

## The chloroplast-animal association in four Iberian Sacoglossan Opisthobranchs: *Elysia timida*, *Elysia translucens*, *Thuridilla hopei* and *Bosellia mimetica*

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**SUMMARY:** The Opisthobranch Molluscs *Elysia timida*, *E. translucens*, *Thuridilla hopei* and *Bosellia mimetica* feed suctorially on the algae *Acetabularia acetabulum*, *Udotea petiolata*, *Cladophora vagabunda* and *Halimeda tuna*, respectively. The animals retain functional chloroplasts from their food. Symbiotic retention of functional algal chloroplasts in these Sacoglossan species is proved by electron micrography of the digestive gland, and the activity of the organelles by means of incubations with  $\text{NaH}^{14}\text{CO}_3$  in the light and in the dark for varying periods of time. In *Elysia timida*, fixation rates were measured at different light intensities and between 10-35 °C. Carbon incorporation after a 40 days starvation period is registered. About 7-8% of the net  $^{14}\text{C}$  photosynthetically fixed by functional chloroplasts was secreted in mucus by the molluscs. After 12 days, juveniles retained chloroplasts in the digestive gland. Chloroplasts associated with *Thuridilla hopei* lose their functional ability after 5 days. The results are discussed in the general frame of the meaning of this symbiosis between algae and molluscs.

**Key words:** symbiosis, Sacoglossa, chloroplasts, *Elysia timida*.

### INTRODUCTION

The sea slugs of the order Sacoglossa (Mollusca: Gastropoda: Opisthobranchia) are highly specific herbivores which feed by puncturing the cells of multicellular algae and suctioning their contents. Chloroplasts of these cells are taken up in the process and stored in the cells of the digestive gland of the mollusc. Thus an intimate, metabolic chloroplast-animal association is established. WAUGH & CLARK (1986) adopt the term "kleptoplastid" (from the Greek "kleptein", to borrow) to refer to the slave algal plastids.

Chloroplast retention is the product of long periods of coevolutionary history between the mollusc and its algal diet. The Sacoglossans have anatomical and behavioural adaptations for the efficient exploitation of the retained chloroplasts (GREENE, 1974; THOMPSON, 1976; TAYLOR, 1981; HINDE, 1983; MARÍN, 1988; MARÍN & ROS, 1988, etc.). Some algae are preferred to others, in relation with taxonomy, morphology and ash content, and the Sacoglossa are

perhaps the most stenophagous gastropods in a group which stands out by its specialized food (CLARK & BUSACCA, 1978; ROS, 1978; JENSEN 1983; CLARK & DEFREESE, 1987; MARÍN, 1988; MARÍN & ROS, 1988; MARÍN *et al.*, in press; etc.).

The chloroplasts are energy sources for the host, providing it with photosynthetic products. However, environmental conditions are correlated with photosynthetic efficiency. Carbon fixation varies with light intensity and temperature (CLARK *et al.*, 1981). There is an equilibrium between the population of the sacoglossan and that of its algal food, with an input of additional energy in the molluscs coming from the "symbiotic" chloroplasts. The life cycle of the sacoglossans is well adapted to that of the algae. Thus, the *Elysia timida* populations studied are narrowly linked to cap initiation and stalk calcification of *Acetabularia acetabulum* (MARÍN, 1988). The turnover rate of chloroplasts may be a function of the tendency of a species to wander to and from its algal food and of its algal food abundance (CLARK & BUSACCA, 1978).

The meaning of this "symbiosis" has been widely discussed, among others, by GREENE (1974), TAYLOR (1981), HINDE (1983), CLARK & DEFRESE (1987), MARÍN (1988), MARÍN & ROS (1988), ROS (in press). The ultimate meaning of the association, however, is still a matter of speculation. According to HINDE (1983) it can hardly be termed a symbiosis a relationship in which one of the partners (the chloroplasts) is not genetically autonomous. In the Discussion we afford some supplementary evidence and add some speculation of our own to the subject.

## MATERIAL AND METHODS

### Provenance of the molluscs

All studied species come from the SE coasts of Spain (Mediterranean Sea). The molluscs were obtained, together with their food algae, from the first, well-lit meters of the medio- and infralittoral levels of the Mediterranean benthos, either on the shores of this sea —*Elysia translucens* Pruvot-Fol, 1957, *Thuridilla hopei* (Vérany, 1853) and *Bosellia mimetica* Trinchese, 1890— or on those of the Mar Menor lagoon —*Elysia timida* (RISSO, 1818) (ROS & RODRÍGUEZ, 1985; MARÍN & ROS, 1988; ROS, in press), during the spring, summer and fall of 1986 and spring and summer of 1987.

In their aquatic environment these molluscs are rather abundant (*E. timida*), occasional (*E. translucens*, *T. hopei*) or very scarce (*B. mimetica*), and the numbers with which we have worked are correspondingly high (*E. timida* and *T. hopei*) and low or very low (the remaining species). Freshly collected, live individuals were maintained in laboratory aquaria (at conditions of light intensity, temperature and salinity similar to those in their natural environment) for short periods after collection, and within 48 hours they were used in one or more of the experimental procedures carried out.

### Incubation of molluscs with $\text{NaH}^{14}\text{CO}_3$

Two animals were incubated for 2 hours in glass flasks containing 3 ml of Millipore-filtered (porosity, 0.45  $\mu\text{m}$ ) seawater with 1  $\mu\text{l}$  of  $\text{NaH}^{14}\text{CO}_3$  (activity, 4  $\mu\text{Ci ml}^{-1}$ ). Measurements of fixation in the dark were carried out under the same conditions but using foil-wrapped flasks. Temperature was maintained constant during incubation by immersing the flasks in a running water bath. Following incubation, animals were rinsed twice with fresh Millipore-filtered seawater. Whole animals were placed in 3 ml cold (4 °C) absolute methanol for several hours until pigment could not be observed in the tissues. Determination of total chlorophyll was made using the method of MACLACHLAN & ZAHLIK (1963). The tissues

were digested in 5 %  $(\text{NH}_4)_2\text{SO}_4$  (100 °C, 1 hour) and 4 M KOH (100 °C, until all tissue had dissolved). Liquid samples were acidified, dried and counted in scintillation fluid in a LKB-WALLAC 1217 RACK-BETA scintillation counter. Counting data were corrected for background and quenching. The total amount of assimilated carbon was calculated using the proportional relationship:

$$\frac{^{14}\text{C available}}{^{14}\text{C assimilated}} = \frac{^{12}\text{C available}}{^{12}\text{C assimilated}}$$

### Light and temperature responses

Light from an incandescent floodlight was filtered through neutral density filters to obtain intensities ranging from 10 to 400  $\mu\text{E m}^{-2} \text{s}^{-1}$ . The effect of temperature was tested from 15 °C to 35 °C in a running water bath (irradiation rate, 60  $\mu\text{E m}^{-2} \text{s}^{-1}$ ). Sacoglossans were used within 48 hours of collection.

### Mucus synthesis

The translocation of  $^{14}\text{C}$ -labelled materials from retained, symbiotic chloroplasts to host animal tissue was studied in *Elysia timida*. Sample specimens were incubated for 2 hours in the light (200  $\mu\text{E m}^{-2} \text{s}^{-1}$ ) and at 25 °C. Animals exposed to  $^{14}\text{CO}_3\text{H}^-$  for 3 hours were temporarily transferred to a dish with a few drops of membrane-filtered seawater. During 15 minutes the mixture of mucus in seawater was removed by pipette and mucus production was stimulated by gently swirling the animal. The sacoglossans were returned to the incubation medium and the sampling was repeated every hour (TRENCH *et al.*, 1970) for 7 hours.

