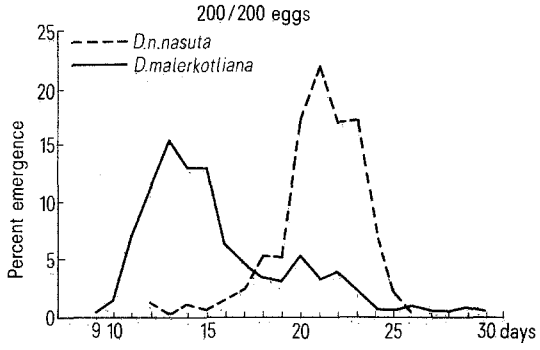

Figure 3. Pattern of adult eclosion in the mixed cultures of *D. malerkotliana*/*D. n. nasuta*.

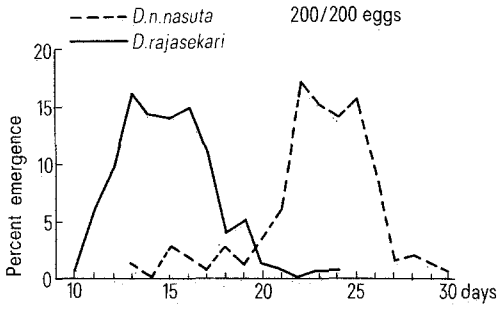

Figure 4. Pattern of adult eclosion in the mixed cultures of *D. rajasekari*/*D. n. nasuta*.

lier than those of its competitors. In the cultures of *D. malerkotliana* and *D. n. nasuta*, the former species demonstrates a significantly faster speed of development than the latter. The findings on egg-to-adult viability are recorded in the table 2. (The tables and figures presented herein provides the data for 200/200 egg densities only. Similar observations were made for 100/100 egg densities). So the present data on the egg-to-adult rate of development and viability reveals the superiority of *D. rajasekari* over *D. malerkotliana* and *D. n. nasuta*.

The field data on the distribution of the 3 species under study suggests that they are sympatric[1,2]. We are of the opinion that the concept of sympatry is a dynamic one and within this apparent sympatric coassociation in natural habitats the exact eco-biological status and relationships are not known. The present experimental results have demonstrated one possible outcome of interspecies interactions under controlled laboratory conditions. This alternation in the peaks of adult emergence can be viewed as an ecological homeostatic set-up of species systems to manifest different speeds of development in mixed cultures.

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Heterogeneous ferritin in the blood of two species of Tyrrhenian limpets, *Patella coerulea* L. and *Patella lusitanica* G.

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Summary. The hemolymph of *Patella* is yellow and contains 30-300 µg/ml of iron. Ferritin was found to be uniquely abundant in the hemolymph, and was identified by electron microscopy and electrophoresis. Electrophoretically it appears to be heterogeneous, with an individually variable number of components with very similar mobilities. Ferritin in the blood of limpets might relate to the turnover of radular denticles.

Among those molluscs, that have an intense iron metabolism, limpets of the genus *Patella* (Archeogasteropoda) deserve further investigation. The capping material of their radular denticles, which are close to 5 in Moh's hardness scale, contains goethite (Fe_2O_3)[1] and their radular muscles contain myoglobin[2]. These features of the buccal apparatus of docoglossan limpets seem to be related to their feeding habits: they are in fact grazers of calcareous algae of the rocky bottoms of the intertidal zone or shallow waters[3,4].

Materials and methods. Patella coerulea L. and *Patella lusitanica* G. collected in the gulf of Naples were fixed whole in Bouin's liquid. Their body was separated from the shell and embedded in paraffin wax. Sections were stained in H and E or by Pearls' reaction for iron. Tissue samples from the mantle edge were fixed in OsO_4 1% or in glutaraldehyde 3% in sea water, dehydrated, sometimes stained in toto by uranyl acetate, and embedded in Epon. Sections were observed with a Philips EM200 electron microscope without further electroncontrast.

Blood was collected from the great pallial vein[5]. The iron content of the hemolymph was determined by the method of Lorber[6], and by atomic absorption spectrophotometry (Densatomic, Optica, Milano, Italy). Electrophoretic analysis of filtered and dialyzed hemolymph was performed in Tris-glycine buffer, pH 8.8 at 300 V on Cellogel strips, using horse-spleen ferritin (Pentex Inc.) as a standard. Strips were stained by amidoblack for proteins and by Pearls' reaction for iron. Diluted and undiluted samples of filtered ferritin were layered on formwar-carbon coated grids and observed with the electron microscope.

Results and discussion. Pearls' reaction revealed ferric iron in some cells of the hepatopancreas, in the odonthogenic epithelium of the radula and in the radular denticles. The intestinal lumen contained loose fragments of worn denticles still intensely reactive for iron.

The amorphous content of all vascular cavities especially the small lacunar spaces of the pallial tentacles, where blood remained entrapped at the time of fixation, stained

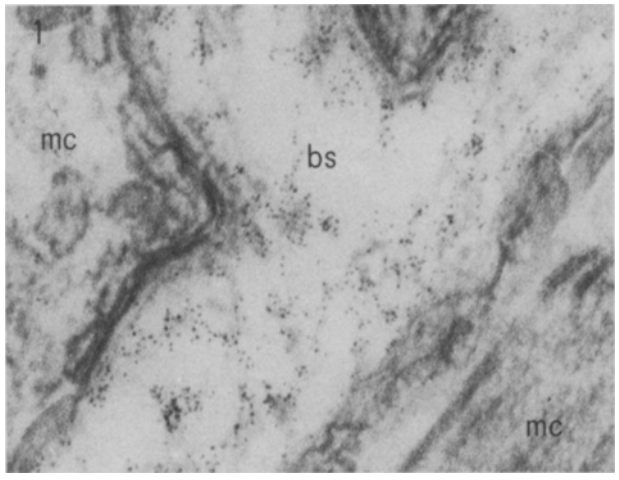

Figure 1. Electrondense ferritin particles in the lumen of a blood space (bs) between muscular cells (mc) of the edge of the mantle. U.A. 'in toto' × 51,500.

