HELGOLÄNDER MEERESUNTERSUCHUNGEN
Helgoländer Meeresunters. 39, 367–374 (1985)

The ultrastructure of the midgut glands in *Ligia italica* (Isopoda) under different nutritional conditions*

J. Štrus[1], P. Burkhardt[2] & V. Storch[2]

[1] *Institute of Biology; Aškerčeva 12, YU-61001 Ljubljana, Yugoslavia*

[2] *Zoologisches Institut I (Morphologie/Ökologie); Im Neuenheimer Feld 230, D-6900 Heidelberg, Federal Republic of Germany*

ABSTRACT: After a period of food deprivation, *Ligia italica* were refed for 2 days with different diets and their midgut glands were examined under the electron microscope with special reference to the large cells. The predominant features are the following: extended glycogen fields after sucrose-diet; numerous lipid droplets and peroxisome-like vesicles after lipid-diet (butter); swollen mitochondria and a great number of pinocytotic vesicles after protein diet (curds); electron dense vesicles and myelin bodies after the uptake of *Escherichia coli*. In contrast to amphipods, the intertidal isopod *L. italica* is not able to digest cellulose, as the cell ultrastructure exhibits all features of starved animals, as well as that following feeding with lignin.

INTRODUCTION

Terrestrial isopods have marked preference for certain types of leaf litter (for review, see Hassall & Rushton, 1984). Such preference, however, may be masked by extraneous factors, such as food deprivation, which in turn induces them to readily accept almost any feed that is offered. It is food quality that causes significant effects on their rates of growth (Debry & Muyango, 1979; Kheirallah & El-Sharkawy, 1981) as well as on the ultrastructure of their midgut glands (Storch, 1984).

Only recently, Carefoot (1984) used an artificial diet to assess the amino acids required for growth in the semi-terrestrial isopod, *Ligia pallasii*. Similar rates or even better growth was obtained for this isopod fed the test diet (nutrient-complete) as compared to that fed on seaweeds. Carefoot's experiments further showed that growth of *L. pallasii* was apparently unaffected by a deficiency of certain amino acids. The presence of microorganisms in their intestinal tract most likely provided them with the lacking amino acids, such that growth was unaffected.

In general, knowledge on the food and feeding habits of semiterrestrial isopods, like *Ligia*, is scant. Members of this genus occur in coastal areas worldwide. They live mostly in rock crevices immediately above the highest high tide and feed on plant material. *Ligia oceanica* (Nicholls, 1931) and *L. pallasii* (Carefoot, 1984), for instance, have been shown to feed mainly on algae. A related species, *L. italica*, was, however, found to be an omnivore (Donadey, 1975). Varying conditions allow these isopods to exhibit cannibalism as well.

* Supported by Deutsche Forschungsgemeinschaft (Sto 75/4–9)

The present work was therefore conducted to find out the effects of different diets on the ultrastructure of the midgut glands in the semi-terrestrial isopod, *L. italica,* and to compare their response with that of true terrestrial isopods.

MATERIAL AND METHODS

The specimens of *Ligia italica* Fab. (Oniscidea, Ligiidae) were collected in the supralittoral zone of the Gulf of Triest in the vicinity of Izola (Slovenia, Yugoslavia) in May 1985. Adult specimens were placed in glass containers and were brought to the laboratory of the Institute of Zoology in Heidelberg. They were kept individually in small plastic containers filled with gypsum and moistened with sea water (18 °C, LD 12:12). Most of the isopods were starved for eight days. The control animals were fed with the brown alga *Fucus virsoides* during the same period. Every day the animals were inspected and the containers were cleaned. After eight days of starvation the isopods were refed with different diets for two days: sucrose (carbohydrate), butter (lipid), curds (protein), filter paper (cellulose), lignin (carbohydrate), *Escherichia coli* (bacteria). The animals were observed several times daily to determine the food intake.

