RELATIONSHIPS BETWEEN SEASONAL BIOCHEMICAL CHANGES AND THE REPRODUCTIVE CYCLE OF THE INTERTIDAL GASTROPOD THAIS LAMELLOSA GMELIN (GASTROPODA, PROSOBRANCHIA)

by

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We accept this thesis as conforming to the
required standard

THE UNIVERSITY OF BRITISH COLUMBIA

June, 1970

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ABSTRACT

The seasonal variation in the major biochemical constituents of T. lamellosa Gmelin have been studied in relation to the reproductive cycle. Digestive gland, foot muscle and gonad were analysed for protein, glycogen, lipid and ash over a period of one year. In addition to biochemical analyses, histological sections of digestive gland and gonad were made throughout the same period. Histological data supplied information on feeding and gamete maturation. Two major periods of feeding activity occurred in April and August. Gametogenesis began in late summer and the peak spawning period was in March. Glycogen is at a maximum in the digestive gland at times of maximum feeding, but food is stored in the digestive gland in the form of lipid. Stored lipid is utilized by the animal during the winter. Glycogen is at a low level in all tissues and appears to be used primarily for lipid and yolk synthesis. The foot muscle does not store either lipid or glycogen to any appreciable extent.

Under normal field conditions during the winter, the digestive gland index decreases as reserves are utilized, while the gonad size is maintained until spawning. Animals which are maintained through the same period under artificial summer conditions, show no loss in the digestive gland index, but a decrease in the size of the gonad. None of the oogonia reach maturity and the mature oocytes are resorbed. The starved animals resorb more material from the gonad than fed animals. The possible role of environmental factors in controlling reproduction is discussed.

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INTRODUCTION

Reproductive cycles of various marine invertebrates based on histological investigations or gonad indices are demonstrated in the literature. Giese (1959) reviewed the work up to that time and since then further species have been investigated: The chitons, Katherina tunicata and Mopalia hindsii (Giese, Tucker, and Boolootian, 1959b) and Cryptochiton stelleri (Lawrence, Lawrence, and Giese, 1965); the abalone, Haliotis cracherodii (Webber and Giese, 1969); the limpets, Acmaea mitra (Fritchman, 1961), Acmaea limatula (Seapy, 1966), and Fissurella barbadensis (Ward, 1966); the littorines, Littorina pintado, L. picta, and L. scabra (Struhsaker, 1966); the bivalves, Crassostrea virginica (Sakuda, 1966), Aequipecten irradians (Sastry, 1966), Spisula solidissma (Ropes, 1968), Mytilus edulis planulatus, Xenostrobus pulex, Septifer bilocularis, Brachidontes variabilis, and Amygdalum glaberrimum (Wilson and Hodgkin, 1967); the asteroids, Odontaster validus (Pearse, 1965) and Pisaster ochraceus (Mauzey, 1966); the ophiuroid, Gorgonocephalus caryi (Patent, 1969).

Biochemical changes associated with the reproductive cycle have not been extensively studied (Giese et al. 1959a; Giese and Araki, 1962; Barnes, Barnes and Finlayson, 1963; Ansell and Lander, 1967; Giese and Hart, 1967; Blackmore, 1969). For the most part, the reproductive cycles in these studies were determined by gonad indices or spawning potential, the latter being determined by standard spawning stimuli. Few studies utilized the more precise method of measuring oocyte sizes in histological sections to determine the stage of the reproductive cycle. The data obtained in these studies provide

a more complete picture of the internal changes, and allows the investigator to postulate the relationship between nutrition and the reproductive cycle. For example, Giese and Hart (1967) found seasonal changes in the gonad and digestive gland indices of Katherina tunicata throughout the year. Analyses showed that most of the body components remained similar in their protein, lipid and total carbohydrate except for the ovary and testis. The latter organs showed marked changes in protein and carbohydrate level, and the ovary showed a change in lipid level which reached a maximum at the height of the breeding season. The carbohydrate level in testis and ovary was maximal during the time of minimum gonad size and minimal at the peak of breeding. From these data, they suggest that as the ovary develops, carbohydrate is utilized by the oocytes. They also suggest that carbohydrate is converted to protein in the testis, while lipid remains fairly constant. It is suggested that carbohydrate is converted to lipid and protein in the ovary. Whether that is in fact what occurs would require more specific investigation, perhaps involving radioactive tracers. However, biochemical studies of this type can at least provide a basis for explaining the mechanisms involved in the timing and control of reproduction.

Early work of this type was done on commercially important bivalves such as the oysters, Ostrea gigas and O. edulis (Mitchell, 1915-16; Russell, 1923; Masumoto, Masumoto, and Hibino, 1934; Hatanaka, 1940) and the mussel, Mytilus edulis (Daniel, 1921), while more recently, biochemical work has been performed on other groups such as echinoderms and chitons. Giese and his colleagues have contributed many papers to this field of invertebrate physiology. There is an obvious lack of

biochemical information on marine prosobranch gastropods except for two recent papers on Patella vulgata (Blackmore, 1969) and on Haliotis cracherodii (Webber and Giese, 1969). Consequently, the marine intertidal gastropod, Thais lamellosa Gmelin, a common species in the area of Vancouver, British Columbia, was chosen for the present study. A study of this type would provide basic information on the changes in biochemical constituents and the reproductive cycle of Thais lamellosa, which might be important to further ecological or physiological investigation.

Some information has been published on the reproduction of T. lamellosa from field observations of breeding aggregations and egg-capsule deposition (Griffith, 1967). Emlen (1966) did an ecological study of T. lamellosa from the point of view of time, energy and risk and provided information on times of capsule laying, length of breeding season, and developmental time in a population from Port Townsend, Washington. Chapman and Banner (1949) report that the native drill, T. lamellosa, lays eggs in Puget Sound between March and June. They also performed some simple experiments on the salinity tolerance of T. lamellosa. After 11 days at 15.2°/oo, 3 out of 10 died, while salinities above this had no observable effect. As the salinity dropped below about 15°/oo the snails tended to close up and remain inactive; hence, the salinity indirectly affected feeding. The diet of T. lamellosa appears to be mussels and barnacles with a preference for the latter (Kincaid, 1957).

Dall (1915) reviewed the taxonomic history of Thais (Nucella) lamellosa Gmelin, and described five varieties with type locations at

four places on the Pacific coast. Kincaid (1957) presented many illustrations of shell variations from different locations on the Pacific coast. For the purposes of this study, animals were collected from one location and identified as Thais lamellosa from the description of Griffith (1967), and no consideration was given to the different varieties.

In this paper, the biochemical constituents: lipid, carbohydrate, protein, and ash; in gonad, digestive gland, and muscle tissue, were determined for monthly samples and, the reproductive cycle was determined from histological data and gonad indices. Environmental parameters of temperature and salinity were recorded concurrently with field collections and, a limited amount of experimentation was performed in the laboratory under controlled conditions. This study was designed to provide some basic information which will serve as the basis for further experimentation on Thais lamellosa.

MATERIAL AND METHODS

Collecting Area

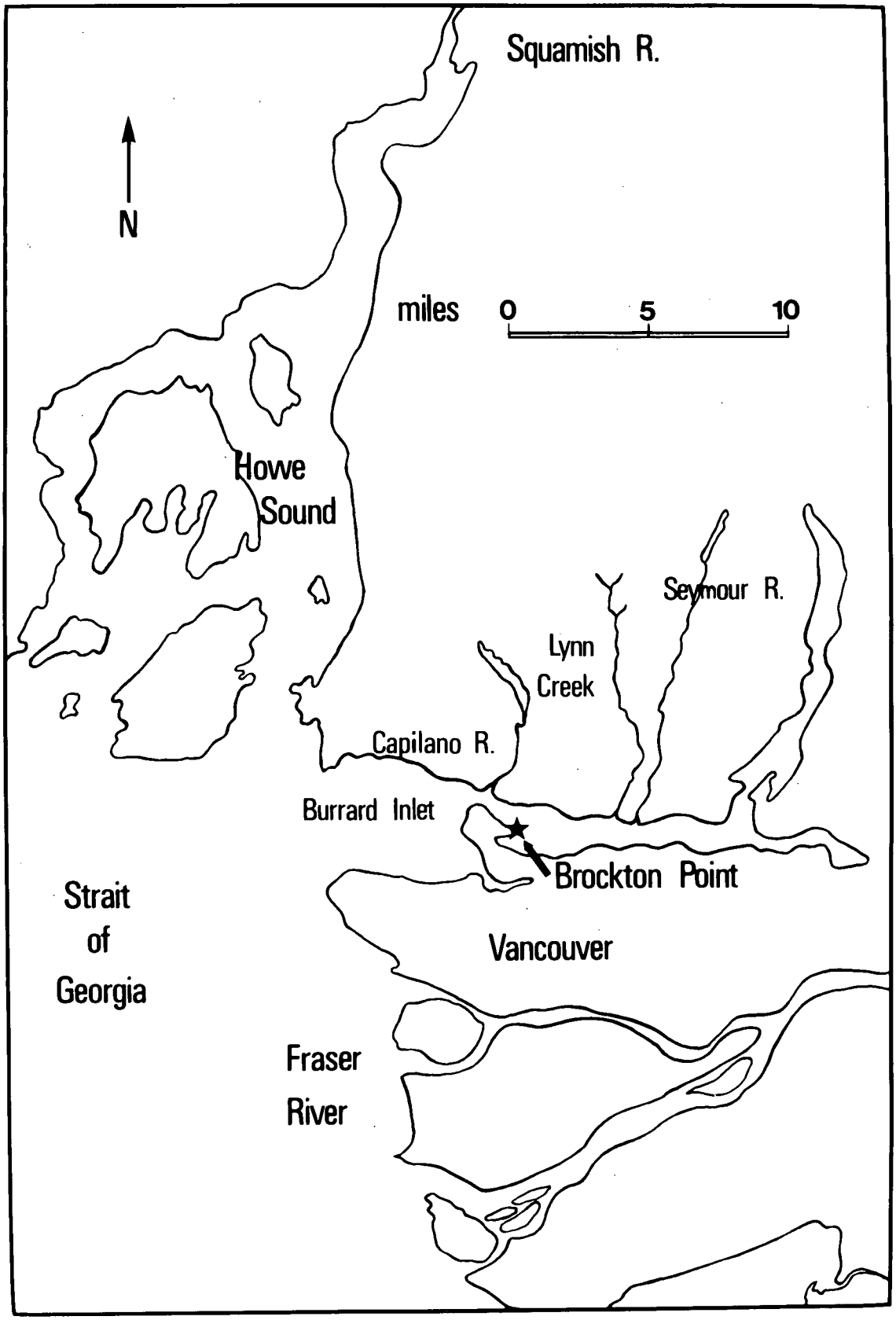
A sample of about 20 Thais lamellosa was collected each month at Brockton Point, Stanley Park, Vancouver, British Columbia, (Fig. 1) and tissue was obtained from 8 females and 2 males. Animals were collected between the 0 and 3 foot tide levels, the maximum tidal height at this location being about 15 feet. Only those animals longer than 4 cm. were chosen, as smaller animals did not yield sufficient tissue for individual chemical analyses. Prior to dissection, animals were kept in aerated sea-water approximating field temperatures, 15°C in summer and 8°C in winter. These temperatures were controlled by the cold tap water on a water table and were subject to the changes in the water supply. The animals were maintained in this way for at least 3 days to allow the gut to empty. Water temperatures were recorded at a depth of 2 feet with a Tempscribe continuous temperature recorder (GBI Scientific) off the Texaco Oil barge adjacent to the collecting area. A water sample was collected once a week and salinity was determined by standard titration methods.

Collection of Samples

The soft parts were removed by cracking the shell open. External water on the whole animal was blotted with absorbant tissue and the total soft parts, including the operculum, were weighed on a top-loading balance to $\pm$ 0.01 grams. A portion of the gonad-digestive gland complex was removed and fixed in Gilson's Fluid (Galigher and Kozloff, 1964) for later sectioning. The gonad, digestive gland and

FIGURE 1

Map of research area showing main sources of run-off.


Squamish R.
N
miles
0
5
10
Howe
Sound
Seymour R.
Lynn
Creek
Capilano R.
Burrard Inlet
Brockton Point
Strait
of
Georgia
Vancouver
Fraser
River

foot muscle were dissected off, transferred to pieces of Parafilm and weighed to ± 0.1 mg. The tissue was dried in a vacuum dessicator over Drierite for 48 hours which removed 98-99% of the water. From this procedure, % dry weight, gonad index, and digestive gland index were obtained. The per cent dry weight is the ratio of dry weight to wet weight and is a measure of the total solids in the wet tissue. Since part of the complex had been removed for fixation, a true component index could not be obtained; however, as near as possible, a proportional amount was removed from each animal. The component index obtained then, was a relative rather than an absolute value. There appears to be some confusion in the literature regarding component index. Some authors calculate the index from the ratio of component volume to total wet weight times 100 (Giese, et al., 1959a; Giese, et al., 1959b; Giese and Araki, 1962), while in more recent papers the ratio of component weight to total wet weight was used (Giese, 1967; Giese and Hart, 1967; Sastry, 1968; Webber and Giese, 1969). The latter method was used in this study.

Histology

Several fixatives were tested, including Baker's, Carnoy's, Bouin's, and Gilson's fixatives. The last was found to give the most satisfactory results and was employed as the fixative for all the tissues. Later in the course of the study, Picro-Nitric fixative was found to give better fixation of yolk but to maintain uniformity of shrinkage of oocytes Gilson's Fluid was used throughout the study. The method of oocyte measurement will be described later.

A segment from the distal end of the gonad-digestive gland complex

was fixed in Gilson's Fluid over night. Sectioning of the whole gonad indicated that development of the oocytes was synchronized throughout. The tissue was then washed for several hours in running tap-water to remove excess fixative. The tissue was dehydrated up to 70% EtOH where it could be stored until processed. From this point the tissue was dehydrated with EtOH, cleared in benzene and embedded in Tissuemat (m.p. 56.7°C). The majority of the sections were cut at 8 microns but some ranged from 5-10 microns depending on factors such as static electricity and sharpness of blade at time of sectioning, and fixation of the specimen. Masson's Trichrome stain (Pantin, 1964) was employed and gave black nuclei, green mucus and connective tissue, pink epithelium, and orange yolk granules. During the staining procedure the sections were exposed to Lugol's Iodine in 70% EtOH which served to remove crystals of mercuric chloride resulting from the fixative.

The gonad sections were analysed by the method of Pearse (1965). With an eyepiece scale calibrated in microns, two measurements of oocyte diameter were made at right angles to each other, these usually being the longest and shortest axes. From these two perpendicular measurements the mean diameter of each oocyte was calculated. These data were obtained for 25 randomly selected oocytes in each of 5 females. Only those oocytes which were sectioned through the nucleus were measured. Some of the large mature oocytes presented problems in measurement since the nucleus was pyknotic and difficult to see among the large yolk granules; furthermore, the membranes of the mature oocytes were difficult to discern so the boundaries often had to be estimated

using clues from the shape of the surrounding tissue and neighbouring oocytes. All the measurements were arranged into size classes and a graph of size distribution was drawn (Fig. 6). The individual graphs or polygons indicate the per cent frequency in each size class for each monthly collection throughout the year.

The sections of digestive gland were analysed for their storage granule content. The columnar cells of the digestive gland tubules were subjectively classified as being full of granules, half full, or empty (Table 2, Plate 1).

Chemical Analyses

Chemical analyses for lipid, protein, and polysaccharide were performed on the dry tissue from individual animals rather than on pooled tissues from several animals. Individual determinations provided information on the amount of variation in the population and the differences between males and females, as well as individual data which could be correlated with the histological results, observations of colour, and per cent water in the tissue. For practical reasons, one determination of each component was performed in each tissue sample, as opposed to pooling the tissues and doing several replicates.

Lipid: Lipid and polysaccharide determinations were performed on the same portion of tissue. Total lipid values were obtained by a method based on that of Sperry and Brand (1955). Approximately 20 mg. of dry tissue were homogenized in 2 ml. of a chloroform-methanol solution (2:1) with a tissue grinder (Pyrex Brand) and transferred to a centrifuge tube. The grinder was rinsed several times and the washings were added to the sample. The homogenate was centrifuged and the

supernatant containing the lipid was transferred to a screw-cap vial. The remaining precipitate was retained for polysaccharide analysis, which will be described later. To the lipid extract, 5 ml. of a wash solution of 1.6% $CaCl_2$ was added which, after shaking for 5 min., caused the separation of the lipid layer. After centrifugation, the upper layer was removed and discarded and about 2 ml. of a second wash solution, chloroform-methanol (2:1) plus 2% $CaCl_2$ (Sperry and Brand, 1955), was layered carefully on the surface to remove any non-lipid impurities remaining at the surface. This wash layer was removed with a Pasteur pipette after centrifugation, and the remaining lipid was evaporated to dryness at 60°C with a stream of nitrogen. This latter procedure was to reduce the possibility of oxidizing the lipid. The lipid remaining was then dissolved with about 1 ml. of ether and transferred to a pre-weighed piece of sponge on a planchet. The tube was rinsed a second time with 1 ml. of ether and was added to the planchet. The sponge allowed for faster evaporation and also prevented spillage of the sample. When dry, the weight of the extracted lipid was determined and the total lipid / unit dry weight of tissue was calculated as a percentage. The percentage lipid present per unit dry weight will be referred to as the lipid level in this paper, following the terminology introduced by Giese (1967).