### Estimation of chloroplast number

Molluscs were homogenized in membrane-filtered seawater with a motor-driven plastic-pestle homogenizer at 0 °C and in the dark. Several 1 ml aliquots of the chloroplasts suspension were filtered onto Nucleopore filter (porosity, 0.2  $\mu\text{m}$ ). The damp filter was then covered by immersion oil and examined by fluorescence microscopy. The chloroplast concentration on the filter was determined by ten replicate counts (about 70 chloroplasts per count). Total number of chloroplasts per sacoglossan individual was calculated from the equation:

$$\begin{aligned} \text{Number of chloroplasts} &= \\ &= \frac{\text{N.}^\circ \text{ chloroplasts per field} \times k}{1 \text{ ml sampling}} \times \\ &\quad \times \text{ml homogenate,} \end{aligned}$$

where  $k$  = surface area of filter/surface area of field (= 8792 in our microscope).

## Tracer chloroplasts

Whole *Elysia timida* slugs were irradiated with UV light, until chlorophyll in chloroplasts was damaged. Afterwards the molluscs were maintained in aquaria containing *Acetabularia acetabulum*. After 4 days, animals were removed and their chloroplasts counted; new plastids were easily recognizable under the fluorescence microscope. Number of irradiated chloroplasts and number of those obtained anew by the molluscs in 4 days were calculated.

## Electron microscopy

Small pieces of mollusc tissue were fixed in 2.5 % Millonig's phosphate-buffered glutaraldehyde for 1 hour. They were then washed in 2.5 % NaHCO<sub>3</sub> and postfixed in 2 % OsO<sub>4</sub> in 1.25 % NaHCO<sub>3</sub> for 1 hour. The tissues were dehydrated in an ethanol series and then embedded in Epon. Ultrathin sections were cut on a glass knife and stained with uranyl acetate and lead citrate. They were observed under an ZEISS EM 10C electron microscope.

## RESULTS

### Chloroplast retention

The four sacoglossan species are highly specific herbivores. *Elysia timida* eats *Acetabularia acetabulum* (L.) Silva (ROS & RODRÍGUEZ, 1985), *Thuridilla hopei* eats *Cladophora vagabunda* (L.) Hoek, *Elysia translucens* feeds on *Udotea petiolata* (Turra) Børgesen, and *Bosellia mimetica* on *Halimeda tuna* (Ellis & Solander) Lamouroux (MARÍN & ROS, 1988; MARÍN, 1988; MARÍN *et al.*, in press).

Electron micrographs of the digestive gland of the slugs show chloroplasts identical with those of the chlorophycean alga used as food (Figs. 7 to 13). The plastids are enclosed by a double chloroplast envelope. The pyrenoids are situated within the chloro-

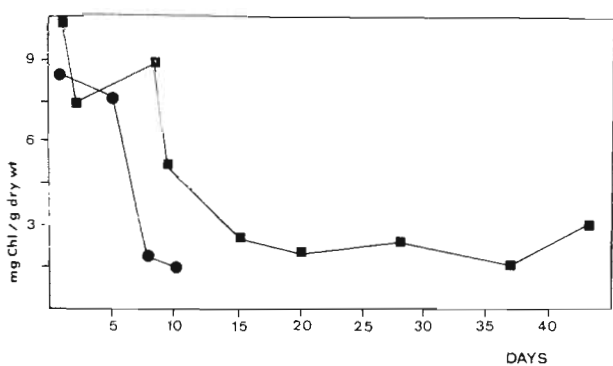


FIG. 1. — Effect of starvation on chlorophyll levels of *Elysia timida* (squares) and *Thuridilla hopei* (dots).

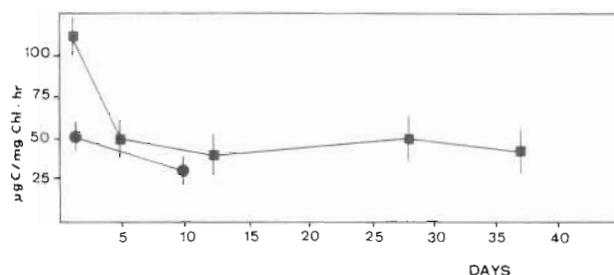


FIG. 2. — Net carbon fixation rate during 40 days of starvation in *Elysia timida* (squares) and during 10 days in *Thuridilla hopei* (dots).

plast, and surrounded by starch grains. In contrast to the green algae, polysaccharide storage occurs in cytoplasmic vesicles in red and brown algae. The chloroplasts of *Udotea petiolata* contain a membrane system termed concentric lamellar system (CLS; Fig. 7) or polar thylakoid organizing body. This membrane system might confer greater osmotic and chemical stability on the chloroplast (WRIGHT & GRANT, 1978; MARÍN, 1988).

In *Elysia timida*, *E. translucens*, *Thuridilla hopei* and *Bosellia mimetica* starved for several days, the chlorophyll level drops sharply (Fig. 1) as the chloroplasts numbers fall. The chloroplasts show also degenerative changes. The chloroplasts in the process of digestion are more electron dense and show thylakoid delamination and interthylakoid vesicle formation (Figs. 9 and 11). All plastids in *T. hopei* and *B. mimetica* are surrounded by a host membrane from the phagocytic vacuole (Figs. 10, 11 and 12). Host membranes around chloroplasts are absent in the two *Elysia* species (Figs. 8, 9 and 13).

### Carbon fixation

There is a significant net carbon fixation in the four sacoglossans studied (Table I). This ranges from 40  $\mu\text{gC mgChl}^{-1} \text{hr}^{-1}$  in *Thuridilla hopei* to nearly

TABLE I. — Carbon fixation rates in several sacoglossan species, in relation to chlorophyll content and illumination. Numbers in the column at right correspond to papers cited in the References: MR refer to this study.

	Carbon fixation, $\mu\text{gC mgChl}^{-1} \text{h}^{-1}$	Illumination $\mu\text{E m}^{-2} \text{s}^{-1}$	Study
A) Mediterranean species			
<i>Elysia timida</i>	144	200	MR
<i>Elysia viridis</i>	95	162	5
<i>Elysia translucens</i>	68	100	MR
<i>Thuridilla hopei</i>	40	100	MR
<i>Bosellia mimetica</i>	45	100	MR
<i>Calliopaea bellula</i>	0	—	6, MR
<i>Limapontia capitata</i>	0	—	9
B) Non Mediterranean species			
<i>Costasiella liliana</i>	200-300	500	4
<i>Elysia tuca</i>	60	—	8

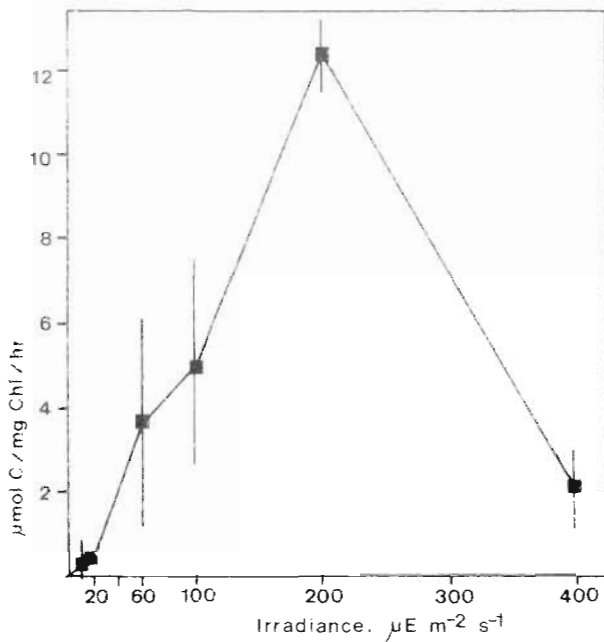


FIG. 3. — Net carbon fixation rate by *Elysia timida* at different light intensities.