After two days of refeeding, the animals were dissected and the midgut glands were fixed. Several fixatives were tested prior to the main fixation (3.5 % glutaraldehyde in 0.1 M cacodylate buffer in sea water, 3.5 % glutaraldehyde in 0.1 M Sörensen phosphate buffer in distilled water, 3.5 % glutaraldehyde with different concentrations of sucrose in Sörensen phosphate buffer). The midgut glands were cut in 1–2 mm pieces and fixed in 3.5 % glutaraldehyde in 0.1 M Sörensen phosphate buffer (pH = 7.4) for two h, rinsed in buffer, and postfixed in 1 % osmium tetroxide. After dehydration in a series of graded ethanol, the glands were transferred to propylenoxide and embedded in araldite. Ultrathin sections were stained with uranyl acetate and lead citrate and examined under a Zeiss 9–2S electron microscope. The experimental work was carried out in the laboratory of the Institute of Zoology, University of Heidelberg, in May 1985.

RESULTS

Animals fed with *Fucus virsoides* (10 days): The isopods ingested the food (numerous faecal pellets were present in the containers; during the dissection we observed that the gut was full of food). The ultrastructures of the large cells of the animals fed with *Fucus* and of the field animals described by Štrus (in press) are practically identical. The apical cytoplasm contains numerous lipid droplets (Fig. 1a) associated with mitochondria. Abundant Golgi fields and rough endoplasmic reticulum are present around the nucleus. Numerous peroxisome-like vesicles were observed in the cytoplasm. The nucleus is large and ovoid having a condensed chromatin pattern. The basal labyrinth is very prominent and associated with small lipid droplets (Fig. 1b).

Starved animals (10 days): Specimens of *Ligia italica* can survive long periods (two months) of food deprivation (Štrus, in press). The ultrastructure of the large cells in starved *L. italica* is very similar to that described by Storch & Lehnert-Moritz (1980). The amount of lipids is reduced and if small amounts are present, they are located in the basal part of the cell. Mitochondria are less numerous. They are, however, slightly swollen. The rough endoplasmic reticulum is in the form of vesicles and closely packed

whorls (Fig. 1c). Golgi bodies are very rare. The nucleus is irregularly shaped having projections. Electron dense vesicles and lamellar bodies are very prominent.

Animals refed with sucrose (8 days of starvation, 2 days of refeeding): The animals were observed to take the food (the gut was partly filled with it). Abundant

