A study of marine fouling ecosystems

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A STUDY OF MARINE FOULING ECOSYSTEMS

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ABSTRACT

The aim of the project was the provision of information regarding the effect of various toxins on members of the fouling ecosystems. This was undertaken in two parts: A background survey to provide information for the main study, the latter consisting of experimental work.

Part 1 - the introduction - deals with fouling, its importance and implications to the Shipping Companies and provides information on the costs and historical aspects of fouling among other things.

Part 2 - the Surveys - provide information on the fouling patterns of ships both laid up and in service, as well as details of the distribution of fouling algae along the British Coast and their fouling systems. The results obtained are discussed in relation to the changes in fouling ecosystems observed with the changes in toxins used in antifouling paints. The implications of this being discussed in Part 4. A summary of the findings is also included.

Part 3 - the experimental work - provides supporting evidence for the changes observed in Part 2 as well as providing other information on the effect of the toxins used on the algae. The results obtained are discussed in relation to the general effect of the toxin; applications of the methods used into future research in marine fouling and possible methods of bioassay.

Part 4 - discusses the implications of the change over in the components of the fouling ecosystem. Explores the possibility of new lines of research into fouling based on the experiments undertaken and discusses the possible occurrence of resistance of algae to toxins being used as antifoulants.

Standard experimental methods are given in the appendix, the remainder being included in the main text.

Part 1

INTRODUCTION

INTRODUCTION

Fouling is a term used to describe the settlement in excess of marine organism, plant or animal, or the hull of ships. The result of such fouling is to increase the drag resistance and results in either a slower speed at the same power, or the same speed but at a higher power output for the engine. This increases costs which ever path is chosen. If a slower speed is maintained the ships availability for cargo carrying is reduced thus resulting in a lower income and higher running costs; if the speed is kept constant increase in power causes an increased fuel consumption. Shell (1964) have shown that an increase of 27% in power was required to maintain the standard speed with a ship which was 'fairly' heavily fouled and one with only slight fouling required a 12.5% increase in power to maintain speed.

A survey published by the Woods Hole Oceanographic Institution (1952) gives the following figures for the effect of fouling:-

Table 1

Effect of Fouling after six months out of dock in Temperate Waters

Type of ship	Displacement	Loss of Max. Speed(knots)	% increase in fuel to maintain 10 knots	20 knots
Battleship	35,000	1.50	45	40
Aircraft Carrier	23,000	1.25	45	40
Cruiser	10,000	1.25	50	45
Destroyer	1,850	2.00	50	35

The figures apply to ships of the Royal Navy in which an allowance is made in the design for an increase in frictional resistance of 0.25% per day in temperate waters and 0.5% per day in tropical waters.

In addition to the direct expense of increased fuel consumption there is also an increase in 'wear and tear' of the machinery to maintain the speed at higher power outputs. The expense of dry-docking must also be taken into consideration. In 1940 this was 4,400 dollars for an 18,000 liner not including the loss of revenue while the ship is out of service.

Analyses have shown (Woods Hole 1952 from Visscher 1928) that there is some relation between fouling and the time spent in port. Fouling being heavier the longer the ship spends in harbour. This is supported (Woods Hole 1952) by the observation that Passenger liners are less liable to fouling than freighters, the latter spending more time in harbour. Among naval vessels Battleships and Aircraft carriers, which spend more time in harbour during peace time, than destroyers or cruisers, are more likely to foul than are the latter.

Some indication of the importance of fouling can thus be obtained the costs must be reduced if a shipping fleet is to be run efficiently. The aim at present must be to reduce the onset and density of fouling until the time that a ship is due to be dry docked for repair purposes not to be de-fouled. This is sufficient for most

passenger and cargo ships but another problem has evolved in recent times: that of the 'giant' oil tanker. These ships of 100,000 to 250,000 tons are so expensive to build and run that they have to be run as efficiently as possible. This requires a very short turn round time at each terminal and thus very little time is spent in harbour or at the oil terminal. This would tend to reduce the density of fouling (see Visscher 1928) but this is complicated by the much greater cost incurred when fouling does occur. These costs exceed those of normal ships and the cost of this is increased by the fact that the return run of the tanker is one in which the ship is not carrying cargo and any increase in the time taken is effectively far more expensive than in normal ships.

Further indication of the cost of fouling to the companies can be seen below (the data being supplied by B.S.R.A. 1969). Shell have shown that the annual operating loss per year is in the order of 0.15 knots. For a tanker of 20,000 D.W.T. this costs £2,000 p.a. due to increase in fuel consumption alone. For one of 80,000 D.W.T. the cost rises to £6,000.

Another operator found that one of their ships required an increase of 20% in the power required to maintain speed, but that another of their vessels in the same time span required an increase in power of 52% to maintain the speed. Other figures given indicate that

for an 80,000 ton tanker fouling results in a cost of £37,000 p.a. of which £10,000 p.a. is due to loss of revenue while out of service; £10,000 p.a. for dock charges and £2,000 for painting. One large tanker operator puts the loss for an 80,000 ton tanker at £30,000 p.a. for loss of speed alone of which 90% is caused by fouling and 10% due to roughening of the surface and not recoverable. On top of this the figure given for dock charges is £1,500 - £3,000. In order to prevent or reduce fouling, ships are coated with a number of compounds aimed at stopping attachment and growth of the fouling organisms. The use of antifoulants dates back to 1000 B.C., the Phoenicians having used pitch and copper on the bottom of their ships (Wood Hole 1952). Lead sheathing was used by the 15th Century Spanish ships but the large weight and softness had its disadvantages.

There is no authentic record of copper being used before the 18th Century. In 1758 H.M.S. 'Alarm' which was plated with copper which kept it weed free during a return voyage to the West Indies.

Difficulty was encountered when the hulls of ships began to be made of iron due to the corrosive action of iron and copper on each other. This resulted in the gradual replacement of copper sheet with paint systems employing an undercoat of anti-corrosive before the antifouling paint, initially copper based,

was added.

Antifouling paint systems have been numerous in design and performance. The general formula of an antifouling paint is the toxin (copper or organometallic) in an organic solvent which is dissolved in a matrix of some sort. The toxin being released as the matric is dissolved. The toxin and matrices used vary, but copper and organometallic compounds are those most often employed. The formula of each paint is critical to enable it to be most efficient in its action. Partington 1964 using a system of Cuprous Oxide, rosin and soluble plasticiser showed that total soluble material must exceed 52% of the total volume of paint for it to be effective. Fisk (1960) suggests that the anticorrosive and matrices may also play some part in supplementing the action of the toxin incorporated in the paint. This was also suggested by Harris (1943).

The basic types of paint systems used today for antifoulant purposes can be described under three headings (Birnbaum et.al 1967) .

Hot plastic : Phenol Formaldehyde resin; rosin; paraffin and Cuprous Oxide applied in a hot metal.

Cold Plastic : liquid phenol formaldehyde resin; rosin; fish oil and Cuprous Oxide

Vinyl : which requires greater surface preparation but gives better results.

Recently organometallic compounds have begun to be used as toxins

and has generally led to an increase in the life of a paint. However, the economic value of this is tied in with a number of factors. Lloyds of London will allow ships to be at sea for a maximum of two years without overhaul. Thus it is in the interests of the owners to aim to get as near to this maximum as possible before having to dry dock the ship.

The copper based paints gave protection for a period of approximately twelve months (this exact period depending on a number of variables: speed, temperature etc.) This necessitated drydocking after only one year at sea. An alternative, taken by certain companies, allows the ships to stay out for two years while being fouled during the second year. This increases fuel bills and reduces the amount of trade but may be compensated by lowered docking costs.

The use of organometallic compounds increases the life of the paint and thus reduces the time spent fouled while at sea for the two years resulting in a saving in operating costs. However, the cost of painting a 200,000 D.W.T. tanker with organometallic antifoulants is nearly £6,000, that for an orthodox paint system £3,200. The reduction in operating costs with the use of organometallic antifoulants must be in the region of £3,000 (Lee 1969 in memorandum to B.S.R.A.). In many cases the saving is obtained from docking once every two years instead of once a year is

in the region of £3,000 alone and thus the organometallic antifoulants would appear to be on a sound economic footing. The use of organometallics as antifoulants provides a difficulty in the analytical stage. Several authors have shown the apparent relationship of the leaching rate of the paint with the effectiveness of fouling. Barnes (1948) showed that with copper based paints a leaching rate of 10 ug cm^{-2} day^{-1} prevented nearly all settlement. This relationship has been used since to provide information on the life of paints.

With copper based paints this minimum leaching rate before fouling starts can be measured accurately by chemical analysis. However, with the organometallic paints the concentration required to prevent fouling is much lower. Rivett (1965) showed that, for Chlamydomonas, growth ceases at 0.005 ppm of tributyl tin oxide. The use of chemical analysis to determine concentrations of toxins as low as this presents difficulties and much reliance is now being placed on biossays for their determination (Rivett 1965).

Just as the methods of antifouling protections have changed over the years so have the fouling communities though these changes are not necessarily concomittant. With wooden hulled ships the boring animals, such as Pholas, used to be the main problem (Woods Hole 1952). The onset of metal hulled ships produced a change to a

community dominated by barnacles and tube worms but also including green and brown algae. When the speed of the ships increased to a rate greater than 15 - 16 knots (B.S.R.A. and Ocean Fleets Ltd. 1969) there was another change. This speed proved too great for the settlement of barnacles and tube worms to be successful and the fouling community changed to one dominated by the green algae (Enteromorpha), Harris 1946. In the last few years reports have suggested that the main fouling community have again changed from the green algal domination to one dominated by brown algae (B.S.R.A. 1969; Shell 1969) this continuing to the present day.

As a result of these changes and the economic importance of fouling to the Shipping Companies the Department of Botany at Durham University was given a grant by the British Ship Research Association to study various aspects of ship fouling.

The aim of this study was the investigation of fouling ecosystems found on ships, in relation to the antifoulant systems in current use.

The research is in two parts: A survey of current fouling organisms and experimental work relating to them. The survey, although providing background material for the project as a whole was one long term basis and as a result experimental work had to be started before the full results from the survey were known. The selection of experimental material was therefore modified as work progressed.

The work will, however, be presented in the logical study sequence.

Part 2

SHIPPING AND COASTAL SURVEYS

THE SURVEY OF SHIP FOULING ORGANISMS AND THEIR COASTAL DISTRIBUTION

Five surveys were carried out to provide background knowledge of present day fouling:-

1) A survey of the distribution of fouling organisms on a ship entering a local port for drydocking after a normal period of service at sea. The study was designed to detect any pattern in the distribution of fouling ecosystems on a ship's hull. The information gained from this would determine the pattern of all further sampling.

2) Survey of Fouling Ecosystems on Ships in Regular Service in order to determine the fouling ecosystems found at present. Earlier surveys have been carried out by Harris 1946, Pyefinch 1948 and Shell 1964. Although there is much evidence of major differences in the composition of contemporary fouling ecosystems there has been no detailed study of this reported in the literature.

3) Survey of the Major Shipping Lines - this was a logical extension of the above in order to obtain as much background data as possible and included both the examination of samples of fouling sent by the companies as well as details of fouling from their own records.

4) A survey of fouling ecosystems present on static objects

The ships investigated in the above surveys are all in regular service and are subject to defouling and replenishing of their antifoulant at regular intervals (usually every twelve to eighteen months). Thi, together with the movement of the ship through the water, prevents the build up of a mature fouling ecosystem. Previous investigations into the mature fouling ecosystems have been carried out using non-toxic test plates, rafts, buoys and other static objects (Stubbing and Houghton 1964; Harris 1946; Allen and Ferguson-Wood 1950 and Summerson et al 1964). It was decided to take the opportunity of studying the fouling on a number of ships of the Royal Navy Reserve Fleet which have been laid up in Portsmouth Harbour for long periods of time. This would provide information on the mature fouling ecosystem appertaining to a ship and provide comparison with that found on other static objects.

5. A survey of the distribution of the fouling organisms around the coast of the British Isles. This was carried out to provide data relating to the possible source of fouling.

The methods used in the above surveys are given in the Appendix S1. The results are presented and discussed below. The main discussion is followed by a summary giving, in outline, the main results of the survey.

Results

The data obtained from the survey carried out are given in tables 2-4. Table 2 shows the variations of the fouling system over the hull of the ship (e.g. Kasimah). It shows that in this vessel and subsequently others have shown that two basic communities exist. One of these communities is dominated by the green algae, mainly of the Enteromorpha genus the second is one of domination by brown algae of the family Fetocarpaceae. The two communities merged about the mid line. This separation of the communities was seen in most of the other vessels seen however there was one basic difference. The 'Kasimah' had the brown algae at the top of the hull and the green at the lower side of the hull. Elsewhere, this position was reversed in that the green tended to be on the light load line and the brown below this.

The above example set the pattern for future sampling. The remainder being sampled, where possible, at two sites on the hull: one near the bilge keel and the other near the light load line.

Before giving the results of the main part of the survey those of the climax fouling community will be given. These samples having been taken from four ships which had been lying in Portsmouth Harbour for varying lengths of time. The results are given in Table 4 in the form of an Association table. The raw table of species and sites with their occurrence values have been resorted following the method

TABLE 2. ASSOCIATION TABLE FOR THE "KAZIMAH"

Side of Ship.	P	P	P	S	S	S	P	S	S	S	P	P	P	P	P
Position along hull	M	A	F	M	A	F	M	M	F	A	M	A	F	A	F
Height of Sample Area	18	18	18	18	18	18	28	28/38	28/38	28/38	38	38	38	28	28
Enteromorpha prolifera.	3·2	3·2	3·2	3·1	3·2	3·1	2·1	1·1	1·1	1·1	3·1	1·1	1·1		
Enteromorpha compressa.	1·1	2·1	2·1	2·2	2·2	2·2	2·1	1·1	1·1	2·1					
Blidingia marginata.	1·1	1·1	1·1	1·1	1·1	1·1		1·1	1·1	1·1	1·1				
Enteromorpha torta	5·5	5·5	5·5	4·2	4·4	4·2	2·2		2·1						
Feldmannia globifera.							5·5	5·5	5·5	5·5	5·5	5·5	5·5	5·5	5·5
Pilyella littoralis.				2·1											
	Zone A						Zone B				Zone C				

KEY:-

P	Port
S	Starboard.
F	Foreward quarter
M	Amidships
A	After quarter.
18	18 feet } above the keel.
28	28 feet } above the keel.
38	38 feet } above the keel.

Zone A - Zone of Green Weed
Zone B - Zone of Green and Brown Weed.
Zone C - Zone of Brown Weed.

TABLE 4 — ASSOCIATION TABLE FOR SHIPS LAID UP IN HARBOUR.

Ship.	AC	AC	D	LC	D	LC	LC	LC
Position of Sample.	B	W	W	W	B	B	B	W
Time laid up (in months).	6	6	24	48	24	48	48	48
Diatoms	1·1	4·2	4·2	1·2				
Ulva lactuca	2·1	1·1	1·1					
Polysiphonia nigra	1·2	2·2	2·2					
Ectocarpus siliculosus	1·1		1·1	1·1				
Insect larvae.		1·1	1·1					
Balanus balanoides	2·2				2·2	2·2	2·2	2·2
Botryllus schlosseri					2·2	2·2	3·2	
Molgula manhattiensis					2·2	2·2	2·2	
Jassa. falcata		1·1	+			1·1	1·1	
Gammarus spp.		1·1				1·1	1·1	
Harmothoë sp.						1·1	1·1	
Ciona intestinalis						1·1		1·1
Diplosoma listerianum	+				+			1·2
Enteromorpha usneoides		1·1						
E. prolifera			1·1					
E. intestinalis			1·1					
Ulothrix sp.		1·1						
Nereid worm.			+					
Idotea sp.		+	+					
Ascidiella aspersa				+				
Blidingia marginata.					+			
Tubularia laryxa.						+		
Enteromorpha compressa.			1·1					

KEY:-

- A.C. Aircraft carrier
- D Destroyer
- LC Landing Craft
- W Waterline } sample
- B Bilge keel }

of Braun-Blanquet 1928 (See Appendix for brief description)

From the table it appears that there are three fouling communities which appear to be temporarily separated.

The first community of Diatoms, Ulva lactuca, Polysiphonia nigra and Ectocarpus siliculosus is found to be dominant on the samples taken from the aircraft carrier and destroyer, these having been in the water for six months and two years respectively.

The second community to appear which tended to be dominant on the four year old ships was one in which Balanus balanoides, Botryllus schlosseri and Molgula manhattiensis were the main organisms present. This would appear to be the basis of the climax fouling ecosystem and is later surplanted by, or incorporated with, an ecosystem (community 3) which consists of Jassa falcata, Harmothoe, Gammarids and Ciona intestinalis.

The remaining species gave only odd occurrences and little can be gained from them.

In general the algae were more abundant in samples taken from the water line than that taken from the bilge keel. This would be expected from the resultant decrease in light intensity with increasing depth. However, the members of the Enteromorpha genus were seen on the bilge keel sample from the destroyer.

From the above results what appears to be happening is that the initial fouling community of algae is replaced by one dominated by Ascidians and Barnacles. Later this community increases in diversity

with the occupation of vacant niches by Crustacea and Polychaetewarus this forming the climax ecosystem.

The data obtained from the ships studies is given in Table 3. This is expressed on cover values for the species in the sample taken. Other information listed is the toxin contained in the antifouling paint, the months elapsed since the last painting and the month of last painting, the general area of trade, site of sample and density of fouling.

After rearrangement there appear to be a number of communities present. One consisting of Blidingia Marginata and Enteromorpha prolifera is one which is present throughout the table but with a greater cover value in the samples taken from the water line.

A second community of Ectocarpus siliculosus, Feldmannia globifera and Giffordia secunda is generally limited to the area of the hull below the light load line. Within this community it can be seen that Feldmannia globifera occurs when the ship is heavily fouled. Ectocarpus reaches its greatest expression when the ship is lightly fouled while Giffordia secunda is more general in its distribution.

A third community is formed by Enteromorpha torta and Enteromorpha compressa. This is generally found in the water line samples. The ships involved had all been on world wide voyages, the Crystal Crown having traded throughout the world although its main route was the Atlantic route.

TABLE 3

ASSOCIATION TABLE of THE FOULING ORGANISMS FOUND ON SHIPS HULLS

Ship	1	2	3	3	4	5	4	6	7	8	8	2	6	9	5	7	1	10	11	12
Route	W	FE			ME	W	ME	US	US	Au	Au	FE	US		W	US	W	W		Co
Months since / Month of last painting	15/2	11/9			7/	13/11	7/	21/9	12/7	3/12	3/12	11/9	21/9		13/11	13/7	15/2	24/4	10/7	
Toxin in paint	C(o)	C(o)			C(o)		C(o)	C(o)	C(o)	Co	Co	C(o)	C(o)		C(o)	C(o)		C(o)	C(o)	Cu
Position of Sample	B	B	R	S	B	B	B	B	B	W	W	S	W	S	W	W	W	S	S	W
Density of fouling	H	L	L	L	H	H	H	M	M	L	L	L	M	L	H	M	H	L	L	L

Blidingia marginata	1-1	2-1	1-1					1-1	+				1-1	1-1	1-1	+				
Enteromorpha prolifera	+					1-1	1-1	1-1	+			1-1	1-1	2-2	3-2	2-1	3-2	+		5-5
Ectocarpus siliculosus	1-1	5-5	5-5	5-4	2-2	1-1	1-1	1-1	4-4											
Feldmannia globifera					2-2	5-5	4-2		+											
Giffordia secunda							1-1	+	1-1	1-1	1-1									
Enteromorpha torta															5-5	1-1	1-1	2-2	1-1	
Enteromorpha compressa															2-1	+	1-1	2-2		
Enteromorpha usneoides		+	+							1-1	1-1									
Enteromorpha clathrata					2-1		2-1													
Enteromorpha ramulosa		+																		
Enteromorpha intestinalis		2-1							+				1-1				1-1			
Ectocarpus fasciculatus													1-1				1-1			
Giffordia sandriana	+											1-1								
Giffordia fenestrata																		+		
Giffordia hincksiae	5-5																			
Giffordia granulosa								+												
Kuetzingiella holmesii													3-2							
Pilyella littoralis					+				+						+					
Barnacles						+			+											
Tubeworm							+		+											
Diatoms							+		+											
Diatomaceous slime																				
Chaetomorpha linum																				

Blidingia marginata																				
Enteromorpha prolifera																				
Ectocarpus siliculosus																				
Feldmannia globifera																				
Giffordia secunda																				
Enteromorpha torta																				
Enteromorpha compressa																				
Enteromorpha usneoides																				
Enteromorpha clathrata																				
Enteromorpha ramulosa																				
Enteromorpha intestinalis																				
Ectocarpus fasciculatus																				
Giffordia sandriana																				
Giffordia fenestrata																				
Giffordia hincksiae																				
Giffordia granulosa																				
Kuetzingiella holmesii																				
Pilyella littoralis																				
Barnacles																				
Tubeworm																				
Diatoms																				
Diatomaceous slime																				
Chaetomorpha linum																				

Table 3

Key:-

Names of Ships

1	St. Margaret	7	Crystal Crown
2	Pando Strait	8	Northern Star
3	Administrator	9	Clan Grant
4	Sugar Importer	10	Foreland
5	Kazimah	11	Athelduke
6	Verena	12	Fulham IX

Route

W	World Wide	US	North America and Caribbean
FE	Far East	Au	Australasia
ME	Middle East	Co	British Coastal Waters

Toxin

Cu	Copper	C(O)	Copper possibly with organotin
CO	Copper plus Organo-tin		

Position of Sample

B	Bilge keel	S	Side
W	Water line	R	Rudder

Density of Fouling

H	Heavy	M	Medium	L	Light

One other tentative community is that of the Barnacles and Tube worms. These were of sporadic occurrence but all the ships involved had been at sea for periods exceeding eleven months. It was rare that the density of these would be great and most individuals were of small size indicating a recent origin of settlement. The remaining species tended to be rather scattered.