Polysaccharide: As mentioned in the lipid analysis method, the residue from the lipid extraction was used for the polysaccharide analysis. This has two advantages: (1) both determinations can be performed on one small amount of tissue, and (2) the prior removal of lipid prevents foaming during the polysaccharide extraction (Giese,

1967). The tissue was, first of all, heated with 4 ml. of 80% EtOH to precipitate the polysaccharide and remove some soluble impurities. The solution was centrifuged and the supernatant discarded. The precipitate was heated with 4 ml. of TCA at 100°C to extract the polysaccharide, the supernatant from the extraction being placed in a 25 ml. volumetric flask. This extraction was performed three times to ensure complete removal of the polysaccharide. A 2 ml. aliquot was then analysed for polysaccharide by the anthrone method of Seifter, et al. (1950) with the modifications of Helbert and Brown (1955). The anthrone method depends on dehydration of the sugar to a furfural derivative by the sulfuric acid component which combines with the anthrone to form a blue-coloured compound. A 0.16% solution of anthrone in 95% sulfuric acid was made up fresh daily. The anthrone solution when 4 hours old, was added to the 2 ml. sample while the latter was in an ice-bath and being agitated with a magnetic stirrer. The tubes were sealed with glass marbles and Parafilm and placed in a boiling water-bath for 10 min. (± 2 secs.). It is important to maintain the bath at a constant temperature and to time the incubation precisely for consistent results. After 10 min. at 100°C the tubes were again placed in the ice-bath to stop the reaction. The tubes were then allowed to equilibrate with room temperature (about 20 min.) and read in a Beckman DU spectrophotometer at a wavelength of 625 millimicrons. An absorption spectrum was run under the same conditions and the point of maximum O.D. was verified as being 625 mμ. The concentration of polysaccharide was calculated from a standard curve prepared from glucose standards. The results were expressed as per cent polysaccharide in the dry tissue and will be referred to as

polysaccharide level.

Protein: The Lowry colorimetric method was used for protein determinations, bovine albumin serving as the standard (Lowry, et al., 1951). Approximately 5 mg. of dry tissue was dissolved in 4 ml. of 1 N. NaOH for several hours and diluted to 25 ml. A 1 ml. aliquot was used for the protein determinations following the procedure of Lowry, et al. (1951). In recent years the Lowry colorimetric method for determining proteins has come into widespread use (Giese, 1967). This method has several advantages. (1) It is as sensitive as Nessler's reagent but requires no digestion. (2) It is 10 or 20 times more sensitive than the measurement of the ultraviolet absorption at 280 mμ and is much more specific and much less liable to disturbance by turbidities. (3) It is several fold more sensitive than the ninhydrin reaction and is somewhat simpler, as well as much easier to adapt to small scale analyses. (4) It is 100 times more sensitive than the biuret reaction. There are two major disadvantages to the Lowry reaction. (1) The amount of colour varies with different proteins; however, in this study the same type of tissue was analysed through the year so only the relative changes were important. (2) The colour is not strictly proportional to concentration. To overcome this difficulty, sample concentrations were kept in the range of 0 to 100 μg./ml. where the standard curve was essentially linear.

The 1 ml. sample aliquot was allowed to sit with 5 ml. of a carbonate-copper solution for 10 min. and then to this mixture was added 0.5 ml. of the Folin-Ciocalteau phenol reagent while the tube was shaken. The colour was allowed to develop at room temperature for 30 min. and was measured in a Beckman DU spectrophotometer at 750 mμ.

An absorption spectrum was obtained to verify 750 mμ as the wavelength of maximum absorption. The concentration was expressed as the per cent protein per unit dry weight of tissue. As with lipid and glycogen, this value will be referred to as the protein level in the tissue.

Ash weights were determined on pooled samples of dry tissue for three periods in the year using the method described in Official Methods of Analysis of the A.O.A.C. (1960, page 419). The pooled sample was divided into two aliquots and the mean of the ash weights calculated. Ashing was done at a temperature of 525°C for about 12 hours to constant weight.

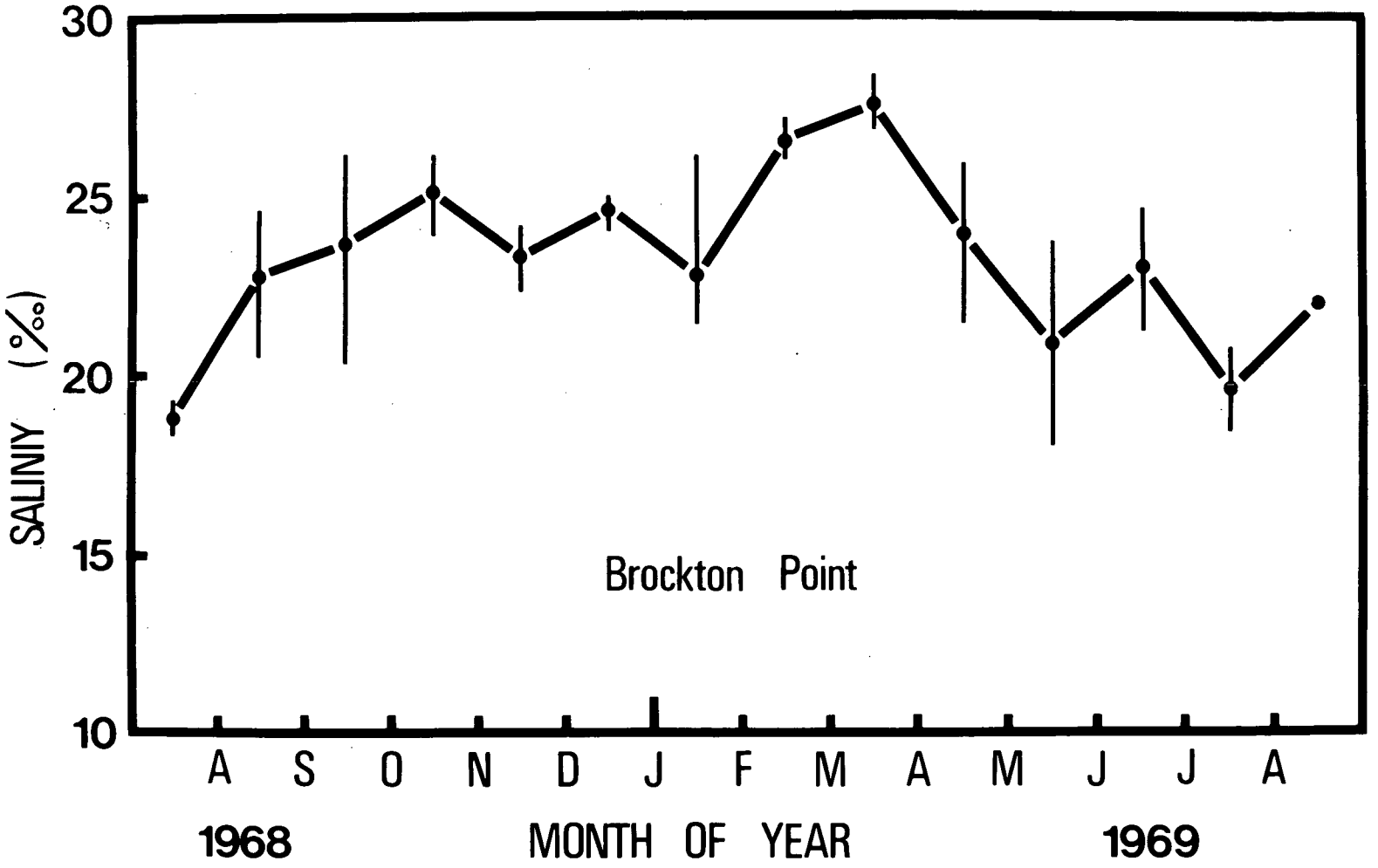
Controlled Conditions

Two groups of Thais lamellosa, about 20 in each, were maintained under controlled "summer" conditions of salinity (18.9°/oo), temperature (15°C) and light (16 hours light; 8 hours dark) in plastic dishes supplied with aerated sea-water. The salinity of 18.9°/oo approximated the low values recorded in May and June (Fig. 2). Full strength sea-water was diluted with glass distilled water to the desired value, and was changed weekly. The temperature of 15°C approximated the field water temperature in the summer (Fig. 4) and was regulated by a thermostatically controlled water-bath (± 1.0°C). The light regime represented the day length in June (Fig. 5) and was automatically controlled by a timing mechanism.

One group of animals was supplied with mussels and barnacles at all times, while the other was starved. Organisms such as barnacles growing on the shells of the starved group were removed and the walls of the dishes were kept clean of algae. At the end of 4 months tissues

FIGURE 2

Mean monthly salinity two feet below the surface at Brockton Point. Vertical lines indicate the range. The first and last points do not represent complete months.


30
25
20
15
10
SALINIY (‰)
Brockton Point
A S O N D J F M A M J J A
1968
MONTH OF YEAR
1969

of 8 females and 2 males were obtained and analysed for protein, lipid, polysaccharide, dry weight, and component index using the methods described previously. Also gonads and digestive glands were sectioned and analysed for oocyte size distribution and storage granule content. Data obtained from regular field samples at the beginning and end of the experimental period represented the normal changes during that time.

Statistical Methods

Student's t-test was used to determine significant differences between mean values. P levels of 5% or less were accepted as significant.

Photographs were taken through a Zeiss Photo-Microscope with bright field optics on Kodak Panatomic-X film. Sections were stained with Masson's Trichrome.

RESULTS

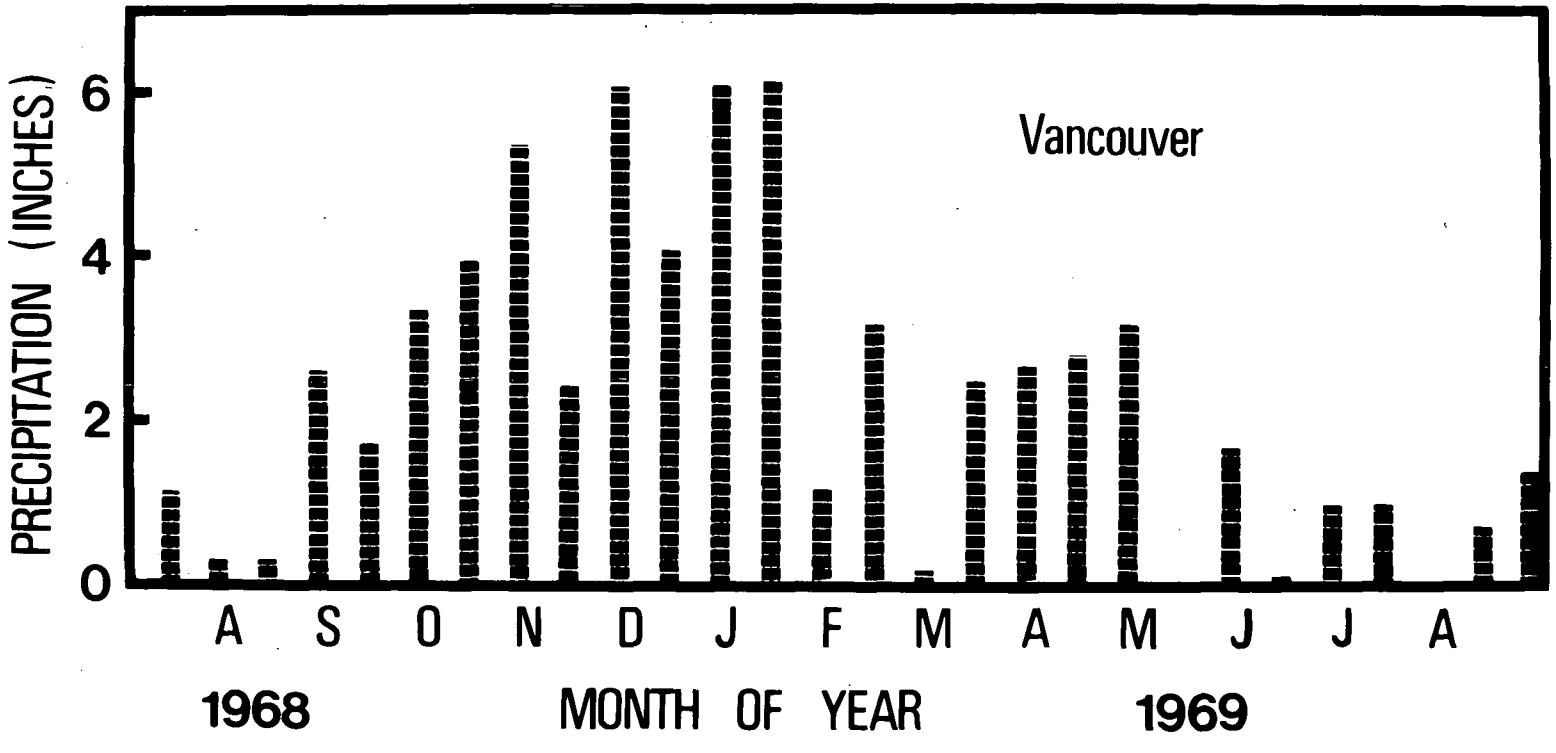
Field Observations

Environmental parameters: Figure 2 shows the salinity changes over a 14 month period from July 1968 until September 1969. The maximum salinity of 28.5°/oo, occurred in the middle of March 1969; the minimum of 18°/oo, at the end of May and the beginning of July. This minimum corresponded to the maximum discharge of the Fraser River. During the winter the Fraser River discharge held steady at about 25,000 cubic feet/sec. and at maximum discharge reached 425,000 cubic feet/sec. (Pacific Oceanographic Group, 1951). The low salinities occurring in September and November can be explained by the rainfall data of Figure 3 (Canada D.O.T. Meteorological Branch, 1968). A peak of rainfall at the beginning of September resulted in a drop of salinity in the second half of September. Similarly, the dip in salinity in November probably resulted from the rainfall peak in early November. From December to March the salinity increased slowly as most of the precipitation fell as snow in the surrounding mountains and run-off was consequently low. From April to July the salinity dropped as snows melted and run-off increased. The main sources of run-off in the collection area are the Squamish River, Capilano River, Fraser River, Seymour Creek, and numerous smaller creeks draining the mountains around Burrard Inlet (Fig. 1).

Figure 4 shows the water temperature variation over a period of 12 months from August 1968 to July 1969. The minimum temperature of 2°C occurred in late December as a result of unseasonally low air temperatures. The maximum of 15.5°C was in July 1969 at the end of

FIGURE 3

Total precipitation for each 15 day period at the Port Meteorological Office, Vancouver. Calculated from tables in the Annual Meteorological Summary (Canada D.O.T. Meteorological Branch, 1969, 1970).


PRECIPITATION (INCHES)
0
2
4
6
Vancouver
A S O N D J F M A M J J A
1968
MONTH OF YEAR
1969

the recording period. A plot of daylight hours (Fig. 5) shows that the minimum water temperature corresponded to the shortest day length, and the maximum water temperature lagged behind the maximum day length by about a month.

Reproductive activities: T. lamellosa was first observed to lay egg-capsules at the beginning of January when the water temperature was near a minimum and the salinity was about 25°/oo. No observations were made on the duration of capsule laying for the population but histological data, which will be described later, indicate the laying continues until about April. The population of T. lamellosa appeared to move to lower tidal levels during the winter and either formed aggregations for reproduction or buried themselves around the bases of rocks. They were found only in the barnacle zone and seldom as high as the Mytilus zone, so it appeared that T. lamellosa was feeding mostly on barnacles; however, these are only casual observations made during collection trips and require more documentation.

Internal Morphology

Gross anatomy: The sexes are separate in T. lamellosa; however, there are no external differences except for the presence of a penis in the male which can sometimes be seen during copulation. The morphology of T. lamellosa is virtually identical to Nucella lapillus, an Atlantic species which is described in detail in Fretter and Graham (1962, pages 332-338). The gonad lies on one side of the digestive gland in the upper coils of the visceral mass. The ovary is usually yellow and granular in appearance, while the testis is more translucent and homogeneous in texture. The digestive gland varies in colour from dark brown to gray-green. Table 1 indicates the range

FIGURE 4

Mean monthly water temperature two feet below surface at Brockton Point. Vertical lines indicate the range.

