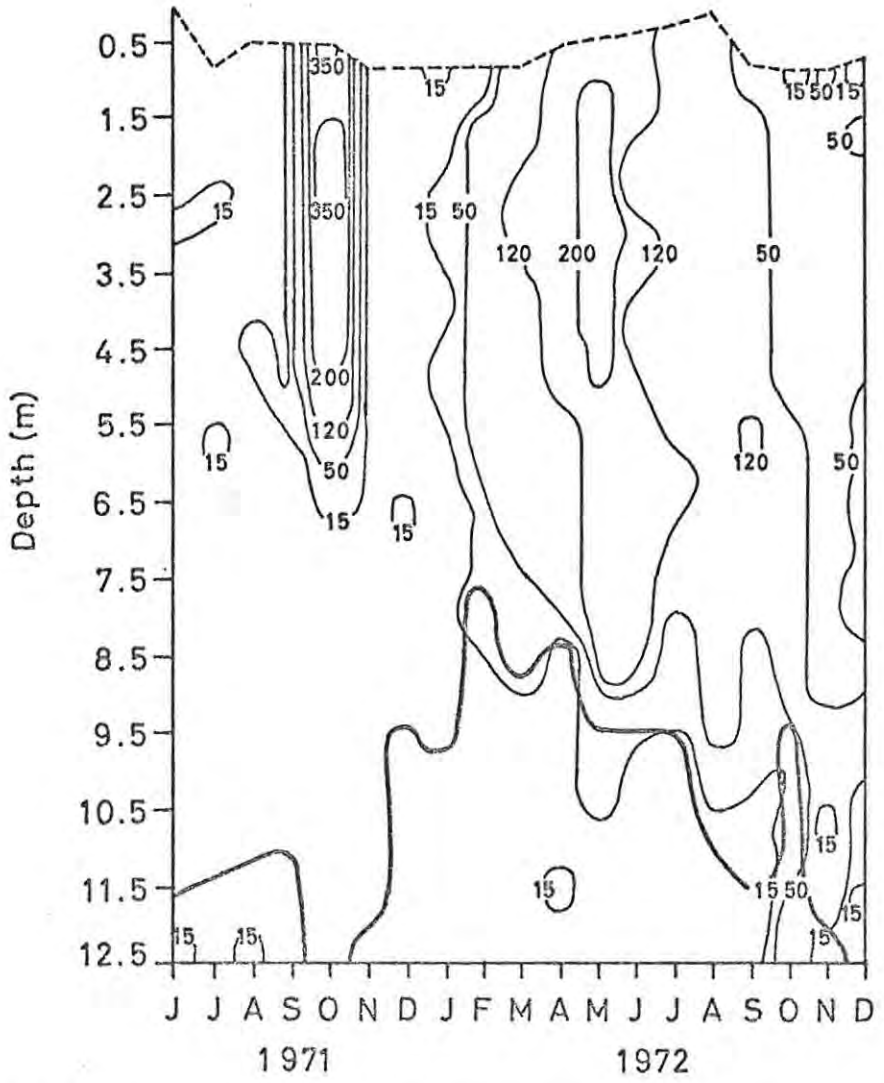


Fig. 31: Density-depth-time diagram of diatoms in the upper reaches of Swartvlei (Org. $l^{-1} \times 10^3$). Thick line represents aerobic-anaerobic interface.

Flagellates found in the anaerobic zone never exceeded 16 x 10^3 cells l^{-1} and represented either dying, sinking cells or flagellate species capable of living in this type of environment. There are a number of such species (Hutner and Provasoli 1951; Brand 1951).

The non-motile diatoms showed a vertical distribution which was the result of the distribution of salinity and oxygen (Fig. 31). The concentration of living diatoms decreased towards the halocline and, with the exception of January-May and October-December 1972, dropped off sharply below it since the halocline usually marked the boundary of the anaerobic zone. Diatoms rely on water currents to maintain themselves in the euphotic zone. The sinking rate of Coscinodiscus is greatly reduced due to its "pancake-like" shape which causes the diatom to sink sidewise with a fluttering motion (Davis 1955).

Between October and December 1972 relatively large numbers of living diatoms were found in the monimolimnion (Fig. 31). In November and December the greatest concentration of the diatom population occurred below the halocline (Fig. 31). Also in November and December, sea water entered the upper reaches mainly below the halocline. It was probable that the monimolimnetic diatoms found in October-December were brought in from the middle reaches and from the sea. The presence of the marine diatom, Grammatophora, in the upper reaches supports this assumption (Giffen 1973; pers.comm.). The two species of Grammatophora could act as a tracer for water movements in Swartvlei.

Primary productivity

Primary productivity in the upper reaches of Swartvlei during 1972 is shown in the depth-time diagram, Fig. 32. The data in this figure have been plotted from the surface down, as in the figures for light transmission and transparency. This was done because primary productivity is not affected by the changes in depth of the system but by the extent of light penetration from the surface. Fig. 32 shows that phytoplankton fixation of inorganic carbon below the 1% level was

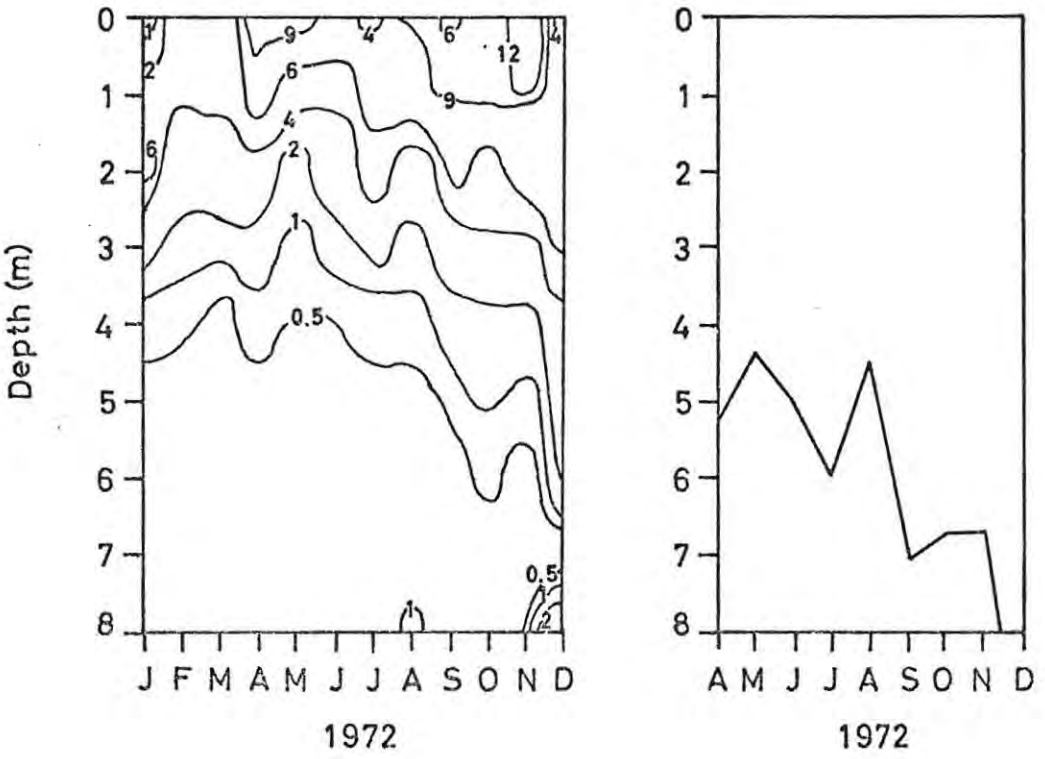


Fig. 32: Depth-time diagram of primary productivity in the upper reaches of Swartvlei (mg C m^{-3} h^{-1}). Right figure, the 1% level of light penetration.

insignificant (See also Table 3). Between January and August 1972 the euphotic zone was approximately 4.5 m deep while from September to the end of the year, with the exception of November, it was extended to deeper levels.

The area enclosed by each depth profile in Fig. 33 is a measure of the integral rate of photosynthesis per unit area of lake surface (Σ a, Table 4). Vollenweider (1965) noted that gross production rates (units of g O_2 m^{-2} h^{-1}) depend, among other factors, upon phytoplankton density, species composition, age and physiological stage as well as on such environmental properties as incident light, light attenuation in the water, temperature and nutrient conditions. Presumably these factors also affect the primary productivity rate as measured by the ^{14}C method, of which the 'gross' or 'net' status is still open to question (Talling et al 1973).

For the comparison of the increasing amount of data on primary productivity and its interpretation Rodhe (1965) suggested that a mathematical but simple rule of general application for the quantitative evaluation of similarities and differences in the pelagic photosynthesis of various water is needed. Although it was not possible to calculate all of the variables suggested by Rodhe for Swartvlei with the available light data, the quotient of maximal assimilation per m^3 (a_{max}) and total assimilation per m^2 (Σ a) was calculated. This quotient is an index of different productivity depth profiles for different lakes and on the whole decreases with increasing transparency (Rodhe 1965). In Table 4 the quotient expressed as a percentage is shown for each month in 1972 and compared to the Secchi disc values. With the exception of October the quotient decreased with increasing transparency. As transparency increases the depth of the euphotic zone increases. Hence a_{max} represents a smaller percentage of the integral photosynthesis in the water column. This is demonstrated by comparing Figs 25 and 32 for August and December 1972. In August a_{max} was 9.946 mg C m^{-3} h^{-1} while Σa was 24.494 mg C m^{-2} h^{-1} for a euphotic zone that extended to 4.5 m. In

TABLE 3

Primary productivity (mg C m^{-3} h^{-1}) down to 8 m in the upper reaches of Swartvlei during 1972.

Depth	January 19 1972	February 23 1972	March 22 1972	April 12 1972	May 17 1972	June 14 1972	July 13 1972	August 16 1972	September 13 1972	October 18 1972	November 15 1972	December 13 1972
Surface	1.003	——	5.223	9.076	10.290	8.475	4.825	9.946	6.675	11.118	13.070	4.544
1 m	2.392	4.518	4.798	8.002	4.905	4.375	6.453	7.745	8.999	9.526	12.719	7.163
2 m	6.028	2.887	2.672	3.404	1.747	2.641	5.466	3.189	7.250	5.332	7.762	8.058
3 m	2.822	1.928	1.200	1.554	0.798	1.182	2.628	1.336	3.554	3.684	3.608	6.822
4 m	0.716	0.612	0.543	0.692	0.533	0.533	0.676	0.632	1.495	1.213	1.366	3.943
5 m	0.276	0	0.211	0.273	0.120	0	0.350	0.427	0.613	1.142	0.924	3.429
6 m	0.111	0	0.028	0.0097	0	0.112	0	0.207	0.158	0.653	0	2.307
7 m	0.207	0	0.015	0.111	0.023	0.083	0	0.250	0.370	0.106	0.110	0.304
8 m	0	0	0	0.362	0	0.042	0.033	0.762	0	0	0.101	2.683

December a_{max} was 8.058 mg C m^{-3} h^{-1} while Σ a was 39.252 mg C m^{-2} h^{-1} for a euphotic zone that reached to greater than 8 m.

TABLE 4

The relationship between the quotient a_{max} (maximal assimilation per m^3) and Σ a (total assimilation per m^2) and Secchi disc transparency in the upper reaches of Swartvlei in 1972.

Month	a_{max}	Σ a	$\frac{a_{max}}{a}$ %	Secchi disc (m)
January	6.0281	13.555	44.47	—
February	5.000	14.945	33.46	—
March	5.223	14.886	35.09	—
April	9.076	23.484	38.65	—
May	10.290	18.416	55.88	2.0
June	8.475	17.443	48.59	2.6
July	6.453	20.431	31.58	3.4
August	9.946	24.494	40.61	2.1
September	8.999	29.114	30.91	3.1
October	11.118	32.774	33.92	3.6
November	13.070	39.660	32.96	2.8
December	8.058	39.253	20.53	4.1

It would seem that this simple calculation is applicable to the pelagic photosynthesis in the upper reaches of an estuary with heavy humate staining. However, a comparison of the quotients calculated by Rodhe for 12 northern hemisphere lakes shows that the quotients for Swartvlei corresponded most closely to those for eutrophic lake Erken (quotient 22) and highly eutrophic Lago di Varese (quotient 35).

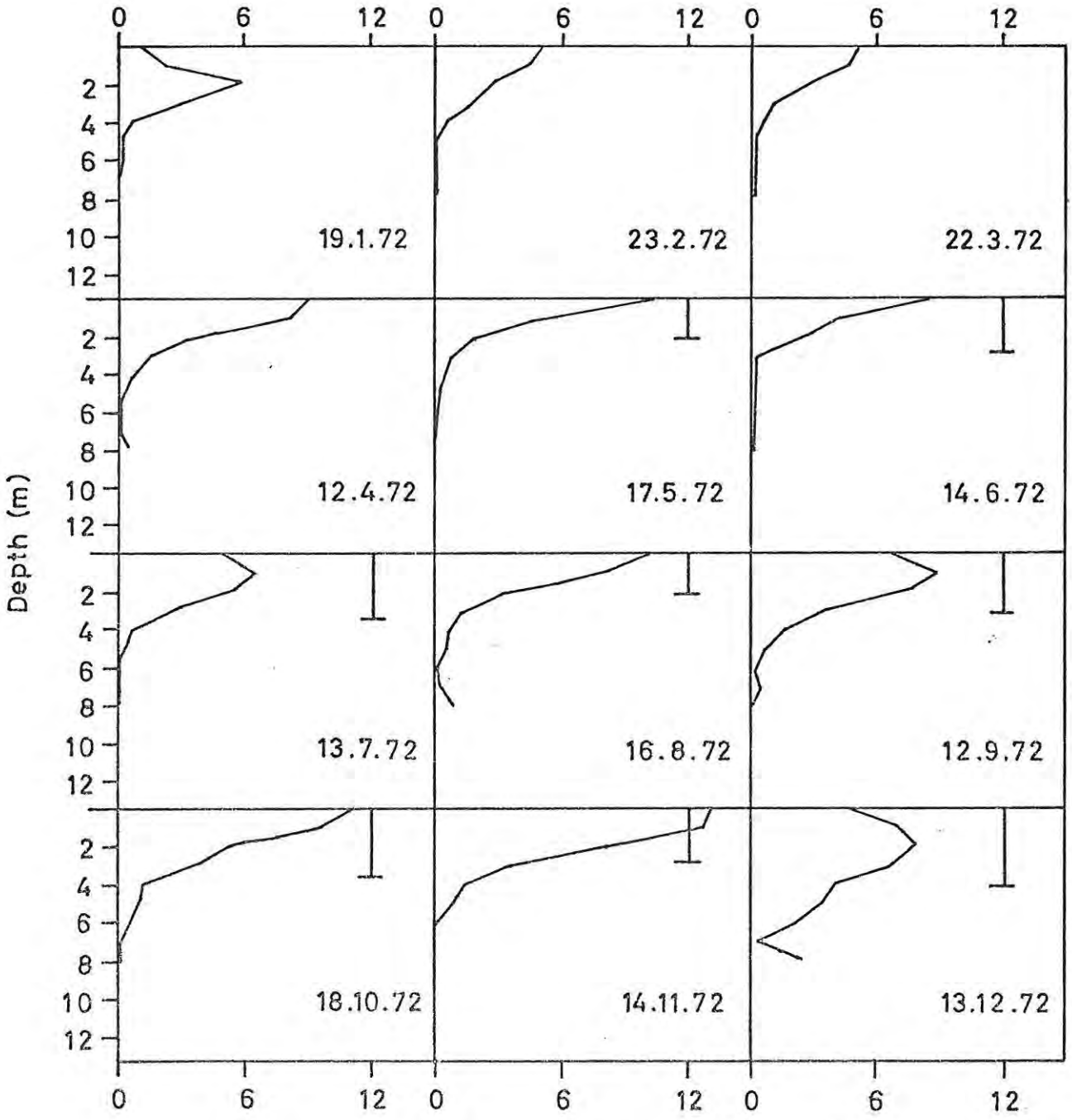


Fig. 33: Depth profiles of primary productivity (mg C m^{-3} h^{-1}) to 8 m the upper reaches of Swartvlei in 1972. Secchi disc reading is also indicated.

Oligotrophic lake Tornetrash in Swedish Lappland had a quotient of 9. According to Rodhe the relationship between the quotient and transparency depends primarily on turbidity and not on trophic level, although the separation of these two effects is difficult, if not impossible. Swartvlei, with its small phytoplankton population, would seem to have light conditions which were usually more severe than in the most eutrophic lakes studied by Rodhe (except perhaps during northern hemisphere winters). This was due to the heavy humate staining and suspended detrital matter in Swartvlei.

The shape of the depth profiles in Swartvlei (Fig. 33) conforms to the general form well estblished in other work on phytoplankton photosynthesis in lakes (Rodhe 1965; Talling et al 1973). In January, July, September and December the curves show a surface depression due to higher light intensities. Fig. 33 indicates that surface light inhibition did not occur when transparency decreased, e.g., July-August, August-September and November-December. This allowed photosynthesis to proceed at maximum rates in the upper few meters with the consequence that although it was reduced at lower levels, the overall assimilation may have increased, e.g., August and November (Figs 32, 34).

