# ASPECTS OF THE BIOLOGY OF THYASIRA GOULDI (PHILIPPI) AND ITS COPEPOD PARASITE AXINOPHILUS THYASIRAE (BRESCIANI AND OCKELMANN)

A thesis submitted for the degree of Doctor of philosophy of the University of Stirling

W.M. Blacknell B.Sc. (London)

Nothing is as simple as when you first look at it.

The work presented in this thesis is the result of my own investigations, it has not been nor will be submitted for any other degree.

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Aspects of the biology of *Thyasira gouldi* (Philippi) and its copepod parasite *Axinophilus thyasirae* (Bresciani and Ockelmann).

A thesis submitted for the degree of Doctor of Philosophy
by

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## Abstract

Taxonomic differences exist between *Thyasira gouldi* and *T. flexuosa* both in conchological features and in the soft parts, perhaps the best of these differences are those concerning the sperm which are easily seen even in badly curated specimens.

The occurrence of a population of *T. gouldi* within Loch Etive on the west coast of Scotland extends the known distribution of this arctic bivalve considerably further south. The development is non-pelagic and this, associated with the non-synchronized reproductive activity , means that independence is gained from plankton blooms and other external triggers allowing the characteristic dense but patchy populations to be built up.

Salinity and sediment particle size both limit the distribution of *T. gouldi* within L. Etive, the

naturally occurring sediment is altered by the inhabitants of the area into a sediment which is better suited to the needs of *T. gouldi*.

Asynchronized breeding, slow growth rate, longevity of life and low adult mortality all combine to give a characteristic bimodal population, the modes of which do not appear to shift with time. A method is described whereby an indication of both the mean state of the population and the state of the individual can be obtained for any period of the year. The biochemical composition varies with size and time of the year as well as with gonad state. Dry weight fluctuations are, however, not entirely explained by variations in the stage of gonad development as seasonal variations in the amount of somatic tissue do exist.

*Thyasira gouldi* is one of the Lucinaceans infected by the much modified copepod parasite *Axinophilus thyasirae*. The life cycle and infective stage of this parasite are described as is the external morphology of the nauplius copopedite and adult. The reproductive system of both the male and the female are also described. The first infected parasite is always female and only rarely does more than one female reach maturity within any one host even though up to five parasites may be present. The parasite has been shown to affect the biochemical composition of the host, reducing the amount of each of the components present, but tending to reduce the amount of nitrogenous material in preference to the amount of carbohydrate.

The parasite affects the gonad of the host resulting in castration probably as an indirect effect of 'food robbing'. The interference to the host's food supply by the parasite is thought to be so great as to reduce the host to starvation levels of food, and tissue reserves, preventing gonad formation.

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# List of Abbreviations used

| | |
|---|---|
| A | acrosome |
| AA | anterior adductor muscle |
| AB | abdomen |
| AD | anterior diverticulum of gut |
| AM | abdominal musculature |
| An | antennae |
| AS | attachment to shell |
| AT | apical tuft |
| C | ctenidium |
| Ce | cephalic region |
| CT | suspected course of ciliary tracts |
| DG | digestive gland |
| ED | ejaculatory duct |
| ES | egg sacs |
| F | foot |
| G | first pair of appendages |
| GA | genital atrium |
| GO | genital opening |
| H | antenna claw |
| J | second pair of appendages |
| L | ligament |
| Lc | living chamber |
| M | mouth |
| ME | mantle edge |
| MT | mucus lined tube |
| Mu | musculature |
| O | oesophagus |
| OH | oral horns |
| OT | oral thickening |
| ot | oviduct |
| oto | oviduct opening |

| | |
|---|---|
| ov | ovary |
| P | prodissoconch |
| PA | posterior adductor muscle |
| Pa | parasite |
| PD | posterior diverticulum to gut |
| PH | point of hatching |
| PRM | pedal retractor muscle |
| R | rectum |
| RA | rejection area |
| RY | reserves of yolk |
| S | stomach |
| So | spermothecal opening |
| Sp | spermatophore sac |
| SS | sediment surface |
| SSc | spermothecal sac |
| ST | sheathless tip |
| T | tail |
| Te | testes |
| Th | thoracic region |
| UT | unlined tube |
| V | velum |
| Vdp | vas deferens procurrens |
| Vdri | vas deferens recurrens inferior |
| Vdrs | vas deferens recurrens superior |

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## General Introduction

In surveys of Scottish sea lochs, both Gage, (1972a, 1972b,) and Pearson (1970, 1971, 1972) found large populations of lucinacean bivalves among the fauna of muddy substrata in the sublittoral benthos. The Lucinacea (Eulamellibranchia) are a relatively small family consisting of about one hundred closely related species (Ockelmann, pers comm.). The family has a widespread distribution, being found from the arctic to the tropics, generally on soft mud/mud sand bottoms, and at all depths.

Among the Lucinacea found by Gage and Pearson was a member of the Thyasiridae: a small translucent shelled bivalve which was identified as *Thyasira flexuosa* (Montagu). This species was regarded as occurring in several of the Scottish sea lochs eg. L.Etive, L. Linnhe and L. Eil in quite dense and often extensive populations. An ideal opportunity thus arose to study these populations and those of other Lucinaceans and to compare them within each loch system.

The literature concerning this family is not very extensive. Allen, 1958, described the basic form and functional morphology of several members of the family but apart from this paper and others by the same author (Allen 1953, 1960) the rest of the literature concerning this family is concerned with distribution e.g. (Thorson 1936, 1946, Zatsepin 1962,

Tebble 1966, Kuznetsov 1970, Kauffman 1967, Pearson 1970, 1971, 1972, Bernard 1972 and Gage 1972a, b) and nomenclature (Tryon 1872, Dall 1901, Lamy 1920, Chavan 1937, 1938, 1952, 1966, Ockelmann 1961).

During the course of investigations of the *Thyasira* of Loch Etive it was established, with the assistance of Dr. K. Ockelmann of the Marine Biological Laboratory, Helsingør, Denmark, that in the upper region of L. Etive, at least, the species present was in fact *T. gouldi*. It was thus possible to study an arctic species which was within easy access of the laboratory and so a detailed investigation of this species formed the major part of the research programme.

The aims of this research programme were therefore altered from a study of the Lucinaceans within several loch systems to the study of *Thyasira gouldi*, and to a lesser extent a comparison of this species with *T. flexuosa*; to provide information on the distribution, ecology and ecological physiology of *T. gouldi*, an arctic species which has survived since the last glaciation in an area south of its normal distribution (Ockelmann 1959, 1961).

# TAXONOMIC AND ECOLOGICAL DIFFERENCES BETWEEN THYASIRA GOULDI AND THYASIRA FLEXUOSA.

The morphological differences between <u>Thyasira gouldi</u> and <u>Thyasira flexuosa</u> are summarized in Table I. There are both conchological differences and differences in the soft parts.

The shell of <u>T. flexuosa</u> has a tendency to be five sided (see Fig. 1 also Merklin 1954) whilst that of <u>T. gouldi</u> is more rounded.

The ligament of <u>T. flexuosa</u> is less pronounced. The tooth found on the left shell valve just below the umbones is more prominent in <u>T. flexuosa</u> being almost non-existent in <u>T. gouldi</u>.

The first fold of the shell is more pronounced in <u>T. flexuosa</u> forming a sharply defined ridge, whereas the fold in <u>T. gouldi</u> is wavy with the ligament more shallowly inset into the resulting depression between the two shells.

The prodissonconch of <u>T. flexuosa</u> is very poorly defined, measuring approximately 160-190 um whilst that of <u>T. gouldi</u> is much clearer and measures 210-250um (Ockelmann 1961), a prodissoconch size range not seen in <u>T. flexuosa</u>. This difference in prodissoconch size is a direct result of developmental differences. <u>Thyasira flexuosa</u> has a pelagic, albeit rather short, larval stage, whereas, as will be shown later, <u>T. gouldi</u> exhibits direct non-pelagic development, as predicted by Thorson (1936, 1946).

There are several differences in the soft parts.

Table 1

Differences between *T. flexuosa* and *T. gouldi*

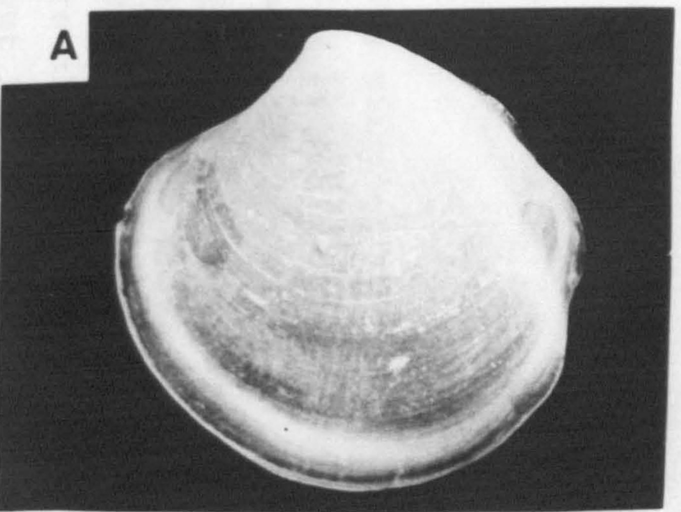
| | *T. flexuosa* | *T. gouldi* |
|---|---|---|
| Conchological features:- | | |
| Shell outline | pentagonal | rounded |
| Ligament | indistinct | well defined |
| Hinge teeth | prominent | not prominent |
| Prodissoconch | poorly defined 160-190µm | distinct 210-240µm |
| Rejection Area | elongate - thin | thick - compact |
| Ctenidia :- | | |
| filaments | 48-52µm thick | 64-66µm thick |
| interfilamental junctions | 104µm apart | 115um apart |
| Palps | 200µm deep | 250-330µm |
| Mantle Edge | thick - dark line | no dark line |
| Egg size | 150-160µm | 170-190µm with capsule = 230µm |
| Sperm :- head | acrosome well defined | acrosome poorly defined |
| tail | short 20-30µm | long - 60µm |

for further details see text

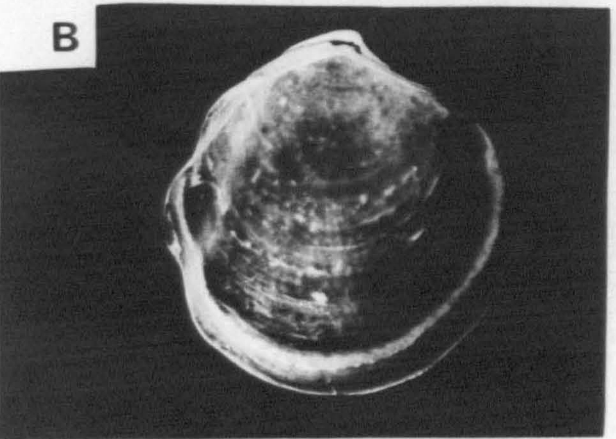
Figure 1

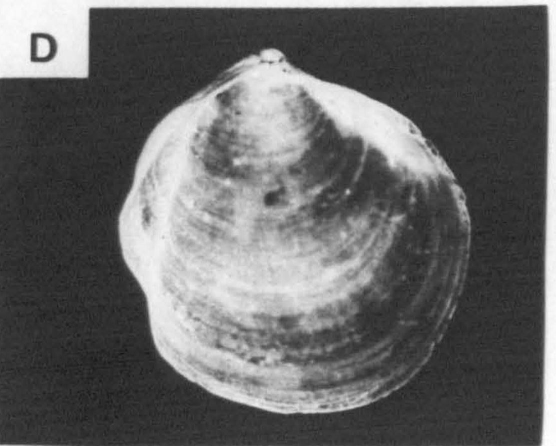
Figure 1a and 1b showing the shell of
*Thyasira gouldi* Philippi

Figure 1c and 1d showing the shell of
*Thyasira flexuosa* Montagu


A

C


B


D

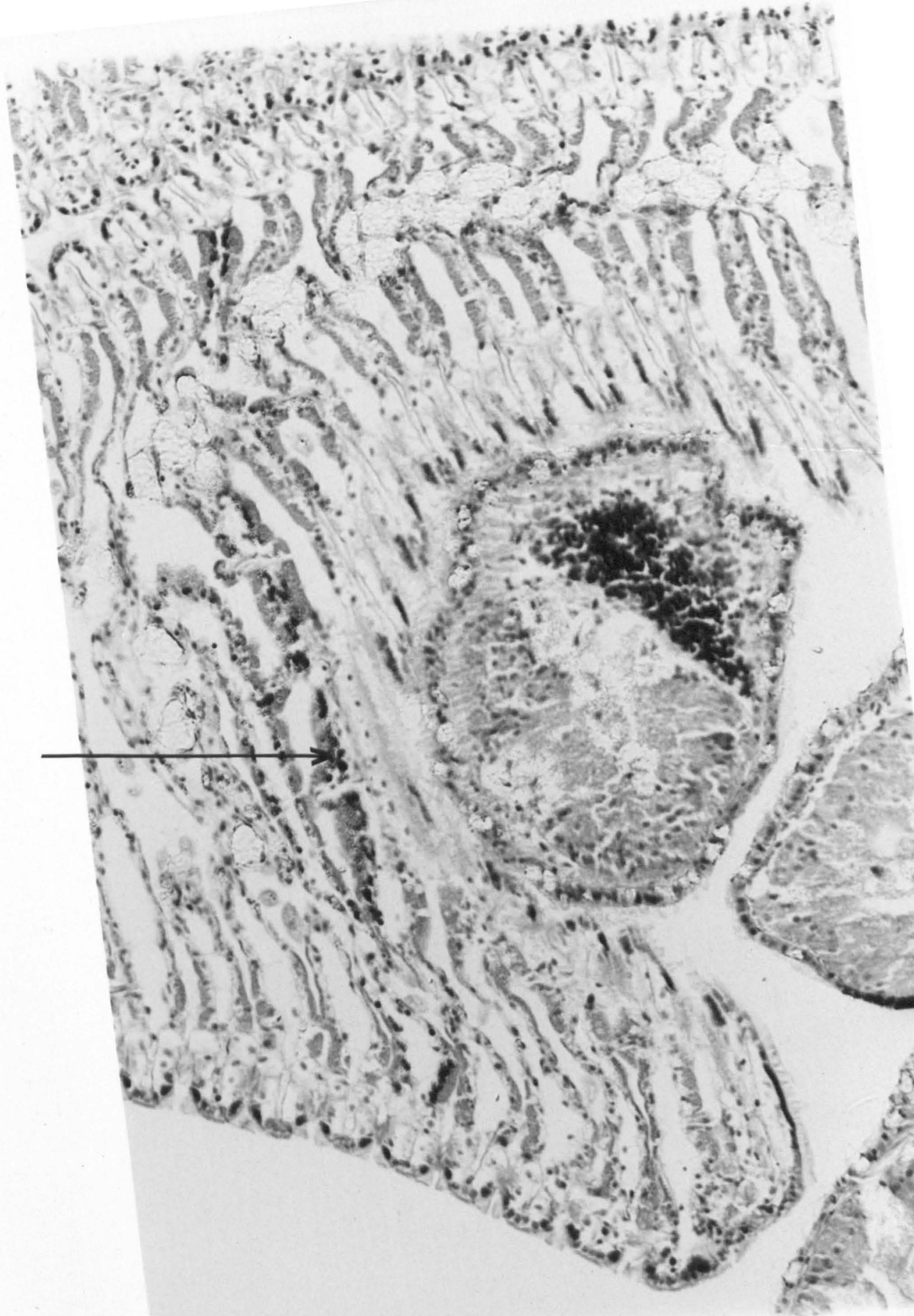
The anterior sorting area (Allen 1958) is much more restricted in <u>T. gouldi</u>              It is probable that the real function of this area is not to sort fine particles, but actually to rid the cavity of large particles which may be brought in (Ockelmann pers. comm.), and it should more correctly be termed an 'anterior rejection area'. The differences between the two species in this area may be related to differences in feeding behaviour.

Pigmentation is often seen along the ctenidia (Allen 1958) and in some animals it can be so extensive as to give the appearance of a black ctenidium. Examination of these ctenidia show that this material is embedded within the gill filaments (Fig. 2 ). It is concluded that these particles are excretory and that they are passed into, and stored in, the ctenidia. In <u>T. gouldi</u> these particles are distributed in patches whilst those of <u>T. flexuosa</u> tend to be aggregated into lines.

The gills of <u>T. gouldi</u> give a general impression of being larger and more strongly built. The gill filaments are much thicker (64-66μm) than those of <u>T. flexuosa</u> which measures 48-52μm. The interfilamental junctions which tend to form lines along the ctenidia are more easily discernible in <u>T. flexuosa</u>, and are closer together, ie. 104μm apart as opposed to about 115um apart in <u>T. gouldi</u>. The palps of <u>T. gouldi</u> are larger. When relaxed in propylene phenoxytol (Owen, 1955) the palps of <u>T. gouldi</u> measure in the region of 250-330μm whilst those of <u>T. flexuosa</u>

Figure 2

The pigmentation deposited within
the ctenidia of *Thyasira gouldi*



are less than 200um in depth.

The colour of the digestive gland is orange/ brown in T. gouldi but is 'dark walnut' in T. flexuosa and is far more convoluted in the latter.

In T. flexuosa a dark line is often seen along the mantle edge in the region of the pedal gape. This is probably a sensory region or an accumulation of mucus producing cells.

In the ripe state the egg of T. flexuosa is only 150-160um in diameter whilst that of T. gouldi is 170-190um, with the capsule in which it develops making a total 'egg' size of up to 230um. The eggs of both species have a tendency to be oval in shape.

Probably the largest single factor as far as soft parts are concerned is the difference of the sperm (Fig. 3). Both species possess the primitive type of sperm associated with release of sperm into the water (Franzèn 1955). However, although the heads of the sperm are the same length in both species they differ greatly in shape. The sperm of T. flexuosa (Fig. 3b) is more slender, with a pointed prominent acrosome, whilst that of T. gouldi (Figs. 3a, 52b) is more robust in appearance with a hardly distinguishable acrosome. The sperm tail of T. gouldi is in the region of 60um in length, whilst the sperm tail of T. flexuosa is only 20-30um in length. This may be associated with differences in motility related to differences in fertilization site (Franzèn 1955).

Figure 3

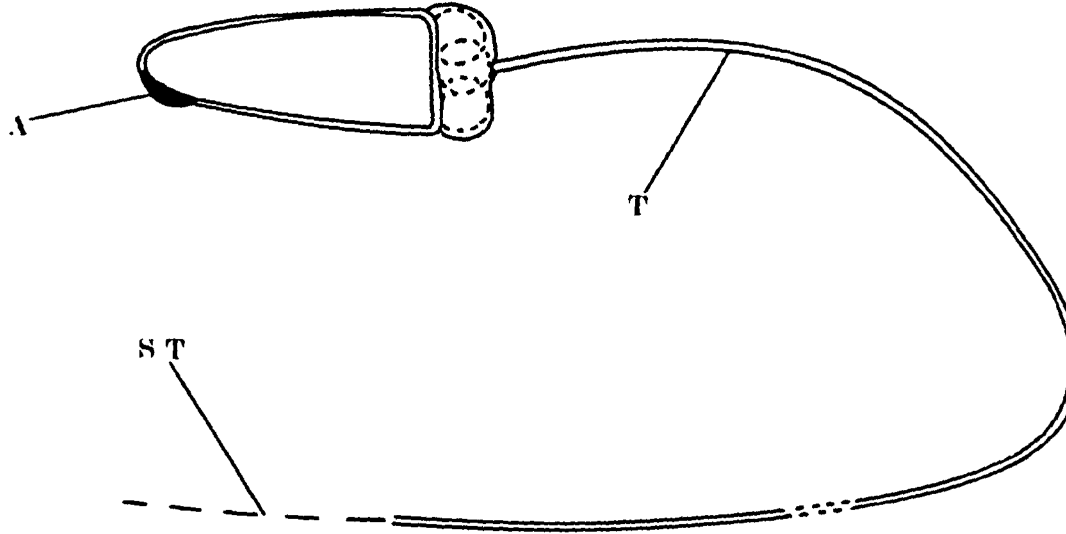
Diagrammatic representation of the sperm of

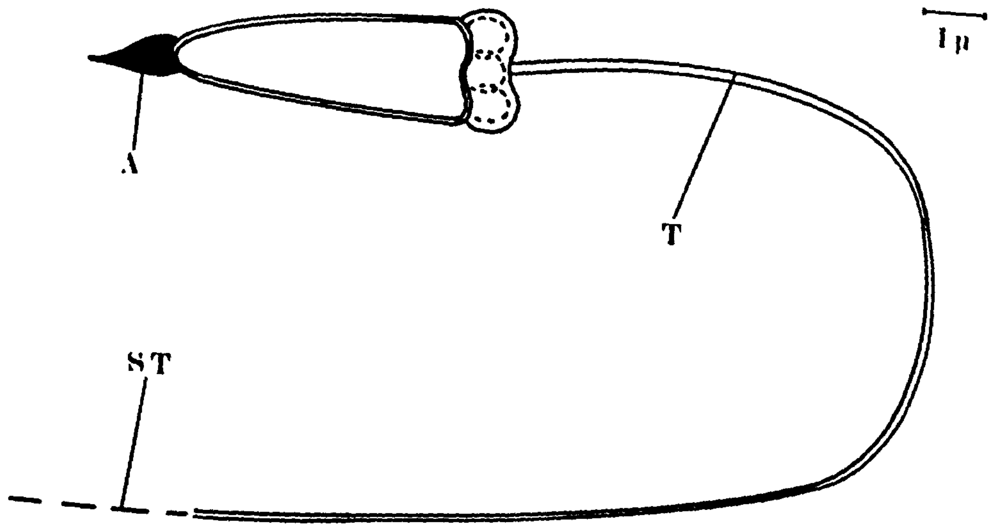
*Thyasira gouldi* Figure 3A

*Thyasira flexuosa* Figure 3B

(For list of abbreviations used see page xvii)

T. gouldi




T. flexuosa

Sufficient differences thus exist to clearly establish the separation of these two species. They can be discerned quite easily on soft parts alone, or if the shell is not too broken or too worn in the region of the umbones, the two species can be distinguished on external features alone. The differences in the sperm are not destroyed by drying (Ockelmann pers. comm.) therefore the two species can still be distinguished even in badly curated museum material.

Both *T. flexuosa* and *T. gouldi* inhabit soft mud to sand/mud sediments generally characterized by a relatively large amount of organic matter and often smelling strongly of hydrogen sulphide (Blegvad 1914, 1922, Bellan 1957, Buchanan 1963, Holme 1966, Bagge 1969, Kuznetsov 1970, Lie and Kisker 1970, Gage 1972a,b). They occur at all depths from a few to several hundred metres (Ockelmann 1959, Tebble 1966 (no distinction made between *T. flexuosa* and *T. gouldi*)) often constituting the bulk of the biomass.

*Thyasira gouldi* is restricted to the waters of the North, it is found in the waters of Russia where it is referred to as *T. flexuosa* (Kuznetsov 1970) an attempt is made by Miloslovskaya (1970) to correct this misidentification. It is found along the northern coast of Norway, around the coast of Greenland (the *T. flexuosa* of Thorson 1936, 1946, see Ockelmann 1958) stretching south as far as the Hebrides with a few odd dead shells having been found around Skye. These latter are thought by Ockelmann (pers. comm.) to be quarternary fossils and not to represent any living population. To this distribution can now be added the population of L. Etive and also a population in a similar loch situation, that of the Borgen fjord, a small fjord off the Trondheim fjord. In view of the population in L. Etive and of a population (which is now thought to be lost) in the Linnhe/Eil loch system, it may be that other populations exist and that the shells of Skye are from a present day population of this adaptable arctic bivalve. On the American coast *T. gouldi* is known from Nova Scotia and the Gulf of

St. Lawrence but due to the cold water currents of this area it is found further south off California on the East coast and Cape Cod on the west (Ockelmann pers. comm.).

*T. flexuosa* is a warmer water species and although quite large populations can be found around the north coast of Scotland it is nearing its northern limit and is not found beyond the Faroes.

Allen (1958, 1960) has described the functional morphology of several species of Lucinacean bivalves, including that of *T. flexuosa*. No attempt has therefore been made to make a detailed study of the anatomy of these species. The following notes refer to aspects of the functional anatomy of the species which are important in investigating their ecology.

Both *T. gouldi* and *T. flexuosa* lack siphons, and maintain communication with the surface by a long tube constructed of mucus rings formed and maintained by the foot (Allen 1953, 1958, Merklin 1954). Allen (1958) found that if the tube was blocked the obstruction was pushed out by vigorous action of the foot. Just prior to spawning the foot also clears the mucus tube probably ensuring that no obstruction will be encountered by the gametes. The action of the foot in cleaning this tube means that the tip is often lost by being eaten by polychaetes (Ockelmann pers. comms.) or sometimes by fish which have been found with thyasirids in their stomachs (Blaney 1904, Clapp 1912, Jones 1952). Allen (1953, 1958) in a figure of the inhalent

tube of *T. flexuosa* shows the tip turning, and running parallel with the substratum for several millimetres, concluding that particles can be drawn in from the sediment surface. This bend in the tube has not been observed in *T. gouldi* and is not thought to occur in this species.

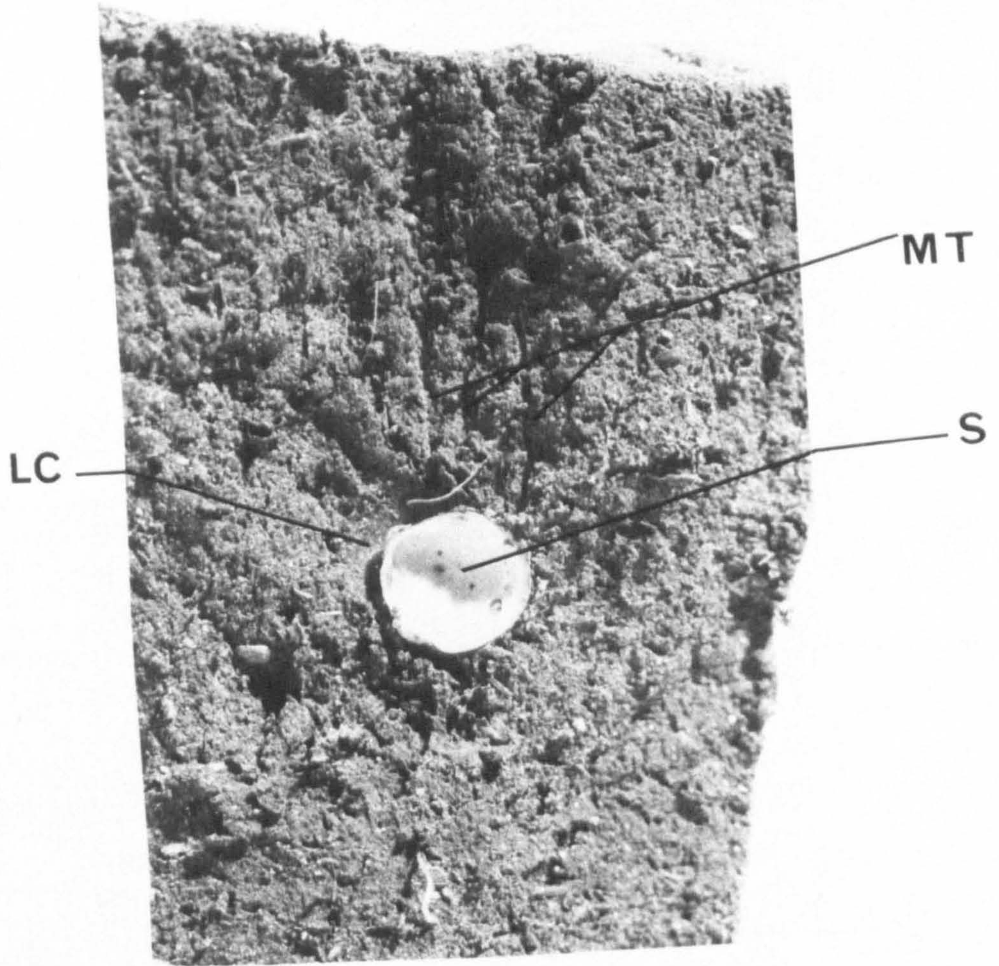
Observations of *T. gouldi* within sediment has shown that quite large movements occur, necessitating the rebuilding of the inhalent tube. Often 'burrows' can be found with several inhalent tubes (Fig. 4 ) though nearly all of these old tubes are blocked off by the mucus lining of the living chamber. Within this chamber the thyasirid tends to lie vertically but at an angle with the surface (Fig. 4 ). In all the burrows studied the 'living chamber' is larger than the shell, is inclined downwards, and is lined with a thin layer of mucus. The animal is always situated at the lower end of the chamber. It is possible that the chambers are formed by slow but continued burial, the upper parts of the chambers subsequently collapsing. It was noted that from the 'living chamber' radiated several randomly placed transitionary tubes similar to those described for other Lucinids including *Diplodonta notata* and *Phacoides pectinatus* (Stanley 1970). When the burrow was first constructed these were quite short separate tubes (Fig.5a ). However, they were subsequently lengthened and interconnected forming quite a large network (Fig.5b). Unlike the inhalent tube, however, they lack a mucus lining. They may be exhalent siphons, taking the place of the single tube depicted for *Loripes lucinalis*

Figure 4

Cut away sections of established burrows of *Thyasira gouldi*

(for list of abbreviations used see page xvii )

A




B

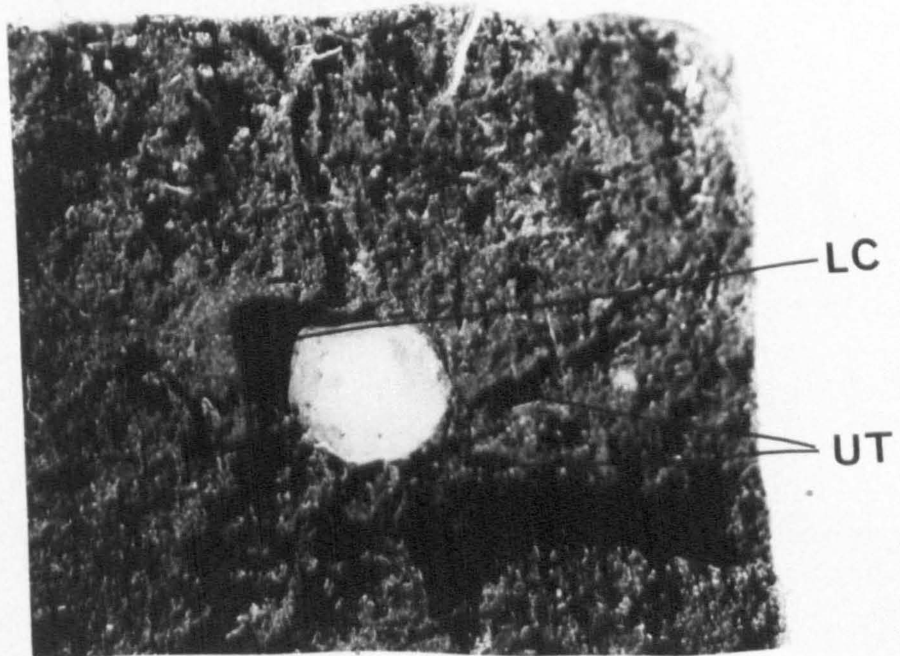
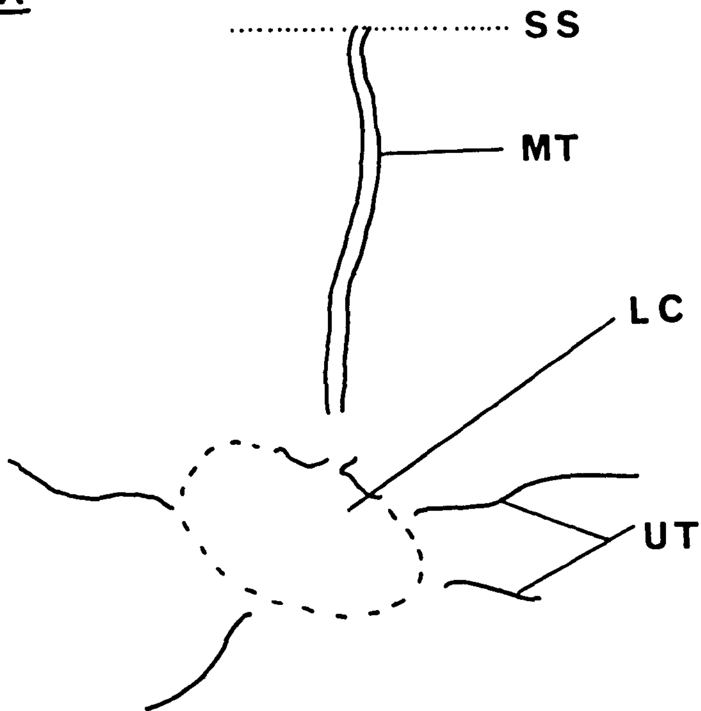


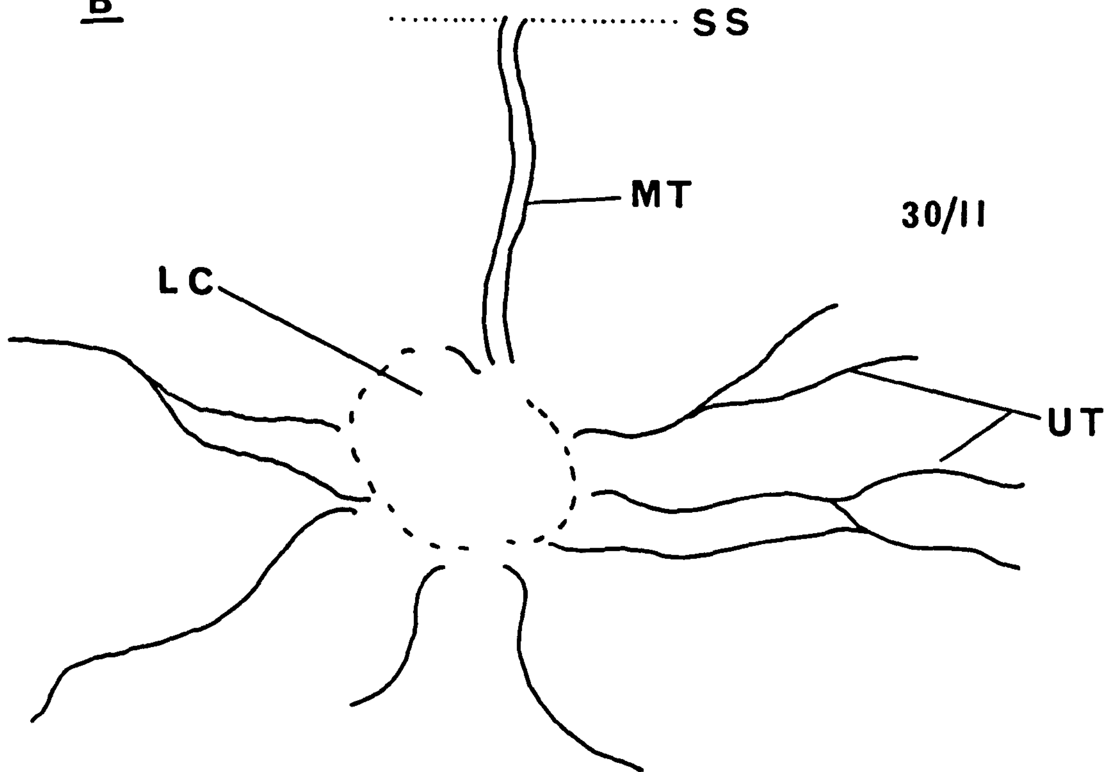
Figure 5

Diagrammatic representation of burrow of
*Thyasira gouldi*

a) just after completion of burial into sediment
b) extension of burrow after two days

(for list of abbreviation used see page xvii )


A
SS
MT
LC
UT
28/11


B
SS
MT
30/11
LC
UT

by Merklin 1954, but Ockelmann (pers. comm.) believes them to be correlated with spawning allowing the rapid ejection of water. However, the possibility that they may be connected in some way with feeding also exists.

The foot is the same in both species and can be extended to ten times the shell length (Rhoads and Young 1971). Distally it enlarges to form a bulbous tip which consists of three regions (see Fig. 6 ), a proximal cuticular region, then a region covered with short cilia capped terminally by a region with very long cilia. In none of the burying activities observed was the foot forced through the substrate; instead the long cilia were used as brushes moving particles out of the way. This gentle method of burial may account for the slowness of the process and account for the need for a loosely compacted substrate.

The animal feeds on the finely divided organic matter within the sediment. Analysis of the gut contents showed that little large material is found in the stomach, contrary to the belief of Young and Rhoads (1971), although small particles are plentiful. The currents of the mantle area are probably concerned with keeping smaller particles in suspension, while larger particles are rejected by the area described by Allen (1958, 1960) as the anterior sorting area (Ockelmann pers. comm.). It is therefore probable that bacteria plays a large role in the food intake of this species, as they do in some other bivalves (ZoBell and Feltham 1938).

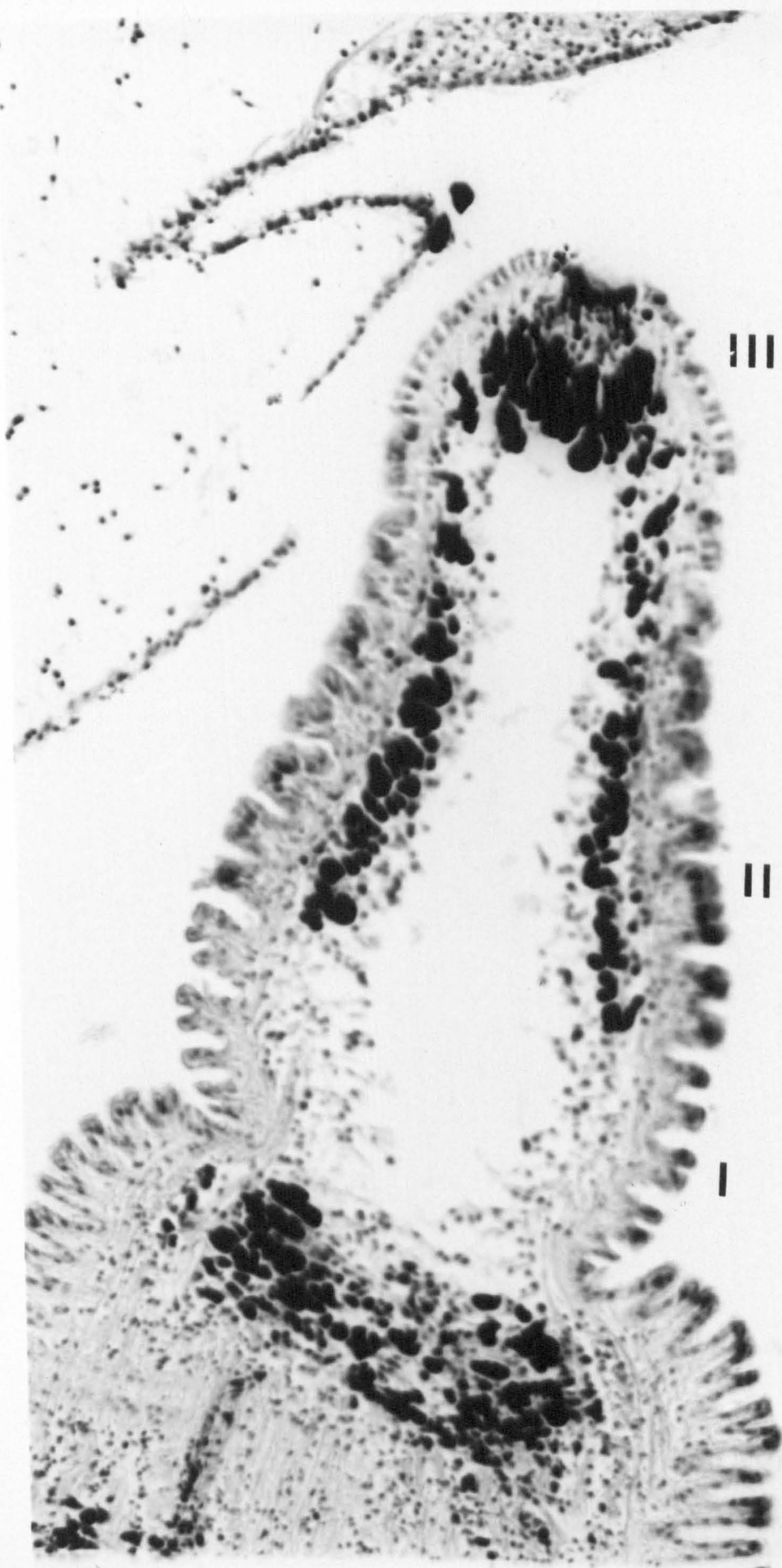
Figure 6

Section across the foot of *Thyasira gouldi* showing division into three regions

I cuticular region

II ciliated ..

III terminal ..


III
II
I

# THE BIOLOGY OF THYASIRA GOULDI IN LOCH ETIVE.

## I) The Study Area

i) General Description

Loch Etive (Fig. 7 ) on the west coast of Scotland, approximately five kilometres from Oban, is a finger-shaped loch, twenty-seven kilometres long, restricted at its seaward end by a narrow sill through which the flow of the tidal currents are modified forming, at certain states of the tide, the so called Falls of Lora.

The loch is effectively separated into two basins by a further shallow narrows at Bonawe. The first basin is about eleven kilometres long with a maximum depth of fifty-five metres, whilst the upper basin has a maximum depth in excess of a hundred and fifty metres. Because of these two sills, sea water interchange within the upper loch is very reduced (Milne 1972) and this isolation associated with high rainfall (273cm in 1970, 240cm in 1971, Meterological Office Monthly Report Summaries) and a large catchment area (McLuskey and Heard 1971) results in the head of Loch Etive being an area of permanently reduced salinity.

The population of _T. gouldi_ studied appears to be restricted to the upper 1-2 kilometres of Loch Etive at depths below 15 metres. The thyasirids collected elsewhere in the Loch were all _T. flexuosa_.

The maximum depth of the study area is about thirty metres (Fig. 8 ). Organic matter, which is mainly of terriginous origin, is plentiful and finely divided. The shallower regions, ie. less than fifteen

Figure 7

General map of west coast of Scotland
in vicinity of Oban to show
position and extent of Loch Etive

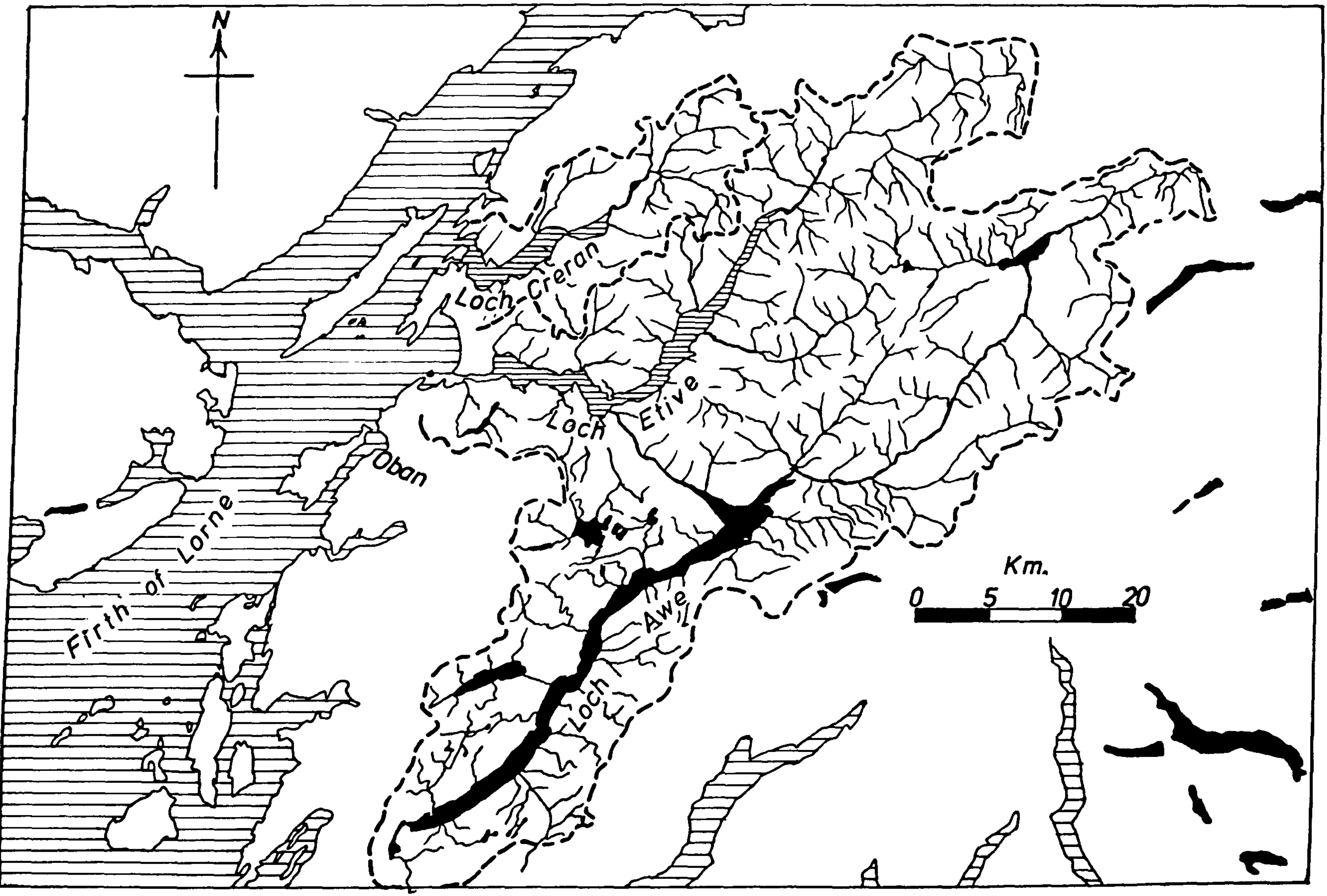
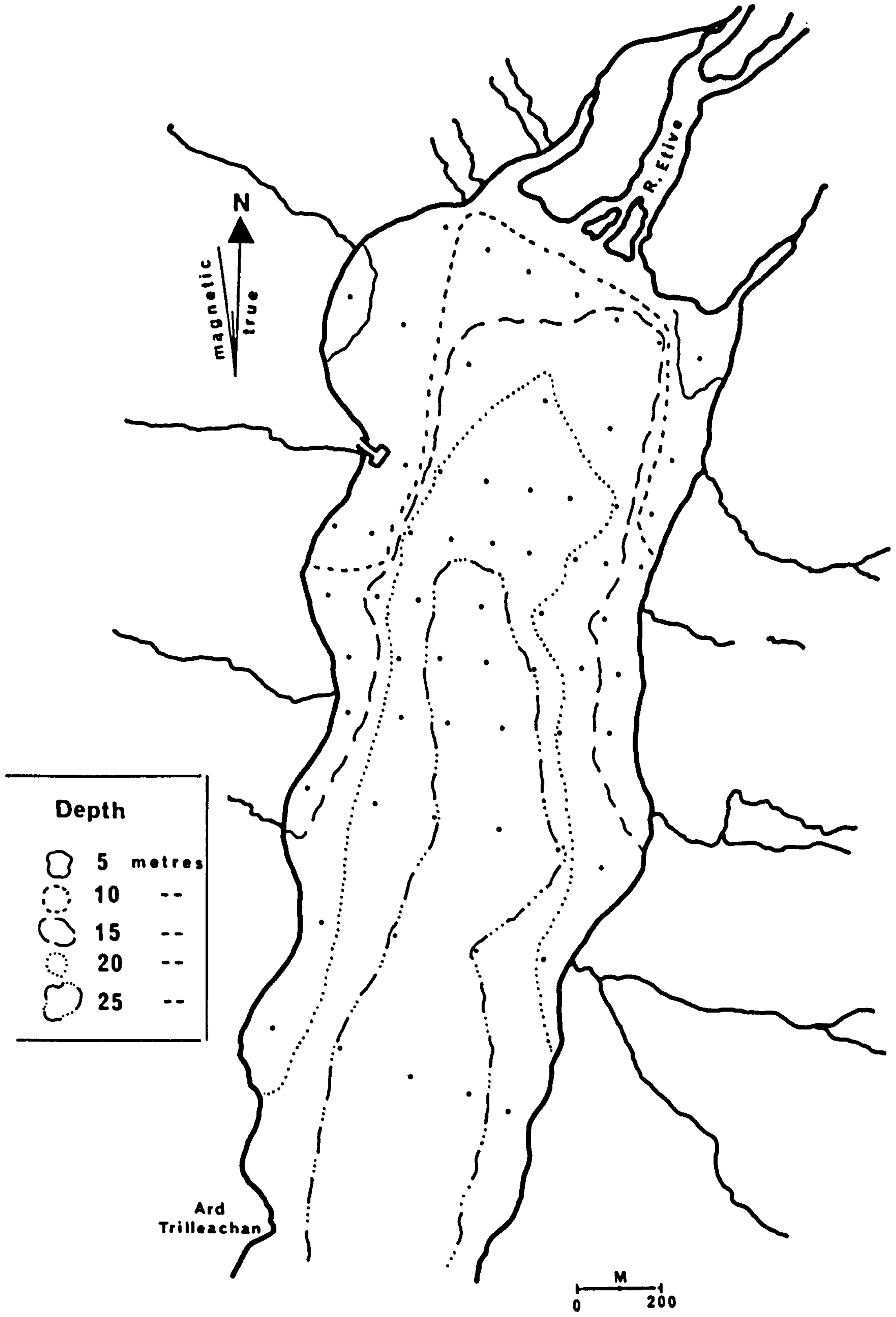

N
Loch Creran
Loch Etive
Oban
Firth of Lorne
Loch Awe
Km.
0 5 10 20

Figure 8

Depth Contour map of Study area at
head of Loch Etive

• - indicate stations for large survey


N
magnetic
true
R. Etive
Depth
5 metres
10 --
15 --
20 --
25 --
Ard
Trilleachan
M
0
200

metres, are marked by the presence of large amounts of leaf litter and small branches.

ii) Hydrographic data

Salinity and temperature data, obtained at fortnightly intervals from several points at the head of the loch (Fig. 9 ), show that generally the salinity and temperature are uniform across the loch, depending upon depth. However, there is a tendency for the water from the River Etive (which has a flow rate of 2-50 cubic metres/sec.) to have a greater effect on the deeper western channel than on the rest of the study area. Salinity and temperature data is summarized in Figs.10 and 11 to which has been added the relevant portion of the unpublished data collected by Mr. B. Grantham and more recently by Mr. A. Edwards (see also Gage 1972 and Milne 1972).

There is a seasonal variation in salinity mainly as a result of variations in the amount of freshwater run off. Surface salinities may vary from virtually fresh water to $25^{o}$/ooS. These very low salinities are not encountered below ten metres although salinities of $20^{o}$/ooS are not uncommon (Fig.10 ). From fifteen metres and below the salinity is less variable, being in the region of 23-$25^{o}$/ooS during the winter months and rising to $27^{o}$/ooS in the summer. However, salinities of less than $23^{o}$/ooS have been recorded (November/ December 1970 and March 1971) whilst the maximum salinity for 1972 was only $25^{o}$/ooS.

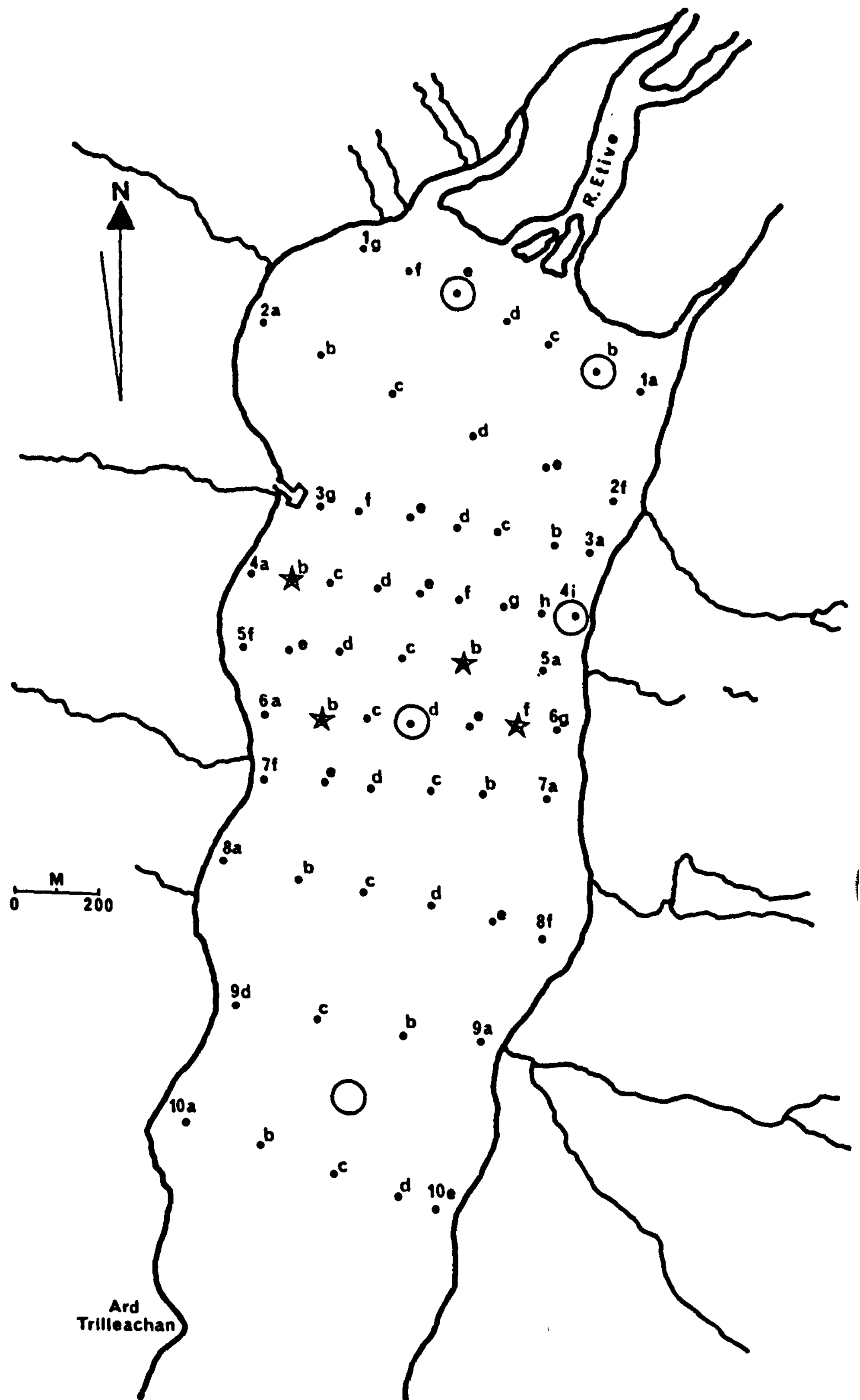
Figure 9

General map of study area to show
position and number of sampling stations

☆ - Stations used for monthly population samples

◯ - Stations used for obtaining salinity and temperature data

• - Stations used for large survey



N
R. Etive
M
0
200
Ard
Trilleachan

Figure 10

Salinity data profile for study area

Data expressed as ‰ - means for all data combined.

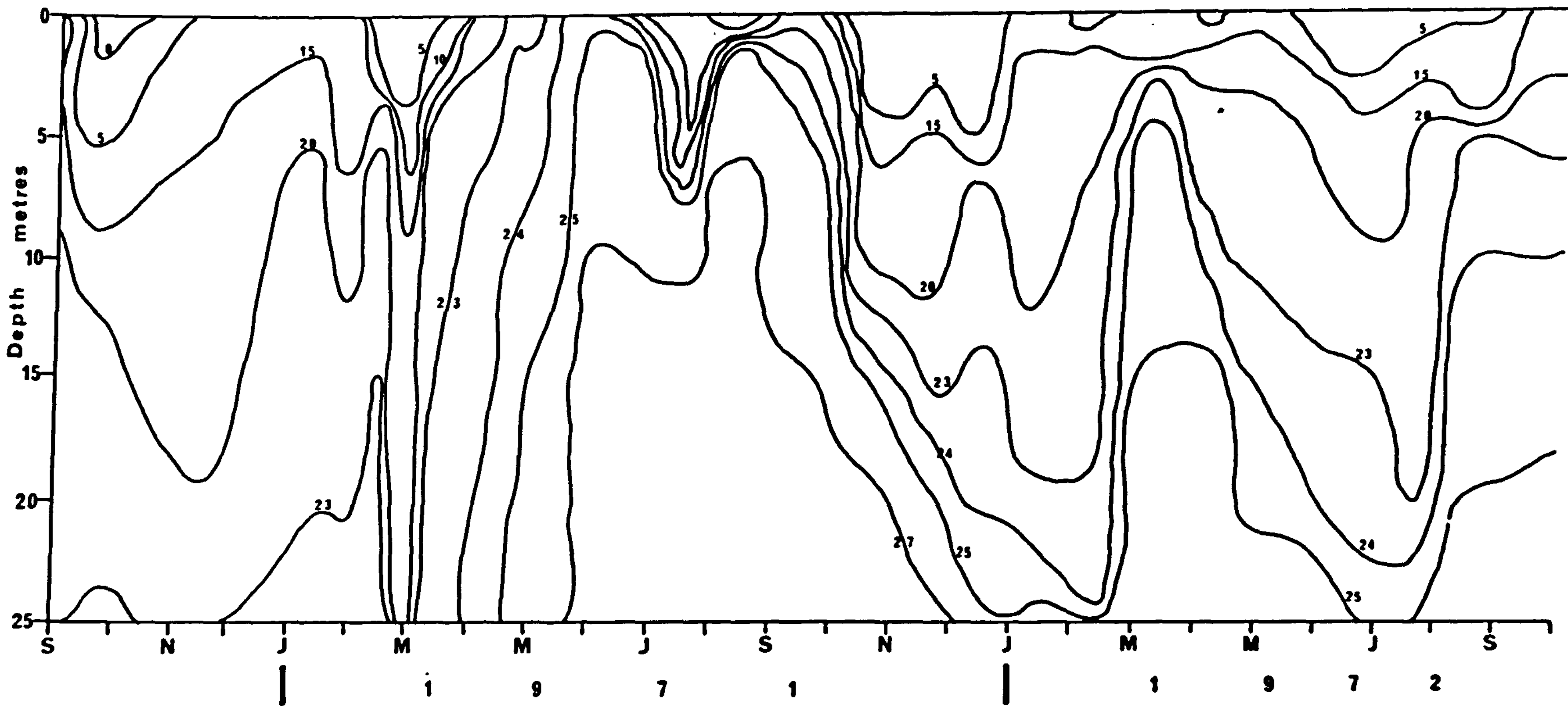
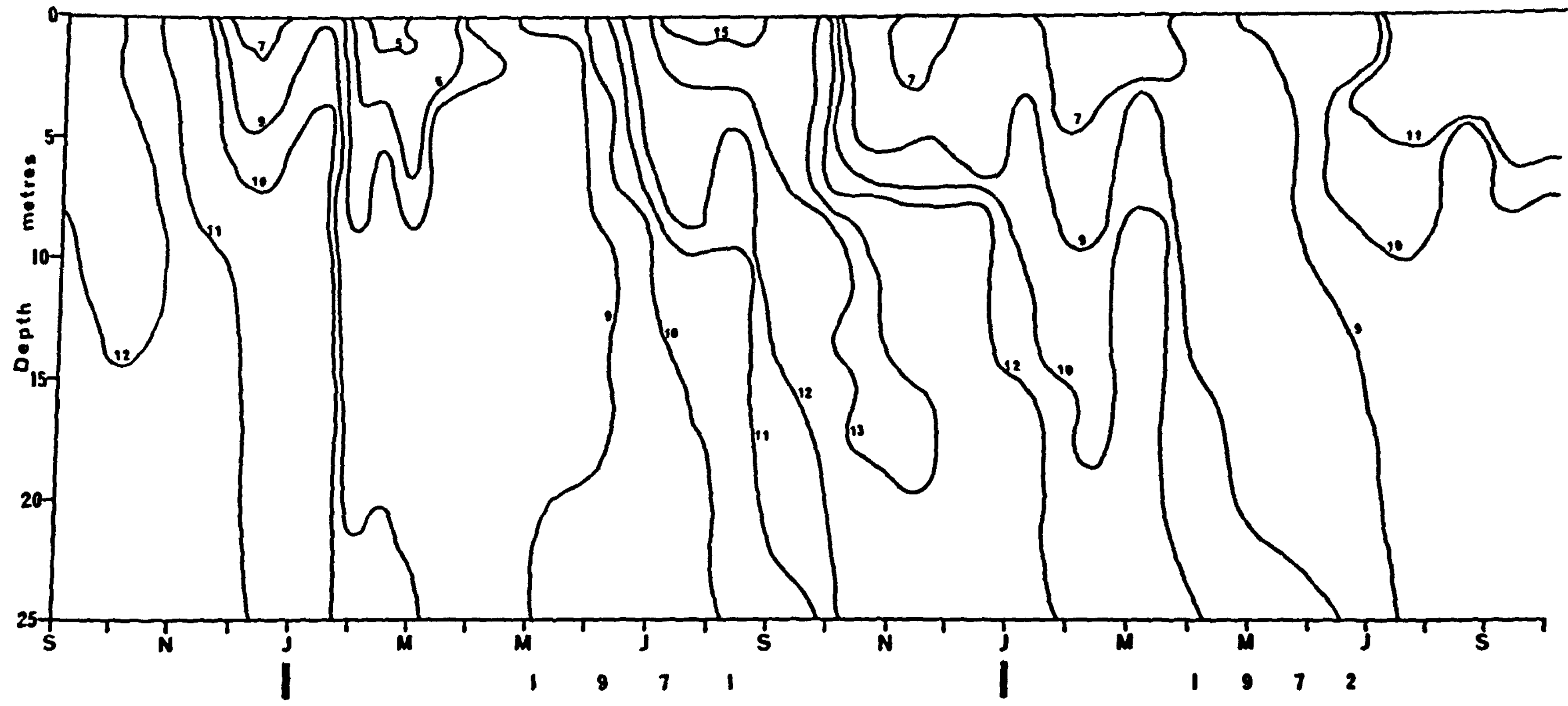

Depth metres
0
5
10
15
20
25
S N J M M J S N J M M J S
1 9 7 1
1 9 7 2

Figure 11

Temperature data profile for study area (°C)

[All data, for study area, combined.]



Depth metres
0
5
10
15
20
25
S N J M M J S N J M M J S
1971
1972

Surface temperatures of nearly 20°C have been recorded during hot spells, whilst in winter the surface water may often freeze. Below fifteen metres, however, these fluctuations are less marked. Temperatures tend towards a minimum from early spring through to mid-summer but only rarely do they drop below 7°C (Fig 11 ). The maximum temperatures 12-13°C are generally seen in November/December, though this is not always so.

Thus, although the surface salinities and temperatures are quite variable this is less marked in deeper water. The area, at the head of Loch Etive, is one of permananently reduced salinity, having a maximum of 27°/ooS but is often below 23°/ooS (Fig. 10 ), with a temperature fluctuation from 7-13°C (Fig. 11 ).

iii) Sediment characteristics

For analysis of the variations in sediment characteristics within the area, four relatively undisturbed sediment samples were taken by means of a 'Craib Corer' (Craib 1965) from five different stations (Fig. 9 ). The top five centimetres of each core were sliced off, the four slices for each station being mixed together, thus minimizing any difference between the cores for each station. The collected sediment was then halved, one half being preserved in neutralized formalin. The other half was divided into two, thus giving duplicate samples for each station. These samples were analysed for sediment size (aggregates size) using gentle wet sieving for larger particles, and pipette analysis

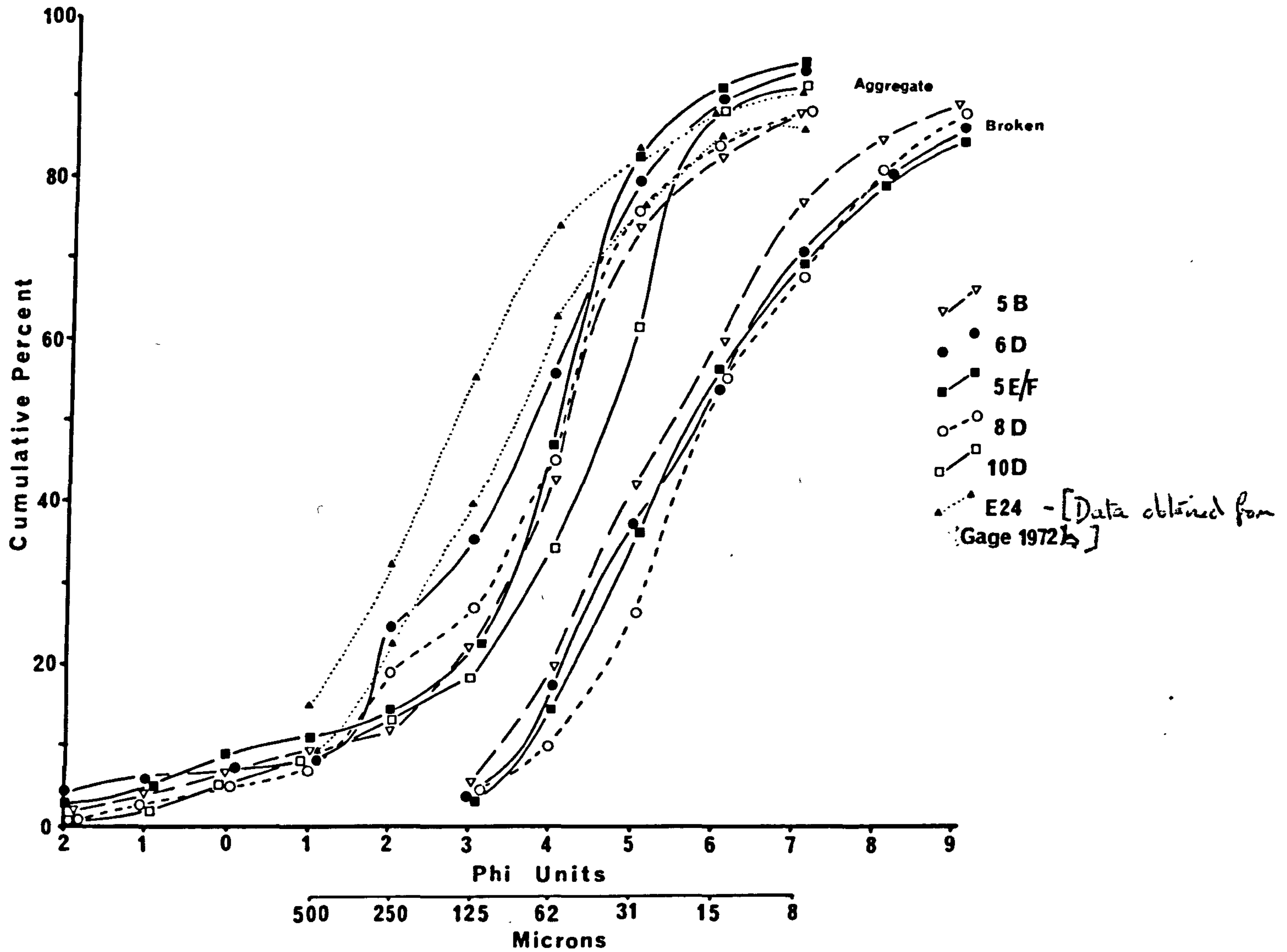
for particles of 62um and below (Krumbien and Pettijohn 1939). 'Natural water', ie. that collected in the immediate vicinity of the sediment, was used in all operations (Morgans 1956). All sub-sample weights were obtained by weighing a beaker of known weight plus wet sediment, then drying to constant weight at 105°C. The resultant difference in weight being due to water loss, the salinity of which was known, thus, the weight of the sediment, minus salts, could be ascertained.

The preserved samples were retained for analysis of particle size per se. The material was oven dried at 105°C. A known weight (approximately 20g) was treated with hydrogen peroxide and centrifuged, this process being repeated until no acid reaction was seen (Leenheer et al 1957, Jackson 1958). Sodium hexametasulphate was added to prevent re-aggregation (Krumbein and Pettijohn 1938). The samples were then sieved, wire brushes being used to ensure that particles were properly broken. The sub-fractions were then made up to one litre and the fine material analysed by pipette analysis (Krumbein and Pettijohn 1938).

Analysis of the details of the sediment analysis using the results of both these methods for the various stations (Fig. 12 ) show that none of the sediments examined vary significantly. Comparison of this data with that given for this area by Gage (1972b) shows certain differences. His data shows a greater predominance in the +1 to +4 Phi range (Fig. 12 ) but less larger than this. His analysis was done on preserved sediment

Figure 12

Analysis of sediment samples
from various stations within study area



Cumulative Percent
100
80
60
40
20
0
Aggregate
Broken
5 B
6 D
5 E/F
8 D
10D
E24 - [Data obtained from Gage 1972b]
2
1
0
1
2
3
4
5
6
7
8
9
Phi Units
500
250
125
62
31
15
8
Microns

in a mechanical shaker and it is possible that this caused the larger aggregates to break up more readily than the smaller ones.

Before comparison of the two methods used here ie. analysis of aggregates and of particle size per se, could be made, the organic matter was removed from the aggregate analysis in a furnace at 650°C. There was no significant difference in the content of organic material in any of the subsamples, thus graphs of the aggregate minus organics should approximate the plot for aggregates already obtained. The matter burnt off at 650°C would include some carbonates, but amounts of these are very low (Ansell pers. comm.). The sediment thus contains in the region of 13-17% organic matter. Conglomerates found in the sediment are largely the result of mineral particles bound together and not solely due to organic material. Microscopic examination of the sediment shows that a very large proportion of it is faecal matter and/or remnants of polychaete tubes.

