UNIVERSITY OF NEW ZEALAND

THESIS

Presented in Partial Fulfilment of the Requirements for
the Degree of Master of Science with Honours.

STUDIES ON THE ECOLOGY OF SOME SUPRALITTORAL POOLS WITH SPECIAL REFERENCE TO THE BIOLOGY OF OPIFEX FUSCUS HUTTON

(DIPTERA: CULICIDAE)

by

D.D. McGREGOR

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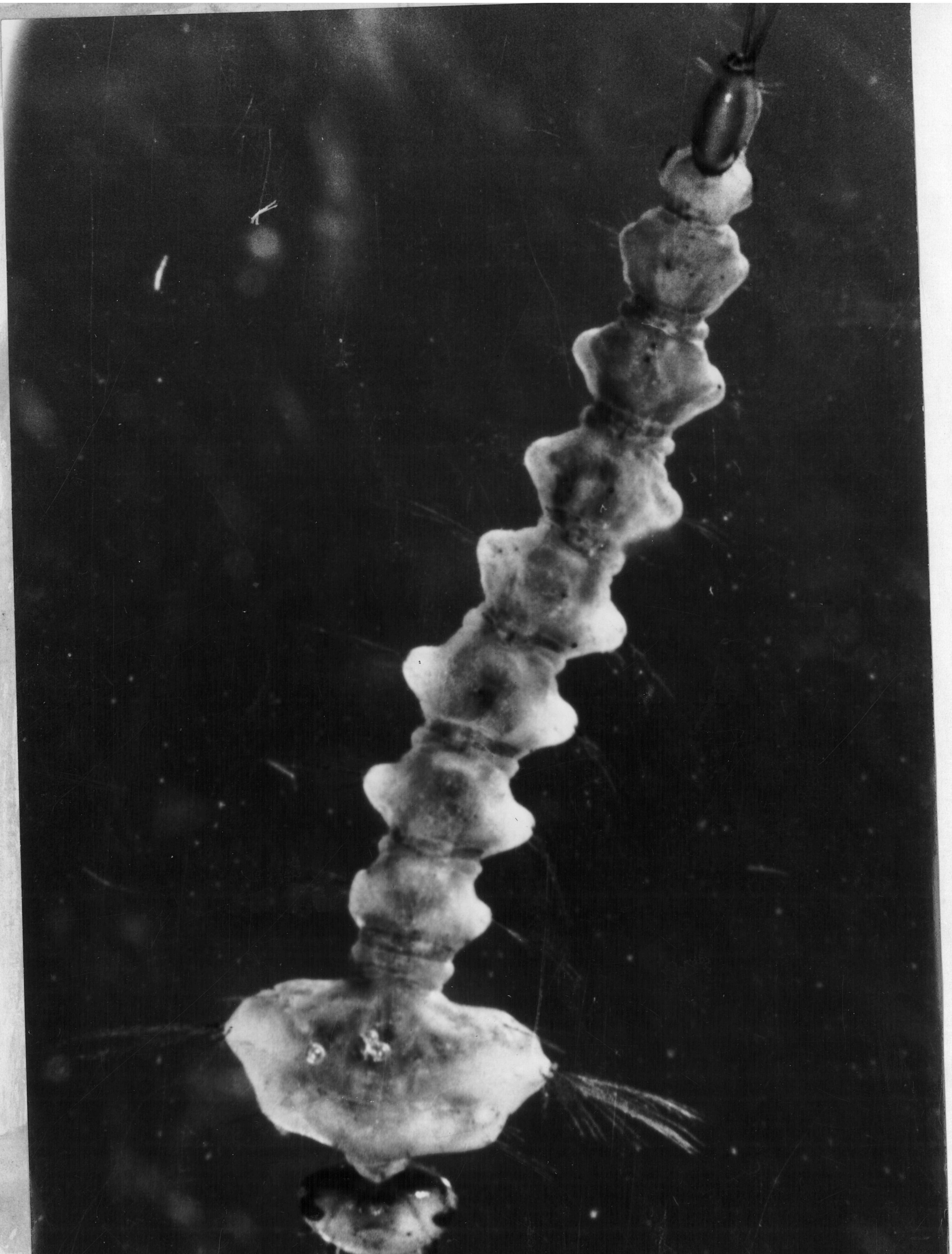
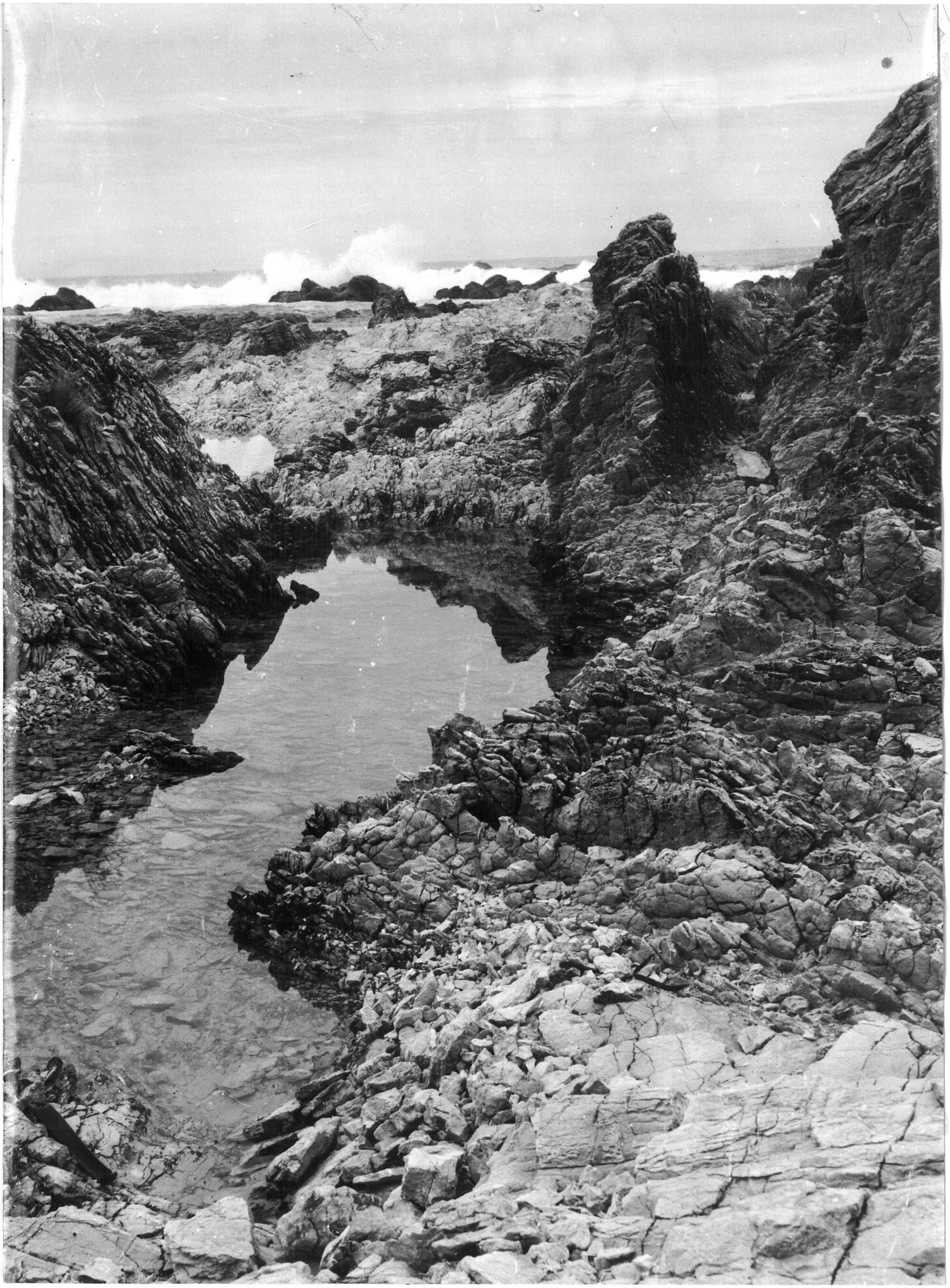
University of
Canterbury



The Animal .....

(Opifex fuscus fourth instar larva)



..... and its Environment
(a supralittoral pool, Kaikoura).

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CHAPTER I

INTRODUCTION

1.1 Supralittoral pools have been largely ignored by littoral ecologists in accounts of the vertical and horizontal distribution of the flora and fauna of coasts. Pools have been regarded as essentially anomalous situations brought about by shore physiography (Doty, 1957), and reference to them in works on intertidal ecology has been mainly incidental.

With the development of New Zealand intertidal ecology the need for detailed studies of specialized communities within the littoral became evident.

One of the most ubiquitous New Zealand supralittoral pool species, the mosquito *Opifex fuscus* Hutton had been shown to be structurally remarkable by Miller (1922), and although Kirk (1923) had described its most peculiar breeding habits, little was known of its biology.

Studies on the ecology of supralittoral pools presented in this thesis provided, both an insight into the physical conditions and the biota of a little known habitat, as well as a description of the environment of *O. fuscus*. Laboratory experiments on the biology of the mosquito complemented field observations so that a stereoscopic picture of the animal within its environment was obtained.

1.2 Review of Previous Work

(a) Supralittoral Pools:

Several workers have presented descriptive accounts of rock pools, particularly those of Scandanavian shores, and made attempts at their classification. Levander (1900) first suggested a classification of supralittoral pools. Since then useful descriptions of Baltic shore pools, and their flora and fauna, have been provided by Gislen (1930), Lindberg (1944), Johnsen (1946), and Droop (1953). The first significant New Zealand contribution to the study of shore pools was made by Cranwell and Moore (1938) who included descriptions of supralittoral and littoral pools in their study of intertidal communities of Poor Knights Island.

An analytical rather than a descriptive approach has been adopted by other ecologists. Klugh (1924) prepared a list of factors controlling the biota of tide pools. Later Gersbacher and Denison (1930) tested their importance. Naylor and Slinn (1958) briefly analysed both the physical properties and the biotic constituents of some supralittoral pools on the Isle of Man. Several accounts of aspects of the ecology of brackish pools, with special reference to their algae, have been given (Yendo, 1914; Feldman and Feldman, 1941; Nasr and Aleem, 1949; Ambler and Chapman, 1950; Wood, 1952). Jarnefelt (1940) examined the hydrology of some Swedish shore pools in considerable detail.

A survey of the literature leads to the following conclusions.

1. Most studies have been short term, pools being examined only occasionally. With few exceptions (Ambler and Chapman, Wood) little assessment of change within pools has been made.

2. Attention has been concentrated on tide pools (sensu strictu), few detailed investigations of supralittoral pools having been made.

3. Usually the number of pools included within each survey has been too small for satisfactory comparisons to be made.

4. Many of the studies have come from the Baltic, where the brackish sea has been responsible for pool salinities lower than those obtaining along open coasts. Assumptions that conditions in Baltic pools apply more widely have led to doubtful generalisations, such as that made by Doty (1957), "the salinities of supralittoral pools are too low to permit their consideration as being truly marine."

(b) Opifex fuscus

Opifex fuscus was first described by Hutton (1902), who classified it as a Tipulid Edwards (1921) examined Hutton's types and transferred Opifex fuscus to the Culicine mosquitoes. Edwards found difficulty in relating Opifex to the other Culicine genera but suggested that it was closer to Aedes than Culex. Miller (1922) gave a fuller description of the external morphology of all the stages of Opifex and erected a new Sub Family, Opificinae, for its reception. However Edwards (1924) regarded this step by Miller as unjustified and the Sub Family has not been recognised.

Kirk (1923) gave a full account of the peculiar mating behaviour of <u>O. fuscus</u>.

In an unpublished thesis Wood (1929) gave an extensive account of the external and internal anatomy of all stages of <u>O. fuscus</u>, as well as making some brief notes on its bionomics.

In 1939 Graham mapped the distribution of mosquitoes, including <u>Opifex</u> in the Auckland Province, while Miller and Phillipps (undated) gave the range of <u>O. fuscus</u> as the rocky coastline of the North Island and the northern part of the South Island.

More recently Knight and Chamberlain (1948) figured the pupa in detail in their revision of the nomenclature of the chaetotaxy of the mosquito pupa, and Roth (1948) reviewed the functional morphology of <u>Opifex</u> in relation to Kirks (1923) observations and his own experimental work on the sexual behaviour of mosquitoes.

Mattingly (in Marks, 1958) observed that some <u>O. fuscus</u> larvae in a collection made in 1921 by G.V. Hudson have pectinate hairs in their mouth-brushes instead of all simple hairs/as described by Miller. In a review of previous work on <u>Opifex</u> Marks (1958) gave some useful suggestions for future research as well as publishing some of her own observations. Dumbleton (1962) examined <u>O. fuscus</u> in relation to a new subgenus of mosquito from the Chatham Islands: Aedes (Nothoskusea) Dumbleton, and the "Caenocephalus" species group of Aedes (Pseudoskusea) Theobald.

Sufficient attention had been directed towards the anatomy and mating behaviour of O. fuscus to reveal that morphologically and biologically it was a decidedly aberrant mosquito, but apart from brief observations by Wood and Marks. little was known of its ecology.

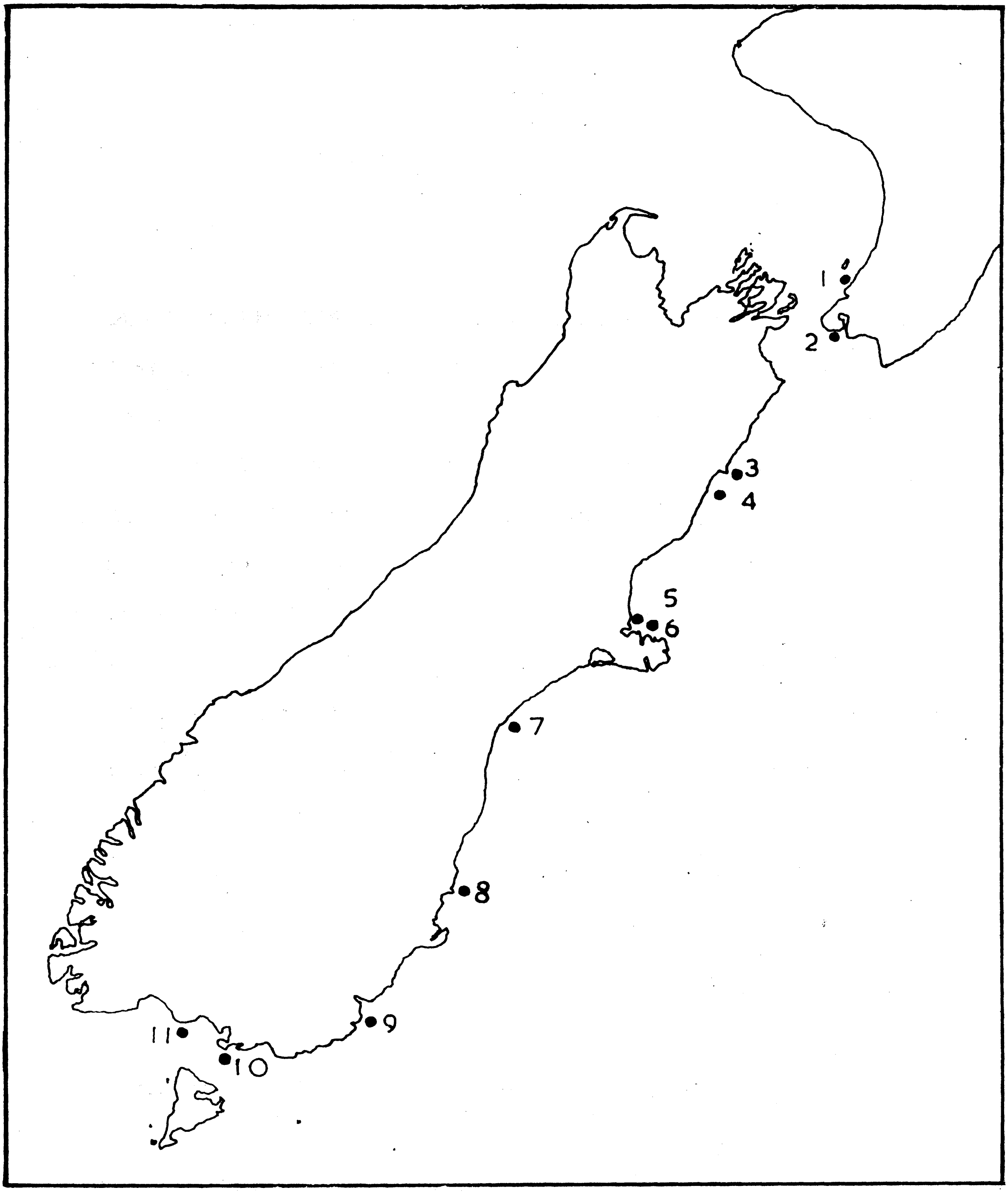


Fig. 1. Southern New Zealand, showing localities at which supralittoral pools were studied. Scale: 1:5,000,000. 1. Plimmerton. 2. Island Bay, 3. Kaikoura. 4. Goose Bay. 5. Taylor's Mistake. 6. Lyttelton Harbour. 7 Timaru. 8 Shag Point. 9 Nugget Point. 10. Bluff. 11. Oraka Point.

CHAPTER 2

THE ECOLOGY OF SUPRALITTORAL POOLS

2.1 Introduction

Sixty supralittoral pools along 500 miles of New Zealand coastline, from Plimmerton in the north to Foveaux in the south, were embraced by this survey (Fig. 1). Twenty pools were visited at regular intervals between January and December 1961, eight at Taylors Mistake (Fig. 2) being examined at least once a fortnight, six in Lyttelton Harbour at approximately monthly intervals and four at Goose Bay once a quarter. Consequently there was an adequate number of pools on which to base comparisons within and between regions.

Data obtained from the pools, including information relating to their physical properties, flora and fauna and Opifex fuscus populations was recorded systematically on cyclostyled sheets.

Part I THE PHYSICAL ENVIRONMENT

Introduction:

Only a limited knowledge of the physical ecology of supralittoral pools could be obtained in the time available, so detailed investigations were restricted to those aspects that were most likely to influence the flora and fauna, especially Opifex fuscus. The properties selected were:

1. Physiography

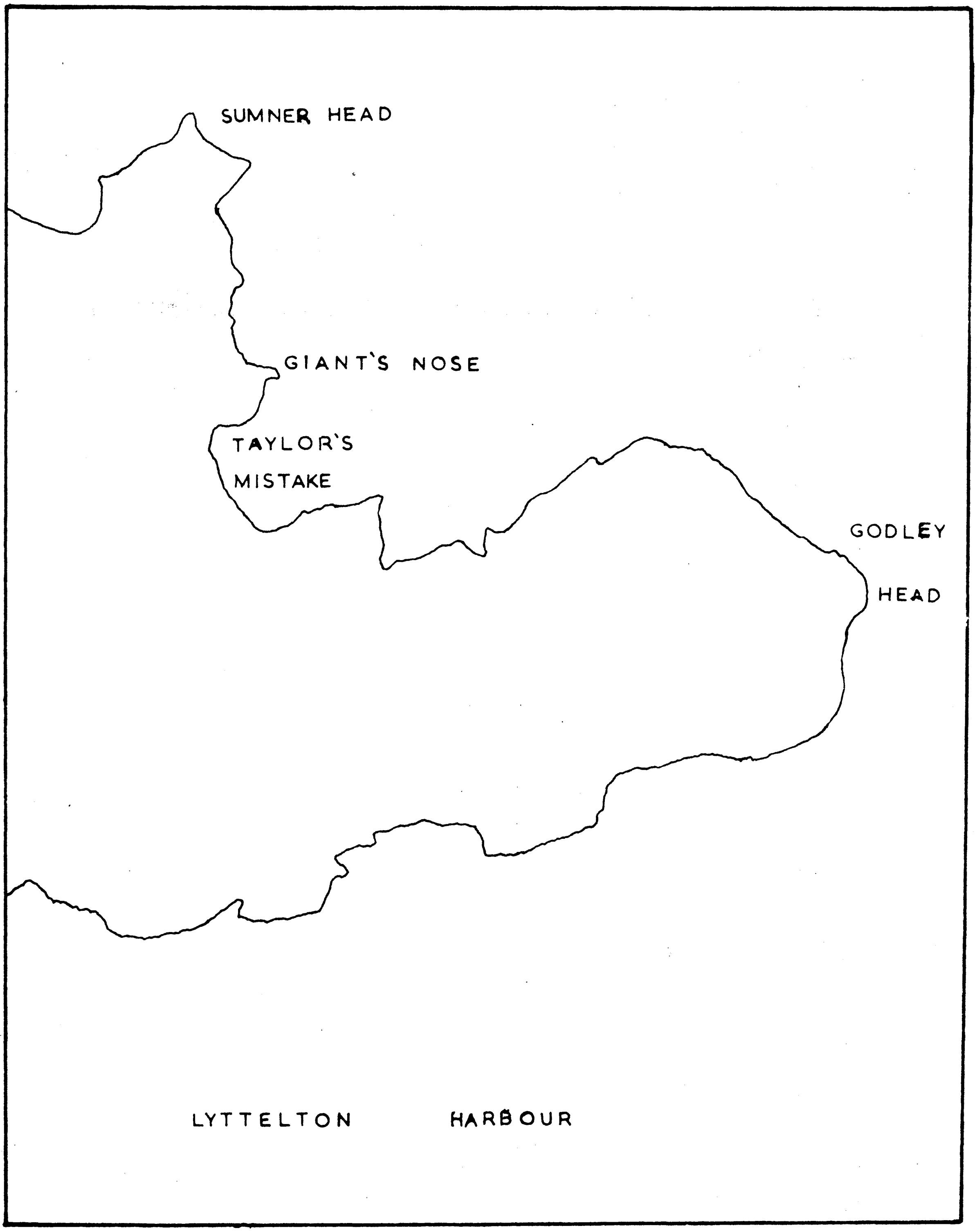


Fig. 2. Map showing location of Giant's Nose, Taylor's Mistake, where detailed studies of eight supra-littoral pools were undertaken during 1961. Scale: 1:25,000.

2. Salinity.
3. Temperature.
4. Oxygen.
5. Hydrogen ion concentration.

In subsequent sections each of these factors is considered in turn.

2.2 Physiography:

Introduction:

The relevance of the situation, shape and size of pools lay not only in their possible effects on other physical properties and the biota of supralittoral pools, but also in the evidence provided of processes of formation and history of the pools.

Wentworth (1938) distinguished four processes which can be involved in the formation of shore platforms.

1. Water layer (water level) weathering.
2. Solution benching.
3. Ramp abrasion.
4. Wave quarrying.

There has been general agreement that all these processes play some part in the erosion of rocky shores, but no unanimity as to their relative importance, or their efficiency at higher levels on the shore.

Methods:

The physiography of a series of pools at Taylors Mistake was studied in detail, although information from other pools was included as well. Measurements of the situation, size, shape, type of sediment, wave exposure, frequency of splashing and fluctuations in level of pools were made. The salinity of water within the pools was determined and the nature of the parent rock, its jointing and fracturing noted.

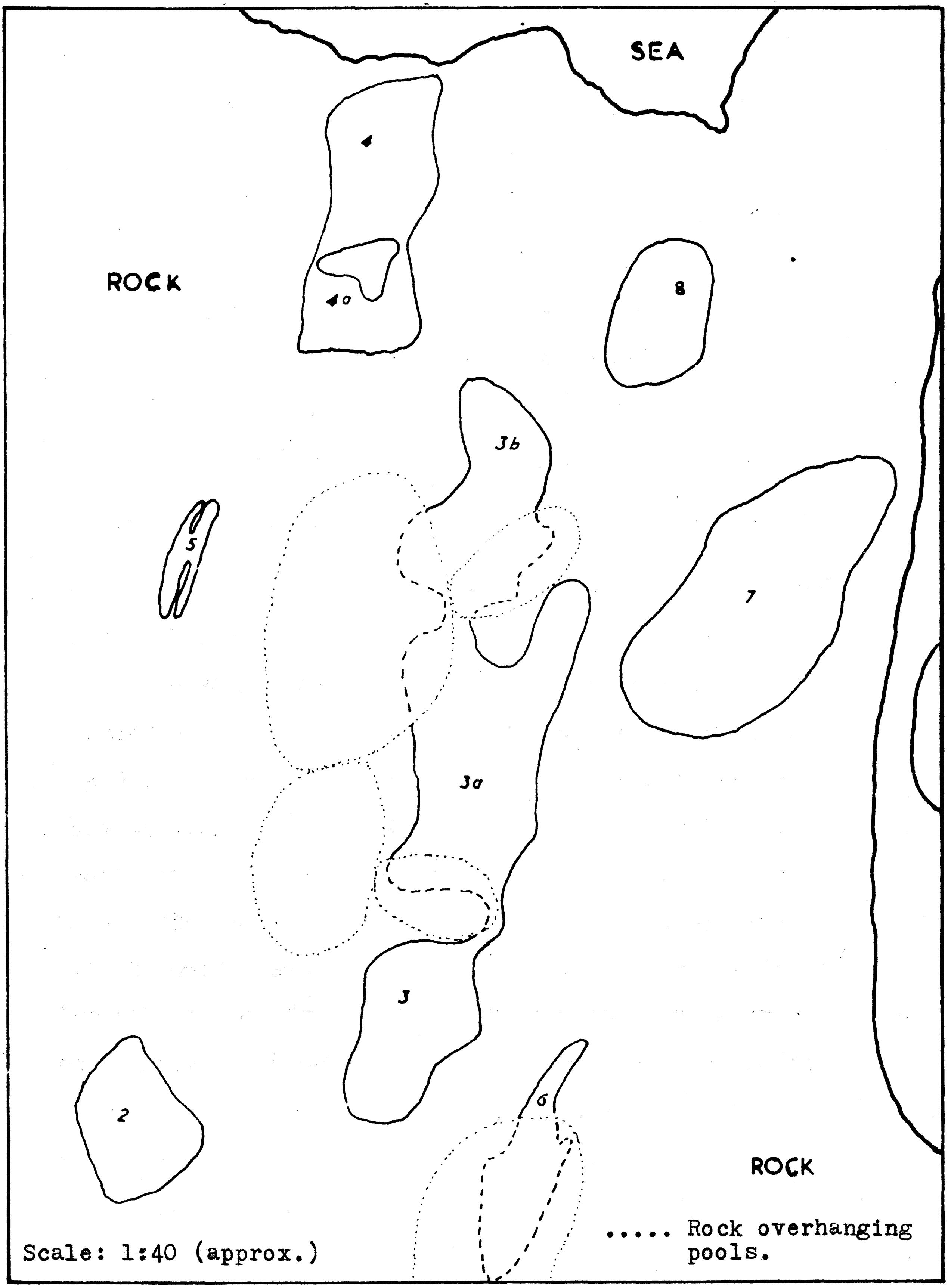


Fig. 3. Diagram showing relationships of supralittoral pools at the Giant's Nose to the sea and one another.

Estimations of the relative wave exposure of pools were based on the following scale:

1. Very exposed.
2. Exposed.
3. Semi-exposed.
4. Sheltered.
5. Very sheltered.

Sediment within pools was classified as follows:

boulders, stones, pebbles, sand, mud and detritus.

Results:

The location of the Giants Nose, Taylors Mistake, where detailed investigations were made, is indicated in Fig. 2, while the relations of the pools there to the shore and each other is mapped in Fig. 3, and illustrated in Fig. 4. Views of each individual pool at Taylors Mistake are provided by Figs. 5 - 12 inclusive. These pools at the Giants Nose lie in platforms between 110 cm. and 470 cm. above mean high water, with extensive midlittoral pools below. Comparison of the volume histograms of supralittoral (Fig. 13) and midlittoral (Fig. 14) pools shows that at the Giants Nose the midlittoral pools were all much larger than those of the supralittoral. The largest supralittoral pools encountered, at Goose Bay and Kaikoura (Figs. 15 - 19), had volumes more comparable with Giants Nose midlittoral pools. (Fig. 20). Boulders and stones covered the bottoms of these large midlittoral pools. Stones were plentiful in the largest supralittoral pools: 25, 31, 32

Fig. 4. The Giants Nose, Taylors Mistake, showing the platforms containing supralittoral pools (foreground), and midlittoral pools (background).

Fig. 5 Pool 1, Taylors Mistake. Note the Opifex fuscus adults upon the water surface.

Fig. 6 Pool 2, Taylors Mistake

Fig. 7 Pool 3, Taylors Mistake

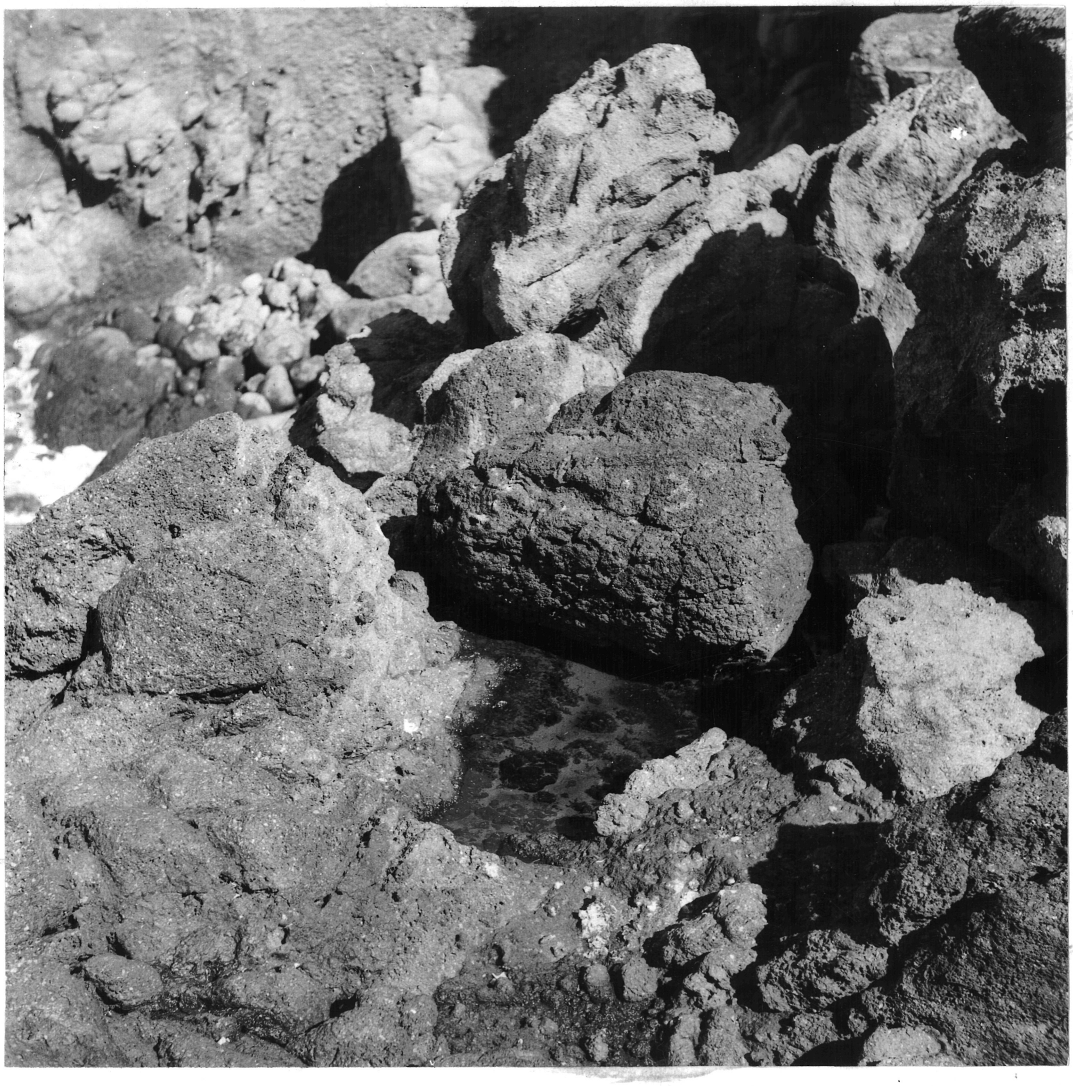


Fig. 8   Pool 3b, Taylors Mistake.

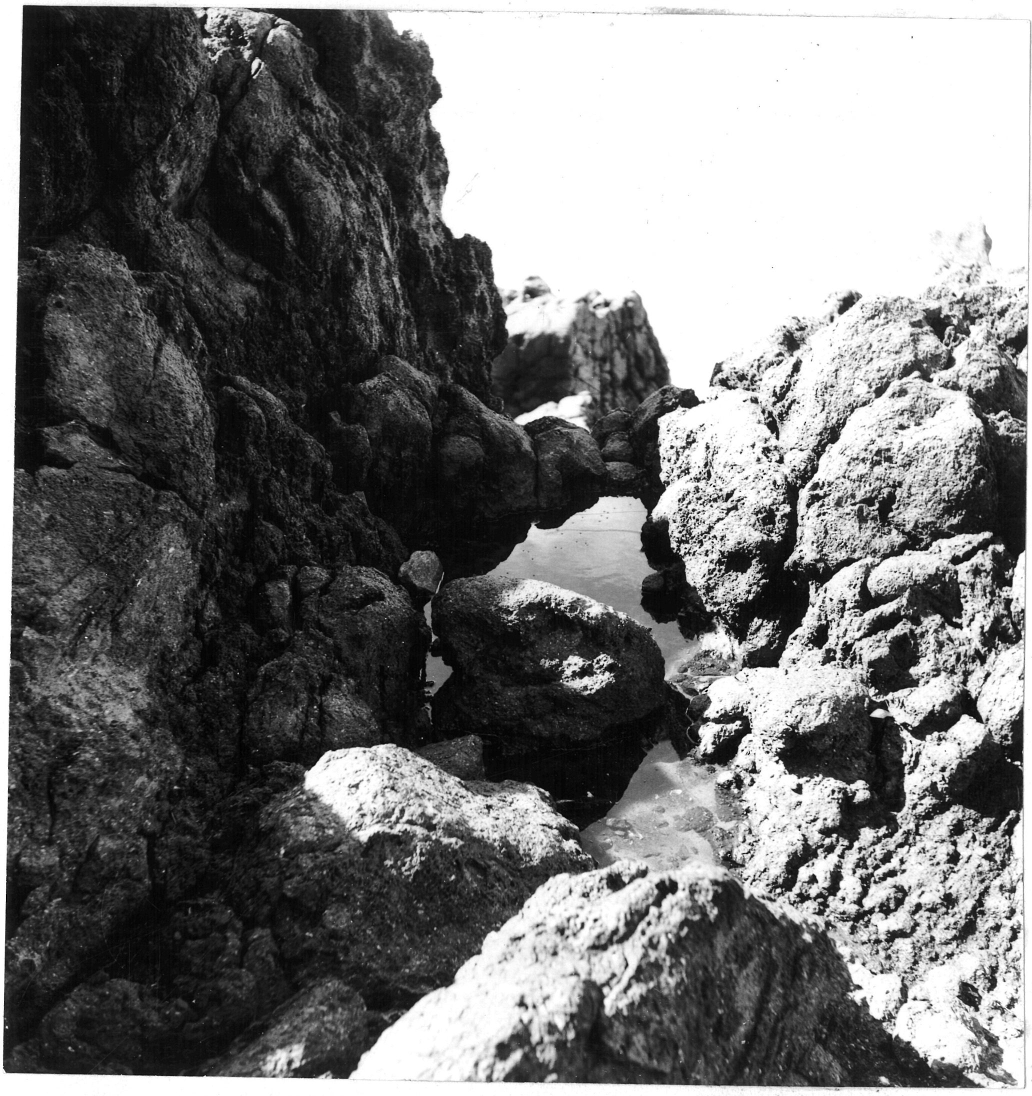


Fig. 9 Pool 4, Taylors Mistake.

Fig. 10 Pool 5 (foreground), Taylors Mistake. Pool 4 is in the background.

Fig. 11 Pool 7, Taylors Mistake

Fig. 12 Pool 8, Taylors Mistake

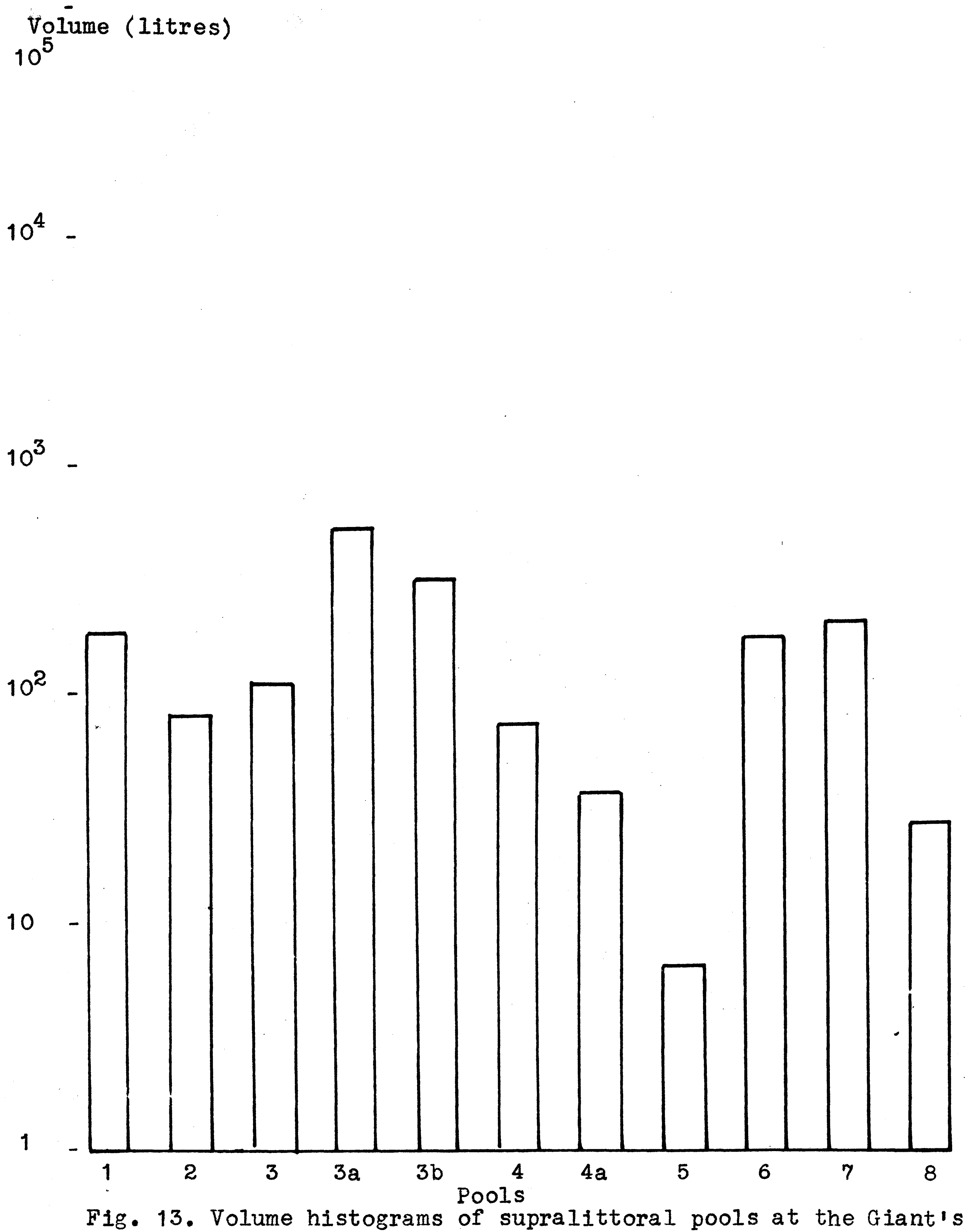


Fig. 13. Volume histograms of supralittoral pools at the Giant's Nose, Taylor's Mistake.

