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EXTENDED ESSAY - TITLE PAGE

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EXAMINATION SESSION: May 19..88.....

NAME OF SCHOOL: LESTER B.PEARSON COLLEGE OF THE PACIFIC
.....COLLEGE.DU.PACIFIQUE.LESTER.B.PEARSON.....

EXTENDED ESSAY

TITLE: A LABORATORY STUDY ON TIDEPOOL PROTISTS.

IB SUBJECT: BIOLOGY, HIGHER.

Louise: This report has been done on the tidepools on Great Race Rocks - It could be added to the collection of student research done on the reserve - Jerry F.

Name of Candidate: CHRISTINA LEE FREDERICKS
Best Language(s): ENGLISH
Language A: ENGLISH
Language B: CHINESE
Approximate number of words: 5000

A L A B O R A T O R Y

S T U D Y O N

T I D E P O O L P R O T I S T S

NAME: Christina Lee Fredericks

SUBJECT: Biology, Higher

BEST LANGUAGE: English

SUPERVISOR: Mr. Garry Fletcher

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INTRODUCTION

The protists studied are of a species yet unclear to me. They are small, green, unicellular flagellates that occupy freshwater upper-level spray tidepools. These experiments were carried out to discover some of their basic physical characteristics, that is, if they displayed any phototactic behaviour and to determine their upper-most salinity tolerance level.

The two questions I wanted to answer concerning the protists were: whether they displayed any phototactic behaviour, and if so, which particular wavelength of light they preferred to orient to, be it the blue or red regions of the spectrum or the green region of the spectrum. The second problem was that I wanted to know their salinity tolerance level in the upper ranges withing a given range of seawater immersions, of which the range they were tested on was from 10%SW to 90%SW. I also wanted to know the effect of an influx of distilled water to protists already immersed in seawater. (N.B. SW = seawater).

HYPOTHESIS

From these questions raised, I began forming a few hypotheses.

Regarding the phototaxis behaviour of the protists:

I. The protists will react differently to different wavelengths of light and will prefer to orient to light of wavelengths in the blue and red region of the spectrum

Salinity Tolerance of the protists:

II: There will be no change in the mobility of the protists during a 12-hour immersion period in seawater in the lower salinity range of 10%SW - 40%SW and in the higher salinity range of 50%SW - 90%SW.

III. There will be no change in the mobility of the protists immersed in the higher salinity range of 50%SW - 90%SW alone, with the mobility of the protists which were exposed to the higher salinity range of 50%SW - 90%SW first, followed by an addition of distilled water.

IV: There will be no change in the mobility of the protists

immersed in the lower salinity range of 10%SW - 40%SW alone, with the mobility of protists which were exposed to the lower salinity range of 10%SW - 40%SW first, followed by an addition of distilled water.

BACKGROUND INFORMATION

These protists are not exclusive to Race Rocks, British Columbia, Canada alone, as they are found in most temperate places at the higher intertidal zones which share the same climactic conditions as British Columbia.

I was first introduced to the protists after trips to Race Rocks where I was shown the emerald-green pools which the protists inhabited. I was impressed by the patterns of green made by the protists and the lack of homogenity of colour in the pools as sunlight shone down on them. This aroused my curiosity regarding their possible phototactic behaviour and to what particular wavelength of light they preferred to orient to. Also, their unique location high up in the intertidal spray zone led me to question their salinity tolerance range. I especially wanted to determine their uppermost limit of salinity tolerance after seeing charts of the salinity tolerance range of other marine and estuarine animal life.

The protists are relatively large enough to be seen under low power magnification of 100x. They are each approximately 46 µm long and 31µm wide and elongate in form (See Figure 1). As they are euglenoid in appearance, many comparisons will be made between the protists and Euglena. The protists appear to be covered by two layers of membrane; the outer one being the plasma membrane, and an inner membrane. Each protist has a single, distinct nucleus

9.5µm in diameter. The dominant pigment appears to be chlorophyll, as the whole protist, nucleus as well as the cytoplasm are a bright green. This chlorophyll is essential for the absorption of light for photosynthesis. An unseen flagella (indistiguishable at 400x magnification) seems to propel the protist while it swims. The presence of a flagella is supected as they frequently change direction while swimming and only occasionally do they rotate.

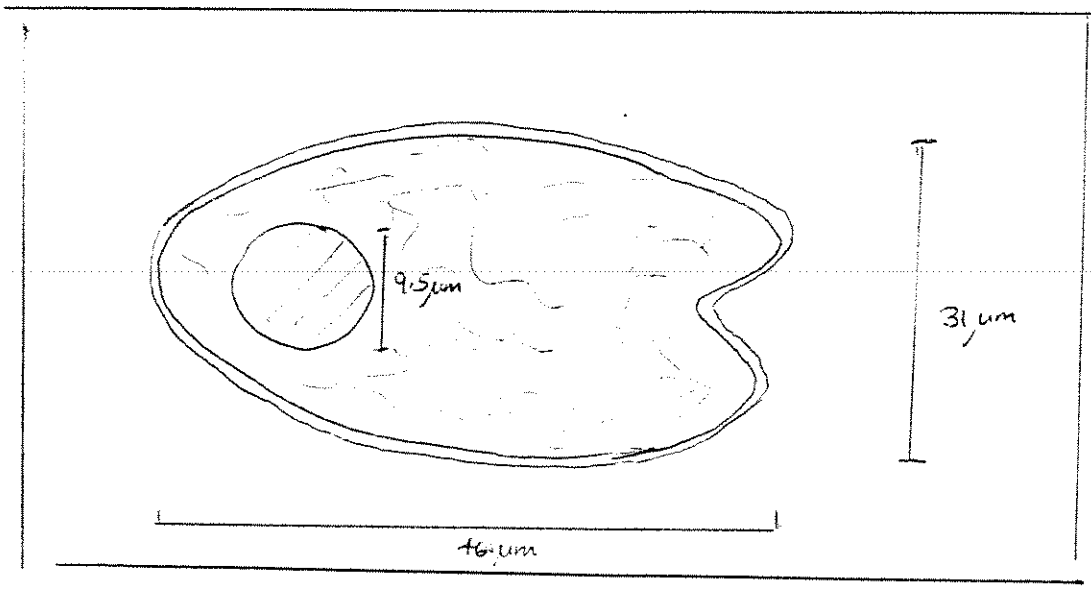


Figure I.

Diagram showing the protist.

The protists are mobile and free-swimming and exist as solitary forms, forming no colonies, although the tidepools which they occupy are deep green because of the millions of protists swimming in them. They appear to be a freshwater organism as these tidepools are low in salinity (1.3%.). These green tidepools are suspected to contain a lot of organic matter in the form of nitrates which usually come from seagull droppings. Most of the protists' nutrition is believed to be obtained through photosynthesis with the aid of chlorophyll within their bodies. Nitrates and other dissolved minerals are believed to be obtained through simple absorption through their plasma membrane and perhaps by diffusion also. This assumption was made because no active ingestion through the engulfing of smaller organisms, pinocytosis or phagocytosis, was observed under the microscope at 400x magnification. Thus, the protists could be saprozoic, photosynthetic organisms that obtain their nutrients from the nitrate-rich freshwater medium in which they dwell and through photosynthesis with the aid of the sun and their chlorophyll.

The protists thrive in shallow freshwater tidepools (maximum depth: 30cm - 35cm) in the upper-level intertidal zone. These tidepools are right next to seagull nests, and, possibly receive fresh nutrients from their droppings. These pools are relatively high up in the intertidal zone and are not immersed in seawater at high tide. They are probably formed by rainwater or accumulated water from surface runoffs, but are also subjected to extremes of temperature and salinity not faced by the lower-level tidepools,

as they are rarely covered by seawater due to their high position. This exposure leads to insolation resulting in evaporation and wide temperature fluctuations due to extreme summer temperatures as high as 30 degrees Celsius and spring temperatures as low as 10 degrees Celsius. This evaporation and high temperature may also lead to dessication of the protists. The protists also have to tolerate variations in salinity caused either by long periods of insolations, which evaporate the water from the pools, or when storms occur in which seawater may wash over the pools, both of which will raise the salinity level of the tidepool. Other than that, dilution may occur through rainfall and freshwater seepage. These extremes of temperature and salinity place alot of stress on the protists. However, the protists appear to have found a niche for themselves in these pools, as these pools are completely dominated by the protists during the spring, summer and early autumn months.

As a result, I set up two experiments to study the protists. For the experiment regarding their phototactic behaviour, I exposed the protists to white light first to see if they displayed phototaxis at all. Then light of different wavelengths were tested on them. For the salinity experiment, I immersed them in different seawater concentrations which ranged from 10%SW to 90%SW to see which percentage of seawater was their limit of tolerance and left them immersed over a set period of time to see if there would be any change in their mobility (or lack of it) after a few hours. In this way, I hoped to replicate what would happen to them in a

storm when waves washed over them and significantly increased the salinity of the pools. The addition of distilled water, meanwhile, would replicate a situation where rain will fall and lower the salinity of the pools after a wave had already washed over them or after evaporation had occured.

Many assumptions were made during the experiment. One important delimitation factor was that the samples of the protists experimented upon came only from the main island at Race Rocks and none from the surrounding islands. Limitation factors included: no controlled temperature in the laboratory was set as I wanted to duplicate the protists' natural enviroment where the temperature was never constant. So the laboratory door was left open to allow for temperature fluctuations during the experiment. Also, the reaction of the protists to salinity stress under laboratory conditions (i.e storage in test tubes, etc.) may be different from the reaction of the protists to salinity stress in their natural environment.

REVIEW OF LITERATURE

Research was done to find out more about the protists by going through books and journal articles regarding previous similar experiments on marine microorganisms such as Euglena and other marine and estuarine organisms such as algae, crabs, fish etc.

Phototaxis:

Before searching for journal articles on phototaxis, research on the basic mechanism of phototaxis on organisms particularly Euglena had to be done by going through textbooks. Clayton (1971) explained how Euglena show phototactic behaviour. he basically said that when Euglena rotates while swimming, a photoreceptor located at the base of the flagella will be shaded by the stigma each time the cell rotates, unless it is swimming directly toward the source of light. With this basic information known, research into journals was then carried out.

Haeder and Mellikonian (1983) conducted an experiment on the phototactic orientation of Euglena mutabilis, which had a different way of orienting themselves toward light. Unlike most species of Euglena, E. mutabilis do not rotate along their longitudinal axis. Therefore their phototactic orientation is not reliant on the periodic shading of their photoreceptors. In fact,

they suspected that:

> "Orientation with respect to the light direction could be achieved during the rotation of the cell around the attached rear end. Since the chloroplasts extend well into the front region, it could be hypothesized that it could function - perhaps in addition to the stigma - as a shading device. During the rotation, the cell could scan the horizon for a maximum in light intensity. When the front end points toward the light source, the PFB receives the highest light intensity not shaded by either stigma nor chloroplasts and this signal could serve as a trigger to induce forward movement" Haeder and Mellikonian, 1983: pg 27-28.

This particular experiment by Haeder and Mellikonian also explored another important requirement (other than photoreceptors) for phototaxis: photoreceptor pigments. These pigments are important for photoperception to specific wavelengths of light. One example stated is the orientation of E. mutabilis to blue light:

> "...the activity in the blue range could be due to an absorption by a flavin photoreceptor" Haeder and Mellikonian, 1983: pg 27.

However, they could not really explain the phototactic activity of E. mutabilis which " extends well in to the red region" (Haeder and Mellikonian, 1983: pg 27), although they did suspect photosynthetic pigments were responsible for this activity.

Haeder and Mellikonian (1983) also brought up another interesting point in their same article, concerning photoreceptor interactions:

> "The fact that white light induces a higher degree of phototactic orientation than any monochromatic irradiation - regardless of its fluence rate - indicates an interaction of more than one photoreceptor" Haeder and Mellikonian, 1983: pg 27.

Blue-green algae also display phototaxis and have specific photoreceptor pigments which trigger phototaxis. Nultsh (1983) stated that chlorophyll and phycobilliproteins triggered positive and negative phototaxis in blue-green algae in wavelength of 440nm and 580nm - 700nm light.

Thus, it would seem that organisms like Euglena orient toward light because of the shading of a photoreceptor, with the help of photoreceptor pigments which allow for the detection of the specific wavelengths of light the organism prefer to orient to.

Salinity

Many journal articles were researched into to obtain some background information for the salinity part of the experiment. Many aspects concerning salinity were explored: i.e. the effects of location of habitat on the salinity tolerance range of marine animals; how marine or estuarine animals and plants osmoregulate their internal osmotic pressure so they can tolerate wide ranges of salinity and what cellular specializations were important for the transportation of ions in and out of the cells.