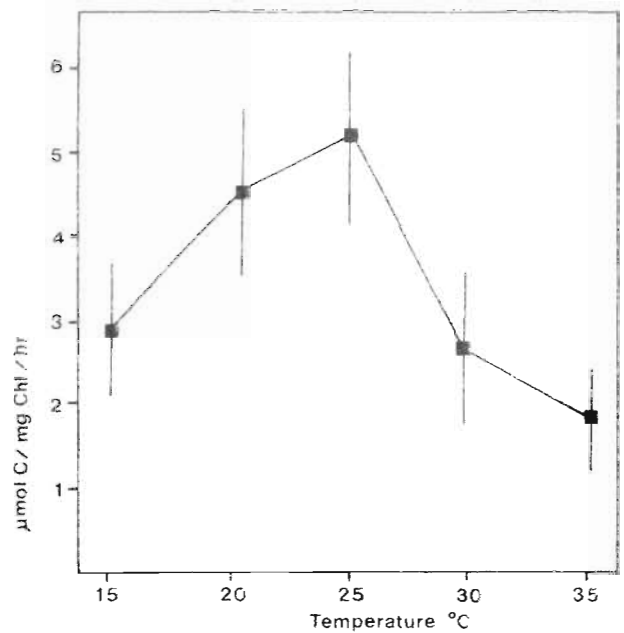


FIG. 4. — Net carbon fixation rate by *Elysia timida* at different temperatures.

four times this amount ( $144 \mu\text{gC mgChl}^{-1} \text{hr}^{-1}$ ) in *Elysia timida*. Carbon fixation by *Elysia timida* varied with light intensity and temperature (Figs. 3 and 4), optimal conditions being at  $25^{\circ}\text{C}$  and  $200 \mu\text{E m}^{-2} \text{s}^{-1}$ . *E. timida* showed photosynthesis inhibition both at high light intensities and at high temperatures.

#### Turnover rate of chloroplasts

The turnover rate of chloroplasts has been calculated through starvation of the molluscs for several days. Chloroplast symbionts in *Thuridilla hopei* lose their functional ability after a starvation period of 5 days, while those of *Elysia timida* maintain their photosynthetic ability for more than 40 days (Fig. 2). After an initial period, *E. timida* retains stable chlorophyll and carbon fixation levels. Because the turnover rate of chloroplasts can be affected by starvation, tracer chloroplasts were also used. UV-irradiated *E. timida*, maintained in aquaria with *A. acetabulum* as has been described before, showed a replacement of about 10 % of the plastids after a 4 day period.

#### Mucus synthesis

Incubation of *E. timida* with  $\text{NaH}^{14}\text{CO}_3$  in the light showed the mucus produced by the slugs had incorporated radiactivity (Fig. 5). Photosynthetic products are used for mucus synthesis in this species, as is the case in others (GREENE, 1970b). The mucus exhibited high percentages of  $^{14}\text{C}$ , about 7-8 % of the net  $^{14}\text{C}$  photosynthetically fixed by the chloroplasts.

#### Development and growth of juveniles: chloroplast acquisition

*E. timida* has direct development (type 3) in winter and spring, while in summer and fall the development is lecithotrophic (type 2; RAHAT, 1976; BONAR, 1978; MARÍN & ROS, 1988; MARÍN, 1988). Probably *E. translucens* is also a directly developing species. *Thuridilla hopei* has lecithotrophic pelagic

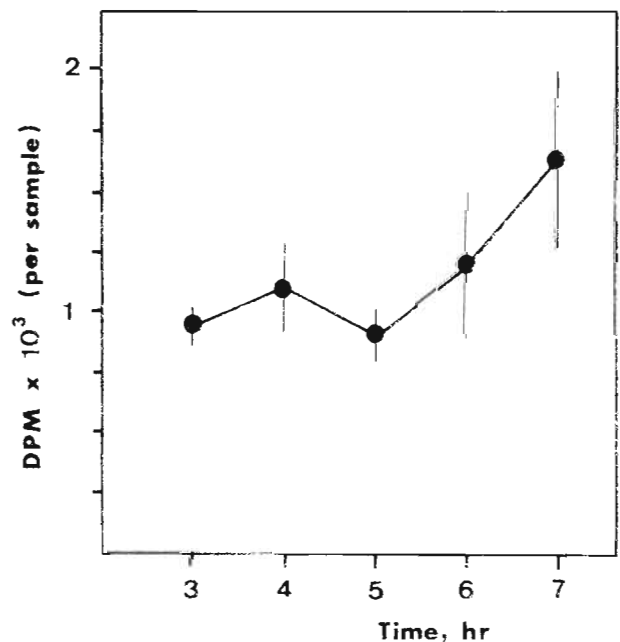


FIG. 5. — Utilization of photosynthetic products in mucus synthesis by *Elysia timida*: appearance of  $^{14}\text{C}$  in mucus, from 15 minutes, hourly samplings.

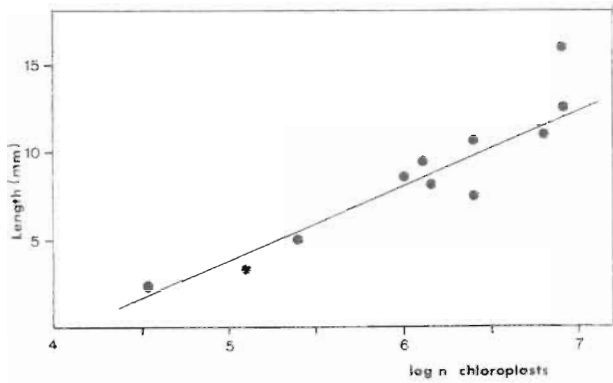


FIG. 6. — Relationship between body length and number of chloroplasts per animal in *Elysia timida*.

larvae (type 2; THOMPSON & SALGHETTI-DRIOLI, 1983), and the development type of *B. mimetica* is unknown (ROS, 1981), but is probably planktotrophic (type 1), since the species is found in the Caribbean, too.

The spawn of *E. timida* is a flat, spiral-shaped band with white eggs 70  $\mu\text{m}$  in diameter. The egg capsules measure 100-125  $\mu\text{m}$ , and egg number per egg mass is low (34-168). Veliger stage and metamorphosis are completed inside the egg capsule, and a benthic juvenile hatches after 16-21 days (RAHAT, 1976; MARIN, 1988).

The chloroplasts are not transmitted in the spawn, and eggs and benthic juveniles examined did not carry chloroplasts. Benthic juveniles were cultivated in Petri dishes with *Acetabularia acetabulum*. In the newly-hatched slug, the radula is functional, with 3 well developed teeth and 5 rudiments. After hatching and until days 4 and 5, the juvenile moves about by cilia on its foot. During this period, the digestive gland is an asymmetrically lobed organ. By day 12, the animal has lost the ciliation and the digestive gland has prolonged into the body. By day 12, *E. timida* juveniles do possess chloroplasts retained in the digestive glands.

The chloroplasts retained in Sacoglossa are limited to the tubules of the digestive gland (GREENE, 1970a). Thus the total number of plastids in a well-fed animal is dependent on surface increase of digestive gland with growth. In *Elysia timida* it can be shown that the number of stored chloroplasts is a function of sacoglossan body length (Fig. 6).

## DISCUSSION

The four studied sacoglossan species appear in their natural marine environment in very low numbers. Only *Elysia timida* in its lagoon habitat forms dense populations (ROS & RODRÍGUEZ, 1985), which has allowed us to carry out replicated and long-

term experiments. The other sacoglossan species have been used in those studies requiring only very few individuals (EM observation,  $^{14}\text{C}$  fixation in short-term experiments, etc.), but only *E. timida* and occasionally *Thuridilla hopei* have allowed experiments requiring many individuals. In spite of this limitation, the results obtained allow a rather broad set of conclusions to be made.