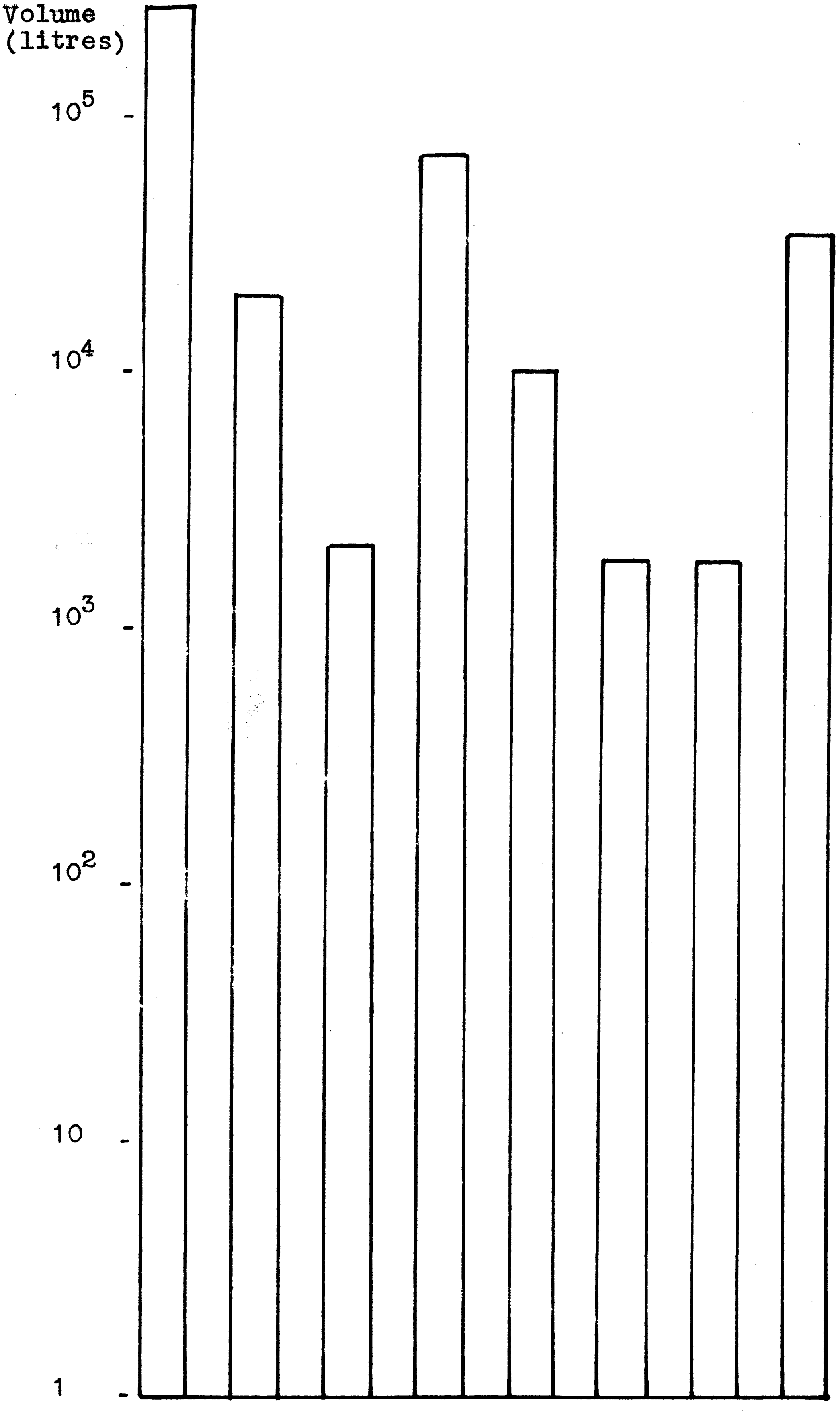


Fig. 14. Volume histograms of midlittoral pools at the Giant's Nose, Taylor's Mistake.

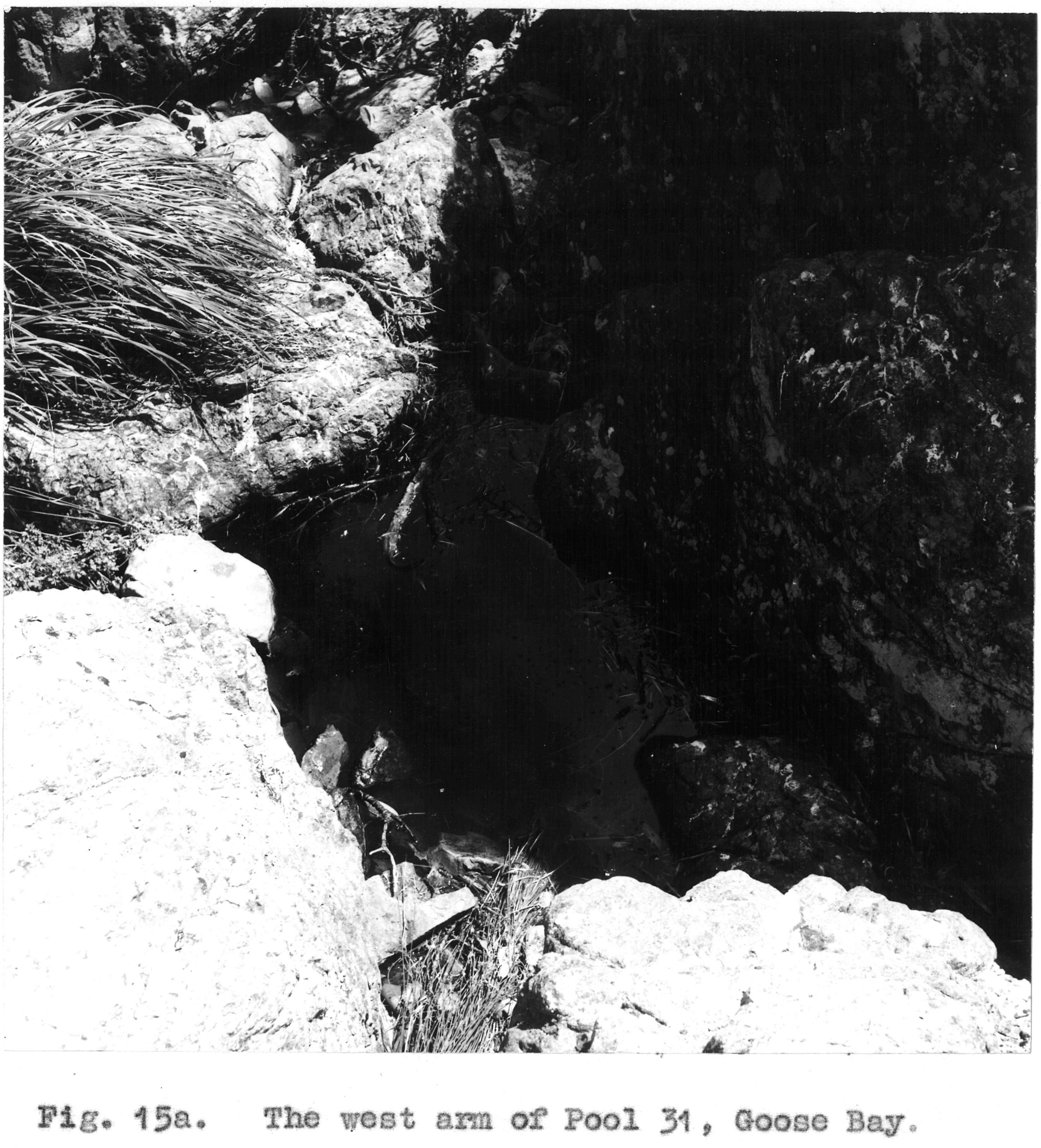


Fig. 15a. The west arm of Pool 31, Goose Bay.

Fig. 15b. The east arm of Pool 31, Goose Bay.

Fig. 16. Pool 32, Goose Bay.

Fig. 17. Pool 33, Goose Bay. Note the Opifex fuscus adults upon the surface of the pool.

Fig. 18. Pool 25, Kaikoura.

Fig. 19. Two Kaikoura supralittoral pools (27 and 28).

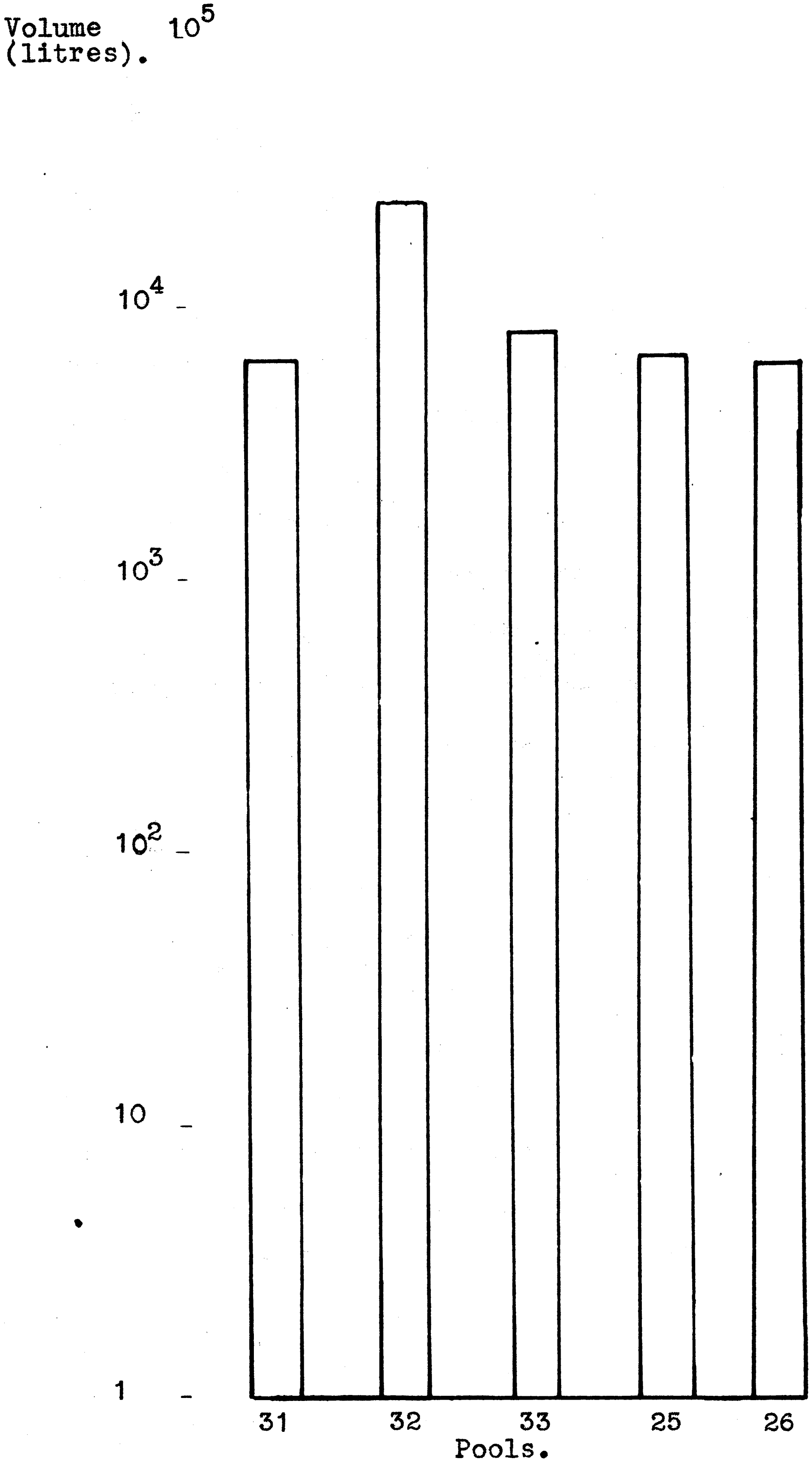


Fig. 20. Volume histograms of supralittoral pools at Goose Bay (31, 32, 33,) and Kaikoura (25, 26,).

and 33 but there were few boulders. The sediment in the smaller pools at Taylors Mistake and elsewhere was mainly sand, mud or detritus, with only occasional stones.

At Taylors Mistake, pools of the midlittoral zone were naturally all more exposed to wave action than those of the supralittoral, but within the latter zone the relative exposure of the various pools differed. The irregular splashing and fluctuations in level of the supralittoral pools caused repeated wetting and drying of their walls. Only the outer lips of the more exposed supralittoral pools were continually moist, whereas the borders of midlittoral pools were seldom dry.

Discussion:

Shore planation at the Giants Nose has been preceeding at two levels; in the midlittoral, and in the supralittoral. The whole area containing pools 2 to 5 is surrounded by a ridge of rock. The shallowness of these pools indicates they are being enlarged faster round their margin than in depth. Wentworth (1938), Bartrum (1938) and Hills (1947) attribute these effects to alternate wetting and drying which is especially characteristic of this area. Water forms a physical shield over the pool bottom hampering the influence of temperature fluctuations except around the margin. While this water layer (or water level) weathering has been chiefly responsible for widening pools, deepening has been induced by mechanical abrasion. The results have shown that the most exposed pools, containing quantities of boulders, stones or pebbles, which are potentially abrasive, were largest both in depth and volume. Pools whose bottoms were covered with sand, mud or detritus have remained shallow.

The geology of an area was found to be important in determining not only where pools occur but also their character. Pools were most frequently found where a soft rock cover had been eroded away from a hard rock undermass, e.g. loess over basalt-andesite on Banks Peninsula; sandstone over argillite at Shag Point. Large pools have been produced in well jointed rock, e.g. Island Bay, Kaikoura, but pools formed in less fragile rocks have remained small, e.g. Bluff.

Studies on salinity (section 2.3) will show that water within pools was seldom of salinity lower than 20^{o}/oo and usually higher. Joly (1901) demonstrated that basalt obsidian, horneblende and orthoclase are three to fourteen times more soluable in salt water than fresh, so it is likely that solution has played at least some part in pool formation.

2.3 Salinity

Introduction:

Supralittoral pools have often been assumed to represent the low end of an onshore-offshore salinity gradient. in some ways analogous to estuaries and lagoons. Ecologists who have worked along Baltic shores have especially favoured the view that supralittoral pools form a transitional environment between sea and fresh water, (Levander, 1900; Jarnefelt, 1940; Lindberg, 1944; Johnsen, 1946). Several North American workers have also shared this belief, (Pearse, 1932; Shelford, 1935; Doty, 1957). All have recognized that super-saline pools occur but these have not been regarded as typical. In this section the salinity of supralittoral pools along the coast of the South Island is examined and discussed in relation to previous work.

Methods:

The chlorinities of pools at Bluff, Shag Point, Akaroa lighthouse, Lyttelton Harbour, Taylors Mistake, Goose Bay and Kaikoura were determined. Taylors Mistake pools were selected for detailed study, chlorinity determination of two pools being made at fortnightly intervals, and on occasions more frequently. Chlorinity values of other pools at Taylors Mistake, Lyttelton Harbour, Goose Bay and Kaikoura were measured on a number of occasions. All these observations were made between November 1960 and November 1961.

The Mohr titrations were used throughout for chlorinity determination. Standard solutions were made up containing 27.25 grams of silver nitrate per litre. When titrated against samples of pool water, using potassium chromate as indicator, the volume of silver nitrate used was equivalent to the salinity of the sample. It was found that the salinity of the pools was usually between the limits of reliability for this method perscribed by Welsh and Smith (1949). Duplicate determinations were always made, and it proved important to measure the chlorinity of both surface and bottom water.

Results:

Salinity determinationsfrom supralittoral pools along the South Island coast led to a number of conclusions.

1. Adjacent pools of similar dimensions at times differed considerably in salinity. For example, on January 24th three pools at Taylors Mistake within an area of ten square metres exhibited the following salinity values:-

Pool 2	26.0°/oo
Pool 3	31.3°/oo
Pool 4	41.3°/oo

On other occasions, e.g. March 29th, the same pools showed greater parity in salinity:

Pool 2	47.5°/oo
Pool 3	41.7°/00
Pool 4	41.7°/oo

2. The above examples illustrate a second characteristic of

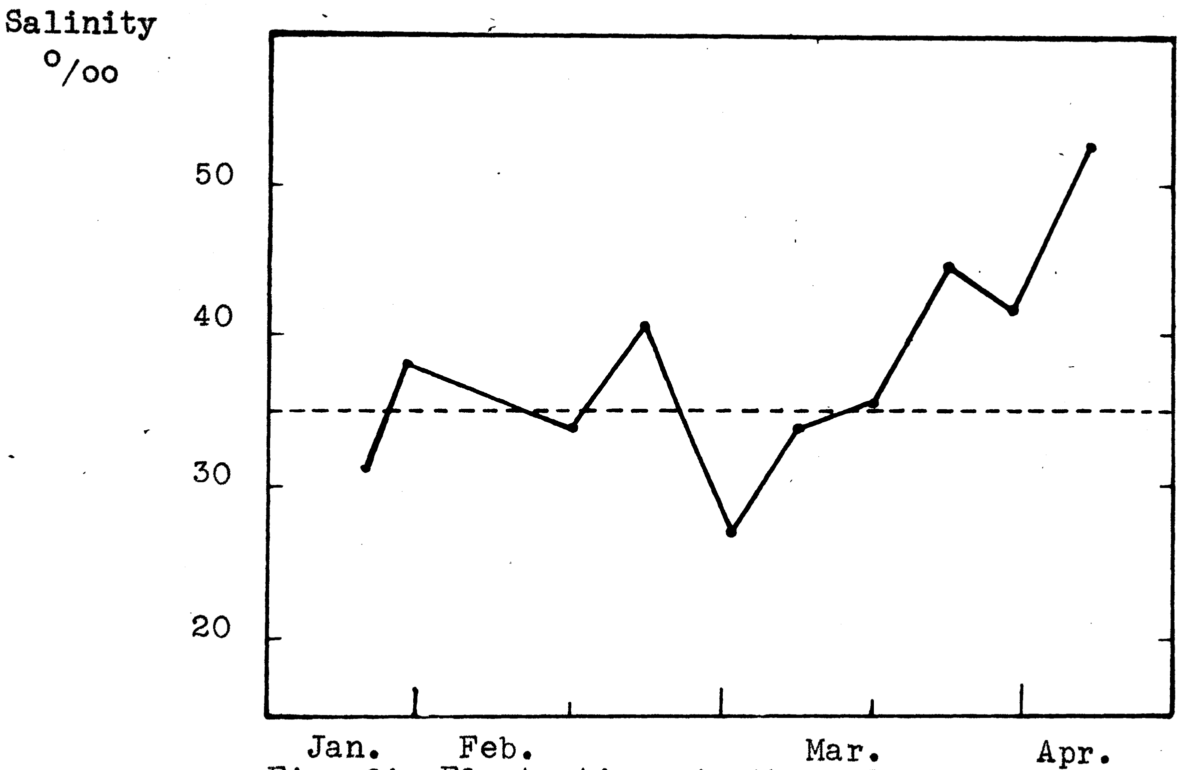


Fig. 21. Fluctuations in the salinity of pool 3 from January 27th to April 5th.

----- Salinity of normal sea water.

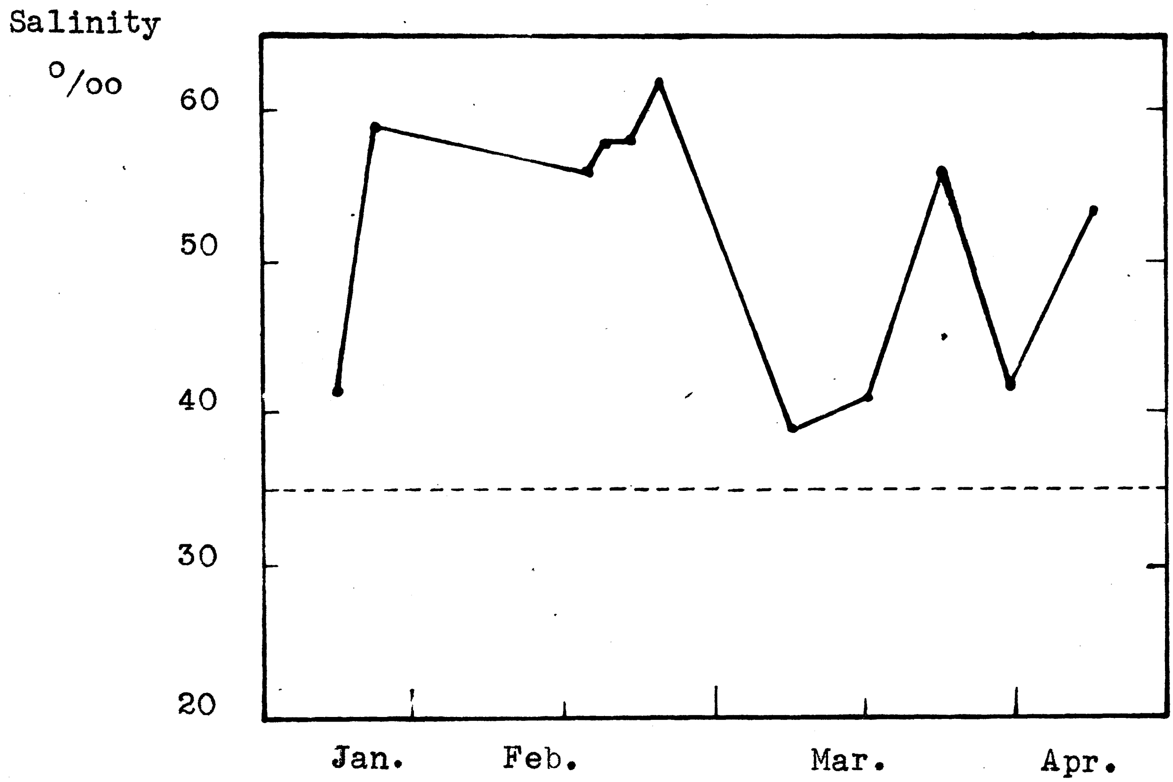


Fig. 22. Fluctuations in the salinity of pool 4 from January 27th to April 5th.

---- Salinity of normal sea water.

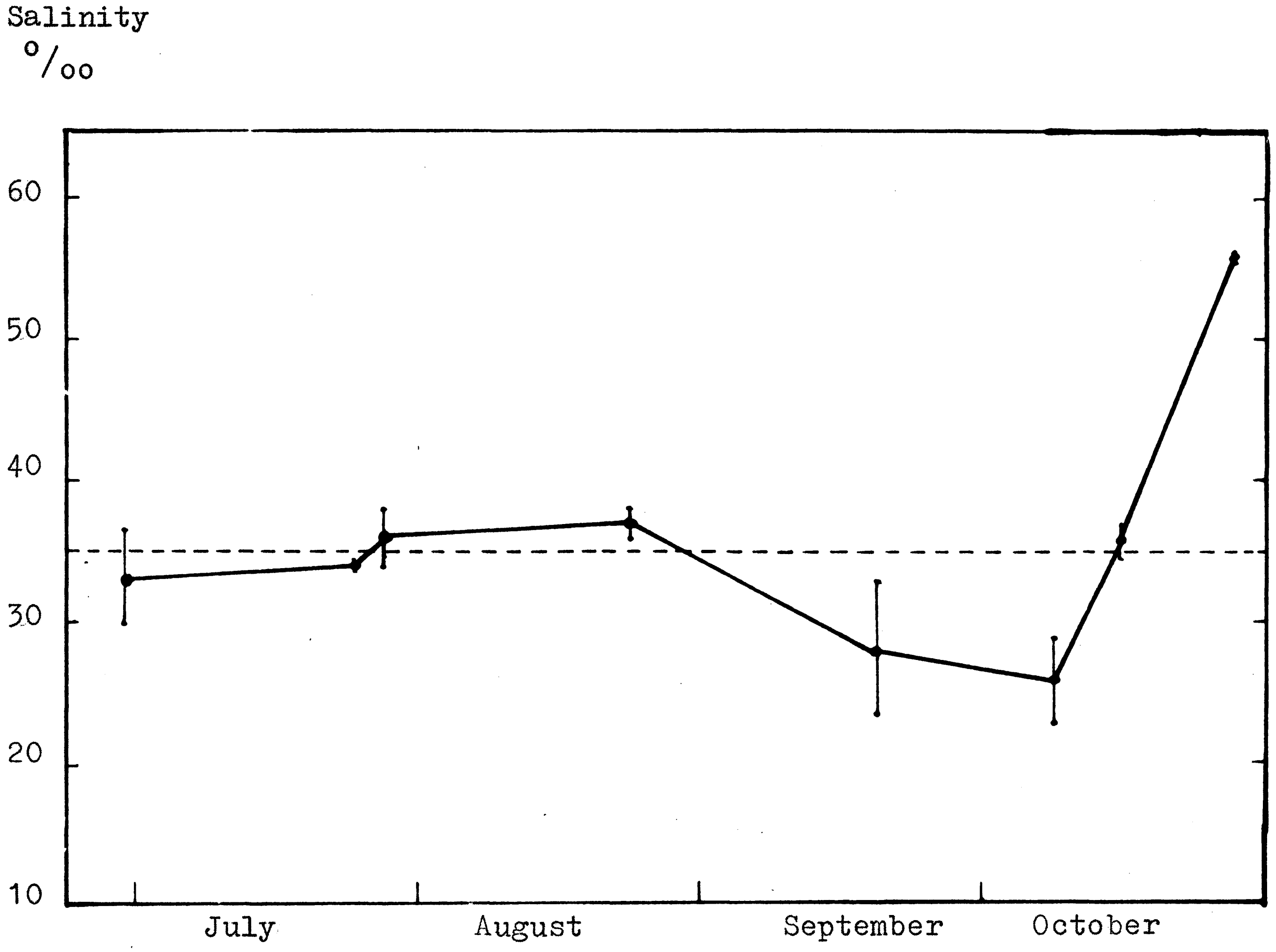


Fig. 23. Fluctuations in the salinity of pool 3 from June 29th to October 28th.

----- Salinity of normal sea water.

Vertical lines indicate range between surface and bottom salinity.

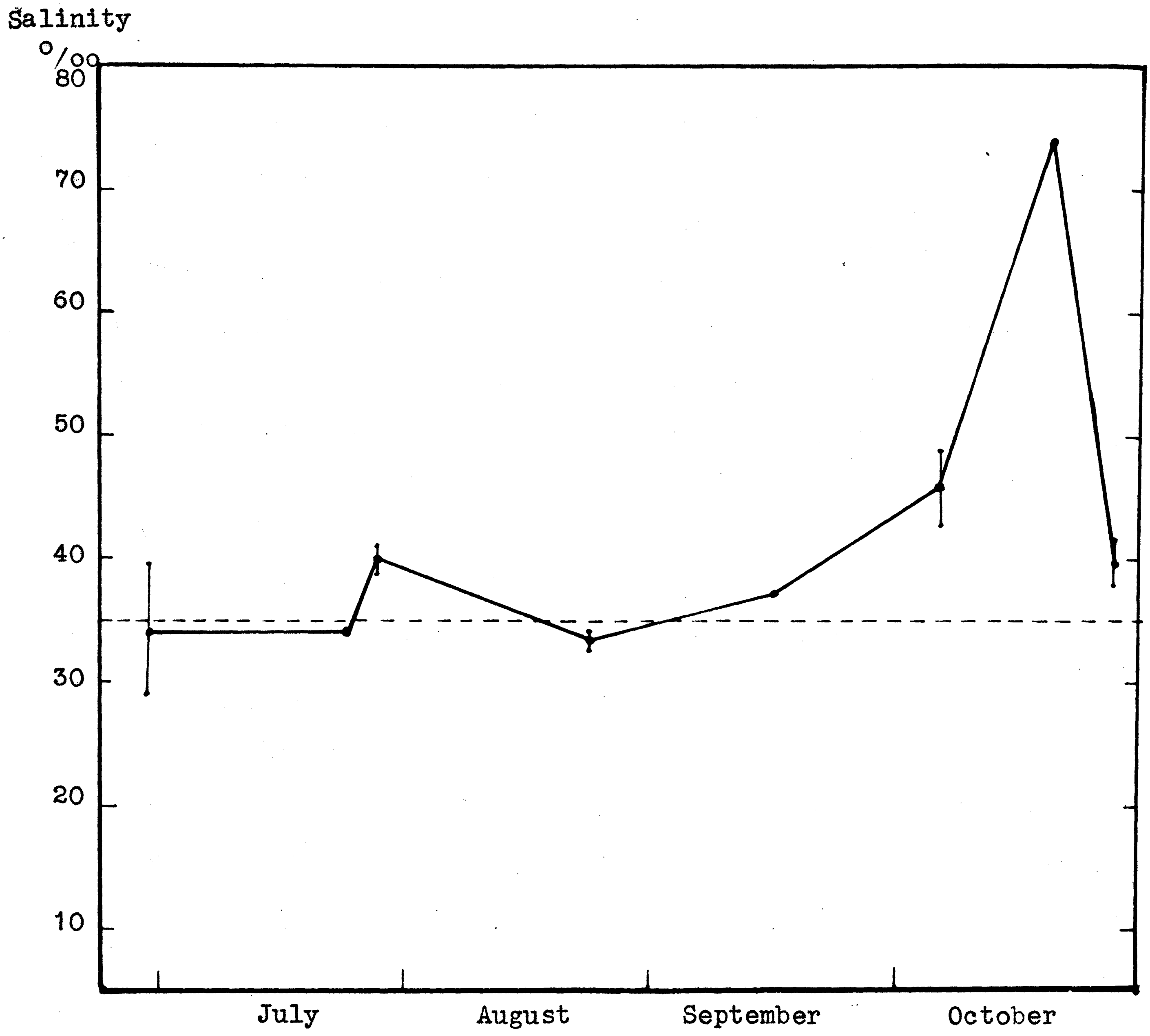


Fig. 24. Fluctuations in the salinity of pool 4 from June 29th to October 28th.

----- Salinity of normal sea water.

Vertical lines indicate range between surface and bottom salinity.

supralittoral pool salinity - fluctuation. In figs. 21 and 23 are salinity fluctuations in Pool 3 from January 27th to April 5th, and June 29th to October 28th respectively. Corresponding fluctuations in Pool 4 salinity over the same periods are plotted in figs.22 and 24. Comparison of the graphs for each pool indicates the influence which the frequency with which determinations were made had on the impression obtained of salinity fluctuations within each pool. Obviously the more frequently the determinations were made the more accurately the resulting graphs portrayed changes in salinity taking place within the pools. Fluctuations in pool salinity could be traced completely only with continuous recording devices, which were unavailable.

From figs. 21 - 24 it can be seen that changes in Pool 3 salinity were usually accompanied by corresponding changes in Pool 4, but as both the graphs and the following table show fluctuations in Pool 4 took place round a higher mean than in Pool 3.

TABLE I

Mean Salinities of Pool 3 and Pool 4,
Taylors Mistake, November 1960 - October 1961

Pool	Number of Determinations	Mean
3	28	36.3 °/oo
4	28	46.7 °/oo

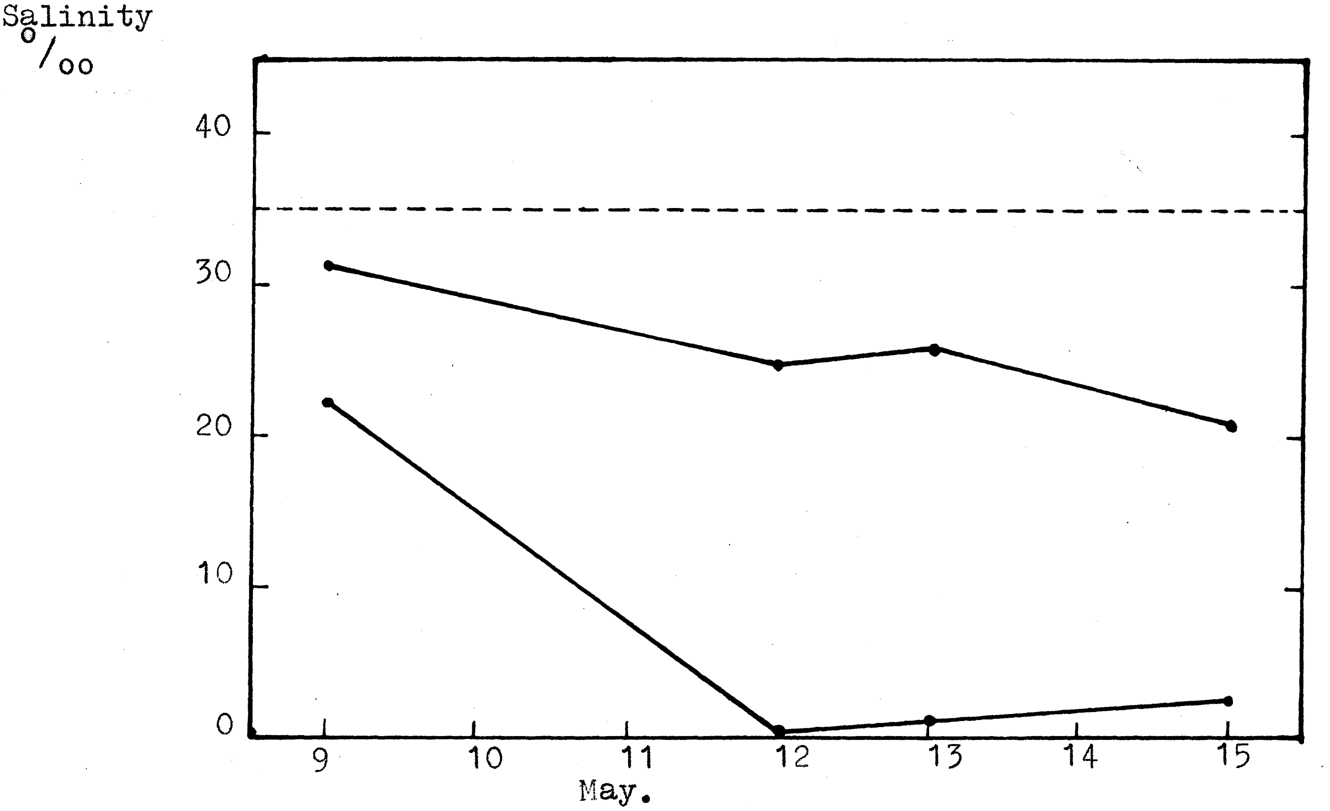


Fig. 26a Salinity layering in pool 25, Kaikoura, May 1961.
The upper line indicates fluctuations in salinity at a depth of 60 centimeters.
The lower line indicates fluctuations in surface salinity.
----- Salinity of normal sea water.

This difference in mean salinities was correlated with differences in the dimensions, type of bottom and temperature regimes of the pools, and will be discussed after temperature has been considered.

Changes in salinity were often rapid. On February 24th Pool 6, Taylors Mistake, was dry. Rain fell on February 28th and by March 1st the pool salinity was 6°/oo, rising within seven days to 28°/oo. A week later the pool was again dry.

3. One of the most notable properties of supralittoral pools was salinity layering. *

The salinity of water samples taken at two different levels in Pool 25, Kaikoura, over a period of six days during May, have been compared in Fig. 26a. One series of samples was taken from the surface, a second series from a depth of 60 centimetres. The contrast in the salinity of the two layers was maintained and accentuated through heavy rain on May 11th and 12th. With the rain there was a sharp decrease in surface salinity from 22.5°/oo on March 9th to 0.9°/oo on March 12th, but by March 15th it had increased to 2.3°/OO. Over the same period the decrease in salinity at a depth of 60 cm. was only half that at the surface, dropping from 31.4°/oo on May 9th to 21.1°/oo on the 15th.

* All previously quoted salinity values are bottom salinity.

Salinity stratification was exhibited by almost all pools studied, both large and small. On some occasions, usually after recent rain, an interface between upper and lower layers of water could be seen within pools. Layering, once established,was not maintained indefinitely and for most pools there were times when little or no difference could be detected between the salinity of surface and bottom waters. When stratification was apparent surface salinity was almost always lower than bottom salinity. There were occasions, however, when surface salinity exceeded that of the bottom by amounts not greater than 0.3°/oo, just outside the possible limits of experimental error (0.2°/oo). This may have been due to recent evaporation from surface water at rates faster than that at which mixing processes were acting.

Differences between surface and bottom salinities were maximal following rain. Extensive algal growth may, as Chapman (1957) suggested, promote layering. A small pool, 26, at Kaikoura, supporting a vigorous growth of Enteromorpha, had a surface salinity of 1.9°/oo and bottom salinity of 6.2°/oo on May 13th. But stratification was equally characteristic of pools which had little or no filamentous algae. On June 29th Pools 3 and 4, Taylors Mistake, contained only scattered algal filaments yet at that time were strongly stratified:

Pool 3	Surface	salinity	28.6°/oo	Bottom	salinity	36.3°/oo
Pool 4	"	"	28.7°/oo	"	"	39.5°/oo

Pool 33, Goose Bay, on October 15th had a thick mat of

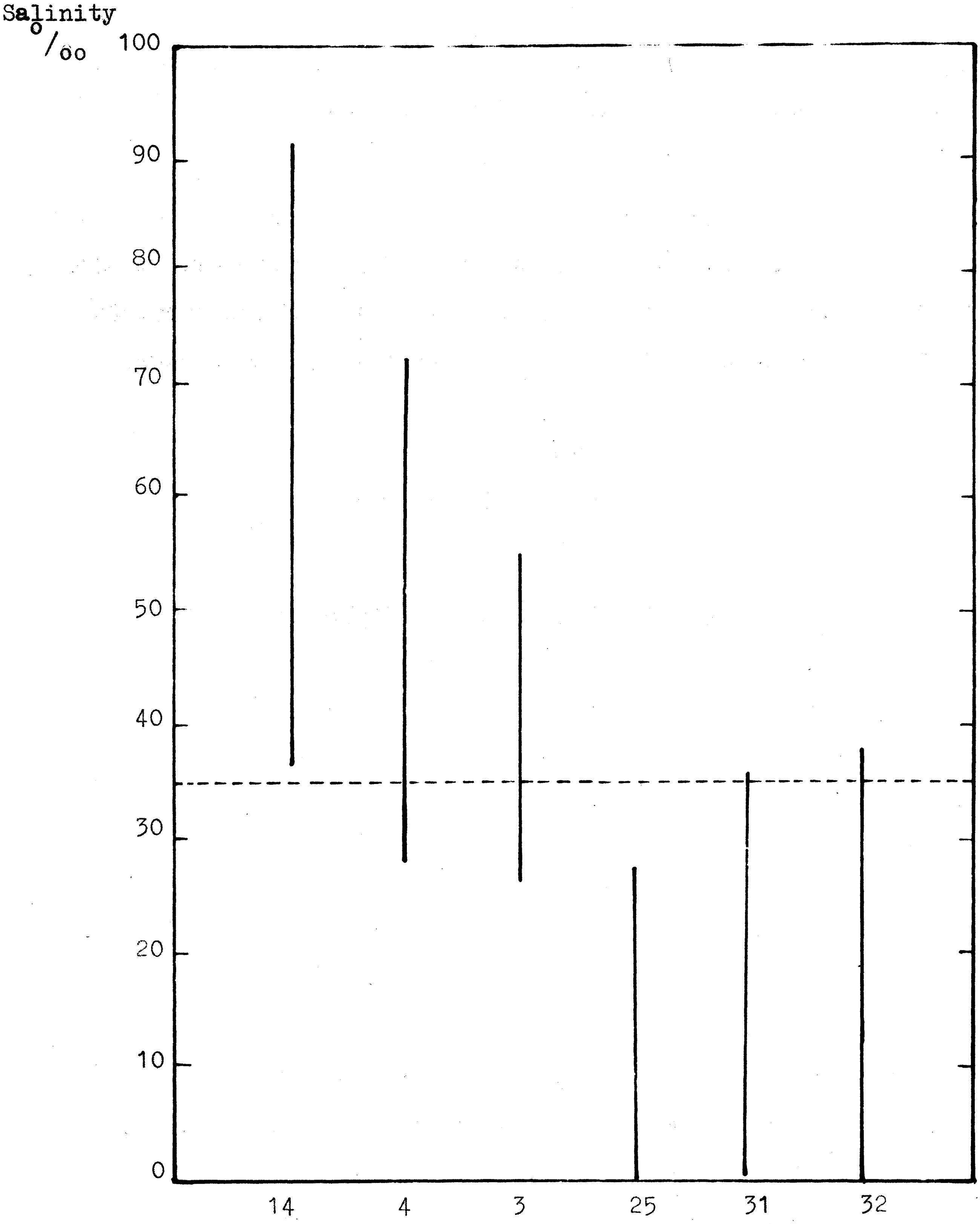


Fig. 25. Ranges of salinity recorded from six supralittoral pools, 1961.

Enteromorpha extending completely across the pool, separating upper and lower water layers. Yet there was no difference between surface and bottom salinity.