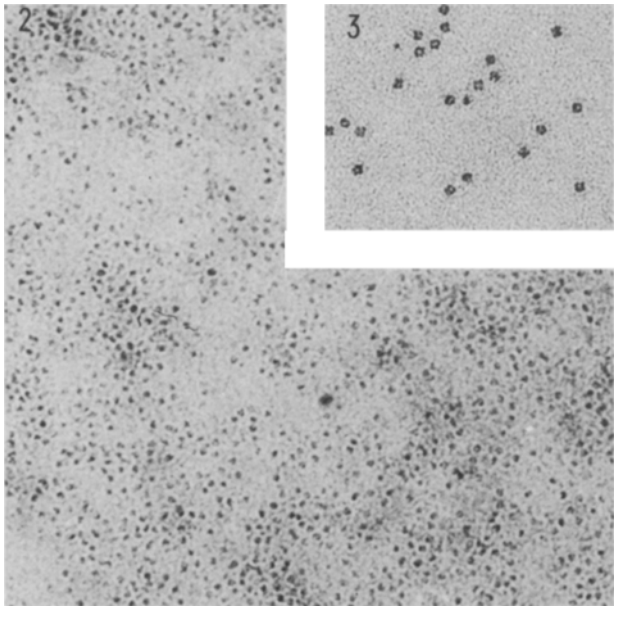

Figure 2. Electrondense ferritin particles in undiluted hemolymph from the great pallial vein. Unstained. × 110,000.

Figure 3. Tetrad structure of ferritin iron cores in hemolymph from the great pallial vein. Diluted 1:5 in water. Unstained. × 153,000.

blue for iron by Pearls' reaction. Electronmicroscopically, ferritin-like electrondense particles, of the order of 10 nm, appeared scattered all over the lumen of blood lacunar spaces between smooth muscle cells at the mantle edge (fig. 1).

The hemolymph, collected by means of bleeding the great pallial vein, varied in colour between pale yellow and dark orange. Each animal, according to size, yielded 0.5–1.3 ml of hemolymph. Electronmicroscopical examination of paper-filtered, unstained, undiluted hemolymph, dried upon the grids, showed a dense carpet of ferritin-like particles (fig. 2). Closer examination of 1:5 water-diluted samples revealed the typical 'tetrad like' picture of the iron core of the ferritin molecule as seen in underfocused micrographs[7]

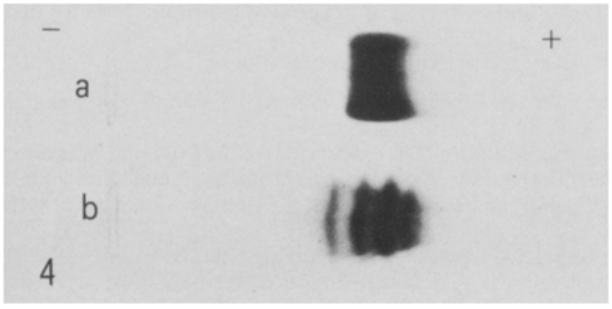

Figure 4. Cellogel electrophoresis of *Patella* hemolymph: Pearls' reaction (exposure to a fresh mixture of equal parts of 2% potassium ferrocyanide and 2% HCl for 5 min) reveals only 1 iron-containing protein band in a (= horse ferritin) and 4 iron-containing protein bands in b (= *Patella* hemolymph).

(fig. 3). Hemolymph total iron content of individual samples (about 20 for each species) varied from 30 to 300 µg/ml. The iron contents were lower in the paler samples and vice-versa.

Electrophoretic analysis of individual samples of hemolymph showed a variable number of bands very close together, which all stained positively – like ferritin – for both proteins and iron. They had an anodal mobility very close to, and within the range of, that of the horse-spleen ferritin run in parallel as a standard (fig. 4). Different individual animals exhibited a number of ferritin bands, variable from 3 to 7, irrespective of the species. No other relevant protein bands were revealed. No correlation of known physiological factors with either iron content or color of the hemolymph was possible.

Electronmicroscopical and electrophoretical results concurrently identified ferritin as a relevant normal component of *Patella*'s hemolymph, where the protein appears to be free in solution and very abundant as compared to normal human serum: here it amounts only to 20–150 µg/l and it is detected by immunoassay techniques[9].

It must be emphasized that so much iron and ferritin in the blood of *Patella* might be related to the processes of formation and turnover of the iron-containing radular denticles. If so it appears strange that neither iron[9] nor ferritin[10] have been detected in the blood of *Chitons* (Mollusca) which also possess iron in their radular denticles[11].

These observations, besides being a contribution to the study of the metabolism of iron in molluscs, invites further investigation on the origin and metabolic significance of blood ferritin and might be of interest to those working in the controversial field of the biochemistry of 'isoferritins'.

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