The relationship between integral primary productivity and the total nannoplankton population in the upper 8 m of the water column in 1972 is shown in Fig. 34. Although the maximum nannoplankton population was recorded in May primary productivity decreased between April and May. Fig. 25 indicates that light penetration was substantially greater in April than in May. The decrease in light penetration reduced significantly the degree of inorganic carbon fixation below the surface (Fig. 32). This effect was unlike that just described for August and November. This would seem to indicate that other factors were involved in the lower primary production at the time of the largest nannoplankton maximum. A large proportion of the algal cells may have been in later growth stages in May and were

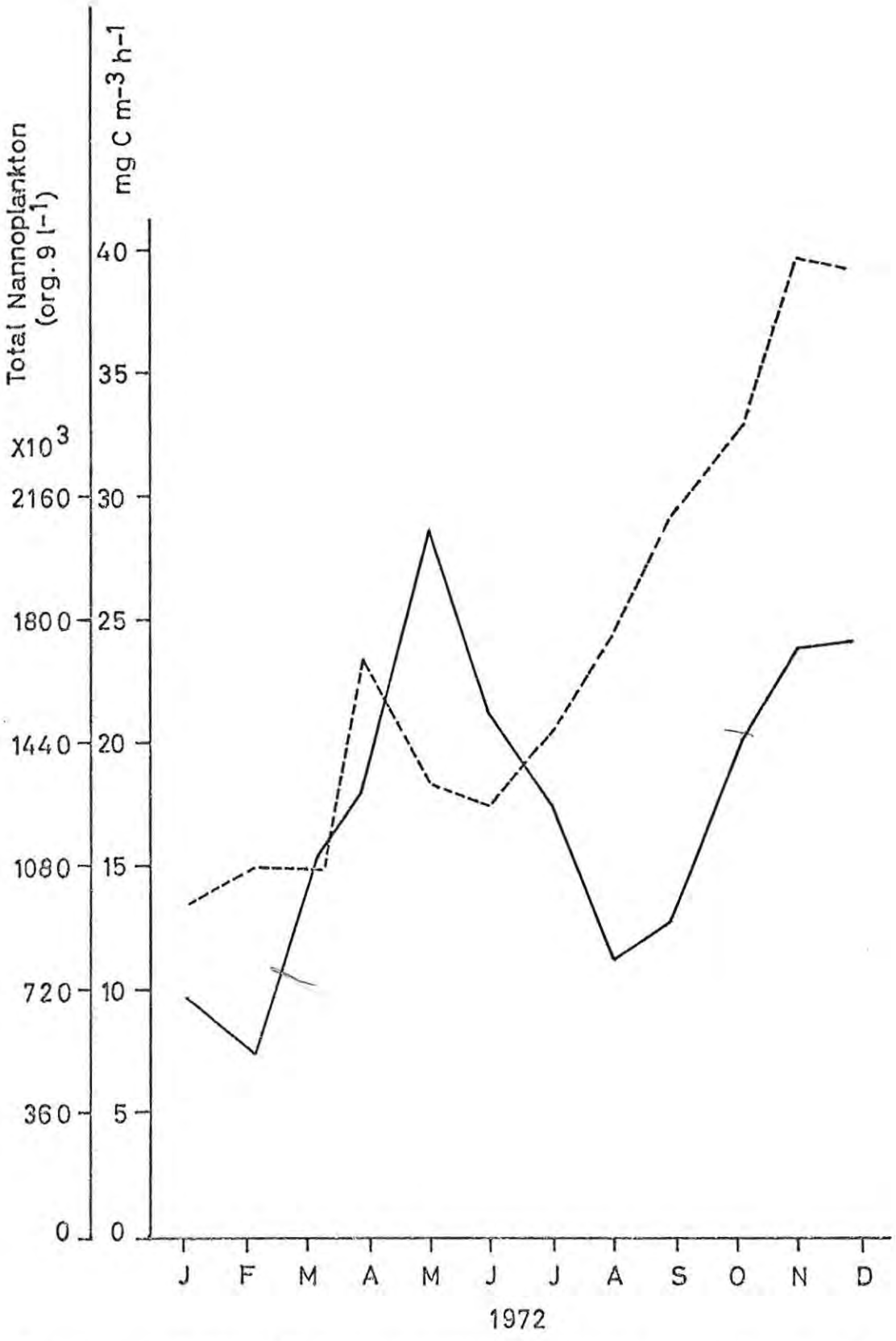


Fig. 34: Relationship between integral primary productivity and total (all groups combined) nannoplankton (to 8 m only). Broken line represents primary productivity and solid line, nannoplankton.

not as photosynthetically active at the time of the experiment. A larger proportion of the total population was located at greater depths in May (Fig. 31). Temperature (Fig. 14) dropped 6°C between April and May. Finally, the phosphate concentration (Fig. 19) was much lower in May.

Talling (1971) pointed out that with increased mixing an increased fraction of the total phytoplankton population comes to lie at levels of lower light intensity which could decrease photosynthesis. Fig. 31 shows that the diatom population between May and September was distributed to deeper levels in the water column. This may partially explain the decreased primary productivity in May and June. In addition, the extremely low PO_4-P concentration, especially in June, may have also had some limiting influence (Fig. 19).

From July to November 1972 the fixation of inorganic carbon increased each month, declining only slightly in December (Fig. 34). The integral primary productivity may have increased in December. This assumption is based on several facts: (1) the 1% light level had extended to 9.6 m and productivity was measured to 8 m only (Fig. 32), (2) Fig. 31 indicates a relatively large diatom population below 8 m, (3) these cells were probably active is shown by the increased productivity at 8 m in Fig. 32 and Table 3.

The primary productivity estimates in this study were for the pelagic phytoplankton only and may have represented only a small percentage of the entire productivity in the upper reaches. From investigations in other aquatic ecosystems of the fixation of inorganic carbon by epiphytic algae, macrophytes and pelagic phytoplankton it is suggested that the large aquatic macrophyte-epiphyte population in the upper reaches of Swartvlei may be more important than the pelagic nannoplankton in the primary productivity of the system (Wetzel 1964; Allen 1971a and c; Rich *et al* 1971; Wetzel and Allen 1972; Wetzel *et al* 1972).

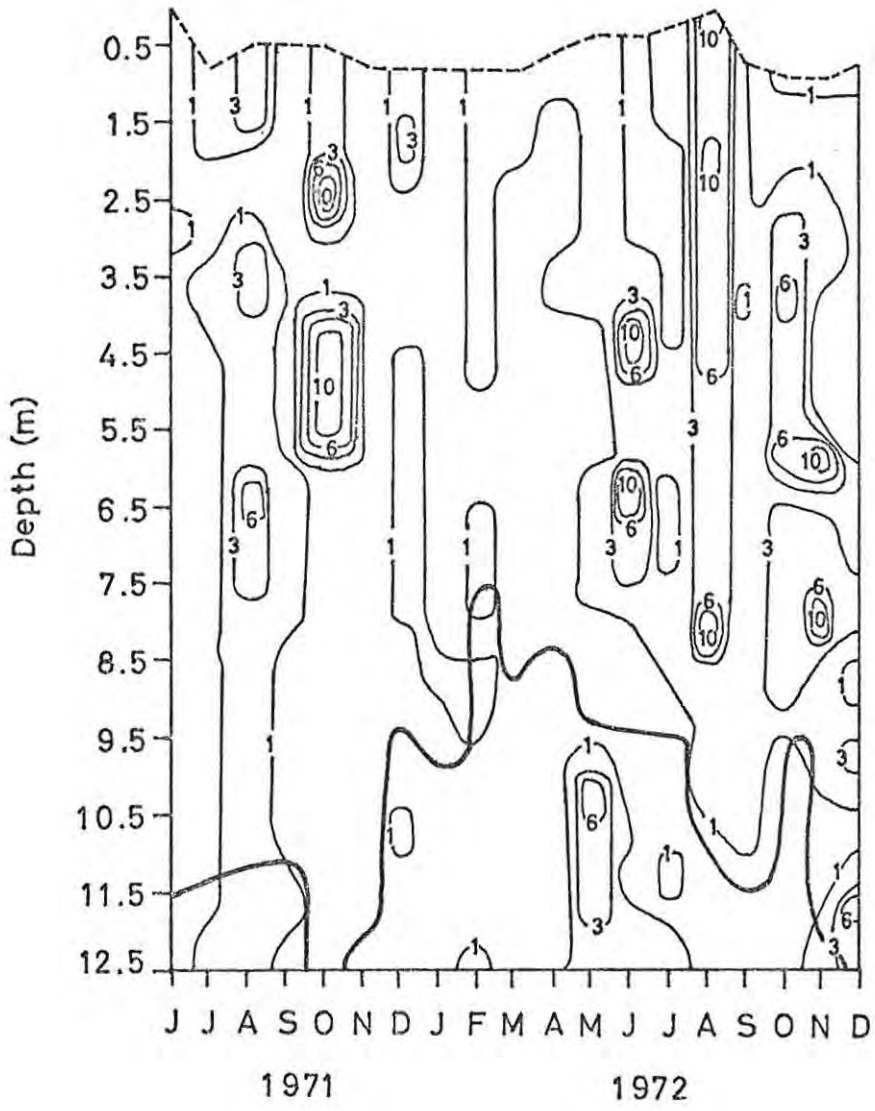


Fig. 35: Density-depth-time diagram of aerobic heterotrophic bacteria (col. $ml^{-1} \times 10^{2}$) obtained on nutrient agar. Thick line represents aerobic-anaerobic interface.

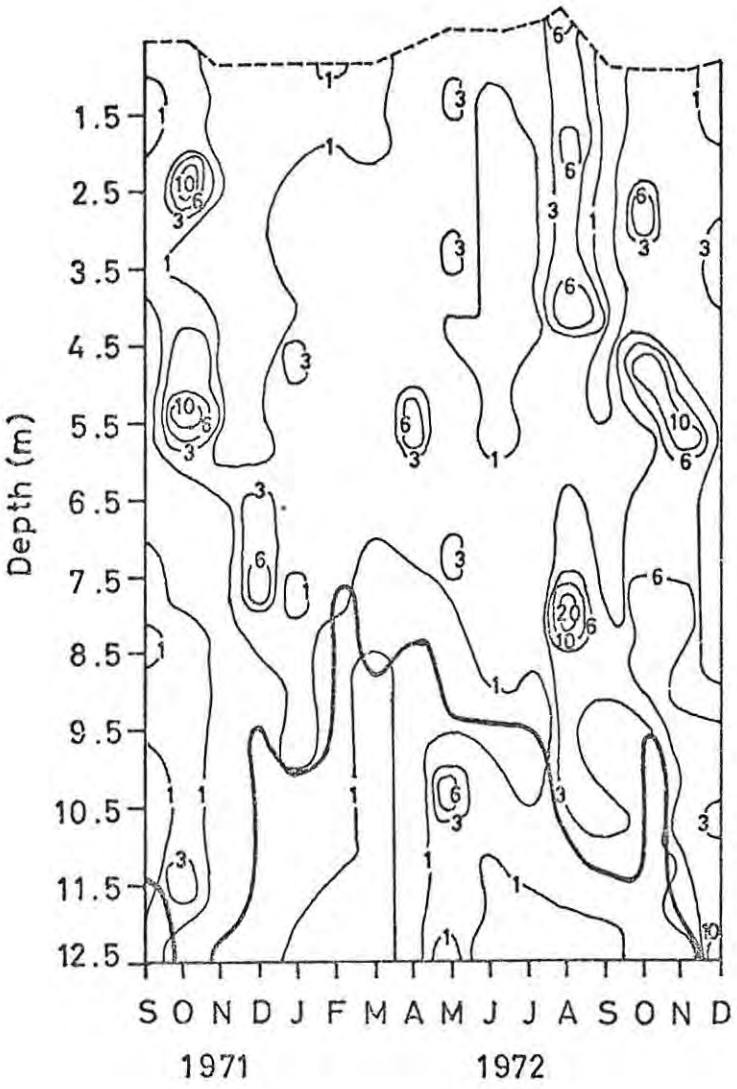


Fig. 36: Density-depth-time diagram of aerobic heterotrophic bacteria obtained on nutrient agar with salt (col. $ml^{-1} \times 10^{2}$). Thick line represents aerobic-anaerobic interface.

Aerobic heterotrophic bacteria-vertical distribution

Several agar media were employed to estimate the size of the aerobic heterotrophic bacterial population in Swartvlei. Bacterial colonies appearing on YPA 25 media and E.coli minimally enriched agar were significantly lower than on nutrient agar and on nutrient agar with 0.85% NaCl. Hence the former two media were not used after October 1971.

Bacterial populations at each depth were recorded as col. ml^{-1}. The total aerobic heterotrophic bacterial population in the water column is indicated as col. 13 ml^{-1} since 13 samples were taken through the water column.

The vertical distribution of the bacterial colonies obtained on nutrient agar (NA) and on nutrient agar with salt (NAS) are shown in the density-depth-time diagrams, Figs 35 and 36. The distribution of the bacteria show a multilamina stratification. This type of layered vertical distribution is perhaps more clearly illustrated in Fig. 37. According to Overbeck (1968b) layering is the result of bacterial maxima forming at levels where the liberation of organic matter from phytoplankton occurs.

Bacterial counts ranged from 0 on both media to maxima of 1890 col. ml^{-1} on nutrient agar and 3805 col. ml^{-1} on NAS media at 8 m in August 1972 (Figs 35-37). Throughout most of the study period counts were normally less than 300 col. ml^{-1}. Since the culturing of bacteria on media automatically imposes a selection factor on the bacteria able to grow on such a substrate, it is probable that the results in Figs 35-37 represent only a small percentage of the actual population (Jannasch 1965; Goldman et al 1968).

The majority of the aerobic heterotrophic bacteria were located in the aerobic zone as expected (Figs 35, 36). However, significant numbers of aerobic bacteria grew on NA media from water taken in the anaerobic zone in July and August 1971; May to July and November 1972 (Fig. 35). On NAS media large counts were obtained for samples from

the anaerobic zone in September 1971 and for all months of 1972 with the exception of April (Fig. 36). The aerobic bacteria were probably carried into the monimolimnion on sinking detritus (cf. Overbeck 1967, 1968a and b) and in October-December 1972 on detritus from the middle reaches by the sea water inflows. Overbeck (1967, 1968a) noted that aerobic heterotrophic bacteria could remain viable in anaerobic regions by utilizing bound oxygen in the form of nitrate producing nitrite.

Due to the selection imposed by the media a true picture of the vertical distribution of the entire bacterial population in the upper reaches of Swartvlei was not obtained. The various types of chemosynthetic and photosynthetic bacteria, especially the sulfur bacteria, would certainly have altered the profile if they had been counted. It is a well known characteristic of meromictic lakes that a large bacterial population or "plate" forms at the O_2-H_2S interface (Hutchinson 1967; Ruttner 1963; Sorokin 1970). If future studies employ specialized media and new equipment for membrane filter-fluorescent-antibody techniques or luminescent microscopy they will produce a more realistic indication of the size of the total bacterial population in Swartvlei. This has not been possible in this study due to the lack of such equipment.

As the counts on NA and NAS media differed, the total population estimated on NAS media usually giving the highest counts (Fig 39), it seemed that the autochthonous bacterial population of Swartvlei had a NaCl requirement. The nutrient agar had a measureable salinity of 0.48 $^{o}/oo$ while the NAS media measured 8.98 $^{o}/oo$. The NAS plates had a salinity which more closely resembled that of the natural environment (Fig. 13a) and could have accounted for the difference in the size of the total population estimated with these two media.

The number of aerobic heterotrophic bacteria in Swartvlei was influenced by the inflowing rivers. Boyd and Boyd (1963) found that bacterial counts in an Arctic coastal lake were highest during periods

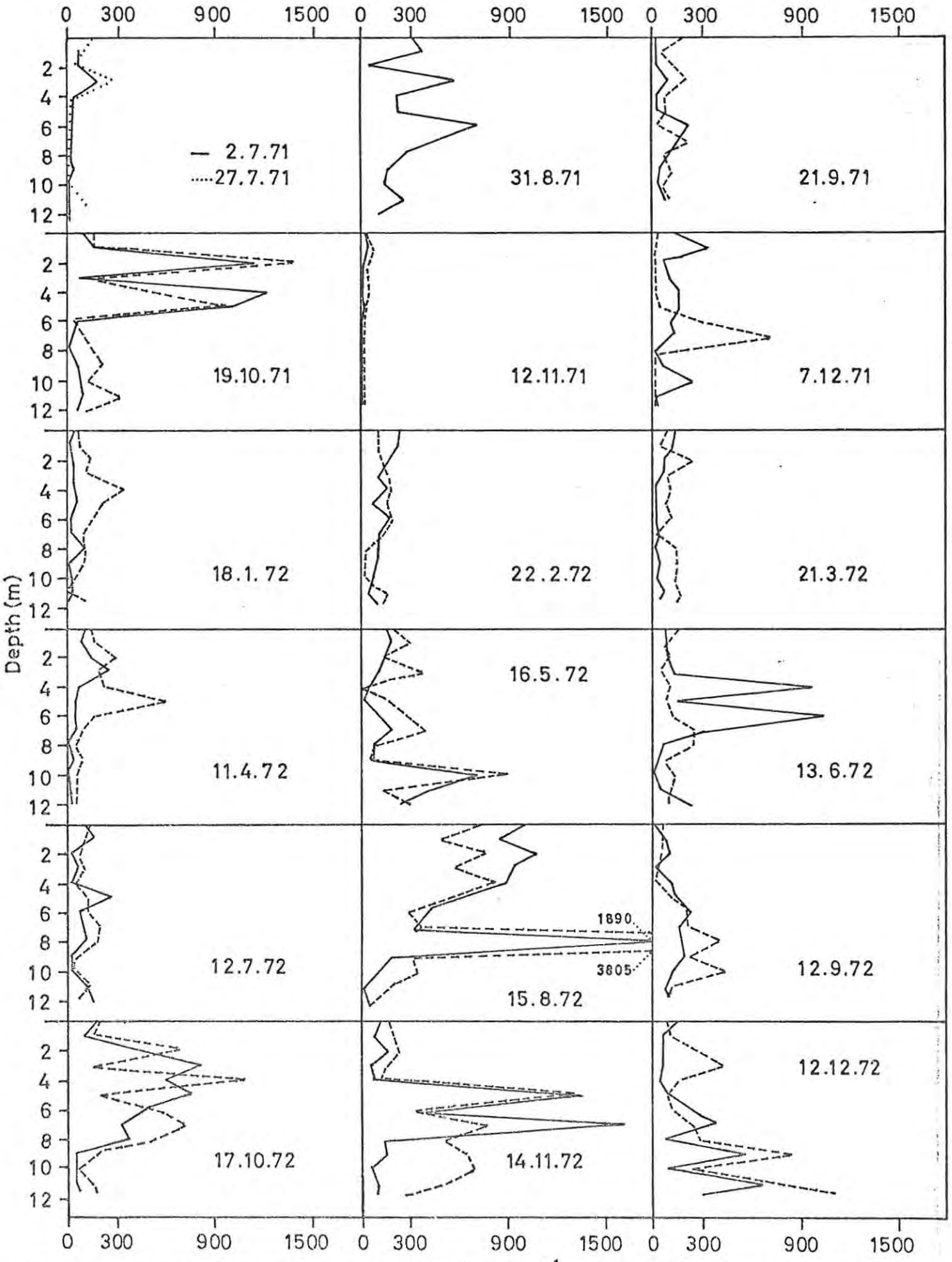


Fig. 37: Aerobic heterotrophic bacteria (col. ml^{-1}) depth profiles. Solid line, nutrient agar; broken line, nutrient agar with salt.