It has previously been demonstrated that fluctuations in the mean salinities of Pool 3 and 4, Taylors Mistake, tended to follow similar trends. The relationship between stratification was much less precise, as the following table shows.

TABLE II

Salinity Stratification in Pool 3 and
Pool 4, Taylors Mistake

Date	Differences between surface and bottom Salinity	
	Pool 3	Pool 4
20/2	0	1.3
26/7	3.7	2.5
23/8	1.0	0.4
19/9	8.6	0.3
5/10	6.0	5.9
8/10	0.3	0
18/10	0.3	0.1
4/11	0.1	1.7

4. Although supralittoral pool salinity varied both in time and with depth, the range of fluctuation differed between pools. The recorded ranges of salinity of six pools from Taylors Mistake, Diamond Harbour, Goose Bay and Kaikoura have been graphed in Fig. 25. In Fig. 26b these ranges are broken down

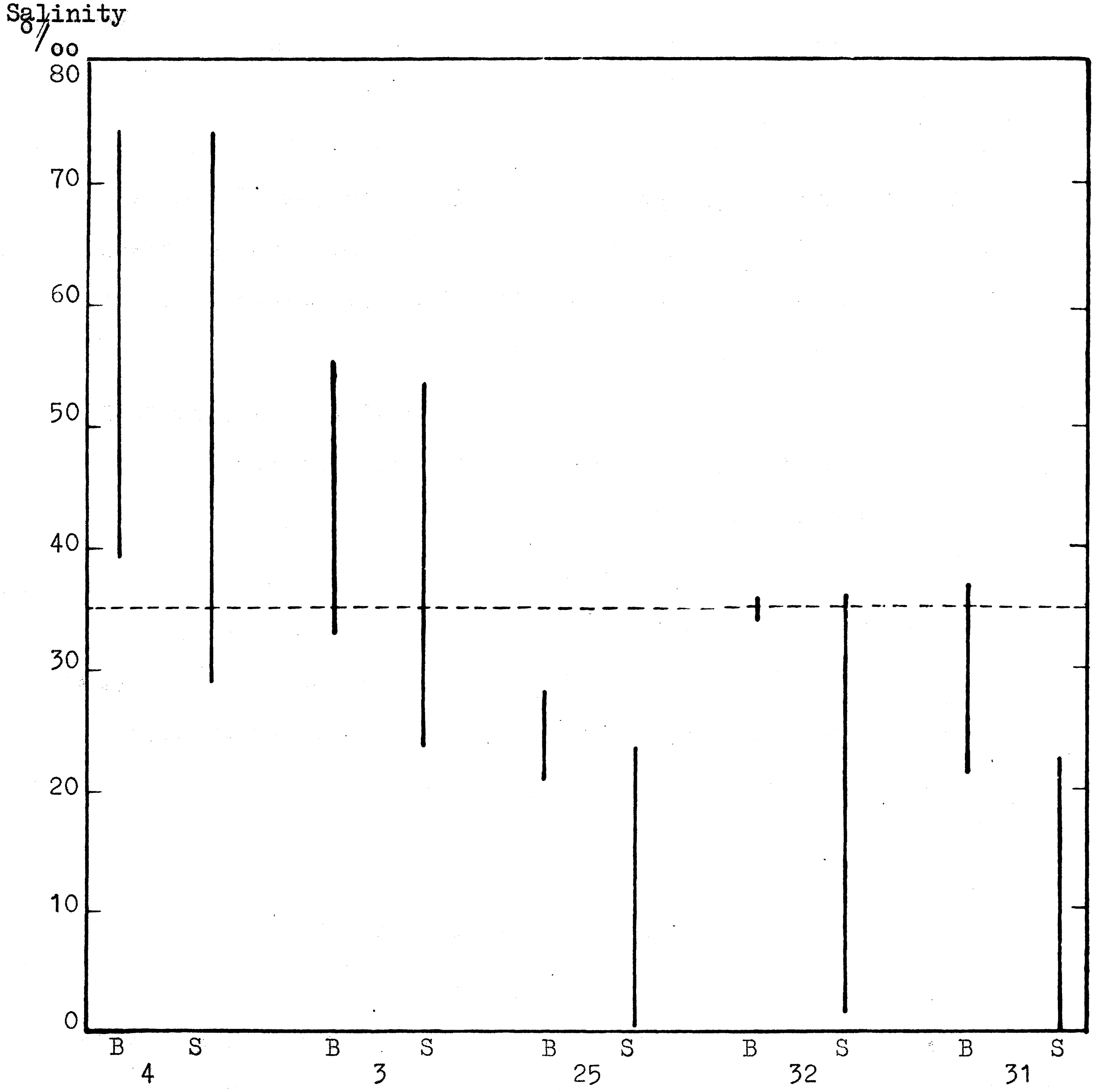


Fig. 26b Ranges of surface and bottom salinity recorded from five supralittoral pools, 1961.
B : Range of bottom salinity. S : Range of surface salinit
---- Salinity of normal sea water.

into those of surface and bottom salinity.

Pools of Lyttelton Harbour, represented by Pool 14, were among the smallest studied, the largest of these being no more than a few litres in volume. Their salinities were higher than those of larger pools. In every one of twenty determinations their salinity was above 35^{o}/oo, and the highest salinity recorded, 90.9^{o}/oo, was from Pool 14, Diamond Harbour on December 18th, 1960.

Only four of twenty eight determinations from Pool 4, Taylors Mistake, were lower than 35.0^{o}/oo, compared with twelve of thirty from Pool 3.

On the other hand the much larger pools of Goose Bay and Kaikoura were never significantly more saline than normal sea water (35^{o}/oo). Although surface salinity of these pools fell after rain to almost fresh water, bottom salinity was never less than 20^{o}/oo.

Discussion:

1. The Causes of Salinity Stratification

A few authors have noted the presence of salinity stratification within supralittoral pools (Gislen,1930; Naylor and Slinn,1958; Remane and Schlieper,1958) but no attempt has been made to explain how it comes about. Estuaries and lagoons have long been known to be stratified. Layering within estuaries and lagoons has been adequately accounted for in terms of outward movement of fresh-water and inward movement of sea water. Emery et al (1957) have drawn attention to the influence which the balance between evaporation, precipitation and rainfall has on the types of stratification found within estuaries. Supralittoral pools, unlike estuaries, are not continually subjected to the flow of stream or tide.

Disturbance of pools by wind and waves broke down density layering. On October 5th Pool 3, Taylors Mistake, was strongly stratified, with surface salinity 22.6°/oo and bottom 28.6°/oo. The water volume within the pool had been considerably increased by October 8th from sea splashing, raising the total salinity, but breaking up the stratification. Surface salinity was 35.0°/oo, bottom 35.3°/oo on October 8th.

Any circulation induced by evaporation or other agents within pools could only act towards the restoration of homogeneous water conditions. The inability of evaporation to promote stratification was demonstrated in an experiment in which a bucket containing a 35 grams per litre (35°/oo) solution of NaCl was left standing for three months. During this time a litre of water evaporated, but no fresh water was added. After three months the surface and bottom salinities had both risen but they were still exactly equal at 37.2°/oo.

After rain contrast between surface and bottom salinity was maximal. On May 9th surface salinity of Pool 25, Kaikoura, was 22.5°/oo, bottom 31.4°/oo. Following rain, by May 12th the respective values were surface 0.9°/oo, bottom 25°/oo, an increase of 15.7°/oo in the difference. Density stratification, it was concluded, is initially established in supralittoral pools when water of lower salinity comes to overly water of higher salinity already within pools. Rain was the most common cause of stratification although seepage of fresh water on to pool surfaces also caused salinity layering. If the layering processes were not accompanied by disturbance of the pool, a sharply defined zone of transition was set up between waters of different density within the pool.

2. Limnological Terminology for Supralittoral Pools.

Adopting Hutchinson's (1937) adaptation of Findenegg's (1935) terminology for chemically stratified masses of standing water, supralittoral pools are meromictic. Just as a normal (holomictic) thermally stratified pond or lake consists of an epilimnion and hypolimnion separated by a thermocline, a meromictic pool consists of an upper region, the mixolimnion and a lower region termed by Findenegg, the monimolimnion. The zone of transition, where the rate of change of concentration with depth is maximal, is termed the chemocline.

3. The Stability of Salinity Stratification

Ruttner (1953) presented a table showing how specific gravity increases with salt content:

TABLE III

Salt Content $^o/oo$ (g. per litre)	Specific Gravity (at 4°C)
0	1.00000
1	1.00085
2	1.00169
3	1.00251
10	1.00818
35	1.02822

It follows from these data that the specific gravity increases nearly linearly with increasing salt content. A salt content of one gram per litre increases the specific gravity by about 0.0008, whereas the difference in density attendent on a change in temperature from 4°C to 5°C is 0.000008. To compensate this difference in density requires an increase in salt concentration of only 10 milligrams per litre.

Thus, in supralittoral pools, salinity stratification overides any tendency for the development of types of thermal stratification often found in bodies of standing fresh water.

4. The Classification of Brackish Waters

Segerstrale recently (1959) presented a comprehensive historical survey of marine water classification as an introduction to the 1958 Venice Symposium on the Classification of Brackish Water. Consequently an historical review of the literature would be superfluous here, but a discussion of the System for the Classification of Marine Waters according to Salinity adopted by the Venice Symposium is revelant.

The system adopted by the Symposium and recommended for universal application was as follows:

TABLE IV

The Venice System for the Classification
of Marine Waters According to Salinity

Zone	Salinity °/oo
Hyperhaline	± 40
Euhaline	± 40 - ± 30
Mixohaline	(± 40) ± 30 - ± 0.8
Mixoeuhaline	± 30 but adjacent euhaline sea
(Mixo -) polyhaline	± 30 - ± 18
(Mixo -) mesohaline	± 18 - ± 5
(Mixo -) oligohaline	± 5 - ± 0.5
Limnetic (freshwater)	± 0.5

(From Remane and Schlieper, 1958)

It was stressed that these figures are approximate, as indicated by the use of the ± sign. In order to indicate waters of unstable or variable salinity (irrespective of mean values) the term "poikilohalinity" has been proposed and for conditions of stable or constant salinity "homoiohalinity".

Relation of Supralittoral Pools to the Venice System of Classification.

From the data gathered during this study it is clear that supralittoral pools, of the South Island coast at least, are poikilohaline. The major divisions of the Venice System can be usefully applied to supralittoral pools but it is doubtful if the proposed subdivisions of mixohalinity can be applied to these pools in temperate regions. The larger pools included in this study, i.e. those of Goose Bay and Kaikoura, can be described as mixohaline, their poikilohalinity ranging from ± 0.6°/oo to ± 35°/oo. The more sheltered small pools, viz. those from Lyttelton Harbour, can be classified as hyperhaline, although values between 30 and 40 °/oo were not uncommon. However, as the Venice Symposium recognized, even the best possible system of classification will never give more than average salinity conditions in a given water mass.

More difficulty is experienced in classifying the Taylors Mistake pools under the Venice System. Nevertheless Pool 4 with a mean salanity of 46.7°/oo can be described as hyperhaline although occasional determinations as low as 28.7°/oo were recorded. With a mean salinity of 36.3°/oo Pool 3 could be regarded as euhaline. But, as Pool 3 ranged from 22.6°/oo to 55.9°/oo, this terminology seriously obscures its essential poikilohalinity.

In any attempt to apply a universal classification to systems as diverse and variable as brackish and marine waters transitional and anomalous situations are bound to be encountered. The merits of the Venice system lie not only in the elasticity with which it can be applied, and the distinction it provides between conditions of unstable or variable salinity and stable salinity, but also more specifically in relation to supralittoral pools, in the useful distinction it provides between the larger mixohaline and the smaller hyperhaline pools.

Conclusions

The largest supralittoral pools can be considered as representing the low end of an onshore - offshore salinity gradient, but pools with volumes under fifty litres, which are much more common in the South Island supralittoral, almost always have salinities similar to, or in excess of, sea water. These small pools form a specialized environment which, with respect to salinity at least, cannot be regarded as transitional between sea and fresh water.

2.4 Temperature

Introduction

Records of temperature from supralittoral pools have chiefly been spot readings, although Ambler and Chapman (1950) published some limited observations on diurnal changes, and O'Gower (1960) investigated both diurnal and seasonal temperature oscillations in some Sydney pools. Variations in temperature with depth in some supralittoral pools were noted by T.S. Austin (in Doty, 1957) and Naylor and Slinn (1958) recognized a relationship between salinity laying and temperature.

In the following pages the results of a year's investigation into the temperature properties of South Island supralittoral pools are presented. Diurnal and nocturnal, as well as seasonal fluctuations are considered and relationships between temperature and some other environmental factors discussed.

Methods:

All readings were taken with a mercury bulb thermometer graduated in degrees Centigrade. A protective cover was fitted to the bulb to shield it from the direct rays of the sun.

Attempts to record diurnal-nocturnal temperature ranges within pools with maximum and minimum thermometers were thwarted, the thermometers either being interfered with or removed.

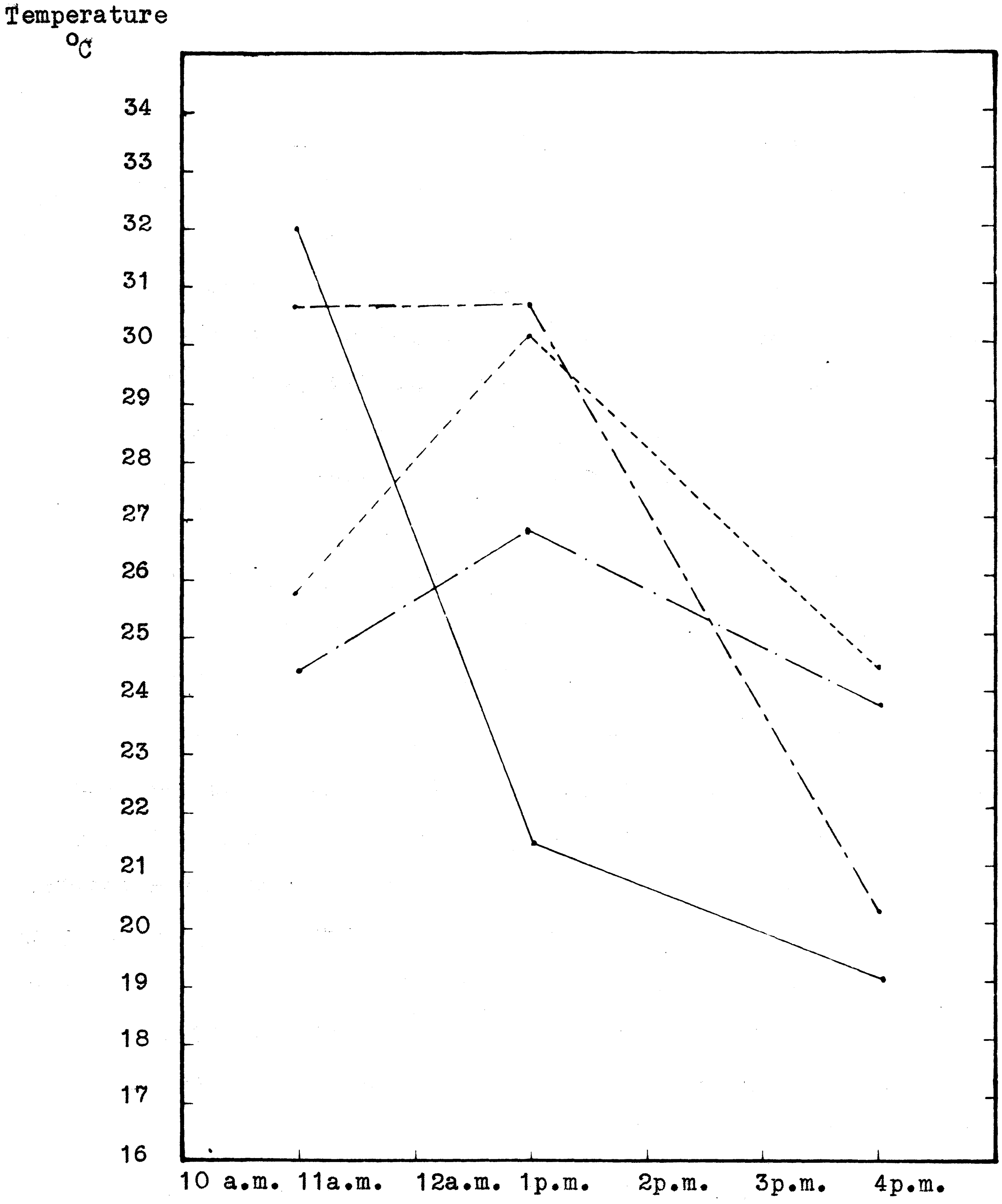


Fig. 27. Fluctuations in temperature within four Taylor's Mistake supralittoral pools over a five hour period on February 9th, 1961.

Results:

1. Temperature, like salinity, was often markedly different in adjacent pools at one time. In Fig. 27 the ranges of temperature in Taylors Mistake pools over a five hour period on February 9th are plotted. The largest recorded range was 13°C at 4.45 p.m. on February 8th when the air temperature was 22°C while the smallest was 1.2°C on March 29th at 3.00 p.m. when the air temperature was 12.1°C. In general the ranges of pool temperatures were greatest when air temperatures were highest. Usually temperatures were spread over a wider range earlier in the day than later. e.g. on February 9th at 10.45 a.m. the range was 12.2°C, at 1.15 p.m. 11°C and 8.3°C at 3.50 p.m.

Differences in pool temperatures were due to the varied situations and dimensions of pools. Each pool received different amounts of insolation and at different periods during the day. Shallow pools were quickly heated to high temperatures, sometimes above air temperature. Temperatures in deeper and larger pools rose more slowly but they retained their heat longer. Contrasts were greatest early in the day, since the differences in the amount of radiation received by pools and their surrounding rock surfaces, were maximal then.

2. As with salinity, stratification was a prominent feature of pool temperature. Differences in temperature between upper and lower water layers was a property of all pools studied. Almost always bottom temperatures were higher

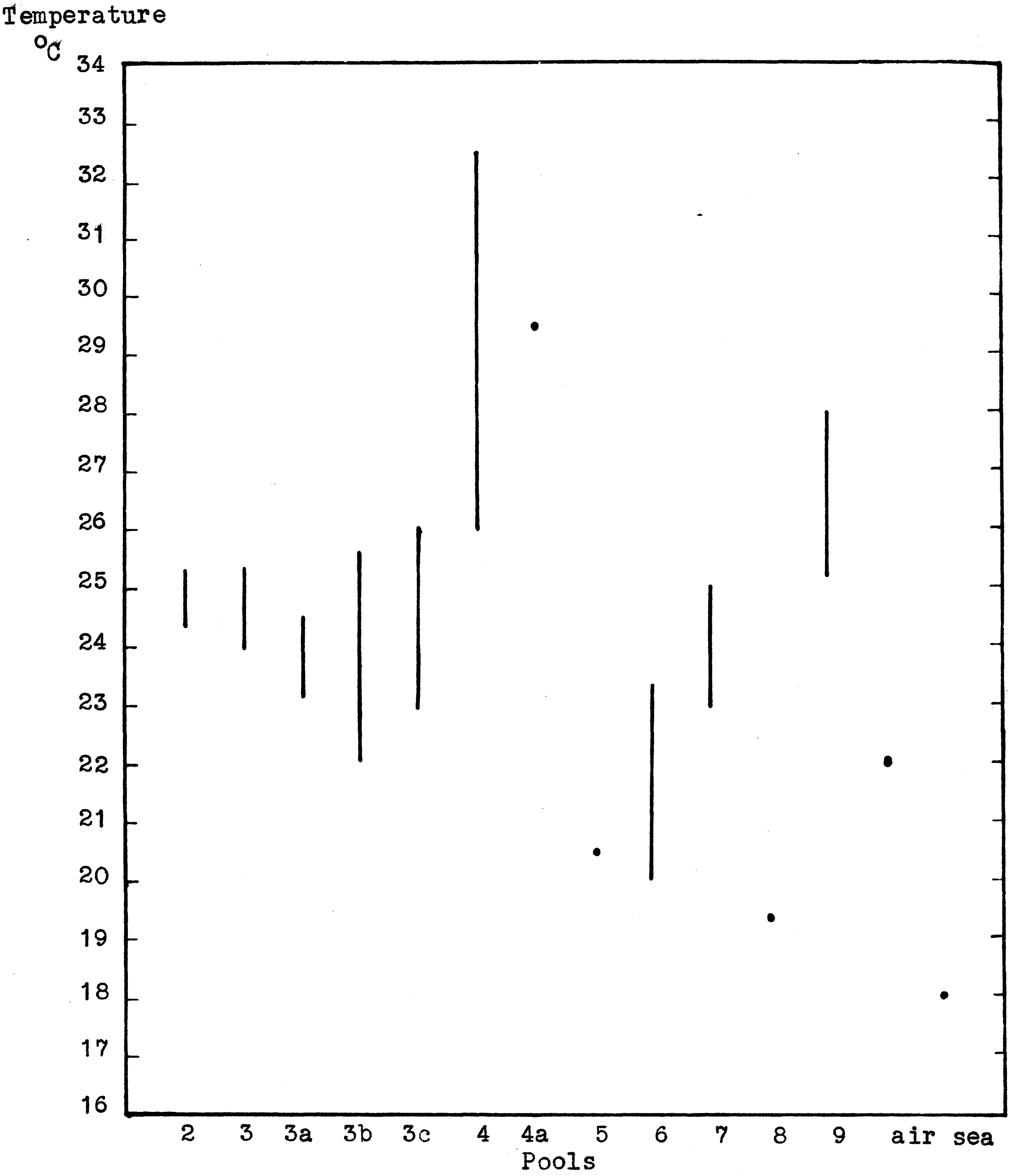


Fig. 28. Ranges of temperature within Taylor's Mistake supralittoral pools at 1.45 p.m. on February 8th, 1961.

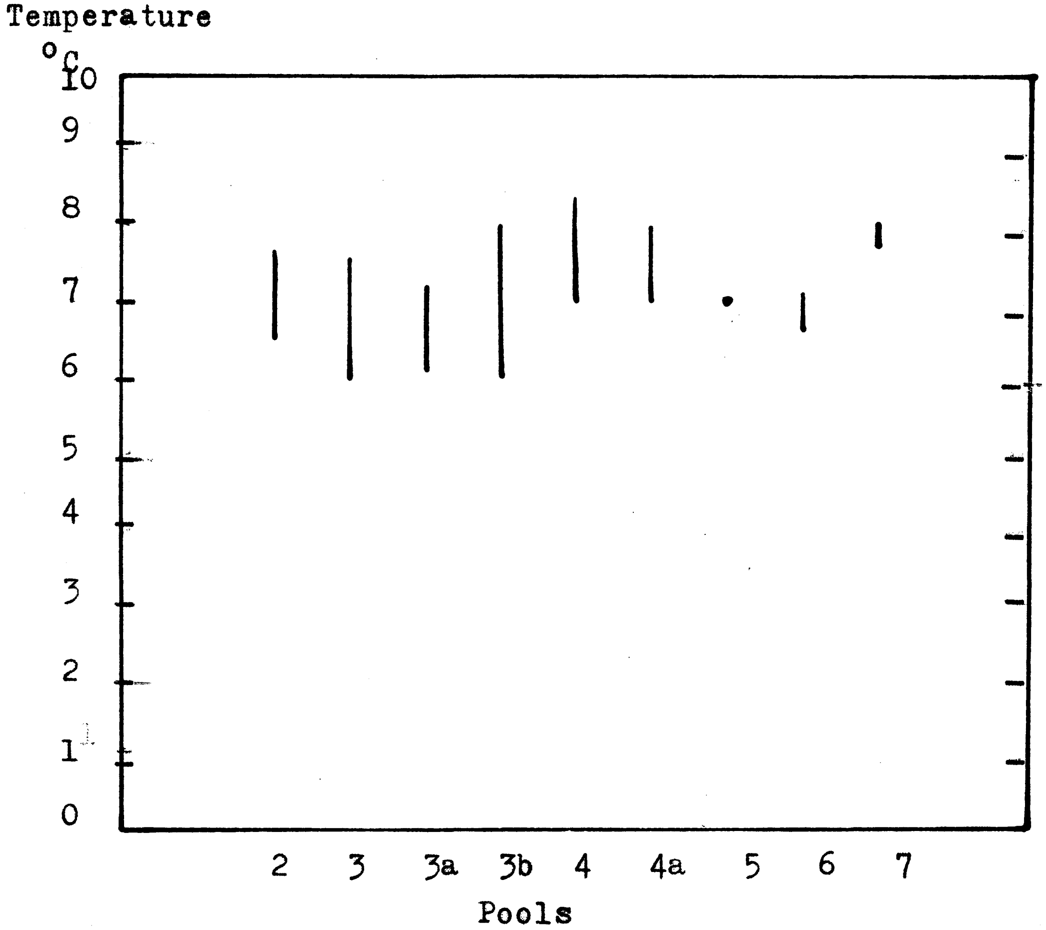


Fig. 29. Ranges of temperature within Taylor's Mistake supralittoral pools at 1.45 p.m. July 26th, 1961.
Air temperature : 14°C.
Sea temperature : 8°C.

than surface. In Fig. 28 the ranges of temperature within Taylors Mistake pools on February 8th at 1.45 p.m. are graphed, while Fig. 29 for comparison gives the ranges within these same pools on July 26th at 1.45 p.m. The maximum recorded ranges of temperature within several Taylors Mistake pools are plotted in Fig. 30.

Temperature stratification was exhibited by pools throughout the year, being equally evident when air temperatures were low or high. For some pools contrasts between surface and bottom temperatures were greatest when temperatures were lowest, as comparison of the following table with Fig. 32 shows.

TABLE V

Temperature Stratification in Taylors Mistake
Supralittoral Pools 10.00 a.m. June 26th

Pool	Temperature		
	Surface	Bottom	Difference
2	3.0 °C	43 °C	1.3 °C
3	2.0	6.3	3.7
3a	2.2	6.0	3.8
3b	2.9	6.0	3.1
4	1.0 °C	5.0 °C	4.0 °C

Although all pools exhibited stratification in all seasons of the year, they were not stratified at all times. The following table records the percentage of times upon which measurements showed pools to be negatively stratified (i.e. surface temperature below bottom temperature.)

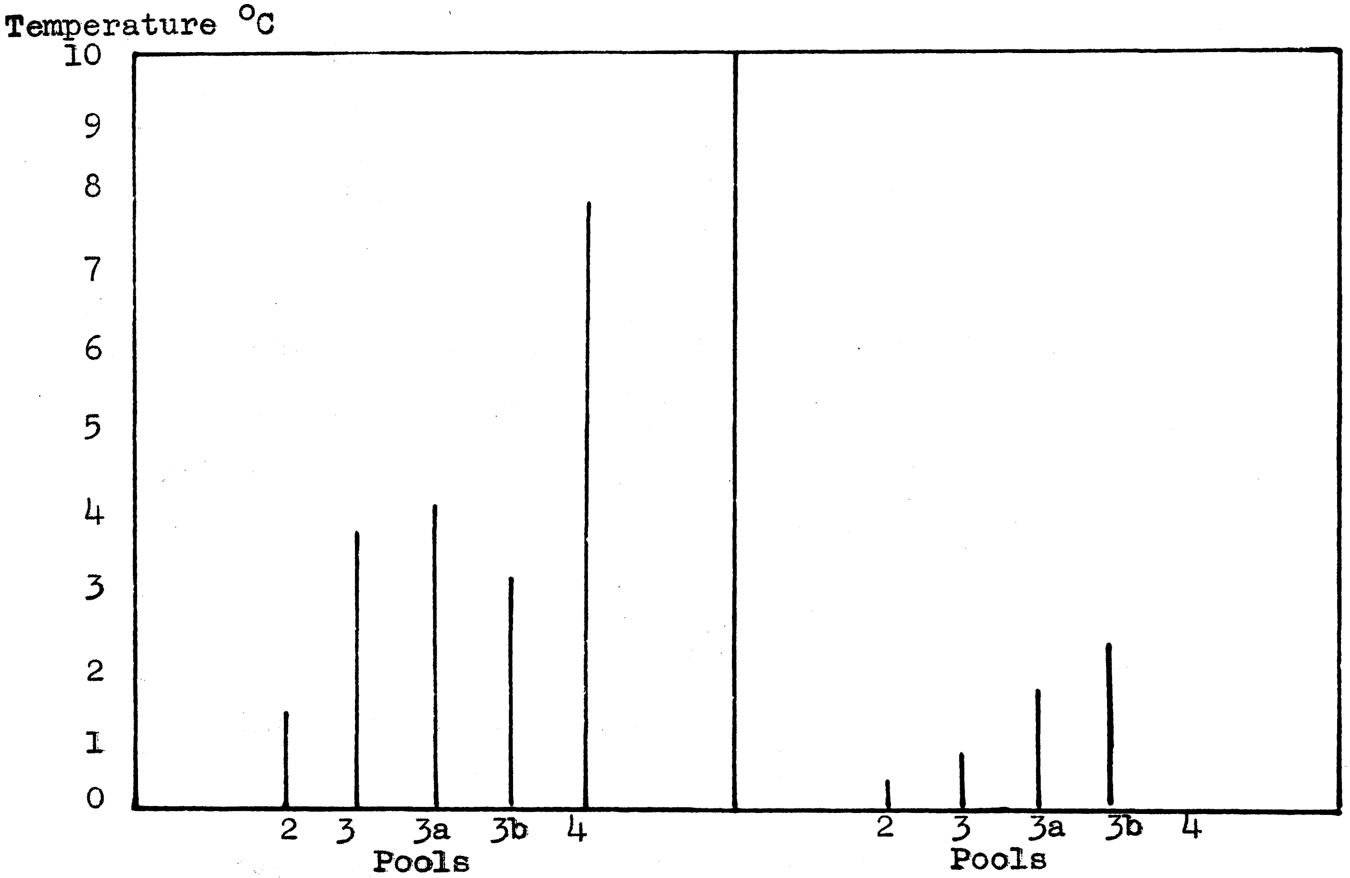


Fig. 30a.

Fig. 30b.

Fig. 30. Maximum recorded ranges of temperature, at any one time, within supralittoral pools at Taylor's Mistake, 1961.
Fig. 30a. gives maximum ranges of negative stratification within pools, (i.e. bottom temperature higher than surface temperature).
Fig. 30b. gives maximum ranges of positive stratification within pools, (i.e. surface temperature higher than bottom temperature).

TABLE VI

Frequency of Negative Temperature Stratification
Taylors Mistake 1961

Pool	Number of Measurements	Percentage Negative Stratification
2	24	34.3
3	29	69.0
3a	29	65.5
3b	29	41.5
4	30	80.0

On other occasions positive temperature stratification (i.e. surface temperature higher than bottom temperature) was recorded. The corresponding percentages for Taylors Mistake pools have been tabulated below.

TABLE VII

Frequency of Positive Temperature Stratification
Taylors Mistake 1961

Pool	Number of Measurements	Percentage Positive Stratification
2	24	4.2
3	29	10.3
3a	29	24.2
3b	29	38.0
4	30	0

The extent of positive temperature stratification was never greater than 2.2°/o , much less than the maximum recorded negative stratification.

Causes of Temperature Stratification in Supralittoral Pools:

Negative temperature stratification is a consequence of density layering within pools. Heat from radiation incident on pool bottoms is trapped in lower waters, density stratification preventing it being distributed evenly throughout the pool by circulation. The amount of radiation absorbed by the rock bottoms and margins of pools is much greater than that absorbed by water. Most of this heat stored in the rock is redistributed to water in contact with it, but because of salinity stratification this heat is retained in lower layers of water. Surface cooling through evaporation or contact with cold air also contributes to negative temperature stratification. In a salinity stratified pool a very large drop in surface temperature would be necessary before the water at the top could sink into the lower layers. Consequently any fall in temperature is confined to the upper layers and its effects are distributed throughout the rest of the pool only gradually. This explains why negative temperature stratification was equally common, provided a pool was density layered, whether air temperatures were high or low.

Positive temperature stratification tended to occur most frequently in pools which were less commonly negatively stratified (compare Tables V and VI), and was caused by heating of surface water through contact with warm air. A large rise

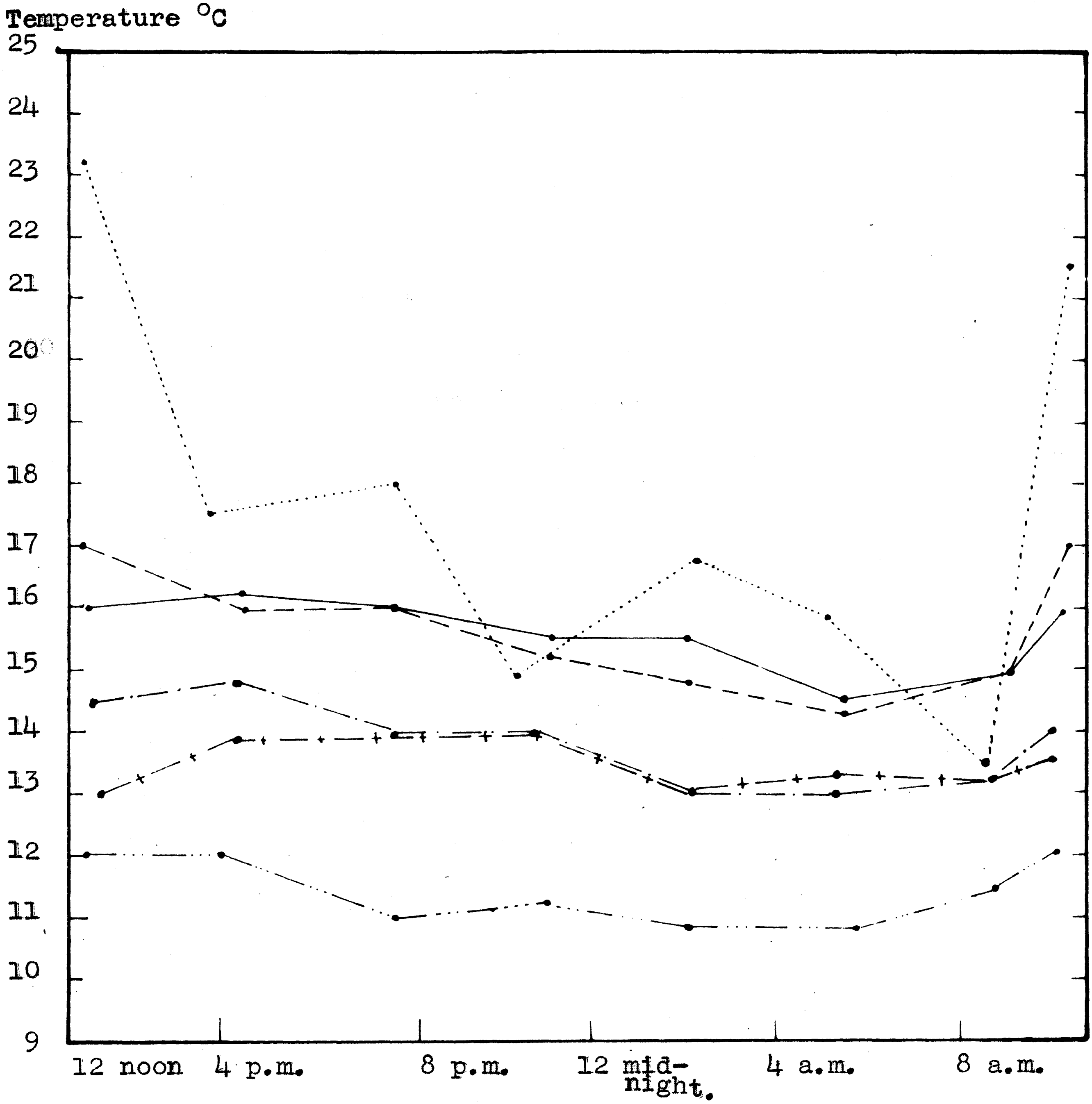


Fig. 31. Fluctuations in temperature in pools 31 and 32, Goose Bay, between mid-day October 14th and mid-day October 15th, 1961.

Air temperature
Sea "
Pool 31 surface temperature.
" " bottom "
" 32 surface "
" " bottom "

in surface temperature would be necessary to convert strongly negatively stratified pools to positive stratification, but a much smaller rise would be sufficient in pools in which the tendency for negative stratification was weak. Pool 3b in which positive stratification was most common, had a light coloured sandy bottom, unlike Pool 4, which never developed positive stratification. The bottom of Pool 4 was bare rock. Much more heat would be reflected from the bottom of Pool 3b than Pool 4 so that negative stratification was more common in the latter pool, and positive stratification in the former.

3. Diurnal-nocturnal fluctuations in the temperature of three Goose Bay pools, 31, 32 and 33 were determined on October 14th and 15th. Both surface and bottom temperatures of all three pools were measured at approximately two hourly intervals over a period of twenty four hours. Recordings of sea and air temperatures were also made at the same intervals. The results of these measurements are plotted in Fig. 31. Pool 33 is not included in the graph as the recordings from it were similar to those from Pool 32.

These three Goose Bay pools all had large volumes (see Fig. 20). Fluctuations in smaller pools would be expected to be of greater amplitude. Nevertheless both surface and bottom temperatures remained remarkably constant over the whole twenty four hours, the difference between maximum and minimum surface temperature for Pool 31 being

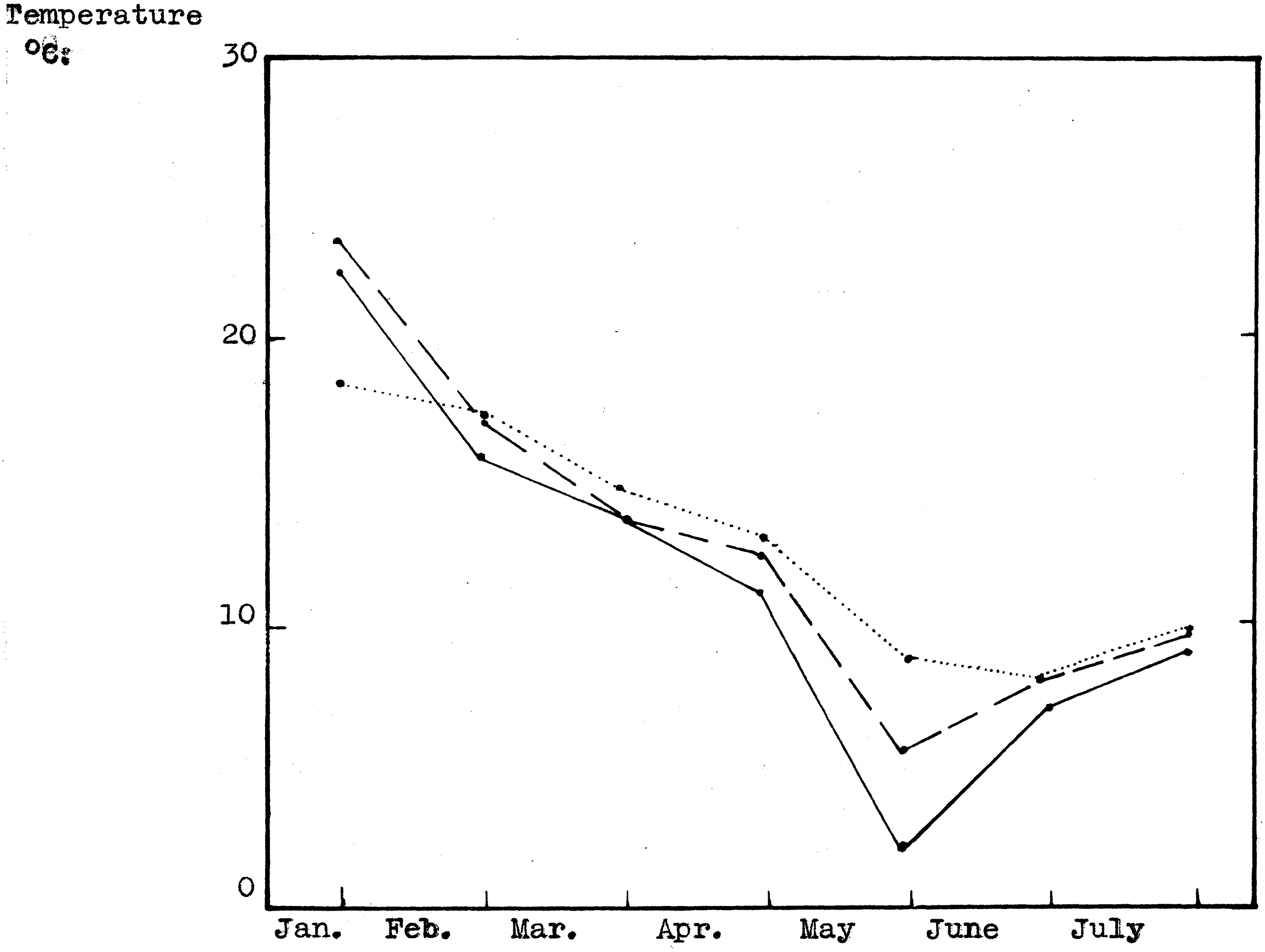


Fig. 32. Mean monthly mid-afternoon temperatures in pool 3, Taylors Mistake, 1961.