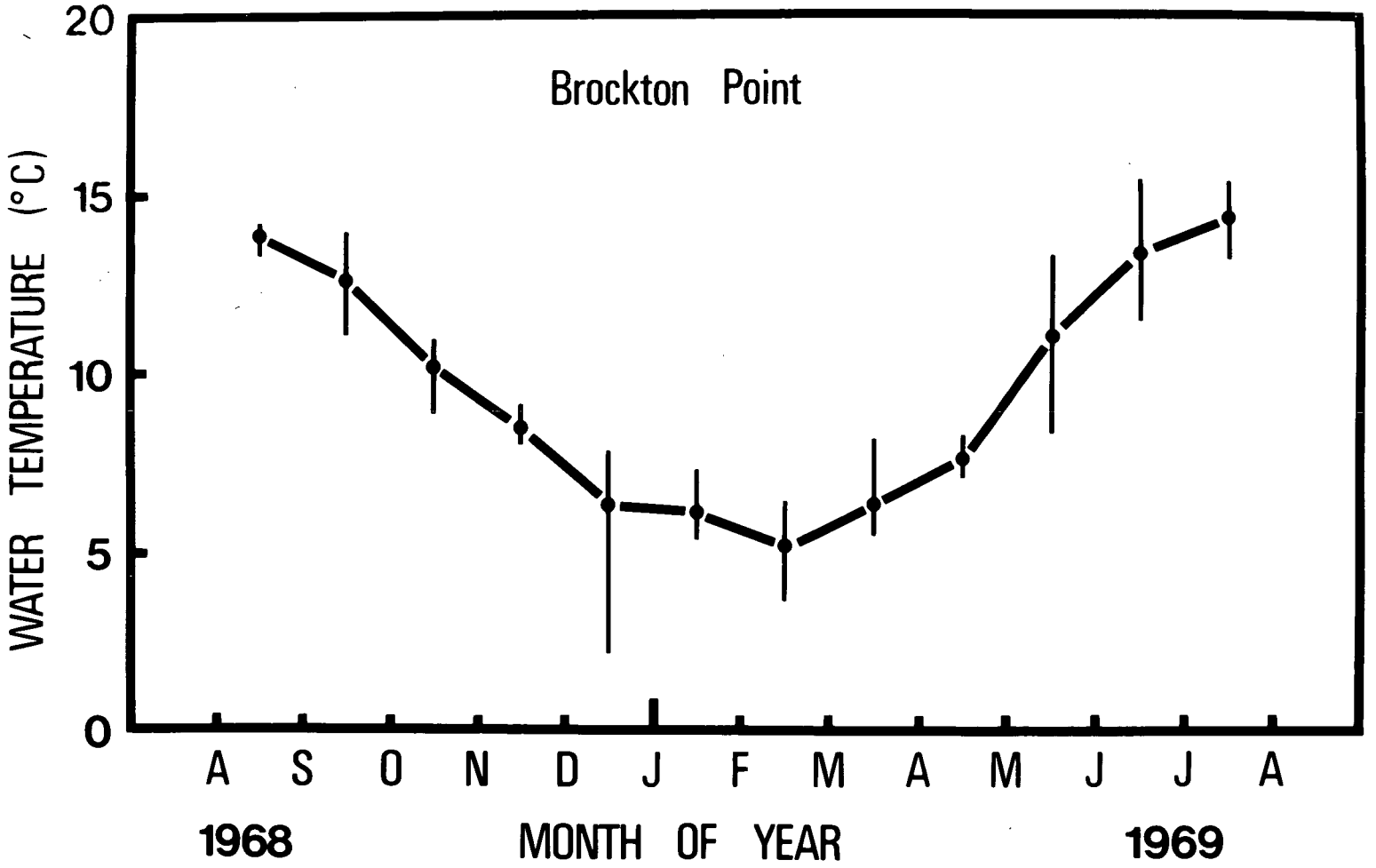
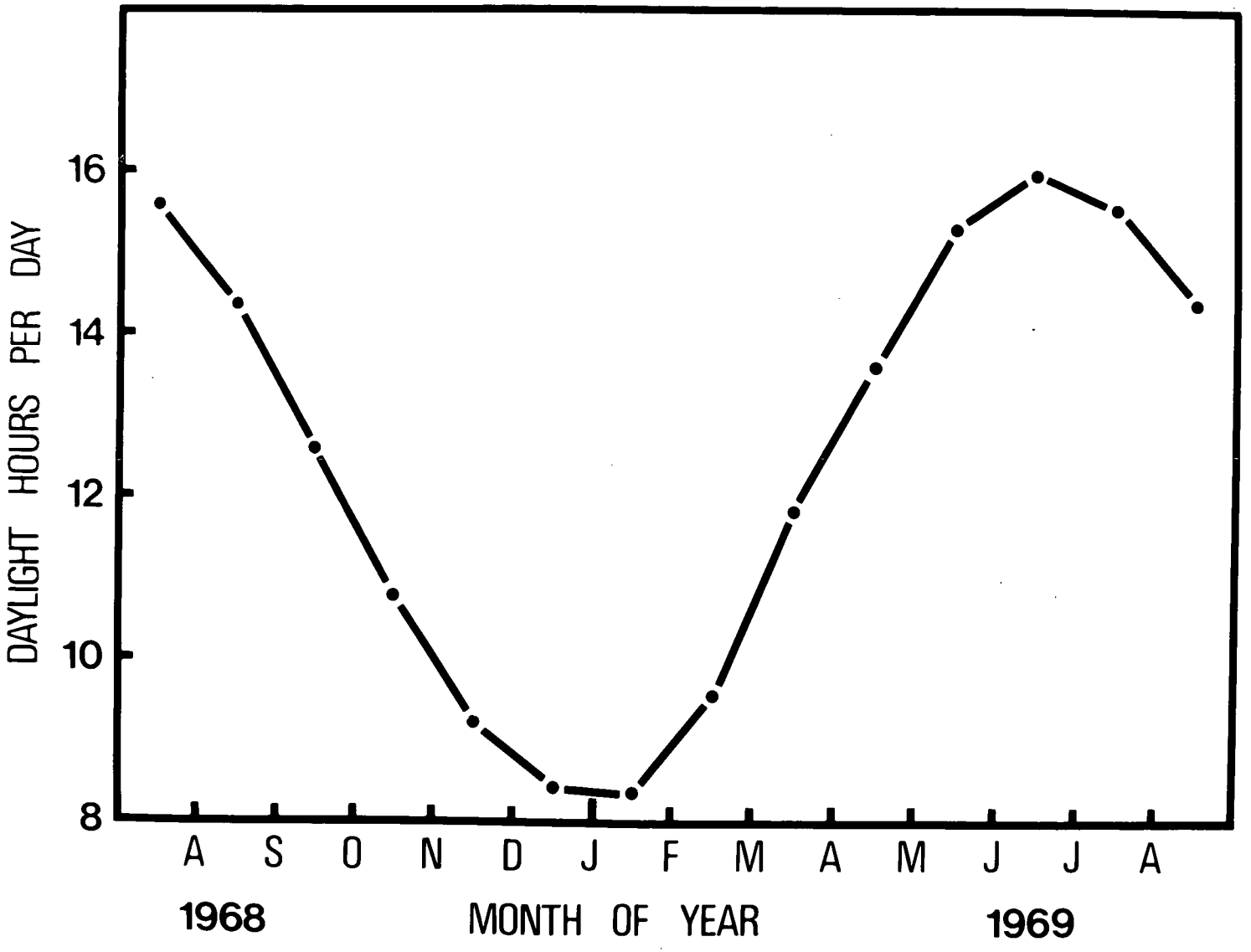

Brockton Point
WATER TEMPERATURE (°C)
20
15
10
5
0
A S O N D J F M A M J J A
1968
MONTH OF YEAR
1969

FIGURE 5

The points represent the average day-light hours per day for each month, calculated from tables in Northcott (1968, 1969).


DAYLIGHT HOURS PER DAY
16
14
12
10
8
A
S
O
N
D
J
F
M
A
M
J
J
A
1968
MONTH OF YEAR
1969

of colour in the digestive gland and the number of animals of a particular colour in each collection. A gray-green digestive gland predominated in the February and May collections, while in August most animals had yellow-brown digestive glands. The gland was predominantly brown to dark brown in the April collection.

Histological observations: Histological sections showed the testis and ovary to consist of many tubules in which the gametes appeared in various stages of maturation. During reproductive periods, from January to March, the tubules were large and closely apposed to each other but in non-reproductive periods the tubules were reduced in size and the connective tissue spaces between tubules increased in size. The histological analysis of the reproductive cycle was confined to females of T. lamellosa.

An attempt was made to classify the ovary on a subjective scale ranging from ripe to spent; however, clear cut changes did not occur in the ovary of T. lamellosa. Large yolk granules were present at all times of the year, as were small immature oocytes. After the reproductive season, oocytes that remained in the ovary appeared to disintegrate and the yolk granules became scattered throughout the tubule. Developing oocytes had prominent nucleoli in a large germinal vescicle and the cytoplasm contained small granules. In large mature oocytes the nucleus became dense and pyknotic and was often difficult to locate among the large yolk granules which completely filled the oocyte.

Determination of reproductive cycle by oocyte diameter: Figure 6 indicates the size distribution of oocytes in each collection throughout the year. Each polygon represents 125 oocytes, 25 from each of

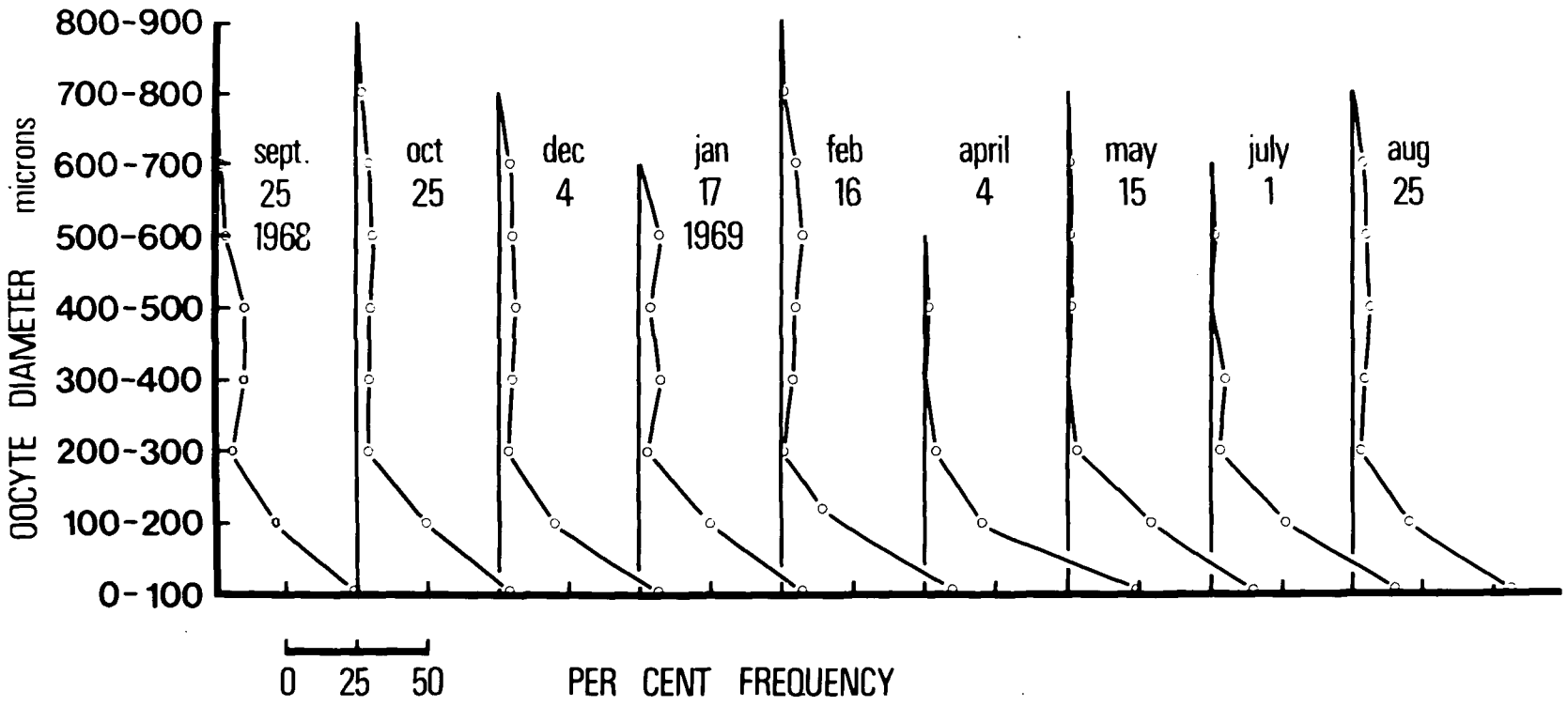
TABLE 1

Subjective classification of digestive gland colour.

Collection Date	Sample Size	Gray-Green	Yellow-Brown	Brown	Dark Brown
October 25, 1968	10	-	8	1	1
December 4	11	1	9	1	-
January 17, 1969	10	4	4	2	-
February 16	19	12	4	3	-
April 4	20	5	1	9	5
May 15	20	11	4	4	1
July 1	20	9	7	4	-
August 25	20	-	17	3	-

FIGURE 6

Oocyte diameter frequency polygons for each monthly collection of T. lamellosa. Each polygon represents a total of 125 oocytes and the points indicate the per cent of the total in each size class. The abscissa is in per cent units.


OOCYTE DIAMETER microns
800-900
700-800
600-700
500-600
400-500
300-400
200-300
100-200
0-100
sept. 25 1968
oct 25
dec 4
jan 17 1969
feb 16
april 4
may 15
july 1
aug 25
0 25 50
PER CENT FREQUENCY

five females, and each point is the percentage of the total, in that size class. For example, on September 25, 1968, 50% of the oocytes measured were in the 0 to 100 μ size class, 22% were in the 100 to 200 μ size class. The graphs indicate that oocytes in the range of 500 μ and greater, occur in significant numbers from September 1968 to February 1969 and again in August 1969. In April the size distribution shows a lack of the larger size classes and probably indicates that spawning has taken place. In May the larger oocytes begin to appear but the small size classes predominate. In July the oocytes appear to be maturing and increasing in size as those in the size classes greater than 300 μ begin to appear in greater numbers. The data indicate that in late March or early April most of the population had spawned and, replenishment of large oocytes began immediately. From August to February mature oocytes, at least from the point of view of size, were present in the ovary.

Parasites: In the April 4 collection, 6 out of 26 T. lamellosa examined were infected by the parasitic trematode of the genus Renicola (Ching, pers. comm.). Infected T. lamellosa were not used for gonad indices or biochemical analyses.

Histology of digestive gland: The digestive gland was examined microscopically and the number of storage granules in the cells of the digestive gland tubules was classified subjectively, full of granules, half full or empty (Table 2, Plate 1). It is assumed that the presence of storage granules indicates that the animals have been feeding. If feeding stops, the granules will gradually diminish in numbers as the stored nutrients are mobilized for body maintenance. The number of storage granules was at a maximum in August and remained fairly high

TABLE 2

Subjective classification of number of granules in the storage cells of the digestive gland of Thais lamellosa.

Collection Date	Sample Size	Full	Half-Full	Empty
August 6, 1968	11	10	1	-
September 25	13	11	1	1
October 25	7	7	-	-
December 4	9	6	3	-
January 17, 1969	9	6	3	-
February 16	10	-	5	5
April 4	9	8	1	-
May 15	9	6	1	2
July 1	10	5	3	2
August 25	10	10	-	-
Experimental Animals				
Aug. 31 to Jan. 6 (fed)	8	5	3	-
Aug. 31 to Jan. 6 (starved)	10	3	5	2

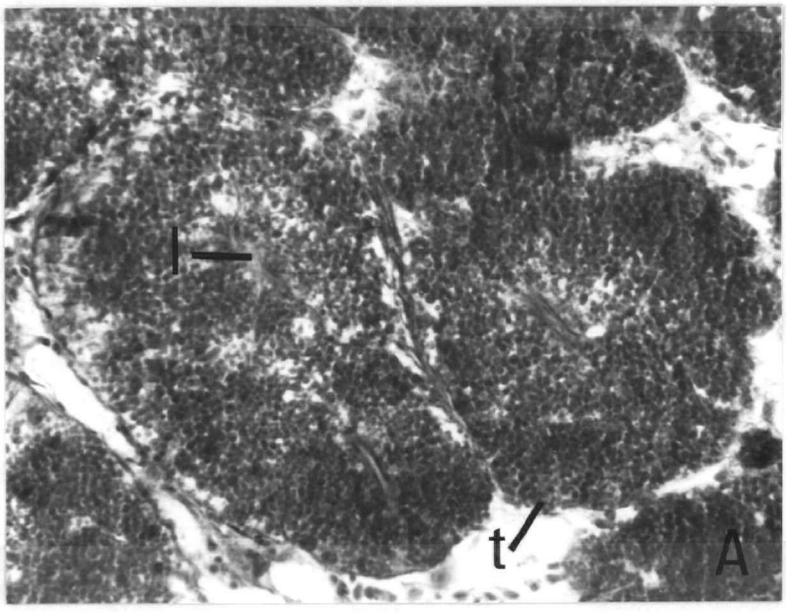
PLATE 1

Cross-section of digestive gland illustrating the subjective classification of granule content. l = lumen, t = tubule, g = storage granule.