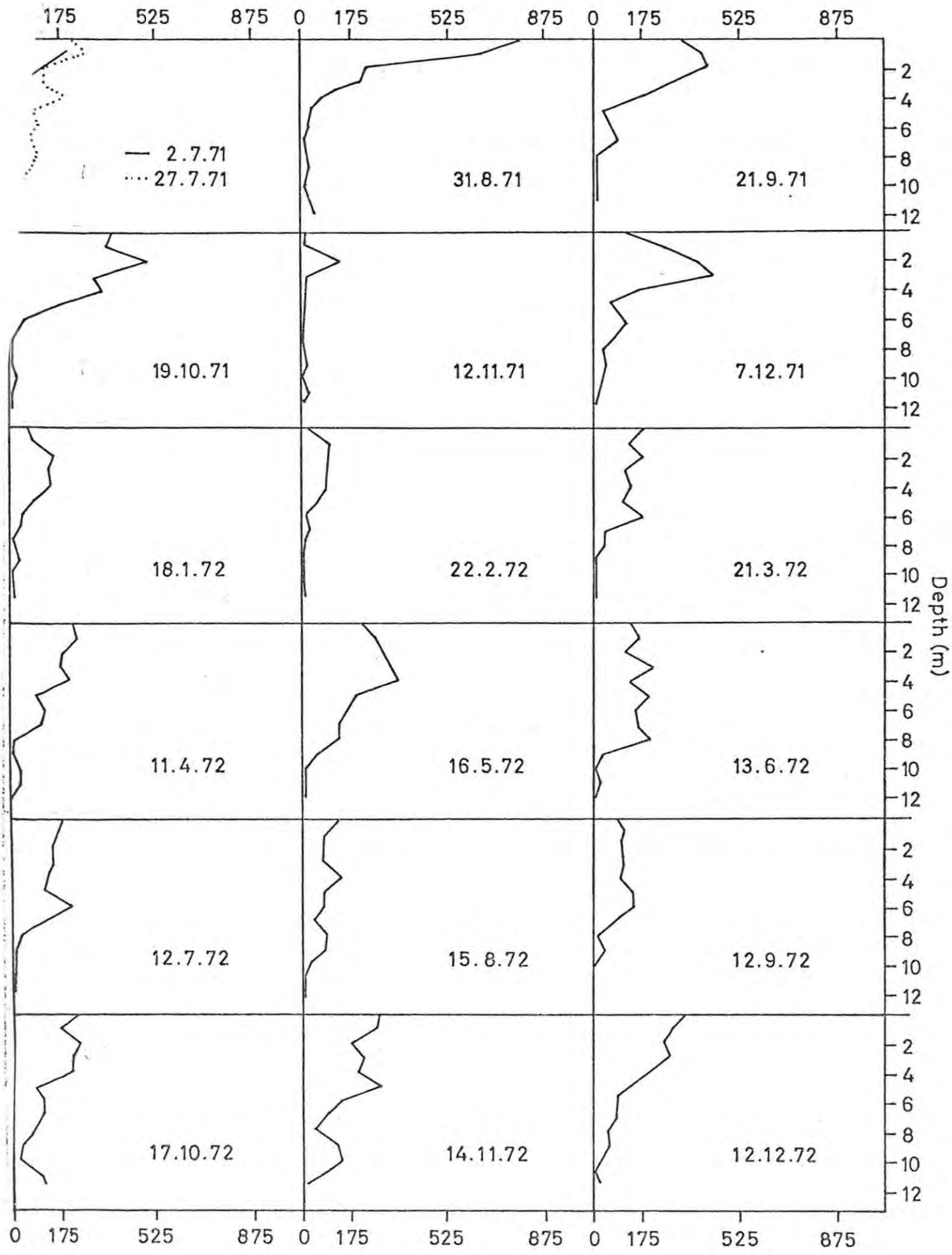


Fig. 38: Total (all groups combined) nannoplankton (Org. l^{-1} x 10^3) depth profiles.

of heavy inlet discharge. Fonden (1969) reported that bacterial numbers (plate counts) from Ekoln Basin, lake Malaren were greatest at sampling stations close to river inflows. Figures 35-37 show that the aerobic heterotrophic bacterial population in Swartvlei increased after the floods in August 1971 and 1972. Bacteria in the rivers washed into the upper reaches as well as a natural response to the visibly increased detritus concentration were two factors probably related to these increases. Another possible source of bacteria for the pelagic zone of the upper reaches of Swartvlei was the weed bed areas which had a bacterial count on nutrient agar of 600 to 2000 col. ml^{-1}. The presence of the littoral diatom Navicula pseudony in the pelagic zone supports this assumption.

Aerobic heterotrophic bacteria-phytoplankton relationship

Overbeck (1967, 1968a and b) has shown that the stratification of the aerobic heterotrophic and direct count bacterial populations in several German lakes followed exactly the profile of the phytoplankton. The seasonal maxima attained by these organisms coincided (Overbeck 1967, 1968b).

If the depth profiles of the total nannoplankton population (Fig. 38) and the aerobic heterotrophic bacterial populations (Fig. 37) are compared the following correlations are shown: October 1971, NA and NAS bacteria and phytoplankton at 2 m and NA bacteria and phytoplankton at 4 m; the nannoplankton maxima at 2 and 4 m in January and in March 1972 at 2 and 6 m and NAS bacteria; NAS bacteria and phytoplankton from the surface to 4 m in July 1972; in August both NA and NAS bacteria and nannoplankton from the surface to 1 m and NAS bacteria and nannoplankton at 4 m; both bacterial populations and nannoplankton at 6 m in September; in October, NAS bacteria and nannoplankton at 2 and 4 m; both bacterial populations and phytoplankton in November 1972 at 5 m and between 8 m and the bottom NAS bacteria and nannoplankton; and finally, in December, NAS bacteria and phytoplankton at 3 m and both bacterial populations and the nannoplankton at 7 and

9 m. These correlations between bacterial and phytoplankton stratification agreed with that described by Overbeck. At all other depths and months during the study there were no significant correlations indicated. These few correlations do not indicate that aerobic heterotrophic bacteria and nannoplankton in Swartvlei were positively correlated. Aerobic heterotrophic bacterial maxima in the upper reaches normally occurred below the phytoplankton maxima (Figs 38, 37) indicating , on the basis of Overbeck's work, that the greatest release of organic compounds from the phytoplankton, either by autolysis or decomposition, usually occurred at a level other than that occupied by the nannoplankton peak.

Overbeck showed that the depth profiles of both direct and plate count bacterial populations corresponded to the phytoplankton profiles. The general lack of correlation between bacteria and nannoplankton in Swartvlei was therefore not likely due to the absence of direct counts, which were not obtained due to the difficulty of distinguishing between bacteria and detritus. There were, however, several other possible reasons for this lack of correlation. The phytoplankton in the German lakes and Swartvlei were different. Overbeck's data referred mainly to a correlation with two species of Oscillatoria and a species of Euglena with bacteria. Olah (1971) reported that a positive correlation between bacteria and phytoplankton from lake Belso could only sometimes be established. This seemed to be partially dependent on the type of phytoplankton present. Overbeck (1968a) noted that the relationship between aquatic bacteria and phytoplankton was influenced by the physiological condition of the phytoplankton. Perhaps most significant were the differences in physical characteristics of Swartvlei and the German lakes. The majority of Overbeck's (1968b) work was done in strongly stratified forest lakes. As Overbeck (1968a) pointed out, one factor influencing the distinct and stable stratification of the bacteria and phytoplankton between 5 and 7 m in Pluss-see was the stable thermal stratification of the lake. In contrast, the stratification

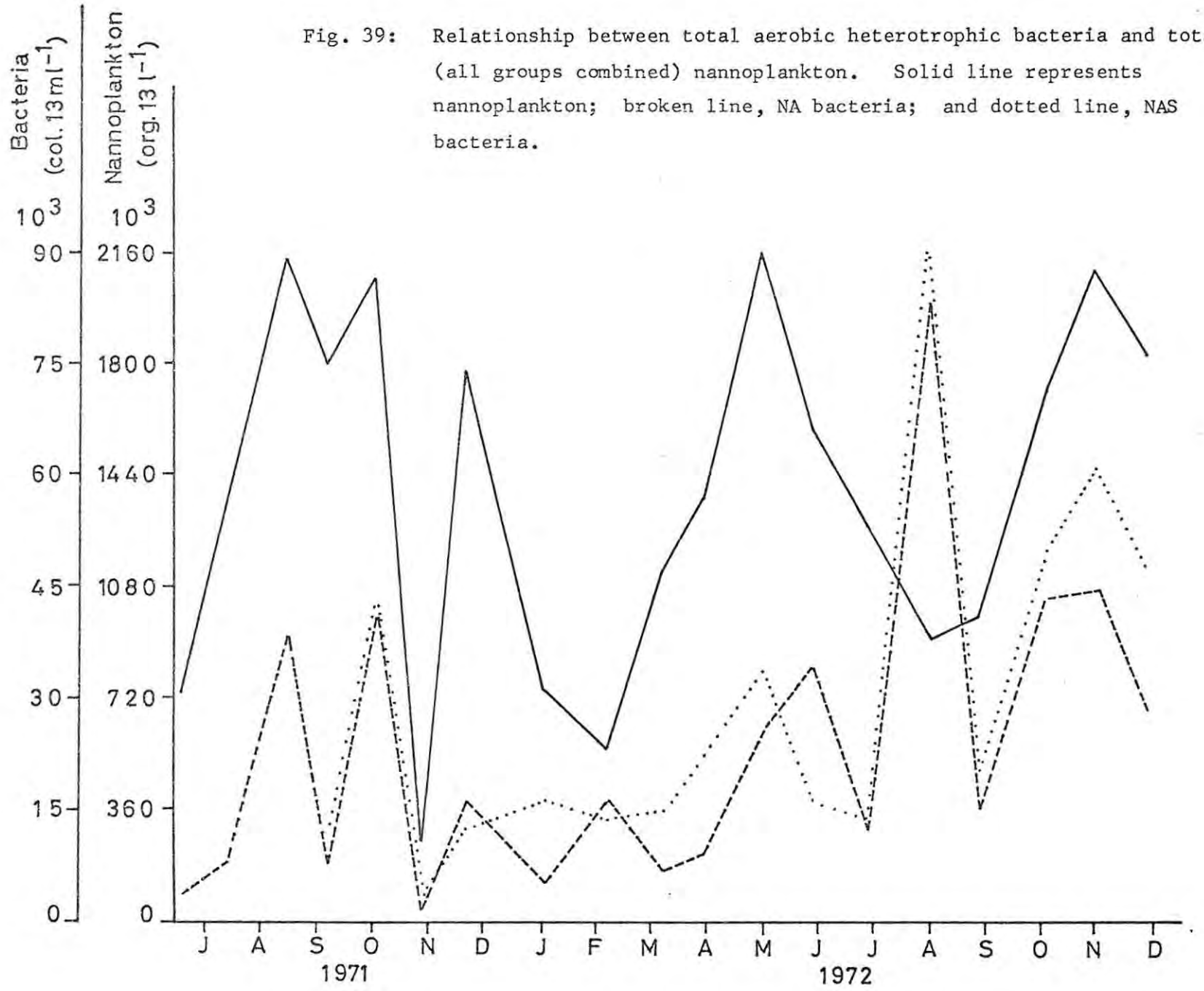


Fig. 39: Relationship between total aerobic heterotrophic bacteria and total (all groups combined) nannoplankton. Solid line represents nannoplankton; broken line, NA bacteria; and dotted line, NAS bacteria.

in Swartvlei was extremely labile as shown by the physico-chemical data. Further studies of the relationship between aquatic bacteria and phytoplankton in systems such as Swartvlei are needed.

The seasonal cycles for the total population of aerobic heterotrophic bacteria on both media are compared to the changes in the total nannoplankton population in Fig. 39. This figure indicates that a bacterial increase might be related to a phytoplankton increase which would agree with, in addition to Overbeck, Waksman et al (1937), Fonden (1969), Schmidt (1969), Olah (1970), Schegg (1971) and Seki (1971). This type of relationship is based on two facts: (1) the bacteria, by rapidly and continuously forming inorganic and organic compounds from waste products, make it possible for the continuous development of phytoplankton (Overbeck 1968a; Kuentzel 1970; Lange 1970) and, (2) the phytoplankton serve as surfaces for attachment and offer a favourable environment for bacterial multiplication (Sverdrup et al 1946; Brock 1966; Seki 1971). Bell and Mitchell (1972) reported that planktonic algae possess a "phycosphere", a zone surrounding them created by the production of extracellular products which may serve as bacterial nutrients. This effect is mediated, in part, by bacterial chemotaxis to the organic material released by the cells, and serves to keep bacteria in proximity to the cells until most of the available organic material has been utilized. Bacterial growth in algal cultures was greatest only as the cultures aged and algal lysis became obvious (Bell and Mitchell 1972).

The correlation indicated in Fig. 39, however, is misleading. In Table 5 the correlation coefficients for the total (col. ml^{-1}) NA and NAS aerobic heterotrophic bacterial populations with the total (org. 13 l^{-1}) nannoplankton population (all three groups combined) indicate that the relationship is not significant. Furthermore, there is no significant correlation between the total flagellate, diatom and dinoflagellate populations with the bacterial populations. There is also no correlation between the bacteria and the different nannoplankton

TABLE 5

Correlation coefficients for the relationship between the total aerobic heterotrophic bacterial population on NA and NAS media and the total nannoplankton population and its components in 1971-72.

	Total aerobic heterotrophic bacteria on nutrient agar		Total aerobic heterotrophic bacteria on nutrient agar with salt	
	n = 19		n = 16	
	Whole water column	down to 6m	Whole water column	down to 6m
Total nannoplankton population	.370	.199	.329	.259
Total flagellate population	.122	.143	.200	.186
Total diatom population	.255	.270	.273	.313
Total dinoflagellate population	.183	.037	.232	.081

groups from the surface to 6 m. It can be concluded that with respect to phytoplankton and aerobic heterotrophic bacteria in the upper reaches of Swartvlei there were additional factors which regulated the size of the bacterial population.

The lack of correlation between aerobic heterotrophic bacteria and nannoplankton in Swartvlei may have been due to the unstable meromixis.

Fonden (1969) pointed out that the ideal lake in which to study bacterial growth is "eutrophic", not "polluted" and has no major influents. In Swartvlei, besides the inflow of three perennial rivers, sea water also enters the upper reaches and these factors in association with wind stress may account for the lack of correlation between the phytoplankton and aerobic heterotrophic bacterial populations.

Uptake of acetate and glucose-kinetic aspects

In order to obtain an indication of the ecological significance of the bacterial population in Swartvlei studies were undertaken involving the uptake of ^{14}C labelled dissolved organic compounds.

Since a sizeable portion of the bacterial population in most aquatic ecosystems may be present in a dormant state, the count of viable organisms is no indication of activity (Jannasch 1965, 1969; Wright and Hobbie 1966). According to Brock (1966) bacterial numbers must be at least 10^6 ml^{-1} or 10^6 g^{-1} before it can be concluded that the organisms are making any significant contribution to the ecosystem. From the bacterial counts in Swartvlei (Figs 35, 36) it would have been assumed that the bacteria were making relatively little, if any, contribution to the functioning of the ecosystem. On account of the selection factor in plate counts it is essential that these counts, as well as direct counts, be supplemented with a measurement of metabolic activity.

Wright and Hobbie (1966) have shown that by adding serially increased amounts of labelled organic compounds at low concentrations to natural planktonic populations a response due to bacteria could be obtained. The kinetics agreed well with those found for transport systems of bacterial cultures analyzed by Michaelis-Menten enzyme kinetics.

Before applying the Wright and Hobbie technique to Swartvlei water samples, preliminary experiments were carried out in situ in a local fishpond during April 1971. The water was definitely eutrophic and was 15-16°C. The first experiment, using low specific activity glucose (29.64 to 237.12 $\mu g\ l^{-1}$), gave a linear plot as expected. The value recorded for T was 4.5 hours; (K+S), 14.0 $\mu g\ l^{-1}$ and V, 3.11×10^{-3} $mg\ l^{-1}\ h^{-1}$. Further experiments were carried out on this pond water with similar results. A mass balance was done to check the accuracy in measuring the uptake of glucose and production of $^{14}CO_2$ in the designed incubation flasks. This was achieved by

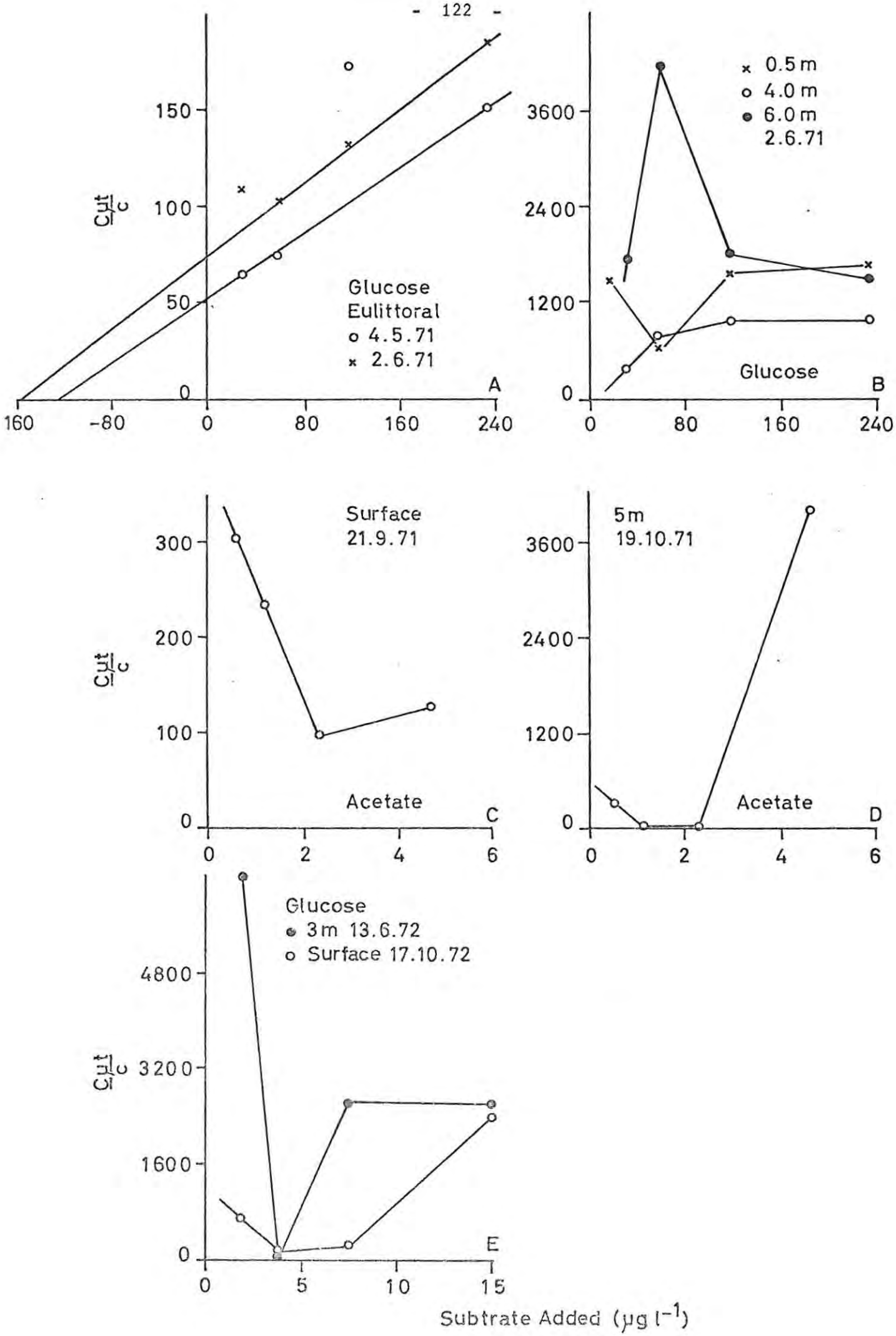


Fig. 40: Representative samples of the data obtained from enzyme mediated experiments in Swartvlei, 1971-72.

totalling the counts obtained from the $^{14}CO_2$ trap, the counts on the Oxoid filter, and the counts from a subsample of the residual ^{14}C-glucose in the filtered water. In this experiment and in subsequent ones the percentage recovery varied between 95.6 and 99.5%. Other preliminary experiments were done in triplicate and the average percentage deviation found on the values of (K+S), T and V was $\pm$ 5.17%. From these preliminary trial runs both the apparatus and procedure were concluded to be working satisfactorily.

