JOURNAL OF CRUSTACEAN BIOLOGY, 15(4): 679–685, 1995

# MIDGUT-GLAND DEVELOPMENT DURING EARLY LIFE-HISTORY STAGES OF THE AMERICAN LOBSTER *HOMARUS AMERICANUS*

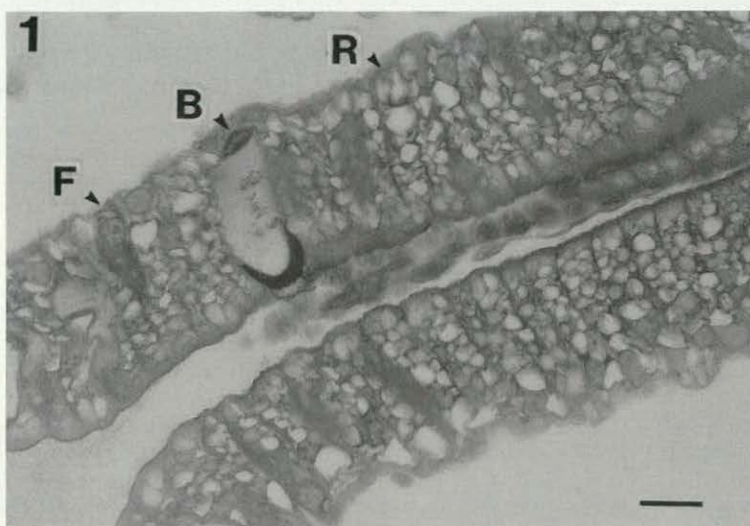
*Patricia M. Biesiot and Judith E. McDowell*

