Sexual and developmental differences in the protein pattern of haemolymph and body tissues of *Streptocephalus dichotomus* Baird (Crustacea: Anostraca)

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**Abstract**

The protein pattern of haemolymph and body tissues of the freshwater fairy shrimp *Streptocephalus dichotomus* has been investigated in both sexes, using polyacrylamide disc gel electrophoresis. The electropho-grams of four developmental stages show variations in number and intensities of protein fractions. In Stage III, two female-specific proteins of glycolipoprotein nature appear. This stage corresponds to maturity: females begin to possess mature oocytes in the ovary. These two vitellogenic proteins are well represented in the female haemolymph, ovary and freshly laid eggs, but are absent in the male haemolymph. A heterosynthetic mode of yolk formation is thus evident in this anostracan. The two sex-limited proteins are only faintly represented in shelled eggs, suggesting an early utilization of these compounds in embryogenesis.

**Introduction**

Among arthropods, early developmental events are accompanied by changes in protein patterns in haemolymph and body tissues (Hudson, 1966; Pasteur & Kastritis, 1974; Engelman, 1969). Such changes are more marked among sexes towards maturity (Engelman, 1969). Associated with maturity, the body fluid circulates a female sex-limited protein not only in insect species (Engelman, 1969; Elliot & Gillot, 1979) but also in several malacostracan crustaceans (Horn & Kerr, 1969; Croisille *et al.*, 1974; Meusy, 1980). Such information in lower crustaceans, especially in anostracans, is limited although they are well suited for such investigations in view of their rapid growth and sexual maturity (Schwab, 1974; De Chaffoy & Kondo, 1980). Hence, the present study uses a freshwater fairy shrimp, *Streptocephalus dichotomus* Baird of which the biology of reproduction is reasonably well understood (Munuswamy, 1982).

**Material and methods**

Specimens of *S. dichotomus* were collected from ephemeral ponds located in Chingleput about 35 km South West of Madras. Four different developmental stages of *S. dichotomus* were classified based on size, nature of ovary, shell glands, eggs in the oviduct or ovisac and other secondary sexual characters as described by Munuswamy (1982), summarized in Table 1.

For electrophoretic analysis, female fairy shrimps in different stages were washed in distilled water. Each tissue was homogenised in 2 ml of 40%
Table 1. Characteristics of reproductive organs in the Post-embryonic development of *S. dichotomus*.

<table>
<thead>
<tr>
<th>Stages</th>
<th>Mean size of animal (mm)</th>
<th>Mean days after hatching</th>
<th>Description of reproductive organs</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>8 ± 1.5</td>
<td>7 ± 1.3</td>
<td>Ovary not visible; devoid of lateral pouch of oviduct and shell glands; ovisac extends up to 1st abdominal segment.</td>
</tr>
<tr>
<td>II</td>
<td>12 ± 2.4</td>
<td>13 ± 2.0</td>
<td>Ovaries appear as white strands of tissue; devoid of any visible oocytes; shell glands are small and transparent, white in colour; ovisac extends up to 4th abdominal segment.</td>
</tr>
<tr>
<td>III</td>
<td>15 ± 2.2</td>
<td>18 ± 3.5</td>
<td>Numerous oocytes visible in ovary; shell glands are light brown in colour; red pigments appear in the dorso lateral walls of ovisac; eggs are seen in the lateral pouch of oviduct. Ovisac extends maximum to 6th abdominal segment.</td>
</tr>
<tr>
<td>IV</td>
<td>18 ± 3.5</td>
<td>25 ± 3.0</td>
<td>Few oocytes in the ovary; no eggs in the lateral pouch of oviduct; eggs are brown in colour and confined to the ovisac.</td>
</tr>
</tbody>
</table>

Sucrose solution and centrifuged at 2000 rpm for 15 minutes. The supernatant, containing soluble protein, was used for electrophoretic analysis. A similar analysis was carried out on pooled male and female haemolymph, ovary, gut, eggs (un-shelled), and shelled eggs.

**Electrophoresis**

Polyacrylamide gel electrophoresis was carried out according to Davis (1964) using original monomer and buffer system but omitting the spacer gel. The preparation of gel and application of samples were outlined by Smith (1968). 7% polyacrylamide gel electrophoresis was conducted at 3 mA per tube for 70 minutes at 4°C.

Simple proteins were stained with Coomassie brilliant blue (Smith, 1968); glycoproteins were detected with the PAS method (Clarke, 1964) and lipoproteins, by the oil red ‘O’ (Kannupandi & Paulpandian, 1975). Scanning of the gel was done using a CHROMOSCAN MK, 11 Double-Beam recording integrating densitometer.

The haemolymph and other body tissues proteins are grouped into slow, middle and fast moving bands (Horn & Kerr, 1969; Fielder et al., 1971). Such a classification is advantageous for the present purpose, namely, the identification of sex-limited vitellogenic proteins in the haemolymph, ovary and other tissues. Numbering of the protein bands in each sample is made in the ascending order from cathodal to anodal region of the electropherogram.

**Results**

Electrophoretic investigations on four different developmental stages of *S. dichotomus* show variations in the number and intensities of protein fractions (Fig. 1). From the electropherogram, it is seen that in immatures (Stage I), there are sixteen fractions, of which the first ten fractions are faintly stained, slow moving, simple proteins. The fractions 11 and 12 are in the middle region and fractions 13 to 16 are fast moving. The fractions 11 and 12 stain for glycoprotein and glycolipoprotein, respectively whereas all the other fast moving fractions are simple proteins.

In Stage II, there is a significant change in the protein pattern. Except fractions 1, 2, 3, 8, 12 and 16 of Stage I, all proteins occupying the slow moving and fast moving regions disappear. However, new proteins appear, especially in the middle zone (Fig. 1). The newly formed proteins in Stage II include lipoprotein (fraction 6), simple proteins (fractions 7, 8 and 11) and glycoprotein (fraction 12), there is intensification of existing proteins such as glycolipoprotein (fraction 10) (Fig. 1).

In Stage III, fractions 4 and 12 of Stage II disappear, but the new fractions that appeared in Stage II are retained (Fig. 1). A remarkable feature of Stage III is the appearance of two new