*Elysia timida*, *E. translucens*, *Bosellia mimetica* and *Thuridilla hopei* retain and exploit algal chloroplasts resistant to digestion by host cells. Plastids of siphonaceous algae, spheroid in shape, are robust and possess greater osmotic and chemical stability than those of septate algae, which generally break during ingestion (HINDE & SMITH, 1974; CLARK & DEFRESE, 1987). The presence of a host membrane surrounding the chloroplasts in *Thuridilla hopei* and *Bosellia mimetica* seems to be closely related to fast plastid turnover. The mechanism by which the chloroplasts are protected from digestion is not understood, but the disappearance of the phagosome membrane may be important (HAWES, 1979; HART, 1979). The turnover rate of chloroplasts is about 10 % in *Elysia timida* maintained for 4 days with *Acetabularia acetabulum*.

Symbiotic plastids of the cerata-bearing sacoglossan *Costasiella lilianae* continued to fix carbon after 65 days of starvation, though efficiency of fixation declined about 87 % (CLARK *et al.*, 1981). The performance of the slug-like, cerata-devoid *Elysia timida* is not so high: the chloroplasts are active after a 45 day period, but the efficiency has dropped by about 60 %. For *Thuridilla hopei* the figures are 10 days and about 85 %. The photosynthetic efficiency decreases considerably in the two species in the early days of starvation (5-10 days in *E. timida*).

Chloroplast retention for long periods could be a security mechanism when food is not available; if this be the case, *E. timida* would behave as a species with a rather high risk of food exhaustion, whereas *T. hopei* would not face similar phases of food scarcity. The differences in habitat stability of each species (the more rigorous infralittoral level of an hypersaline lagoon in *E. timida*, and the more constant photophilic to sciaphilic infra- and circalittoral marine bottoms in *T. hopei*), seem at first glance to account for this pattern. Food availability, however, does not follow the same trend: *A. acetabulum* is present virtually all the year, while *Cladophora vagabunda* occurs only from summer to winter. On this basis, it is not surprising that *Elysia timida* is a nearly year-round species and *Thuridilla hopei* is a summer-fall species.

If availability of food rather than habitat stability is the main factor involved here, one would expect the degree of dependency on their retained chloroplasts would be lower in *Elysia translucens* and

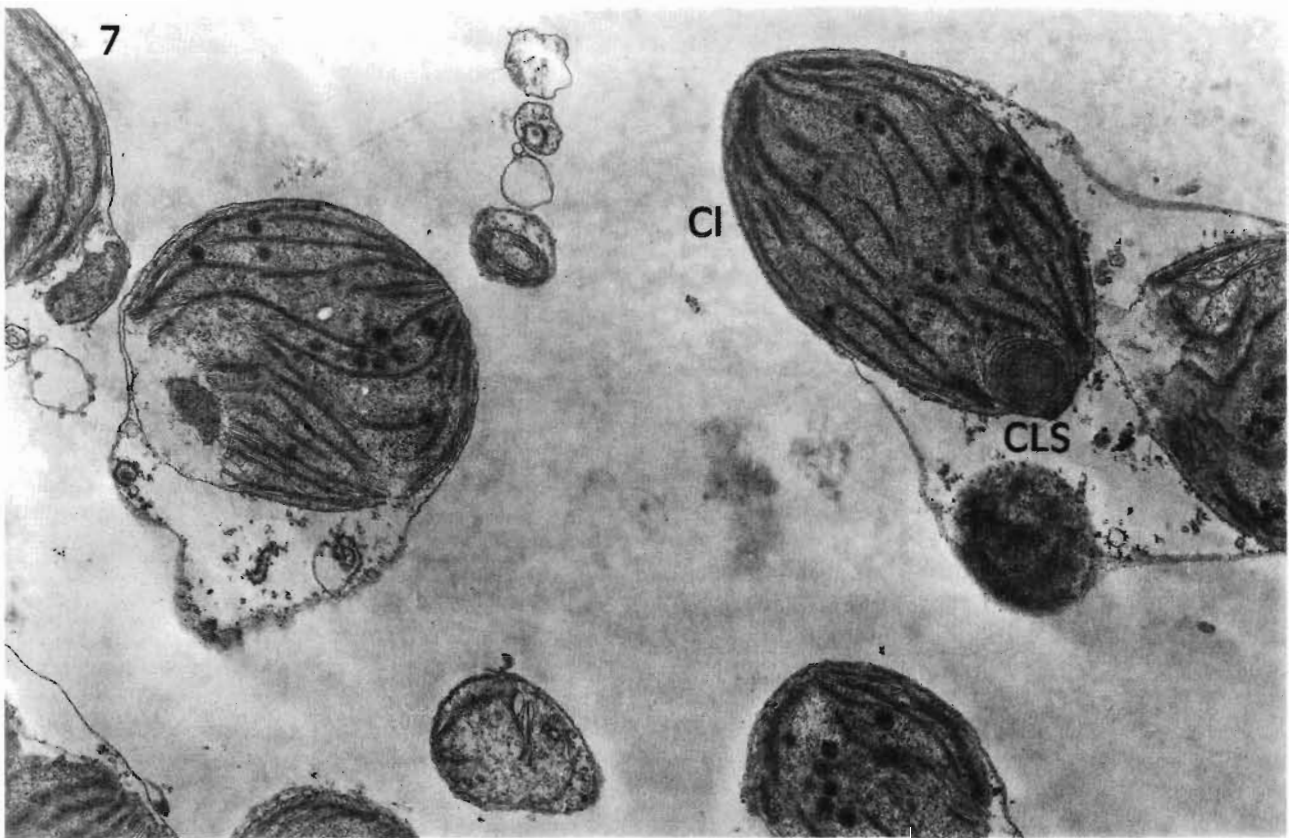


FIG. 7. — Chloroplasts of *Udotea petiolata* ( $\times 5000$ ). Note the concentric lamellar system (CLS). Cl, chloroplast.