The location of an organism's habitat plays an important role in determining the organism's salinity tolerance range. Malli's (1982) experiment on two difference species of limpets which live at different levels in the intertidal zone proposed that he resistance level towards salinity changes correlate to the limpet's position. His experiments on the high-level Cellana radiata and the low-level Siphonaria siphonaria at the Mangrol Coast in western India showed that S. siphonaria showed less tolerance to salinities above and below the natural conditions usually faced by it, as well as dessication, as compared to the higher-level C. radiata.

Osmoregulators or osmoconformers are organisms able to adapt to salinity changes in the external enviroment. One example is the stone crab, Cancer pagurus (Wanson, Pequeux and Gilles, 1983). Young crabs were found to survive 50%SW after a direct transfer

from seawater for 15 days. C. pagurus is described as an osmoconformer; they display an ability to regulate their cell's volume. This high tolerance for cellular swelling allows the crabs to survive in lower salinities.

Another osmoregulator is the snakehead (Woo and Tong, 1983). This showed that the snakehead could penetrate slightly hyperosmotic media by both tolerance and osmoregulation. This regulation in the snakehead was acheived through a stimulation of Na^+-K^+-ATPase activity. This enzymic activity resulted in Na^+-K^+-ATPase activity being pushed out across the gills. The snakehead's osmotic pressure also conformed to the salinity of the enviroment.

Plants can also be osmoregulators. An estuarine plant, Polysimphonia lanosa is a good example. When P. lanosa is subjected to changes in salinity (Reed, 1983) it reacts by regulating its cellular turgor. Reed explains thus:

> "...strict regulation of turgor does not occur in either marine or estuarine cells of P. lanosa ...the effector mechanisms responsible for changing cellular concentrations of solutes (and consequently osmotic potential) do not produce a 1:1 reaction in response to a change in external salt concentration...turgor does not remain constant in the face of changes in external salinity and cells of P. lanosa must be able to withstand a range of cell turgor potentials" Reed, 1983:

pg 190.

Another osmoregulating plant is the red alga Gracilara tikvahiae. In response to hypoosmotic shock from 26%. to 16%., the alga was discovered to have retained an excess of inorganic anions (Lapointe, Rice and Lawrence , 1984). G. tikvahiae, as an estuarine alga is subjected to wide salinity fluctuations in their habitat. They cope by developing a biochemical response to displace an influx of water from a less saline external medium to the more saline internal medium.

Certain cellular specializations also appear to be significant for osmoregulatory mechanisms such as active transport and cellular secretions. Finol and Croghan's (1983) experiment on the gill of an amphibious crab found that:

> "A feature of the crustacean bronchial epithelial cell is the very large number of mitochondria present...these cells have a very high metabolic activity and, if this is principally in relation to transport function, the energy requirements for this must be comparable in both concentrated and dilute media...the significance of rough endoplasmic reticulum is also unknown by might be related to cuticular secretion" Finol and Croghan, 1984: pg 73 -74.

Another form of cellular specialization is to have an efficient transport system from within the cytoplasm to reach out of the cell. Hatae, Tanenori and Benedetti (1983), in the examination of lamprey chloride cells revealed a complex membrane system in the cytoplasmic tubules which are also related to the transport of cellular products. The last example concerns compartmentalization. Compartmentalization provides a unique and effective way of osmoregulation. This specialization was found in the green alga Dunliella tertiolecta (Ehrenfield and Cousin, 1982). With this mechanism, the concentration of salt within the cell was lower than the surrounding medium.

Thus, through research into both areas of the experiment, the basic information as well as the complexitites regarding phototaxis and salinity was known. In this way, a proper analysis of results and a discussion could be made concerning the experiment.

PROCEDURE

I: Examining the phototactic behaviour of the protists.

The protists were going to be tested on their phototactic responses to red light, green light, blue light and white light. First, the protists were poured into four $50cm^3$ measuring cylinders. Then, a box was placed over each measuring cylinder. The boxes were then checked for any openings or holes which might allow light through and distract the protists. These openings were then sealed with black tape. Then a hole was punched with a knife into each box directly in front of each measuring cylinder, the hole made at a mark about half the height of the measuring cylinder. A piece of cellophane of the appropriate colour was then placed over the hole just made in each of the boxes and attached firmly with tape. The cellophanes used were: red (700nm) for the first box; green (550nm) for the second box; blue (450nm) for the third box; and a colourless cellophane was placed over the hole of the fourth box to simulate white light. A light bulb was then placed right in front of the each of the cellophanes, switched on, and left lighted for 6 hours. Figure 2 depicting the experiment appears overleaf.

After 6 hours, the light bulbs were switched off and the boxes removed. Tracing paper was then placed over the dark green patch in the middle of each measuring cylinder where the protists

accumulated in order to trace out the outline of the patch. The darkness or intensity of the patch was also recorded to determine the extent of the phototaxis exhibited by the protists.

Five repetitions of the experiment were conducted.

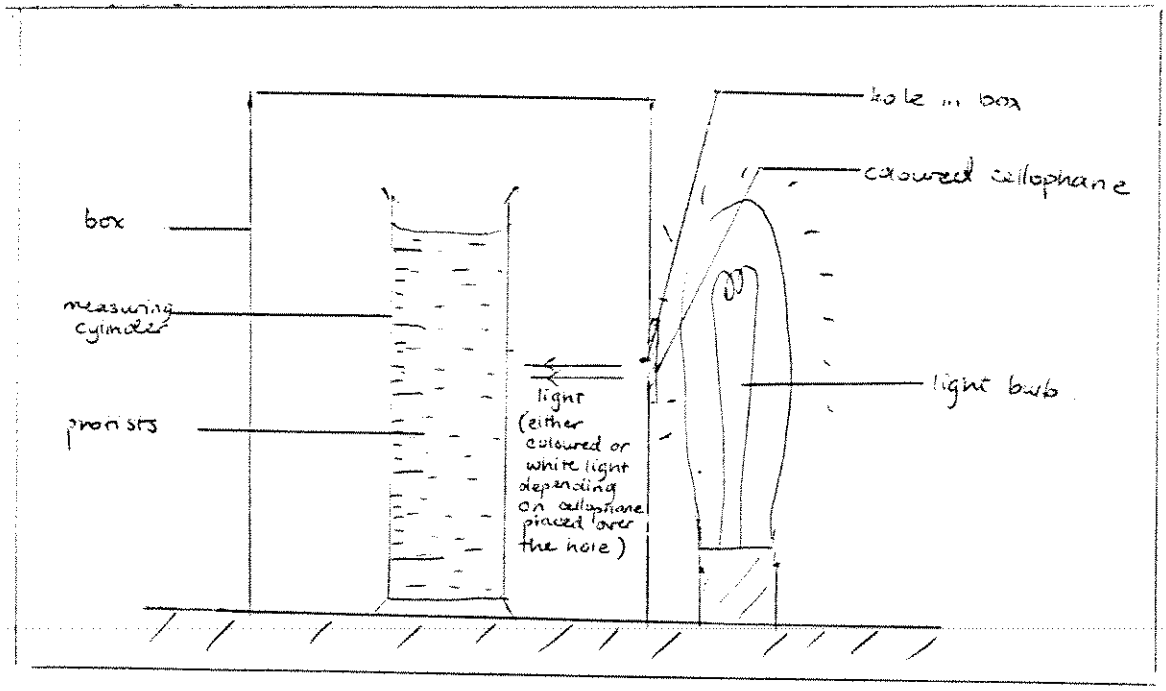


Figure 2

Diagram showing the set-up of the apparatus used for the phototaxis experiment

II: Determining the upper range of salinity tolerated by the protists

This experiment was conducted in small test tubes, in which samples of the freshwater from the tidepools containing the protists were diluted with seawater in order to stimulate conditions ranging from 10%SW to 90%SW. The immediate result of the adding of the seawater and the effect of dilution of the seawater-protist solutions with distilled water for 12 hours was also recorded.

In order to obtain the percentages of seawater required, seawater (which was obtained from Pedder Bay, British Columbia, and then carefully strained using a plankton net to prevent the protists from being ingested by plankton) was mixed with the protists in different ratios. The ratios used are as follows:

10%SW was obtained by mixing $1cm^3$ seawater to $9cm^3$ protists
20%SW was obtained by mixing $2cm^3$ seawater to $8cm^3$ protists
30%SW was obtained by mixing $3cm^3$ seawater to $7cm^3$ protists
40%SW was obtained by mixing $4cm^3$ seawater to $6cm^3$ protists
50%Sw was obtained by mixing $5cm^3$ seawater to $5cm^3$ protists
60%SW was obtained by mixing $6cm^3$ seawater to $4cm^3$ protists
70%SW was obtained by mixing $7cm^3$ seawater to $3cm^3$ protists
80%SW was obtained by mixing $8cm^3$ seawater to $2cm^3$ protists
90%SW was obtained by mixing $9cm^3$ seawater to $1cm^3$ protists

After the addition of seawater to the protists were done, the

solutions were thoroughly mixed.

Before examining the effects of the seawater on the protists, the salinity of the freshwater medium of the protists and salinity of seawater had to be quantified along with the salinity of the solutions in each of the nine test tubes. The method used for quantifying the salinity was the titration method. This titration method requires 5ml of the solution of unknown salinity to be taken with a pipette and emptied into a 450ml flask. Then, distilled water is added to the 50ml mark. After that, 17ml of potassium chromate ($K_2Cr_2O_4$), which is yellow, is added to the solution in the flask. Then, silver nitrate ($AgNO_3$) is titrated using a burette into the flask and swirled continuously until the solution turns a murky orange, the colour of potassium dichromate ($K_2Cr_2O_7$) which does not disappear even after continuous swirling. The volume of the silver nitrate titrated would be the salinity of the solution in parts per thousand(‰). The salinities of the tidepool water, seawater and of the nine test tubes containing 10%SW to 90%SW were quantified using this titration method.

After the salinities were quantified, the samples were thrown away. New samples were made using the method mentioned in the second paragraph. The nine test tubes were then labelled 10%SW.....90%SW accordingly. In order to determine the effect of seawater on the protists or on their state of health, the ratio of the numbers that were sessile to the total population of protists in a volume of $0.2mm^3$ was used. (N.B: Total population = number of protists sessile [not moving] + number of protists mobile [freely

swimming]). The protists were counted using a blood hemacytometer. The first count was done by placing a small drop of solution on the notch at one end of the hemacytometer and covered with the coverslip. Using the grid which marked out an area where the volume of the solution would be $0.1mm^3$, the sessile protists were counted first, followed by the mobile protists. The results were then recorded. The second count was done and then recorded. The results of the numbers sessile and the total population from both the counts were then added together to obtain the numbers sessile and the total population in a volume of $0.2mm^3$. This was done to involve a larger number of protists and obtain greater accuracy for the experiment.

This particular method was used as the protists unable to tolerate or adapt to salinity stress would be sessile while those able to tolerate or adapt would continue swimming. Thus, the greater the numbers of protists sessile to the total population (number of protists sessile plus number of protists mobile) was used as the population of the samples at each dilution was not constant. Therefore: the ratio of the numbers sessile to the total population of protists in $0.2mm^3$ was used as a way of determining the state of the health of the protists. A number close to 1 would denote low health, meaning fewer were mobile in proportion to the population, showing a greater amount of salinity stress on the protists as a whole. A number close to 0 denotes good health, meaning more were still mobile in proportion to the population, showing a lesser amount of salinity stress.

These counts were done immediately after each mixing of the protists and the seawater, and then counted after 2 hours. After the count for the 2-hour exposure was completed, I decided to compare the effects of exposure to seawater after 12 hours with the effect on the protists after the addition of distilled water to the seawater-protist solution after 2 hours. The addition of distilled water stimulated an influx of freshwater to the enviroment of the protists already exposed to seawater. To do this, 5ml of the solutions from the old test tubes was removed and placed into new test tubes. The old test tubes would now only have 5ml of the seawater-protist solution, and would be left for another 12 hours. The new test tubes with the 5ml of seawater-protists from the old test tubes were then added with 5ml of distilled water and stirred. The test tubes were then labelled 10%SW + distilled water.....90%SW + distilled water accordingly. These test tubes were left for 12 hours. Then a count was made from the test tubes containing only seawater-protists as well as the test tubes that had distilled water added to them.

These counts for the four separate conditions (i) the immediate effect in seawater, (ii) the effect after 2 hours in seawater, (iii) the effect after 12 hours in seawater and (iv) the effect after 12 hours with the addition of distilled water. They were then recorded for each of the nine different percentages of seawater in the four different conditions. This experiment was also repeated five times.

STATISTICS

The statistics were only conducted on the salinity part of the experiment. They were conducted to examine any variances between the samples of different time levels of the experiment as well as within samples of the same time levels. The ANOVAS (analysis of variance) tests from the "Stats Plus" program (1982) allowed me to check for sure whether any set of samples from the different time levels were statistically different (or not) from one another. The results from which the statistics were run on and the comparisons made was from the value of the ratio number of protists that were sessile: total population of the protists (i.e. sessile + mobile protists). As five repetitions of the experiment were done, there was enough data for statistics to be run.