Thus the natural occurring sediment is of fine particles uniformily distributed with a certain amount of organic matter. This sediment is, however, greatly modified by its inhabitants (Sanders 1960, Rhoads and Young 1970) with the result that the particle size is altered from a mean size of 20um (5.5-5.7 Ø) by aggregation to a median particle size of 62um (4 Ø units).

Conditions within the study area are of fluctuating

salinity (20-27$^{o}$/ooS) and temperatures (7-13$^{o}$C). Much of this fluctuation is due to freshwater run off. This brings with it a certain amount of organic matter which characterizes the sediment. The sediment itself is one of basically fine particles, however, these are reworked by the inhabitants of the sediment such that faecal material is plentiful.

## II) The Population Structure

Several species of Thyasira have been found around the coast of Scotland by many workers (Scott 1897, Knight and Frank 1893, more recently McIntyre 1961, Pearson 1972). Gage (1972a, b) sampled the population at present under study (termed T. flexuosa). However, all these studies have been faunistic surveys and not concerned with the Thyasira population to any great extent.

For the study of age, structure and distribution of the population, lines of stations were established each marked by paired shore bearings on either side of the loch. The positions of stations along each transect were determined by the availability of further adequate paired shore marks. By this method the stations (Fig. 9 ) could be revisited with ease, the three sets of shore marks ensuring a high degree of accuracy in positioning in this small area of approximately 2x½ kilometres. In all sixty-three stations (Fig. 9 ) were established on ten transects across the loch.

The number of Thyasira obtained in grabs taken in the same area vary greatly (Pearson 1970, 1971, 1972, Gage 1972a, b) and this, associated with the time taken to pick out the animals from the large amount of residue retained on the 1mm sieve used, led to the adoption of the following sampling programme:-

The sixty-three stations selected were visited twice yearly to determine population density and distribution, only one grab sample being taken at each station at each visit. Four of the stations

(Fig. 9 ) were visited initially at monthly and latterly at bimonthly intervals to determine spawning period, recruitment and variation in population size range. Animals used for other observations, though of necessity taken from within the population area, were taken from the areas between the transect lines, generally between transects four and five. Salinity and temperature data (see earlier) were taken at five metre depth intervals at five selected stations. From this data the salinity/temperature perameters for any of the sixty-three stations could be ascertained.

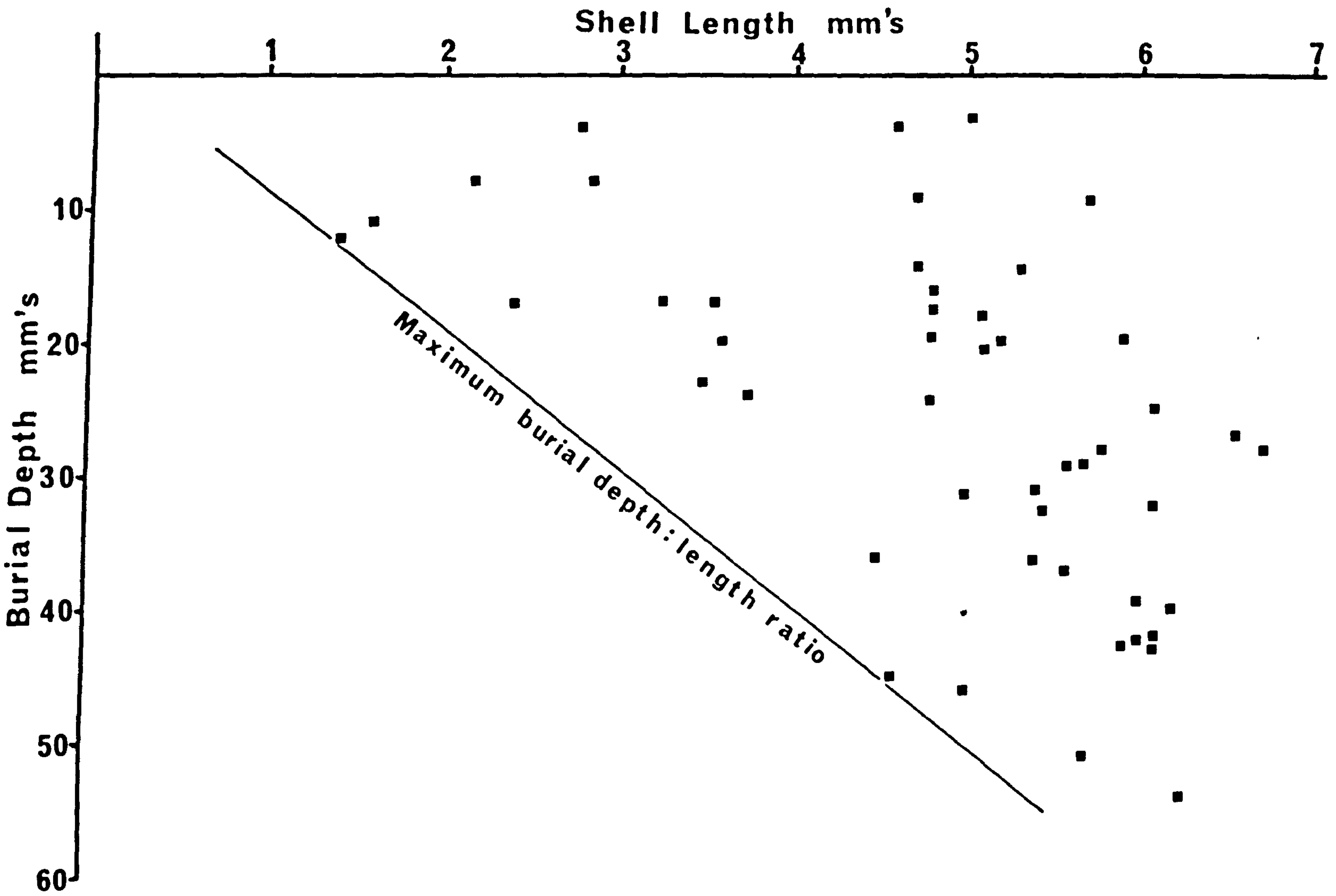
Samples were initially taken from 'R.V. Calanus', but later, use was made of the smaller and more manoeverable 'Beaver' as soon as this was available. All samples were taken with a 1/10mm$^2$ Van Veen grab, samples so collected being sieved on a 1mm sieve, making use of the hopper described by Holme (1959). The samples were preserved in neutralized formalin, the Thyasira being picked out and a note made of the other most abundant species present in each sample.

As found by Gage (1972b) the Van Veen grab in this area was always filled to its maximum capacity (a depth of bite of 20cms). Young and Rhoads (1971) gave the maximum depth of burial for T. gouldi as being in the region of ten times the shell length. It would thus appear that the grab was sampling the whole population. However, as a check on this, the depth of burial was determined. Cores of approximately 30cms length were taken at random throughout the study area using the Craib corer (Craib 1956). By slicing these at

0.5cms intervals the depth of any T. gouldi within the sediment could be ascertained. The maximum depth of burial observed for T. gouldi (Fig. 13 ) agrees with the data of Young and Rhoads (1971) and the sampling method employed can thus be accepted as sampling the whole population. Early observations within the aquarium suggested that the depth of burial was in the order of six times the shell length. However, these were the result of studying only short term aquarium observations. It is possible that the greater burying depth attained in the wild is the result of slow but prolonged burial (see earlier p. 10).

Figure 13

Analysis of burial depth to
shell length for *Thyasira gouldi*


Shell Length mm's
1
2
3
4
5
6
7
Burial Depth mm's
10
20
30
40
50
60
Maximum burial depth: length ratio

i) Distribution and Population Density of T. gouldi

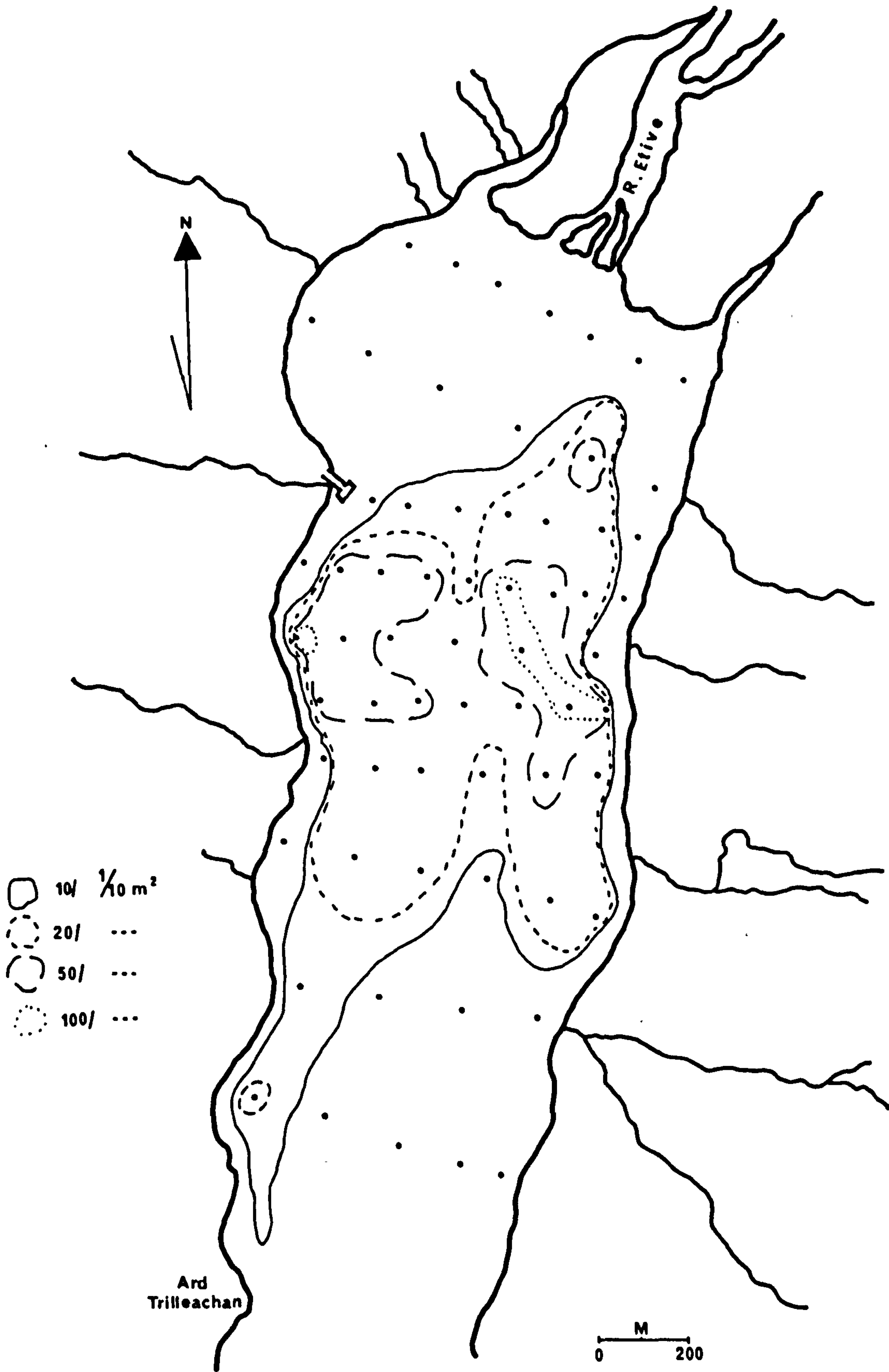
Complete surveys were made in January and September 1971 and again in January 1972. The results were used to construct contoured distribution maps for T. gouldi in the area for these three dates. Quite a large variation was found between sampling dates but the general picture which emerged was the same. This is shown in Fig. 14 which is formed from a combination of all the surveys and shows the mean contours for the study period. The population of T. gouldi extends from transect 2 southwards down the loch to transect 10. The main concentration is focused on two roughly central areas within this area. Here the numbers exceed 1,000m$^2$ but are separated by a central, less densely populated area. This central area has been sampled quite extensively in the past (Ansell, Pearson both unpublished, Gage 1972) and this may account for some, but not all, of this reduction in population.

From the mean contour diagram (Fig. 14 ) a total population of ≈1,500,000 animals can be calculated. This is however a conservative estimate of the total population of T. gouldi at the head of L. Etive as those animals below about 1.5mm which are not retained by the sieve are ignored.

The sediment over much of the area is fairly uniform (see earlier) being soft, thick, black mud, smelling strongly of Hydrogen Sulphide. Above transect 2, where T. gouldi was absent, large pieces of organic matter, including small branches, were seen. Along

Figure 14

General map of study area showing
population density contours of *Thyasira gouldi*



N
R. Etive
10/ 1/10 m²
20/ ---
50/ ---
100/ ---
Ard
Trilleachan
M
0
200

the western side of the loch (stations 7f and 8a) there appears to be an area of quite strong current, probably caused by fresh water run off. This is reflected in a very coarse sand/gravel sediment in which few Thyasira were found. The sediment in the middle of the lower study area is relatively free of organic matter, being also to a large extent free of T. gouldi, this is also probably correlated with fresh water run off at least as regards the eastern bank.

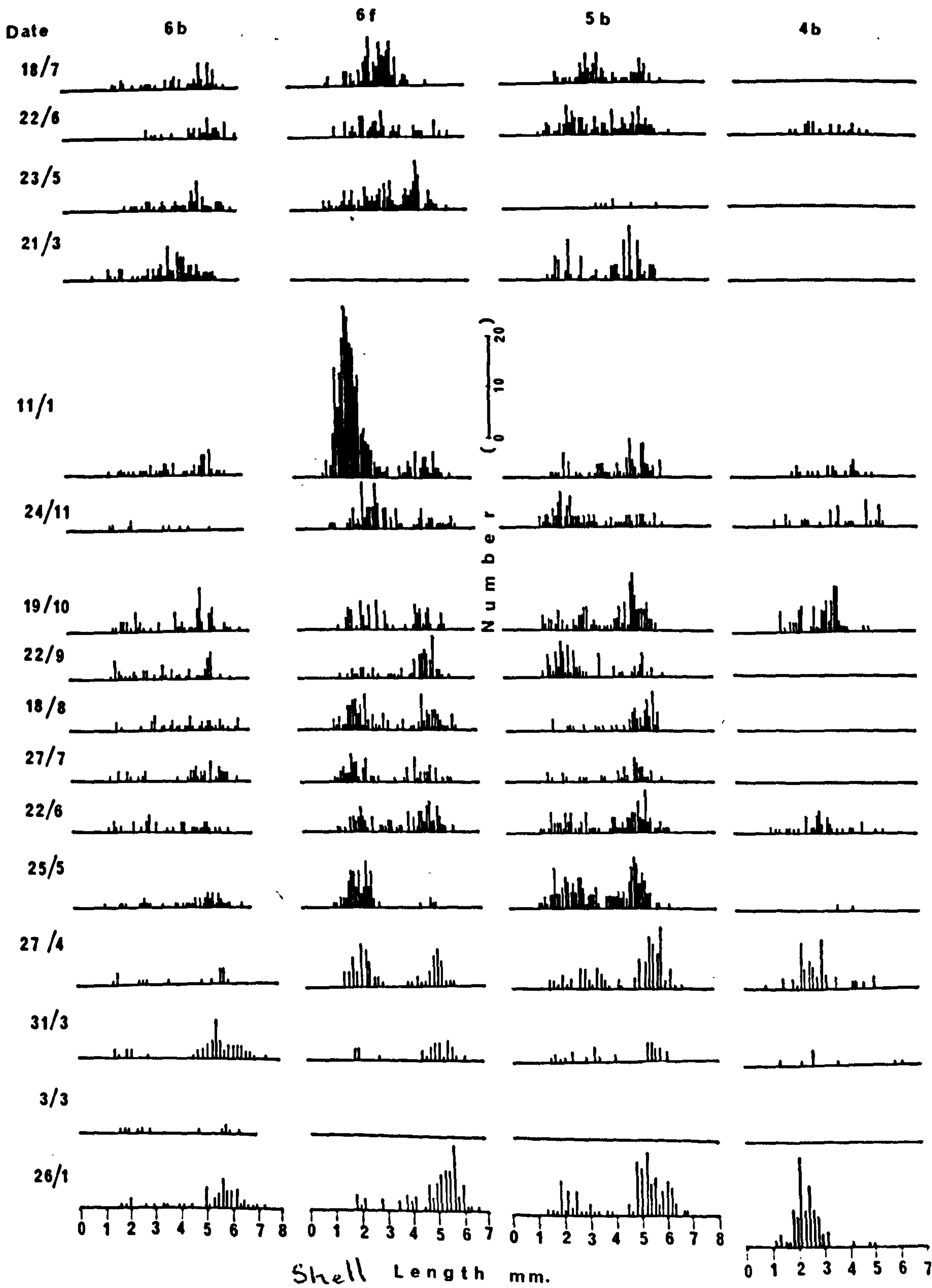
The population as a whole appears to be aggregated towards the edge of the loch. Sedimentation whether of organic matter and/or of particle size is important in the distribution of T. gouldi (see also Bellan 1957). The current flow has to be such that settling material is finely divided, this generally resulting in a degree of organic deposit which may be important. The population is further restricted to areas below fifteen metres, probably due to variations in salinity and temperature (see later).

Analysis of the monthly samples (Fig. 15 ) again showed the great variation in numbers. It can be presumed that the population is not randomly distributed but a series of often quite dense patches. These patches are probably small as they are apparent even when the station is clearly defined. They may be related to localized modifications of the habitat by other animals (Rhoads and Young 1971) or a direct result of the mode of development (see later).

Figure 15

Size frequency histogrammes for *Thyasira gouldi* throughout study period at selected stations ie. 4b, 5b, 6b and 6f

at given dates.



Date
6b
6f
5b
4b
18/7
22/6
23/5
'72
21/3
11/1
24/11
19/10
22/9
18/8
27/7
22/6
25/5
'71
27/4
31/3
3/3
26/1
Number
0
10
20
0 1 2 3 4 5 6 7 8
0 1 2 3 4 5 6 7
0 1 2 3 4 5 6 7 8
0 1 2 3 4 5 6 7
Shell Length mm.

ii) Other species present

Gage in his survey of the whole loch gives a species list for this area for all animals retained by a 1mm sieve (see Gage 1972a station 24). The community structure is outside the scope of this work and only the most abundant species were noted. (For further details of animals co-occuring with *T. gouldi* the reader is referred to the data of Thorson 1936, 1946, Einarsson 1941, Ockelmann 1959, Filatova and Barsonova 1964, Pearson 1970 (stations 1 and 2 where *T. gouldi* and *T. flexuosa* co-occur), Kuznetsov 1970, and Gage 1972a (station E24) ).

The distribution of the major species do, however, give some indication of the general conditions within this area. Table 2 shows the occurrence of the major dominant species, suffice to add the following notes concerning the more frequent species:-

*Arenicola marina* (L) Restricted to transects 2 and 3, probably indicating that stations 2a and 2b are more similar to transect 1 than to the rest of transect 2.

*Hydrobia ulvae* (Pennant) Frequent in transects 1-4 but lower than this confined to the edge stations.

*Macoma balthica* (L) Like *Hydrobia* confined to upper stations though less widely dispersed.

*Mya truncata* (L) Two defined patches viz.

Distribution of Most Abundant Species

| Species | 1a | 1b | 1c | 1d | 1e | 1f | 1g | 2a | 2b | 2c | 2d | 2e | 2f | 2g | 3a | 3b | 3c | 3d | 3e | 3f | 3g | 4a | 4b | 4c | 4d | 4e | 4f | 4g | 4h | 4i |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Pennatulacea sp. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Nemertea sp. | | o | | | | | | | | + | + | | | o | | | | | | | | o | | | | | | | | |
| Annelida | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Oligochaeta sp. | | | | | | | | + | | | | | | | | | | | | | | | | | | | | | | |
| Ancistryllis groenlandica McIntosh | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Amphitrite sp. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Arenicola marina (Linne) | | | | | | | | | | + | | | | + | | | o+ | | | | | | | | | | | | o | |
| Castala punctata O.F. Muller | | | | | | | | | | | | | | | | | | | | | + | | | | | + | | | | |
| Eteone sp. | | | | | + | | | | | | | | | | | | | | | | | | | | | | | | | |
| Glycera alba Rathbe | | | + | | | | | | | | o+ | | + | o | o+ | o | o+ | | | | | | | | | | | | | |
| Goniada maculata Orsted | | | o+ | o | o+ | | | | o+ | + | o+ | o+ | o+ | | o | o+ | o+ | o | + | o+ | | o | o+ | | | o+ | | | o+ | o+ |
| Goniada norvegica Orsted | | | | | | | | | | | | | | | | | | o | o | o+ | | o | o | | o | o+ | + | o+ | o+ | + |
| Nephthys hombergii Audoin et M. Edwards | | | | + | + | | | | | | | + | | | | o | | | | | | | | | | | | | | |
| Pectinaria belgica Pallas | | | | | | | | | | | | | | | | | | | | o | | | | | | | o | + | | o |
| Phyllodoce sp. | | | | | | | | | | o | | | | | o+ | o | | | | | | | | | | | | | | |
| Pista cristata (Muller) | | | | | | | | | | | | | | | | | | | | | | | | o | | | | | | |
| Praxillura longissma Arwidsson | | | | | | | | | | | | | | | | | | | | | | | | | o | | | | | |
| Rhodine gracilior (Tauber) | o | o+ | o | + | + | | | o+ | o | | + | o | o | o+ | o | o | o+ | | | | | | | | + | | | | | o |
| Sosane sulcata Malmgren | | | | | | | | | | | | | | | | | | o+ | o | | + | o+ | + | o | o | | o+ | o | | o |
| Spirochaetopterus typicus Sars | | | | | | | | | | | | | | | | | | | o | | | o | | | | | | | | |
| Terebellida stroemi Sars | | | | | | | | | | | | | | | | | | | o | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | + | | | | o | | | + | | | o | | + |
| Arthropoda | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Chironomid larvae | + | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mollusca | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Akera bullata Muller | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Hydrobia ulvae (Pennant) | o+ | o+ | + | | o+ | | | o | o | + | o+ | | o+ | o+ | o | o+ | o | | | | | | | | | | | + | | |
| | | | | | | | | | | | | | | | | | | | | + | + | o+ | o+ | o | o+ | o | + | + | + | o+ |
| Astarte spp. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Crenella decusata Montagu | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Lucinoma borealis L. | | | | | | | | | | | | | | | | | | | | | | | | | | o | | + | | |
| Macoma balthica L. | o+ | o+ | o+ | o+ | o+ | | | o+ | o+ | + | | | o | o+ | o+ | | | | | | | | | | | | | | | |
| Mya truncata L. | | | | | | | | | | | | | | | + | | | | + | | + | o+ | o+ | | | | | | | o |
| Mytilus edulis L. | o+ | | | | | | | o | | | | | | | | | | | | | | + | o+ | | | | | | | |
| Nucula tenuis Montagu | | | | | | | | | | | | | | | | | | | | | | o | | | | | | | | |
| Nucula sulcata Bronn | | | | | | | | | | | | | | | | | | | | | | | | | | + | o+ | | | |
| Parvicardium sp. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Echinoderms | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Amphiura filiformis (Muller) | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Ophiura albida Forbes | | + | | | | | | | | | + | | + | | | | o | + | + | o | | | | | | | | + | + | + |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Leptosynapta inhaerns O.F. Muller | | | | | | | | | | | | | | | | | | | | | | | | | | | + | | | |

| Species | 5a | 5b | 5c | 5d | 5e | 5f | 6a | 6b | 6c | 6d | 6e | 6f | 6g | 7a | 7b | 7c | 7d | 7e | 7f | 8a | 8b | 8c | 8d | 8e | 8f | 9a | 9b | 9c | 9d | 10a | 10b | 10c | 10d | 10e |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Pennatulacea sp. | | | | | | | | | | | | | | | | | | | | | | | | | | o | | | | | | | | |
| Nemertea sp. | | | + | | | | | | | | | o | | | | | | | | | | | | | | | | o | o | | | | | |
| Annelida | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Oligochaeta sp. | | | | | | | | | | | | | | | | | | | | | | | | o | | | | | | | | | | |
| Ancistryllis groenlandica McIntosh | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | + | | |
| Amphitrite sp. | | | | | | | | | | | | | | | | | | o+ | + | + | + | | | | | | | | | | | | | |
| Arenicola marina (Linne) | | | | | | o | + | | | | | + | | | | | | | | | | | | | | | | | | | | | | |
| Castala punctata O.F. Muller | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Eteone sp. | + | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Glycera alba Rathbe | | | | | | | | | | | | | o | | | | | o | o+ | o | o | + | | o | o | | | | | | | o | o | |
| Goniada maculata Orsted | + | o | | o | | | o+ | o | | o | o | | o | + | + | o+ | + | o+ | | o+ | | | + | o+ | o+ | o | o+ | o+ | + | o+ | o | | o | o |
| Goniada norvegica Orsted | + | o+ | o+ | o+ | o+ | o+ | o | o+ | + | o+ | o | o+ | | | | | | | | | | | | | | | | | | | | | | |
| Nephthys hombergii Audoin et M. Edwards | | | | | | | | | | | | + | | | o | | | | | | | | | | o | | | | | | | | | o |
| Pectinaria belgica Pallas | | | | | | | | | | | o | | | | | | | | | | | | | | | | | | | | + | + | + | + |
| Phyllodoce sp. | | | | | | | | | | | | | + | o | o | | | | o | o | | | | | o | o | | | | | | | | |
| Pista cristata (Muller) | | | | | | o | | + | | | o | | | | | | | | | | | | | | | | | | | | | | | |
| Praxillura longissma Arwidsson | | + | | | | | + | | | | | | | | + | | + | + | | | | | | | | | | | | | | + | | |
| Rhodine gracilior (Tauber) | | + | | | + | | | + | | | + | | o | | o+ | o+ | o+ | o+ | | | o | o+ | o+ | o+ | o | o+ | o+ | o+ | o | o+ | o+ | o+ | o+ | o |
| Sosane sulcata Malmgren | | + | o+ | o+ | + | | | o+ | o+ | o+ | o+ | + | | | | | | | | | | | | | | | | | | | | | | |
| Spirochaetopterus typicus Sars | | | | | | | | | | | | | | | | | | o | | | | | o | | | o | | | | o | o | | | o+ |
| Terebellida stroemi Sars | | | | | | | | | | | | | o+ | + | o | | | | o+ | + | + | | | | o+ | o | | + | o+ | | + | | o | |
| | + | o+ | + | | | o+ | o+ | | + | | | o+ | | | | | | | | | | | | | | | | | | | | | | |
| Arthropoda | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Chironomid larvae | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | + | | | + |
| Mollusca | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Akera bullata Muller | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Hydrobia ulvae (Pennant) | | | | | | | | | | | | | o | o | | | o | | o+ | o | + | | | | o | + | | | | | | | | |
| | o | + | | | | + | + | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Astarte spp. | | | | | | | | | | | | | o | | | | | | o | o | | | | | | | | | | | | | | |
| Crenella decusata Montagu | | o | | | | o | o | o | | | | o | | | | | | | | | | | | | | | | o | | | | o | | |
| Lucinoma borealis L. | o | o | o | | | | | | o | | | | | | | + | | | | | | | | | | | | | | | | | + | |
| Macoma balthica L. | | | | | | | | | | + | | | | | | | | | | | | | | | | | | | | | | | | |
| Mya truncata L. | | | | | | | | | | | | | o+ | o | | | | | o | o+ | + | | | | | | | | o+ | | | | | |
| Mytilus edulis L. | o+ | | | | | o+ | o | | | | | + | | o | | | | | o | | | | | | | | | | | | | | | |
| Nucula tenuis Montagu | | | | | | | | | | | | | | | + | o+ | o+ | o | | | o+ | o+ | o+ | + | + | o+ | o+ | o+ | | + | o+ | o+ | o+ | + |
| Nucula sulcata Bronn | | o+ | o+ | o | | | | o+ | o+ | o+ | o | + | | | | | | | | | | | | | | | | | | | | | + | |
| Parvicardium sp. | | | | | | | | | | | | | | o | | | | | | o | | | | | | | | | | | | | | |
| Echinoderms | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Amphiura filiformis (Muller) | | | | | | | | | | | | | + | + | | | o+ | o | | + | | | | + | + | + | | | o+ | | | + | | |
| Ophiura albida Forbes | + | o+ | | + | | + | + | | o+ | o+ | o | + | | | | | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | + | | | | | | | | | + | + | | | | | | | | | |
| Leptosynapta inhaerns O.F. Muller | | + | + | | | | | o+ | + | + | | o+ | | | | | | | | | | | | | | | | | | | | | | |

o Abundant 3.9.71
\+ Abundant 11.1.72

stations 4a-8a on western side, 4i-7a on eastern side.

Mytilus edulis (L) Found around 1a and 2a, also 4a, 7f, and 7a, all areas of great freshwater effect.

Nephthys sp. Restricted to the edge of the area but found along edges of nearly all transects.

Nucula tenuis (Montagu) Central distribution from transect 4 downwards. Found closer in shore on eastern side after transect 8.

Ophiura affinis (Lutken) Central patch from transect 4-9.

Rhodine lavine, (Malmgren), Glycera alba (Muller), Goniada maculata (Oersted). All appear to be relatively evenly distributed throughout the area. Glycera being less frequent than the other two.

The affect of freshwater is reflected to some extent in the major species present. None of these other major species, however, give any clearly defined reason for the limits to the extent of the population of T. gouldi. It would thus appear that T. gouldi is affected more by physical factors than by any interspecific interaction.

iii) Size distribution and rate of growth

All the animals collected from each sample were

measured and their shell length determined to the nearest 0.1mm.

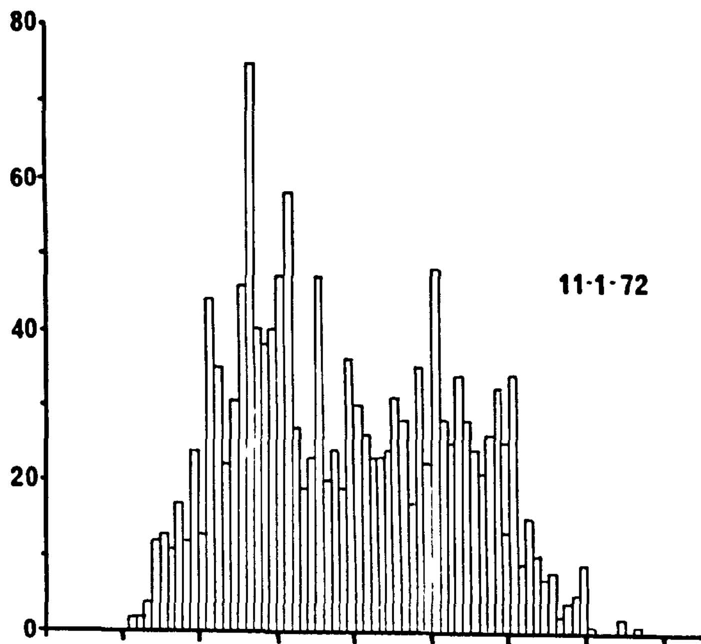
The results of monthly sampling at stations 4b,5b, 6b and 6f (Fig. 9 ) is summarized in Fig. 15 which shows size frequency histograms for each station for each sampling date. There is a bimodal distribution at all times in all stations where sufficient numbers exist. This phenomenum, which is even more pronounced if the samples collected are combined for any one of the dates when all the 63 stations were sampled (Fig. 16 ), was not apparent in earlier samples collected in this area (Ansell pers. comm.) but is present in all the samples collected in Lochs Linnhe and Eil by Pearson. The initial region of any of these size distribution data is probably missed due to sampling - the small animals being lost through the 1mm mesh sieve. There is no clear evidence of any consistent shift in the modes of the size frequency distributions with time, either in the L. Etive or the L. Linnhe/Eil samples. These data, therefore, do not give any indication on the rate of growth of T. gouldi.

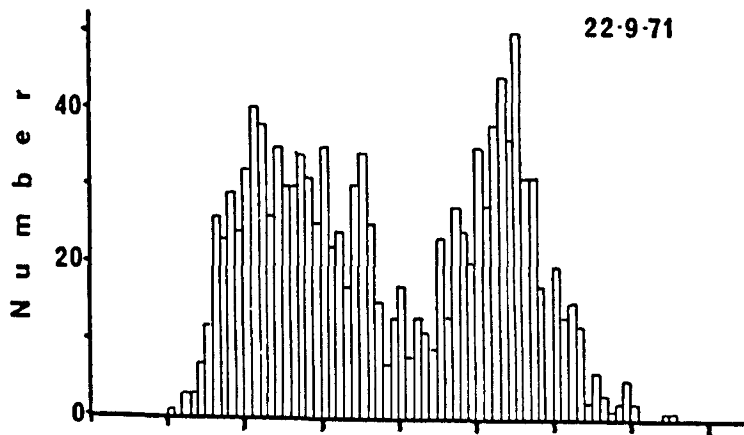
The age of molluscs is often obtained by counting the concentric growth checks on the shell (Hopkins 1941, Haskin 1954). Very few T. gouldi showed any such growth checks, and analysis of those that were present may have been biased, due to extra slow growth, or some other factor causing the checks to become apparent. Attempts were therefore made to establish the growth rate by placing measured animals into pots of mud

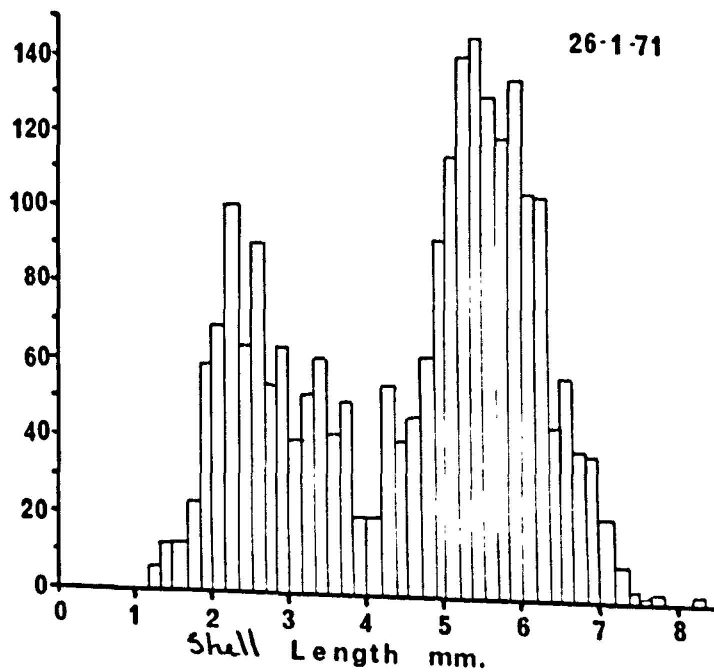
Figure 16

Size frequency histogrammes
for all stations combined
for each of three sampling dates

ie. 26/1/71, 22/9/71, 11/1/71


80
60
40
20
0
11·1·72


Number
40
20
0
22·9·71


140
120
100
80
60
40
20
0
26·1·71
0
1
2
3
4
5
6
7
8
Shell Length mm.

placed within the population area. Twenty-four such pots were placed into a framework, each pot containing twenty animals within a closely defined size range (0.5mm). The jars were sampled at monthly intervals, but the results obtained were disappointing. Very few living animals or shells were recovered from the pots. The few that did survive were measured, but the results were not significant. Although spider crabs (Hyas sp.) were not caught in any of the grab samples, large numbers (15-20) were seen aroung the frame within the first two days, and it may have been these crabs which ate the Thyasira.

Read (1960) used a method of embedding and grinding barnacle plates, this method was tried but met with little success in showing any growth checks. However, growth checks were seen in shells decalcified by using a weak solution of R.D.S. (Rapid DeCalcifier, manufactured by Bethlehem Instruments of Hertfordshire, made known to me by Mr. A. Bullock of this Laboratory) in alcohol or an alcohol formalin mixture. Rings were apparent in the organic layer left after the calcium of the shell had been dissolved and after separation from the mantle tissue. Staining with Rose Bengal made these even more visible. After studying a large number of these rings it was concluded that they were laid down annually, and that they correspond to slight and often indistinct rings upon the shell, caused by a slowing of growth, due to variations in the environment.

Between three and four hundred animals were treated

with R.D.C. and the number of rings counted.

The results are summarized in Fig. 17 . They suggest that T. gouldi grows at an annual rate in the region of 1mm per annum. This rate is very low but agrees well with the other data known about Thyasira. Comparison of the growth rate of T. gouldi with that of T. flexuosa of Lochs Linnhe/Eil shows that the growth rate of the latter is slightly higher (Fig. 17 ). This higher rate for T. flexuosa has also more recently been found to be the case in the populations around Denmark (Ockelmann pers. comm.).

After five years the growth rate tends to decline, leveling off after nine or ten years (Fig. 17 ). Analysis of the number of rings in animals of this age is very difficult as the rings are so close together. It is assumed, therefore that virtually no growth occurs after ten years. Nicol (1960) relates size to habitat for several species of bivalve. The small size of T. gouldi (ie. maximum 10mm) may be a result of its arctic habitat.

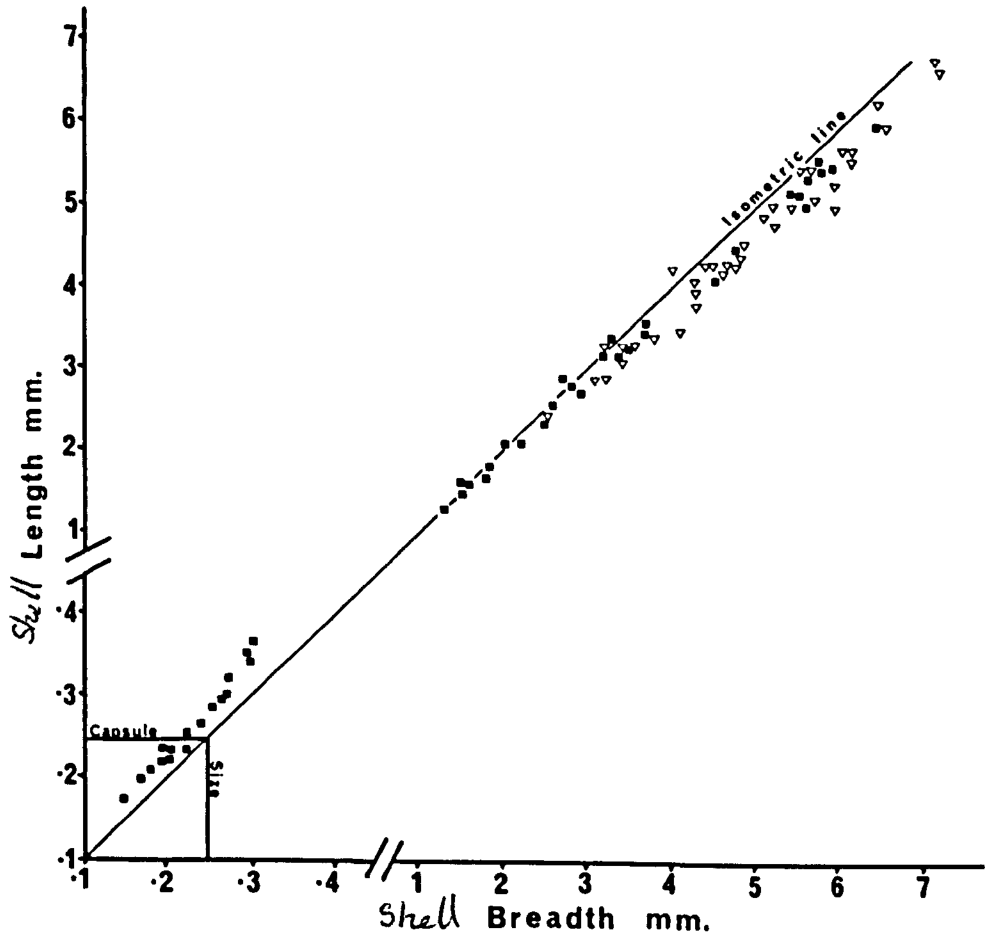
## Discussion

Several factors concerning the bimodal population structure of T. gouldi have been described. The size frequency modes do not shift, this coupled with the growth rate data rules out the predominance of

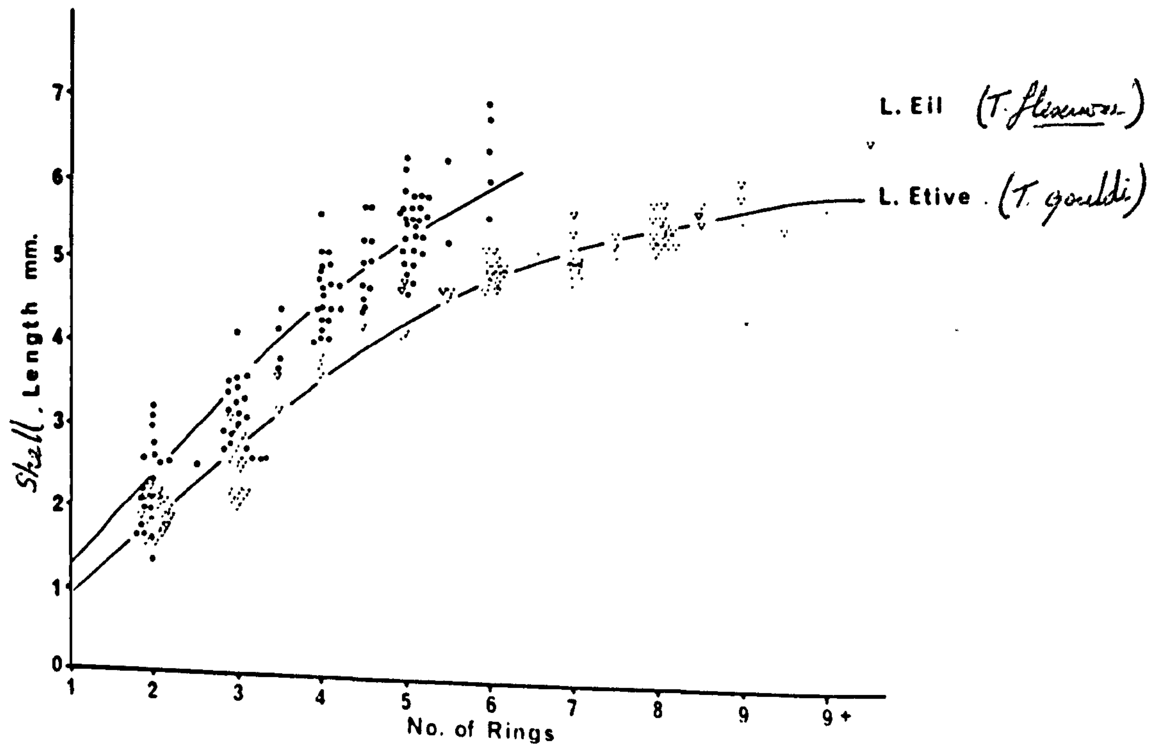
Figure 17

Fig. 17a Growth rate of *Thyasira gouldi* from ~~Loch Eil and~~ Loch Etive and of *T. flexuosa* from L. Eil.

Fig. 17b Relationship of shell length to shell breadth for *Thyasira gouldi*



Shell Length mm.
Shell Breadth mm.
Isometric line
Capsule
Size

Shell. Length mm.
No. of Rings
L. Eil (T. fleemingi)
L. Etive . (T. gouldi)

one or two year classes accounting for the population structure. The growth rate data (Fig. 17 ) shows a large overlap of the size range of successive year classes which is more noticeable in older animals. If the mean size and total size range (Table 3) are taken for each size class, and an annual mortality, after the first one or two years, of 10% is assumed, a survival or catch curve can be constructed. Fig. 18a shows such a survival curve, whilst Fig. 18 b shows the type of histogram which can be obtained by the summation of individuals, of the different year classes, for selected sizes. The form of this histogram gives a close approximation with the actual histogram obtained from direct measurement of the natural population. No data is available for the smaller sizes (ie. below 1mm), however, mortalities will be higher as the young *Thyasira* will form part of the prey of a range of deposit or detritus feeders (Eioma 1966, Ockelmann pers. comm.).

The population of *T. gouldi* within L. Etive is composed of a large number of year classes. Due to a combination of slow growth and low adult mortality the larger sizes are composed of several year classes so that a bimodal population structure results.

Table 3

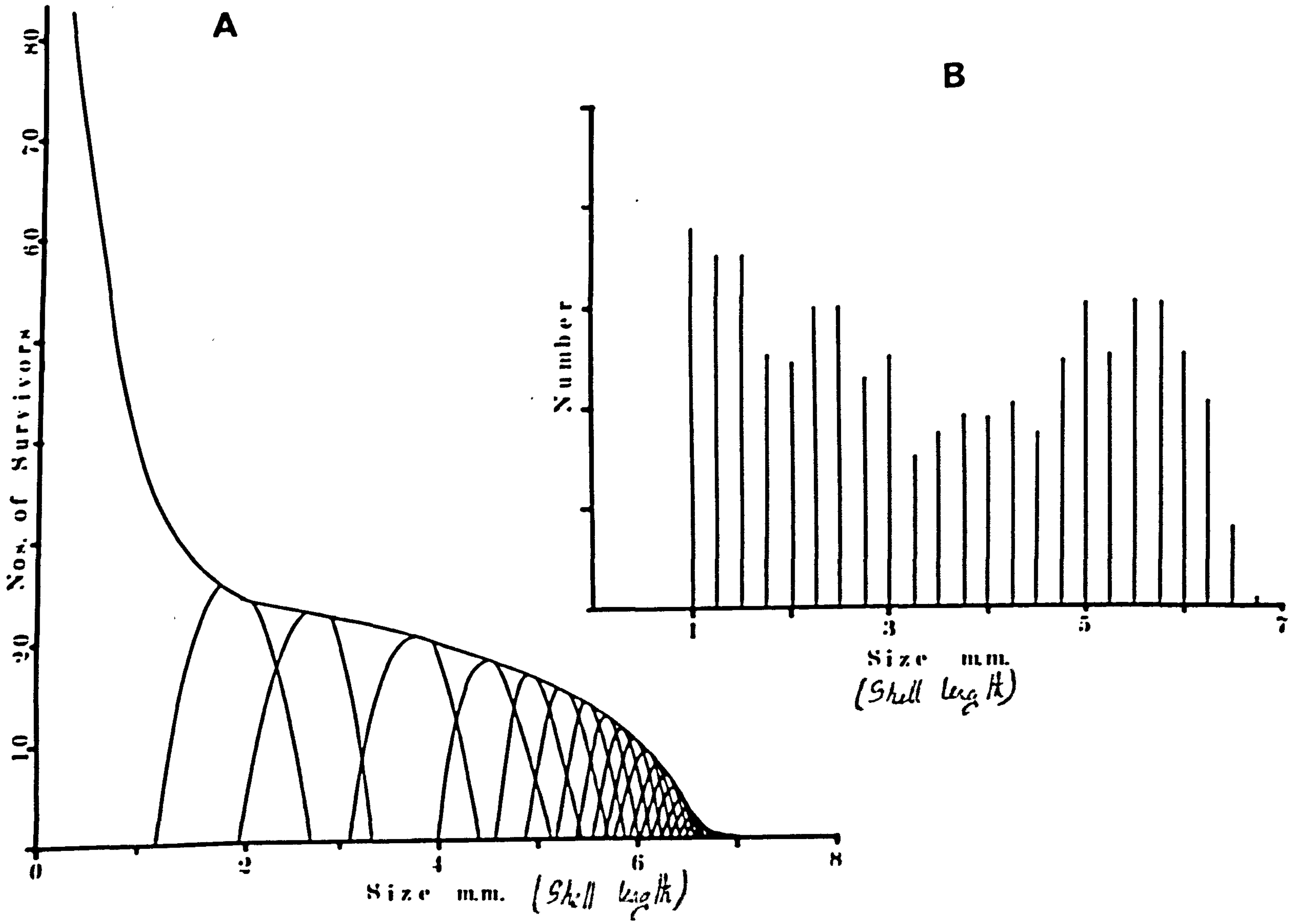
Correlation of shell length to number of growth rings, used in construction of survival model, based on animals from Loch Etive

| Age (years) | Size<br>Smallest | <br>Largest | <br>Mean |
|---|---|---|---|
| 0 | | | |
| 1 | 7 | 17 | 10 |
| 2 | 14 | 26 | 18 |
| 3 | 19 | 32 | 28 |
| 4 | 32 | 48 | 37 |
| 5 | 42 | 50 | 44 |
| 6 | 47 | 52 | 49 |
| 7 | 48 | 57 | 53 |
| 8 | 52 | 58 | 55 |
| 9 | 53 | 61 | 57 |
| 10? | 55 | 61 | 58.5 |
| 11? | 57 | 63 | 60 |

Figure 18

Fig. 18A Survival curve constructed from growth data, and inset :-

Fig. 18B Size frequency histograms obtained from survival curve


A
Nos. of Survivors
80
70
60
10
0
2
4
6
8
Size mm. (Shell length)
B
Number
1
3
5
7
Size mm.
(Shell length)

## III) Factors affecting the distribution of adults

*Thyasira gouldi* is an arctic species occurring around Iceland, Greenland and the north Norwegian coast, but due to cold currents extends further south around the west coast of America to California (Thorson 1936, 1946, Ockelmann 1958, Kuznetsov 1970, Young and Rhoads 1971, Bernard 1972) where salinities are generally about 33$^{o}$/ooS and temperatures are barely positive.

The large population at the head of L. Etive exists where salinities rarely exceed 28$^{o}$/ooS (see earlier) whilst temperatures are often above 12$^{o}$C. The affect of temperature and salinity upon adults from this population was therefore studied, and in addition some observations have been made on the animals' responses to variations in sediment.

A) Tolerance to salinity

The tolerance to salinity of adult *T. gouldi* from L. Etive was investigated by three methods, namely:-

1) The mortality of animals buried in natural sediment,

2) The mortality of animals with no sediment, and

3) The ability of animals to burrow into natural sediment.

In addition a number of measurements of the depression of freezing point of the body fluid of animals kept

at different salinities were also made.

For the study of mortality in animals allowed to bury into natural sediment several loads of mud were collected from within the sampling area by means of a Van Veen grab. These were then mixed together and divided to fill six baths, each bath containing sufficient mud to fill twenty 100ml beakers. The baths were then filled with water of a pre-determined salinity (12, 16, 20, 25, 30 or 35°/ooS) and agitated. The salinity was re-adjusted as necessary, until the interstitial salinity of the mud was brought to that of the test salinity. Twenty glass beakers were then filled with mud for each of the six salinities, placed into a larger bowl, water added of that test salinity and aerated.

After sufficient time had been allowed for any fine material to settle, the salinity was rechecked and ten animals placed onto the sediment contained in each beaker. The bowls were covered to cut down evaporation, and the water changed every other day.

The animals used (ie. 1,200) were all collected on the same day and from the same area between transects 4 and 5 (Fig. 9 ) having been removed from the sediment by gentle sieving using a benthic hopper (Holme 1959). Only animals over 4mm in length were used. All experiments were conducted at 10°C $\pm$ 1°C. The experiment was conducted in October 1970 and repeated in August 1971, when 'natural' salinities were 23°/ooS and 27°/ooS respectively.

The percentage mortality for each salinity (Fig. 19 ) was calculated from samples taken at intervals throughout the experiment. The animals from two randomly selected beakers constituted each sample, the criterion of death being taken as animals in which the shell gaped and showed no response when the foot was prodded. (Pierce 1970, Gilles 1972).

During both these experiments on the salinity tolerance of animals allowed to bury, the rate of burial (the percentage of the total animals present which had buried at any one time) was also studied. Thus the rate of burial could be compared with the longer term percentage mortality.

For the experiments on the salinity tolerance of animals without sediment ten T. gouldi were placed into each of six salinities 12,16, 20, 25, 30 and $35^{o}$/ooS and the time for death recorded using the criteria used above. The water was aerated and after death dead animals were removed and the water renewed. Again all experimentation was conducted at $10^{o}C \pm 1^{o}C$.

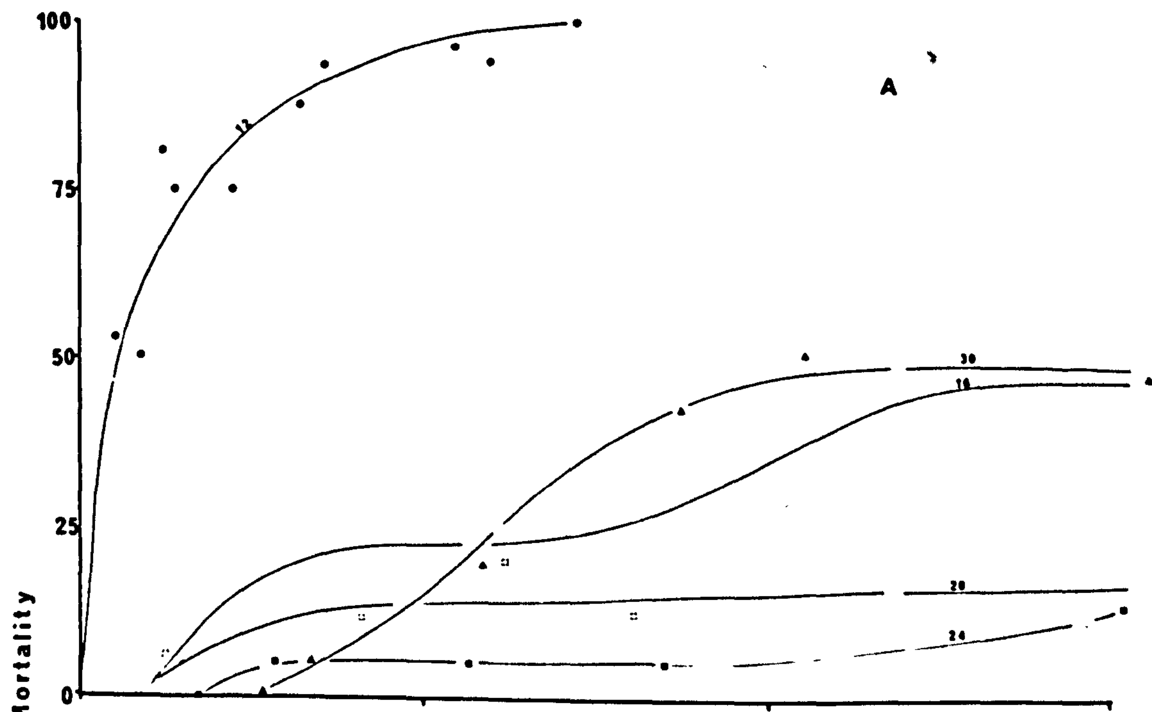
The results of the experiments in which sediment was provided are shown in Fig. 19 . There was a decrease in mortality with increase of salinity up to $25^{o}$/ooS after which the mortality increased. There was a large variation in the percentage mortality between the two dates (Fig. 19 ), but the general trend is the same. In October 1970 (Fig. 19a) mortality below $25^{o}$/ooS was lower than that obtained in August 1971 (Fig. 19b ). The reverse was true for salinities

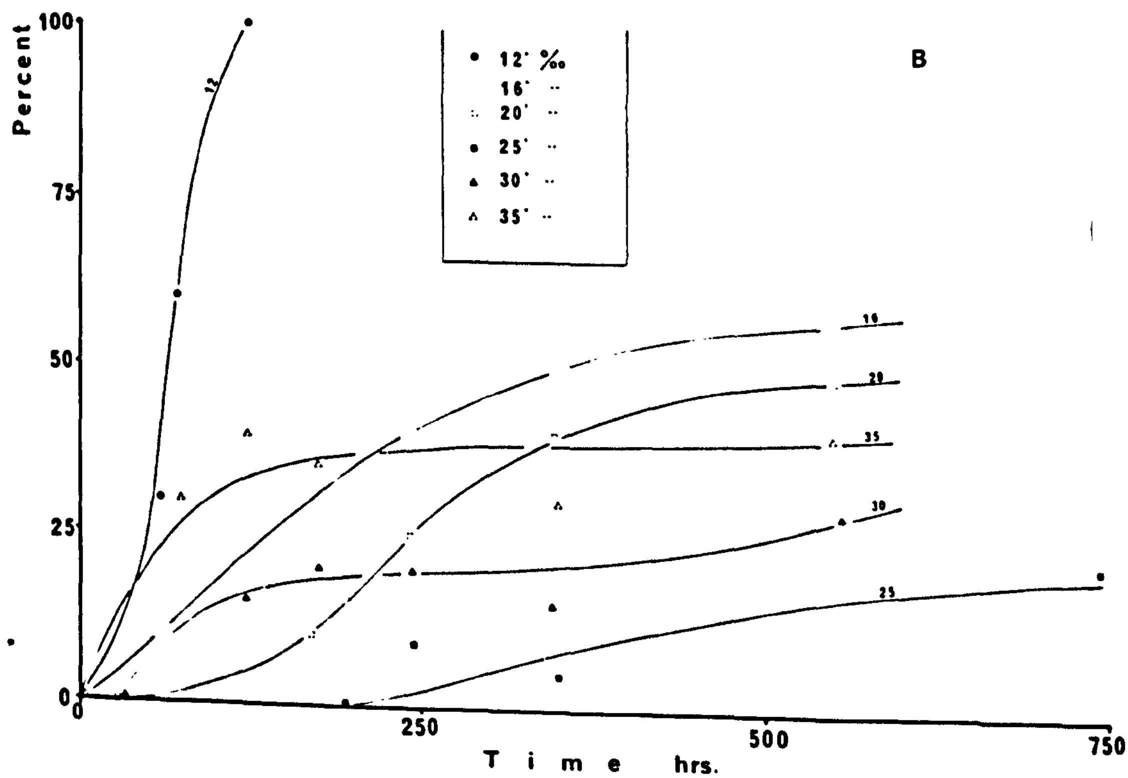
Figure 19

Salinity tolerance of *Thyasira gouldi* allowed to bury expressed as percentage mortality conducted in:

October 1970 Fig. 19A

August 1971 Fig. 19B


A
100
75
50
25
0
Percent Mortality
12
30
16
20


B
100
75
50
25
0
12
16
20
35
30
25
12° ‰
16° "
20° "
25° "
30° "
35° "
0
250
500
750
T i m e hrs.

above 25$^{o}$/ooS. At 12$^{o}$/ooS all animals had died within 375 hours (Fig. 19a ) whilst in August 1971 all had died within only 125 hours. Similarly at 20$^{o}$/ooS in October 1970 only 15% had died after 700 hours compared with 50% over a similar period when the experiment was repeated in August 1971. However, the percentage mortality at 30$^{o}$/ooS was higher in 1970 (50%) than in August 1971 (25%).

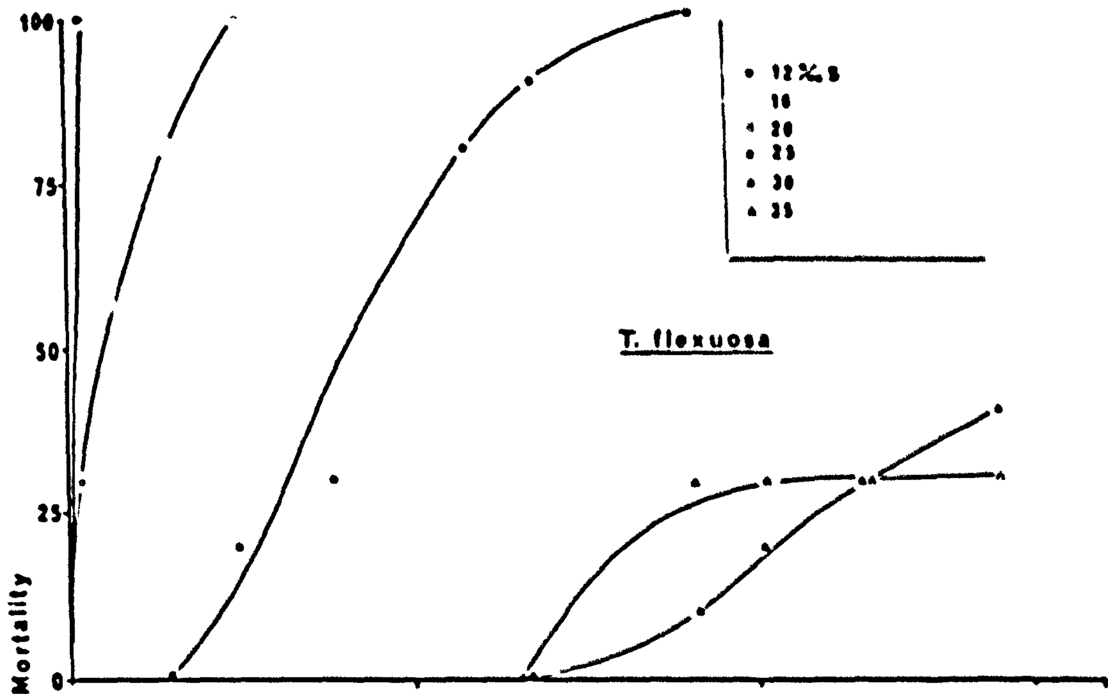
It would thus appear that whereas in October 1970 the optimum salinity for survival was around 25$^{o}$/ooS, in August 1971 it was higher than this lying between 25$^{o}$/ooS and 30$^{o}$/ooS. These two dates were chosen as being the periods when natural salinities were at their lowest (October 1970) and highest (August 1971). Prehistory (Fry 1947, Loosanoff and Davis 1952) is therefore clearly important in the salinity tolerances of the adult: the adults were pre-conditioned to a higher salinity in August 1971 and were better able to tolerate higher salinities.

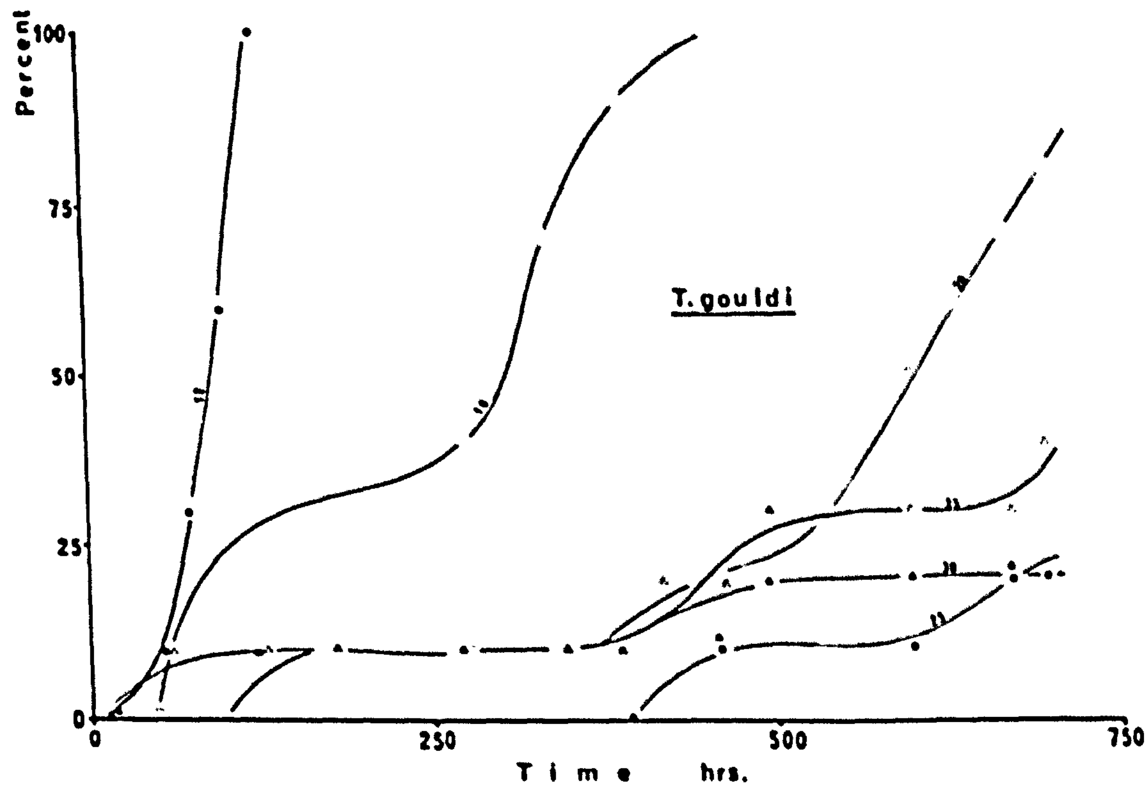
In the experiments with animals without sediment present, which were conducted at the same time, there is an almost linear relationship of mortality with time at 12$^{o}$/ooS (Fig. 20a ). A 50% mortality was reached at this salinity after only 85 hours, and all had died after 125 hours. At all other salinities a sigmoid effect was seen (Fig. 20a ). Mortality decreased with increasing salinity up to 25$^{o}$/ooS after which further increase of salinity resulted in an increase in mortality.

The salinity/mortality effect seen here is thus

Figure 20

Salinity tolerance of *Thyasira flexuosa* (Fig. 20A) and *Thyasira gouldi* (Fig. 20B) expressed as percentage mortality without sediment.


100
75
50
25
0
12‰S
16
20
25
30
35
T. flexuosa
Mortality


Percent
100
75
50
25
0
T. gouldi
0
250
500
750
Time hrs.

largely similar to that obtained when sediment was provided. The percentage mortality at $12^{o}/ooS$ is the same whether sediment is present or not. This probably indicates the time for which shells can remain closed (Kinne 1964, Gilles 1972) rather than any effect due to presence or absence of sediment. At $16^{o}/ooS$ mortality without sediment is higher (Fig.20a ) absence of sediment thus apparently has a detrimental effect. This is confirmed by the results obtained at $20^{o}/ooS$ which gives an 80% mortality after seven hundred hours without sediment compared with only 50% if sediment was present. These experiments were conducted at the same period using animals collected at the same time and place. The lack of sediment therefore appears to add to the stress upon the animals, thus increasing the mortality rate (McLusky 1967).

These methods of analysing the effect of environmental conditions upon survival have certain disadvantages. The latter method is influenced by a detrimental effect of lack of sediment, whilst the former method only sub-samples the animals used. To avoid these disadvantages the rate of burial was studied. This gives an indication of the response to salinity at less cost to the population, and provides a cumulative response curve with time based upon all the animals being used in the experiment and not samples of such a population. It also provides a quick measure of stress which could be applied to adults used in gamete production and the setting up of an aquarium stock.

The results of the observations on the rate of

burial are given in Fig. 21 . In October 1970 the rate of burial increased with increase in salinity. After fifty hours 5% had buried at $12^{o}$/ooS, 45% at $16^{o}$/ooS and 85% at both $20^{o}$/ooS and $24^{o}$/ooS, whilst slightly lower burying rates were seen at $28^{o}$/ooS and $30^{o}$/ooS, ie. 75%.

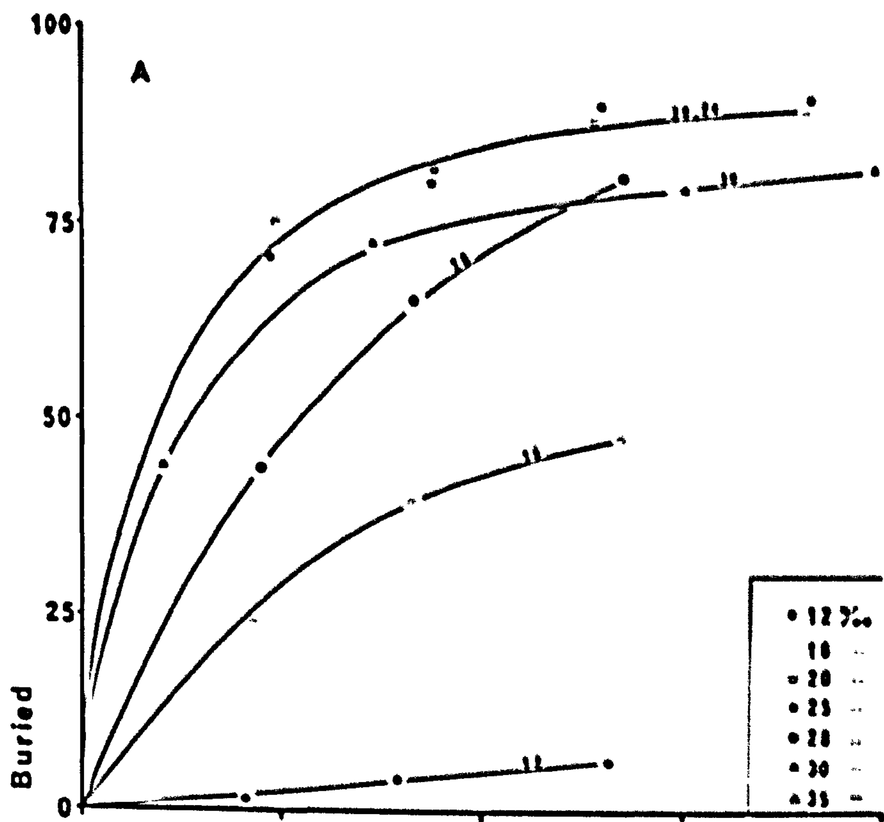
This pattern very closely followed the percentage mortality (Fig.19a ) with the peak performance found at the optimum declining on either side (Chanley 1958). At $16^{o}$/ooS the agreement is not quite so good. The percentage mortality shows a long plateau from one hundred to four hundred hours (Fig.19a ) which is thought to be an artifact of the sampling procedure. The burying rate analysis probably gives a better idea of the stress of the situation as it is an analysis of the whole and not of just a part sample.