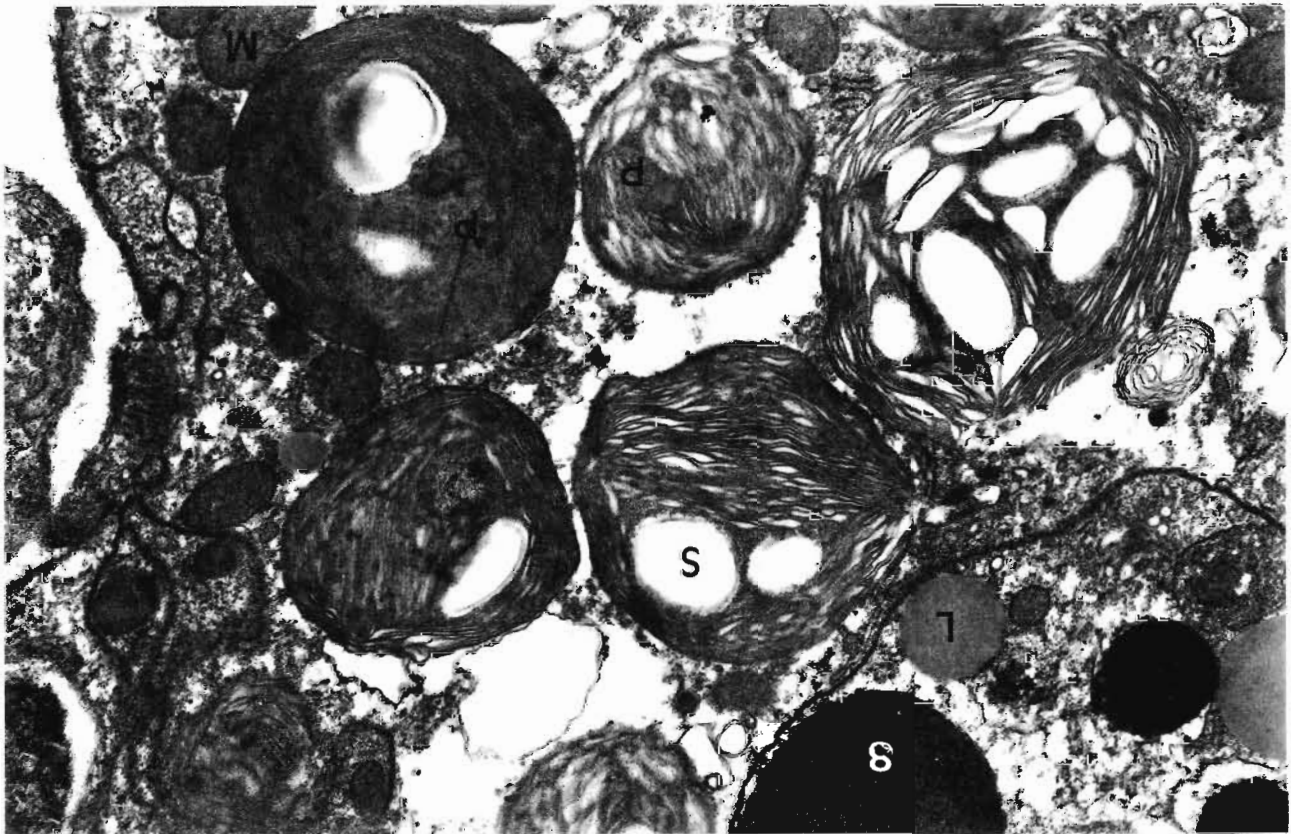


FIG. 8. — Electron micrograph of the digestive gland of unstarved *Elysia timida* ( $\times 5000$ ). Cl, chloroplast; L, lipid droplet; M, mitochondria; P, pyrenoid; S, starch.

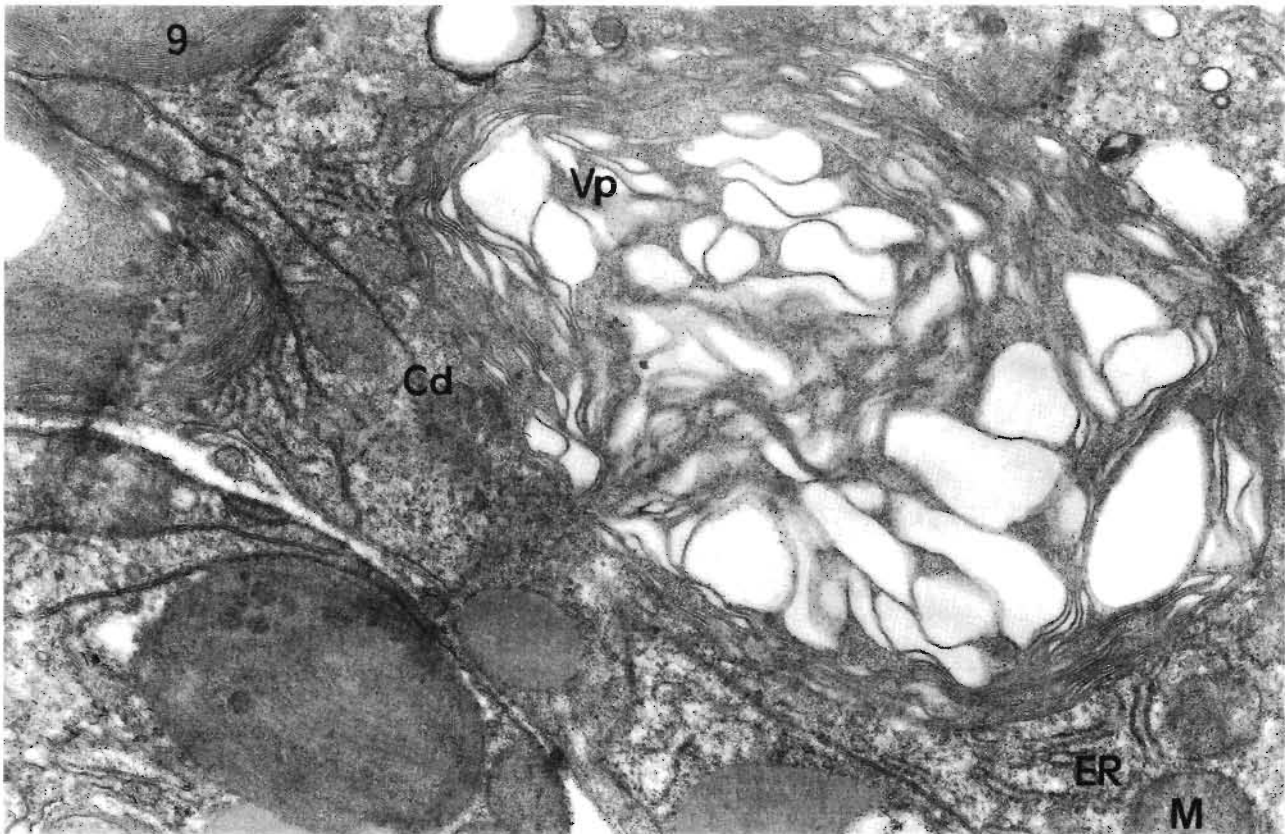


FIG. 9. — Tissues of *Elysia timida* showing early degenerative changes ( $\times 8000$ ). ER, rough endoplasmic reticulum; M, mitochondria; Vp, vesiculated plastid.

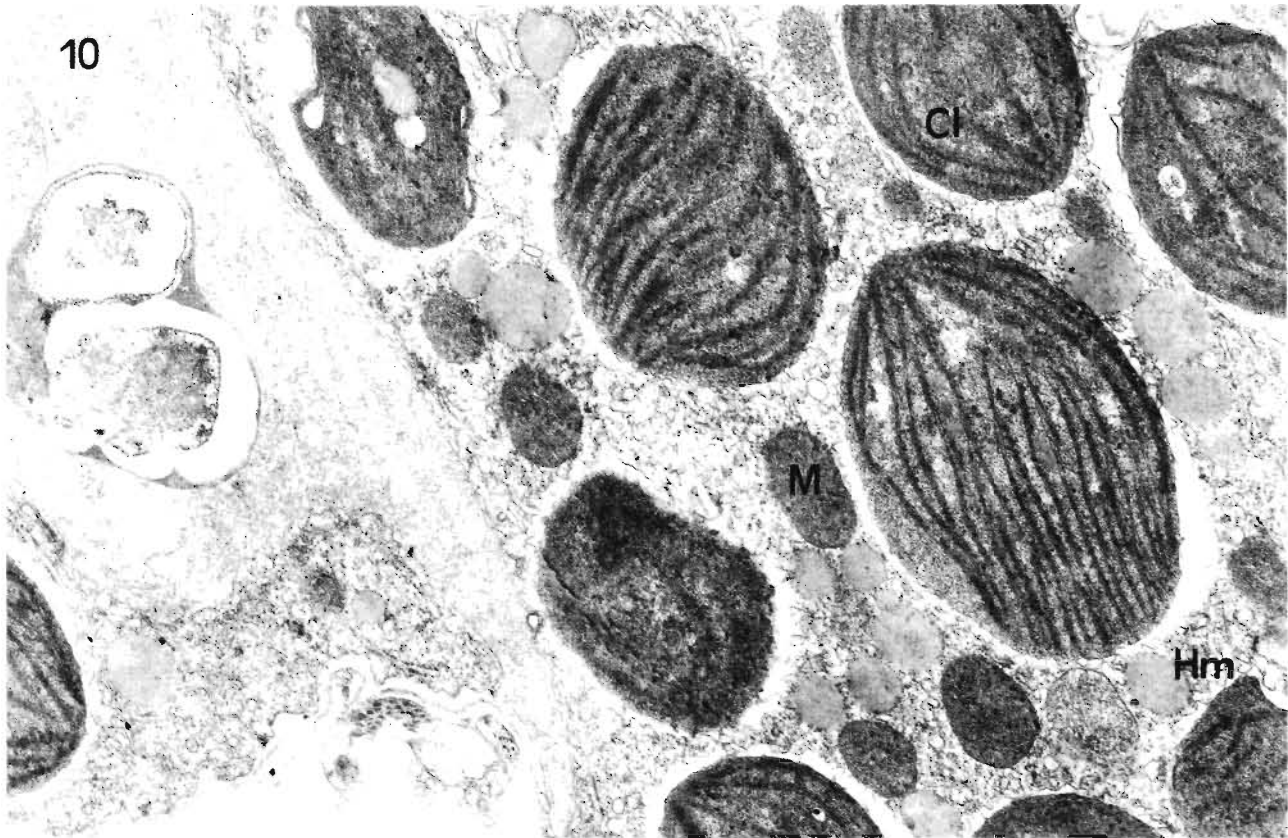


FIG. — 10. Intact chloroplast from digestive gland cell of *Thuridilla hopei* ( $\times 6200$ ). Cl, chloroplast; Hm, host membrane; M, mitochondria.