The "Fulham 9" gave an anomalous result. Here the fouling community was one of diatomaceous slime and Chaetomorpha linum. This differed from the remainder on being an entirely coastal vessel probably spending much of its time in esturine waters, these algae being typical of esturine waters.

Coastal Distribution

The sites from which the algal lists were taken are given in Fig. 1. The lists are given in Table 5. They have not been re-arranged forming a "community" type arrangement unlike the data from the ships hulls. The sites have been arranged clockwise around the coast starting in the Shetlands.

From the results it can be seen that Ectocarpus siliculosus, Enteromorpha compressa, Enteromorpha intestinalis, Enteromorpha linza and Pilyella littoralis are of general occurrence throughout the British Isles. The remainder vary from localised to scattered occurrence.

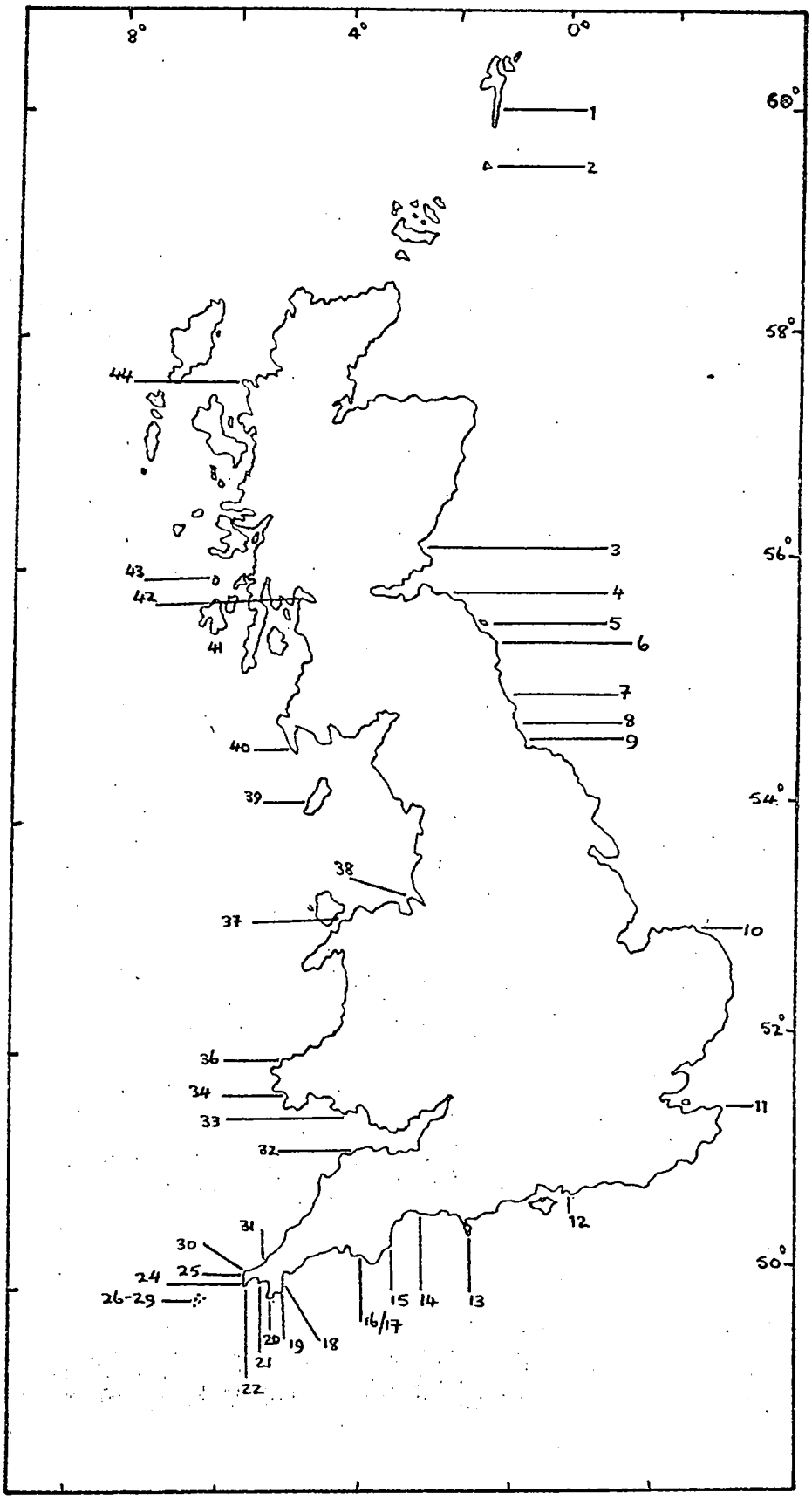
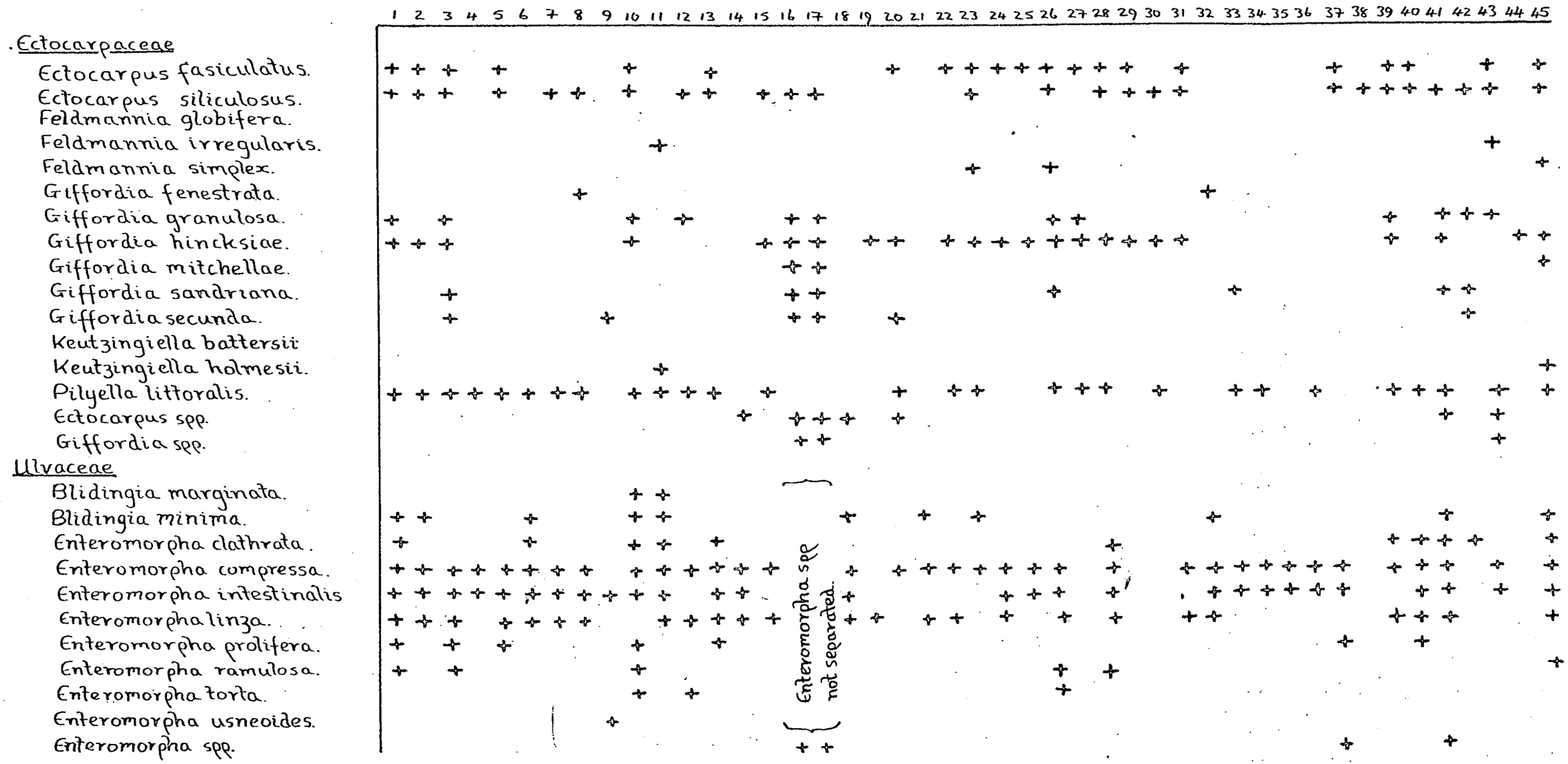


Figure 1 Sites of Coastal Surveys.

Key to Sites of Coastal Survey

1 Shetland Islands
2 Fair Isle
3 St. Andrews
4 St. Abbs Head
5 Holy Island
6 The Northumberland Coast
7 St. Mary's Island
8 Souter Point (Sunderland)
9 Hartlepools
10 The Norfolk Coast
11 Westgate
12 Chichester Harbour
13 The Dorset Coast
14 Beer Bay
15 Brixham
16 River Yealm
17
18 Porthallow
19 The Lizard
20 Porthmellin
21 Penzance
22 Roskilly
23 Porthcotham
24 Lamorna
25 Sennen Cove
26 Isles of Scilly - St. Mary's
27 Isles of Scilly - St. Agnes
28 Isles of Scilly - Bryher
29 Isles of Scilly - St. Martin
30 Cape Cornwall
31 Porthreath
32 Combe Martin
33 Gower Peninsular
34 Milford Haven
35 Cwm-Yr-Eglwys
36 Martin's Haven
37 North Wales and Anglesea
38 Mersey and New Brighton
39 Isle of Man
40 Galloway
41 West Coast of Scotland
42 Firth of Clyde
43 Colonosay (Inner Hebrides)
44 Gair Loch and Loch Torridon
45 The West Coast of Ireland

Table 5. THE COASTAL DISTRIBUTION OF MARINE FOULING ALGAE



Ectocarpus fasiculatus is generally restricted to the west coast of Britain with a few scattered localities on the east site although this may be due to sampling in the north east and the bias of the lists to the south and west. 4

Giffordia Hincksiae shows a more consistant occurrence on the south west coast than elsewhere although it does occur on the west coast of Scotland and Ireland.

The remainder on the list are of limited occurrence or of scattered occurrence throughout the British coasts.

The fouling periods of algae in the River Yealm are given in Table 6, from the data of G.T. Boalch. Unfortunately, the genus Enteromorpha was not split to species level.

The results are given on:-

a) a monthly basis
b) a seasonal basis

The former were replaced at monthly intervals and inspected. The latter were inspected at monthly intervals but not replaced. Also one series was placed at the surface (30 cm depth) and was placed at an angle of 45° to the surface at a depth of 60 cm., thus being in portal shade.

The results obtained from this show that Ectocarpus spp, Enteromorpha spp, and Polysiphonia spp the fouling season extends from March to October. The frequency of occurrence of these is also high throughout the season. Giffordia spp has a season extending

Table 6 ALGAL SETTLEMENT AT NEWTON FERRERS 1959 – 1962. (LABORATORY SITE)

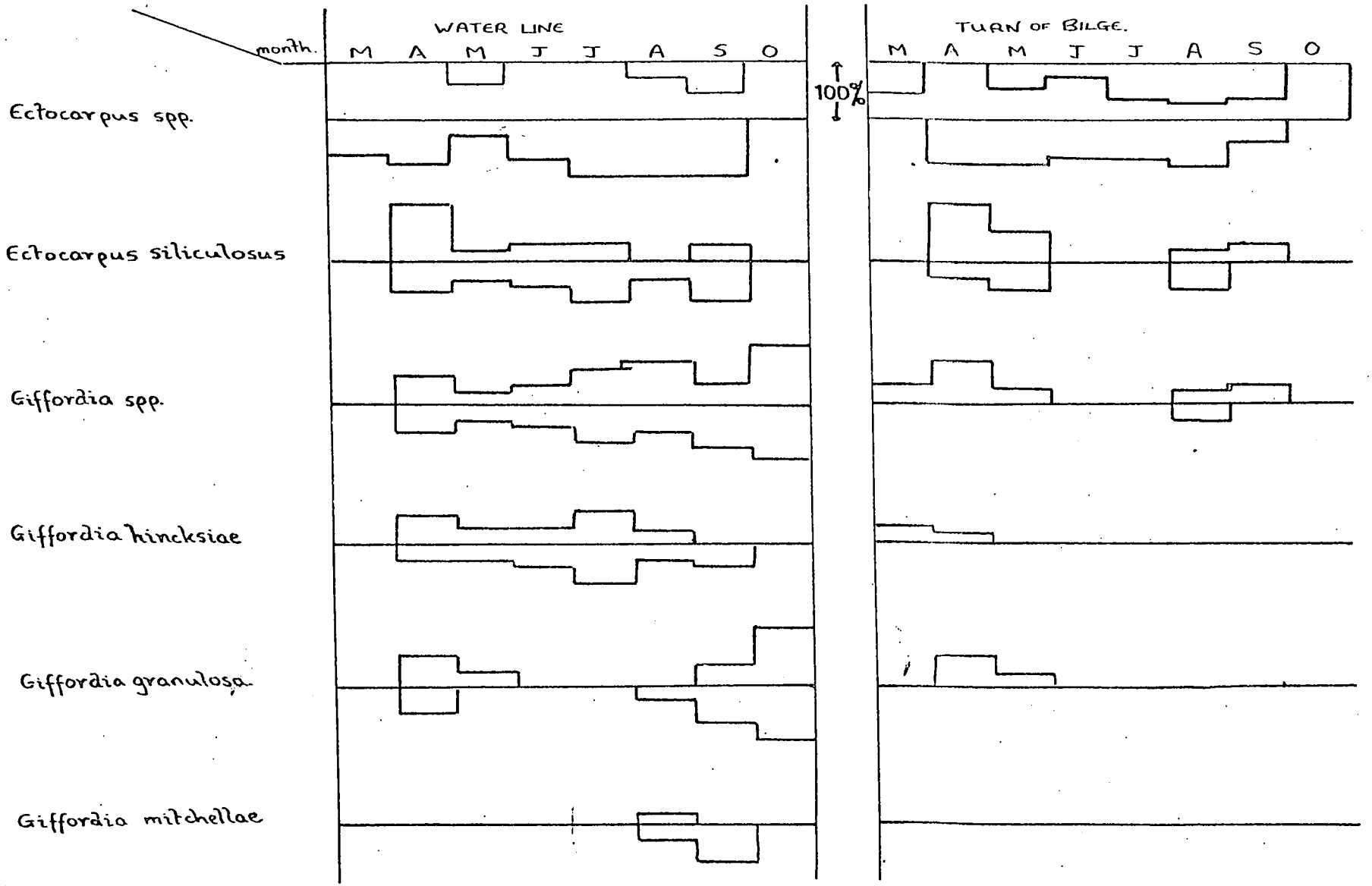
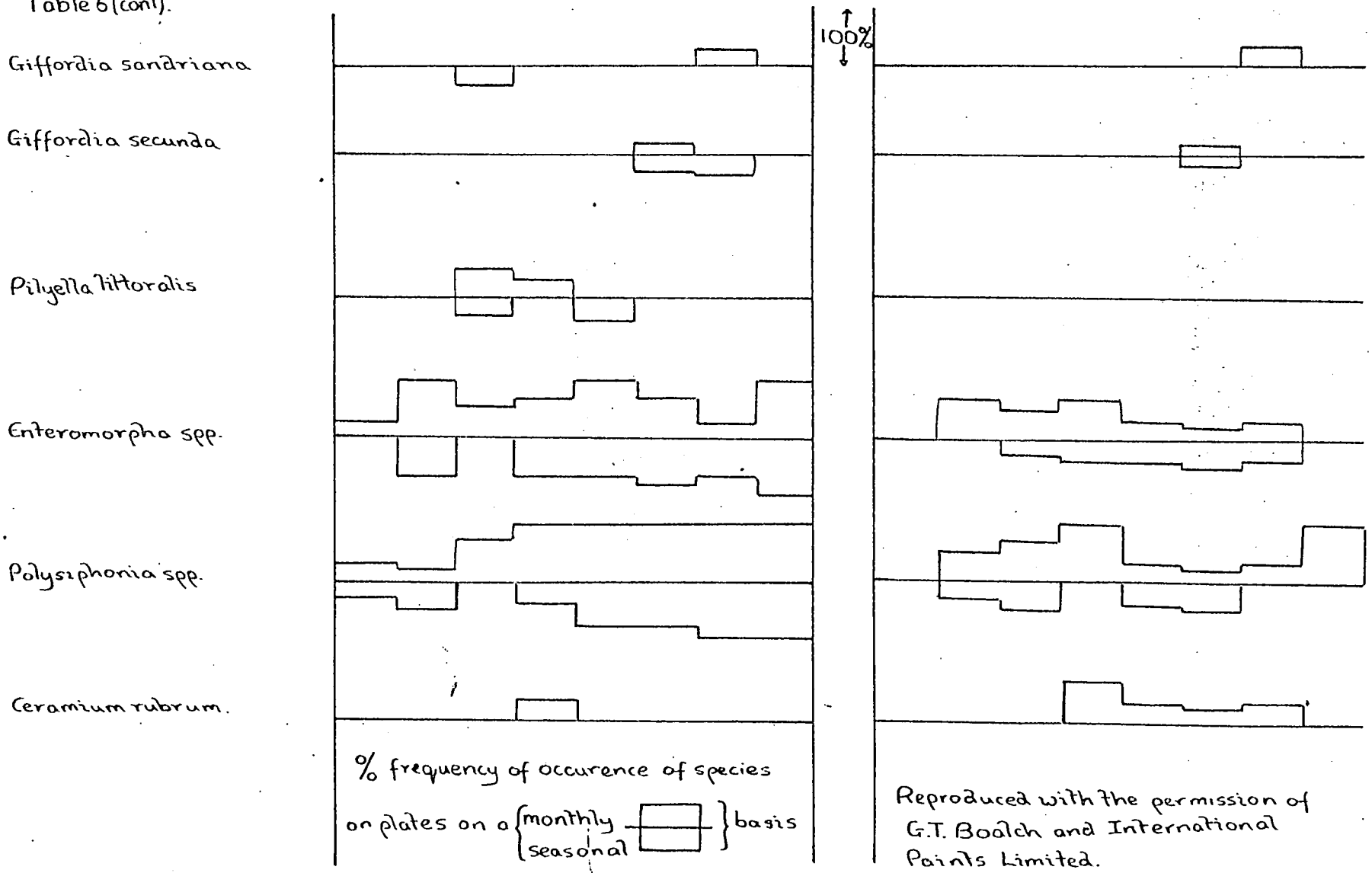


Table 6 (cont).



from April to October but is of lower frequency than the above three genera. The remainder have a limited fouling season generally restricted to one or two months of the year. Gifferdia granulosa has a bimodal fouling season, one part in April and May, the second in August to October. The remaining species of the genus Gifferdia tend to have their season in August to September. Pilyella littoralis, however, while being ubiquitous on the littoral zone is limited in its fouling period to May, June and July.

In general the seasonal plates follow the above pattern from the monthly plates. The plates angled at 45^{o} show some variation. There is a reduction in frequency of occurrence and also in the length of the season, but that of Ceramium rubrum is lengthened. This would seem to be a result of the drop in light intensity due to the angling of the plates.

Discussion

The results of the ship fouling survey show two clear areas of fouling. One band of green weed at or near the light load line and below this an area of brown weed. Tube worms and barnacles were generally absent or of low incidence.

Previous surveys have shown that the green weeds, (Enteromorpha spp) tended to be the dominant form of fouling when the vessels were running at sufficiently high speeds to prevent barnacle settlement. Harris 1946 showed that in samples from a large number of vessels Enteromorpha and Ectocarpus were the most abundant genera. He states that Enteromorpha formed the bulk on all ships. The same author gives details of a series of samples from ships taken at Liverpool and Glasgow from 1942 to 1944. In these he showed that at the waterline Enteromorpha was the dominant form and this merged gradually to an animal fouling nearer the keel.

Data from the Woods Hole Oceanographic Institution 1952, show that while on Buoys and Test plates Ectocarpus occurs with the same frequency as Enteromorpha, on ships Enteromorpha is almost twice as common. This is also supported in data from Hentschell 1923 and Visscher 1927 in the above report.

The data from Boalch shows that both Enteromorpha and Ectocarpus favour high light intensities but Ectocarpus is less sensitive to a drop in light intensity also shown by Barashkov and Fedyakina 1965.

Ivanova (1967) in 32 samples from 9 vessels showed that Enteromorpha and Cladorpha were the most common algae and gives details of certain vessels with zinc plated iron being fouled with Enteromorpha and Ectocarpus after 2 - 4 months. One vessel painted with a copper-mercury antifoulant was fouled with Enteromorpha after one year. Kingcome (1959) states that Enteromorpha was less sensitive to copper than Ectocarpus.

The main toxin used in antifouling paints prior to 1960 was copper on cuprous oxide. It would appear from the above reports that, with this paint, ships would tend to be fouled by green weed when the paint is exhausted. There would be some brown weed but this would be limited to the lower parts of the hull due to the dominance of the green weed at high light intensities.

Recent reports have indicated that there has been a shift during the last few years from green to brown weed as the main fouling type. This is correllated with the introduction of organometallic compounds as suitable toxins. This change over has been noted by a number of shipping concerns and was thought by at least one to be due to this use of organometallic compounds in antifouling paints. Experimental evidence cited later would seem to support this view and if one assumes it to be so one can explain the results obtained in this survey and those given elsewhere.

One fact that has to be taken note of is that many ships are only covered with antifouling paint as far as the light load line. Only

in a small number of vessels is the antifouling carried on above this level. This allows the assumption, that with organometallic toxins the brown weeds are the chief fouling organisms, to seem a reasonable one.

The water line samples were taken from near to the light load line and probably above the zone of antifoulant. This area was predominantly of green weed with only the occasional occurrence of brown weed. This is what is expected if no antifouling (A/F) paint had been used.