______ Surface temperature
— — Bottom "
..... Sea "

1.8°C and for Pool 32 2.8°C. Sea surface temperature changed by 1.2°C over the same period, while air temperature fluctuated over a range of 10.2°C. Pool temperatures fell slowly from a mid-day maximum to a minimum for Pool 31 in the early hours of the morning, and in Pool 32 just at sunrise. In the morning there was a sharp rise in the temperature of Pool 32, but a slower one in Pool 31. Surface and bottom temperatures of Pool 32 were consistantly warmer than those of Pool 31. Differences in the temperature properties of the two pools can be ascribed to their situations. Pool 32 was exposed to the sun's direct rays for almost all the daylight hours, whereas Pool 31 was shaded for several hours at different periods during the day. Consequently more heat was received by Pool 32, and its volume was large enough to maintain higher temperatures during the hours of darkness.

4. Regular temperature recordings taken at Taylors Mistake in mid-afternoon for a period of ten months gave an indication of seasonal temperature oscillations. In Figs. 32 and 33 the monthly mean pool surface and bottom temperatures are plotted for Pools 3 and 4. The monthly mean recorded sea and air temperatures are included in Figs. 32. Unfortunately for completeness Pool 3 was dry for much of August, September, October and November. Temperatures were at a peak in the summer months and dropped to a minimum in June. Similar trends were found in all pools. For all pools bottom temperature was higher than surface temperature during most

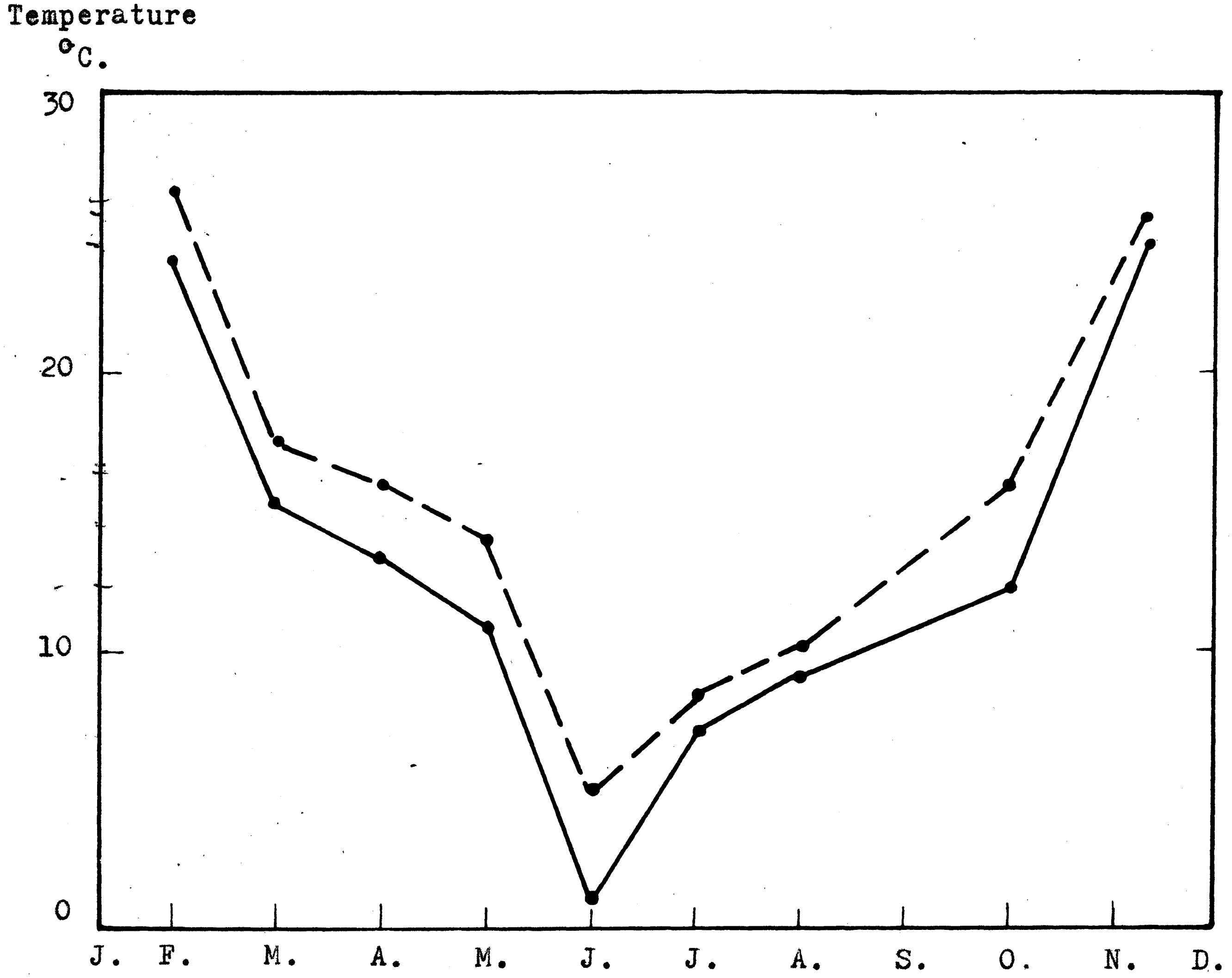


Fig. 33. Mean monthly mid-afternoon temperatures in pool 4, Taylor's Mistake, 1961.

— — — Bottom temperature.

——— Surface temperature.

months. In February the mean afternoon pool temperatures were higher than mean sea temperature, but in March mean pool temperatures dropped below sea temperature till in June mean pool temperatures were three to five degrees below sea temperature.

Conclusions:

As with salinity, the outstanding characteristic of supralittoral pool temperatures was variability, both with time and depth. Whereas surface salinity was almost always lower than bottom salinity, surface temperature could be either higher or lower than bottom temperature, although the maximum range of positive stratification was much less than that of negative. Seasonally pool temperatures oscillated over a wider range than sea temperatures.

2.5 Oxygen

Introduction:

Dissolved oxygen has long been recognized as important in the biology of lakes and oceans. Surprisingly few workers have included oxygen tension determination in investigations of rock pool ecology. Ambler and Chapman (1950) and Naylor and Slinn (1958) made occasional spot determinations, but diurnal fluctuations in oxygen tension have not previously been studied. Pools had been found to be often supersaturated with oxygen during the day. Naylor and Slinn observed that surface oxygen tension may be similar, higher or lower than that at the bottom of pools and suggested these differences were related to density layering.

Methods:

The Winkler method (see Appendix) was employed throughout in estimating the dissolved oxygen tension of pools. Samples were taken in 250 millilitre bottles. By using 100 ml aliquots from these samples duplicate determinations were made. All titrations were completed as soon as practicable after sampling.

Determinations were made from Pools 3 and 4 at Taylors Mistake and Pools 31, 32 and 33 at Goose Bay. Diurnal nocturnal fluctuations were examined in these three Goose Bay pools.

TABLE VIII

Oxygen tension in Supralittoral Pools, Taylors Mistake

A. Pool 3

Date	Time	Value mg.atom 1^{-1}	Salinity $^{o}/oo$	Temp. ^{o}C	% Saturation.
5/4	1515	0.226	52.0	14.5	70.7
7/5	1700	1.009		11.7	
22/7	1500	0.690	33.8	12.0	167
27/7	1240	0.748	35.0	6.5	158
27/7	1515	0.845	35.0	6.5	179
22/8	1500	0.750	36.3	9.5	239
15/9	1300	0.789	29.0	12.7	176
5/10	1320	0.855	26.0	12.5	183
29/6	1000	0.416	32.0	5.0	80.5

Pool 4

Date	Time	Value mg.atom 1^{-1}	Salinity $^{o}/oo$	Temp. ^{o}C	% Saturation.
5/4	1515	0.146	54.0	14.5	51.0
7/5	1300	0.683		12.0	
22/7	1500	0.710	56.3	8.5	240
27/7	1240	0.670	33.0	7.5	182
22/7	1515	0.730	33.0	7.5	198
22/8	1500	0.587	33.4	9.5	128
29/6	1000	0.719	34.0	3.0	138
15/9	1300	0.616	36.8	14.1	170
5/10	1320	0.695	45.0	12.8	201

TABLE IX

Oxygen tension in Supralittoral Pools, Goose Bay

A G31

Date	Time	Value mg atom l^{-1}	Salinity o/oo	Temperature °C	% Saturation
15/5		0.0182			
2/8	Surface	0.836			
	Bottom	0.192			
14/10	1.00 pm	0.830	27.2	13.7	185
	3.50 "	0.740		14.4	165
	7.15 "	0.625		14.0	139
	10.15 "	0.593		14.0	132
15/10	12.45 am	0.440		13.0	96
	5.15 "	0.345		13.2	75
	8.30 "	0.383		13.2	83
	11.00 "	0.431		13.7	96

G32

Date	Time	Value mg atom l^{-1}	Salinity o/oo	Temperature °C	% Saturation
15/5		0.523			
2/8		0.622			
14/10	1.30 pm	0.754	35.5	16.5	198
	4.30 "	0.619		16.1	162
	7.35 "	0.728		16.0	192
	10.55 "	0.664		15.4	168
15/10	2.10 am	0.575		15.2	146
	5.30 "	0.527	35.5	14.5	134
	8.50 "	0.549		15.0	139
	10.10 "	0.626		16.5	164

G33

Date	Time	Value mg atom l^{-1}	Salinity o/oo	Temperature °C	% Saturation
15/5		0.516			
14/10	1.50 pm	1.245	35.8	16.1	327
	4.45 "	0.613		16.1	161
	7.50 "	0.842		15.2	214
	11.10 "	0.729		14.8	185
15/10	2.30 am	0.574		14.3	147
	5.45	0.270		13.7	69
	9.00	0.931		13.5	238
	10.10	0.985		16.5	268

Results:

Oxygen values from Taylors Mistake pools have been recorded in Table VIII and those from Goose Bay pools in Table VIIII. Corresponding salinity and temperature recordings made at the time oxygen tension was determined have been included. The oxygen content of samples was calculated by substitution in the formula

$$C = \frac{n. F. 1000}{2. f}$$

where C = oxygen content, n = volume of thiosulphate of normality F used in litration and f. a constant of 99.2 (Barnes 1959) The percentage saturation was obtained from a Table in Barnes (1959).

1. Both Pool 3 and Pool 4, Taylors Mistake, were almost always supersaturated with oxygen during the middle of the day when samples were taken, the variations in values being due to fluctuation in photo-synthesizing populations, prevailing weather conditions, and pool volumes. When strong seas were running, with pools frequently being splashed with drops of supersaturated spray, oxygen tension was at its highest; e.g. on August 22nd the percentage saturation of Pool 4 was 234%. On that day the sea was splashing vigorously into Pool 4, but no drops reached Pool 3 in which the percentage saturation was only 128%.

2. Oxygen tension also varied with depth, for example

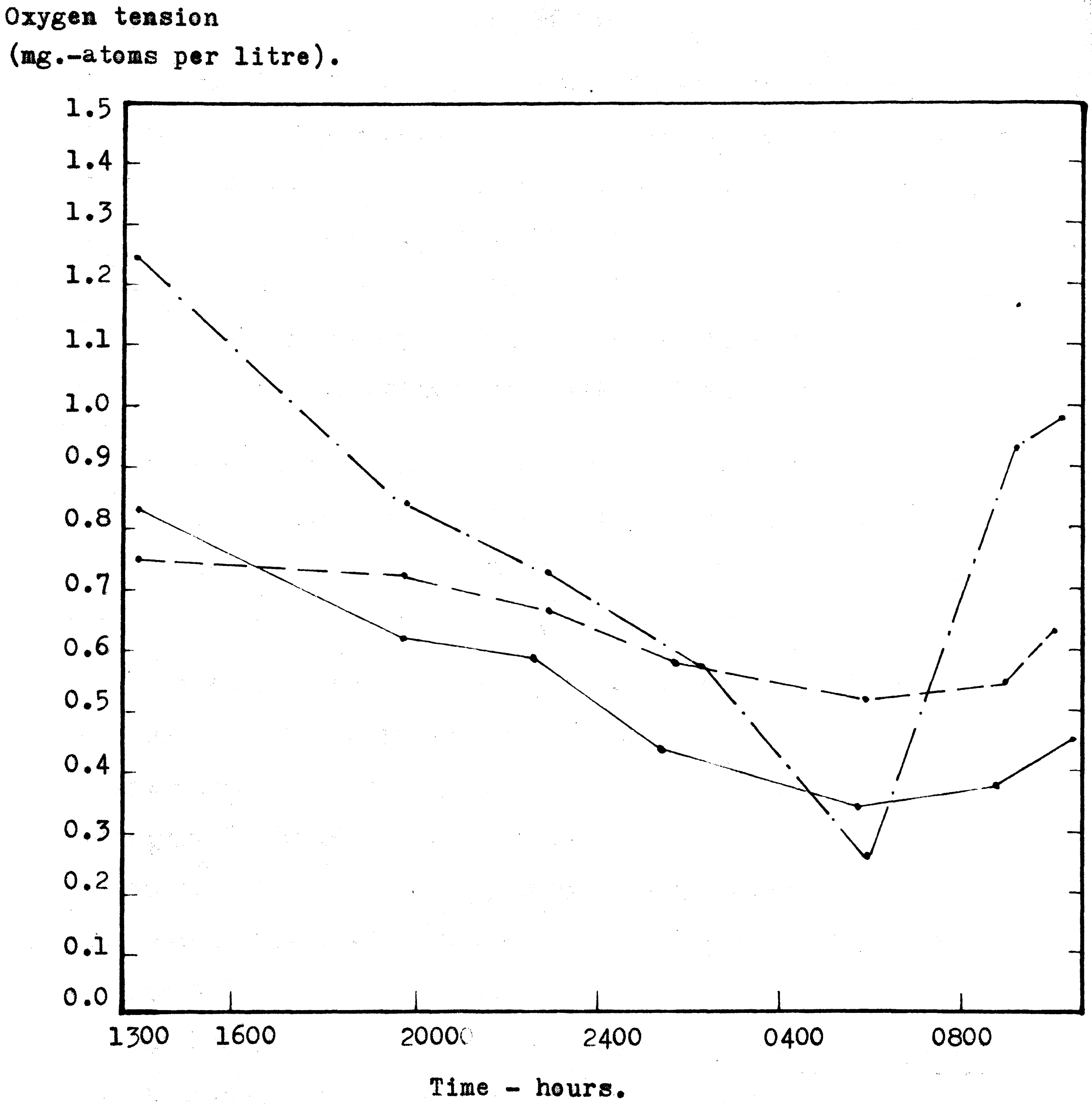


Fig. 34. Fluctuations in the oxygen content of the surface layers of three Goose Bay supralittoral pools, between 1 p.m. on October 14th and 11.a.m. October 15th, 1961.

— · — pool 33.
— — — pool 31.
——— pool 32.

measurements showed surface oxygen tension to be 0.836 milligram atoms per litre in Pool 31, Goose Bay on August 8th, while at the pool bottom tension was only 0.192 mg. atom per litre. At that time Pool 31 exhibited strong salinity stratification, surface salinity being 4.4°/oo lower than bottom. Clearly density layering prevented even distribution of dissolved oxygen throughout the pool.

3. On October 14th and 15th the oxygen tensions of three Goose Bay pools were measured at approximately two hourly intervals over a twenty four hour period. The results of this survey have been plotted in Fig. 34.

All three pools showed peaks in oxygen tension in the middle of the day and minima at sunrise. Oxygen tension within Pool 33 rose much higher than in the other pools and fell at a greater rate to a lower minimum. Pool 33 supported a vigorous growth of <u>Enteromorpha</u> which covered much of the pool surface, whereas in the other pools algal growth was less extensive being confined to walls and bottom. Production of oxygen from photosynthesis would have been greatest during the day in Pool 33 while oxygen consumption at a faster rate through respiration in the hours of darkness would have been responsible for the greater depletion of oxygen in this pool.

<u>Conclusions</u>:

As well as varying in time and with depth as did salinity and temperature, oxygen tension in supralittoral pools also varied according to the algal growth pools supported. Salinity stratification influenced oxygen tension at different levels within pools and diurnal-nocturnal oscillations were sharpest in pools bearing extensive growths of algae. Pools were commonly supersaturated with oxygen during daylight hours.

2.6 Hydrogen Ion Concentration

Introduction:

Hydrogen ion concentration of water masses has received considerable attention, not only because it is easily measured, but also as Allee et al (1949) observed biologists with a physiological training expected that hydrogen ion concentration of environments might prove to be of outstanding importance in the control of distribution. This expectation has not in general been realized (Allee et al,1949). Reed and Klugh (1924) suggested that pH was important in the control of the distribution of animals and plants in pools, but the restricted scope of their investigations has thrown doubt upon their conclusions.

Methods:

Determinations of hydrogen concentration in South Island supralittoral pools were carried out with a Beckman pH meter.

Results:

The range of pH readings from pools at Taylors

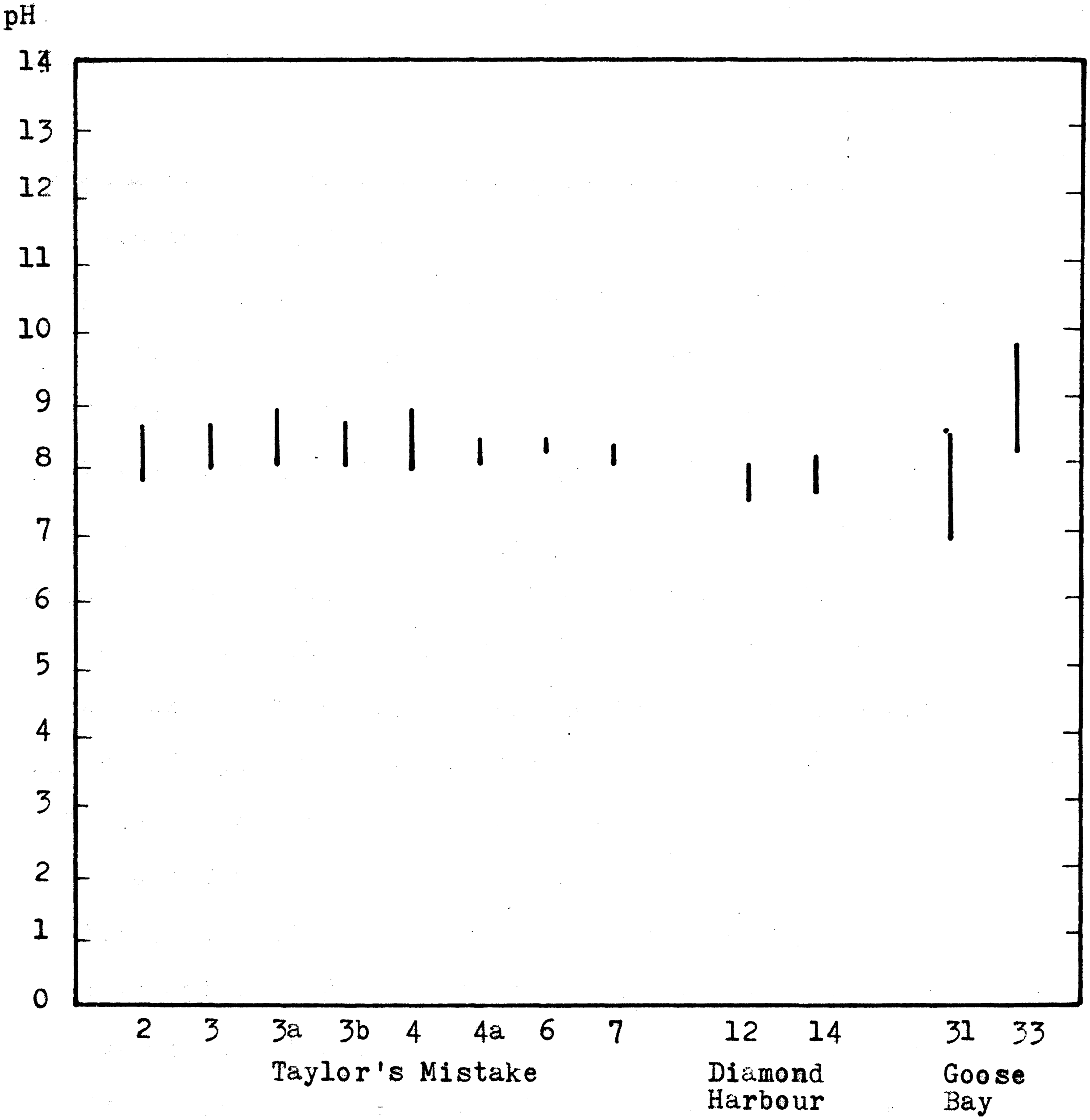


Fig. 35. Recorded ranges of hydrogen ion concentration from supralittoral pools, 1961.

Mistake, Diamond Harbour and Goose Bay have been plotted in Fig. 35.

Hydrogen ion concentrations ranging from 6.9 to 9.8 were encountered, while the mean of the sixty five determinations made was 8.2.

All Taylors Mistake pools had a similar pH ranges, their lowest recorded value being 7.8 and the higest 8.8. At times the hydrogen ion concentrations of pools were similar, while on other occasions values were more widely spread. For example, on April 5th at Taylors Mistake the range of pH was 8.0 - 8.2 but on September 15th concentrations from 8.1 to 8.8 were recorded.

Diamond Harbour pools tended to have lower hydrogen ion concentrations than those at Taylors Mistake. Both lowest and highest pH readings were from Goose Bay pools. When the lowest value, 6.9, was recorded from Pool 31 on May 15th oxygen tension was also at an abnormally low level of 0.018 milligram atoms per litre. The highest pH value, 9.8, came from Pool 33 on October 14th when the highest record of oxygen tension was also made from this pool. Allee et al (1949) had previously noted that regions of relatively low or high pH are also frequently regions of low or high oxygen content.

Conclusion:

Water in supralittoral pools was found to be always alkaline, with a single exception of a recording of pH 6.9.

Hydrogen ion concentration in pools was correlated with oxygen tension, high and low values of both occurring together.

CHAPTER 3

THE BIOTA OF SUPRALITTORAL POOLS

3.1 Introduction

Approaches to the study of the flora and fauna of supralittoral pools have been varied. Levander (1900) and Johnsen (1946) included supralittoral pools in surveys which extended inland to embrace fresh water ponds and pools, while Gislen (1930), Shelford (1935) and Cranwell and Moore (1938) have described the biota of supralittoral pools in their examinations of littoral communities. Pearce (1932) regarded the fauna of supralittoral pools and similar bodies of brackish water to be in some ways transitional between that of marine and fresh waters. Emery (1946) examined the biota of some shore pools in relation to their geological processes of formation. Supralittoral pools were included by Ambler and Chapman (1950) in studies on the ecology of shore pools. Some observations on the ecology of supralittoral pools organisms were made by Naylor and Slinn (1958).

Several workers have investigated the ecology of algae occurring in supralittoral pools and similar masses of saline water. (Yendo, 1914; Setchell, 1926; Johnson and Skutch, 1928; Feldman and Feldman, 1941; Nasr and Aleem, 1949; Wood, 1952; Chapman, 1957.) Others have examined the ecology of particular groups of animals found in supralittoral pools:

Droop (1953) flagellates, Faure-Fremiet (1948) ciliates, Fraser (1936) copepods, Lindeberg (1944) insects.

There is a large literature on mosquito ecology, but only a few papers on the bionomies of mosquitoes breeding in salt water pools, notably by King and Del Rosinio (1935), Margaleft (1949), Laird (1956) and O'Gower (1961).

Firstly the ecology of algae found in South Island supralittoral pools will be discussed, then the fauna of these pools and finally the biology of *Opifex fuscus*.

3.2 The Algae of Supralittoral Pools.

Introduction:

In the available time it was clearly impossible to analyse thoroughly the trends exhibited by all the biotic elements of pools. Selective study of those facets of the ecology of the flora and fauna most pertinent to an understanding of the supralittoral pool environment was imperative.

Filamentous algae were chosen for detailed ecological investigation for two reasons.

Firstly, they were usually the most obvious component of the supralittoral pool biota. As such they were potentially the most useful indicators of the properties of pools.

Secondly, filamentous algae have been used extensively by littoral ecologists in the erection of systems of zonation. It was thus logical to enquire into the ecological factors controlling algae in pools.

Methods:

An analysis of filamentous algae present in Taylors Mistake supralittoral pools was completed monthly. A similar survey of pools in Lyttelton Harbour and on the Kaikoura Coast was made at quarterly intervals. The algae present in the pools were identified at generic level. In view of the uncertain state of the systematics of some groups of Chlorophyceae, especially the Ulvaceae, there could be little justification for specific identification. Moreover the reproductive phase is required for specific identification of most Chlorophyceae and this was available only for short periods of the year and then only in some pools.

The abundance of the green alga Enteromorpha in pools was recorded on the following arbitrary scale -

0	None present.
1	Present.
2	Common
3	Abundant
4	Very abundant

The relative exposure of pools to splashing and washing by the sea was classified as follows:

1. Very sheltered.
2. Sheltered
3. Semi - exposed
4. Exposed

Class 1 pools were splashed only when the heaviest seas were running, while class 4 pools were splashed by all but the calmest seas.

Estimates of pool volumes were made from measurements of their diameter and depth to give the following volume index:

Index		Volume
1	less than	50 litres
2	from	50 to 500 litres
3	from	500 to 1000 litres
4	greater than	1000 litres

An index of the ratio of estimated surface area of pools to their depth was also erected, under the following scale:

Index		Surface Area : Depth
1	less than	500 cm
2	from	500 to 1000 cm
3	from	1000 to 2000 cm
4	greater than	2000 cm

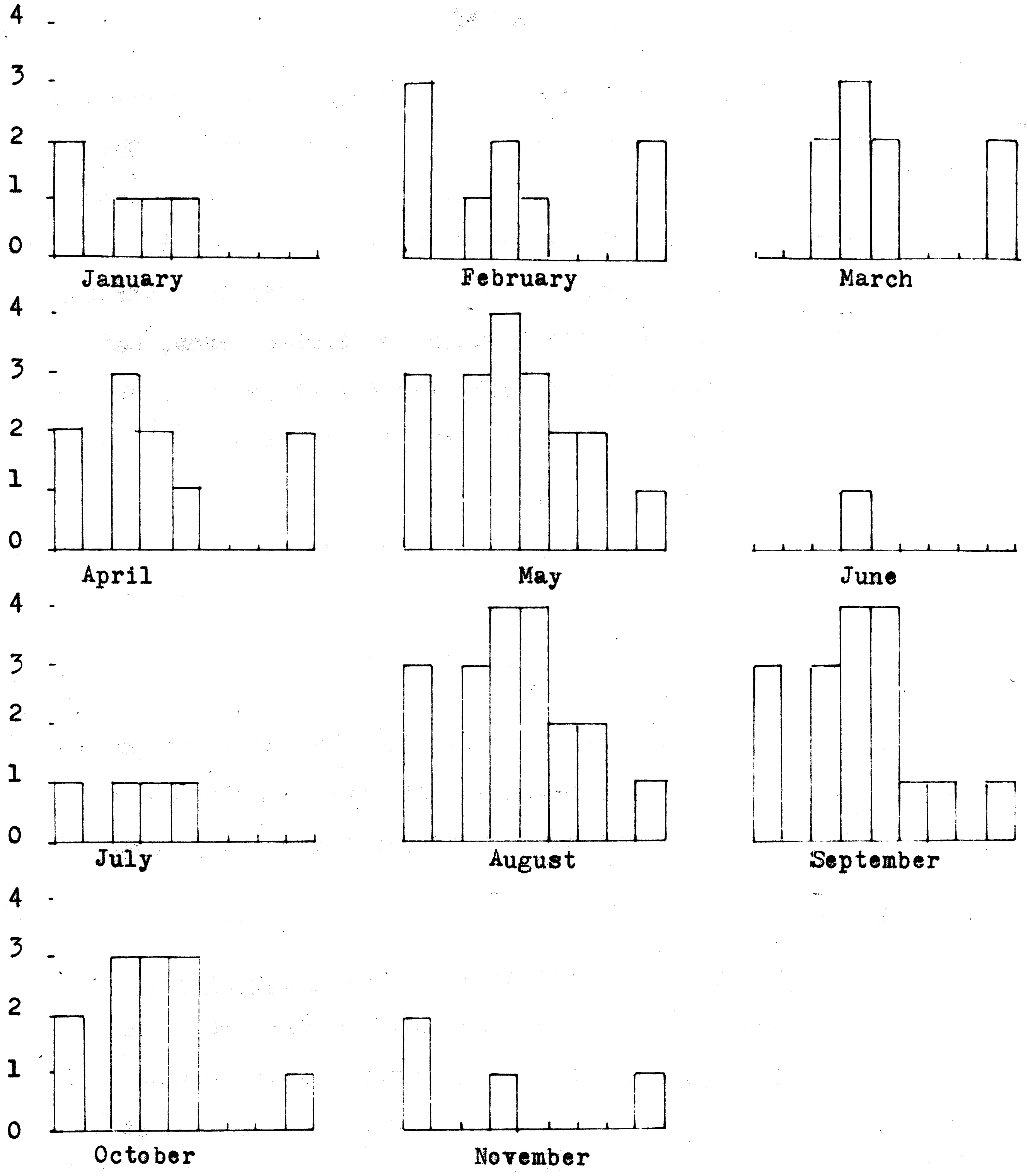


Fig. 36. Fluctuations in the abundance of *Enteromorpha* in Taylor's Mistake supralittoral pools, 1961.

The pools represented in each histogram are, in order from the left:- 1, 2, 3, 3a, 3b, 4, 5, 6, 7.

Droop (1953) has established a relationship between temperature and pools dimensions. Rise in temperature during the day depends on heat received at the surface, less heat lost at the surface and on the volume of water to be heated. Since both heat gained due to radiation and heat lost through evaporation depends to a large extent on surface area, and the volume is some factor of depth times surface area, the following relationships might be expected to exist.

$$\text{Heat change} = \frac{\text{Surface area}}{\text{Depth} \times \text{Surface area}} = \frac{1}{\text{Depth}}$$

A pool, which was found dry for more than one period between November 1960 and November 1961, was regarded as temporary (T), while other pools were classified as permanent.

Results:

Fluctuations in the abundance of *Enteromorpha* in Taylors Mistake pools have been recorded in Fig. 36. In these pools *Enteromorpha* was most abundant in the autumn months of March, April and May, and again in August, September and October. It did not occur at all in Pools 2 and 6, while it was only present in one pool throughout the year, 3a.

TABLE X

Filamentous Algae and Ecological Factors in South Island Supralittoral Pools

Pools	Algae	Permanency	Exposure	Volume	Surface Depth	Salinity
TM 1	*Enteromorpha*	P	3	2	1	Euhaline
2	-	T	1	2	2	Euhaline
3	*Enteromorpha*, *Chaetomorpha*, *Rhizoclonium*	P	3	3	2	Euhaline
4	*Enteromorpha*	P	4	3	3	Hyperhaline
5	*Enteromorpha*	T	2	1	2	Euhaline
6	-	T	1	2	2	Euhaline
7	*Enteromorpha*, *Chaetomorpha*	P	2	2	4	Hyperhaline
8	*Enteromorpha*	P	4	1	3	Euhaline
20	*Enteromorpha*, *Oscillatoria*, *Lyngbya*	P	4	2	1	Hyperhaline
21	*Enteromorpha*	P	2	2	2	Euhaline
14	-	P	3	1	2	Hyperhaline
31	*Enteromorpha*	P	1	4	2	Mixohaline
32	*Enteromorpha*, *Chaetomorpha*, *Cladophora*, *Rhizoclonium*, *Rhodochorton*	P	4	4	3	Euhaline
33	*Enteromorpha*	P	3	4	1	Euhaline
25	-	P	1	4	3	Mixohaline

Factors Affecting the Occurrence and Abundance of Algae in Supralittoral Pools:

Table X summarizes the ecological factors considered most relevant to the occurrence and abundance of algae in pools studied, as well as listing the algalgenera found in those pools. The information has been presented under the scales previously enumerated, and the Venice System has been used to classify the pools according to salinity as accurately as possible, remembering that all pools were poikilohaline.

1. Permanency:

Of the three temporary pools no filamentous algae were present at any time in two, and Enteromorpha was found in the third only during two months. By contrast filamentous algae were totally absent from only two permanent pools.

2. Exposure:

Only a single pool with an exposure value of 1 (least exposed) supported filamentous algae. The growth of Enteromorpha in this particular pool, 31, was stunted. On the other hand the pool with greatest variety of algae had an exposure index of 4, while the two pools which contained three genera of filamentous algae were both semi-exposed.(3)

Volume:

Algae were recorded from pools over all volume ranges, and were also absent from pools whose volume indices were 1, 2, and 4. However of the three smallest pools (volume 1), algae were never present in Pool 14, and were only once recorded from Pool 8, and twice from Pool 5.

Surface Depth Ratio:

There was no direct correlation between surface-depth ratio, alone, and the occurrence and abundance of algae in supralittoral pools.

Salinity:

No relationship was found between the salinity and the occurrence of Enteromorpha in pools. There was insufficient evidence to indicate whether other genera were limited by salinity.

Combination of Factors:

Pool 32 with high indices in every category: exposure 4, volume 4, surface depth ratio 3 supported more genera than any other pool. Pool 25, similar in dimensions to Pool 32 , but very sheltered and with lower salinity, did not support any filamentous algae. The only other permanent pool which did not contain filamentous algae at any time during the year, Pool 14, had a very small volume, while of the temporary pools algae appeared only in the most exposed of the three , Pool 5.

Conclusions:

The occurrence and abundance of filamentous algae in supralittoral pools is controlled in a complex way by a number of ecological factors, which are interwoven to produce a unique environment in each pool. Of these factors, permanency of pools and their exposure to the influence of the sea are the most important. In the smallest pools volume is a limiting factor.

Enteromorpha was found to tolerate a wide range of ecological conditions, but its greater prevalence in autumn and spring indicated that in summer and winter its distribution and abundance were limited by temperature.

3.3 The Fauna of Supralittoral Pools:

Introduction

The most extensive descriptions of fauna of supralittoral pools have come from Scandanavian ecologists (Levander, 1900; Gislen, 1930; Johnsen 1946.) Less detailed accounts have been presented by Shelford (1935), United States; Naylor and Slinn (1958), United Kingdom; and Cranwell and Moore (1938) and Ambler and Chapman (1950), New Zealand.

The ecology of supralittoral pool flagellates in some northern European pools was thoroughly examined by Droop (1953), and Faure-Fremiet (1948) investigated ciliates in some pools on the coast of France.

In this section the fauna of supralittoral pools along the South Island's east coast is examined and discussed in relation to the pools' ecology.

TABLE XI

DISTRIBUTION OF ANIMALS IN SUPRALITTORAL POOLS OF KAIKOURA, GOOSE BAY, TAYLORS MISTAKE AND DIAMOND HARBOUR

	Kaikoura		Goose Bay			Taylors Mistake								Diamond Harbour		
Pool	25	26	31	32	33	1	2	3	4	5	6	7	8	12	13	14
1. Parozoa (unidentified)				X												
2. Actinozoa																
Actinia tenebrosa Farquhar				X												
Anthopleura Duch and Mich				X												
Epiactus thompsoni (Coughtrey)				X												
3. Nemertea																
Lineus Sowerby				X												
4. Nematoda	X	X	X	X	X	X	X	X	X		X	X				
5. Tubificidae	X	X	X	X												
6. Polychaeta																
Galeolaria hysterix Morch				X												
Sabella Savigny				X												
7. Sipunculoidea																
Phascolosoma annulatum Hutton				X												
8. Amphineura																
Ischnochiton maorianus Iredale				X												
9. Gastropoda																
Scutus breviculus (Blainville)				X												
Cellana radians radians (Gmelin)													X			
Melarhaphe cineta (Quoy and Gaimard)				X	X			X	X	X		X	X			
Melarhaphe oliveri Finlay				X	X				X			X	X			

TABLE XI (continued)

	Kaikoura		Goose Bay			Taylors Mistake								Diamond Harbour		
Pool	25	26	31	32	33	1	2	3	4	5	6	7	8	12	13	14
Zeacumantus subcarinatus (Sowerby)				X	X											
Stiliger felinus (Hutton)				X	X											
Pleurobrachaea novaezelandiae novaezelandiae Cheesman				X												
10. Lamellibranchia																
Perna canaliculus (Gmelin)				X				X	X							
Mytilus edulis aoteanus Powel				X				X	X			X				
Aulacomya maoriana (Iredale)								X	X			X				
11. Ostracoda (Unidentified)				X	X			X								
12. Copepoda																
Tigriopus fulvus (Fischer)	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X
Amphiascus Sars	X	X	X	X	X											
13. Amphipoda																
Hyale grandicornis Kryer	X			X	X											
Paracorophium excavatum Thomson	X							X	X			X				
14. Decapoda																
Palaemon affinis Milne-Edwards	X	X		X	X											
Hemigrapsus edwardsii Hilgendorf	X	X		X	X	X		X	X		X	X				
Heterorozius rotundifrons Milne-Edwards				X												
Cancer novae-zealandiae Jacquinot and Lucas				X												
15. Hemiptera: Heteroptera																
Anisops assimilis Buchanan-White				X												
16. Diptera: Ephydridae																
Ephydrella novae-zealandiae Tonnoir and Malloch	X	X	X		X	X	X	X	X	X	X	X		X	X	X

TABLE XI (continued)

	Kaikoura		Goose Bay			Taylors Mistake								Diamond Harbour		
Pool	25	26	31	32	33	1	2	3	4	5	6	7	8	12	13	14
17. Diptera: Culicidae *Opifex fuscus* Hutton	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X
18. Ophiuroidea *Pectinura maculata* Verrill				X												
19. Ascidacea *Corella eymyota* Transted	X			X												
20. Teleostei *Tripterygion varium* (Forster)				X												

Results:

The distribution of animals in pools at Taylors Mistake, Diamond Harbour and Goose Bay and Kaikoura has been tabulated in Table XI.

From this table it can be seen firstly, that there were sharp contrasts between pools in the variety of animals they supported. Thirty two species, representing almost every major phylum in the animal kingdom were found in Pool 32, Goose Bay, while only three species, all arthropods were recorded from Pool 12, Diamond Harbour.

Secondly, certain groups of animals and species were present in almost all pools, e.g. Nematodes, Amphipods, *Tigriopsus fulvus*,(Copepoda) and the Dipterans *Ephydrella novae-zealandiae* and *Opifex fuscus*.

Thirdly a number of species were found only in pools in which water conditions most closely resembled those of the sea, i.e. in pools such as Pool 32, with large volumes and well exposed to the sea. Among these animals were sponges; anemones (*Actinia tenebrosa*, *Anthopleura* sp, *Epiactus thompsoni*); polychaetes (*Galeolaria hysterix*, *Sabella* sp);the sipunculid *Phascolosoma annulatum*; the nemertean *Lineus* sp, some decapods (*Cancer novae zealandiae*, *Heterorozius* rotun difrons); the notonected *Anisops assimilis* (Hemiptera; Heteroptera); the amphineuran *Ischnochiton maorianus*; the gastropoda *Scutus breviculus*, *Pleurobranchaea novae-zelandiae*, and *Stiliger felinus*; *Pectinura maculata* (Ophiuroidea); *Corella eumyota* (Ascidacea); and the cockabully *Tripterygion varium*.