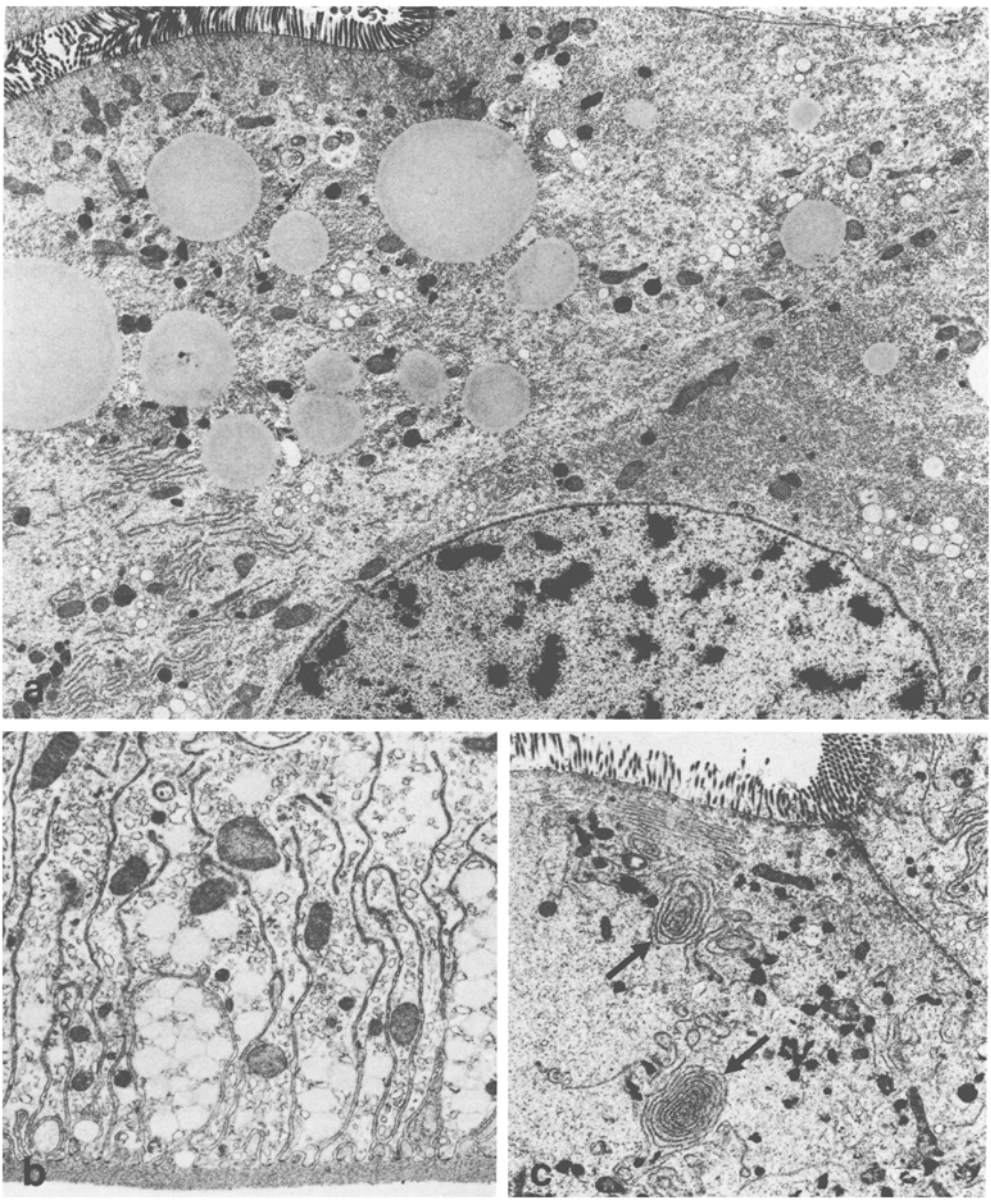

Fig. 1. Large cell (a, b) after 10 days feeding with *Fucus virsoides*. (a) apical cytoplasm (4750 : 1); (b) basal labyrinth with small lipid droplets (11 900 : 1); (c) starvation period 10 days, ER in whorls (arrow) (4750 : 1)

glycogen fields associated with numerous electron-lucent vacuoles are present in the cytoplasm (Fig. 2a). There are only a few small lipid droplets in the apical part of the cell. Mitochondria are slightly swollen. The rough endoplasmic reticulum is mainly in the