A representative sample of results obtained from Swartvlei water is shown in Fig. 40. Fig. 40 illustrates the linear plots for May and June 1971 using low specific activity glucose and water from the western eulittoral area. In May the maximum substrate concentration, (K+S) was 118.0 $\mu g\ l^{-1}$ while in June it was 154.0 $\mu g\ l^{-1}$. Turnover time, T, in May was 51.3 hours and in June 73.75 hours while V, the maximum velocity of uptake, was 2.38 and 2.09 $\mu g\ l^{-1}\ h^{-1}$ for May and June, respectively. The fact that the water temperature was 17.2°C in May and 16.0°C in June was reflected in the higher T and lower V values for June. These values are temperature sensitive (Hobbie and Wright 1968). For example, Hobbie (1967) reported for lake Erken a T of 10 hours in summer and ranging close to 1000 hours in winter and a V of 45×10^{-5} to 1×10^{-5} mg glucose $l^{-1}\ h^{-1}$ for summer and winter.

Other samples for enzyme mediated uptake measurements in Swartvlei were taken from the surface to the bottom at different points in the western littoral region. Still others were taken at all depths in the open water of the upper reaches and from surface to bottom in the eastern rivers entrance and from the middle reaches (railbridge area). The results, in Fig. 40b, for low specific activity glucose are typical of the plots obtained. No linear responses were recorded.

Further attempts to obtain linear kinetic uptake plots were made at various times during 1971 and 1972 from different depths at the raft station using high specific activity glucose and acetate (Fig. 40c-e). The results were similar to those obtained using low specific activity

glucose. This indicated that the higher substrate concentrations (which were much lower than 0.5 mg l^{-1}, the concentration at which Wright and Hobbie found algal diffusion became operative) used earlier had no bearing on the non-linear results.

The results from a surface sample taken in September 1971, in which the flagellates were abundant (316 x 10^3 cells l^{-1}, Fig. 28), produced a non-linear response (Fig. 40c). Samples tested in October 1971 from 5 m, in June 1972 from 3 m and in October 1972 from the surface when the flagellates were recorded at less than 5258 cells l^{-1}, also gave non-linear plots (Figs 40d-e). This indicated that another factor or other factors were responsible for the results obtained.

The small bacterial population of the open water (Figs 35, 36) as compared to the high counts (600-2000 col. ml^{-1}) of the eulittoral, was also suspected of being responsible for the non-linear uptake plots. However, a plot obtained in October 1971 from a 5 m sample taken at the raft station, in which the bacterial count was high (NA: 1030 col. ml^{-1}; NAS: 1005 col. ml^{-1}; Figs 35, 36) also produced a non-linear kinetic plot (Fig. 40d). The size of the bacterial population did not seem to be the factor responsible for the non-linear responses.

The data plotted in Figs 40e-f does not take into account the $^{14}CO_2$ produced. Hobbie and Crawford (1969b) noted that the correction for respired ^{14}C gives a better fit of the data to a straight line. This correction, however, did not influence the response obtained in Figs 40b-d. The measurement of carbon dioxide was discontinued. Due to extremely rough weather conditions at Swartvlei and a rough road from Swartvlei to the field station the labelled water sample usually contaminated the $^{14}CO_2$-filter paper.

Non-linear responses from enzyme mediated uptake studies have been reported for oceanic water samples by several authors although positive responses were usually noted for coastal water samples (Vaccaro and Jannasch 1967; Vaccaro 1969; Hamilton and Preslan 1970). Hobbie *et al* (1968) reported a linear response for the uptake of amino acids in an

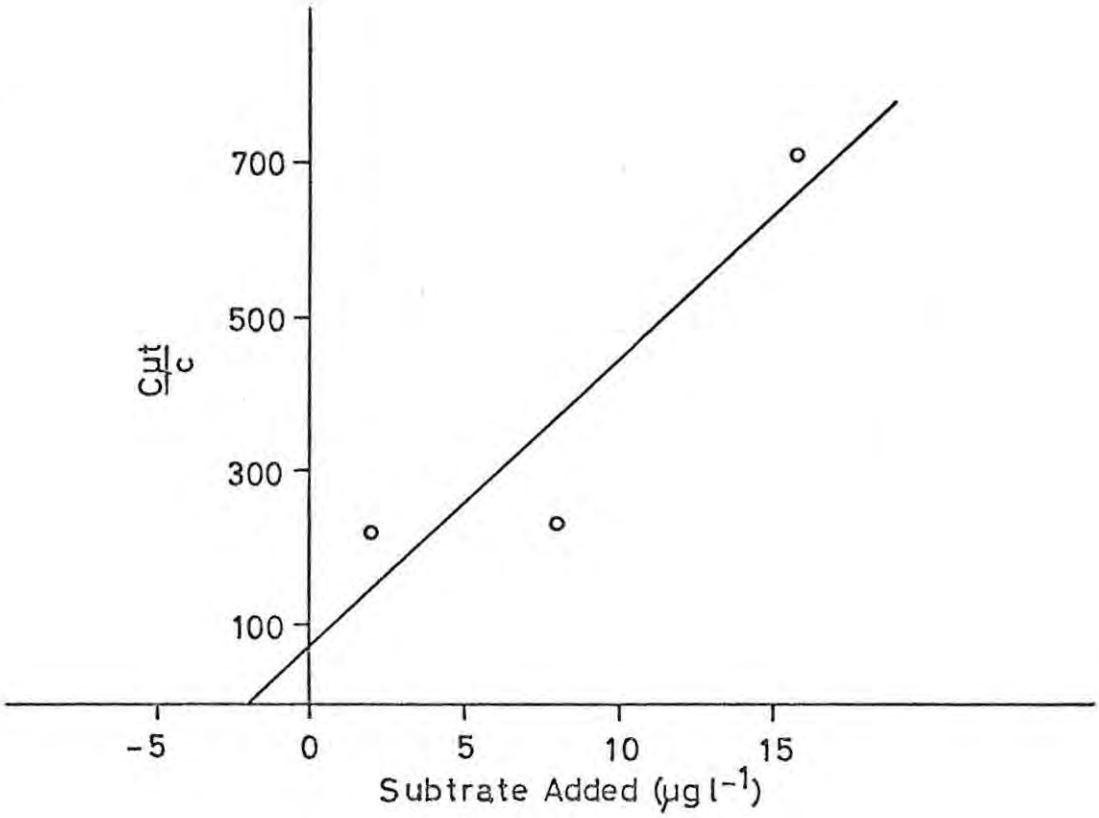


Fig. 41: Uptake plot for glucose experiment with surface water sample incubated 24 hours, October 17, 1972. Values recorded are: (K+S), 1.893 µg l^{-1}; T, 70.25 hours and V, 2.695 x 10^{-5} mg l^{-1} h^{-1}.

estuary. The plots published by Vaccaro and Jannasch were similar to those given in Figs 40b-e. Vaccaro and Jannasch showed that this type of response was not dependent upon an unusual environmental effect on the substrate. These authors pointed out that a plot of V against substrate concentration for a natural heterogeneous population should give a non-linear function since presumably, uptake constants differ for different bacterial species. That linear responses were obtained indicated that the response was probably due to a single predominant species.

Vaccaro (1969) found that by increasing incubation time to 24 hours a linear plot could be obtained for samples that would otherwise give a non-linear function. This finding showed that organic enrichment of a natural water sample reduced the number of functional species while permitting one species to dominate the total microbial population.

In October 1972 a surface sample from Swartvlei was incubated for 3 and 24 hours with high specific activity glucose. The 3 hour incubation produced the usual non-linear response (Fig. 40e). The 24 hour incubation gave a response more typical of the theoretical although the data were scattered (Fig. 41). Due to experimental error the value from the second flask was erroneous and has not been plotted in Fig. 41.

The slope of the line for the other points was calculated by regression analysis. Turnover time for this sample was 70.25 hours while (K+S) was 1.893 $\mu g\ l^{-1}$ and V was 2.695×10^{-5} mg $l^{-1}\ h^{-1}$. These data are questionable because of the large degree of scatter even though the values obtained were within the wide ranges obtained by others such as Hobbie (1971).

Induction of transport systems by extended incubation times seems to apply only to oligotrophic water where the bacteria are apparently in some resting state most of the time (Williams 1970; Hobbie 1971). The V obtained from such experiments gives an indication of heterotrophic potential of a sample. Samples incubated for extended periods of time

do not give the heterotrophic activity of their microbial populations at the time of sampling. It is essential that disturbance be kept minimal and hence the incubation time should be as short as possible. Since V could only be obtained for Swartvlei samples using 24 hour incubations the following method for measuring relative heterotrophic capability was used.

Relative heterotrophic capability

The method, like the method used by Wright and Hobbie (1966), relies on the use of very low added substrate concentrations to separate bacterial and algal uptake. All uptake was assumed to be due to the bacteria at the concentrations used (< 10 $\mu g\ l^{-1}$) unless a comparison of the uptake curve with the vertical profile of a nannoplankton group suggested otherwise. It was possible to indicate phytoplankton uptake of dissolved organic compounds if this uptake dominated the uptake of the bacteria.

Unlike the Wright and Hobbie method the uptake rate obtained in these experiments was probably not equal to V, the maximum velocity of uptake. The relative heterotrophic capability measurement was an underestimate of the actual rate of uptake at the added concentration used since $^{14}CO_2$ production was not monitored (cf. Hobbie and Crawford 1969a and b) and the natural substrate concentration was unknown, as in the Wright and Hobbie procedure. Hence, the uptake rate obtained in the relative heterotrophic capability experiments was a measurement indicative of the heterotrophic capability of the microbial population of a water sample at the time of sampling. An increase or decrease in this capability with time could only be indicated if the concentration added to all samples was the same. Therefore, the uptake rate obtained was a measurement relating the activity of microbial populations at various depths, and from month to month, at a particular concentration. Since the glucose concentration used was 3.6x as great as the acetate concentration caution must be exercised in comparing the uptake rates obtained. In order for a difference between the uptake rates of

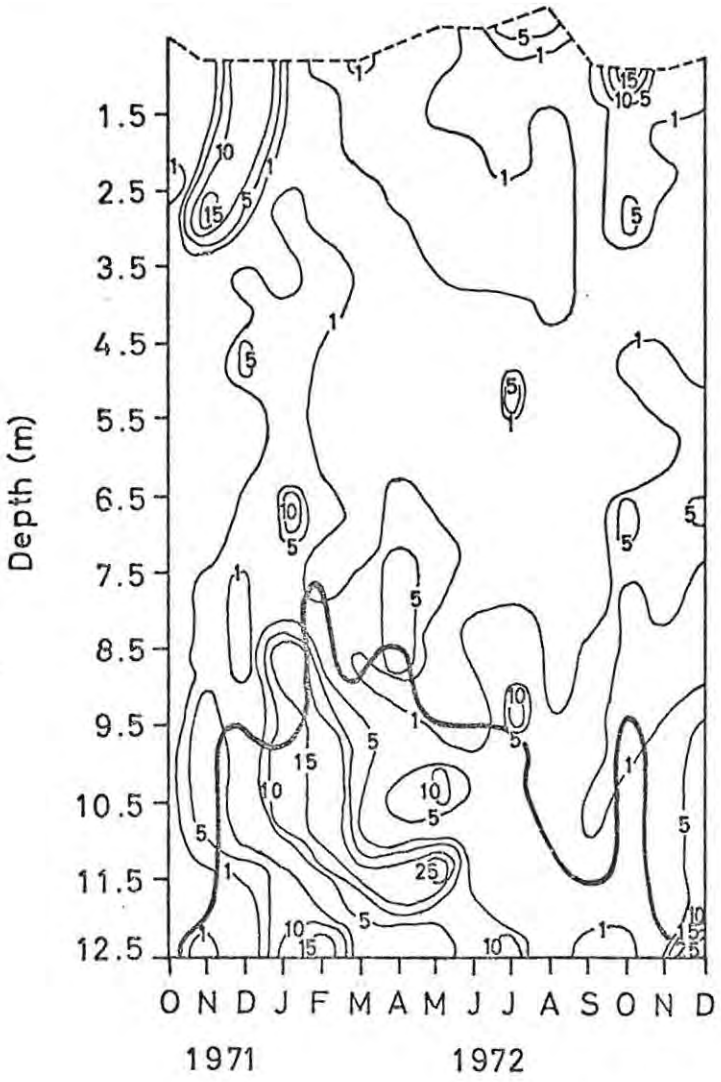


Fig. 42: Uptake of ^{14}C -glucose (µg C m^{-3} h^{-1}) depth-time diagram. Thick line represents aerobic-anaerobic interface.

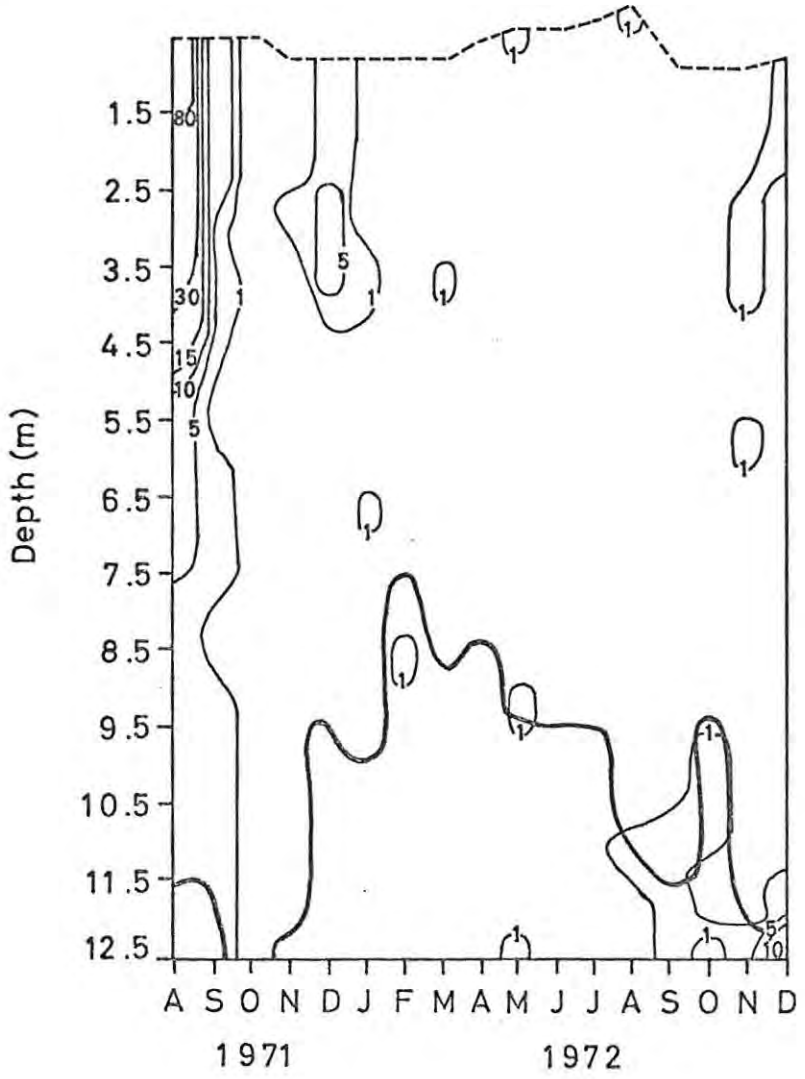


Fig. 43: Uptake of ^{14}C -acetate (µg C m^{-3} h^{-1}) depth-time diagram. Thick line represents aerobic-anaerobic interface.

glucose and acetate to be significant the glucose uptake should be approximately 4x as great as that of acetate. Differences less than this may be questionable. The uptake rate obtained, as is true of V, cannot be compared to productivity since glucose and acetate are only two of many compounds that can be taken up heterotrophically (Parsons and Strickland 1962).

The uptake of glucose and acetate in the upper reaches of Swartvlei is shown in the depth-dime diagrams, Figs 42 and 43. These graphs indicate that glucose uptake was usually significantly greater than acetate uptake (See also Tables 6 and 7). However, in August and September 1971, acetate uptake was extremely high. No comparable data on the uptake of glucose at this time are available. Low specific activity glucose was used prior to October 1971. Higher glucose concentrations were added to the samples hence the results are not comparable to those obtained later with high specific activity label. The uptake rates (Table 8) were much higher than those obtained with high specific activity glucose. Table 8 does show that there was an increase in glucose uptake between July and August 1971 but whether it would have equalled or surpassed that of acetate uptake is not known. The large uptake of acetate in 1971 and its decline to lower values throughout the rest of the study is shown in Fig. 44. In Fig. 46a a large increase in acetate uptake was indicated for December 1971. From November 1971 integral glucose uptake, taking into account the higher concentration of labelled compound added, was usually greater than that of acetate (Fig. 44; Tables 9a and 9b).

Although relative heterotrophic capability uptake rates might not be equated directly with V, a comparison of these values gives some idea of the similarity between them. The V value obtained for the 24 hour experiment in October 1972 was 2.695×10^{-5} mg glucose l^{-1} h^{-1} (Fig. 41). The relative heterotrophic capability rate was 3.91×10^{-5} mg glucose l^{-1} h^{-1} for the same sample.

It is not possible to compare the relative heterotrophic capability

TABLE 6

Relative heterotrophic capability uptake of ^{14}C-Glucose ($\mu g\ C\ m^{-3}\ h^{-1}$). Thick line represents aerobic-anaerobic interface.