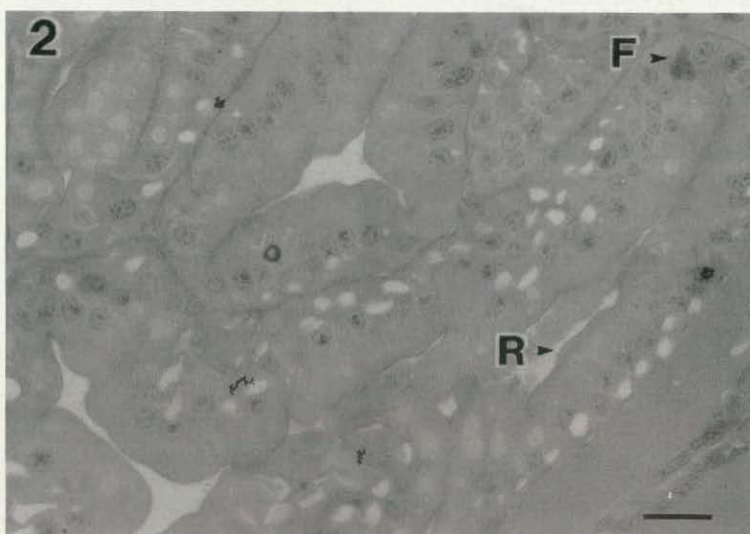
ABSTRACT

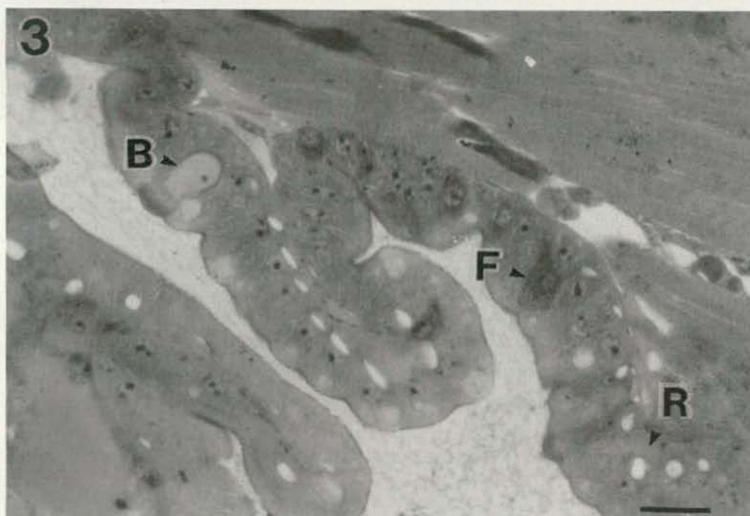
Currently there are at least 2 hypotheses regarding function of the different cell types in the midgut gland of decapod crustaceans. Both agree that E-cells are undifferentiated cells which mature into the other cell types. One hypothesis holds that F-cells synthesize digestive enzymes and subsequently differentiate into B-cells, which, in turn, secrete the enzymes into the midgut-gland lumen. R-cells are believed to function in the absorption of digested nutrients, intracellular digestion, and storage of lipid and glycogen, among other roles. The alternate hypothesis states that F-cells synthesize and secrete digestive enzymes and then take up partially digested material to yield B-cells, which are involved in intracellular digestion. B-cells are later discharged into the lumen; R-cells subsequently absorb nutrients from the gut lumen and store lipid and glycogen. In the present study, the midgut glands of embryos, prelarvae, and stage I larvae of the American lobster *Homarus americanus* were examined histologically. R-cells of all the early developmental stages sampled had only 1 or 2 lipid vacuoles, presumably representing resorbed yolk lipids, in contrast to the numerous lipid vacuoles in R-cells of adult lobsters. E-, R-, and F-cells, but not B-cells, were present in embryos sampled approximately 3 days before their siblings hatched as stage I larvae. B-cells had developed in prelarvae sampled approximately 12 h before their siblings hatched and also occurred in prelarvae sampled approximately 3 h before hatching and in newly hatched stage I larvae. B-cell numbers increased by the time stage I larvae reached intermolt, regardless of whether they were fed or starved. Since embryos, prelarvae, and newly hatched larvae have not ingested exogenous nutrients, the presence of B-cells in these early developmental stages was triggered by something other than uptake of partially digested food and is probably genetically programmed. These results provide indirect evidence supporting 1 hypothesis regarding midgut-gland cell function: that F-cells mature into B-cells before, rather than after, digestion has begun.

The midgut gland (also called the hepatopancreas or digestive gland) of decapod crustaceans is responsible, among other functions, for the synthesis and secretion of digestive enzymes, for resorption of digested nutrients, and for storage of energy reserves (cf. review articles of Gibson and Barker, 1979; Dall and Moriarty, 1983; Icely and Nott, 1992). Although considerable information about various aspects of the midgut gland exists, there is still some discussion about the roles played by the different types of cells that comprise the epithelium of this organ. These cells include E-cells (embryonic), F-cells (fibrillar), B-cells (blister), and R-cells (originally, "the rest" but later, resorptive) (Jacobs, 1928; Hirsch and Jacobs, 1928). An additional cell type, the M-cell (midget), has been described from the shrimp *Penaeus semisulcatus* de Haan (see Al-Mohanna *et al.*, 1985b; Al-Mohanna and Nott, 1987a). M-cells do not, apparently, occur in all decapods (Caceci *et al.*, 1988).

A current paradigm (Gibson and Barker, 1979; Dall and Moriarty, 1983; Icely and Nott, 1992) about midgut-gland cell functions holds that E-cells are produced continuously by mitosis at the distal, closed ends of the tubules that make up the midgut gland. As they grow away from the tubule tips, E-cells differentiate into either R- or F-cells. R-cells are the most abundant cell type and function in the absorption of digested nutrient molecules and in the storage of both lipid and glycogen, among other roles. These cells characteristically contain large numbers of irregularly shaped lipid vacuoles. F-cells synthesize digestive enzymes and store them in supranuclear vacuoles that enlarge by pinocytosis of fluids from the tubule lumen. F-cells appear striated due to an extensive rough endoplasmic reticulum; the cells are basophilic because there are large numbers of ribosomes. B-cells are considered to be mature F-cells; they are the largest midgut-gland cell type and contain a huge vacuole which is formed by


1
R
B
F


2
F
R


3
B
F
R

continued enlargement and coalescence of the F-cell vacuoles. B-cells function to secrete the digestive enzymes produced during the F-cell stage.