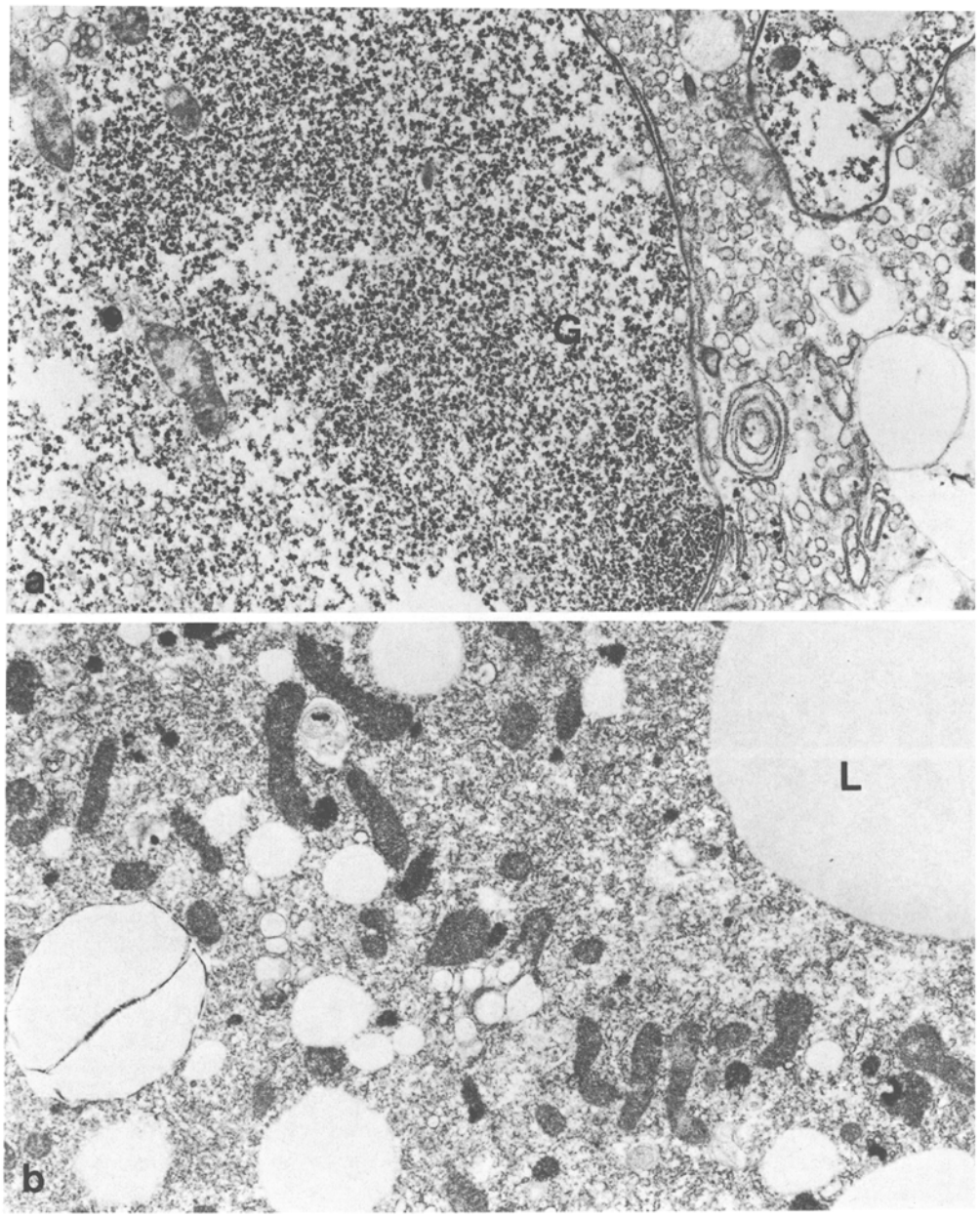

Fig. 2. Large cell after 8 days starvation, 2 days refeeding with (a) sucrose; and (b) lipid diet (butter). G: glycogen L: lipid droplet (11 900 : 1)

form of vesicles; Golgi fields are very frequent. The nucleus has numerous small projections; chromatin is condensed.

Animals refed with butter (8 days of starvation, 2 days of refeeding): Animals ingested the food (faecal pellets were present in the containers). Lipid droplets associated with peroxisome-like vesicles are the most prominent features of the large cells (Fig. 2b). Numerous mitochondria are present. They sometimes assume a ring-shaped form enclosing large vacuoles. Golgi fields are abundant; the rough endoplasmic reticulum is in the form of short cisternae. Pinocytotic vesicles were observed beneath the microvillous border.

Animals refed with curds (8 days of starvation, 2 days of refeeding): Animals were not observed to ingest the food (the gut was empty at the time of dissection). Few lipid droplets are present in the large cells. Mitochondria are light and swollen. A great number of pinocytotic vesicles were detected under the microvillous border. Golgi fields are concentrated around the nucleus. The rough endoplasmic reticulum is in the form of short cisternae and vesicles. Numerous peroxisome-like vesicles are present in the apical cytoplasm. Nuclei with projections were observed.

Animals refed with lignin (8 days of starvation, 2 days of refeeding): The animals were observed to ingest lignin (the gut was full of food). Myelin bodies are very frequent. Lipid droplets are concentrated in the basal part of the cell. Mitochondria are often ring-shaped or elongated with a dark matrix. Peroxisome-like vesicles are present in the cytoplasm. The rough endoplasmic reticulum is in the form of short cisternae. The nucleus has numerous projections (Fig. 3a).

Animals refed with cellulose (8 days of starvation, 2 days of refeeding): The animals were observed to take small pieces of filter paper (the gut was empty at the time of dissection). The ultrastructure of the large cells resembles that in the starved animals.

Animals refed with *Escherichia coli* (8 days of starvation, 2 days of refeeding): The animals were observed to take the food (numerous faecal pellets were present in the containers; the gut was partly filled with food). The ultrastructure of the large cells is very specific. Numerous electron-dense vesicles resembling peroxisomes are concentrated in the apical cytoplasm (Fig. 3b). We observed large amounts of small lipid droplets. Mitochondria are slightly swollen. Abundant Golgi fields are present. The rough endoplasmic reticulum is vesicular or in the form of short cisternae. Myelin bodies are frequent. The light oval nucleus has no projections. The nuclear envelope has dense pore complexes. Nucleoli are very large with prominent granular and fibrillar parts. Pinocytotic vesicles are present under the microvillous border.