Depth (m)	Oct. 19 1971	Nov. 12 1971	Dec. 7 1971	Jan. 18 1972	Feb. 22 1972	March 21 1972	April 11 1972	May 16 1972	June 13 1972	July 12 1972	August 15 1972	Sept. 12 1972	Oct. 17 1972	Nov. 14 1972	Dec. 12 1972
Surface	0.748	0.0202	7.80	0.340	1.07	0.796	0.956	1.04	0.568	5.72	7.56	0.848	15.64	2.45	2.21
1	0.193	0.952	10.44	0.393	0	1.29	1.15	0.380	0	0.912	1.02	0.780	4.12	0.536	0.238
2	2.76	19.04	0.524	1.54	0	0.379	1.09	4.12	0.170	0.304	1.67	0	6.96	1.036	0.576
3	0.154	0	1.60	0.800	3.05	0.330	0.704	1.996	1.45	3.21	5.00	0	0.276	0.251	0.716
4	0.776	0.357	7.40	1.76	0	0.203	0.0996	0.844	0.260	0.632	4.00	0	3.24	1.55	0.952
5	1.76	0.254	0	2.69	0.600	0.120	0.137	0.500	0.0313	5.28	0.492	0.520	0	0.116	1.54
6	0.168	0.604	4.08	12.56	2.52	0.337	2.32	0	0	0	0.996	0	9.52	2.284	5.28
7	0.156	1.088	0	3.72	0.544	3.03	6.84	1.80	0.0812	0.368	0.348	0.632	1.02	1.596	0.472
8	0.696	4.64	0.355	18.84	3.96	0.740	7.32	0.374	1.59	2.47	0.356	2.82	0.816	0.464	0.532
9	0.291	8.36	3.17	12.96	17.00	5.28	1.76	0	0.984	12.92	0.608	1.44	0.268	3.45	7.65
10	-	7.12	1.52	13.00	20.46	1.97	5.96	14.28	1.12	1.72	1.36	0	1.66	1.75	7.25
11	0	-	0.528	7.92	3.88	5.64	24.56	26.72	3.19	2.31	2.65	1.16	1.50	2.02	5.40
Bottom 11.6-12.5	0.029	1.14	0.996	14.64	16.78	1.73	1.29	3.81	6.52	14.44	2.26	0.672	0.134	1.88	26.10

TABLE 7

Relative heterotrophic capability uptake of ^{14}C -acetate ($\mu g\ C\ m^{-3}\ h^{-1}$). Thick line represents aerobic-anaerobic interface.

Depth (m)	Aug. 31 1971	Sept. 21 1971	Oct. 19 1971	Nov. 12 1971	Dec. 7 1971	Jan. 18 1972	Feb. 22 1972	Mar. 21 1972	Apr. 11 1972	May 16 1972	June 13 1972	July 12 1972	Aug. 15 1972	Sept. 12 1972	Oct. 17 1972	Nov. 14 1972	Dec. 12 1972
Surface	81.60	7.96	0.336	0.234	—	0.612	0.224	0.094	0.824	1.78	0.452	0.452	4.28	0.357	0.956	0.596	1.096
1	80.40	8.88	0.928	0.408	2.93	0.504	0.353	0.544	0.472	0.520	0	0.240	0.296	0.238	0.321	0.780	1.30
2	73.60	7.92	0.408	1.42	7.08	0.560	0	0	0.556	0.512	0.0208	0.259	0	0.520	0.620	1.078	0.740
3	68.80	2.24	0.424	0.147	8.12	1.09	0.118	1.09	0.0764	0.303	0.588	0.284	0.0748	0.588	0.122	1.41	0.317
4	27.08	3.82	0.178	0.960	0.084	0.708	0.900	0	0.600	0.820	0.376	0.207	0.492	0.108	0.384	0.600	0.420
5	8.60	0.708	0	0.504	0.365	0.980	0.117	0.0428	0.206	0.368	0.178	0.242	0.412	0.373	0.168	4.194	0.356
6	6.44	1.52	0.042	0.146	0.0604	3.99	0.372	0	0.238	0.552	0.008	0.532	0.343	0.369	0.117	0.528	0.389
7	5.00	2.81	0.201	0.102	0.828	0.732	0	0	0.288	0.408	0.064	0.222	0.512	0.343	0.152	0.202	0.145
8	3.36	0.660	0.214	0.193	0.768	0.496	2.04	0.390	0.108	0.315	0.219	0.323	0.0428	0.316	0.0592	0.219	0.351
9	3.17	2.42	0	0.176	0.0788	0.194	0.219	0	0.184	1.62	0.544	0.364	0.342	0.321	1.75	0.229	0.988
10	2.82	1.48	0	0.968	0.265	0.270	0.412	0.0476	0.230	0.852	0.171	0.187	0.824	0.311	4.44	0.233	0.616
11	4.88	3.07	0.0912	0.230	0.0174	0.145	0.291	0.340	0.628	0.2996	0.0382	0.291	2.50	1.97	0.107	0.564	2.24
Bottom 11.6-12.5	—	—	0	0.322	0.0428	0.203	0.728	0.428	0.224	1.15	0.0130	0.464	0.206	3.68	0.748	1.52	10.29

TABLE 8

Relative heterotrophic capability uptake of ^{14}C - Glucose - low specific activity ($\mu g\ C\ m^{-3}\ h^{-1}$)
Thick line represents aerobic anaerobic interface.
1971.

Depth (m)	July 2 1971	July 27 1971	August 31 1971	Sept. 21 1971
Surface	—	23.96	69.60	28.92
1	74.80	14.40	66.80	32.08
2	34.00	48.00	40.40	37.60
3	21.36	45.60	23.28	21.88
4	27.60	15.32	24.72	29.00
5	23.52	29.40	21.88	13.00
6	28.00	14.92	13.60	27.84
7	12.28	10.88	6.40	39.84
8	14.64	14.84	23.32	11.92
9	8.56	18.44	24.24	12.84
10	14.80	13.96	10.40	35.48
11	20.28	—	42.80	31.32
Bottom 11.6-12.5	39.44	21.12	—	—

TABLE 9a

Total uptake of glucose in the aerobic and anaerobic zones and for the whole water column ($\mu g\ C\ m^{-2}\ h^{-1}$).

	Oct. 19 1971	Nov. 12 1971	Dec. 7 1971	Jan. 18 1972	Feb. 22 1972	March 21 1972	April 11 1972	May 16 1972	June 13 1972	July 12 1972	August 15 1972	Sept. 12 1972	Oct. 17 1972	Nov. 14 1972	Dec. 12 1972
Aerobic zone	8.028	43.56	32.21	42.64	7.24	6.48	13.29	11.05	4.15	18.90	23.41	7.04	41.59	17.504	58.913
Anaerobic zone	0	1.14	6.22	48.52	62.64	15.36	40.90	44.81	11.82	31.39	4.91	1.83	3.56	1.878	0
Total	8.028	44.70	38.43	91.16	69.88	21.84	54.19	55.86	15.97	50.29	28.32	8.87	45.15	19.382	58.913

TABLE 9b

Total uptake of acetate in the aerobic and anaerobic zones and for the whole water column ($\mu g\ C\ m^{-2}\ h^{-1}$).

	Aug. 31 1971	Sept. 21 1971	Oct. 19 1971	Nov. 12 1971	Dec. 7 1971	Jan. 18 1972	Feb. 22 1972	Mar. 21 1972	Apr. 11 1972	May 16 1972	June 13 1972	July 12 1972	Aug. 15 1972	Sept 12 1972	Oct. 17 1972	Nov. 14 1972	Dec. 12 1972
Aerobic zone	356.01	40.40	2.82	5.48	29.59	9.67	2.08	1.77	3.26	5.58	1.91	2.76	7.62	6.64	2.90	10.635	19.24
Anaerobic zone	9.76	3.07	0	0.322	0.404	0.811	3.69	1.21	1.37	3.92	0.766	1.31	2.70	5.66	7.05	1.52	0
Total	365.77	43.48	2.82	5.81	29.99	10.48	5.77	2.98	4.64	9.49	2.67	4.07	10.32	12.30	9.95	12.155	19.24

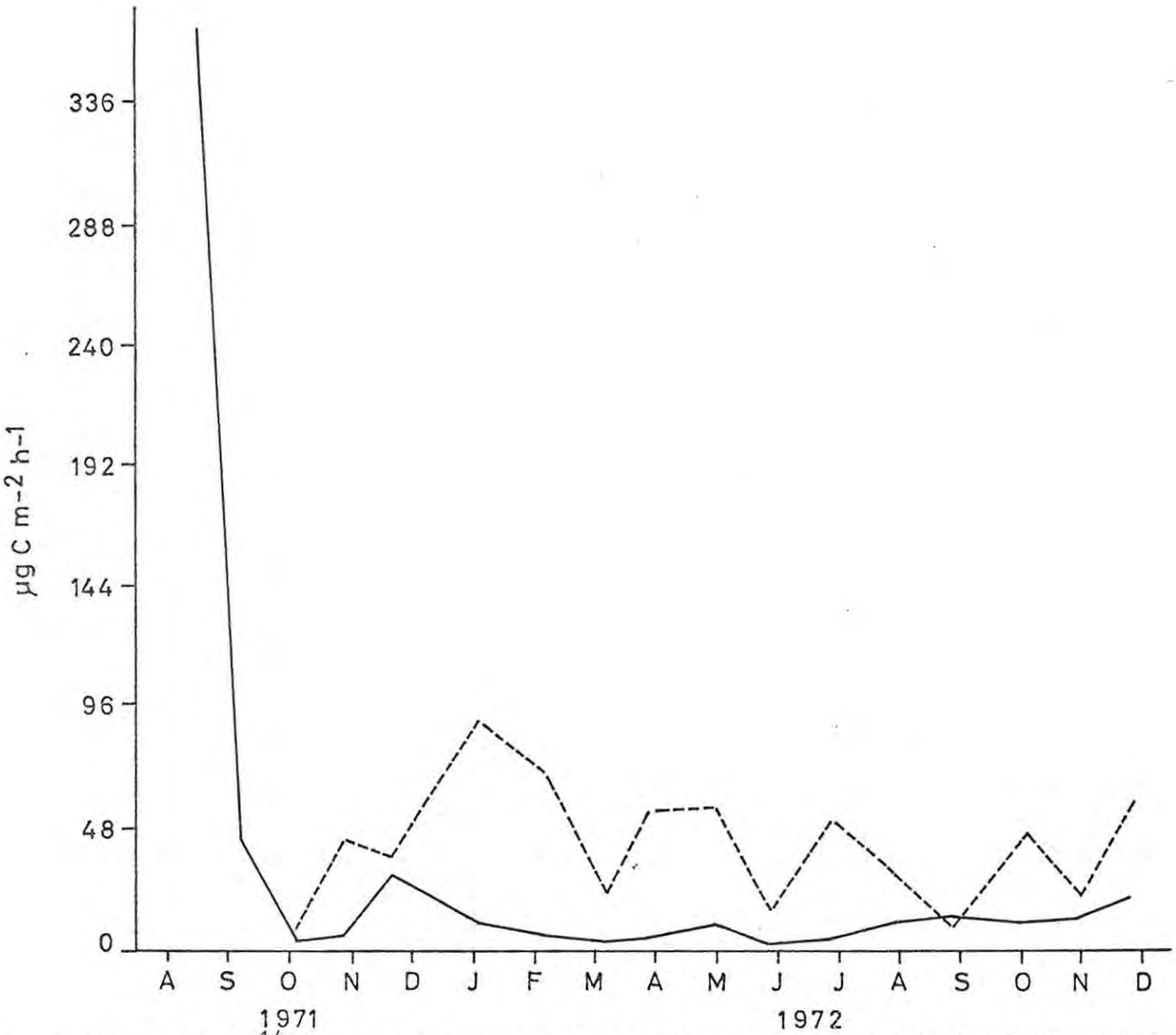


Fig. 44: Total uptake of ^{14}C glucose and acetate in the water column from relative heterotrophic capability experiments. Broken line, glucose; solid line, acetate.

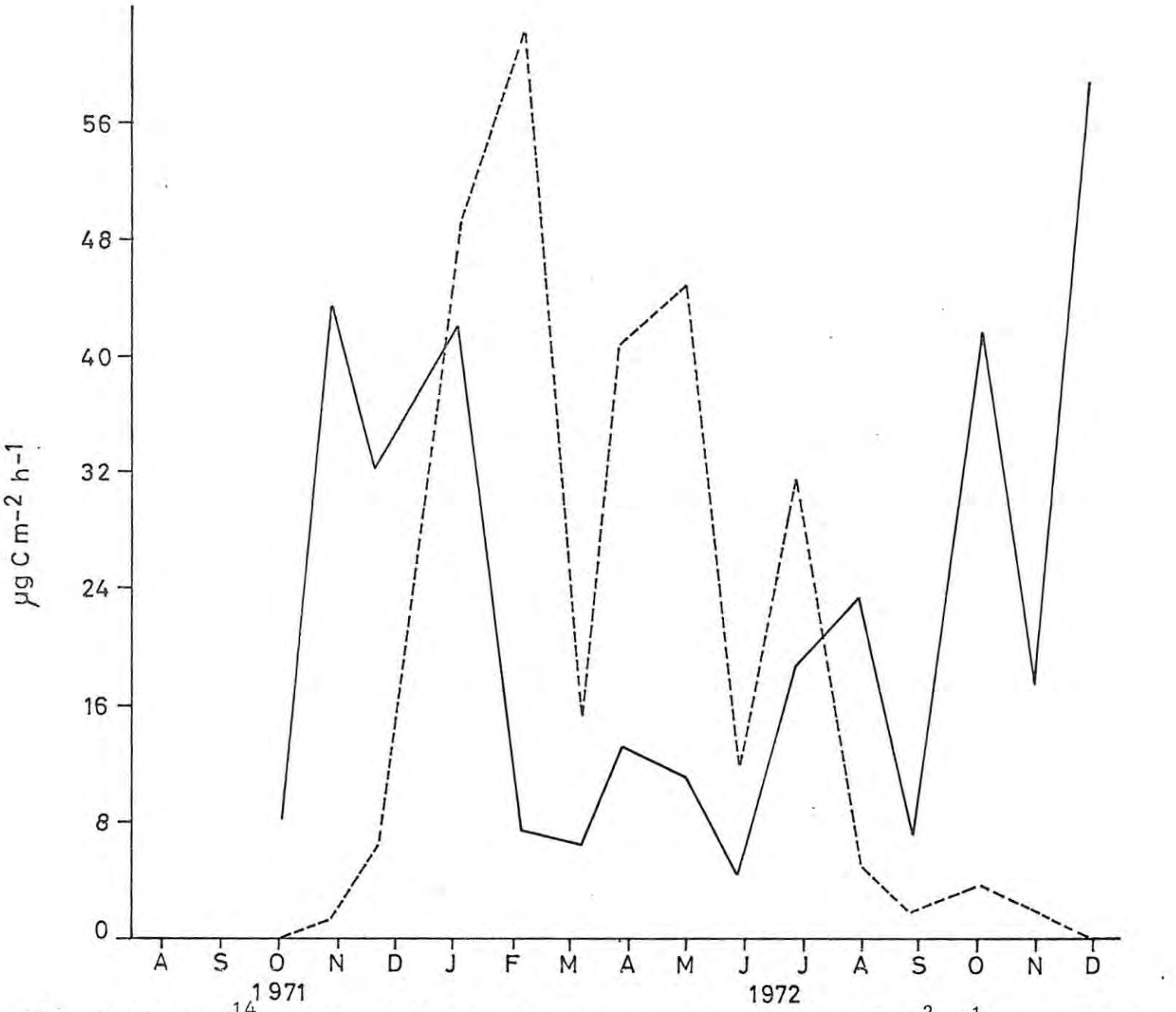


Fig. 45: Uptake of C^{14} -glucose in the aerobic and anaerobic zones (µg C m^{-2} h^{-1}). Solid line represents aerobic uptake; broken line, anaerobic uptake.

uptake rates for the warm oligotrophic waters of Swartvlei with the majority of V values obtained in other studies since, most studies were done in either eutrophic lakes or in cold water oligotrophic lakes. These factors affect the V value (Hobbie 1971). However, some V values have been obtained for warm oligotrophic waters. Seki et al (1972) have published V values for samples obtained at 50 and 100 m from the western north Pacific ocean and from the Kuroshio current during June-July 1971. Temperatures at these stations were approximately 18-22°C. The maximum velocity of uptake, V, for these samples ranged from 4.06 to 14.1 µg glucose m^{-3} h^{-1} which is equal to 1.624 to 5.64 µg C m^{-3} h^{-1}. The aerobic, glucose uptake values for Swartvlei in the months with equivalent temperatures were: October-December 1971, 0 to 19.04 µg C m^{-3} h^{-1}; May 1972, 0.120 to 3.03 µg C m^{-3} h^{-1}; and October-November, 0 to 15.64 µg C m^{-3} h^{-1} (Table 6). It would seem that the relative heterotrophic capability measurement was similar to V for waters of comparable temperature and trophic status. It was, therefore, probably a realistic indication of the uptake of dissolved organic compounds by the microbial organisms in Swartvlei.

Uptake of acetate and glucose in the aerobic vs. anaerobic zone

The relative uptake rates of both glucose and acetate in the aerobic and anaerobic zones of Swartvlei are shown in Figs 45 and 46a. Fig. 45 shows that the uptake of glucose between October-December 1971 and August-December 1972 was higher in the aerobic zone while during the rest of the year glucose was taken up predominantly in the anaerobic zone. In Fig. 42 the aerobic-anaerobic interface line shows that from January to July 1972 the anaerobic zone occupied a large proportion of the water column and thus partially accounted for the importance of the anaerobic uptake of glucose during this time. It also indicated that a very large and active microbial population was present in this region. A sharply stratified bacterial maxima or plate usually occurs at the O_2-H_2S interface in meromictic lakes (Hutchinson 1957; Ruttner 1963;

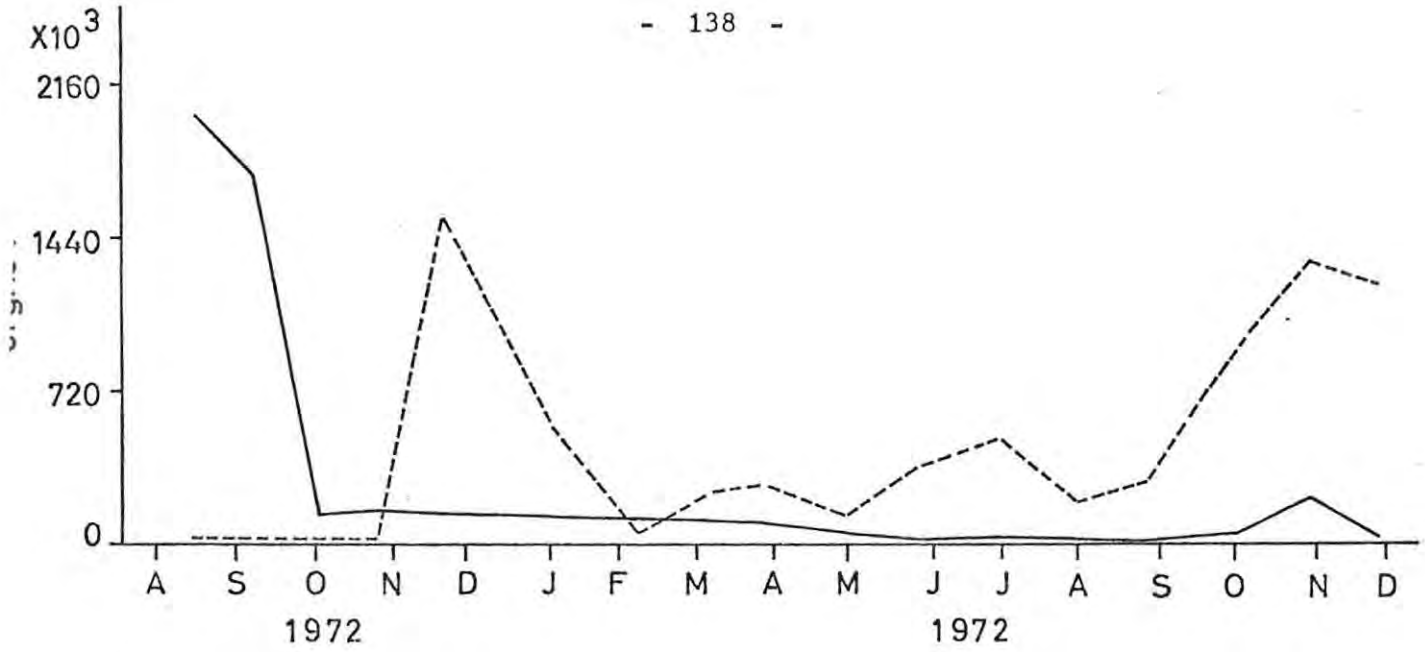


Fig. 46b: Seasonal cycles of total flagellate and dinoflagellate populations. Solid line represents flagellates; broken line, dinoflagellates.