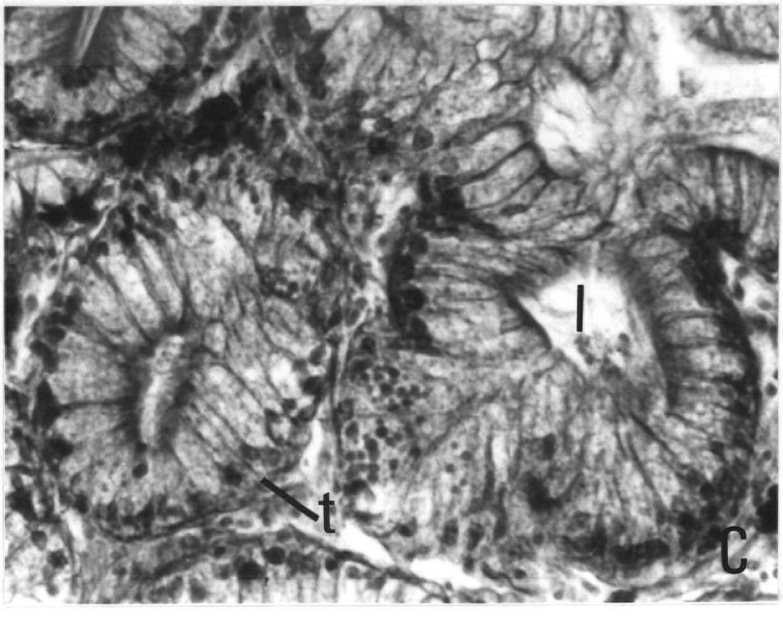
A. tubules full of granules (2600 X)

B. tubules half full (3400 X)

C. tubules empty (3400 X)


l
t
A


l
g
t
B


l
t
C

until October 25. From December until February the number of granules in the cells dropped, probably indicating that the animals were feeding less and utilizing stored nutrients. The number of granules increased in April and in May and July the numbers dropped again.

Per Cent Dry Weight

The per cent dry weights of three body organs; gonad, digestive gland, and muscle, over a 12 month period are plotted in Figure 7. The per cent dry weight of muscle remained essentially constant at about 27.5%. The per cent dry weight of ovary ranged from a maximum of 63% in December to a minimum of 40% in July. The per cent dry weight of digestive gland ranged from a maximum of 55% in December to a minimum of 39% in July with a further low value of 40% in February. The per cent dry weight indicates the amount of solid material in proportion to water content; consequently, when the gonads are full of mature eggs and the inter-tubule spaces are smallest, the per cent dry weight is at a maximum. Similarly, when the animal has been feeding, all the cells of the digestive gland are full of stored material and no empty spaces are evident in the histological sections, resulting in a high per cent dry weight.

Gonad Index

Another method of representing the reproductive cycle is by the gonad index. The gonad index cycle is shown in Figure 8. Only the females were sampled in quantities which allowed calculation of mean and standard error. For this population, the ovary index dropped from about 7 during the months of October to February, down to 2 in April, probably indicating spawning. This correlates well with the data from egg size frequency (Fig. 6) and per cent dry weight (Fig. 7) which both

FIGURE 7

Per cent of dry material in a unit weight of fresh tissue of T. lamellosa. The vertical lines indicate ± 1 S.E. Data for females only.

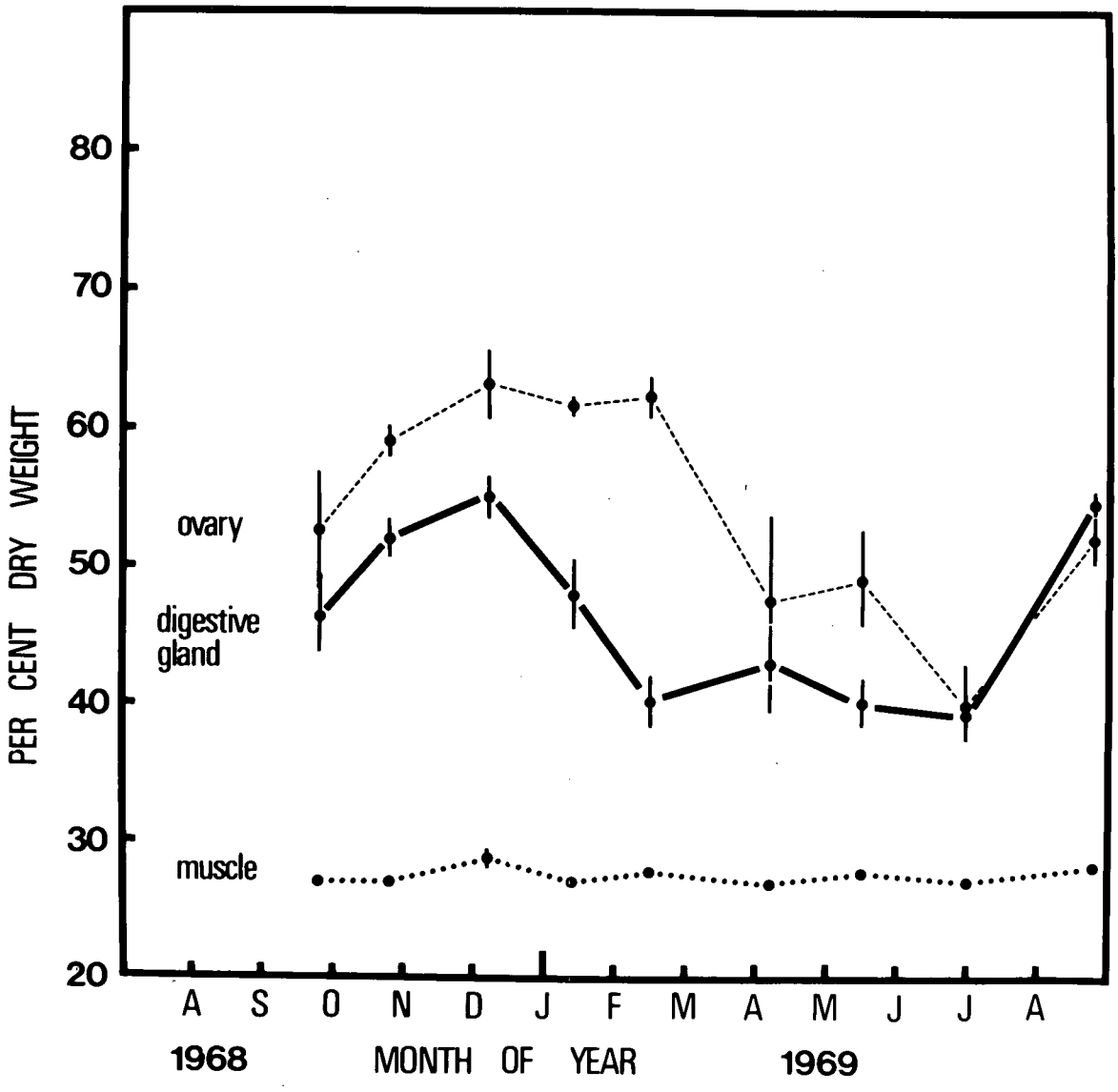
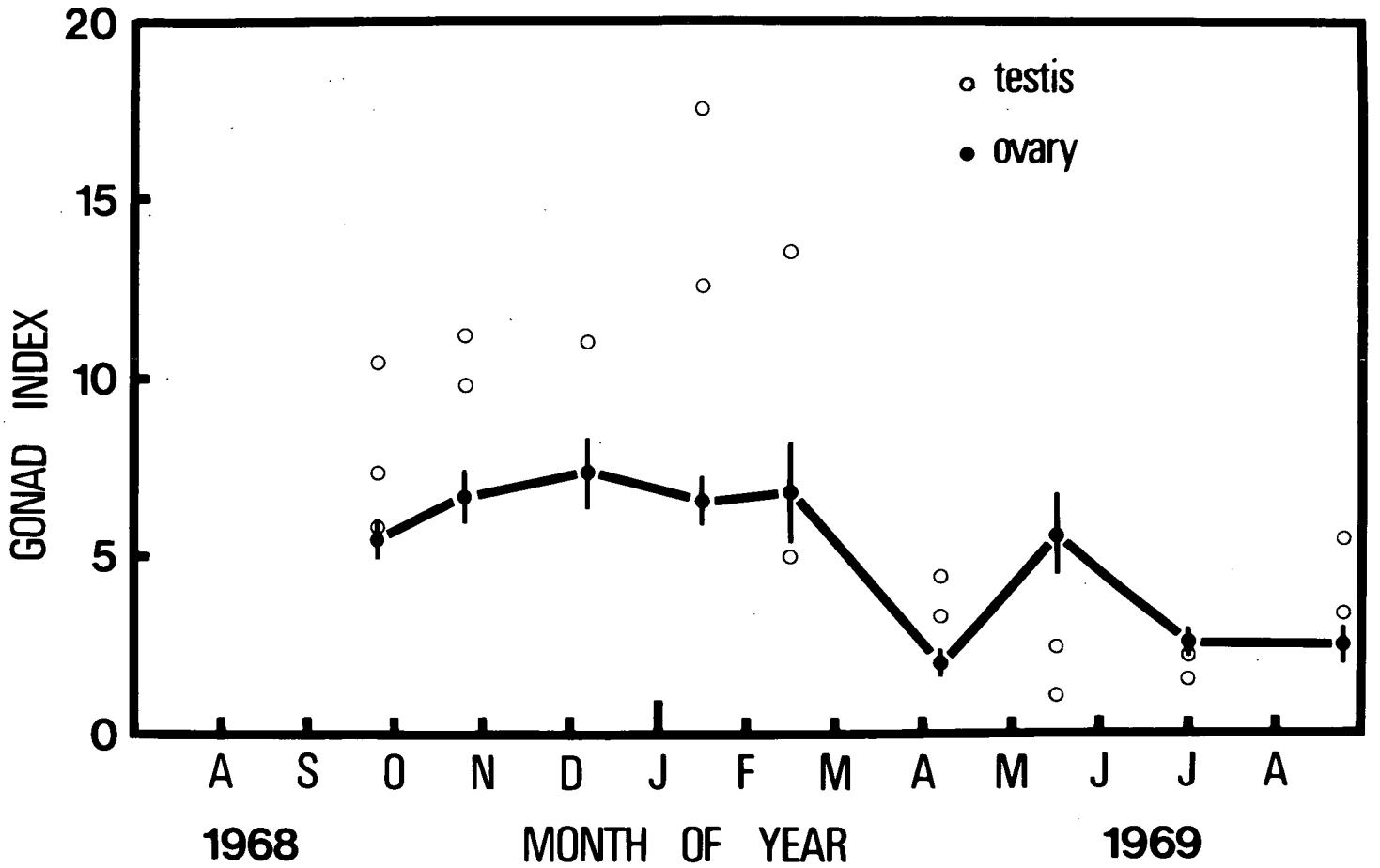

80
70
60
50
40
30
20
PER CENT DRY WEIGHT
ovary
digestive gland
muscle
A S O N D J F M A M J J A
1968
MONTH OF YEAR
1969

FIGURE 8

Gonad indices of male and female T. lamellosa as a function of month of year. Closed circles (●) represent the mean ovary index and open circles (○) represent individual males. Vertical lines indicate ± 1 S.E.


20
15
10
5
0
GONAD INDEX
testis
ovary
A
S
O
N
D
J
F
M
A
M
J
J
A
1968
MONTH OF YEAR
1969

dropped during this same period. From April to May the ovary index rose again from 2 up to 5.5, and this is reflected in the appearance of oocyte size classes above 500 μ in the May 15 collection (Fig. 6). The testis index appears to follow a similar pattern to the ovaries but with higher absolute values during the winter months.

Digestive Gland Index

The changes in the digestive gland indices are shown in Figure 9. The maximum index of 22.5 for the females occurred in late August 1969 and the minimum of 7 in February. The changes in the digestive gland index were parallelled by the per cent dry weight data in Figure 7 and the storage granule data in Table 2. The maximum index in August corresponded to the greatest accumulation of storage granules, and the minimum index in February corresponded to the lowest storage granule content. The male digestive gland index showed similar changes to the female but due to the small sample size minor differences could not be determined. It should also be emphasized that due to the method of sampling as described previously, the indices arrived at are only relative values and they underestimate the true index.

Constituent Levels

Digestive gland: Figure 10 shows the levels of protein, lipid, and glycogen for the digestive gland; Figure 11 shows the levels in the foot muscle; and Figure 12, the gonad. The changes in the lipid will be considered first. The greatest variation in lipid level occurred in the female digestive gland (Fig. 10), which went from 38.5% in December to 20% in April, followed by another increase to 35% in July. The lipid level in the muscle remained essentially constant at 6 to 8%. The lipid levels in the male digestive gland and

FIGURE 9

Digestive gland indices of male and female T. lamellosa as a function of month of year. Closed circles (●) represent the mean of 8 females and the open circles (○) represent individual males. Vertical lines indicate ± 1 S.E.