Before the statistics were run, I had to divide the 10%SW to the 90%SW samples into two so that comparisons of mobility or the state of health of the protists between two salinity ranges could be done. The devider was deduced from Fig. 6 (Graph I) concerning the effect immediately after the SW addition. There is a cleardivision between the 10%SW - 40%SW samples where the ratio of numbers sessile: total population were approximately half and the 50%SW - 90%SW samples where the ratio of numbers of protists sessile: total population was very high. Therefore, the 10%SW - 40%SW range was treated as a block of results and so was the 50%SW - 90%SW range.

With these two sets of results, comparisons between and within the results of different time levels of the seawater

addition during the experiment (i.e. the change in mobility or state of health in the protists immediately after the seawater addition, the change in mobility after 2 hours in seawter, after 12 hours in seawater) as well as after the 12-hour immersion in distilled water.

Regarding the 10%SW - 40%SW range

These samples were compared amongst themselves within each of their own time levels. For example, the effect of the immediate addition of seawater on the protists' state of health in the 10%SW, 20%SW, 30%SW and 40%SW samples were compared amongst themselves. This was repeated in the 2-hour immersion in seawater time level, the immersion for 12 hours in seawater alone and the immersion for 12 hours in distilled water.

They were also compared as a whole set between each of the different time levels of the seawater immersion as well as the distiled water immersion. For example, the first time level of the immediate addition of seawater was compared with the immersion after 2 hours then with the 12 hours in seawater, [redacted]. This was then repeated with the 2-hour immersion being compared with the 12-hour seawater [redacted], and the 12-hour immersion in seawater was compared with the immersion in distilled water for 12 hours.

Regarding the 50%SW - 90%SW range

This whole procedure was repeated in the 50%SW - 90%SW range.

The 10%SW - 40%SW and the 50%SW -90%SW range was also compared within their own time levels of the seawater and distilled water immersions to check for any statistical differences in the state of health of the protists.

To see if there was any statistical difference between sets of samples, the F-test was used. However, if a statistical difference between samples was found, the F-test does not indicate which of the samples specifically differ. This problem was alleviated by looking at the graphs (Figs. 6, 7, 8 and 9) and checking their patterns of behaviour.

The results were then fed into a computer program that ran the statistics on the results which I wanted statistically analysed. The program would automatically calculate the value of F. If the calculated value of F was greater than the table value, this shows a statistical difference between samples. If the calculated F value was smaller than the table F value, there was no statistical difference between samples.

RESULTS

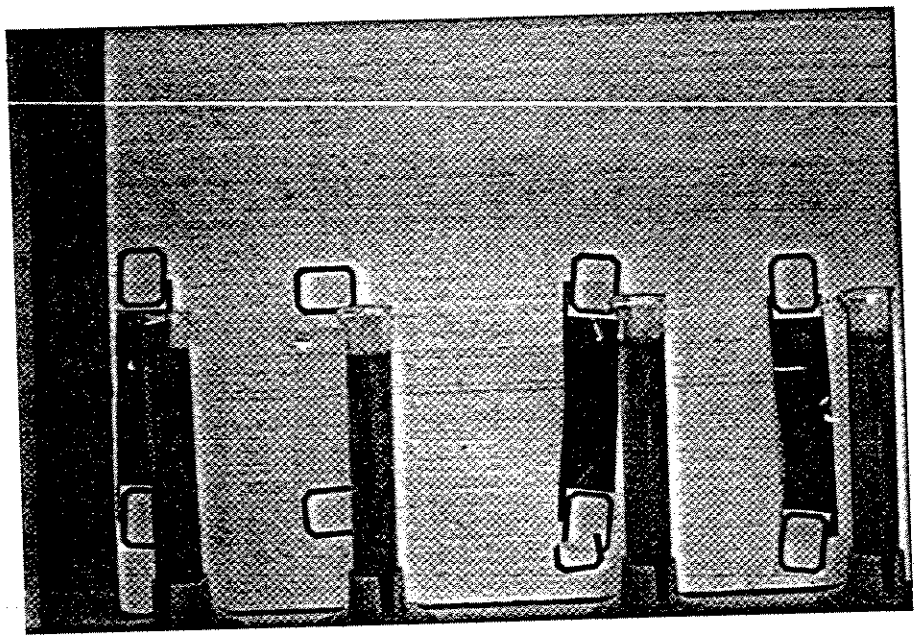
\&8

\&10

TABLE 1: Phototactic Orientation - Area and Darkness

REPITITION #	WHITE LIGHT		RED LIGHT		BLUE LIGHT		GREEN LIGHT	
	AREA cm_2	DARKNESS	AREA cm_2	DARKNESS	AREA cm_2	DARKNESS	AREA cm_2	DARKNESS
1	9.75	V.Dark	3.00	Dark	0.75	Medium	1.50	Faint
2	14.00	V.Dark	7.50	Dark	3.00	Medium	5.00	Faint
3	9.00	V.Dark	4.50	Dark	2.25	Medium	1.25	Faint
4	10.00	V.Dark	3.00	Dark	1.00	Medium	1.25	Faint
5	9.00	V.Dark	3.75	Dark	2.00	Medium	1.50	Faint
Average	10.35		4.35		1.8		1.8	

Faure 3 : Photograph showing the results of the phenotaxis of the protists in response to green, white, blue and red light.
(Note the green patches on the measuring cylinders. The coloured cellophanes used for the experiment are placed beside the appropriate measuring cylinder.



protists exposed to green light (550nm) A green cellophane was used.

Protists exposed to white light. A clear cellophane was used.

Protists exposed to blue light. (450nm). A blue cellophane was used.

Protists exposed to red light (700nm). A red cellophane was used.

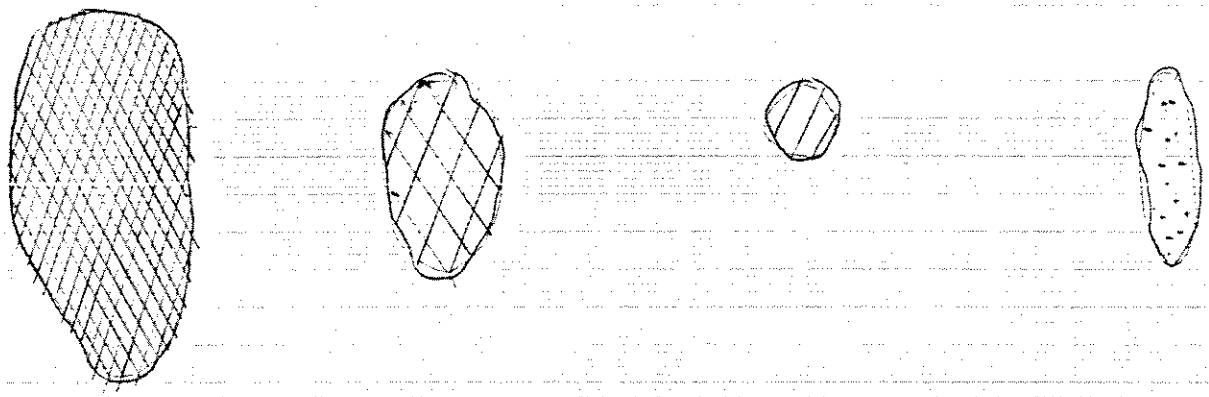
Phototactic Responses

Results of the experiment showing the area and darkness of the patches of protists in the measuring cylinder.

Repetitions I and II

White light	Red light 700nm	Blue light 450 nm	Green light 550nm

Repetition I



Repetition II

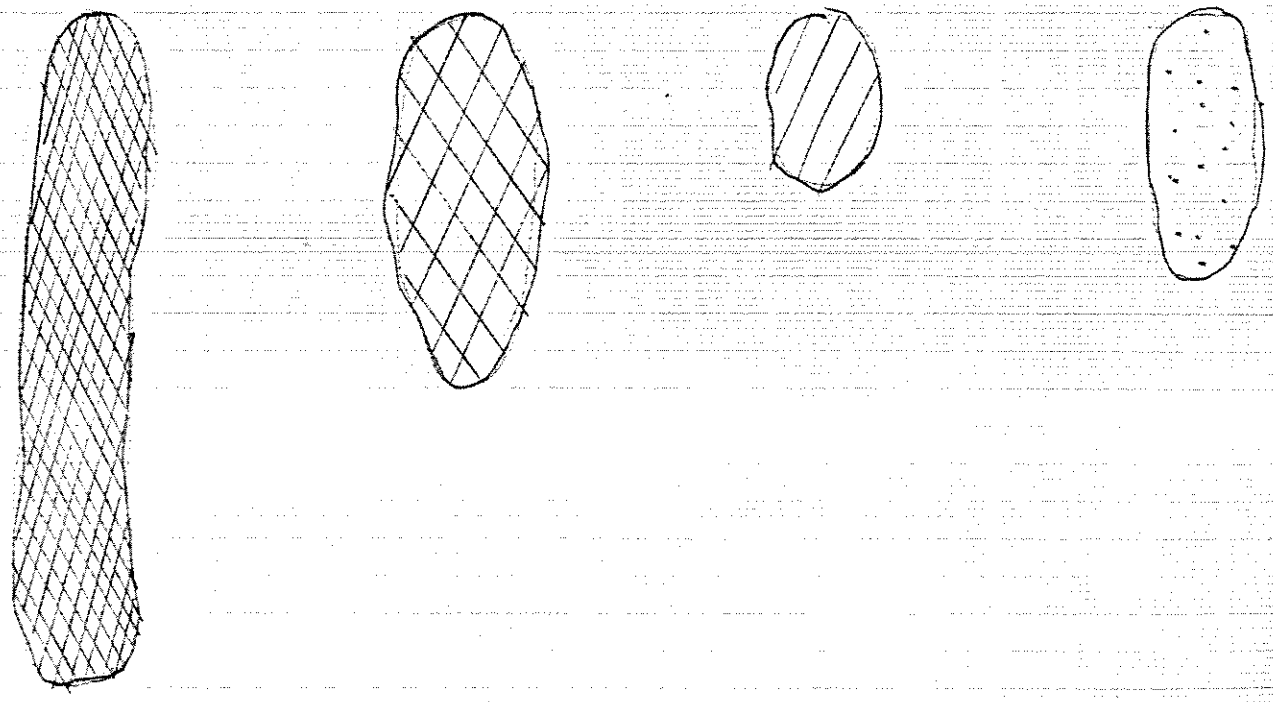


Figure 4

N.B: Very dark; Dark; Medium dark; faint

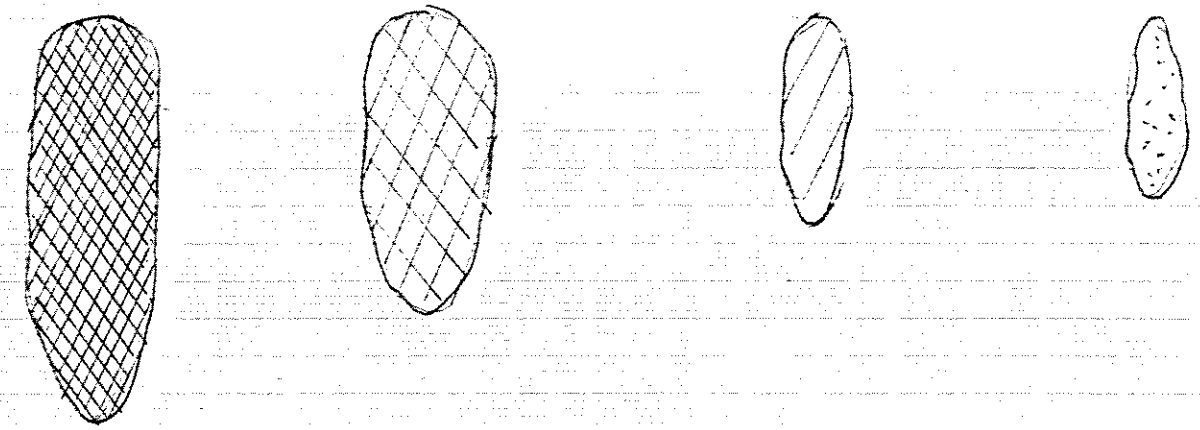
Phototactic Responses

Repetitions 3, 4 and 5

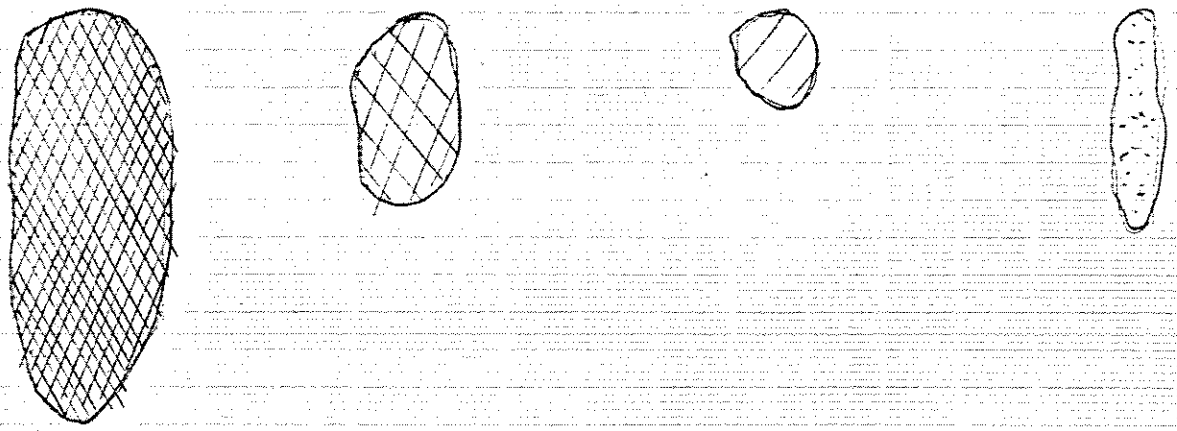
Results of the experiment showing the area and darkness of the zones of protists on the measuring cylinder.