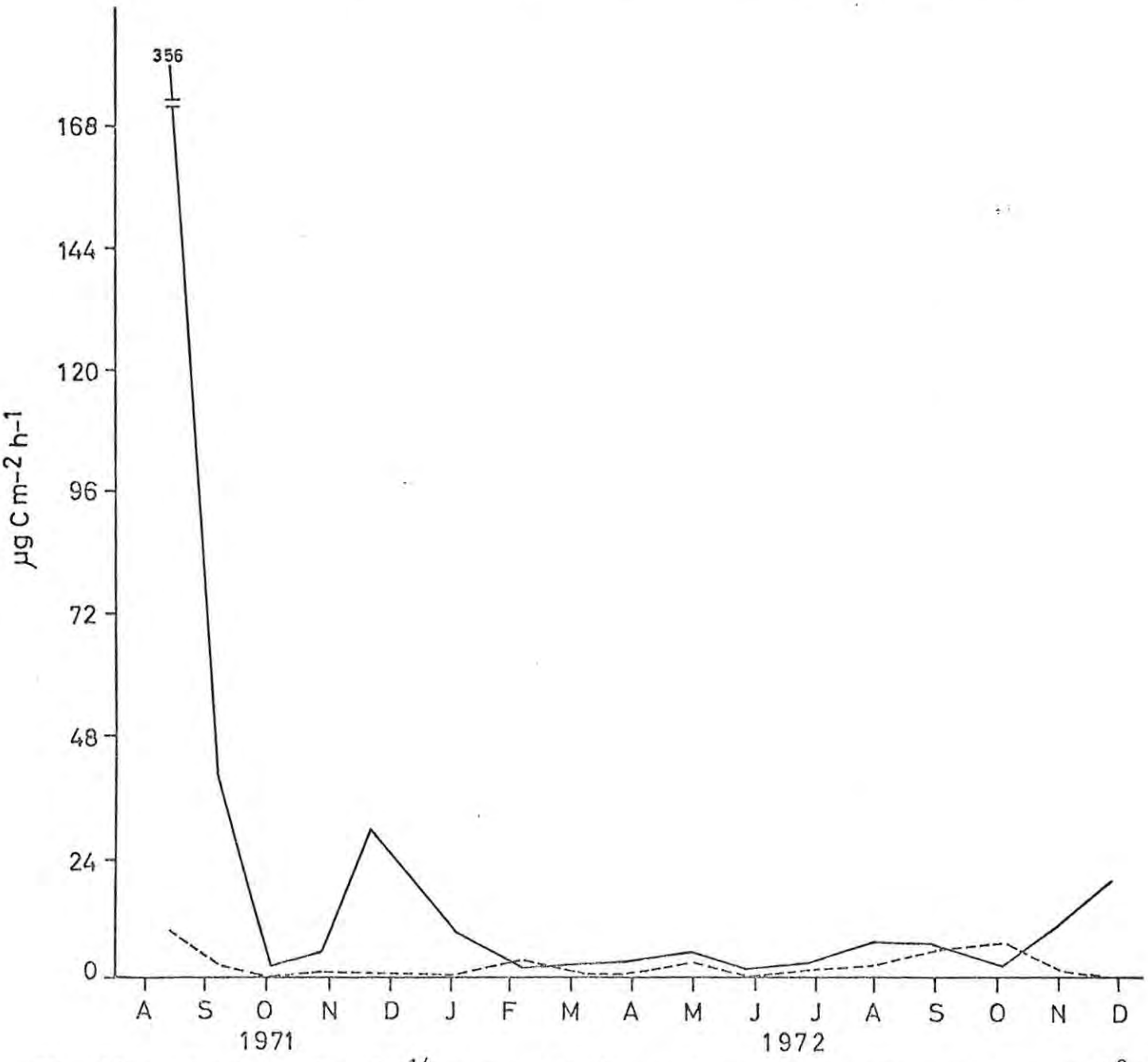


Fig. 46a: Total uptake of C^{14}-acetate in the aerobic and anaerobic zones (µg C $m^{-2}h^{-1}$). Solid line represents aerobic uptake; broken line, anaerobic uptake.

Sorokin 1970). In this layer, and in the anaerobic zone generally, a great number of chemosynthetic and photosynthetic bacteria may be found.

According to Pfennig (1967) the Athiorhodaceae, or purple and brown nonsulphur bacteria can photometabolize simple organic substances as can the Thiorhodaceae, or purple sulphur bacteria. Whether or not photosynthetic bacteria can utilize organic compounds in the dark does not seem to be known. The existing work on the dark metabolism of Athiorhodaceae is small but it is known that dark metabolism depends on the presence of stored sulphur (Pfennig 1967). It must be assumed, therefore, that the photosynthetic bacteria were not responsible in Swartvlei for the uptake of glucose and acetate in the dark experiments performed. The uptake of glucose and acetate was probably due to heterotrophic chemosynthetic bacteria in these experiments.

Fig. 46a shows that the uptake of acetate was greatest in the aerobic zone and most noticeably in August 1971 when 356 $\mu g\ C\ m^{-2}\ h^{-1}$ were taken up.

Little is known of the production of dissolved organic matter by microbial decomposition of dead algal cells under anaerobic conditions (Otsuki and Hanya 1972b). Otsuki and Hanya studied the anerobic decomposition of dead cells of Scenedesmus and found that the organic compounds produced consisted mainly of lower fatty acids (predominantly acetic acid) and "yellowish acidic substances". Brock (1966) noted that many simple organic substrates (e.g., formate, acetate) are fermented with difficulty and are relatively stable anaerobically. Thus, under anaerobic conditions, many organic molecules are "relatively resistant" to biological attack. Vallentyne (1962) reported that dissolved organic compounds can accumulate to a certain extent and resist decomposition in environments that are either abiotic or nearly so, or are lacking organisms with the appropriate enzyme systems. This type of phenomenon is assumed to occur in the anaerobic bottom waters of meromictic lakes where soluble compounds may be formed by

chemical decomposition processes. Although the bottom waters of meromictic lakes are often cited as favourable preservation sites for organic compounds, Vallentyne (1969) noted that direct evidence for this has not been presented. Sugars, however, readily ferment and rarely accumulate even in anaerobic conditions (Brock 1966).

The fact that sugars readily ferment under anaerobic conditions and acetate does not, may have been a major factor responsible for the low uptake of acetate measured in the anaerobic monimolimnion of Swartvlei. The measurement of the uptake of acetate and glucose in the anaerobic zone of Swartvlei seems to be the first attempt to measure the anaerobic uptake of dissolved organic compounds. A possible exception, however, may be the work of Wetzel (1967) which suggests he measured acetate, fructose, galactose and glucose uptake in the anaerobic hypolimnion of Crooked lake. The high uptake rates of glucose in the anaerobic zone of Swartvlei indicated that glucose would probably not accumulate. This agrees with Brock (1966). Although the anaerobic uptake of acetate was not large this compound apparently did not accumulate. This was indicated by the fact that when phosphate was released from the monimolimnion between June-July 1972 there was no increased acetate uptake in the aerobic water column (Fig. 43). Since an increased acetate concentration would have resulted in an increased bacterial biomass and consequently an increased rate of uptake (Wright and Hobbie 1966; Allen 1967), acetate was probably not released from the monimolimnion. It would seem, therefore, that acetate production in the anaerobic zone of Swartvlei was small.

Relationship between the aerobic heterotrophic bacterial population and the uptake of acetate and glucose

There was no correlation between the aerobic heterotrophic bacterial population and glucose and acetate uptake in Swartvlei (Figs 35, 36, 42, 43). This point underlines the fact that the count of viable organisms is no indication of activity.

The multilamina vertical profile of the uptake rate of glucose and

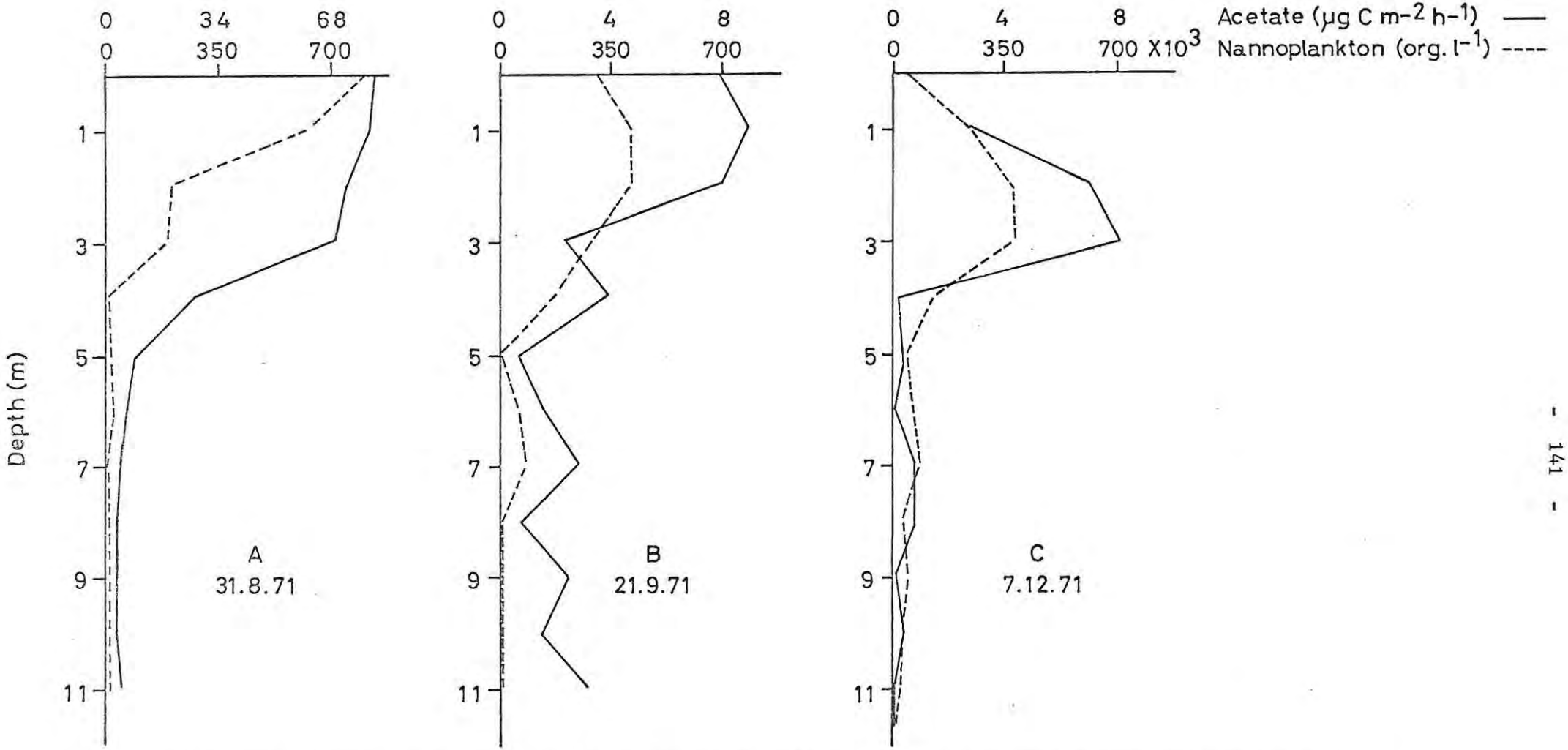


Fig. 47: Vertical profile of flagellates (A+B) and dinoflagellates (C) compared to the vertical profile of acetate uptake.

acetate in Swartvlei suggested a definite physiological and quantitative stratification in the total bacterial population (cf. Wetzel 1967). The variations in the utilization of glucose and acetate for the same sample at one particular depth was similar to the findings of Wetzel (1967) for two marl lakes. Wetzel noted that this type of result indicated specific uptake mechanisms were present amongst the microbial population.

Flagellate and dinoflagellate uptake of acetate

The work of Wright and Hobbie (1966, etc) indicated that bacteria in Swartvlei would be responsible for the uptake of glucose and acetate. However, Wright and Hobbie cautioned that small flagellates (10-15 µm diameter) may be able to compete successfully with bacteria for dissolved organic compounds. The recent work of Allen (1971b) indicated that nannoplanktonic flagellates of 4-8 µm were responsible for the majority of the active uptake of glucose and acetate in Star Lake, Vermont. This work has particular significance to the results obtained in Swartvlei which had a large population of 10-12 µm flagellates in 1971. Lackey (1967) noted that not only flagellates but also dinoflagellates are capable of utilizing soluble organic compounds.

No correlation was found between flagellate, diatom and dinoflagellate vertical distribution and the uptake of glucose in Swartvlei (Figs 28, 30, 31, 42). There was also no correlation between acetate uptake and the diatom population (Figs 31, 43). However, there was a correlation with acetate uptake and the vertical distribution of flagellates in August-September 1971 and with the dinoflagellate population in December 1971 (Fig. 47). The correlation coefficient for the flagellate vertical profile and acetate uptake profile in August was $r = 0.842$ and in September, $r = 0.864$. The correlation coefficient for the dinoflagellate vertical profile and acetate uptake profile was $r = 0.951$ ($n = 12$).

Uptake of acetate in the water column in August, September and December 1971 was also partially due to the bacteria. With the

relative heterotrophic capability technique it was not possible to separate bacterial and phytoplankton uptake but only indicate the dominant group of organisms responsible for the uptake.

In Fig. 46a and b the flagellates are strongly associated with acetate uptake. In the months that the flagellates dominated the phytoplankton population (August-September 1971; Fig. 27) acetate was at maximum values in the aerobic zone. After the majority of the flagellates left the water column in October 1971 acetate uptake dropped to very low levels. In November 1972 a peak of flagellates at 5.5 m corresponded to an acetate uptake peak (Figs 28, 43). The appearance of the first species of dinoflagellate in December 1971 corresponded to an increased uptake of acetate (Figs 46a and b). With the species change and population decrease in January 1972 acetate uptake decreased. The increased acetate uptake in December 1972 was in the aerobic monimolimnion and, since no flagellates or dinoflagellates were present, this uptake was assumed to be due to bacteria.

Since the flagellates did not have an affinity for glucose it may well be that these organisms belonged to the acetate flagellate group that, by definition, cannot take up glucose due to a deficiency of hexokinase (Hutner and Provasoli 1951; Danforth 1962). Danforth suggested that the failure by acetate flagellates to utilize sugars might be due to permeability limitations. If hexokinase and other kinases are actually components of the permease system, and if the flagellates do not posess these enzymes, sugars would be expected to penetrate flagellate cells with difficulty. Euglena gracilis var. bacillaris can take up glucose at higher pH values (7-8) and it was shown that hexokinase is a constitutive enzyme of these organisms (Hurlbert and Bates 1971). Whether or not the flagellates in Swartvlei removed the acetate by active transport is not known. In September 1971 a surface sample in which the flagellate population was large (Fig. 28) was incubated according to the technique used by Wright and Hobbie (1966). This sample produced a non-linear uptake response

(Fig. 40c) as did other samples in which bacteria were assumed to be taking up the label by active transport (Figs 40b,d,e).

Micro-autoradiography

In October 1971 samples from all depth stations were incubated in triplicate with ^{14}C-acetate as described in the relative heterotrophic capability methods section. In order to provide conclusive proof of nannoplankton heterotrophy in Swartvlei two of these samples were prepared as micro-autoradiographs following the method used by Brock and Brock (1968). However, after examining the phytoplankton samples in the Institute it was found that the flagellates had all but left the water column (Fig. 28). When the micro-autoradiographs were developed (exposure time: set 1 - 3 days; set 2 - 1 week) it was found that uptake had not been great enough to expose the nuclear track emulsion. Further attempts with this technique were planned for July-August 1972 when the flagellates were expected to return. Since they did not, further evidence for flagellate and dinoflagellate heterotrophy in Swartvlei was not obtained.

Production of acetate in Swartvlei

Acetate uptake usually increased after a flood (Figs 13a, 43) and more generally, acetate uptake was greatest in 1971 when rainfall was heaviest (Figs 13b, 43). Carbohydrate, and thus glucose, has already been suggested to be present in Swartvlei from phytoplankton excretion and possibly an unknown allochthonous source. Acetate, however, does not appear to have been identified as an extracellular product of phytoplankton, except in the anaerobic decomposition of dead algal cells (Otsuki and Hanya 1972b). The velocity of uptake is proportional to bacterial biomass. Aquatic bacteria increase in biomass rather than increase their velocity of uptake in response to an increase in substrate concentration (Wright and Hobbie 1966; Allen 1967). It would seem then that the increased uptake of acetate in Swartvlei noted after a flood indicated an increased acetate concentration. Allen (1967) suggested that the acetate concentration in lake Lotsjon,

Sweden may have been due to, besides the possible excretory and decomposition sources, extraneous material from outside the pond or dissolved organic matter which was possibly excreted into the water by submerged or emergent aquatic vegetation.