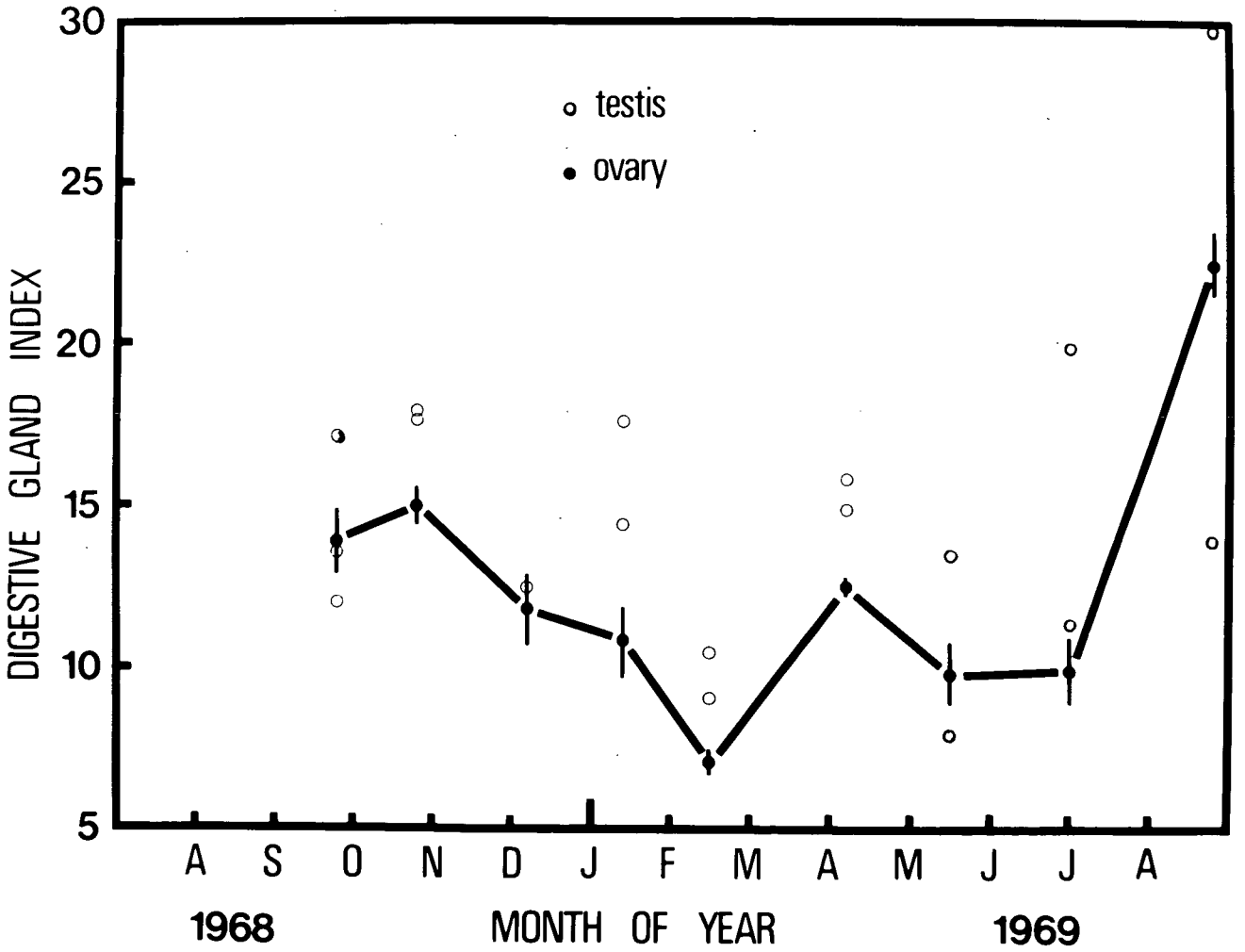
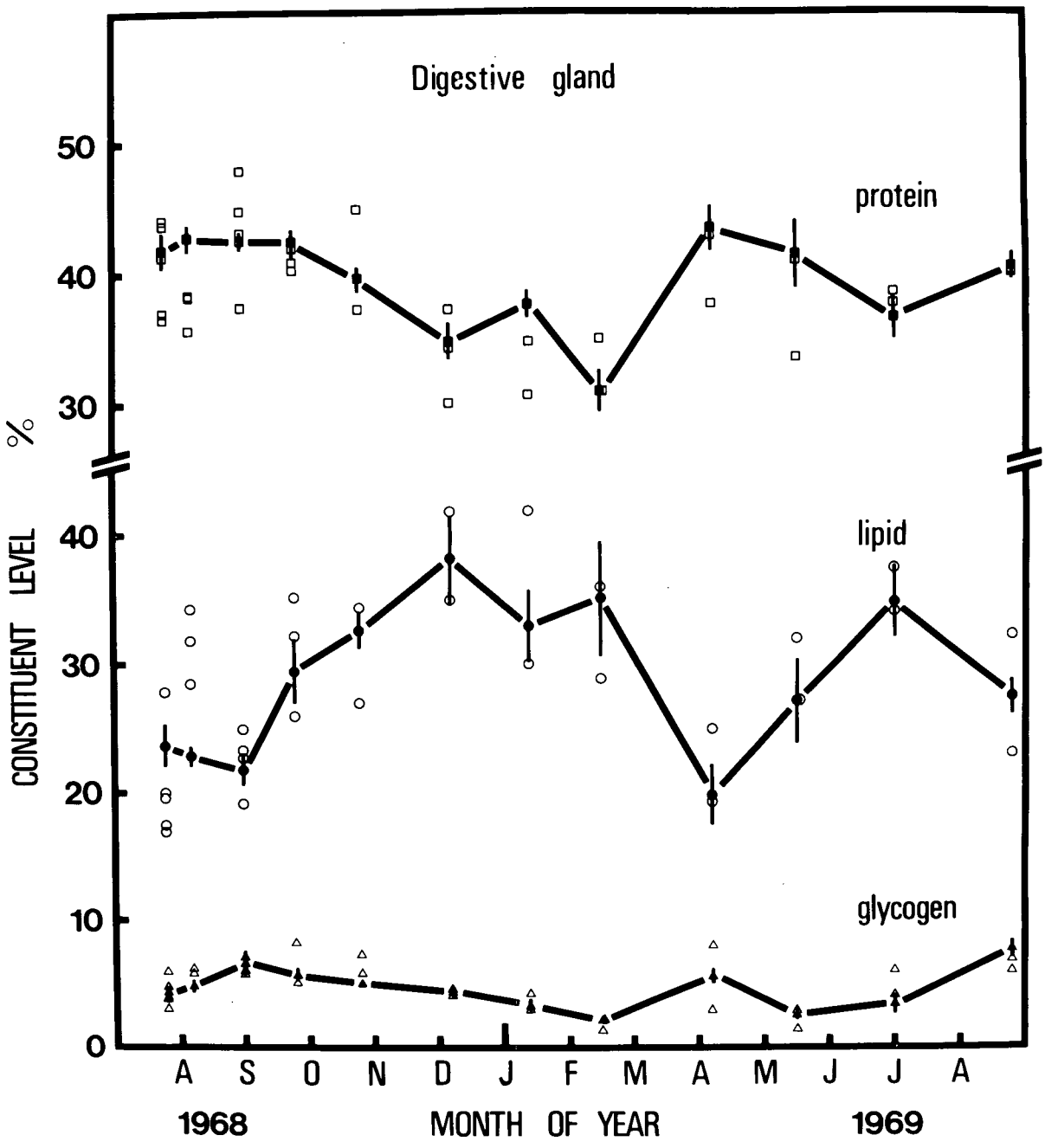

30
25
20
15
10
5
DIGESTIVE GLAND INDEX
testis
ovary
A
S
O
N
D
J
F
M
A
M
J
J
A
1968
MONTH OF YEAR
1969

FIGURE 10

Levels of protein, lipid and glycogen in the digestive gland of T. lamellosa as a function of month of the year. Each point represents the per cent of a constituent in a unit weight of dry tissue. Closed symbols represent female means and open symbols represent individual males. Vertical lines indicate ± 1 S.E.


Digestive gland
protein
lipid
glycogen
CONSTITUENT LEVEL %
50
40
30
40
30
20
10
0
A S O N D J F M A M J J A
1968
MONTH OF YEAR
1969

muscle appeared to follow those of the females. The ovary lipid level fluctuated several times during the year with the most significant change occurring between October and December from 26% down to 18% ($p < .05$). Fluctuations in February, April and May were also significant ($p < .05$). The testis lipid appeared to drop down to about 10% in the winter with possibly a slight rise in July.

Protein levels: In female tissue the highest protein levels occurred in muscle (Fig. 11) with a high of 64% in April and a low of 46.6% in July 1968. Ovary was slightly lower with a maximum of 56% in October and a general downward trend to April where the protein level was 46% (Fig. 12). The digestive gland showed the greatest range in protein level from a maximum of 43.5% in April to a minimum of 31% in February. The protein levels in male muscle and digestive glands were, as near as could be determined by the small sample size, the same; but in the testis, protein values were generally lower and averaged about 40%.

Glycogen and ash levels: Emerson (1966) showed that the polysaccharide of T. lamellosa is 100% glycogen; hence, in this study the polysaccharide will be referred to as glycogen. Glycogen was at very low levels in all three tissues. In the ovary it ranged from 2% to 4%; in the muscle it fluctuated from 2% to 4%; and in the digestive gland the range was slightly greater, from 2% to 7.5%. Again, the glycogen level in the male tissues was essentially the same. Ash weights for the three tissues at three times during the year are represented in Figure 13. Male and female tissues were combined in all

FIGURE 11

Levels of protein, lipid and glycogen in the foot muscle of T. lamellosa as a function of month of year. Each point represents the per cent of a constituent in a unit weight of dry tissue. Closed symbols represent female means and open symbols represent individual males. Vertical lines indicate ± 1 S.E.

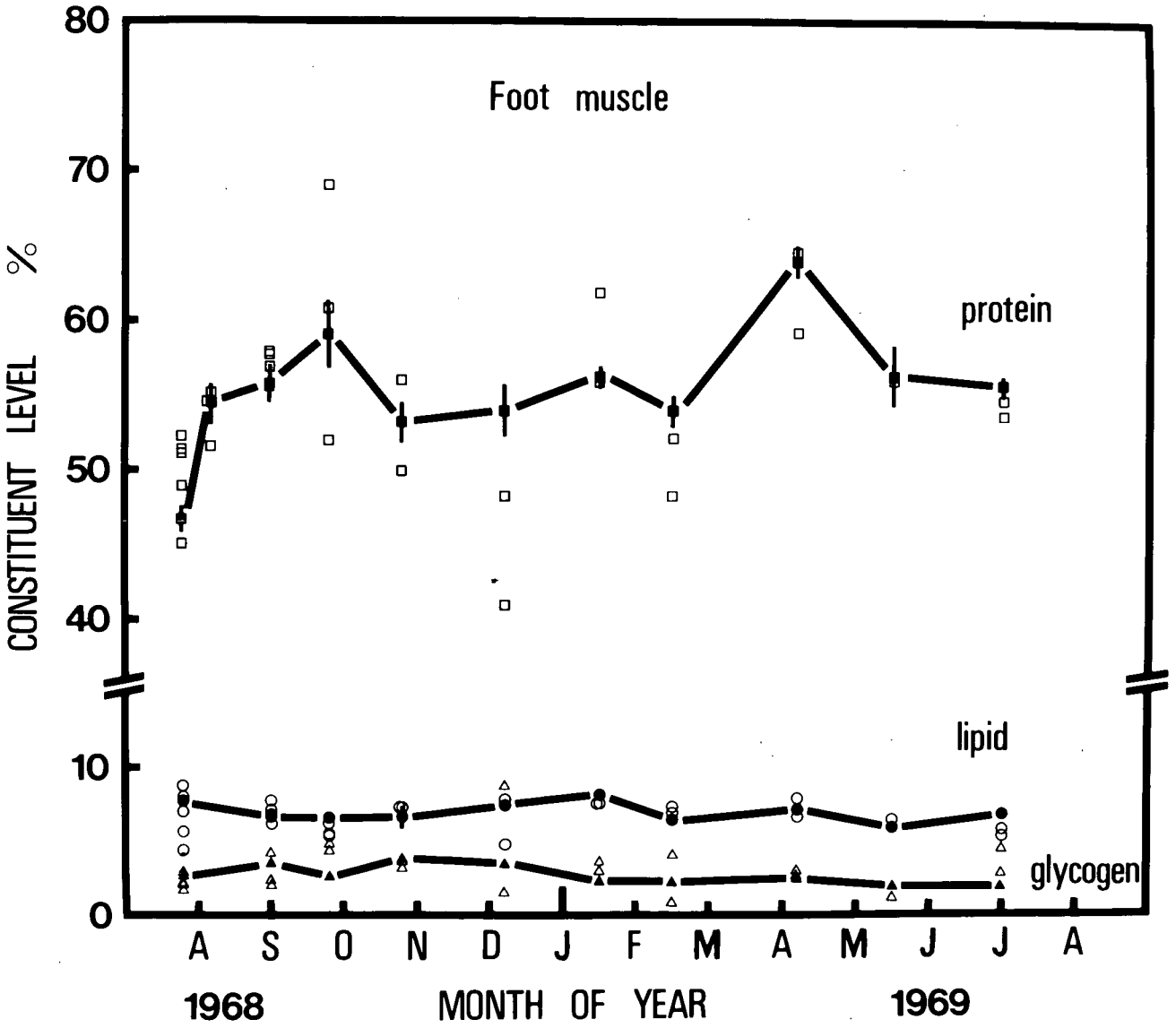
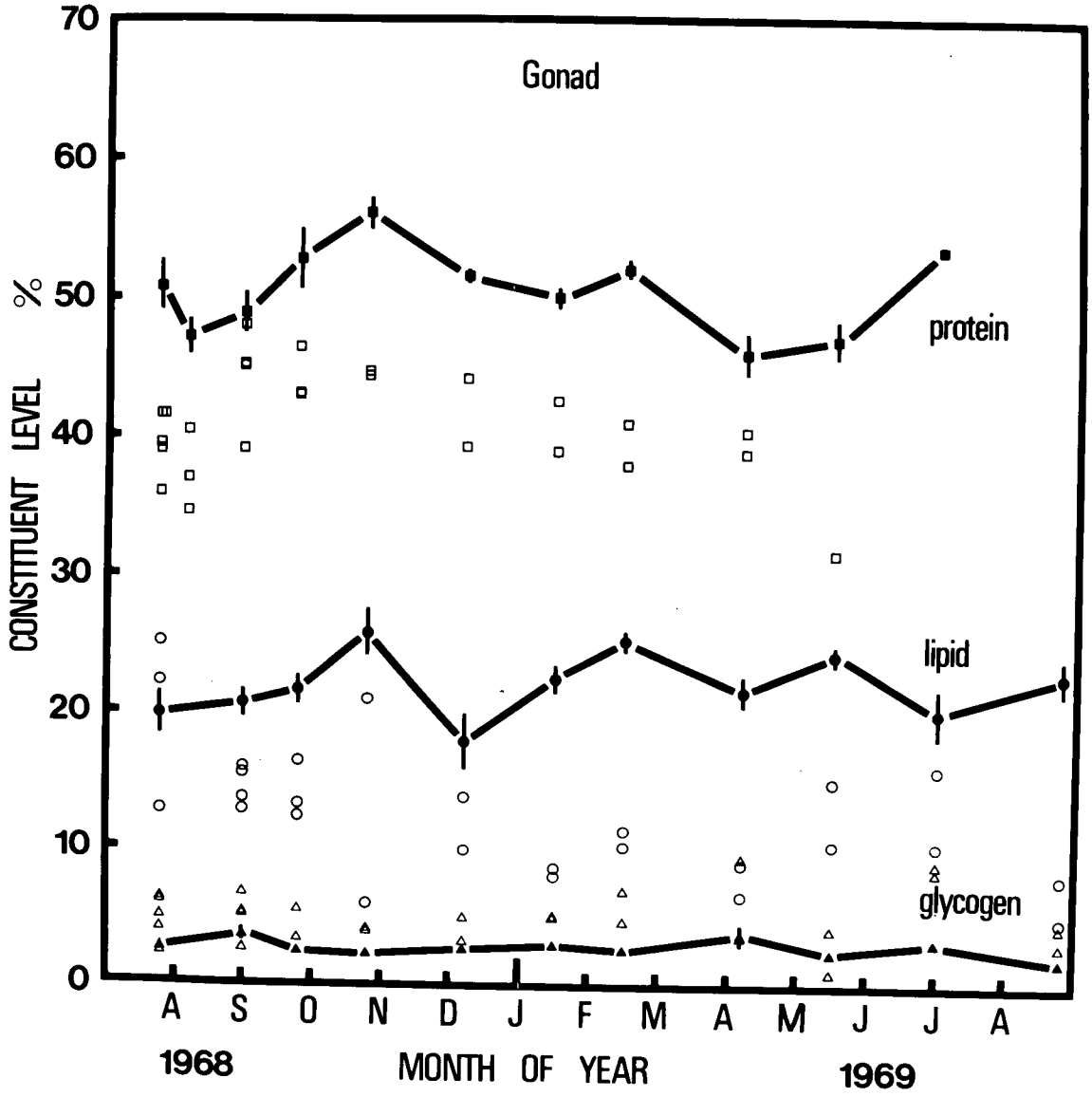

Foot muscle
CONSTITUENT LEVEL %
80
70
60
50
40
10
0
protein
lipid
glycogen
A S O N D J F M A M J J A
1968
MONTH OF YEAR
1969

FIGURE 12

Levels of protein, lipid and glycogen in the gonad of *T. lamellosa* as a function of month of year. Each point represents the per cent of a constituent in a unit weight of dry tissue. Closed symbols represent female means and open symbols represent individual males. Vertical lines indicate ± 1 S.E.


Gonad
70
60
50
40
30
20
10
0
%
CONSTITUENT LEVEL
protein
lipid
glycogen
A S O N D J F M A M J J A
1968
MONTH OF YEAR
1969

cases except the gonad which represents only the females. Both muscle and gonad have similar ash levels at the three collection dates; however, the ash level in the digestive gland rises from 3.4% in August to 7% in March.

Figure 14 shows the amount of dry weight accounted for by lipid, glycogen, and protein analyses in the digestive gland each month. In September, January and May, the ash weight values have been added. It can be seen that these analyses account for only about 80% of the dry weight.

Experimental Results

As described in the methods section, two groups of T. lamellosa were maintained in summer conditions from August 31, 1968 until January 6, 1969; one group being fed, the other, starved. A regular collection, made at the beginning of the experiment from the same location (Aug. 31) served to establish the starting levels of protein, lipid, glycogen and dry weight. The experimental animals at the start were assumed to have the same biochemical levels as the field group since they were collected from the same population. At the end of the experimental period another field collection was analysed; hence, the changes in the normal animals during the experimental period were determined. At the end of the experiment, the fed and the starved groups were analysed for the various biochemical constituents. Table 3 summarizes the data, giving levels of lipid, glycogen, protein, dry weight and component indices for the field controls and the two experimental groups. The starting values for the dry weight level and component indices were not obtained on August 31 so the data from the September 25 collection were used as starting values.

FIGURE 13

Per cent ash per unit weight of dry tissue in three tissues of the body. The data for digestive gland and foot muscle are means of pooled male and female tissue.