The bilge keel sample was taken in the zone of A/F paint and here the predominate forms were the brown algae and although green algae did occur occasionally, it was with only low cover values. The information obtained from B.S.R.A. concerning the toxin contained in the A/F paint was that most contained copper although a number of manufacturers had been experimenting with organometallic toxins, few had given positive indication of its use in their paint. However, from the data obtained here, and from the experimental results, suggest that most of the paints contained organometallic (probably organotins) toxins. If this is so it would explain the dominance of brown weeds on the lower part of the hull below the light load line. This observation is not solely explained by a light gradient as generally there is a district boundary between the greens and browns not a gradation as one should expect if there was no distinct

physical or chemical boundary.

These assumptions, based on experimental evidence, can be used to explain the distribution of fouling organisms found on the 'Kasimah'. Here the green weeds were dominant on the lower surface of the hull below the light load line, and the brown weeds were dominant above the light load line. This is the reverse to that found elsewhere. Unlike the other cases there was a gradual merging of the two communities. This suggests that there was no abrupt physical or chemical changes suggested for the other ships. One assumes from this that the A/F paint continues up the sides of the hull to a point above the heavy load line. The distribution of the algae also supports this if it is assumed that the organotins favour brown weed fouling. The green weeds occur below the light load line and this is subject therefore to continuous leaching by the sea. The area of the hull above this level will only be subject to intermittant leaching and as this vessel is a tanker this intermittant leaching will last roughly for 50% of its total time at sea. One would expect from this that at any given time the area below the light load line would contain less toxin than above that level and therefore would be expended before the latter level.

Once the paint has been exhausted it would be expected that the green weed would become the dominant form and this would be expected to occur first on the lower part of the hull as seen here. Thus one

can explain the apparent anomaly of the 'Kazimah' by suggesting that she had been coated with an organometallic paint further up the hull than is normal and that the differential erosion of this paint has resulted in the two communities found. The green weed being as the lower exhausted surfaces, the brown on the upper still active surfaces.

Using the same assumption it may be possible to explain the results found by a major shipping organisation in the mid 1960's. A number of ships were surveyed and inspected visually at roughly monthly intervals. The paint systems used varies from only anticorrosive to supertropical A/F paints.

The use of a number of paints suggests that there would be a similar variety of compositions and these would be expected to give different results. Where brown weed is the first fouling noted it can be assumed that these paints contained organotin compounds. These were generally seen in supertropical and epoxy A/F paints. The change over to green weed could be ascribed to the exhaustion of the paint at least of the organotin contained therein. The variability in the change over could be due to the differing compositions and their decay times and not necessarily any differences in routes. Although in certain cases this may not hold. Support for this is seen in that the weed at the end of the voyage, in the ships which passed through the brown weed fouling stage, was very heavy, an indication of exhaustion of a paint but before any settlement of barnacles.

This would also have been seen in the 'Kazimah' if only half the hull had been painted. The fact that most of these ships were fouling after only a few months at sea suggests that their formulation is unsatisfactory.

While barnacles and tube worms seem to be not as frequent as they used to they still occur. Ocean Fleet Ltd., report that shell (Barnacle and tube worm) fouling is greater in their ships working below a speed of 16 knots. In the survey carried out the barnacles and tube worms were of small size and probably recent origin but samples returned from the second survey show that barnacles still cause a problem to certain owners. In one case hydroids were seen suggesting virtually total exhaustion of the paint.

The fouling intensity can be modified by a number of factors. Blue Star Lines report that fouling does not affect them greatly as their ships often pass through fresh water or silt laden water or over abrasive surfaces (South American Trade) and this prevents long term settlement of algae. However, recently occurrences of oysters have been reported and this gives rise to concern among certain companies. The fact that fresh or silty water can remove fouling has been known for many years but not all ships can make use of these.

Turning to the difference observed in the climax fouling ecosystem and that generally found on ships, the climax in Portsmouth Harbour appears to be one of Barnacles and Tunicates as the

dominant organisms with the insect larvae and crustaceans also coming in at the dominant stage. A zonation of fouling was observed and the algae were generally limited to the upper areas of the hull, the animals to the lower areas. The chief algae involved were Ulva lactuca, Polysiphonia nigra, Diatoms and Ectocarpus siliculosus, greatly different from the fouling seen on ships. This climax is seen in Plymouth (Harris 1946) where Ascidians are the dominant form. Stubbings and Houghton (1964) have shown that in Chichester Harbour and adjacent harbours on the south coast the dominant forms in midsummer are the barnacles but in late summer tunicates become the dominant form but generally die off over winter.

The difference between the climax and general fouling ecosystems can readily be explained. Barnacles are only serious when the speed of the ship is below 16 knots, and as most of the ships investigated exceed this speed they do not constitute a problem. Pyefinch (1948) gave details of the fouling sequence due to decreased toxin concentration in the paint. This sequence, from most resistant to least resistant, is as follows:-

Brown mats (Ectocarpus type) - Hydroids - Enteromorpha - Ectocarpus - Barnacles --- Ascidians Ulva and Laminaria. Harris (1946) shows that Polysiphonia and Ceramium are very sensitive to copper.

Thus with fast ships and a toxin concentration just below the maximum to allow fouling one would expect it to produce a fouling ecosystem of Enteromorpha and Ectocarpus the hydroids being too fragile

to withstand the movement through the sea.

The importance of the fouling season of the algae is that it is only at this time that fouling can occur; when the algae liberate their spores. Thus by knowing the fouling seasons of the ports of call during a voyage one can estimate when the fouling is most likely to start and, with data from thelife of the paints, take additional steps to prevent it.

Results show that the distance from the shore is in inverse relation to the degree of fouling (Ivanova 1961) and thus protection would be required in harbour. This would take the form of a temporary structure of tubes releasing low concentrations of toxins to prevent attachment. This idea has been tried on a permanent basis on ships (Iskra 1960, Fisk 1960). Other ideas that have been tried include Ultrasonic vibrations (Fisk 1960, Iskra 1960, Aksel'Band 1960) but the energies required for large ships are too great to be economic. Yoshi and Vedak (1966) have experimented with electrolytic methods but corrosion of the metal is a possible point on which this will not succeed. Thus paints would appear to be the main antifouling system used for some time to come.

The coastal survey has shown that many of the normal littoral and sublittoral members of the genus Enteromorpha and Ectocarpaceae are found on fouling organisms. It might be possible, therefore, to estimate where the failure of the paint has started by comparing the flora of the ships hull with that of the coast around the port of

call. This, of course, has severe limitations as the data amassed here is restricted to the British Coast. However, if lists could be compiled at the other major shipping ports then this could be tested. Analysis would show, if this assumption is correct, the date of commencement of fouling. This would provide useful information on the life and behaviour of the paint of importance to both the shipowners and paint manufacturers. One example we have obtained the m.v. 'Calchas' docked at Birkenhead and Liverpool during one voyage. The length of time spent in Liverpool was 14 days. The flora was found to be:- Enteromorpha compressa, E. ramulosa, E. prolifera, Ectocarpus siliculosus, Giffordia secunda and G. sandriana. The local flora includes E. compressa, E. prolifera and Ectocarpus siliculosus. These are very widespread algae and any conclusion would be very suspect, but is worth careful note.

SUMMARY OF THE FINDINGS OF THE SURVEY WORK

1. The results from the 'Kazimah' show that there is a definite pattern of fouling, which bears a relation to the displacement of the vessel. The absolute height of the fouling band, while dependent on the displacement, fluctuates in any one ship along with the bow wave caused by the passage of the ship through the water.

The pattern revealed is a result of a difference in the vertical distribution of the fouling organisms, there being no variation horizontally and takes the form of two bands of fouling, (the components of which vary) which merge gradually at the light load line. The only other observable patterns are those relating to bilge exits and other obstructions on the ships hull. Providing these last mentioned are avoided and sampling restricted to the major zones of fouling, the samples would appear to be ecologically comparative.

The fouling ecosystems from ships with different routes have remarkable similarities which are summarised in Table 3.

The main fouling organisms found are:-

a) Blidingia marginata; Enteromorpha prolifera - of general occurrence through the hull.

b) Ectocarpus siliculosus; Feldmannia globifera and Giffordia secunda limited to regions of A/F paint if organotin is a constituent.

c) Enteromorpha compressa and E. torta limited to areas near the water line but above regions of A/F paint if organotin is a constituent. On non-toxic or with copper on the toxin Enteromorpha spp dominates over Ectocarpus spp as seen here and in Harris (1946).

d) Barnacles and tube worms, these only occur in low densities and generally as immature animals and are thought to have originated while the ship had been travelling at slow speeds or had been in harbour recently. From the above data and from correspondence with shipping companies there has been a major change in the components of the fouling ecosystem. Where as previously the green algae predominated, the brown algae now do so. It appears that this change occurred at the same time as the introduction of organometallic toxins into antifouling paints.

While the hull below the light load line is covered with an antifoulant paint and weed free for 12-18 months, that above the light load line is subject to rapid fouling due to the lack of protection. Extension of antifoulant paints to the level of maximum displacement might reduce the cost due to fouling on vessels fully loaded in use (if oil tankers 50% of the time are empty).

While the major fouling algae are of widespread occurrence around the British Coast, ships anywhere in British waters during the fouling seasons of the more restricted algae (Table 6) could receive an inoculum of the fouling algae of a specific district. Exact information

regarding the life history of the propagules of the fouling organisms is required before fouling organisms with restricted natural distribution can be as indicator organisms.

Part 3

EXPERIMENTAL WORK

Experimental Work

The experimental work carried out had three basic aims:-

1) To investigate the action of toxins on the major compounds of fouling systems in an attempt to isolate the cause of the shift, in fouling, from green to brown weed.
2) To use experimental techniques in the above investigation which would allow the assessment of antifouling properties of new compounds which are toxic to biotic systems.
3) To develop these methods as potential bioassay techniques for use in the estimation of leaching rates of the antifouling paints.

Material

The algae used in the experimental work were:-

Giffordia secunda (Kutz) Batt

Giffordia fenestrata (Berk ex Harv.) Batt

Ectocarpus siliculosus (Dillw.) Lyngb.

Pilyella littoralis (L) Kjellm

Enteromorpha intestinalis (L) Link

E. compressa (L) Grev

E. linza (L) J.Ag.

These algae were collected from various sites along the coast of Northumberland and Durham from both the littoral and sub-littoral zones. They were stored in sea water culture solution in a constant environment room until required. Collection generally being

arranged so that this period was as short as possible.

Details of the culture solution and culture techniques are given in the Appendix - E1.

Four experimental procedures were carried out:-

A Warburg Manometry

The aim of this experiment was to show the effect of the toxin used on the metabolism of the algae involved. This should provide a rapid technique for toxin assessment.

The Warburg apparatus is used to give a measurement of the respiration and photosynthesis, both net and gross, of a plant. Details of the methods involved are given in Appendix E2-1.

Results

The results of this experiment are given in Appendix E3.1-E3.3 and summarised in graphic form in Figures 2-12.

Tributyl tin hydroxide (T.B.T.OH) appears to act upon the alga's metabolism producing increased respiration and net photosynthesis. The increase in the former being greater than that in the latter bring about a resultant decrease in gross photosynthesis (Figures 2,3,5 and 6). This effect is far more marked in the green algae (Enteromorpha spp) than in the brown algae (family Ectocarpaceae) as in Enteromorpha compressa and Enteromorpha intestinalis their compensation point is reached with a solution of T.B.T.OH of 1-2% saturation in sea water. In the Ectocarpaceae tested the compensation point is either not

Figure 2. The Effect of Tributyl Tin Hydroxide on the Photosynthesis and Respiration of Enteromorpha Compressa.

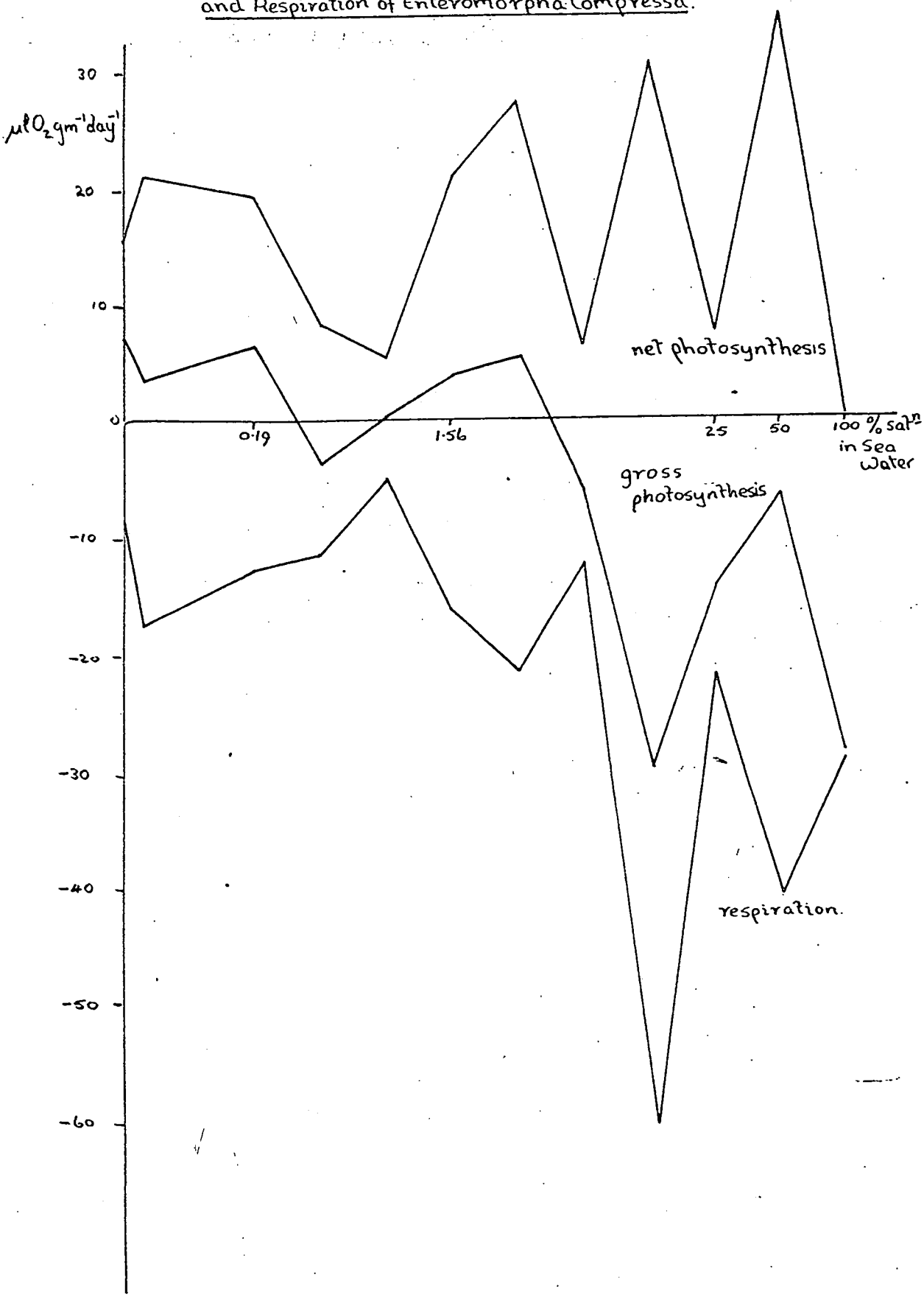


Figure 3. The Effect of Tributyl tin hydroxide on the Photosynthesis and Respiration of Enteromorpha intestinalis.

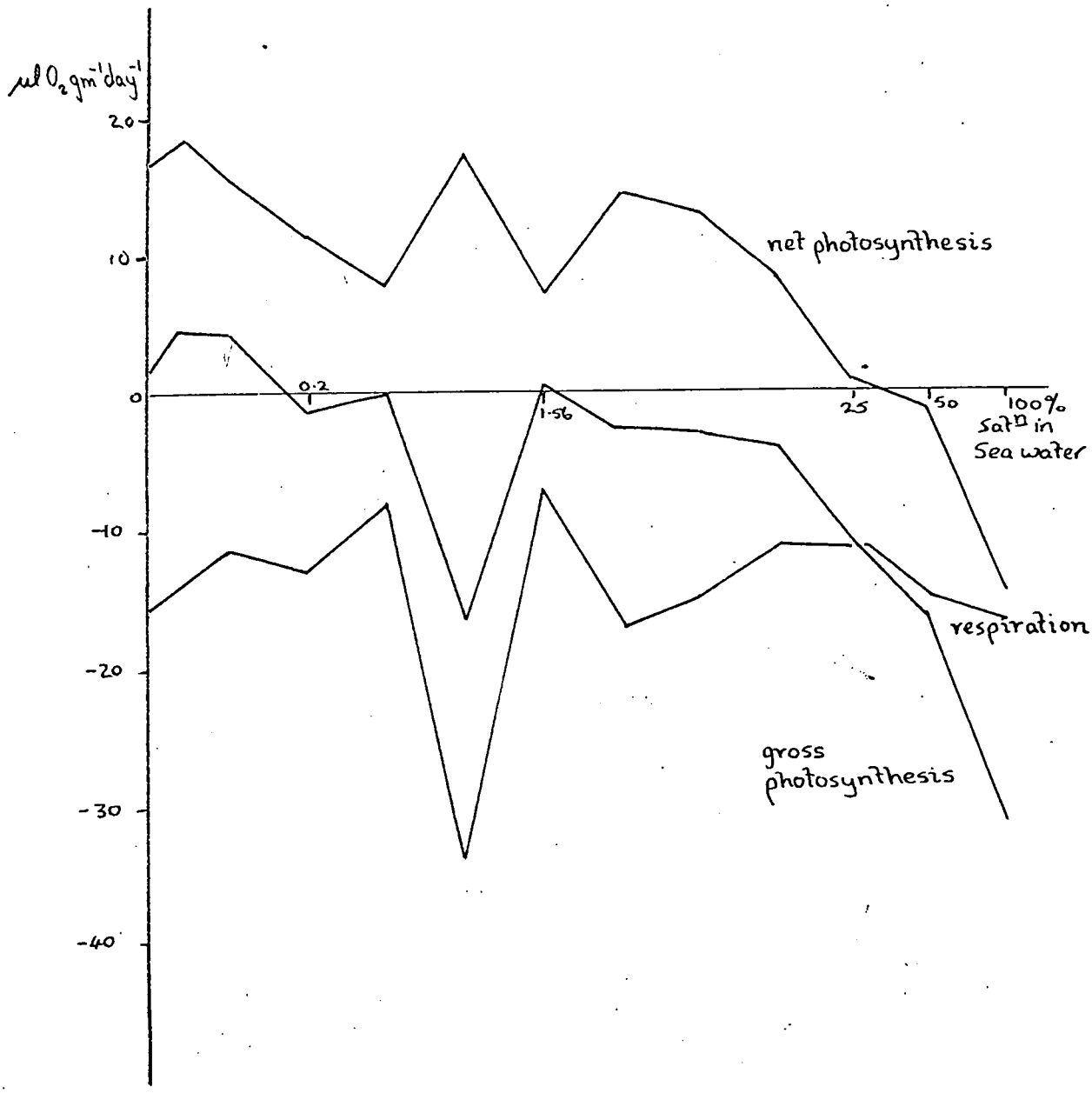


Figure 4

The Effect of Tributyl tin Hydroxide on the Photosynthesis and Respiration of Enteromorpha linza.