Notes on the Ecology of Animals from Supralittoral Pools:

1. *Protozoa:*

Two species of flagellates appeared with remarkable regularity and in considerable quantities in supralittoral pools. They were *Oxyrrhis mariana* Dujardin, (Dinoflagellata, Pronoctilucidae) and a species of *Branchimonas* Bohlin (Phytomonadina, Chlamydomonadidae). Both these species occurred in pools at Lyttelton Harbour, Taylors Mistake, Goose Bay and Kaikoura. Species of *Euglena*, Ehrenberg were found in Pools 31, 32 (Goose Bay) and 25 (Kaikoura) but not in Taylors Mistake or Diamond Harbour pools. On occasions Pool 14, Diamond Harbour, was coloured dark green with myriads of a species of *Pyramimonas* Schmarda (Phytomonadina, Carteriidae). After the pool water had been disturbed by rough seas their numbers were reduced to a few individuals within a sample. *Pyramimonas* also appeared in Pool 5 (Taylors Mistake) in high density during August 1961, but was never found in numbers in any other pools.

Ciliates were present in all pools but were never as numerous as flagillates. Species of the Holotrichs *Amphileptus*, Ehrenberg, *Paramecium* Hill, and *Sonderia* Kahl occurred in pools of Banks Peninsula and the Kaikoura Coast.

Stylonychia Ehrenberg (Spirotrichia, Hypotrichia) were common amongst filamentous algae although they also thrived in Pool 25, which supported no filamentous algae.

2. Actinozoa:

Anemones were found in only two supralittoral pools, Pool 32 and Pool 34 at Goose Bay. In each pool there were no more than a dozen individuals, all of which were confined to the most shaded corners of the pools.

3. Annelida, Nemertea and Nematoda:

Tubificid oligochaetes were found in the largest pools; the exposed Pool 32 and the more sheltered Pool 31, 26 and 25, but not in any of the smaller pools. Two species were recognized from Pool 25, one of these species being that found in the other pools. Alsterberg's (1922) description of the respiratory behaviour of Tubificids was confirmed for these species. On several occasions large numbers of worms were seen oscillating their tails in frequent and rapid rhythmical movements. However when oxygen tension was very high or low these movements ceased.

The only polychaetes found in supralittoral pools were scattered individuals of Galeolaria hysterix and Sabella sp in Pools 32 and 34 (Goose Bay). Amongst the filamentous green algae in Pool 32 the nemertean Lineus sp was common but it was not seen in any other pools.

Nematodes were among the commonest aminals in supralittoral pools. Apart from the work of Wieser (1959) on nematodes of Puget Sound, littoral nematodes have received little attention and no systematic studies of New Zealand shore nematodes have yet been undertaken. The nematodes found in pools studied belonged to the Orders Chromodoridea and Enoploidea, but specific identification was not possible. Nematodes were found both in the bottom ooze of pools where oxygen content was low and amongst filamentous algae, regions of high oxygen tension. Different species of nematodes were associated with each species of alga, and several species were characteristically found in bottom ooze and detritus.

4. Mollusca:

Scutus breviculus and Pleurobranchaea novae-zelandiae were numerous in Pool 32, the only pool in which they were found. The small nudibranch Stiliger felinus was at times abundant upon the green filamentous algae of Pools 32, 33 and 34. Only a few individuals of S. felinus were seen in these Goose Bay pools in March, but they were present in numbers in May and most abundant in August, but by October their numbers had again dropped.

The only species not in Pool 32 which was found in but a single supralittoral pool was a solitary specimen of Cellana radians from Pool 8, Taylors Mistake. Although Pool 8 was among the smallest of supralittoral pools, it was splashed more than any other pool studied.

The lamellibranchs Perna canaliculus and Mytilus edulis aoteanus were also present in Pool 32, but it was more surprising to find them in the smaller supralittoral pools of Taylors Mistake as well. Aulacomya maoriana, Iredale P. canaliculus and M. edulis aoteanus first appeared in Pools 3 and 4 at Taylors Mistake in the third week of July after very heavy seas had been running. Individuals of all three species had been dislodged from lower down the shore and hurled into these supralittoral pools by wave action. Once within the pools these mussels quickly settled down upon their new substrate. P. canaliculus and M. edulis aoteanus in Pool 32 were two or three years old, but none of the mussels washed in to Taylors Mistake pools were more than two years old (I.H. McDonald, personal communication). Six individuals of A. maoriana, four of M. edulis aoteanus and three of P. canaliculus became established in Pool 4, while the corresponding numbers in Pool 3 were five, nine and two respectively. All three species survived in these pools for almost three months but by the second week of October all the A. maoriana were dead. A week later the last P. canaliculus died. Mytilus edulis aoteanus survived a further week. Records of the salinity of these pools during the three months are listed below.

TABLE XII

date	Pool 3		Pool 4	
	Surface	Bottom	Surface	Bottom
23/7	- °/oo	33.9 °/oo	- °/oo	33.6 °/oo
26/7	34.0	37.7	38.3	40.8
23/8	36.4	37.3	33.2	33.6
19/9	24.5	33.1	36.8	36.9
5/10	22.6	28.6	42.5	48.4
8/10	35.0	35.3	50.7	50.7
18/10	-	-	74.1	74.0
28/10	- °/oo	55.9 °/oo	38.9 °/oo	42.0 °/oo

During most of the period in which these species of mussel survived in the Taylors Mistake pools the water was euhaline. None of the species was able to survive the desiccation of Pool 3 or the hyperhalinity of Pool 4 during October.

Melarhaphe oliveri and Melarhaphe cincta:

Both these species of littorinids were frequently found in and around supralittoral pools. Investigations were carried out on the Melarhaphe population of two Diamond Harbour Pools, Pool 13 and Pool 15. The numbers of M. oliveri and M. cincta found in each pool are tabulated below, together with their numbers in an area of one square metre around each pool.

TABLE XIII

Numbers of Melarhaphe in and around Supralittoral Pools at Diamond Harbour - September, 1961

	M. oliveri	M. cincta
Pool 13	5	18
Around Pool 13	17	10
Pool 15	49	0
Around Pool 15	0	0

From this Table it can be seen that within Pool 13, which although not a truly permanent pool usually contained water, there were three times as many M. cincta as M. oliveri. But in Pool 15, which had water in it only after heavy rain or strong seas, only M. oliveri was present. There were no littornids in the square metre around Pool 15. The proportion of M. oliveri on the rock surface surrounding Pool 13 was very much greater compared with M. cincta than actually within the pool. The salinities of the two pools were similar.

Pool 13	Surface	$37.8^{o}/oo$
	Bottom	$37.8^{o}/oo$
Pool 15	Surface	$38.8^{o}/oo$
	Bottom	$38.8^{o}/oo$

so the differences in distribution of the two species were clearly not correlated with salinity.

These observations illustrated the patchiness with which both species of Melarhaphe were distributed in the supralittoral. Never at any time during the year did Pool 13 contain more M. oliveri than M. cincta and often only the latter species was found within the pool. At Taylors Mistake too, there were always fewer M. oliveri than M. Cincta in pools. Only in the most temporary pools, such as Pool 15, did M. oliveri predominate.

5. Crustacea:

Amphipods were present in most supralittoral pools. Hyale grandicornis was the commonest species in Pools 32, 33 and 34 (Goose Bay). Paracorophium excavatium was present in Taylors Mistake pools during autumn and spring but was rarely found in summer or winter. The common rock hopper Orchestia chiliensis Milne-Edwards was often seen around pool margins at Goose Bay and Kaikoura, as was the isopod Ligia novae-zealandiae Dana, although in much smaller numbers. In dry pools L. novae-zealandiae was observed foraging upon stranded and desiccated Opifex fuscus larvae.

Hemigrapsus edwardsii (Decapoda) was present in all the larger pools except the most stagnant: Pool 31 Goose Bay. It was only occasionally found in the smaller Taylors Mistake pools and never in hyperhaline pools.

Unquestionably the most ubiquitous macroscopic species in New Zealand supralittoral pools is the bright orange Harpacticid Copepod Tigriopsus fulvus. Not only was it in almost every supralittoral pool from Plimmerton to

Bluff, but it has also been found on the Snares Is. (Professor G.A. Knox, pers. comm.) and Campbell Is. (P.M. Johns pers. comm.) The life history and ecology, including the remarkable powers of survival of this cosmopolitan species have been investigated by Fraser (1935, 1936). A second Harpacticid Copepod, a species of Amphiascus Sars was commonly found among the filamentous green algae, although it did occur also in some pools in which there were no filamentous algae, e.g. Pool 25, Kaikoura.

6. Insecta:

Notonectidae:

It was somewhat surprising to find several individuals of Anisops assimilis in Pool 32 on October 15th. At that time this pool had a surface salinity of 35.7°/oo and a bottom salinity of 35.5°/oo. Notonectids have previously been recorded from brackish pools of lower salinity along Baltic coasts. Tillyard (1924) remarks that "all the New Zealand Notonectidae are carnivorous being especially partial to a diet of mosquito or other Dipterous larvae." It is possible that A. assimilis had been partially instrumental in controlling the population of Opifex fuscus in Pool 32. In March 1961 Pool 32 had a dense population of O. fuscus larvae and pupae of the order of 250 per litre. No A. assimilis were then found in the pool, but in October when this Notonectid was present the O. fuscus population was reduced to a very low level of less than one larva per litre.

Diptera Ephydridae:

Larvae of *Ephydrella novae-zealandiae*, a Cyclorrhaphan fly, were common in small shallow pools with mud silt or sand bottoms. Unlike *Opifex fuscus* larvae, those of *E. novae-zealandiae* withstood desiccation when pools dried up. In the laboratory several larvae survived a month in dry conditions. When pools became dry larvae sought refuge in rock crevices or by burrowing in the mud and sand on pool bottoms. Larvae remained active during these dry periods while their exoskeleton became progressively more wrinkled, but on immersion in water they soon regained their original shape. This ability to withstand desiccation has been an important contribution to the success of *E. novae-zealandiae* in a temporary environment.

Ephydrella novae-zealandiae is a multivoltine species, larvae and adults being present in every month of the year, though with varying abundance. Copulation was most frequently observed in September, October and November, when the level of the adult population was also at a peak. In February, March and April there was a second smaller maximum in adult numbers.

Conclusions:

In discussing the occurrence and abundance of algae in supralittoral pools it was concluded that the most important limiting factors were permanency of pools, their exposure to the influence of the sea, and in the smaller pools, volume. The fauna of supralittoral pools was found to be controlled by the same series of factors. Pools, such as 32, with the greatest variety of algae also had the most varied fauna. Few species of animals were to be found in the smallest pools, which were usually lacking in algae also. The fauna of the most sheltered pools such as Pool 31, Goose Bay, was restricted to a few of the most tolerant species. Tubificids, Nematodes, Tigriopsus fulvus and Opifex fuscus. Although the successful species in such pools were few, they were present in large numbers. At times the density of Tigriopsus fulvus in Pool 31 exceeded ten per cubic centimeter. Tigriopsus, Ephydrella and Opifex which thrived in the most stagnent and smallest pools, permanent and temporary, were usually less numerous in pools with a more varied fauna where they met greater competition. Only for one period during the year was there a high density of O. fuscus within Pool 32.

None of the factors controlling the biota of supralittoral pools are independent:- volume, permanency, exposure to the sea, temperature and salinity all bear some relation to each other. These and other factors interact to produce an inconstant and unique environment with each pool. Pools are oases of diversity within the supralittoral zone.

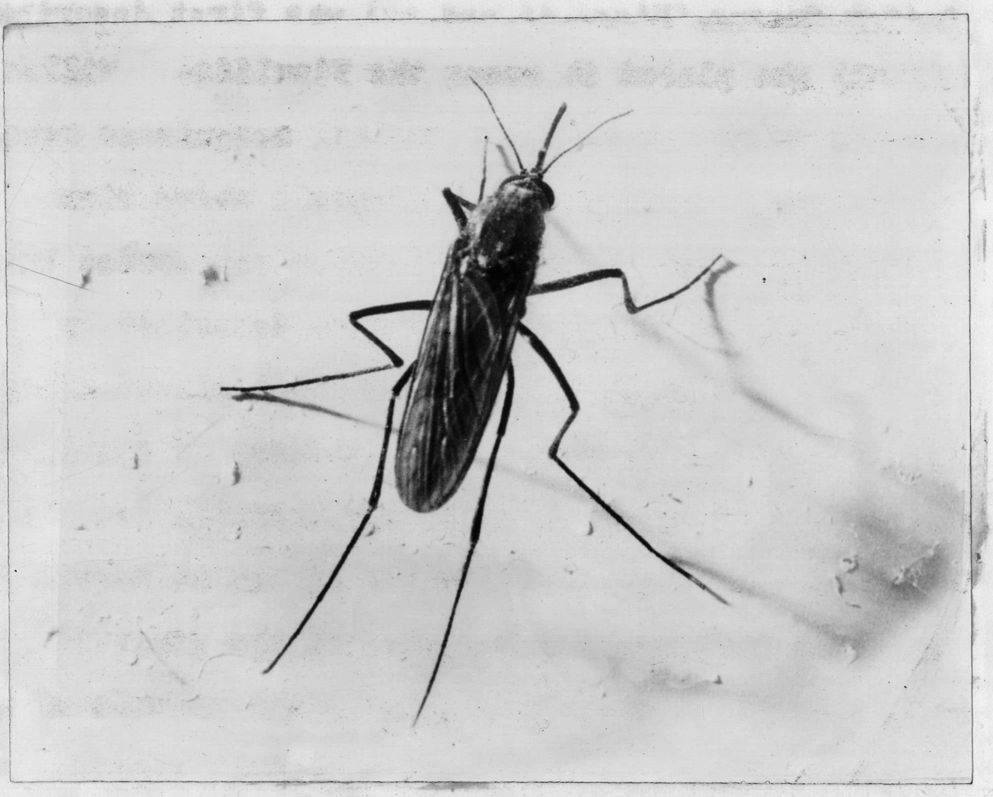


Fig. 41. <u>Opifex fuscus</u> adult. X 7.

CHAPTER 4

THE BIOLOGY OF OPIFEX FUSCUS HUTTON

4.1. Systematics

Opifex fuscus (Figs. 41 and 42) was first described by Hutton (1902) who placed it among the Tipulids. Miller (1920) in an investigation into New Zealand mosquitoes reported a common species, breeding in saline pools above high water mark. He presumed this species to be unrecorded but G.V. Hudson pointed out it had already been described by Hutton as a Tipulid. *Opifex fuscus* was first placed among the Culicine mosquitoes by Edwards (1921) after an examination of Hutton's types. Edwards found *Opifex* difficult to place in relation to the other genera of Culicini but it seemed nearer to *Aedes* than *Culex*. A revision of the generic characters was made by Miller (1922) who on the grounds of the remarkable characters of the adult and pre-adult stages erected a new sub family, Opificinae, for this one species. In a further account of *O. fuscus* Edwards (1924) considered that the creation of the sub family Opificinae by Miller was unjustified, and it has not been recognized by subsequent authors. Edwards (1932) in a later review held that *Opifex*, apart from its secondary sexual characters, did not differg greatly from *Aedes*, and concluded that it "may well be included in the same group of genera."

The now accepted systematic position of *Opifex fuscus*

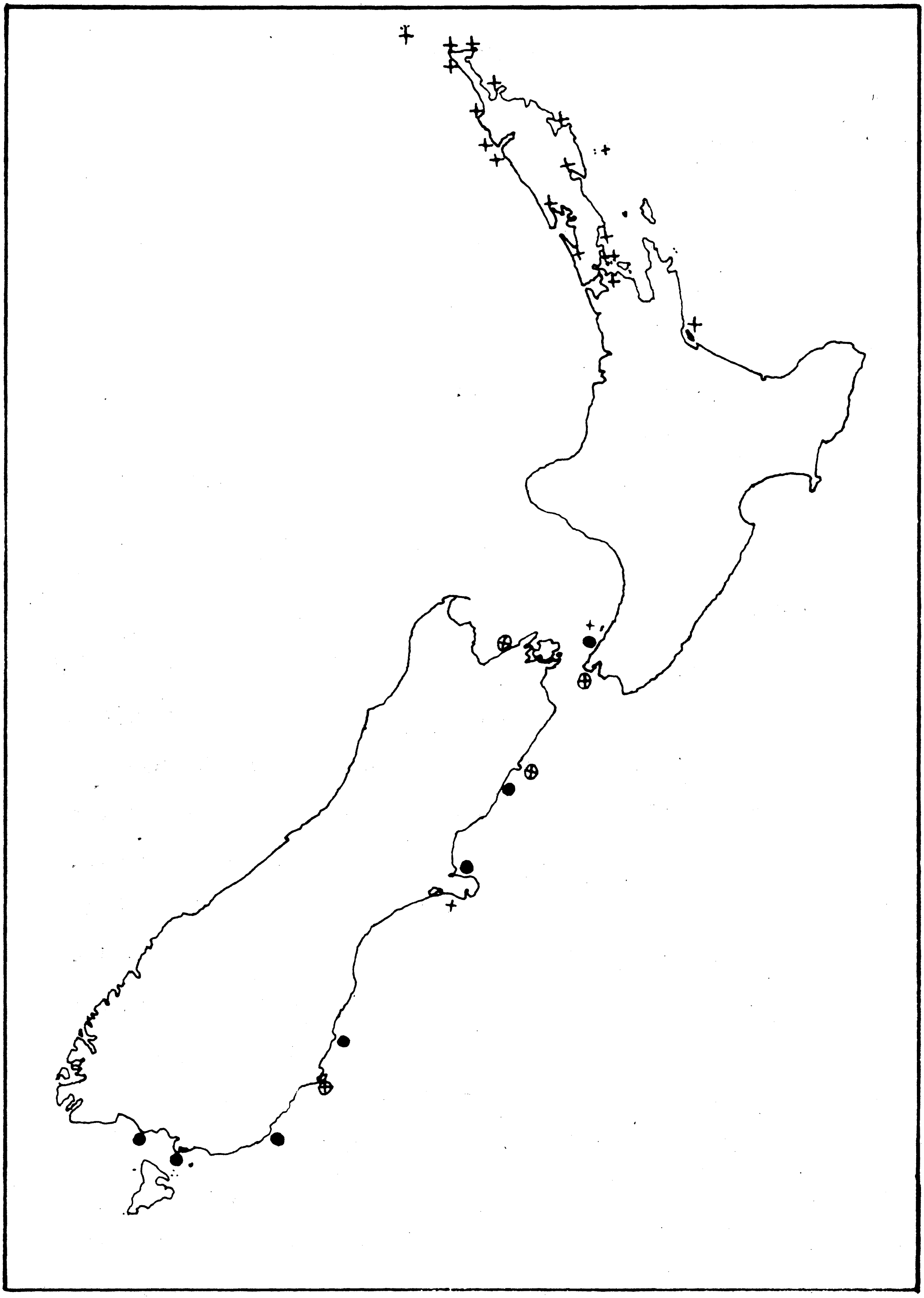


Fig. 43. New Zealand, showing distribution of Opifex fuscus.
Scale: 1;6,000,000.

● New records made in the course of this study.
⊕ Published records confirmed during this study.
\+ Published records.

is as follows:-

Phylum: Arthropoda
Sub-phylum: Insecta
Family: Culicidae
Sub-family: Culicinae
Tribe: Culicini
"Group": *Aedes*
Genus: *Opifex* Hutton 1902
fuscus Hutton 1902

4.2. Distribution:

Opifex fuscus is the only species of mosquito endemic to New Zealand. Previously *O. fuscus* was known to occur from North Cape (Graham, 1939) to Otago Peninsula (Marks, 1958) along the coast in pools above high water mark. It has also been reported from Three Kings Islands, (Marks, 1958), Poor Knights Island (Cranwell and Moore, 1938), Hen Island, Kapiti Island, (Dumbleton 1962) but not from Chatham Islands, Snares Island, Campbell Island or Auckland Islands. The distribution has now been extended south of Otago Peninsula to Bluff and Oraka Point (Colac Bay), Foveaux Strait, and new records have also been made along the east coast of the South Island. A map of the distribution of *O. fuscus* has been compiled from data provided in previous works as well as collections made in the course of this study (Fig. 43).

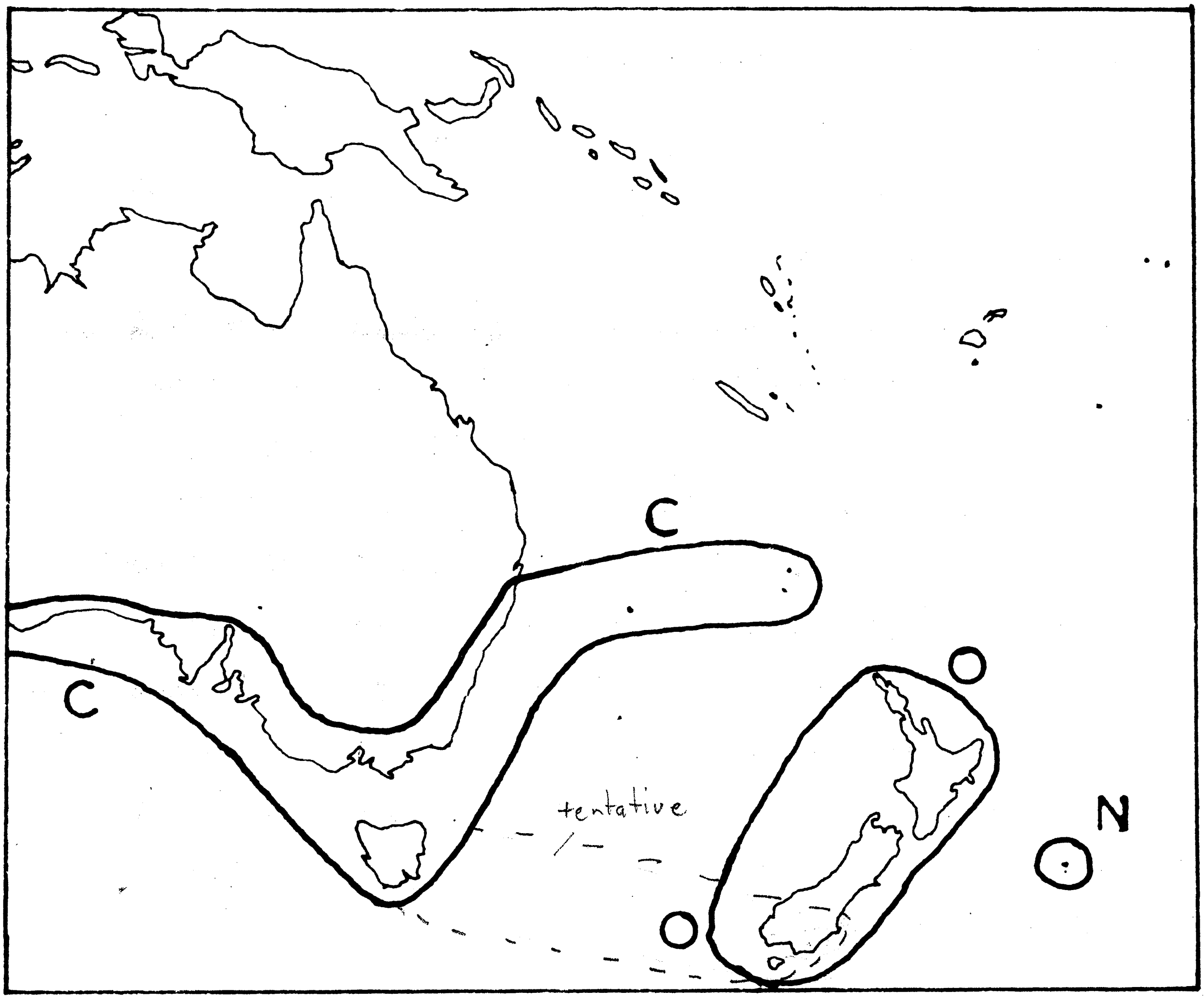


Fig. 44. Australasia, showing the distribution of <u>Opifex fuscus</u> and closely related groups.

O :- Distribution of <u>Opifex fuscus</u> (New Zealand).

N :- Distribution of <u>Aedes</u> (<u>Nothoskusea</u>) <u>chathamiscus</u> (Chatham Islands).

C :- Distribution of the "<u>Caenocephalus</u>" species group of <u>Aedes (Pseudoskusea)</u>.

1. <u>A. (P.) australis</u> (New South Wales Tasmania, Norfolk Island, Lord Howe Island).
2. <u>A. (P) ashworthi</u> (West Australia).

Distribution in relation to <u>A. australis</u> needs to revised. ~~[illegible]~~

DD McGregor.

The Relationships of *Opifex fuscus*:

The relationships of *Opifex fuscus* and the New Zealand culicine fauna in general have been recently discussed by Dumbleton (1962).

The closest affinities of *O. fuscus* are with *Aedes* (*Nothoskusea*) *chathamicus* which occupies a similar niche to *Opifex* on the Chatham Islands, and the "*Caenocephalus*" species group of *Aedes* (*Pseudoskusea*) which ranges from coastal New South Wales, Tasmania, Norfolk Island and Lord Howe Island (*australis*) to West Australia (*ashworthi*) Dumbleton 1962), (Fig 44). Neither *Opifex*, nor any close relative has so far been recorded from South America. Of the New Zealand culicine fauna, only *Aedes (Ochlerotatus)* shows even slight faunal affinity with South America. (Dumbleton, 1962).

The relationships and evolutionary position of *O. fuscus* will be further discussed after the morphology of larval mouthparts has been considered, but it is clear from published work that the relationships of the New Zealand culicine fauna, in general, and *Opifex fuscus* in particular are consistent with an origin and distribution from the north and west.

4.3. The Mating Behaviour of *Opifex fuscus*.

Kirk (1923) reported a long series of observations he had made on the peculiar mating habits of *Opifex fuscus* both in the laboratory and out in the field. He described how the very active adult males dart about on the pool surface after rising pupae. The male uses his anal forceps to capture the pupa and the puparium is ruptured along one side of its frontal ridge. Movement of the anal forceps extends the slit backwards. If the young imago is a female connection with the male is effected before her abdomen is clear of the puparium.

Reference was also made by Kirk to the struggle which may take place when a number of males compete for a single pupa. When there were swarms of males upon pools such struggles occurred over every pupa. The male which made the original capture was submerged by his competitors which often numbered up to a dozen. Sometimes a second male wrested a pupa from its original captor. On other occasions pupae made clear escapes while males battled on. If after approximately ten seconds a captor male still remained in possession of the captured pupa, the thwarted males retired to search for other pupae. Then the victorious male, grasping the frontal ridge of the puparium firmly with his anal forceps, would stride jerkily across the pool dragging his passive prize through the water behind. Emergence of the head, followed by the thorax of the young imago, proceeded with the puparium still just below the water surface. When this was completed the male often ceased active movement as Kirk observed, and rested upon the pool shore just above the water surface as the slow steady emergence of the abdomen from the puparium continued. However on occasions the emergence of the young imago was completed before the male left the pool surface. Emergence was completed any time between five minutes and thirty minutes after capture. Although the male was active in rupturing the puparium it did not assist the emergence of the young imago until connection was established, usually some time before the abdomen was fully clear of the puparium. As emergence began the head of the young imago faced in the same direction as that of the captor, but the plane of the emerging body was inclined at almost 45° from the longitudinal axis of the male. By the time emergence had been completed the two adults were in an end to end position with heads facing in opposite directions. After having emerged the young imago darkened in colour so that within two or three minutes its body was the brownish black of a mature adult.

Connection, which was established before emergence was completed, lasted only a few minutes with some pairs, but up to fifty minutes with others. Commonly connection was broken about twenty minutes after establishment. Establishment of connection was never seen between two adults, although Kirk once observed this when he placed a male and female within a glass phial. As soon as connection was broken the male would fly off. The female would sometimes fly too, but usually it rested for a few minutes before moving. Females were capable of flight within two minutes of completion of emergence. This was verified by disturbing young imagines.

Males always emerged without assistance and females were quite capable of doing so, but rarely had the opportunity in the field. Active males would sometimes mistake a male pupa for a female but on discovering their error would release the pupa as Kirk remarked, "with, one could imagine, an expression of disgust."

Biological Significance of the Mating Behaviour of Opifex Fuscus:

Within the supralittoral pool environment Opifex fuscus is exposed to several hazards, the most serious of which is drying up of pools. A second threat is the washing out of pools during rough weather. For an animal living in a temporary environment the conservation of time in the completion of its life cycle is of paramount importance. The significance of the mating behaviour of O. fuscus lies in the reduction of the time between maturation of the female mosquito, and its fertilization, to an absolute minimum. As there was no evidence to indicate that the sex ratio of O. fuscus departs radically from unity, the competition between males for females was not an expression of the inequality of the sexes, but rather a hypertrophy of the male sex instinct which ensures rapid and efficient fertilization of the available females, so that there is minimal wastage of reproductive potential within populations.

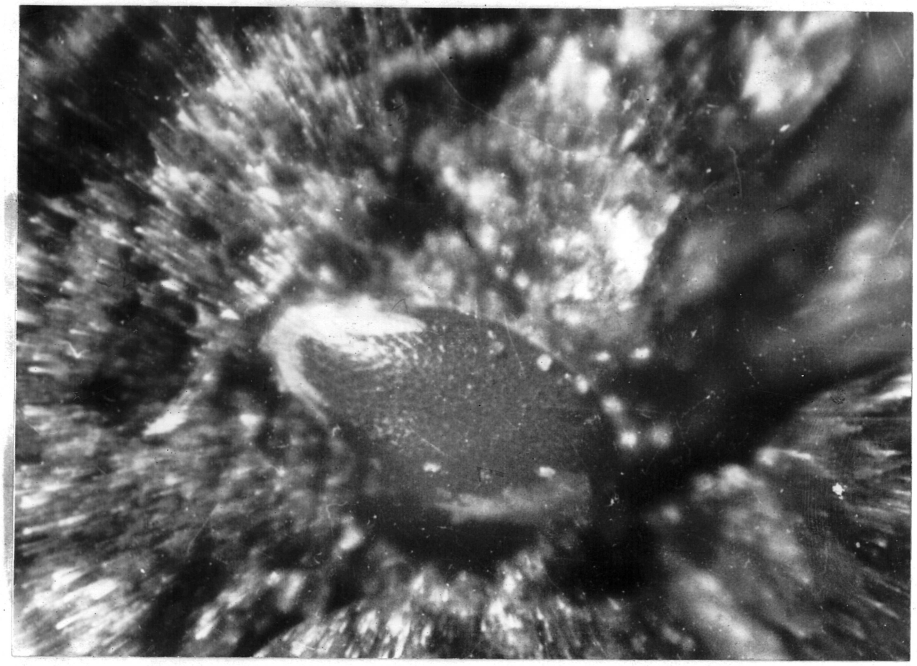


Fig. 45. <u>Opifex fuscus</u> egg upon rock surface. X65.

4.4. Oviposition:

For the deposition of their eggs (Fig. 45) female Opifex select a suitable moist spot around the pool margin. Around the shore of each pool there is a damp zone which may be anything between one to five centimetres in width. This band is maintained in a moist condition by splashing and washing from the pool water and by capillary action. Flying females may alight directly upon this zone, or they may land on the water below, or the rock surface above and walk onto it. Once on this moist strip they begin searching for a suitable place to lay their eggs. Small cracks and crannies or rough patches of rock are preferred. If none is available in the immediate vicinity the females may fly off to other parts of the same pool or other pools, or they may deposit their eggs upon smooth sites, especially if the population density is high and competition for egg-laying sites keen. The eggs are not exclusively laid on solid rock faces. Females have been observed depositing eggs on small stones and pebbles, pieces of wood, shells and even filaments of Enteromorpha. The essential criterion for oviposition sites is that they be moist.

Having selected a suitable site the female moves down so that her hind legs are must at the edge of the pool. These legs are then extended while the front legs are slightly relaxed. The head is then close to the substrate with the antennae and often the proboscis moving rapidly over the surface, presumably in search of the most suitable site for deposition of the eggs. Then the abdomen is arched under the thorax so that its tip is just below the head. The rapid vibration of the abdomen which accompanies this movement of antennae and proboscis now ceases as its tip is applied to the substrate. This is followed by a slow relaxation of the abdomen as the egg is expelled. The whole process may be repeated at the same site, as many as six or eight eggs being laid in quick succession adjacent to each other. On the other hand the female may move off to some other site to continue her egg laying.

Each egg is covered by a sticky secretion by which it adheres to the substrate or to adjacent eggs if they are laid close together. This secretion is variable in its effectiveness, some eggs, especially those on smoother surfaces where the area of attachment is small, being easily dislodged, others being difficult to move without imparting damage. It is thus not uncommon to find eggs floating on the pool surface or lying loosely on the mud and sand of dried up pools.

When the eggs are laid they are, as Wood (1929) noted, almost white, but they darken on exposure to light. This darkening process may take several hours if the eggs remain in the shade. An accurate description of the structure of O. fuscus eggs has been provided by Wood (1929) who figured the egg and described the hatching process.

4.5. The Hatching Mechanism of *Opifix fuscus* Eggs.

Gjullin et al (1941) and independently, Gander (1951) demonstrated that hatching of eggs of various species of aedine mosquitoes was induced by a lowered dissolved oxygen level of the flooding medium. These results have been extended by others who have indicated that hatching of multivoltine species can be brought about by biological (i.e. bacterial respiration), chemical or physical removal of dissolved oxygen. Judson (1961) demonstrated that decreasing oxygen tension is much more effective in stimulating hatching than is a similar but constant concentration. Borg and Horsfall (1953)and especially Horsfall (1956) have emphasized the importance of conditioning, (the preflooding exposure of eggs to specific environmental conditions) in readying aedine eggs to hatch on flooding.

Properly conditioned *Opifex fuscus* eggs hatched between twelve and twenty four hours of flooding. If the eggs had not been subject to specific environmental conditions necessary for conditioning they failed to hatch on submergence. When *O. fuscus* eggs were flooded by a solution in which dissolved oxygen was maintained at a high level by pumping compressed air through the medium, none hatched. Twelve of twenty eggs kept out of water for six months hatched within twenty four hours of flooding.* The remaining eight failed to hatch.

These results indicate that the hatching mechanism

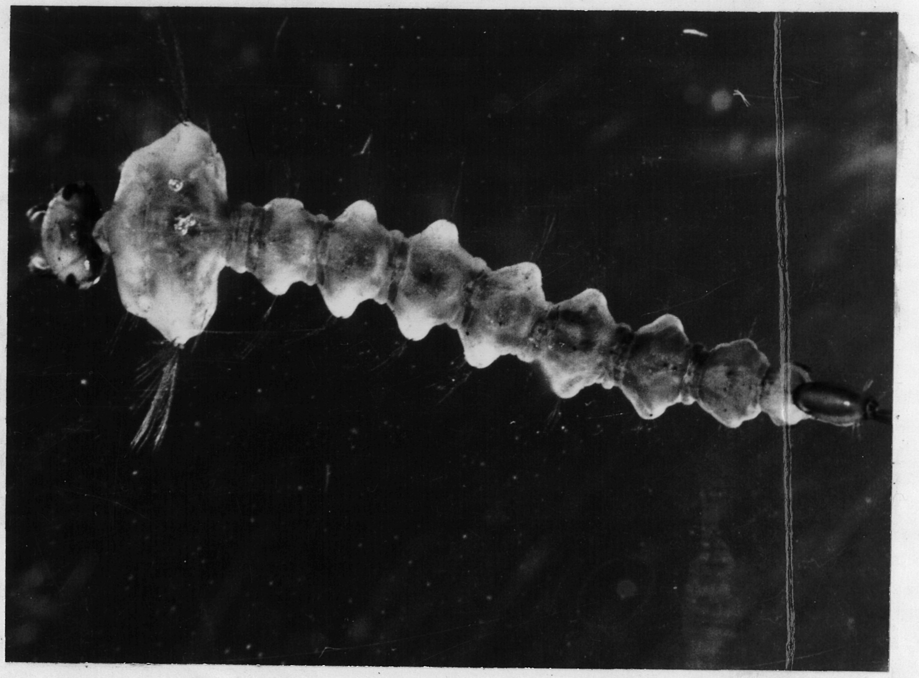


Fig. 46. Opifex fuscus, fourth instar larva. X15.

for O. fuscus eggs is similar to that for other aedine mosquito eggs. Opifex fuscus eggs are able to remain quiescent for long periods. This is most advantageous for an animal occupying supralittoral pools which are frequently dry.

4. 6. The Larval Ecology of Opifex fuscus

Introduction

Opifex fuscus like all mosquitoes has four larval instars. The larvae (Fig 46) are aquatic, metapneustic and very active. The morphology of O. fuscus has been examined by Edwards (1921, 1930, 1932), Miller (1922), Wood (1929) and Dumbleton (1962). In this study aspects of larval morphology most intimately related to the central theme, the relationships between Opifex fuscus and its environment, were investigated. Whereas previous work has been upon individuals, this thesis is mainly concerned with populations of O. fuscus.

4. 6.1 Population Studies

One of the many striking features of the biology of Opifex fuscus is the high density with which larvae may occur. Pools at Taylors Mistake were selected as sites for detailed investigation of the population dynamics of the species. These pools had two main advantages for such a study. Firstly they were easily accessible and could be visited daily if need be. Secondly they were small and hence fluctuations could be detected more readily and with greater certainty than in larger pools.

Sampling Methods:

No population studies of mosquitoes in supra-littoral pools had been previously undertaken. Various sampling methods were considered in relation to the problems involved in population studies in this particular environment.

1. Direct counting:

This is the most reliable method to employ in population surveys, but was impracticable when dealing with larval O. fuscus populations for several reasons. The chief difficulties were movement of larvae and the high densities with which they frequently occurred. It was also difficult to differentiate between instars without examining each larva individually. However direct counting was successful for pupae, as they did not move as frequently as larvae, nor did they occur in such numbers. Direct counting of adults upon pool surfaces provided a valuable index of the level of adult populations.

2. Marking of Larvae:

A widely employed method of estimating animal populations involves the capture, marking, release and recapture of a proportion of a population. The most popular variant of this technique is the "Lincoln Index" method. With insects such as O. fuscus in which the duration of instars may be as short as two days it is a problem finding a suitable method of marking. External marking by amputation, labelling, spot marking, dust and spray pigments or stains, was unsuitable because of the short time between moults. Internal marking appeared to offer more hope. But feeding larvae on stained or coloured food was not a satisfactory method as the gut contents were shed with the peritrophic membrane every twenty four hours. Serious consideration was given to marking larvae with a radioactive tracer. Baldwin, et al. (1955) had considerable success in population studies of several species of mosquitoes by labelling larvae and pupae with P_{32}. However Welch (1960) drew attention to some of the limitations inherent in this method: a large number of larvae should be tagged; the experiment must be of short duration otherwise the whole population becomes contaminated; and the technique in general is more satisfactory in permanent or discrete pools. Moreover Baldwin et al. were dealing with univoltine populations. The application of this method to multivoltine populations such as <u>Opifex fuscus</u> would introduce considerable complications both in sampling, and mathematical treatment of data. It was evident that even if a sophisticated modification of the methods of Baldwin et al. was used, there could be no guarantee that an adequate interpretation of the population dynamics of <u>O. fuscus</u> could be obtained from the data collected.

3. <u>Dipping</u>:

Dipping is a standard method of sampling populations

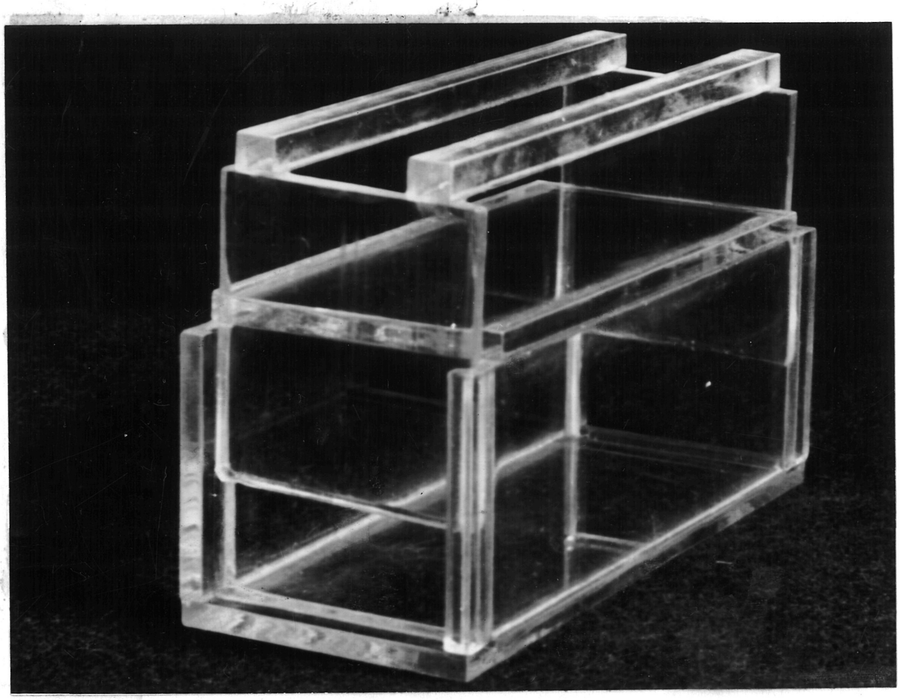


Fig. 46a. Box for volume sampling of larval populations.

of mosquito larvae. However Horsfall (1946) has found that larval densities determined by this method deviate with observers, and often comparable densities may appear divergent when recorded by the same observer in different places. For these reasons dipping was not employed as a method of determining O. fuscus larval densities.