An alternative hypothesis (Al-Mohanna *et al.,* 1985a), modified from the one described above and based on work with the shrimp *Penaeus semisulcatus,* states that F-cells have two roles: they synthesize digestive enzymes and later secrete those enzymes during the extracellular phase of digestion. After enzyme secretion, F-cells function in the uptake of partially digested nutrients from the lumen of the gut; they subsequently mature into B-cells, which, in turn, are involved in intracellular digestion, assimilation, and, finally, elimination of undigested materials (Al-Mohanna *et al.,* 1985a; Al-Mohanna and Nott, 1986, 1989). The role of R-cells is to absorb soluble nutrients from the lumen of the gut and to store lipid and glycogen; these reserves are mobilized to sustain the organism during periods of nonfeeding (Al-Mohanna and Nott, 1987b, 1989).

It is the precise relationship between, and function of, F- and B-cells that is at issue. There is consensus that F-cells synthesize digestive enzymes and many, but not all, workers believe that B-cells are mature F-cells. Vogt *et al.* (1985), for example, held the opinion that all cell types in the midgut gland, including B-cells, derive from E-cells. The fundamental difference between the two main hypotheses regarding F- and B-cell function is whether F-cells differentiate into B-cells before (null hypothesis) or after (alternate hypothesis) secretion of the digestive enzymes.

Most of what is known or suspected about the morphology and function of midgut-gland cells has been based on studies and observations using adult decapods. Ontogenetic changes in the digestive tract have been described for larval stages of a few species of decapod crustaceans, including the white shrimp *Penaeus setiferus* (L.) (see Lovett and Felder, 1989), the grass shrimp *Palaemonetes varians* (Leach) (see Le Roux, 1971a, b), the American lobster *Homarus americanus* Milne Edwards (see Herrick, 1911; Williams, 1907; Hinton and Corey, 1979; Factor, 1981), and the stone crab *Menippe mercenaria* (Say) (see Factor, 1982). These studies included cursory mention of morphological changes in the midgut gland and none specifically addressed the different cell types comprising the midgut gland. Sasaki *et al.* (1986) briefly described morphological changes occurring in the midgut gland during both embryogenesis and larval development of *H. americanus.* In this paper we discuss morphological changes occurring in the midgut gland of early developmental stages of the American lobster *H. americanus* that should be considered in the interpretation of F- and B-cell structure and function.

## Materials and Methods

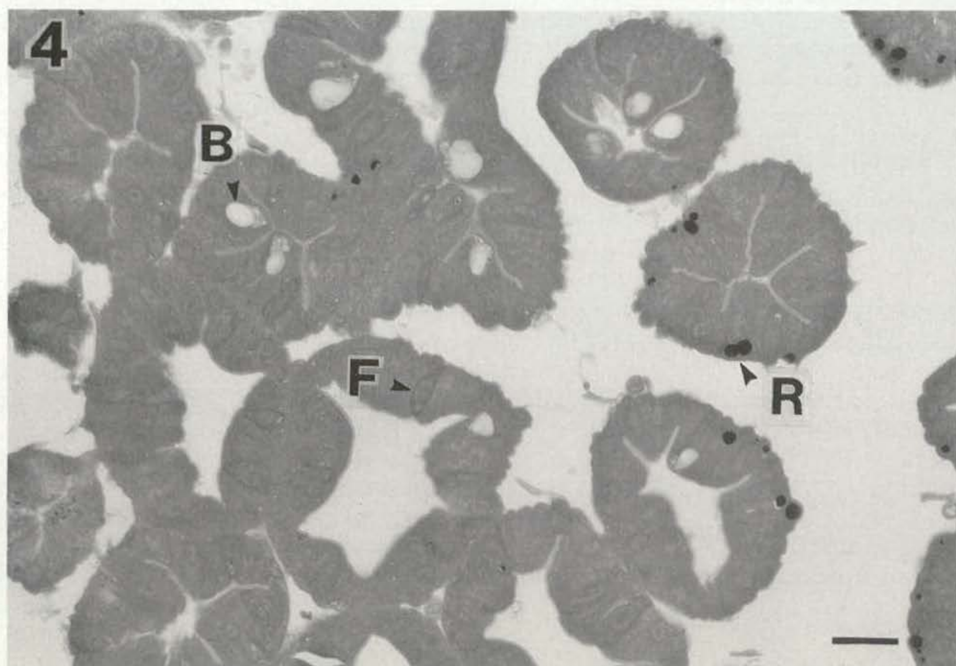
Ovigerous female lobsters, *Homarus americanus,* were obtained offshore from New Bedford, Massachusetts, U.S.A., and were maintained in the laboratory in separate flow-through aquaria at 22°C and ambient salinity (30–31 ppt); the sea water was passed through a 10-μm filter before entering the aquaria to exclude larger plankton and detritus.