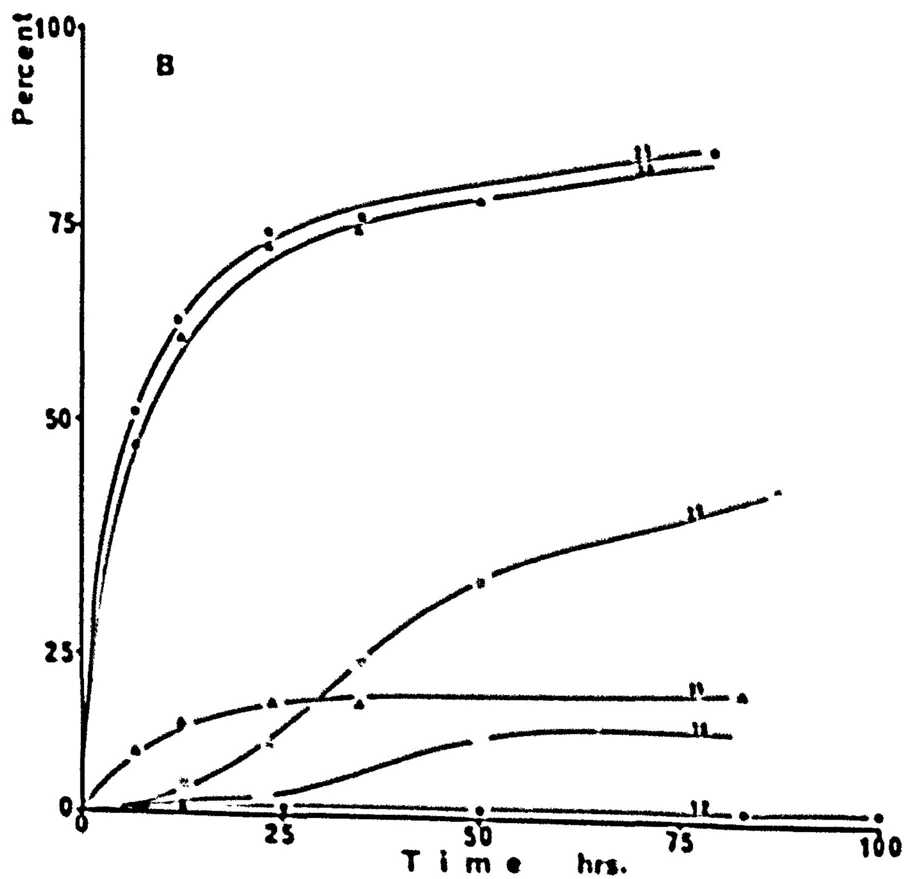
A repeat of the experiment in August 1971 (Fig.21b ) using salinities of 12, 16, 20, 25, 30 and $35^{o}$/ooS again gave a good correlation with the percentage mortality curve obtained at this time (see earlier + Fig. 19b).

Many molluscs are able to isolate the flesh from adverse conditions by withdrawing into the shell (Kinne 1964, Gilles 1972), in gastropods the operculum often sealing the body aperture (Todd 1964), or, by the secretion of mucus (Kinne 1964). It has been shown that although salinities of $12^{o}$/ooS are lethal to <u>T. gouldi</u> such salinities are not experienced by the natural population. Salinities of $16^{o}$/ooS

Figure 21

Salinity tolerance of *Thyasira gouldi*
expressed as rate of burial conducted in
October 1970 (Fig. 21A) and August 1971 (Fig. 21B)


A
100
75
50
25
0
Buried


B
Percent
100
75
50
25
0
0
25
50
75
100
T i m e hrs.

however, which can result in mortalities of 50%, may well be experienced after heavy rain in shallow areas (eg. 4b Fig. 9 ) and areas close to inflowing streams (stations 7f, 6a, 5a). The effect of exposure to $16^{o}$/ooS for varying periods of time may therefore be important in limiting the population, whilst the effect of periods of exposure to $12^{o}$/ooS may give an indication of the importance of the shell in isolating the flesh from adverse conditions (Kinne 1964, Gilles 1972).

A supplementary experiment was therefore devised to study the effect of short periods of exposure to reduced salinity. Groups of approximately one hundred animals in batches of twenty each were taken from the holding salinity ($25^{o}$/ooS) and placed into water of $12^{o}$/ooS or $16^{o}$/ooS for varying periods of time (7, 24 and 46 hours) before being replaced into the holding salinity. Other groups were placed into water of $12^{o}$/ooS but replaced not into the holding salinity but into water of 16 or $20^{o}$/ooS. After these transfers, the burying rate and later the percentage mortality were noted.

The results are summarized in Fig. 22 . Immersion of <u>T. gouldi</u> in water of $12^{o}$/ooS even for seven hours greatly impaired the rate of burial (Fig. 22a) and increased the mortality (Fig. 22b) compared with those not so immersed. Longer periods of immersion resulted in an even greater increase in mortality, so that a mortality rate of 20% was obtained after immersion for seven hours, 85% after twenty-four hours

Figure 22

The effect upon *Thyasira gouldi* of varying periods of immersion into water of reduced salinity ie. $12^{o}$/ooS and $16^{o}$/ooS expressed as effect upon rate of burial (Fig. 22A) and percentage mortality (Fig. 22B)

Percent Buried
100
75
50
25
0
0
25
50
75
100
Time hrs.
kept at 25‰
7 hrs. at 16‰
25 -- -- --
7 -- -- 12‰
24 -- -- --
kept at 12‰
46 hrs at 12‰

Percent Mortality
100
50
0
kept at 12‰
-- -- 25‰
46 hrs. at 12‰
24 -- -- --
7 -- -- --
100
50
0
kept at 16‰
-- -- 25‰
25 hrs. at 16‰
7 -- -- --
0
50
100
150
200
250
Time hrs.

and 100% after forty-six hours immersion. If animals were kept at 12°/ooS for seven hours, then transferred to 16°/ooS none subsequently buried and a mortality rate of 90% was seen compared with 40% in animals kept at 16°/ooS (Fig. 22a). Burying was also curtailed if, after being kept at 12°/ooS for seven hours, the animals were transferred to 20°/ooS. Mortalities were, however, not different from animals kept at 20°/ooS although twenty-four hours at 12°/ooS did result in an increased death rate.

If the duration of immersion at 16°/ooS was only seven hours, then, although the rate of burial was slower (Fig.22b), it eventually became equal to that of the untransferred stock. A percentage burial of 80% was reached after 117 hours compared with fifty hours for untransferred stock. If the time at 16°/ooS was increased to twenty-five hours the burying rate never reached that of the control, mortalities also being higher (Fig.22b) ie. 36% after 250 hours compared with only 10% in the control. An immersion period of forty-six hours at 16°/ooS was as lethal as being left at 16°/ooS. If, however, T. gouldi was immersed for twenty-five hours in water of 20°/ooS prior to being placed into water of 16°/ooS mortality was decreased and burying rate slightly improved when compared with animals placed directly into water of 16°/ooS from 25°/ooS.

Similar experiments have been carried out with T. flexuosa (Fig.20 ) which indicate that the salinity

optimum of this species is above $30^{o}$/ooS. Certain differences are also evident as regards the importance of sediment (see later section). It appears, however, that the salinity tolerance of T. gouldi has been 'altered' due to prevalent conditions at the head of Loch Etive so that salinities of areas in which it normally occurs and co-occurs with T. flexuosa are lethal. Arnold (1972) showed that the salinity tolerance of Patella could be altered and extended according to the salinity range likely to be encountered and this is apparently true of T. gouldi within L. Etive. Thyasira gouldi has been kept in water of $32^{o}$/ooS by slowly raising the salinity $1^{o}$/ooS every three weeks. Many animals died and others shed gametes, but some were kept at this salinity for several months. It is therefore probable that the salinity tolerances shown are not due to any genetical change in the population, but linked with prevaling conditions (Topping and Fuller 1942, Nicol 1960).

Apart from the tolerance to salinity per se, the osmotic pressure of the body fluid, in water of various salinities, was also studied.

The majority of marine invertebrates are poikilosmotic (Beadle 1931, 1937, Robertson 1964, Krogh 1939) although several have been found to exhibit a certain degree of osmoregulation (Beadle 1931, 1937, Pearse 1928, Nicol 1960, Robertson 1964, Potts 1968) including several members of the mollusca (Federighi 1931, Allen 1960, Kinne 1964, Arnold 1972). Thyasira gouldi has been shown to be able to tolerate a large

variation in salinity (16-30$^{o}$/ooS) a range which can be altered by pre-acclimation (16-35$^{o}$/ooS see earlier). This could be achieved either by tolerance of cells to reduced salinity, or by regulation of the internal osmotic pressure, or a combination of both.

The variation of osmotic pressure within the body, compared with that of the environment, expressed as depression of freezing point, has been studied for several bivalves (Anderson and Prosser 1953, Freeman and Rigler 1957, Pierce 1970, Gilles 1972), the samples of blood for such analyses generally being taken from the heart or a blood space close to it. Attempts were made to sample this region in T. gouldi, but, due to the small size of the heart and to contamination caused by granules within the kidney, (Allen 1958) this had to be abandoned, use being made instead of the blood space in the foot. Due to the relatively small size of the blood space, the method of Ramsay and Brown (1955) was used for the determination of the freezing point. The sample size necessary was only about $10^{-3}mm^{3}$.

Silica glass capillary tubing, of wall thickness 0.5mm and bore size 1mm, was heated in an oxyacetylene torch to the point of collapse, and drawn out to a fine capillary (bore size 120-140um ) capable of penetrating the foot muscle but small enough to enter the blood space. After puncturing the musculature of the foot, the sample tube was pushed along the blood space while maintaining a slight pressure so that a thin stream of paraffin oil was lost. In this way

contamination of the sample was reduced and the fine bore of the sampling tube was not clogged by mucus. Any samples contaminated with mucus or cells were discarded. Uncontaminated valid samples were frozen immediately in a dry ice/alcohol mixture at -70$^{o}$C then stored under paraffin oil at -20$^{o}$C for subsequent analysis. (Storage in this fashion had been found by Blaxter (pers. comm.) not to effect the samples.)

It had been found (see earlier) that any effect of salinity upon the flesh would be evident within forty-eight hours. Consequently samples were taken over a two day period, using a series of samples obtained just prior to the commencement of the experiment as the starting point. Only one sample was withdrawn from each animal, animals from which unsatisfactory samples were obtained were also discarded.

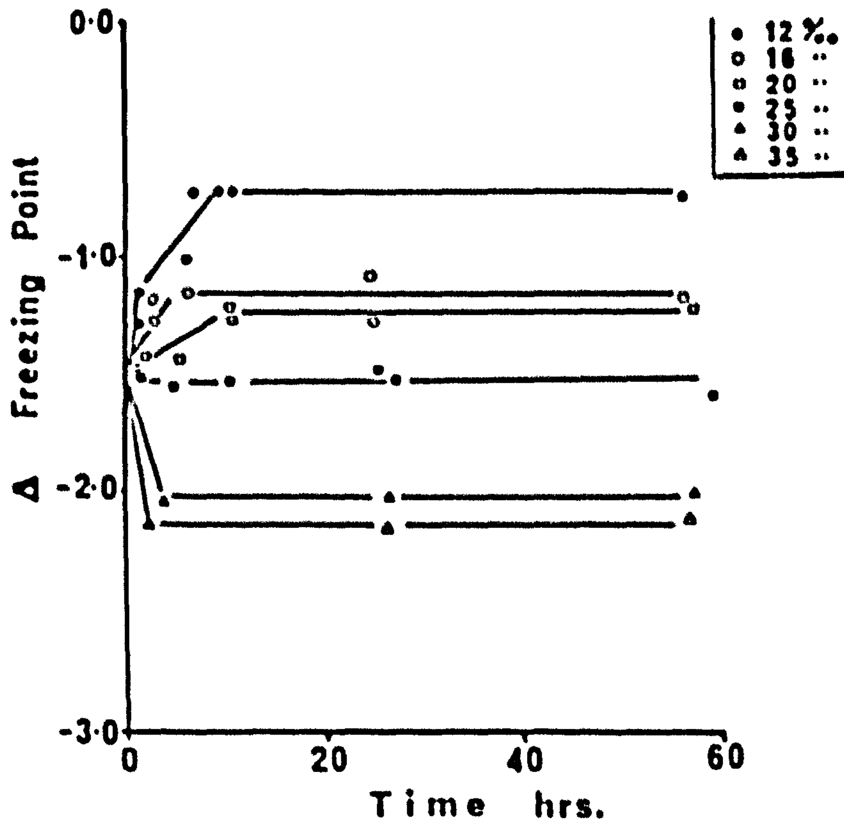
The results of the depression of freezing point determination are summarized in Fig. 23 . The effect of any change in environmental osmotic pressure (salinity) is rapid, a steady level $\Delta$ i being seen within ten hours of being placed into the test salinity (Fig. 23a). The <u>T. gouldi</u> used in this study have a blood concentration isotonic with that of the sea water ie. 25$^{o}$/ooS. The concentration of the blood ($\Delta$i) appears to alter with that of the environment ($\Delta$ e) within the range of 1.20 to 1.60 (Fig. 23b). Outside this range, however, the blood concentration ($\Delta$ i) differs from that of the environment ($\Delta$ e) such that between $\Delta$ e of 1.75 and 1.20 and again between $\Delta$ e of 1.60 and 2.10 the blood is hypertonic

Figure 23

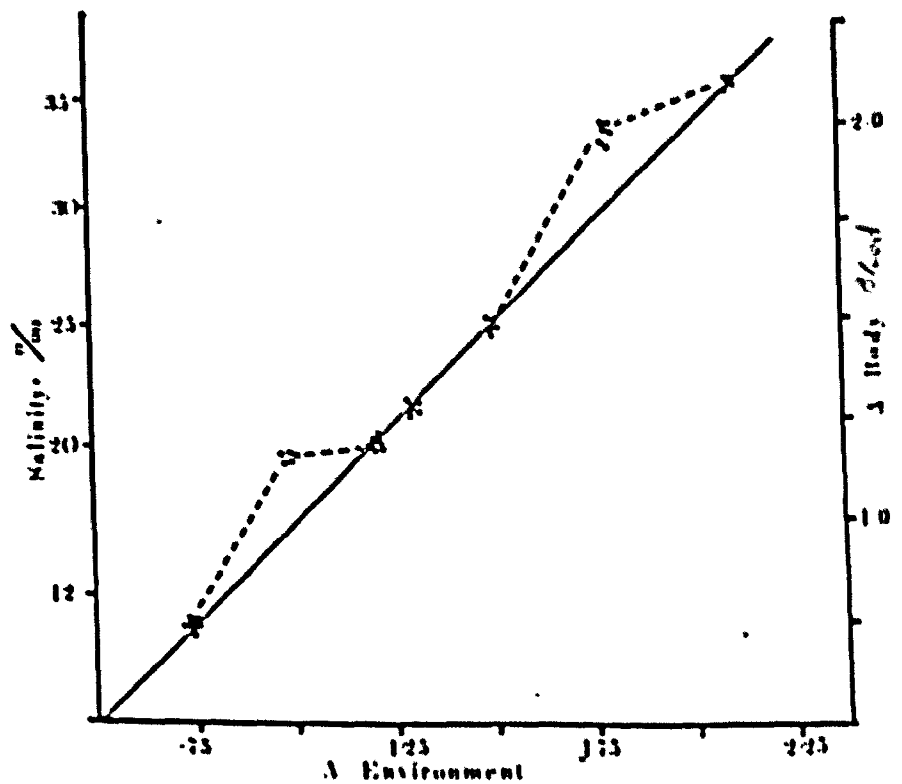
Osmotic pressure of the blood compared to that of the environment

Fig. 23A depression of freezing point of blood with time

Fig. 23B resulting correlation of blood o.p. to o.p. of environment



0·0
-1·0
-2·0
-3·0
Δ Freezing Point
0
20
40
60
Time hrs.
12 ‰
16 ..
20 ..
25 ..
30 ..
35 ..

2·0
1·0
Δ Environment

to the environment. In keeping with other marine animals capable of regulating their blood osmotic pressure (Anderson and Prosser 1953, Prosser 1955, Nicol 1960, Kinne 1964, Todd 1964, McLusky 1967, Tucker 1970) *T. gouldi* is hyperosmotic at lower salinities, ie. below $20^{o}$/ooS. This regulatory capacity breaks down below $16^{o}$/ooS, the blood varying with the environmental salinity. Todd (1964) working with *Littorina littorea* found that there was a slight rise in blood osmotic pressure, compared to that of the environment, at higher salinities, but this rise is not as marked as in *T. gouldi*.

In *T. gouldi* two thresholds of regulation appear to be present, one regulating the blood pressure to around $34^{o}$/ooS, the salinity of the open oceans; the other coming in to action when environmental salinities approach $20^{o}$/ooS. Although this means that, in a habitat where salinities vary from $30\text{-}35^{o}$/ooS the internal osmotic pressure is not going to alter very much, drops in salinity below this will result in a very large decrease in blood osmotic pressure. This change over between two methods of regulation may explain the mortalities experienced in the salinity tolerance experiment.

Without further experimentation it is possible only to speculate on the method by which this regulation is obtained. The regulation at higher salinities could be the result of an active excretion of water allowing a stable internal state, and would be the only method involved for the majority of populations

of T. gouldi, occurring as they do in water of 32-34°/ooS. This capacity is soon overtaken so that in lower salinities the blood is isosmotic with the environment, and would probably continue to be so if not for the powers of volume regulation (Kinne 1964). At an Δ i of 1.2 (20 ‰) water uptake is reduced due to the inherent strength of the cell walls, however, although this allows animals to survive in water of 16°/ooS, at 12°/ooS this strength is overcome, resulting in the rupture of cells and organs and so death (Lange 1970).

Several authors (Kinne 1964, Gilles 1972) describe the importance of the shell of bivalves in resisting adverse conditions. This does not appear to be of very great importance to T. gouldi the effect of such adverse conditions being seen within seven hours, as compared with the ninety-six hours shown for Mytilus edulis (Gilles 1972). This may be related to habitat, T. gouldi being an infaunal species where sustained closure of the shell is not a part of everyday life, and cannot be maintained for long periods.

B) Tolerance to temperature

Thyasira gouldi has already been shown to have become acclimated to the salinities of L. Etive, which are outwith the range encountered in those regions where T. gouldi is generally found. Temperatures of L. Etive are also markedly different from the near zero temperatures generally encountered in the

main area of distribution, in that they can reach $13^{o}C$ but do not fall lower than $6^{o}C$. It has been shown by several authors (Bêlehrádk 1935, Fox 1936, Gunter 1957, Kinne 1963, Ansell 1968, Kennedy and Mihursky 1971, see also the bibliography of Kennedy and Mihursky 1967) that temperature often limits the distribution of animals. It was thought that it would prove interesting to investigate the temperature tolerance of T. gouldi, and to try of correlate this with its distribution and that of its close relative T. flexuosa.

The percentage burial gave a good correlation with the degree of stress caused by varying salinity (see earlier). Both this and the percentage mortality were used to determine the effect of temperature upon T. gouldi. Thyasira gouldi were obtained from within the sample area at the head of L. Etive (Fig. 9 ) and T. flexuosa from Loch Linnhe (Pearson 1970). The temperature was regulated to $\pm$ 0.2$^{o}$C of the test temperature (ie. 1, 4, 8, 12, 16, 20$^{o}$C). Salinity was kept at the mean for the area from which the animals were collected, ie. 30$^{o}$/ooS for L. Linnhe animals and 25$^{o}$/ooS for L. Etive animals, to minimise any effect on temperature tolerance of differences in salinity tolerance.

Ten animals were placed on the surface of each of ten beakers of mud in each of six, lagged water-baths. The rate of burial was noted and two beakers were withdrawn at intervals, from which the percentage mortality was determined using the criteria for death

used previously.

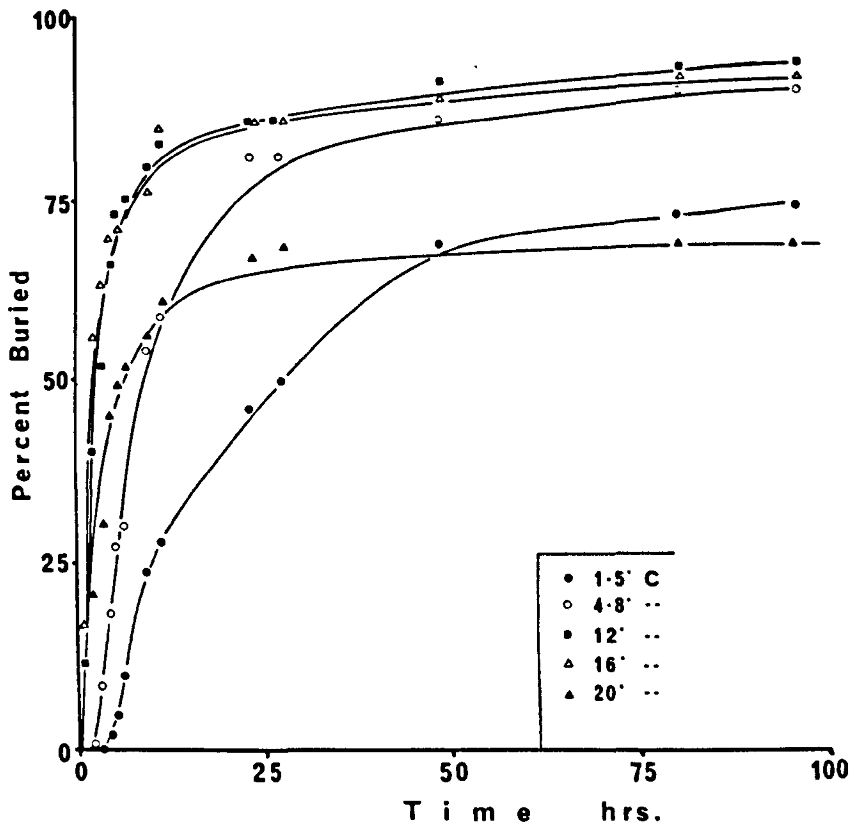
Acclimation had proved important in the salinity tolerances of T. gouldi and to allow for any effect of this factor on temperature tolerances, the experiments were conducted in December/January (1971/1972) and April/May (1972) when temperatures were at their maximum and minimum respectively (Fig. 11 ). The temperatures for both L. Etive and L. Linnhe were approximately the same, varying from 6°C to 12°C (Milne 1972, Topping and Johnson in press, R.E. Edwards unpublished data).

For T. gouldi analysis of the percentage of animals buried (Fig. 24 ) shows that burial at all temperatures was fairly rapid, burial at 20°C being the lowest and all other temperatures being roughly equal except for at 1.5°C. At this temperature burial was initially slow, but after a hundred hours a value intermediate between 20°C and the other temperatures is seen. The same general picture is seen in April/ May except that burial rates were slightly lower (Fig. 24b). These results are not reflected in the percentage mortality (Fig. 25 ). In December (1971) very few animals died, only at 16°C were any mortalitie seen. After a thousand hours at 16°C only 30% of the animals had died, at 20°C, however, all animals had died within two hundred hours. Similar results were obtained at 20°C in April (1972) (Fig.25b ), although the mortality rate was higher.

Quite large mortalities were seen at all

Figure 24

Tolerance to temperature of *Thyasira gouldi*
as shown by rate of burial
Fig. 24A December 1971
Fig. 24B April 1972


Percent Buried
100
75
50
25
0
0
25
50
75
100
T i m e hrs.
1·5° C
4·8° --
12° --
16° --
20° --

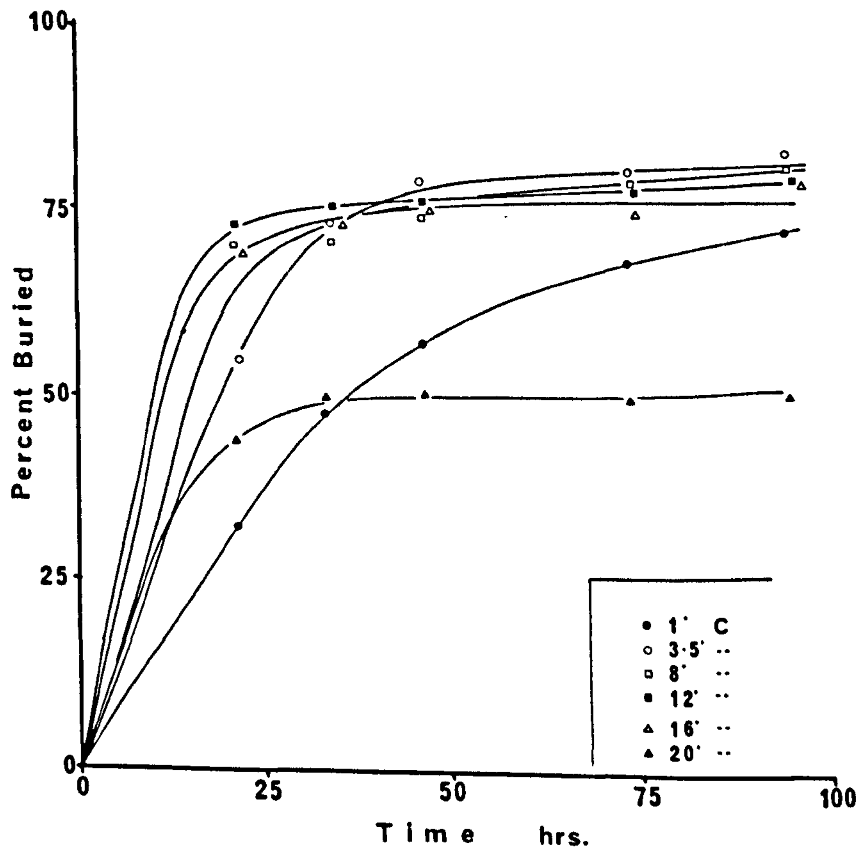
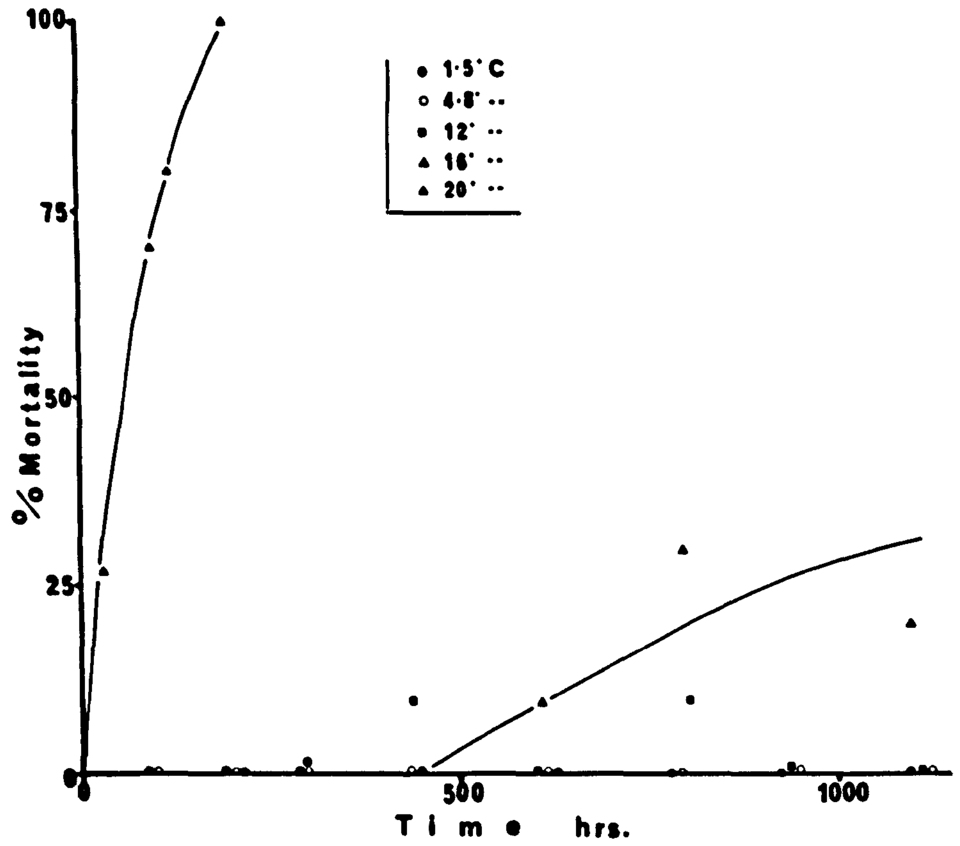
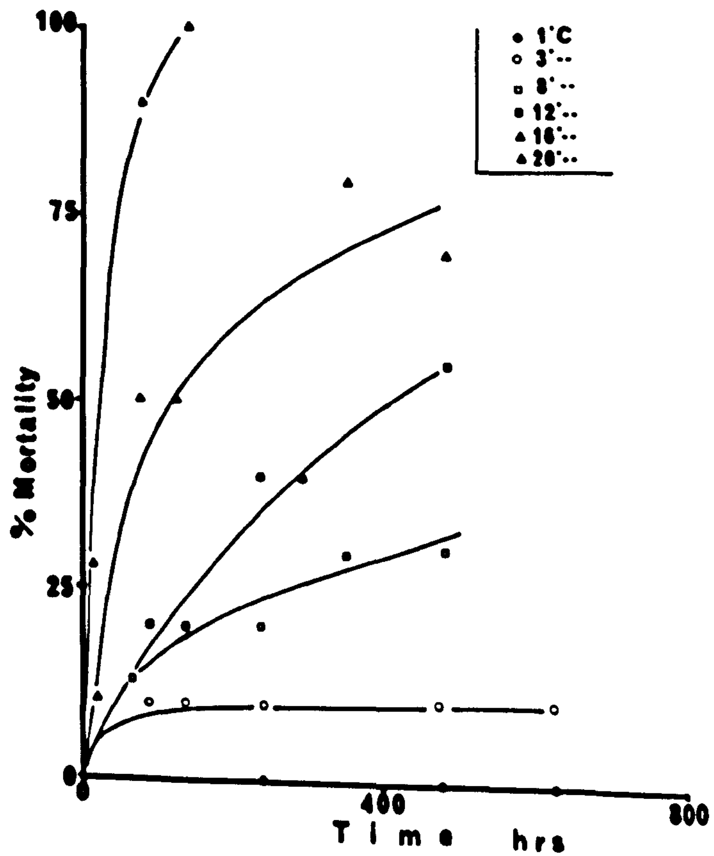

Percent Buried
100
75
50
25
0
0
25
50
75
100
T i m e hrs.
1° C
3·5° --
8° --
12° --
16° --
20° --

Figure 25

Tolerance to temperature of *Thyasira gouldi*
as shown by percentage mortality
Fig. 25A December 1971
Fig. 25B April 1972



100
75
50
25
0
% Mortality
1·5° C
4·8° --
12° --
16° --
20° --
0
500
1000
T i m e hrs.

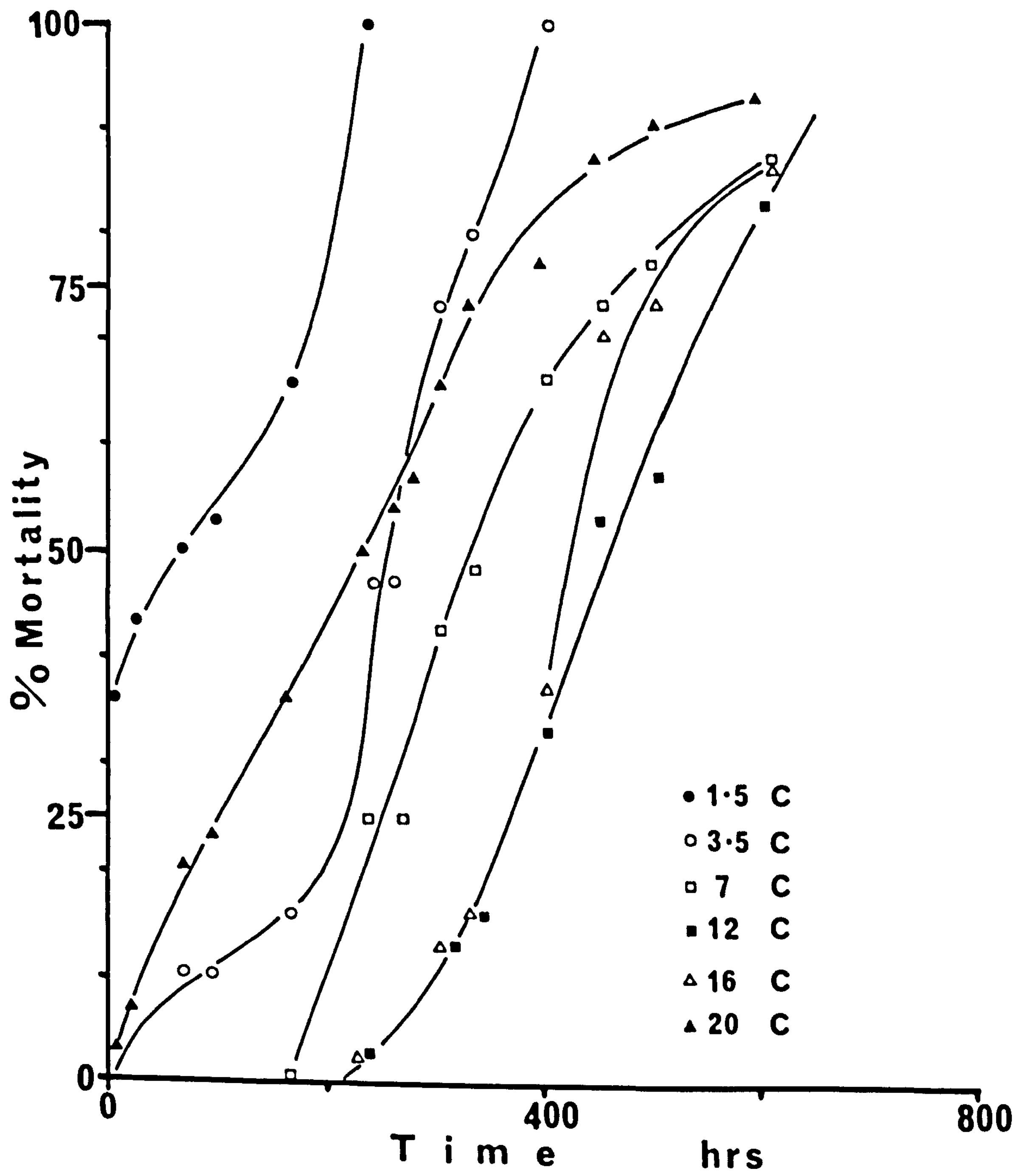
100
75
50
25
0
% Mortality
1°C
3°--
8°--
12°--
16°--
20°--
0
400
800
T i m e hrs

temperatures in April, so that, after five hundred hours, even at 12$^{o}$C, 50% mortality was passed. Only at 3$^{o}$C were mortalities of 10% or less obtained. At 1$^{o}$C no mortalities at all were suffered.

Although T. flexuosa, obtained from L. Linnhe and brought back to the laboratory, suffered little mortality even after eight hundred hours at 30$^{o}$/ooS with no sediment, (see earlier salinity tolerance data), high mortalities were suffered in April 1972 when placed onto sediment from L. Etive. It would appear that some factor, apart from temperature, was responsible for much of this mortality. Whether this was due to the sediment used (eg. L. Etive sediment has a strong smell of $H_2S$) is not known, but this could well have been a contributory factor. The results obtained (Fig.26 ) do indicate the relative stress caused by each temperature. Thyasira flexuosa is intolerant of temperatures of 1.5$^{o}$C. The mortality decreases with increasing temperature, and the lowest mortalities were obtained at 12$^{o}$C and 16$^{o}$C which would thus appear to be the optimum for this species at this time. The experiments were conducted in April 1972 when environmental temperatures were at their lowest (Fig. 11 ), but if acclimation is important then this figure would represent the 'lower temperature acclimated optimum' a higher figure would be obtained for animals acclimated to the warmer temperatures of November/December.

Figure 26

Temperature tolerance of *Thyasira flexuosa*
without sediment
shown as percentage mortality



100
75
50
25
0
% Mortality
0
400
800
T i m e hrs
1·5 C
3·5 C
7 C
12 C
16 C
20 C

## Discussion

Acclimation (see reviews of Berg 1953, Prosser 1955, Bullock 1955) is again shown to be important as in tolerance to salinity. When the environmental temperatures were in the region of 13°C (November 1971) higher temperatures could be withstood than when the environmental temperature was only 8-9°C (April 1972). Temperatures of 1°C can easily be withstood by T. gouldi, which released viable eggs after several weeks at this temperature, but not by T. flexuosa. It has been shown that although exposure to higher temperatures pre-acclimates animals to better survival at even higher temperatures (Kennedy and Mihursky 1971) acclimation at lower temperatures pre-adapts the mollusc to better survival at even lower temperatures than equal ones (Vernberg, Schlieper and Schneider 1963).

Neither of the two species survived very well in water of 20°C though probably this temperature could be survived by T. flexuosa adapted to a slightly warmer temperature than was the case here. The probable optimum temperature for T. flexuosa appears to be about 12-16°C, whilst temperatures of 1°C are lethal. This may be due to an adverse effect of lowered temperature upon ciliary action, so that at low temperatures there is insufficient water current to maintain life (Galstoff 1928). At 1.5°C ciliary action was observed to have effectively ceased.

Attempts at slowly increasing the water temperature over a period of several weeks did little to improve

the tolerance of T. gouldi to 20°C. It is therefore considered that this is, or is close to, the upper lethal temperature for this species. Henderson (1929) found that the upper lethal limit for molluscs was in the region of 30-40°C. However, Wells and Gray (1960) pointed out the errors involved in using his method, finding that, as here, upper lethal limits of 20°C were the norm for temperate species.

Temperature tolerances are often a reflection of the normal temperature regimes of an animal's habitat (Evans 1948, Zhirmunsky 1967) those which are found in the tropics often living close to their upper lethal limit (Mayer 1914). This is seen to be the case with the boreo-lusitanian T. flexuosa, tolerance to low temperatures is very poor, whilst the upper lethal limit is only slightly above those seen at the southern limits of its distribution. The distribution of T. flexuosa, in keeping with many other marine animals (Evans 1948, Gunter 1957, Remane and Schlieper 1958) is thus probably restricted by its tolerance to temperature. This is not true of T. gouldi which not only occurs in arctic waters but can survive, spawn and develop in water of 16°C. The observed geographical distribution of this species is probably a result of a combination of lack of dispersal ability - there being no planktonic developmental phase-and the adult being a weak competitor (Molander1928, Allen 1958) rather than to any restriction due to temperature.

C) Sediment and Particle size importance

Preliminary experiments in keeping T. gouldi in the aquarium showed that the presence of sediment had a marked effect upon survival. It was apparent that if no mud was present, survival was not very good, 50% mortality being reached after only four days (Fig.27 ). A thin film of mud present enhanced survival (50% mortality in nine days), while allowing animals to bury gave a survival time of twelve days. This was in contrast to T. flexuosa which could live far in excess of thirty days without sediment. It would thus appear that certain differences exist in the modes of life of these two species.

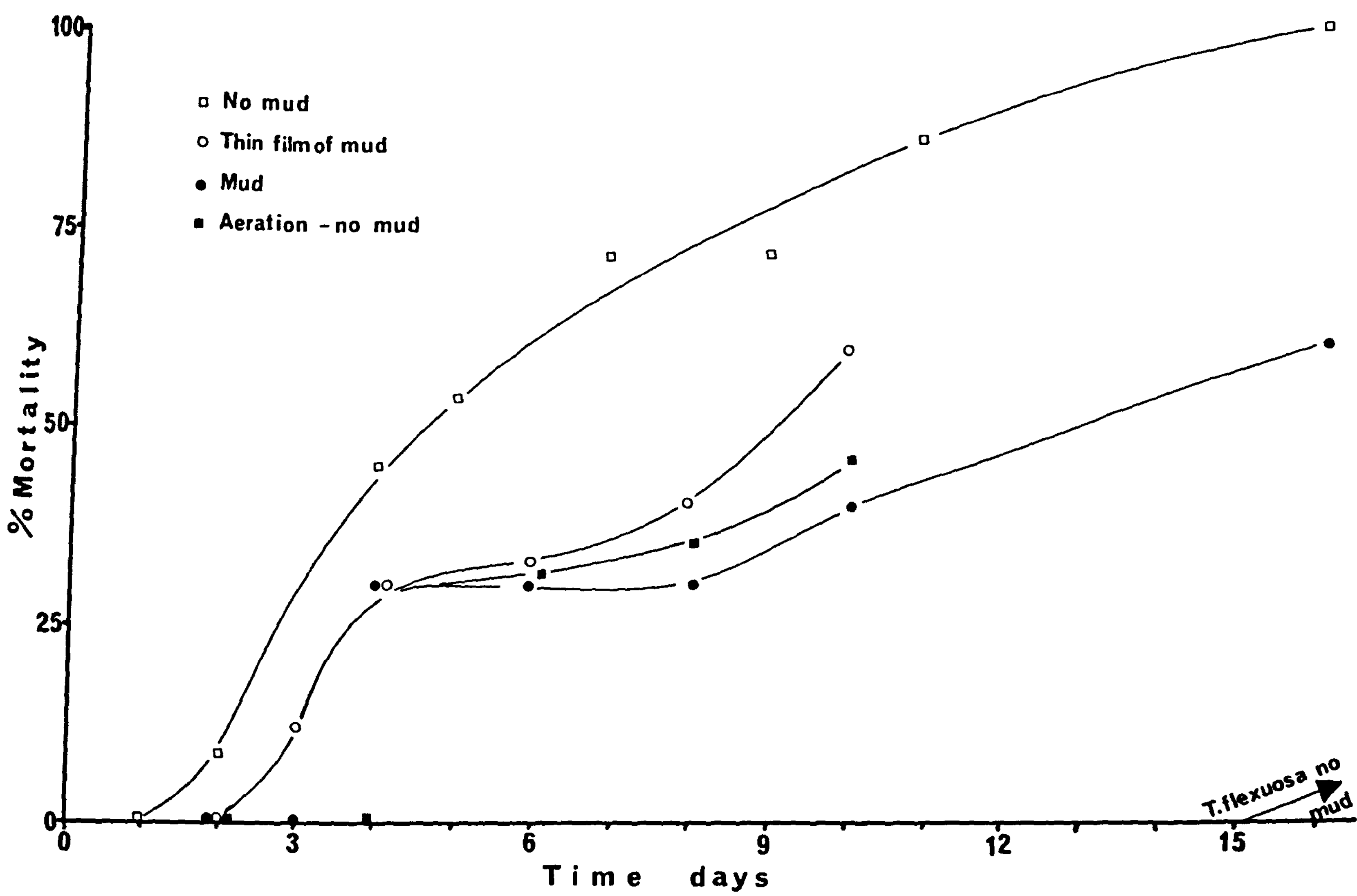
Aeration of the water led to an increase in survival of T. gouldi kept without sediment (Fig. 27 ). Thus although food may have been introduced with the thin film of mud, sediment is necessary for the construction of an inhalent tube, either for respiration or dispersal of metabolites, or combination of these: agitating or aerating the water serves this same purpose.

Using filtered water with aeration, survival was best in groups of animals provided with 'normal mud', worst with no mud, a level between the two being obtained with baked mud formed by heating normal mud at 700°C to burn off all the organic matter. Not allowing animals to bury, but aerating the water apparently causes more stress that allowing burial. The expenditure of energy due to burrowing is outweighed by the benefits of having a burrow. The low mortality in the

Figure 27

Relative importance of sediment
to _Thyasira gouldi_ and _Thyasira flexuosa_

Fig. 27A Percentage mortality
with and without sediment
maintained at 'natural' salinities



% Mortality
100
75
50
25
0
No mud
Thin filmof mud
Mud
Aeration - no mud
T.flexuosa no mud
0
3
6
9
12
15
Time days

normal mud may be associated with available food in this mud. Attempts to feed T. gouldi on a variety of phytoplanktonic organisms, kindly supplied by Mr. M. Scott, proved unsuccessful, often resulting in a decreased survival. It was concluded from stomach content analysis (see earlier) that T. gouldi probably feeds not on large particles (Young and Rhoads 1971) but on organisms it collects from the sediment, which is characteristicly high in organic matter (Blegvad 1914, Bellan 1957, Holme 1966, Kuznetsov 1970).

Thyasira gouldi is generally found on soft mud, silt mud or clay mud bottoms (Bagge 1969, Lie and Kisker 1970, Gage 1972) and although some have been recorded on gravelly bottoms, these have often proved to be only dead shells (Ockelmann pers comm.)

A preliminary experiment was devised to determine the general range of particle size into which T. gouldi could burrow and to establish whether or not the adults were capable of any lateral movement from an unsuitable sediment to a more suitable one. This preliminary experiment consisted of placing four different sediments (normal mud, baked mud, fine sand (BDH fine sifted) and gravel particle size 0.5 - 1.0mm (obtained by sieving through both a 0.5mm sieve and a 1.0mm sieve using the fraction retained in the 0.5mm sieve) ) into a two litre beaker of water, the sediments being initially separated by perspex partitions which were subsequently removed leaving four equal sections of sediment with no physical barrier between them. Fifteen T. gouldi of approximately equal length were

placed evenly onto the surface of each sediment type and the time taken to bury plus any lateral movement from one sediment to another was noted.

During the period of the experiment, ie. sixty-five hours, 93% had buried in the soft mud, 13.3% into the sand, but only 6.7% into the gravel. The rate seen on the baked mud (Fig. 28a) is perhaps odd. However, unless great care was taken this sediment solidified to form a clay into which the animals could not bury due to compaction. This was a direct effect of burning off the organic matter as it was not seen when air dried mud was used. Before solidification took place a rapid burial rate far in excess of that seen in the sand was noted. Although no barriers were present between the sediments, only three animals crossed from one sediment to another. Allmoved into the 'normal mud', one from the baked mud, the two others from the sand. These animals were, however, on the normal mud/baked mud or normal mud/sand interfaces and thus do not represent any great lateral movement.

Thyasira gouldi thus appears capable of burrowing into mud and to a lesser extent sand, a few being able to burrow into gravel. It requires a sediment of fine particles but this has to have a relatively high organic content to prevent it solidifying. Virtually no lateral movement occurs: if the adults are placed onto a sediment of the wrong type they remain there until death.

A further experiment was conducted to define

Figure 28

Ability of <u>Thyasira</u> <u>gouldi</u> to bury into various sediments

Fig. 28A Coarsely defined sediments used in preliminary experiments

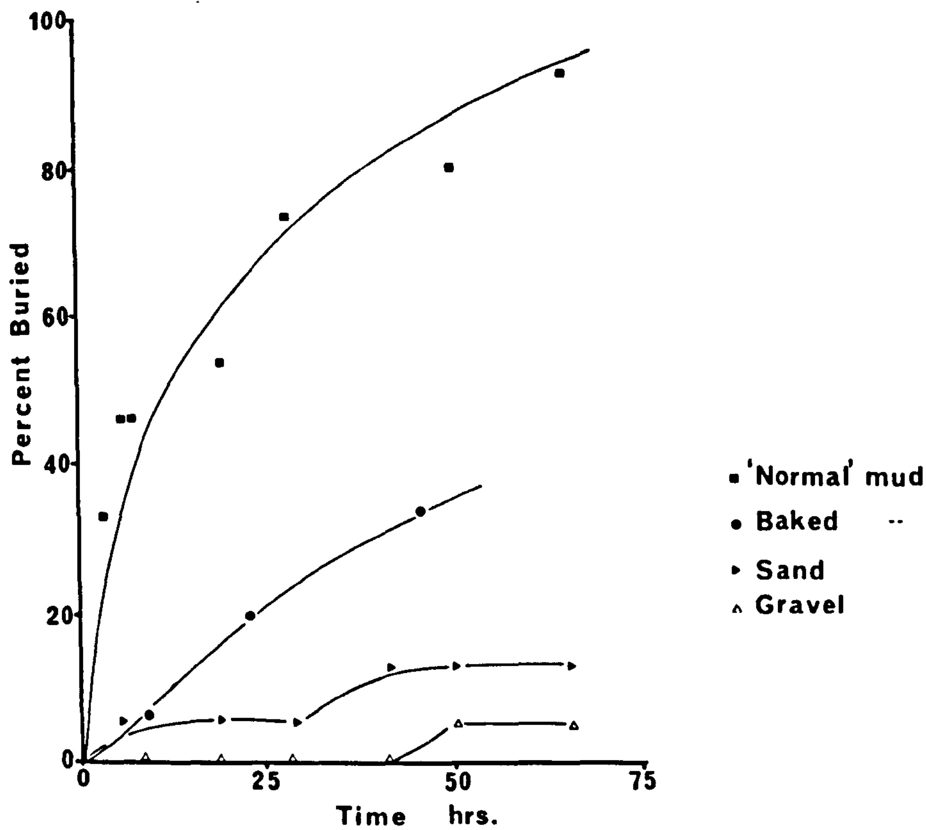
Fig. 28B More closely defined sediments used in later experiment

S : Sand

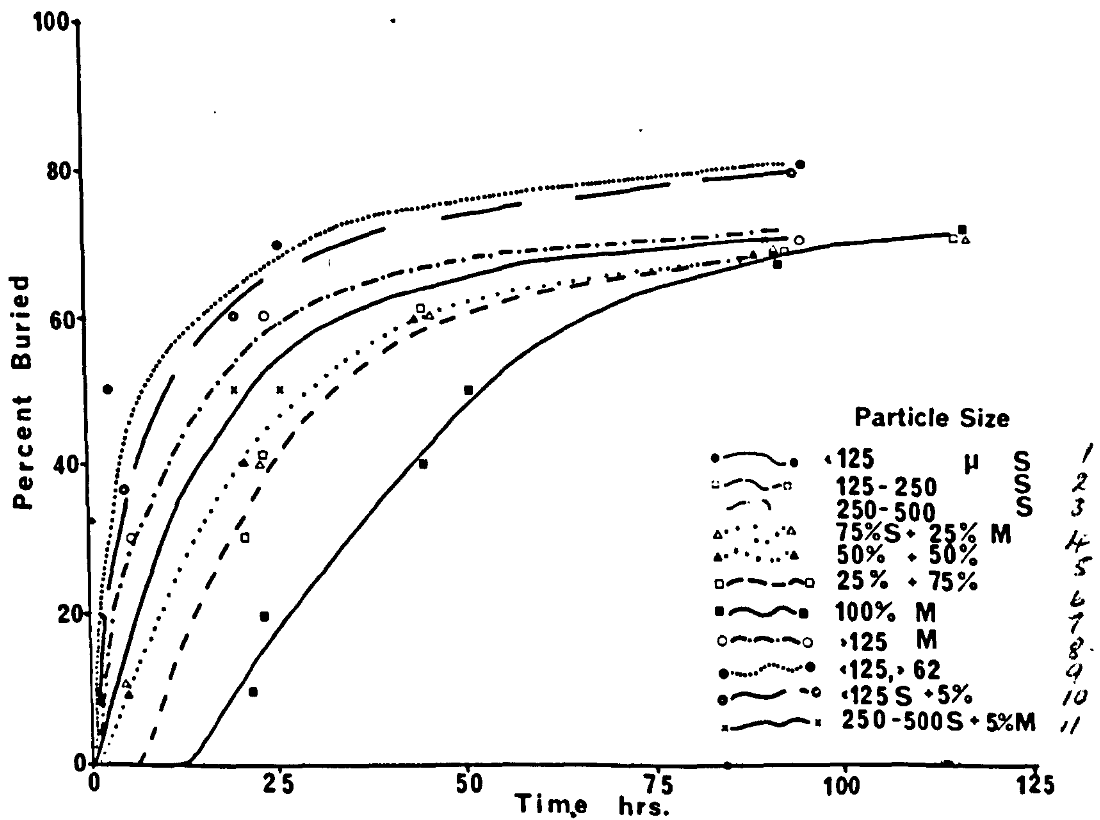
M - 'Natural' mud obtained from centre of study area

Lines for sediments 4 - 5 combined

" " " 1, 2, 3 along base line.



Percent Buried
Time hrs.
0
20
40
60
80
100
25
50
75
'Normal' mud
Baked --
Sand
Gravel

Percent Buried
Time hrs.
0
20
40
60
80
100
25
50
75
125
Particle Size
<125 μ S
125-250 S
250-500 S
75%S + 25% M
50% + 50%
25% + 75%
100% M
>125 M
<125, >62
<125 S + 5%
250-500S + 5%M

more closely the particle size and the amount of organic material necessary to allow T. gouldi to bury successfully.

Sediments of various particle size were made up using both fine sand and mud as outlined in Table 4. Eleven beakers were filled with different sediment, three being clean sand fractions, the rest mixtures of these sand fractions with varying amounts of mud. The particle fractions were obtained by sieving, dry sieving being used for the sand (BDH fine sifted) and wet sieving for the natural sediment. The term 'mud' with no indication of particle size being used to represent the 'natural mud' from the head of L. Etive (for particle size analysis of which see Fig. 12). The beakers of sediment were placed into a large bath of water of $25^{o}$/ooS, ie. the optimum salinity for the population (see earlier), twenty T. gouldi being placed onto the surface of each sediment and the relative rates of burial noted.

The results are summarized in Fig. 28b. From the cumulative percentage buried per unit time it can be seen that in none of the solely sand sediments did any animals manage to burrow. In the finest sand fraction ie. < 125um there were signs of burrowing - ie. disturbances caused by the activity of the foot upon the sand surface, but the sand soon compacted and no animals actually buried.

A decrease in the rate of burial is seen with respect to sediments 10, 4, 5, 6 and 7 ie. with increasing

mud content (Table 4). The rate of burial in sediments 4 and 5 were equal. There wasalower rate of burial in sediment 6 and an even lower rate in sediment 7 (100% natural mud). Sediment 10 which had only 5% of mud added to it gave the highest rate of burial for that sand fraction. In the other sediments 8, 9, and 11 a high burial rate is seen, but of these only sediment 9 gave a rate of burial higher than that seen for sediment 10.

*Thyasira gouldi* is only able to burrow into a sand sediment if a certain degree of mud or organic matter is present. If the amount of naturally occurring mud is increased above this level then the efficiency with which *T. gouldi* can burrow decreases (viz. gradation from sediment 10-4-5-6 and thus to 100% natural mud, ie. sediment 7). This increase in natural sediment reflects an effective increase in the percentage fine material, ie. less than 62um, as approximately 50% of the 'natural mud' is less than 62um . A high rate of burial was seen in the fine mud sediment (sediment 8), however, it is probable that much of the finer material was lost from this sediment during sieving, the highest rate of burial being found on the coarser mud (sediment 9).

It would appear that although *T. gouldi* forms quite a dense population at the head of L. Etive, conditions are not ideal. The very fine particles forming the sediment (treated analysis Fig. 12 ) are modified by the inhabitants (Young and Rhoads 1971) so that a much coarser sediment (aggregate

Table 4

Sediment choice

| Sediment type<br><br>Code No. | Composition<br>ie. particle size |
|---|---|
| 1 | <125um |
| 2 | 125 - 250um |
| 3 | 250 - 500um |
| 4 | 75% (2) + 25% (7) |
| 5 | 50% (2) + 50% (7) |
| 6 | 25% (2) + 75% (7) |
| 7 | E24 'natural mud' |
| 8 | Mud >125um |
| 9 | Mud < 125um > 62um |
| 10 | Sand < 125um + 5% (7) |
| 11 | Sand 250 - 500um + 5% (7) |

analysis Fig. 12 ) is actually encountered by the *Thyasira*. Even so this sediment is not ideal, the rate of burial being more rapid in a coarser sediment, ie. one with particles above 62um (sediment 9). *Thyasira gouldi* is able to burrow into sand only if a certain amount of organic/fine material is present. Its inability to burrow into clean sand is not due to any lack of burial response, but to the sediment compacting to such an extent that burrowing of the type exhibited by *T. gouldi* is not possible. The distribution of *T. gouldi* is related in a broad sense to particle size (Lie and Kisker 1970) but a certain amount of organic/silt fraction is necessary. It is probably this fraction of organic matter which is responsible for the distribution (Bagge 1969), too much organic material having an adverse effect. Several authors have analysed the sediment populated by *Thyasira* (Buchanan 1963, Lie and Kisker 1970, Young and Rhoads 1971). However, this analysis is on the broken down particles, into which it is dubious whether *Thyasira* could bury if they actually occurred, rather than the particles actually encountered. They are probably not too different from this sediment but like it are modified by the inhabitants (Young and Rhoads 1971) so that less than 10% of it is actually less than 15um in diameter. This sediment is however not the best possible, a sediment rich in organic material but of particles greater than 62um giving the best burial rates.

Discussion

Thyasiri gouldi within L. Etive is restricted to the head of the loch. It is further restricted to areas where the depth of water exceeds fifteen metres. Temperature is not thought to be a factor in limiting this distribution but sediment and salinity are important. It is concluded from this study that T. gouldi is restricted to a sediment rich in organic matter, if the size of such organic material is too large clogging of the burrow occurs. Too large a sediment particle size prevents burrowing and thus areas of coarse sediment or gravel are not colonized. The sediment at the head of L. Etive is generally too fine, however, this is altered by the inhabitants of the sediment giving a more suitable sediment for T. gouldi.

Acclimation to salinity and its effect upon subsequent tolerances has been shown to be important for other bivalves, (Anderson and Prosser 1953, Bedford and Anderson 1972, Gilles 1972) as well as for other invertebrates (Marshall and Orr 1955, Nicol 1960, Davenport 1972). The tolerances exhibited by this population of T. gouldi are the result of such acclimation and not thought to be due to any genetical change. Topping and Fuller (1942) showed that the salinity tolerance range of Nereis virens could be extended by a gradual alteration of salinity. It is thought that the tolerance range of T. gouldi occuring in L. Etive has similarly been extended. The salinity tolerance of T. gouldi from water of

more stable salinity has not been investigated, but tolerances are generally related to the range of salinity normally experienced (Federighi 1931, Nicol 1960). It is therefore considered that the salinity tolerance of T. gouldi in L. Etive has been altered due to the lowered salinities of this area, such that salinities above $30^{o}$/ooS are lethal unless the change in salinity is very gradual. Salinities of $12^{o}$/ooS are lethal and effectively limit the extent of this population of T. gouldi, as although the bivalve can avoid these salinities by closing the shell, (Kinne 1964, Gilles 1972) this ability is not very efficient and gaping of the shell is soon seen followed by death, probably as a result of the breakdown of any volume regulatory ability (Lange 1970).

## IV) The consumption of oxygen by *Thyasira gouldi*

References to the oxygen consumptions of marine invertebrates are plentiful in the literature (Wingfield 1939, Zuethen 1947, 1953, Walshe-Maetz 1952, Vernberg 1956, Nicol 1960, Vernberg and Vernberg 1969, Staaland 1972) as are references to the oxygen consumption of the lamellibranchs (Sparck 1936, Thorson 1936, Dam Van 1954, Bielawski 1961, Read 1962, Vernberg et al 1963). However, references to arctic forms are not so plentiful (Sparck 1936, Thorson 1936, Scholander et al 1953) whilst there are no references to the oxygen consumption of Thyasira.

Thyasira gouldi generally inhabits areas of open water, the salinity of which is always above 30°/ooS and usually above 33°/ooS (Thorson 1936, Bernard 1972, Lie and Kisker 1970). These areas are of stable conditions with a variation in salinity of often only 1°/ooS (Buchanan 1963) and a variation of temperature of 1-2°C. The general temperatures experienced are around 1°C in the arctic waters of Greenland (Thorson 1936) though T. gouldi is found in warmer waters of 6-8°C (Buchanan 1963, Bagge 1969, Lie and Kisker 1970) or up to 10°C in Cape Cod Bay (Young and Rhoads 1971). In L. Etive, however, conditions are far more variable, salinities of 20-28°/ooS are commonly experienced, as are temperatures of up to 13°C. Salinities and temperatures such as these are, however, well within the tolerance limits of T. gouldi from this area (see earlier).

All the animals used for the determination of rates of oxygen consumption were collected from

between transects 4 and 5 of the study area (Fig. 9 )
by means of a Van Veen grab. The animals were removed
from the mud as quickly as possible and transported
back to the laboratory contained in a thermos flask
in water of 25°/ooS. They were then placed into clean
water of 25°/ooS and allowed to stand overnight at 10°C
to allow the gut to empty.

Initially attempts were made to use the Scholander
Respirometer (Scholander et al 1952, Scholander and
Iversen 1958). However, although this 'micro-respiro-
meter' produced good and reproducable results for
large animals, the difference in the level of mano-
metric fluid between the control and the experimental
flasks was too small to be reliable when using small
animals (less than 5mm), or for temperatures lower
than 10°C. These respirometers could have been used
if more than one animal was used in each flask,
but then there are difficulties in determining the
effects of size, sex or variation in activity level
of the animals within each flask. The former can
easily be overcome by grouping according to both
length and weight, but the latter factors cannot be
evaluated until the experiment is terminated. Although
it is probably better to use a flow method of ascertain-
ing oxygen consumption (Keys 1930, Kamler 1969),
the early experiments showed that the levels of
oxygen consumed by <u>T. gouldi</u> would be very low, conse-
quently a closed system was adopted. Small pyrex
vials (capacity 3-5ml) with a double top (see Appendix
1) were used for the determinations of oxygen consumption
so that, after shaking the flask, the smaller cap

could be removed causing the minimum of disturbance to the contained water when the nozzle of the Micro-pippette (Fox and Wingfield 1937) was inserted to sample the water. The oxygen content of the 2ml sample was determined by the method of Fox and Wingfield (1937). The volume of water used was obtained accurately by weighing the vial + caps, empty and full of water, and the volume occupied by the animal was separately determined using the apparatus described in Appendix 2.

The oxygen consumption was measured for whole animals, both acclimated and unacclimated to the test conditions, and for animals removed from their shells. Measurements were made at a variety of salinities (16, 20, 25 and 30$^{o}$/ooS) and for three temperatures (ie. 5, 10, 15$^{o}$C) at the optimum salinity (25$^{o}$/ooS).

The water used in all experiments was filtered on Whatman'C' filters then aerated using a gas distribution tube and bacterial air filters, all tubing used being Latex autoclavable tubing. The experiments to test the effect of salinity were carried out with animals collected when the environmental water temperature was 9$^{o}$C and the salinity was 27$^{o}$/ooS. Those to test the effect of temperature when the temperature of the environmental water was at 13$^{o}$C and repeated when the water temperature was 6$^{o}$C (ie. in the months of November and July.

Blank determinations of the oxygen level showed that the level of accuracy obtained was well within the 2% given by Fox and Wingfield (1937) and was

generally less than 1.5%. To obtain the oxygen consumption of the animal the oxygen content of the control flask was subtracted from that of the test flask, resulting in a cumulative error of approximately 5% which is well within the limits of accuracy of other methods.

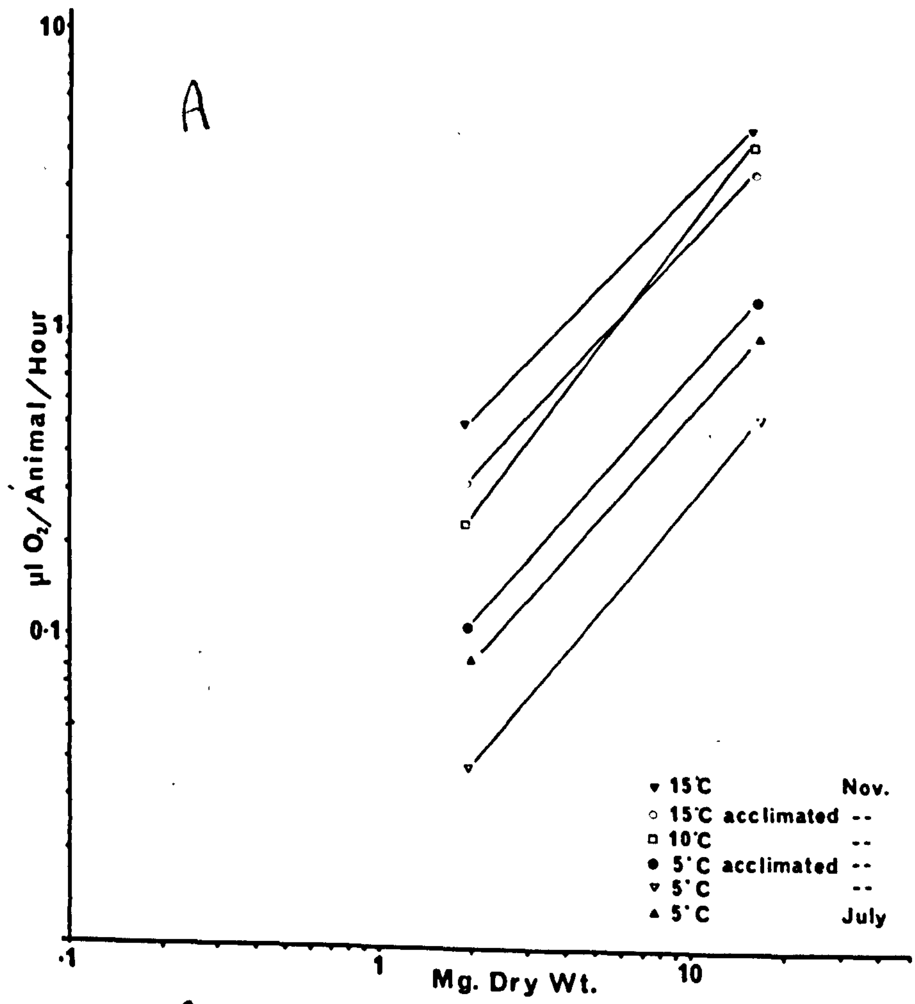
Thyasira gouldi is known to live in areas of relatively low water current and thus low oxygen content, values as low as 0.4ml/litre having been reported (Bagge 1969). It would thus appear that no great effect upon oxygen consumption would occur due to any decline in the oxygen level when using the closed bottle system. The oxygen consumptions of various size ranges of animals were determined over varying periods ie. 6, 12 and 24 hours. Individual rates of oxygen consumption were quite variable, but no constant differences appeared which could be attributed to the incubation period. An incubation period of twelve hours was thus adopted ensuring no effect due to oxygen decline, but giving an oxygen decrease which was easily readable. Comparison of the oxygen consumption obtained when some flasks were shaken, whilst others were not, showed no effect upon the oxygen consumption due to shaking, unless this was vigorous, in which case the oxygen consumption decreased, probably due to closure of the shell.

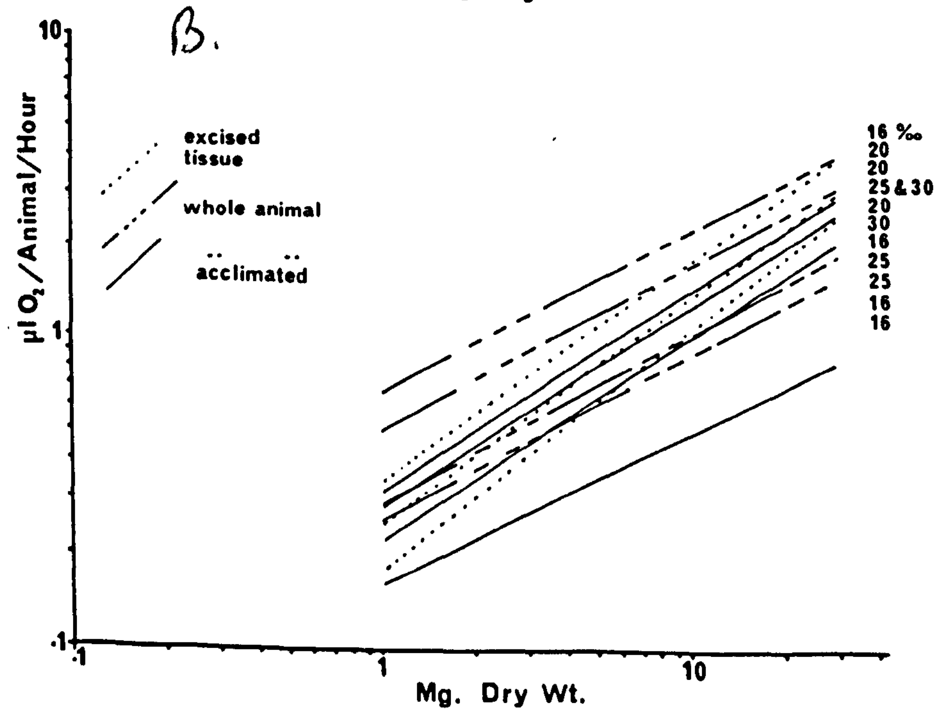
The results of all determinations of oxygen consumption for T. gouldi are summarized in Figs.29 . In all cases larger animals consumed more oxygen than smaller ones. However, this relationship between

Figure 29

Regression lines obtained from oxygen consumption experiments for varying temperature at $25^{o}$/ooS (Fig. 29A) and for varying salinity at $10^{o}C$ (Fig. 29B)

(Each line based on at least 20 animals)




listed in sequential order.

size and oxygen consumption is generally inverse when calculated per unit weight (Regneault and Risset 1849, Krogh 1941, Zuethen 1947, 1953, 1970, Bullock 1955, Leffler 1973). Analysis of covariance showed that within each group of experiments, ie. whether the animals are acclimated or not or whether excised tissue is used, the lines can be pooled to have the same slope. This could not be done between the groups as there were significant differences. Comparisons have therefore been made by taking values for a standard size animal.