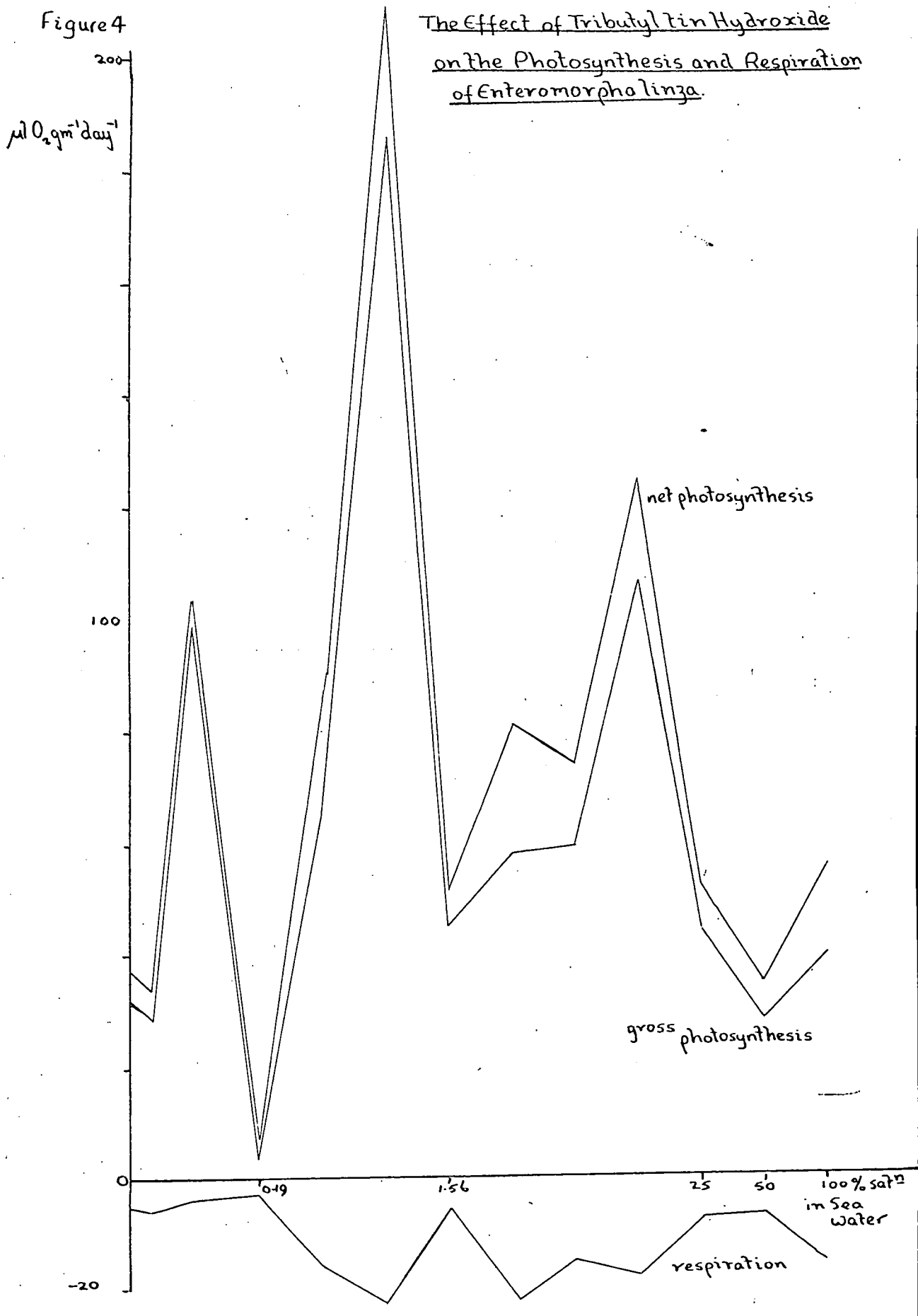


Figure 5. The Effect of Tributyl tin hydroxide on the Photosynthesis and Respiration of Pilyella littoralis

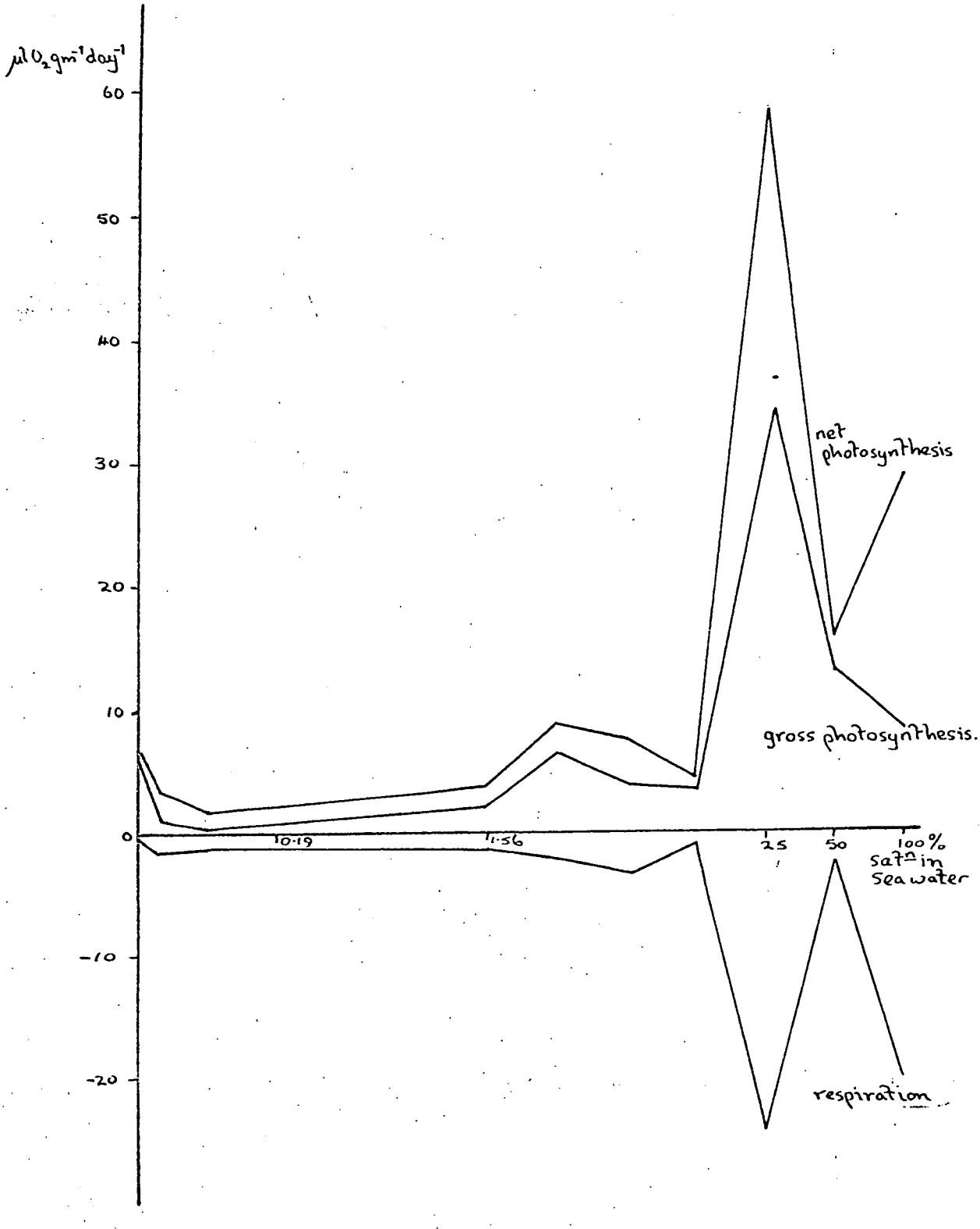


Figure 6 The Effect of Tributyl tin hydroxide on the Photosynthesis and Respiration of Ectocarpus siliculosus.

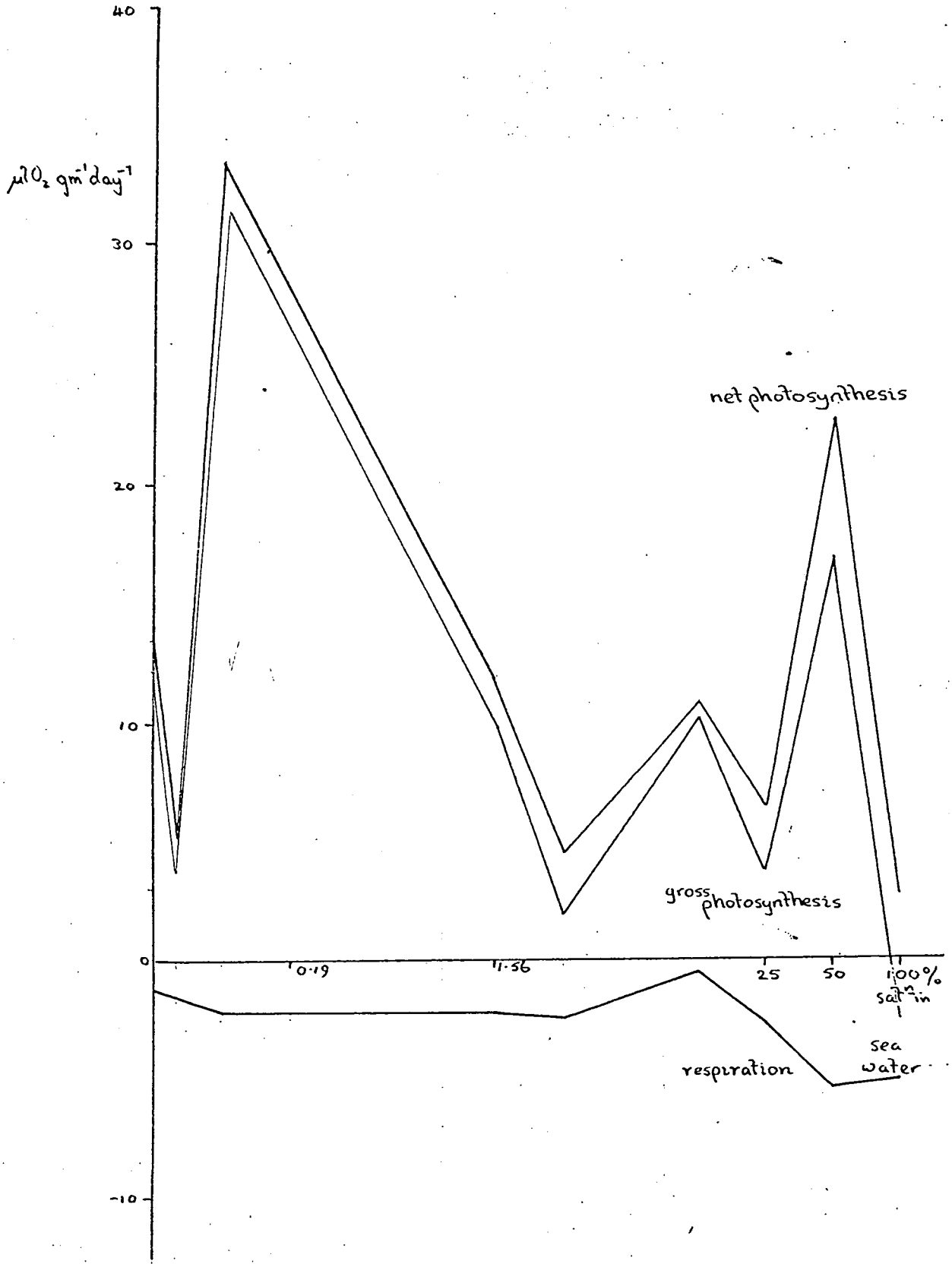


Figure 7. The Effect of Cuprous Oxide on the Photosynthesis and Respiration of Enteromorpha intestinalis.

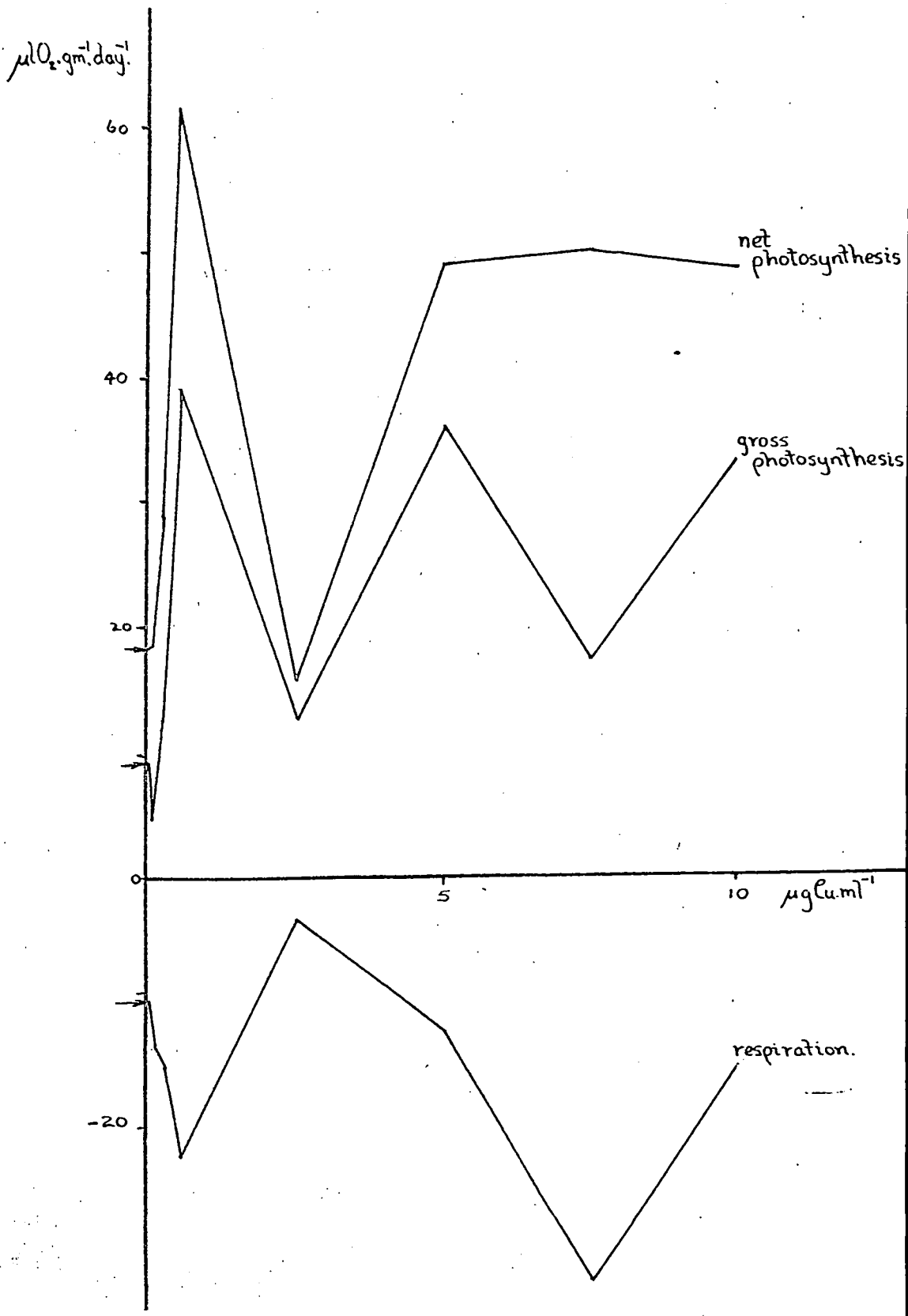


Figure 8 The Effect of Cuprous Oxide on the Photosynthesis and Respiration of Pilyella littoralis

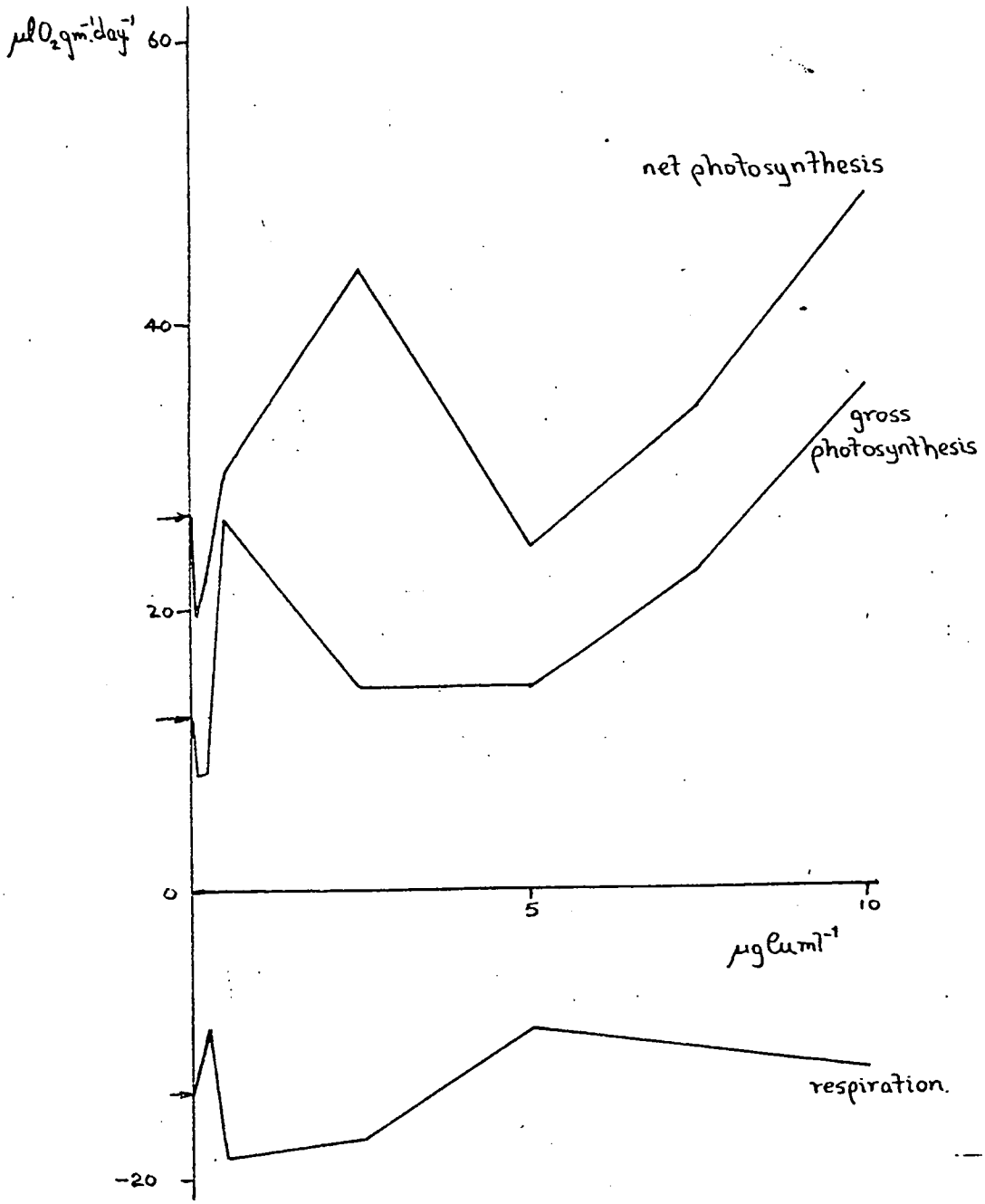


Figure 9. The Effect of Cuprous Oxide on the Photosynthesis and Respiration of Giffordia fenestrata.

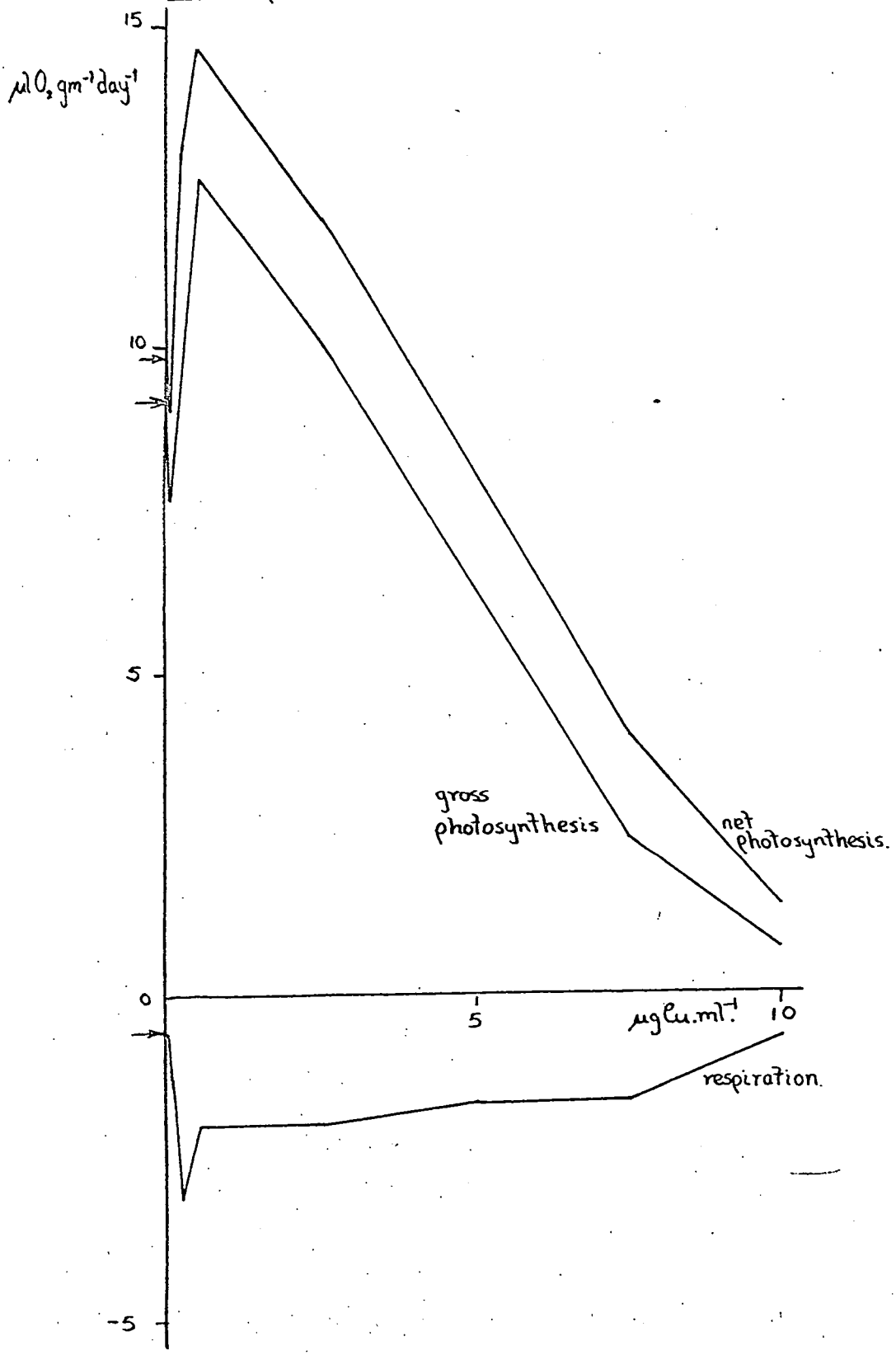


Figure 10. The Effect of Cuprous Oxide on the Photosynthesis and Respiration of Ectocarpus siliculosus.