DISCUSSION

Ligia italica can survive long periods of food deprivation. Starvation mainly affects the ultrastructure of the large cells in the midgut glands, and as already reported by Storch & Lehnert-Moritz (1980) and Štrus (in press), it results in a reduction of energy-rich compounds, mitochondrial hydration, and the transformation of the rough endoplasmic reticulum into whorls and vesicles. Gas & Noaillac-Depeyre (1976), while studying the intestinal epithelia of fasting carps, suggest that such morphological transformation may be due to changes in the permeability of membranes. On the other hand, Taira et al.

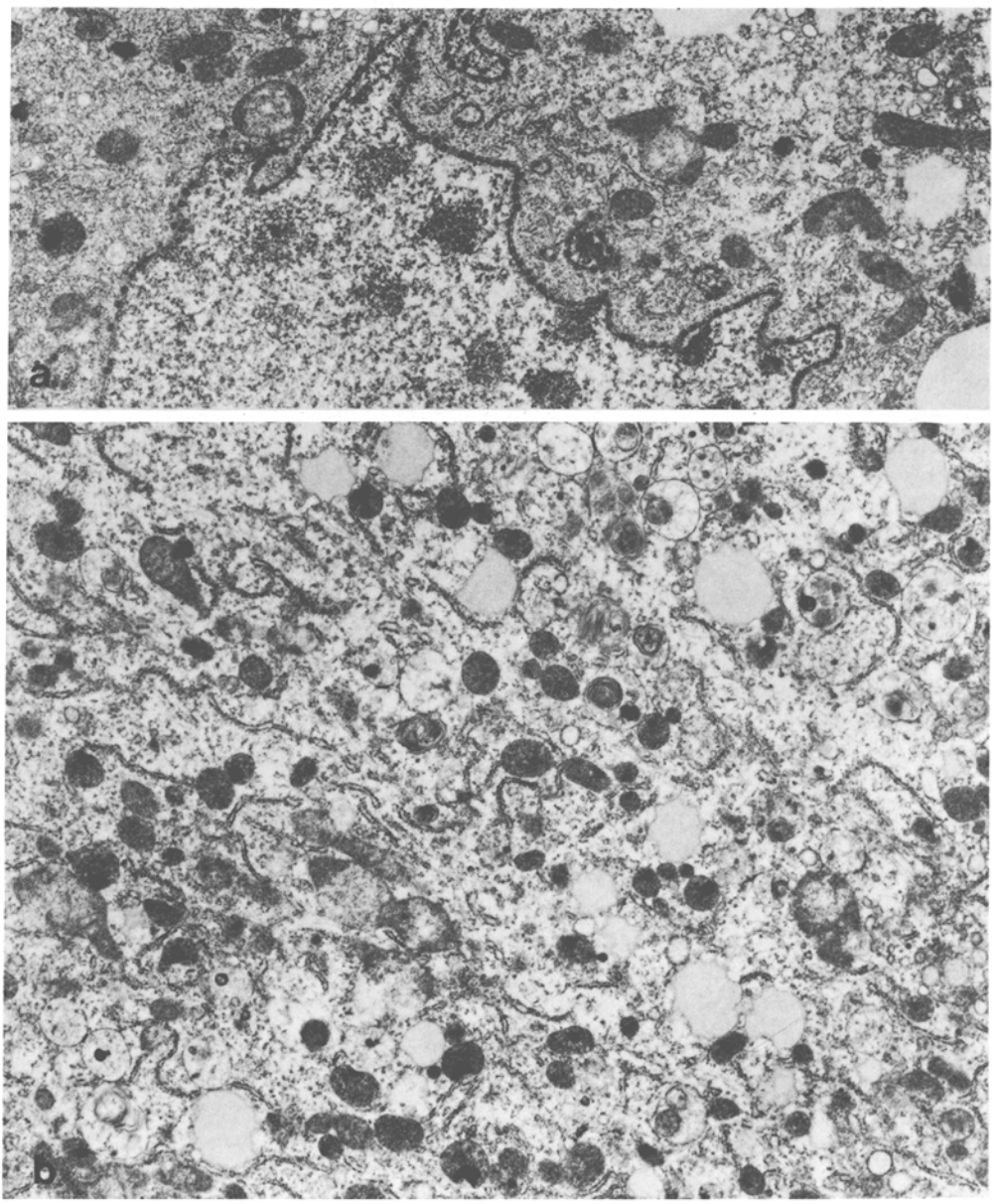

Fig. 3. Large cell after 8 days starvation, 2 days refeeding with (a) lignin, showing nucleus with projections; and (b) *Escherichia coli*, showing peroxisome-like vesicles and myelin bodies (11 900 : 1)

(1981) observed a similar whorled arrangement of the rough ER in the exocrine cells of the pancreas in the starved Japanese newt and African clawed toad, which condition was reversed upon resumption of feeding. Thus, the same investigators implied that the

whorled configuration of the rough ER served as ER reservoir and actually represented the inactive physiological state of that organelle. While both of the foregoing contentions appear to rest on a good basis, there are modest possibilities that such alterations could have also been due to the effects of the fixatives used.

Intracellular lysis of cells in starving animals is demonstrated by the frequent appearance of myelin bodies. It is deduced that these bodies consist of fibrils derived from the ER (Gas & Noaillac-Depeyre, 1976). A longer starvation period results in nuclear pycnosis. As described by Storch (1984), this is characterized by a progressive transformation of the nuclei into irregular forms, with their periphery developing small projections and the chromatin assuming a network pattern. Refeeding previously starved *L. italica* with diets results in either complete or partial regeneration of cell structure similar to that described by Storch (1984) for terrestrial isopods.