Comparison of the variation in oxygen consumption due to salinity for a standard animal of 5mg dry tissue weight is summarized in Fig. 30. The rate of oxygen consumption at $25^{o}$/ooS did not vary greatly whether taken for whole animals, excised tissue or for whole animals kept in the aquarium for three days. At $20^{o}$/ooS (Fig. 30b) oxygen consumption was slightly higher than at $25^{o}$/ooS (1.0um $O_2$/hour/animal as compared to 0.86um $O_2$/hour/animal for an animal of 5mg dry weight), and although there was little difference between the tissue and unacclimated animals the rate for animals left at $20^{o}$/ooS for three days was far higher. At $16^{o}$/ooS there appeared to be two rates for the acclimated animals, one being identical to that obtained for animals placed directly into this salinity (Fig. 30b), the other being far in excess of this rate. At $30^{o}$/ooS rates of oxygen consumption almost identical with those obtained at $25^{o}$/ooS were obtained.

The effect of temperature upon the standard

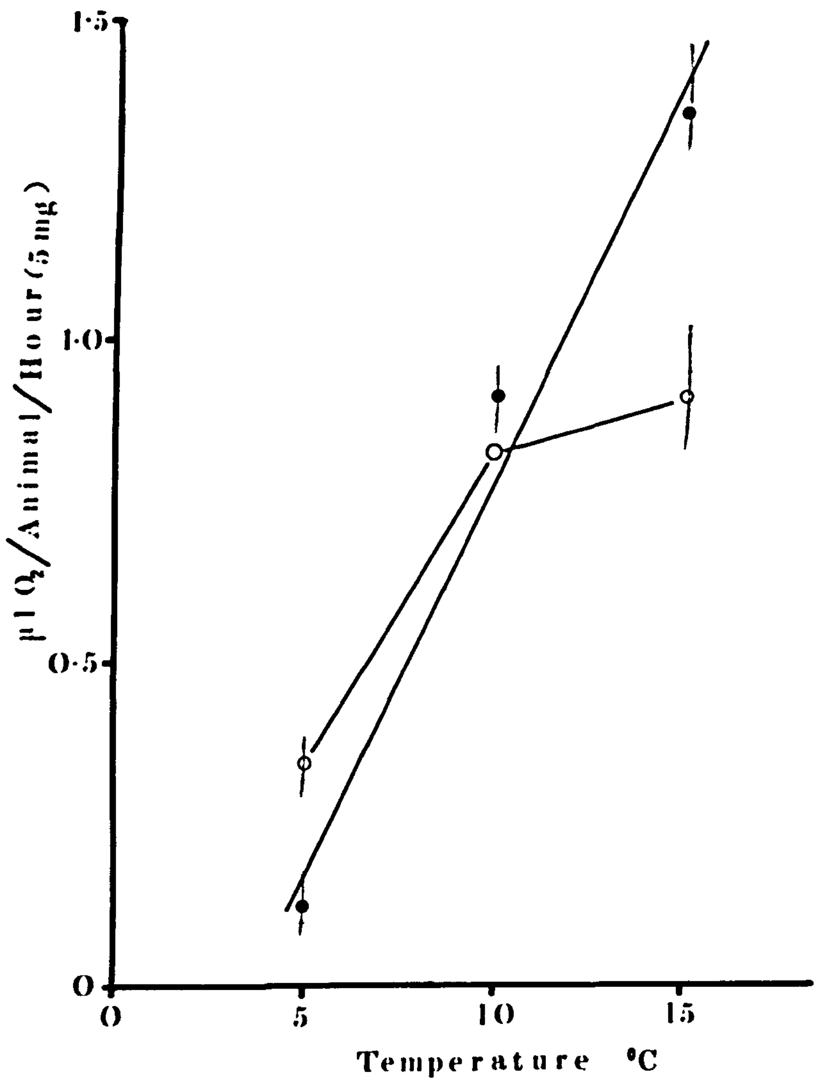
Figure 30

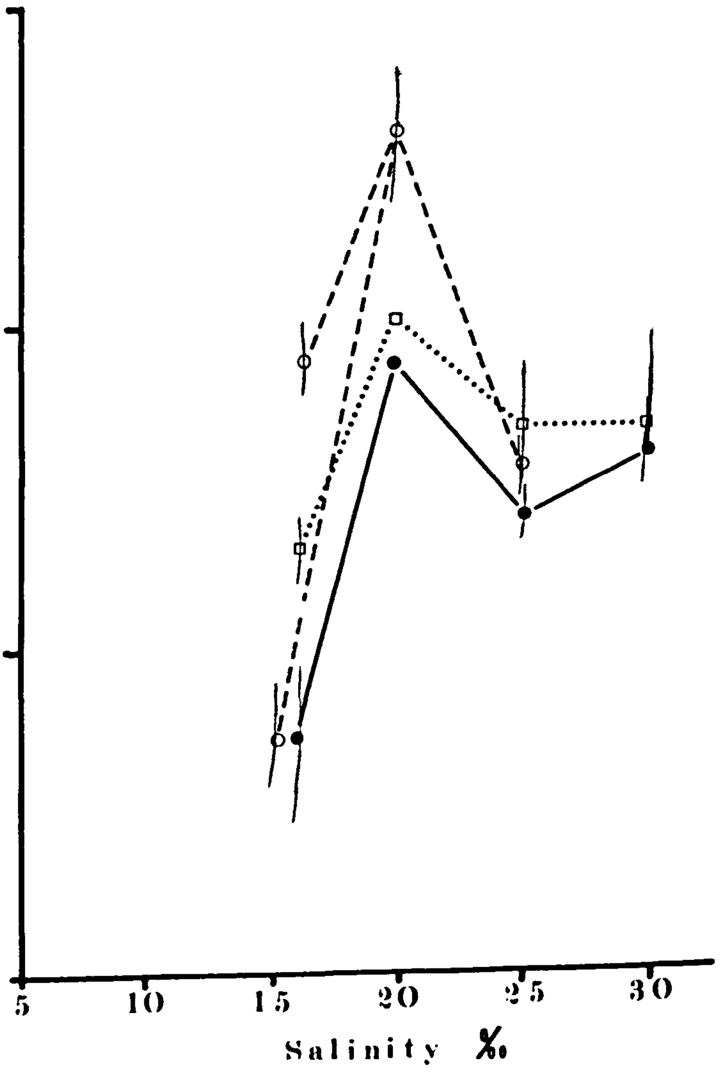
Variation in oxygen consumption
for animal of 5mg dry tissue weight

o - whole animal

□ - excised tissue

O - acclimated whole animal


μl O₂/Animal/Hour (5 mg)
1·5
1·0
0·5
0
0
5
10
15
Temperature °C


5
10
15
20
25
30
Salinity ‰

animal is summarized in Fig. 30. For animals placed straight into the test temperature, the rate of oxygen consumption varied directly with temperature over the range tested. For acclimated animals however the rates were different, the rate at 15°C being significantly reduced, while the rate at 5°C was slightly increased. In July when temperatures of the environment were lower, a slightly higher respiration rate was obtained at 5°C than in November, showing once more the significance of acclimation.

Discussion

'The metabolic rate is generally lowest at the salinity at which the organism naturally occurs' (Kutty et al 1971). Although generally true, this is not the whole story as regards T. gouldi. A small decrease in salinity, ie. down to 20°/ooS, results in quite a large and sustained rise in oxygen consumption, probably related to stress (Schlieper 1955) and active osmoregulation (Hopkins 1941, Bullock 1955, Nicol 1960, Prosser and Brown 1961, Negus 1968) the body fluid being at a higher osmotic pressure than the environment. 16°/ooS has been found to mark the limit of salinity tolerance, and at this salinity two levels of oxygen uptake are seen. The cost of active regulation is very great and whilst some individuals can maintain this level for several days, this is not true of all the individuals tested; these others apparently rely more upon a passive volume regulation due to the inherent strength of the cell

walls. Animals placed directly into this salinity from the holding salinity of the aquarium gave a lower rate of oxygen consumption probably due to sustained shell closure (Kinne 1967), which can reduce oxygen uptake to at least a third or less (Hiscock 1953b).

Acclimation which is important in the survival of many animals (Prosser 1955, Bullock 1955, Berg et al 1958, Lange 1968, Chin 1972) is not seen as regards salinity effect upon oxygen consumption in T. gouldi, however, it is seen with regard to temperature. Acclimation to ~~raised~~ increased temperature results in a decrease in the oxygen consumption obtained at high temperatures (Tribe and Bowler 1968) whilst acclimation to lowered temperatures tends to raise the oxygen consumption rates from those otherwise expected. Cold water species are generally as active as warmer water species at their naturally occurring temperatures (Sparck 1936, Berg 1953, Bullock 1955) even though such cold waters are lethal to tropical species (Scholander et al 1953). The arctic T. gouldi has been found to be able to tolerate temperatures of 1°C which are lethal to its warmer water relative T. flexuosa. It may be that the acclimation to lowered temperatures is important in this respect allowing an oxygen consumption in excess of that which would otherwise be possible.

Thyasira gouldi is an inhabitant of deep waters, areas where the sediment is soft mud rich in sulphides, areas thus of low oxygen content (Føyn 1969, Beyer 1970, Bagge 1969, Staaland 1972) indicating, as has

been found, that its oxygen consumption would be quite low. Comparison with other arctic bivalves is rather difficult as respiration rates are given as cc's per Kilo of flesh generally with no indication of size of animal used (Sparck 1936, Thorson 1936). Raising the respiration rate of T. gouldi to such limits exaggerates any errors in determination, however, a rate of about 10-15cc/Kilo would probably serve for comparison. Pecten groenlandicus has an oxygen consumption of about double that of T. gouldi (Thorson 1936), much of this difference probably being related to differences in mode of life (Sparck 1936). Comparison with the more sedentary bivalve Saxicava artica, (see Sparck 1936) shows quite close agreement with the rate obtained for T. gouldi, the values for both these species being near the lowest respiration rates for arctic bivalves (see Fig. 22 in Thorson 1936).

The opportunity arose recently to compare this data with that obtained by using an L.K.B. batch microcalorimeter. For six animals together this gave a direct estimate of 13 cal per sec at 20°C which is equivalent to 1.63μm of oxygen per animal per hour using the oxycalorific value of 3.34. Although this gives quite good comparison with the above data, the oxygen consumption rate is somewhat lower, probably due to the high temperature and difficulties of using six animals at the same time. Oxygen consumption rates for T. gouldi are however low, an ideal adaptation to habitat. No effect, due to decreased oxygen tension, was apparent even if the oxygen content of the water was in the region

of 1ml/litre. Molander (1928) and Allen (1958) concluded that *Thyasira* are found in areas where competition is not great, *T. gouldi*'s ability to live in areas of variable salinity and low oxygen tensions thus opens up large, relatively unpopulated, areas for it to colonize.

## V) Seasonal Cycles

## Introduction

Analysis of the size distribution of the population of *T. gouldi* within L. Etive, (see earlier) and of the rather sparse data of both *T. gouldi* and *T. flexuosa* from the L. Linnhe/Eil complex, has shown that small individuals can be found at all times of the year. This would suggest either that spawning occurs at any time of the year, or that the slow growth rate, (1mm per annum) coupled with a fairly long spawning period ensures that animals of less than 1mm are always present.

Three main methods were used to investigate the reproductive cycle of individuals within the population, and other changes associated with this cycle. These were:-

a) a study of the stage of gonadal development reached by individuals in samples of the population collected at regular intervals throughout the year, to ascertain the mean stage of gonad development and individual variation in gonadal development,

b) a study of the responses of groups of animals when retained in the laboratory at intervals through the year, to ascertain the spawning response, and

c) a study of the seasonal changes in body weight and of changes in the biochemical composition of the tissue, since these changes normally reflect changes associated with gonad development and with

the state of reserve materials within the body.

Method

For the study of the gonadal cycle, the stage of development of the gonad, which was readily visible amongst the dark brown digestive tubules was assessed in samples collected from between transects 4 and 5 (Fig. 9 ).

Animals were collected by means of a Van Veen grab, the sample being sieved on deck making use of a hopper such that the outflow of mud into the sieve could be controlled (Holme 1959). The material retained by the sieve was then sorted, the Thyasira picked out and placed into a thermos flask containing water of $25^{o}$/ooS. The salinity of the surface water at the head of L. Etive often approaches that of freshwater, consequently all stages of sieving and picking out were carried out as rapidly as possible, the Thyasira being washed in water of optimum salinity ($25^{o}$/ooS) before being transported back to the laboratory.

Seven stages in the development of the gonad (see Table 5) could be easily distinguished by eye, aided by low power magnification and/or smears where necessary. Stage O, (Fig. 31a ) had no gonad material visible at all, whilst stage 4 was nearly mature (Fig. 31b ). Stage 5 (Fig. 31c ) was the fully mature stage, thought to be almost ready to spawn. The spent stage (stage 6) differed from stage O in that the digestive tubules had a milky or opaque appearance,

Table 5

Description of gonad stages adopted

| Stage | |
|---|---|
| 0. | No gonad material present |
| 1. | Slight gonad material on underneath of digestive tubules |
| 2.<br>3. | Increasing amount of gonad material covering underneath inside of digestive area |
| 4. | Gonad material now seen on edge of digestive material but not on top of digestive tubules |
| 5. | White gonad material meeting over the top of digestive gland practically obscuting digestive gland. |
| 6. | Milky or opaque appearance to digestive gland indicating recent swelling due to gonad. |

Stages three to five inclusive were discernable into male and female gonads due to the granular appearance of the female gonad at this stage.

Figure 31

Diagrammatic representation of the gonad/digestive gland of *Thyasira gouldi* at various stages of gonad development

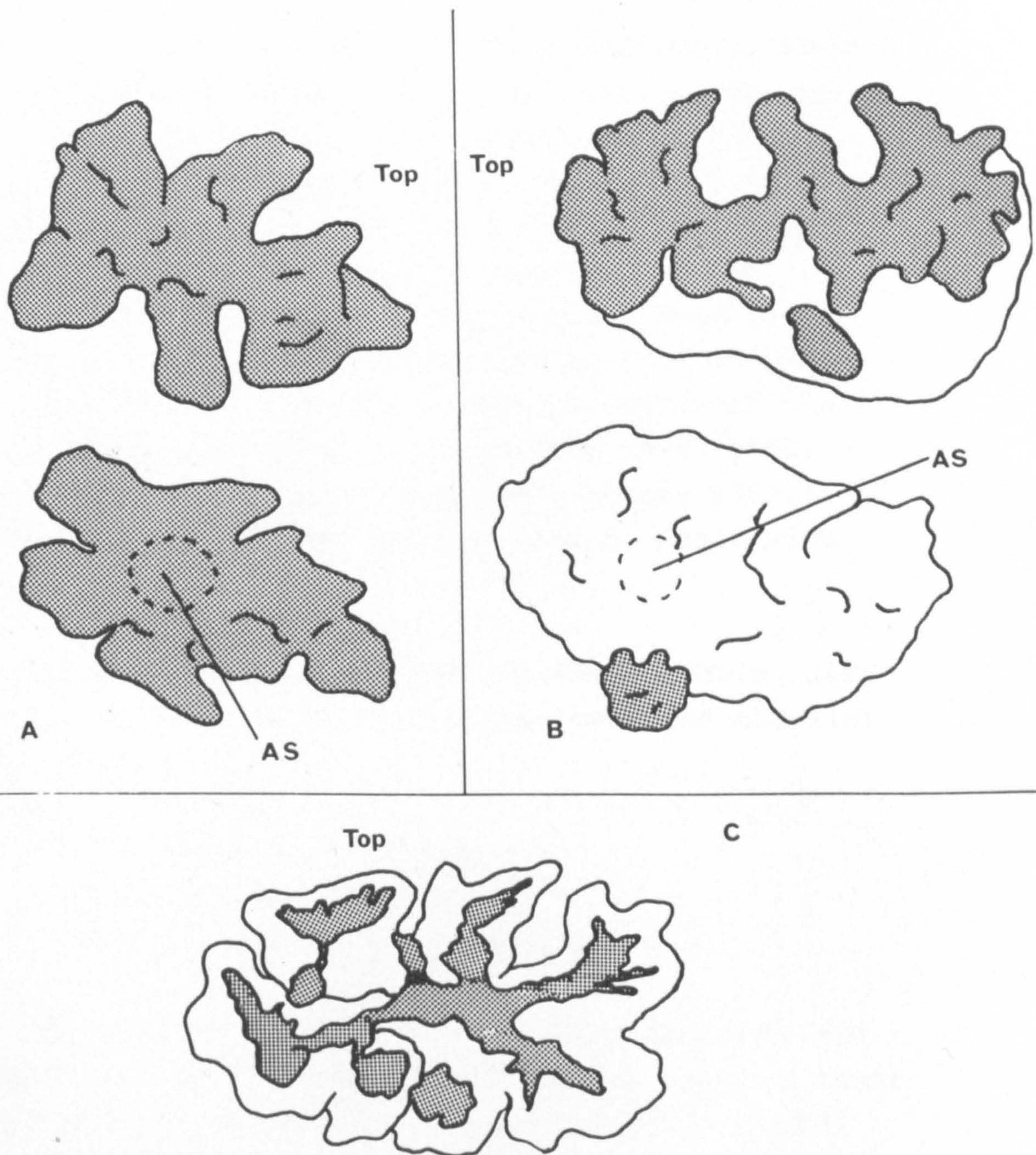
31a Stage 0

31b Stage 4

31c Stage 5

viewed from above and below - shaded area represents digestive gland, plain area represents overlying white gonadal tissue.

AS. Attachment to 'stomach'



Top
Top
AS
A
AS
B
Top
C

being the site of the recently ejected gonadial material.

From January 1971 samples were obtained at fortnightly intervals, taken back to the laboratory, the shell measured, opened, and the state of the gonad ascertained. T. gouldi is one of the species of Thyasira infected with the copepod parasite Axinophilus thyasirae, and the presence, or absence, of this parasite was noted. Data on parasitized animals were not included with the rest of the data in case of any effect by the parasite upon the gonad of the host. (Scott and Scott 1913, Caullery 1908, 1952, Brand 1952, Fretter and Graham 1962, Rogers 1962.) The data for parasitized animals will be dealt with later.

The animals were grouped into the following size classes, based on shell length (ie. measured parallel to the hinge line):-

i) Over 6mm in length
ii) 5-6mm in length
iii) less than 5mm in length.

The gonad state of at least fifty unparasitized animals was ascertained on any sampling data, at least twenty being taken from the two larger size groups, and at least ten from the smallest size group.

For the study of egg production samples of fifty to a hundred animals were collected at fortnightly intervals and allowed to bury in mud in the

aquarium. Often eggs were released just after the animals were placed into the aquarium. Although these may have been 'normal' releases from ripe females, they were ignored as they could have been the result of disturbance by collection. All other releases of eggs were, however, noted and the number of eggs released by each female counted. This was made possible as the dense eggs sink rapidly (see later), eggs from any one female being located within a centimetre of the inhalent aperture of the parent's burrow.

From an examination of sections and of smears of the gonads of T. gouldi it appeared that the female contained eggs at very different stages of development. As batches of eggs ripened they were released, if stimulated, while others continued their development. Attempts at inducing spawning by the use of chemicals and changes of temperature (Loosanoff and Davis 1963) proved unsuccessful, but the introduction of ripe sperm often worked. Even this, however, was haphazard in its results and as an idea of the number of eggs released in each spawning under natural conditions was required, it was thought desirable to employ a method involving no form of added inducement. However, males brought into the aquarium often spawned without inducement, resulting in a later 'natural' spawning by the females.

For the study of the seasonal variation in body weight and biochemical composition, samples of one

hundred to one hundred and fifty animals were collected at fortnightly intervals. These were placed in the aquarium (10°C) and left overnight. Three size groups were again selected (3-5mm, 5-6mm and greater than 6mm) covering the whole size range from the smallest that could be worked with to the largest found. An even distribution of animals were taken within each size range, twenty animals from each of the larger size ranges and ten to twenty animals from the smallest size range. The total weight, wet and dry tissue weights and the weight of the dried shell were determined as well as the stage of gonadal development. The presence or absence and gonad state of the parasite Axinophilus thyasirae (Bresciani and Ockelmann 1966) were also noted. The levels of lipid,carbohydrate and Nitrogen in the dry tissue were determined for the majority of the samples.

For the body weight determinations the total weight (ie. whole air dried animals) was first determined, and then the shell length was measured and the weight of the wet tissue as removed from the shell determined for each animal. The tissue was then rapidly frozen, placed into a freeze drier, dried and the dry tissue weight and the weight of the dry shell determined. In this determination, the wet tissue was first put into pre-weighed aluminium foil dishes and then weighed. The foil dishes and tissue were then placed into glass vials before being freeze dried for the dry weight determination. If this was not done the small foil dishes were easily lost or upset when the vacuum in the freeze drier was broken.

Samples taken directly from the freeze drier and weighed showed different results from those weighed later, due to the uptake of water. Determinations of the 'actual dry weights' are difficult and require extrapolation backwards of weight obtained at set time intervals to a zero time. All dry material was thus weighed after sitting for one hour on the bench after which time water uptake from the air is complete (see Appendix 1). The dry weights obtained were thus 'actual dry weight' plus the water taken up from the surrounding atmosphere (ie. approximately 4%). Some of the earlier samples were oven dried and although these results have been found to be comparable (see Appendix) certain differences do exist. The freeze-dried material takes up slightly more water than oven dried material. This is thought to be due to the difference in surface area and the number of air pockets resulting from the two drying methods. Certain differences were also present in weighing flesh at different temperatures. All weights of dried material are therefore taken after sufficient time has elapsed to allow the dry tissue to equilibrate to both vapour pressure and temperature of the surrounding atmosphere. Such weights obtained are designated for the purpose of this study as the 'dry' weight.

After weighing, the tissue was re-dried in the freeze drier and stored over dessicant in a deep freeze for subsequent analysis of the major biochemical components.

Samples of the dry tissue were taken from animals

grouped within each size class according to gonad state. Parasitized animals were grouped separately in case of any effect upon the biochemical composition due to the presence of the parasite, the results of these animals are given later.

The grouped material was then ground by hand in a pestle and mortar and subsamples taken for the quantitative determination of carbohydrate, lipid and nitrogen.

The method of Dubois et al (1956) was used for the determination of carbohydrate. This method relies upon the conversion of carbohydrate to furforal in the presence of phenol and sulphuric acid. The sulphuric acid has to be added as rapidly as possible ie. 0.5 sec., to supply the necessary heat of reaction and this rapid addition of acid to 'water' results in a degree of spluttering which necessitates the use of long tubes (Ehrlich 1972). The modification recommended by Barnes (pers. comm., see also Ehrlich 1972) in which a non-phenol blank is added to compensate for any colour due to charring by the sulphuric acid was also followed.

Reasonably high levels of carbohydrate were found within the tissue thus the amount of dry flesh necessary for a determination could be lowered from those used by Ehrlich (1972). The non-phenol blank was, however, still the limiting factor, but a level of 200mg of dry tissue could be easily used.

Glucose standards were taken through the entire reaction and a glucose calibration curve drawn for each set of determinations.

Flow chart for carbohydrate determination

| Phenol Blank | Test Sample | Standard |
|---|---|---|
| Weighed ground sample | Weighed ground sample | known quantity of glucose solution |
| 2ml $H_2O$ | 1ml $H_2O$ | 1ml $H_2O$ |
| | 1ml 2.5% Phenol | 1ml 2.5% Phenol |
| 5ml conc. $H_2SO_4$ | 5ml conc. $H_2SO_4$ | 5ml conc. $H_2SO_4$ |
| When cool read at 490um in 1cm cell | When cool read in 1cm cell at 490um | When cool read at 490um in 1cm cell |

Total lipid was estimated using total lipid test kit - Biochemica Test Combination - Boehringer Mannheim GMBH - which uses a phosphoric acie/vanillin reaction with lipid (Zollner and Kirsch 1962). The colour obtained by the reaction of sulphuric acid, phosphoric acid and vanillin upon the lipid was read at 520um on a Unicam S.P. 600 spectrophotometer and compared with the reaction of a known amount of lipid. Although primarily a method for the determination of total lipid of blood it is recommended by Barnes and Blackstock (1973) as a simple, sensitive, relatively stable

method giving results which compare closely with gravimetric analysis for a wide range of the tissues tested.

The test kit, however, uses Cholesterol as a reference lipid and whilst this is a major constituent of human blood lipid it is not the major constituent of molluscan lipid. This difference in blood constituents accounts for the differences in the results for the test kit and gravimetric determinations of lipid content of tissue of Mytilus edulis found by Barnes and Blackstock (1973). Following their recommendation a calibration curve was determined relating the lipid content, determined gravimetrically using the washing modification of Folch, Lees and Sloane-Stanley (1957), to analysis of the same extracted lipid using the test kit. This relationship was used to provide a conversion factor (Fig. 32a) wh ich was applied to all the lipid determinations obtained by use of the test kit. (The absorption maxima for Thyasira lipid and the test kit standard were previously found to be the same (Fig. 32b).)

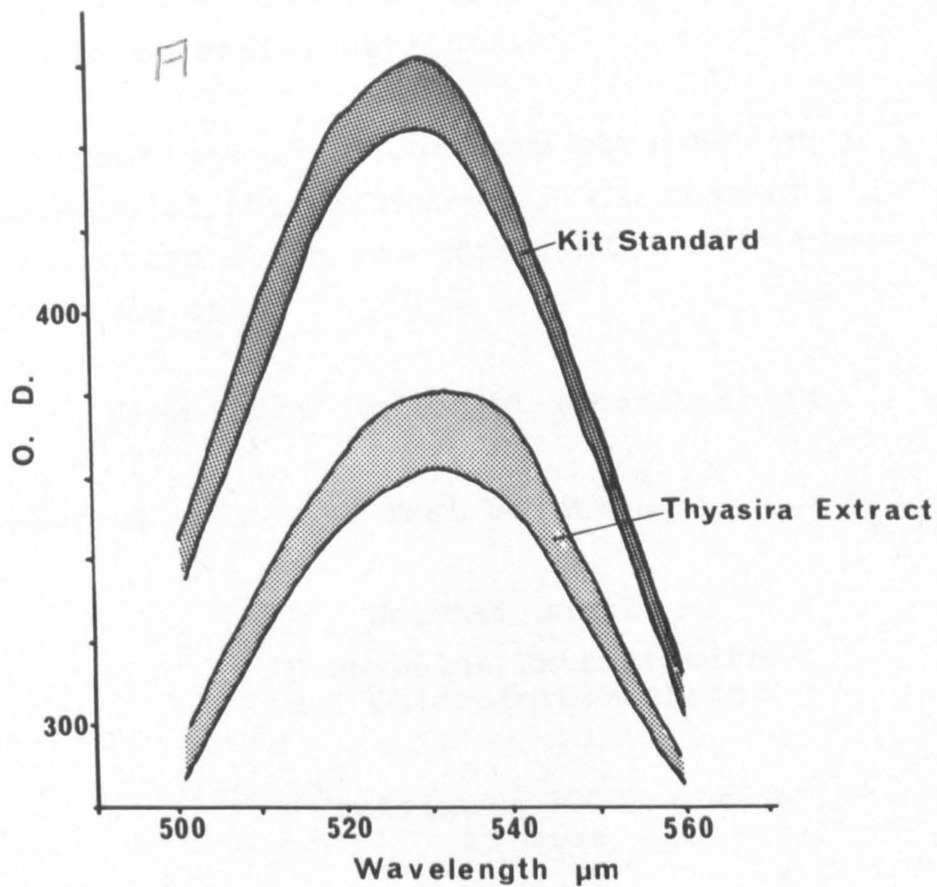
By using the system outlined in the flowchart below, levels of tissue as low as 300um could be used quite easily. For each set of determinations a blank was run - shown to the left in the flow chart. As the colour obtained was dependent upon the boiling time, standard solutions were also run for each determination. The standards were made up by dilutions of a stock solution which consisted of the supplied cholesterol solution diluted 9:1 as recommended by

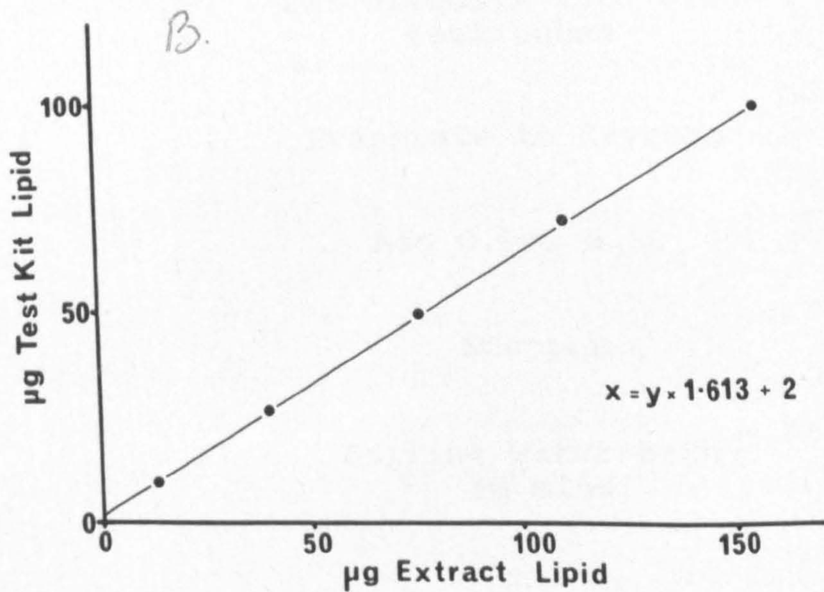
Figure 32

Relationship between test kit standard lipid and lipid extracted from *Thyasira gouldi*

Fig. 32A absorption spectra

Fig. 32B correlation of test kit lipid to *Thyasira gouldi* lipid

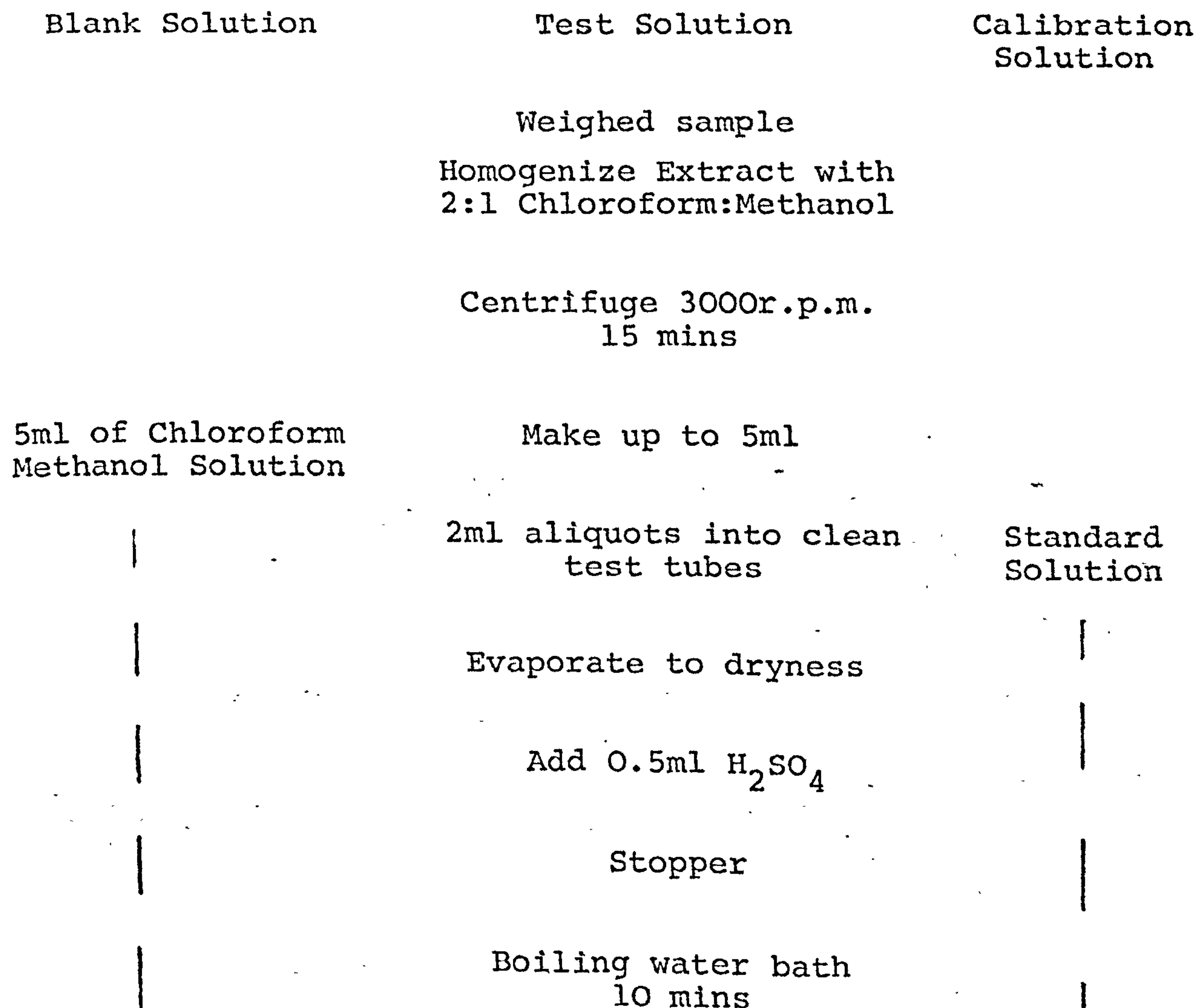

A
400
300
O. D.
Kit Standard
Thyasira Extract
500
520
540
560
Wavelength µm


B.
100
50
0
µg Test Kit Lipid
x = y × 1·613 + 2
0
50
100
150
µg Extract Lipid

Barnes and Blackstock (1973) so that lipid levels of 0-100um/ml could be easily obtained.

Slight variations of colour were obtained in different batches of the phospho-vanillin reagent, so a new calibration curve was constructed each time a new test kit was used.

Flow chart for lipid determination



Rapidly cool

0.2ml aliquot into clean
dry test tubes

Add 5ml phospho-vanillin reagent

Stand for 15-30 mins

Read at 520um in 1cm cell

Duplicates were initially taken through the entire process. However, in most cases no difference between spectrophotometer readings could be discerned. In others the total variation between duplicate samples was equivalent to less than 3% in lipid variation decreasing with the amount of lipid present. Running duplicates was therefore largely discontinued thus saving on the often rather sparse material, some duplicates were taken where material was plentiful as a check on the method.

Total nitrogen was estimated by the use of a Perkin Elmer Elemental analyser which also gave the carbon content. As found by Ehrlich (1972) and Mrs. L. Robb (pers. comm) the agreement shown by this machine was such that duplicates were not necessary, a few being taken as a check on the functioning of the machine.

All cleansing of glassware was carried out by boiling for ten minutes in a 2% solution of R.B.S. 25 (Chemical Concentrates Ltd.) in water in a stainless steel bucket, then rinsed repeatedly and oven dried. Distilled water was used throughout for both the detergent solutions and all rinsing water.

Results

a) Seasonal changes in gonad state

The percentage of animals in each gonadal stage for each size class at each sampling date is summarized in Figs. 33 a,b,c . There is no clear indication of a seasonal variation such as occurs in many bivalves in temperate waters (Quayle 1942, Loosanoff 1953, Mason 1958, Ansell 1962, 1967, Ropes 1968, Calabrese 1970, Hughes 1971 and the review by Allen 1963). It is apparent that even in the smallest size group there were a number of almost mature individuals. There was a peak in the number of early stages ( 1, 2, 3) in May/June (Fig. 33c ). This may reflect a peak of spawning activity at this time, but it was not reflected in the larger sizes. In these sizes (Fig.33 a,b) there was a reduction in the percentage of mature animals (stage 5) in the months of November and December. This reduction may be due to a decrease in the build up of gonad material or, more probably, to an increase in spawning, as this period was followed by an increase in the proportion of spent animals. In the larger size groups, ie. animals greater than 5mm in length (Fig.33a,b), a further peak of spawning was seen in the spring. In general, however, mature animals were present throughout the year. There was no indication of a restricted period of spawning, and little indication of any widespread synchrony of breeding activity of individuals within the population.

A summary of the size to gonad state relationship

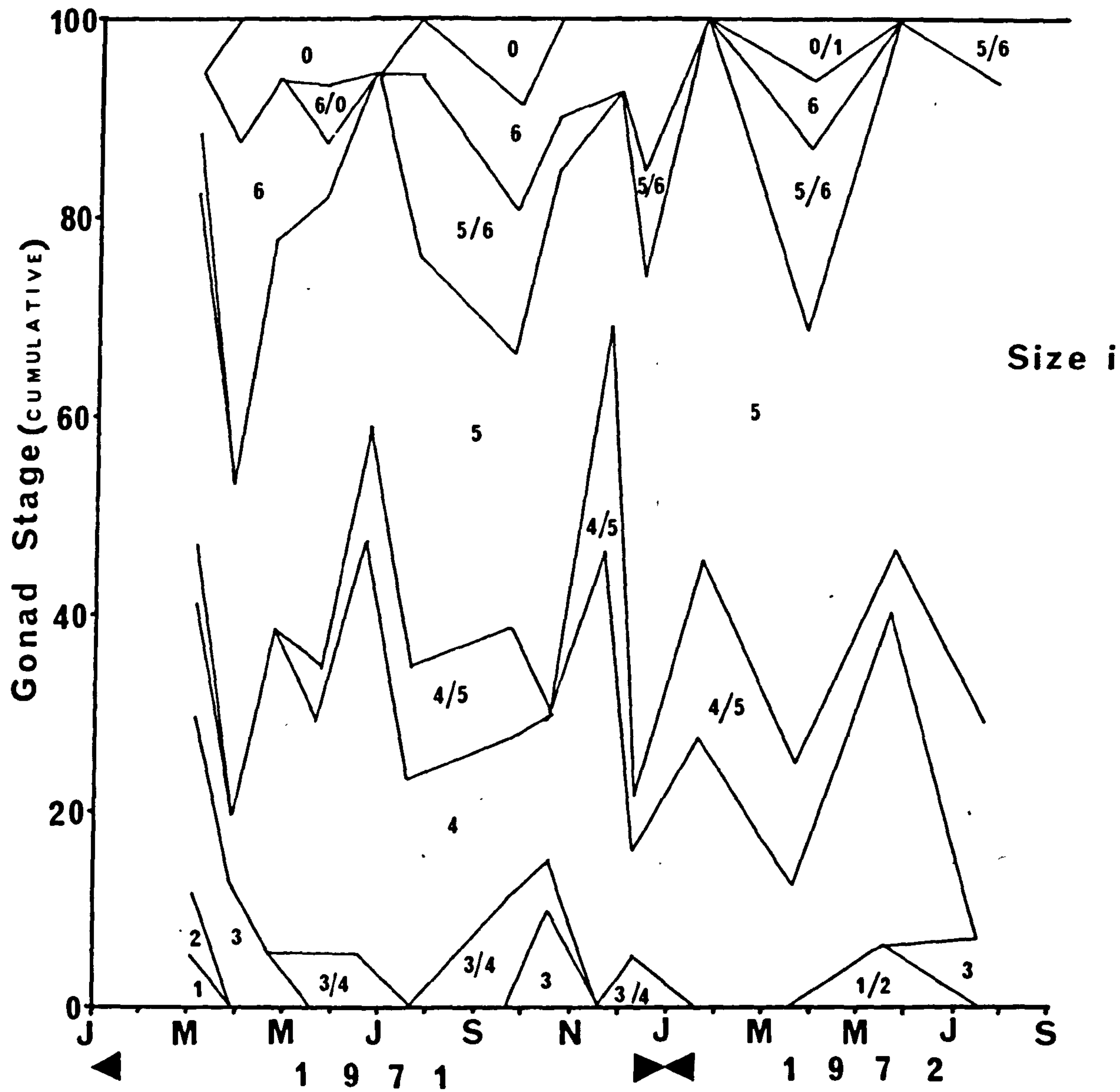
Figure 33

Variation in gonad stage throughout study period

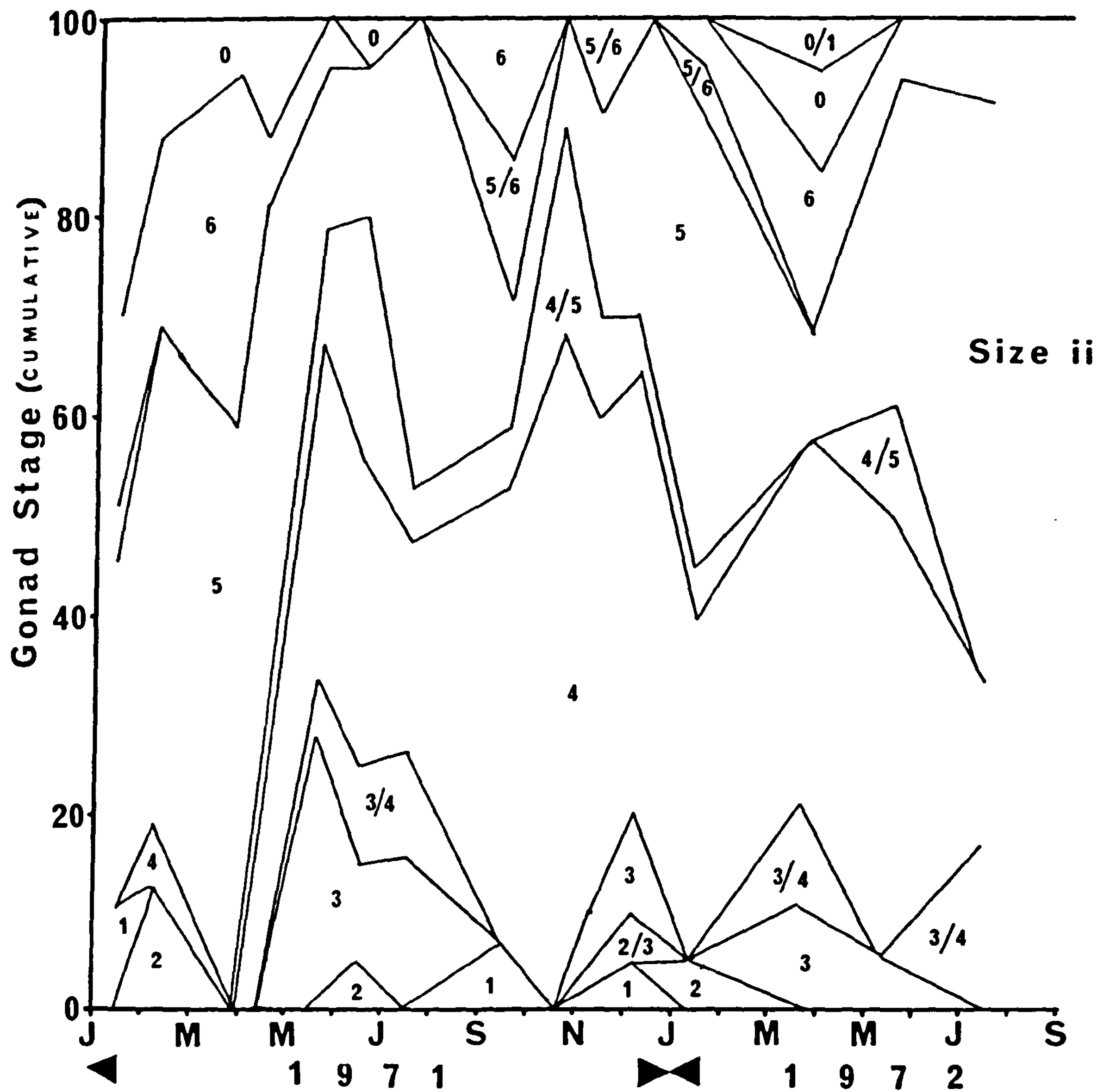
Fig. 33A Size (i) animals

Fig. 33B Size (ii) animals

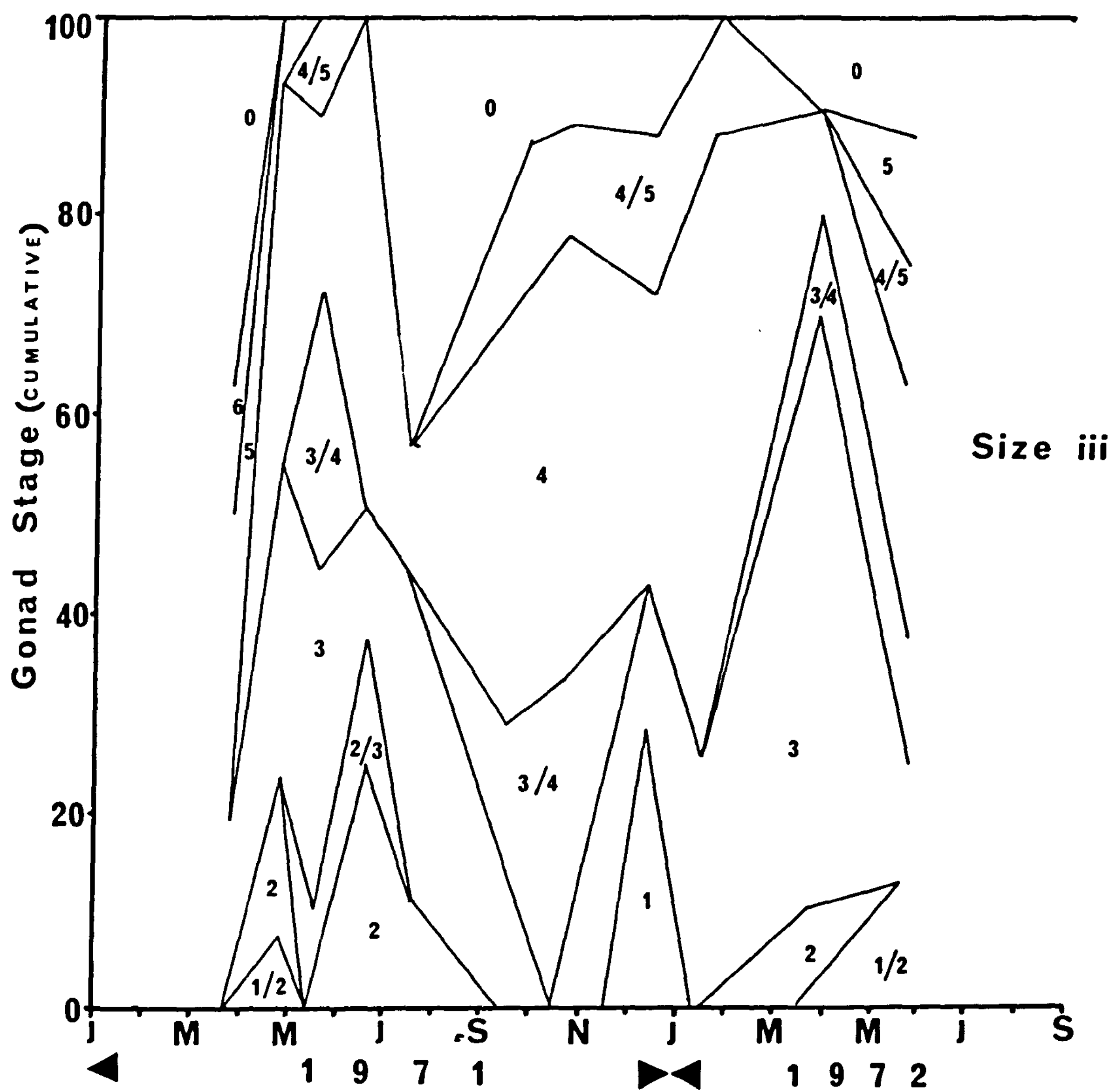
Fig. 33C Size (iii) animals


A.
Size i
Gonad Stage (CUMULATIVE)
100
80
60
40
20
0
0
0
0/1
5/6
6/0
6
6
6
5/6
5/6
5/6
5/6
5
5
4/5
4/5
4/5
4
2
3
1
3/4
3/4
3
3/4
1/2
3
J
M
M
J
S
N
J
M
M
J
S
1 9 7 1
1 9 7 2

β



C.



(Fig. 34 ), shows that the majority of these animals, below 3.5mm, were in the immature state. The first stage 4 individuals were found at a length of over 4.00mm, whilst the first fully mature animal (as designated by stage 5) was over 4.25mm. It would thus appear that maturity is not reached until the animal is at least three years old. These data are based on macroscopic examination and comparasion of the extent of the gonad with larger animals, but smear preparations did not significantly alter the picture.

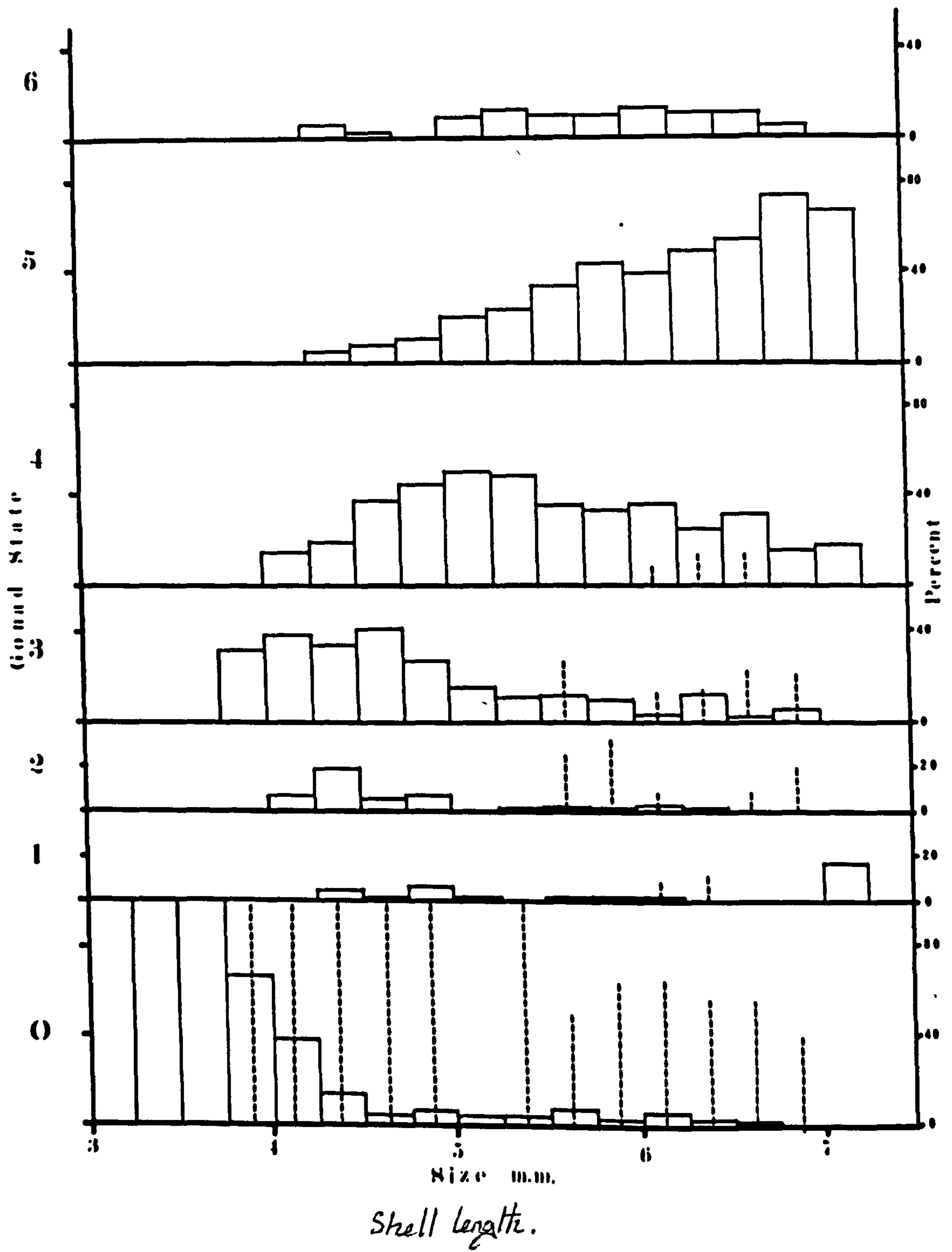
The percentage of stage 5 steadily increased with size so that there were very few large immature animals. This suggests that once maturity is reached not all the gametes are shed at any one time, but that small batches ripen and are released at intervals throughout the year. Sections made of various stages in the development of the gonad support this hypothesis, since there is a great variation in the state of development of the eggs (Fig. 35 ). It would appear that the spent stage occurs only rarely once maturity is reached. The release of small batches of gametes at intervals, probably results in a degree of interchange between stages 4 and 5 and these should in fact be linked as normal fluctuations of the adult state. (The large percentage of stage 1 in the 7mm+ size range is due to small sample size and is best ignored.)

Analysis of the adult population, ie. those over 5mm, showed that there were slightly more males than females (50%:40% (Fig. 36 ) ). However, a larger proportion of males (30%) than females (15%) were in

Figure 34

Size to gonad state relationship for
*Thyasira gouldi*

/ Indicates parasitised animals.




*Shell length.*

Figure 35

Section of the ovary of <u>Thyasira gouldi</u>

(Mature state)

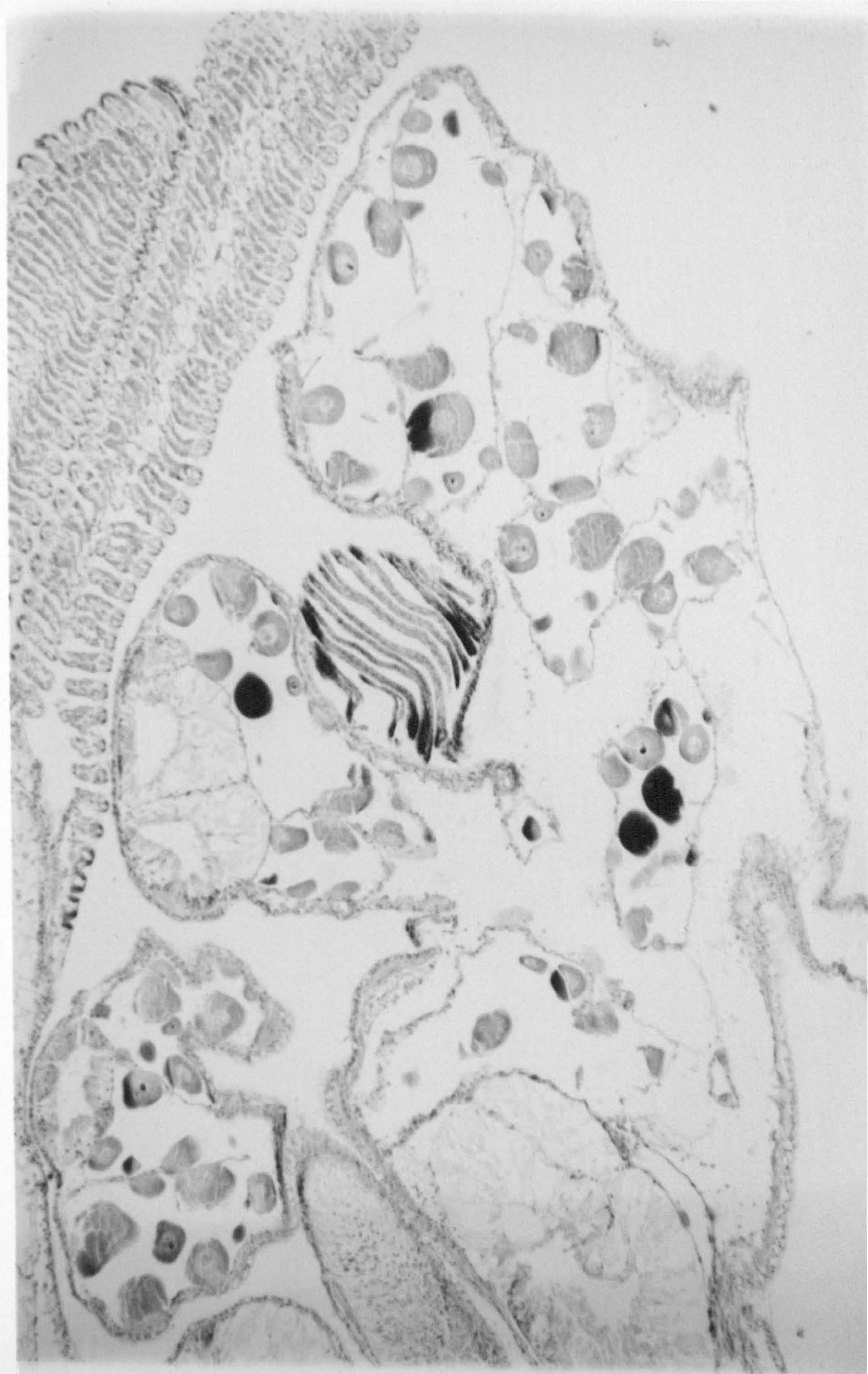
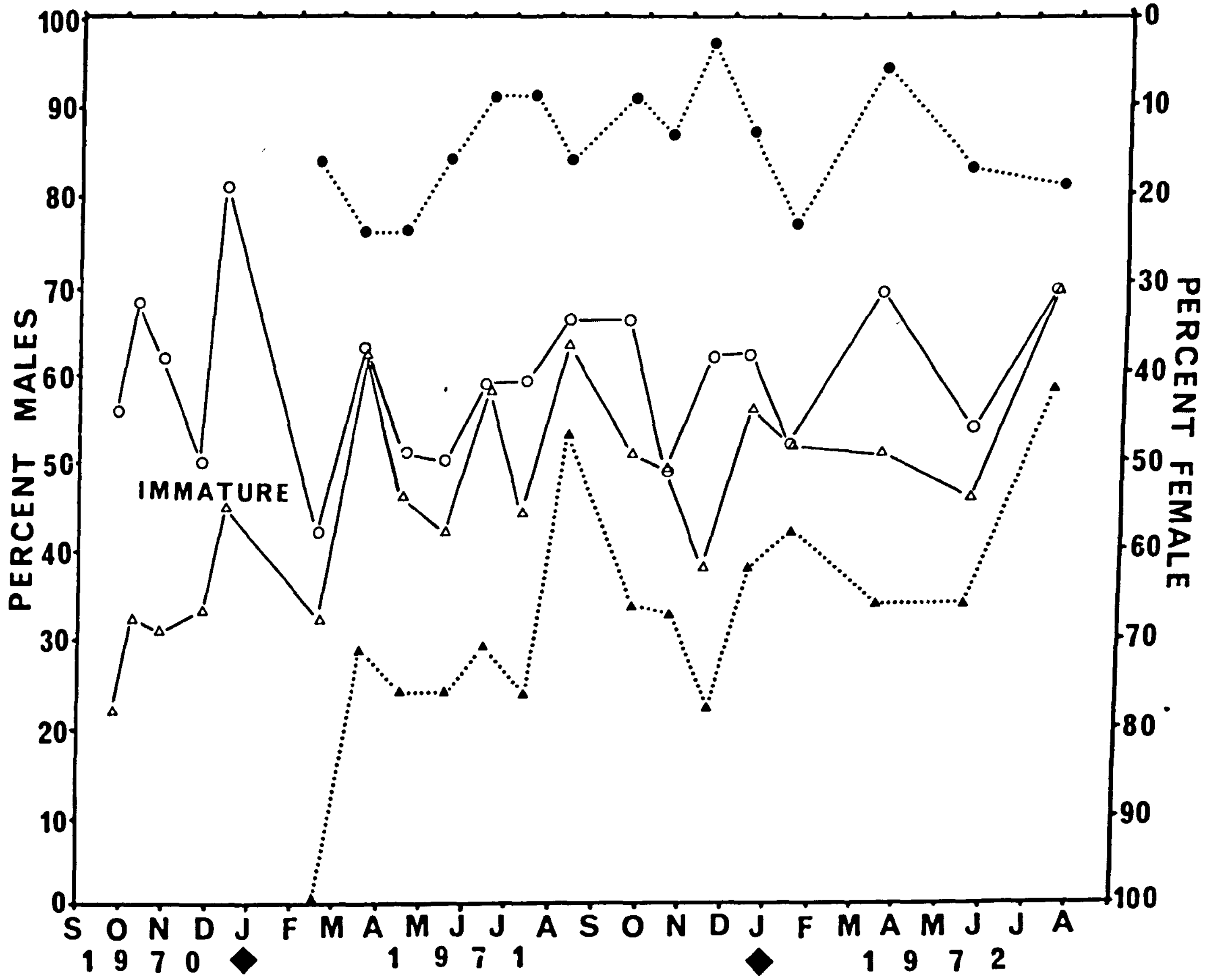


Figure 36

Relative proportion of males to females
within population of *Thyasira gouldi*

● % Mature ie. stage 5 ♀
○ Total % ♀
△ Total % ♂
▲ % Mature ie. stage 5 ♂

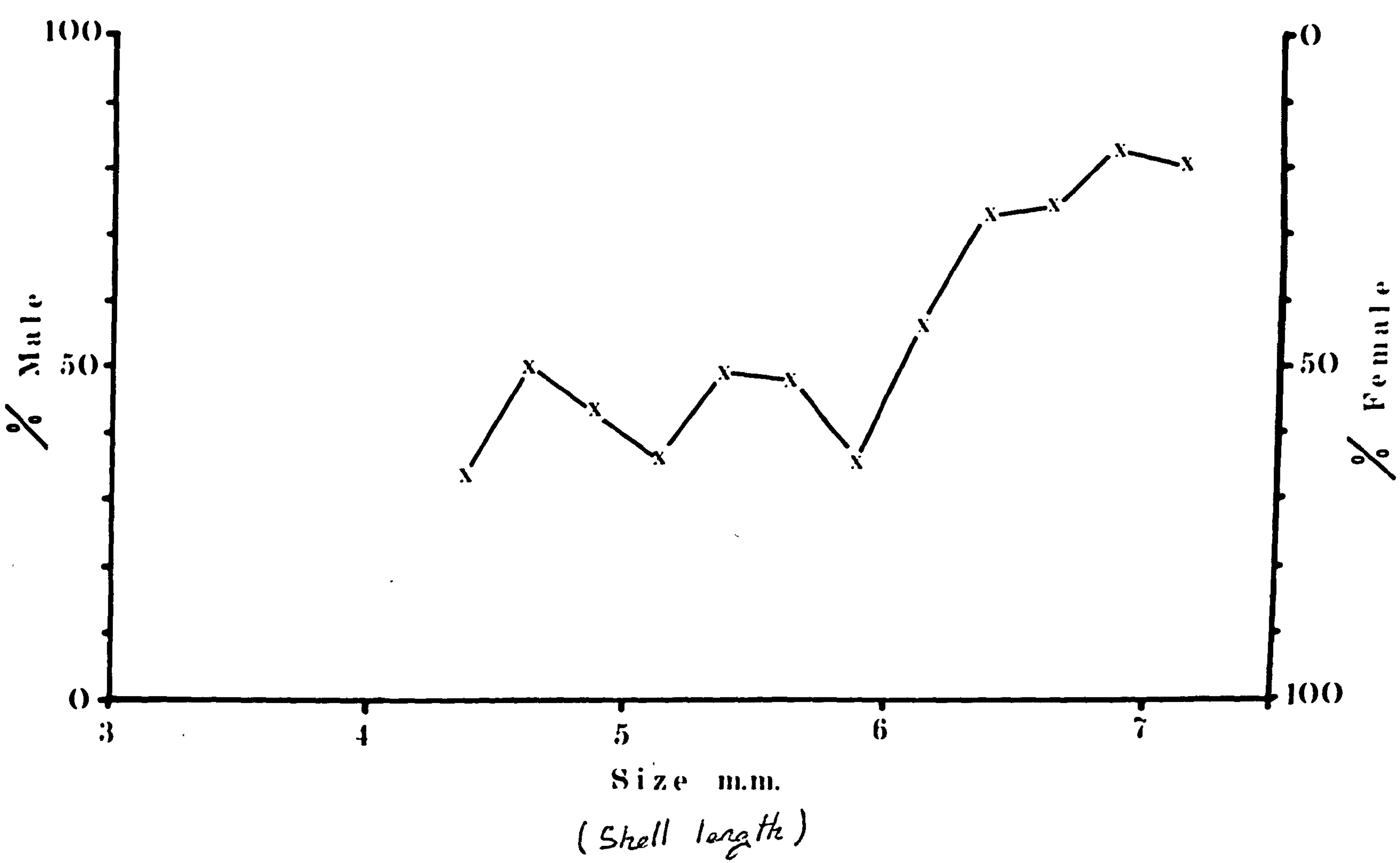

PERCENT MALES
PERCENT FEMALE
IMMATURE
100
90
80
70
60
50
40
30
20
10
0
S O N D J F M A M J J A S O N D J F M A M J J A
1970
1971
1972

the mature (stage 5) state. The males are thought to be capable of spawning at any time of the year, but are influenced when ripe by changes of salinity and temperature. Females, however, have only been induced to spawn viable eggs in the presence of sperm. Eggs released from buried animals if washed in filtered sea water immediately after their release all develop. It is thus probable that fertilization takes place before the eggs are ejected. This explains how fertilization occurs in eggs which rapidly settle (see later), and may also account for the need for more males than females within the population. The ratio of mature males to females may, however, be a result of gamete release, a release of eggs having a greater visual effect than the release of sperm, giving for the females, a fluctuation between stages 4 and 5.

If the data for all stage 4, 5 and 6 animals (approximately 400 animals) for the period January 1971 to July 1972 is combined (Fig. 37 ), it is evident that the ratio of males to females varies with size. In the smaller sizes this ratio was even, but above 6mm, the percentage of males increased, until, in the larger size range, there were four times as many males as there were females. This may explain, at least in part, why there are more mature males than females. It may be due to a higher mortality, or to decreased growth rate by the female, or a combination of these factors.

Figure 37

Variation of percentage Males:Females
with size for *Thyasira gouldi*


% Male
100
50
0
% Female
0
50
100
3
4
5
6
7
Size m.m.
(Shell length)

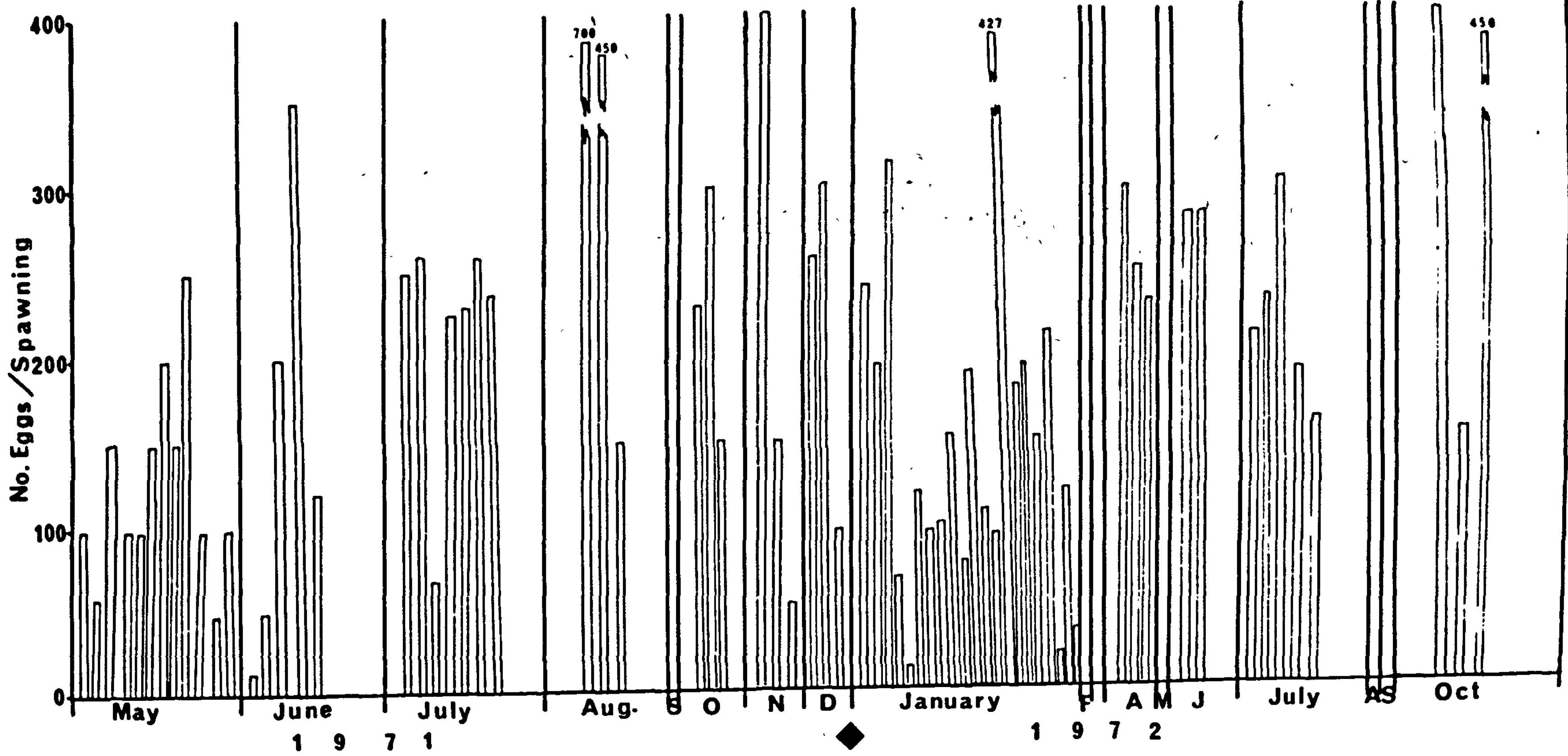
b) Seasonal changes in egg production

Releases of fertilized eggs were obtained from animals collected at fortnightly intervals and brought back to and kept in the aquarium, in all months from January 1971 through to October 1972. The occasional release of unfertilized eggs was thought to be the result of stress due to variation of salinity or temperature when the death point was approached (see later). Eggs released under these conditions soon fragmented and died, often in extreme cases the eggs were not fully formed and lacked a capsule. The number of eggs released in any spawning was variable, depending upon time of year or salinity or the period since the last spawning.