White light	Red light 700nm	Blue light 450nm	Green light 550nm

Repetition 3



Repetition 4



Repetition 5

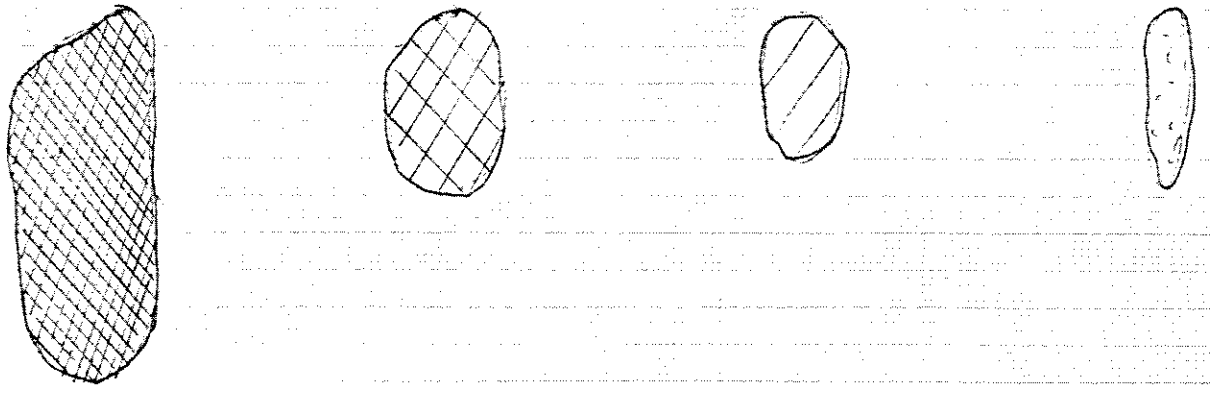


Figure 5

N.B. very dark; medium dark; Dark; faint

DATA: SALINITY TOLERANCE

TABLE 2

SOLUTIONS	SALINITY %
TIDEPOOLS	1.3
SEAWATER	31.0
10%SW	4.1
20%SW	8.2
30%SW	10.2
40%SW	13.8
50%SW	16.8
60%SW	19.8
70%SW	23.3
80%SW	27.8
90%SW	30.5

Time Level 1: The result immediately after immersion in seawater

TABLE 3: 10%SW

REPETITION #	COUNT	# SESSILE PER $0.1MM_3$	TOTAL POPULATION PER $0.1MM_3$	# SESSILE PER $0.2MM_3$	TOTAL POPULATION PER $0.2MM_3$	RATIO OF # SESSILE: TOTAL POPULATION
1	(i)	25	44	60	88	0.68
	(ii)	35	44			
2	(i)	20	25	32	42	0.76
	(ii)	12	17			
3	(i)	21	66	36	105	0.34
	(ii)	15	39			
4	(i)	30	51	55	85	0.65
	(ii)	25	34			
5	(i)	52	78	92	128	0.72
	(ii)	40	50			

TABLE 4: 20%SW

REPETION #	COUNT #	# SESSILE PER $0.1MM_3$	TOTAL POPULATION PER $0.1MM_3$	# SESSILE PER $0.2MM_3$	TOTAL POPULATION PER $0.2MM_3$	RATIO OF # SESSILE: TOTAL POPULATION
1	(i)	15	42	45	91	0.49
	(ii)	30	49			
2	(i)	29	70	66	144	0.46
	(ii)	37	74			
3	(i)	46	64	78	125	0.62
	(ii)	32	61			
4	(i)	39	64	75	115	0.65
	(ii)	36	55			
5	(i)	48	60	98	118	0.83
	(ii)	50	58			

TABLE 5: 30%SW

REPETITION #	COUNT #	# SESSILE PER $0.1MM_3$	TOTAL POPULATION PER $0.1MM_3$	# SESSILE PER $0.2MM_3$	TOTAL POPULATION PER $0.2MM_3$	RATIO OF # SESSILE: TOTAL POPULATION
1	(i)	26	41	50	83	0.60
	(ii)	24	42			
2	(i)	22	44	36	78	0.46
	(ii)	14	34			
3	(i)	7	22	15	42	0.36
	(ii)	8	20			
4	(i)	15	32	30	73	0.41
	(ii)	15	41			
5	(i)	12	28	40	69	0.58
	(ii)	28	41			

TABLE 6: 40%SW

REPETITION #	COUNT #	# SESSILE PER 0.1MM$_3$	TOTAL POPULATION PER 0.1MM$_3$	# SESSILE PER 0.2MM$_3$	TOTAL POPULATION PER 0.2MM$_3$	RATIO OF # SESSILE: TOTAL POPULATION
1	(i)	5	59	60	126	0.48
	(ii)	55	67			
2	(i)	15	59	41	122	0.34
	(ii)	26	63			
3	(i)	19	41	46	81	0.34
	(ii)	27	40			
4	(i)	19	45	57	97	0.59
	(ii)	38	52			
5	(i)	26	36	42	69	0.61
	(ii)	16	33			

TABLE 7: 50%SW

REPETITION #	COUNT #	# SESSILE PER 0.1MM$_3$	TOTAL POPULATION PER 0.1MM$_3$	# SESSILE PER 0.2MM$_3$	TOTAL POPULATION PER 0.2MM$_3$	RATIO OF # SESSILE: TOTAL POPULATION
1	(i)	24	25	50	51	0.98
	(ii)	26	26			
2	(i)	27	27	55	55	1.00
	(ii)	28	28			
3	(i)	31	32	62	63	0.98
	(ii)	31	31			
4	(i)	33	35	59	62	0.95
	(ii)	26	27			
5	(i)	21	21	40	40	1.00
	(ii)	19	19			

TABLE 8: 60%SW

REPETITION #	COUNT #	# SESSILE PER 0.1MM$_3$	TOTAL POPULATION PER 0.1MM$_3$	# SESSILE PER 0.2MM$_3$	TOTAL POPULATION PER 0.2MM$_3$	RATIO OF # SESSILE: TOTAL POPULATION
1	(I)	34	34	60	61	0.98
	(ii)	26	27			
2	(i)	38	38	71	71	1.00
	(ii)	33	33			
3	(i)	25	27	49	52	0.94
	(ii)	24	25			
4	(i)	28	29	59	60	0.98
	(ii)	31	31			
5	(i)	19	19	43	43	1.00
	(ii)	24	24			

TABLE 9: 70%SW

REPETITION #	COUNT #	# SESSILE PER 0.1MM$_3$	TOTAL POPULATION PER 0.1MM$_3$	# SESSILE PER 0.2MM$_3$	TOTAL POPULATION PER 0.2MM$_3$	RATIO OF # SESSILE: TOTAL POPULATION
1	(i)	16	16	36	37	0.97
	(ii)	20	21			
2	(i)	15	16	32	33	0.97
	(ii)	17	17			
3	(i)	19	19	39	39	1.00
	(ii)	20	20			
4	(i)	11	12	22	23	0.96
	(ii)	11	11			
5	(i)	24	24	43	43	1.00
	(ii)	19	19			

TABLE 10: 80%SW

REPETITION #	COUNT #	# SESSILE PER 0.1MM$_3$	TOTAL POPULATION PER 0.1MM$_3$	# SESSILE PER 0.2MM$_3$	TOTAL POPULATION PER 0.2MM$_3$	RATIO OF # SESSILE: TOTAL POPULATION
1	(i)	14	14	27	27	1.00
	(ii)	13	13			
2	(i)	12	13	23	24	0.96
	(ii)	11	11			
3	(i)	11	12	24	25	0.96
	(ii)	13	13			
4	(i)	10	10	22	22	1.00
	(ii)	12	12			
5	(i)	13	13	27	27	1.00
	(ii)	14	14			

TABLE 11: 90%SW

REPETITION #	COUNT #	# SESSILE PER 0.1MM$_3$	TOTAL POPULATION PER 0.1MM$_3$	# SESSILE PER 0.2MM$_3$	TOTAL POPULATION PER 0.2MM$_3$	RATIO OF # SESSILE: TOTAL POPULATION
1	(i)	8	8	16	17	0.94
	(ii)	8	9			
2	(i)	12	12	25	25	1.00
	(ii)	13	13			
3	(i)	11	11	21	21	1.00
	(ii)	10	10			
4	(i)	10	10	21	21	1.00
	(ii)	11	11			
5	(i)	15	15	27	27	1.00
	(ii)	12	12			

Time Level 2: The results after a 2-hour immersion in seawater

TABLE 12: 10%SW

REPETITION #	COUNT #	# SESSILE PER 0.1MM$_3$	TOTAL POPULATION PER 0.1MM$_3$	# SESSILE PER 0.2MM$_3$	TOTAL POPULATION PER 0.2MM$_3$	RATIO OF # SESSILE: TOTAL POPULATION
1	(i)	4	8	6	13	0.46
	(ii)	2	5			
2	(i)	10	25	27	53	0.57
	(ii)	17	28			
3	(i)	21	52	51	111	0.46
	(ii)	31	59			
4	(i)	19	34	35	69	0.57
	(ii)	16	35			
5	(i)	8	22	19	44	0.43
	(ii)	11	22			

TABLE 13: 20%SW

REPETITION #	COUNT #	# SESSILE PER 0.1MM$_3$	TOTAL POPULATION PER 0.1MM$_3$	# SESSILE PER 0.2MM$_3$	TOTAL POPULATION PER 0.2MM$_3$	RATIO OF # SESSILE: TOTAL POPULATION
1	(i)	18	26	36	53	0.68
	(ii)	18	27			
2	(i)	15	28	23	55	0.42
	(ii)	8	27			
3	(i)	20	38	42	87	0.48
	(ii)	22	49			
4	(i)	13	40	20	74	0.27
	(ii)	7	34			
5	(i)	14	23	28	61	0.46
	(ii)	14	38			

TABLE 14: 30%SW

REPETITION #	COUNT #	# SESSILE PER 0.1MM$_3$	TOTAL POPULATION PER 0.1MM$_3$	# SESSILE PER 0.2MM$_3$	TOTAL POPULATION PER 0.2MM$_3$	RATIO OF # SESSILE: TOTAL POPULATION
1	(i)	24	39	54	29	0.61
	(ii)	12	34			
2	(i)	31	39	56	92	0.61
	(ii)	25	53			
3	(i)	26	46	53	98	0.54
	(ii)	27	52			
4	(i)	25	53	57	128	0.40
	(ii)	26	75			
5	(i)	46	76	87	153	0.57
	(ii)	41	77			

TABLE 15: 40%SW

REPETITION #	COUNT #	# SESSILE PER 0.1MM$_3$	TOTAL POPULATION PER 0.1MM$_3$	# SESSILE PER 0.2MM$_3$	TOTAL POPULATION PER 0.2MM$_3$	RATIO OF # SESSILE: TOTAL POPULATION
1	(i)	13	36	25	70	0.37
	(ii)	12	34			
2	(i0	12	37	16	56	0.29
	(ii)	4	19			
3	(i)	14	37	38	79	0.48
	(ii)	24	42			
4	(i)	24	32	42	58	0.72
	(ii)	18	25			
5	(i)	6	8	15	27	0.56
	(ii)	9	19			

TABLE 16: 50%SW

REPETITON #	COUNT #	# SESSILE PER 0.1MM$_3$	TOTAL POPULATION PER 0.1MM$_3$	# SESSILE PER 0.2MM$_3$	TOTAL POPULATION PER 0.2MM$_3$	RATIO OF # SESSILE: TOTAL POPULATION
1	(i)	16	31	27	63	0.43
	(ii)	11	32			
2	(i)	14	24	36	53	0.68
	(ii)	22	29			
3	(i)	14	34	36	63	0.57
	(ii)	22	29			
4	(i)	9	27	23	55	0.42
	(ii)	14	28			
5	(i)	3	8	14	30	0.47
	(ii)	11	22			