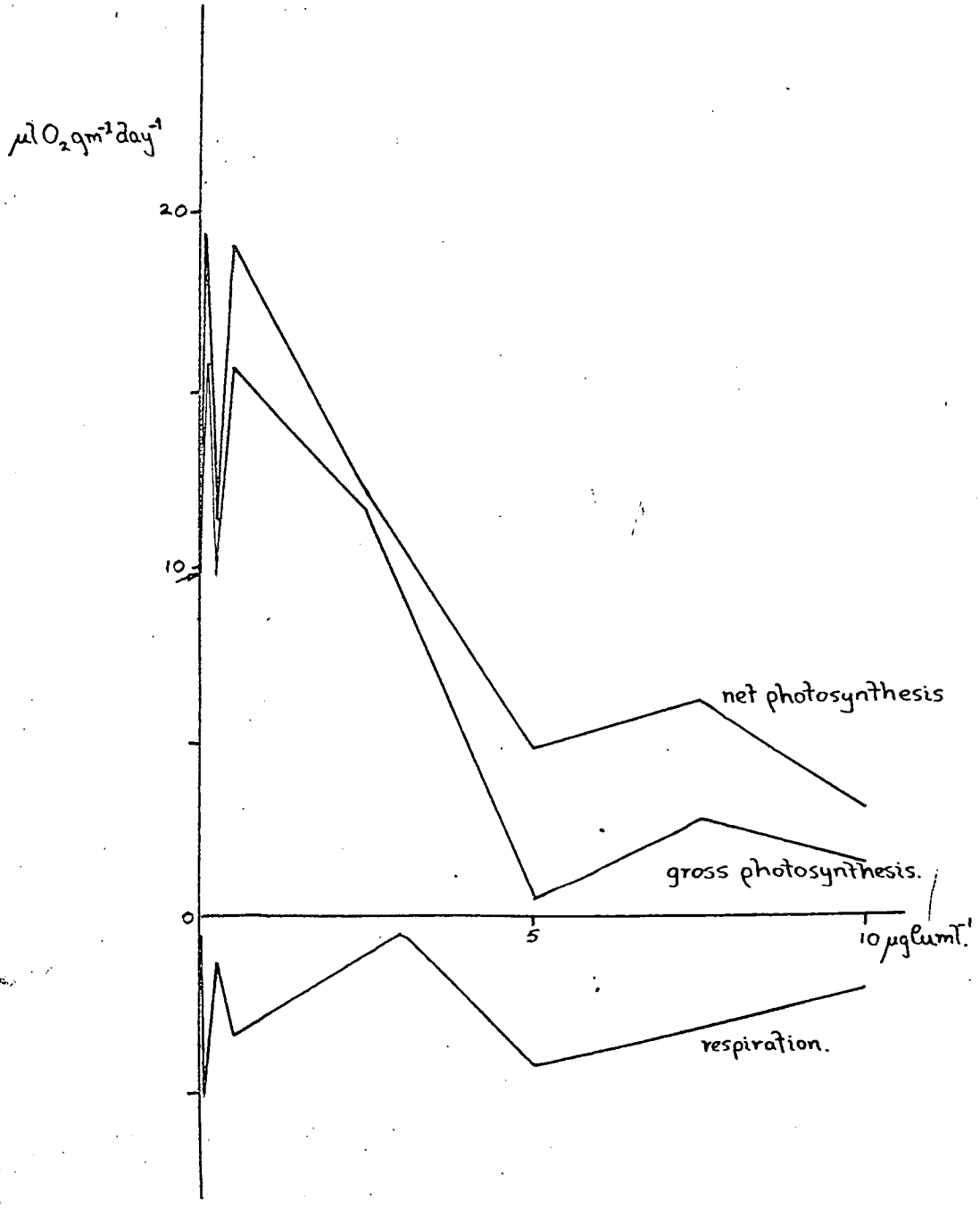


Figure 11. The Effect of Copper on the Photosynthesis and Respiration of Pilyella littoralis.

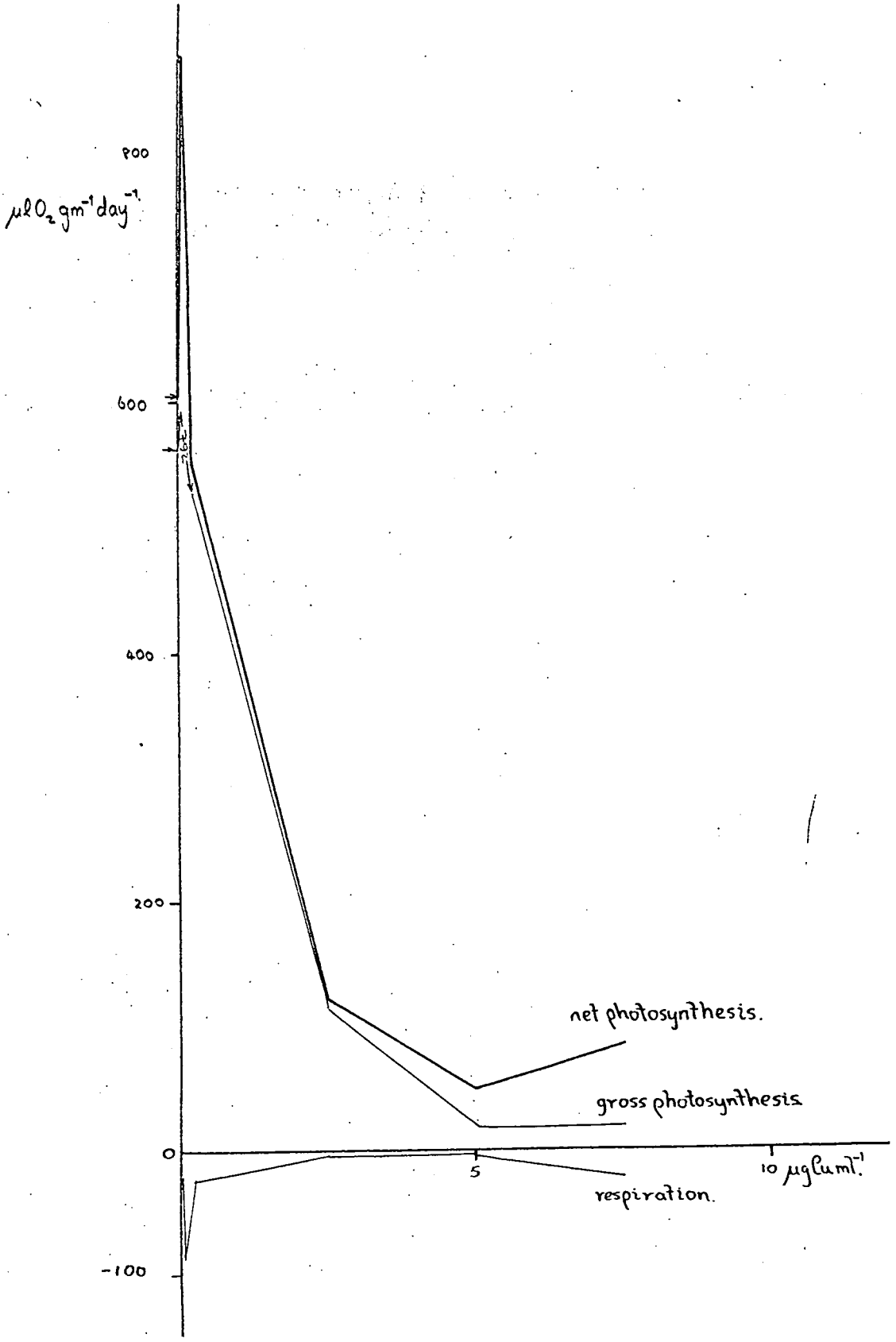
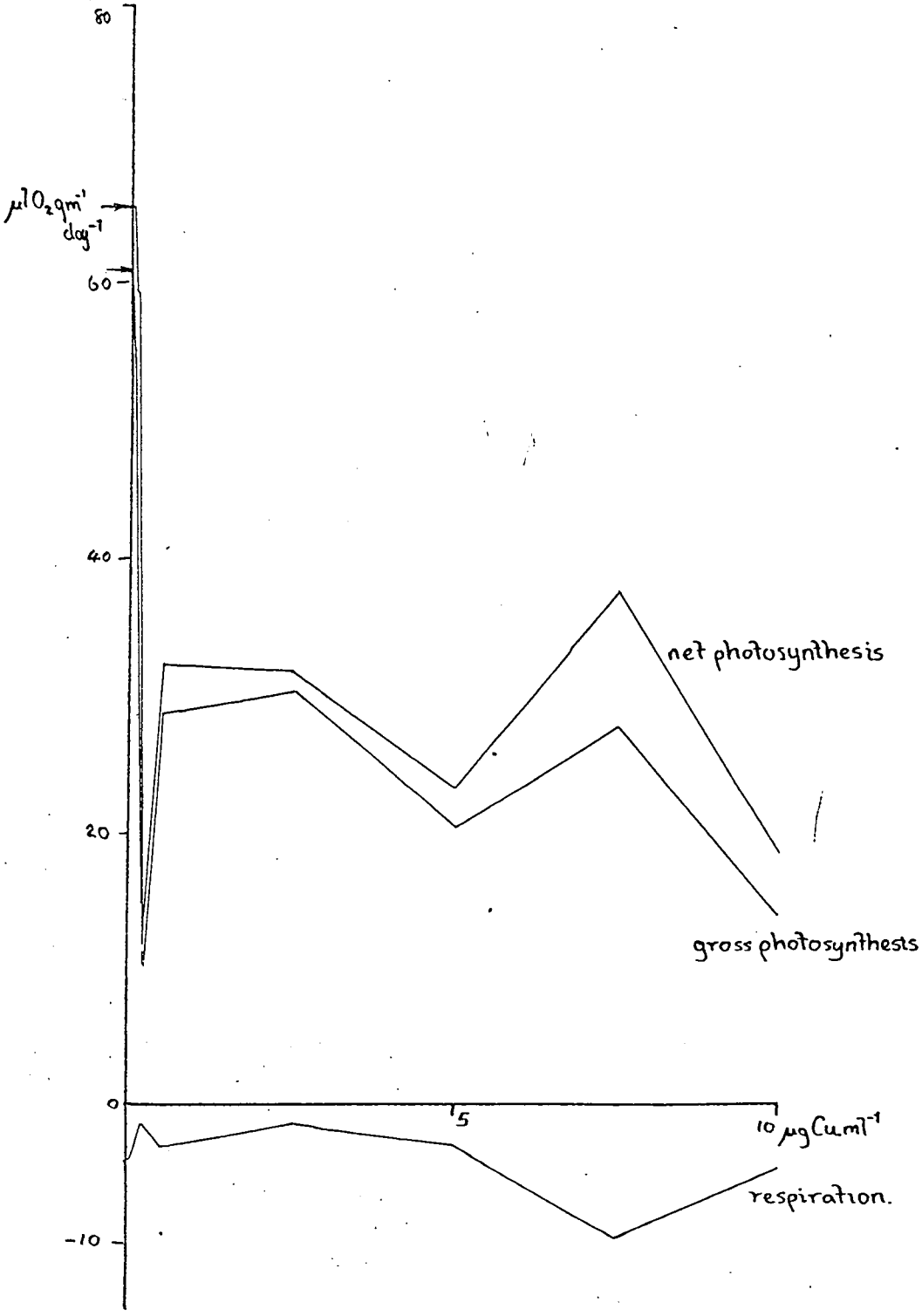


Figure 12. The Effect of Copper on the Photosynthesis and Respiration of Enteromorpha intestinalis.



reached or crossed only at solutions nearly saturated with T.B.T.OH. Enteromorpha linza proved to be an exception producing random fluctuations in photosynthesis and to a lesser extent, respiration. Concentrations of T.B.T.OH of between 0 and 0.05% saturated solution would appear to reduce both photosynthesis and respiration in a number of the algae tested. This is followed by an increase, rapid in some cases, in both at 0.1-0.2% saturated solution. Between 0.2 and 12.5% there is a gradual fall followed by another increase which continues to the higher concentrations. It is this increase which reduces gross photosynthesis to a great extent in the brown algae but the first named increase which causes the reaching of the compensation point by the green algae.

Cuprous oxide would seem to act in a different way to T.B.T.OH. Here there is an initial stimulation in gross photosynthesis between concentrations of 0-0.5ugCuml^{-1} as a result of increased net photosynthesis and respiration, the increase in the former exceeding the latter. Normally, there follows a gradual decrease in gross, net photosynthesis and respiration as the toxin concentration increases. This is exemplified by Giffordia fenestrata and Ectocarpus siliculosus (Figs 9-10). With Enteromorpha intestinalis and Pilyella littoralis there is the initial stimulation followed by a decrease in the three components between 1-5ugCuml^{-1}. Above this concentration there is a second increase in photosynthesis, that of gross photosynthesis in

Enteromorpha intestinalis being due to a decrease in respiration at higher concentrations. At the higher copper concentrations the green alga Enteromorpha appear to be growing at a greater rate than most of the brown algae tested. This is the reverse situation to that found with tributyl tin hydroxide and will be discussed in detail later.

The effect of finely divided copper on two algae was also tested. The test material was Enteromorpha intestinalis and Pilyella littoralis. It was found that the general trend seen with cuprous oxide was followed here. The initial increase at 0.5ugCuml^{-1} followed by a gradual decrease in all three components as the copper concentration increased. As with cuprous oxide photosynthesis of Enteromorpha intestinalis was the higher at most copper concentrations, a fact which, as will be shown later, ties up with studies of the flora of the ships hulls.

The actual quantative values given for the Warburg experiment are however of limited use as the number of runs carried out with each species was low.

B Metabolic Flux

This experiment was designed to obtain the same information as the Warburg but using less sophisticated equipment. Photosynthesis and respiration were measured by the use of pH measuring indicating change in carbon dioxide concentration and microwinkler techniques to record changes in oxygen. The methods which follow Verdium 1960 and Beyers et al 1963 for carbon dioxide, and Fox and Wingfield 1938, for oxygen, are given in Appendix E2-2.

The results are given in Table 7. These suggest that there is no logical output of carbon dioxide with respiration or output with photosynthesis and likewise for oxygen, although there is the assumption that only the carbon dioxide flux will affect pH, which cannot be held absolutely. But the results of metabolic studies on lake systems and other equatic environments (Teal 1957, Odum 1957, Odum and Odum 1955) give good results using basically the same method.

Not only does there appear to be no relation of gas flux with toxin concentration but there would appear to be no relation of carbon dioxide flux with oxygen flux, a situation that is stoichiometrically unlikely. The difference between the two varying between a factor of 1 to 16. The differences resulting from the use of two methods of calibration of pH with with carbon dioxide concentration are of a lesser nature, generally there being no more than a factor of 2 difference.

Table 7 The Effect of Toxins on the Metabolism of Some Marine Fouling Algae - Measurement of O_2 and CO_2 concentrations

A.TRIBUTYL TIN HYDROXIDE */ (all measured as ml. gm.(f.w.)$^{-1}$ day.$^{-1}$)		0	0.5	2.5	5.0	25.0
Ectocarpus siliculosus						
GROSS PHOTOSYNTHESIS	i	16.819	-249.882	69.518	-4.884	37.535
	ii	- 5.806	30.967	-26.667	-28.852	- 55.433
	iii	-7.258	40.645	-15.000	-14.754	- 14.173
RESPIRATION	iv	-92.010	22.486	48.054	83.422	- 3.385
	v	76.47	-1.585	-1.806	-226.400	-166.364
	vi	132.353	-8.490	-36.290	-198.000	-225.000
Pilyella littoralis						
GROSS PHOTOSYNTHESIS	i	21.562	23.839	22.469	-10.537	- 42.853
	ii	-49.435	-10.313	-71.256	-87.190	-114.244
	iii	-65.134	-52.373	-104.072	-84.806	- 84.142
RESPIRATION	iv	- 8.250	11.070	29.460	- 2.428	14.170
	v	-66.634	-14.163	-14.396	-36.612	- 11.053
	vi	-102.804	-11.576	10.539	-34.230	- 14.269
Enteromorpha intestinalis						
GROSS PHOTOSYNTHESIS	i	-5.822	0.897	-1.290	-2.455	- 2.673
	ii	3.139	-0.056	5.940	7.193	2.888
	iii	5.862	16.268	11.201	13.904	14.133
RESPIRATION	iv	-1.789	-5.469	-6.735	-2.091	-32.867
	v	1.865	10.833	8.420	4.601	7.472
	vi	11.792	18.021	20.910	12.985	14.732
Enteromorpha compressa						
GROSS PHOTOSYNTHESIS	i	-1.202	7.407	1.515	1.280	0.056
	ii	-0.048	-2.984	-1.160	-5.670	-6.504
	iii	0.363	4.475	-0.915	-10.631	2.219
RESPIRATION	iv	1.932	1.780	2.957	4.199	0.593
	v	-1.550	-3.136	2.912	-3.273	-1.126
	vi	1.824	-8.066	4.913	-3.459	-0.448

B.CUPRIC OXIDE		0	0.1	0.5	1.0	5.0
Giffordia secunda						
GROSS PHOTOSYNTHESIS	i	-66.151	-28.214	0	-21.762	-5.399
	ii	43.084	-14.141	34.054	0.872	-107.630
RESPIRATION	iii	15.726	-44.529	-18.538	-32.986	-7.874
RESPIRATION	iv	-142.268	-199.965	-128.640	-129.457	-100.396
	v					
	vi					
pillyella littoralis						
GROSS PHOTOSYNTHESIS	i	-46.897	-10.110	-1.453	0.864	-1.482
	ii	35.881	-82.060	32.761	20.243	15.478
RESPIRATION	iv	27.689	-58.636	-3.360	-14.383	15.724
	v	-28.717	-86.500	-17.075	-11.500	-10.750
Enteromorpha compressa						
GROSS PHOTOSYNTHESIS	i	-2.452	-0.463	-0.077	0.212	-0.985
	ii	-1.043	-1.132	-1.442	-16.270	1.576
RESPIRATION	iv	0.954	-0.418	-0.517	0.744	-17.990
	v	-4.615	-6.258	-8.535	-1.856	2.735

i and iv - Oxygen flux (Fox & Wingfield 1938)
ii and v - CO_2 flux - (Verdium 1956)
iii and vi - CO_2 flux - (Beyer et al. 1963)
* percentage saturation in sea water 9 ug Cu. ml^{-1}.

The values given for the flux are in the order of 1,000 times greater than those taken from the Warburg experiment and 10 times greater than obtained in the growth experiments below.

C Growth Experiment

This experiment was designed to obtain data on the effect of the toxin on algae in a less refined manner using growth as an index of gross photosynthesis.

The method follows that of Lindahl (1962) and is given in Appendix E2-3. The results are given in detail in Appendix E3-4 and summarised in graphical form in Figures 13-15.

Pilyella littoralis and Giffordia secunda have a high natural growth rate in pure culture solution. This drops rapidly as the concentration of the toxin is increased. Between 0.005 and 0.25% saturated colution of T.B.T.OH there is a transient increase in growth, but in Giffordia secunda this then decreases and reaches its compensation point at 0.5% saturation. Pilyella littoralis decreases rapidly as the concentration is increased to 0.1% saturated solution and then rises but only to a value of (0.33 x control).

Enteromorpha intestinalis reaches its compensation point at much lower concentrations of tributyl tin hydroxide, about 0.01% saturated solution, and then continues to act as an increasing respiratory drain on the system.

With cuprous oxide a similar trend is observed to that of the Warburg. Giffordia secunda reacted to increase from a rate of $2.5ul0_2gm^{-1}day^{-1}$ at 0.05% $ugCuml^{-1}$ and then decreased to a minimum of $0.3ml0_2gm^{-1}day^{-1}$ at $5ugCuml^{-1}$, after which the photosynthesis rate rose.

Figure 13. / The Effect of Tributyl tin hydroxide on the Growth of some Marine Algae.