The number of spawnings obtained from each group of animals and the number of eggs spawned by each animal throughout the study period is summarized in Fig.38 . Earlier data indicated that two peaks of spawning activity may be present. The number of spawnings obtained in this analysis are not directly comparable as the number of mature females per sample may alter, nevertheless, the number of eggs released per spawning in the months of May, June and July was quite low. This may be a reflection of the gonad activity as a whole, if males are spawning fairly frequently sperm would be plentiful resulting in the stimulation of the female to release of eggs as they ripened. This frequent release of eggs resulted in low numbers of eggs per spawning. The largest number of eggs released in any one spawning (ie. 750)

Figure 38

Variation in number of spawnings and number of eggs per spawning obtained from May 1971 - October 1972.


No. Eggs / Spawning
0
100
200
300
400
700
450
427
450
May
June
July
Aug.
S
O
N
D
January
F
A
M
J
July
A
S
Oct
1 9 7 1
1 9 7 2

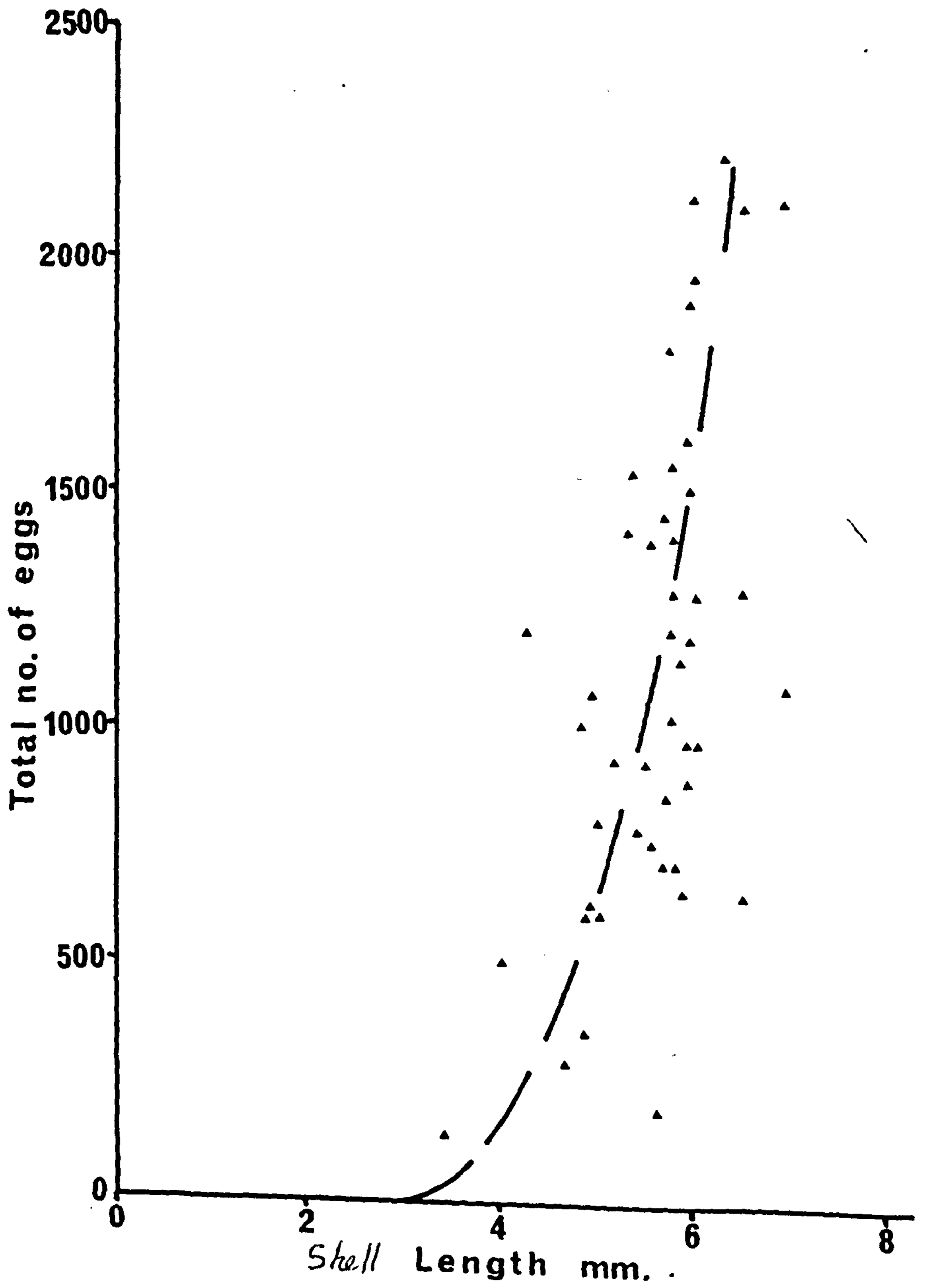
was seen in August. This increase in the number of eggs per spawning may reflect a decrease in male spawning activity, or, due to a lessening of water currents, an increase in the localization of any spawning activity allowing a build up of mature eggs within the females. No spawnings at all were seen in September, which thus may be a period of low natural spawning activity. Spawning activity is resumed in October/November, which may be a re-extension of the localized epidemic spawnings, induced by the turn over of water at this period, the associated currents enlarging the effect of any spawning activity.

The total number of eggs contained within stage 5 females, estimated from direct counts of eggs from dissected gonads, may exceed 2,000 (Fig.39 ). This is probably an underestimation as large numbers of small oogonia were present not all of which were counted. It shows that the number of eggs released at any one time is only a fraction of the total present since the maximum released by a female in any one spawning was only 750 (Fig.38 ).

Thus, in summarizing the data concerning gonad state, it would appear that animals of over 4mm in length are capable of attaining sexual maturity. Once maturity has been reached, gonadial material is released at intervals and only rarely does the individual regress to an immature state. Even though spawning is asynchronous, minor peaks of gonad activity are probably associated with the effect of variations of temperature, and/or salinity, upon the male which,

Figure 39

Relationship between size and
number of eggs contained within the gonad



Total no. of eggs
2500
2000
1500
1000
500
0
0
2
4
6
8
Shell Length mm.

in turn, by the release of sperm or some other agent causes the female to release eggs. Fertilization is probably within the hypobranchial ~~mantle~~ cavity, a process which can be envisaged as a form of energy conservation in that only ripe, fertilized eggs are released. The total number of males to females within the population tends to equality, but the number of mature females is less than the number of mature males. This may be due to the differing effects of release of eggs or sperm upon the visual appearance of the gonad, or to there being a greater percentage of males in the larger size ranges. Spawning by one male results in a localized 'epidemic' of spawning activity (Thorson 1946, Ansell 1959) amongst the surrounding males. This 'epidemic' effect, associated with the high percentage of males present, ensures that the level of sperm necessary for the release of eggs is obtained. The number of eggs released at any one time is dependent upon the time since the last spawning and the amount of sperm available for fertilization. Excessively frequent or heavy spawnings may occur for one reason or another, resulting in the spent state (stage 6) which is sometimes seen (Fig.33 ).

c) Seasonal variations in body weight and biochemical composition.

Seasonal variations in weight and changes in the biochemical composition of the flesh have been studied for several invertebrate phyla, (Atwater 1892, Greenfield et al 1958, Barnes et al 1963, Lovern 1964, Giese 1966a,

Boddington and Mettrick 1971, Lee and Chin 1971, Moss and Lawrence 1972) as well as in the mollusca (Nicol 1967, review of Giese 1969). Most of these concern synchronized populations in which the variations found are the result of spawning and other reproductive processes. Members of the population of Thyasira gouldi are not synchronized so that at any time adults in all stages of the reproductive cycle can be found (Fig. 33 a,b,c, ). In this species the mean population changes therefore would not reflect individual changes but by differentiating between the various gonad states, individual variations in dry weight and chemical composition of the flesh can be obtained for animals with or without gonad at all times of the year.

This method which is described in more detail later thus enables an indication of the individual's variation in dry weight and biochemical composition to be made in an asynchronous population, and this allows a more direct comparison with animals from populations in which these activities are synchronized.

As in all sampling for other than population and distributional studies, the T. gouldi for this study were taken from between transects 4 and 5 of the grid (Fig. 9 ).

Changes in weight

In analysing the weight data, the animals were grouped according to their respective gonad state.

For the estimation of the weight of a standard animal, all the data for each sampling date, with the exception of that for the parasitized animals, was pooled and regressions of the log weight on log of the shell length fitted. Analyses of covariance were used to compare these regressions, testing for homogeneity of variance and for differences in slope and intercept of the lines. From these, the data for individual dates can be pooled into groups of lines with the same slope, and within these groups, sub-groups can be made consisting of lines which do not differ significantly in their intercept. From these regressions the weight for standard length animals were obtained for each of the sampling dates.

By grouping all the animals for the whole sampling period which were of the same gonad state and carrying out the same analysis the weight equivalent to any particular gonad stage (gonad weight index) was obtained (Table 7 ). A gonad weight index could then be assigned to each sampling date and so variations in weight for any size of animals at any gonad state determined for any time of the year.

The treatment of all data was greatly aided by use of a Hewlett Packard 9100b desk-top computer, statistical methods being taken from Snedecor (1959).

The results of the analysis of co-variance of the total weight to length data is given in Fig.40a which gives the slope and intercept relationships. The variance was found to be homogenous for all the dates

Table 6

Codes of samples used in log weight on log length regressions

| Monthly samples | | Gonad influence | |
|---|---|---|---|
| Code No. | Sampling date | Code No. | Gonad stage |
| 1 | 11.1.71 | A | All parasatized animals |
| 2 | 3.2.71 | B | All stage 0, 1, 2 |
| 3 | 3.3.71 | C | All stage 5 males |
| 4 | 31.3.71 | D | All stage 5 females |
| 5 | 27.4.71 | E | All stage 0 |
| 6 | 25.5.71 | F | All stage 1 |
| 7 | 21.6.71 | G | All stage 2 |
| 8 | 21.7.71 | H | All parasitized, parasites with eggs |
| 9 | 22.9.71 | I | All parasitized, parasites with nauplii |
| 10 | 19.10.71 | J | All parasitized, parasites immature |
| 11 | 9.11.71 | K | All parasitized |
| 12 | 8.12.71 | L | All stage 3 |
| 13 | 11.1.72 | M | All stage 4 females |
| 14 | 21.3.72 | N | All stage 4 males |
| 15 | 23.5.72 | O | All stage 6 |
| 16 | 17.7.72 | | |

Table 7

Gonad Weight Index for various sampling dates (merely to obtain gonad weight index)

No. at each gonad stage

| Sample Code No. (Table 6) | Size Range (Page 72) | 1 | 1/2 | 2 | 2/3 | 3 | 3/4 | 4 | 4/5 | 5 | 5/6 | 6 | 6/0 | 0 | 0/1 | 6/1 | Total No. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | (ii) | 2 | | | | | | | | 7 | 1 | 4 | | 6 | | | 20 |
| 2 | (ii) | | | 2 | | | | 1 | | 8 | | 3 | | 2 | | | 16 |
| 3 | (i) | 1 | | 1 | | 3 | | 2 | 1 | 6 | 1 | 1 | | | 1 | | 17 |
| 4 | (i) | | | | | 2 | | | | 5 | | 6 | | 2 | | | 15 |
| | (ii) | | | | | | | | | 10 | | 6 | | 1 | | | 17 |
| | (iii) | | | | | 3 | | | | 5 | | 2 | | 6 | | | 16 |
| 5 | (i) | | | | | 1 | | 6 | | 7 | | 3 | | 1 | | | 18 |
| | (ii) | | | | | | | 3 | 1 | 9 | | 1 | | 2 | | | 16 |
| | (iii) | | 1 | 2 | | 4 | | 5 | | 1 | | | | | | | 13 |
| 6 | (i) | | | | | | 1 | 4 | 1 | 8 | | 1 | 1 | 1 | | | 17 |
| | (ii) | | | | | 5 | 1 | 6 | 2 | 3 | | 1 | | | | | 18 |
| | (iii) | | | | 1 | 3 | 2 | 2 | 1 | | | | | | | | 9 |
| 7 | (i) | | | | | | 1 | 7 | 2 | 6 | | | | 1 | | | 17 |
| | (ii) | | | 1 | | 2 | 2 | 6 | 5 | 3 | | | | 1 | | | 20 |
| | (iii) | | | 2 | 1 | 1 | | 4 | | | | | | | | | 8 |
| 8 | (i) | | | | | | | 4 | 2 | 7 | 3 | 1 | | | | | 17 |
| | (ii) | | | | | 3 | 2 | 4 | 1 | 9 | | | | | | | 19 |
| | (iii) | | | 1 | | 3 | | 1 | | | | | | 4 | | | 9 |
| 9 | (i) | | | | | | 2 | 3 | 2 | 5 | 3 | 2 | | 1 | | | 18 |
| | (ii) | | 1 | | | | | 7 | 1 | 2 | 2 | 2 | | | | | 15 |
| | (iii) | | | | | 1 | 1 | 3 | 1 | | | | | | 1 | | 7 |
| 10 | (i) | | | | | 2 | 1 | 3 | | 11 | 1 | 2 | | | | | 20 |
| | (ii) | | | | | | | 13 | 4 | 2 | | | | | | | 19 |
| | (iii) | | | | | | 3 | 4 | 1 | | | | | 1 | | | 9 |
| 11 | (i) | | | | | | | 6 | 3 | 3 | | 1 | | | | | 13 |
| | (ii) | | | | | 1 | | 5 | 1 | 2 | 1 | | | | | | 10 |
| 12 | (i) | | | | | | 1 | 2 | 1 | 10 | 2 | 3 | | | | | 19 |
| | (ii) | 1 | | | 1 | 2 | | 9 | 1 | 6 | | | | | | | 20 |
| | (iii) | 2 | | | | 1 | | 2 | 1 | | | | | 1 | | | 7 |
| 13 | (i) | | | | | | | 3 | 2 | 6 | | | | | | | 11 |
| | (ii) | | | 1 | | | | 7 | 1 | 9 | 1 | 1 | | | | | 20 |
| | (iii) | | | | | 2 | | 5 | 1 | | | | | | | | 8 |
| 14 | (i) | | | | | | | 2 | 2 | 7 | 3 | 1 | | | | 1 | 16 |
| | (ii) | | | | | 2 | 2 | 7 | | 2 | | 3 | | 2 | | 1 | 19 |
| | (iii) | | | | 1 | 6 | 1 | 1 | | | | | | 1 | | | 10 |
| 15 | (i) | | 1 | | | | | 5 | 1 | 8 | | | | | | | 15 |
| | (ii) | | | | | 1 | | 8 | 2 | 6 | | 1 | | | | | 18 |
| | (iii) | | 1 | | | 1 | 1 | 2 | 1 | 1 | | | | 1 | | | 8 |
| 16 | (i) | | | | | 1 | | | 3 | 9 | 1 | | | | | | 14 |
| | (ii) | | | | | | 2 | 2 | | 7 | | 1 | | | | | 12 |

Percentage at each gonad stage

| Sample Code No. (Table 6) | Size Range (Page 72) | 1 | 1/2 | 2 | 2/3 | 3 | 3/4 | 4 | 4/5 | 5 | 5/6 | 6 | 6/0 | 0 | 0/1 | 6/1 | Gonad weight index (values from Table 10) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | (ii) | 10 | | | | | | | | 35 | 5 | 20 | | 30 | | | 0.6356 |
| 2 | (ii) | | | 12.5 | | | | 6.3 | | 50 | | 18.8 | | 12.5 | | | 0.6476 |
| 3 | (i) | 5.9 | | 5.9 | | 17.7 | | 11.8 | 5.9 | 35.3 | 5.9 | 5.9 | | | 5.9 | | 0.8410 |
| 4 | (i) | | | | | 13.3 | | | | 33.3 | | 40 | | 13.3 | | | 0.8435 |
| | (ii) | | | | | | | | | 58.8 | | 35.3 | | 5.9 | | | 0.6628 |
| | (iii) | | | | | 18.8 | | | | 31.3 | | 12.5 | | 37.5 | | | 0.3896 |
| 5 | (i) | | | | | 5.6 | | 33.3 | | 38.9 | | 16.7 | | 5.6 | | | 0.8531 |
| | (ii) | | | | | | | 18.8 | 6.3 | 56.3 | | 6.3 | | 12.5 | | | 0.6607 |
| | (iii) | | 7.7 | 15.4 | | 30.8 | | 38.5 | | 7.7 | | | | | | | 0.3776 |
| 6 | (i) | | | | | | 5.9 | 23.5 | 5.9 | 47.1 | | 5.9 | 5.9 | 5.9 | | | 0.8574 |
| | (ii) | | | | | 27.8 | 5.6 | 33.3 | 11.1 | 16.7 | | 5.5 | | | | | 0.6366 |
| | (iii) | | | | 11.1 | 33.3 | 22.3 | 22.3 | 11.1 | | | | | | | | 0.3823 |
| 7 | (i) | | | | | | 5.9 | 41.8 | 11.8 | 35.3 | | | | 5.9 | | | 0.8554 |
| | (ii) | | | 5 | | 10 | 10 | 30 | 25 | 15 | | | | 5 | | | 0.6423 |
| | (iii) | | | 25 | 12.5 | 12.5 | | 50 | | | | | | | | | 0.3728 |
| 8 | (i) | | | | | | | 23.5 | 11.8 | 41.2 | 17.6 | 5.9 | | | | | 0.8650 |
| | (ii) | | | | | 15.8 | 10.5 | 21.1 | 5.3 | 47.4 | | | | | | | 0.6556 |
| | (iii) | | | 11.1 | | 33.3 | | 11.1 | | | | | | 44.4 | | | 0.3605 |
| 9 | (i) | | | | | | 11.1 | 16.7 | 11.1 | 27.8 | 16.7 | 11.1 | | 5.6 | | | 0.8542 |
| | (ii) | | 6.7 | | | | | 46.7 | 6.7 | 13.3 | 13.3 | 13.3 | | | | | 0.6464 |
| | (iii) | | | | | 14.3 | 14.3 | 42.9 | 14.3 | | | | | | 14.3 | | 0.3884 |
| 10 | (i) | | | | | 10 | 5 | 15 | | 55 | 5 | 10 | | | | | 0.8614 |
| | (ii) | | | | | | | 68.4 | 21.1 | 10.5 | | | | | | | 0.6507 |
| | (iii) | | | | | | 33.3 | 44.4 | 11.1 | | | | | 11.1 | | | 0.3925 |
| 11 | (i) | | | | | | | 46.2 | 23.1 | 23.1 | | 7.7 | | | | | 0.8562 |
| | (ii) | | | | | 10 | | 50 | 10 | 20 | 10 | | | | | | 0.6508 |
| 12 | (i) | | | | | | 5.3 | 10.5 | 5.3 | 52.6 | 10.5 | 15.8 | | | | | 0.8658 |
| | (ii) | 5 | | | 5 | 10 | | 45 | 5 | 30 | | | | | | | 0.6459 |
| | (iii) | 28.6 | | | | 14.3 | | 28.6 | 14.3 | | | | | 14.3 | | | 0.3724 |
| 13 | (i) | | | | | | | 27.3 | 18.2 | 54.6 | | | | | | | 0.8687 |
| | (ii) | | | 5 | | | | 35 | 5 | 45 | 5 | 5 | | | | | 0.6592 |
| | (iii) | | | | | 25 | | 62.5 | 12.5 | | | | | | | | 0.3996 |
| 14 | (i) | | | | | | | 12.5 | 12.5 | 43.8 | 18.8 | 6.3 | | | | | 0.8641 |
| | (ii) | | | | | 10.5 | 10.5 | 36.8 | | 10.5 | | 15.8 | | 10.5 | | 5.3 | 0.6333 |
| | (iii) | | | | 10 | 60 | 10 | 10 | | | | | | 10 | | | 0.3685 |
| 15 | (i) | | 16.7 | | | | | 33.3 | 6.7 | 53.3 | | | | | | | 0.8611 |
| | (ii) | | | | | 5.6 | 44.4 | 11.1 | 33.3 | | 5.6 | | | | | | 0.6561 |
| | (iii) | | | | | 12.5 | 12.5 | 25 | 12.5 | 12.5 | | | | 12.5 | | | 0.3879 |
| 16 | (i) | | | | | 7.1 | | | 21.4 | 64.3 | 7.1 | | | | | | 0.8708 |
| | (ii) | | | | | | 16.7 | 16.7 | | 58.3 | | 8.3 | | | | | 0.6633 |

Figure 40

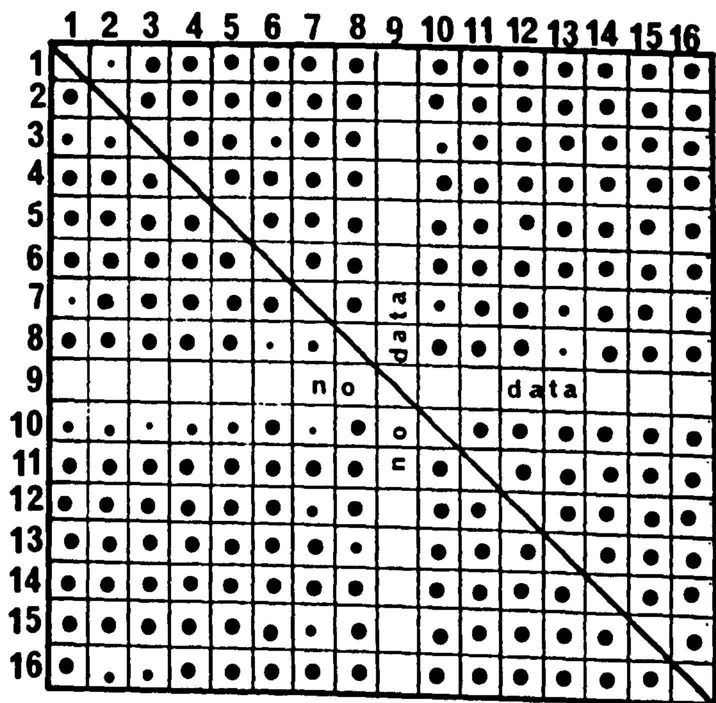
Relationships between regression lines from analysis of covariance

40a) log total weight on log length

40b) log shell weight on log length

For code numbers of lines see Table VI

Total Wt./Length




Slope

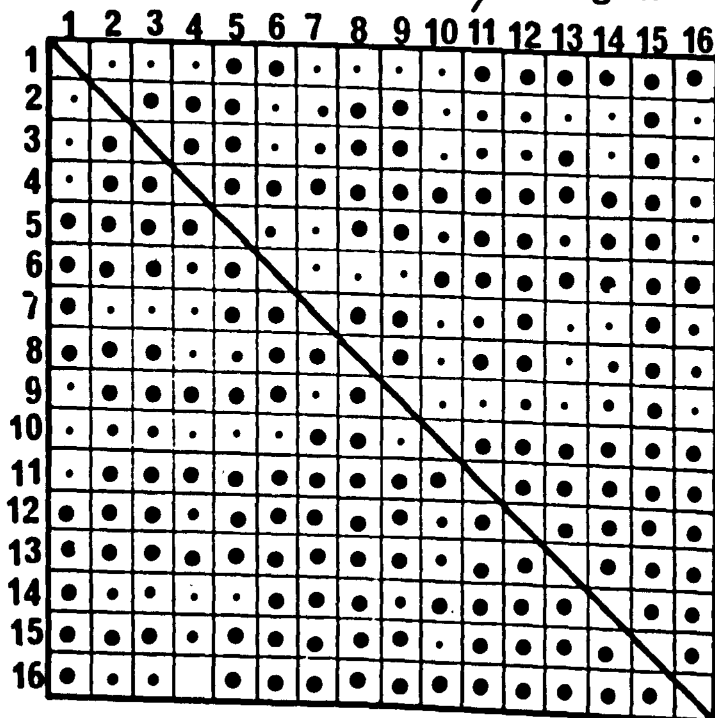
Position

Groups
1; 2
3
4,5,6
7
8
10
11, 12, 13, 14, 15, 16

• No difference

• Significant difference

Shell Wt./Length




Slope

Position

Groups
1
2,3,9
14; 7
6; 8; 12,15
4
10
13, 11, 16
5

Figure 41

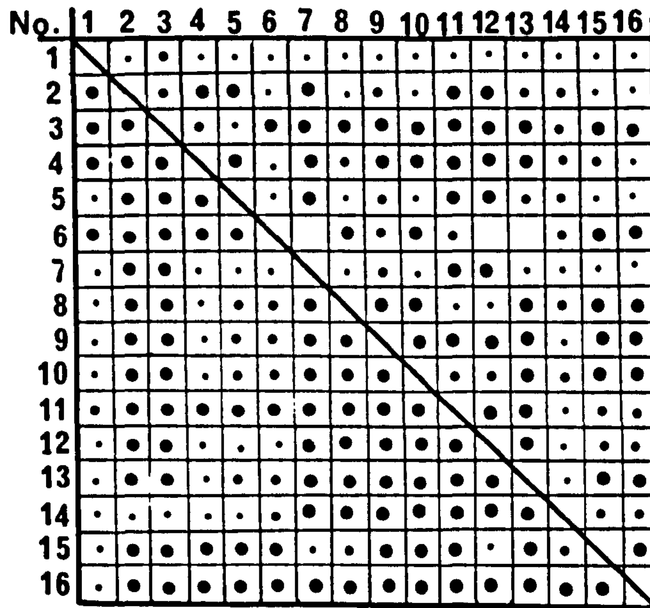
Relationship of regression lines from analysis of covariance

41a) log Dry Tissue weight/log length

41b) log Dry Tissue weight for animals of various gonad state on log length

For code numbers of lines see Table VI

Dry Wt./Length




Slope

Groups

1

2;3

4,5;6

9,12,14;7 ;8;10;13;

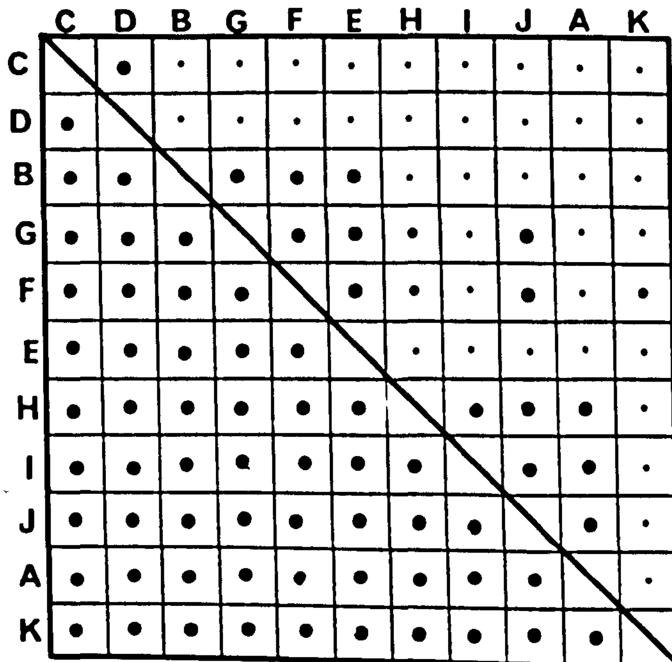
11,16;

15;

• No difference

•

• Significant difference




Position

Slope

Groups CD; BE; GF; AHI; J; K;

tested. The data from the sixteen sampling dates combine to form nine lines which differ from each other according to slope and/or position. There is little difference between the total weights for each of the sampling dates, at least as regards the larger animals (Fig 42b ) any changes in flesh content being taken up by a roughly equal change in water content. In the smallest size class larger differences are apparent, but these are mainly attributable to variations in shape of the shell resulting in slight variations in volume. On the whole total weight is quite uniform with no strong seasonal sequence in variation. However, there may be some change in early summer (ie. April, May, June) which is particularly reflected in the smallest size classes.

The results of the analysis of covariance of the data for shell weight are given in Fig. 40b . Variance was again found to be homogenous. Many of the regression lines for shell weight appear to pivot about the centres of the size ranges used. Much of this variation is the result of difficulties in extracting tissue from very small and often fragile shells, coupled with the fact that slight differences in shape and/or shell thickness will lead to relatively large variations in weight of these small shells. In general there is little change in shell weight for the standard length animal (Fig. 42b ) although there may be some deposition of shell material in the early winter months (ie. October through to January), perhaps connected with the laying down of growth rings.

Figure 42

log weight for animals of standard shell length from October 1970 to August 1972

42a) log total weight

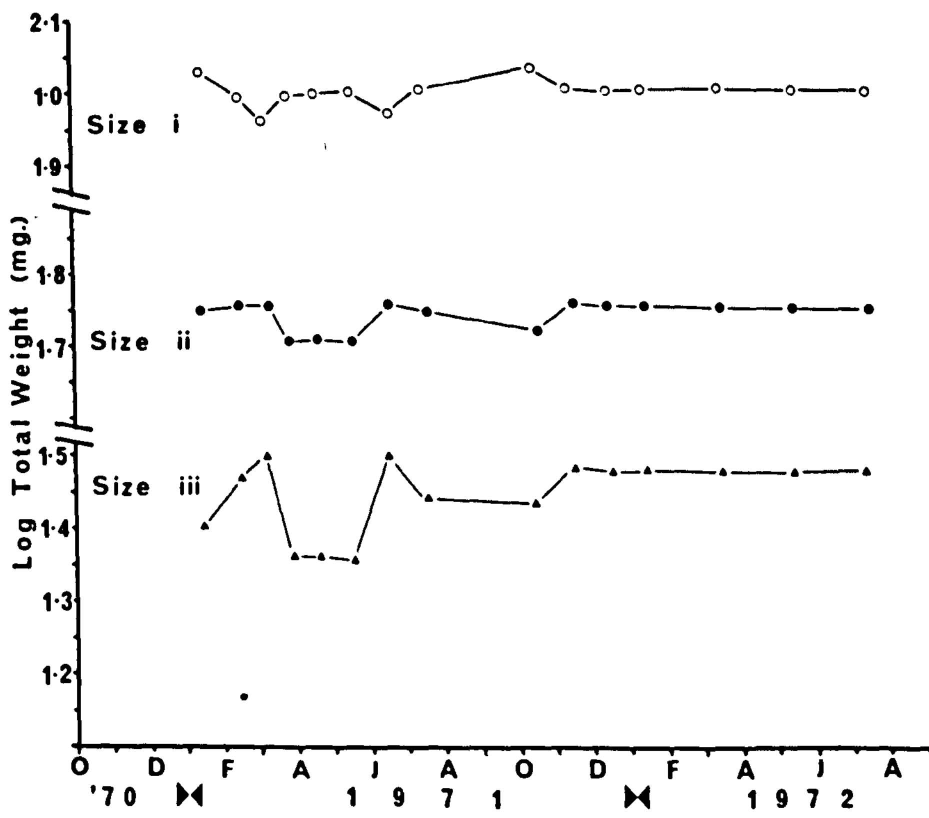
42b) log shell weight

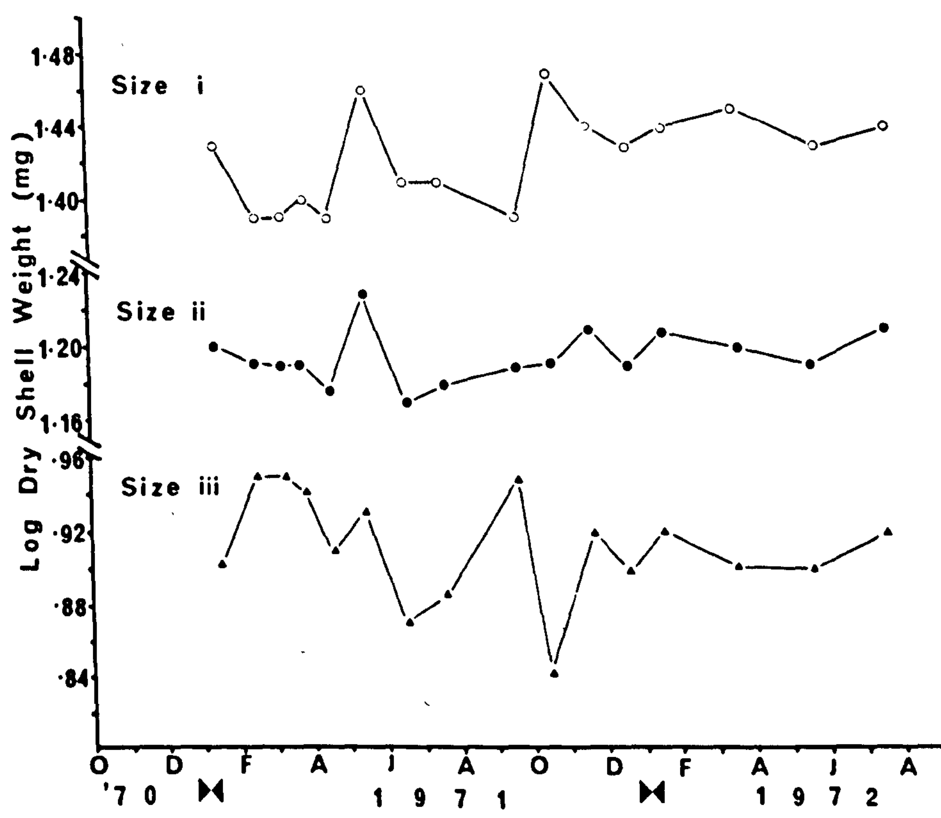
for animals of standard shell lengths

i) over 6 mm.

ii) 5 to 6 mm.

iii) less than 5 mm.


A.
Size i
Size ii
Size iii
Log Total Weight (mg.)
2·1
1·0
1·9
1·8
1·7
1·5
1·4
1·3
1·2
O D F A J A O D F A J A
'70 1 9 7 1 1 9 7 2


B.
Size i
Size ii
Size iii
Log Dry Shell Weight (mg)
1·48
1·44
1·40
1·24
1·20
1·16
·96
·92
·88
·84
O D F A J A O D F A J A
'70 1 9 7 1 1 9 7 2

The results of the analysis of covariance of the data for dry tissue weight are given by Fig. 41a . Again variance was found to be homogenous. Significant differences in slope divide the data into six groups each with the same slope (Fig. 41a ). These groups contain in all twelve sub-groups due to position differences, thus twelve lines can be used to give the length to dry weight relationships for the period studied.

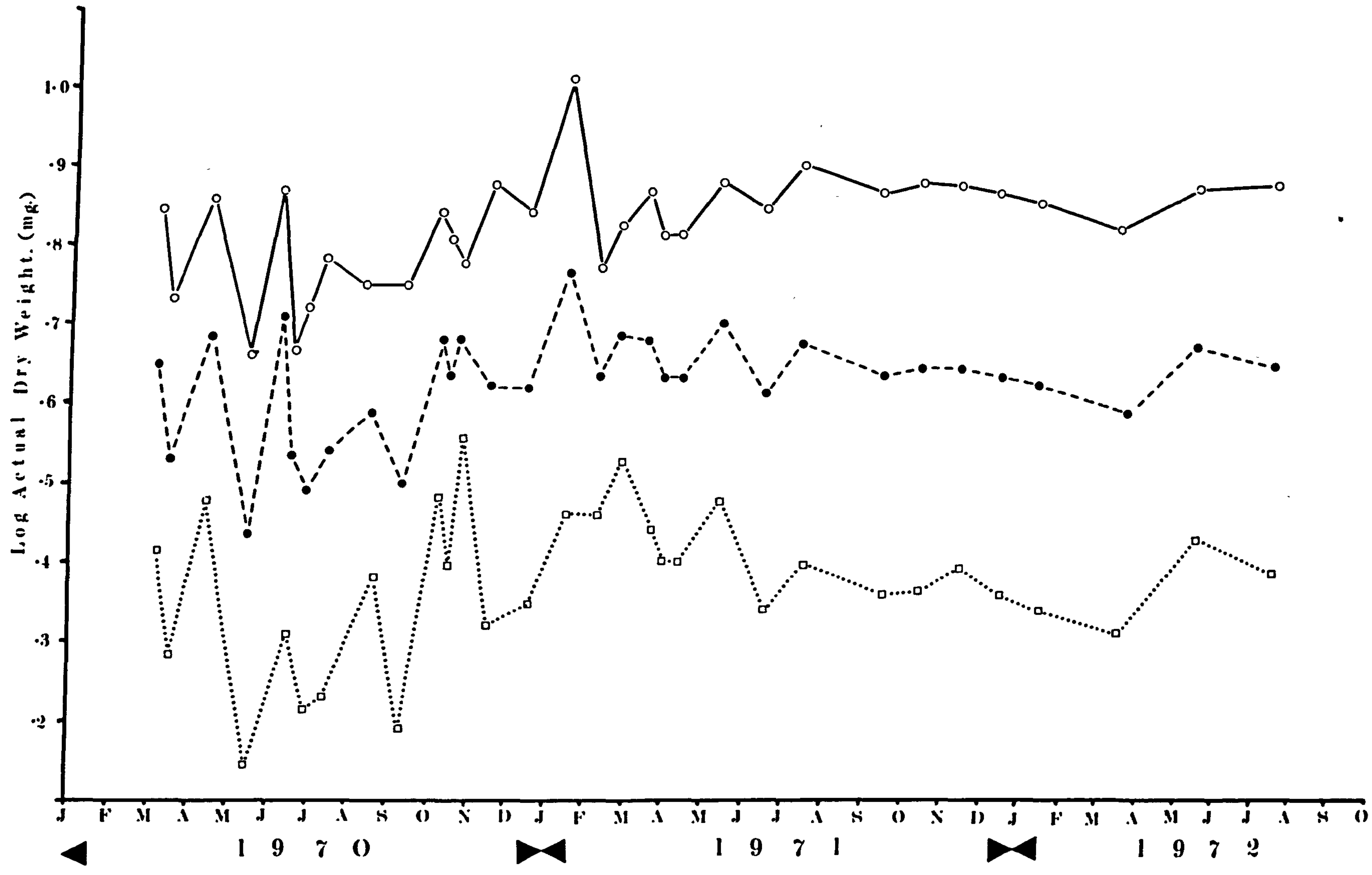
Taking the standard lengths (ie. 4.5, 5.5 and 6.5mm) a plot of the variation in dry tissue weight throughout the study period for each of the size groups is obtained (Fig. 43 ). Variations in the early period (1970) are quite large, due to the small sample size used during this period, but, a general trend in dry weight variations is apparent which is reflected in each of the size classes. In 1970 there is a build up of material around the beginning of August which extends through to January before falling again, giving a period of lowered dry weight in April/May 1971. The rest of 1971 differs from 1970 and is characterized by a relatively stable or slightly falling dry weight, depending upon size, although several fluctuations in dry weight are seen. The dry tissue weight does not show any marked increase until April 1972 but in none of the size ranges does the dry tissue weight reach the low levels seen in the summer of 1970.

Calculations of the length/dry tissue weight regression equations for animals of the same gonad

Figure 43

Log of dry tissue weight for standard animals based on regression lines

| | |
|---|---|
| Size (i) | ie. 6.5mm shell length |
| Size (ii) | ie. 5.5mm shell length |
| Size (iii) | ie. 4.5mm shell length |


Log Actual Dry Weight. (mg.)
1·0
·9
·8
·7
·6
·5
·4
·3
·2
J F M A M J J A S O N D J F M A M J J A S O N D J F M A M J J A S O
1 9 7 0
1 9 7 1
1 9 7 2

state, grouped for the whole sampling period, give an indication of the weight due to each of these gonad stages. Such regressions (Table 8 ) show that there are consistent weight differences between gonads at different stages of development. Analysis of covariance of these regressions is summarized in Fig. 41 b . Stage 5 males have a regression equation which is identical to that for the same stage females for both slope and position. Stage 6's are identical to 4's again with no differences between the sexes. Comparison of these equations show that for any given length, as expected, the fully mature stage (stage 5) will have the greatest dry weight (Fig. 44 ) with a progressive decrease in weight to stages 1 and 2. Stage 0, however, has a greater dry weight indicating a greater utilization of stored material in reaching stages 1 and 2 than the presence of such a gonad contributes to the dry weight.

Using these general regressions of dry weight on length for each individual gonad stage we can derive for each standard animal a value for the mean body weight corresponding to each gonadal stage (Table 10 ) and thence calculate a mean body weight for the population based on the percentage occurence of each gonad stage (Table 7). The result is a gonad weight index for the population (Fig. 45 a). The 'average' gonad state for the two larger size groups tends to remain the same, the middle size group showing two peaks of spawning activity, indicated by a reduction in the gonad index, in the early part of the year and again in late spring. An average gonad

Table 8

Equations obtained from covariance analysis of regressions of log weight to log length for varying samples

**Monthly samples**

| Date | Code | Dry tissue weight | Shell weight | Total weight | Wet weight |
|---|---|---|---|---|---|
| 11.1.71 | 1 | 3.42x + (-1.7792) | 3.34x + (-1.2784) | 3.93x + (-1.1568) | |
| 3.2.71 | 2 | 2.64x + (-1.3292) | 2.769x +(-0.8608) | 3.93x + (-1.1954) | |
| 3.3.71 | 3 | 2.64x + (-1.2820) | 2.769x +(-0.8608) | 2.77x + (-0.2860) | 2.73x + (-0.6350) |
| 31.3.71 | 4 | 2.509x +(-1.2315) | 2.85x + (-0.9105) | 3.269x +(-0.6559) | 3.597x +(-1.2960) |
| 27.4.71 | 5 | 2.509x +(-1.2315) | 3.00x + (-1.0400) | 3.269x +(-0.6559) | 3.367x +(-1.1752) |
| 25.5.71 | 6 | 2.509x +(-1.1665) | 3.307x +(-1.2274) | 3.269x +(-0.6559) | 3.367x +(-1.0916) |
| 21.6.71 | 7 | 3.183x +(-1.7473) | 3.475x +(-1.3964) | 2.50x + (-0.0650) | 3.572x +(-1.2589) |
| 21.7.71 | 8 | 3.183x +(-1.6892) | 3.307x +(-1.2574) | 3.40x + (-0.7560) | 3.572x +(-1.2832) |
| 22.9.71 | 9 | 3.183x +(-1.7278) | 2.769x +(-0.8608) | | |
| 19.10.71 | 10 | 3.183x +(-1.7173) | 3.92x + (-1.7100) | 3.67x + (-0.9440) | 3.597x +(-1.2479) |
| 9.11.71 | 11 | 3.198x +(-1.7298) | 3.245x +(-1.1902) | 3.228x +(-0.6232) | 3.367x +(-1.0916) |
| 8.12.71 | 12 | 3.183x +(-1.7278) | 3.307x +(-1.2520) | 3.228x +(-0.6232) | 3.367x +(-1.0997) |
| 11.1.72 | 13 | 3.183x +(-1.7397) | 3.245x +(-1.1902) | 3.228x +(-0.6232) | 3.367x +(-1.0916) |
| 21.3.72 | 14 | 3.183x +(-1.7731) | 3.475x +(-1.3723) | 3.228x +(-0.6232) | 3.125x +(-0.9127) |
| 23.5.72 | 15 | 2.714x +(-1.3443) | 3.307x +(-1.2520) | 3.228x +(-0.6232) | 3.125x +(-0.9127) |
| 17.7.72 | 16 | 3.198x +(-1.7298) | 3.245x +(-1.1902) | 3.228x +(-0.6232) | 3.283x +(-1.0415) |

**Influence of Gonad state**

| Code | Dry tissue weight |
|---|---|
| A | 2.758x + (-1.5779) |
| B | 2.758x + (-1.4481) |
| C | 2.758x + (-1.3579) |
| D | 2.758x + (-1.3579) |
| E | 2.758x + (-1.4481) |
| F | 2.758x + (-1.4704) |
| G | 2.758x + (-1.4704) |
| H | 2.758x + (-1.5779) |
| I | 2.758x + (-1.5779) |
| J | 2.758x + (-1.5644) |
| K | 2.758x + (-1.3889) |
| L | 2.758x + (-1.4352) |
| M | 2.758x + (-1.4002) |
| N | 2.758x + (-1.4002) |
| O | 2.758x + (-1.4033) |

Table 9

Population mean log dry weight calculations

| | For population, gonad state as found ie. regressions Table 8 | | | For population if gonad stage 1 | | |
|---|---|---|---|---|---|---|
| | Size (iii) | Size (ii) | Size (i) | Size (iii) | Size (ii) | Size (i) |
| Log length | 0.6532 | 0.7404 | 0.8129 | 0.6532 | 0.7404 | 0.8129 |
| Sample code No. | | | | | | |
| 1 | - | 0.7530 | - | - | 0.6990 | - |
| 2 | - | 0.6255 | - | - | 0.5499 | - |
| 3 | - | - | 0.8640 | - | - | 0.7966 |
| 4 | 0.4072 | 0.6260 | 0.8079 | 0.3476 | 0.5352 | 0.7360 |
| 5 | 0.4072 | 0.6260 | 0.8079 | 0.3596 | 0.5373 | 0.7264 |
| 6 | 0.4722 | 0.6910 | 0.8729 | 0.4210 | 0.6264 | 0.7871 |
| 7 | 0.3319 | 0.6095 | 0.8402 | 0.2891 | 0.5392 | 0.7564 |
| 8 | 0.3900 | 0.6676 | 0.8983 | 0.3595 | 0.5840 | 0.8050 |
| 9 | 0.3514 | 0.6290 | 0.8597 | 0.2930 | 0.5546 | 0.7771 |
| 10 | 0.3619 | 0.6395 | 0.8702 | 0.2994 | 0.5608 | 0.7804 |
| 11 | - | 0.6379 | 0.8698 | - | 0.5591 | 0.7852 |
| 12 | 0.3514 | 0.6290 | 0.8597 | 0.3090 | 0.5551 | 0.7655 |
| 13 | 0.3395 | 0.6171 | 0.8478 | 0.2699 | 0.5299 | 0.7507 |
| 14 | 0.3061 | 0.5837 | 0.8144 | 0.2676 | 0.5224 | 0.7219 |
| 15 | 0.4282 | 0.6648 | 0.8616 | 0.3703 | 0.5807 | 0.7721 |
| 16 | - | 0.6379 | 0.8698 | - | 0.5466 | 0.7706 |

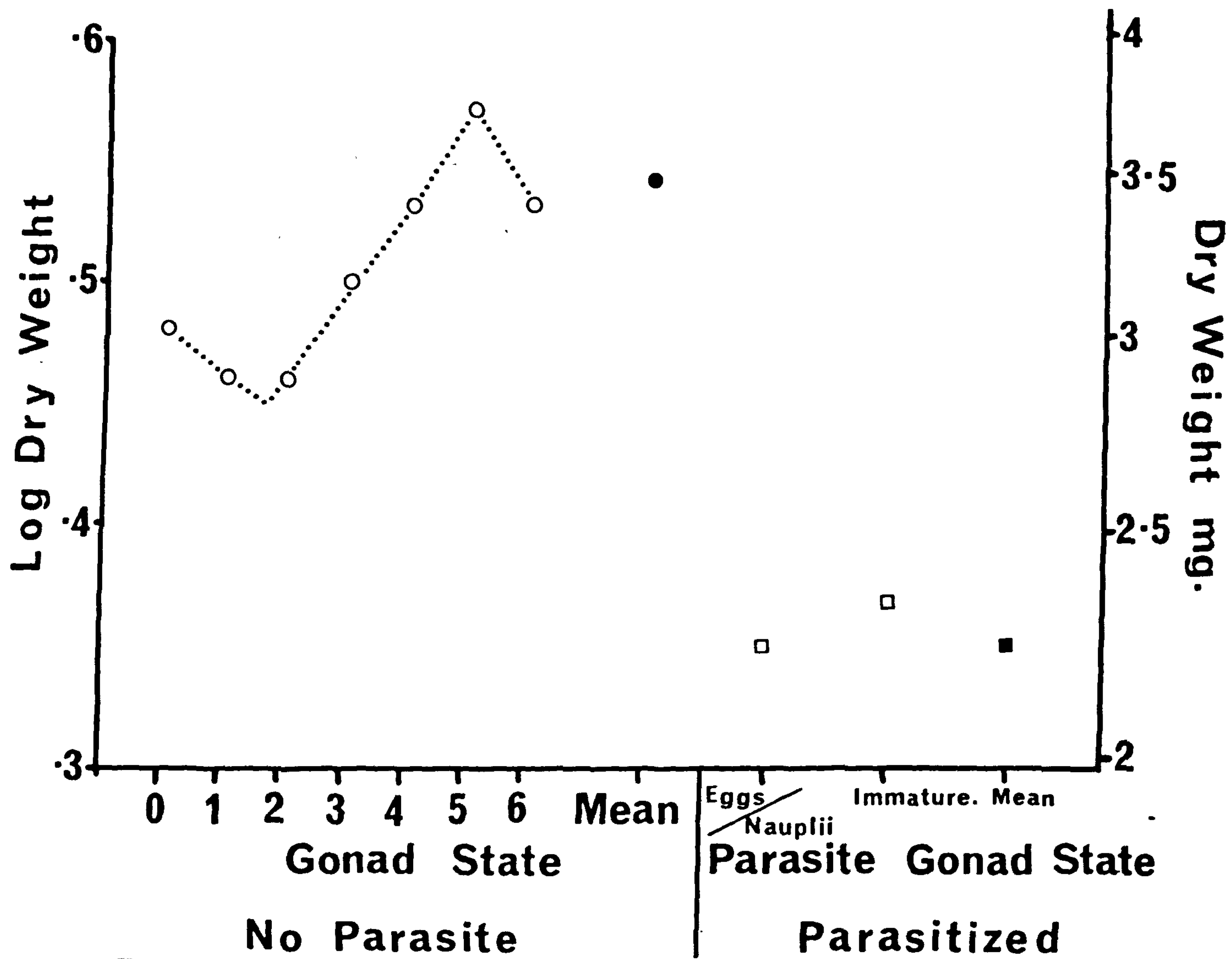
## Table 10

Log mean dry tissue weight for each gonad stage

| Size | Log length | Gonad stage | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 1/2 | 2 | 2/3 | 3 | 3/4 | 4 | 4/5 | 5 | 5/6 | 6 | 6/0 | 0 | 0/1 | 6/1 |
| (iii) | 0.6532 | .3311 | .3311 | .3311 | .3487 | .3663 | .3838 | .4013 | .4225 | .4436 | .4209 | .3982 | .3758 | .3534 | .3423 | .3647 |
| (ii) | 0.7404 | .5716 | .5716 | .5716 | .5892 | .6068 | .6243 | .6418 | .6630 | .6841 | .6614 | .6387 | .6163 | .5939 | .5828 | .6052 |
| (i) | 0.8129 | .7716 | .7716 | .7716 | .7892 | .8068 | .8243 | .8418 | .8629 | .8841 | .8614 | .8387 | .8163 | .7939 | .7828 | .8052 |

Figure 44

Mean dry tissue weight for standard animal of shell length 5mm for the various stages of gonad development and degree of parasite infection



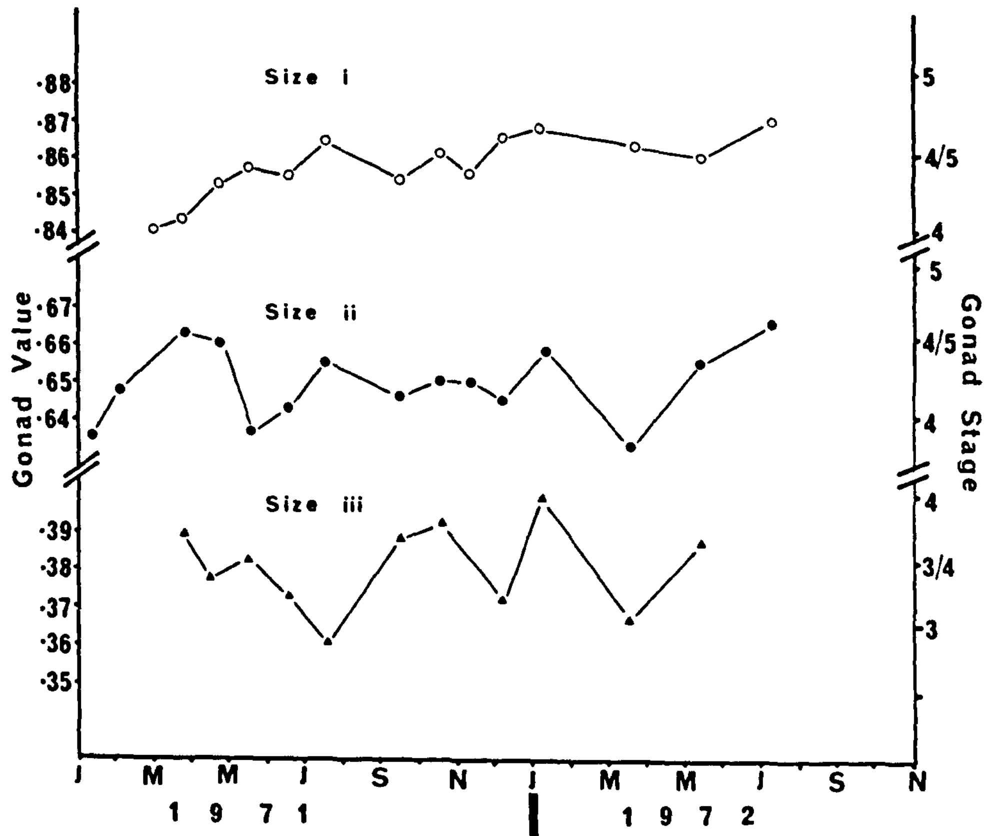
Log Dry Weight
·6
·5
·4
·3
Dry Weight mg.
4
3·5
3
2·5
2
0 1 2 3 4 5 6 Mean
Gonad State
No Parasite
Eggs
Nauplii
Immature. Mean
Parasite Gonad State
Parasitized

Figure 45

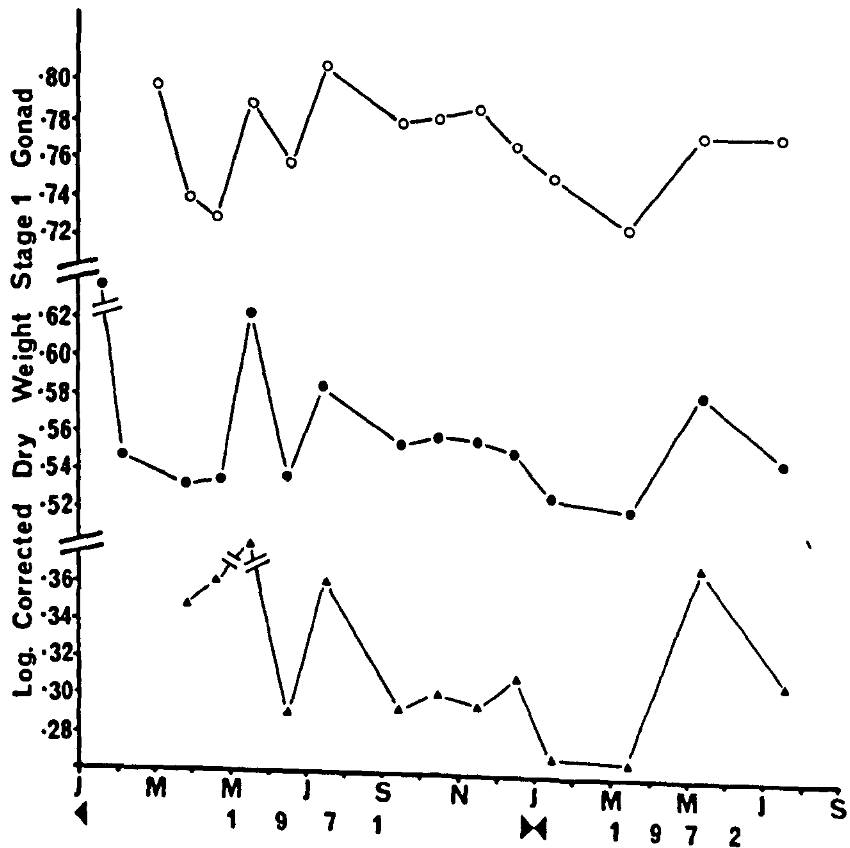
Fig. 45a Gonad weight index (Gonad Value) of standard animals from January 1971 to November 1972

Fig. 45b Log of dry tissue weight for animals of standard lengths if a standard gonad state ie. Stage I is imposed.

A.




B.



state of between stages 4 and 5 is shown for both the two larger size groups. The smallest size class shows a somewhat larger variation alternating between stages 3 and 4 (Fig. 45a).

If variations in percentage occurrence of the different gonad stages accounted for all the observed seasonal changes in body weight (Fig. 43 ), the calculated gonad weight index (Fig. 45a) should correspond closely with the mean body weight. This is not the case. The difference between the gonad weight index and the observed mean body weight must reflect consistent seasonal differences in the body weight, independent of the stage of gonadal development. The most likely explanation for such differences are best illustrated as fluctuations in the somatic body weight, obtained by subtracting the gonad weight index (Fig. 45a ) from the observed mean dry weight (Fig. 43 ) based on regressions, and adding to the resulting value the mean value for a stage 1 animal of that standard size (Table 10 ). Stage 1 is chosen because it has the lowest dry weight for any gonad stage and hence the contribution to the dry weight of gonadal material for animals of this stage is minimal. An example of this calculation is as follows:-

eg. 3.3.71 (Table 9)

| | |
|---|---|
| Observed dry weight from regression for animal of 6.5mm | 0.8660 |
| Gonad weight index for animal of length 6.5mm | 0.8410 |
| | 0.0250 |
| Mean weight stage 1 of length 6.5mm | 0.7716 |
| | 0.7966 |

The results of such a calculation for all the size groups is shown in Fig. 45b. Large fluctuations are seen in the somatic body weight for all the standard sizes used, however the general picture is much the same. The somatic body weight is low in March/April 1971, but then rises giving two peaks, one in May the other in July. From July to the following March the weight of the dried somatic body tissue falls, rising again in March to give the May peak. This pattern shows considerable correlation with the actual dry tissue weight obtained from regressions. Fig.43 shows the actual dry tissue weight so obtained for a similar time span. The population dry tissue weight variations are therefore apparently mainly due to variations in the amount of stored material and appear to be only influenced by gonad activity per se to a very small degree.

d) Changes in biochemical composition.

The changes in biochemical composition are presented in two ways:

1) as changes in the percentage composition

and 2) as changes in the composition of animals of given shell lengths, hereafter referred to as standard animals.

Where percentages are used to describe biochemical composition an increase or decrease in one component will produce a proportional effect in all other components, percentages being values of one component relative to all others (Barnes, Barnes and Finlayson

1963, Ehrlich 1972). On the other hand, the values for a standard animal are greatly influenced by choice of size and are difficult to compare for different size classes. The two methods together give a complete picture of both actual changes of each component and the relative degree of change of each component in relation to all the others.

The composition of the standard animal in each gonadal stage was estimated by multiplying the dry weight for the standard animal of that gonadal stage by the values of percentage composition found by analysis of the dried tissue obtained by grouping animals of the same gonadal stage for each sampling date. The dry weights of the standard animals were obtained from the seasonal values for stage 1 animals derived earlier (Fig. 45 b) and applying correction factors based on the general regressions of dry weight on length for each gonadial stage, ie. for stage 5 adult +0.1125 and for immature animals +0.0223 being the difference between the stage 1 weights and the stage 5 and immature, ie. stage 0, weights. These calculations were applied to give values of dry flesh weight and hence biochemical composition both in actual amounts and percentages for standard animals of the three size lengths referred to previously (Table 11 ).