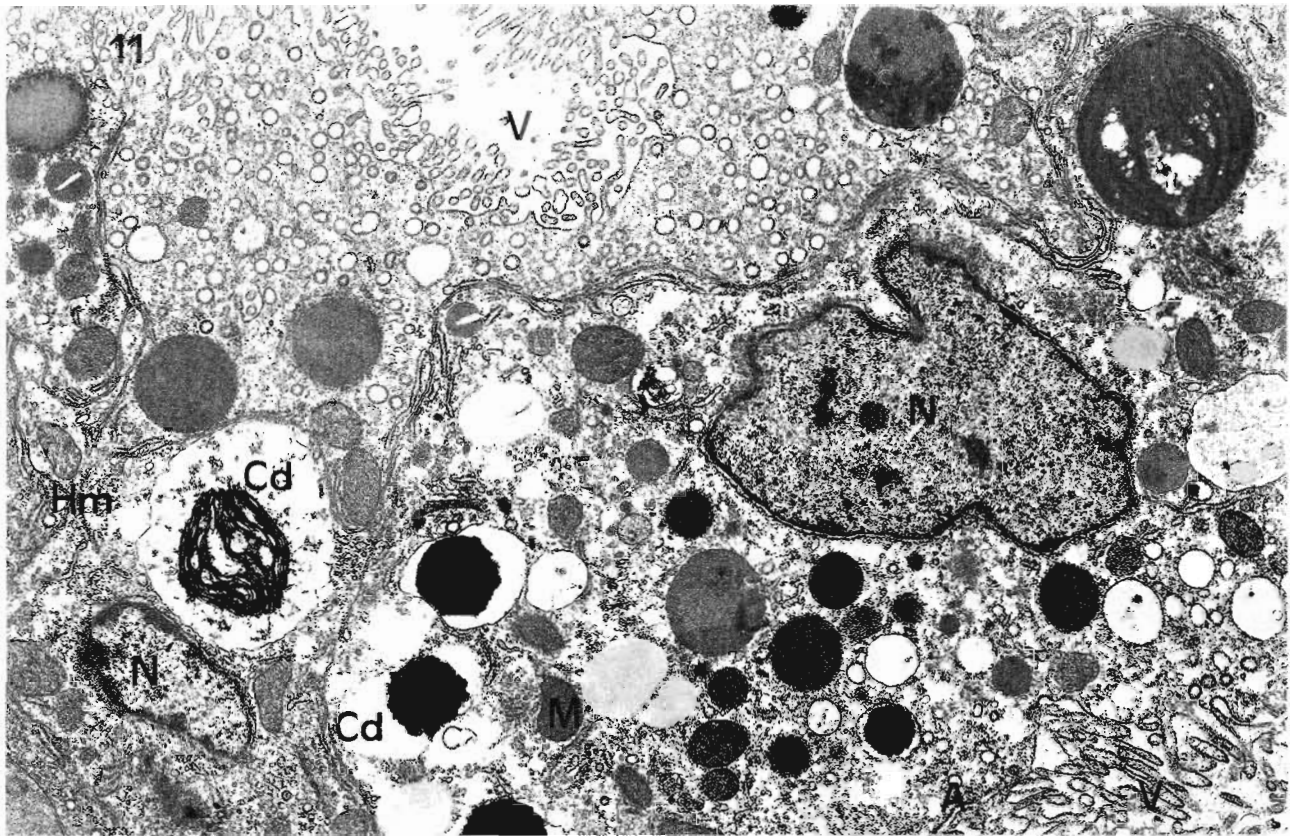


FIG. 11. — Chloroplasts from intestinal epithelium of 4-days starved *Thuridilla hopei* showing degenerative changes ( $\times 2440$ ). A. cilia; Cd, chloroplast showing degenerative changes; G, Golgi complex; Hm, host membrane; L, lipid droplet; M, mitochondria; N, nucleus; V, microvilli.

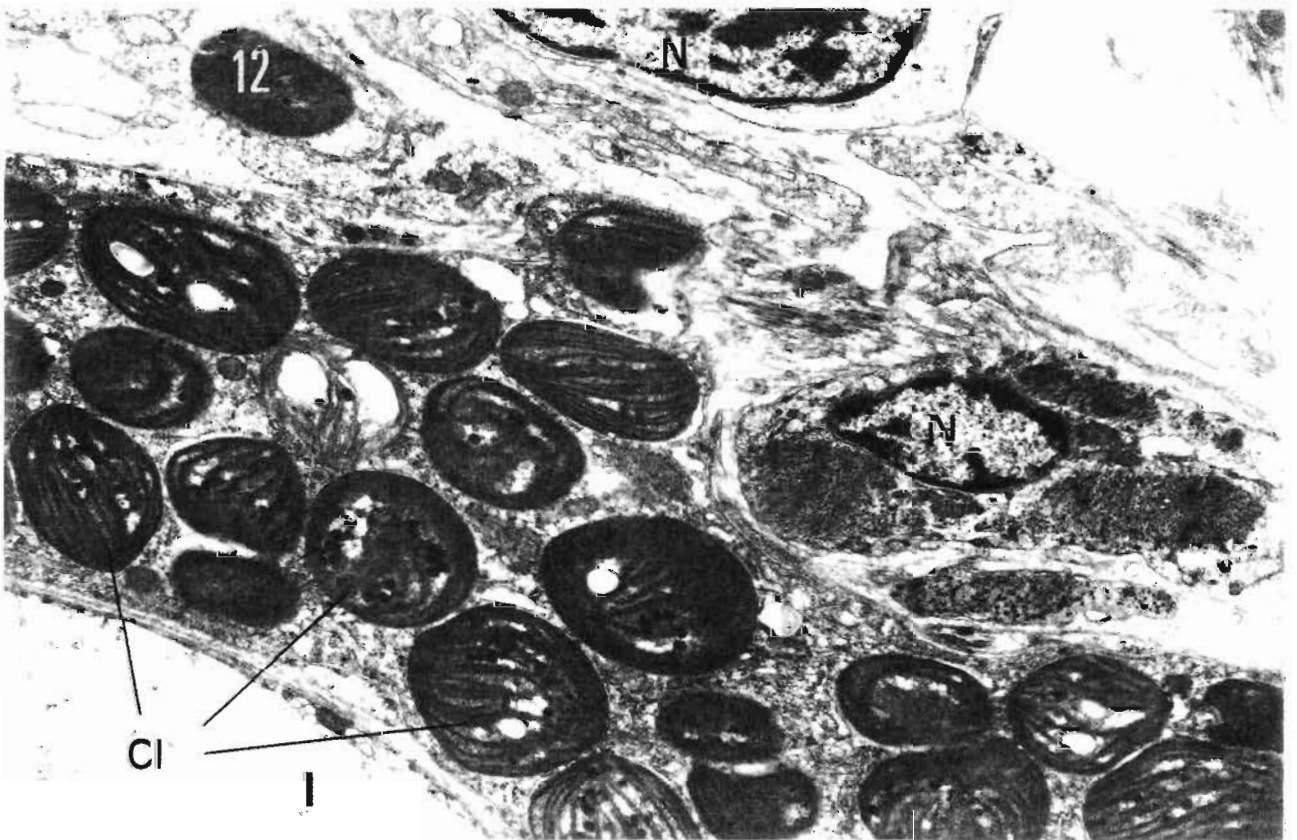


FIG. 12. — Electron micrograph of the digestive gland of *Bosellia mimetica* ( $\times 3160$ ). Cl, chloroplasts; I, intestinal lumen; N, nucleus.