4. Area and Volume Sampling:

Horsfall (1946) developed a technique of area sampling of mosquito larvae populations. Subsequently Bidlingmayer (1954) and Welsh and James (1960) introduced modification and improvements to Horsfall's method. The area sampling principle makes use of the reaction of mosquito larvae to disturbances. Most Aedes, Culex and Anopheles larvae dive to the bottom of a pool when disturbed and then slowly rise to the surface again. When a cylinder is dropped into a pool it encloses these larvae within a given area. The number of larvae caught may be used as a measure of density.

Unfortunately the irregular bottom of most supralittoral pools precluded the use of such an area sampling device with O. fuscus populations.

This difficulty was overcome by developing a device which would sample a given volume of water rather than an area of bottom. A box of the following dimensions was constructed: 11 x 4.5 x 4.5 centimetres (see Fig. 46a). The box was open at both ends, but could be closed quickly by inserting two sliding doors into grooves at each end. The entire construction was of perspex, so that the larvae and pupae would not be disturbed by any change in light intensity during sampling.

For a sample the open box was carefully inserted into the pool with as little disturbance of the water as possible. Unlike most mosquito larvae <u>O. fuscus</u> do not suspend themselves from the surface for long periods, but are distributed throughout a pool. Also they react differently to disturbance. Instead of diving to the bottom they move away from the centre of disturbance in all directions. With practice the box could be inserted into a pool without noticeably disturbing the larvae or pupae. After the box had been placed in the pool with a minimum of disturbance the larvae and pupae within the volume of the box were enclosed by quickly sliding the doors down. Then the box and its contents were removed from the pool and each larva and pupa within this fixed volume counted and its instar group noted. The number of larvae and pupae within the box gave a measurement of density. Owing to the time involved in counting and placing larvae and pupae in instar groups it was not possible on any one occasion to undertake a number of samples sufficient for subjection to rigorous satistical analysis. Nevertheless by combining data from several successive sampling periods, statistical techniques could be applied. The volume sampling technique provided a useful and reliable indication of population trends within, and contrasts between, pools. The

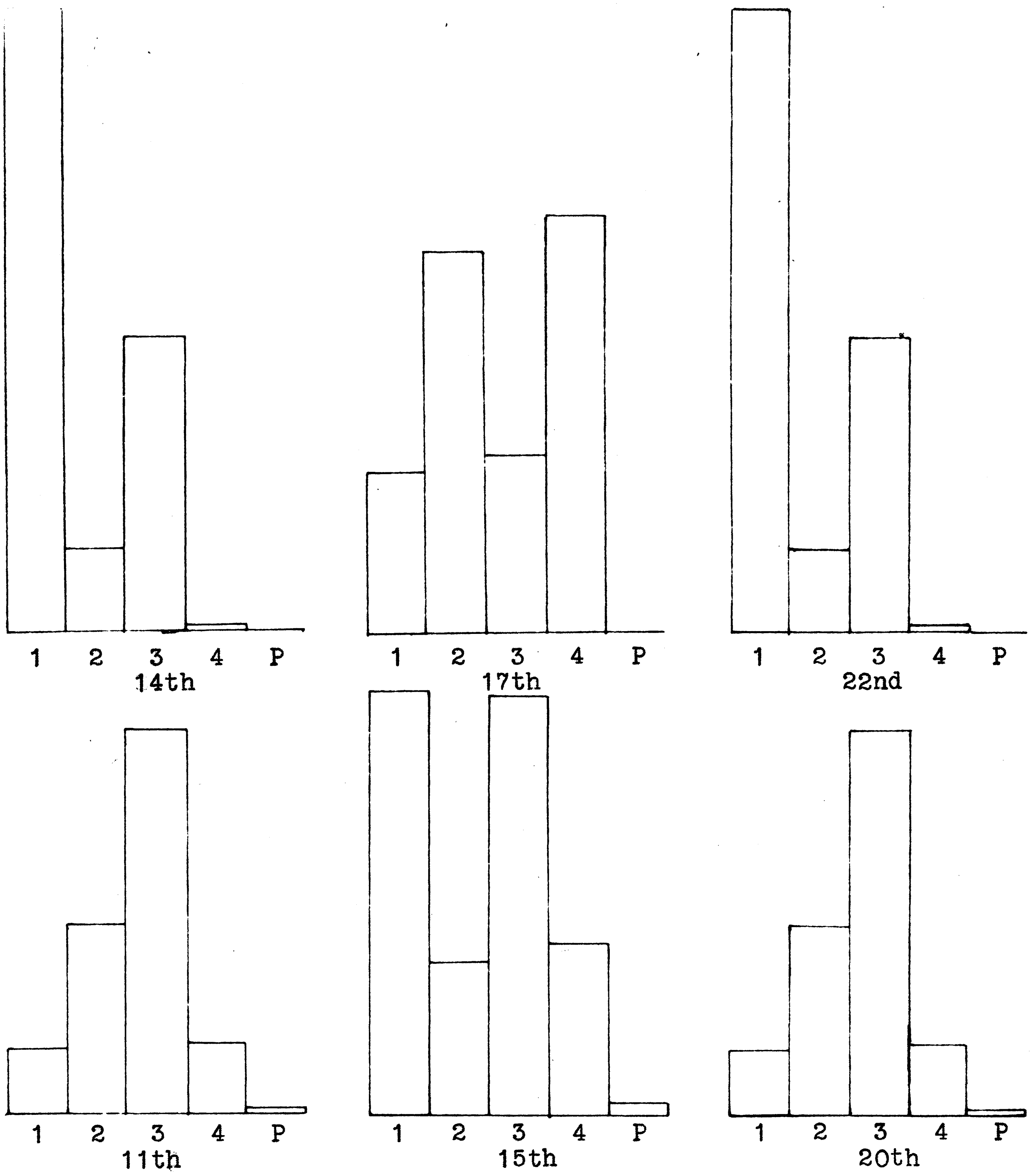


Fig. 47. Larval and pupal population densities in pool 2, Taylor's Mistake, February 11th to 22nd, 1961.

One centimetre on the vertical scale represents ten individuals. Each histogram records the number of individuals per volume sample (223 millilitres). 1,2,3, 4, and P represent the successive larval instars and pupal phase.

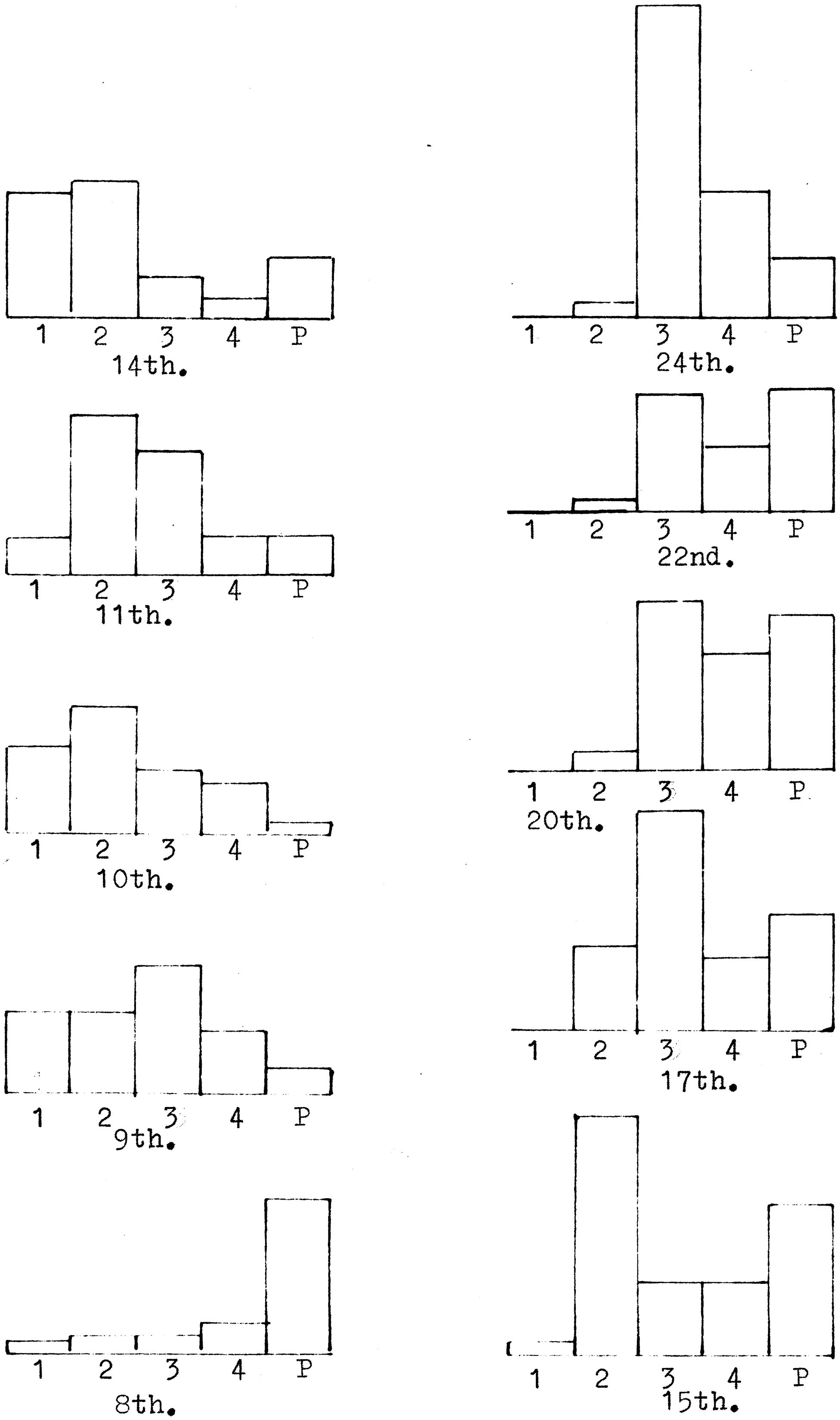


Fig. 48. Larval and pupal population densities in pool 3, Taylor's Mistake, February 8th to 24th, 1961.

population structure and dynamics of Pools 2, 3 and 4 at Taylors Mistake were investigated in detail during February and March, 1961, by volume sampling.

Results:

Histograms of data obtained from volume samples of the larval and pupal populations of Pools 2, 3 and 4 at Taylors Mistake between February 8th and February 24th, 1961 are presented in Figs. 47, 48 and 49.

All the histograms were drawn to the same scale.

This series of histograms illustrate the differences in the densities of the O. fuscus populations within the pools. Detailed comparisons between the population densities of both Pools 2 and 3, and Pools 3 and 4 for the months of February and March are given in Tables XIV and XV.

TABLE XIV

Comparison of Population Densities of Pool 2 and Pool 3
February - March 1961

No. of Samples	Mean	S^2	S_x
I Pool 3			
17	68.00	574.25	- 5.81
II Pool 2			
11	113	4826.7	24.563

"t" = 4.8376

df = 26

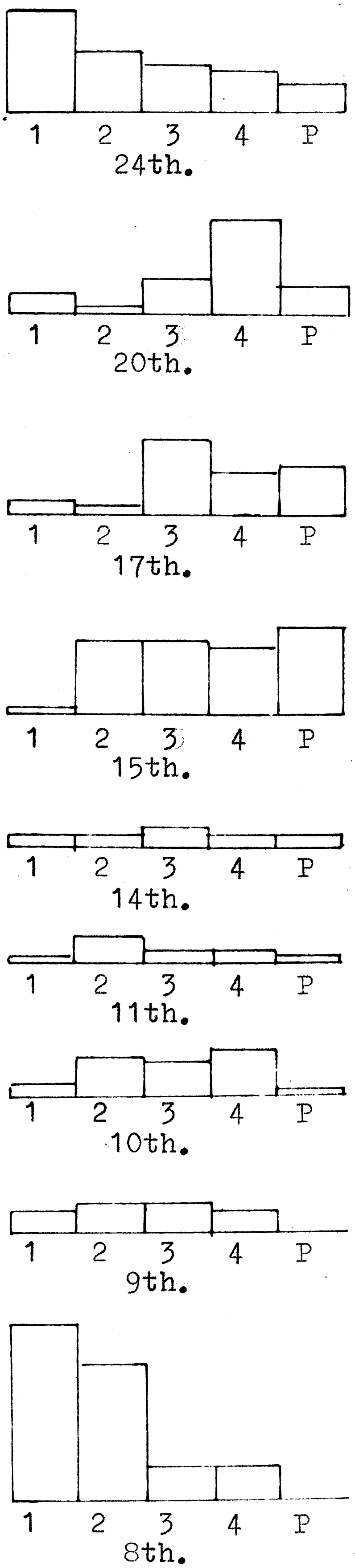
Significance probability less than 0.001

i.e. Difference between population densities highly significant.

S^2 = Sample estimate of the variance of population

S_x = Standard error of mean.

df = degrees of freedom.



Scale and legend as for Figs. 47 and 48.

Fig. 49. Larval and pupal population densities pool 4, Feb. 8-24th.

TABLE XV

Comparison of Population densities of Pools 3 and 4

February - March 1961

Pool	No. of Samples	Mean of Samples	S^2	S_x
3	17	68.00	574.25	- 5.81
4	18	24.94	251.706	- 3.739

"t" = 47.203

df = 33

Significance probability less than 0.001

i.e. Difference between population densities highly significant.

From these tables it emerges that differences in population density between the pools were highly significant. Apart from differences between the mean densities of each pool there were also equally striking contrasts in the relative densities of the various instars, both between pools and within each pool over a number of days.

The most substantial changes in density of first instar larvae occurred in Pool 2. This was found to be associated with the water level within the pool. On the 11th February when the density of first instar larvae was low Pool 2 was very nearly dry. But following rain between the 11th and 14th the pool became filled with water. The high density of first instar larvae following the rise in pool level was evidence of an association between submergence of eggs and their hatching mechanism. As the level of Pool 2 fell again after February 14th so did the density of the first stage larvae in samples, despite the concentrating effect of a decrease in pool volume.

Similarly in Pool 3 between February 11th and 14th there was a rise increase in the density of first instar larvae coincident with a rise in pool level. However the density increment was not as extreme as that in Pool 2.

Emerging from this consideration of changing 1st instar density is the fluctuating population structure of the *O. fuscus* as a whole. Even allowing for sampling error, the histograms reveal rapid changes in the relative proportions of the instars. Subsequent laboratory determinations of growth rates lead to fuller understanding of the significance of these fluctuations. The fluctuations have two main causes -

1. Irregular hatching of eggs.
2. Unequal duration of instars as revealed by growth rate studies.

The truncation of Pool 2 population histograms at later instars and the pupal phase can be ascribed to mortality due to the desiccation of the pool. Commonly only earlier instars were represented in temporary pools. In these pools the life cycle of *O. fuscus* only rarely proceeded to completion and tremendous wastage of breeding potential resulted.

Table XVIpresents comparisons of different sampling localities within the same pools.

TABLE XVI

Comparison of Population in Parts of Same Pool

1. Pool 3a South End and Pool 3a East End.

Pool	No. of Samples	Mean of Samples	S^2	S_x
3 South End	15	63.4	1069.286	8.45
3 East End	9	50	762.5	3.2044

t = 3.094
df = 22

Significance probability lies between 0.01 and 0.001 i.e. Difference between population densities is highly significant.

2. Pool 3 South End Pool 3 East End

Pool	No. of Samples	Mean of Samples	S^2	S_x
3 South end	17	68	574.25	5.81
3 East end	10	53.6	416.22	6.45

t = 1.5888
df = 25

Significance probability lies between 0.2 and 0.1 i.e. Difference between populations is only just significant

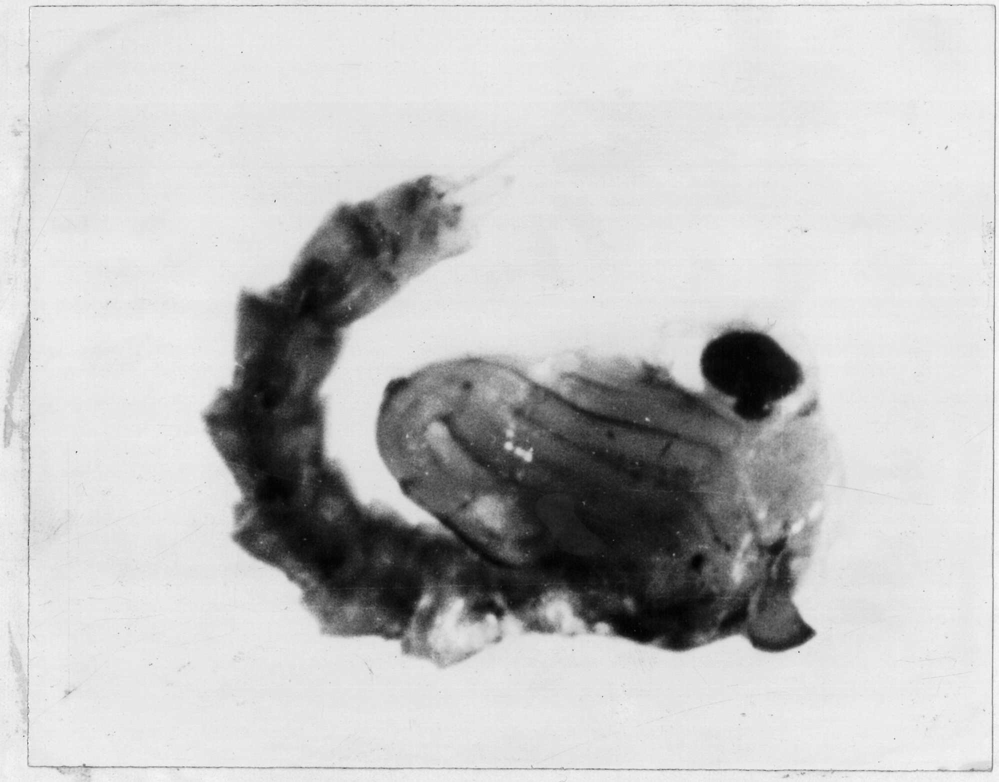


Fig. 50. Opifex fuscus pupa. X16.

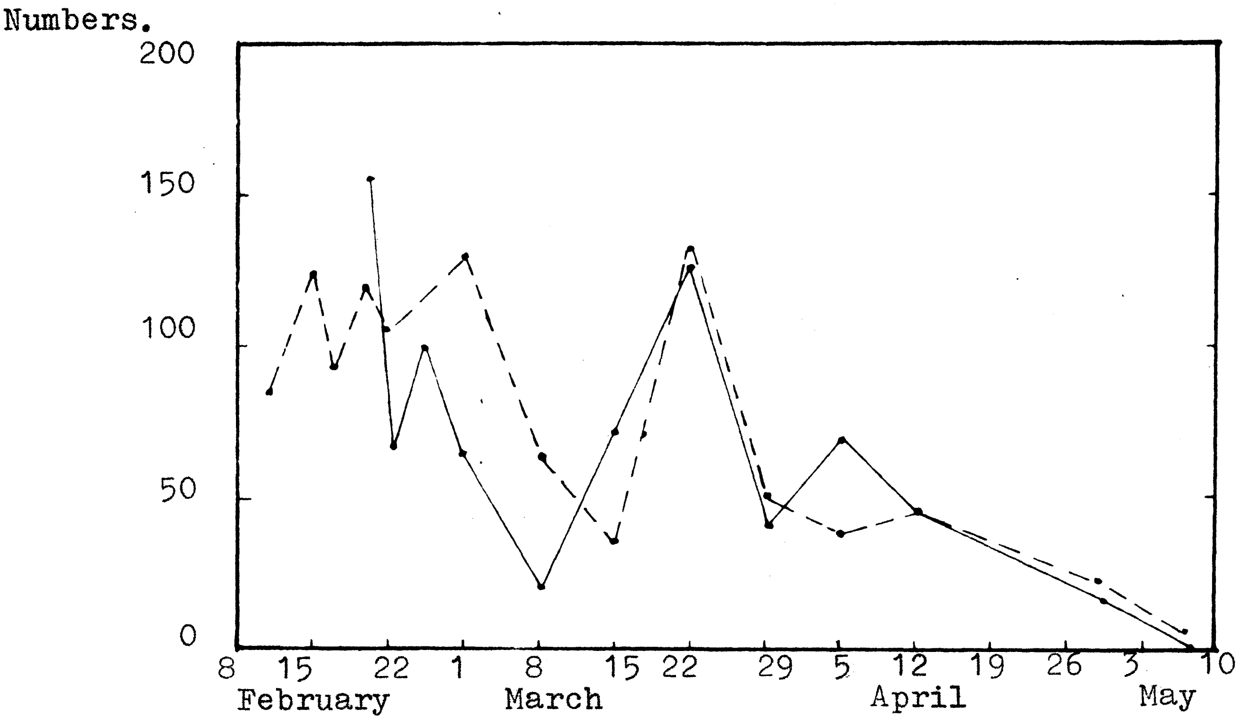


Fig. 51a. Fluctuations in pupal and adult populations pool 3, February to May, 1961.

— — Adult numbers.

——— Pupal "

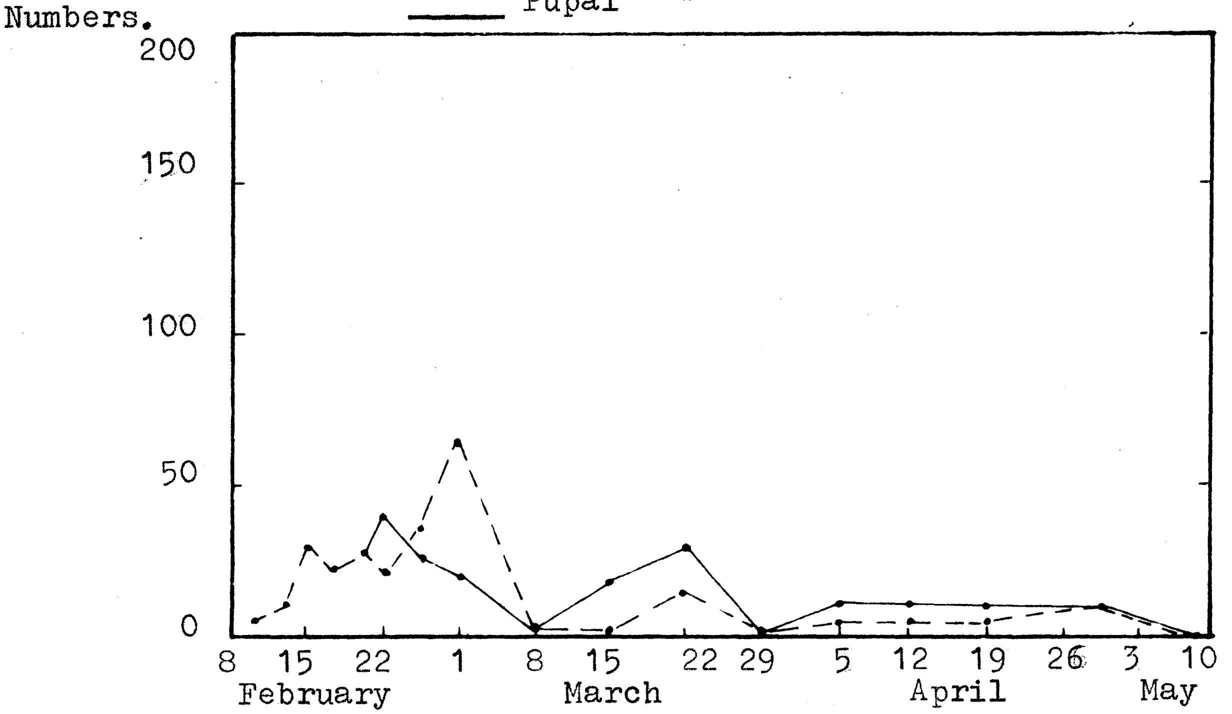


Fig. 51b. Fluctuations in pupal and adult populations pool 4, February to May, 1961.

— — Adult numbers

——— Pupal "

OPIFEX FUSCUS POPULATIONS

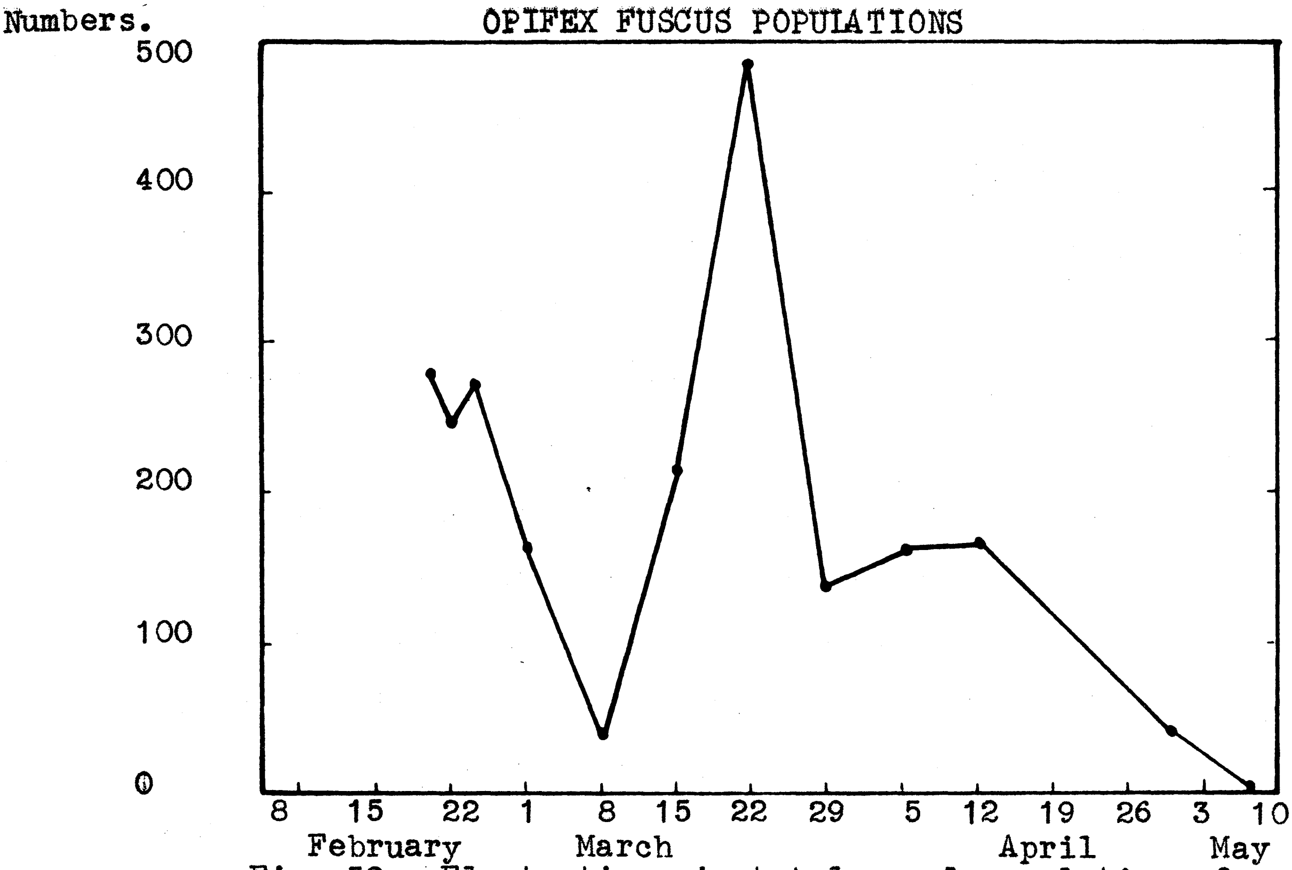


Fig. 52a. Fluctuations in total pupal populations from all Taylor's Mistake supralittoral pools, February to May 1961.

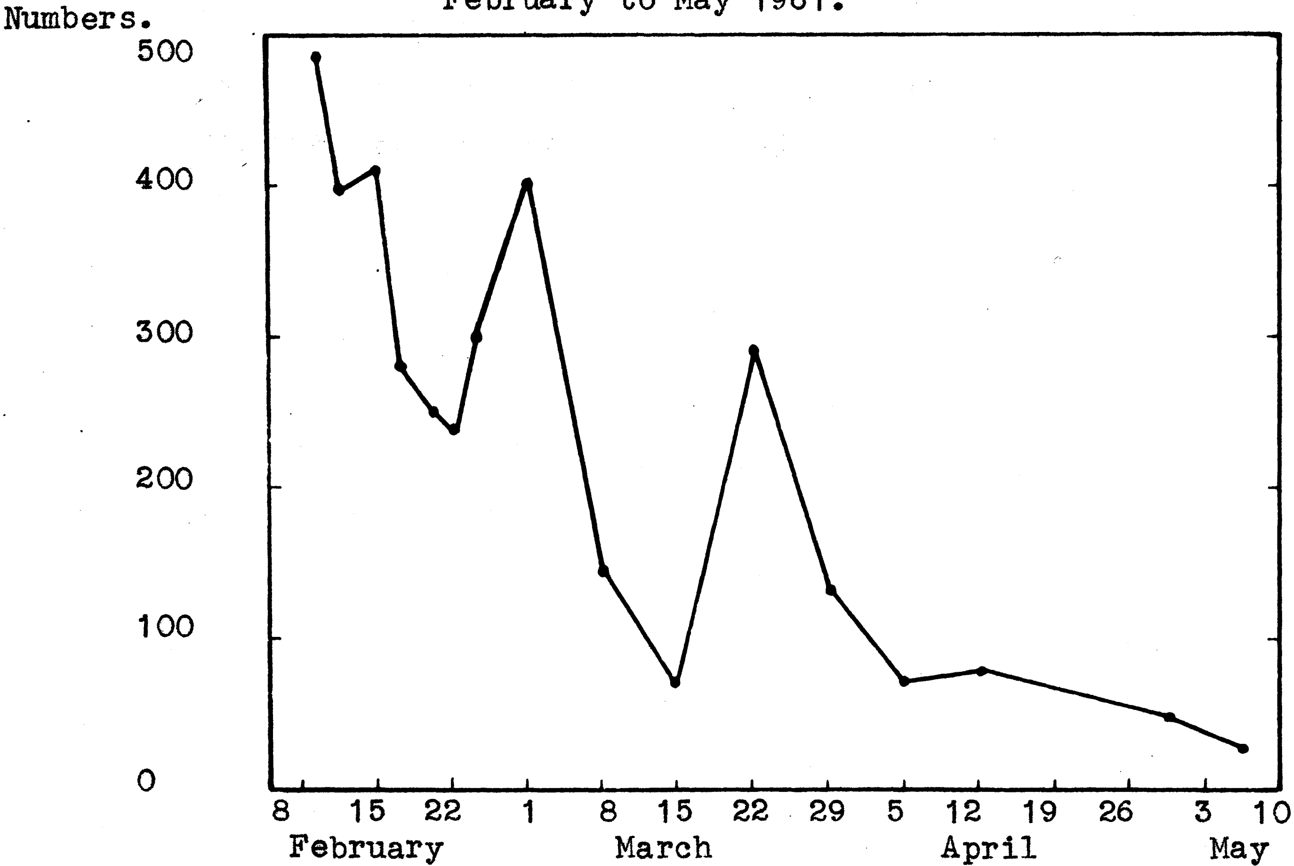


Fig. 52b. Fluctuations in total adult numbers on supralittoral pools at Taylor's Mistake, February to May, 1961.

During February and March there were highly significant differences in the population density of south and east end sampling areas of Pool 3a. However the difference between the density in south and east ends of Pool 3 over the same period was only just significant, the probability of "t" lying between 0.2 and 0.1.

Population analysis using the volume sampling method was augmented by direct counting of pupae (Fig. 50) and adults. The results of these direct counts made during the months from February to May are graphed in Figs. 51 and 52. In February, March and April there were considerable fluctuations, both in the total pupal population at Taylors Mistake and in pupal numbers in Pools 3 and 4. After falling from 277 on 20th February to 38 on 3rd March the total pupal population recovered to 481 on 22nd March, only to be down to 142 a week later. The amplitude of the fluctuations was substantially less in Pool 4 than Pool 3 which supported the larger Opifex population. During the second half of April the total pupal population fell steadily till by May 7th there were only three pupae in Taylors Mistake pools. By May 28th no pupae were left in these pools. Similar fluctuations were recorded in the adult population. However whereas the pupal counts were complete including every individual in the locality, the adult counts only provided an index to the adult population. Only those adults upon the water surface of pools were counted. Collections showed these adults were predominantly male, although females were not uncommonly found upon pools. As Figs. 51 and 52 show the fluctations of adult population tend to lag behind corresponding fluctuations in pupal numbers. The adult population index was of a similar order to the pupal population. On May 28th there were still seven adults, but none were seen in June.

Larval population also fell substantially during April and May but the decrease was modified by the continued hatching of eggs. For example a volume sample from Pool 3 on May 28th when no pupae and only seven adults were seen, revealed seventeen first instar, four second instar, sixteen third instar and five fourth instar larvae. Other pools exhibited a similar reduction in larval density. Taylors Mistake populations were maintained around this reduced level of density during June and the first half of July, but the O. fuscus population suffered severe depredation during a storm in the third week of July. On July 22nd after the worst of this onslaught had passed no first or second instar larvae were seen in any of the Taylors Mistake pools. No larvae survived in Pools 2 or 4 at all. The only remnants of the previously well established population were five third stage and two fourth stage larvae in Pool 3. This differential survival within Pool 3 may be ascribed to the ability of the larger larvae to shelter amongst the small rocks and stones upon the bottom of Pool 3. No similar shelter was available in either Pool 2 or Pool 4. The discovery of one live fourth instar larva secreted tail first with only its head protruding from a narrow tube within a stone gave some indication of how these few managed to survive. By August 16th the Opifex population had become re-established from eggs which had remained quiescent through the winter. First instar larvae were numerous in all pools. A volume sample from Pool 3 on September 15th revealed the following population structure:-

1st Instar	2nd Instar	3rd Instar	4th Instar	Pupae
198	30	6	8	0

Although many eggs had hatched since July, few moults and no metamorphosis had taken place. A similar situation prevailed in other pools. It was not until October 2nd that the first pupa reappeared at Taylors Mistake: a solitary specimen within Pool 3. On October 1st there were over one hundred pupae in Pool 23, Pile Bay, and more than seventy adults upon the pool surface. Temperature conditions in Pile Bay and Taylors Mistake pools were likely to be similar, but Pile Bay is much more sheltered during rough weather. Little mortality had been suffered by the Pool 23 population during the winter, and consequently pupal and adult populations were re-established sooner than at Taylors Mistake.

A second serious setback was suffered by the Taylors Mistake O. fuscus populations during October. For three weeks from October 5th all the pools were dry except Pool 4. At a time when, given favourable conditions, the breeding population would be approaching a maximum level after the winter, metamorphosis, mating and oviposition were restricted to one small, low population density pool. When the pools were filled again at the end of October the population recovery was, surprisingly, even more spectacular than the previous one in August. No eggs had been laid around any pool except possibly a few round Pool 4 for at least five months. Yet the number of larvae which hatched in late October and early November was greater than in August. The water level within the pools was no higher ih November than in August, so the eggs which were submerged in the later month would also have been submerged earlier. Borg and Horsfall (1953) and especially Horsfall (1956) have emphasized the importance of conditioning (the presubmergence exposure of eggs to specific environmental conditions) in readying aedine mosquito eggs to hatching on submergence. Thus the adequacy of conditioning has been shown to be important in determining the eggs'response to the hatching stimulus. It is suggested that the failure of many eggs to hatch when first submerged was due to inadequate conditioning. Subsequent environmental conditions prepared the eggs to respond favourably to later hatching stimuli.

Populations in Other Pools:

Population structure and fluctuation of Opifex fuscus in other pools were not examined in detail as at

Taylors Mistake. However, observation confirmed that the major trends recorded from Taylors Mistake pools also took place in other South Island pools.

Firstly larval pupal and adult density was always highest during summer months. No pupae or adults were seen at any pools during the winter although Wood found occasional pupae and adults at Island Bay in the winter of 1929.

Secondly there were marked contrasts between pools in the amplitude of population fluctuations. For instance, one of the largest pools, 32 Goose Bay, on March 3rd, supported an enormous Opifex population. On that day the number of adults upon its surface was estimated at between nine and eleven thousand. Yet on May 10th only a single adult was seen upon its surface. Heavy seas washing into the pool were no doubt responsible for the devastation of the O. fuscus population. Unlike Taylors Mistake pools, the O. fuscus population was only very slowly re-established. As late as October 15th there were no more than thirty larvae within the pool 32. Apparently continued disturbance by the sea, predation by Anisops assimilis and possibly by the cockabully Tripterygion varium, had prevented any resurgence of population numbers. On the other hand the larval density of Pool 31, Goose Bay, remained much steadier throughout the year, although again it was highest in the summer months.

In a third large pool, 25 Kaikoura, larvae congregated in preferred regions, where density was maintained around twenty or thirty per volume sample, (224 c.c.s.) while larvae were absent from other parts of the pool. Density under these conditions had only a limited meaning. Larval numbers in Pool 25 never approached the maximum levels in 31 or 32.

4.6.2 Larval Food:

Sources of larval food were determined by examination of the gut contents of larvae of all four instars. First stage larvae appear to gain most of their nourishment from yolk reserves enclosed within the hind gut. Occasional small diatoms were found within the hindgut of first instar larva but the plantids were not digested. Some fine sand grains were usually present as well.

No yolk was retained in the gut of second instar. Gut contents of second, third and fourth instar larvae from each pool were similar in nature but there was a progressive increase in the maximum size of acceptable particles with each instar. Commonly the gut contents of larvae consisted of a conglomeration of diatoms, unicellular green algae, filaments of blue green algae and detritus particles. The relative abundance of these various components in gut contents was primarily determined by their availability. However there were two distinct types of feeding behaviour associated with morphological differences in larval mouth parts. This will be discussed later.

Occasionally partially digested protozoa and fragments of *O. fuscus* exoskeleton were also found within the larval hindgut. Miller (1922) and Wood (1929) have presented evidence of cannibalism among *O. fuscus* larvae. Cannibalism was not a common occurrence, and only injured larvae were attacked, and only when other food supplies were inadequate. Such feeding habits are an extension of the scavenging behaviour of larvae. Dead larvae, and decaying plants and animals were frequently attacked.

Bacteria and yeasts probably form an important part of the diet of *O. fuscus* as with most mosquito larvae. No direct evidence of their presence in gut contents was obtained, but with high magnification and critical lighting both bacteria and yeasts were observed in supralittoral pool water which larvae had been filtering. The experiments of Trager (1936) indicate that *Aedes aegypti* is able to grow on materials in solution only, and it is consequently widely held that most mosquito larvae depend in part, at least, on food materials in solution in water.

4.6.3 Methods of Feeding and Larval Mouthparts:

Introduction:

In his description of the larval morphology of *Opifex fuscus*, Miller (1922) described the pre-mandibular mouthbrushes as being made up of simple hairs. Wood (1929) did not challenge Miller's description although his own account differed somewhat. According to Wood, each mouthbrush consists of hairs of two kinds. The outer ones were long and curved; the inner ones were shorter than the outside ones, and were toothed. In an examination of specimens of O. fuscus collected in 1921 by G.V. Hudson P.F. Mattingly (in Marks, 1958) discovered that some mouthbrushes conformed to Miller's description (all simple hairs) while others had pectinate hairs corresponding to Woods account. Marks collected larvae at Island Bay and found that this population also was comprised of a mixture of larvae with simple and pectinate types of mouthbrushes. As early as 1921 Wesenberg-Lund had recognized that there were these two basic types of mosquito mouthbrushes, but the existence of both types within a single species has not previously been reported.