Coler and Gunner (1969) noted that an abundant population of epiphytes formed on aquatic macrophytes. This phenomenon seemed similar to the rhizosphere effect in terrestrial plants, i.e., the enhancement of microbial activity by the exudation of organic substances from plant roots. In view of the universal distribution of aquatic macrophytes Coler and Gunner suggested this arrangement should be investigated further.

The epiphytic population on aquatic macrophytes has recently been reported on by Allen (1971a and c) and Wetzel and Allen (1972). In the carbonate-muco-organic complex attached to macrophytes the sources which contributed to the dissolved organic matter (DOM) pool were (1) extracellular release from the macrophyte (2) active excretion by attached algae and bacterial flora (3) decomposition products following autolysis of epiphytes (4) dissolved carbon compounds present in the littoral water column of allochthonous and autochthonous origin (Allen 1971a). The macrophytes and their attached periphytic communities are capable of making a significant contribution to the total DOM in a lake. However, considerable utilization and transformation of the macrophytically produced DOM is likely to occur prior to its movement into the littoral and pelagial areas (Allen 1971a and c). This presumably also applies to the compounds extracted from the littoral water column by the epiphytic populations. It may be expected that the DOM in the littoral water column consists mainly of the simpler organic compounds such as acetate etc. Allen (1971a and c) studied the uptake of acetate by the epiphytic bacteria and by the epiphyte community as a whole and found it was considerably higher than the uptake of glucose which indicated a high acetate concentration. The effect of a large volume of water flowing through the littoral of

Swartvlei may mean an increased release of acetate and other compounds to the pelagial DOM pool. An increased rate of acetate uptake in the pelagic zone after a flood would indicate that such a release had occurred and may partially explain the values obtained in Swartvlei. The very low levels of acetate uptake in the upper reaches during 1972 (Fig. 43) may have been due to the lower rainfall (Fig. 13b) and the macrophyte-epiphyte complex removing the majority of DOM in the river water before it could enter the pelagic zone (cf. Wetzel and Allen 1972). High acetate uptake rates, other than after a flood such as in September 1972, may have been due to washed-in suspended matter settling out and being decomposed to form acetate. In October-December 1972 the inflow of salt water may have produced results similar to a flood since this water flows through weed beds and a salt marsh area (See Fig. 4).

Hobbie and Crawford (1969b) and Paerl and Goldman (1972) have used the measurement of V, the maximum velocity of uptake, to indicate nutrient enrichment. Hobbie and Crawford were able to detect the inflow from a sewage lagoon and a phosphate mine into an estuary by noting the increase in V at two places in the estuary, although no physico-chemical changes could be noted in the water and bacterial counts indicated no significant change. Paerl and Goldman traced the inflow of the Upper Truckee River into lake Tahoe by a similar means.

Acetate determination

According to Riley (1965) Koyama and Thompson in 1959 extracted acetic acid from sea water at pH 3 with ether or chloroform and identified it using partition chromatography. A technique to determine the acetate concentration in the littoral and pelagic regions of the upper reaches of Swartvlei was developed on this limited information and with further suggestions offered by Dr H. Parolis, Department of Chemistry, Rhodes University. The procedure involved fixing the pH of a 1 litre sample at 8 with NaOH so that any acetic acid would be held as sodium acetate. The sample was then evaporated to dryness in a rotary evaporator. The sediment was dissolved in 10% H_2SO_4

to convert the acetate to acetic acid which was then to be extracted with $CHCl_3$ in a separatory funnel. To the chloroform mixture, anhydrous Na_2SO_3 was added to remove any water. The $CHCl_3$ was then analysed for acetic acid in a gas chromatograph equipped with a 150 cm glass column (3 mm ID and 6 mm OD) packed with chromosorb 101. The carrier gas was nitrogen and the oven temperature was 190°C.

The technique, however, did not produce the desired results as the majority of the acetic acid in prepared high concentration standards was not recoverable. It was thought that the loss occurred in the concentration step. A better column packing will also be needed as the impurities in analar chloroform, at the high sensitivity needed, trailed into the acetic acid peak. Van Huyssteen (1970) has found chromosorb 101 coated with 3% F.F.A.P. very effective for measuring acetic acid in aqueous solution. It may be possible to eliminate the extraction procedure if a suitable concentration technique can be found. Concentration of acetate in Swartvlei water will likely remain necessary since the lowest concentration Van Huyssteen used was 25 mg l^{-1}. It is probable that this concentration was considerably higher than that in Swartvlei.

Proof of the movement of acetate from the littoral to the pelagic zone of Swartvlei will have to wait until further fulltime research can develop the above procedure or until another method is devised.

Zooplankton

The zooplankton were identified by Dr J.R. Grindley, T. Wooldridge and D. Smith of the Port Elizabeth Museum. The plankton was comprised almost entirely of copepods and the dominant animals were species of Acartia and Halicyclops. The other copepods found were Pseudodiaptomus hessei and an unidentified Harpacticoid. Acartia is often represented in estuaries (McLusky 1971; Riley 1967) and occurs widely in south Cape estuaries, as does Halicyclops (Grindley 1972; pers.comm.). Zooplankton other than copepods included in the zooplankton count were the zoea larvae of Hymenosoma which from January to March 1972 constituted

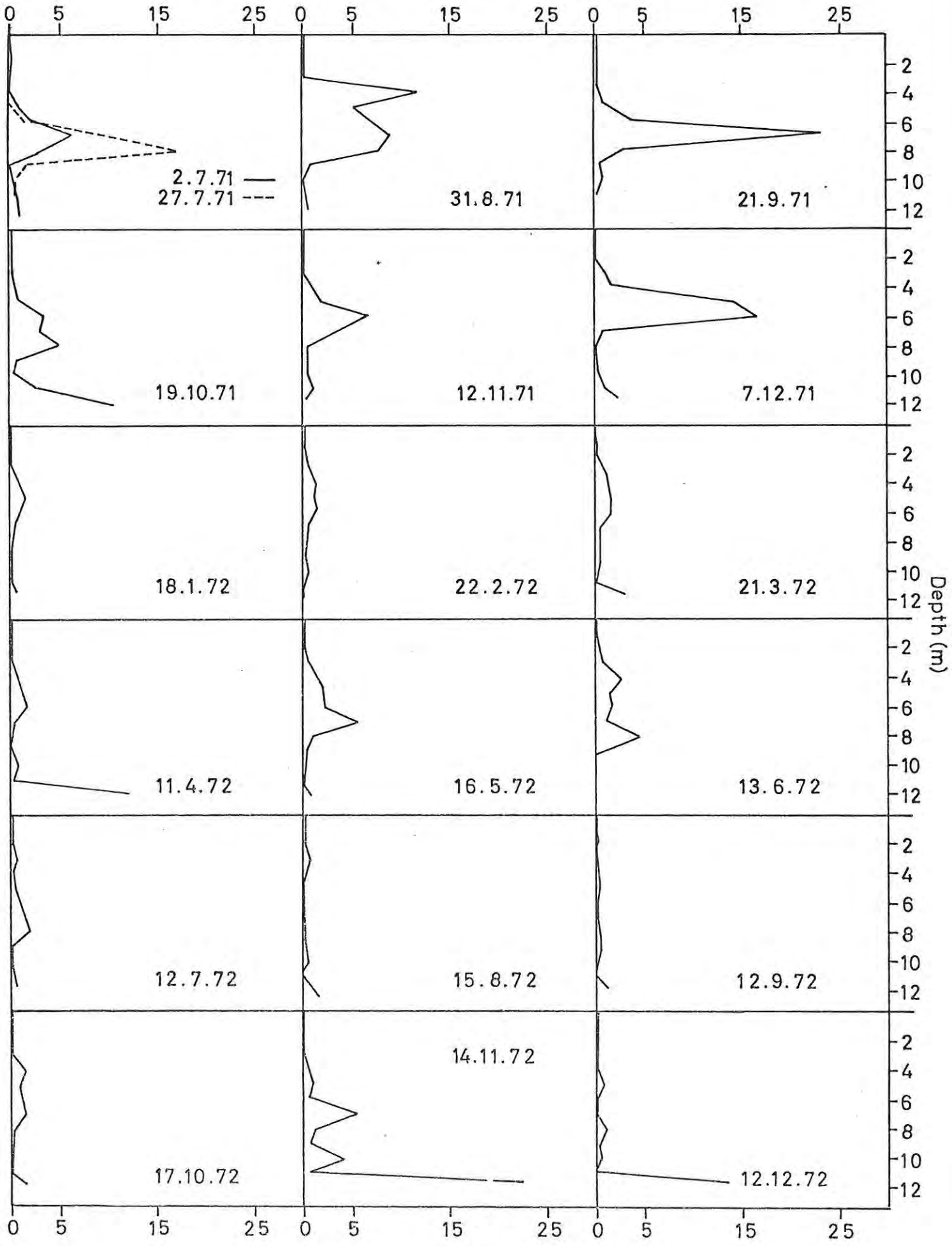


Fig. 48: Zooplankton distribution (org. l^{-1}). Daytime population at the raft station in the upper reaches of Swartvlei.

only an insignificant part of the total daytime zooplankton population (2 org. 120 1^{-1} per month), and polychaete larvae and adults which, in many cases, formed the dominant zooplankton population from 8 or 9 m to the bottom (July 1971 to May 1972 and October-December 1972).

Zooplankton counts are expressed as organisms 1^{-1} in Fig. 48. In Fig. 49 the total daytime, pelagic zooplankton count is expressed as organisms 12 1^{-1}. There were 12 samples taken through the water column with the 13th being a bottom sample. As the 13th was a benthic sample the counts obtained were not included in the total pelagic population numbers although the organisms found are indicated in Fig. 48.

The vertical distribution of the total daytime, pelagic zooplankton population is shown in Fig. 48. The zooplankton maximum was usually located below 5 m. After the flood in August 1971 the zooplankton peak moved upwards to 4 m indicating that the daytime position may have been controlled by light penetration. From July to October 1971 no copepods were found in the bottom sediment. However, after the bottom water became aerobic in October (Fig. 15) copepods were found in significant numbers in the bottom sediments. Although anaerobic conditions were found again in November 1971 and were a permanent characteristic of the water column until December 1972, copepods were always found in the bottom sediments. These copepods were Acartia and Halicyclops, the former copepod being noted for its ability to penetrate completely deoxygenated water (Grindley 1972; pers.comm.).

The daytime zooplankton population was considerably smaller in the water column after January 1972 and until the end of the study (Fig. 48). In Fig. 49 the seasonal zooplankton population changes are compared to the seasonal cycles of the total (org. 13 1^{-1}; all groups combined) nannoplankton and total (org. 13 1^{-1}) flagellate populations.

In oligotrophic lakes nannoplankton represent 44% of the food consumed by zooplankton (Gliwicz 1969a and b; Kajak 1970) since the bacterial population is not large enough to support it (Gliwicz 1969b). Fig. 49 indicates that the total daytime, pelagic zooplankton population

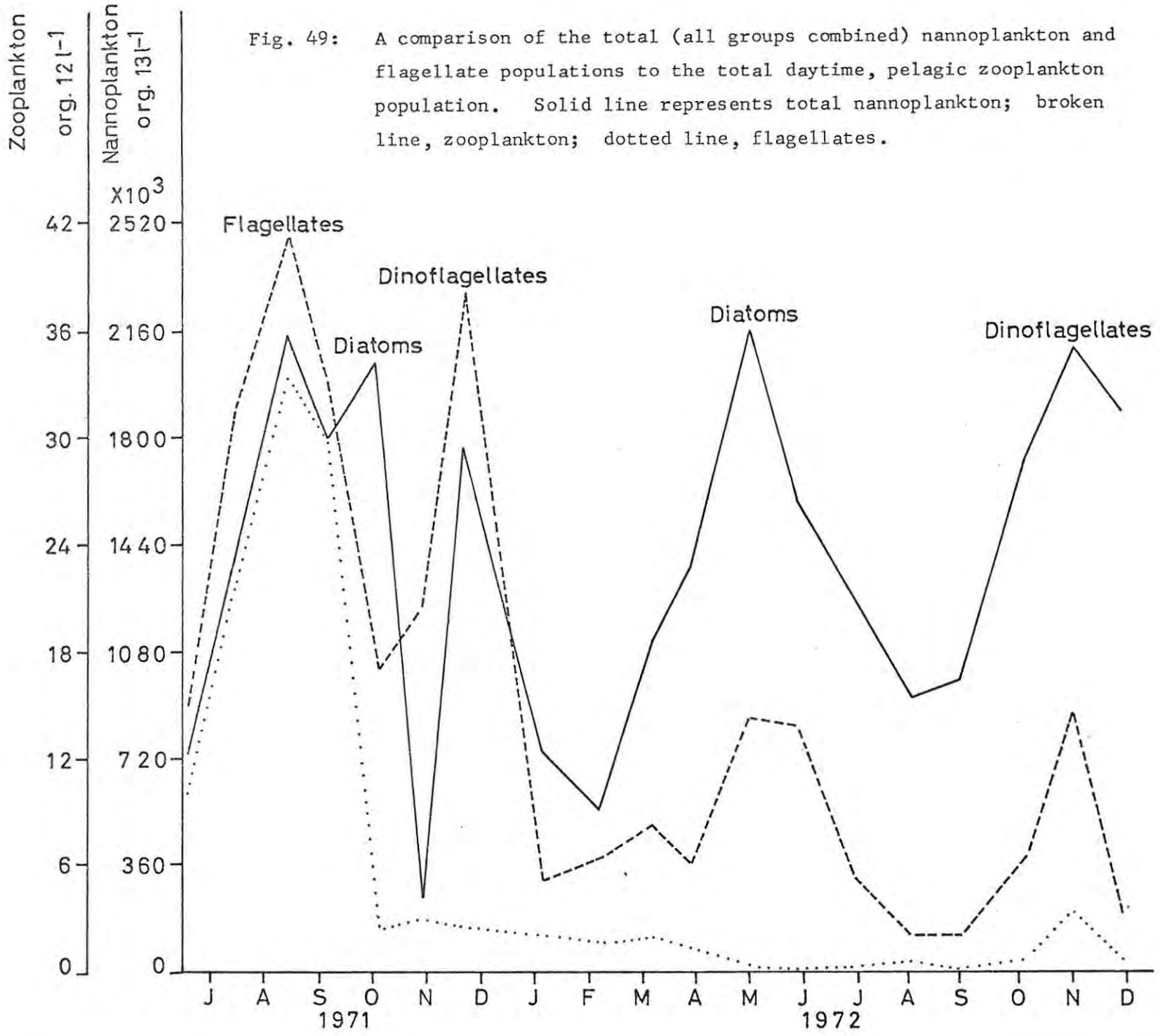


Fig. 49: A comparison of the total (all groups combined) nannoplankton and flagellate populations to the total daytime, pelagic zooplankton population. Solid line represents total nannoplankton; broken line, zooplankton; dotted line, flagellates.

size was related to the size of the nannoplankton populations. This correlation, however, is not significant ($r = 0.4423$; $n = 19$).

According to Straskraba (1966) phytoplankton species composition is an important factor in regulating the ratio between phytoplankton and zooplankton. In Swartvlei, the correlation coefficient for the relationship between diatoms and zooplankton is $r = .2934$ ($n = 19$) and for dinoflagellates and zooplankton, $r = .1052$ ($n = 19$) indicating that there was no significant correlation between the increases of these populations. The flagellate population, on the other hand, was highly significant in its relationship to the zooplankton ($r = .7655$; $n = 19$) (Fig. 49). It would seem that the flagellate population in the upper reaches of Swartvlei was a major factor in regulating the daytime zooplankton population size.

These findings agree with those of Nauwerck (1963) who found that in lake Erken small crysomonads were the most important food source for the zooplankton, followed by cryptomonads, small green algae and considerably less important, small diatoms. The Peridineae, which were one of the dominant algae, were not at all important as a food source.

Nothlich (1972) reported that in the lower reaches of the Elbe estuary that although there was a mass development of centric diatoms in the summer there was no corresponding increase in the herbivores. These primary producers, especially Actinocyclus and Coscinodiscus lacustris, contributed little to the nutritional base of the herbivorous zooplankton because they were too large for filter feeders to eat. The food of the herbivores in the Elbe estuary consisted mainly of flagellates, chlorophyceae and small diatoms. A decrease in these primary producers was accompanied by a decrease in the herbivores.

Since the diatom population in Swartvlei had a large size variation (18 to 60 µm) some of the smaller diatoms were probably acceptable as a food source and would partially explain the zooplankton increase in May 1972 which coincided with the diatom maximum (Fig. 49).

Other factors which may have influenced the size of the daytime zooplankton population in Swartvlei are bacteria, detritus and light. According to Gliwicz (1969a and b) and Kajak (1970) bacteria represent 37.5% of the food filtered by zooplankton in oligotrophic lakes. In Swartvlei, with the dinoflagellate maximum in December 1971 and the diatom peak in May 1972 there was an increase in the total aerobic heterotrophic bacterial population (Fig. 39). These bacterial increases may partially explain the zooplankton increases at these times (Fig. 49). However, in August 1972 when the largest bacterial population was recorded the zooplankton decreased. Since the plate counts did not represent the total bacterial population, the importance of bacteria in the zooplankton diet in Swartvlei cannot be ascertained from these data.

In August 1972, after the flood, the detritus content of the upper reaches increased. Gliwicz (1969a and b) and Kajak (1970) noted that detritus in an oligotrophic lake is only a minor food source for zooplankton which seems to be the case in Swartvlei as the zooplankton remained at low numbers in August. Detritus, however, may be more important because of its effect on light penetration and transparency. Although the decreased light penetration in August 1972 did not cause a daytime, pelagic zooplankton population increase, the population was low in April when light penetration was greater than in May. In May, when light penetration decreased, the zooplankton increased (Figs 24, 49).

In 1971 light penetration was probably poorer than it was in 1972. In 1971 zooplankton were certainly more abundant. Since light has been indicated as the major factor influencing the presence of the flagellate population, and the flagellates were significantly correlated with the size of the zooplankton population, detritus (and humate staining) may have been more influential in regulating the size of the zooplankton through its effect on light than as a food source.

DISCUSSION

The analysis of the data from this study of Swartvlei has raised points which require further discussion. First, the cell size of the phytoplankton found in Swartvlei may have many adaptive advantages. It is possible that the factors determining phytoplankton cell size in Swartvlei may be similar to those initiating the trend towards smaller cell size noted in phytoplankton populations of northern hemisphere lakes with the approach of winter. Phytoplankton heterotrophy may be significant in this type of succession. Finally, bacterial chemosynthesis and photosynthesis in the anaerobic monimolimnion may contribute considerably to the productivity of the pelagic zone.