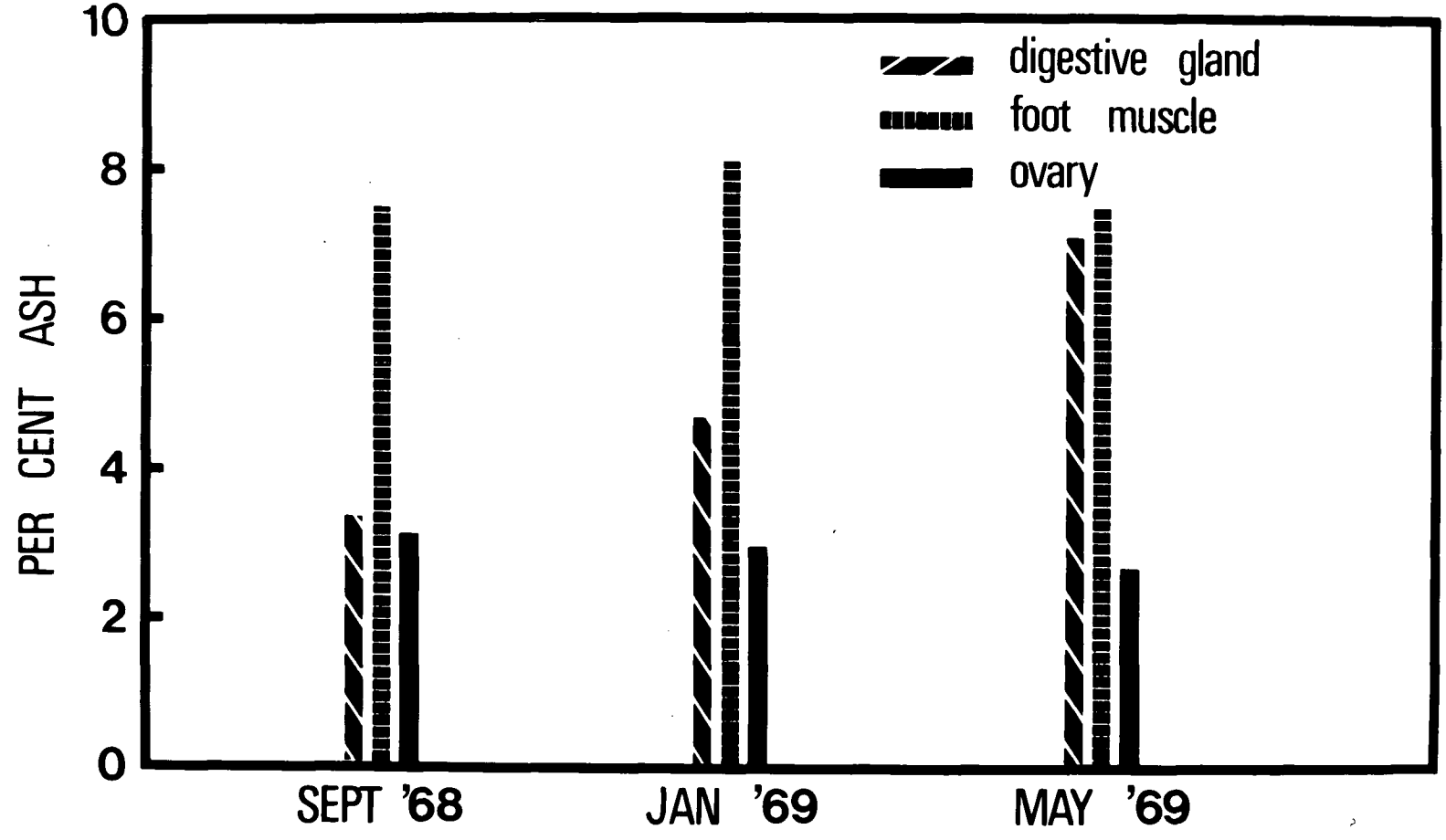

10
8
6
4
2
0
PER CENT ASH
digestive gland
foot muscle
ovary
SEPT '68
JAN '69
MAY '69

FIGURE 14

Histograms represent the amount of dry tissue accounted for by the protein, lipid and glycogen determinations. Data for ash is available for only three of the collections.

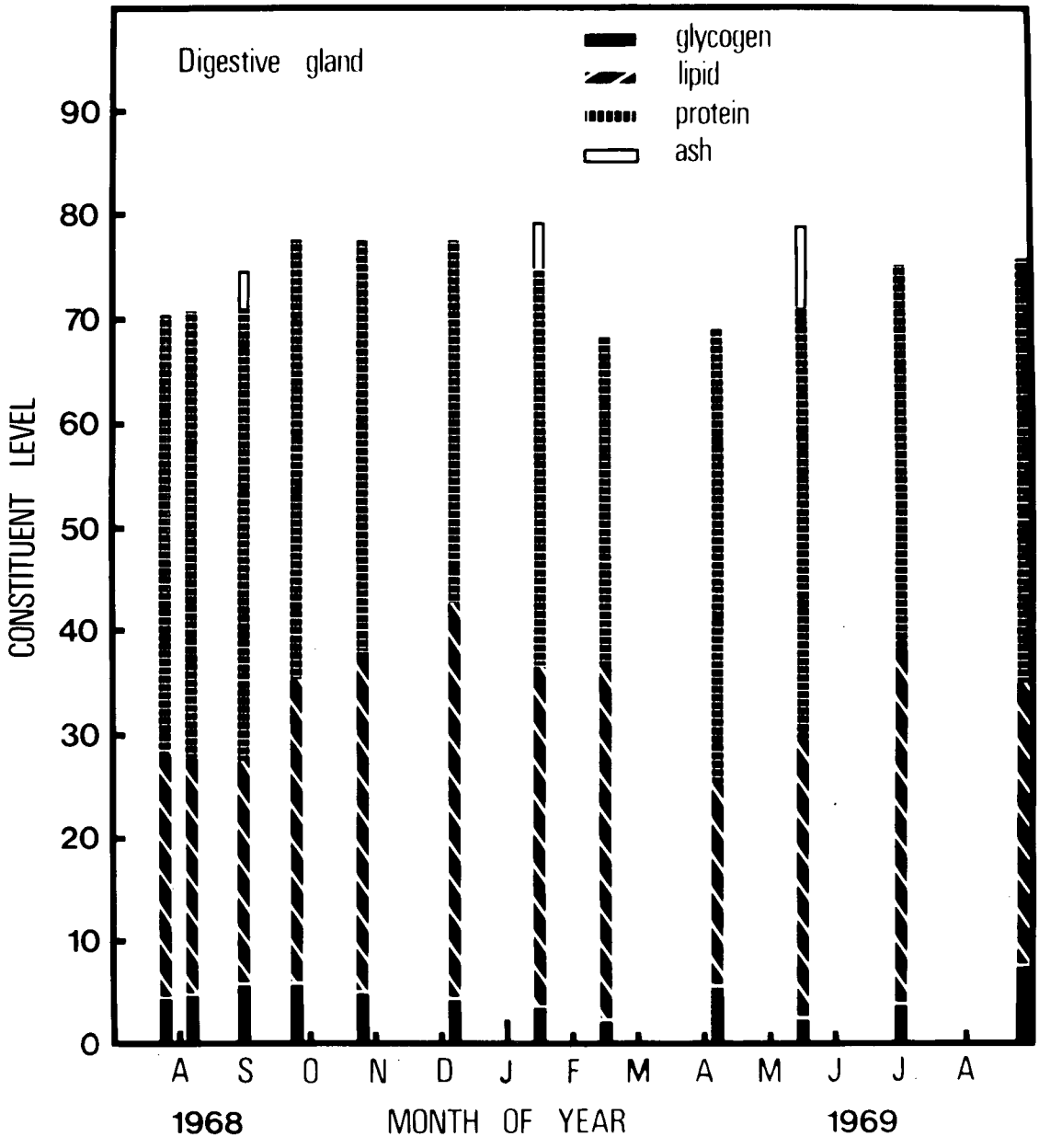

Digestive gland
glycogen
lipid
protein
ash
CONSTITUENT LEVEL
0
10
20
30
40
50
60
70
80
90
A
S
O
N
D
J
F
M
A
M
J
J
A
1968
MONTH OF YEAR
1969

TABLE 3

Summary of data for two experimental groups of T. lamellosa maintained in summer conditions from August 31, 1968 to January 6, 1969. Data for females only. Numbers in brackets are 1 S.E.

c_1 = field sample at start of experiment (August 31).

c_2 = field sample at end of experiment (January 6).

f = experimental group fed, and sampled at conclusion.

s = experimental group starved, and sampled at conclusion.

Tissue		Lipid	Glycogen	Protein	% Dry Weight	Component Index
Digestive Gland	c_1	21.8(±1.2)	6.6(±0.9)	42.4(±0.7)	46.6(±3.0) (Sept. 25)	13.9(±1.0) (Sept. 25)
	c_2	33.0(±2.9)	3.4(±0.4)	37.7(0.9)	47.9(2.6)	10.8(1.1)
	f	32.9(±2.1)	7.0(0.6)	37.7(1.9)	46.2(2.2)	13.8(1.0)
	s	35.6(±1.9)	6.3(0.9)	33.8(1.9)	43.5(1.5)	12.4(1.1)
Ovary	c_1	20.7(±1.0)	3.6(0.5)	48.8(1.4)	52.6(4.3)	5.5(0.6)
	c_2	22.5(±1.0)	3.0(0.2)	49.9(0.7)	61.5(0.6)	6.6(0.6)
	f	31.4(±1.1)	4.2(0.5)	42.3(1.3)	54.4(3.4)	4.1(0.8)
	s	32.5(±1.9)	4.0(0.4)	42.2(2.1)	52.1(2.9)	2.5(0.4)
Foot Muscle	c_1	6.7(±0.2)	3.5(0.3)	55.7(1.3)	27.0(0.2)	
	c_2	8.1(±0.1)	2.3(0.3)	56.2(0.7)	26.9(0.5)	
	f	6.7(±0.3)	4.2(0.6)	60.4(1.0)	25.6(0.7)	
	s	6.6(±0.2)	3.4(0.2)	53.6(1.4)	24.0(0.6)	

The digestive gland index of the field population dropped from 13.9 to 10.8 ($p < .05$) during the experimental period (Table 3). However, the digestive gland indices of the fed and starved animals did not change. Table 2 shows the storage granule data for the two experimental groups. Most of the fed individuals were full of storage granules, while the digestive glands of the majority of starved animals were half-full of granules. There was also a noticeable increase in the number of mucous cells in starved animals.

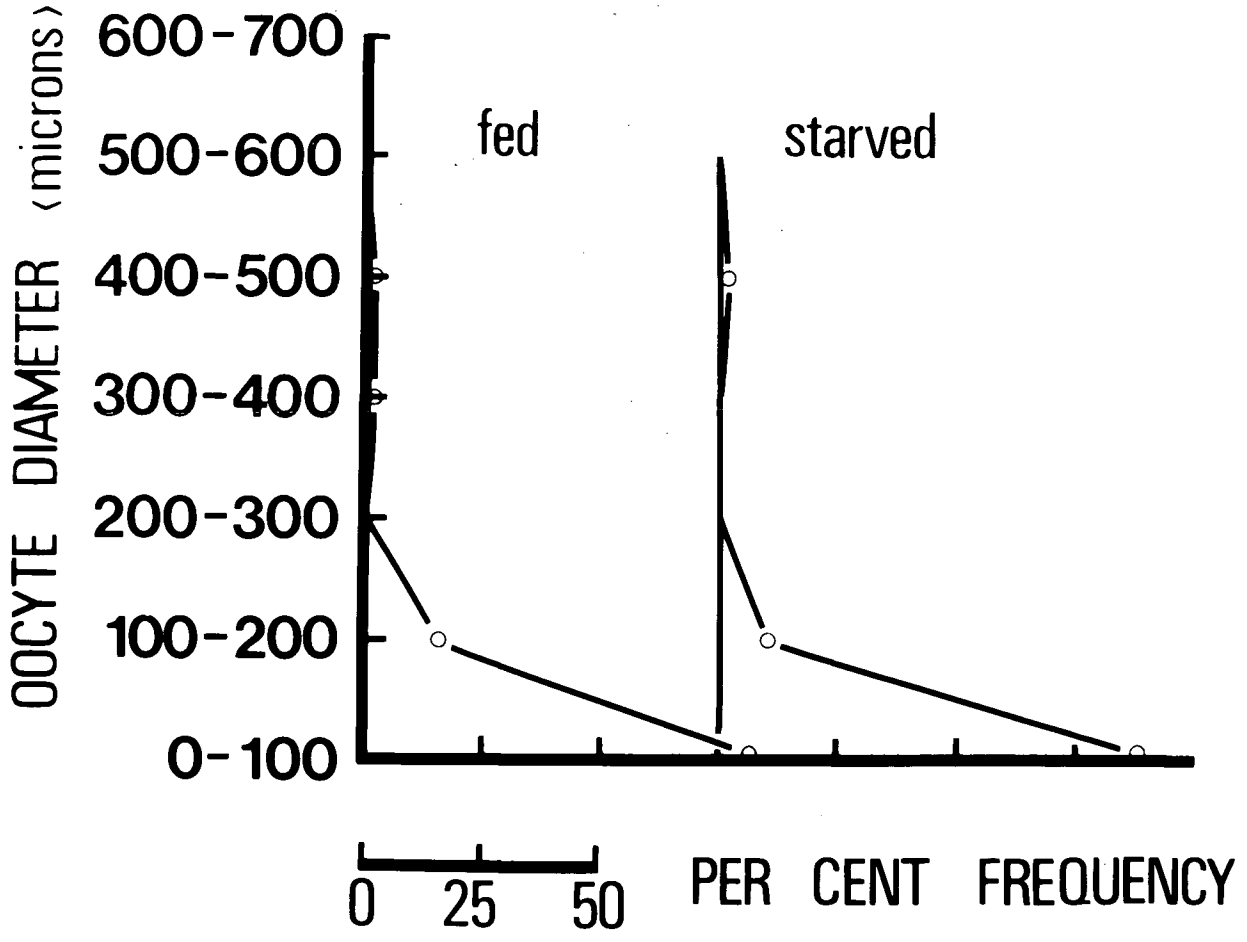
The mean ovary index of the field population and the fed group did not change significantly during the experimental period. However, there was a significant drop in the ovary index of the starved group from 5.5 to 2.5 ($p < .05$). Figure 15 shows the per cent frequency of oocytes in each size class for the two experimental groups. In both cases more than 75% of the oocytes measured are in the range of 0 to 100 microns, and none occur in the classes greater than 500 μ.

In the digestive glands of the field controls the protein and glycogen levels dropped ($p < .05$), lipid increased ($p < .05$), and the dry weight level remained constant. In the fed individuals the protein level dropped significantly ($p < .05$) and lipid increased significantly ($p < .05$), while glycogen and dry weight remained the same. As in the fed individuals the digestive glands of the starved animals showed a significant drop in protein ($p < .05$), an increase in lipid ($p < .05$) and no change in glycogen or dry weight.

The ovaries of the field controls showed no significant change in the lipid, protein, or glycogen levels. In both the fed and starved animals significant changes occurred in lipid and protein ($p < .05$)

FIGURE 15

Oocyte diameter frequency polygons for two experimental groups. Explanation as in Figure 6. Animals kept under summer conditions from August 31, 1968 until January 6, 1969.


OOCYTE DIAMETER (microns)
600-700
500-600
400-500
300-400
200-300
100-200
0-100
fed
starved
0
25
50
PER CENT FREQUENCY

but glycogen and dry weight remained the same.

During the experimental period the muscle tissue of the field animals showed small changes in the lipid and glycogen levels which were significant at the 5% level. In the muscle tissue of the fed individuals, lipid and glycogen levels remained the same but the protein level increased significantly ($p < .05$). The starved individuals on the other hand, showed no change in protein, lipid, or glycogen levels.

DISCUSSION

Reproduction and Development

In all higher prosobranchs, fertilization is by copulation (Thorson, 1950). The fertilized eggs then proceed through development in one of several ways. They may either develop inside the female by viviparity or some form of internal brood protection; they may be shed singly into the water and proceed through development as pelagic larvae; they may develop in a gelatinous mass or string; or, as in Thais lamellosa, be deposited on the substrate within a capsule (Thorson, 1946). Larvae which develop inside capsules leave after a certain period and complete development as pelagic or non-pelagic young.

The eggs of T. lamellosa, when laid, are in the order of 500 microns in diameter. This large size is apparently indicative of non-pelagic development (Thorson, 1946). Some examples of egg sizes for species with non-pelagic development described by Thorson, are Trophon hanleyi (500 μ), Brachystomia rissoides (380 μ), Amauropsis islandica (1500 μ), and Neptunea antiqua (300 μ). Development within a capsule has several advantages. Since every egg deposited in the capsule is fertilized, there is less "waste" than in broadcast fertilization. The large supply of yolk enables the larva to develop to an advanced stage within the capsule, thereby reducing predation encountered by pelagic species. Thorson (1950) discusses to a greater extent the relative advantages of the different types of larval development in relation to the environmental conditions.

The capsules of some species contain nurse eggs, which are

oocytes that do not develop but become a food source for the other larvae (Fretter, 1941). The stage of development at hatching reached by the larvae which utilize nurse eggs, varies greatly, depending on the ratio of nurse eggs to larvae. Embryos will leave the capsule when they have reached the stage of development that the nurse eggs will support. Because of this, embryos of a species in one locality may hatch as non-pelagic young while in another locality where fewer nurse eggs were produced, the embryos may hatch as pelagic larvae (Thorson, 1950). T. lamellosa does not produce nurse eggs (Ahmed and Sparks, 1970); hence the larvae always leave the capsule at the same stage of development. Emlen (1966) found that egg-capsules of T. lamellosa contained from 20 to 150 eggs with a mean of 81. He also estimated that a female can lay about 200 capsules a year and assuming a spawning period of 5 months, 1.35 capsules per day.