TABLE 17: 60%SW

REPETITION #	COUNT #	# SESSILE PER 0.1MM$_3$	TOTAL POPULATION PER 0.1MM$_3$	# SESSILE PER 0.2MM$_3$	TOTAL POPULATION PER 0.2MM$_3$	RATIO OF # SESSILE: TOTAL POPULATION
1	(i)	12	17	24	37	0.65
	(ii)	12	20			
2	(i)	8	18	18	39	0.46
	(ii)	10	21			
3	(i)	6	45	20	75	0.27
	(ii)	14	30			
4	(i)	5	21	15	55	0.27
	(ii)	10	34			
5	(i)	7	26	12	47	0.26
	(ii)	5	21			

TABLE 18: 70%SW

REPETITION #	COUNT #	# SESSILE PER 0.1MM$_3$	TOTAL POPULATION PER 0.1MM$_3$	# SESSILE PER 0.2MM$_3$	TOTAL POPULATION PER 0.2MM$_3$	RATIO OF # SESSILE: TOTAL POPULATION
1	(i)	7	16	13	40	0.33
	(ii)	6	24			
2	(i)	9	17	16	32	0.50
	(ii)	7	15			
3	(i)	4	9	10	31	0.32
	(ii)	6	22			
4	(i)	5	11	10	24	0.42
	(ii)	5	13			
5	(i)	12	25	23	44	0.52
	(ii)	11	19			

TABLE 19: 80%SW

REPETITION #	COUNT #	# SESSILE PER 0.1MM$_3$	TOTAL POPULATION PER 0.1MM$_3$	# SESSILE PER 0.2MM$_3$	TOTAL POPULATION PER 0.2MM$_3$	RATIO OF # SESSILE: TOTAL POPULATION
1	(i)	2	13	7	28	0.25
	(ii)	5	15			
2	(i)	2	14	3	30	0.10
	(ii)	1	16			
3	(i)	4	13	8	26	0.31
	(ii)	2	13			
4	(i)	7	18	17	38	0.45
	(ii)	10	20			
5	(i)	7	14	13	25	0.52
	(ii)	6	11			

TABLE 20: 90%SW

REPETITION #	COUNT #	# SESSILE PER 0.1MM$_3$	TOTAL POPULATION PER 0.1MM$_3$	# SESSILE PER 0.2MM$_3$	TOTAL POPULATION PER 0.2MM$_3$	RATIO OF # SESSILE: TOTAL POPULATION
1	(i)	2	9	3	16	0.19
	(ii)	1	7			
2	(i)	2	12	5	23	0.22
	(ii)	3	11			
3	(i)	4	14	7	29	0.24
	(ii)	3	15			
4	(i)	2	12	3	22	0.14
	(ii)	1	10			
5	(i)	1	4	3	11	0.27
	(ii)	2	7			

Time Level 3: The results after a 12-hour immersion in saltwater

TABLE 21: 10%SW

REPETITION #	COUNT #	# SESSILE PER 0.1MM$_3$	TOTAL POPULATION PER 0.1MM$_3$	# SESSILE PER 0.2MM$_3$	TOTAL POPULATION PER 0.2MM$_3$	RATIO OF # SESSILE: TOTAL POPULATION
1	(i)	3	19	21	51	0.41
	(ii)	18	32			
2	(i)	17	29	25	57	0.44
	(ii)	8	28			
3	(i)	14	41	49	100	0.49
	(ii)	35	59			
4	(i)	18	36	38	76	0.50
	(ii)	20	40			
5	(i)	14	34	38	72	0.53
	(ii)	24	38			

TABLE 22: 20%SW

REPETITION #	COUNT #	# SESSILE PER 0.1MM$_3$	TOTAL POPULATION PER 0.1MM$_3$	# SESSILE PER 0.2MM$_3$	TOTAL POPULATION PER 0.2MM$_3$	RATIO OF # SESSILE: TOTAL POPULATION
1	(i)	19	39	62	108	0.57
	(ii)	43	69			
2	(i)	2	33	5	75	0.07
	(ii)	3	42			
3	(i)	9	39	30	77	0.39
	(ii)	21	38			
4	(i)	1	30	3	64	0.05
	(ii)	2	34			
5	(i)	5	18	22	41	0.45
	(ii)	17	31			

TABLE 23: 30%SW

REPETITION #	COUNT #	# SESSILE PER 0.1MM$_3$	TOTAL POPULATION PER 0.1MM$_3$	# SESSILE PER 0.2MM$_3$	TOTAL POPULATION PER 0.2MM$_3$	RATIO OF # SESSILE: TOTAL POPULATION
1	(i)	3	10	13	36	0.36
	(ii)	10	26			
2	(i)	5	30	13	63	0.21
	(ii)	8	33			
3	(i)	8	33	13	62	0.26
	(ii)	10	35			
4	(i)	7	29	11	52	0.21
	(ii)	4	23			
5	(i)	7	27	18	63	0.29
	(ii)	11	36			

TABLE 24: 40%SW

REPETITION #	COUNT #	# SESSILE PER $0.1MM_3$	TOTAL POPULATION PER $0.1MM_3$	# SESSILE PER $0.2MM_3$	TOTAL POPULATION PER $0.2MM_3$	RATIO OF # SESSILE: TOTAL POPULATION
1	(i)	4	26	9	54	0.17
	(ii)	5	28			
2	(i)	8	39	13	53	0.25
	(ii)	5	14			
3	(i)	7	39	22	74	0.30
	(ii)	15	35			
4	(i)	3	8	11	32	0.34
	(ii)	8	24			
5	(i)	2	10	4	24	0.17
	(ii)	2	14			

TABLE 25: 50%SW

REPETITION #	COUNT #	# SESSILE PER $0.1MM_3$	TOTAL POPULATION PER $0.1MM_3$	# SESSILE PER $0.2MM_3$	TOTAL POPULATION PER $0.2MM_3$	RATIO OF # SESSILE: TOTAL POPULATION
1	(i)	23	63	42	113	0.37
	(ii)	19	50			
2	(i)	17	35	35	74	0.47
	(ii)	18	39			
3	(i)	13	38	40	72	0.56
	(ii)	27	54			
4	(i)	24	82	35	156	0.34
	(ii)	29	74			
5	(i)	24	63	45	113	0.40
	(ii)	21	50			

TABLE 26: 60%SW

REPETITION #	COUNT #	# SESSILE PER 0.1MM$_3$	TOTAL POPULATION PER 0.1MM$_3$	# SESSILE PER 0.2MM$_3$	TOTAL POPULATION PER 0.2MM$_3$	RATIO OF # SESSILE: TOTAL POPULATION
1	(i)	8	19	14	47	0.30
	(ii)	6	28			
2	(i)	10	34	16	65	0.25
	(ii)	6	31			
3	(i)	7	32	12	73	0.16
	(ii)	5	41			
4	(i)	5	31	19	70	0.27
	(ii)	14	39			
5	(i)	12	43	21	87	0.27
	(ii)	9	44			

TABLE 27: 70%SW

REPETITION #	COUNT #	# SESSILE PER 0.1MM$_3$	TOTAL POPULATION PER 0.1MM3	# SESSILE PER 0.2MM$_3$	TOTAL POPULATION PER 0.2MM^3	RATIO OF # SESSILE: TOTAL POPULATION
1	(i)	2	18	4	34	0.12
	(ii)	2	16			
2	(i)	3	25	13	57	0.23
	(ii)	10	32			
3	(i)	2	24	4	46	0.09
	(ii)	2	22			
4	(i)	1	26	4	22	0.18
	(ii)	3	16			
5	(i)	1	15	4	38	0.11
	(ii)	3	23			

TABLE 28: 80%SW

REPETITION #	COUNT #	# SESSILE PER 0.1MM^3	TOTAL POPULATION PER 0.1MM^3	# SESSILE PER 0.2MM^3	TOTAL POPULATION PER 0.2MM^3	RATIO OF # SESSILE: TOTAL POPULATION
1	(i)	1	12	3	25	0.12
	(ii)	2	13			
2	(i)	2	17	4	37	0.11
	(ii)	2	20			
3	(i)	3	15	5	33	0.15
	(ii)	2	18			
4	(i)	0	15	10	34	0.29
	(ii)	10	19			
5	(i)	4	18	5	35	0.14
	(ii)	1	17			

TABLE 29: 90%SW

REPETITION #	COUNT #	# SESSILE PER 0.1MM^3	TOTAL POPULATION PER 0.1MM^3	# SESSILE PER 0.2MM^3	TOTAL POPULATION PER 0.2MM^3	RATIO OF # SESSILE: TOTAL POPULATION
1	(i)	2	17	5	36	0.14
	(ii)	3	19			
2	(i)	2	15	3	29	0.10
	(ii)	1	14			
3	(i)	2	22	7	49	0.14
	(ii)	5	27			
4	(i)	2	20	4	44	0.09
	(ii)	2	24			
5	(i)	2	24	3	40	0.08
	(ii)	1	16			

Time Level 4: Results after a 12-hour immersion in distilled water

TABLE 30: 10%SW

REPETITION #	COUNT #	# SESSILE PER 0.1MM^3	TOTAL POPULATION PER 0.1MM^3	# SESSILE PER 0.2MM^3	TOTAL POPULATION PER 0.2MM^3	RATIO OF # SESSILE: TOTAL POPULATION
1	(i)	3	9	4	16	0.25
	(ii)	1	7			
2	(i)	3	7	9	21	0.43
	(ii)	6	14			
3	(i)	2	14	7	29	0.24
	(ii)	5	15			
4	(i)	5	14	11	28	0.39
	(ii)	6	14			
5	(i)	10	25	25	51	0.49
	(ii)	15	26			

TABLE 31: 20%SW

REPETITION #	COUNT #	# SESSILE PER 0.1MM^3	TOTAL POPULATION PER 0.1MM^3	# SESSILE PER 0.2MM^3	TOTAL POPULATION PER 0.2MM^3	RATIO OF # SESSILE: TOTAL POPULATION
1	(i)	0	6	1	22	0.05
	(ii)	1	14			
2	(i)	2	12	4	29	0.14
	(ii)	2	16			
3	(i)	12	22	19	39	0.49
	(ii)	7	17			
4	(i)	3	17	11	33	0.33
	(ii)	8	16			
5	(i)	1	17	2	33	0.06
	(ii)	1	16			

TABLE 32: 30%SW

REPETITION #	COUNT #	# SESSILE PER 0.1MM^3	TOTAL POPULATION PER 0.1M±^{3}	# SESSILE PER 0.2MM^3	TOTAL POPULATION PER 0.2M±^{3}	RATIO OF # SESSILE: TOTAL POPULATION
1	(i)	5	20	6	33	0.24
	(ii)	5	12			
2	(i)	3	20	10	39	0.26
	(ii)	7	19			
3	(i)	1	16	2	27	0.07
	(ii)	1	11			
4	(i)	2	12	5	30	0.17
	(ii)	3	18			
5	(i)	4	14	5	24	0.21
	(ii)	1	10			

TABLE 33: 40%SW

REPETITION #	COUNT #	# SESSILE PER 0.1MM^3	TOTAL POPULATION PER 0.1MM^3	# SESSILE PER 0.2MM^3	TOTAL POPULATION PER 0.2MM^3	RATIO OF # SESSILE: TOTAL POPULATION
1	(i)	2	22	8	34	0.24
	(ii)	5	12			
2	(i)	11	17	16	29	0.55
	(ii)	5	12			
3	(i)	2	17	4	25	0.16
	(ii)	2	8			
4	(i)	4	13	5	25	0.20
	(ii)	1	12			
5	(i)	2	8	6	20	0.30
	(ii)	4	12			

TABLE 34: 50%SW

REPETITION #	COUNT #	# SESSILE PER 0.1MM^3	TOTAL POPULATION PER 0.1MM^3	# SESSILE PER 0.2MM^3	TOTAL POPULATION PER 0.2MM^3	RATIO OF # SESSILE: TOTAL POPULATION
1	(i)	5	14	9	28	0.29
	(ii)	4	14			
2	(i)	10	20	29	53	0.55
	(ii)	19	33			
3	(i)	1	14	1	26	0.04
	(ii)	0	12			
4	(i)	1	14	7	29	0.24
	(ii)	6	15			
5	(i)	9	22	17	43	0.40
	(ii)	8	21			