Fasted animals accumulate enormous deposits of glycogen in the large cells of their midgut glands when refed with sucrose, although starvation symptoms are still evident. This lends support to Carefoot's (1984) contention that an artificial diet containing a high percentage of carbohydrates (42.84 % dry weight of starch and sugars, and 32.28 % of cellulose) was good. Small amounts of carbohydrates (glycogen) were observed in the midgut glands of newly-caught specimens of *L. italica* (Štrus, in press). Whereas sucrose promotes only partial regeneration of the cells, the specimens fed with cellulose exhibited cell ultrastructures similar to that in starved animals, suggesting that cellulose was not utilized by *L. italica.*

The results obtained for butter and *Fucus virsoides* are similar. Several workers have already shown that lipids play a major role in the metabolism of isopods (Stanier et al., 1968; Steeves, 1963; Vonk, 1960). Following absorption of lipids in a manner described by Komnick et al. (1984), deposition of lipid droplets occurs mainly near the centre of the cell. In dragonfly larvae, ingested fats are processed by luminal lipolysis, the end products of which are delivered across the membranes of the enterocytes by active transport and resynthesized into lipid droplets in the ground plasm.

The importance of proteins in crustacean nutrition has already been discussed by Neiland & Scheer (1953) and Boghen & Castell (1981). Slight regeneration of cell structure was obtained after refeeding *L. italica* with curds. This is consistent with the result obtained for casein-fed *Penaeus monodon* postlarvae suggesting to Vogt et al. (1985) that casein was not well utilized by the young prawns.

The literature provides substantial information on the significance of fungi and bacteria in the nutrition of crustacea (Bärlocher & Kendrick, 1973; Hassall & Rushton, 1984; Wieser, 1979; Coughtrey et al., 1980; Donadey, 1975; Carefoot, 1984). Results obtained in this study showed that the large cells of the midgut glands were rapidly regenerated after feeding *L. italica* with *Escherichia coli.*

In general, this study has revealed that *L. italica* is omnivorous, at least after a period of starvation, and that this species readily accepts several feeds offered. Cellulose, however, is not assimilated by *L. italica,* in contrast to the semi-terrestrial amphipod, *Orchestia cavimana,* whose R-cells exhibit large amounts of glycogen after feeding on cellulose (own observations).

Acknowledgements. Financial support was received in the form of a grant for Jasna Štrus from the Carl Duisberg Gesellschaft.

LITERATURE CITED

Bärlocher, F. & Kendrick, B., 1973. Fungi in the diet of *Gammarus pseudolimnaeus* (Amphipoda). – Oikos *24,* 295–300.