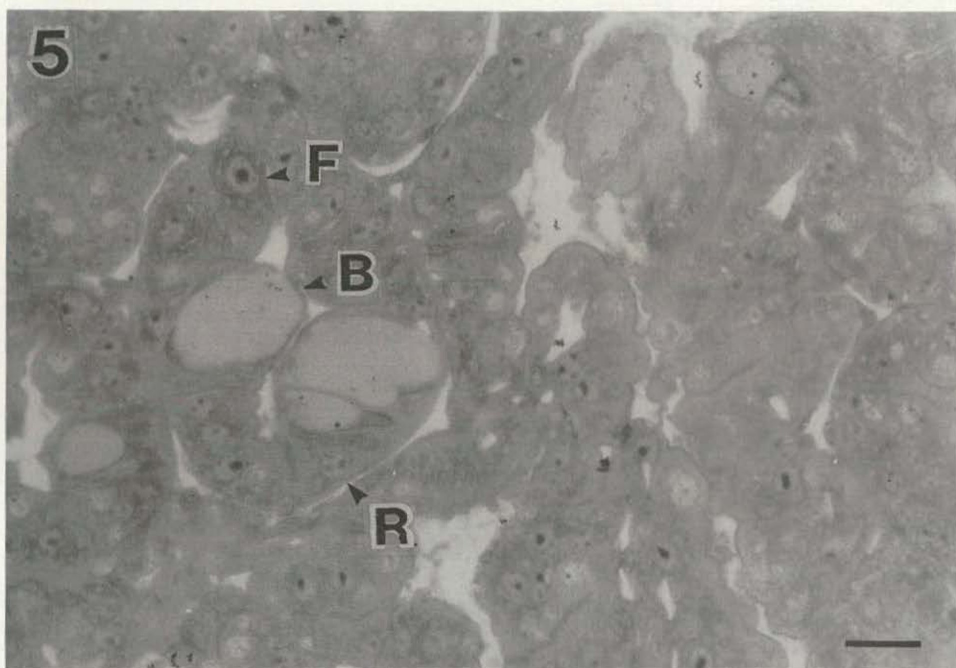
Newly extruded lobster eggs are dark green, but soon become black. The black color persists until about a month prior to hatching when the eggs take on a golden color. A final color change to pale blue signals initiation of the hatching process; the eggs swell slightly and turn blue approximately 1–2 days before free-swimming larvae are released. Near the end of the blue-egg stage, the outer egg envelope ruptures, although the prelarva is retained within an inner egg envelope and can remain attached to the female for hours. The prelarva is incapable of swimming, even if removed from the inner egg envelope, until ecdysis occurs and the free-swimming stage I larva is released. Details regarding egg color and the hatching process in *H. americanus* are given in Davis (1964).

Well-advanced gold eggs, blue eggs, prelarvae, and stage I larvae were examined in the present study. An

←

Figs. 1–3. Cell types comprising the midgut gland of the American lobster. Fig. 1. The adult. Numerous heavily vacuolated R-cells (R), several striated, dark-staining F-cells (F), and one mature B-cell (B) are shown. Scale bar = 5 μm. Fig. 2. The advanced gold-egg stage (sampled three days prior to hatching of sibling stage I larvae). There are primarily R-cells (R) and a few F-cells (F). R-cells differ from those of adult lobsters in having only one or two large lipid vacuoles. F-cells are not very well developed. Scale bar = 5 μm. Fig. 3. The blue-egg stage (sampled approximately 12 h prior to hatching of sibling larvae). Mature B-cells (B) are present, along with R-cells (R) and F-cells (F). Scale bar = 5 μm.




