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The biochemical composition of the cypris larva of the barnacle *Balanus balanoides* L.

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The biochemical composition of the cyprids of *Balanus balanoides* is described. The mean total freeze-dried weight per cyprid was 37·7 µg. Mean values, as percentages of total dry weight were 9·2% neutral lipid, 4·8% phospholipid, 0·8% polysaccharide, 2·7% free sugars, 6·8% protein nitrogen, 8·2% total nitrogen, 3·2% chitin and 12·4% ash.

Free-swimming cyprids, prevented from settling for up to 8 weeks at 8°C utilised their neutral lipid (triglyceride) reserves. At the end of this period 90% of the initial neutral lipid reserves were depleted. There was only a slight decrease in the phospholipid, free sugar and nitrogen content of the cyprids, and the polysaccharide content remained comparatively constant.

Introduction

Barnes, Barnes and Finlayson (1963) and Barnes (1965) have described the seasonal changes in biochemical composition of adult barnacles and the changes in composition of the developing eggs of both *Balanus balanoides* and *B. balanus*. Little is known however, about the biochemical composition of the cyprid and nauplius stages of barnacles. The cypris is the final larval stage and is specificially adapted to locate and explore a suitable site for settlement (see Crisp, 1974); when this is achieved the cyprids metamorphose into the attached adult form.

Cyprids do not feed, so that survival and success at metamorphosis is entirely dependent on stored energy reserves. In this paper, we have determined the biochemical composition of *B. balanoides* cyprids and described the loss of energy reserves in cyprids prevented from settling for up to eight weeks.

Materials and methods

Collection of cyprids

Plankton hauls were taken in the Menai Strait between late April and early May 1972. In the laboratory, samples of the plankton were pipetted onto filter paper and *B. balanoides* cyprids counted under a binocular microscope and collected. Approximately 10 000 cyprids from the collection on 2 May 1972 were maintained at 8°C in the laboratory in a 3 l glass cylinder containing u.v.-irradiated, filtered sea water. The sea water was replenished twice a week and was kept strongly agitated by means of a filtered compressed air flow. Approximately 1000 free-swimming cyprids were sampled at weekly intervals for biochemical analysis over a period of eight weeks.

Biochemical analysis

The cyprids were washed free of sea water by rinsing with 0·9% aq. ammonium formate on a sieve and then freeze-dried. The freeze-dried samples were weighed and the dry weight per cyprid calculated. Between 2 and 4 mg of dried cyprids were milled and protein nitrogen, total carbohydrate (sub-divided into free sugars and polysaccharide) and total lipid (sub-divided into neutral lipid and phospholipid) were determined by the methods described previously by Holland and Gabbott (1971) and Holland and Hannant (1973). In addition total nitrogen in a sample of the aqueous homogenate was determined by micro-Kjeldahl digestion in the same way as protein nitrogen.

Ash weight was determined by heating replicate samples of an aqueous homogenate of cyprids at 500°C for 5 h in a muffle furnace.

Determination of chitin

Replicate samples (each 500 mg dry weight) of milled cyprids were homogenised in 10 ml cold 5% aq.

Table 1. The total dry weight and biochemical composition, expressed as a percentage of total dry weight, of *B. balanoides* cyprids.

Date of collection 1972	Mean total dry weight µg	Total lipid Neutral lipid	Total lipid Phospho-lipid	Total carbohydrate Polysacc-haride	Total carbohydrate Free sugars	Protein N	Total N	Chitin	Ash
27 April	34·8	8·1	4·7	0·8	2·4	7·5	8·4		
2 May	40·5	10·4	4·4	0·5	3·7	5·6	8·2	3·2*	12·4*
8 May	37·8	9·0	5·3	1·2	2·1	7·2	8·1		
Mean	37·7	9·2	4·8	0·8	2·7	6·8	8·2	3·2	12·4

* The three replicate samples of cyprids were pooled for determination of chitin and ash.

TCA and then allowed to stand for 30 min at 4°C. The homogenates were centrifuged for 15 min at 800 *g* and the precipitates washed twice (by resuspension and centrifugation) with cold 5% TCA. Lipids were extracted from the precipitates by washing twice with chloroform : methanol (2 : 1 v/v). The residues were washed twice with methanol and air dried. The material, consisting mainly of protein and chitin, was heated at 100°C in a water bath with 75 ml 10N NaOH for 2 h, to hydrolyse protein. The reaction mixture was allowed to cool and then centrifuged at 800 *g* for 30 min. The remaining precipitate was washed with distilled water and freeze-dried. The product, consisting of chitin with, perhaps, some residual protein was light grey in colour.

Results and discussion

Biochemical composition of *B. balanoides* cyprids

Table 1 shows the total dry weight and biochemical composition of cyprids collected from the plankton in the Menai Strait.

Total lipid accounted for 14·0% but carbohydrate only 3·5% of the total dry weight of the cyprids. Similarly, the larvae of *O. edulis* (Millar and Scott, 1967; Holland and Gabbott, 1971), and several species of planktonic crustacea (Raymont, Srinivasagam, and Raymont, 1969) also store more lipid than carbohydrate.

In *B. balanoides* cyprids, neutral lipids formed 66%, by weight, of the total lipid fraction (Table 1). In deep water planktonic crustacea wax esters form a large proportion of the neutral lipid fraction and apparently function as secondary energy reserves when the triglycerides are exhausted (Lee, Hirota and Barnett, 1971; Morris, 1972). Analysis of the neutral lipid fraction from *B. balanoides* cyprids, by thin-layer chromatography on Silica gel G, showed the presence of triglycerides but wax esters were absent (Holland, unpublished observation).