TABLE 35: 60%SW

REPETITION #	COUNT #	# SESSILE PER 0.1MM^3	TOTAL POPULATION PER 0.1MM^3	# SESSILE PER 0.2MM^3	TOTAL POPULATION PER 0.2MM^3	RATIO OF # SESSILE: TOTAL POPULATION
1	(i)	0	4	2	10	0.20
	(ii)	2	6			
2	(i)	1	11	2	18	0.11
	(ii)	1	7			
3	(i)	7	41	16	48	0.21
	(ii)	7	27			
4	(i)	3	15	18	40	0.45
	(ii)	15	25			
5	(i)	6	18	14	33	0.42
	(ii)	8	15			

TABLE 36: 70%SW

REPETITION #	COUNT #	# SESSILE PER 0.1MM^3	TOTAL POPULATION PER 0.1MM^3	# SESSILE PER 0.2MM^3	TOTAL POPULATION PER 0.2MM^3	RATIO OF # SESSILE: TOTAL POPULATION
1	(i)	9	14	15	29	0.52
	(ii)	6	15			
2	(i)	0	5	1	9	0.11
	(ii)	1	4			
3	(i)	0	7	3	15	0.20
	(ii)	3	8			
4	(i)	3	6	6	12	0.50
	(ii)	3	6			
5	(i)	3	7	6	15	0.40
	(ii)	3	8			

TABLE 37: 80%SW

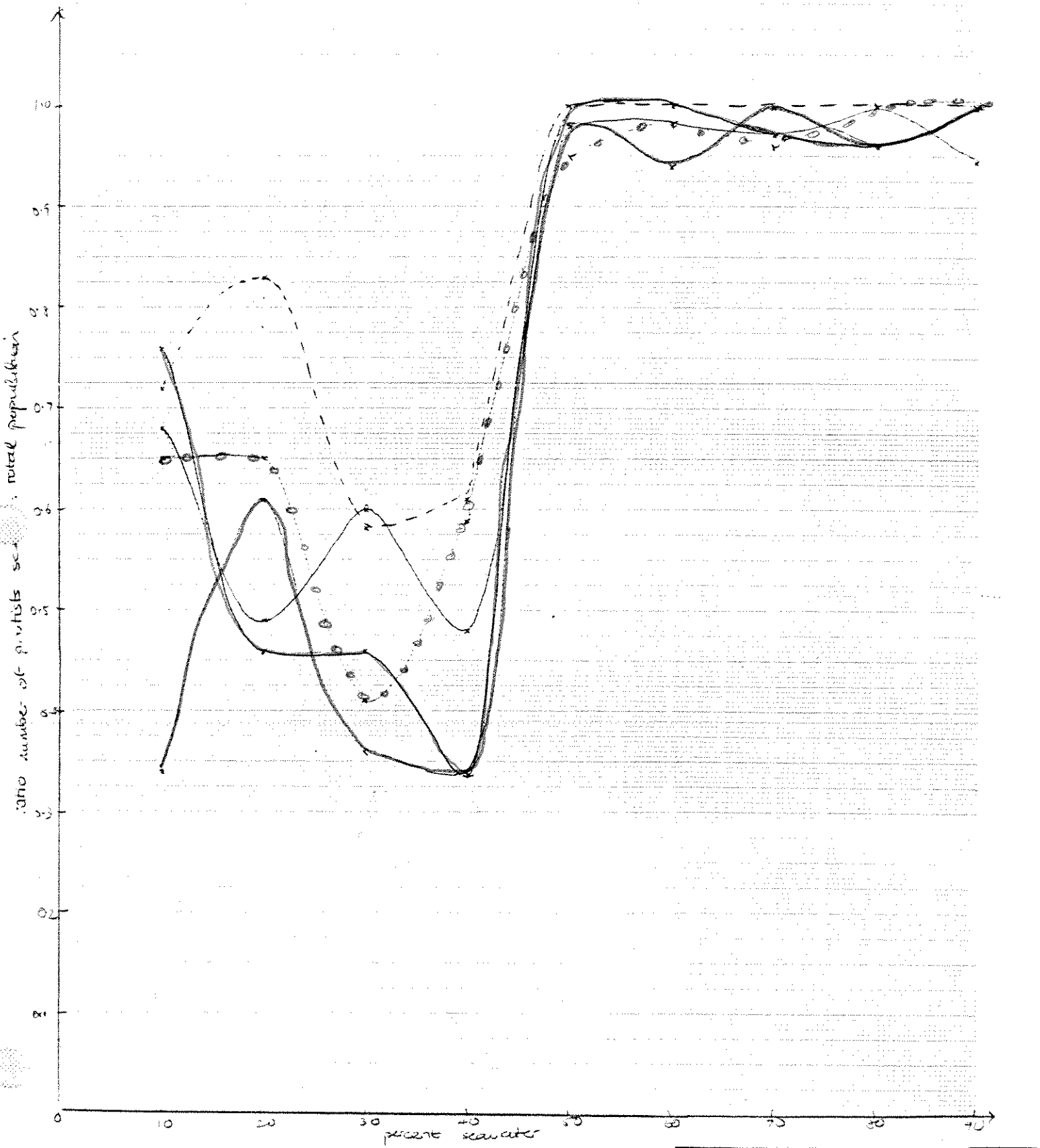
REPETITION #	COUNT #	# SESSILE PER 0.1MM^3	TOTAL POPULATION PER 0.1MM^3	# SESSILE PER 0.2MM^3	TOTAL POPULATION PER 0.2MM^3	RATIO OF # SESSILE: TOTAL POPULATION
1	(i)	1	6	2	12	0.17
	(ii)	1	6			
2	(i)	0	4	2	12	0.17
	(ii)	2	8			
3	(i)	1	7	1	10	0.10
	(ii)	0	3			
4	(i)	0	1	5	11	0.45
	(ii)	5	10			
5	(i)	2	4	7	12	0.58
	(ii)	5	8			

TABLE 38: 90%SW

REPETITION #	COUNT #	# SESSILE PER 0.1MM^3	TOTAL POPULATION PER 0.1MM^3	# SESSILE PER 0.2MM^3	TOTAL POPULATION PER 0.2MM^3	RATIO OF # SESSILE: TOTAL POPULATION
1	(i)	1	7	2	15	0.13
	(ii)	1	8			
2	(i)	4	8	4	11	0.36
	(ii)	0	3			
3	(i)	1	5	2	10	0.20
	(ii)	1	5			
4	(i)	2	6	3	12	0.25
	(ii)	1	6			
5	(i)	0	6	1	10	0.10
	(ii)	1	4			

Fig. 6 : Results immediately after the addition of seawater (Time level I)

Graph of ratio number of protists : total population vs percent seawater.



NOTE:
repetition I
repetition II
repetition III
repetition IV
repetition V

Fig. 7 : Results after 2 hours in seawater (Time level II)

Graph of ratio number of plants sessile : total population vs. percent seawater.

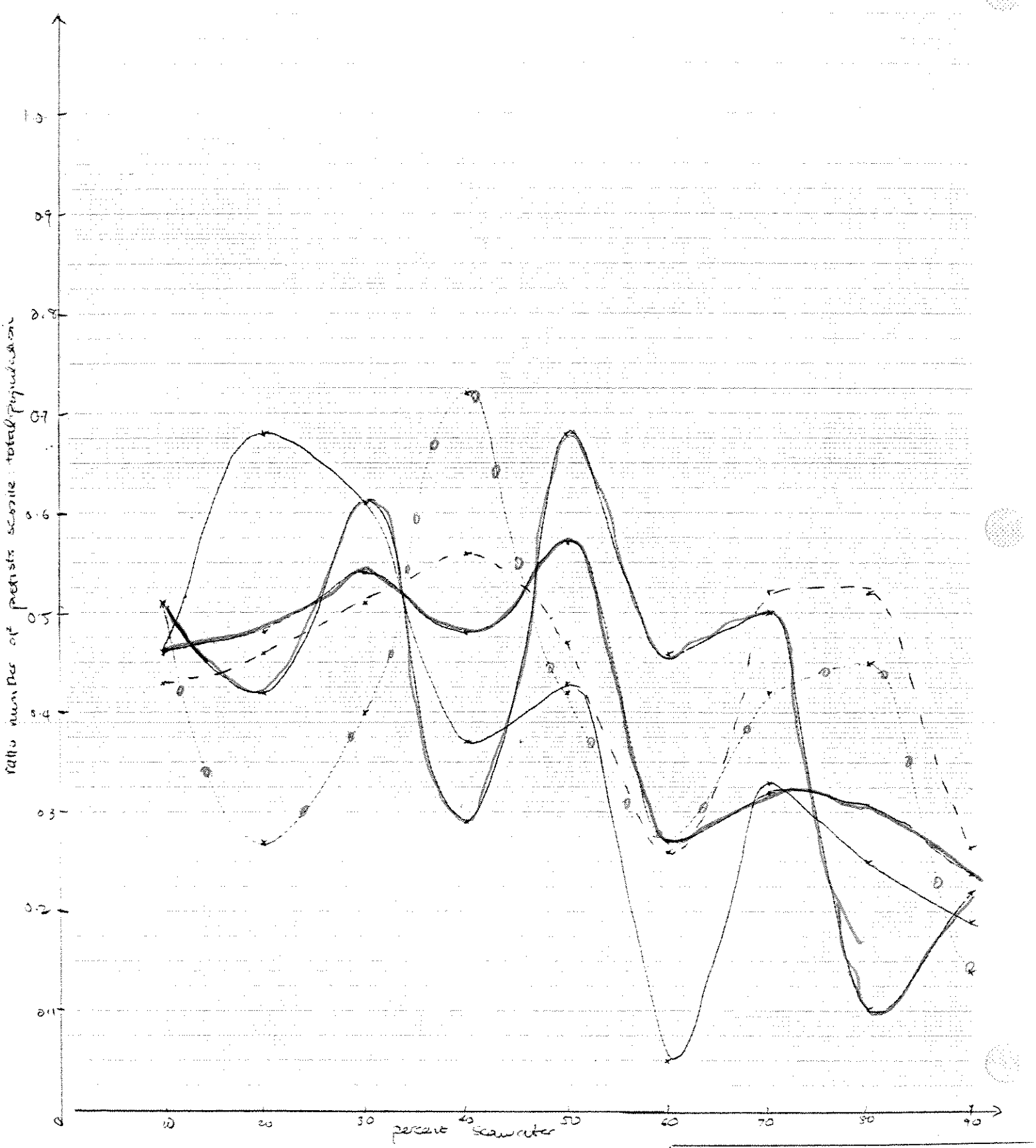


Fig. 3 : Results after 12 hours in seawater (Time level III)

Graph of ratio number of protists sessile : total population vs percent seawater.

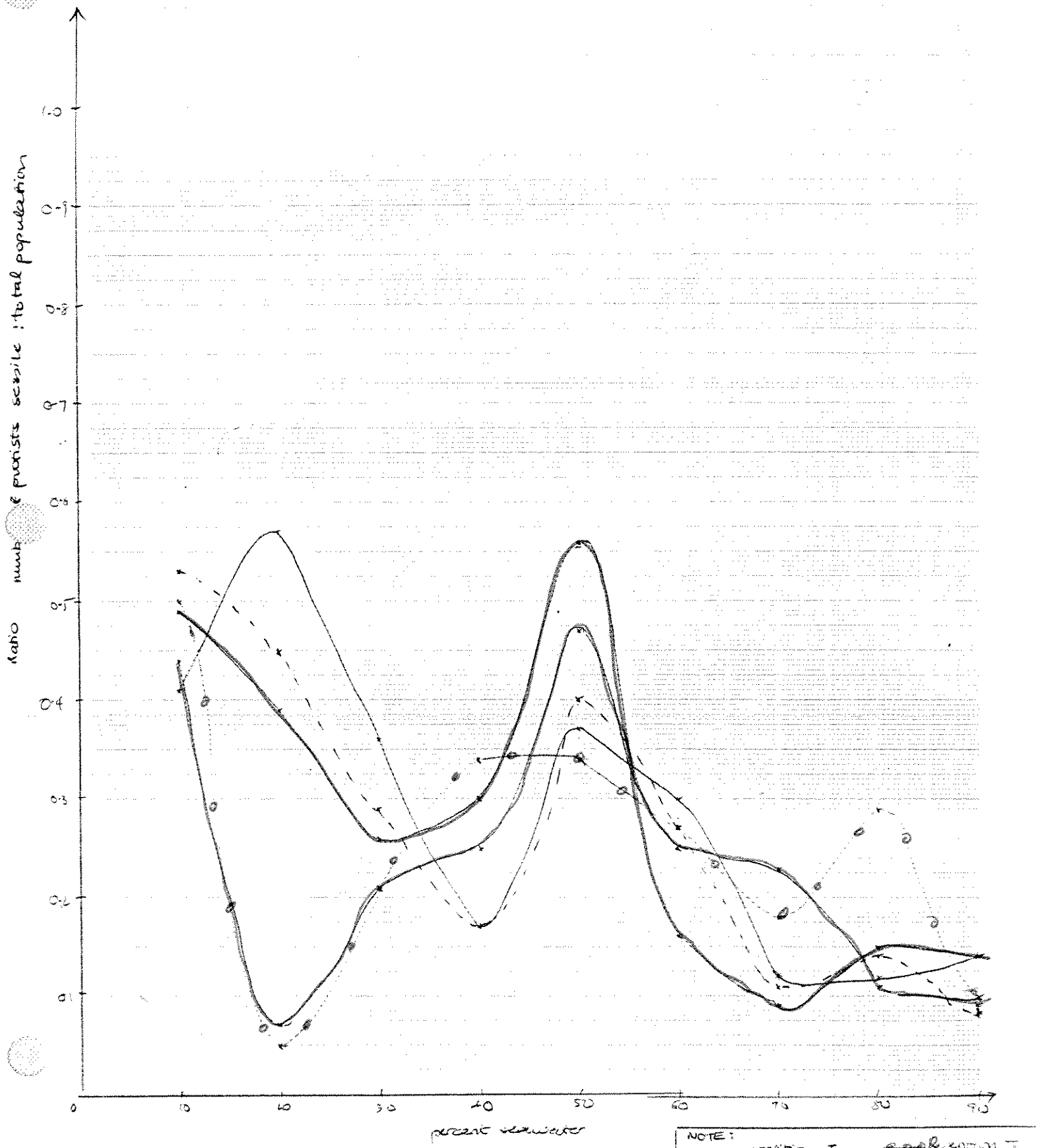


Fig. 9 : Results after 12 hours in distilled water (Time level 4)

Graph of ratio number of protists sessile : total population vs percent seawater.