Methods:

The structure of both simple and pectinate types of mouthbrushes was examined. Larvae and adults were searched for possible linked morphological features. Comparisons were made of the function of the two types of mouthbrushes. The proportion of both types in several pools was examined, and possibility of a relationship between sex and frequency of the two kinds of mouthbrush was checked. A series of laboratory experiments was undertaken to determine the bearing which a variety of environmental conditions might have on the morphology of the mouthparts.

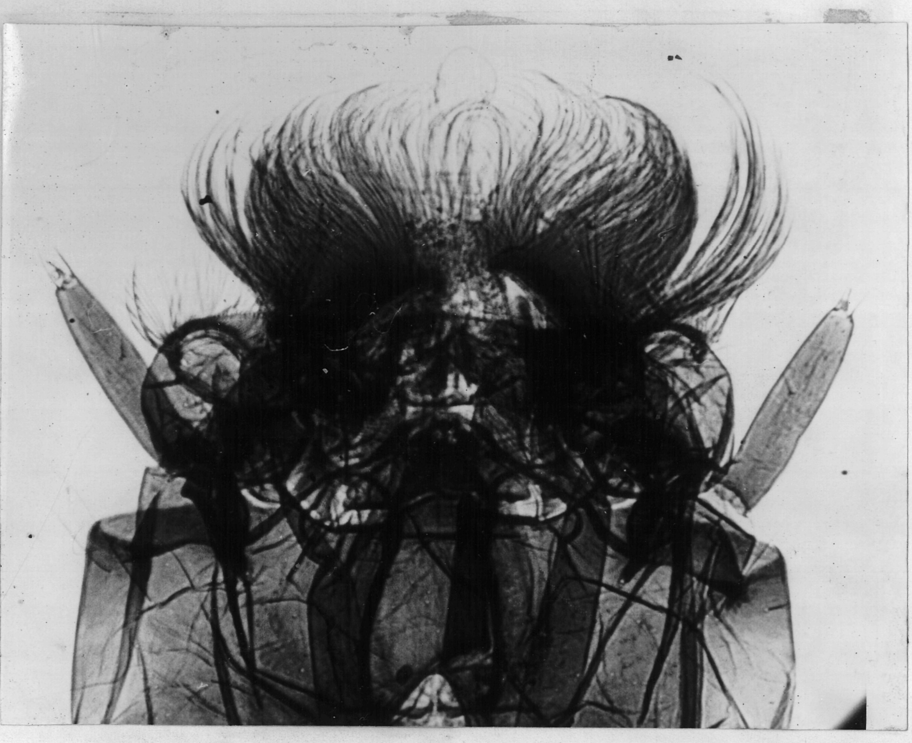


Fig. 53a. Opifex fuscus, fourth instar larva, head, dorsal aspect, showing mouthbrushes with only simple hairs.

Fig. 53b. Opifex fuscus, larval mouthbrushes, simple type.

Fig. 54a. Opifex fuscus, fourth instar larva, head, ventral aspect, showing mouthbrushes with pectinate bristles.

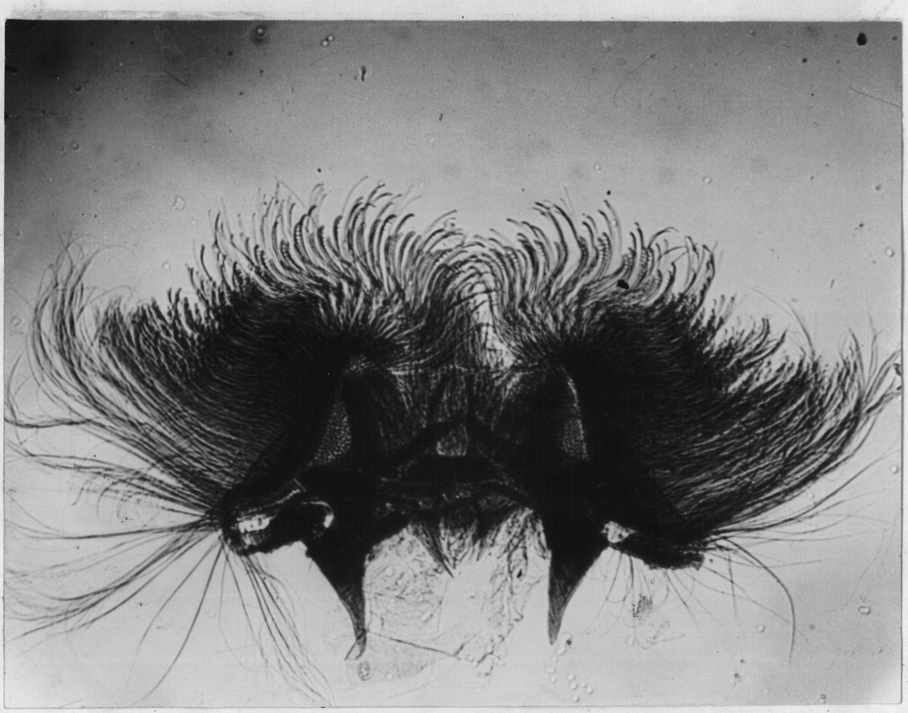


Fig. 54b. Opifex fuscus larval mouthbrushes, pectinate type.

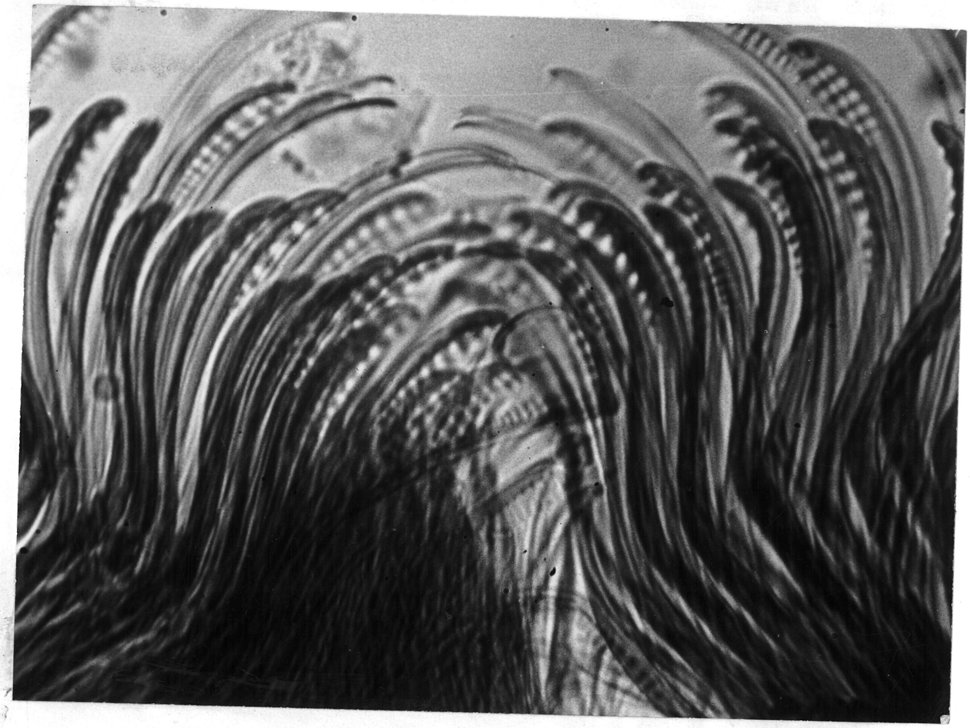


Fig. 55. Opifex fuscus larva. Pectinate bristles of mouthbrushes.

Results:

The difference between simple and pectinate bristles is sufficient to produce two very distinct types of brushes (Figs. 53 and 54). Pectinate bristles (Fig. 55) are sickle shaped with teeth on the inside edge of the curve. They are rigid, up to ten times the width of the simple hairs and no more than two thirds their length. In a brush with pectinate bristles, the outer quarter of the flabella supports simple hairs. The density of hairs in this outer quarter is greater than in the inner three quarters of the brush whether the other hairs are pectinate or simple. Similar numbers of hairs are present both in the simple and pectinate types of brushes. There is considerable variation in pectinate bristles. In fourth instar larvae the most strongly developed pectinate bristles are between 140 and 170 μ in length and between 2 and 5 μ in width. The longest teeth are 12 μ and the broadest 3 μ wide. All teeth on one bristle are not of equal length. Usually the third or fourth tooth from the tip is the longest and there is a gradual decrease in length towards the base of the bristle. With wear, teeth may be reduced to mere stumps no more than 1.5 μ long. Bristles on the ventral surface of the brushes are the most strongly developed. As they become worn and broken off the more dorsal bristles are strengthened and functionally replace them. Developing pectinate bristles differ from simple hairs in being shorter, slightly broader, and have minute serrations along their inner margin. The teeth reach their full length before broadening out. Thus dorsal to the sturdy ventral bristles are several rows of bristles bearing fine combs. Though the teeth within these combs may be 10 or 12 μ in length, in width they are no more than 0.6 μ . There is also variation in the number of teeth upon bristles. Commonly there are between twenty and thirty teeth along the outer third of the inside edge of each bristle. But there may be no more than ten teeth on a bristle.

Larvae and adults were carefully searched for other morphological characters that might be associated with variation in the types of mouthbrushes. Apart from the generally heavier chitinization of the mouthparts, evidence of their greater wear in larvae within pectinate type of mouthbrushes, no difference could be detected. Adults which had been produced from simple and pectinate types of larvae were indistinguishable.

The Functions of Mouthbrushes:

Mosquito larvae have several different methods of feeding (Bates, 1949). These are:-

(a) Gnawing hard submerged objects.

(b) Scraping off the periphyton on the surface of such objects.

(c) Swallowing comparably large floating bodies.

(d) Filtering small suspended particles from the water.

Mouthbrushes are involved in each of these methods, all of which are employed by Opifex fuscus.

Larvae that were observed gnawing or scraping were found always to have pectinate brushes. Particles were grasped and held between the brushes on each side while the brushes were rasped quickly and repeatedly over them. Frequently detritus particles were held between the brushes and carried through the pool. Larvae were seen with a firm grip on debris with their brushes struggling and pulling until a particle was loosened and then swallowing it, if it were of suitable size.

Nevertheless, despite the ability of O. fuscus to gnaw objects, scraping surfaces was a much more popular feeding method for larvae with pectinate brushes. Almost any object appeared to provide a surface acceptable for scraping. The widely extended brushes were applied with their ventral face against the surface. Then there was a sharp posterio-ventral and central movement of the brushes in unison. This movement was continued until the bristles were in intimate contact with hairs and bristles of the mandibles and maxillae. A short sharp lateral movement of the brushes coupled with a slight central movement of the maxillae resulted in the particles gathered by the brushes being transferred to the mandibles and maxillae. As the brushes were withdrawn from contact with the mandibles and maxillae the latter structures were vigorously thrust into the oral cavity. Then the mentum was thrust dorsally and forward reducing the size of the mouth as the mandibles and maxillae were withdrawn. During their withdrawal they scraped against the toothed mentum leaving their load of particles within the oral cavity. At the same time, particles which were too large to enter the mouth were rejected. As the mandibles and maxillae were withdrawn the pharynx enlarged and the food was sucked into the gut. Meanwhile, the inward and posterior movement of the mouthbrushes had resulted in the larva being propelled forward so that the surface to which they were next applied was new.

Examination of gut contents showed that the swallowing of large particles was a regular occurrence both with larvae having pectinate and simple types of mouthbrush when such particles were available. Selection acted through particle size rather than on potential food value. Bits of wood, detritus, filamentous algae and cuticle appeared within the gut of both types of larvae.

For larvae with exclusively simple hairs in their mouthbrushes, filtering was the most favoured feeding method. Larvae with pectinate mouthbrushes also fed by filtering small particles from the water but they were predominately scraping feeders. With their longer hairs, larvae with the simple type of mouthbrush were able to filter a greater volume of water in a given time than pectinate types, and were therefore more efficient filter-feeders. The mechanism of filter-feeding in <u>Opifex fuscus</u> larvae is identical with that of the scraping method of feeding. Indeed filter-feeding can be regarded as scraping the water for food. Exactly the same sequence of movements of the mouthparts are involved in each method. The proportion of small particles such as unicellular algae and diatoms was much higher in the gut contents of filter feeding simple mouthbrush larvae than in pectinate mouthbrush larvae.

A variation of the common method of filter-feeding is interfacial feeding (Renn quoted in Bates 1949). O. fuscus larvae frequently feed in this way while breathing by suspending themselves from the water surface by their anal siphon and arching their abdomen so that their head is also at the surface. In this position the mouthbrushes sweep particles from the surface film into the larval mouth. As a result of the various currents thus created the larva rotate about the siphonal pivot point at a rate determined by the vigour of the mouthbrush movements.

The volume of water exploited by filter feeding is surprisingly large, Senior White (1928, in Bates) estimating it at ranging between from 0.5 to 2.0 litres per day for third and fourth stage larvae of various mosquito species.

Behavioural Adaptations:

Associated with these various feeding habits are behavioural adaptations. O. fuscus larvae are gregarious. Frequently larvae were concentrated in a few areas within a pool while the rest of the pool was completely void of larvae. This gregariousness was particularly evident in large pools such as Pool 25 where larval numbers were not excessive. On May 10th volume samples were taken amidst the two largest concentrations of larvae within Pool 25, Kaikoura. The first sample was taken in the middle of a group of larvae filter feeding, while the second sample composed larvae hovering around a decaying <u>Lessonia</u> frond at the opposite end of the pool. The mouthbrush types of the two samples are given below.

<u>Sample 1</u> Mid Water: Filtering

Number of Simple larvae	Number of Pectinate larvae
20	2

(All Third Instar)

<u>Sample 2</u> Over <u>Lessonia</u>: Browsing

Number of Simple larvae	Number of Pectinate larvae
1	10

(All Third Instar)

Obviously within this pool there was habitat selection associated with the type of mouth-brushes which the larvae possessed and the kind of available food.

Proportions of Mouthbrush Types in Other Populations:

The mouthbrush types of larval samples taken from other pools were also determined. In Table XVIIthe results of these examinations are recorded by instars.

TABLE XVII

Mouthbrush Types in Opifex fuscus Population

Locality	Date	Instar 2nd Simple	2nd Pectinate	3rd Simple	3rd Pectinate	4th Simple	4th Pectinate
Island Bay	8.1.61	2	9	1	10	0	10
G. 32	10.5.61	0	0	7	1	0	0
G. 31	10.5.61	0	0	20	21	13	26
TM.3	15.2.61	20	4	2	11	0	0
TM.3	8.3.61	12	13	3	11	2	12

G = Goose Bay Pool
T.M. = Taylors Mistake Pool

This table shows that larvae of each instar, except the first, had either simple or pectinate types of mouthbrushes. All first stage larvae had simple mouthbrushes. The proportion of simple and pectinate types varied between pools and within different samples from the same pool.

• It was possible that mouthbrush type might have been linked with the sex of the larvae. Even in early stage larvae it was possible to distinguish the sex of larvae as the rudimentary male gonads are dense black in colour while the female rudiments are not pigmented. Thirty-two larvae of second, third and fourth instars were investigated for mouthbrush type and sex. The results are tabulated below in Table XVIII.

TABLE XVIII

Sex and Mouthbrush Types in Opifex fuscus

Instar	Mouthbrush Type	Male	Female
2	Simple	2	4
2	Pectinate	0	1
3	Simple	5	8
3	Pectinate	1	0
4	Simple	1	0
4	Pectinate	3	7

There was no correlation between mouthbrush type and the sex of larvae.

Laboratory Experiments on Mouthbrush Type:

To gain a deeper understanding of this morphological variation in the types of hairs within the larval mouthbrushes it was important to conduct laboratory experiments with a view to establishing possible causes. Experiments were designed initially to determine whether the primary control was genetic or environmental. The influence of different kinds and particle size of food, temperature, salinity, and light on the structure of the mouthbrushes was tested.

Methods:

First instar larvae, collected in the field were grown individually in test tubes. After each moult the sloughed exo - skeleton from the previous instar was examined and the type of hairs in the mouthbrushes determined. The water in which the larvae were raised was changed several times each week.

Results:

The life histories of five selected larvae provided a key to the field data. The sequences of mouthbrush types within these life histories are tabulated below.

TABLE XIX

Mouthbrush Types of Instars of Five Larvae.

Larvae Number	Instar 1st	2nd	3rd	4th
1	S	S	S	S
2	S	P	P	P
3	S	S	P	P
4	S	P	P	S
5	S	S	P	S

In this table S = Simple hairs only in mouthbrushes.

P = Pectinate bristles in mouthbrushes.

These larvae were grown at 25°C and fed on powdered fish food.

Mouthbrush types of <u>Opifex fuscus</u> may remain the same in successive instars or they may change either from simple to pectinate or vice versa.

Since this experiment had demonstrated that the morphological variation in larval structure was not due to genetic differences between larvae, further experiments were designed to determine environmental stimuli which might produce larvae with simple or pectinate bristles in mouthbrushes.

Field observations had suggested that food supply might contribute to the morphological variation. Larvae with

TABLE XX

Mouthbrush Types by Instars of Larvae fed on Loefflers Dehydrated Blood Serum at 25°C

Larva Number	Instar 1st	 2nd	 3rd	 4th
6	S	S	S	S
7	S	S	S	S
8	S	S	P	P
9	S	S	S	S
10	S	S	S	S
11	S	S	S	S
12	S	S	S	S
13	S	S	S	S
14	S	S	S	P
15	S	S	S	S
16	S	S	S	S
17	S	S	S	S
18	S	S	S	S

only simple hairs in their brushes had been found to be almost exclusively filter-feeders while those with pectinate bristles were browsers. Moreover the occurrence of each type of larvae was correlated with the availability of suitable food. Powdering of the fish food used in the initial experiment produced a wide range of particle size. It was suspected that the varied results of the first experiment may have been associated with the chance supply of particles of different sizes in unequal quantities to each larva.

A second experiment was performed to test this hypothesis. Loeffler's dehydrated blood serum, which had been successfully used by Wigglesworth (1933) for feeding mosquito larvae, was selected as a suitable food. Its chief advantages were that the particles were of uniform size and because this was small most remained in suspension between water changes. Consequently larvae were dependent on filtering for obtaining most of their nourishment. The results of this experiment are given in Table XX .

With almost all the available food in suspension and accessible only by filtering the mouthbrushes developed by the larvae were almost exclusively simple.

A further experiment was initiated using much larger sized particles which could only be manipulated by scraping or gnawing and not by filtering. Fine grain fish food that had not been powdered was used as food for the larvae, which were grown in a constant temperature at 25°C. The mouthbrush

TABLE XXI

Mouthbrush Types by Instars, of Larvae fed on commercial Fish Food

Instar	1	2	3	4
Larval Mouthbrush Types	S	P	P	P
	S	P	P	P
	S	P	P	P
	S	P	P	P
	S	P	P	P
	S	P	P	P
	S	S	P	P
	S	S	P	P
	S	S	P	P
	S	P	P	P
	S	P	P	P
	S	S	S	S
	S	P	P	P
	S	S	P	P
	S	P	P	P

S = Simple hairs only in mouthbrushes

P = Pectinate bristles in mouthbrushes larvae.

All Larvae were grown at 25 - 27 °C

TABLE XXII

Mouthbrush Types, by Instars, of Larvae supplied with Fish Food during Second Instar, and Loefflers Dried Blood Serum during Third and Fourth Instars.

Instar	1	2	3	4
Larval	S	P	S	S
Mouthbrush	S	P	P	S
Types	S	P	P	S
	S	P	S	S
	S	P	S	S
	S	P	P	S
	S	P	P	S
	S	P	P	S
	S	P	S	S
	S	P	P	S
	S	P	P	S
	S	P	P	S
	S	P	P	S
	S	P	P	S
	S	P	S	S
	S	P	S	S

S = Simple hairs only in mouthbrushes

P = Pectinate bristles in mouthbrushes

All larvae were grown at Room Temperature types of the larvae grown under these conditions are presented in Table XXI.

In contrast to the previous experiment these larve predominantly developed pectinate type of mouthbrushes. However, one larva possessed simple brushes in all instars. This may have been associated with feeding on bacteria and protozoa which developed within the culture medium.

An experiment was also performed in which supplied food was changed during the third instar, from large particle size fish food to the finely particulate Loeffler's dried blood serum. From Table XXIIit is clear that corresponding with the decrease in the particle size of the food there was a change from pectinate to simple types of brushes. The change in food supply to all larvae was made at the same time, but as the third stage larvae were at different stages of development, their response to the change was varied. However, all the fourth instar larvae developed simple brushes. In a further experiment larvae were grown in a waterbath at 15°C - 1°C, at room temperature and at 25°C - 2°C, some being fed on fish food and some on Loefflers blood serum, but temperature had no apparent effect on the type of mouthbrushes developed. Similarly there was no difference in the types of brushes developed in complete darkness and normal days at room temperature.

It was possible, but unlikely that differences in the chemical constitution of food were responsible for the development of brushes. In the field larvae had been found to respond to a variety of foods, whose chemical compositions were undoubtedly different from those used in the laboratory, by developing the type of brushes better suited to the exploitation of the available food. Moreover the experiments had shown that mouthbrushes of either type may be developed if larvae are grown upon the same food supplied in a range of particle size.

It can therefore be concluded that pectinate or simple types of mouthbrush are developed in each instar, except the first in response to the available food. If food is in suspension and can be gathered more efficiently by filtering, simple types of hairs only, are developed within the mouthbrushes of O. fuscus. When the food supply is of longer particles, or can be exploited only by scraping or browsing pectinate bristles are developed in the mouthbrushes. In a population the proportion of larvae with each type of brush is adjusted so that the available food is most efficiently utilized.

Mechanism Controlling Polymorphism in Opifex fuscus.

Polymorphism is not uncommon among insects. In many insects which occur naturally in more than one form the characters are controlled by genetic factors, but there are well known examples in which polymorphism may be brought about in the course of development by environmental factors acting upon insects of uniform constitution, e.g. seasonal dimorphism in butterflies; head types of polymorphism in ants; polymorphism in honey-bee colonies; and in aphids differences on the one hand between parthenogenic and gamic generations; and on the other between alate and apterous forms among the parthenogenic individuals. In many instances the primary environmental stimuli responsible for the development of the different forms have been determined, but little is known of how these stimuli influence the structure of animals.

It has been established that mouthbrush polymorphism in Opifex fuscus is determined by the available food but there is no evidence, at present, as to how this stimulus acts upon the animal.

Biological Significance of Larval Polymorphism:

Polymorphism of larval mouthbrushes is of adaptive value to Opifex fuscus enabling the larvae to exploit most fully the food resources of the environment. The ability to respond morphologically to the available food ensures that it is efficiently utilized so that a maximum number of individuals develop to form a breeding population as quickly as possible.

In no species of mosquito other than O. fuscus has the occurrence of two distinct types of larval mouthbrushes been reported. However Rosen and Rozeboom (1954) have shown that at least two species of the scuttelaris group of Aedes in Polynesia, A polynesiensis and A. pseudoscutellaris may have either hairy or non hairy larvae. Although Rosen and Rozeboom established that the control of this variation was environmental they did not elucidate the particular factor responsible.

Evolutionary Significance of Larval Morphological Variations

In a survey of larval mouthparts of Culicinae Surtees (1959) concluded that filter-feeding was primitive among mosquito larvae while other feeding habits have been more recent developments. This theory was based on a comparative study of the structure and feeding habits of a number of culicine species.

Surtees described the typical filter-feeding facies as follows: long fine unserrated mouthbrushes, large maxillae bearing many fine setae, small weakly chitinized mandibles, a weakly chitinized mentum possessing a large number of very small teeth, and associated with these features, large subapical tufts on the antennae. Larvae of the typical browsing facies have short stout antennae, with no subapical tuft of setae, and distally serrated mouthbrushes, strong mandibles, reduced maxillae with well developed setae. A series of species with features intermediate between these two facies was described by Surtees.

Apart from the radical differences in the structure of mouthbrushes in the two types of <u>Opifex fuscus</u> larvae, the mouthparts of filter-feeders and those larvae which are predominantly browsers are almost identical, except that in larvae with pectinate brushes other mouthparts tend to be more heavily chitinized and setae stouter. Moreover the structure

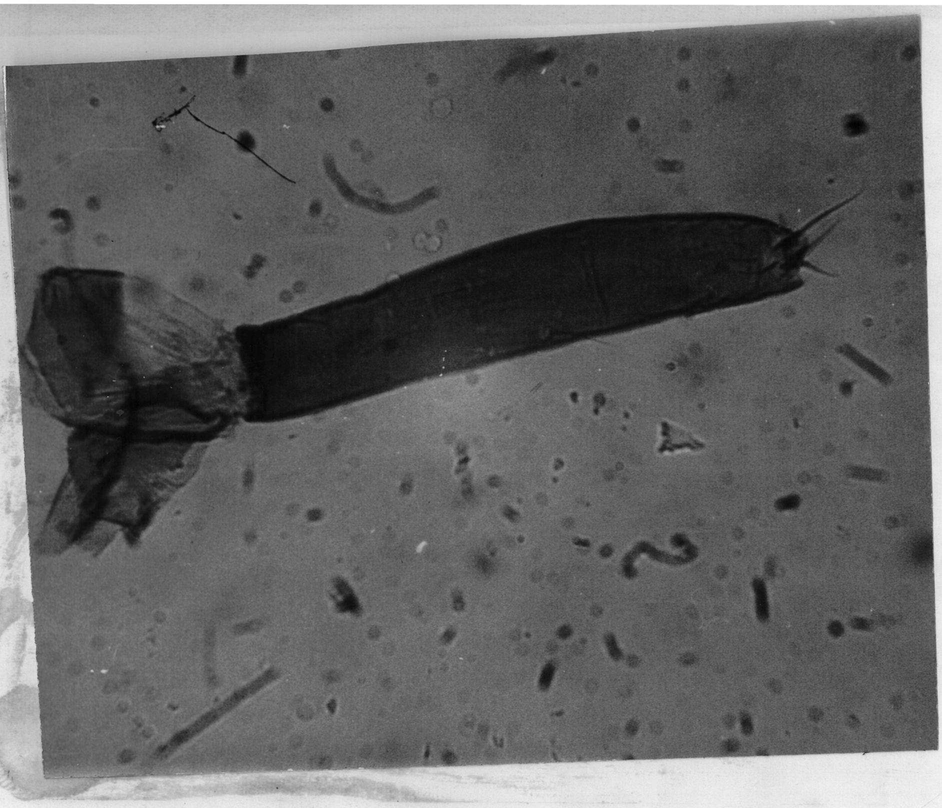


Fig. 56. Opifex fuscus. Antenna of a fourth instar larva. X100.

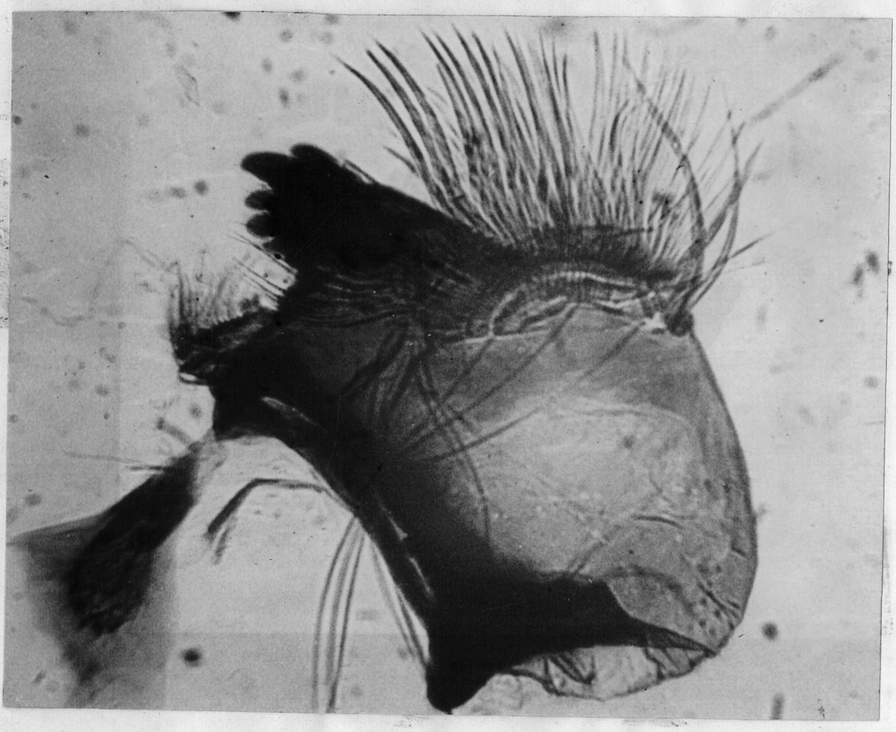


Fig. 57. Opifex fuscus. Mandible of a fourth instar larva. X100.

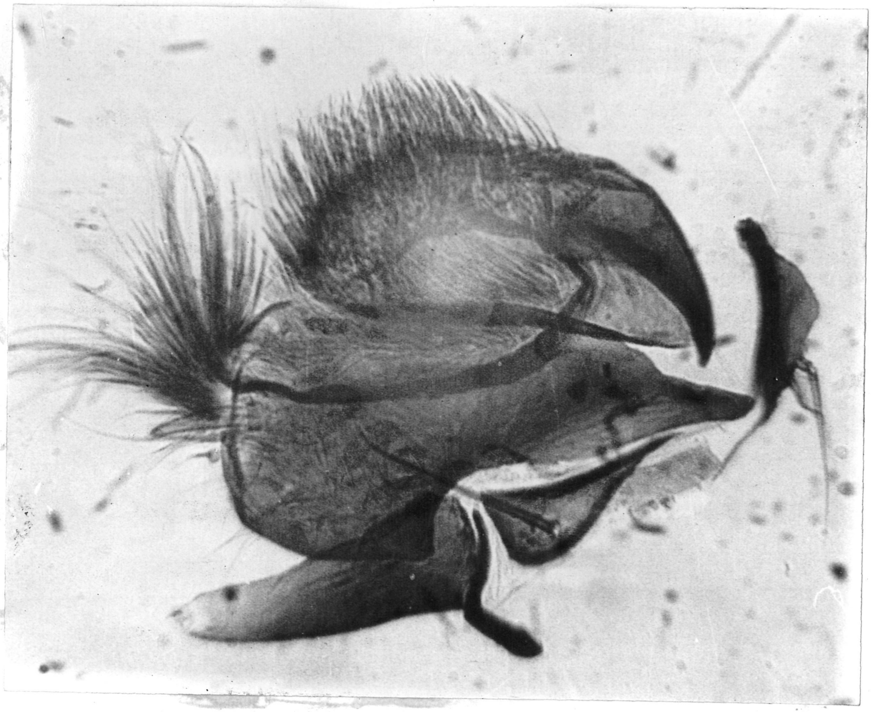


Fig. 58. Opifex fuscus. Maxilla of a fourth instar larva. X100.

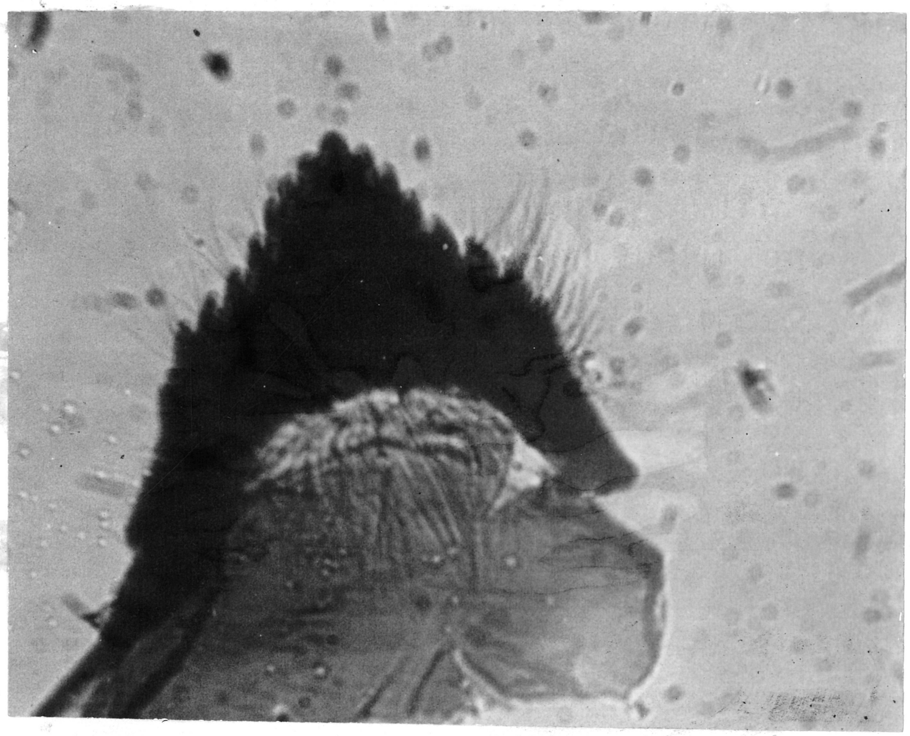


Fig. 59. Opifex fuscus. Mentum of a fourth instar larva. X100.

of O. fuscus mouthparts corresponds more closely with Surtees criteria of the browsing facies than the filtering facies. The antennae (Fig. 56) have a single subapical bristle; the mandibles (Fig. 57) are strong; the maxillae (Fig. 58) are similar in size to the mandibles, smaller than is common in filter feeders, and have well developed setae; the mentum (Fig. 59) is strongly chitinized and well provided with teeth.

While this evidence does not invalidate Surtees' theory it does suggest that it should be regarded with caution until more intensive work is done on species from a greater variety of localities. Even when the mouthbrushes in O. fuscus are simple, the mouthparts as a whole are of a typical browsing facies. This combination functions as an efficient filtering system and it may well be that the development of the full filter-feeding facies, which Surtees regarded as primitive, is in fact a later adaptation to environments in which suspended particles formed the bulk of the available food. In such habitats the retention of the browsing facies would be detrimental to reproductive success. But in supralittoral pools and similar environments where food may be obtained either by filtering or browsing, or both, a feeding apparatus which permits the exploitation of both sources is most advantageous. Consequently it is surprising that the kind of morphological variation found in O. fuscus is not more widespread.

Both Edwards (1924) and Wood (1929) considered O. fuscus exhibited primitive features. Edwards regarded the absence of upright forked scales on the vertex of the head as primitive, while Wood considered the following as primitives:- the enlarged scape and reduced torus of the antennae, the paired condition of both the oesophogeal ganglion and the last abdominal ganglion in the adult; the distinct antennal and optic lobes of the brain, and the presence of two sub-oesophogeal ganglia in the larvae.

The evidence from the nervous system presented by Wood is particularly convincing, but if indeed _O. fuscus_ is primitive one would expect polymorphism in mouthbrushes, because of its adaptive value, to have been recorded in at least some other species. That _Opifex_ appears unique in this respect adds weight to Dumbleton's (1962) suggestion that it may not be a relict genus which has been isolated in New Zealand since the Cretaceous, but a strongly deviant form of later origin. It is difficult to see how the ability to respond morphologically to the available food so that it can be exploited more fully, once established within a group could be lost in related species occupying similar niches.

6.4 Growth Rates:

Growth rates of the immature stages of mosquitoes, especially in relation to temperature and food have been investigated by a number of authors notably Hinman (1930), Hurlbut (1943), Huffaker (1944) and Nielson and Evans (1960). Huffaker examined the temperature relations of larval and

and pupal phases of *Anopheles quadrimaculates* in detail, while Nielson and Evans determined the effect of temperature in the duration of the pupal stage of *Aedes taemorphynchus*. In both works mathematical relationships between temperature and development were formulated.

The degree of precision reached by Huffaker and Nielson and Evans was unobtainable with the facilities available for studies on *Opifex fuscus*. However these experiments provided an adequate insight into the relationship between the growth of *O. fuscus* and the temperature of the environment.

Methods:

Larvae were grown individually in test tubes containing 25 ml of sea water. Equal quantities of commercial fish food or Loeffler's blood serum were added after each water change every other day, in quantities sufficient to ensure that a surplus was available to larvae throughout the two day period, without the culture medium becoming contaminated with fungal and bacterial growths. Larvae were grown at the following temperatures:-

1. At 13°C - 1°C, in a constant temperature bath using running tap water.
2. At room temperature (mean maximum 20.8°C, mean minimum 14.4°C).
3. At 25°C - 2°C in a constant temperature bath.
4. At 27°C - 2° in an oven.

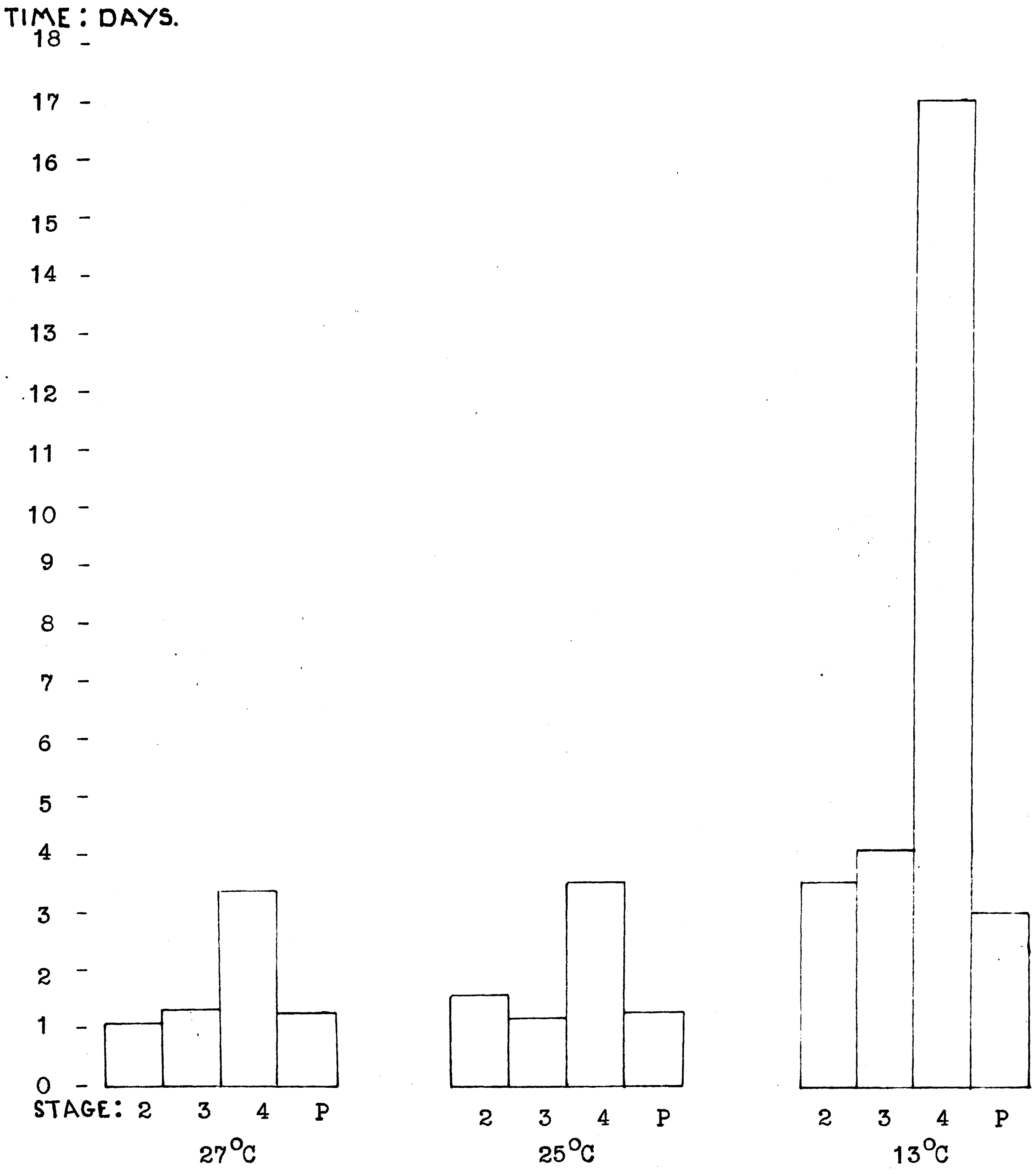


Fig. 60 Growth rates of immature stages of <u>O. fuscus</u> at constant temperature.