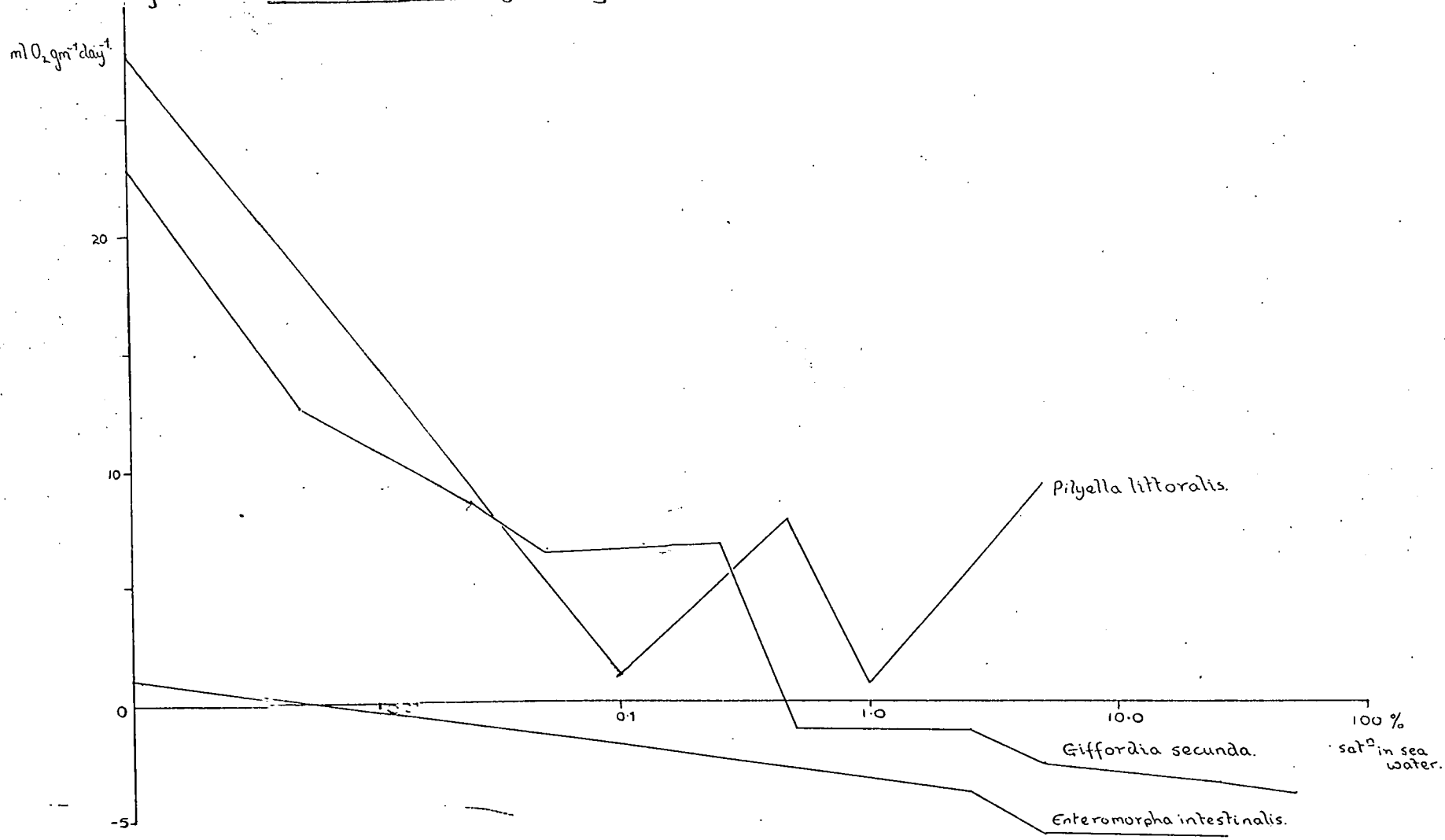


Figure 14. The Effect of Copper on the Growth of some Marine Algae

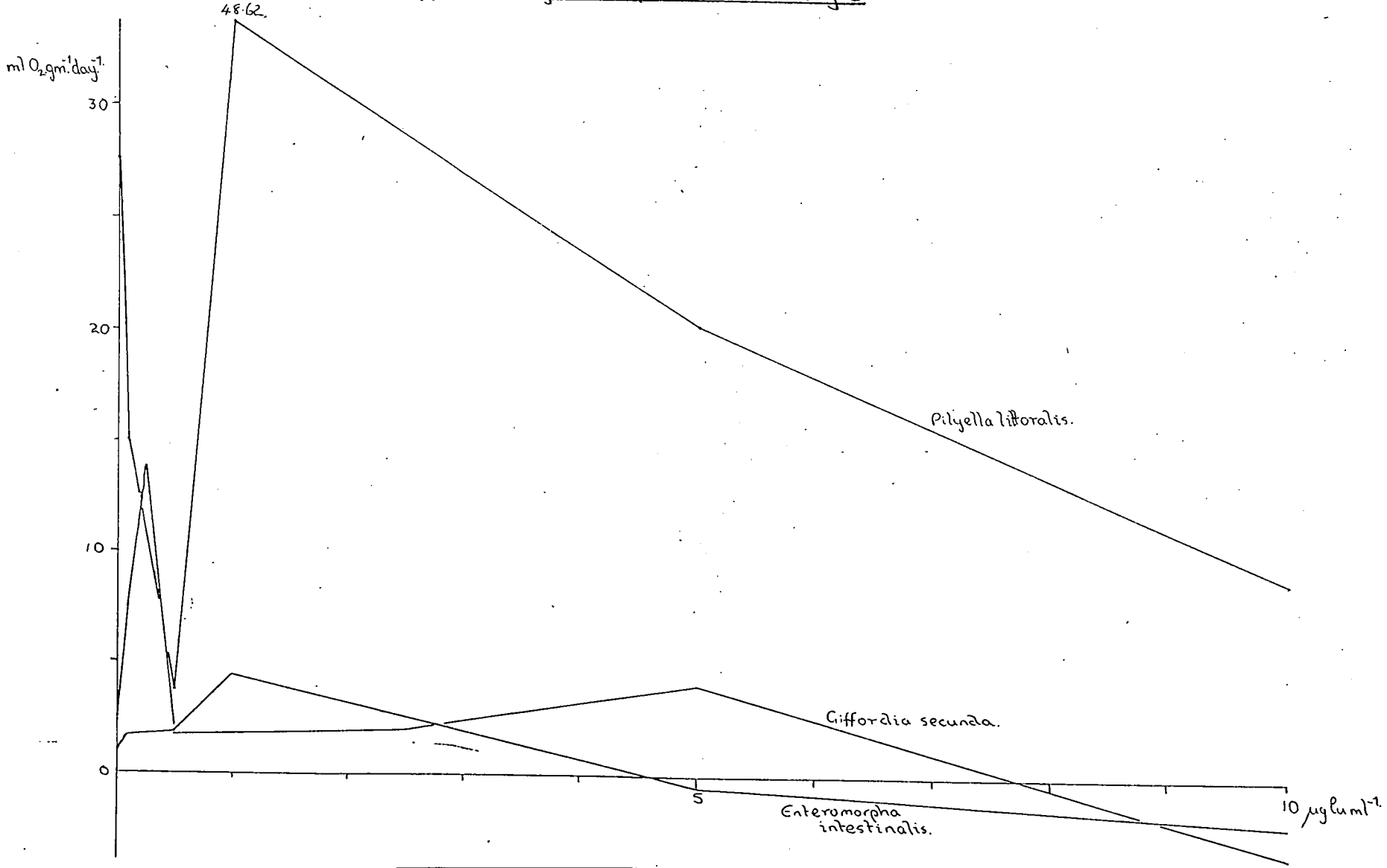
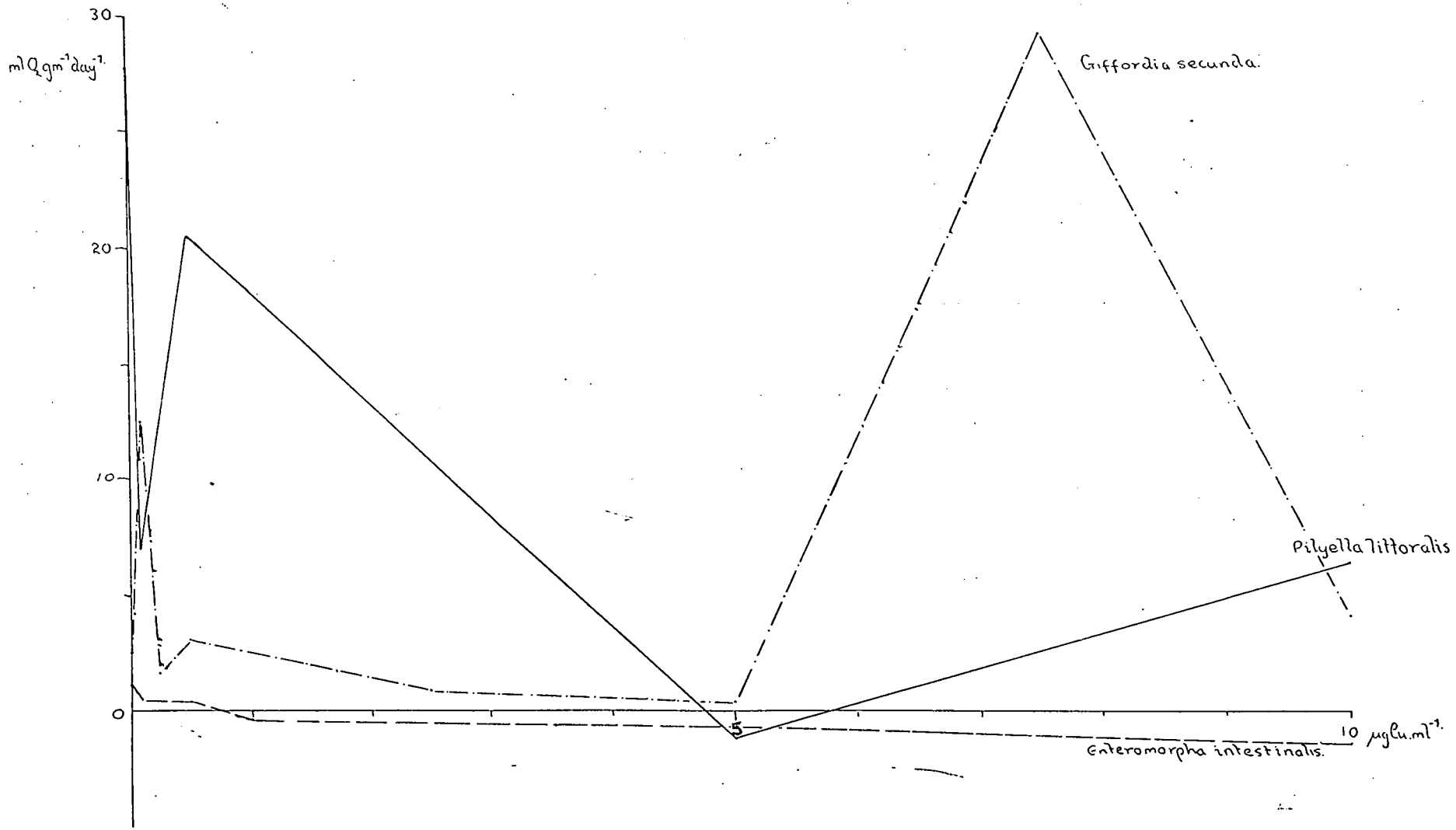


Figure 15. The Effect of Cuprous oxide on the Growth of some Marine Algae.



Pilyella littoralis decreases rapidly with increasing concentration from a control photosynthesis rate of $28ml O_2 gm^{-1} day^{-1}$ to one of $6.4ml O_2 gm^{-1} day^{-1}$ at $10ugCuml^{-1}$. There appeared to be no initial stimulation.

Enteromorpha intestinalis has a much lower photosynthetic rate of only $1ml O_2 gm^{-1} day^{-1}$ and this decreases, again without the initial stimulation reaching a compensation point at about $1.0ugCuml^{-1}$. This is very unlike the results obtained from the Warburg apparatus.

The copper solution gave the general trend seen using the Warburg technique with very low concentrations of copper up to $0.5ugCuml^{-1}$ there is an increase in growth. This is from $1-4ml O_2 gm^{-1} day^{-1}$ for Enteromorpha and $2.5-13ul O_2 gm^{-1} day^{-1}$ for Giffordia. Pilyella behaves more randomly reaching a peak at $1.0ugCuml^{-1}$ and then decreasing. After the initial peak both Giffordia and Enteromorpha have a decreased growth rate but the latter reaches the compensation point at $5ugCuml^{-1}$ while Giffordia reaches it at $7.5ugCuml^{-1}$. Pilyella does not appear to reach its compensation point as even at $10ugCuml^{-1}$ there is still growth.

The most unusual feature of these is the fact that the algae would appear to act as a respiratory drain on the system at much lower concentrations than are indicated by the Warburg experiments.

GERMINATION EXPERIMENT

The germination of spores is a critical event in the life cycle of an alga. It has been shown by Christie and Shaw 1968 that in Enteromorpha the settlement of zoospores is not passive, active swimming occurs in order to find a suitable point for settlement. It would thus be expected that this stage in the life cycle of an alga would be one which was very sensitive to environmental effects (e.g. toxins).

The following experiment was designed in order to test the sensitivity of algae to various toxins and evaluation of this as a bioassay technique with a high degree of sensitivity.

Method

The experiment was carried out using Giffordia secunda, Pilyella littoralis and Enteromorpha intestinalis. The last named failed to produce viable spores and is now being repeated following the method of Evans and Christie 1962. (The results of this not being available due to the periodicity of sporulating exhibited by this alga).

The algae were weighed to obtain their fresh weight and placed into a sterile petri dish with a disk of filter paper at the bottom to assist the attachment of spores. 20 ml of test solution was then added. The petri dishes were then placed in the constant environment room at 18°C and a light intensity of 4000lux. The algae were reweighed at weekly intervals and the number of germinating spores

Figure 16 The Effect of Various Toxins on the Germination of Giffordia Secunda.

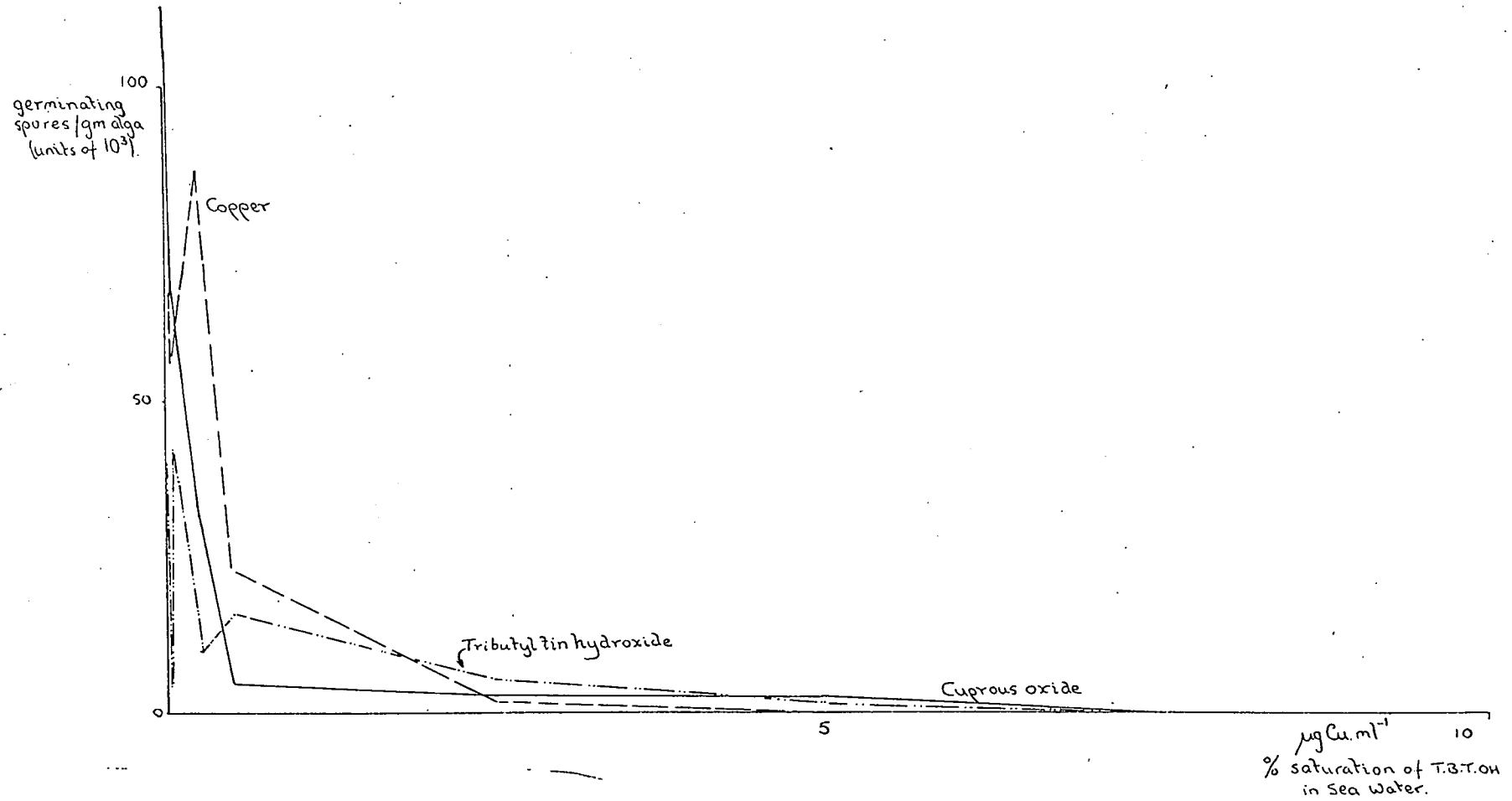
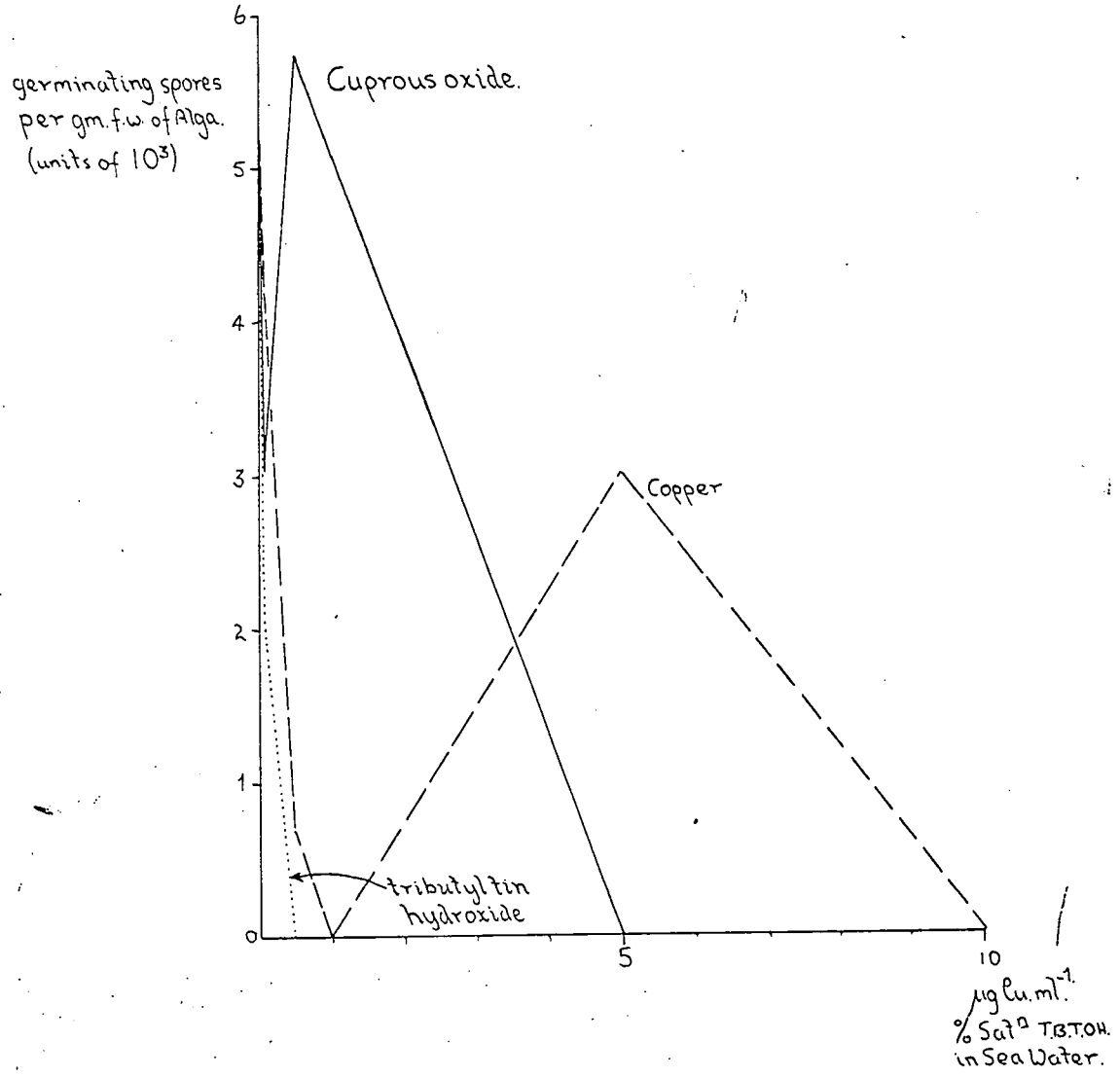


Figure 17 The Effect of Various Toxins on the Germination of Pilyella littoralis.



counted and expressed as spores per gramme of alga. The test solutions used were Copper and Cuprous oxide 0; 0.05; 0.25; 0.5; 2.5; 5; 7.5; 10ugCuml^{-1} T.B.T.OH. 0; 0.005; 0.025; 0.05; 0.25; 0.50; 2.50; 5.00; 25; 50 percent saturated solution.

Results

Figures 16, 17 and Appendix E5 give the results obtained in the germination experiments. In general the trends observed for Giffordia secunda follow those obtained in the previous experiment. With tributyl tin hydroxide there is an initial decrease from 40 x 10^3 to 4.4 x 10^3 spores gm^{-1} and then an increase to 42 x 10^3 spores gm^{-1}. This then decreases gradually with a slight but transient rise at 0.5% saturated solution until after 5% saturated solution no more germination occurs.

With Cuprous oxide solution there is a gradual decrease from 112.5 x 10^3 spores gm^{-1} at zero concentration of toxin to 4.27 x 10^3 spores gm^{-1} at 0.5ugCuml^{-1} the decrease in germination success after that is more gradual and above 5.0ugCuml^{-1} no germination occurs.

Copper shows a similar pattern but at 0.25ugCuml^{-1} there is a transient increase in germination success reaching 87 x 10^3 spores gm^{-1}. There follows a rapid decrease and above 2.5ugCuml^{-1} there appears to be no germination occurring.

With Pilyella littoralis there is a stimulation in germination success at 0.5ugCuml^{-1} of 5.71 x 10^3 spores gm^{-1} and then decreases

rapidly such that after 5.00ugCuml^{-1} there is no germination at all. With copper there is a rapid drop in germination success for a control of 5.18 x 10^3 spores gm^{-1} to 0.67 x 10^3 spores gm^{-1} at 0.5ugCugm^{-1}. There is then a sudden rise to 3.0 x 10^3 spores gm^{-1} at 5ugCugm^{-1} after which no germination occurs.

Tributyl tin hydroxide was only observed to allow germination at control and 0.1% saturated solution. This is in contrast to Giffordia secunda in which germination occurred right up to 5% saturated solution.

Unfortunately during the present series of experiments it was found that Enteromorpha could not be made to germinate in culture but this is now being repeated using a refined technique.

Discussion

The experimental work was carried out with three aims which have been listed previously. The discussion will be divided into three parts, each part will discuss the results obtained in light of one of these aims.

Section One

The Effect of the Toxin on the Major Components of the Fouling Ecosystem

There are two phases in the life cycle of an alga which can be attacked by the use of toxins. The first is at the stage of germination of spores, the second is during the growth phase. The experiments carried out investigate the effect of three toxins on both of these phases, they being copper, cuprous oxide and tributyl tin hydroxide.

The results show that, with copper and cuprous oxide growth will occur throughout the concentration range used (from zero to fully saturated solutions) but at higher concentrations there is a lowering of the growth rate. The initial increase in growth at very low concentrations is thought to be equated with the use of copper as a micronutrient, but like a number of micronutrients it acts on a toxin in high concentration (Steward 1963).

However, in Giffordia secunda, germination would appear to cease at concentrations of copper above 5ugCuml^{-1} (as Cu^{I}) and 7.5ugCuml^{-1} as copper metal. It would appear that in this case, copper attacks the stage of germination in algae. If copper does prevent germinations at higher concentrations than 5.0ug per ml, while growth can continue to fully saturated solutions it would seem that it also acts in a similar way in Enteromorpha. This alga has a greater tolerance to copper than Ectocarpus in high light intensity (Harris 1946) and one would expect Enteromorpha to germinate at similar or higher concentrations especially as its growth rate, from Warburg operations, is lower than that of the Ectocarpaceae. The lower growth rate suggests that some exclusion mechanism might be involved in the dominance of Enteromorpha over Ectocarpus.