Figs. 4, 5. Cell types comprising the midgut gland of *Homarus americanus*. Fig. 4. The newly hatched (molt stage A) stage I larva. Tissue was postfixed with osmium tetroxide to demonstrate the presence of lipid vacuoles (small round black vacuoles) in the R-cells (R). B-cells (B) and F-cells (F) are also present. Scale bar = 5 $\mu$m. Fig. 5. The intermolt (molt stage C) stage I larva. B-cells (B), F-cells (F), and R-cells (R) are similar to those of earlier developmental stages. Scale bar = 5 $\mu$m.

aliquot of gold-colored embryos was sampled 3 days before siblings in the same brood hatched. Blue eggs were collected at 12 h and again at 3 h prior to hatching of their siblings. Prelarvae are defined as late-stage embryos which are in the process of molting to the stage I larva; they were obtained from blue-colored eggs. All eggs were examined for viability using a binocular dissecting microscope and only those embryos having a strong heart beat were used for this study. Prelarvae were either netted from the bottom of the aquaria, where they sometimes dropped from the egg mass before molting to the stage I larva, or they were removed directly from the egg mass using small forceps. Newly-hatched stage I larvae (molt stage A) were netted from the aquarium.

Larvae from one brood were segregated into individual containers within 15 min of hatching. These larvae were maintained in flowing, 1-$\mu$m filtered sea water for approximately 30 h until the larvae were in intermolt (molt stage C); they were not fed during this time. Other larvae were collected within 1–2 h of hatching and were reared communally in 40-l plankton kreisals (Hughes *et al.,* 1974); these larvae were fed adult brine shrimp, *Artemia* sp., ad libitum until attaining intermolt. Molt-staging was accomplished according to the method of Sasaki (1984).

Whole embryos and prelarvae and the midgut glands dissected from stage I larvae were used. For comparison, we also sampled midgut-gland tissue from an adult female lobster which had been starved for 3 days prior to sacrifice. All tissues were fixed in separate vials with 3% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2, for 1.5–2.0 h at 7°C. The tissues were subsequently washed in cold buffer, dehydrated in ethanol at room temperature, and infiltrated and embedded in plastic embedding medium, either Sorvall or JB-4, according to the manufacturers' specifications. Tissues were sectioned at 2 or 3 $\mu$m on a Sorvall JB-4 microtome with ⅜ inch-wide glass knives and mounted on clean glass microscope slides. The sections were stained with Lee's methylene blue-basic fuchsin (Bennett *et al.,* 1976), which gives results similar to hematoxylin and eosin.

Newly hatched (molt stage A), unfed, stage I lobster larvae were used in experiments to demonstrate the presence of lipid vacuoles in putative R-cells. These larvae were fixed with 3% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, with 0.75 g/ml sucrose for 1–2 h on ice. After fixation, the midgut gland was dissected out, washed in buffer, and stored in the final buffer wash at 7°C for a maximum of 2 days. The tissues were postfixed for 2 h on ice with 1% osmium tetroxide in 0.1 M phosphate buffer, pH 7.4, with 0.75 g/ml sucrose and then washed in cold buffer. The tissues were dehydrated, infiltrated, embedded in plastic, and sectioned as described above. We tried to examine late-stage gold embryos after similar osmium tetroxide treatment, but they were too brittle to section properly; the yolk remnants in and around the midgut gland were more extensive than in the stage I larvae.

## RESULTS

Morphology of the midgut-gland cells of adult American lobsters (Fig. 1) fits the characteristic description for each cell type (cf. Gibson and Barker, 1979; Dall and Moriarty, 1983; Icely and Nott, 1992). B-cells have one huge vacuole; the nucleus is compressed against the proximal cell border and an apical complex comprised of an accumulation of numerous small vacuoles and pinocytotic vesicles is found at the distal end of the cell, next to the midgut-gland lumen. The F-cells are smaller, striated, and darkly stained. R-cells are the most abundant cell type and contain numerous irregularly shaped lipid vacuoles. The terminal web, an area nearly free of intracellular organelles, is located just beneath the brush border of each R-cell. E-cells occur at the distal tips of the midgut-gland tubules (data not shown).