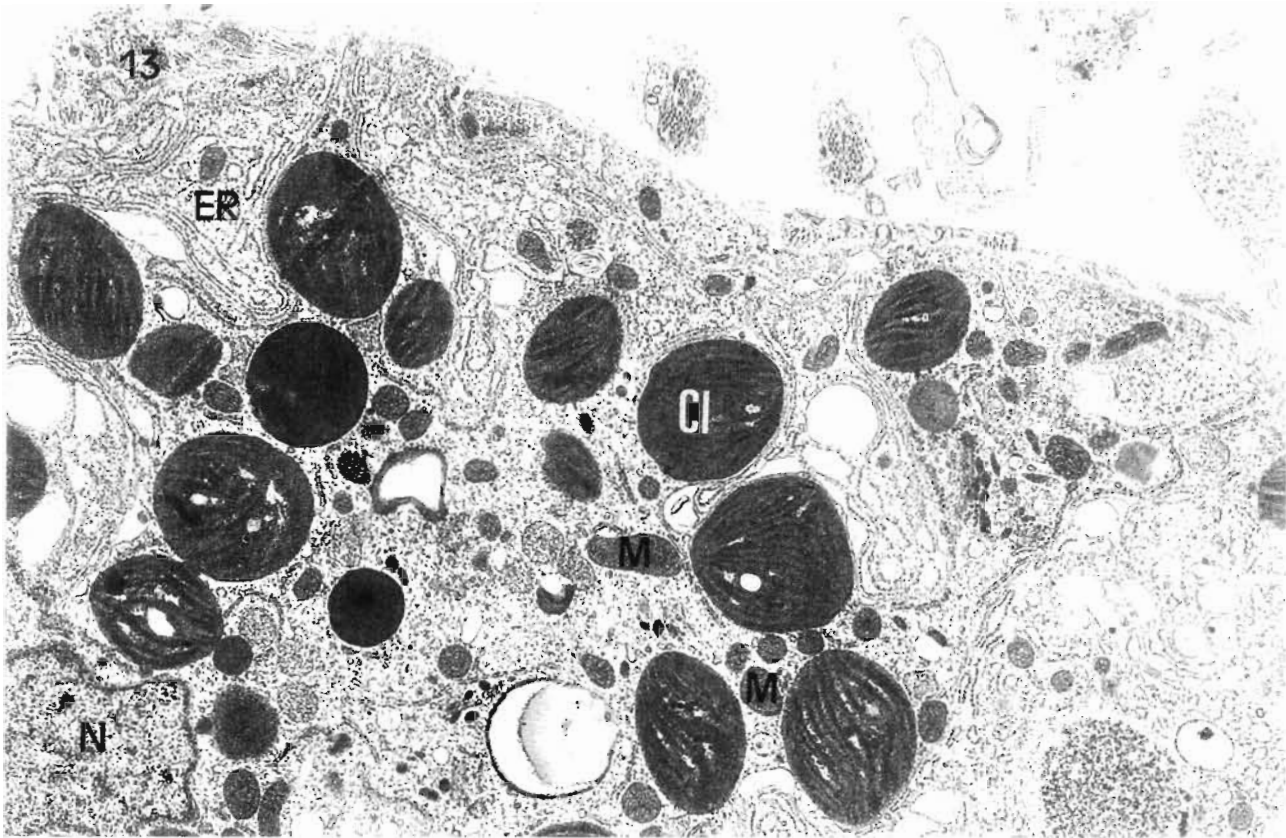


FIG. 13. — Electron micrograph of the digestive gland of *Elysia translucens* ( $\times 1600$ ). Cl, chloroplasts; ER, rough endoplasmic reticulum; M, mitochondria; N, nucleus.

*Bosellia mimetica* (two infra- and circalittoral species feeding on two sciaphilic, long-lived algae present all the year round in their relatively stable habitats: *Udotea petiolata* and *Halimeda tuna*). The carbon fixation data of table I seem to confirm this view, but it has not been possible to follow the decrease in photosynthetic efficiency in *E. translucens* nor *B. mimetica*, because of their scarce numbers, as stated before.

Development type is also related to food availability. When in fall *Acetabularia acetabulum* begins to grow in the Mar Menor bottoms, and the algal stalks are not yet calcified, *Elysia timida* shows lecithotrophic, type 2 development; this allows the colonization by the young slugs of the scattered incipient *A. acetabulum* populations. In winter and spring the development of the sacoglossan is direct (type 3); the *Acetabularia* stalks begin to grow and not going far away from readily edible food is advantageous. Late in spring and in summer the development is again lecithotrophic; *A. acetabulum* stalks have calcified and cap formation begins: now the advantageous strategy is to explore new and distant, not yet calcified, stands of *A. acetabulum* (MARÍN, 1988).

CLARK & DEFRESE (1987) link the energetic benefit of chloroplast retention by Sacoglossans not to food scarcity, but to algal resistance to feeding

(which, from an energetic point of view, is the same thing). They note that "kleptoplastid retention is apparently absent among the Conchoidea, is relatively common among ascoglossans that feed upon high-ash algae (Elysiidae), and uncommon among those feeding upon low-ash algae (e.g. Stiligeridae)". The data on table II, showing the ash-content of the food algae of the four studied species, together with that of some other Mediterranean Sacoglossans retaining chloroplasts (MARÍN & ROS, 1988), are in agreement with this view.

The carbon fixation rate for *Elysia timida* is the highest so far for an elysiid sacoglossan, while those obtained for the remaining molluscs are of the same order of magnitude than those recorded in the literature data (Table I). Former figures for *Elysia timida* (ROS & RODRÍGUEZ, 1985) are in agreement with those shown here.

As was to be expected, temperature and illumination optima for carbon fixation correspond to those in the natural environment of *E. timida*, the well lit and warm waters of the Mar Menor lagoon (ROS, 1987; ROS *et al.*, 1987). The behavior of the molluscs can adjust the optimal exposure to sunlight of symbiotic chloroplasts. RAHAT & MONSELISE (1979) have examined the photoreactive behavior of *E. timida*. In the dark the parapodia are closed, but they

TABLE II. — Ash content and organic matter content of the algal species eaten by the four sacoglossans studied here and by other Iberian cryptogamic slugs (MARÍN & ROS, 1988). Data are original or from the literature (see Reference list); some figures have been estimated by the authors of the references (\*) or by us (\*\*). When there are two figures, they correspond to young and old plants, respectively.