In the case of tributyl tin hydroxide it was found that while the Ectocarpaceae would remain growing throughout the concentration range, although as with copper tending to zero at the highest concentrations, Enteromorpha, two species found on ships, would only show growth at concentration less than 1-2% saturation in sea water. The germination experiments showed that germination in Giffordia secunda occurs up to concentrations of 5% saturation. Thus one would expect from this that at concentrations of tributyl tin hydroxide of between 2-5% saturation Giffordia and possibly the other Ectocarpaceae would both germinate and grow while Enteromorpha (E. compressa and

E. intestinalis) could not grow even if the germination could occur.

The conclusion drawn from these results is that on ships with copper or cuprous oxide on the toxin the green algae would probably dominate and with paints based on tributyl tin hydroxide one would expect the members of the Ectocarpaceae to dominate.

That this might be so is upheld by reference to survey of ship fouling. Harris (1946) found green algae to predominate although the brown algae were present. The results presented in the current survey show that the Ectocarpaceae tend to be the dominant type. However, one survey carried out by one of the major shipping companies showed that ships might be covered initially with brown weed and this may later be replaced by green weed. This would be expected if the green weeds compete successfully for the same niche as the brown weeds. This is known to be so for regions of high light intensity (Harris 1946) and may be the reason for the change although we have no proof of it as the experiments were not designed with this in mind. A possible way in which the dominance of Enteromorpha over Ectocarpus is brought about is in the light of the lower growth rate, by some exclusion mechanism. This replacement of brown weed by green could also be explained on the basis that the copper retained in the antifoulant paint after all the organotin had gone was too high to allow the germination of Ectocarpus but too low to prevent the germination of Enteromorpha and replacement occurred either naturally

by replacement after death or by some exclusion mechanism as above.

The results from the growth experiment show that the algae reach their compensation point at lower concentrations than in the Warburg experiments. It is possible that this is a result of ageing of the solution but may also be real. It is Enteromorpha which appears to suffer most in this manner, however, and it might be that it is the solution ageing that is producing these results. The members of the Ectocarpaceae follow in general the pattern observed in the Warburg operation even to the extent of replicating the "micronutrient" peak with copper. The tributyl tin hydroxide run closely parallel to that obtained from the Warburg. It is suggested that Enteromorpha is more sensitive to changes in the solution involved and this might cause the lowering of the toxin required to produce a respiratory drain on the alga.

Section Two

The Use of These Experimental Techniques in the Assessment of New Compounds with Antifouland Properties

The application of the experimental technique described in relation to a future research programme into the evaluation of compounds with antifouling potential is given in the General Discussion. This section will deal with their use in isolation not as part of a combined programme.

The Warburg apparatus produced results which, in the case of Enteromorpha compressa and Enteromorpha intestinalis were remarkably similar when using tributyl tin hydroxide. The remaining examples were sufficiently alike to predict trends in photosynthesis and respiration caused by the toxin. The apparatus, although elaborate, is simple to operate and a large number of samples can be used at once. The production of a set of figures for net, gross photosynthesis and respiration takes in the region of 2 hours including a calibration to allow for photosynthesis or respiration in the solution alone. This method was found to give the most consistent results for the expression of growth. The growth experiments, although giving a similar picture were less consistent in the case of Enteromorpha and required fourteen days for completion. This increase in the time of the experiment is compensated by the extreme reduction in experimental cost. The

apparatus consisting simply of petri dishes, and requiring a room of uniform environment, which is generally available in most research institutions, and an accurate balance.

The results from the metabolic flux experiment suggest that it should be carried no further, even allowing for its use in the studies on energy flow in ecosystems (Teal 1957; Odum 1957).

The germination experiment provided useful information on the tolerance of algal zoospores to toxins in sea water. This in itself warrants further work into this aspect. It provides a simple explanation for patterns observed in the surveys carried out in part one; its potential as a bioassay technique will be discussed later. It requires the same apparatus as the growth experiment and takes approximately the same time and could present a simple and sensitive estimation of the effect of a potential antifoulant.

Section Three

The Extension of the Experimental Techniques as Bioassay Methods

The aim of a biological assay is to provide an index of the concentration of a compound, which is too low for accurate chemical analysis, through the use of a parameter of a biological nature. This parameter has, in the field of marine fouling, been limited to the growth rate of organisms. The methods of Fitzgerald (1963) with Chlorella and Rivett (1965) with Chlamydomonas measure the change in cell density over a period of about four days. This is compared to a series of growth rates prepared from standard concentrations of toxin.

The aim of this section is to provide other methods of bioassay which are either quicker, while maintaining the accuracy required, or slower and more sensitive. Two methods used come intp these categories. The Warburg manometric technique requires 2 hours for a series of fourteen estimations followed by a longer time span to dry the alga and weigh it if the dry weight is required. This last mentioned could be read from a correction table of fresh weights to dry weights. These problems can be overcome by the use of a unicellular alga such as Chlamydomonas. This can be injected in known concentrations into the flasks and thus reduce the time required per run to the two hours. Additional runs will require less time as the calibration for biological activity in the solution will have

been calculated previously.

Also, Chlorella can be kept as viable pure strains for periods up to 1 year - Ammain & Fraser Smith (1968).

The results obtained from using a variety of non-uniform stocks show that trends can be shown even under these conditions. With a standard stock of a unicell and allowing time for equilbration there is no reason why this should not provide an accurate bioassay.

The germination experiment takes longer than the Rivett method but it is believed would be much more sensitive. Experimental work by Christie and Shaw (1968) show that in Enteromorpha the zoospores are capable of some choice of settlement area in response to environmental influences, and it seems likely that this stage might show more sensitive reactions to any toxins in the water.

The importance of this lies in the very low concentrations at which these recent toxins (organometallics etc) work, if even more effective toxins were developed there will be a requirement for more sensitive methods of bioassay, which could be provided by the germination experiment.

The equipment required is not very elaborate and provided a fairly uniform environment is maintained, the experiment is easy to run. The results with Giffordia secunda providing a fairly reliable indication of its sensitivity.

Part 4

GENERAL DISCUSSION

General Discussion

The following discussion is aimed at the combination of experimental and field data discussed in detail previously and to suggest some of its implications in the field or marine fouling, and possible future avenues of research.

At present, paints incorporating various toxins are the only methods of preventing ship fouling that are used on a large scale. Much of the formulation of the paint would appear to follow a pattern of introducing a trial toxin into a paint and testing it out on raft or ship trials. On rafts these trial plates are removed at regular intervals for the leaching rate to be determined. The leaching rate at the commencement of fouling being used as an index of toxicity of the compound and the concentration required in the paint to prevent fouling. Raft trials can take up to four years before fouling occurs, this being equated with a ship life of two years.

One way in which this situation might be improved is by the investigation, biochemically, of the effect of toxin on the fouling ecosystems - the green and brown algae. This would enable certain of the proposed toxins to be eliminated and would only take a short time to complete. The toxin can then be suggested to the paint chemist who would prepare a suitable carrier for it. This paint could then be applied in various thicknesses to test plates and subjected to

accelerated leaching rate tests. This would provide information on the erosion of the paint and the time taken to provide a minimum concentration of toxin to prevent fouling. This latter would be calculated from experiments such as the Warburg respiratory and germination experiments. The time taken to reach this would be much shorter than test trials or raft trials, and would eliminate unsatisfactory paints before there had been any extensive manufacturing of them.

Once these accelerated leaching rate trials had been carried out the suitable formulation could then be applied to ship tests or raft tests. The former being preferred as these resemble actual conditions more accurately. The performance of these paints on the ships could be followed and any fouling could be described to enable any indicator organisms to be detected and used to get an indication of the performance of the paint. This would rule out the need for elaborate chemical or physical tests to be carried out on the trials, the results being used for the indicator organisms being gained by the initial biochemical analysis of the effect of the toxins on the fouling systems.

The toxins that will be used will be ones which are toxic at far lower concentrations than those used today. This in itself requires bioassay techniques in order to detect the very low concentrations required and development of these is necessary. This being one of the

aims of this project, another being to show how integration of biochemical and visual experiments can play a part in the subject of marine fouling.

The discussion following the work on the ships survey indicates how the experimental work carried out helps to explain the results of the survey. The importance of this lies in the effect of the fouling on the speed of the ship. If the green weed produces more drag on a ship than the brown weed, all other things being equal, then a paint favouring the brown weed would be preferable, or vice-versa (Experiments are now being devised to test this point). This factor is complicated by the fact that the organometallic paints last up to 18 months before fouling is heavy whereas the copper paints will last only 12 months. If the brown weed produces more drag than the green the increased cost due to this must be balanced against the decrease in cost due to the longer life of the paint. The relative costs of the paints must also be taken into account before the true significance of the change over can be perceived.

Calculations based only on one sample show that the fouling on the St. Margaret was 456gm^{-2} (365gmm^{-2}yr^{-1}). Ivanova (1961) gives a figure of 770-1,000gmm^{-2} for fouling. If this is multiplied by the size of the vessel and then corrected to give the drag imposed by the weed then one can calculate the ton of speed and hence the increase cost of running the ship. However, more samples of fouling must be taken before accurate calculations can be applied.

The figure given by Ivanova (1961) compares with that of Barashkov and Fedyakina (1965) which gives the following values of weed growing on immersed concrete blocks:-

Sea of Azov - Enteromorpha prolifera $1000gm^{-2}yr^{-1}$

All algae (average) $1860gmm^{-2}yr^{-1}$

and Zenkevitch (1963) who gives a figure for Enteromorpha intestinalis of $209.5gmm^{-2}yr^{-1}$ for the Black Sea. These would seem to suggest that there may be some difference in the productivity on ship and shore but this requires more samples before any case can be stated.

Future lines of attack, many of which have been tried out, could bear repeating limiting their use to that period coinciding with the fouling organisms. Fisk 1960 stated that the expense of running ultrasonic equipment is a deterrant. Costs might be reduced by only using this equipment in port and only during the fouling season. The equipment however, must not be of a frequency which could cause the self distruction of the ship. The use of piped toxins could be applied during the fouling season but it has to be expelled in a finely divided form, the apertures for its dispersal thus might be susceptible to clogging by material in the water.

The greatest advantage of the paint is that a coating of paint is required to prevent the steel hull from corrosion by the sea water, and since the ship has to be docked for this, the additional application of an antifoulant does not prove too expensive.

Finally, there is the question of algae evolving resistance to the toxins used. Resistant strains have developed in most types of animals and plants. Bacteria are known to rapidly build up resistance. Cases are known of resistance to heavy metals occurring in Angiosperms (Jain and Bradshaw 1966) and in fungi. The speed of building up the resistance depends upon the selection pressure to do so and the speed of reproduction.

The algae involved reproduce quite rapidly although do so in marked seasons. This would produce some limit on the speed of setting up resistance. The selection pressure on the hull of the ship is great but on the shore it will depend on the advantages, if any, of this resistance. If this resistance is genetically determined and also confers increased viability to the alga then its spread would be rapid. However, if there was no advantage its spread would be slow or in the extreme if it was less viable than the non-resistant strains there would be a tendancy for it to die on the shore. If this last named were true then the only source of replenishment would be the ships themselves.

The transference of spores of these algae is from ship to ship either directly or indirectly via the ship-shore-ship link. The ship transference is the simplest and could readily occur in harbours and result in quite rapid build up of resistance. The ship-shore-ship would result in a build up if there was no disadvantage

in having the resistant gene in an environment not requiring it (i.e. the shore).

There would appear to be little known on the subject for resistant strains could cause the costs of research into new toxins to increase in relation to the speed at which resistance is built up. It is also important where the mechanism of resistance is involved if the mechanism involved deals with a type of compound rather than a specific compound it makes prevention more difficult and more expensive and as a result this could well be one field which could suffer some research in the near future.

Part 5

APPENDICES

Appendix S1

SHIPPING AND COASTAL SURVEYS - METHODS

Section S1-1

Section S1-1

Fouling Ecosystems present on the "Kazimah"

This study was undertaken in order to detect any basic pattern in the distribution of fouling ecosystems on the ships hull. The information gained would form the basis for future sampling.

Sampling was taken at three heights on the hull, 18; 28 and 38 feet above the keel. These fell into the two major zones which were visible and in the junction between the two at the light load line. Any gradual or disjunct separation of the two zones would thus be detected.

Sampling at each of these heights was carried out at three sites along the hull, one each side of the ship. This would detect any variation in pattern along the length of the ship and on either side of the same.

The samples were then subjected to microscopic analysis to determine their algal components. Identification of the algae was from Newton (1931); Eifon Jones (1964) Blding (1963). The nomenclature follows that of Parke and Dixon (1968).

The data is displayed on Table 2 in a form convenient for inspection.

Section S1-2

Section S1-2

Fouling Ecosystems on Ships in Regular Service

The sampling procedure used was modified from that used for the 'Kazimah', based on the results from that vessel.. Two samples were taken from each vessel. One was taken from the light load line or just above; the second from the hull near the bilge keel. Bilge exits and other similar sites on the hull were avoided.

The samples were stored in sea water containing 5% formalin until identified. Indentification was from Newton (1931); Bliding (1963); Eifon Jones (1964).

Other details which were obtained for each ship were:-

i) period elapsed since last scraped

ii) antifouling paint used

iii) routes covered

iv) position of samples

v) notes: any relevant points appertaining to fouling.

The analysis of the data followed that of Braun-Blanquet 1928 (see Appendix S2. for description of method). The data being displayed as an association table - Table 3 - for convenient inspection.

On one ship, the 'St. Margaret', an area of fouling of 89 sq.cm., was dried and weighed and corrected to give weight per square metre. This was to provide some indication of the biomass that fouling organisms can produce.

Section S1-3

Section S1-3

Fouling Ecosystems from the Main Shipping Lines

This was carried out as an extension of Section S1-3. The first stage was to send a letter (Fig. S1-3a) to a number of shipping companies outlining the aims of the study and asking for their co-operation. If this was forthcoming, a second letter (Fig. S1-3b) was sent explaining in detail what was required, and enclosing a number of sample bags with attached data sheets (Fig. S1-3c).

The shipping companies were asked to obtain samples of fouling from the light load line and near the bilge keel. These were to be returned in the bags supplied together with the completed data sheet. Identification procedure follows that of Sections S1-1 and S1-2. The data required is shown on Fig. S1-3c and is similar to that taken in Section S1-2.

There was some delay in starting this survey and results are still coming in. The results, obtained to date (August 1969) are depicted in Table S1-3a in a similar fashion to those of Sections S1-1 and S1-2.

Details were also obtained from a number of companies concerning their past fouling histories.

UNIVERSITY OF DURHAM

Department of Botany,
Science Laboratories,
South Road, Durham City.

Dear Sir,

We are conducting a survey as to the main type of ship fouling at the present day, and would be grateful if we could have your co-operation in this.

It would entail us sending you some sample bags in which we would like a sample of the fouling on the ship, together with a slip of paper which would give:-

(a) the name of the vessel;
(b) dates of this and previous scraping;
(c) anti-foulant used during period of (b);
(d) routes used (British, continental, world wide).

And then any other details as may be thought helpful such as if there has been any changes in fouling organisms during the last ten or twenty years, and if this is associated with a change in the toxic contained in the paint.

The aim of this survey is to evaluate any change that might have occurred in the fouling organisms in recent years with any coincident change in paint toxins or in the ports of call used. It is not intended as evaluation of the brands of paints used, except insofar as it is concerned with the toxin used not any other ingredients.

If you are unable to participate in this, would it be possible for you to send us a report on subjects (a) through (d), but without the samples. We would be grateful for any information received and would give, in return, any assistance we can with regard to this.

Could you please reply on the postcard enclosed.

Yours faithfully,

Richard Sims.

figure S1-3a

U N I V E R S I T Y O F D U R H A M

DEPARTMENT OF BOTANY
SCIENCE LABORATORIES
SOUTH ROAD
DURHAM CITY

Dear Sir,

Thank you for your reply indicating that you are willing to take part in this survey. Enclosed are a number of sample bags each attached to a post card on which the following details should be noted:-

i) Shipping line.
ii) Name of Ship.
iii) Type (Tanker, Freighter etc.)
iv) Routes used (Coastal, Continental, Atlantic etc.)
v) Dates of this and previous scraping coincident with application of antifoulant.
vi) Paint used.
vii) Cover of algae, barnacles or tube worms.
viii) Notes (if any).

We would be grateful if you could send us two samples per ship at a) water line, b) bilge keel levels. Each sample should be placed in a bag (which may be sealed after) and posted to Mr. R.E. Sims at the above address preferably by first class mail to prevent undue deterioration of the sample.

Yours faithfully,

RICHARD E. SIMS

figure S1-3b

Shipping Line:- OCEAN STEAM SHIP CO.

Name of Ship:- "AENEAS"

Type:- CARGO

Route:- FAR EAST

Dates of Scraping

Last:- AUGUST 1968

Present:- JULY 1969

Sample:- ~~Waterline~~ or Bilge Keel

Paint:- LEIGH-DAVISON TROPICAL

Cover of Fouling	Light	Moderate	Heavy	None
Algae(Weed) Brown	✓			
Green				
Barnacles		✓		
Tubeworm	✓			

(Please tick under appropriate boxes.

NOTES

UNDER WATER FORM FOULED MORE THAN USUAL FOR VESSEL ON FAR EAST RUN.

Figure 51-3c

Table S1-3a — Fouling Ecosystems from the Shipping Lines

Name of Ship	Aeneas	Selby	Selby	Calchas	Diomed	Diomed	Paparoa
Type	C	C	C	C	C	C	C
Route	FE	C	C	FE	FE	FE	*
Months since/month of last paint coating.	12/8	·	·	12/8	14/5	14/5	6/2
Toxin.	Cu	·	·	·	Cu	Cu	Cu
Position of sample	B	W	B	W	B	W	W
Weed Brown	L	M	M	H		M	
Green		P	P	H			
Barnacles	M	M	M	L†	L	M	H
Tube worm	L			L†	L	M	
Enteromorpha compressa.		+	+	+			
ramulosa.				+			
prolifera.				+			
Ectocarpus siliculosus				+			
Giffordia secunda				+			
sandriana.				+			

KEY:-

Type: C — Cargo.

Route FE Far East.
C Coastal
* Vessel became fouled while laid up in R. Blackwater. 27-v-69 to 7-viii-69.

Toxin Cu Copper based.

Position of Sample B - Bilge keel
W - Waterline.

Fouling Ecosystem:-
L - Light.
M - Moderate.
H - Heavy
† in sea boxes only.

Section S1-4

Section S1-4

Fouling Ecosystems on Ships laid up in Harbour for long periods

The study was carried out at Portsmouth in a marine-brackish water harbour. Samples were taken, with the aid of a diver, from a series of four ships which had been laid up for different lengths of time (six to fourtyeight months). Two samples were taken from each ship. One from just below the waterline, the second from the level of the bilge keel.

The samples were preserved in sea water containing 5% sea water until identified from Barrett and Yonge (1958), and Alder and Hancock (1907). The data was processed following Braun-Blanquet (1928) being presented in Table 4 in a convenient form for inspection.

Section SI-5

Section S1-5

Coastal Distribution of Fouling Algae

The table was compiled from a number of algal lists published in the literature, where possible these were taken only if published after 1960. Other lists were compiled by the author from samples taken while out collecting for experimental material. A third source was from species lists sent i n from various Marine Laboratories in reply to a request for information on the distribution of these algae.

Table 5 depicts the results obtained. The sites are in order to make any geographical distribution evident.

It had been planned to provide information on the fouling periods of these algae, and successional sequence to a mature fouling ecosystem, by the use of a raft anchored in a harbour on the North East Coast. However, this part was unable to be completed in the available time. Instead, information on the fouling periods of algae in the River Yealm has been included with the permission of Dr. G.T. Boalch and International Paints Ltd. This information is given in Table 6.

Appendix S2

THE ZURICH-MONTPELLIER SCHOOL OF PHYTOSOCIOLOGY

The Zurich-Montpellier School of Phytosociology

A plant community may be considered to be a group of plants, the area of which is sharply delimited by physico-chemical boundaries, similar groups of plants occur in other areas of similar physical or chemical constitution. Such communities can be classified. Where the boundary is one of a gradual change in nature then the change in plant types will also be gradual and a continuum (Curtis 1959) will be evident and ordination is used in its description.

The classification of plant communities has been attempted using a variety of methods since those of Braun-Blanquet (1928). Examples of these may be seen in Poore (1956); Williams and Lambert (1959; 1961; 1962) and Goodall (1953). The method used here follows that of Braun-Blanquet (1928).

A plant community has certain synthetic characters. These include presence and degree of presence, fidelity and sociological indicator values. Presence can be measured subjectively in terms of cover values from I-V representing increments of 20%, or as directly a percentage; or it can be measured objectively using parameters such as biomass per unit area, basal height or density among others. The former usually lends itself to classifacatory techniques, the latter to ordination (Bray and Curtis 1957)

The Sociability of a plant is measured subjectively according to the following:-

Sociability 1 - growing singly
2 - grouped or tufted
3 - in patches or cushions
4 - in small colonies, extensive patches or carpets
5 - pure crowds

Fidelity is no longer used due to its uncertain value in plant description.

In the Braun-Blanquet School of Phytosociology lists of plant communities are taken using presence (as a cover index) and sociability. Each list is taken from an area in a plant community which is the least area required for the expression of that community (Minimal area). Other physical and chemical factors are also taken into account such as the slope; aspect of soil type among others. These are transferred to a table which has one axis for the species and another for the quadrats. This table, the raw table, is then rearranged in order to produce an association table in which groups of species and quadrats are ordered together to produce an abstract which can be related to the plant community in the field (Tables 2,3 and 4) by returning to the site and testing the data in the association table with that from the plan community.