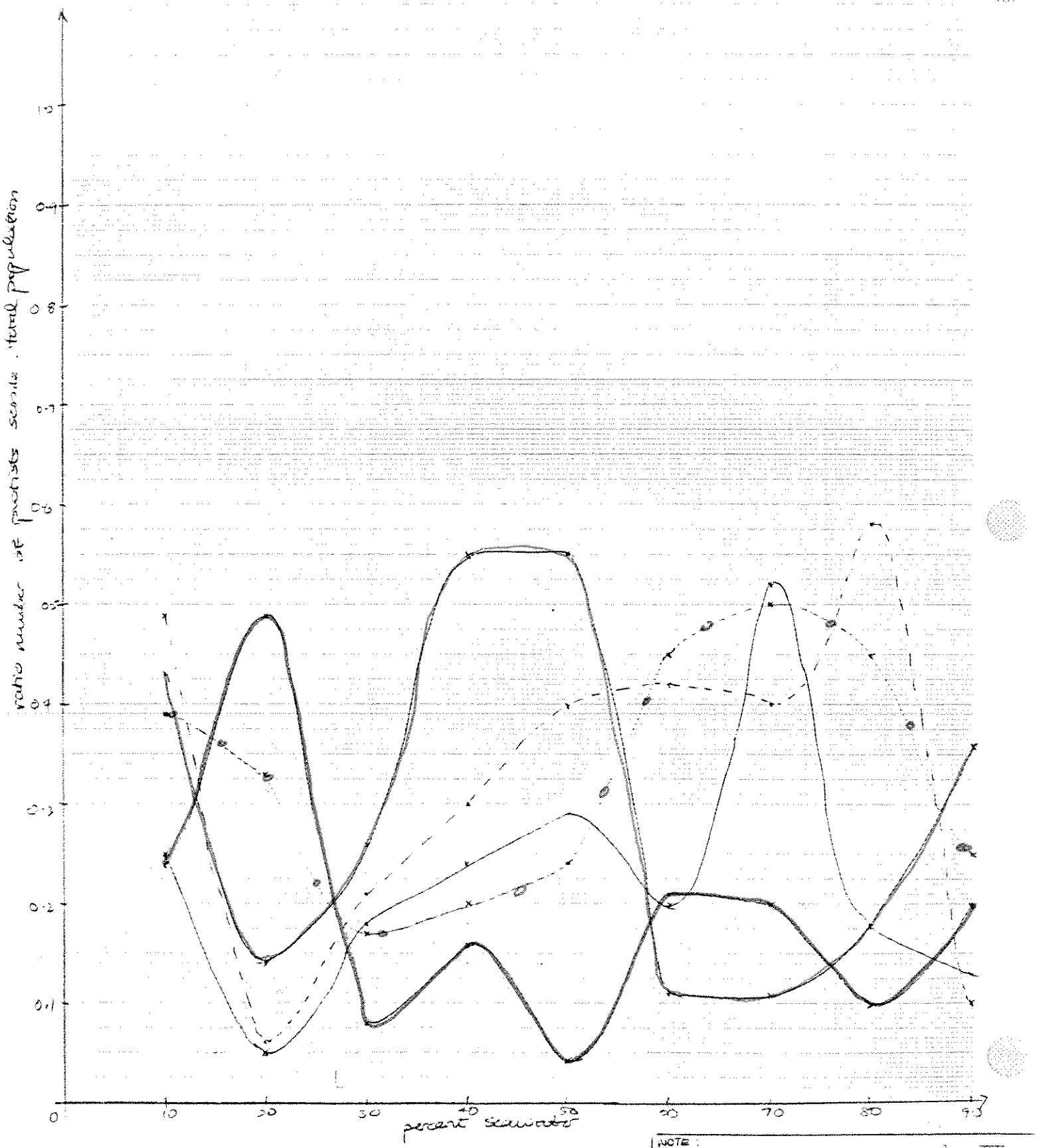


TABLE 39: STATISTICS

RUN #	STATISTICS RUN ON:	CALCULATED F	TABLE F	STATISTICAL DIFFERENCE?
01	Immediate effect of addition of seawater in the 10%SW vs 20%SW vs 30%SW vs 40%SW samples	1.78	5.29	NO
02	Immediate effect of addition of seawater in the 50%SW vs 60%SW vs 70%SW vs 80%SW vs 90%SW samples	0.11	4.43	NO
03	Immediate effect of addition of seawater in the 10%SW -40%SW range vs immediate effect of addition of seawater in the 50%SW - 90%SW range	212.06	7.31	YES
04	Result after 2 hours in seawater in the 10%SW vs 20%SW vs 30%SW vs 40%SW samples	0.48	5.29	NO
05	Result after 2 hours in 50%SW vs 60%SW vs 70%SW vs 80%SW vs 90%SW samples	5.12	4.43	YES

06	Result after 2 hours in 10%SW - 40%SW ranges vs result after 2 hours in 50%SW -90%SW range	12.03	7.31	YES
07	Immediate result of addition of seawater in 10%SW - 40%SW range vs results after 2 hours in 10%SW - 40%SW range	1.85	7.31	NO
08	Immediate result of addition of seawater in 50%SW - 90%SW range vs results after 2 hours in 50%SW - 90%SW range	410.56	7.31	YES
09	Results after 12 hours in seawater in 10%SW vs 20%SW vs 30%SW vs 40%SW samples	3.22	5.29	NO
10	Results after 12 hours in seawater in 50%SW vs 60%SW vs 70%SW vs 80%SW vs 90%SW samples	20.24	4.43	YES
11	REsults after 12 hours in seawater in the 10%SW - 40%SW range vs results after 12 hours in seawater in the 50%SW - 90%SW range	6.33	7.31	NO
12	Immediate effect of addition of seawater in 10%SW - 40%SW range vs results after 12 hours in seawater in 10%SW - 40%SW range	22.93	7.31	YES

13	Results after 2 hours in seawater in 10%SW - 40%SW range vs results after 12 hours in seawater in 10%SW - 40%SW range	15.79	7.31	YES
14	Immediate effect of addition of saltwater in 50%SW - 90%SW vs results after 12 hours in seawater in 50%SW - 90%SW range	847.09	7.31	YES
15	Results after 2 hours in seawater in 50%SW - 90%SW range vs results after 12 hours in seawater in 50%SW - 90%SW range	10.04	7.31	YES
16.	Results after 12 hours in distilled water in 10%SW vs 20%SW vs 30%SW vs 40%SW samples	1.70	5.29	NO
17	Results after 12 hours in distilled water in 50%SW vs 60%SW vs 70%SW vs 80%SW vs 90%SW samples	0.43	4.43	NO
18	Results after 12 hours in distilled water in 10%SW - 40%SW range vs results after 12 hours in distilled water in 50%SW - 90%SW range	0.15	7.31	NO
19	Results after 12 hours in seawater in 10%SW - 40%SW range vs results after 12 hours in distilled water in 10%SW - 40%SW range	1.77	7.31	NO

20	Results after 12 hours in seawater in 50%SW - 90%SW range vs results after 12 hours in distilled water in 50%SW - 90%SW range	2.67	7.31	NO

DISCUSSION

I: Phototactic behaviour

The exposure of the protists to light of different wavelengths showed different degrees of phototactic orientation. The protists would orient themselves toward the light source and cling to the side of the measuring cylinder which was exposed to the light. A green patch would form which would not dissipate even when the light was switch off (See Figure 3). In fact, the protists had to be scraped off the sides of the glass cylinder in order to remove them.

The outline of the patches were traced out on graph paper in order to estimate the area of the patch, and also shaded in accordance to the darkness of the patches on the measuring cylinders. The larger the area and the darker the patch, the stronger phototactic response. The results obtained are illustrated in Figures 4 and 5 and are written out in Table 1.

From the results, it can be seen that white light elicits the greatest amount of phototactic activity because the area of the patches are the largest and darkest (average: 10.35cm^2, very dark patches, see Table 1). Regarding the phototactic orientation of the protists toward the monochromatic irradiation of the red light

(700nm), blue light (450nm) and green light (530nm), the protists most actively respond to red light. In comparison to the responses seen in blue and green light, the patch of protists in the cylinder exposed to red light is the largest and darkest (average: 4.35cm^2, dark, see Table 1). This means that the protists are strongly attracted to the red region of the spectrum. It does not show as much activity in the blue light and even less in green light. While the average area of the patch in blue light is the same as the average area in green light (both averaging 1.8cm^2), the patch in blue light is medium dark and much more distinct than the very faint patch obtained in the cylinders exposed to green light. Thus it can be surmized that the protists show a medium amount of orientation to blue light but show very little, almost negligible, orientation in the green region of the spectrum.

Unfortunately, the results of this experiment could not be statistically analysed as the holes in the boxes were not precisely the same size and the distance between the measuring cylinders and the sources of light were not precisely the same length. However, even in these shortcomings, it is clear that the protists show the highest degree of phototactic orientation to white light, followed by red light, then blue light, and finally, green light.

The protists display a distinct topic phototactic orientation because they orient themselves directly toward the source of light. This explains the lack of homogenity in the pools, as some parts of the pools are shaded while other parts are directly

exposed to sunlight. The patterns seen on the pools were a result of the protists' attraction to sunlight.

This strong topic phototaxis exhibited by the protists must be caused by their need to photosynthesize. If this is so, they appear to prefer the blue and, especially, the red region of the spectrum and not so much the green region of the spectrum for photosynthesis. The reason for this is that only organisms in the deep depths of the sea use green light to photosynthesize as it is the only light that is able to pass through very deep water. Blue and red light would be absorbed as depth increases. Thus, as the protists live in such shallow pools, they have easy access to blue and red light, preferring to photosynthesize with light of these wavelengths.

These results support Hypothesis I. The question that arises is: How do the protists exhibit such strong topic phototaxis? As was mentioned before, the protists flagella has yet to be seen (even under 400x magnification), and it does not rotate very often during locomotion. The protists are probably very similar to E. mutabilis (Haeder and Mellikonian, 1983), and the way in which E. mutabilis moves to light may well be applicable to the protists.

However, what makes the protists orient toward red light? In the case of the protists, the probable answer to the question of which photosynthetic pigment or pigments were responsible would probably be chlorophyll, as it is the dominant pigment. If this theory is true, then chlorolphyll is the only pigment involved in triggering the protists' phototactic orientation towards the blue,

green and red regions of the spectrum. The strong orientation towards white light may be caused by the chlorophyll responding to stimulus from the red, green and blue regions of the spectrum.

What if there are other pigments present in the photoreceptors that may also trigger phototaxis? If this is so, then Haeder's theory of the interaction of many photoreceptor pigments resulting in a strong orientation to white light may be valid. Only if tests are conducted to see if any other pigment is present in the photoreceptor, or tests done to confirm if chlorophyll is really the pigment responsible for phototaxis and an extensive electron microscopic study on the protists as yet unseen flagellum, paraflagellar body and stigma were conducted, will all these questions be answered. As yet, I am only making speculations concerning the protists based on account of the protists physical similaritites to Euglena. At present, I can only assume that the pigment chlorophyll is responsible for the protists' phototactic orientation because I am certain of its presence.