The midgut gland of late gold embryos (sampled three days before free-swimming sibling larvae were released from the brood mass) is composed primarily of R-cells (Fig. 2); E-cells occur at the distal tips of the tubules (data not shown). F-cells, although rare and not very darkly stained, are also present during this stage. R-cells in the embryonic lobster midgut gland have an unusual feature. They lack the numerous, irregular lipid vacuoles seen in adults; sections through these cells show only a few round or oval vacuoles, located at the proximal end of the cell. However, these cells do have an organelle-free terminal web immediately below the brush border and they are the most numerous cell type, both of which are classic characteristics of R-cells (Gibson and Barker, 1979; Dall and Moriarty, 1983; Icely and Nott, 1992). Since F-cells do not have a terminal web, they can be distinguished from R-cells of the gold embryos by that lack.

By the latter part of the blue-egg stage, approximately 12 h before free-swimming stage I larvae were released, B-cells have appeared in the midgut gland (Fig. 3). They are similar in appearance to those of the adult lobster. B-cells are also present in the prelarvae sampled approximately 3 h before hatching (data not shown). F-cells of embryos in the blue-egg stage are more darkly stained than those of the gold-egg stage and thus appear more like those of the adult. R-cells are similar in appearance to those of the gold-egg stage.

Midgut-gland morphology of the newly hatched (molt stage A or postmolt) stage I larva is shown in Fig. 4. This tissue had been

postfixed in osmium tetroxide to confirm the presence of lipid in the R-cells, primarily because the morphology of R-cells from embryos, prelarvae, and stage I larvae is so different from that of adults. Black vacuoles, indicating the formation of a lipid-osmium complex, are present in the proximal end of putative R-cells. Care was taken to collect newly hatched larvae as soon as they hatched and before they could have fed. It is therefore presumed that the lipid present in the R-cells of this, and earlier developmental stages, is derived from resorption of yolk material. F-cells are faintly discernable after osmium treatment and B-cells are classified as such by the presence of the apical complex and the large, unstained vacuole.

Cell types comprising the midgut gland of intermolt (molt stage C) stage I larvae are shown in Fig. 5. The diameter of the midgut-gland lumen appears to have decreased during the period between postmolt and intermolt. No obvious changes occur in F- or R-cell morphology between postmolt and intermolt. The number of B-cells in the midgut gland of the intermolt stage I larva increases, however, compared to those in prelarvae and newly hatched stage I larvae; this increase occurs among both fed and starved stage I larvae (data not shown).

## Discussion

Lobster larvae are planktotrophic and we have observed them feeding as soon as 15–30 min after molting from the prelarva to the free-swimming stage I larva. The presence of F-cells in the midgut gland of embryos in the well-advanced gold-egg stage and of F- and B-cells in the hatching stages (blue eggs and prelarvae) indicates the potential for imminent digestive capabilities. In another study (Biesiot and Capuzzo, 1990), we have shown an increase in total activities of nonspecific protease and amylase, but not lipase, during the hatching process. Very low levels of enzyme activity among late-stage gold eggs and blue eggs increased to higher levels among newly hatched and intermolt stage I lobster larvae, even if the stage I larvae were starved. Having functional digestive capability at the time of hatching, but before feeding has occurred, is an advantage. Larvae that are successful in capturing and ingesting food particles are able to derive nutritional benefit from their first meal.

We believe that the presence of B-cells in the midgut gland of the hatching stages (blue eggs and prelarvae) and the unfed stage I larvae is evidence that B-cells develop their distinctive morphology by increased synthesis of digestive enzymes and not by resorption of partially digested food. Embryos are incapable of ingesting exogenous food and the starved stage I larvae were reared individually in filtered sea water specifically to preclude their feeding on particulate organic matter. B-cell production could not have been triggered by absorption of partially digested food in either of these instances. Thus, at least for the early developmental stages of the American lobster, our results support the null hypothesis regarding B-cell function, that they secrete digestive enzymes, rather than the alternate hypothesis that B-cells resorb partially digested food. B-cell production must be triggered by some other cue and is probably genetically programmed.

