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BODY DISTRIBUTION AND SEASONAL CHANGES IN THE GLYCOGEN CONTENT OF THE COMMON SEA MUSSEL *MYTILUS EDULIS*

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Abstract—1. Glycogen content was measured in the sea mussel *Mytilus edulis*, and found to be present in amounts ranging from 10 to 35 per cent dry weight of the soft parts.

2. The annual glycogen cycle was followed for five different fractions: the digestive gland, muscles (including those of the foot), gills, mantle and residue. Seasonal changes in gills and muscles were much smaller than in the other tissues.

3. The annual glycogen cycle for the total animal consisted of a rapid increase in spring and early summer, followed by small fluctuations in the late summer. In autumn and early winter there was a gradual decline reaching a minimum of about 10 per cent in February and March.

4. There was an interrelation between seasonal changes in dry weight and the percentage of glycogen.

INTRODUCTION

THE COMMON sea mussel *Mytilus edulis* is able to withstand anaerobic conditions for many days depending on the temperature of its environment. When the tide recedes leaving the mussel uncovered it closes its shell thus reducing or checking its supply of oxygen (Newell, 1964). Studies on the metabolic changes which enable bivalves to survive such a stressful environment have been carried out recently by many investigators. It became clear that there is a heavy dependance on glycolysis during anaerobic periods. As most recent research concerns intermediary carbohydrate metabolism using radioactive precursors (Simpson & Awapara, 1966) or studying the presence of enzymes (Bennett *et al.*, 1968; O'Doherty *et al.*, 1971; de Zwaan, 1971a, b) it seemed useful to study normal levels of glycogen in a bivalve. Literature covering this field is mostly confined to old data and gives information about total glycogen content but not about glycogen distribution over the body. Moreover, studies about seasonal variations are rare and incomplete with the exception of some oyster species.

This paper gives an account of stored glycogen and its distribution between various organs in *M. edulis* during 14 successive months in a population of mussels from the Waddenzee (northern part of the Netherlands).

MATERIALS AND METHODS

Selection of the samples

At approximately monthly intervals about 650 animals were collected from the same natural mussel bank in the Waddenzee. The samples were received approximately 15 hr after being taken from the sea. On arrival the mussels were cleaned of encrusting epifauna and placed in running sea water for about 24 hr. The length of the shell of each mussel was measured and only animals of $5{\cdot}5 \pm 0{\cdot}2$ cm were pooled. This group, accounting for about one-third of the original sample, was weighed and the average weight was calculated. The twenty-five specimens having a weight nearest to this average were used for the analysis.

Preparation of the samples

The soft parts of the mussels were removed from the shells and divided as follows: hepatopancreas, muscles (anterior and posterior adductor muscles, anterior and posterior byssal retractor muscles, pallial muscle, retractor pedis muscle and foot), gills, mantle and residue.

Preparation was carried out on a watch-glass so that the intra- and extracellular liquid and enclosed sea water could be collected. These were added to the residue as well as 2 ml of distilled water used for rinsing the watch-glass.

Corresponding parts of each of the twenty-five mussels were pooled together. The residue sample was centrifuged and the supernatant was deproteinized by trichloroacetic acid (to an end concentration of 3 per cent), and 1 ml was taken for glycogen determination. The sediment from the residue sample and all other fractions were saponified in 10 ml 30% KOH for 1 hr at 50°C. Each hydrolysate was made up to 60 ml with distilled water. Twenty-five ml from each sample were dried on a disc (dia. 10 cm) in an oven at 100°C for 24 hr, cooled in a vacuum-desiccator containing P_2O_5 and then weighed. Thus the dry weights were calculated. (This procedure was tested with twenty replicate samples from one hydrolysate. The average value was 0·848 g with S.E. 0·010. After further drying for 24 hr there was an average loss in weight of 2·3 mg.) From the remaining 35 ml of each hydrolysate the following procedure was carried out in triplicate. Three ml was neutralized with 5 N HCl, deproteinized with trichloroacetic acid to an end concentration of 3 per cent and centrifuged for 20 min at 10,000 *g*. The supernatant was then diluted 100 to 200 times, depending upon the season, and 1 ml was used for glycogen determination.

Estimation of glycogen

Estimation was based on a photometric method using anthrone reagent as described by van Handel (1965). The anthrone reagent was prepared by dissolving 0·15 g in 100 ml of diluted sulphuric acid (76 ml sulphuric acid, d = 1·84, poured into 30 ml water while stirring and cooling). A 1-ml sample was measured into a centrifuge tube and stirred with 0·05 ml of a saturated solution of Na_2SO_4, followed by 3 ml of ethanol. The tube was placed in a boiling water-bath for 3 min and cooled in an ice-bath for at least 1 hour and then centrifuged. The ethanol was carefully decanted and the glycogen pellet ($+Na_2SO_4$) after drying was dissolved in 0·05 ml water. Three ml of freshly prepared anthrone reagent was added and the tube was heated at 90°C for 20 min, cooled in ice water and measured at 620 nm.