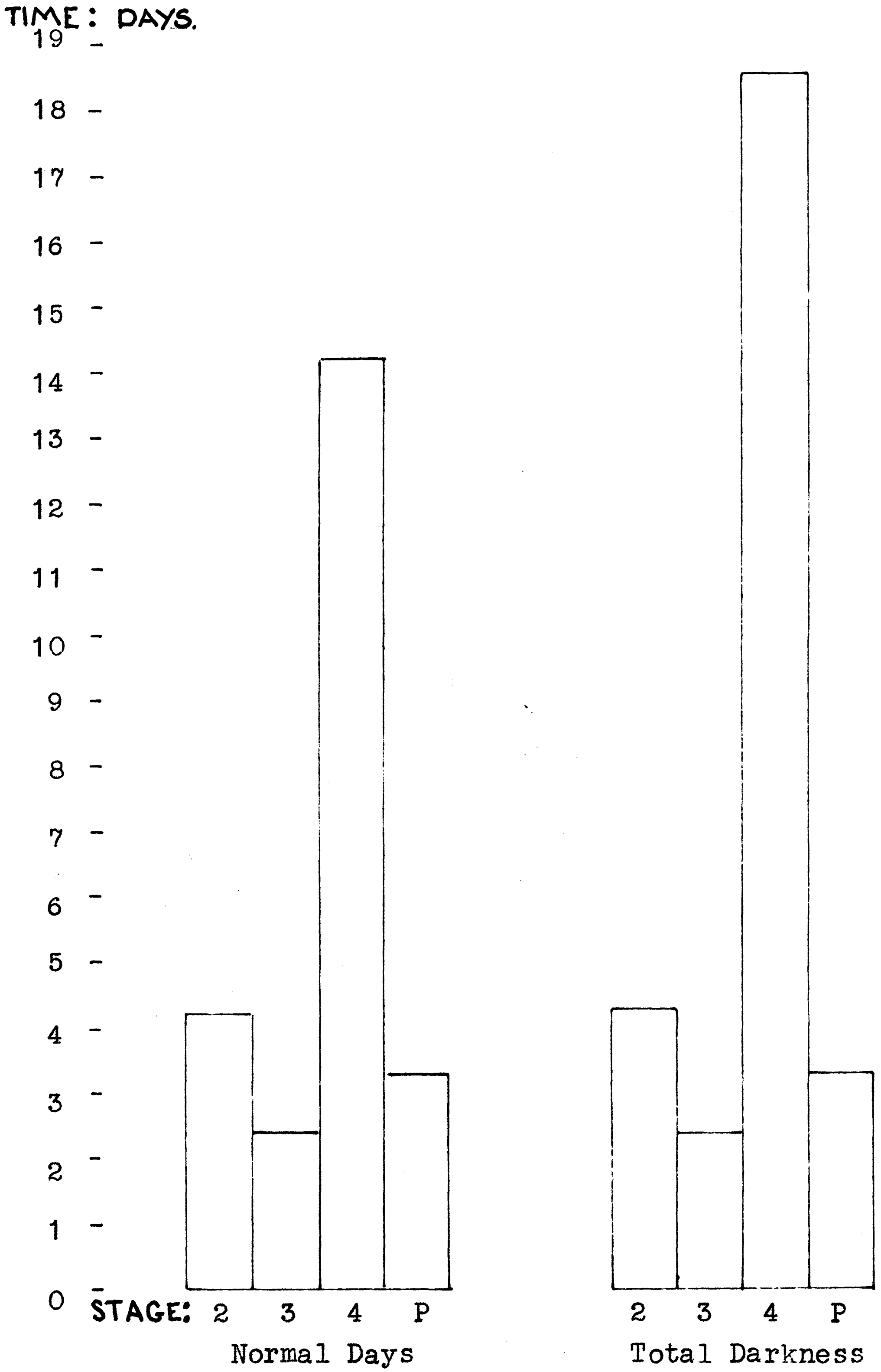


Fig. 61 Growth rates of O. fuscus, at room temperature, in normal days, and complete darkness.

Of the two batches of larvae grown at room temperature one was kept in complete darkness, except when being fed; the other was exposed to normal length days.

Attempts were made to grow larvae in an oven at 32°C. Larvae were also kept in a refrigerator at 3°C - 2°C.

Between fifteen and twenty five larvae were included in each experiment. All the tubes were checked daily when those larvae which had moulted in the previous twenty-four hours were recorded. The duration of the second, third and fourth instar, as well as the pupal phase, of each individual animal was recorded. As each experiment was begun with first instar larvae the duration of this stage could not be recorded.

Results:

Growth rates at various temperatures and conditions of light and darkness are plotted in Figures 60 and 61. From Fig. 60 it can been seen that the growth rates in the oven at 27°C - 2°C in almost complete darkness, and in the water bath at 25°C - 2°C with normal length days were almost identical. The small differences between the duration of second and third instars in the two experiments cannot be considered as significant.

At all temperatures the stage of longest duration was the fourth instar. As Huffaker (1944) has pointed out the growth of the fourth instar involves by far the greatest increase in mass, as well as basic tissue transformations, so that it is not surprising that it should require the longest time.

The duration of all stages was longer at room temperature and at 13°C than at higher temperatures. Almost identical rates were recorded for second, third and pupal stages with larvae grown in normal days and in almost complete darkness at room temperature. That the fourth instar was longer in darkness can probably be attributed to the effect of light upon the food supply. Although an excess of Loefflers blood serum and fish food was available at all times to all larvae, lighted conditions were more favourable for the development of micro-organisms. Bacteria and protozoa are known to be normal food of mosquito larvae (Bates 1949). On the other hand the delayed growth of fourth instar larvae in darkness may have been due to the unfavourable effect of some toxic material in the media, rather than to dietary deficiency.

Previous work has shown that light is an unimportant direct factor in mosquito development. Thus Fielding (1919) found that the larvae of *Aedes aegypti* grow equally well in the presence or absence of light; Jobling (1937) obtained similar results with *Aedes aegypti*, *Culex pipens* and *C. fatigans*. The length of the fourth instar at room temperature and at 13°C indicated that these larvae were near their lower temperature threshold for pupation. Since earlier stages moulted with comparative facility under the same conditions, it appears that the lower threshold for pupation is higher than the corresponding threshold for larval development. Field observations substantiate this for, whereas larvae continued to moult in South Island pools during winter months, no pupae were seen till spring.

Some first and second instar larvae survived but many died in a refrigerator maintained at 3°C - 2°C. Third and fourth instar larvae readily tolerated this temperature surviving for several months but no pupae survived more than three days. No larvae exhibited any signs of development at this temperature.

Ability to withstand low temperatures is necessary for animals inhabiting supralittoral pools which follow fluctuations in atmospheric temperature more closely than larger bodies of water.

Attempts to raise larvae at 32°C were unsuccessful owing to contamination of culture by micro-organisms. Larvae survived between instars, but died immediately after moulting. That larvae could tolerate higher temperatures, at least for short periods, was apparent from field observations, which revealed temperatures as high as 36°C in pools containing O. fuscus.

Mouthbrush types were examined after each moult during growth experiments. There was no tendency for larvae with one type of brushes to develop faster than those with the other. Rather the type of brushes most suitable for the utilization of the available food was developed.

4.6.5 Salinity Tolerance of Opifex fuscus.

Opifex fuscus occurred in all pools from which salinity data are recorded in section 2.3. The maximum recorded salinity was $90.9^{\circ}/oo$, the minimum $0.40^{\circ}/oo$. Dumbleton (1962) observed that O. fuscus had been bred from a freshwater stream at Murray's Mistake, Banks Peninsula. Larvae were also found in a land locked lagoon at Moeraki, North Otago.

Experiments on the salinity tolerances of O. fuscus were undertaken in the laboratory.

Methods:

Larvae were grown individually in tubes containing solutions of a wide range of salinity. The reactions of larvae to each solution and their ability to withstand transference from one solution to another was recorded. Solutions used during experiments were:

1. Distilled water.
2. Tap water.
3. Sea water diluted to $3^{\circ}/oo$ with tap water.
4. Sea water diluted to $18^{\circ}/oo$ with tap water.
5. Normal sea water.
6. Artificial sea water prepared by the method developed by Dakin and Edwards (1931) and used by Woodhill (1936)

for experiments on the tolerances of Aedes (P) australis. The following quantities of salts were first added to 900 ml of distilled water: 28.15 gm NaCl, 3.67 gm KCl, 5.51 gm $MgCl_2$, 6.92 gm $MgSO_4$, $7H_2O$. When these salts were dissolved, 1.45 gm of $CaCl_2$, $6H_2O$ was added, followed by 0.25 gm $NaHCO_3$. Following the addition of a trace of iodien the whole was made up to 1000 ml with distilled water. With a salinity of 35.7^{o}/oo the physical and chemical properties of the solution closely approximated natural seawater.

7. A method suggested by Dr. R.L.C. Pilgrim (pers. comm.) was adopted for the preparation of concentrated artificial seawater. The composition of the solution was

KCl	2.4M	1.8 ml
$CaCl_2$	1.6M	2.8 ml
$MgCl_2$	1.6M	14.5 ml
NaCl	2.4M	to 100.0 ml

Its concentration, in respect to the major cations, was four times that of seawater. Any required lower concentration was produced by dilution with M/400 $NaHCO_3$.

Commercial fish food was fed to all larvae in salinity tolerance experiments. Culture media were changed every other day. When larvae were changed from one solution to another they were pipetted, with as little water as possible, from the initial solution to one of the required salinity, in which they were left for several minutes before being finally shifted to another tube containing a similar solution. Ten larvae were used in each experiment.

Results:

1. All stages survived and developed in distilled water. The addition of fish food would have supplied some salts, but the tolerance of Opifex fuscus larvae for negligible salinity was remarkable.

2. The immature stages of O. fuscus all developed in tap water, seawater diluted to 3°/oo, 18°/oo, in normal seawater and in artificial seawater (35.7°/oo).

3. No larvae survived direct transference from distilled water to normal seawater, but three out of ten survived a change in the opposite direction. All possible combinations of changes of larvae between tap water, and salt water of salinity of 3°/oo, 18°/oo and 35°/oo were tried, but no mortality resulted.

4. Six of ten first instar larvae and four of ten second instar larvae were dead within twenty four hours of transference from artificial seawater, salinity 35°/oo to 70°/oo. All twenty third and fourth instar larvae survived a similar change. Ten larvae of each instar were transferred from artificial seawater, 35°/oo to 105°/oo but only one, a fourth stage, survived. When twenty first and second instar larvae were moved from artificial seawater, salinity 35°/oo

through solutions of 45°/oo and 55°/oo, remaining in each for several hours, to 70°/oo, four first stage and seven second stage larvae survived.

5. Larvae were kept in a dish to which normal seawater was added as the volume fell with evaporation. In this way the salinity gradually increased while the volume of water within the dish was kept approximately constant. Growth and metamorphosis continued until the salinity reached approximately 130°/oo. After that no larvae pupated but adults emerged from pupae present. When the last of the larvae died the salinity was between 160 and 170°/oo. Lack of sufficient food was unlikely to have been a primary cause of death as larvae at lower salinities were kept for months without being supplied with food.

Discussion:

These experiments confirmed field observations showing that Opifex fuscus has wide salinity tolerance and powers of acclimatization. In this Opifex is not unique for mosquitoes occupying similar niches elsewhere behave in a like manner (King and del Rosaria 1935, Woodhill 1936, Beadle 1939, Margalef 1949, Horsfall 1949, O'Gower 1960).

Opifex fuscus resembles Aedes (Pseudoskusea) australis in that the salinity tolerance, and powers of acclimatization, of first stage larvae are less than those of fourth stage larvae. O'Gower showed that fourth instar larvae of *A. australis* could tolerate salinities up to a maximum of approximately 240°/oo which is greater than the recorded maximum of *O. fuscus*, 170°/oo. Salinities in Australian supralittoral pools are likely to reach higher values more frequently than in New Zealand pools, and hence the toleration of high salinities would be more critical for *A. australis* than *O. fuscus*. The upper salinity threshold for pupation is below the survival threshold for both species also, but again the value for *A. australis* is higher than *O. fuscus*. For *A. australis* Woodhill (1936) found that the maximum salinity for pupation was 200°/oo. The corresponding value for *O. fuscus* was 150°/oo.

4.6.6 Osmoregulation in Mosquito Larvae

Osmoregulation in mosquito larvae has been investigated in detail by Wigglesworth (1933, 1938), Beadle (1939, and Ramsay (1950). Wigglesworth demonstrated that the anal papillae (or "gills") of fresh water species function for the absorption of water, and there is some evidence that the mechanism by which they can concentrate chloride from very dilute solutions is seated in these gills. Neither of these functions is required in saline waters, and species such as *O. fuscus* have much reduced anal papillae. The possession of large gills permeable to water would proveaa positive disadvantage under such conditions. Beadle, studying the saline water species *Aedes detritus*, found that its very small anal papillae were impermeable to water and salts. He

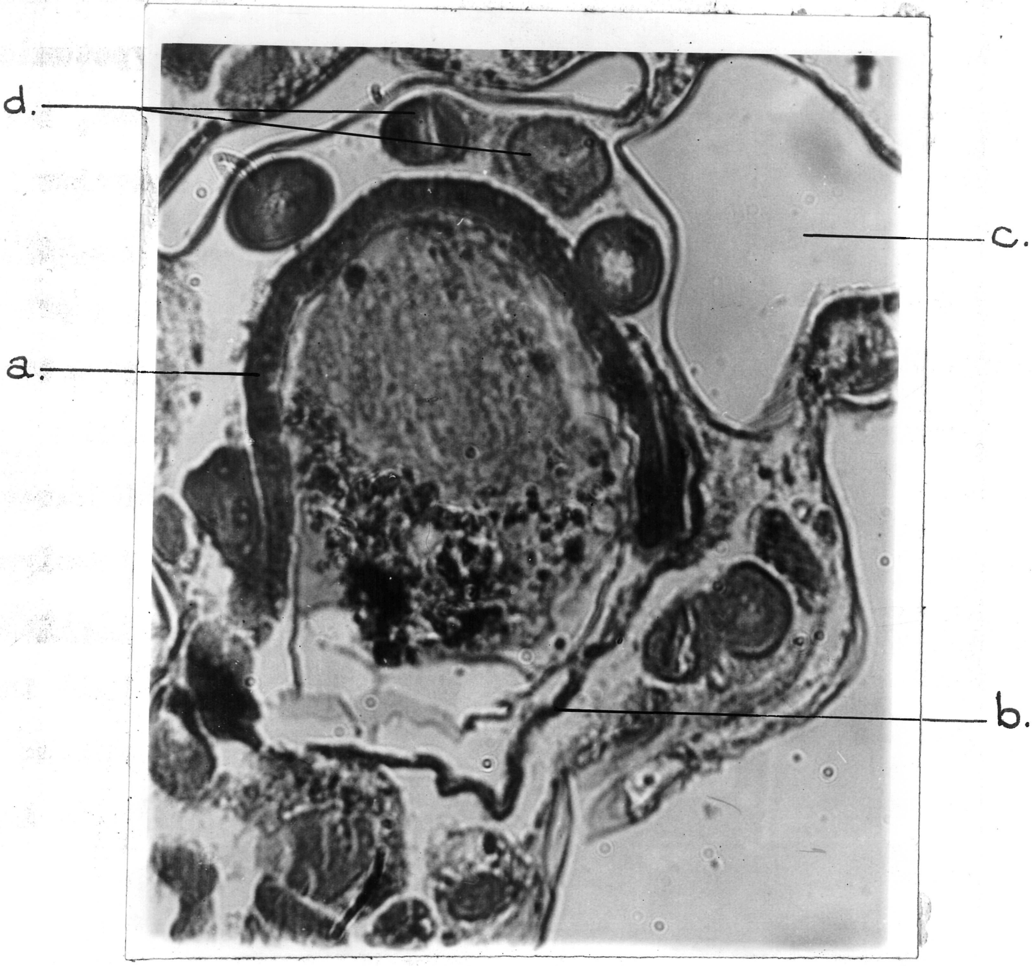


Fig. 62. Transverse section (somewhat oblique) through rectum of <u>O. fuscus</u> showing transition from anterior epithelium a, to posterior epithelium, b.
c, tracheal trunks.
d, malpighian tubules.

found that salt exchange with the environment took place via the gut, the body surface being impermeable to salts and water. Ligature experiment suggested that the Malpighian tubules excreted salt. In sea water of varying salinity *A. detritus* regulated both the total osmotic pressure and the chloride content of the haemolyph, while in hypotonic solutions and distilled water much chloride was lost, but this was compensated by an increase in the non-chloride fraction. Subsequently Ramsay (1950) found that *A. detritus*, unlike the normally fresh water species, *A. aegypti*, produces in sea water a fluid in the rectum which becomes hypertonic to the haemolymph, and approximately isotonic with the external medium, before being eliminated. In fresh water, rectal fluid of *A. detritus* was hypotonic to the haemolymph. Ramsay tentatively associated this ability of *A. detritus*, not possessed by *A. aegypti*, to produce a hypertonic fluid in the rectum, with a region in the anterior part of the rectum lined with an epitheliumdistinctly different from that in the remainder of the rectum.

Osmoregulation in Opifex fuscus

Sections of fourth instar *O. fuscus* larvae cut at 10 µ and stained with Delafields Haematoxylin showed that the rectum of *O. fuscus* (Fig. 62) like that of *A. detritus* is divisible into two histologically distinct regions. This indicates that osmoregulatory mechanisms in the two species may be similar.

4.6.7 Respiration

Wood (1929) examined and figured the larval tracheal system in detail. The tracheal trunks in late stage larvae are very large, their breadth being such that they extend half way round to the ventral surface. Although, in the majority of mosquito species larvae regularly come to the surface to breathe, respiratory behaviour is varied. It is clear that a certain amount of respiration continues even when larvae are deprived access to the surface; such respiration is probably a function of the general integument (Bates 1949), since Wigglesworth has shown that the so-called anal gills function for the regulation of osmatic pressure at least in species in which they are well developed. The larvae of the North American species *Psorophora discolor*, which has very large anal gills, never come to the surface for air. Larvae of all species of *Mansonia* derive their air from the roots of water plants, which they pierce by means of the saw apparatus in the siphon.

The respiratory behaviour of fourth instar *Opifex fuscus* larvae was examined. Experiments were designed to determine the reaction of larvae to deprivation of access to the water surface.

Methods

The activity of a fourth instar larva was observed by placing it in a measuring cylinder containing 25 ml of

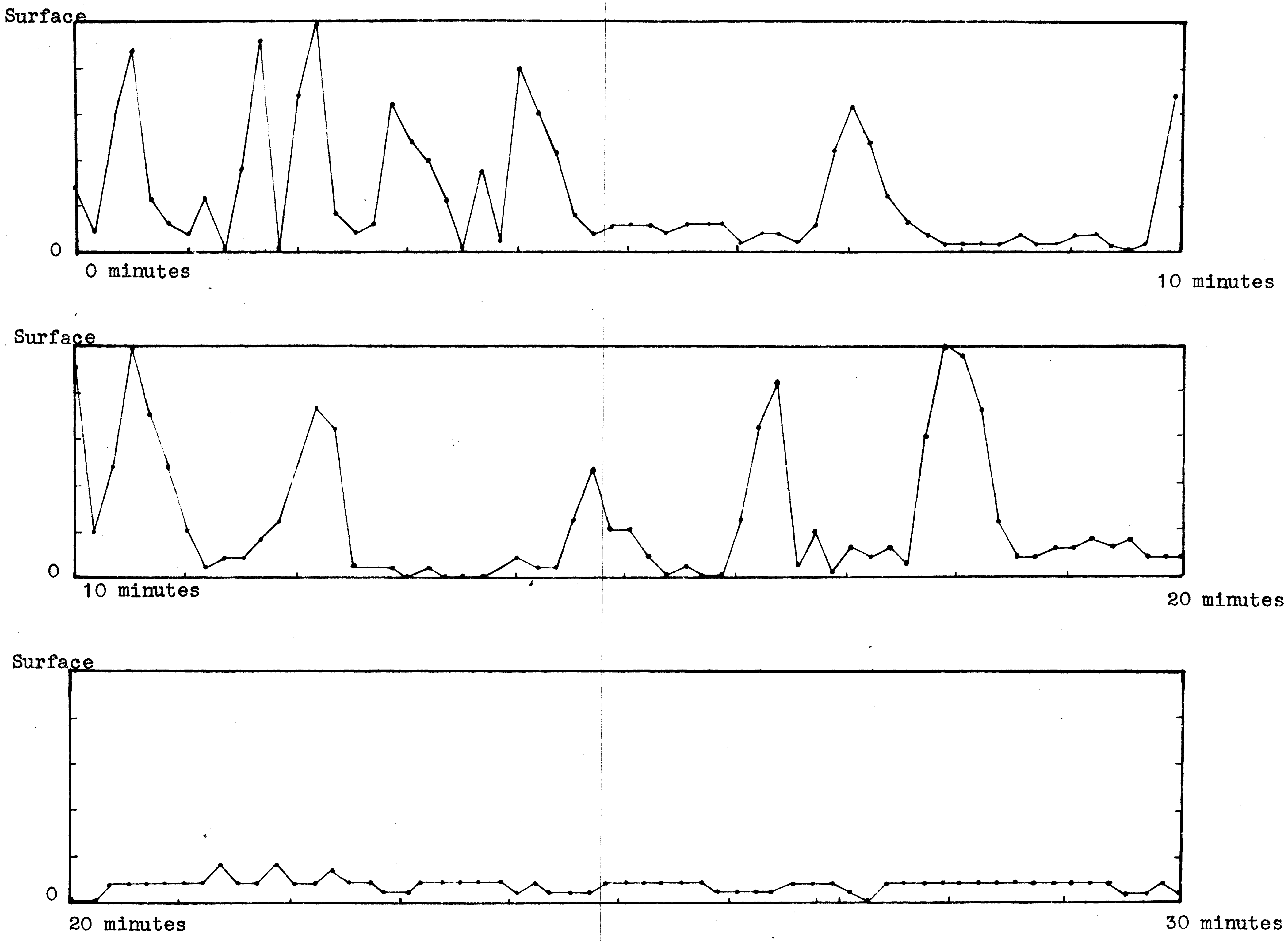
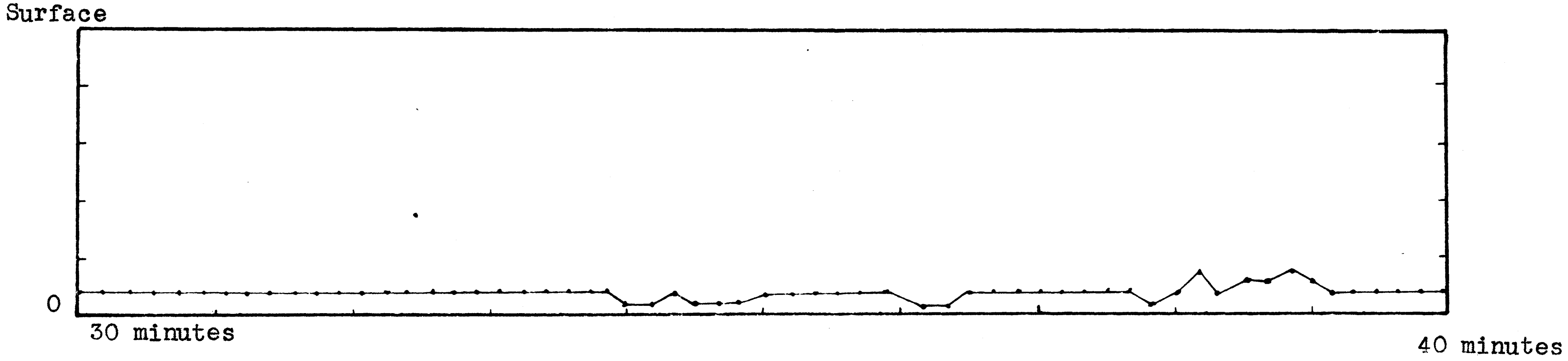


Fig. 63 Activity of a fourth instar larva, O. fuscus, introduced into a tube containing 25 ml. of seawater. The tube was 10 cm. in height.



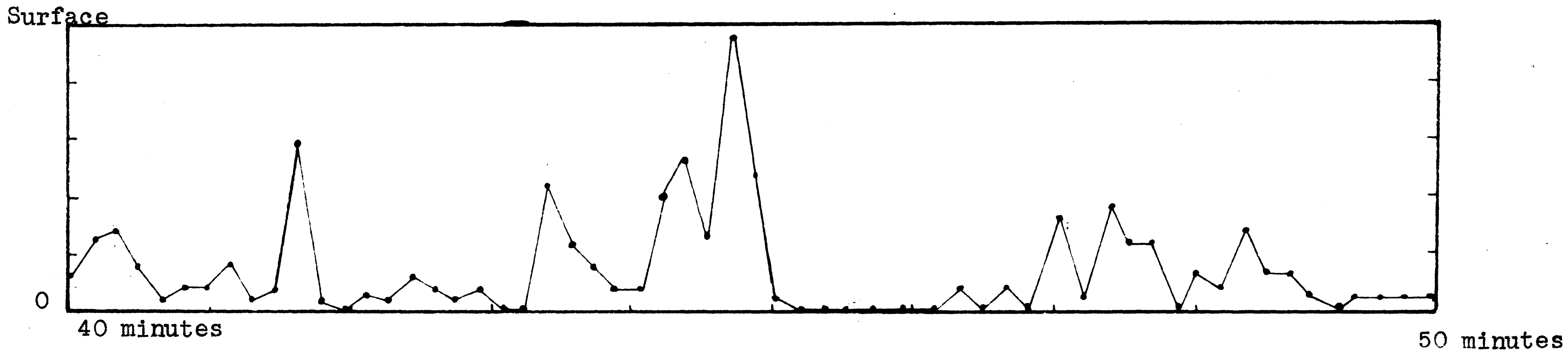


Fig. 63 Continued

seawater without any solid food. Every ten seconds the position of the larva in the cylinder was noted and the results plotted as an actograph.

To test the effect of the deprivation of access to the water-air interface twenty larvae were placed in individual tubes containing 25 mls. of seawater. A close fitting plug of fine nylon mesh was then placed below the water surface in each tube, confining the larvae below.

Results

The activity over a fifty minute period is plotted in Fig. 63, each section representing a ten minute period. When the larva was first placed in the cylinder, represented by the first section of Fig.63 it was most active, rapidly moving up and down the tube in an exploring reaction. After about five minutes the larva settled down and during the next five minutes only twice moved to a little more than halfway up the cylinder, but in the subsequent ten minutes it continued to move freely about. For the following twenty minutes the larva remained in the bottom few centimetres, almost immobile apart from the filtering movements of its mouthbrushes. Succeeding this was a period of increased activity, but not of the intensity of the initial movements.

During the whole fifty minutes the anal siphon pierced the surface only three times, after two minutes immersion, eleven minutes and eighteen minutes. The larva remained below the surface for the remaining thirty two minutes of the experiment.

On another occasion a fourth instar larva was observed for one hour and twenty minutes without coming to surface. Conversely, in a quarter hour period a similar larva surfaced twenty three times.

Field observations confirmed that the frequency with which larvae surfaced was most variable, as was the time for which they remained at the surface. Often larvae remained for two or three minutes surface-feeding, as they breathed, while at other times the siphon would pierce the surface for only a second or two. Pupae never stayed at the surface for more than a few seconds, except when they were ready to emerge.

Of ten fourth instar larvae deprived of access to the surface, all survived forty eight hours, but eight were dead after sixty hours. One of the survivors died after 190 hours of submersion but the other lasted under the surface for 570 hours. Four first instar larvae in the same experiments lasted between 80 and 110 hours during which time they moulted. None of six third instar larvae survived more than 48 hours.

Discussion

The large volume of the tracheal trunks can be correlated with the ability of Opifex fuscus larvae to remain submerged for long periods. Among mosquito larvae, which are normally surface breathers, *O. fuscus* is not unique in this ability, Horsfall (1955) recording that larvae of *Aedes aegypti* have survived denial of access to the surface for nearly 600 hours. Presumably gaseous exchange took place through the integument of *O. fuscus* while submerged, but on several occasions, both in the laboratory and the field, the valves of the siphon were observed in contact with air bubbles adhering to objects below the surface; so these, too, were probably used as a source of oxygen.

CHAPTER 5

SUMMARY

In reviewing previous work on supralittoral pools in the Introduction it was observed that littoral ecologists, in concentrating on describing and accounting for the vertical and horizontal distribution of shore biota have given scant attention to pools, treating them as essentially anomolous situations brought about by shore physiography. Many of the specialized studies on pools have been confined mainly to tide pools (sensu strictu). Investigations of supralittoral pools have often been restrictive in time, number and variety of pools studied, and in geographical areas embraced.

This study of some supralittoral pools of New Zealand's coasts, the first intensive one of its kind undertaken in the Southern Hemisphere, was designed, firstly, to provide an insight into the more important physical properties of the pools. A sufficient number and variety of pools, over 500 miles of coast was included within the survey to enable distinction of local variation from geographical differences. By extending the studies over a year seasonal oscillations in properties were determined.

Examinations of shore physiography showed that planationwas proceeding at different levels along the same shores. On more exposed coasts, especially lower on the shore, rock abrasion and wave quarrying were found to be the most important agents of erosion. Where coasts were more sheltered, and particularly in the supralittoral, water-layering and solution benching have been the dominant processes. While water-layering has been chiefly responsible for widening supralittoral pools, mechanical abrasion has played an important part in theïr deepening. The physiography of coastlines, and of the pools along them, has been strongly influenced by rock type, and investigations by Emery and Joly have shown that in suitable rock, solution may be an important process of pool formation.

The outstanding salinity property of pools was fluctuation. Adjacent pools differed considerably in salinity. Each pool exhibited salinity fluctuations whose frequendy and amplitude were dependent on weather and sea conditions, as well as on the pool's situation and dimensions. There was some, but no absolute correlation between pool level and salinity. Salinity tended to be high when water level was low.

The most striking salinity property of supralittoral pools was stratification, which was exhibited by almost all pools studied. Its extent was most variable. Water of lower salinity coming to overlie more saline water was established as the cause of density stratification within supralittoral pools. Other external and internal disturbances acted towards the restoration of homogeneity within pools.

Supralittoral pools can be usefully classified under the Venice System for the Classification of Marine Waters according to Salinity, recently recommended for universal application. Using the Venice System terminology all pools studied were poikilohaline. Larger pools were usually mixohaline, smaller pools euhaline or hyperhaline. However as pool salinity varied, both in time and with depth, any classification of pools according to salinity must be accepted with caution.

Temperature properties of supralittoral pools were in many ways similar to their salinity properties. Again fluctuation was the chief characteristic, temperature often being markedly different in adjacent pools at one time. Air temperature fluctuations were reflected more closely in pools than in the sea, and more closely in smaller than larger pools. Density layering controlled temperature stratification in pools, bottom temperature usually being higher than surface temperature, although occasionally surface layers were warmer. Mean temperatures of pools differed with situation and dimensions as did salinity.

During the day most supralittoral pools were supersaturated with oxygen, whether they supported growths of filamentous algae or not. As with salinity and temperature, there were fluctuations in oxygen tension within pools, contrasts between pools, and variation with depth. Oxygen tension reached a peak in the early afternoon, decreasing through the rest of the day and night to fall to a minimum at sunrise.

The recorded range of pH from the pools was 6.9 to 9.8, with a mean of 8.5. Some correlation was observed between pH and oxygen tension, high and low value of both occurring together.

Supralittoral pools studied were remarkable for the diversity and variability of their physical properties. In most pools, if colonization is to be successful, species must be tolerant of a wide range of conditions. Not unexpectedly, the biota of most pools was limited to a few species, although where conditions were more stable and favourable, e.g. Pool G32, there was a wealth of species almost every major phylum in the Animal Kingdom being represented. The few species well adapted to the supralittoral pool environment were often present in very large numbers.

Enteromorpha was the most common genus of filamentous algae in supralittoral pools. Its abundance reached peaks in autumn and spring. The most important factors influencing the occurrence of algae in rock pools were the permanency of pools and their exposure to the influence of the sea, algae being most abundant, in permanent pools most exposed to the sea action.

The fauna of supralittoral pools was found to be controlled by the same series of factors as the flora:- exposure to the sea, permanency and dimensions being the most important. Most of the other limiting properties of pools stem from these. Salinity and temperature, as primarily limiting factors, have been over-emphasized by previous authors. The temperature properties of pools are closely related to their dimensions, which together with exposure largely controls salinity. Density stratification provides a vertical range of salinity, but there was no evidence of species being restricted to certain levels within pools. No single factor can be considered as controlling the biota of supralittoral pools. Rather, a variety of factors interact to produce a unique environment within each pool. The outstanding feature of pools of the supralittoral zone is diversity.

Although the systematics and morphology of _Opifex fuscus_ had received considerable attention, little was known of its biology apart from Kirk's work on mating behaviour. Studies on the physical ecology of supralittoral pools, in Chapter Two, provided an insight into properties of the environment of the immature stages of _O. fuscus_. In this environment the mosquito may be associated with a variety of algae and animals but few exhibited as wide a range of tolerances as _O. fuscus_.

Kirk's observations on the peculiar mating habits of O. fuscus have been confirmed and extended. The significance of this mating behaviour lies in the reduction of the time between maturation of the females and their fertilization to an absolute minimum. For an animal living in a temporary environment the conservation of time in the completion of its life cycle is of considerable importance. Rapid and efficient fertilization of available females is ensured by their precociousness, and hypertrophy of the male sex instinct, so that there is minimal wastage of reproductive potential within populations.

The essential criterion of oviposition sites was that they should be moist. Eggs were laid just above the water surface, rock crevices being preferred, but if they were not available a variety of moist surfaces served as suitable substitutes. On submergence eggs hatched within twenty hours, provided they had been suitably conditioned. Judson established that the actual hatching stimulus for aedine mosquito eggs was decrease in oxygen tension of the surrounding water, and field observations and laboratory experiments indicated that O. fuscus eggs hatched in response to a similar stimulus. The oviposition behaviour and hatching mechanism ensure that larvae were only introduced into pools which have recently increased in volume, thus giving larvae a better chance of surviving through to maturity without pools drying up.

Differences in larval densities, both between pools and within each pool, over periods of time were due to differences in the numbers of eggs hatching, and numbers of individuals metamorphising. *Opifex fuscus* populations were at a peak during February, declining to a minimum by July. Pupae and adults were absent from South Island pools from June to September. Although the population density in many pools was very high during summer months, severe reduction resulted, both from the drying up of pools, and flushing by heavy seas, several times during the year. But the populations were almost always re-established from quiescent eggs lining the pool margins.

Examination of gut contents revealed that first instar larvae gained most of their nourishment from yolk enclosed within the hindgut. The other three instars contained an assortment of diatoms, unicellular green algae, protozoa, filaments of blue green alga, detritus, sand, and even fragments of *O. fuscus* larval cuticle within their gut. Bacteria and yeast probably form an important part of the diet of *O. fuscus*, and mosquito larvae are known to be able to grow on materials in solution only.

One of the most significant findings to emerge from this study was that *Opifex fuscus* larvae may develop pectinate or simple types of mouthbrushes in each instar, depending on the particle size and accessibility of available food. In this *O. fuscus* is unique among the Culicidae. It has been clearly established that this balanced polymorphism is biologically rather than genetically controlled. A balance is maintained between the two polymorphs, so that the available food may be exploited to the best advantage of the population. The presence and efficient functioning of simple filter-feeding types of mouthbrushes in association with other mouthparts of a typical browsing facies, suggests that the full filter-feeding facies may not be primitive as Surtees postulated, but a later adaptation to environments in which suspended particles formed the bulk of the available food. Both Edwards and Wood considered O. fuscus exhibited primitive features. If their evidence is accepted the mouthparts of O. fuscus larvae may be regarded as resembling the ancestral form, from which the full filter-feeding facies, and the complete browsing facies are specialization in diverging directions. On the other hand the apparent absence of the clearly advantageous mouthbrush polymorphism from genera which are considered Opifex's closest relatives, and which occupy similar niches, adds weight to Dumbleton's suggestion that Opifex may not be a relict genus, but a strong deviant of later origin. Before any firm conclusion can be reached more detailed study of the larval morphology and biology of other genera, especially of the Chatham Islands Aedes (Nothoskusea) Dumbleton, is required.

The results of the growth rate studies of the immature stages of Opifex fuscus closely conformed to those on other mosquito species. Provided larvae were adequately fed, rates of growth increased with temperature. Light had no effect on growth. As well as being the stage of longest duration at all temperatures, the fourth instar was most affected by changes in temperature. The lower threshold for pupation was below 13°C, but above 5°C.

Opifex fuscus larvae exhibited remarkably wide salinity tolerances and powers of acclimatization, being able to withstand distilled water as well as salinities up to 170°/oo. It was not as toleralant of high salinity as its Australian counterpart *Aedes australis* which has been reported from salinities of 240°/oo. Like *A. australis*, the salinity tolerance and powers of acclimitization of *O. fuscus* first stage larvae are less than those of fourth stage larvae, and for both species the upper salinity threshold for pupation is below the survival threshold.

Histological evidence indicates that osmoregulatory mechanisms in *Opifex fuscus* are similar to those in *Aedes detritus*, salt exchange with the environment taking place via the rectum.

Experiments showed that the frequency with which the anal siphon of *O. fuscus* larvae penetrated the water surface was most variable. One larva survived deprivation of access to the surface for 570 hours. Clearly some exchange took place through the larval integument.

CONCLUSION

Supralittoral pools, where fluctuations in physical properties, especially salinity, may be rapid and extensive, form difficult environments for plants and animals. Each pool is unique, and the pools bring diversity to a zone otherwise noted for its uniformity.

The animals which have successfully colonized supralittoral pools are often present in large numbers, for their competitors are few.

Opifex fuscus exhibits many adaptations which have contributed to its success in a difficult environment. Several of these adaptations are common in other mosquito species occupying similar niches in other parts of the world; e.g. the oviposition behaviour and probable hatching mechanism, salinity and temperature tolerances; but in many respects O. fuscus is a peculiar and aberrent mosquito, not only structurally as Edwards, Miller and Wood have shown, but also biologically with its remarkable mating habits, first described by Kirk, and the unique balanced polymorphism of the larval mouthbrushes.

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APPENDIX

THE WINKLER METHOD.

This method employs the oxidation of manganous hydroxide, by dissolved oxygen to give tetravalent manganese. Upon acidification in the presence of iodide ion, free iodine (or I_3^- ion), is formed, equivalent to the amount of dissolved oxygen.

$$Mn^{++} + 2OH^- \longrightarrow Mn(OH)_2$$

$$2Mn(OH)_2 + O_2 \longrightarrow 2\ MnO(OH)_2$$

$$Mn(OH)_2 + 2H^+ \longrightarrow Mn^{++} \quad 2H_2O$$

$$MnO(OH)_2 + 4H^+ + 3I^- \longrightarrow Mn^{++} + I_3^- + 3H_2O$$

The complex iodine-iodide ion (I_3^-) thus released is then titrated against thiosulphate solution using starch as indicator.

$$I_3^- + 2S_2O_3^= \longrightarrow 3I^- + S_4O_6^=$$

A committee of the Association Oceanographie Physique on Chemical Methods and Units has recommended that the oxygen content be reported as the amount in milligram-atoms, dissolved in an amount of sea water that at 20^o has the volume of 1 litre, or in ml. at N.T.P. dissolved in a similar amount of water. The molecular volume of O_2 is 22.393 litres, its atom weight is 16.00. Therefore the atomic volume of oxygen is 11.1965 litres, and the units can be interconverted as follows:

11.1965 x milligram atom/litre = ml/litre.

To convert oxygen concentrations to parts per million

mg-a/litre = ml/litre

11·1965 mg - a/litre/ml/litre = 0.179144 gm/l

= 179.144 gm/1000l.