In Borax lake, California, Wetzel (1964) found that during the warmer seasons of the year most of the phytoplankton carbon fixation was by phytoplankton forms of a size greater than 5-10 µm. During the winter months the smaller forms dominated. This type of succession towards smaller cell size in winter has also been noted in other systems (Rodhe et al 1960; Lund 1961; Goldman and Wetzel 1963; Olah 1970; Kalff 1972). During this study the pelagic phytoplankton of the upper reaches of Swartvlei was characterized by nannoplanktonic diatoms, dinoflagellates and flagellates. Of these phytoplankton groups the flagellates were the smallest (10-12 µm) and dominated the total population in the winter months of 1971. In the winter of 1972 the phytoplankton was composed of diatoms and dinoflagellates. The diatoms were the dominant organisms. In 1971 light penetration in the water column was considered to be poorer, particularly in August, than for the same period in 1972. This was related to the heavier rainfall in 1971 and consequently increased humate and suspended matter concentration in the water. In 1972 rainfall was relatively low and, with the exception of August, light conditions in the water column improved throughout the winter.

Wetzel (1964) suggested three possible reasons for the tendency towards decreased size with seasonal variations in environmental conditions:

(1) inherent advantages of surface area to volume ratios during periods when light and nutrients can become limiting, (2) a change from a dominance of autotrophic productivity to one of heterotrophy and, (3) the amount of chlorophyll per unit of planktonic dry weight decreasing and a corresponding shift from autotrophic to heterotrophic dominance occurring.

In aquatic ecosystems where light conditions are extremely sensitive to the amount of suspended matter there are a number of adaptive advantages in small cell size. The chemical analysis done in Swartvlei in 1972 has shown that nitrate was always available in relatively large concentrations. Phosphate, on the other hand, reached almost zero concentration in June 1972 but at all other times was readily available. The absence of larger, net phytoplankton forms in the upper reaches seems unlikely to be due to a nutrient limitation. It is not unusual for workers to report large nannoplankton populations under the ice in northern hemisphere lakes where light was considered limiting in the fixation of inorganic carbon (Wright 1964; Nauwerck 1966; Rodhe et al 1966; Pennak 1968; Kalff 1970). In Swartvlei light penetration has been shown to be extremely sensitive to changes in water colour and the amount of suspended matter. That phytoplankton populations can significantly reduce the penetration of light in lakes is well known (Findenegg 1965a; Talling 1971). The formation of a net phytoplankton population in Swartvlei could possibly severely reduce light penetration and transparency to such a degree that it would inhibit its own growth. The small cell size of the nannoplankton in Swartvlei may therefore have a distinct adaptive advantage over larger forms.

Small cell size means that the nannoplankton would not sink out of the euphotic zone as quickly as larger phytoplankton forms (Goldman and Wetzel 1963; Pechlaner 1967; Hulburt 1970). A slower sinking rate also means a lesser degree of nutrient contact. This problem would be overcome by a more favourable ratio of surface area to volume

ratio in small cells which facilitates the uptake of nutrients (Goldman and Wetzel 1963; Findenegg 1965b). A further advantage of nannoplankton, according to Goldman and Wetzel, is that the efficiency of light utilization is increased by decreased size. Nannoplankton in lakes and oceans have been shown to be more efficient primary producers than the net plankton, possibly for this reason (Rodhe et al 1960; Goldman and Wetzel 1963; Findenegg 1965b; Gelin 1971; Malone 1971; Kalff 1972). The flagellates and dinoflagellates in Swartvlei were probably capable of migrating to optimum light and nutrient conditions. This would be an advantage for survival in an ecosystem with poor light conditions (Pechlaner 1967).

The recording of only nannoplankton in the pelagic zone of the upper reaches of Swartvlei may indicate a similar adaptive process as that found in freshwater phytoplankton populations in environments which alter with the seasons and become more restrictive (Wetzel 1964).

Although the phytoplankton in Swartvlei appeared well adapted to their environment it was only the flagellates that were able to maintain a large population during the winter months of 1971. A similar condition was found in lakes with thick ice and snow covers where flagellated nannoplankton were commonly found in large numbers (Rodhe et al 1960; Wright 1964; Kalff 1967b). In these northern hemisphere lakes light was considered to be a major factor limiting primary productivity. These organisms were assumed to survive times of total darkness through heterotrophy. Light conditions during the 1971 winter in Swartvlei may have been similar to those of such ecosystems due to heavy humate staining and a large concentration of suspended matter in the water.

The presence of flagellates in Swartvlei has been associated with poor light conditions. The reasons for these organisms becoming abundant under these conditions may be related to a versatile nutrient uptake capability. According to Wood (1965) the presence of flagellates, especially coloured flagellates, in the aphotic zone of marine

environments suggested that alternate modes of nutrition may be significant. Hutner and Provasoli (1951) noted that nearly all the chlorophyllous phytoflagellates which had been grown in darkness were those already showing pronounced stimulation to substrates in the light. Most of the organisms investigated belonged to the acetate flagellates as, it seems, did the flagellates found in Swartvlei. The versatility of these organisms may have been the most important factor involved in their abundance during the 1971 winter. The photosynthetic potenial of these organisms in Swartvlei under conditions such as in August 1971 is unknown and is an important topic for future research.

Hobbie and Wright (Rodhe et al 1966) have cautioned against assuming that heterotrophy can explain the survival of phytoplankton under extreme environmental conditions. In August 1971, however, the aerobic assimilation of acetate in the upper 8 m of the water column of Swartvlei was 0.355 mg C m^{-2} h^{-1} as measured by the relative heterotrophic capability method. This uptake was correlated with the flagellates. The lowest value of primary productivity measured in 1972 was in January when 13.56 mg C m^{-2} h^{-1} (down to 8 m) were fixed. The uptake of acetate in August 1971 represented 2.62% of this value. Assuming that light conditions were significantly improved in January compared to August it is possible that the uptake of acetate was a more important mode of nutrition for the flagellates than photosynthesis since the rate of inorganic carbon fixation in August would have been much lower than the January value. Unlike primary productivity, chemo-organotrophy can continue at night so that there could be a source of energy and carbon throughout the day (Saunders 1972). Furthermore, the heterotrophic capability uptake rate cannot be equated with productivity since acetate is only one of numerous other compounds that can be utilized heterotrophically. Acetate flagellates are known to be able to utilize various other fatty acids, Krebs cycle acids, alcohols and related compounds (Hutner and Provasoli 1951; Danforth 1962). Hence

heterotrophic assimilation might have been significantly greater than the 0.355 mg C m^{-2} h^{-1} recorded if other labelled compounds had been added to the water sample.

In September 1971 only 0.0365 mg C m^{-2} h^{-1} as acetate was aerobically utilized in the upper 8 m of the water column. This large decrease which occurred with only a small drop in flagellate numbers indicated that the flagellates were primarily autotrophic and that heterotrophy was only a supplementary mode of nutrition, presumably because light penetration in the water column had improved. Allen (1971b) noted that most algae in natural conditions are probably capable of easily switching from autotrophy to heterotrophy with a combination of these two types of metabolism often being simultaneously functional. Kalff (1967b) noted that in an Arctic pond the sequence of events appeared to be a widespread heterotrophic capability of the phytoplankton in the dark, shifting to a heterotrophic-photoheterotrophic-autotrophic existence under a very low light regime, with photosynthesis possibly enhanced by the availability of organic carbon sources. Although a progressively larger proportion of the carbon was fixed autotrophically as the light intensity increased, a certain amount of organic carbon fixation continued in the light.

Only the first dinoflagellate species noted in Swartvlei showed any heterotrophic capability and then only for acetate. The aerobic uptake of acetate down to 8 m due mainly to the dinoflagellates amounted to only 0.0217 mg C m^{-2} h^{-1} in December 1971. The dinoflagellate population in this region was 18.13% smaller than the flagellate population for the same region in September. The integral uptake rate of acetate (to 8 m) was 40.61% lower in December. There are two possible explanations: (1) the dinoflagellates may have had a less effective uptake system than the flagellates and (2) because light conditions were improved in December photosynthesis may have all but replaced heterotrophy in the assimilation of carbon, which is similar to an assumption made by Kalff (1967b) for Arctic phytoplankton.

Lackey (1967) noted that besides important roles as reaerators and as food organisms some flagellates and dinoflagellates in estuaries must have an important but imperfectly understood role in the degradation of organic matter. The data from the relative heterotrophic capability experiments in Swartvlei indicated that the flagellates and one dinoflagellate species when they were present were the major organisms utilizing dissolved organic carbon compounds such as acetate in the pelagic zone. The bacteria were responsible throughout the study for the assimilation of dissolved organic carbon compounds such as glucose. In the absence of heterotrophic flagellates and dinoflagellates the bacteria were also responsible for the uptake of acetate.

Some flagellates and dinoflagellates can ingest solid food (Lackey 1967). The importance of this process by these organisms in an ecosystem such as Swartvlei which may contain a large concentration of suspended matter is unknown.

Phytoplankton heterotrophy in the upper reaches of Swartvlei appears to serve as a survival adaptation during short periods of extreme environmental conditions. In addition, it would appear to be of undetermined importance to the flagellates and one species of dinoflagellate under less severe conditions as a supplementary form of nutrition to photoautotrophy. Significant indications of phytoplankton heterotrophy were only noted in August-September and December 1971. There was only one correlation of acetate uptake with a flagellate peak at 5 m in November 1972. On an annual basis algal heterotrophy would amount to only a minor form of carbon fixation compared to photosynthesis in the aerobic pelagic portion of the upper reaches. According to Martin (1960) rainfall at Swartvlei is not usually irregular or seasonal but occasional erratic dry spells and periods of torrential rains may occur as in August 1971. It is not possible to predict when an equivalent flagellate population possessing an efficient uptake system for acetate as that found in August 1971 will again occur in Swartvlei. Other estuarine systems in South Africa which are also

subject to these heavy periods of rain may be excellent ecosystems in which to look for examples of effective phytoplankton heterotrophy.

Saunders (1972) has recently presented evidence for the uptake of glucose and acetate within natural substrate concentrations by Oscillatoria agardhii var. isothrix and two other blue-green algae. A third blue-green could assimilate glucose but not acetate. All of these species occur in a widely distributed yet rather specialized habitat. Saunders found that they occurred maximally in the interphase between aerobic and anaerobic conditions which may lie near the bottom of the photic zone or in the aphotic zone. This work by Saunders is the best evidence published so far of phytoplankton heterotrophy at low substrate concentrations in natural aquatic ecosystems. Such a study has been called for by Allen (1971b).

Saunders noted that phytoplankton heterotrophy may occur generally since O.agardhii and lakes with clinograde oxygen curves have worldwide distributions. In other systems where there is no population of O.agardhii or in those which are not characterized by a clinograde oxygen curve the possibility of phytoplankton heterotrophy should not be completely ruled out.

If this study on Swartvlei had begun in January 1972 rather than in mid 1971 no significant indication of phytoplankton heterotrophy would have been obtained. It would have been assumed that this phenomenon did not occur in the upper reaches amongst the planktonic algal populations. In fact, under particular environmental conditions significant phytoplankton heterotrophy did occur. It is possible that data on this phenomenon in other ecosystems has not been obtained because either investigators had not sampled throughout an entire year or simply because they were in the wrong place at the wrong time.

One of the points raised by Pechlaner (1971) against the hypothesis for a general heterotrophic growth of alpine phytoplankton during winter was that because aerobic heterotrophic bacteria compete successfully for dissolved organic compounds there would be no adequate

substrate for osmotrophic heterotrophy. Similarly, Hobbie and Wright (1965a; 1968) and Wright and Hobbie (1965b; 1966) noted that at very low substrate concentrations algal uptake by diffusion was ineffecitve. Since the bacteria maintain dissolved organic compounds at very low concentrations in natural systems they prevent heterotrophy in most forms of planktonic algae. While this may hold true for the majority of phytoplankton the combined results of the work by Allen (1971b), Saunders (1972) and the present study of Swartvlei has indicated that this is a generalized conclusion that should be applied with caution. Phytoplankton such as O.agardhii and small nannoplanktonic forms, living in specialized habitats, may quite possibly have uptake systems effective at low concentrations. Indeed, Wright and Hobbie (1966) and Hobbie and Wright (Rodhe et al 1966) have suggested this for nannoplankton.

The anaerobic zone in Swartvlei extended up to 7.5 m in the water column and was present almost continually throughout the year in the pelagic zone of the upper reaches. The possible contribution of the photosynthetic and chemosynthetic processes in this region to the total productivity of the pelagic zone should be examined.

According to Takahashi and Ichimura (1970) the amount of organic matter produced by bacterial photosynthesis is too high to be overlooked in aquatic ecosystems containing H_2S. Photosynthetic bacteria normally appear at the boundary layer of the oxidative and reductive zones where H_2S is present and the light intensity is lower than 10% of the surface value (Takahashi and Ichimura 1968; 1970).

Culver and Brunskill (1969) measured bacterial and phytoplankton photosynthesis in a meromictic marl lake in New York. They found that 83% of the annual production in the pelagic zone was due to photosynthetic sulfide-oxidizing bacteria in the chemocline at 18-20 m depth. A similar study was undertaken by Takahashi and Ichimura (1968) in a number of Japanese lakes. They reported that the organic matter synthesized by photosynthetic bacteria in the brackish water lakes with a permanent concentration of H_2S could be expected to be 9-25%

of the total annual primary productivity of the pelagic zone.

Both Culver and Brunskill and Takahashi and Ichimura found that the zooplankton population maxima was usually located just above the zone where the photosynthetic sulfur bacteria grew. These bacteria were assumed to form an important part of the food in the zooplankton diet.

Takahashi and Ichimura (1970) reported that the main factors determining the growth of photosynthetic sulfur bacteria in lakes are the H_2S concentration in the upper layer and the light conditions in the deeper layer. In the Japanese lakes studied by Takahashi and Ichimura (1968) approximately 0.5 to 10% of the surface illumination penetrated into the upper layer of the reductive zone. Culver and Brunskill (1969) reported that in meromictic Green lake the photosynthetic bacteria seemed severely limited by light only during periods of snow and ice cover. Bacterial photosynthetic fixation of carbon at the chemocline increased into summer as Secchi disc transparency decreased.

In Swartvlei, production by photosynthetic bacteria may not be that important to the total pelagic primary productivity. The aerobic-anaerobic interface from April to December 1972 was located at a level with less than 1% of the total surface illumination. Light penetration in 1972 was assumed to have been better than in 1971 and yet less than 1% illumination reached the aerobic-anaerobic interface. The level of this interface was not constant. H_2S was present up to only 10.5 m in August-September 1972 and not present at all in October 1971 and December 1972. However, in months such as February 1972 when the interface was at about 7.5 m bacterial photosynthesis may have represented a large proportion of the total pelagic primary production. The importance of bacterial photosynthesis in the productivity of the upper reaches requires investigation.

Another group of organisms in the anaerobic monimolimnion of meromictic lakes is the chemosynthetic bacteria. According to Sorokin (1965) chemosynthesis plays an important role in the productivity of

the organic matter in meromictic lakes. Full oxidation of reduced products of the anaerobic decomposition of organic matter, such as NH_4, CH_4, H_2S, H_2, takes place in the water column and in the surface layer of bottom sediments and is carried out by chemoautotrophic bacteria (Sorokin 1964). The trophic role of chemosynthesis lies in the utilization of the energy which is bound in the end products of anaerobiosis for the biosynthesis of the bacterial biomass, an important source of particulate food for aquatic invertebrates (Sorokin 1964, 1965). Sorokin noted that the energetic connection between the processes of destruction in sediments and the process of biological productivity, and between the aerobic and anaerobic zones in aquatic ecosystems is accomplished through the activity of chemoautotrophs. The ecological peculiarity of chemosynthetic production of particulate food is demonstrated by the fact that this process can be found in those layers of a basin and in those times of the year where and when primary photosynthetic production is low or absent (Sorokin 1965).

In meromictic lake Belovod, Sorokin (1965), found that H_2S strongly increased at 13 m, the limit of light penetration. Large daily changes in H_2S and O_2 concentrations and in Eh occurred between 10-13 m due to bacterial activity. The microflora was abundant in this layer. Experiments showed that the maximum rate of chemosynthesis and of photoautotrophic assimilation of CO_2 occurred between 10-13 m. A similar situation was found in the Black Sea between 160-200 m (Sorokin 1965).

The maximum zooplankton population in lake Belovod was found just above the aerobic-anaerobic interface (Sorokin 1965). With the lowering of the hydrogen sulphide zone in daytime the zooplankton descended. In the layer populated with purple bacteria Sorokin found crustaceans at midday when the H_2S had been completely oxidized by bacterial photosynthesis. Experiments in lake Belovod indicated that in the feeding of crustacean plankton, bacteria were nore effective as a food for filtrators than phytoplankton.

In Swartvlei the daytime zooplankton population size was correlated with flagellate numbers. However, from October 1971 to the end of the study zooplankton were always found in the bottom water and sediments which, with the exception of October 1971 and December 1972, was anaerobic with H_2S present. Prior to October 1971 samples indicated no zooplankton in the anaerobic bottom. The two copepods found in the anaerobic monimolimnion were the animals that dominated the total population, Acartia and Halicyclops. The presence of these zooplankton in the anaerobic monimolimnion after October 1971, when the flagellates had left the water column, may indicate that chemosynthetic and photosynthetic bacteria had become a major food source for them. Hart (1973, pers.comm.) found Pseudodiaptomus hessei in the aerobic bottom of lake Sibaya during the day. Experiments indicated these animals were not feeding until they migrated into the upper water column at night. The vertical migration and feeding habits of Acartia and Halicyclops in Swartvlei needs to be studied in relation to the anaerobic bacteria.

Even in 1972 when a large population of zooplankton was found in the bottom of Swartvlei there was always a pelagic daytime population (mainly Acartia). This population always occurred above the aerobic-anaerobic interface. Whether this daytime position was a result of light penetration, as has been suggested, or related to the boundary of the anaerobic monimolimnion as assumed by Sorokin (1965), Takahashi and Ichimura (1968) and Culver and Brunskill (1969) is not known.

According to Sorokin (1965) chemosynthesis and heterotrophic carbon dioxide assimilation is one aspect of the bacterial biosynthesis of particulate food which plays not a less, but in some cases, a more important role in the regeneration of initial food resources in aquatic ecosystems than photosynthetic production. The importance of chemosynthetic and photosynthetic bacteria found in the anaerobic monimolimnion of meromictic lakes underlines the need for these processes to be investigated in Swartvlei. With the very low light levels penetrating

to the anaerobic monimolimnion chemosynthesis may be the more important process.

It is hoped that the interest of other limnologists in further studies of the microbial ecology of aquatic ecosystems in southern Africa will be stimulated by this study of the upper reaches of Swartvlei. It is further hoped that this work will provide a broad foundation for additional and more sophisticated studies on the planktonic microbial populations in Swartvlei. As Odum (1971) pointed out, many of the most important physical and biological attributes of estuaries are not transitional but unique. This is so of Swartvlei. Odum stressed that the increasing use and abuse of estuaries make it vital that the unique features of these systems be widely understood.

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