Boghen, A. D. & Castell, J. D., 1981. Nutritional value of different dietary proteins to juvenile lobsters, *Homarus americanus.* – Aquaculture *22,* 343–351.

Carefoot, T. H., 1984. Nutrition and growth of *Ligia pallasii.* – Symp. zool. Soc. Lond. *53,* 455–467.

Coughtrey, P. J., Martin, M. H., Chard, J. & Shales, S. W., 1980. Micro-organisms and metal retention in the woodlouse *Oniscus asellus.* – Soil Biol. Biochem. *12,* 23–27.

Debry, J. M. & Muyango, S., 1979. Effects du cuivre sur le bilan alimentaire de *Oniscus asellus* (Isopoda) avec référence particulière au cuivre contenu dans le lisier de pores. – Pedobiologia *19,* 129–137.

Donadey, C., 1975. Présence de peroxysomes dans les caecums digestifs des Crustacés Isopodes. – J. Microsc. Biol. cell. *24,* 377–382.

Gas, N. & Noaillac-Depeyre, J., 1976. Studies on intestinal epithelium involution during prolonged fasting. – J. Ultrastruct. Res. *56,* 137–151.

Hassall, M. & Rushton, S. P., 1984. Feeding behaviour of terrestrial isopods in relation to plant defences and microbial activity. – Symp. zool. Soc. Lond. *53,* 487–505.

Kheirallah, A. M. & El-Sharkawy, K., 1981. Growth rate and natality of *Porcellio olivieri* (Crustacea: Isopoda) on different foods. – Pedobiologia *22,* 262–267.

Komnick, H., Kukulies, J., Bongers, J., Fischer, W., 1984. Absorption of dietary triacylglycerol by lipolysis and lipid resynthesis in the mesenteron of larval *Aeshna cyanea* (Insecta, Odonata). – Protoplasma *123,* 57–69.

Neiland, K. A. & Scheer, B. T., 1953. The hormonal regulation of metabolism in crustaceans. V. The influence of fasting and sinus gland removal on body composition of *Hemigrapsus nudus.* – Physiologia comp. Oecol. *3,* 321–326.

Nicholls, A. G., 1931. Studies on *Ligia oceanica.* II. The processes of feeding, digestion, and absorption, with a description of the foregut. – J. mar. biol. Ass. U. K. *17,* 675–706.

Stanier, J. E., Woodhouse, M. A. & Griffin, R. L., 1968. The fine structure of the hepatopancreas of *Carcinus maenas* (L.) (Decapoda, Brachyura) – Crustaceana *14,* 56–66.

Steeves, H. R., 1963. The effects of starvation on glycogen and lipid metabolism in the isopod *Lirceus brachyurus* (Harger). – J. exp. Zool. *154,* 21–37.

Storch, V. & Lehnert-Moritz, K., 1980. The effects of starvation on the hepatopancreas of the isopod *Ligia oceanica.* – Zool. Anz. *204,* 137–146.

Storch, V., 1984. The influence of nutritional stress on the ultrastructure of the hepatopancreas of terrestrial isopods. – Symp. zool. Soc. Lond. *53,* 167–184.

Štrus, J., 1985. The effects of starvation on the structure and function of the hepatopancreas in the isopod *Ligia italica.* – III. Colloquium Crust. Dec. Mediterr., Barcelona (in press).

Taira, K., Mutoh, H. & Shibashi, S., 1981. Freeze-fracture study of the whorls of rough endoplasmic reticulum in the exocrine pancreas cells of the Japanese newt and African clawed toad. – Cell Tissue Res. *220,* 669–672.

Vogt, G., Storch, V., Quinitio, E. T. & Pascual, F. P., 1985. Midgut gland as monitor organ for the nutritional value of diets in *Penaeus monodon* (Decapoda). – Aquaculture *48,* 1–12.

Vonk, H. J., 1960. Digestion and metabolism. – In: The physiology of the Crustacea. Ed. by T. H. Waterman. Acad. Press, New York, *1,* 291–311.

Wieser, W., 1979. The flow of copper through a terrestrial food web. In: Copper in the environment. Ed. by J. O. Nriagu. London, *1,* 325–355.