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Digestive-Gland Histology in Paralarval Squids (Cephalopoda: Loliginidae).

The transition from hatchling to adult in cephalopods does not involve a radical metamorphosis as is found in many other marine invertebrates (Boletzky 1974), but distinctive changes occur early in development (Vecchione 1979, 1981, 1982), similar to those found in fishes. The highest, and perhaps the most variable, rates of prespawning mortality in cephalopods occur during this paralarval development. A recent review of the early life history of cephalopods (Vecchione 1987) presented evidence that starvation, resulting from failure to feed successfully after absorption of the internal yolk, may be a major cause of paralarval mortality. However, other explanations, such as predation or suboptimal environmental conditions, may also explain high paralarval mortality rates. To test among these alternatives, methods must be developed to determine whether paralarval squids are suffering from starvation.

Similar problems exist in ichthyoplankton

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ecology. Histology has been used to determine whether larval fishes are starving at sea (O'Connell 1976, 1980; Theilacker 1978, 1986; Govoni 1980; Eldridge et al. 1981; Kashuba and Matthews 1984). In cephalopods, the digestive gland is uniquely suited to be a target for a histological study of feeding history. The cells of the digestive gland can be categorized along a developmental continuum of immature, synthesizing, mature, and resting cells of a single type (Boucaud-Camou and Yim 1980; Boucaud-Camou and Boucher-Rodoni 1983; Boucher-Rodoni et al. 1987). Boucher-Rodoni et al. (1987) proposed that the developmental condition of the cells of the digestive glands of very young *Sepia officinalis* and other cephalopod paralarvae could be used as an indicator of successful first-feeding.

The digestive-gland cells in the genus *Loligo* undergo a developmental sequence similar to that of other cephalopods (Portmann and Bidder 1928), although Boucher-Rodoni and Boucaud-Camou (in press) have found that the digestive gland in *Loligo* differs substantially from that of other cephalopods. The cells of the loliginid digestive gland are characterized by large apical vacuoles containing lipids and carbohydrates (Bidder 1950, 1966).

We examined the digestive glands of paralarval *Loligo*, both *L. pealei* from field collections and *L. forbesi* that had been hatched and maintained in the laboratory under known nutritional conditions. Specifically, we wanted to see whether the presence of mature digestive-gland cells could be associated with successful first-feeding in the commercially important squid family Loliginidae.

Materials and Methods

Laboratory squids were obtained from an experiment in culturing the eastern Atlantic species *Loligo forbesi* (Hanlon et al. 1985). Because the primary objective of the experiment was to determine methods for successfully culturing squids, the squids could not be sacrificed on an optimum schedule for determination of starvation. Furthermore, because the scope of the feeding experiments was limited, the sample available to us (other investigators were interested in other problems) was quite small.

These squids included hatchlings (<1 d old), some of which had been offered zooplankton as food and some that had been kept without food. Also included were squids >1 wk old. These older squids had survived past the point at which death from starvation normally occurs in unfed squids. Among these older squids were some that had been offered zooplankton as food and some that had been kept without food in a seawater culture medium containing a high concentration (10 mg C per L) of dissolved organic material (DOM). Squids collected in the field were *Loligo pealei* sorted from zooplankton samples from the western North Atlantic. The *L. pealei* were chosen to represent the entire paralarval size range (Vecchione 1981) (Table 1).

TABLE 1.—Paralarval squids examined for digestive-gland histology. DOM = Dissolved Organic Material.

Loligo pealei Dorsal mantle length	*Loligo forbesi* Dorsal mantle length	Age	Treatment
2.0 mm	3.8 mm	<1 day	nonfed
2.0 mm	4.1 mm	<1 day	nonfed
2.6 mm	3.3 mm	<1 day	fed
3.0 mm	3.9 mm	<1 day	fed
3.4 mm	3.4 mm	>1 week	fed
5.1 mm	4.0 mm	>1 week	fed
6.4 mm	4.2 mm	>1 week	DOM
6.9 mm	4.4 mm	>1 week	DOM

The field-collected squids were fixed and preserved in 4% formaldehyde in seawater, whereas those cultured in the laboratory were fixed in Bouin's solution and sectioned shortly after fixation. For all squids, 10 μm horizontal sections were prepared after having been embedded in paraffin. Sections were cleared with oil of wintergreen and stained with hematoxylin and eosin. Observations were standardized by selecting sections that, as much as possible, were ventral to the posterior salivary gland and dorsal to the ink sac, although in some squids these organs overlapped dorsoventrally.