The groups are classificatory and form classes, orders, and alliances (in descending order of merit) and the name given to them

is taken from the Charakterarten (species which occur most regularly in the group e.g. Order Seslerietalia coeruleae - Charakterarten : Potentilla crantzii, Aster alpinus) (Braun-Blanquet 1928).

This method of classification, although a subjective one, has been found to give similar results to the newer methods such as that of Williams and Lambert (1962); Moore (1956) while being quicker to reorganise the data. Examples of its use applied to the marine field are seen here in Tables 2-4, and Johns (1968) the latter reviews its application in the marine field.

The results depicted in Tables 2-4 seem to be of ecological value and correspond to data given elsewhere by other methods and present the data in a way which can be readily explained.

Appendix E1

ALGAL CULTURES - MATERIALS AND METHODS

Cultures of Algae

Material

The algae used in the experimental work were collected from various sites on the coasts of Northumberland and Durham. Enteromorpha sp were collected from Souter Point (Sunderland), Crester and Hartlepool. Pilyella and the Ectocarpus complex were collected from Souter Point and St. Abbs Head (Berwickshire). The algae which were used in the experiments were:-

Giffordia secunda (Kutz) Batt.

Ectocarpus siliculosus (Dillw). Lyngb

Giffordia fenestrata (Berk ex Harv.) Batt

Pilyella littoralis (L) Kjellm

Enteromorpha intestinalis (L) Link

Enteromorpha compressa (L) Grev.

Enteromorpha linza (L) J.Ag.

These were kept in the culture solution in the constant environment room between collection and use in an experiment.

Culture Vessels

Cultures were grown in sterile petri dishes with 25 ml of culture solution added. A disc of filter paper was kept in the dish to provide an attachment area for germinating spores.

Media

Natural sea water, from Hartlepools, provided the base for the

culture solution. This was filtered through Whatman No. 1 and stored in the dark until required.

The basic culture medium was enriched with the following nutrients:-

potassium nitrate (KNO_3) 202 mg/L

potassium phosphate (K_2HPO_4) 34.8 mg/L

feonic chloride ($FeCl_3.6H_2O$) 2.7 mg/L

Mangamese chloride ($MnCl_2.4H_2O$) 0.2 mg/L

and then autoclaved for 15 minutes at 15 lbs $sq.in^{-1}$ pressure. The phosphate was autoclaved separately from the rest and added before innoculation to prevent precipitation. The pH of the solution was in the region 7.7-8.0 when used. Ampicillin was also added to reduce bacterial activity in the culture solution. (Ampicillin 6.43 mg/h).

Culture Apparatus

The petri dishes containing the samples in the culture solution were placed on a white background in a constant environment room at 18°C and continuous light from fluorescent tubes giving 4000lux. The constant environment room was also used for germination and growth experiments. The culturing of algae proved successful and it was found possible to obtain sporelings of all the Ectocarpaceae used, but with Enteromorpha it proved difficult to obtain sporelings though growth did occur. This was unfortunate in that a possibility of any comparison of

germination success in relation to toxin concentration, in the Ulvaceae and Ectocarpaceae, was lost.

Unialgal Cultures

Giffordia secunda was used to provide the source for the first series of growth experiments. This source was the speciments grown in culture from a plating technique following Boalch 1961.

Specimens of Giffordia secunda were placed on a moist filter paper and left overnight in the dark. The next morning the release of spores was brought about by flooding the system with sterile culture medium. After a period of 15 minutes the algal material was removed. The dish was covered and placed in the constant environment room until required. Germination occurred after 10-14 days.

Appendix E2

EXPERIMENTAL METHODS

Section E2-1

Section E2-1

Warburg Manometry

The Warburg apparatus is designed to measure gas change at constant volume by means of the change in pressure when gas is absorbed or evolved. This pressure change is recorded on the manometer and can be converted to volume change by use of a standard formula. The apparatus can be illuminated to allow the measurement of photosynthesis or kept in the dark to measure respiration. The design of the flask is such that toxins or stimulants can be added without the need of resetting the apparatus. The use of this apparatus in the measurement of photosynthesis and respiration follows that of Lindahl (1963).

The experimental material used consisted of the following algae:-

Enteromorpha intestinalis
Enteromorpha compressa
Enteromorpha linza
Ectocarpus siliculosus
Giffordia fenestrata
Pilyella littoralis

Photosynthesis and respiration were measured by a direct method due to the difficulty of using identical quantities of alga in the flasks. The equipment used was that manufactured in Germany by B. Braun. Conical flasks with a single side arm and central reservoir were used and the volume of these including that of the manometer used ranged from 7 - 14 ml. The flasks were illuminated from below by a series of incandescent bulbs. These posed some problems due to the heat also emitted and ice had to be used to keep the temperature of the water both down to the temperature of the experiment 23°C.

After consideration of the discussion on buffering capacity, in Lindahl (1963), it was decided not to add any carbon dioxide as even due to the lack of buffering the variation is pH during the run of the experiment was not thought to be exceedingly large; the results obtained would seem to agree with this.

The flasks were set up using 2 ml of the solution under test in the outer well into which the algae were placed when used. Each run consisted of 13 or 14 flasks, 12 - 13 test solutions and a thermobarometer. Three toxins were subjected to testing:- finely divided copper in sea water (20 ug/ml); cuprous oxide in sea water (10 ug/ml); and tributyl tin hydroxide (the oxide of which has a solubility of 20(ppm and forms the hydroxide when added to water). The Copper and Cuprous oxide were tested at concentrations of 10.; 7.5; 5.0; 2.5;

1.0; 0.5; 0.1; 0.05 ug/ml; and the tributyl tin hydroxide at the following (as a percentage of saturation in sea water):- 100; 50; 25; 12.5; 6.7; 3.3; 1.6; 0.8; 0.8; 0.2; 0.1; 0.05. Each run had a control with 2 ml of sea water with no toxin added.

It was found before running the experiment that the culture solution has some inherrant gas flux due probably to certain microorganisms. It was thus decided to run the experiment in the following manner:-

In order to assess the compensation that would be required to allow for the ambient gas flux of the system, a dummy run, identical to that normally used but without the added algae, was carried out.

The flasks were equilibrated for 20 minutes with the taps open and then the manometer set at zero and the taps closed. Readings were taken at 5 minute intervals for 20 minutes, the flasks being shaken except while readings were being taken. The experiment was run twice, once with the lights switched on to record photosynthesis and then with them off and the apparatus covered with aluminium foil, to ensure minimum light entry into the flasks, to allow respiration to be recorded.

After the dummy run the algae to be used were added and the experiment being repeated using different algae in each run.

At the end of the experiment the algae were rinsed quickly in

distilled water and damp dried, weighed to obtain the fresh weight, then dried to constant weight at 105°C.

Photosyntehsis and respiration were expressed in terms of ul O_2 evolved or absorbed per gm day not of alga per day after correction for flask volume.

Section E2-2

Section E2-2

Metabolic Flux

Photosynthesis and respiration result in changes of oxygen and carbon dioxide concentrations. By estimation of these one can obtain some idea of the effect of the toxin on the growth of the organism, in this case algae. Measurement of both oxygen and carbon dioxide will provide a close estimation of growth and it is possible that each can be used to provide a relative estimation of the validity of the other technique.

The use of pH and oxygen flux as a measurement of growth was chosen for its simplicity experimentally and that the estimation of carbon dioxide and oxygen flux had been used in a large number of cases in metabolic studies both in fresh water and sea water as well as in terrestrial habitats (Odum 1957; Odum & Odum 1955; Teal 1957) (Verdium 1956). There has been some controversy on the use of pH for the estimation of carbon dioxide concentration, however, it was decided to follow in outline the methods of both Verdium (1956) and Beyers et al 1963; and to use oxygen measurements as a rough check to their accuracy. For further discussion of both points of view see Beyers et al 1963. It was also hoped that some support might come from the Warburg results as to the accuracy of the method.

Materials used:- The algae used in the experiment were as follows:-

Enteromorpha compressa

Pilyella littoralis

Ectocarpus siliculosus

Giffordia secunda

50 mls of test solution were pipetted into a 50 ml beaker. The pH of this solution was measured using an E.I.L. pH meter which had been standardised with buffer solutions at pH 4.0 and pH 9.2. Then, the oxygen concentration was measured using a modification of the microwinkler technique (see details below).

The algal specimen was then added and one of the two replicates was placed in light at 4000 lux and a temperature of 18°C for 24 hours: The other in the dark at 18°C. The dark series was placed in a box of aluminium foil which had to be constructed as the dark room was not available for use. After 24 hours the pH and Oxygen concentration were estimated again. The experiment was repeated three times for each test solution (once for three different algae), there being two test solutions used :- Cupric oxide in sea water and tributyl tin hydroxide in sea water.

Cupric oxide was used at 5; 1; 0.5; 0.1 and 0 ugCu ml^{-1}

Tributyl tin hydroxide at concentrations of 25; 5; 2.5; 0.5 and 0% of saturation in sea water.

Estimation of Oxygen by a modification of the Microwinkler technique

The estimation of oxygen dissolved in sea water was carried out

using a modification of the Winkler reaction diluting all solutions by a factor of 100 and using 2.5 ml of sea water instead of 125 ml of sea water. The reason for using this modification of the Microwinkler technique (Foxe Wingfield 1938) is that the limitations placed on the quantity of specimens and culture solution available required a technique using only small amounts of sea water.

Method

2.5 ml of sea water were carefully pipetted into a sample tube. To this was added 2 ml of manganous sulphate solution, 2 ml of alkyl azide solution. Time was allowed for the precipitate produced to absorb the oxygen in the sea water and then 6 drops of concentrated sulphuric acid added. This liberated iodine which was titrated by 0.01 N sodium thiosulphate solution using starch as an indicator.

The reactions involved are as follows. The alkyl azide solution precipitates manganous hydroxide from the solution. This is oxidised by oxygen in the sea water to manganic hydroxide. The acid oxidise the solution of manganic hydroxide and potassium iodide and releases iodine proportional to the oxygen concentration of the water and this iodine is then titrated by standard sodium thiosulphate.

$MnSO_4 + 2NaOH \qquad Na_2SO_4 + Mn(OH)_2$

$2MN(OH)_2 + H_2O + O \qquad 2Mn(OH)_3$

$2Mn(OH)_3 + 2KI + 3H_2SO_4 \qquad 2MnSO_4 + K_2SO_4 + 6H_2O + I_2$

1 ml Sodium thiosulphate (2.482g/L) 82.76ul O_2.

Solutions used :- (all in distilled water)

Manganous sulphate:-	4.80 g/L of $MnSO_4$ $4H_2O$
Alkyl-iodide-azide	5.00 g NaOH + 1.35 g NaI in 1 liter of distilled water and add 0.1 g NaN_3 in 40 ml water.
Sodium thiosulphate	2.482 g/L of $Na_2S_2O_3$ $5H_2O$.

Measurement of Carbon dioxide flux using pH

Carbon dioxide when dissolved in water results in an acid solution. At low carbon dioxide concentrations the gas goes into solution as a bicarbonate but as the concentration of gas increases more gas goes into the solution as the carbonate ion

$$CO_2 + H_2O \quad H^+ + NCO_3^- \quad 2H^+ + CO_3^{2-}$$

Thus it can be seen that addition of carbon dioxide into solution results in an increase in the hydrogen ion concentration in solution and a resultant lowering of pH.

This decrease in pH is not linear with the increase of carbon dioxide due to the two forms which carbon dioxide may exhibit itself as in solution. This non-linearity results in the curve found in Beyer et al 1963, when plotting pH against carbon dioxide concentration.

Method

The method of CO_2 analysis follows in outline that of Verdium 1956 and Beyers et al 1963. Since there is the non-linear relation of pH with CO_2 concentration calibration curves must be drawn. (This being

the cause of the difference in the views of Verdium 1956 and Beyers et al 1963).

The calibration curve is drawn by writing the pH of the solution adding successive increments of 1 ml of 0.01 N NaOH solution and noting their respective pH values. According to Verdium this addition of sodium hydroxide results in the change in pH identical to that caused by the removal of 10uh moles of CO_2 by photosynthesis. This is plotted on a graph of pH against effective CO_2 removal and the pH values obtained in solution read of this to obtain the changes in carbon dioxide concentration. The validity of this method has been questioned and will be discussed later. The reason for its use was its simplicity and it could be compared with figures obtained for oxygen flux.

Each speciment was titrated by the sodium hydroxide until there was clear evidence that the curve did not alter with the concentration of toxin used. It was repeated for each toxin used. The change in pH due to CO_2 flux was also estimated in a manner similar to that of Beyers et al (1963). In this some sea water was shaken with a constant flow of carbon dioxide (obtained from the action of diluted acid on calcium carbonate) until saturated. The pH of this solution was taken using an E.E.L. pH meter. This solution was then diluted to 90, 80, 70, 60, 50, 40, 30, 20, 10 and 0% saturated % CO_2 by the replacement of certain quantity of the solution by gas free sea water.

The solubility of carbon dioxide in sea water was obtained in Beyers et al (1963) and a calibration curve was constructed of pH against CO_2 concentration. This being used to estimate the CO_2 flux obtained in the case of Tributyl tin hydroxide.

Section E2-3

Section E2-3

Growth Experiments

This experiment was designed to follow the changes in growth rate with toxin concentration.

The experimental material used was samples of Giffordia secunda Enteromorpha intestinalis and Pilyella littoralis.

Enteromorpha and Pilyella had been collected from the sea shore and kept in culture solution at 18°C and in constant light before use. The Giffordia was grown from spores germinated in culture.

The method follows that of Lindahl (1966).

The algae were grown in sterile petri dishes with 20 ml of test solution at a temperature of 18°C and continuous light intensity of 4000 lux. The fresh weight of the algae were recorded at weekly intervals over a number of weeks but the growth data was obtained after 14 days. After this all began to loose weight. The change in weight was expressed as grammes per gramme fresh weight.

A correction to produce an equivalent of growth in oxygen output was also undertaken although this will not apply absolutely it gives an indication of the comparison between the methods used before.

The test solutions used were:-

Copper and Cuprous oxide : 0; 0.05; 0.10; 0.50; 1.00; 5.00; 7.50; 10.0 $ugCuml^{-1}$

Tributyl tin hydroxide : 0; 0.05; 0.1; 0.2; 0.4; 0.8; 1.6; 3.3; 6.7; 12.5; 25; 50 and 100% saturated solution in sea water.

Appendix E3

Tables of Experimental Results

Section E3-1

Table E3-1

The Effect of Toxins on the Photosynthesis and Respiration of some Marine Algae.

A) Tri butyl tin hydroxide	photosynthesis net	photosynthesis gross	respiration
	(as $\mu l O_2 gm(d.w.)^{-1}.day^{-1}$)		
Enteromorpha intestinalis			
0*	16·949	1·457	-15·492
0·05	18·635	4·658	-13·977
0·10	15·602	4·334	-11·268
0·19	11·370	- 1·372	- 12·742
0·39	7·892	0	- 7·892
0·78	17·564	-16·425	- 33·988
1·56	7·389	0·450	- 6·939
3·12	14·532	- 2·564	- 17·097
6·25	12·054	- 2·939	- 14·993
12·50	7·316	- 3·872	- 11·188
25·00	0·883	-10·684	- 11·567
50·00	- 1·509	-16·577	- 15·068
100·00	-14·679	-31·360	- 16·681
E. compressa			
0*	15·274	7·354	- 7·920
0·05	21·069	3·523	- 17·546
0·10	—	—	—
0·19	19·294	6·413	- 12·881
0·39	7·999	- 3·686	- 11·687
0·78	5·256	0·202	- 5·054
1·56	20·938	4·320	- 16·618
3·12	27·091	5·203	- 21·888
6·25	6·312	- 6·029	- 12·341
12·50	30·535	-30·096	- 60·631

Table E 3-1 (cont)

	NP	GP	R
25·00	7·577	- 14·506	- 22·082
50·00	34·145	- 6·557	- 40·702
100·00	0·228	- 28·867	- 29·095
E. linza.			
0*	37·143	32·055	- 5·088
0·05	33·817	28·134	- 5·683
0·10	103·454	99·965	- 3·489
0·19	7·033	4·626	- 2·417
0·39	82·239	66·438	- 15·801
0·78	208·800	186·336	- 22·464
1·56	51·464	46·165	- 5·399
3·12	80·965	58·294	- 22·671
6·25	74·672	59·498	- 15·174
12·50	124·873	106·722	- 18·151
25·00	52·434	44·677	- 7·757
50·00	34·960	27·934	- 7·026
100·00	55·731	39·625	- 16·106
Ectocarpus siliculosus			
0*	13·525	12·282	- 1·242
0·05	5·191	3·730	- 1·461
0·10	33·598	31·367	- 2·231
1·56	12·083	9·883	- 2·200
3·12	4·438	1·931	- 2·507
12·50	10·961	10·354	- 0·607
25·00	6·547	3·777	- 2·673
50·00	22·734	17·241	- 5·492
100·00	2·781	- 2·261	- 5·041

Table E3-1(cont)

	NP	GP	R
Pilyella littoralis			
0*	7·043	6·498	- 0·545
0·05	3·461	1·879	- 1·582
0·10	1·664	0·377	- 1·264
1·56	4·081	2·767	- 1·314
3·12	8·827	6·587	- 2·239
6·25	7·702	4·018	- 3·683
12·50	4·341	3·451	- 0·889
25·00	58·821	34·432	- 24·389
50·00	15·860	13·263	- 2·597
100·00	28·841	8·482	- 20·359

* % saturation in sea water.

B) Cuprous oxide

Enteromorpha intestinalis			
0 μg Cu ml^{-1}	18·345	9·168	- 9·177
0·05	18·400	4·602	- 13·798
0·25	28·084	12·764	- 15·320
0·50	61·642	39·228	- 22·414
2·50	15·724	12·288	- 3496
5·00	48·800	36·074	- 12·726
7·50	49·978	17·252	- 32·950
10·00	48·697	33·207	- 15·490

Table E 3-1(cont)

	NP	GP	R
Pilyella littoralis			
0 μg Cu ml^{-1}	26·633	12·678	- 13·955
0·05	19·427	8·171	- 11·256
0·25	22·097	12·680	- 9·417
0·50	29·665	11·038	- 18·627
2·50	43·774	26·342	- 17·432
5·00	24·380	14·524	- 9·586
7·50	33·935	22·429	- 11·056
10·00	48·515	35·560	- 12·955
Giffordia fenestrata			
0	9·848	9·269	- 0·579
0·05	9·029	7·661	- 1·368
0·25	13·156	10·047	- 3·109
0·50	14·631	12·627	- 2·004
2·50	12·047	10·040	- 2·007
7·50	4·040	2·424	- 1·616
10·00	1·395	0·719	- 0·676
Ectocarpus siliculosus			
0	9·815	8·645	- 0·540
0·05	20·908	15·899	- 5·009
0·25	11·306	9·806	- 1·500
0·50	19·068	15·752	- 3·316
2·50	12·135	11·677	- 0·458
5·00	4·822	0·467	- 4·355
7·50	6·097	2·771	- 3·326
10·00	3·164	1·449	- 2·165

Table E 3-1(cont)

	NP	GP	R
C) Copper			
Enteromorpha intestinalis			
0 µg Cu ml^{-1}	65·23	60·96	- 4·27
0·05	59·23	55·20	- 4·03
0·25	11·90	10·32	- 1·58
0·50	32·13	28·80	- 3·33
2·50	31·70	30·24	- 1·46
5·00	23·37	20·40	- 2·97
7·50	37·79	27·85	- 9·94
10·00	18·59	13·74	- 4·85
Pilyella littoralis			
0	605·04	582·24	- 22·80
0·05	876·44	792·48	- 83·76
0·25	553·44	529·68	- 23·76
2·50	119·76	116·88	- 2·88
5·00	23·69	22·56	- 1·13
7·50	42·06	19·79	- 22·27

Section E3-3

Table E3-3 The Effect of Toxins on the Growth

A) tributyl tin hydroxide

*	0	0·005	0·025	0·05
Giffordia secunda i	17·14	0·95	6·35	4·76
ii	22·72	12·63	8·42	6·32
Enteromorpha intestinalis i	0·82			
ii	1·09			
			0·10*	
Pilyella littoralis i	20·97		0·85	
ii	27·82		1·14	

* % saturation in sea w
i expressed as mg.g
ii " " $\mu l O_2$

Table E 3-3(cont)

B) Cuprous oxide.		0	0·05	0·10	0·25
Giffordia secunda	i	1·89	9·44		1·20
	ii	2·50	12·52		1·59
Enteromorpha intestinalis	i	0·82		0·33	
	ii	1·09		0·44	
Pilyella littoralis	i	20·97		5·28	
	ii	27·82		7·00	
C) Copper					
Giffordia secunda	i	1·89	5·83		10·48
	ii	2·50	7·73		13·90
Enteromorpha intestinalis	i	0·82		1·07	
	ii	1·09		1·42	
Pilyella littoralis	i	20·97		11·31	
	ii	27·82		15·00	

Section E3-4

Table E3 - 4. The Effect of Toxins on the Germination of Some Marine Fouling Algae

Giffordia secunda	Cuprous Oxide @	Copper @	Tributyl tin Hydroxide @
0	112.5	112.5	40.00
// *			
0.05 (0.005)	67.14	54.80	
0.25 (0.025)	30.53	87.00	4.44
0.50 (0.050)	4.27	23.07	42.50
2.50 (0.250)	2.72	1.67	I0.00
5.00 (0.500)	2.I0	-	16.00
7.50 (2.500)	-	-	5.71
I0.00(5.000)	-	-	1.90
Pilyella littoralis			
0	5.18	5.18	5.18
// *			
0.I (0.005)	3.04	4.17	2.00
0.5 (0.025)	5.71	0.67	-
I.0 (0.050)	5.07	-	-
5.0 (0.250)	-	3.00	-
I0.0 (0.500)	-	-	-

ϕ/ copper as

* tributyl tin as percentage saturated solution in sea water

@ in units of I000

Part 6

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