RESULTS AND DISCUSSION

Figure 1 shows the annual glycogen cycle in various parts of the sea mussel as mean percentages of dry tissue weight. It is clear that *M. edulis* stores large amounts of glycogen, especially during the time of the year when there is an abundance of food (summer and early autumn). The graph for the total animal (dotted curve) shows the maximum value was about 35 per cent (mid-July) and

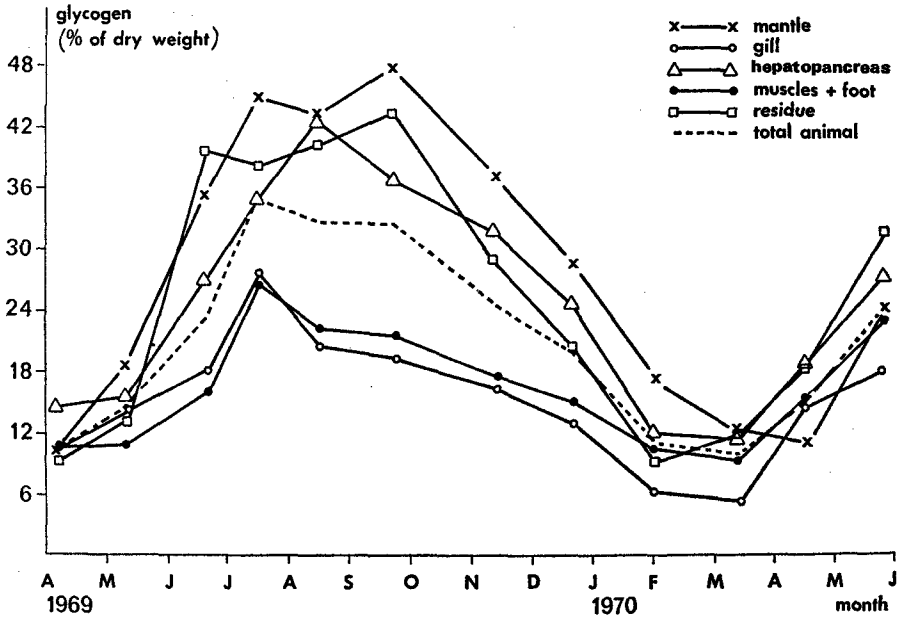

FIG. 1. Concentrations of glycogen as percentages of the dry weight during 14 successive months in five parts of the sea mussel *M. edulis* (4 April 1969–25 May 1970).

the minimum about 10 per cent (mid-March). The five fractions are sharply divided into two groups with respect to their glycogen percentages (Fig. 1). The first group consists of the mantle, hepatopancreas and residue, all having a similar pattern in glycogen percentage throughout the year. The second group consisting of gills and muscles plus foot also has a similar pattern but changes in their glycogen content are much less marked than in the first group.

Figure 2 gives the absolute glycogen values in mg. The seasonal variation is different in the various fractions. The greatest variation being found in the mantle (13–145 mg glycogen) and the smallest in the gills (6–26 mg). The other organs showing seasonal variations are the hepatopancreas (7–57 mg), muscles and foot (30–85 mg) and the residue fraction (8–60 mg). The glycogen values of the residue fraction include the glycogen assays from the supernatant after the first deproteinization and centrifugation (see under Materials and Methods). The amounts found in the supernatants were very low and therefore not shown separately.

The annual cycle can be summarized from Figs. 1 and 2 as follows: in the spring and early summer there is a rapid increase in glycogen content followed by small fluctuations in the late summer (July–September). During the autumn and early winter (October–mid-January) there is a gradual decline leading to the minimum glycogen content being reached in February–March. The period of glycogen