I. Salinity

This part of the experiment was especially significant because the results did not correspond with my predictions. For example, in the 10%SW - 40%SW range, I did not expect any recovery in the mobility of the protists after 12 hours in seawater. I certainly did not expect the protists in the 50%SW - 90%SW range

of samples to recover after their "shock", which left them sessile, when saltwater was added to their enviroment. Recovery was seen (i.e. they began swimming actively) after only 2 hours in seawater and most of them (approximately 30% of them, see: Figure 8) recovered from their "shock" and swam normally again after 12 hours. Also the protists in the 10%SW - 40%SW range and the 50%SW - 90%SW range still continued to survive despite an addition of distilled water which lowered the surrounding salinity albeit a negligible amount of lysis was detected.

These rather surprising responses elicited from the protists stems from the organisms' remarkable ability to both adapt and tolerate salinity stress. In order to help me explain the protists' osmoregulatory behaviour, the statistics from Table 39 and the graphs from Figures 6 to 9 were used. While the graphs would show me the general pattern of behaviour of the protists, the statistics would prove whether there was a statistical difference between samples or not, while the graphs would show me which samples specifically differed.

From the statistics table (Table 39), numbers 1, 2 and 3 showed that the 10%SW - 40%SW range and the 50%SW - 90%SW range were divided accurately as there was no statistical difference within the 10%SW - 40%SW range and the 50%SW - 90%SW range, whereas there was a statistical difference between the 10%SW - 40%SW range and the 50%SW - 90%SW range in the first time level, that is, the immediate result upon the addition of saltwater.

Concerning the 10%SW - 40%SW range, the protists appear to be

able to tolerate the lower salinity levels of this range. The immediate effect of this slight salinity "upshock" resulted in about half the protists being unable to tolerate the salinity stress to become sessile, while those able to tolerate it continued swimming (see Figure 6).

They were sessile because it is suspected that they could not withstand a change in osmotic pressure because of the influx of excess anions into their bodies from the surrounding seawater. What led me to believe that the protists could tolerate a salinity of up to 40%SW, was because after 2 hours, there was no apparent recovery, but there was no deterioration in their mobility either as graph (II), Figure 7 indicates. Number 7 of Table 39 also shows that there is no statistical difference between the mobility (or lack of) of the 10%SW - 40%SW range in the first time level (the immediate addition of seawater) and the second time level, where the protists were in seawater for 2 hours. This proves that there is no difference in the state of health of the protists in the 10%SW - 40%SW range between the times of the immediate addition of seawater and the immersion after 2 hours in seawater, as there was no improvement or deterioration in their mobility.

Recovery was only apparent in the 10%SW - 40%SW range after 12 hours in seawater in those protists that became sessile first after the immediate addition of seawater. We can see this by comparing graphs 7 and 8. There is a jump downwards in the numbers that were sessile in this range between the 2 hour immersion in seawater and the 12 hour immersion. Number 13 of Table 39 shows

indeed, there is a statistical difference in the samples mentioned, showing a change in the state of health, the change being an improvement in mobility in the 10%SW - 40%SW range after 12 hours in seawater. Meanwhile, those protists that survived the addition of seawater from the very beginning still kept going on strong - perhaps showing still a high tolerance for their changed enviroment.

On the other hand, some that were initially sessile, recovered after 12 hours, thus showing that time was needed for the "shocked" protists to adapt to the new high-saline enviromenò. They may have adapted by altering their internal osmotic pressure to suit their new higher salinity enviroment. Some, however, did not recover at all. So it seems that those protists are exhibiting an amazing ability to adapt to their enviromen if given time to do so.

In the higher salinities of 50%SW - 90%SW, it is clear that the protists had to adapt to these hypersaline conditions, as most of them (95% of them, see Figure 6) were in a state of shock after the immediate addition of seawater. However, they did recover and became active again after 2 hours and were almost back to normal 12 hours later.

This can be deduced from numbers 8, 14 and 15 of Table 39, which show a statistical difference between the state of health in the 50%SW -90%SW range after 2 hours and after 12 hours in seqateò from the result immediately after the addition of seawater. Comparisons between Figures 6 and 7 show that, indeed, a recovery

occured over the time period as he ratio of the number of sessile to total population was reduced.

This lapse of time allowed the protists to cope with the hypersaline medium by perhaps altering their own internal osmotic pressure. Evidence of this adaptation can be deduced by observing the miraculous recovery of most of the protists from being sessile to becoming mobile again and swimming actively once more. Another interesting point to note is that the protists appear to require a certain level of high-salinity influx in order to quicly adapt to the new seawater enviroment. This level of "critical salinity" has been ascertained to be 50%SW or 16.8%., because from 50%SW upwards, recovery was seen after only 2 hours.

Also, recovery was much faster a s the salinities were raised from 50%SW to 90%SW. This 50%SW mark was also the level in which many of the protists were knocked sessile after the immediate addition of seawater. From the 40%SW level downwards, recovery could only be seen after 12 hours. This means that the protists reaction rates to adapt is slower at lower salinities than at higher salinities. In the lower range of 10%SW - 40%SW, the enviroment was not saline enough to trigger a rapid adaptation to the hypersaline enviroment. This slower rate of adaptation could be because of their ability to tolerate the lower salinity range. Some of those which could not tolerate this lower range would adapt after some time, in this case, 12 hours later. This resulted in the slower recovery rate in the 10%SW - 40%SW range than in the 50%SW - 90%SW range.

Thus, it can be said that Hypothesis II had to be rejected as there was a change in the mobility of the protists after they were immersed for 12 hours in the lower salinity range of 10%SW - 40%SW while a change in the mobility of the protists immersed in the higher salinity range of 50%SW - 90%SW was already apparent after only 2 hours. The implication would be that some of the protists exposed to a salinity lower than the "critical salinity" of 50%SW (16.8%.) will tolerate the environment, while others will slowly adapt to their new enviroment given time. Those protists exposed to a salinity higher than the "critical salinity" level would quickly adapt to their new, high-saline enviroment.

Statistics and graphs support Hypothesis III. Number 20 in Table 33 shows no statistical difference between the mobility of the protists in the 50%SW - 90%SW range that were left in seawater alone with the mobility of the protists in the 50%SW - 90%SW range in which distilled water was added. Figures 7 and 8 further show no average change in the mobility of the protists left in seawater alone with the protists in which distilled water was added to.

The now possible saltwater protists in the 50%SW - 90%SW range in which distilled water was added to had to either tolerate the influx of distilled water into their bodies or re-alter their internal osmotic pressure to now suit a lower salinity enviroment, as no deterioration in the mobility was found when the salinity of their environment was lowered. However a drastic alteration was not required as the distilled water addition only lowered the salinity by a few degrees and not entirely returned the protists

to a freshwater environment.

This implies that the protists in this range of 50%SW - 90%SW can survive equally well when left in seawater alone or when distilled water is added to the saltwater enviroment.

Number 19 of TAble 39 confirms no statistical difference in the mobility of the protists in the 10%SW - 40%SW range left in seawater alone and the mobility of the protists in the 10%SW - 40%SW range in which distilled water was added to. Figures 7 and 8 also show no average change in mobility in the protists in seawater alone with the protists in which distilled water was added to. Thus, statistics and graphs support hypothesis IV.

This also implis that the protists in the 10%SW - 40%SW range can survive equally well when left in seawater alone or when distilled water is added after an exposure to saltwater. This shows that the protists in which distilled water was added to could also either tolerate or adapt to the new surrounding environment by perhaps altering their internal osmotic pressure as there was no deterioration in their mobility in response to the lowering of the surrounding salinity.

From the experiments carried out, it can be seen that the protists are a euryhaline species which are able to tolerate wide fluctuations in salinity. This is because of the protists' high-level habitat. They are subjected to frequent changes in salinity because of salt water influxes during storms when the waves invade the tidepools and from dilution when rainwater and freshwater streams enter their enviroment. Dessication and

evaporation are a big problem especially in the hot summer months. They have to be able to tolerate these salinity changes and to be able to adapt very quickly in order to survive in their high-level enviroment.

The next paragraphs deal with questions that arise concerning the protists' remarkable ability to tolerate and adapt to salinity fluctuations, namely:

How does the protist actually deal with salinity stress?

What are the mechanisms involved? (The actual mechanisms involved are unknown, as there is very little known about the protist itself.)

In order to be able to tolerate or adapt to changes in salinity, the protists may have to be able to withstand any cellular swelling which occurs when its enviroment changes from a hypersaline medium to a hyposaline medium. If it cannot tolerate it, lysis will occur. This behaviour could explain how the protists were sole to tolerate the addition of distilled water after being immersed in salt water for 2 hours, as only a few of the animals has lysed during that part of the experiment while the rest survivied. The protists will also have to tolerate some cellular shrinkage caused by plasmolysis which occurs when seawater is added to the protists. Many animals have displayed osmoregulation character, and the protist is probably among them. As information is scarce on the effect of salinity change on

protist, and there is none on my particular protist which is yet unidentified and researched before, I have had to turn to research on other euryhaline marine and estuarine animals, such as fish, crabs and algae in order to draw parallel observations.

Another way to adapt to falling salinity levels is to be able to retain excess salt or anions when distilled water is added to the protists which are now accustomed to saltwater conditions after their 2-hour immersion. These anions have to be retained to balance any influx of water into the cell through osmosis so that the protists can deal with the new freshwater medium.

On the other hand, adaptation to a more hypersaline enviroment requires an ability to tolerate an influx of salt which is inevitable because of the diffusion of salts from the external medium into the cells. Therefore, some kind of mechanism to remove, not retain, the excess salts is required. Also, the organism should be able to tolerate a certain amount of loss of water to the enviroment.

One effective mechanism for getting rid of excess ions within the cell to the outside enviroment is active transport. This may explain why the protists adapt so well to salinity fluctuations, allows ions to move from a low concentration of ions to a high concentration of ions. Active transport may be responsible for removing salt from within the protists to hypersaline surroundings to counteract diffusion of salts from the hypersaline medium into the cells. Active transport also requires a large amount of energy, which is primarily obtained from the mitochondria.

Other than that, an extensive network of endoplasmic reticulum, microtubules and golgi complexes are required for the transport of cellular secretions. I suspect that the protists possess cellular specializations such as mitochondria for active transport and an extensive cytoplasmic membrane system to allow the transport of salts in and out of the protists.

CONCLUSION

This study began as a project to seek out two characteristics of the protists: their phototaxis behaviour and their uppermost salinity tolerance level. However, the protists displayed euryhaline characteristics and their salinity tolerance level is not known yet as they are osmoregulators who are most efficient at the highest salinity level tested on them. i.e. 90%SW.

Although many theories based on other organisms possessing similar capabilities have been proposed in an attempt to explain the protists' behaviour, they have been left unproved. Very little is known about the animal, and this study has only managed to raise more questions concerning these protists. One thing is clear though, that the protists are unique, possessing many unexpected qualitites which allow them to reveal their phototactic behaviour and surprising responses to salinity fluctuations. Chlorophyll, responsible for triggering phototactic responses to light in the 450nm and 500nm - 700nm bands in blue-green algae, corresponds to the phototactic behaviour of the protists, although the possible

presence of other pigments cannot be ignored at this stage. Even the mechanisms for osmoregulation are unknown, and the protists appear to be at home in both seawater and freshwater.

Further studies are required to answer the questions raised. An extensive e.m study is needed to find out if the protists possesses the cellular specialization that other cells able to cope with salinity stress have. For example, a large number of mitochondria to generate energy for active transport, golgi bodies and a complex network of cytoplasmic membranes to see if this active transport assists the protists to secrete cellular products such as salt, as well as seeing if compartments exist in the protists. Tests have to be carried out to see if excess anions are expelled from the protists when immersed in seawater and if excess anions are retained by the protists when it is transferred to distilled water after being previously immersed in seawater. Regarding its phototactic orientation, testing if other pigments are present in them and examining the structure of their elusive flagella and their photoreceptors would explain their behaviour.

These protists open many avenues for other research. Experiments could be carries out to see if there is a relation betwei temperature and osmoregulation. Barker and Wilhm (1982) concluded that temperature did affect metabolism for they discovered that osmoregulation declines in Cironomus riparius and Chaoborus punctipennis at higher temperatures (38 degrees Celsius). Tests could be conducted if the protists displayed any negative phototaxis. At present, nothing is known about how the

protists reproduce, what nutrients they require, their temperature tolerance range, etc. We could even see if the protists could tolerate salinities of over 100%SW. Different methods could be used to determine the health of the protists. One method could be to measure its level of photosynthesis using O_2 electrodes, or to measure its respiration level. So many other areas have yet to be explored.

We need not conduct experiments under laboratory conditions either; experiment could be conducted on the tidepools themselves, which are inhabited by the protists, to explore their reactions to different variable (i.e. salinity, pH, temperature, etc.) in their own natural enviroment. One could determine its pH tolerance range and see if it can be used as an indicator for acid rain.

These protists have much potential for research, for they already have exhibited many unique characteristics which allow them to thrive in their ecological niche.

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