Algal species (eaten by sacoglossan species)	Ash, %	OM, %	Ref.
<i>Griffithsia flosculosa</i> ( <i>Hermatea bifida</i> )	30*	70*	1
<i>Enteromorpha intestinalis</i> ( <i>Limapontia capitata</i> )	39*	61*	1
<i>Chaetomorpha aerea</i> ( <i>Ercolania funerea</i> )	30**	70**	
<i>C. linum</i> ( <i>Calliopaera bellula</i> , <i>E. funerea</i> )	30**	70**	
<i>Cladophora arcta</i> ( <i>Limapontia capitata</i> )	40*	60*	1
<i>C. rupestris</i> ( <i>L. capitata</i> , <i>Elysia gordanae</i> )	40*	60*	1
<i>C. sericea</i> ( <i>L. capitata</i> , <i>Elysia flava</i> )	32*	68*	1
<i>C. vagabunda</i> ( <i>L. capitata</i> , <i>Thuridilla hopei</i> )	31.7*	68.3*	1
<i>Valonia utricularis</i> ( <i>Ercolania coerulea</i> )	64.7	35.3	1
<i>Acetabularia acetabulum</i> ( <i>Elysia timida</i> )	34.5	65.5	2
<i>Bryopsis plumosa</i> ( <i>Placida dendritica</i> , <i>P. viridis</i> )	32.4*	67.6*	1
<i>Codium adhaerens</i> ( <i>Placida verticillata</i> )	55*	45*	1
<i>C. bursa</i> ( <i>Elysia viridis</i> )	54.7	45.3	1
<i>C. fragile</i> ( <i>E. viridis</i> , <i>Placida dendritica</i> , <i>Hermatea paucicirra</i> )	42.0	58.0	7
<i>C. vermicularis</i> ( <i>E. viridis</i> , <i>H. paucicirra</i> , <i>P. dendritica</i> , <i>P. verticillata</i> )	47.1/52.3	47.7/52.9	1
<i>Halimeda simulans</i> ( <i>Bosellia mimetica</i> , <i>Elysia flava</i> )	88.0	12.0	3
<i>H. tuna</i> ( <i>Bosellia mimetica</i> )	55.1/80.2	19.8/44.9	1
<i>Udotea petiolata</i> ( <i>Elysia translucens</i> )	50.0	50.0	1
<i>Caulerpa prolifera</i> ( <i>Ascobulla fragilis</i> , <i>Oxynoe olivacea</i> , <i>Lobiger serradifalci</i> )	14.9	85.1	3

open in light (at  $3 \times 10^3$  to  $3 \times 10^5$  ergs  $\text{cm}^{-2} \text{s}^{-1}$ ). At higher light intensities (up to  $6 \times 10^5$  ergs  $\text{cm}^{-2} \text{s}^{-1}$ ) the parapodia close up again. In strong light (as is the case with the first, well lit meters of the Mar Menor lagoon bottoms) the sacoglossans can be exposed to potentially toxic hyperbaric molecular oxygen. The sea anemone *Anthopleura elegantissima* and the scyphomedusa *Cassiopeia xamachana* maintain superoxide dismutase (SOD) activity in direct proportion to their chlorophyll content, as an enzymatic defense against this oxygen toxicity (DYKENS, 1984). No estimate of SOD levels has been made in *E. timida*. Both in the wild and in the laboratory, however, the *E. timida* from Iberian waters behave according to the presence of algal chloroplasts: the populations both in the sea and in the lagoon are restricted to the shallow, well lit habitats; the yearly life cycle of the sacoglossans is narrowly bound to seasonal (spring) cap initiation and stalk calcification of *Acetabularia acetabulum* (MARÍN, 1988); the animals show positive phototropism: its crawling is rhythmic and intermittent, favoring the water renewal of the mantle cavity, etc. (BOUCHET, 1984; ROS & RODRÍGUEZ, 1985).

The chlorophyll level of the North-american Atlantic species *Elysia tuca* varies with geographic location and seasonally (WAUGH & CLARK, 1986). In the individuals of *Elysia timida* and *Thuridilla hopei*

studied there is no evidence of such a variation, but it must be remembered that the sacoglossans studied come from the same geographical area: the *E. timida* specimens all come from the same lagoon populations, and the *T. hopei* specimens are all of the "green" type (ROS, 1975; THOMPSON, 1981; THOMPSON & SALGHETTI-DRIOLI, 1984; THOMPSON & JAKLIN, 1988) and not of the "blue" variety. In a recent survey by one of the authors (MARÍN, 1988), *E. timida* specimens from a fully Mediterranean population have been studied, and no differences have been found relating to photosynthetic pigment. In the near future this comparison of different populations is to be further expanded; in addition to *E. timida* from lagoon and sea locations, we intend to compare *B. mimetica* from southern, shallower stands of *Halimeda tuna* and from northern, deeper ones.

As for the typification and ecological meaning of the chloroplast association or "symbiosis" in these and other sacoglossan species, the following can be said. The algal species *H. tuna*, *A. acetabulum* and *U. petiolata* are exploited by the sacoglossan species having a longer period of functional chloroplast retention (*B. mimetica*, *E. timida* and *E. translucens*, respectively). These algae have an annual life cycle and they show calcification of the cell walls (*H. tuna* and *A. acetabulum*) or have repellent substances discouraging herbivores (*H. tuna* and *U. petiolata*). These defensive adaptations makes them resistant to sacoglossan exploitation; in short, what the algae attain is a decrease in the biomass turnover rate. These algae are of tropical affinities, that is, they belong to rather stable environments, where symbiosis and other mutualistic relationships are common.

Sacoglossans feeding on these chlorophytes are characterized by a low rate of food uptake, spawn masses with low egg numbers, direct or lecythotrophic development and retention of functional chloroplasts for long periods. The retained chloroplasts can serve as an additional food source and as a food reserve when molluscs are travelling from one individual alga to another. Furthermore, the protection they obtain in being colored like their food (crypsis by homochromy; ROS, 1976, 1977) enhances their adaptive fitness. Thus, chloroplast retention in these molluscs stabilizes and increases the energy flow in the system they exploit. The consortium between calcified Chlorophyceae and those sacoglossan species able to retain chloroplasts for long periods follows what MacArthur termed K strategy (MACARTHUR & WILSON, 1967; ROS, 1981, 1982).

Another group is formed by those algae, generally soft, septated and seasonal, eaten by sacoglossans which maintain functional for only a few hours their chloroplasts (or rhodoplasts), or have lost the ability to retain them. These seasonal algae have a high turnover rate, do not show calcification and are

able to maintain a high sacoglossan population during the few months in which they occur. This is the case of the sacoglossan *Ercolania funerea* and the alga *Chaetomorpha linum* (MARÍN, 1988; MARÍN *et al.*, in press). In this case the sacoglossans have a high rate of food intake, retain plastids during short periods or lose this ability; they lay large numbers of eggs of generally planktonic (types 1 and 2) development. The organelle retention in these r-strategists (sensu MacArthur) can have a different meaning, serving to maintain a high reproductive rate. This is in good accordance with the food algae being characteristic of unstable or unpredictable environments, and with a high and exploitable primary production.

Between these two extremes of chloroplast retention in an r-K continuum of ecological strategies, there can be intermediate situations; this could be the case with *Thuridilla hopei*, having lecithotrophic development but eating cladophorales with a seasonal life cycle (*Cladophora vagabunda*).

Chloroplast retention by sacoglossans is the result of a long, coevolutionary process between the Chlorophycean algae and the molluscs. The existence itself of this association pleads for its adaptive significance. Nevertheless, while the advantages for the molluscs are obvious, there are doubts as to the benefits for their algal counterparts. As has been said, it is not easy to postulate that such benefits exist when one of the consorts in the algal-mollusc association is not a complete organism but only a non reproductive part of it (HINDE, 1983).

We think that from an ecological point of view a clear benefit for the algal partner can also be found. If a fraction of the mollusc metabolic needs are provided for by their captive chloroplasts, this same amount of energy (in the form of photosynthetates translocated from the organelles to the animal) is spared to the algal food population. Thus, if we compare two ideal sacoglossan species feeding on the same alga species, one of them with chloroplast retention and the other without it, the former can build a larger population, or invest more resources in reproduction, than the latter from the same algal food population (if it is an r-strategist); or, alternatively, it can maintain the same population with a lower grazing rate, that is, save energy in food obtained or reduce in some other way their turnover rate (if it is a K-strategist; ROS, in press). (The discussion by MARGALEF, 1974, on the evolution of consortia applies very well here.) The algal population would also benefit more from an herbivore which manages to obtain more food from the same algal diet, than from another not having this ability and, thus, damaging a higher number of individual plants.

If this is the case (and some preliminary studies with *Elysia timida* and *Acetabularia acetabulum* seem

to confirm it; MARÍN, 1988), we see no reason to reject the term "symbiosis" for the relationship studied. The only condition would be to apply it to the consortium mollusc-alga, and not to the association mollusc-organelle.

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