The carbohydrate content of a standard animal (Fig. 46 a,b,c) shows a general rise in level from April/May reaching a peak in June/July before falling off again. Where the data overlaps two years ie. Fig. 46 b it can be seen that the levels of carbohydrate are

Table 11

Biochemical Composition

| Date | Size | Sample | Percent from Analysis | | | | log dry wt. | Amount in microgrammes | | | | Dry weight (mg) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Carbohydrate | Lipid | Nitrogen | Carbon | | Carbohydrate | Lipid | Nitrogen | Carbon | |
| 30.10.70 | (ii) | Immature | 11.27 | 11.632 | 9.42 | 39.45 | .6622 | 517.744 | 534.374 | 432.755 | 1812.333 | 4.594 |
| " | (ii) | Male | 14.92 | 12.305 | 8.30 | 36.31 | .7524 | 843.428 | 695.602 | 469.200 | 2052.604 | 5.653 |
| " | (ii) | Female | 14.00 | 11.529 | 9.10 | 39.04 | .7524 | 791.942 | 651.734 | 514.423 | 2206.931 | 5.653 |
| 24.11.70 | (ii) | Immature | 13.99 | 8.90 | 8.85 | 37.39 | .7493 | 785.399 | 499.365 | 496.839 | 2099.075 | 5.614 |
| " | (ii) | Male | 8.12 | 10.11 | 8.23 | 36.77 | .8395 | 561.092 | 698.532 | 568.693 | 2540.807 | 6.910 |
| " | (ii) | Female | 9.37 | 10.17 | 8.51 | 37.03 | .8395 | 647.467 | 702.816 | 588.041 | 2558.773 | 6.910 |
| 7.12.70 | (ii) | Male | 14.05 | 8.55 | 9.12 | 38.15 | .7590 | 806.612 | - | 523.579 | 2190.192 | 5.741 |
| " | (ii) | Immature | 11.81 | 8.85 | 8.94 | 37.72 | .6688 | 550.937 | 398.744 | 417.051 | 1759.638 | 4.665 |
| " | (ii) | Female | 13.63 | 11.62 | 8.62 | 38.42 | .7590 | 782.498 | 667.104 | 494.874 | 2209.137 | 5.741 |
| " | (ii) | Parasite immature | 13.23 | - | 8.07 | 36.61 | .5565 | 476.280 | - | 290.520 | 1317.960 | 3.600 |
| 12. 1.71 | (ii) | Immature | 10.62 | 10.82 | 8.34 | 35.95 | .7127 | 547.992 | 558.209 | 430.344 | 1855.020 | 5.160 |
| " | (ii) | Male | 12.43 | - | 8.97 | 36.51 | .8019 | 787.689 | - | 568.429 | 2313.639 | 6.337 |
| 17. 2.71 | (ii) | Immature | 10.34 | 12.74 | 8.66 | 37.94 | .5722 | 386.199 | 475.727 | 323.451 | 1417.059 | 3.735 |
| " | (ii) | Female | 16.28 | 11.57 | 8.44 | 36.98 | .6634 | 750.020 | 533.030 | 388.831 | 1703.669 | 4.607 |
| " | (ii) | Parasite immature | 14.40 | - | 7.87 | 37.31 | .4599 | 415.152 | - | 226.892 | 1075.647 | 2.883 |
| 17. 3.71 | (i) | Male | 13.13 | 9.99 | 8.40 | 37.26 | .8485 | 926.059 | 704.454 | 592.452 | 2627.948 | 7.053 |
| " | (i) | Female | 15.02 | 16.47 | 8.04 | 37.85 | .8485 | 1059.361 | 1161.629 | 526.154 | 2669.561 | 7.053 |
| " | (i) | Parasite immature | 16.91 | 11.85 | 7.46 | 37.20 | .7047 | 856.661 | 600.169 | 377.924 | 1884.552 | 5.066 |
| " | (ii) | Male | 14.14 | 11.31 | 8.68 | 37.65 | .6477 | 628.240 | 502.414 | 385.652 | 1672.790 | 4.443 |
| " | (ii) | Female | 11.97 | 13.33 | 8.27 | 38.27 | .6477 | 531.827 | 592.207 | 367.436 | 1700.336 | 4.443 |
| " | (iii) | Immature | 12.98 | 11.04 | 8.55 | 37.71 | .3679 | 302.823 | 257.563 | 199.472 | 879.774 | 2.333 |
| " | (iii) | Male | 13.00 | 11.13 | 8.79 | 37.54 | .4601 | 375.050 | 321.129 | 253.592 | 1083.029 | 2.885 |
| " | (iii) | Female | 11.45 | 14.25 | 8.11 | 37.31 | .4601 | 330.333 | 411.084 | 233.974 | 1076.394 | 2.885 |
| " | (iii) | Parasite immature | 17.36 | 14.81 | 7.99 | 37.55 | .2576 | 314.042 | 267.822 | 144.539 | 679.280 | 1.809 |
| 15. 4.71 | (i) | Male | 16.08 | 13.02 | 8.31 | 37.52 | .8389 | 1109.681 | 898.579 | 573.473 | 2589.255 | 6.901 |
| " | (i) | Female | 14.80 | 15.33 | 8.36 | 38.16 | .8381 | 1021.348 | 1057.578 | 576.924 | 2633.422 | 6.901 |
| " | (i) | Parasite immature | 16.74 | 12.15 | 7.97 | 38.25 | .6364 | 724.675 | 525.974 | 345.021 | 1655.843 | 4.329 |

/cont'd

Table 11 (cont'd)

| Date | Size | Sample | Percent from Analysis | | | | log dry wt. | Amount in microgrammes | | | | Dry weight (mg) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Carbohydrate | Lipid | Nitrogen | Carbon | | Carbohydrate | Lipid | Nitrogen | Carbon | |
| 15. 4.71 | (ii) | Male | 11.04 | 13.06 | 8.17 | 35.87 | .6498 | 492.936 | 583.174 | 364.791 | 1601.596 | 4.465 |
| " | (ii) | Female | 12.62 | 13.08 | 8.35 | 35.80 | .6498 | 563.483 | 583.843 | 372.828 | 1598.470 | 4.465 |
| " | (ii) | Parasite immature | 13.38 | 21.68 | 7.64 | 37.64 | .4473 | 374.774 | 606.613 | 213.996 | 1054.296 | 2.801 |
| " | (iii) | Immature | 16.09 | 14.55 | 7.58 | 35.99 | .3819 | 387.447 | 350.292 | 182.526 | 866.639 | 2.408 |
| " | (iii) | Female | 12.92 | 21.77 | - | - | .4721 | 383.207 | 645.758 | - | - | 2.966 |
| " | (iii) | Parasite immature | 17.19 | - | 7.84 | 37.56 | .2696 | 319.906 | - | 145.902 | 698.992 | 1.861 |
| 13. 5.71 | (i) | Male | 13.24 | 12.42 | 8.83 | 37.23 | .8996 | 1050.726 | 985.492 | 700.749 | 2954.573 | 7.936 |
| " | (i) | Female | 13.92 | 13.91 | 8.34 | 38.31 | .8996 | 1104.691 | 1104.215 | 661.862 | 3040.282 | 7.936 |
| " | (i) | Parasite immature | 14.52 | 12.22 | 8.06 | 38.24 | .6971 | 722.206 | 608.262 | 401.227 | 1903.587 | 4.978 |
| " | (ii) | Immature | 14.73 | 11.30 | - | - | .6487 | 655.927 | 503.055 | - | - | 4.453 |
| " | (ii) | Male | 13.68 | 11.43 | 9.11 | 37.69 | .7389 | 749.801 | 627.684 | 499.319 | 2065.789 | 5.481 |
| " | (ii) | Female | 13.87 | 11.97 | 8.59 | 37.75 | .7389 | 760.215 | 656.240 | 470.818 | 2069.078 | 5.481 |
| " | (iii) | Immature | 15.53 | 15.09 | 8.97 | 38.38 | .4422 | 429.870 | 417.608 | 248.290 | 1062.358 | 2.768 |
| " | (iii) | Parasite immature | 12.74 | - | - | - | .3299 | 272.254 | - | - | - | 2.137 |
| 8. 6.71 | (i) | Male | 15.82 | 13.01 | 9.32 | 38.98 | .8996 | 1255.475 | 1032.315 | 739.635 | 3093.453 | 7.936 |
| " | (i) | Parasite immature | 17.76 | 12.70 | 8.27 | 38.76 | .6971 | 884.093 | 632.007 | 411.681 | 1929.473 | 4.978 |
| " | (ii) | Male | 12.26 | 12.86 | 9.04 | 37.32 | .7389 | 671.971 | 704.911 | 495.482 | 2045.509 | 5.481 |
| " | (ii) | Female | 7.83 | 17.95 | 8.57 | 39.51 | .7389 | 429.162 | 964.327 | 469.722 | 2165.543 | 5.481 |
| " | (ii) | Parasite immature | 15.67 | 13.11 | 8.02 | 36.73 | .5364 | 538.891 | 450.715 | 255.808 | 1263.145 | 3.493 |
| 6. 7.71 | (i) | Male | 16.01 | 15.86 | 9.39 | 38.97 | .8689 | 1183.779 | 1172.541 | 694.297 | 2881.442 | 7.394 |
| " | (i) | Parasite immature | 15.53 | 12.21 | 8.54 | 37.67 | .6664 | 720.281 | 566.346 | 396.085 | 1747.135 | 4.638 |
| " | (ii) | Immature | 13.18 | 16.00 | 8.99 | 38.74 | .5615 | 480.147 | 582.989 | 327.508 | 1411.298 | 3.643 |
| " | (ii) | Male | 13.44 | 13.83 | 9.63 | 38.82 | .6517 | 602.650 | 620.137 | 431.809 | 1740.689 | 4.484 |
| " | (ii) | Female | 15.29 | 15.01 | 8.73 | 38.78 | .6517 | 685.604 | 673.183 | 391.453 | 1738.895 | 4.484 |
| " | (iii) | Immature | 15.28 | 12.89 | 8.53 | 37.78 | .3114 | 312.934 | 263.905 | 174.694 | 775.578 | 2.048 |
| " | (iii) | Male | 14.03 | 11.88 | 8.79 | 38.82 | .4016 | 353.837 | 299.513 | 221.684 | 979.040 | 2.522 |
| " | (iii) | Parasite immature | 14.29 | - | - | - | .1991 | 225.925 | - | - | - | 1.581 |

/cont'd

Table 11 (cont'd)

| Date | Size | Sample | Percent from Analysis | | | | log dry wt. | Amount in microgrammes | | | | Dry weight (mg) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Carbohydrate | Lipid | Nitrogen | Carbon | | Carbohydrate | Lipid | Nitrogen | Carbon | |
| 3. 8.71 | (i) | Immature | 15.56 | 9.49 | 7.40 | 34.63 | .8273 | 1045.476 | 637.297 | 497.206 | 2326.790 | 6.719 |
| " | (i) | Male | 12.50 | 12.10 | 9.72 | 38.94 | .9175 | 1033.625 | 1003.301 | 803.747 | 3219.949 | 8.269 |
| " | (i) | Female | 14.60 | 12.03 | 8.65 | 38.43 | .9175 | 1207.274 | 995.009 | 715.269 | 3177.777 | 8.269 |
| " | (i) | Parasite immature | 14.19 | 12.54 | - | - | .7150 | 736.177 | 650.627 | - | - | 5.188 |
| " | (ii) | Male | 13.49 | 9.10 | 8.31 | 38.44 | .6965 | 670.723 | 452.054 | 413.173 | 1911.237 | 4.972 |
| " | (ii) | Female | 13.90 | 12.18 | 8.95 | 39.33 | .6965 | 691.108 | 605.391 | 444.994 | 1955.488 | 4.972 |
| " | (ii) | Parasite immature | 13.24 | 13.40 | 7.54 | 37.93 | .4840 | 403.555 | 408.462 | 229.819 | 1156.106 | 3.048 |
| " | (iii) | Immature | 13.87 | 13.10 | - | - | .3818 | 333.990 | 315.304 | - | - | 2.408 |
| " | (iii) | Female | 11.32 | 17.13 | 9.04 | 39.37 | .4720 | 335.638 | 507.934 | 267.947 | 1167.321 | 2.965 |
| " | (iii) | Parasite immature | 11.95 | - | - | - | .2695 | 222.270 | - | - | - | 1.860 |
| 23. 9.71 | (i) | Male | 13.04 | 10.98 | 9.27 | 38.24 | .8896 | 1011.382 | 851.066 | 718.981 | 2965.894 | 7.756 |
| " | (i) | Parasite immature | 14.40 | 8.89 | 8.44 | 38.00 | .6871 | 700.560 | 432.596 | 410.606 | 1848.700 | 4.865 |
| " | (ii) | Immature | 10.40 | 9.26 | - | - | .5769 | 392.600 | 349.414 | - | - | 3.775 |
| " | (ii) | Female | 13.23 | 12.45 | 9.05 | 38.59 | .6671 | 614.666 | 578.241 | 420.463 | 1792.891 | 4.646 |
| " | (ii) | Parasite immature | 15.38 | 11.33 | 8.59 | 38.97 | .4646 | 448.327 | 330.299 | 250.399 | 1135.976 | 2.915 |
| " | (iii) | Immature | 15.34 | 16.67 | 7.90 | 37.61 | .3153 | 316.924 | 344.423 | 163.214 | 777.023 | 2.066 |
| " | (iii) | Male | 14.59 | 8.97 | - | - | .4075 | 372.920 | 229.197 | - | - | 2.556 |
| " | (iii) | Parasite immature | 14.09 | - | - | - | .2030 | 224.876 | - | - | - | 1.596 |
| 19.10.71 | (i) | Male | 13.10 | 11.01 | 9.18 | 37.79 | .8929 | 1023.634 | 859.931 | 717.325 | 2952.911 | 7.814 |
| " | (i) | Female | 13.64 | 13.36 | 9.03 | 40.01 | .8929 | 1065.830 | 1043.560 | 705.604 | 3126.381 | 7.814 |
| " | (ii) | Male | 12.86 | 10.35 | 9.20 | 38.17 | .6733 | 606.092 | 487.654 | 433.596 | 1798.952 | 4.713 |
| " | (ii) | Female | 12.71 | 12.00 | 8.45 | 38.04 | .6733 | 599.022 | 565.749 | 398.249 | 1792.825 | 4.713 |
| " | (ii) | Parasite immature | 13.52 | 6.24 | 8.12 | 37.65 | .4708 | 399.651 | 184.336 | 240.027 | 1112.934 | 2.956 |
| " | (iii) | Female | 14.61 | 15.67 | 8.81 | 40.53 | .4119 | 377.084 | 404.339 | 227.886 | 1046.079 | 2.581 |
| " | (iii) | Immature | 15.80 | 13.62 | - | - | .3217 | 331.326 | 285.569 | - | - | 2.097 |
| " | (iii) | Parasite immature | 15.11 | - | 6.15 | 31.74 | .2094 | 244.631 | - | 99.56 | 513.871 | 1.619 |
| 9.11.71 | (i) | Male | 13.37 | 10.68 | 9.57 | 38.26 | .8977 | 1056.497 | 843.539 | 756.221 | 3023.305 | 7.902 |
| " | (i) | Female | 13.81 | 9.70 | 8.54 | 38.33 | .8977 | 1091.266 | 766.573 | 674.831 | 3028.837 | 7.902 |

/cont'd

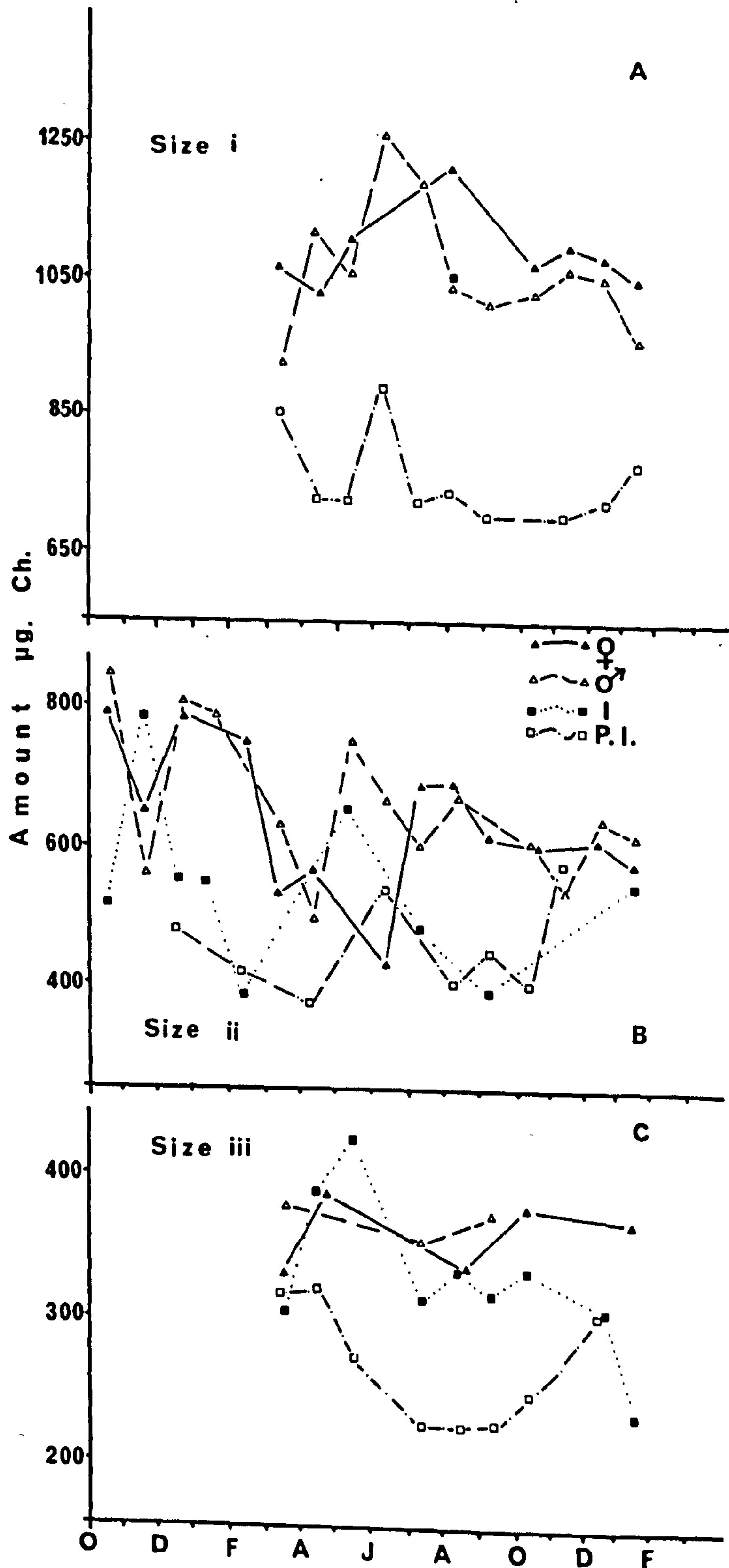
Table 11 (cont'd)

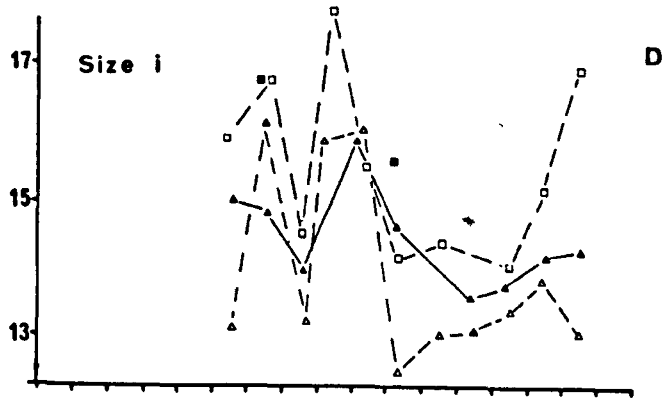
| Date | Size | Sample | Percent from Analysis | | | | log dry wt. | Amount in microgrammes | | | | Dry weight (mg) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Carbohydrate | Lipid | Nitrogen | Carbon | | Carbohydrate | Lipid | Nitrogen | Carbon | |
| 9.11.71 | (i) | Parasite immature | 14.11 | 12.52 | 8.30 | 37.17 | .6952 | 699.433 | 620.616 | 411.431 | 1842.517 | 4.957 |
| " | (ii) | Male | 11.42 | 12.45 | 9.33 | 38.43 | .6716 | 536.169 | 584.715 | 438.044 | 1804.289 | 4.695 |
| " | (ii) | Parasite immature | 19.57 | 13.30 | 8.47 | 38.65 | .4691 | 576.337 | 391.567 | 249.442 | 1138.243 | 2.945 |
| 9.12.71 | (i) | Male | 13.82 | 10.71 | 9.00 | 37.54 | .8780 | 1043.548 | 808.863 | 679.590 | 2834.645 | 7.551 |
| " | (i) | Female | 14.20 | 9.29 | 8.29 | 38.67 | .8780 | 1072.246 | 701.412 | 625.978 | 2919.972 | 7.551 |
| " | (i) | Parasite immature | 15.21 | 11.49 | 8.28 | 37.73 | .6755 | 720.498 | 544.281 | 392.224 | 1787.270 | 4.737 |
| " | (ii) | Immature | 16.84 | 11.12 | 8.68 | 37.24 | .5774 | 636.384 | 420.074 | 328.017 | 1407.300 | 3.779 |
| " | (ii) | Male | 13.73 | 12.87 | 9.01 | 37.99 | .6676 | 638.582 | 598.351 | 419.055 | 1766.915 | 4.651 |
| " | (ii) | Female | 13.07 | 9.82 | 7.81 | 35.41 | .6676 | 607.886 | 456.496 | 363.243 | 1646.919 | 4.651 |
| " | (iii) | Immature | 14.27 | 9.48 | 8.48 | 36.54 | .3313 | 305.949 | 203.294 | 181.811 | 783.418 | 2.144 |
| " | (iii) | Male | 11.57 | - | 9.40 | 38.64 | .4215 | 305.332 | - | 248.066 | 1019.710 | 2.639 |
| " | (iii) | Parasite immature | 18.18 | 13.64 | 8.12 | 38.86 | .2910 | 301.061 | 225.829 | 134.467 | 643,522 | 1.656 |
| 11. 1.72 | (i) | Male | 13.07 | 11.21 | 8.74 | 37.29 | .8632 | 953.849 | 818.398 | 637.845 | 2721.424 | 7.298 |
| " | (i) | Female | 14.36 | 13.34 | 8.22 | 38.39 | .8632 | 1047.993 | 973.407 | 599.896 | 2801.702 | 7.298 |
| " | (i) | Parasite immature | 16.96 | 11.27 | 8.16 | 36.89 | .6607 | 776.429 | 516.032 | 373.565 | 1688.824 | 4.578 |
| " | (ii) | Immature | 15.23 | 16.93 | 8.00 | 37.01 | .5522 | 543.254 | 604.000 | 285.360 | 1320.147 | 3.567 |
| " | (ii) | Male | 14.04 | 10.33 | 9.06 | 37.89 | .6424 | 616.216 | 453.515 | 397.643 | 1662.992 | 4.389 |
| " | (ii) | Female | 13.09 | 12.81 | 8.54 | 38.14 | .6424 | 574.520 | 562.055 | 374.821 | 1673.965 | 4.389 |
| " | (iii) | Female | 15.16 | 13.88 | 8.30 | 38.72 | .3824 | 365.659 | 334.833 | 200.196 | 933.926 | 2.412 |
| " | (iii) | Parasite immature | 15.22 | - | 8.40 | 37.97 | .1799 | 230.279 | - | 127.092 | 574.486 | 1.513 |

Figure 46

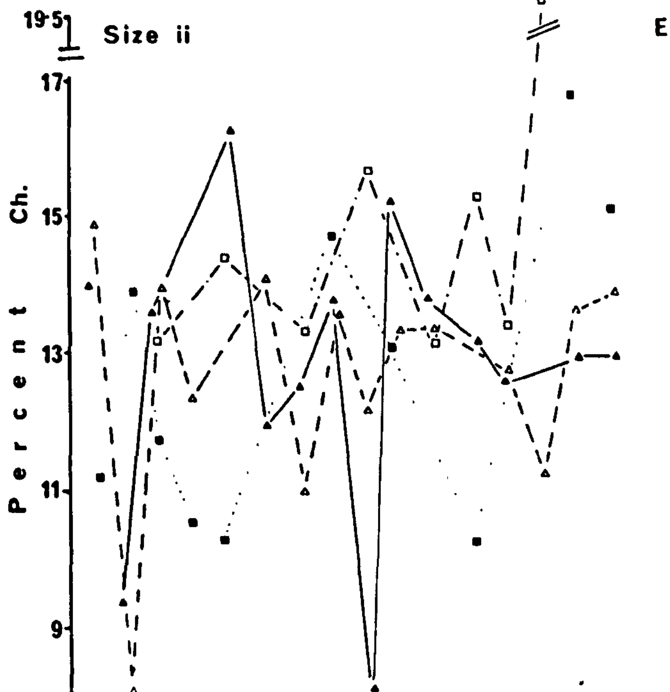
Fig. 46a-c:- carbohydrate content and Fig. 46d-f:- percentage carbohydrate for animals of standard length at varying stages of gonad development.

Symbols as inset on Fig. 46b

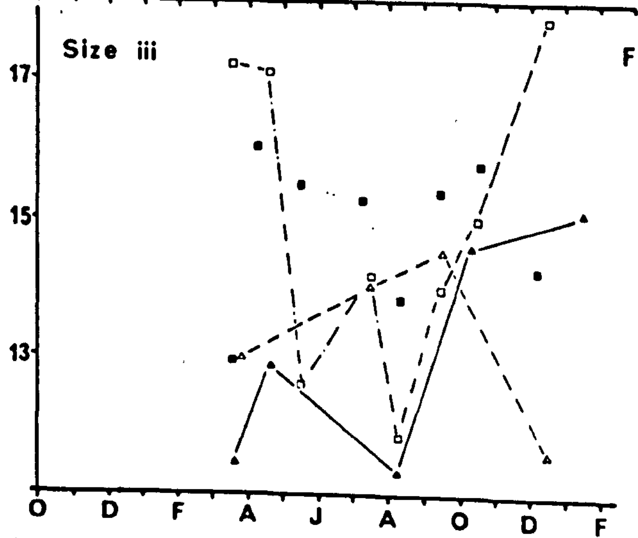

A
Size i
1250
1050
850
650
Amount µg. Ch.
♀
♂
I
P.I.
800
600
400
Size ii
B
C
Size iii
400
300
200
O
D
F
A
J
A
O
D
F



17
15
13
Size i
D

19·5
17
15
13
11
9
Size ii
E
Percent Ch.

17
15
13
Size iii
F
O
D
F
A
J
A
O
D
F

lower in 1971 than they were in October/December of the previous year. The carbohydrate content was approximately the same for both the mature males and the mature females. Changes in the carbohydrate content reflect the changes in dry weight. However, changes in the percentage of carbohydrate occur so that in April/May the relative importance of carbohydrate within the body increases.

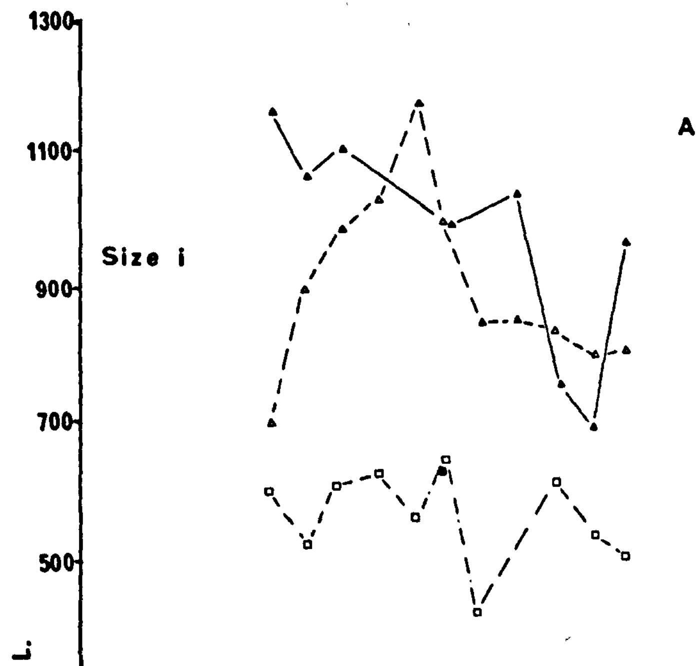
The lipid content of the mature males shows a seasonal cycle reaching a peak in July, (Fig.47b,c). This increase is largely associated with dry weight changes but is reflected to a certain degree in fluctuations in the percentage of this component, at least in the intermediate size range (Fig.47e). Mature females within this size group tend to follow the fluctuations shown by the males, as do the immature animals, both as regards actual amount changes (Fig.47b) and percentage (Fig.47e). This is however not true of the largest size group. Within this group, females do not show any increase in lipid in July/August but rather lipid tends to decline throughout the year. Initially, lipid of the mature females is higher than that in the males, but rapidly falls off, reaching the same level as in the males later in the year (Fig. 47a). Too little data is available for the smallest size group to ascertain any details of variations in lipid content.

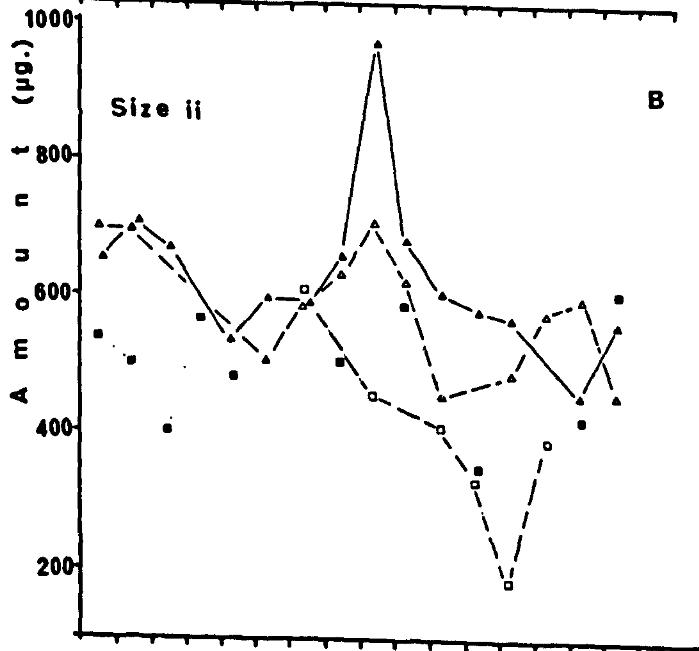
As with the other components measured, variations in the amount of nitrogen present are largely due to dry weight changes. The mature males and mature

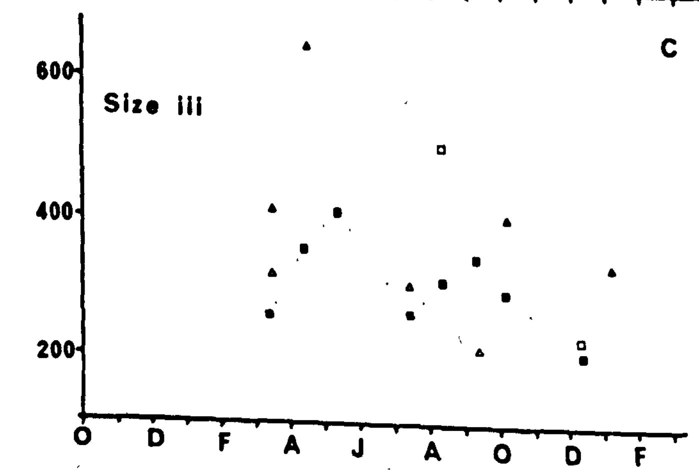
Figure 47

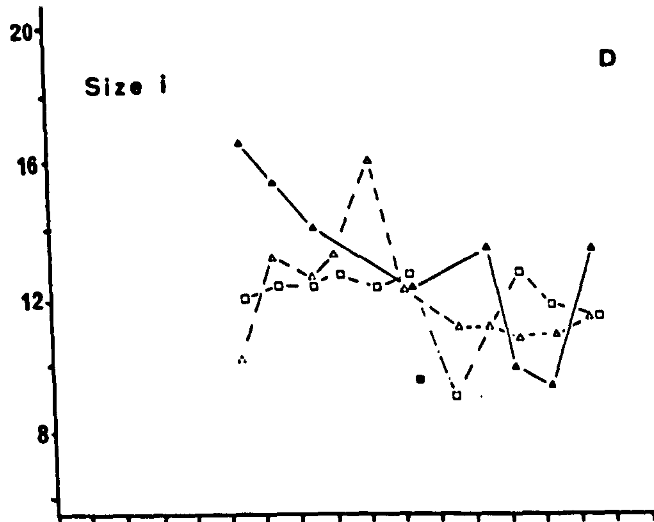
Fig. 47a-c lipid content and Fig. 47d-f percentage lipid in animals of standard lengths at varying stages of gonadal development.

For meaning of symbols used see Fig. 46b.

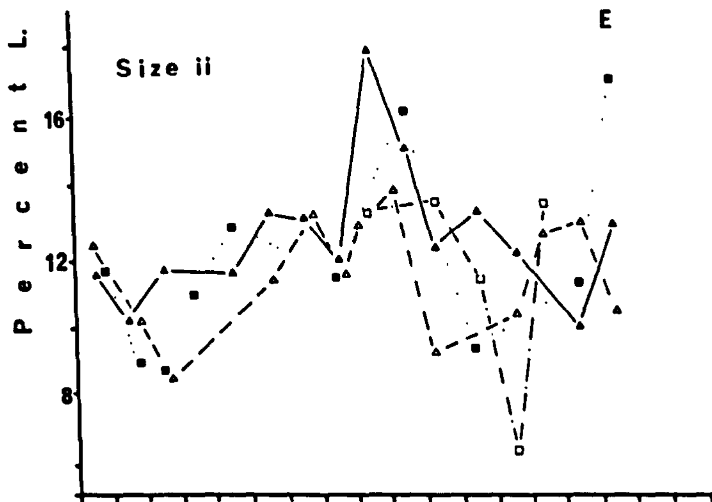

1300
1100
900
700
500
Size i
A


Amount (μg.)
1000
800
600
400
200
Size ii
B

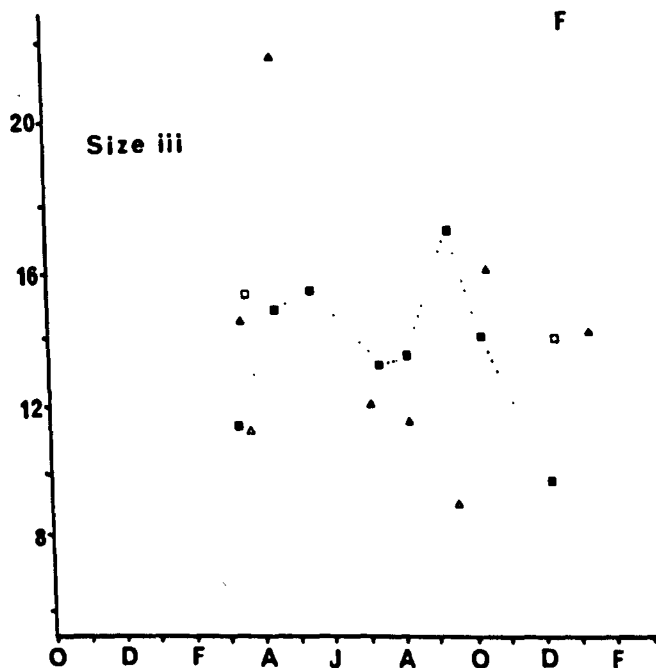

600
400
200
Size iii
C
O
D
F
A
J
A
O
D
F



20
16
12
8
Size i
D

Percent L.
16
12
8
Size ii
E

20
16
12
8
Size iii
F
O
D
F
A
J
A
O
D
F

females show the same basic pattern within each size group with regard to nitrogen levels (Fig. 48 ). This is a rise in nitrogen in March/April which is sustained util October/November before falling again (Fig. 48a). Within the smaller sized animals (Fig. 48b) there appears to be an initial large peak in nitrogen level in May/June followed by a fall and a subsequent less pronounced rise in September/October. This may also be true of the smallest size range, though too little data is available to be more certain (Fig. 48c).

The fluctuations seen as regards carbon (Fig. 49 ) are similar to those seen for nitrogen (Fig. 48 ). The level of carbon is similar for both the mature males and mature females. The differences between size groups noted for nitrogen apply also to carbon, the percentage of carbon is, however, approximately the same for all animals whatever their size or gonad state (Fig. 49c,d,e).

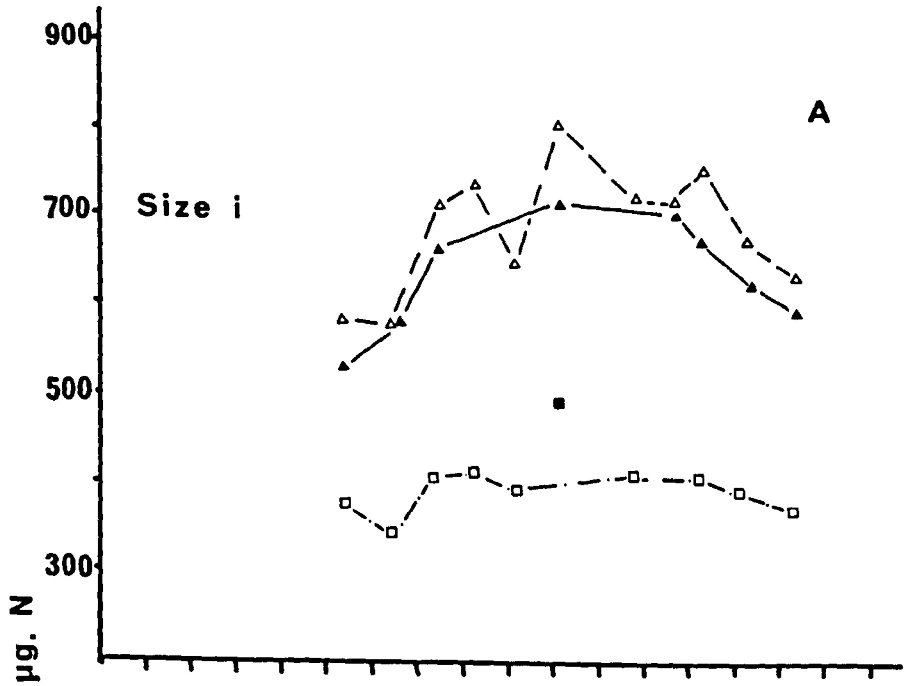
The calorific value of the dried flesh can be obtained by conversion of the biochemical data using the relative calorific values ie. 4.2 for carbohydrate, 9.45 for lipid and 5.7 for protein (ie. nitrogen + 6.25).

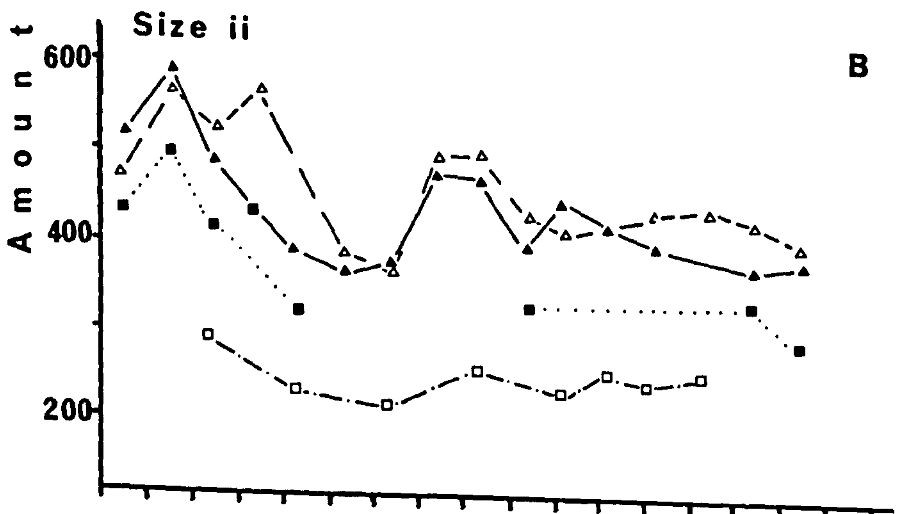
The total calorific values for the dried tissue show a certain degree of difference between the various size groups (Fig. 50 ). In general however a level of 4-5.5 K cal per gram dry weight is seen, a value comparable to other bivalves (Ansell and Trevallion 1967, Ansell 1972). There are two peaks in the energy content of the male, one in summer the other in the

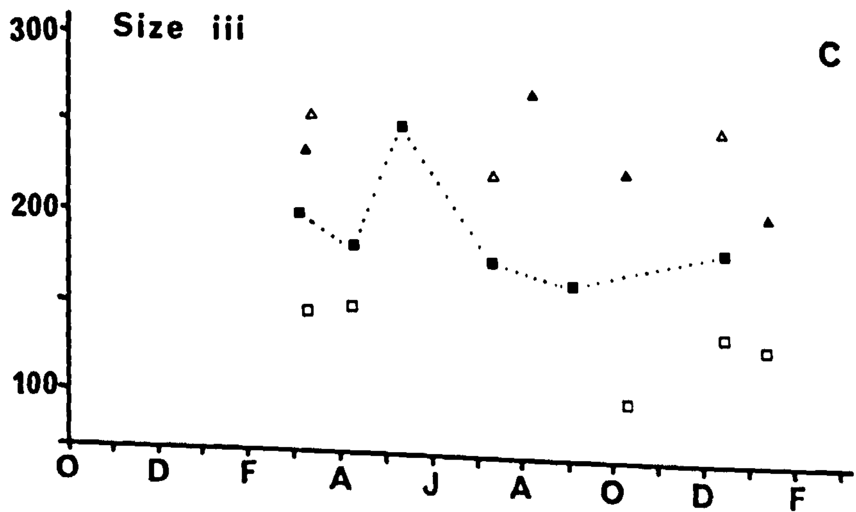
Figure 48

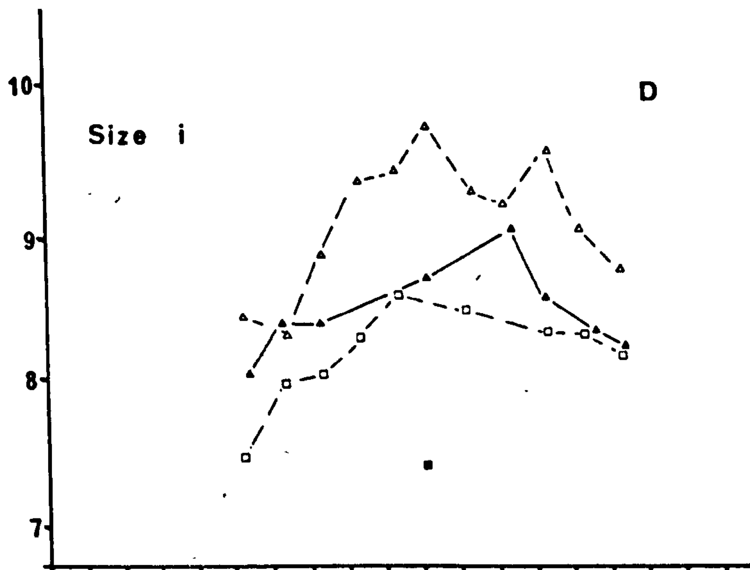
Fig. 48a-c Nitrogen content and Fig. 48d-f percentage nitrogen in animals of standard lengths at varying stages of gonadal development.

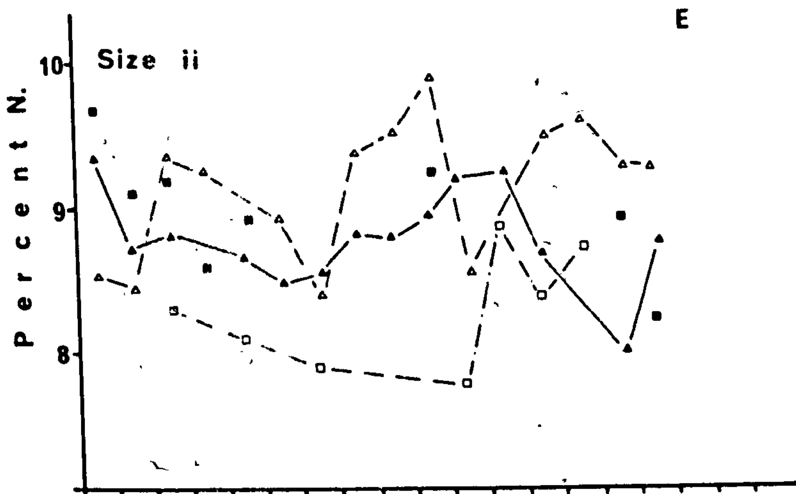
For meaning of symbols used see Fig. 46b.


900
700
500
300
A
Size i
μg. N


Amount
Size ii
600
400
200
B


300
200
100
Size iii
C
O
D
F
A
J
A
O
D
F


D
Size i
10
9
8
7


E
Size ii
Percent N.
10
9
8

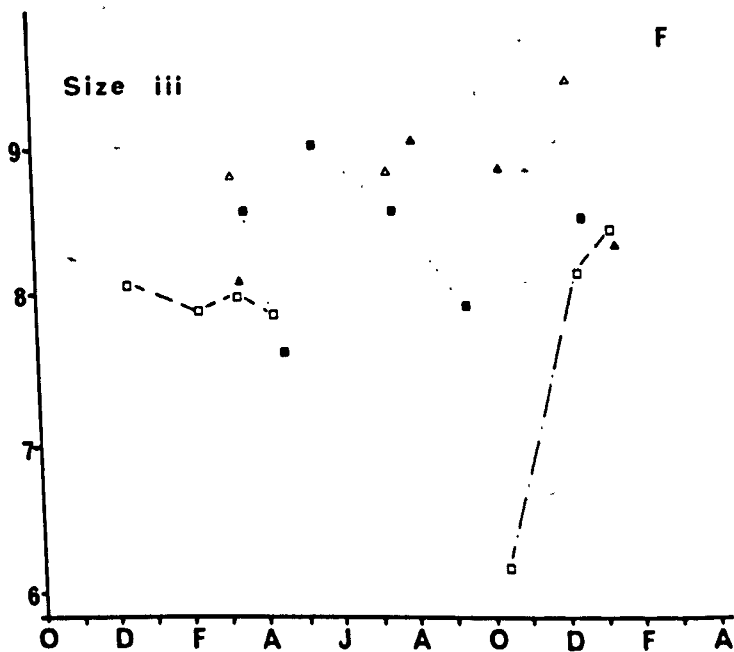
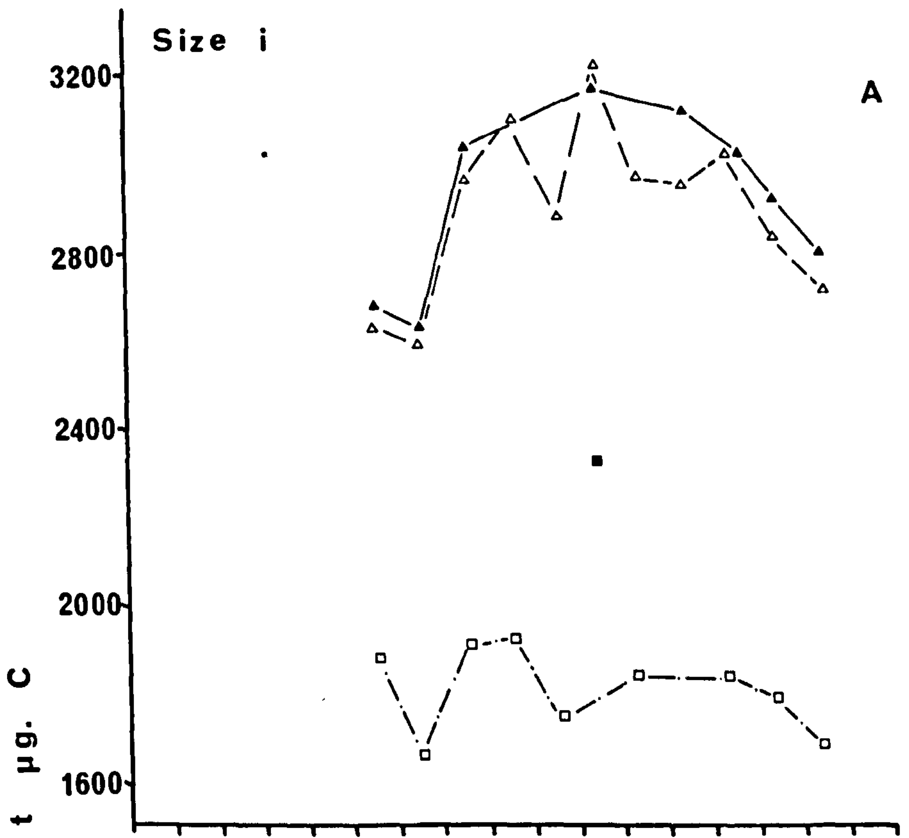
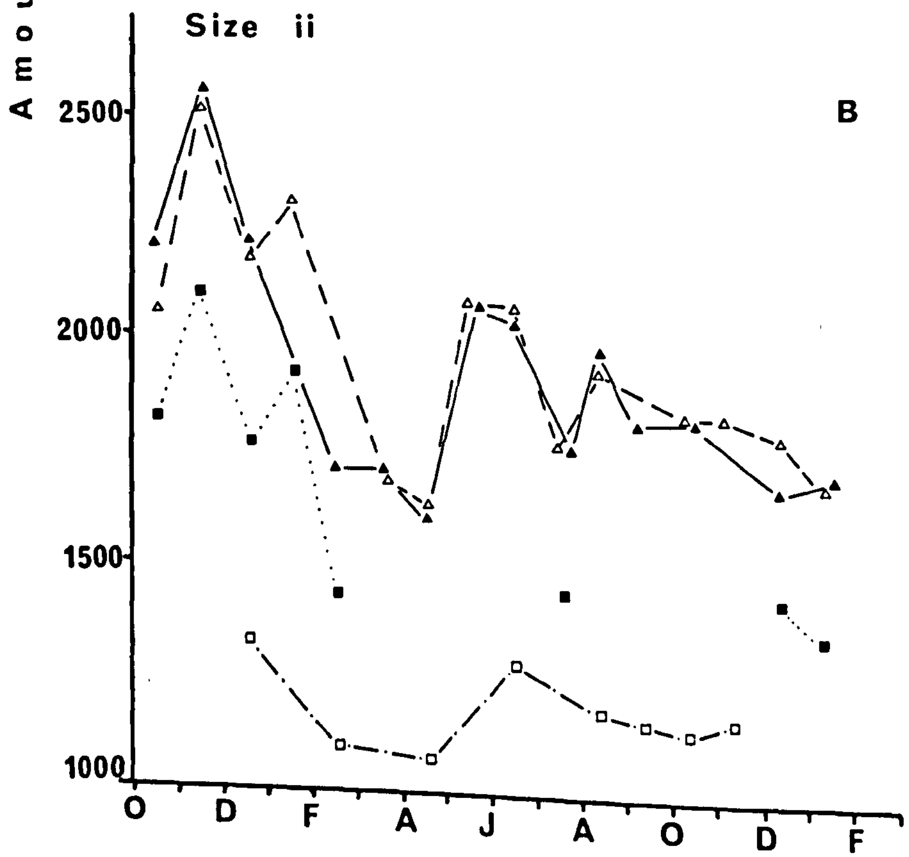

F
Size iii
9
8
7
6
O D F A J A O D F A

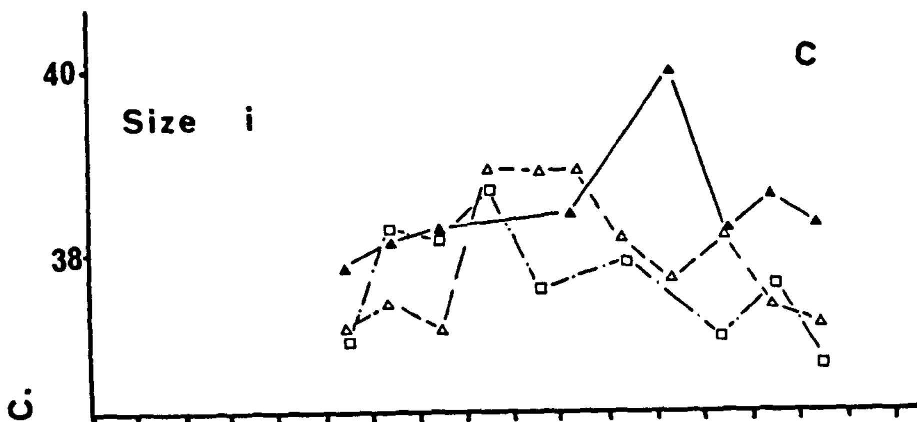
Figure 49

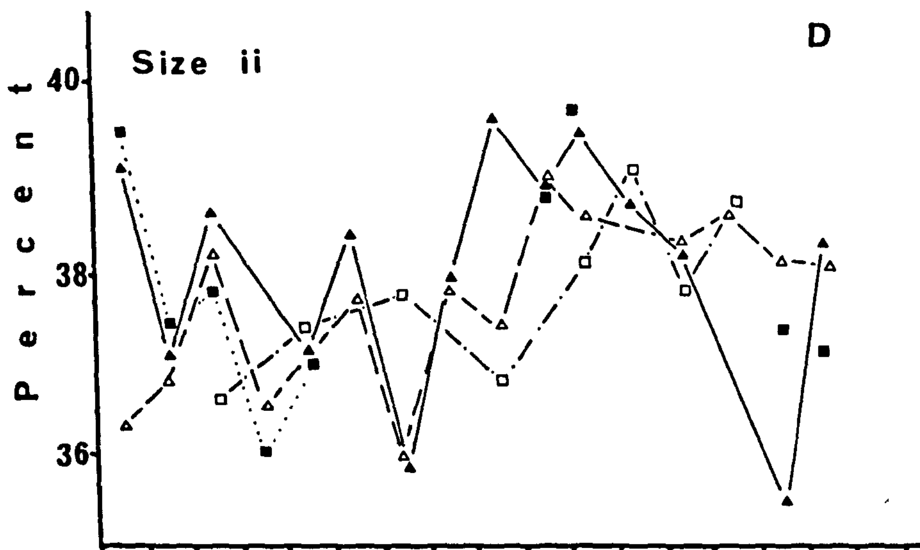
Fig. 49a-b carbon content and Fig. 49c-e percentage carbon in animals of standard length at differing stages of gonadal development.

For meaning of symbols used see Fig. 46b.


Size i
A
3200
2800
2400
2000
1600
Amount µg. C


Size ii
B
2500
2000
1500
1000
O
D
F
A
J
A
O
D
F


C
Size i
40
38
C.


D
Size ii
40
38
36
Percent

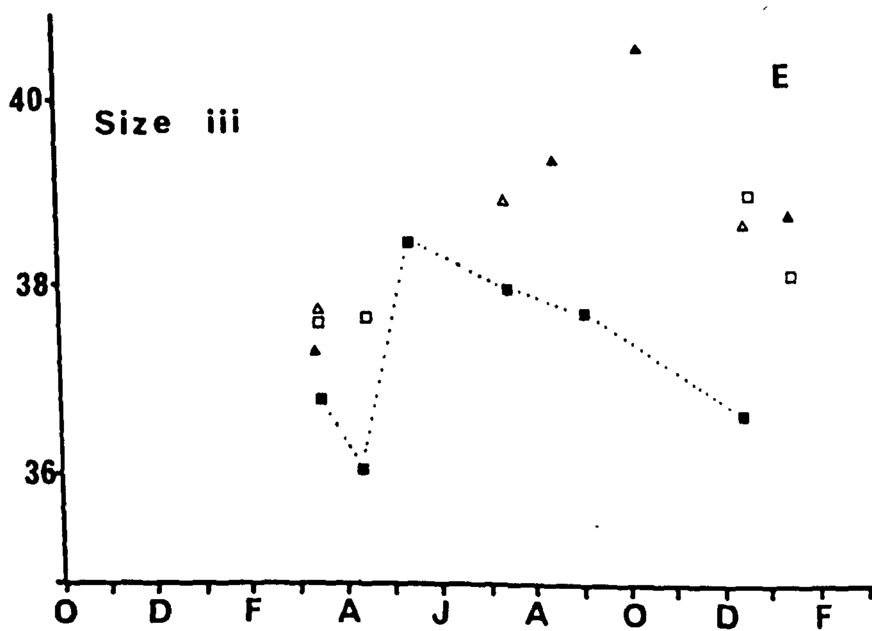
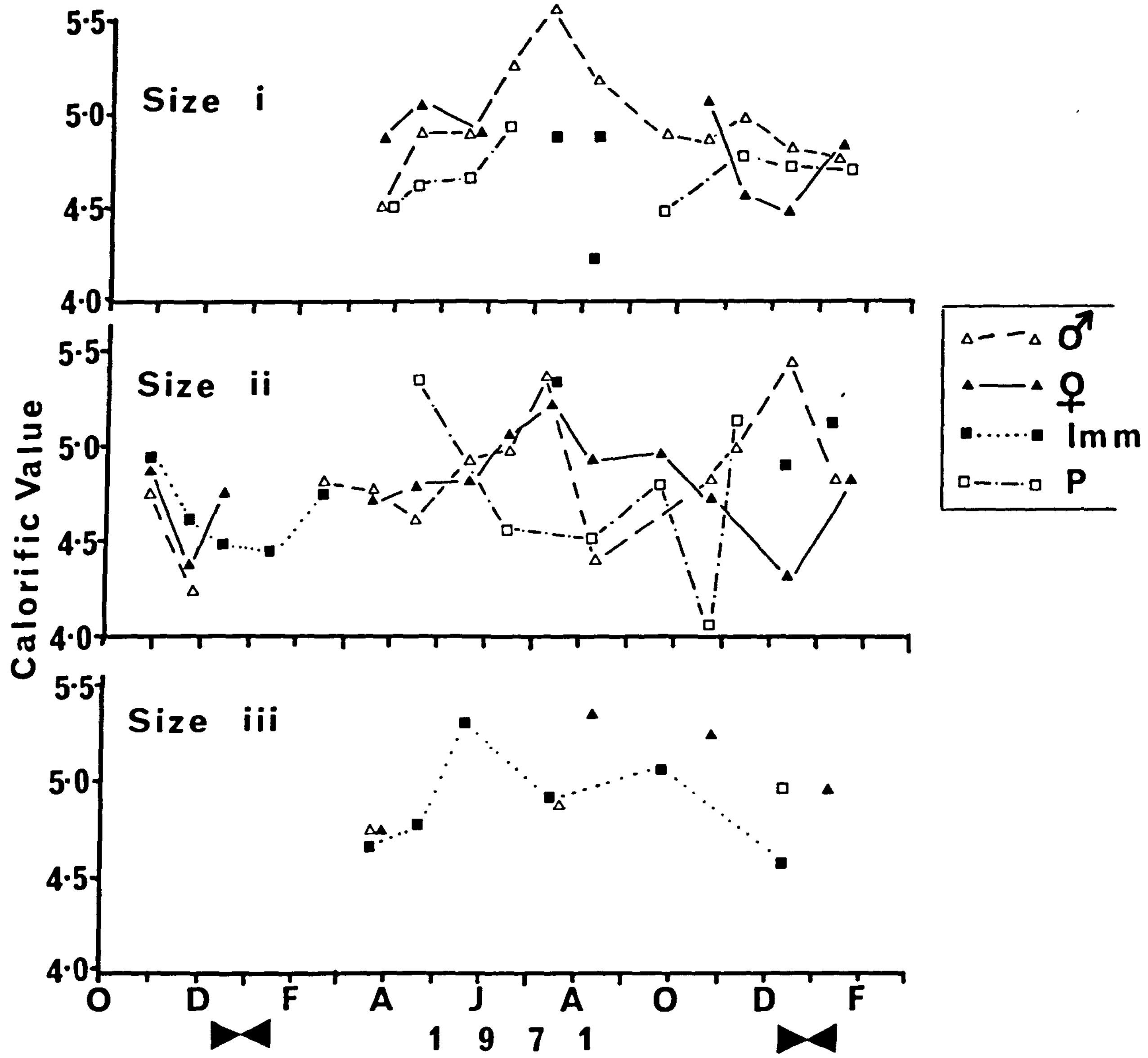

E
Size iii
40
38
36
O
D
F
A
J
A
O
D
F

Figure 50

Calorific value of animals of different standard lengths from October 1970 to February 1972.


Size i
Size ii
Size iii
Calorific Value
5·5
5·0
4·5
4·0
O
D
F
A
J
A
O
D
F
1 9 7 1
♂
♀
Imm
P

early winter. Only the former of these is shown by the female although it is of greater extent, not falling off until October/November.

These variations in dry weight and biochemical composition are not due to gonad accumulation and are still present if a value for the gonad is subtracted from the dry tissue weight (Fig.45b). Such variations resemble the dry tissue weight variations seen in other populations (Orr 1934, Barnes, Barnes and Finlayson 1963, Ansell et al 1964, Omori 1968, Boddington and Mettrick 1971, Moss and Lawrence 1972). Increases represent accumulations of material during peak periods over and above that necessary for gonad and everyday use (Barnes, Barnes and Finlayson 1963) while decreases represent utilization of these reserves in times of stress. As in cirrepedes (Barnes 1972) and other bivalves (Ansell 1973) all components are apparently utilized under such circumstances. In T. gouldi two features emerge as regards the seasonal cycle, these are:-

1) a tendency for all components to increase during the period April to June

and 2) a tendency for all components, but especially carbohydrate and lipid, to decrease during the period November to February.

In these two respects this cycle resembles that described for other bivalves in boreal waters (Ansell and Trevallion 1967, Ansell 1972, Ansell in prep.)

Certain differences are apparent due to size and to gonad state. Mature males and mature females generally have the same carbohydrate, carbon and nitrogen content. The amount of lipid is more variable but taken as a whole there is no consistent difference in lipid content between mature animals, whether males or females, as seen in other animals (Bogorov 1934, Marshall and Orr 1955, Greenfield at al 1958, Lee and Chin 1971, Pillary and Nair 1973). In the early spring, however, the females contain considerably more lipid than the males, this may be related to spawning frequency on the part of the females (see earlier).

The presence of a mature gonad does not lead to an accumulation of any one component (Korringa 1952a, Ansell et al 1964, Arinwasan 1966, Nicol 1967, Giese 1969, Cook and Gabbott 1972) but all four measured components are found in roughly the same percentage as they are in the general body.

Summary

The population of T. gouldi under study does not exhibit any clearly defined seasonal cycle as far as reproductive processes are concerned. The individual members of the population are not synchronized so that at all times of the year animals in all stages of gonadial development are present. Once the gonad has reached maturity spawning results in the release of only a small proportion of the total gonadal material present so that only rarely does the adult

regress to the immature state. Spawning by the female is under the control of the male which is itself influenced by variations in temperature and/or salinity. There are slightly more males than females, however, a greater percentage of the males are mature thus ensuring a good supply of sperm for the females. Fertilization of the eggs is thought to occur within the mantle cavity of the female.

In the majority of marine animals, changes in the population mean dry tissue weight are linked with changes in the reproductive cycle, This is not true of T. gouldi. The changes in dry tissue weight are the result of variations in the level of stored material and not to variations in gonadal material. Changes in the dry tissue weight are due to changes in the level of all the biochemical components measured, however, changes in the relative importance of each of the components do occur throughout the year. The importance of any one component is influenced by size, season and also gonad state.

## VI) Aspects concerning the development of *Thyasira gouldi*

Although rearing of bivalve larvae probably started in the middle 19C, it did not really become established until the successful rearing of oysters by Wells in 1927. Since that date few bivalve eggs have been reared through the larval stage within the laboratory, many workers relying on a correlation of larvae collected in the plankton with the occurrence of the adult populations (Stafford 1912, Thorson 1936, 1946, Rees 1950, 1951, 1954). This indirect method, (Loosanoff, Davis and Chanley 1966) although useful, has its limitations as not all larvae are planktonic, also bivalve larvae tend to be very similar in appearence. It is therefore far better, although often very tedious, to rear larvae from known adults.

The majority of larvae reared in the laboratory have been commercially important species (Loosanoff and co-workers, also the review by Loosanoff and Davis 1963, Loosanoff, Davis and Chanley 1966). The only references to development in the Lucinacea are those of Thorson (Thorson 1936, 1946) and later those of Ockelmann (1958, 1961). Thorson inferred from the egg size (170-190um) that the development of <u>T. gouldi</u> (referred to as <u>Axinus flexuosa</u> see Ockelmann 1958) was 'lecitrophic with a short or non-existent planktonic stage'.

All the observations on the development of <u>T. gouldi</u> recorded here were made on embryos developing within the laboratory, from eggs naturally spawned from adults collected in the course of the study of

the reproductive cycle described earlier.

Artificial methods of inducing spawning by raising the temperature, adding chemicals, sperm etc. (Elsey 1936, Loosanoff 1954, Costello et al 1957) generally proved ineffective, the eggs rupturing or the capsule being incomplete. Loosanoff in his studies of bivalve larvae often found that it was impossible to obtain normal eggs by stripping the female, since the final maturation division of the egg occurs just prior to release during natural spawnings. This is also apparently the case with T. gouldi. Stripped eggs always fragmented or were devoid of a capsule.

Thorson (1946) and Ockelmann (1958) have both found eggs within the gill, concluding from this that brooding takes place. In all the T. gouldi I have studied only rarely were eggs found in the gill. It is my opinion that this is due to rupture of the tissue of this area and that brooding does not occur, the eggs being held in the gonad and ejected in the presence of sperm onto the mud surface. Fertilization presumably takes place within the suprabranchial chamber or the mantle cavity of the female, since even eggs collected immediately after their ejection from the burrow were found to be fertilized.

i) The eggs

The eggs are white, slightly oval in shape having a largest dimension of 160-190um and surrounded by a

capsule so that the total size is in the region of 210-260um (Fig. 51 ).

The capsule is initially quite thick (25um). and gelatinous, (Fig. 51a ) resembling the gelatinous membrane surrounding the eggs of other bivalves. Soon after spawning the limits of the capsule become more defined, probably due to a combination of sea water effects, ageing and fertilization changes, giving a more rigid sticky elastic coat 10-13um in thickness. (Fig. 51b ) As development proceeds the capsule tends to become opaque, later forming 'tubercles' which enlarge and obscure the developing larva.

A number of methods were used in attempts to remove the capsule from developing eggs. These gave interesting information on the possible structure and function of the capsule though none met with any real success.

Berg (1967) and Wessel (1967) have reviewed the literature concerning the chemical removal of egg membranes. Most of the methods reviewed were tried with the capsules of <u>T. gouldi</u> but none proved successful. Pepsin and trypsin (Runnstrom 1948a) as well as starch enzymes all destroyed the larvae leaving the capsule apparently unharmed. The plant enzyme Papain (Northrup 1947, Spiegel 1954) and also sodium thioglycolate were also used but again with no success. Ethylenediaminetetra-acetic acid (E.D.T.A.) (Engstrom 1971) similarly had little effect upon the capsule. Hyaluronidase (Monroy and Ruffo 1947), the amphibian

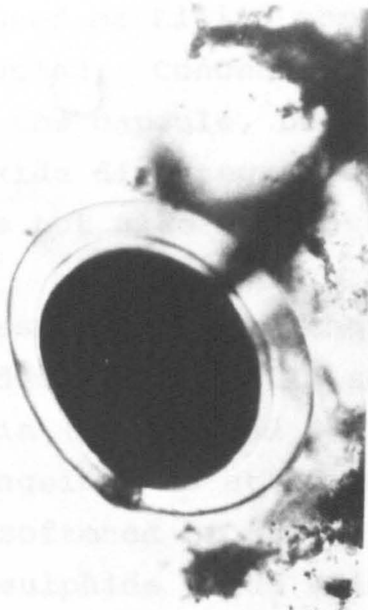
Figure 51

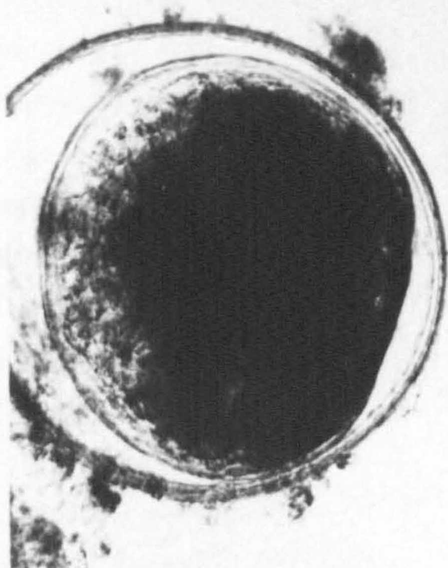
a) Eggs of *Thyasira gouldi* just ejected from burrow of female

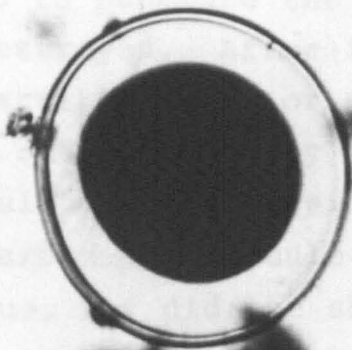
b) Egg after 24 hours in 25$^{o}$/ooS showing 'ageing' of capsule wall

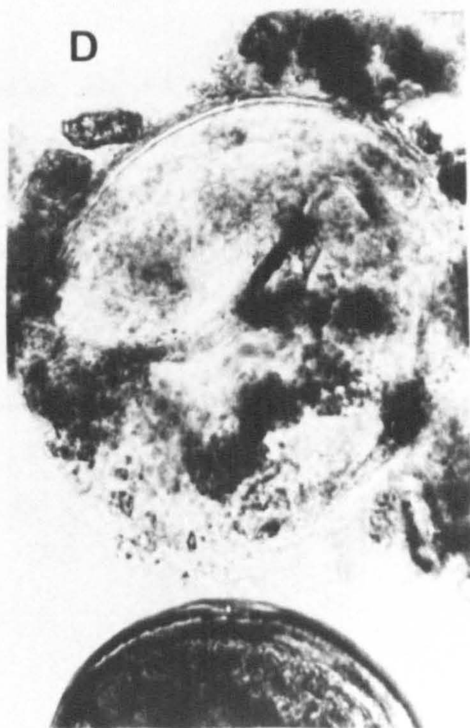
c) Larva at straight hinge stage still contained within capsule

d) Juvenile emerging from capsule


A


C


B


D

hatching enzyme, toluene, cysteine and albumen all either disrupted or killed the larvae leaving the capsule untouched. Concentrated Sulphuric acid had no effect on the capsule, but a strong solution of sodium hydroxide disintegrated the capsule within a few minutes but also destroyed the larva.

These results suggest that the capsule is a sulphide bonded keratin-like substance or some other tanned protein (Monroy and Runnstromm 1948, Hunt 1971) as it shows ageing, is attacked by strong alkali and is slightly softened by thioglycollic acid, which acts on the sulphide bonds which give the capsule its rigidity (Krauss 1950a). None of the chemicals used had any effect upon the capsule without also destroying the larvae.

Attempts to remove the capsule with micro-manipulators also were initially unsuccessful as so much force was required to puncture the capsule that the larvae were also destroyed. After immersion in Carriker's solution (Carriker 1950) or alcohol for several hours the capsule could be easily removed with the aid of micro-manipulators. Immersion in Carriker's solution tended to harden the capsule without causing hardening of the tissue, nor did the shells decay even if left in this solution for several months. Carriker's solution was therefore used throughout the study period as a preservative allowing the study of dead larvae from which the capsule could be easily removed.

The fact that the various chemicals used in trying

to destroy the capsule were able to pass through it and attack the larvae, indicate that the protection afforded by the capsule is that of a 'leaky sieve' rather than a solid wall. This was supported from observations of the effect of salinity upon the larvae described later. The capsule's function, therefore, is not to isolate the larvae from the environment; it may act as a physical barrier to the entry of protozoa or bacteria, or may afford some degree of protection in enlarging the diameter of the egg. Alternatively its function may be merely to entrap the larva, (which resembles that of planktonic forms) ensuring that development is non pelagic.

In the study of development although the sticky capsule made observations difficult, it did facilitate changing the water. Also no additives eg. Sulmet (Calabrese 1969) were necessary to combat bacterial attack (Walne 1958, Guillard 1959).

Dispersal of the egg

In most non-motile marine forms, the dispersal of the species is achieved by the dispersal capacities of the egg and/or the larvae.

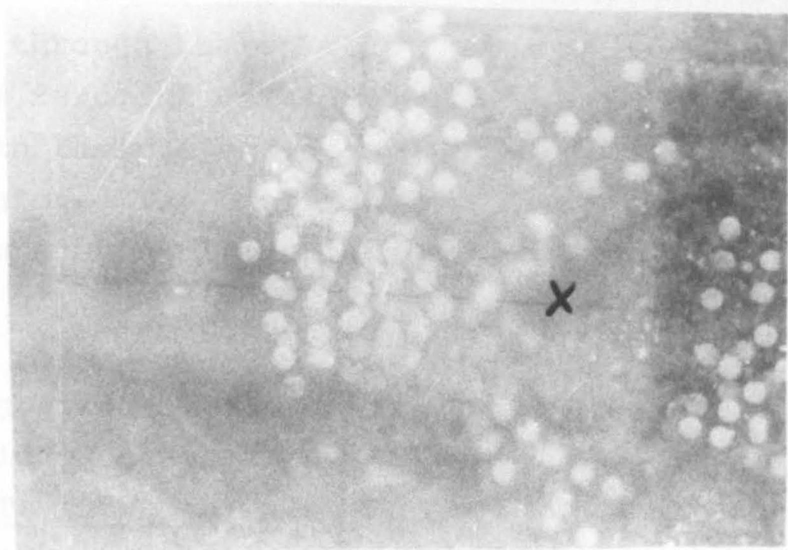
In all observations of the eggs of _T. gouldi_ it was noted that the eggs rapidly settled and were, when ejected from the inhalent aperture of the burrow, only scattered within 1cm of this aperture (Fig. 52a). It is probable that the eggs after their release into

Figure 52

A) Eggs on sediment surface as ejected from burrow
X - inhalent aperture to burrow.

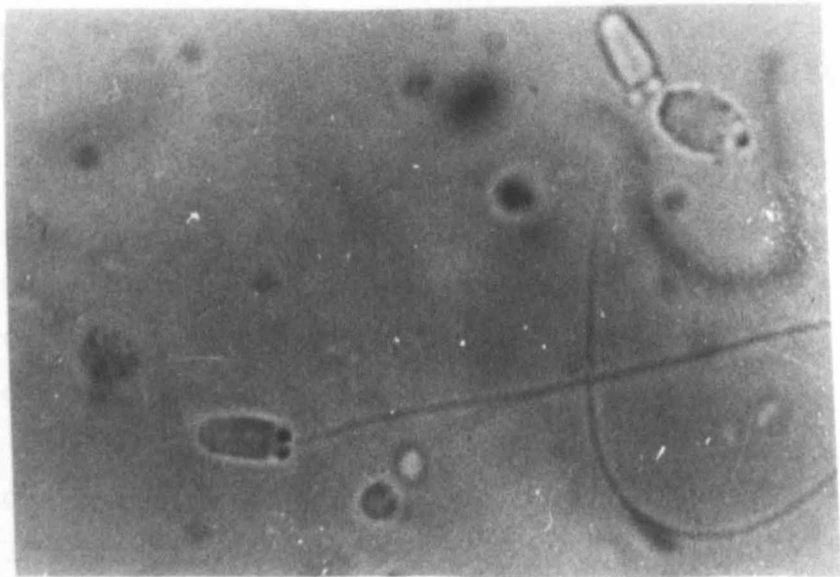
B) Sperm of *Thyasira gouldi*

A



1 mm.

B



5 μ

the suprabranchial chamber are carried by some mechanism, though not through the gills, into the mantle cavity, from here forceful contraction of the adductor muscles results in their ejection out through the normally inhalent region. The ejection force is sufficient to shoot the eggs about ½-1cm up into the overlying water column.

From the egg size (170-190um) Thorson (Thorson 1936, 1946) concluded that the development of T. gouldi (referred to as T. flexuosa) must be lecitrophic with a short or nonexistent pelagic phase, this has now been shown to actually be the case. The egg, therefore, apparently does not aid in the dispersal of the species at all. However, to determine the possibility of the egg being carried by currents within L. Etive the settling velocity of the egg in water of various salinities was determined.

Because the capsule in the early stages of development is very sticky such eggs were very difficult to handle, thus eggs of two to three days old were used in all experiments. These were carefully washed and as much foreign matter as possible removed from the capsule. They were then quickly washed in water in the test salinity and transferred with the smallest amount of liquid possible into a 5ml graduated cylinder of water of the test salinity. The time taken to drop 3cms was noted and transformed into a sinking rate expressed in cms/sec. Five test salinities were used, 33.5, 40, 50, 60 and $70^{o}$/ooS, all made by the addition of hypersaline solution, formed by the slow

evaporation of sea water to water of $32^{o}$/ooS. Eggs were dropped into the test salinity in batches of two or three, the slowest sinking rate (the egg most readily carried by any current) being determined for each batch. Ten such determinations were made for each of the five test salinities, the eggs being allowed to fall at least 1cm before any readings were taken. All the experimentation was carried out in a room the temperature of which was controlled at $10^{o}C \pm \frac{1}{2}{}^{o}C$.

The results of these experiments are summarized in Fig. 53 . There was quite large variation in the sinking rates obtained, probably due, at least in part, to small particles adhering to the capsule. When the average sinking rates obtained for each salinity are extrapolated to zero (Fig. 53 ) a salinity equivalent to $110^{o}$/ooS is indicated as necessary to ensure flotation. The eggs therefore have an average density of 1.0883 (Admiralty Nomograms). Even if the slowest sinking rates are used, a salinity of $100^{o}$/ooS is indicated as being required for flotation (ie. eggs have a density of 1.080).