The eggs of T. lamellosa are larger and more yolky than many other species, so oogenesis is probably a longer process. It is reasonable to expect then, that T. lamellosa spawns only once a year, while some broadcast fertilizers which produce much smaller gametes, may spawn more often. The bivalve, Spisula solidissima (Ropes, 1968) and the hard-shell clam, Venus mercenaria (Ansell and Lander, 1967) have two spawning periods in a year. According to Webber and Giese (1969), the abalone, Haliotis cracherodii, which is a broadcast fertilizer, had two periods of gametogenesis in the year although there was probably only one spawning period. The ovary showed an increase in size only during one gametogenic period. The second gametogenic period, which was said to have occurred after spawning, may have been an artifact of the sampling technique, as explained

below. In the present study, gametogenesis occurred later in the summer (Fig. 6), and one spawning peak occurred in March and April. The sudden increase in the smaller size classes in April may not have necessarily been due to an immediate increase in their numbers but rather to a loss of the large mature oocytes. The data are in percentages; hence if no large oocytes are present the percentage of small oocytes appears to increase. This perhaps explains the conclusion by Webber and Giese (1969) that there is a second gametogenic period immediately after spawning in H. cracherodii.

Emlen (1966) determined the developmental time within the capsules of T. lamellosa to be 140 days. If that is true for the Brockton Point population the eggs laid in March would hatch in mid-summer. A subjective estimate, made on July 1, 1969 indicated that about 30 to 40 per cent of the capsules checked were empty. Emlen (1966), studying a population at Port Townsend, Washington, reported the first appearance of capsules on November 20 in 1964 and on December 10 in 1965. Animals were still spawning on March 12, 1966 and had ceased by April 21, 1966. The breeding period for the population in the present study agrees with that reported by Emlen, although the beginning of the capsule laying may have been slightly later. The conditions at Port Townsend are probably more constant with respect to salinity and temperature, since the area is more directly influenced by the Pacific Ocean through the Juan de Fuca Strait. The exact period and extent of spawning by T. lamellosa at Brockton Point was not determined by extensive field observations, but merely by observation at the time of regular sampling. It is possible that capsules were laid at very low tidal levels (zero feet) before those first

observed in January, 1969. The average size of T. lamellosa from Port Townsend was 60 mm while those from Brockton Point averaged between 45 and 50 mm in length.

One individual of T. lamellosa collected on October 25, 1968, and kept in an aquarium, laid three egg-capsules on November 16, 1968. Whether this incident can be extended to the field population is difficult to say. A few individuals in the field were observed copulating on December 4, 1968 so conceivably, egg-laying may have taken place soon after.

It is assumed that an oocyte requires one year to mature; however, there is no direct evidence to support this conclusion. Nimitz and Giese (1964) report that oogenesis in Katherina tunicata is a two year process, but they do not offer evidence to support this statement. The egg diameter frequency data (Fig. 6) indicate that mature size oocytes (500 μ) are present in the ovary from September to the time of the February collection. Furthermore, the gonad indices (Fig. 8) during this time are essentially the same. On the basis of oocyte size and gonad index, it appears that T. lamellosa has the potential to spawn during the period from September to April.

There are possibly two periods of stimulation which may affect the timing of the breeding cycle. The first induces gametogenesis and oocyte development, and the second triggers actual spawning or egg laying. Thorson (1946) maintains that the inducement for gametogenesis is a temperature different from that required to induce spawning; hence at the edge of a species' geographical range an animal could conceivably ripen its sexual products but not spawn them. Orton, Southward, and Dodd (1956) studying the limpet, Patella vulgata, con-

cluded that the breeding cycle of a population is relatively constant from year to year; that is, the population is potentially ready to spawn during a specific time in the year, e.g. September to April, but the spawning stimulus seems to over-ride the breeding cycle. Depending on the year or the locality, the time of the release of gametes may be quite variable. In the case of P. vulgata spawning appeared to correspond to rough seas rather than to temperature, tides, or phases of the moon (Orton, et al., 1956).

Seasonal Biochemical Changes

The results of the experimentation in this study suggest that temperature, light, or salinity rather than food might induce gametogenesis and egg laying in T. lamellosa, but it will be necessary to perform controlled experiments in order to conclude which stimuli are involved. The work presented here describes the biochemical changes which took place in this animal during the breeding cycle of 1968-69. From these data, conclusions will be drawn about the transfer of storage products within the various organs of the snail, and the relationships among feeding, gametogenesis, spawning and the biochemical levels. Biochemical analyses were performed on whole tissues; hence any internal transfer of constituents could not be measured but only suggested. Histochemical data from related species of mollusks and the morphological changes observed in this study will be employed to explain the gross biochemical changes.

Biochemical constituents of oocytes: Raven (1966) reviewed the types of yolk formed during vitellogenesis in mollusks. In the nudibranch, Aplysia and the pulmonate snails, Limnaea, Helix, and Eremina, the first to be formed was protein yolk and in later stages lipid was

added. Nudibranch eggs are 66% to 75% by volume protein yolk and only about 2% to 5% lipid yolk. However, some of the protein yolk may have lipid associated with it. Crepidula eggs are reported to be 75% protein yolk (Raven, 1966). T. lamellosa eggs are probably similar to those of nudibranchs and Crepidula, but since the eggs are larger and the larvae must live off the yolk content for a longer time than most other species, it would seem economical to add more lipid. Lipid provides more than twice as many calories per gram than either carbohydrate or protein (White, Handler, and Smith, 1964). The levels of the biochemical constitutents in T. lamellosa eggs were not determined, but Bayne (1968) found that free lipids were present in the nutrient reserve of the eggs of Nucella lapillus, a closely related species. For the maturation of oocytes, it appears that protein and lipid are the essential constituents.

In order to supply the oocytes with the required biochemical constituents the animal may ingest food and transfer it directly to the gonad as in Strongylocentrotus purpuratus (Giese et al., 1959a), or it may store food in another part of the body and transfer the reserves to the gonad when required. Pisaster ochraceus stores food reserves in the pyloric caeca and transfers them to the gonads during the breeding period (Farmanfarmaian et al., 1958). Balanus balanoides and B. balanus store material during the spring when food is abundant and transfer it to the gonads in the winter (Barnes et al., 1963).

Feeding: It is generally accepted that the chief site of digestion and storage of ingested food in gastropods is the digestive gland (Yonge, 1937; Millott, 1938; Graham, 1939; Howells, 1942; and Morton, 1951). In this study, the presence of storage granules in

the digestive gland diverticula was used as an indication of feeding. This method was used by Mauzey (1966) as an indication of feeding in Pisaster ochraceus. Table 2 indicates that T. lamellosa was feeding in August, September, and October, 1968 and to a lesser extent in December and January. In the February collection, the number of storage granules was least, suggesting the least amount of feeding. This period corresponded to the breeding time when most of the snails were at the zero tide level forming breeding aggregations, and were presumably not actively feeding. Thorson (1958) documents "passive periods" in several predator species of bottom-living invertebrates, especially during breeding. In the April collection, when most individuals had completed spawning, the increase in storage granules suggest that feeding had resumed. The decrease of storage granules on May 15 and July 1 imply that feeding was reduced. A reduction in feeding might be explained by a decrease in salinity at the collection site due to run-off (Fig. 2). As mentioned in the introduction, Chapman and Banner (1949) found that reduced salinity (15°/oo) caused a reduction in motility and even withdrawal into the shell; thereby indirectly reducing feeding. The effect of salinity on the present population was not tested, although present work on this aspect supports the suggestion (Johannsson, pers. comm.).

The conclusions about feeding, based on the storage granule content, are also supported by the digestive gland indices (Fig. 9). The index drops from October until February then rises in April when feeding resumes after spawning. There was a drop in the index during the period of low salinity in May and June, followed by a large increase in the latter part of the summer. The index showed an increase cor-

responding to the increase in the number of storage granules.

Explanation of terms: Before discussing the data on biochemical changes, several points should be made clear. When presenting the levels of polysaccharide in T. lamellosa, determined in the present work, they will be referred to as glycogen, since Emerson (1965) has shown by chromatography that the polysaccharide in T. lamellosa is 100 per cent glycogen. However, when reference is made to other papers, the general term polysaccharide will be used unless the author stated the specific polysaccharide which was measured.

The constituent levels in this study are expressed as per cent of a unit weight of dry tissue; therefore care should be exercised in interpreting the data. For example, if one constituent is utilized from a tissue, the remaining constituents, on a per cent basis, may appear to increase. The change in size of an organ must be considered when interpreting the per cent data. Changes in constituent levels in conjunction with a decrease in organ size, may indicate that the constituents have all been utilized but at different rates. Per cent data then, when considered with changes in organ size, can indicate approximate rates of utilization.

Constituents of digestive gland: If ingested food is first stored in the digestive gland, there should be a parallel increase in one or more of the biochemical constituents and the digestive gland index. If the constituent levels in the gland (Fig. 10) are compared to the digestive gland indices (Fig. 9), it will be noted that the protein and glycogen levels peak at times corresponding to the index peaks, especially in April and August, 1969. There was a protein and glycogen peak in September, 1969, but no digestive gland index was obtained.

Lipid levels peak about 3 months after the September and April peaks, on December 4, 1968 and July 1, 1969, respectively. Starting on August 31, 1969, the lipid level began to increase and the glycogen level began to decrease. Since feeding was still occurring through August, September, and October, and the digestive gland was enlarging, the food was most likely being stored as lipid. The apparent drop in protein is probably a passive result of the increase in lipid. Protein makes up the structural parts of tissues; hence an increase in stored material, such as lipid, will result in a drop in protein on a per cent basis. As a rule, there is a net loss of protein only under starvation conditions (White, Handler, and Smith, 1964). The fact that the protein curve in Figure 10 is the reciprocal of the lipid curve probably indicates the passive nature of the protein changes in relation to the stored lipid.

The biochemical composition of barnacles, the food of T. lamellosa, based on the data from Barnes et al. (1963) for two species of Balanus, show that the ratio of lipid to polysaccharide is about 2:1. If the barnacles eaten by T. lamellosa have a similar composition to those reported by Barnes et al. (1963) then the snails will ingest twice as much lipid as polysaccharide. The glycogen level in the digestive gland does not reach more than 7%, while the lipid level does not fall below 20%. This suggests that ingested glycogen is converted to lipid fairly quickly, at least in the fall when the animal is preparing for reproduction. During the summer, when food is plentiful, the animal may utilize glycogen directly for energy rather than convert it to lipid. In late August, when feeding is great, lipid is at its lowest level and glycogen is at a maximum, but

as fall and winter approach, reproductive demands increase and lipid becomes more prominent in the snail's economy.

Emerson (1965) analysed several Pacific west coast prosobranchs for their polysaccharide content and found a positive correlation between those with a carnivorous diet and the largest polysaccharide content. He reported a glycogen level of 6.05 (± 1.16, 95% confidence limit) per cent of the dry weight for the combined soft parts of T. lamellosa collected in July-August. This glycogen level reported by Emerson is in the same range as levels in this study, but a direct comparison cannot be made, since his determinations were performed on the total soft parts rather than on individual tissues. The herbivorous prosobranchs had polysaccharide values in the range of 1 to 3 per cent. Emerson (1965) also determined the total ether extractable material in the herbivorous top shell, Tegula funebralis, as being 12.1 (± 4.7)% of the dry weight in the female visceral mass, and 2.3 (± 0.9)% in the male. The lipid in the foot muscle was 4.3 (± 2.4)% in the female and 5.5 (± 4.2)% in the male. The difference in the lipid levels of the visceral mass between males and females was probably due to oocytes in the ovary. The lipid level in the visceral mass of Tegula funebralis is much lower than that in T. lamellosa, and is perhaps a reflection of the difference in diet.

Glycogen in the digestive gland: The maximum level of glycogen in T. lamellosa digestive gland is twice as great as the maximum polysaccharide level of 3% in the "gonad-free" tissue of the limpet, Patella vulgata (Blackmore, 1969). The low polysaccharide level in P. vulgata would be expected on the basis of the figures quoted by Emerson (1965) for the herbivorous limpets. Blackmore (1969) found

large amounts of ash in P. vulgata, ranging from 15 to 37% of the total dry weight. He concluded that the ash content was highest when the algal cover was at a minimum, and the limpet ingested relatively more inorganic material along with the food. In T. lamellosa the highest ash weights were 8% in the foot muscle and about 7% in the digestive gland.

Role of glycogen in reproduction: Barry and Munday (1959) showed that in Patella glycogen levels in the digestive gland were closely correlated with seasonal variations in blood glucose, and they concluded that the increased glycogen levels were probably indicative of feeding. There was a decrease in glycogen level in all tissues of Patella coinciding with the shedding of gametes in January. However, after spawning, the resultant low level of glycogen remained constant throughout the winter. It was concluded that Patella did not utilize glycogen to maintain metabolism during the winter. The gonad of Patella, during the year, never contained appreciable glycogen reserves as compared to the pulmonate, Helix, or the oyster, Ostrea gigas. Barry and Munday (1959) and Blackmore (1969) both concluded that in Patella the major food reserve is lipid, and that polysaccharides are not significant as food reserves but vary with feeding. Giese and Hart (1967) found that polysaccharide levels in Katherina tunicata gonad were highest when the gonad was small and inactive. Blackmore (1969) also found this in Patella vulgata, and came to the conclusion that polysaccharide occurred in the interstitial tissue rather than in the oocytes. As stated previously, the amount of polysaccharide in marine gastropod eggs is low, most of the reserves being in the form of protein yolk and lipid yolk. There is an

increase in the glycogen level of the ovary of T. lamellosa after spawning, which like Patella and Katherina, suggest that glycogen is situated in the interstitial tissue of the ovary. What role this glycogen has in the ovary is not known, but Nimitz and Giese (1964), using histochemical techniques on the ovary of K. tunicata, found that glycogen in the epithelium of the ovary gradually decreased to its lowest value in May when spawning took place. Despite the decrease of glycogen in the epithelium, no glycogen appeared in the oocytes. There was a similar decrease of lipid in the epithelium, but unlike the glycogen, lipid began to appear in the growing oocytes. Presumably, the glycogen was used in the synthesis of lipid and protein yolk. A similar situation occurs in the ovary of the oyster, Ostrea gigas. Glycogen reached a maximum in April then began to decline as the lipid level increased. The lipid level peaked in June after which spawning occurred. It was postulated that glycogen in the storage tissue of the gonad was converted into lipid and transferred to the sexual products (Masumoto, Masumoto, and Hibino, 1934). Other histochemical investigations have shown that the follicle cells around oocytes in chitons can resorb nutrients from unshed oocytes, store them, and later pass them on to growing oocytes (Gabe and Prenant, 1949; Selwood, 1970). A histochemical study of T. lamellosa would reveal the role of the various accessory cells in the movement and storage of nutrients.

The glycogen level in the ovary of T. lamellosa peaked in September and April at the same time as in the digestive gland. This suggests that glycogen from ingested food is incorporated into the ovary as well as into the digestive gland, although not to the same extent. The glycogen level dropped in both ovary and digestive gland

following the September collection and at the same time protein and lipid levels in the ovary increased (Fig. 12). This may have been due to the utilization of glycogen in the synthesis of protein yolk and lipid yolk in the oocytes. If glycogen reserves were being used to synthesize yolk, it would be expected that the glycogen level would drop and the protein and lipid levels in the ovary would increase. Unlike the digestive gland, the protein and lipid levels of the ovary change in a parallel manner, since both constituents are major contributors to yolk.

Constituents of the ovary: At the time of spawning, the ovary index dropped, but the corresponding drop in lipid and protein level in the ovary was not dramatic (Fig. 12) as found in some other invertebrates (Giese and Hart, 1967; Giese, Hart, Smith and Cheung, 1967; Blackmore, 1969). The relatively small biochemical changes in the ovary of T. lamellosa might be explained by morphological evidence. Although the egg size data showed loss of mature oocytes at spawning, in fact some were still present in the form of disintegrating aggregations of yolk granules. They were not considered oocytes as the criterion required the presence of a nucleus. Unshed oocytes appeared to disintegrate and be resorbed. It has been shown in the chiton, Sypharochiton septentriones, that all the cell types in the ovary, apart from the oocytes and late follicle cells, possess lysosomes, and are capable of resorbing unshed, mature oocytes and later passing the nutrients to new oocytes (Selwood, 1968). Some yolk may be digested by the ingesting gland of T. lamellosa, situated between the albumen gland and the capsule gland. Sectioning of this gland showed the presence of a few yolk granules. Fretter (1941) also gave

evidence for the role of the ingesting gland in digesting unused yolk. The yolk granules in a disintegrating oocyte would probably yield similar results to the yolk in a mature oocyte. The fact that the ovary is never completely emptied of mature oocytes probably explains why the biochemical constituents did not show dramatic changes.