The present data may not, however, completely rule out the alternate hypothesis. Little is known about the mechanism of yolk resorption and utilization during crustacean embryogenesis, including the role of the midgut gland. We assume that R-cells absorb some material from the yolk as it is degraded during embryogenesis, because the embryonic R-cells contain one or more lipid vacuoles. It is conceivable that the mechanism of nutrient resorption by cells in the midgut gland could change from resorption by R-cells to resorption by B-cells during the ontogenetic shift from yolk resorption to exogenous feeding. However, since it is not a major change in function to progress from resorbing yolk lipids to resorbing digested exogenous nutrients, it is more likely that R-cells would continue to function in nutrient resorption.

## Acknowledgements

This research was supported in part by the Massachusetts Institute of Technology/Woods Hole Oceanographic Institution Joint Program in Oceanography, a Tai-Ping Foundation Predoctoral Fellowship (to PMB), and the U.S. Department of Commerce, National Oceanic and Atmospheric Administration, Office of Sea Grant (Grant No. NA80-AA-D-00077 to JEM). This is Contribution No. 8904 of the Woods Hole Oceanographic Institution.

## Literature Cited


Al-Mohanna, S. Y., and J. A. Nott. 1986. B-cells and digestion in the hepatopancreas of *Penaeus semisulcatus* (Crustacea: Decapoda).—Journal of the Marine Biological Association of the United Kingdom 66: 403–414.

———, and ———. 1987a. M-"midget" cells and moult cycle in *Penaeus semisulcatus* (Crustacea: Decapoda).—Journal of the Marine Biological Association of the United Kingdom 67: 803–813.

———, and ———. 1987b. R-cells and the digestive cycle in *Penaeus semisulcatus* (Crustacea: Decapoda).—Marine Biology 95: 129–137.

———, and ———. 1989. Functional cytology of the hepatopancreas of *Penaeus semisulcatus* (Crustacea: Decapoda) during the moult cycle.—Marine Biology 101: 535–544.

———, ———, and D. J. W. Lane. 1985a. Mitotic E- and secretory F-cells in the hepatopancreas of the shrimp *Penaeus semisulcatus* (Crustacea: Decapoda).—Journal of the Marine Biological Association of the United Kingdom 65: 901–910.

———, ———, and ———. 1985b. M-'midget' cells in the hepatopancreas of the shrimp, *Penaeus semisulcatus* De Haan, 1844 (Decapoda, Natantia).—Crustaceana 48: 260–268.

Bennett, H. S., A. D. Wyrick, S. W. Lee, and J. H. McNeil, Jr. 1976. Science and art in preparing tissues embedded in plastic for light microscopy, with special reference to glycol methacrylate, glass knives and simple stains.—Stain Technology 51: 71–97.

Biesiot, P. M., and J. M. Capuzzo. 1990. Changes in digestive enzyme activities during early development of the American lobster *Homarus americanus* Milne Edwards.—Journal of Experimental Marine Biology and Ecology 136: 107–122.

Caceci, T., K. F. Neck, D. H. Lewis, and R. F. Sis. 1988. Ultrastructure of the hepatopancreas of the Pacific white shrimp, *Penaeus vannamei* (Crustacea: Decapoda).—Journal of the Marine Biological Association of the United Kingdom 68: 323–337.

Dall, W., and D. J. W. Moriarty. 1983. Functional aspects of nutrition and digestion.—*In:* D. E. Bliss, ed.-in-chief, The biology of Crustacea. Vol. 5. L. H. Mantel, ed., Internal anatomy and physiological regulation. Pp. 215–261. Academic Press, New York, New York. Pp. i–xxvii, 1–471.