Stokes Law states that the settling velocity of particles can be obtained (Krumbein and Pettijohn 1939) from the formula:

$$v = cr^2$$

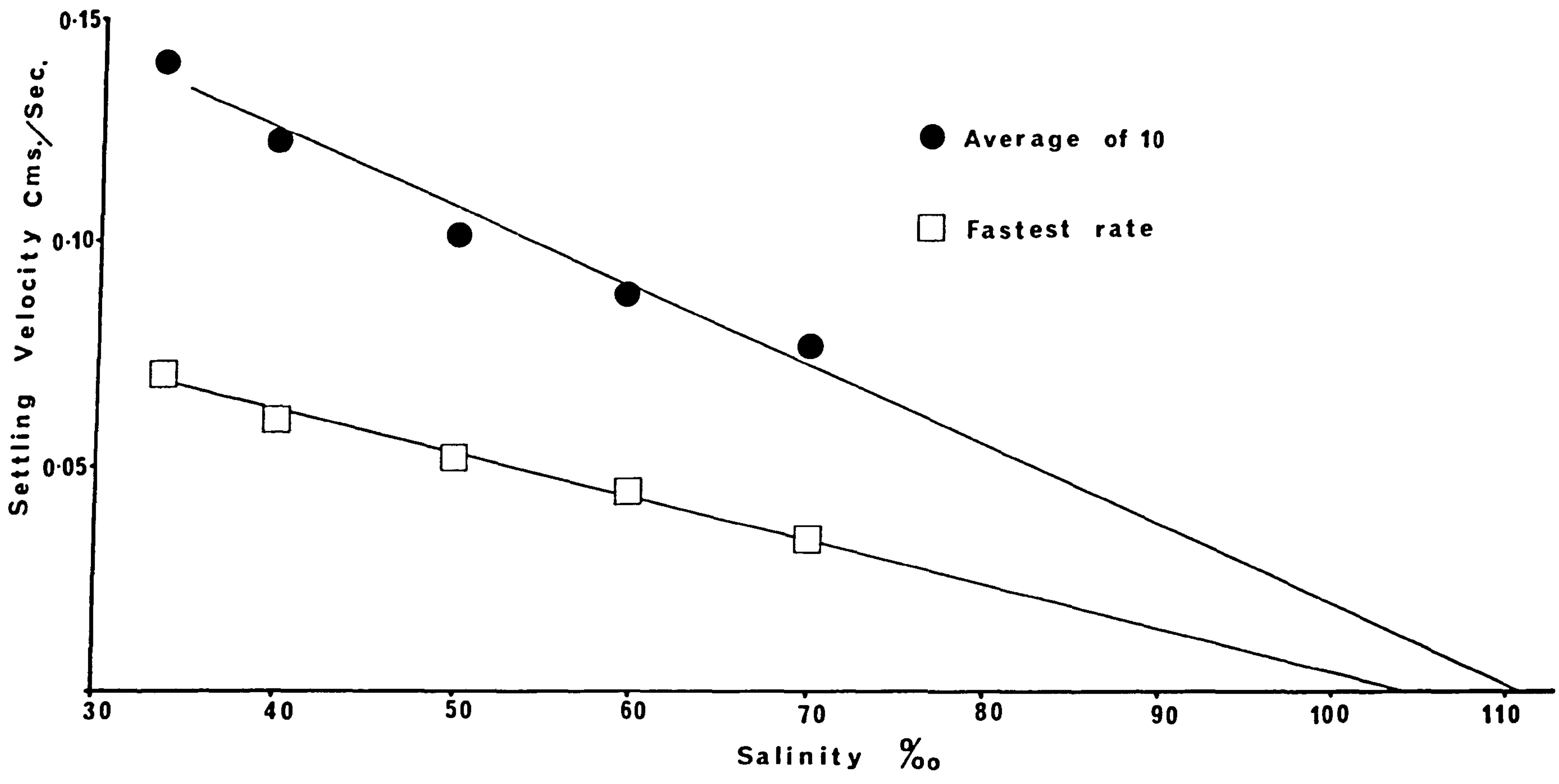
where v = settling velocity, r = the radius of the particle, and:

$$\text{the constant } c = \frac{2(d_1 - d_2)g}{9}$$

with $d_1$ = density of particle, $d_2$ = density of fluid,

Figure 53

Settling velocity of eggs of *Thyasira gouldi* in water of different salinities.



Settling Velocity Cms./Sec.
0·15
0·10
0·05
Average of 10
Fastest rate
30
40
50
60
70
80
90
100
110
Salinity ‰

g = force of gravity, = viscosity of fluid. Thus in the case of the egg of T. gouldi:

$$c = \frac{2(1.084-1)\ 980.6}{9 \quad 0.013}$$

For an average egg size of 220um (the range of egg size with capsule being 210-230um) the settling velocity in fresh water at $10^{O}C$ would be 0.1704 cms/sec.

An average specific gravity for the majority of sediment particles is given by Krumbein and Pettijohn (1939) as 2.65. Assuming this value for the sediment in L. Etive, a particle with a settling velocity of 0.170cm/sec would have a diameter of $4.96 \times 10^{-3}$ or 49.6um or 4.33Ø (Ø = -log Krumbein and Pettijohn 1939),

$$\text{ie. } 49.6\text{um} = \frac{1}{20.16\text{mm}} \doteq \frac{1}{2\text{x}2\text{x}2\text{x}2\text{x}\sqrt[3]{2}} = 4.33Ø$$

From the particle size analysis ie. disaggretated analysis (Fig. 12 ) over 75% of the settling particles are smaller than 4.33Ø. This strongly suggests that in this area the effect of current upon the dispersal of the egg is small. Particles adhering to the very sticky capsule will further restrict any dispersal effect ensuring that development, within the capsule at least, will take place on the bottom in close proximity to the parent. It has been established from a study of development (see later) that there is no free pelagic state, a shelled juvenile emerging

from the capsule. Thus there is no liberation of gametes or larvae (except for sperm) into the water column, an obvious adaptation do the deep water benthic mode of life. The direct benthic development with its associated lack of waste of gonadial material provides a very strong link and reminder of *T. gouldi*'s arctic affinities (Thorson 1936, 1946, Ockelmann 1958), and accounts for the dense but patchy populations found.

ii) The Sperm

The structure of the sperm of *T. gouldi* has already been briefly described in the section dealing with the differences between this species and the closely related *T. flexuosa*.

The sperm of *T. gouldi* which is of the primitive type (Franzen 1953) is 60-70μm in length. The head (5-5.5μm) is rounded without a prominent acrosome, the unsheathed tail tip is about 4.5μm (Fig.3.52b ). Four large mitochondria typical of the majority of bivalves are present.

iii) Development of the egg within the capsule

Several spawnings of eggs were reared until hatching within the laboratory upon which observations are based the following general notes.

At 10°C the first division of the egg occurs

after 16-19 hours. After a further 9-10 hours eight cells were present, the large 'D' cell formed at the first division remaining far larger than the other cells (Fig. 54a ). Within three days the egg had formed into a slowly rotating ball of cells apparently uniformly covered by very short cilia. Thereafter the speed of rotation increased rapidly until after approximately four days the embryo had developed into a typical pear-shaped trocophore. This showed several major ciliary tracts, the apical tuft being formed of the longest cilia (15um in length). (Fig.54 b ) The larva continued to rotate within the capsule until the ninth day when the rotation became very jerky and invagination of the gut began. By the twelfth day a typical straight hinge larva was beginning to form

At around the sixteenth day after release the velum had started to fragment, though the remains persisted for some time. The adductor muscles had become noticeable by the twenty-second day, and by the twenty-sixth day the foot had elongated quite markedly. Gill filaments were noticed on the thirty-fourth day in larvae at 16°C. At this temperature juveniles had started to hatch after the thirty-sixth day although the majority had not hatched until they were thirty-eight days old. At 10°C gill filaments did not form until after thirty-eight days and no indication of the breakdown of the capsule was seen until after the fifty-first day.

Hatching

Observations of the living larvae showed that the

Figure 54

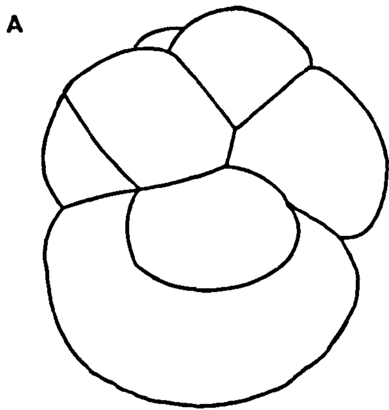
Diagrams of the larvae of _Thyasira_ _gouldi_

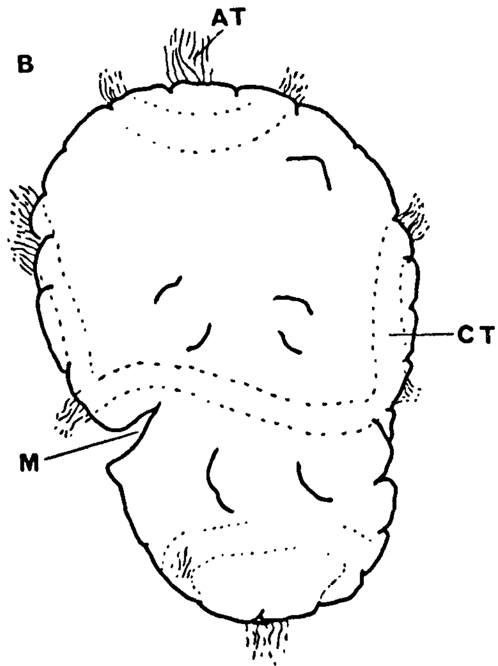
A 28 hours old

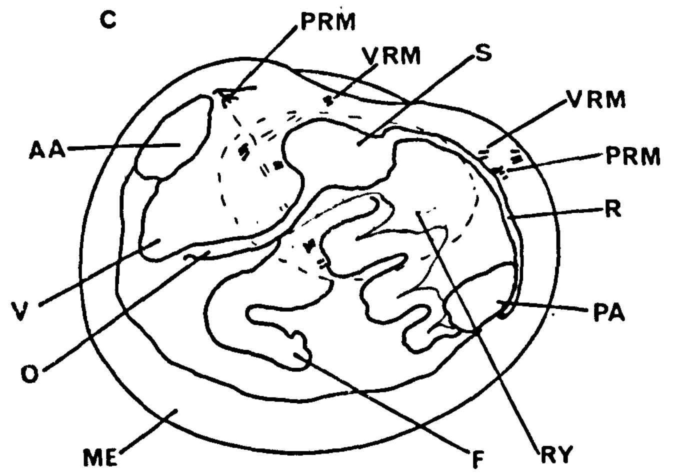
B 4 days old

C 130 days old

For list of abbreviations used see page xviii


A
50 µ


AT
B
CT
M


C
PRM
VRM
S
VRM
AA
PRM
R
V
PA
O
ME
F
RY

mantle often protruded beyond the edge of the shell onto the capsule wall, and that in this region the capsule wall appeared to be thin. It is suggested that the release of the larvae results from secretion of an enzyme from the mantle area which partially digests the capsule wall allowing the larva to emerge (Fig.51d ).

iv) Subsequent development

By the fifty-second day nearly all the larvae at 10$^{o}$C had hatched and a third gill filament had formed. The velum was still present to some degree, and in fact was not entirely lost until the young *Thyasira* was about one hundred and thirty days old. Immediately after hatching the foot was very active probing the substratum. By this time the juvenile was in the region of 220-230um in length with a width of 180-212um. The elasticity of the capsule, which remains for quite some time after hatching, is shown by the fact that the aperture through which the young *Thyasira* escaped is only 50-60um at the most.

By the fifty-sixth day the umbones had begun to develop, the size having increased to 230-237um. The mantle, however, had thickened quite considerably so that observations could no longer be made except by sectioning.

Examination of serial sections of later larvae showed that the velum still appears to be present up

to one hundred and thirty days after release, although it is very reduced. (Fig. 54 c ). Three well developed gill filaments are present and musculature is well formed. The young Thyasira at this stage still however contains a certain amount of yolk. Thorson (1936) showed the relationship of nutritive matter to larval shell size for bivalves. The position of T. gouldi is well above this curve. Thus whilst it is true generally that 'nonpelagic forms hatch at a later stage of development' (Thorson 1936) the young of T. gouldi hatch at a smaller size than would be expected, their growth apparently being restricted by the presence of a capsule. It may be that feeding following hatching is not a matter of great urgency, however, as quite large yolk deposits remain long after the juvenile has hatched.

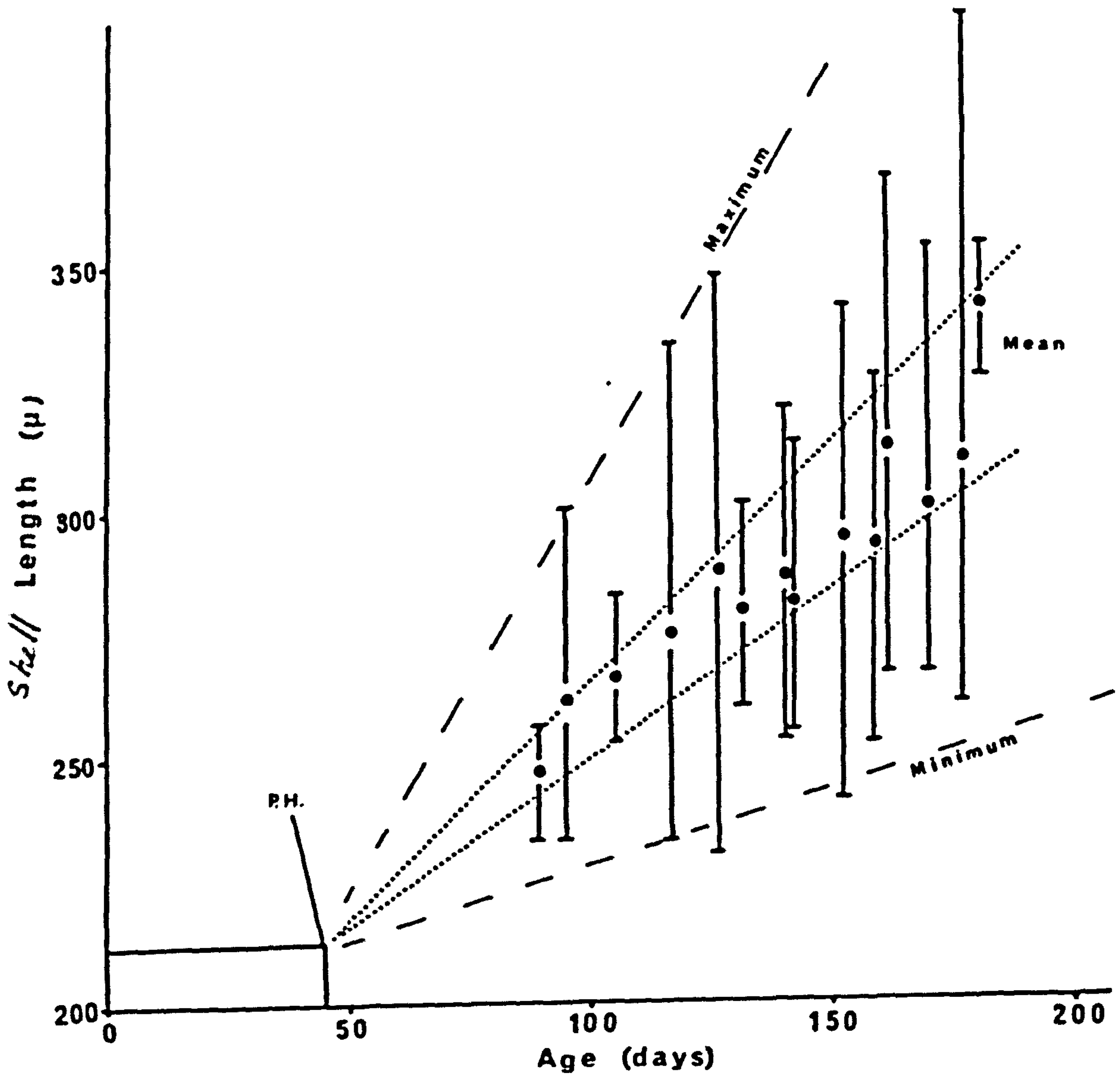
Although the larvae were reared for about two hundred days, little further development was noted, save for the loss of the velum and the commencement of another gill filament.

v) Growth of the juvenile

Groups of approximately fifteen larvae were measured at intervals throughout the two hundred days they were reared. There was quite large variation in size as expected (Loosanoff and Davis 1963). A maximum growth rate within the laboratory at 30°/ooS and 10°C of 100um in fifty days was found (Fig. 55 ), indicating a growth rate of about 1mm per annum.

Figure 55

Growth rate of animals reared within the aquarium



Maximum
Mean
Minimum
P.H.
Shell Length (µ)
350
300
250
200
0
50
100
150
200
Age (days)

This figure agrees with earlier data of the adult growth rings and has subsequently been confirmed by Ockelmann (pers. comm.). During early larval development, growth is quite slow indicating perhaps that growth is restricted, development taking precedence in the encapsulated larva.

Measurements of the larval shell showed that the longest axis is parallel to the hinge line. This is however not true of the adult in which the longest axis is not the 'length' measured parallel to the hinge line but the width, ie. the dimension of the shell measured perpendicular to the hinge line. There is thus a stage when length and width are equal. From the analysis of measurements made of larvae and adult shells (Fig. 17 ) this stage of changeover of shape is apparently reached at about 1.5mm. The reason for this changeover is not certain but may be connected with burrowing, and in some way associated with the 'folds' of the shell resulting in strengthening and better penetration of the substrate. (Stanley 1970).

## Discussion

Although many people have studied the development of bivalve larvae (Carriker 1957, 1959, Creek 1960, Allen 1961a, and a review Allen 1963, Ansell 1962, Loosanoff and associated workers 1963, 1966) few references have been found to work on encapsulated development in bivalves (Drew 1901, Oldfield 1955). From studies of the adult gonad, development within a sticky capsule is thought to occur in some species

of Astarte, Macoma and also Thracia from East Greenland (Thorson 1936, 1946, Ockelmann 1958 and pers. comm.), however, none of these have been followed through the larval stage. Turtonia (Oldfield 1955) and Nucula (Drew 1901) which form the only published work on encapsulated larval development in bivalves, differ in that several eggs are contained within each capsule. In T. gouldi each egg has its own capsule from which it hatches as a fully formed juvenile. The capsule appears to act as a leaky sieve, development continuing within it through the normal trocophore stage for forty to eighty days (probably the slowest developing bivalve yet recorded).

Several genera are mentioned by Thorson (1936, 1946) in which development of southern species is pelagic, whilst that of arctic forms is direct. In the gastropods this is shown by Natica the lamellaridae, and other genera such as Acmaea. Among the lamellibranchs the genus Modiolaria shows direct development in M. discors var laevigata and in M. nigra whilst the boreo-lusitanian M. marmorata has a pelagic larva (Thorson 1936). To these examples we can now add the thyasiridae, the arctic T. gouldi having direct development, whilst the boreal T. flexuosa has a short pelagic stage (Ockelmann pers. comm.). Thorson (1936, 1946, 1950) and Ockelmann (1958, 1962) from their work around Greenland show that the more typically arctic a species so the greater the tendency for lecitrophic direct development. The majority of deep sea forms show lecithrotropic development with a short pelagic phase, although some have direct development

(Knudsen 1961, 1967, 1970), many fresh water bivalves show direct development (Wesenburg-Lund 1909). Direct lecitrophic development frees the larvae from any reliance upon plankton blooms allowing development to proceed at a slow rate (an obvious advantage in cold areas). It does mean that juveniles are deposited close to the adult and it is economical in ensuring the larvae land in suitable areas. Although population increase can be quite rapid the dispersal properties of a planktonic phase are lost and the onus of providing the larvae with food is placed upon the adult. Animals with a planktonic phase which invade arctic areas often are very successful and can inhabit areas where they are incapable of reproducing. This is not possible for the direct developer, where there is little chance of either recolonization or fresh colonization (Ockelmann 1962). The occurrence of T. gouldi in L. Etive is as a contained isolated population. Because of the form of development, the fourth type possible (Mileikovsky 1971) there can be little or no outside recruitment, and it can thus be concluded that this population has existed since the last glaciation when this arctic species was able to colonize areas south of its present day distribution.

Several arctic coastal species have also been found in the tropics, but only at a great depth (Fischer 1883, cited in Thorson 1936). It may well be that T. gouldi will be found yet farther south, but restricted to deeper, thus colder waters.

## VII) Salinity tolerances of Embryos and Juveniles

The distribution of <u>T. gouldi</u> in L. Etive (Fig. 14 ) is apparently restricted to depths below fifteen metres probably due to the effect of salinity. Observations in the laboratory showed that the adult was incapable of any great movement ( see sediment preference) this associated with the direct mode of development means that the larvae and adult are subjected to the same environmental factors. The reason for the limitation of distribution could be due to an effect of adverse salinities upon the adult or upon the larvae, the tolerances of which may or may not be different (Broekhusen 1936, Fox 1941, Rao 1951, Kinne 1953).

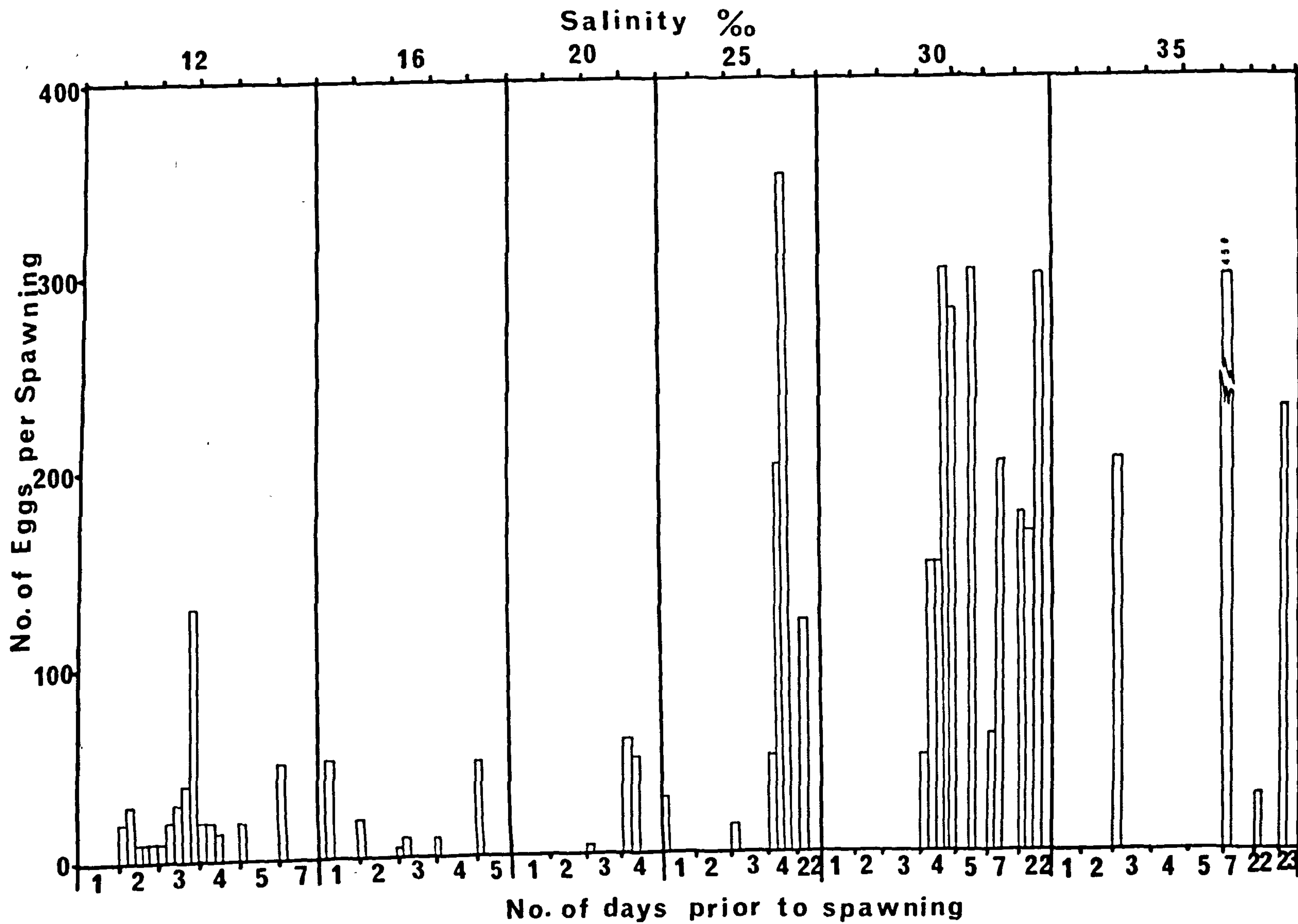
In July 1971 when experiments were being conducted on the salinity tolerances of the adult in each of six test salinities, ie. 12, 16, 20, 25, 30 and 35$^{o}$/ooS (formed by the addition of distilled water or of 'super sea water' formed by the slow evaporation of normal sea water) several hundred eggs were released at different salinities after different periods of time. This provided the opportunity to study the effect of salinity on larval development and also the effect on larval development of the salinity at which the gonad had developed (Davis 1958).

The number of eggs released per spawning, and the time of release at the various test salinities varied (Fig. 56 ).

Fewer eggs were released at salinities below 25$^{o}$/ooS than at either 25$^{o}$/ooS or 30$^{o}$/ooS, the majority

Figure 56

Number of eggs per spawning and number of spawnings obtained for animals maintained in water of various salinity


Salinity ‰
12
16
20
25
30
35
No. of Eggs per Spawning
400
300
200
100
0
1 2 3 4 5 7 1 2 3 4 5 1 2 3 4 1 2 3 4 22 1 2 3 4 5 7 22 1 2 3 4 5 7 22 23
No. of days prior to spawning

of these releases at these lower salinities were within the first few days. This may well be due to stress upon the adult, the lower salinities causing the premature release of gemetes.

In a first series of experiments, eggs from animals kept at the various test salinities were reared at 10°C in water of the salinity in which the adult had been kept. The water was changed daily. The results are summarized in Table 12.

In water of 12°/ooS no eggs lived more than twelve days. Many were released without a capsule and these disintegrated within a few hours. The capsule, when present, at release resembled the capsules of eggs in normal sea water (see earlier), but it failed to contract to form the resilient more permanent structure seen in these other eggs. It may well be that the contraction of the wall is in some way influenced by salts in the water (Chambers 1949).

At 16°/ooS the eggs lived slightly longer, but died within thirteen days. In both 12°/ooS and 16°/ooS there was a relationship between the period the adult had spent in the test salinity before spawning, and the survival time of the released egg. The longer the period of adult exposure to the reduced salinity, the shorter the period of survival of the released egg.

At 20°/ooS development did not proceed beyond the gastrula stage, all the eggs having died by the

Table 12

Summary of Development of *T. gouldi* spawned and reared at the same salinity

| Salinity | Time adult in test salinity before spawning | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 10 | 12 | 20 | 25 | 30 | 35 | 40 | 47 | Comments |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Stage at various time intervals after spawning (days) | | | | | | | | | | | | | | | |
| $12^{o}/oo$ | 24 hrs | | $M_2$ | | $M_3$ | | | | Z | | | | | | | | All larvae spawned at $12^{o}/oo$ fragmented - some were spawned without a capsule. |
| " | 36 hrs | | $M_3$ | | | | | Z | | | | | | | | | |
| " | 48 hrs | | $M_4$ | | | | Z | | | | | | | | | | |
| " | 72 hrs | | $M_4$ | | | | Z | | | | | | | | | | |
| " | 102 hrs | $M_3$ | $M_4$ | | | Z | | | | | | | | | | | |
| $16^{o}/oo$ | 10 hrs | | | | | $M_1$ | | | | | Z | | | | | | lasted longer than larvae at $12^{o}/oo$ but all soon died |
| " | 24 hrs | | | | $M_2$ | | | | | Z | | | | | | | |
| " | 36 hrs | | | | $M_3$ | | | $M_4$ | | Z | | | | | | | |
| " | 48 hrs | | | $M_3$ | | | $M_4$ | | Z | | | | | | | | |
| " | 72 hrs | | | | | $M_4$ | | | Z | | | | | | | | |
| $20^{o}/oo$ | 48 hrs | | B | | | C | | $M_2$ | | Z | | | | | | | Some reached gastrula stage - majority only reached 8-16 cell stage |
| $25^{o}/oo$ | 36 hrs | | B | C | D | | $E_1$ | | $E_2$ | $E_3$ | $F_1$ | $F_2$ | $G_1$ | | $G_2H_O$ | | Later spawnings developed faster - those spawned after 22 days died for some reason |
| " | 48 hrs | A | | C | D | | | | | | | | | | | | |
| " | 22 days | B | C | | D | | | $E_3$ | | Z | | | | | | | |
| $30^{o}/oo$ | 48 hrs | A | | C | | D | | | $E_1$ | $E_2$ | $E_3$ | $M_2$ | $H_1$ | $G_1$ | $G_1/G_2$ | $H_3$ | Later spawnings developed faster - many hatched - though often less developed than those at $25^{o}/oo$. |
| " | 120 hrs | | D | | | | | $E_1$ | $E_3$ | | | | | | $G_2/G_3$ | $H_3$ | |
| " | 22 days | | | | | | $E_1$ | $E_2$ | | | | | | | | $H_4$ | |
| $35^{o}/oo$ | 36 hrs | | | | | $M_2$ | $M_3$ | | $M_4$ | Z | | | | | | | Lived longer than at $12^{o}/oo$ or $16^{o}/oo$ - none developed a shell. |
| " | 48 hrs | | | | | | D | | $M_2$ | | Z | | | | | | |

Meaning of Symbols Used

A - 4 cell stage

B - 8 cell stage

C - Slowly rotating gastrula

D - Rapidly rotating - trocophore

E - Stages in formation of shell
- $E_1$ - shell beginning to form
- $E_2$ - shell nearly complete
- $E_3$ - shell complete

F - Stages in development of foot
- $F_1$ - foot obvious
- $F_2$ - foot well developed

G - Gill filament development
- $G_1$ - 1 gill filament present
- $G_2$ - 2 gill filaments present
- $G_3$ - 3 gill filaments present

H - Hatching process
- $H_O$ - capsule breakdown
- $H_1$ - less than 10% hatched
- $H_2$ - 10-25% hatched
- $H_3$ - 25-50% hatched

M - Moribund
- $M_1$ - less than 10% fragmented or rotting
- $M_2$ - 10-25% fragmented or rotting
- $M_3$ - 25-50% fragmented or rotting
- $M_4$ - 50%+ fragmented or rotting

Z - All dead

eleventh day.

At 35$^{o}$/ooS the eggs lived longer, however, although a rotating trocophore was observed, none developed a shell. In those that spawned later, it was again observed that the eggs died quicker. However, they also developed faster, such that the early trocophore stage was reached by the sixth day and not the ninth day.

At both 25$^{o}$/ooS and 30$^{o}$/ooS eggs survived for the duration of the experiment. Again eggs spawned later, developed more quickly. In those spawned after thirty-six hours at 25$^{o}$/ooS eight cells were present after two days, and by the fourth day a ciliated ball of cells had developed. The shell was formed by the twelfth day, the foot clearly visible after a further eight days, gill filaments were present after thirty-six days. None, however, had hatched before the experiment was terminated, ie. fifty days. In those spawned after forty-eight hours at this same salinity, gill filaments were present after thirty-three days. Those spawned after twenty-two days in this salinity reached the straight hinge stage in only seven days but then died. At 30$^{o}$/ooS where eggs survived from each spawning, there was a similar relationship between the rate of development and the duration of exposure, prior to spawning, of the adult at this salinity as there was at 25$^{o}$/ooS. However, even though larvae hatched at this salinity after only thirty days they were not at such an advanced state of development as those which were of an equal age.

Development of the internal organs is more rapid at 25$^{o}$/ooS, being retarded above or below this salinity which thus appears to be the optimum salinity for development (Ameniya 1926, Ong and Costlow 1970, Lough and Connor 1971). Eggs developed slower, but nevertheless hatched earlier at 30$^{o}$/ooS than they did at 25$^{o}$/ooS, this may be a reflection of the relevant degree of stress caused by these two salinities. Hatching, at least to some extent, seems to be determined by the abundance of yolk within the juvenile which was noticeably less for any given age at 30$^{o}$/ooS than at 25$^{o}$/ooS.

The longer the adult was left in the test salinity before the eggs were released, so the faster the egg developed when released. Earlier releases may have been forced, or conditions may have prevented yolk being deposited in the egg, this was found to affect hatching time and may also affect development.

In the earlier spawnings at 30$^{o}$/ooS on which the description of development was based, hatching was not seen until after the fiftieth day. In these experiments however, again at 30$^{o}$/ooS, hatching had started after only thirty days. This difference is almost certainly connected with the salinity before capture, which was several parts per thousand lower for the former spawnings. This effect of pre-history was also evident in the latter part of 1970 when environment salinities were about 20$^{o}$/ooS. Larvae at this time could not be maintained at 30$^{o}$/ooS but could still be reared at 25$^{o}$/ooS.

Thus salinity changes can be tolerated if these are not too drastic. Spawning may occur if the change in salinity is too great, however, premature eggs incapable of developing are ejected.

Development of the larvae is effected by the salinity at which the gonad matures. (Ameniya 1926, Davis 1958, Stickney 1964).

Hatching appears to be influenced by stress and may well be a reflection of the amount of yolk remaining within the larva.

The capsule shows ageing and its function is not to isolate the larvae from the environment but probably to protect it from protozoa and other small organisms. Pre-history is important, (Fry 1947, Loosanoff and Davis 1952) the higher the environmental salinity so the higher the lethal salinity and vice versa.

Although salinity is seen to effect the development of the larvae, quite a lot of this is thought to be an indirect effect through an effect upon the adult. This is reflected to some extent in the number of eggs released per spawning at the various test salinities (Fig. 56 ).

In a second series of experiments, to avoid the effects resulting from previous of exposure of the adult to differing salinities, eggs spawned from adults maintained within the optimum salinity range (25-30$^{o}$/ooS) were placed into water of various salinities

and the direct effect of salinity upon development thus observed.

Adults were maintained at both 25°/ooS and 30°/ooS at 10°C. Eggs released from these adults were reared at the same salinity until the ciliated gastrula stage, (ie. approximately five days) at which stage they were placed into various salinities and the course of their development followed. Eggs spawned and initially reared at 25°/ooS were placed into water of 12, 16, 20, 30 and 35°/ooS, a control group being kept at 25°/ooS. Those spawned at 30°/ooS were, when five days old, placed into water of 16, 20 and 35°/ooS again maintaining a control group at 30°/ooS. The major changes in development were noted (Table 13 ) the time given being the time following transfer to the test salinity not the age of the eggs (the latter can be obtained by the addition of five days to the time notation in the table).

In the eggs spawned at 30°/ooS transfer to the extremes of the test salinity greatly affected development. Although the capsule remained unaltered in size when placed in water of 16°/ooS the larvae rapidly swelled, reaching a size of 180-220um within the first day. After three days, at a size of 220-230um they could only just rotate, being pressed hard against the capsule wall. The eggs soon fragmented and all were dead within ten days of transfer to this salinity. The eggs placed in to water of 35°/ooS shrank in size and only after a further ten days were they of a size equal to what they were before they were placed

Table 13

Development of T. gouldi spawned at $25^{o}/ooS$ or $30^{o}/ooS$ but reared at various salinities

| Spawning Salinity | Rearing Salinity | Stage of development after transference to rearing salinity (days) 1 | 3 | 5 | 10 | 15 | 18 | 21 | 25 | 28 | 33 | 38 | 41 | Comments |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $30^{o}/oo$ | $16^{o}/oo$ | Egg 183-220um | $M_3$ | $M_4$ | Z | | | | | | | | | Large water intake but eggs soon fragmented - lived less than 10 days. |
| " | $20^{o}/oo$ | D | C | $M_2$,$E_2$ | $E_3$ | $M_3$ | Larvae large - shell pressed against capsule | | | | F | | $G_1$ | 50% fragmented - some however formed a gill filament. |
| " | $30^{o}/oo$ | D | $E_1$ | $E_2$ | $E_3$ $F_1$ | $F_2$ | $H_O$ | $H_1$ | $G_1$ | $G_1/G_2$ | $H_2$ | | $G_2H_3$ | Lowest mortality of this set of expts. 2 gill filaments formed - 30% hatched. |
| " | $35^{o}/oo$ | $M_1$ | | $M_2$,$E_1$ | $E_2$ | $M_3$ | | | $M_4$ | | Z | | | Nearly all formed some shell but in none was a complete shell seen. All dead within 33 days. |
| $25^{o}/oo$ | $12^{o}/oo$ | $M_1$ | $M_3$ Egg 220um+ | | Z | | | | | | | | | Failed to divide properly All dead within 10 days. |
| " | $16^{o}/oo$ | Egg 190-210um | $M_1$ | | $M_2$ 196-230um | $M_3$ | | $M_4$ | | Z | | | | Divided but failed to form a shell - dead within 28 days. |
| " | $20^{o}/oo$ | D | 190-205um | $E_3$ 200-226um | $M_2$ | 216-229um $F_1$ | $F_2$ | | | | $G_1$ | $G_1/G_2$ | $G_3$ | 3 gill filaments seen but none had hatched. |
| " | $25^{o}/oo$ | D,$E_1$ | $E_2$ 190-205um | $E_3$ 200-226um | $F_1$ | $F_2$ | | | | $G_1$ | $G_2$ | $G_3$,$H_O$ | | 3 gill filaments seen but none had hatched - capsule breakdown was seen. |
| " | $30^{o}/oo$ | D | $E_2$ 183-196um | $E_3$ 196-202um | $M_1$ | $M_2$ | $F_2$ | | | | $G_1$,$H_O$ | $G_1/G_2$ | $H_1$ | Some had formed 2 gill filaments 3% had hatched. |
| " | $35^{o}/oo$ | C 157-170um | D 160-170um | $M_3$ 190-196um | $E_1$ | | $E_2$ | | | | $M_4$ | Z | | Slow to develop, none had complete shell, all died within 38 days. |

Meaning of Symbols Used

A - 4 cell stage

B - 8 cell stage

C - Slowly rotating gastrula

D - Rapidly rotating - trocophore

E - Stages in formation of shell
- $E_1$ - shell beginning to form
- $E_2$ - shell nearly complete
- $E_3$ - shell complete

F - Stages in development of foot
- $F_1$ - foot obvious
- $F_2$ - foot well developed

G - Gill filament development
- $G_1$ - 1 gill filament present
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- $H_O$ - capsule breakdown
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- $H_3$ - 25-50% hatched

M - Moribund
- $M_1$ - less than 10% fragmented or rotting
- $M_2$ - 10-25% fragmented or rotting
- $M_3$ - 25-50% fragmented or rotting
- $M_4$ - 50%+ fragmented or rotting

Z - All dead

into this salinity. Development however did not progress very far. Although signs of shell formation were seen, none showed a completely developed larval shell. The larval size in those transferred from 30°/ooS into both 20°/ooS and kept at 30°/ooS tended to be the same. Some of the larvae in both these salinities survived the duration of the experiment, those in water of 20°/ooS being slightly larger but developing slightly more slowly. The shell in those at 30°/ooS was complete by the fifth day as compared with the tenth day in those at 20°/ooS. Similarly, at the termination of the experiment (41 days) the majority of the larvae at 30°/ooS had two gill filaments and one third had hatched, while at 20°/ooS few showed any sign of gill filaments and none had hatched.

Of those spawned at 25°/ooS and transferred to 12°/ooS, all died within ten days, having swollen so that they pressed against the capsule wall. Those transferred to 16°/ooS all died within twenty-one days. Larvae transferred from 25°/ooS to 20°/ooS and to 30°/ooS developed normally, the former developing more rapidly so that by the forty-first day three gill filaments were present.

Of the larvae transferred to water of 35°/ooS none formed a complete larval shell. Thus of the larvae spawned and initially reared at 25°/ooS, only those kept at within 5°/ooS of this salinity survived. Those at 20°/ooS and 25°/ooS reached the most advanced state, showing three gill filaments by the termination

of the experiment.

Larvae at 30°/ooS though reaching a less advanced state of development than larvae at the optimum salinity (25°/ooS) hatched earlier, supporting the earlier belief that yolk store influences hatching time.

From this data it is probable that many of the deaths seen in the earlier experiment were due not to the effect upon the larvae, but to the effect of salinity variation upon the gonad (Davis 1958). The population in L. Etive has adapted to the prevalent low salinities (see earlier) to such an extent that salinities in excess of 30°/ooS (prevalent in areas where T. gouldi is generally found) cannot be tolerated by the larvae. A drop in salinity of up to 10°/ooS from the spawned salinity can be withstood by the larvae, but a rise of 5°/ooS cannot, if this takes the salinity above 30°/ooS.

Time of hatching is not directly linked to development but appears to be a result of stress and food supply, larvae at 30°/ooS being under greater stress and using up yolk reserves quicker than larvae at 25°/ooS.

Pre-history is important, such that the salinity at which the larvae are spawned, and the degree of maturation of the gonad at a salinity, play an important part in the subsequent development of the larvae.

In conclusion, although this population was believed to be.

originally of the same stock as those of areas of higher salinity (33-34$^{o}$/ooS) they have acclimated to such an extent that the larvae cannot tolerate these salinities. Indications are that these high salinities cannot be tolerated by the adult, resulting in premature ejection of gametes and other 'stress' symptoms. This population could therefore be considered as a physiologically different race (Korringa 1957) but, in all probability could re-adapt to higher salinities if the changes in salinity were gradual enough.

# THE BIOLOGY OF THE COPEPOD AXINOPHILUS THYASIRAE (BRESCIANI AND OCKELMANN) PARASITIC ON THYASIRA GOULDI, AND THE EFFECT OF THIS PARASITE UPON ITS HOST

*Axinophilus thyasirae* is a much modified dioecious copepod parasite first described in 1966 by Bresciani and Ockelmann and found in several members of the Thyasiradae (Bresciani and Ockelmann 1966). Its occurrence within the population of *T. gouldi* under study was first noted by Ansell (pers. comm.). His attention to the parasite was, however, scant and thus it was not until this work that it received further and more detailed attention.

### (i) Morphology of the adult

The description of the external morphology of the adult given by Bresciani and Ockelmann (1966) was confirmed although certain additional factors can be added.

The parasite generally attaches to the anterior adductor muscle of its host, the bulk of the body of the parasite lying within the mantle cavity (Bresciani and Ockelmann 1966) (Fig. 57 ). In some cases, however, single parasites have been found in other regions, ie. on the outer demibranch or even between the two demibranchs or attached to the outside of the digestive region. A few were found attached to the mantle where they were often covered in pseudofaeces. One parasite, found close to the inhalent region, was in fact covered with a brown deposit similar in appearance to that often found encrusting the shell. None of these misplaced parasites were mature, mature parasites were found only on the anterior adductor muscles.

The living animal is white and semi-transparent although a certain degree of colouration is provided by the gonads. The body is comprised of three regions (Fig. 58 ).

The cephalic region is weakly segmented into three regions but there are no other outward signs of segmentation. The first region bears two pairs

Figure 57

Diagram of the position and relative size of
*Axinophilus thyasirae* within its host

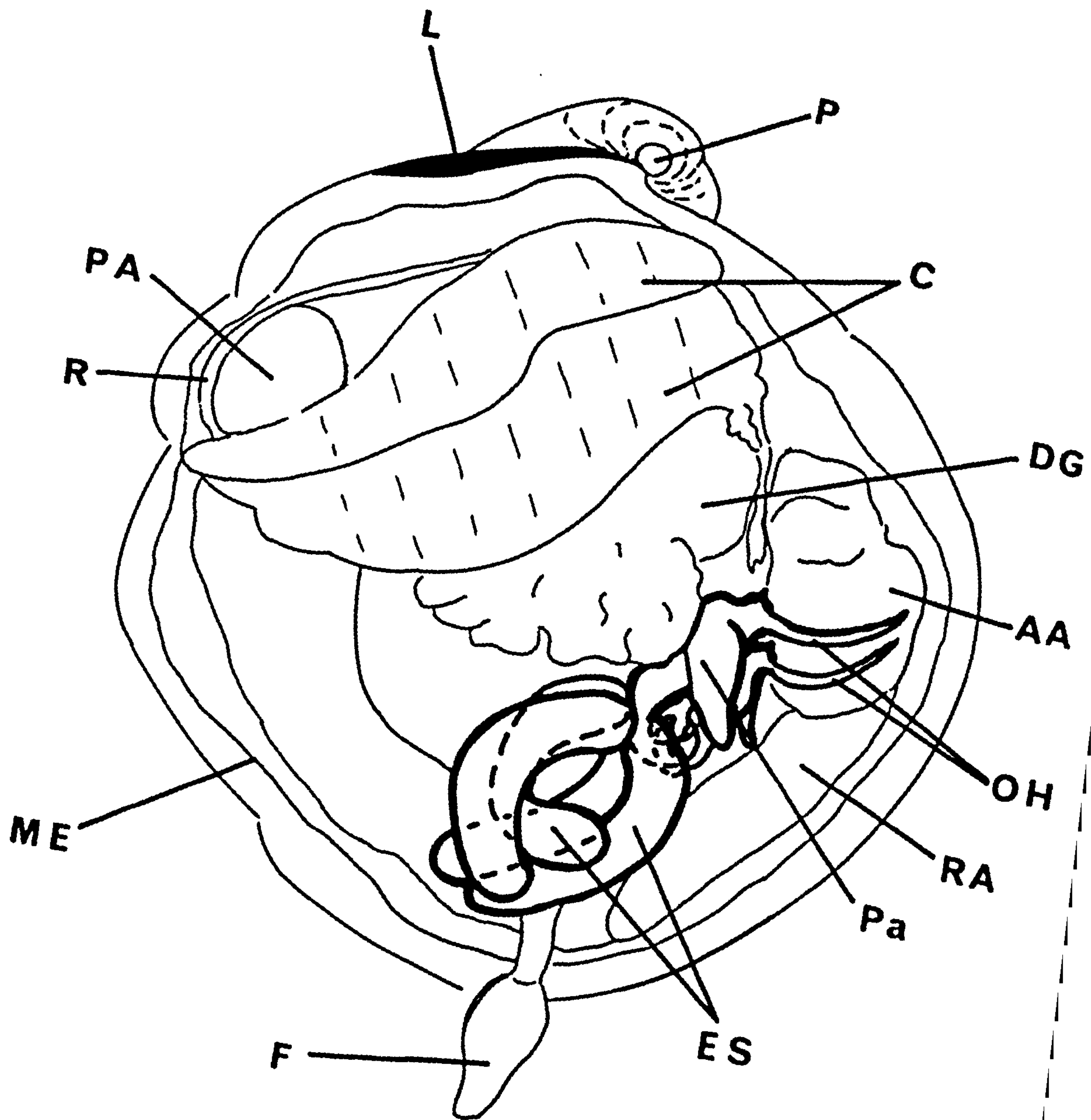

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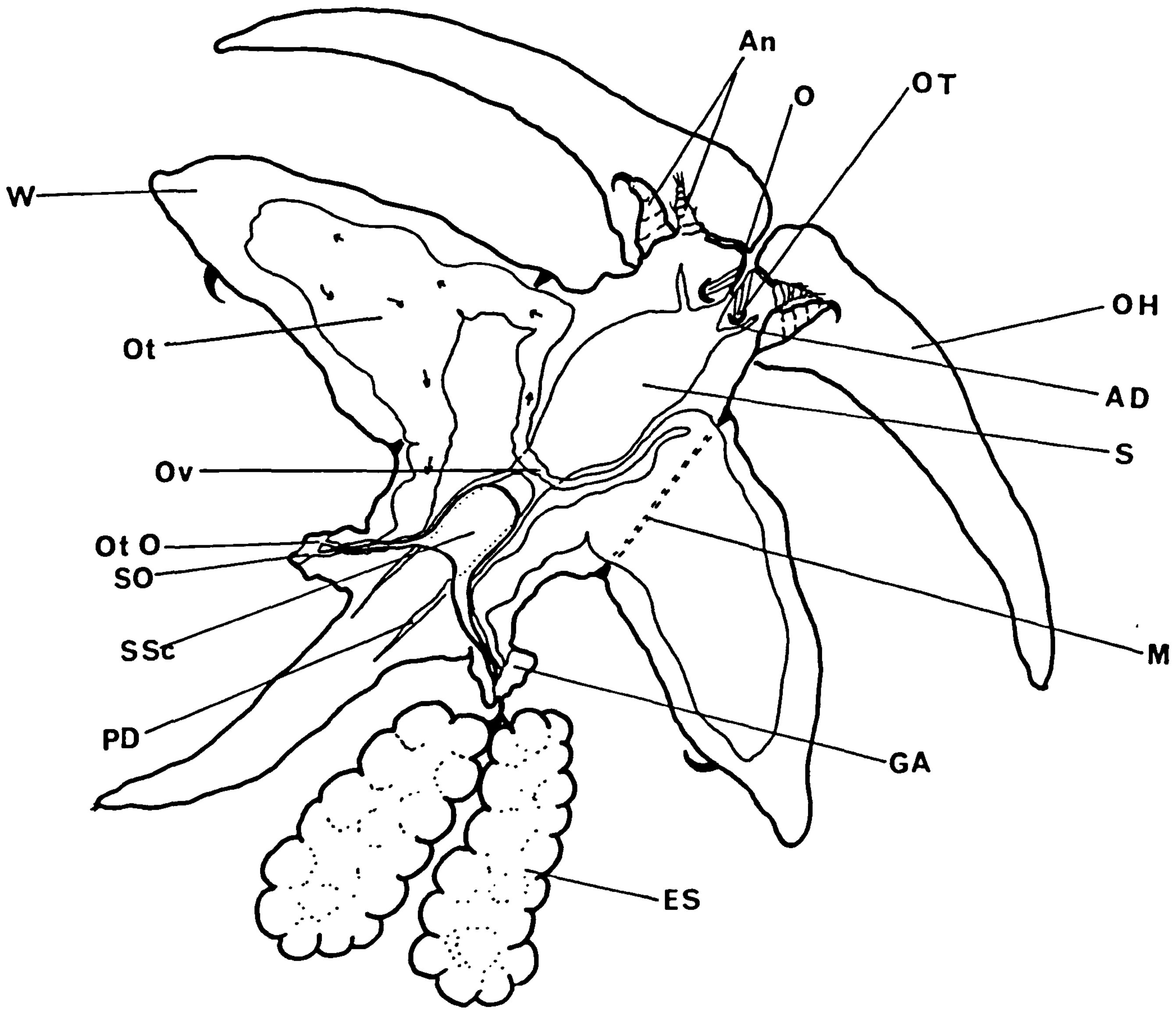
Figure 58

The reproductive system of *Axinophilus thyasirae* reconstructed from serial sections.

Fig. 58A The female reproductive system (dorsal view)

Fig. 58B The male reproductive system (dorsal view)

Fig. 58C The male reproductive system (lateral view)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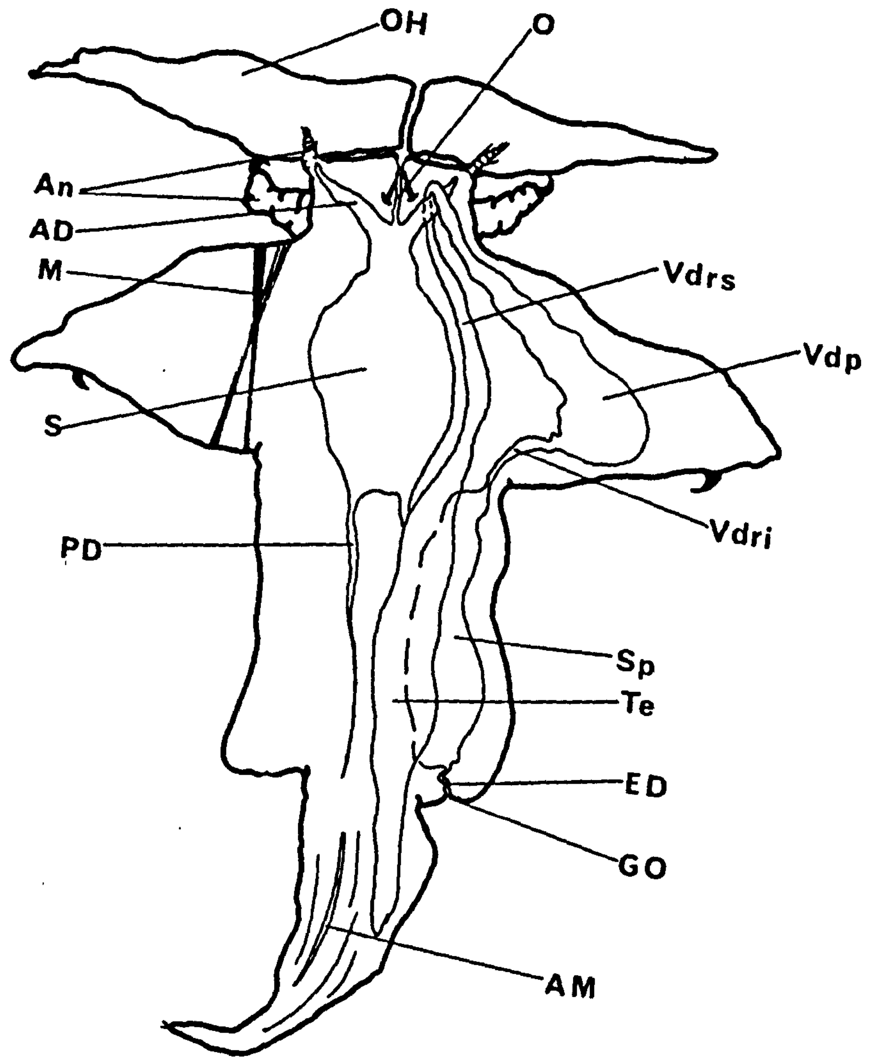

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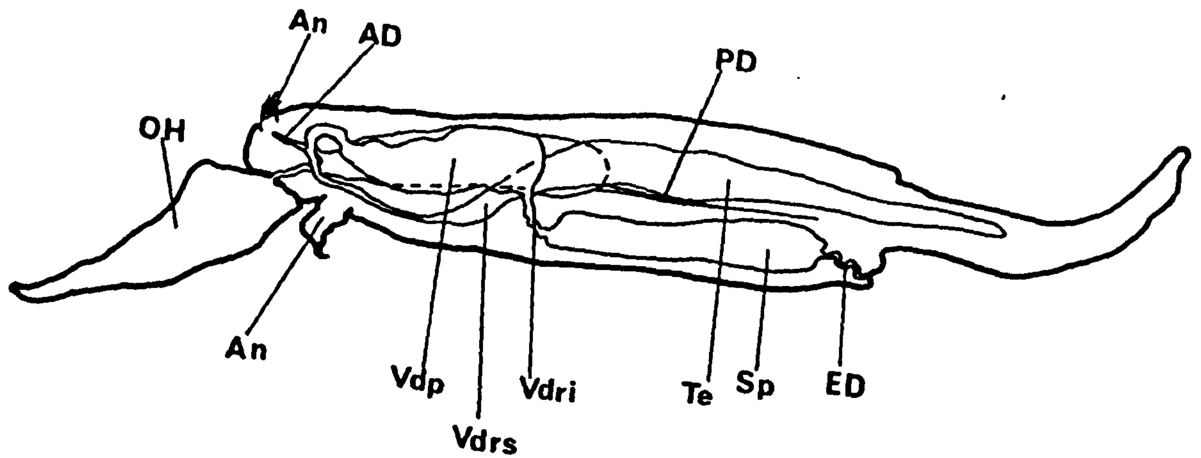

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of antennae, the first pair of which are of four segments terminating in a robust claw, and the second pair of six segments some of which bear bristles. In the adult two large horns are present in the oral region and it is with these oral horns that contact with the tissue of the host is maintained.

The metasoma is larger than the cephalic region and bears a pair of wing-like processes on the posterior borders of which are found a single incurved hook.

The metasoma/abdomenal border is marked by the genital atria. The rest of the abdomen, which lacks any appendages, tapers posteriorally.

Externally the mature female differs from the mature male in that the lateral wings of the metasoma enlarge due to the presence of ripening eggs - these give a yellow-orange colour to the wings. The female carries the double egg sacs which are attached to the atrium. The nauplii remain attached to the central mass of the egg sac, and thus to the female, for some time before they are released.

Oral region and gut

The length of the oral horns has been found not to be related to the length of the parasite, so much as to the size of the host. In parasites which are off centre in their attachment to the anterior adductor muscle, the oral horns are of uneven length. It would thus appear that attachment has to reach the extremity

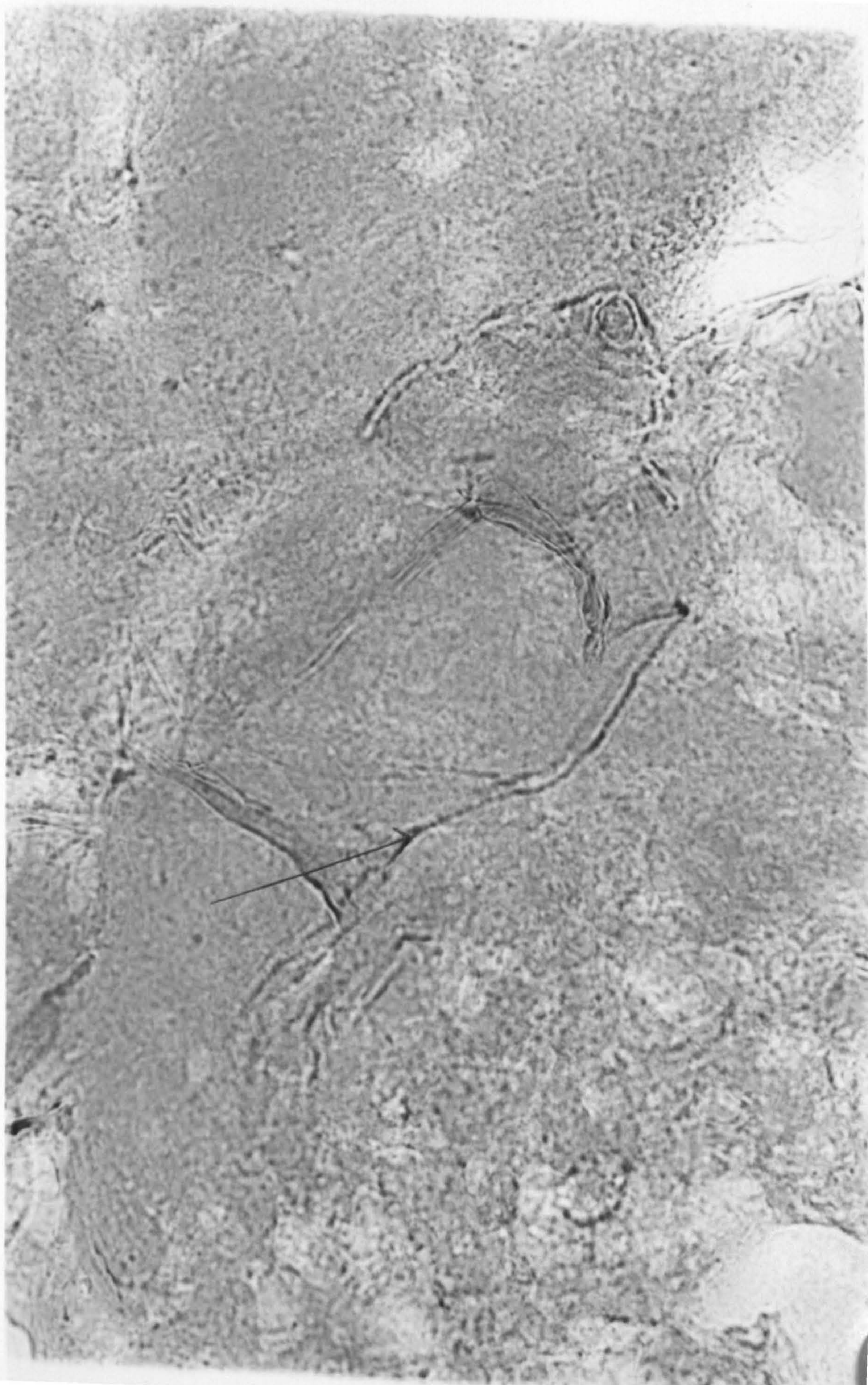
of the adductor muscle, the tip of the oral horns being closely applied to the shell. Such a mode of attachment can be imagined as greatly strengthening the attachment and may effect the activity of the adductor muscle. From serial sections of the parasite it can be seen that the two large oral horns appear to grow out from a chitinized thickening (Fig. 59 ) similar to that found in the Lamippidae (Hockley 1951, Humes 1953, Bresciani and Lutzen 1962, Kabata 1965) and which is probably, as in this family, formed by the fusion of the labrum, labium and mandibles. The oral horns may also be homologous with one or more of these mouth parts.

The mouth, which is found in all stages except the naupliar, is situated between the horns but dorsal to them, so that any suction by the mouth operates along the outer surface of the adductor muscle. The parasite feeds by interrupting the passage of material across the adductor muscle, and an examination of the internal anatomy of the oral region reveals quite large muscles attached to the chitinized buccal area, indicating that the mouth region may be capable of a quite strong sucking action.

The mouth connects by means of a thin oesophagus into a wide gut which extends through much of the metasome. Two diverticulae are given off anteriorly into the paired second antennae and two more appear to be given off into the abdomen. No anus was found.

Figure 59

Whole mount preparation of *Axinophilus thyasirae* showing the chitinized thickening in the oral region. as arrowed.



## Reproductive Organs

The nomenclature of Heberer (1932) and also Fahrenbach (1962) has been followed for the reproductive system.

The anatomy of the female reproductive system, reconstructed from serial sections is given in Fig. 58a.

The paired ovaries are ventral to the gut, the youngest stages of oogenesis being found at the most posterior end of the ovary, that is close to the metasoma/abdomenal border. The developing eggs pass anteriorly into the oviduct, which then runs posteriorly out into the 'wings' (Fig. 58a). Examination of the 'wings' shows a succession of batches of eggs at different stages of development, such that as one batch is released to form the egg sacs, another group ripens, a sequence of batches of eggs thus being produced by the ovary. Much of the differentiation of the egg mass takes place along this length of oviduct, each egg becoming enveloped in large masses of yolk. The oviduct fills the 'wings' so that in mature females these are swollen and extended with eggs immediately distinguishing such females from males or immature animals. The eggs continue their passage from the 'wings' through a thin walled region of the oviduct and out via the atria to form the egg masses (Fig. 58a).

The spermotheca is a large central 'sac', a duct on each side connecting with the atrium.

Fertilization probably occurs in the genital atrium as the eggs pass the opening of the spermotheca. In all those animals studied which carried egg sacs, the spermotheca still contained sperm, and it is thus probable that one pair of spermatophores can fertilize more than one batch of eggs.

The anatomy of the male reproductive system, again reconstructed from serial sections, is shown in Fig. 58 b.c.

In the fully developed male the paired testes are long, slightly pear-shaped organs, extending from the metasoma/abdomenal junction into the tail-like region of the abdomen. As in other copepods the testes can be divided into three regions depending upon the development of the spermatozoa (Fahrenbach 1962). The most anterior membranous walled section of the testes leads smoothly into the vas deferens recurrens superior (v.d.r.s.). This duct is quite thin walled (approximately 5um) with a lumen of roughly 15um diameter. Initially the duct passes antero/ventrally then anteriorly along the ventro/lateral edge of the stomach, decreasing in size along its length until, in the region of the oesophagus, its wall is only 2-3um in thickness. It then turns dorsally, forming a loop around the anterior diverticulum of the gut, before enlarging markedly to give rise to the vas deferens procurrens (v.d.p.). This region of the male genital system is very thick walled, the wall being about three times the thickness of the lumen, which itself is 12um thick. The v.d.p. forms a loop out into the 'wing' so forming the vas deferens

recurrens inferior. This is only a short duct and quickly gives rise to the very large spermatophore sac which extends from the metasoma/abdomenal junction to the atrium, being connected to the latter by a thin sinuous tube, the ejaculatory duct.

Copulation

Both the male and the female have the same orientation within the host, with their dorsal sides uppermost. The male, however, lies ventral to the female. In all the parasites examined, the abdomen curled characteristically in each sex. In the male the abdomen curls dorsally, whilst in the female it curls ventrally. It is probable that this difference is significant in copulation, since by this curling of the abdomen, the genital atria of the two parasites come to lie close together - a necessary process in copepods without any limbs to manipulate the spermatophore. Prior to copulation, which must involve active participation on the part of both partners, the spermatophore is apparently turned within the spermatophore sac, as no manipulatory appendages are present for orientating the spermatophore (Fahrenbach 1962 describes this process in _Diarthrodes cystoecus_). The neck of the spermatophore is then cemented to the atrium and the contents discharged into the atrium (Park 1966).

ii) Development Stages

The double ovisacs each contain between 50 and 250, depending on parasite size, roughly spherical white eggs of 127-155um in diameter. These remain within the egg sac and give rise to the first naupliar stage. This first nauplius measures from 200-225um in length and appears to be a 'normal' copepod nauplius of the cyclopid type (Hardy pers. comm.) (Fig.60 ).