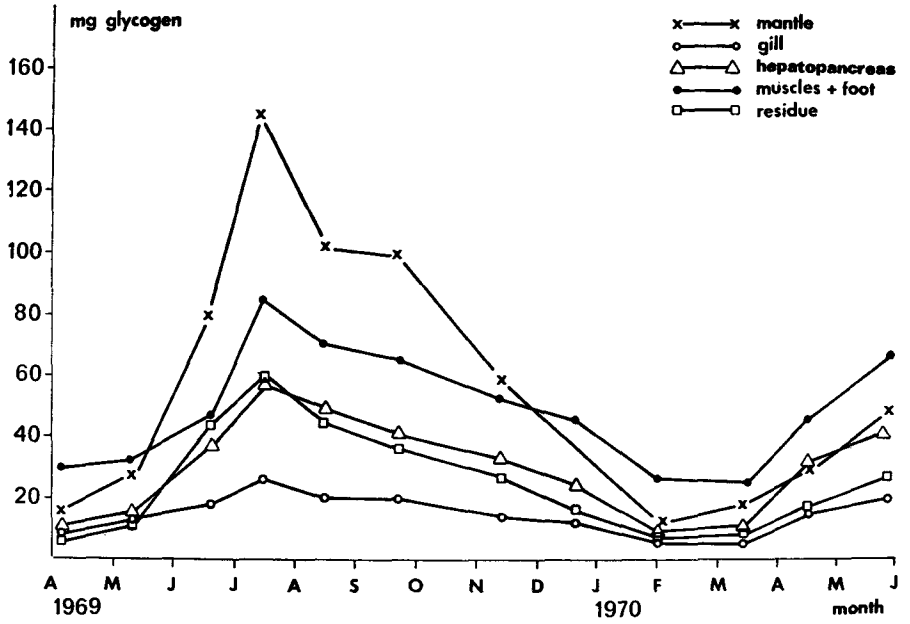

FIG. 2. Absolute glycogen concentrations in mg during 14 successive months in five parts of the sea mussel *M. edulis* (4 April 1969–25 May 1970).

accumulation covers the main growth season, whereas the period of glycogen consumption coincides with winter dormancy.

Figure 3 shows the interrelationship between seasonal changes in dry weight and glycogen content. During autumn and winter there is a somewhat greater loss in dry weight than in glycogen content and in the months of January and February changes are mostly restricted to the dry weight. Over the whole period both curves have roughly the same pattern which shows that variations in dry weight are mainly the result of similar variations in glycogen content. This illustrates the importance of glycogen for the mussel.

The glycogen values given here agree with those in the literature data. Calvin (1931) reported an average glycogen content of 27·6 per cent of the dry weight in the hepatopancreas of a fresh-water mussel and Lane *et al.* (1952) found 50 per cent in the same organ from *Teredo pediculata*. More recently Favretto (1958) found values of 2–5 per cent glycogen/100 g of wet edible parts.

For *Mytilus* subspecies the following data are available: *M. galloprovinciales*, 2·25 (Renzoni, 1963) and 2·83 (Manier *et al.*, 1958; Monnier *et al.*, 1958) and *M. edulis*, 2·39 (Frage 1956); all figures are given as percentages of wet tissue.

Seasonal changes have also been studied in oysters. Seven populations of *Ostrea edulis* from different parts of the world were studied for 1 year and the results showed large geographical differences between the cycles (Walne, 1970).

Fluctuations in chemical composition of the edible parts of *M. edulis* from the southern Baltic sea during the period 1959–60 were observed by Drzycimsky

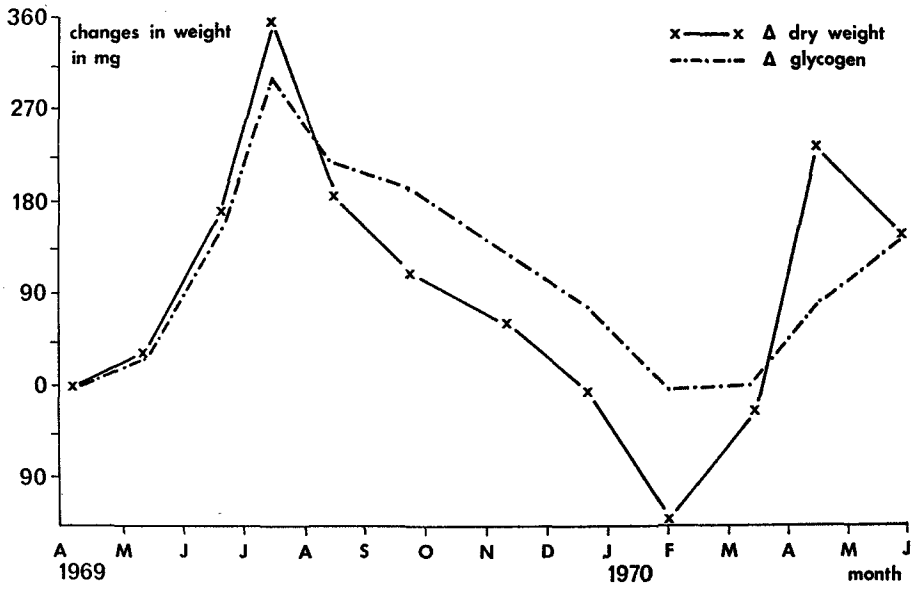

FIG. 3. Alterations in weight (Δ) during 14 successive months in the total dry weight and the total glycogen content of the sea mussel *M. edulis* (4 April 1969–25 May 1970). The alterations are given with regard to the situation on 4 April 1969.

(1961). The percentage of dry weight of carbohydrates was in the range 0·21–36·79. The variations in the chemical composition of the edible parts were associated with the kind of food found in the alimentary tract.

The aim of this paper has been to show that *M. edulis* accumulates glycogen to serve as an energy store during winter dormancy. Furthermore, it shows that the whole body is involved in the accumulation and release of glycogen. The role of glycogen during anaerobiosis is undergoing study by the authors and will be published presently.

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Key Word Index—Sea mussel; *Mytilus edulis*; molluscs; carbohydrates; glycogen; annual cycle.