Davis, C. C. 1964. A study of the hatching process in aquatic invertebrates. XIII. Events of eclosion in the American lobster, *Homarus americanus* Milne-Edwards (Astacura, Homaridae).—American Midland Naturalist 72: 203–210.

Factor, J. R. 1981. Development and metamorphosis of the digestive system of larval lobsters, *Homarus americanus* (Decapoda: Nephropidae).—Journal of Morphology 169: 225–242.

———. 1982. Development and metamorphosis of the feeding apparatus of the stone crab, *Menippe mercenaria* (Brachyura, Xanthidae).—Journal of Morphology 172: 299–312.

Gibson, R., and P. L. Barker. 1979. The decapod hepatopancreas.—Oceanography and Marine Biology Annual Review 17: 285–346.

Herrick, F. H. 1911. Natural history of the American lobster.—Bulletin of the United States Bureau of Fisheries 29: 149–408.

Hinton, D. J., and S. Corey. 1979. The mouthparts and digestive tract in the larval stages of *Homarus americanus*.—Canadian Journal of Zoology 57: 1413–1423.

Hirsch, G. C., and W. Jacobs. 1928. Der Arbeitsrhythmus der Mitteldamdrüse von *Astacus leptodactylus*. I. Teil: Methodik und Technik der Beweis der Periodezitat.—Zeitschrift für vergleichende Physiologie 8: 102–144.

Hughes, J. T., R. A. Shleser, and G. Tchobanoglous. 1974. A rearing tank for lobster larvae and other aquatic species.—Progressive Fish Culturist 36: 129–132.

Icely, J. D., and J. A. Nott. 1992. Digestion and absorption: digestive system and associated organs.—*In:* F. W. Harrison, treatise ed., Microscopic anatomy of invertebrates. F. W. Harrison and A. G. Humes, ed., vol. 10, Decapod Crustacea. Pp. 147–201. Wiley-Liss, Inc., New York, New York. Pp. i–xiv, 1–459.

Jacobs, W. 1928. Untersuchungen über die Cytologie der Sekretbildung in der Mitteldarmdrüse von *Astacus leptodactylus*.—Zeitschrift für Zellforschung and mikroskopische Anatomie 8: 1–62.

Le Roux, A. A. 1971a. Etude anatomique et fonctionnelle du proventricule des larves et des juveniles du premier stade de *Palaemonetes varians* (Leach) (Décapode, Natantia). 1. Données anatomiques.—Bulletin de la Société Zoologique de France 96: 127–240.

———. 1971b. Etude anatomique et fonctionnelle du proventricule des larves et des juveniles du premier stade de *Palaemonetes varians* (Leach) (Décapode, Natantia). 2. Les phenomènes mécaniques de la digestion.—Bulletin de la Société Zoologique de France 96: 141–149.

Lovett, D. L., and D. L. Felder. 1989. Ontogeny of gut morphology in the white shrimp *Penaeus setiferus* (Decapoda, Penaeidae).—Journal of Morphology 201: 253–272.

Sasaki, G. C. 1984. Biochemical changes associated with embryonic and larval development in the American lobster *Homarus americanus* Milne Edwards.—Ph.D. dissertation, Massachusetts Institute of Technology/Woods Hole Oceanographic Institution, WHOI-84-8. Pp. 1–457.

———, J. M. Capuzzo, and P. Biesiot. 1986. Nutritional and bioenergetic considerations in the development of the American lobster *Homarus americanus*.—Canadian Journal of Fisheries and Aquatic Sciences 43: 2311–2319.

Vogt, G., W. Stocker, V. Storch, and R. Zwilling. 1985. Biosynthesis of *Astacus* protease, a digestive enzyme from crayfish.—Histochemistry 91: 373–381.

Williams, L. W. 1907. The stomach of the lobster and the food of larval lobsters.—Annual Report of the Commissioners of Inland Fisheries: State of Rhode Island and Providence Plantation 37: 153–180.


Received: 27 January 1995.
Accepted: 13 March 1995.

Addresses: (PMB, current address) Department of Biological Sciences, University of Southern Mississippi, Hattiesburg, Mississippi 39406-5018, U.S.A.; (PMB and JEM) Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543, U.S.A.