Polysaccharide as a storage product: In animals which use polysaccharides as a storage product, the levels are much higher than the maximum glycogen level of 7% in T. lamellosa. The clam, Tivela stultorum, has from 10 to 25% glycogen in the non-gonad tissue, and 25 to 50% in the gonads (Giese et al., 1967). The abalone, Haliotis cracherodii, has polysaccharide levels ranging from 5 to 25% in the foot muscle (Webber and Giese, 1969). In the sea urchin, Strongylocentrotus purpuratus, the gonad appears to be the major site of food storage. Here the maximum glycogen content was 10% at the time of highest gonad index, and the minimum was 1% (Giese et al., 1959a). Judging from the relatively low glycogen levels in the gonad and foot muscle of T. lamellosa, and the low levels of glycogen in the digestive gland, which fluctuate with feeding, glycogen is not an important food reserve. After ingestion it may be stored briefly then synthesized into either lipid, protein, or yolk.

Further evidence for the role of glycogen in T. lamellosa can be obtained from the experimental data in Table 3. In the three tissues analysed, the difference in glycogen level between fed and starved animals was almost negligible. However, the glycogen levels in digestive gland and foot muscle from the field population were lower on January 17, 1969, than at the beginning of the experiment on August 31, 1968. This drop in glycogen may have been due to either conversion to lipid, or

utilization by the developing oocytes. In the fed and starved groups, the gonads did not develop. There was no requirement for glycogen in the synthesis of protein or lipid yolk, so the glycogen levels did not decrease. It is possible that glycogen is not readily used during starvation, but is preferentially used for the developing oocytes. Martin (1961) described the work of May (1934) on the pulmonate, Helix pomatia. May found that glycogen was utilized by the snail during starvation, but galactogen was only drawn upon when glycogen was depleted. It was speculated that the role of galactogen was primarily as a reserve for reproduction, and because it was not easily mobilized, the snail was driven to exert itself in finding food and thus, carried through the demanding reproductive process. This may be the case in T. lamellosa except that protein and lipid is used during starvation, and that glycogen is used for the developing oocytes. However, more data are required to substantiate this hypothesis.

Lipid levels in T. lamellosa: The sites of lipid storage are probably the digestive gland and the gonad. The level of lipid in the foot muscle was about 7% throughout the year (Fig. 11). Giese (1966) states that the lipid level in crab muscle cells, which histochemically appeared to store no lipid, was 5.2%. Therefore, it was concluded that a level of 5.2% represented only structural lipid, and anything above this probably represents stored lipid. The level of lipid in the foot muscle of T. lamellosa did not exceed 8%, so storage of lipid in the foot muscle probably does not occur to any great extent. Species which histochemically have been shown to possess lipid vacuoles in the foot muscle, have much larger lipid

values. For example, the foot of the chiton, Mopalia hindsii, has a lipid level of 21% and 17% in gravid and spent animals, respectively (Giese and Araki, 1962). The chiton, Cryptochiton stelleri, which spawns in April-May, has a lipid level in the foot muscle of 5% or less, and in the digestive gland, from 2% to 10% (Tucker and Giese, 1962). T. lamellosa, on the other hand, has a greater level of lipid in the digestive gland, from 20% to 38%. These differences may be a reflection of their diets. C. stelleri is mainly herbivorous and therefore, ingests more polysaccharide, whereas T. lamellosa is carnivorous and takes in more lipid. Prolonged starvation in the sipunculid, Phascolosoma gouldii, caused a drop in all lipid except in the muscle. In fact, the level of lipid increased, suggesting that protein or some other constituent was utilized at a greater rate (Wilber, 1947).

Constituents of the foot muscle: The constituent of the foot muscle of T. lamellosa which did appear to change significantly was the protein. There was a high protein level in the September collection (Fig. 11) followed by a drop in October. The high level in September might have been due to the incorporation of ingested food into tissue protein in the foot muscle. Similarly, the peak in April was probably due to the resumption of feeding at that time. The protein level was probably not changing in response to a lipid change, as in the digestive gland, since the lipid level remained fairly stable. The drop in October corresponded to an increase of protein in the ovary, which resulted from the formation of protein yolk. This decrease in foot muscle protein also occurred during the time when feeding was reduced, which suggests that protein may be either metabolized as a

source of energy, or utilized by the oocytes. The experimental data in Table 3 show that protein in the muscle of the starved animals did not change but in the fed group it increased significantly ($p < .05$). Further, in the two experimental groups the ovaries did not increase in size and no mature oocytes developed. Hence, protein was probably not required for yolk production as in the normal field population where the ovaries developed normally. The increase in the protein level of the fed group may have been a result of feeding. From this evidence, it is suggested that the drop in foot muscle protein after Octoberand the peaks in September and April were a result of different rates of feeding by T. lamellosa, rather than as a result of transfer to growing oocytes.

Conclusions about nutrient transfer: The collection on October 25, 1968, showed that the digestive gland index was at a peak. From this data until February 16, 1969 collection, the digestive gland decreased in size, suggesting that reserves were being utilized from that organ. Analyses of the animals from the collection following the digestive gland peak showed that the lipid had increased and the glycogen had decreased. It is speculated that the increase in lipid was a response to a reduction of food availability. With the onset of a reduced food supply ingested nutrients and polysaccharide reserves may be directed into lipid synthesis for storage. The loss of weight from the digestive gland was probably due to glycogen utilization for lipid or yolk synthesis, and later to utilization of lipid reserves during winter. The snails presumably lived off the accumulated lipid during the winter, and by April the lipid was at its lowest level. The glycogen level

had already begun to increase by April as feeding had resumed. When glycogen reaches a certain level due to feeding, some of it may then be converted into lipid for storage. This may explain the lag in the lipid peak following the onset of feeding in April.

Conclusions drawn from experimental observations: Data from the experimental work (Table 3) suggest what roles the digestive gland and ovary play in the metabolism of the snail under normal reproductive demands and starvation. The mean index of the digestive glands of the field group decreased significantly during the experimental period, but the ovary index did not change. Thus, in the field population the size of the ovary was maintained, but the digestive gland decreased. Biochemical analyses have shown that the weight loss was due to utilization of glycogen, lipid, and to some extent, protein. However, if one looks at the two experimental groups, which due to the imposed conditions did not follow the normal reproductive pattern, it was the digestive gland which remained at a constant size, and rather the ovary size which decreased. If the fed and starved groups are compared it will be noted that the digestive gland index of both did not decrease, but the ovary index of the starved animals did. Further, the ovary index of the starved group decreased more than the fed group. It seems that under normal conditions, reserves in the digestive gland are used, but during starvation nutrients are resorbed from the ovary. However, the starvation experiment was performed under abnormal conditions of light, temperature, and salinity, so the effect of starvation under normal field conditions is not known. In both the experimental groups the glycogen level did not decrease, not even in the starved animals. This supports the suggestion that the primary

function of glycogen is to supply the developing oocytes rather than to serve as a general energy reserve. Emerson and Duerr (1967) also came to the conclusion that little glycogen is used during starvation in Littorina planaxis, and suggested that it might be a reserve for anaerobiosis if starvation was coupled with dessication. In summary, under normal conditions, the data suggest that the digestive gland supplies nutrients to the developing oocytes and for general body maintenance, as shown by the reduction of the size of the gland and the decrease in glycogen and lipid. Under abnormal conditions, when the ovary is prevented from developing, it appears that the animal draws on the reserves of the ovary to maintain its body functions.

Von Brand, McMahon, and Nolan (1957) showed that starvation in the pulmonate snail, Australorbis glabratus, resulted in a small loss of lipid and polysaccharide, but the majority of the weight loss was due to utilization of protein. They did not measure protein directly, but on the basis of oxygen consumption data, the amount of oxygen consumed did not account for the small loss in lipid and polysaccharide. Data for a cephalopod indicated that protein as well as other reserves were metabolized, since the lipid level remained the same while the animal decreased in size (Giese, 1966). Emerson and Duerr (1967), on the other hand, found that after starving the prosobranch, Littorina planaxis, total lipid decreased significantly, while protein and polysaccharide did not. The experimental data for T. lamellosa do not seem to suggest utilization of lipid during starvation. The conditions of the experiment, however, should be considered. Under the experimental conditions, the starved animals withdrew nutrients from the ovary rather than from the digestive gland. During the winter, when

food intake is low, lipid is utilized from the digestive gland.

In a review of lipids in marine invertebrates, Giese (1966) concluded that although lipid has been shown to be an important reserve material, protein is generally present in all tissues in a quantity greater than either polysaccharide or lipid, and may be used when the need arises. Giese mentions the following species as utilizing protein during starvation: the sipunculid, Phascolosoma agassizi, the chiton, Katherina tunicata, and the shore crab, Hemigrapsus nudus. As mentioned previously, when an organ decreases in size, protein is usually metabolized along with lipid and glycogen. The protein level in the digestive gland of the starved T. lamellosa showed more of a loss than the fed animals.

Effect of Environmental Factors on Gonad Development

Lack of food: The ovary index of the starved animals decreased, partly because the experimental conditions did not allow maturation of the oocytes, and partly because of a lack of food. The ovary index of the fed animals also declined but not the same extent. Hence, availability of food allowed the ovary to maintain its size, but the physical conditions prevented maturation of the oocytes. Mature oocytes which were present at the beginning were in the process of being resorbed. The size of the ovary of the starved animals was less, presumably due to the greater demand on the reserves in the ovary. Sastry (1966) found that starvation of scallops during the period of gonad growth resulted in an absorption of oogonia and oocytes, but if the animals had already accumulated gonad reserves, they released gametes, regardless. The pulmonate, Lymnaea stagnalis, if starved

for six weeks, maintained all stages of oogenesis and spermatogenesis in the ovotestis; however the number of sex cells and the size of the ovotestis was reduced. A large number of mature oocytes degenerated and were resorbed by the nurse cells, but developing oocytes did not seem to be affected (Joosse, Boer, and Cornelisse, 1968).

Temperature: In T. lamellosa, maintained under summer conditions of light, temperature, and salinity, the sex cells proliferated more in fed than in starved animals, but in neither case did the oocytes mature. Thus, it could be said that the condition for gonad maturation is a factor other than food supply. Since only one combination of light, temperature, and salinity was applied in the experiment, it was not possible to conclude which was the controlling factor. Sastry (1968) showed that there was a relationship between food supply and temperature and the reproductive activity of the scallop, Aequipecten irradians. Optimum temperatures with food seemed to initiate gonad development. When temperatures were below optimum, the reserves from the ingested food seemed to accumulate more in the digestive gland than in the gonad. In the present study, experimental animals in temperatures higher than the field, maintained the digestive gland size, and utilized reserves from the gonad. If the temperature was far above the optimum, the reserves of A. irradians did not seem to accumulate in the gonad either because of increased metabolic utilization, failure to regulate the synthetic processes of the growing oocytes, or death of the scallops. Furthermore, although oogonia may develop below the optimum temperature when food was supplied, oocyte development did not seem to occur. This may be the case in T. lamellosa, but in the opposite way. These snails breed at the

lowest temperatures of the year; therefore an optimum low temperature may be required to allow the oocytes to mature. Two species of barnacles, Balanus balanoides and B. balanus, which breed in November and February, respectively, did not breed at temperatures of 14 to 18°C but did in the range of 3 to 10°C (Crisp, 1957). The dog whelk, Thais lapillus, under artificial laboratory conditions, required 9°C to stimulate egg deposition. No information, however was given about the effect of temperature on gametogenesis or oocyte maturation (Largen, 1967).

Day length: Day length has been implicated in the control of breeding cycles. Boolootian (1963) maintained male purple sea urchins at a constant temperature of 15°C and varied the day-length. On a 14-hour day, initial development of gonial cells began but no mature sperm resulted. On a 6-hour day, gonial cells were reduced in number as they developed into spermatocytes. The purple sea urchin used in the study normally reproduces in the winter. Barnes (1963) performed similar experiments on winter-breeding B. balanus. Constant illumination inhibited breeding, especially the later stages of development. A period of 4 to 6 weeks at less than 12 hours light per day was required for maturation of gametes.

Webber and Giese (1969) analysed breeding data for Haliotis cracherodii and Katherina tunicata collected over several years, and concluded that temperature did not act as an exogenous control of gonad growth. Photoperiod did not show a correlation with the increase in gonad size, but it was suggested that gametogenesis was initiated by day lengths of greater than approximately 12 hours. It was emphasized that mere correlation of environmental changes cannot be viewed

as conclusive, and data from controlled experiments should be obtained to establish causal factors. The role of the endocrine system was also suggested as an important consideration for the future study of mollusk reproductive cycles.

Salinity: Salinity has not generally been considered as a stimulus for reproduction, but in situations such as Brockton Point, where relatively large salinity fluctuations take place (17 to 29°/oo), it should not be discounted. It could be argued that in regions on the open coast temperature and salinity fluctuations are very low, yet breeding occurs at regular times. It may be that populations of a species in different localities adapt to that particular environment, and utilize the most obvious regular changes in the environment, to time the breeding cycle. For example, the population of H. cracherodii, studied by Webber and Giese (1969), encountered only small fluctuations in temperature (range: 5°C) and salinity, yet appeared to breed regularly. There was a correlation between day length and initiation of gametogenesis, however. As mentioned in the previous paragraph, endogenous rhythms in the endocrine system are probably important in the regulation of the breeding cycle.

The results cited in the previous few paragraphs for other invertebrates, give evidence for the role of feeding, temperature, and light, in the control of breeding cycles. Until controlled experiments, using various combinations of temperature, salinity and light can be performed no conclusions can be drawn about the factors controlling reproduction in Thais lamellosa.

Suggestions for Further Study

The data collected in this study provide a basis for further

studies into T. lamellosa reproduction. For example, histochemical data would elucidate the distribution of nutrients within the tissues, which so far can only be speculated. The techniques of radioactive labelling could be employed to show the sites of synthetic activity and the sources of precursors in relation to food. "Tracer experiments are needed to determine the movement of nutrient in marine invertebrates" (Giese, 1966, p. 286). This method was also suggested by Barnes et al. (1963) as a means of tracing the partition of the various materials during growth and development of the gonads. A more accurate determination of feeding times during the year could be obtained by measuring the blood glucose levels in the manner described by Barry and Munday (1959). Also, biochemical analyses of the eggs would help to explain further the gross changes in the gonad. Very little work has been done on the endocrine system of molluscs and its role in the control of reproduction (Boer, Douma, and Koksma, 1968; Webber and Giese, 1969). A study of the effects of environmental factors on reproduction, using controlled conditions, would also provide a good opportunity to look at the role of neurosecretion in controlling the breeding cycles.

Several authors have pointed out the need for further investigation into seasonal biochemical changes, and the relationships between nutrition and reproduction in mollusks (Barnes et al., 1963; Ansell and Lander, 1967; Emerson, 1965, 1967; Giese, 1966). The present work has attempted to answer some of these questions with regard to the prosobranch, Thais lamellosa. The data show the general similarities between T. lamellosa and other prosobranchs with regard to biochemical constituents and reproduction.

SUMMARY

1. A population of Thais lamellosa, from Brockton Point, Vancouver, British Columbia, was sampled approximately monthly from July 28, 1968, until August 25, 1969. A study was made of the biochemical changes associated with the reproductive cycle.

2. From the three tissues, digestive gland, foot muscle and gonad; per cent protein, glycogen, lipid, and ash were obtained. As well, gonad and digestive gland indices and histological sections of the gonad and digestive gland were obtained.

3. The reproductive habits of T. lamellosa and some of the properties of the eggs were discussed in relation to length of breeding season, gametogenesis, developmental time within the capsule, and timing of the breeding cycle.

4. Estimates of storage granules in the digestive gland diverticulae were used to determine periods of feeding activity. There was a feeding peak in April and another in August. During the winter months until spawning in March, there was a decrease in the amount of feeding.

5. In the fall ingested food is accumulated in the digestive gland as lipid, but in periods of active feeding, April and August, glycogen is highest and lipid is at a minimum.

6. It was concluded that glycogen is not used as an energy reserve, but is either converted to lipid in the digestive gland or used by the growing oocytes for yolk production.

7. The foot muscle does not store lipid or glycogen to any appreciable extent, but the protein appears to fluctuate in response to food intake. The protein level increased during maximum feeding

and decreased when feeding was low.

8. In the field population, the digestive gland index decreased as reserves were used, but the size of the ovary was maintained from about October until spawning in March.

9. Under the experimental conditions, the digestive gland of both fed and starved animals maintained its size, while the ovary decreased in size. The starved animals withdrew more material from the ovary than the fed animals.

10. Mature oocytes in the ovary of the starved animals appeared to be resorbed, leaving the smaller oogonia, in the range of 0 to 200 microns. Likewise, after spawning, unshed mature oocytes were resorbed.

11. The role of temperature, light, and salinity, in timing reproduction was discussed, but conclusions could not be drawn based on correlations with field data.

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