In the first nauplius the first pair of antennae are 83um in length and are uniramous, composed of four segments. The armament upon these appendages is not very great, there being in all only four setae. The penultimate segment bears one seta and the terminal segment two long setae with a shorter central bristle between them (Fig.61a ). The second pair of appendages are biramous, the exopodite having eight segments with bristles on the last five segments and a long seta on the terminal segment (Fig.61b ). The endopodite is of only six segments of which the terminal segment bears two bristles, the only other bristle found is the single one on the fourth segment. The mandibles which are the shortest appendages are also biramous the endopodite has three bristles whilst the exopodite has four. The nauplius lacks a mouth living on its yolk store. After its release from the central mass of the egg sac, to which it is attached by its two terminal furcae (Fig. 60 ) release being achieved by the breakdown of the central mass, it swims freely in the water for a few hours. However, the swimming activity is not of long duration, the nauplius soon

Figure 60

# The nauplius of *Axinophilus thyasirae*

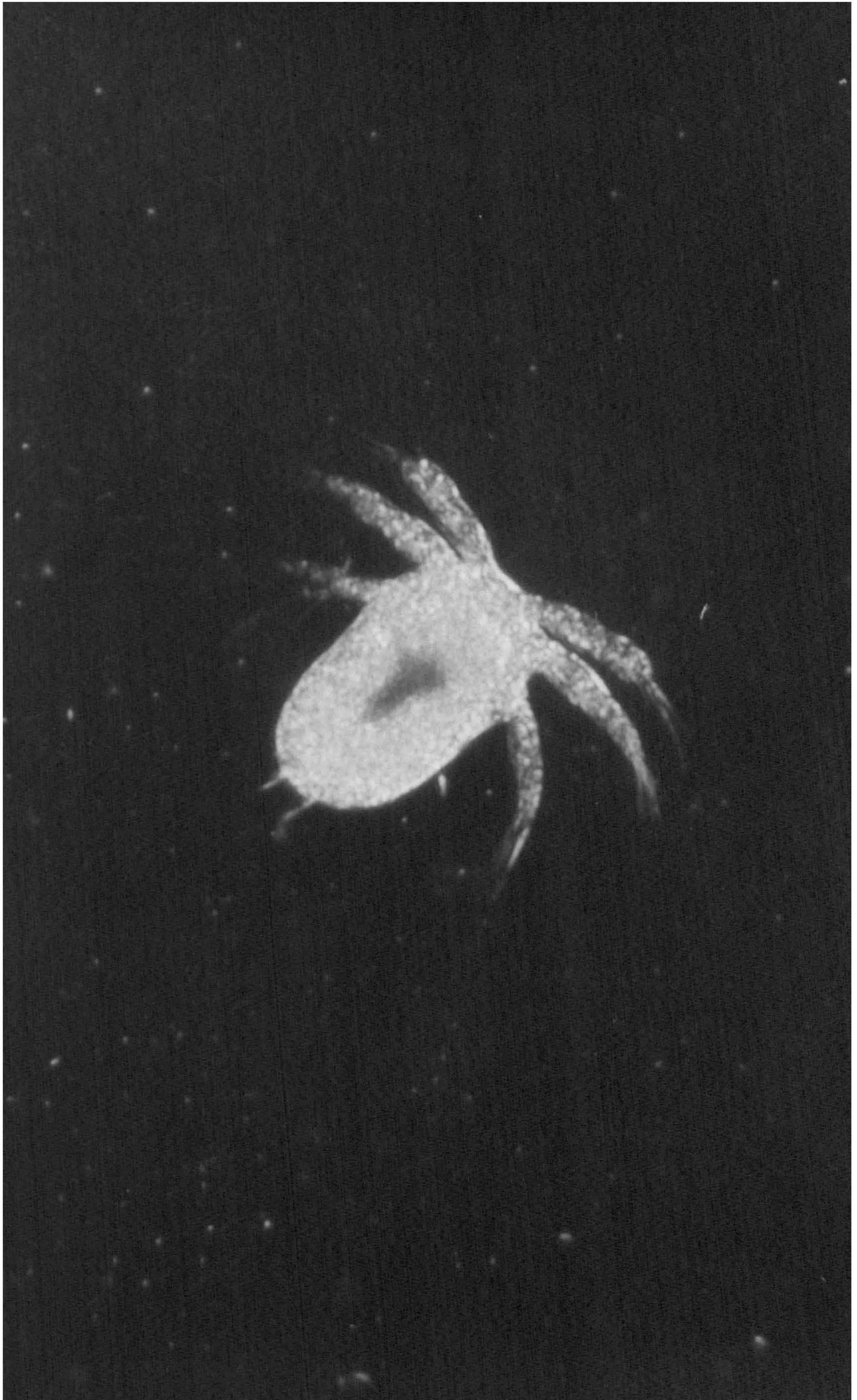


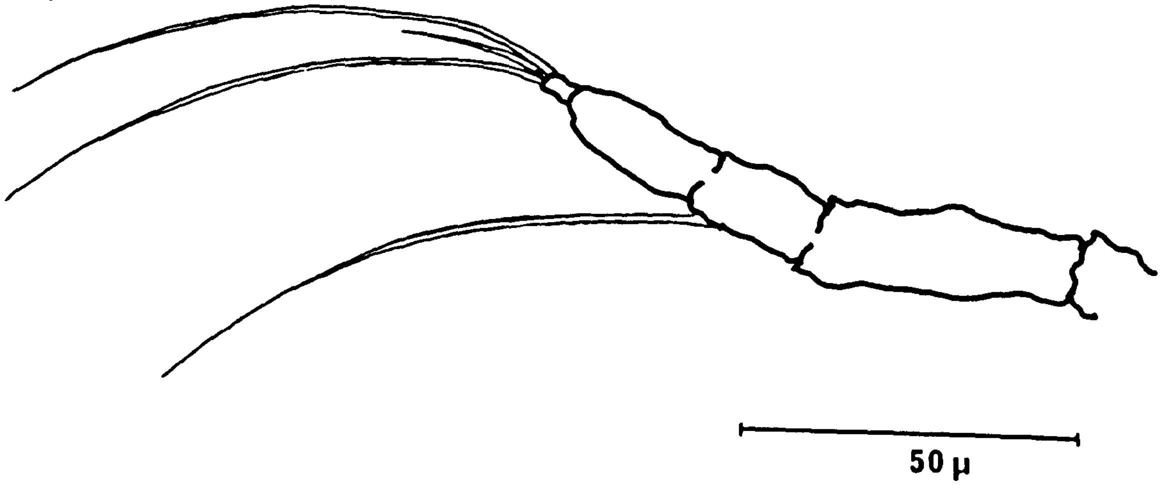
Figure 61

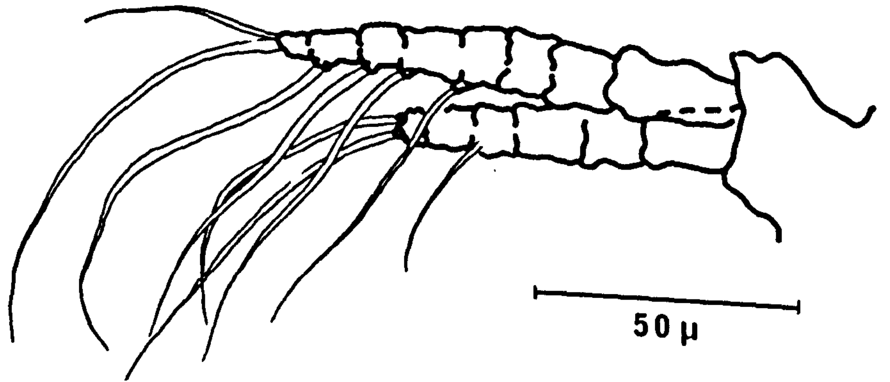
The naupliar appendages

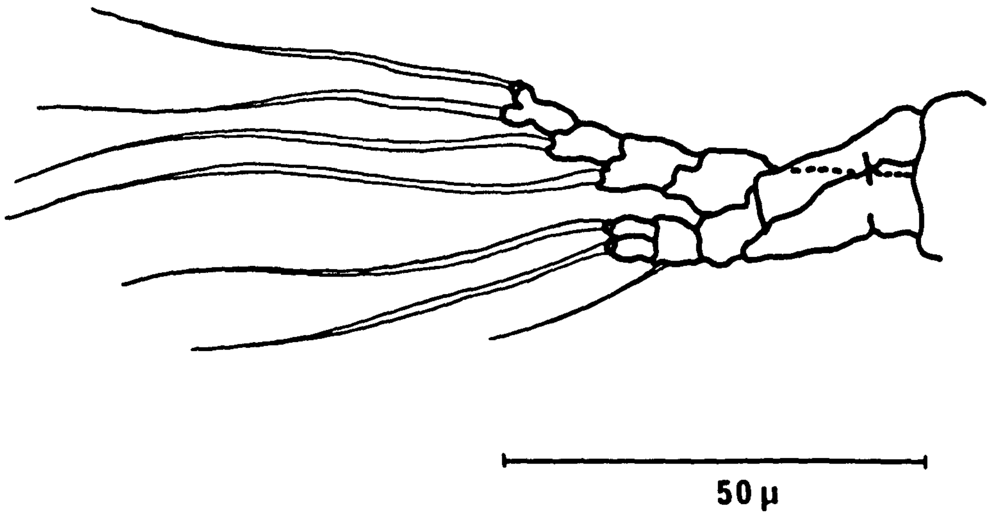
A) 1st Antenna (left)

B) 2nd Antenna (left)

C) Mandibles (left)


A
50 μ


B
50 μ

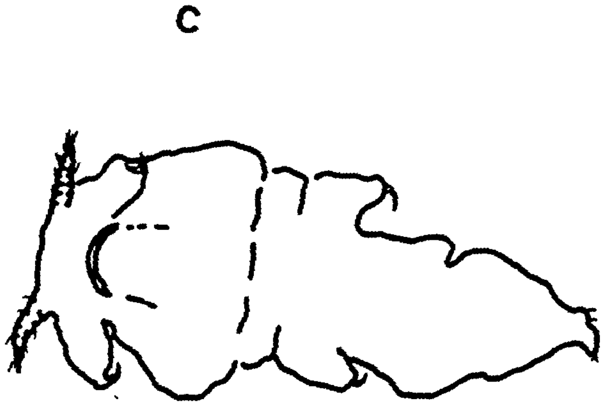

C
50 μ

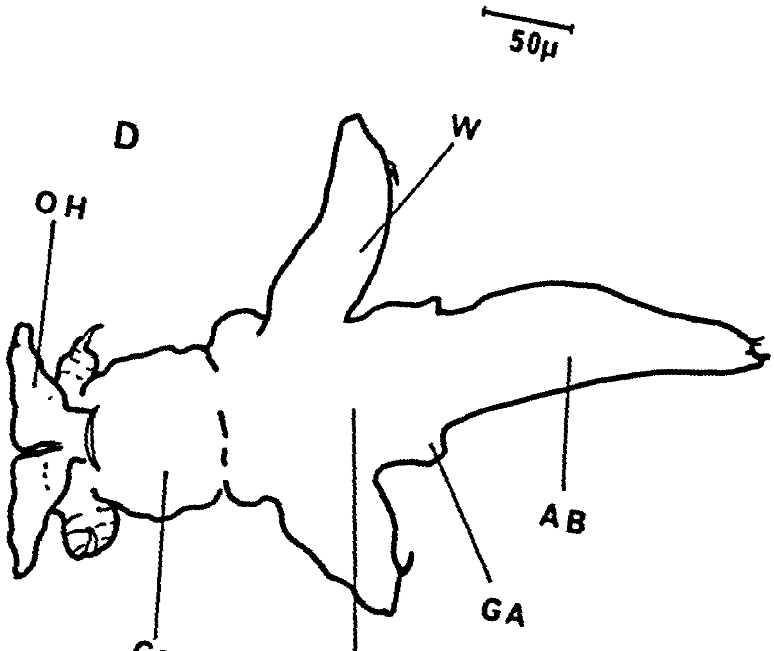
settling and crawling about on the mud surface. It would thus appear that this stage at least aids in dispersal. Whether there is more than one naupliar stage is not known, but the smallest copopedite measures only 226um suggesting a reduction in the number of naupliar stages.

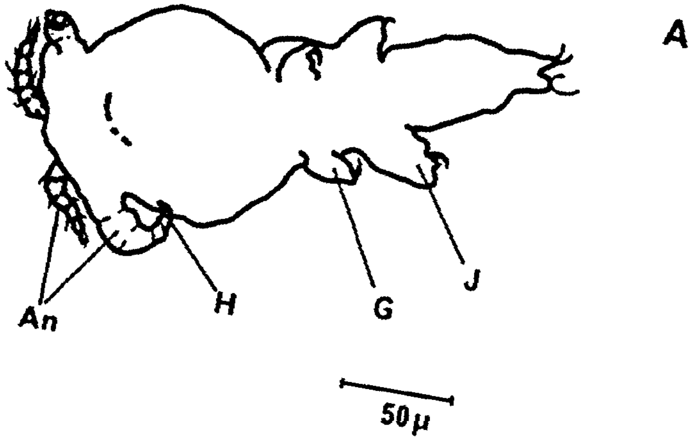
Four copopedite stages were found. These bear, apart from the two pairs of antennae which are similar to those of the adult, only two other pairs of appendages. The first antennae are very reduced, being of six segments with very few bristles, apart from the four terminal bristles or aesthetes. The second antennae are of only four segments terminating in a robust claw. The only other obvious segmentation appears to be a division of the cephalic region into three segments, an outward sign of segmentation which is retained in the adult form. In all the copopedites the abdomen is divided into two terminally, each of these bearing two terminal furcae. The other appendages are very simple and are best seen in the first two copopedite stages (Fig. 62 ). The anterior region of the metasoma bears one pair of these simple appendages, these resemble wing-like structures bearing a single hook. The other pair of appendages are similar though initially larger and found more posteriorly. With development these appendages as such are lost. The latter pair appear to decrease in importance in the adult, their place being taken by the genital swellings. Probably this region, designated by Bresciani and Ockelmann (1966) as the abdomen, is in reality a fusion of thoracic and abdomenal segments.

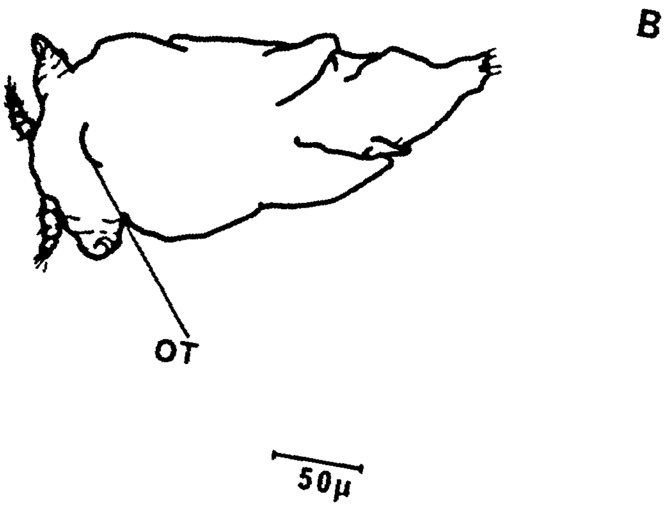
Figure 62

Four of the copopedite stages of
*Axinophilus thyasirae*


A
An
H
G
J
50μ


C
50μ


B
OT
50μ


D
OH
W
AB
GA

The anterior appendages, however, increase and form the 'wings' seen in the adult, bearing the single strongly developed hook which characterizes these appendages in the early copopedites. Large increases of size are seen after the fourth copopedite stage. It is unlikely that this is obtained by moulting, but the body is not chitonized and increase of size may occur without ecdysis. After infection the oral horns begin to form. These are embedded in the host tissue between the muscle fibres and the overlying connective tissue of the anterior adductor muscle, the rest of the parasite lying free in the mantle cavity. It is probable that once the oral horns are embedded the point of attachment is maintained for the life of the parasite.

Initial attachment of the copopedites is obtained by the hooks of these two pairs of appendages as well as by the strong claw-like second antennae. The second antennae appear to retain this function and can be seen to dig into the overlying connective tissue of the anterior adductor muscle of the host. They may, by retaining this point of attachment, ensure a close connection of the anterior region to the host, and also of the mouth, and thus be important in feeding.

Maturity of the female parasite is first reached at a size of 1.5mm. In the male sexual maturity is also reached at a similar size. Although the female can attain a length of over 4.5mm, such a size is never reached by the male which at most reaches just over 3mm.

Bresciani and Ockelmann (1966) touch lightly upon the question of the affinities of this parasite to other copepod parasites. *Axinophilus thyasirae* does have certain similarities with other families, eg. the Lamippidae (Bouligand 1960b, Bresciani and Lutzen 1962), the Notodelphoidea (Gray 1933, Gotto 1954) and to the genus Hatschekia (Scott 1913). However, none of these are very strong. It therefore seems best to place this parasite in the group labelled by Bresciani and Lutzen (1962) as "Incertae Sedis"...... copepods of doubtful systematic position.

(iii) The Life Cycle of the Parasite

The life cycle of *Axinophilus thyasirae* in *T. gouldi* was studied using the data on parasitized animals obtained during the course of investigations of *T. gouldi* described earlier.

From each collection, the total number of *T. gouldi* parasitized was noted. The number of parasites in each host, the gonad state of the host and of the parasite was also noted. Four stages of the female parasite were discernable, based upon whether egg sacs or nauplii were present and whether the female contained ripe eggs, indicated by enlargement of the 'wings'. Also noted was the number of cases in which copopedites were present, the length of the parasite and the length of the host's shell. In addition, for each date when samples of the *Thyasira* population were taken for body weight, or biochemical analysis, similar samples were obtained, as far as possible, for parasitized animals. The data was used to provide information on the life history of the parasite, and on the effects of the parasite upon the host.

The seasonal data for the period August 1970 - October 1971 is summarized in Fig. 63 . This shows the percentage of the monthly samples which were parasitized (ie. percentage infection) and the percentage of parasitized animals which had small parasites (ie. parasites of less than 1mm). It also shows the percentage of parasitized animals which contained a

Figure 63

The seasonal cycle of <u>Axinophilus</u> <u>thyasirae</u>

A) % infection

B) % with copopedites present

C) % of parasites with 'swollen wings'

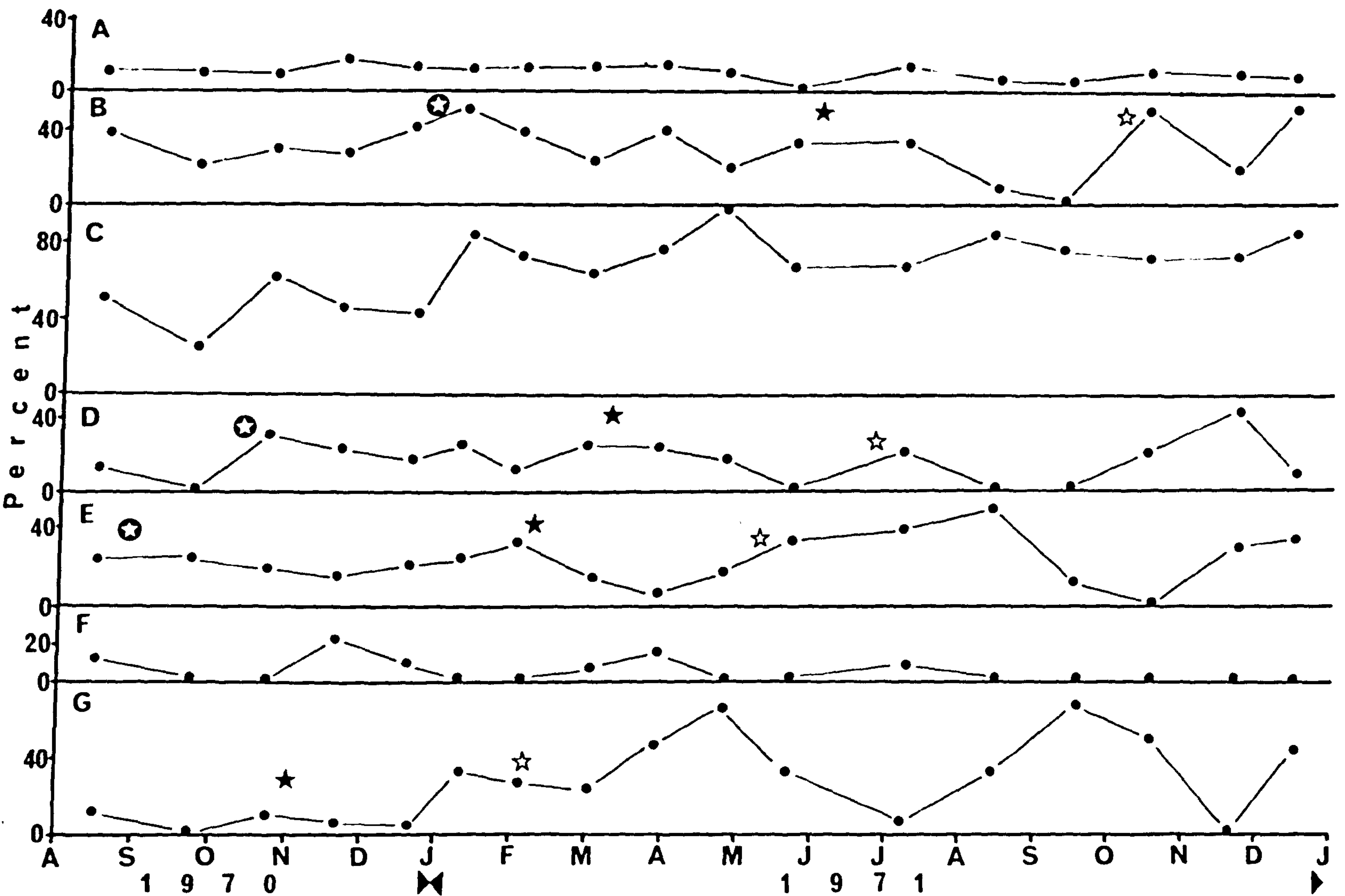
D) % of parasites with nauplii and 'swollen wings'

E) % of parasites with egg sacs and 'swollen wings'

F) % of parasites with egg sacs only

G) % of parasites with 'swollen wings' but without eggs or nauplii

Other symbols indicate supposed sequence of peaks used in calculation of duration of stages of life cycle.



A
B
C
D
E
F
G
Percent
40
0
80
20
A S O N D J F M A M J J A S O N D J
1 9 7 0
1 9 7 1

female which had swollen 'wings', egg sacs or nauplii. This data was based on at least a hundred animals taken from grab samples from a station centrally positioned within the study area (ie. Station E24 Gage 1972).

The major stages of this copepod have been described and the data from the seasonal samples may be used to define the length of life and the relationships between the different stages.

Experiments conducted within the laboratory in which the development of Axinophilus thyasirae was followed within the host (the shell of young T. gouldi being quite thin allows the white parasite to be easily seen), showed that the period to hatching of the egg was at least four weeks, and that the life of the first nauplius stage, which is thought to be the only one present, was at least eight weeks. An indirect method of assessing the number of animals at each stage of development at various times of the year and following the successive peaks of such numbers was relied upon to furnish further details of the life span. The parasites found at each monthly sampling date were classified according to the females' gonad state.

From the data given in Fig. 63 it would appear that the eggs take in the region of 4-5 weeks to hatch to give the nauplius. After about three months, the nauplii have undergone their first moult and are seen as infected copopedites. About half of the

naupliar period is probably spent attached to the remains of the egg sac by the two terminal bristles. The nauplius itself is not thought capable of infection, lacking any sustained motility and also any attachment hooks, and even if placed into the mantle cavity of a host, the nauplii are soon ejected. To this period of development can be added the time taken for the almost ripe gonad - as indicated by full 'wings' - to relaease the eggs into the egg sacs. This would appear to be in the region of two to three months.

After the egg sacs are formed the ovary continues the production of eggs, such that by the time the nauplii are released the 'wings' are swollen by a further batch of eggs ready to be released to form a new egg sac. The period of egg production up to the full 'wing' state can thus be estimated as being in the region of two months. The total development time, from the commencement of gonad activity to the copopedite thus appears to be in the region of eight to nine months. To this can be added the life span of the copopedite stages (3-4 months), thus making a total development time of over a year.

Once maturity is reached, however, the gonad must produce eggs almost continuously, as only rarely were parasites found which were carrying egg sacs but had not got swollen 'wings', whilst when nauplii were present the 'wings' were always swollen due to the presence of mature eggs. It would therefore appear that at least two if not three broods of eggs are produced each year. A further estimate of mortality

and total life span can be deduced from the data given in Fig. 63. If the year April 1970 to April 1971 is studied, the infection rate averaged for the whole year is 10.5%, [≈ 500 animals sampled = all.] which gave 126 parasite infected animals. Of these 70% contained mature females, to give 88 female parasites. If we assume that each female produces two broods of nauplii per year, then as each brood contains at least 200 nauplii, the total production of nauplii for the year would be in excess of 35,200.

Of the animals actually sampled, 16% were carrying nauplii, whilst only forty copopedites were found, giving a ratio of nauplii to copopedite of 8,000:40 or 200:1, representing a mortality of 99.5% between naupliar and copopedite stages. If the total production of 35,200 nauplii possible from 1,200 _T. gouldi_ suffer a mortality of 99.5%, this leaves a total copopedite production of 176, this would replace the adult population of 258 parasites in about one and a half years.

The ratio of the number of copopedites actually found (40) to the number of parasites present (258, there being on average 2.02 parasites per host) indicates that the adult parasite life is in the region of 6.4 times greater than that of the copopedite. The copopedite is thought to live for three to four months (Fig. 63 ) thus an adult life span of 1½-2 years is suggested. It would thus appear that the copepod _Axinophilus thyasirae_ has a total life span from egg to adult to egg of 2½-3 years, of which at

least half is spent in the adult state.

Only in arctic or other cold water forms of copepod is a longevity approaching that of *Axinophilus thyasirae* seen (Brodsky 1950, Digby 1954, Fish 1955, Marshall and Orr 1955, Carter 1969). It may therefore be that this species like its host has some arctic affinities. It parasitizes several members of the Lucinacea (Bresciani and Ockelmann 1966) as well as *T. gouldi*, however, little can be said of its preferences for any one species, as conditions here are different from those studied by Bresciani and Ockelmann (1966). From their work it would appear that *T. gouldi* is not its prime host if several species of Lucinaceans co-occur.

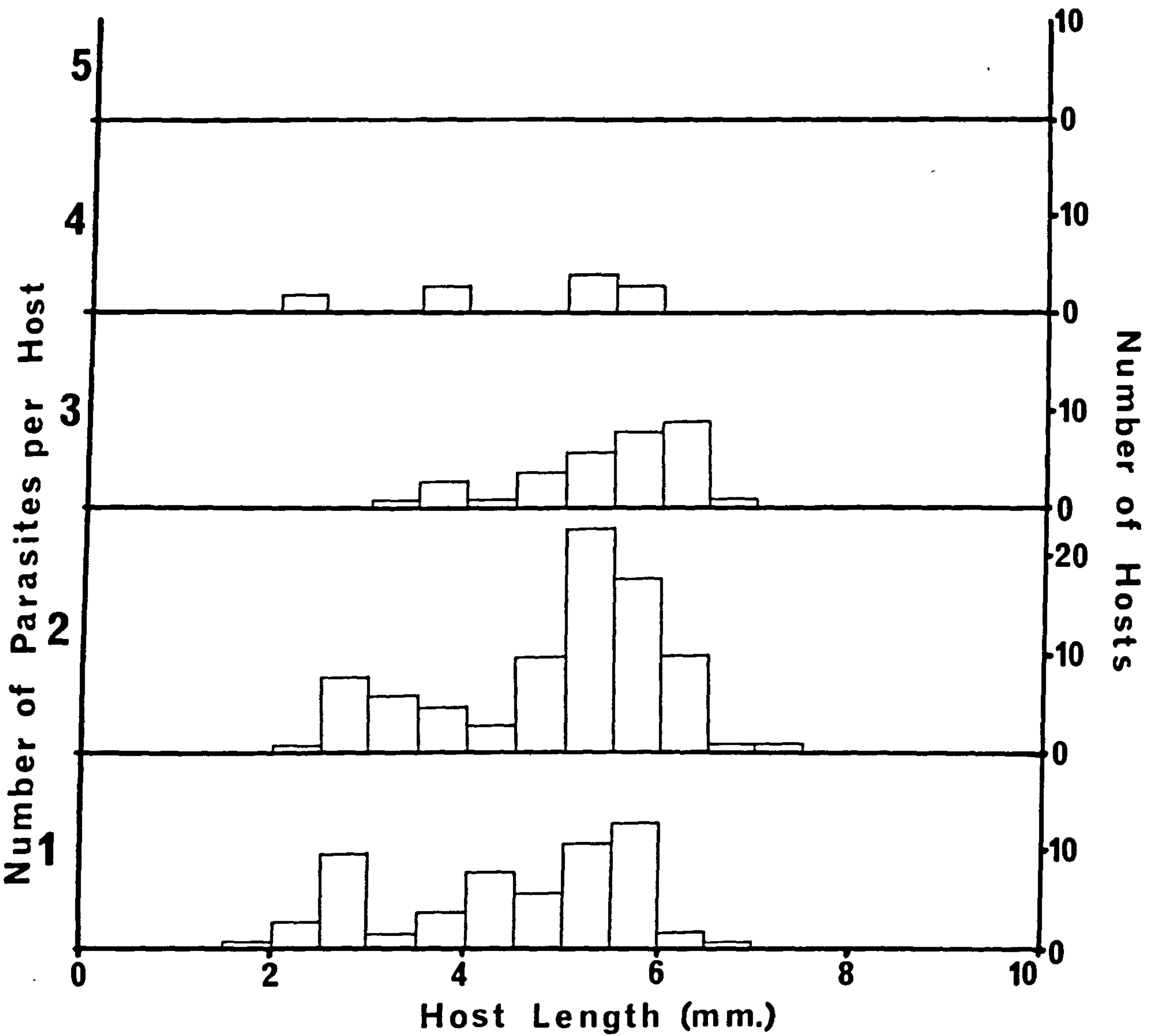
### iv) Parasite/Host Relationship

The host can contain one, two, three, four or even, though rarely, five parasites. The proportions of hosts with each of these numbers are shown in Fig. 64 . The greatest number of hosts have two parasites. In fact more than twice the number of parasitized hosts studied had two parasites, than had a single parasite. This suggests that there is some form of attraction resulting in the attraction of a second parasite to a host already infected, in preference to an infection of a new host, since chance would result in there being a far greater percentage of hosts with a single parasite where there is such a low infection rate.

There is apparently a prime position of attachment within the host, that is in the mid-line across the anterior adductor muscle just ventral to the mouth region. The largest parasite is always found attached in this position, while subsequent parasites lie more ventrally. The second parasite lies immediately ventral to the first while any other parasites lie further ventrally, and are generally displaced from the mid-line. Rarely is a parasite found attached to other than the anterior adductor. It seems probable therefore that when the large parasite dies all the other parasites infecting that host die also. It is difficult to imagine any movement of a parasite which has become firmly attached by the development of the oral horns.

Figure 64

Number of parasites per host


Number of Parasites per Host
5
4
3
2
1
Number of Hosts
10
0
10
0
10
0
20
10
0
10
0
0
2
4
6
8
10
Host Length (mm.)

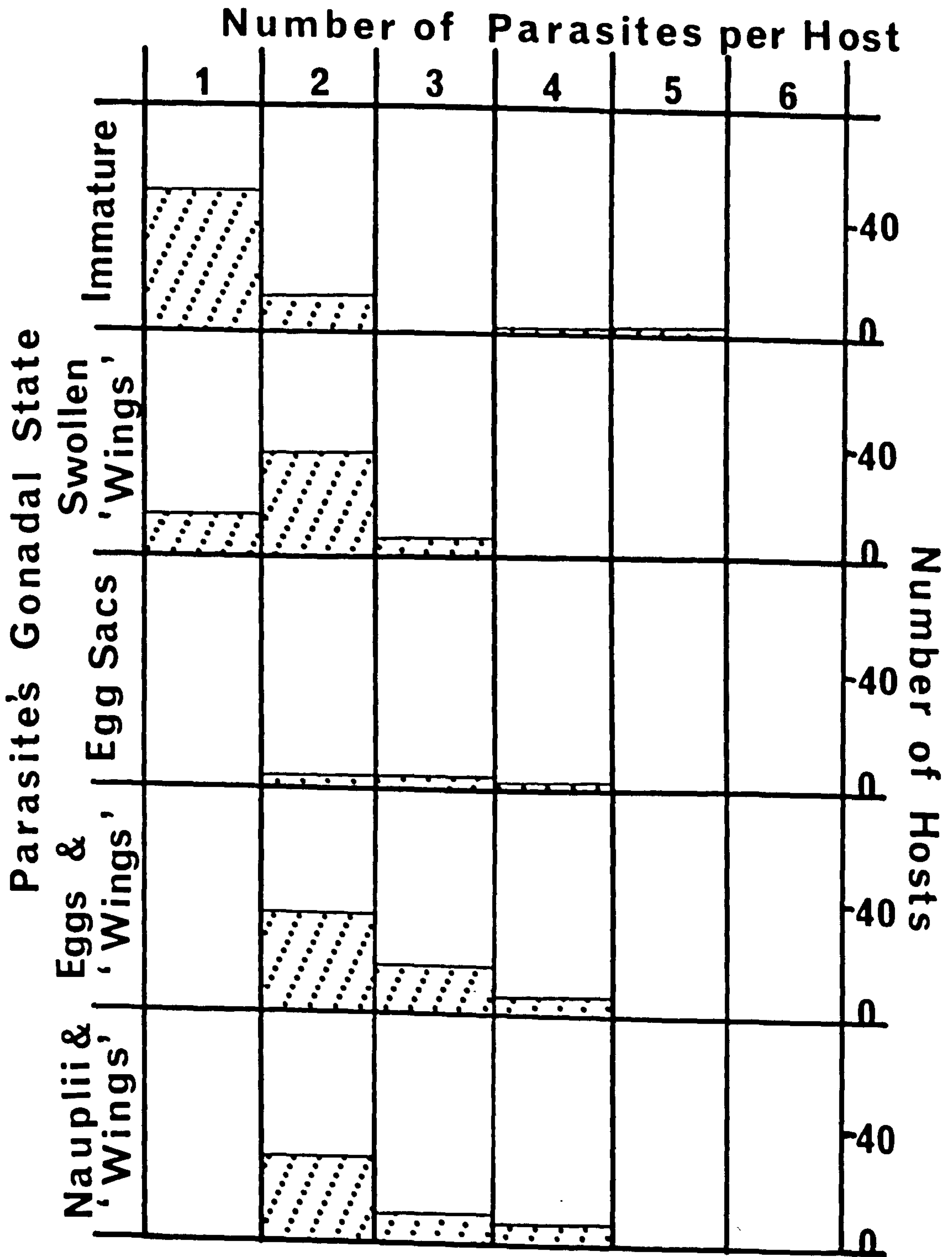
The effect of one parasite upon another goes further than inducing further infection. The first parasite present, ie. the one in the central or 'prime' position is always female. Only rarely is another female parasite present, although occasionally if four parasites are present two effective pairs may be seen.

The distribution of females and males suggests that the sex of the parasite is determined after attachment and depends on the presence or absence in the host of a previous parasite. Alternatively the already infected female may release a substance which whilst attracting males has an opposite effect upon female copopedites, the males lacking the ability to find the host without this substance being produced. Whichever of these two hypotheses is correct the outcome is that a solitary male within a host is never found and that the first infecting parasite is always female. When three or four parasites are present, and when there is not a second female, as is generally the case, it is doubtful if any but the male nearest the female actually reaches maturity or takes part in the fertilization of the female.

Analysis of the gonad state of the female parasite present showed that the ovary could develop so that the 'wings' were swollen with nearly ripe eggs, but that a second parasite is essential before development could proceed beyond this (Fig. 65 ). No examples were found of infection by a solitary mature female. There is thus an arrest of gonad activity until a

Figure 65

Relationship between number of parasites infecting any one host and the stage of development of the first parasite.


Number of Parasites per Host
1
2
3
4
5
6
Parasite's Gonadal State
Immature
Swollen 'Wings'
Egg Sacs
Eggs & 'Wings'
Nauplii & 'Wings'
40
0
Number of Hosts

second parasite can reach the mature male state. The release of any attracting chemical must therefore take place before this stage, probably commencing just after the copopedite is established.

Axinophilus thyasirae can infect its host at any size and time during the host's life. Infection is thought to be by the copopedite stage and these were found in all size ranges of the hosts studied (Fig.66b ). The copopedite infected animals comprise the whole of any infected animals in the small size ranges probably due to a combination of the host's small size and relatively rapid growth. At sizes approaching, or above 4mm in length, the copopedite infected hosts comprise 20-30% of the total infected. It would appear that there is a period of increased susceptibility to infection between 2.5 and 4.0um in length (Fig.66 ) but even so, infection levels are not very high, averaging for a random sample of T. gouldi at about 11% for stations in the centre of the study area. Samples taken from other areas, particularly close to the edges of the Thyasira population (Fig. 14 ), gave quite variable results (Table 14 ). It would thus appear that the tolerance levels of the host and of the parasite are not completely integrated, the relationship between density of the population of the host and degree of infection may be related to the relatively poor locomotory powers of the free-living stages of the parasite.

Analysis of the regular samples of Axinophilus thyasirae revealed a number of ways in which parasitism

Table 14

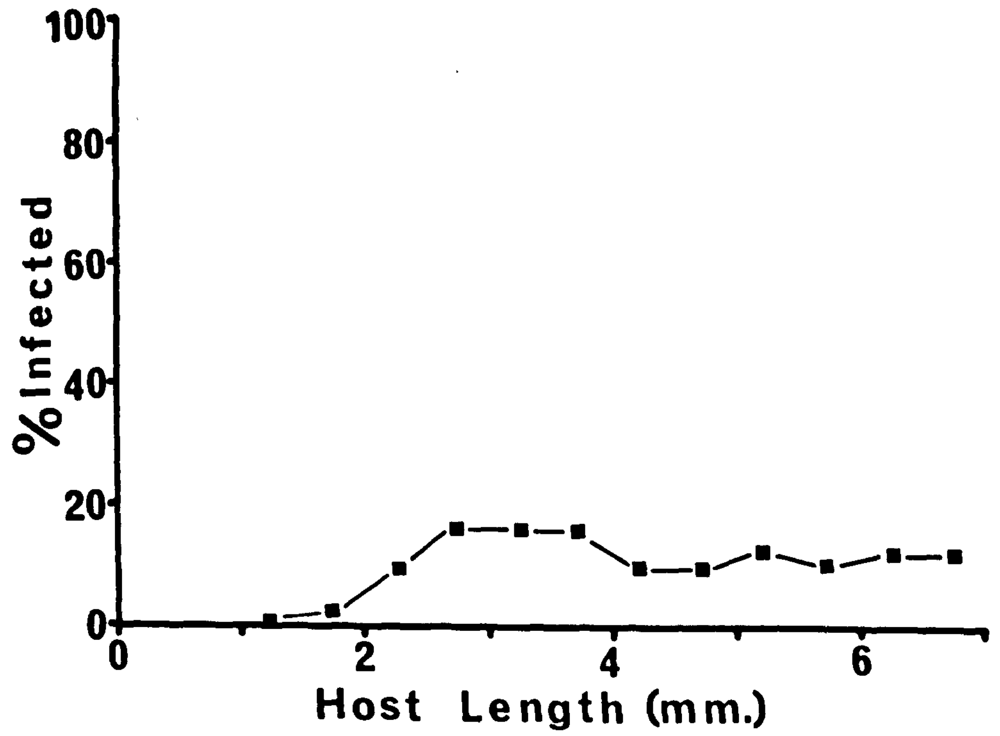
Variation in percentage infection of *Axinophilus thyasirae* within study area

| Date | Station | % Infection |
|---|---|---|
| 26.1.71 | 2e | 12.7 |
| | 4c | 10.8 |
| | 4f | 20.8 |
| | 5b | 9.2 |
| | 5d | 21.4 |
| | 5f | 13.6 |
| | 6b | 17.8 |
| | 6c | 9.1 |
| | 6f | 27.5 |
| | 7b | 22.4 |
| | 8e | 27.5 |
| 27.4.71 | 5c | 19.5 |
| | 6g | 1.4 |
| 3.9.71 | 5b | 4.2 |
| | 6c | 6.5 |
| | 6g | 4.2 |
| 11.1.72 | 6a | 2.4 |
| | 6g | 4.2 |

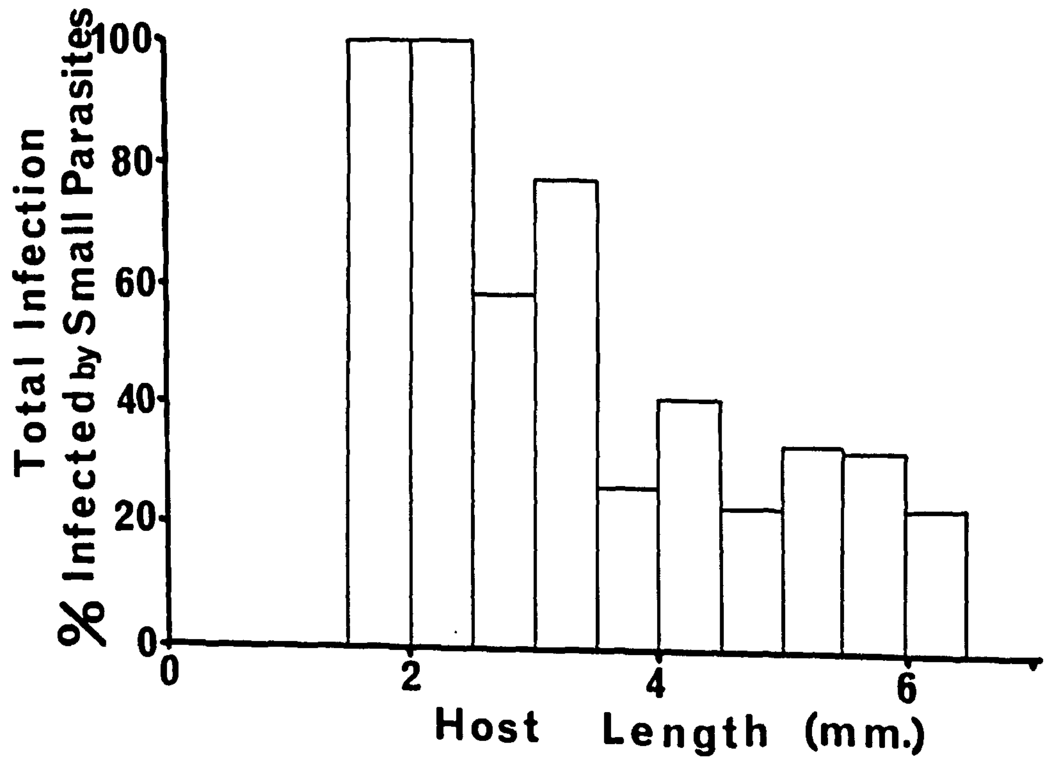
Figure 66

Fig. 66A The incidence of infection of *Thyasira gouldi* of varying shell lengths by the copepod parasite *Axinophilus thyasirae*

Fig. 66B The incidence of copopedites within hosts of varying size as a percentage of the total infection.



% Infected
100
80
60
40
20
0
0
2
4
6
Host Length (mm.)

Total Infection
% Infected by Small Parasites
100
80
60
40
20
0
0
2
4
6
Host Length (mm.)

by this organism effects the host. Clearly this is to be expected in the case of a parasite in which the female grows to a length of 4.5mm and the male to a length of 3.0+mm, and which is found in numbers of up to five per host. The mantle cavity is greatly restricted by such an infection (Fig. 57 ). It also follows that feeding is effected, particularly if, as believed by Ockelmann (pers. comm), currents within the mantle cavity are used to separate food particles. This effect upon feeding is reflected in the dry weight of infected animals. When the regressions of the log of the dry weight on log of the shell length of parasitized animals is compared with that of non-parasitized animals (Table 8 Fig. 44 ) it is clear that for a given length the dry weight of an infected animal is much lower than that of an uninfected animal, even an immature uninfected animal.

The seasonal changes in body weight and biochemical composition of parasitized Thyasira were examined using the same methods as for non-parasitized animals (see earlier). The results are summarized in Fig.46-49 where the composition of the parasitized animals is compared with that of the non-parasitized thus generally immature animals.

The percentage biochemical composition of parasitized animals is very little different from that of the unparasitized ones. There seems to be a tendency for the parasite to consume nitrogenous material in preference to carbohydrate, but it does not noticeably effect the percentage of lipid (Majumdar 1970).

Some of this variation may be due to an indirect effect upon the host's metabolism (Von Brand 1952). The absolute quantities of all components are significantly reduced when compared with those of a non-parasitized _Thyasira_ of similar length.

Examination of the stage of development of the gonad of parasitized animals suggests also that parasitism results in indirect castration of the host, similar to that seen in other cases of parasitism (Caullery 1908, 1952, Giard 1911-1913) probably again through 'food robbing'. This effect is summarized in Table which shows the stage of gonadial development of animals combined from various collections. If only one parasite is present the effect upon the host is not very great (Table 15), however, this effect is increased with increasing numbers of parasites. With two or three parasites present the gonad only matures in about 10% of the infected animals. If four parasites are present the effect is however again lessened, but this is because in the majority of cases the four parasites were all small. Whereas only 6.3% of the Thyasirids would be expected to be without gonad material present, if a parasite were not present, in parasitized animals this percentage is 56.5%. This effect is better illustrated in Fig. 67 in which only animals of adult size are used in the analysis. Although at all times of the year the majority of unparasitized animals of adult size are in the mature state, only rarely do parasitized animals reach maturity.

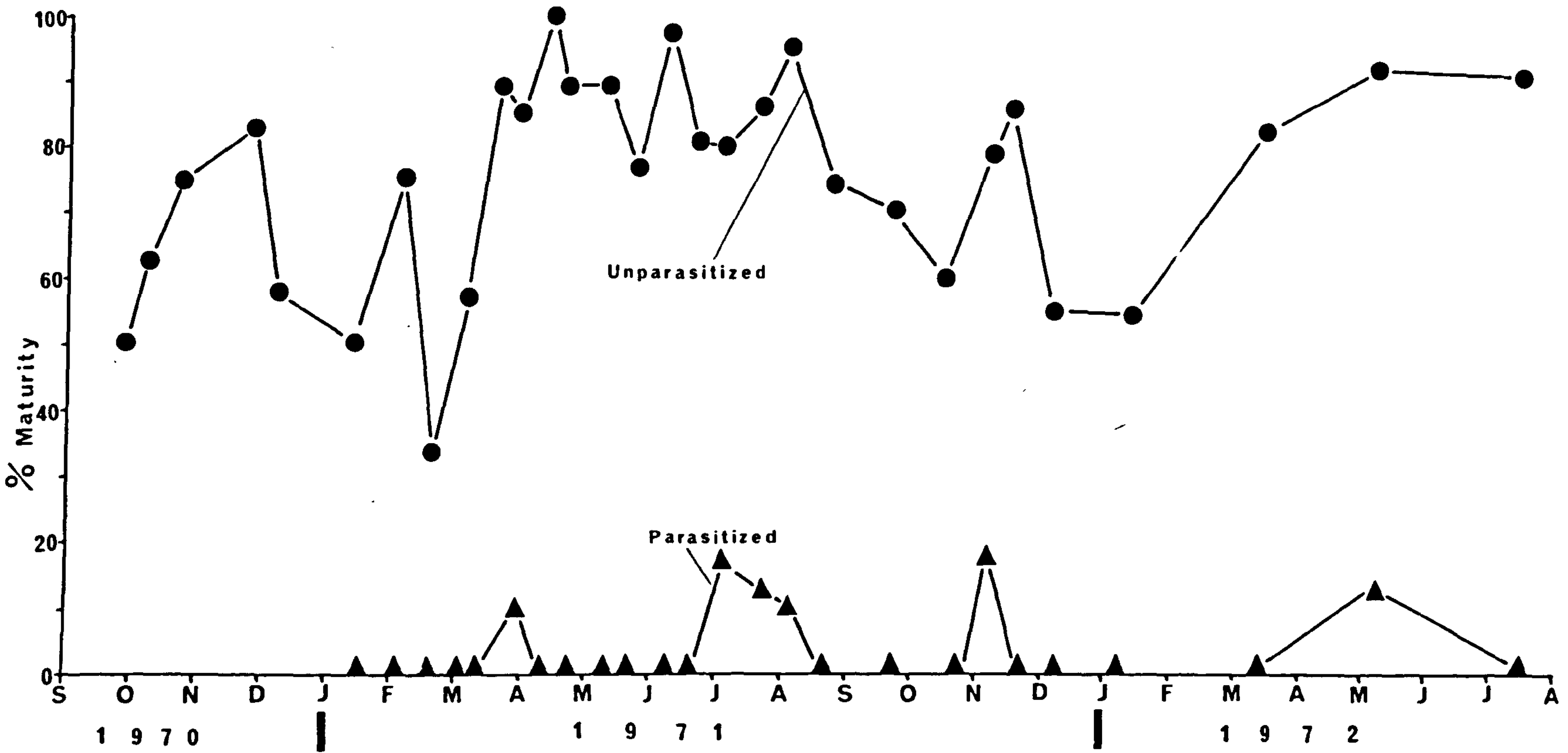
Table 15

Analysis of gonad state to show effect of parasite

| | 1) % with No gonad present | 2) % with Gonad present | 3) % with Little gonad present | 2) + 3) |
|---|---|---|---|---|
| All animals | 12.8 | 83.4 | 3.8 | 87.2 |
| Unparasitized only | 6.3 | 91.0 | 2.7 | 93.7 |
| Parasitized only | | | | |
| 1 parasite present | 16.6 | 66.8 | 16.6 | 83.4 |
| 2 parasites present | 71.2 | 17.3 | 11.5 | 28.8 |
| 3 parasites present | 71.4 | 10.7 | 17.9 | 28.6 |
| 4 parasites present | 66.6 | 33.3 | - | 33.3 |
| 5 parasites present | | insufficient data | | |
| Mean parasitized | 56.5 | 32.0 | 11.5 | 43.5 |

Figure 67

The effect of *Axinophilus thyasirae*
upon the gonad of its host


% Maturity
100
80
60
40
20
0
Unparasitized
Parasitized
S O N D J F M A M J J A S O N D J F M A M J J A
1 9 7 0
1 9 7 1
1 9 7 2

Summary

The morphology of the adult has been described as have the reproductive organs of both the male and female parasite. The life cycle has been described and as far as is known only a single naupliar stage exists, although the copopedite passes through four stages the first of which is the infection stage. Probable durations of each of the stages have been given and it is thought that the copepod has a total life span of 2½-3 years.

A certain order of position upon the adductor muscle according to the number of parasites present is prevalent. The first parasite or the parasite in the 'prime' position is always female. This is thought to affect the sex of any other parasites also releasing some form of attracting chemical such that in the majority of cases more than one parasite is present within any one host.

The parasite effects the dry weight of its host and from this 'food robbing' or upset of the currents of the mantle cavity leads to indirect castration of its host. The parasite apparently utilizes all components of its host's food in roughly the same proportion but due to the effect upon the dry weight the absolute quantity of any of these components is reduced in the parasitized animals when compared to unparasitized animals of similar size.

## General Discussion

The population of T. gouldi under study is restricted to the upper region of L. Etive but is in places quite dense so that numbers in excess of 1,000/$m^2$ are not uncommon. This density and patchiness of distribution is a direct result of non-pelagic development, the larvae completing its development within a tough elastic capsule, hatching as a completely shelled juvenile.

The extent of the population would appear to be the result of the balance between, in the first instance, the very limited powers of dispersal of this lecithotrophic non-pelatic bivalve and to sediment particle size and its tolerance to salinity and temperature. The latter, within the study area, is of only minor importance as before any temperature limitation is seen the prevalent salinities of areas in which the temperature is likely to fluctuate outside the tolerance range of T. gouldi would result in death. The tolerance to salinity exhibited by T. gouldi is quite large and can be increased by pre-history, however, salinity effectively restricts the population to areas below fifteen metres and away from the emergence point of the many run off streams.

There is a strong tendency towards a bimodal size frequency which is noticeable in all samples of the population which are of sufficient size. These modes are due to a longevity of life, low mortality in the larger size ranges, and a slow growth

rate (1 mm per annum).

These factors coupled with the fact that the population as a whole is asynchronous so that at all times of the year fertilized eggs are released means that the successive year classes overlap as regards their size distribution. There is thus an accumulation of year classes in the larger size ranges so that each size class is composed not of one or two but of five or six age classes resulting in a summation of numbers in these size classes.

Thyasira gouldi is one of the members of the Lucinaceans which is prone to infection by the highly modified copepod parasite Axinophilus thyasirae. The degree of infection is closely linked with the areas most highly populated by T. gouldi and may well indicate that the locomotory powers of this parasite are poorly developed, thus supporting laboratory data. Although the number of naupliar stages is not known it seems probable that only one or two may exist, ther are, however, four copopedite stages and it is probably the first of these which infects the host. Infection ~~is~~ may be controlled to some extent by hormones such that there is a tendency for more that one parasite to be present within any one host.

Seasonal cycles are seen in the dry tissue weight and also the biochemical composition of T. gouldi the variation in dry tissue weight being independent of the gonadal cycle. The parasite effects all biochemical components, reducing all of them, although

there is a tendency for a greater reduction in the amount of nitrogenous material rather than carbohydrate. The effect on the tissue weight of this food interference is to reduce the host to starvation levels and prevent gonadal development.

The occurrence of T. gouldi in L. Etive has effectively extended south from the known distribution of this small Lucinacean which is generally found only in arctic waters. Certain factors associated with the arctic way of life have enabled this bivalve to be so successful within L. Etive. The direct development and asynchronous reproduction ensures that numbers can be built up quite easily with little loss from the population. Independence is gained from plankton and also from the necessity of stimulation due to variation in external factors. Salinity and temperature do however result in a slight degree of synchrony in that they can induce ripe males to spawn, which in turn results in the spawning of the ripe females. The low oxygen assumption generally associated with arctic life also aids the bivalve in existing in this area which is often subjected to low oxygen tensions in the overlying water, whilst the mud layer smells strongly of Hydrogen sulphide.

Environmental conditions are generally fairly stable in arctic waters, however, T. gouldi shows large salinity and temperature tolerances further altered by pre-history so that the fluctuating perameters at the head of L. Etive are not lethal and allow the establishment and build up of this bivalve in an

otherwise relatively poorly populated area.

## General Summary

1) Certain differences both as regards conchological differences and differences in the soft parts have been found between *T. flexuosa* and *T. gouldi*. These have been described in the hope that they may assist others to distinguish between these closely related species.

2) The occurrence of *T. gouldi* within L. Etive effectively extends south the known distribution of this arctic bivalve. Details of the extent of the population and of the salinity, temperature and sediments of the populated area are given.

3) The population of *T. gouldi* within L. Etive has been shown to be bimodal due to a combination of slow growth rate, asynchronous reproduction, low mortality and longevity of life. A model has been constructed to show how these factors can combine to give a bimodal population.

4) The growth rate of *T. gouldi* both in L. Etive and in Lochs Linnhe and Eil have been studied, growth rates of 1mm per annum appear to be the maximum, decreasing with increasing age until almost no increase of size is seen after a length of 7mm has been attained.

5) The other most abundant species within the study area have been listed and from contours of the distribution of these species data concerning the study

area has been supplemented.

6) The tolerance levels to both salinity and temperature have been investigated for both *T. gouldi* and *T. flexuosa* and correlated with their distribution.

7) The effect of the external salinity upon the osmotic pressure of the blood, investigated as depressions of freezing point, have been studied. Two mechanism of control of the body osmotic pressure appear to exist.

8) The relative importance of sediment in the life of *T. gouldi* and of *T. flexuosa* was investigated as was the ability of *T. gouldi* to live and bury into sediments of various particle sizes.

9) The effect of salinity and temperature upon oxygen consumption was studied and the ability of *T. gouldi* to live in waters of low oxygen tensions established.

10) The effect of salinity upon larval development and the maturation of the gonad were investigated.

11) Further details, supplementing the early paper of Bresciani and Ockelmann (1966), of the external morphology of *Axinophilus thyasirae* are given and the life history and internal anatomy of this copepod parasite are described.

12) The reproductive system and process of

*Axinophilus thyasirae* with respect to both the male and the female are described. The hypothesis is put forward that chemical 'attractors' are produced ensuring that more than one parasite is present within each host.

13) The variation in the level of infection of the copepod *Axinophilus thyasirae* was investigated as was the time and size of infection.

14) The effect of the parasite upon the biochemical composition and the stage of gonadal development of the host was investigated.

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# Appendix I

## The Effect of Freeze Drying or Oven Drying

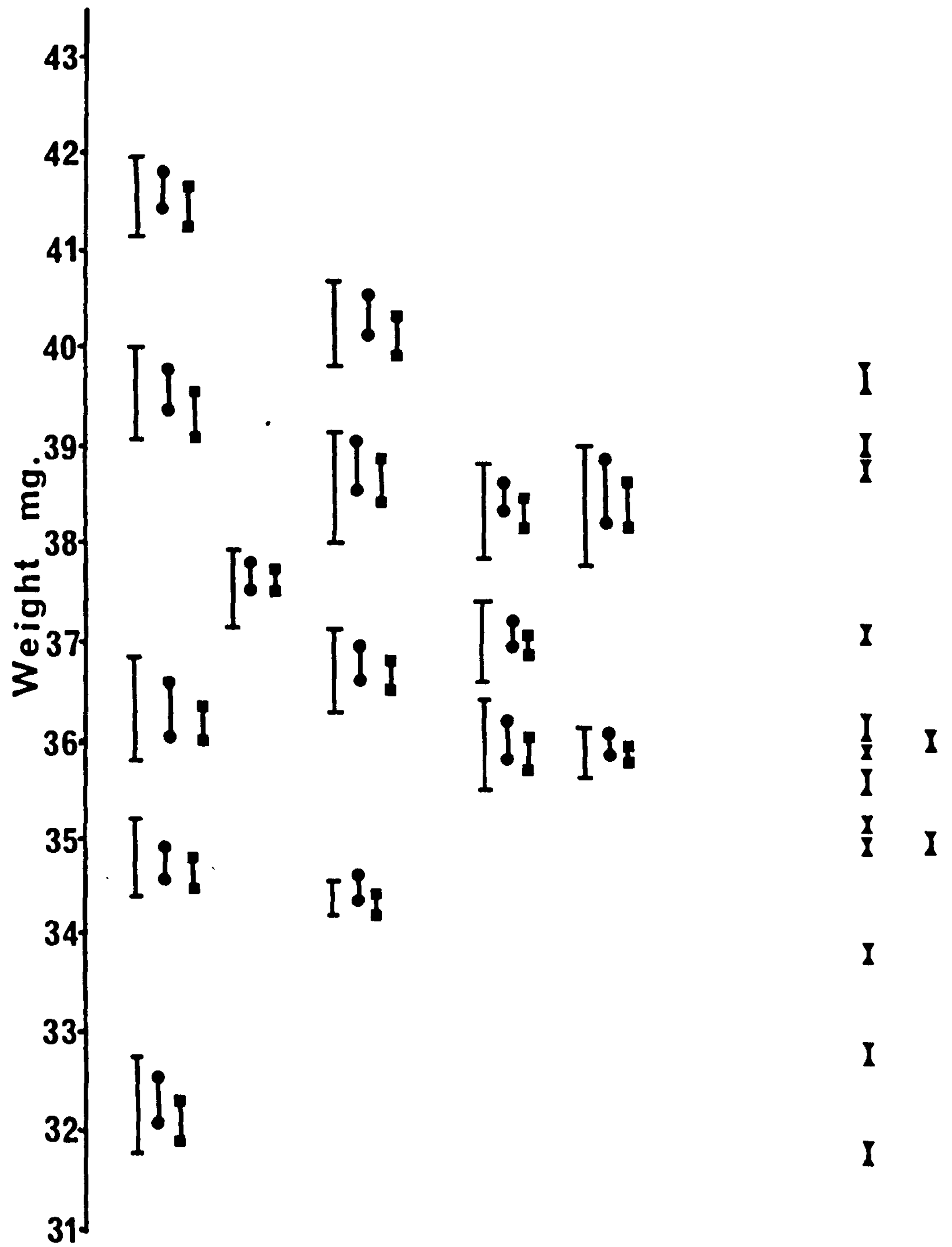
Before any of the dry weights were taken, the difference between dry weight obtained by oven drying, ie. at 85°C for 36 hours, or freeze drying and thus the interchangeability of these was required. Also the uptake of water by dry tissue and time needed to be allowed for a stable 'dry' weight to be obtained so that all weights obtained were at the same stable basis.

The normal procedure of opening shells and placing the flesh into weighed tin boats was adopted. The flesh was then either freeze dried or oven dried. Samples from the freeze drier were placed into dessicators in the deep freeze for several hours after which time they were removed and weighed at ten minute intervals. Samples from the oven were weighed at ten minute intervals. The oven dried samples were then replaced into the oven so that any moisture they had picked up was again driven off, then placed into a dessicator and brought to room temperature before being weighed again at ten minute intervals. Thus the difference between the weights of oven dried material obtained from hot samples (ie. straight from the oven) and samples at room temperature could be ascertained. The freeze dried samples were similarly treated, ie. again freeze dried, placed into the deep freeze in a dessicator but then the dessicator was brought out and allowed to equate with room temperature before the samples were again weighed every ten minutes. Freeze dried samples were then oven dried, and oven

Figure A1

The variation in weight of freeze dried and oven dried tissue.

- Freeze dried then oven dried weighed straight from oven
- Freeze dried - weighed directly from freeze drier
- Freeze dried - weighed after equilibration with room temperature
- Oven dried - weighed directly from oven



Weight mg.
43
42
41
40
39
38
37
36
35
34
33
32
31

dried samples were freeze dried, the same processes being repeated. All weights were extrapolated back to zero time.

The results obtained are summarized in Fig. A1 and show that taking freeze dried samples straight from the fridge leads to a very great variation in weight obtained. They all increased in weight but stabilized after one hour. The weights obtained were all higher than those obtained when samples were brought to room temperature within the dessicator. The extent of variation in oven dried tissue was far less and was not effected if samples were weighed either straight from the oven or after equilibration with room temperature. No variation was seen when samples were oven dried then freeze dried. However, samples which were' freeze dried then oven dried but weighed straight from the oven took up far more water than any of the other freeze dried samples, but if weighed at room temperature they varied to the same extent as freeze dried only samples weighed at room temperature.

It is thus clear that the temperature and time lapse between bringing samples into air is going to effect the weight of the sample. For all dried tissue weights, whether oven dried or freeze dried, all samples were brought to room temperature within a dessicator, after which they were left in air for one hour before being weighed. It is shown that there is no difference in freeze dried or oven dried samples under these conditions, thus these are interchangeable

methods. The fact that freeze dried material takes up more water than oven dried is probably related to the form of these samples; those freeze dried are full of air spaces and pockets, whereas the oven dried are a solid mass with far less surface area available for water uptake. It was found that the tin boats did not alter in weight with time, water uptake by the tissue, however, amounted to between 4 and 9% of the dry tissue weight.

# Appendix II

## Method for the determination of volume of small animals

In monitoring the oxygen consumption of <u>Thyasira</u> a need arose to determine its volume. As none of the available apparatus for measuring small volumes (Persoune 1971, Langvatin 1972) were sufficiently sensitive, the following apparatus based on the simple principle of displacement of water was devised. The sensitivity of the apparatus is within that of the Alga Microburette used, was easy to construct and was very rapid in usage.

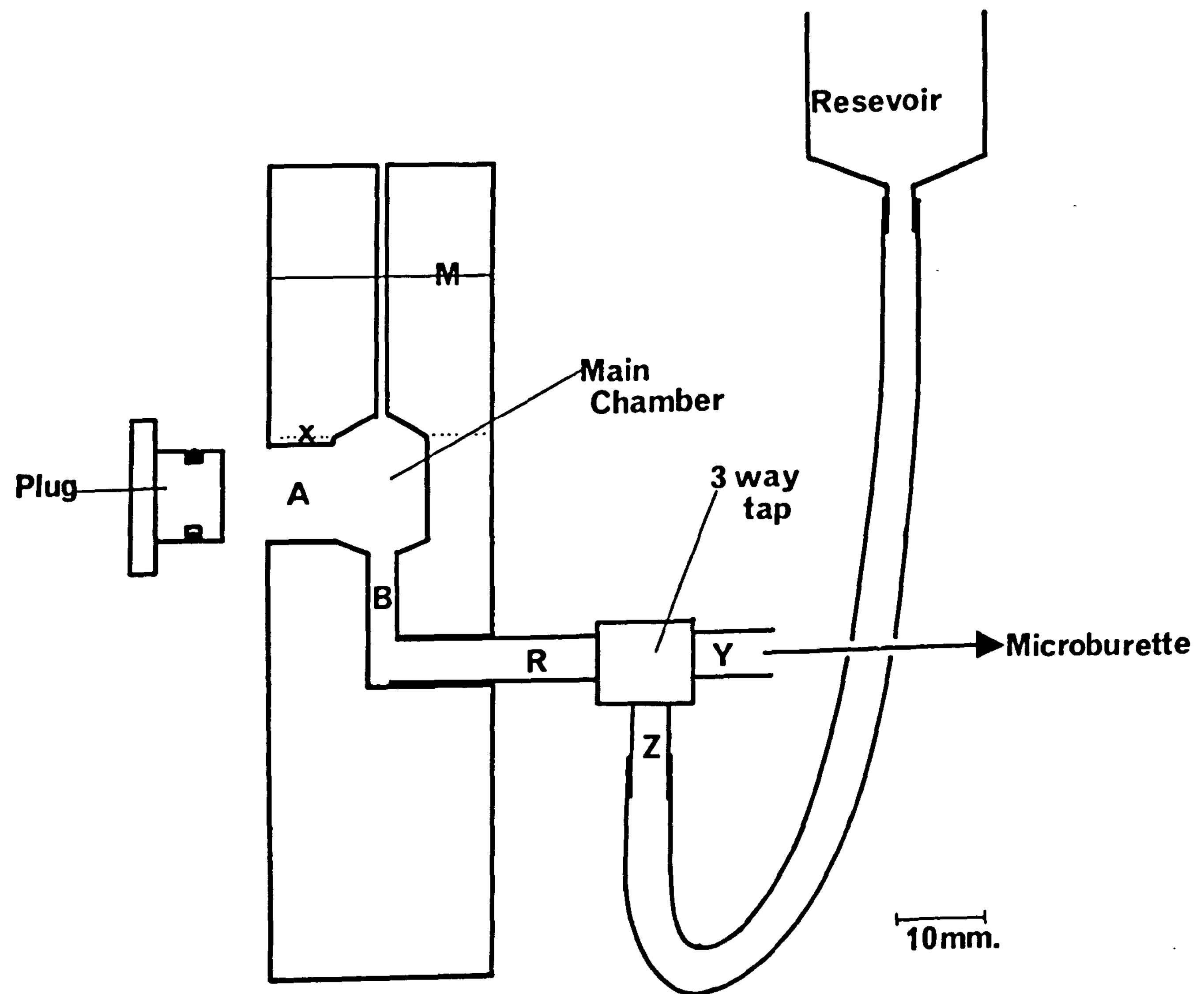
The construction of the apparatus is simple, all that is required is a piece of 2½cm solid clear acrylic tube, a 2.5ml plastic syringe, a 3-way tap and a small piece of stiff tubing plus the micro-burette (Alga Microburette from Burrough's Welcome Laboratory was used).

The apparatus (Fig. A2) was constructed in two halves being joined at the point X. In the lower region the bore 'A' was machined out first and the plug made such that a good fit within this bore was obtained. The 'O' ring was then placed onto the plug 3mm along it. The plug was then inserted into the bore 'A' and the main chamber of diameter 9mm carefully bored out, the other bores being subsequently machined. By boring out the main chamber with the plug inserted, the plug forms part of the wall of the main chamber but does not obstruct it in any way.

Figure A2

Diagrammatic representation of the apparatus
for measuring the volume of *Thyasira gouldi*

For symbol meaning see.text of Appendix 2


Resevoir
M
Main
Chamber
X
Plug
A
3 way
tap
B
R
Y
Microburette
Z
10mm.

All the bores were then polished with soft tissue and Brasso and the two faces to be joined machined, polished and glued with perspex cement. The three way tap was then connected into place and connected to the reservoir and to the microburette as shown (Fig. A2).

In operation the reservoir is filled with sea water to which photo-flo is added. This ensures easy flow of the water but does not appear to adversely affect the animals in any way. Raising and lowering the reservoir with the appropriate parts of the three-way valve open facilitates flushing of the system. The Microburette is carefully filled before attaching it to the rest of the apparatus.

Once all the air bubbles have been expessed, the volumes of animals can be determined very readily, approximately 3-5 minutes was found adequate for each determination.

With the apparatus full of water the procedure is as follows. With the ports R and Y open the bulk of water is ejected from the Microburette, the reservoir is then raised or lowered with only the port Z open thus bringing the water level to the mark M. The ports R and Y only are then opened and the water level checked and adjusted as necessary with the Microburette. The reading on the burette is then taken, the water is then drawn into the Microburette until the level in the main chamber falls below 'B'. The plug is removed and the animal inserted, the plug is then replaced and the water expelled from the

Microburette until the mark M is reached and the reading on the Microburette taken. The difference in these two burette readings thus indicates the volume due to the animal. Initially the water was again brought to the mark M after removal of the animal but no significant difference in burette readings was obtained after such an operation. Raising and lowering the water level to the mark M also gave no perceptable change in the Microburette reading, nor did control runs of plug removal.

Errors involved in the used of this piece of apparatus which is easy to construct and rapid in usage are less than the sensitivity of the Microburette (the Alga Microburette is calibrated in volumes of 0.0002cc).

# Appendix III

## Construction of flasks for the measurement of oxygen consumption

In designing these flasks consideration had to be given to the following factors:-

1) The flasks had to allow easy entry and observation of the test animal.

2) The design must be such that a representative quantity of unpolluted liquid can be removed.

3) The oxygen content of the sample has to be sufficient to be descernible from the controls but at the same time the flask must be large enough so that no effect of oxygen lack is seen.

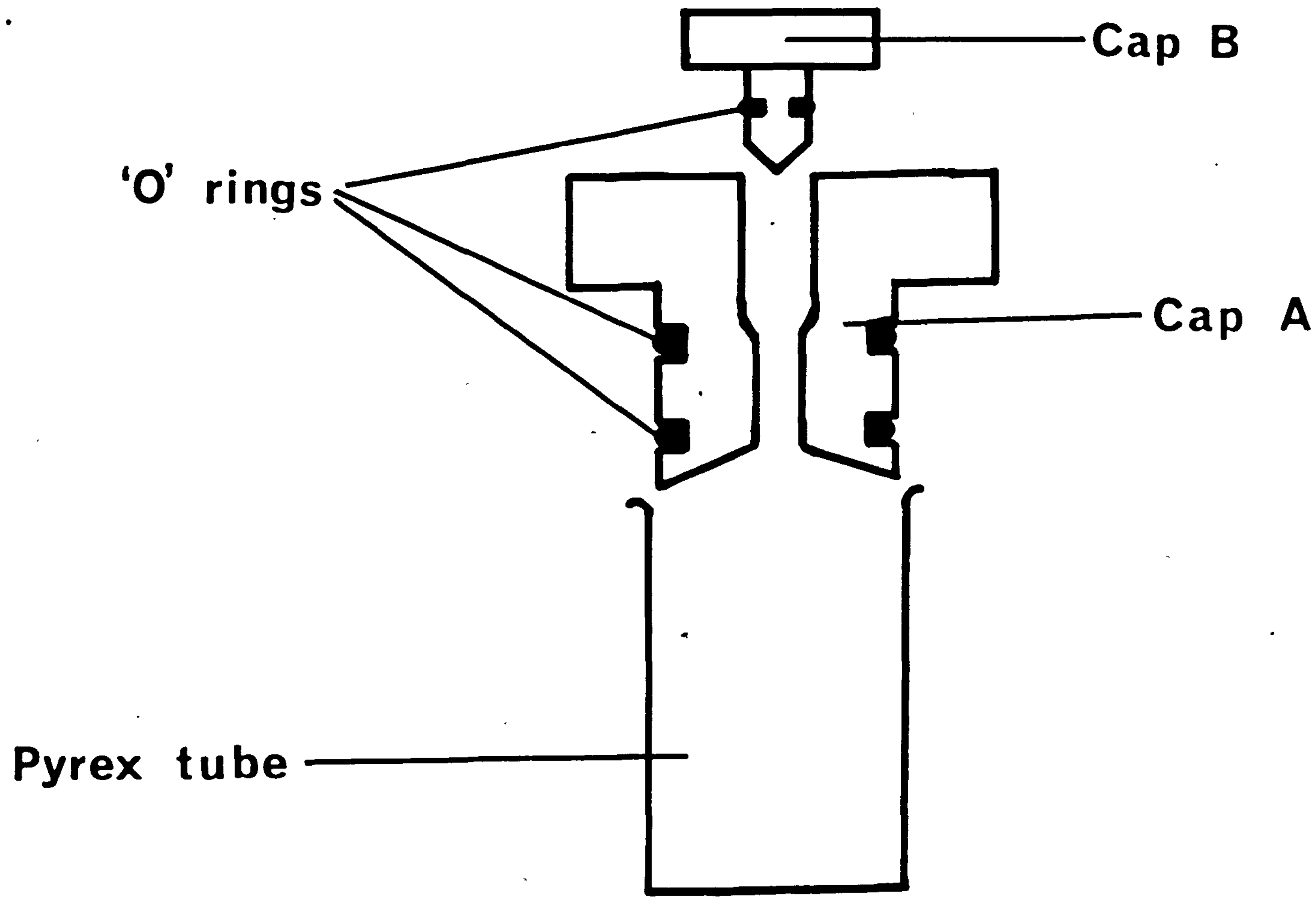
The basic design of the flasks was thus of a 'double capped' pyrex tube, lipped to allow easy entry of the'O' ring seals on the cap (Fig. A3). The first cap 'A' allows easy entry of the test animal and easy filling and cleaning of the flask. The second cap 'B' allows the insertion of the nozzle of the Fox and Wingfield reaction vessel (Fox and Wingfield 1938) with the minimum of pollution from the surrounding air. The fitting of 'O' seals to the two caps and the use of silicone grease ensured the necessary air tight seal.

In operation insertion of the cap 'A' into the water filled flask forces water up and out through the

Figure A3

Incubation flasks used in measurement of
oxygen consumption

For symbol meaning see text of Appendix 3


Cap B
'O' rings
Cap A
Pyrex tube

bore in the cap. The tapered and polished surface of this cap and cap 'B' prevent any air being trapped, insertion of cap 'B' completing the setting up procedure.

After incubation the flask is inverted several times so that the oxygen content within the flask can be assumed uniform. Cap 'B' is carefully removed and the nozzle of the reaction vessel inserted, the water sample being obtained from near the bottom of the respiration flask.

Blanks were run as controls of the oxygen levels, the volume of the flask being obtained by weighing, the volume due to the test animal being determined as in Appendix II. The average flask size was 3.5-5ml this being a balance of incubation time and oxygen consumption rate of *T. gouldi*.