Chitin forms 3·2% of the total dry weight of the cypris larva (Table 1) and this value lies within the normal range of 3–6% by weight already reported for planktonic copepods, decapods and euphausids (see Raymont, Austin and Linford, 1963; 1967; Raymont, Srinivasagam and Raymont, 1971). The ash weight of cyprids was 12·4% of the total dry weight (Table 1). The percentage of ash is within the comparatively wide range of ash weights, 2 to 21% of the total dry weight, quoted for several species of crustacean zooplankton (Raymont et al. 1963, 1967; and 1971).

Biochemical changes in *B. balanoides* cyprids prevented from settling

The total dry weight and percentage biochemical composition of free-swimming cyprids prevented from settling for up to eight weeks is shown in Table 2. Figure 1 shows the content per cyprid of each biochemical constituent over the eight week period. Although there was a slight decrease in the nitrogen, phospholipid and free sugar content of the cyprids, the polysaccharide content remained comparatively constant. On the other hand the amount of neutral lipid fell dramatically. Four weeks after capture more than 60% of the neutral lipid reserves had been lost and at the end of the experiment the loss of neutral lipid had increased to 90% of the initial value.

During the development of the egg masses of *B. balanoides*, Barnes (1965) found that in the early stages there was a loss of protein followed by a loss of carbohydrate. Only in the later stages of egg development was there any use of lipid reserves. In the cyprids of *B. balanoides* however neutral lipid (triglycerides) is clearly the main energy reserve. Neutral lipid has also been shown to be the main reserve in oyster larvae (Holland and Spencer, 1973), although, according to Corner and Cowey (1968), the importance of lipid as a food reserve may vary in other zooplankton.

The mass of *B. balanoides* cyprids is present in the

Table 2. The total dry weight and biochemical composition, expressed as a percentage of total dry weight, of *B. balanoides* cyprids kept in the laboratory and prevented from settling for up to eight weeks.

Duration of experiment in weeks after capture	Date 1972	Mean total dry weight µg	Total lipid Neutral lipid	Total lipid Phospho-lipid	Total carbohydrate Polysacc-haride	Total carbohydrate Free sugars	Protein N	Total N
Start	2 May	40·5	10·4	4·4	0·5	3·7	5·6	8·2
1	9 May	36·6	11·7	3·8	1·6	3·5	5·7	9·1
2	16 May	35·2	10·5	4·5	1·4	3·4	4·1	8·9
3	23 May	33·5	9·3	3·6	0·6	3·3	5·5	9·5
4	30 May	31·5	5·1	3·8	2·2	2·5	5·6	9·5
5	6 June	30·4	3·9	3·6	1·3	3·3	5·2	9·1
6	13 June	28·5	3·7	3·7	1·4	3·1	6·0	10·1
7	20 June	26·5	2·3	4·5	0·8	3·4	6·0	10·8
8	27 June	27·2	1·5	3·3	1·1	3·3	5·4	9·9

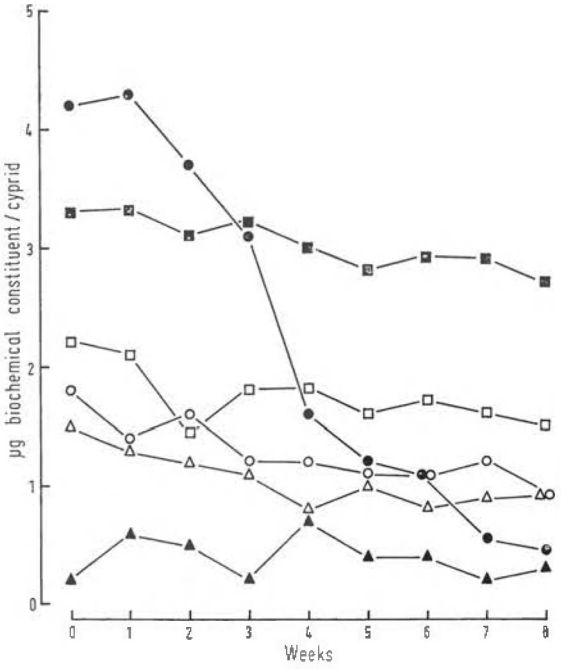

Figure 1. The lipid, carbohydrate and nitrogen content of *B. balanoides* cyprids kept in the laboratory and prevented from settling for up to eight weeks. ■ total nitrogen, □ protein nitrogen, ● neutral lipid, ○ phospholipid, ▲ polysaccharide, △ free sugars.

Menai Strait for about four weeks (mid April–mid May) and during this period settlement takes place. In fact the literature indicates that cyprids of littoral species of barnacles may settle well within 14 days of planktonic life (see Wolf, 1973; Table XIX). Thus although *B. balanoides* cyprids were successfully maintained in the laboratory for eight weeks this is probably an abnormally long time for these cyprids to be in the plankton. Their ability to settle successfully during this period was not however tested experimentally. The energy cost of metamorphosis in terms of lipid reserves is not known. It may be that the free-swimming cyprids collected towards the end of the eight week period had lipid reserves so depleted that successful metamorphosis could not be achieved.

There must be a time when the advantages to the cyprids of remaining in the plankton is outweighed by the depletion of their energy reserves. This implies that utilisation of energy reserves has perhaps some influence on the drive to settle in cyprids, which has hitherto not been considered, although Crisp and Meadows (1963) found that the rate of settlement in cyprids increased with age.

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