Results

Some mature cells containing conspicuous apical vacuoles were found in all squids. Numbers and sizes of vacoules varied as did the relative volume of the glandular epithelium of the digestive gland.

Hatchlings of *L. forbesi* were all similar in appearance, both nonfed (Fig. 1A) and those that had been offered food and therefore may have fed (Fig. 1B, C). The digestive glands appeared to be robust, with thick glandular epithelium that occupied more volume than the

lumen. Numerous small vacuoles were seen throughout the tissue of both the fed and the nonfed squids. Thus, a large percentage of the cells of squids from both treatments could be considered to be mature. Additionally, a few medium-sized and large vacuoles were found in the nonfed hatchlings (Fig. 1A).

The greatest differences between feeding-treatments were found in the *L. forbesi* that had survived for more than 1 week. The digestive-gland tissue of the squids that had been offered zooplankton, and presumably had fed because of their longevity, consisted of thin walls with many long, thin lamellae that extended into a very large lumen (Fig. 2A, B). The volume of the lumen was much greater than that of the digestive-gland tissue. This tissue was characterized by vacuoles that were few but very large (Fig. 2B). Conversely, the digestive glands of the squids that had been raised on DOM had grown but had retained an overall appearance very similar to that of the hatchlings. Digestive-gland tissue was massive, occupying much more volume than the lumen; furthermore, it was

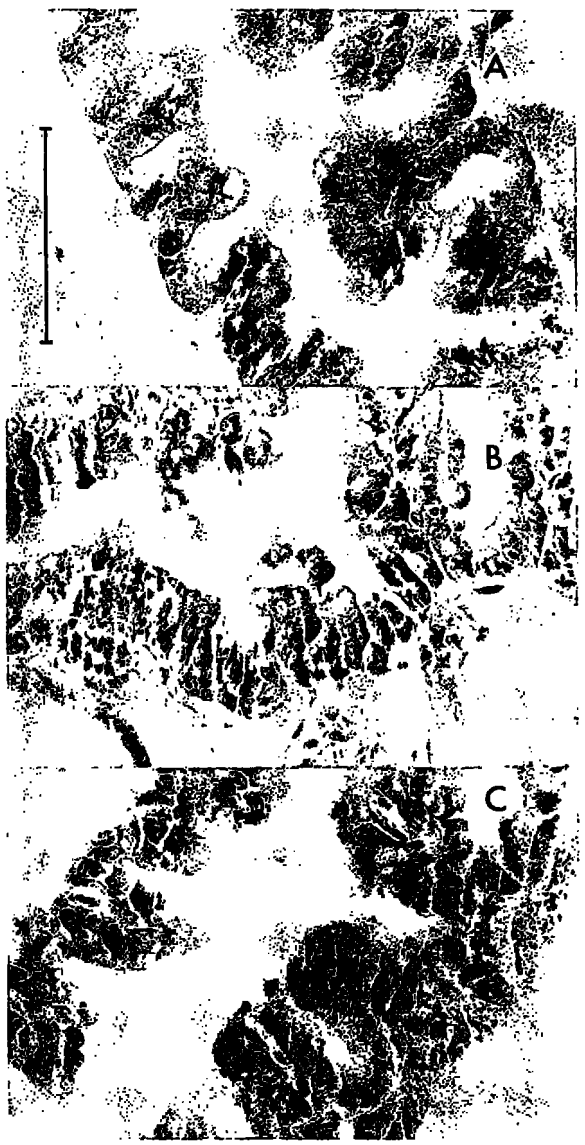

FIGURE 1.—Laboratory-hatched *Loligo forbesi*, < 1 day old: A, 3.8 mm dorsal mantle length (DML), nonfed; B, 3.3 mm DML, from a container with zooplankton food organisms present; C, 3.9 mm DML, from a container with zooplankton food organisms present. Scale bar = 100 μm.

FIGURE 2.—Laboratory-reared *Loligo forbesi*, > 1 week old: A, 3.4 mm dorsal mantle length (DML), fed; B, 4.0 mm DML, fed; C, 4.2 mm DML, from a container with elevated concentrations of dissolved organic matter. Scale bar = 100 μm.

characterized by numerous small to medium-sized vacuoles (Fig. 2C).

Differences between feeding-treatments in the gross morphology of the digestive gland were dramatic (Fig. 3). The digestive glands of squids that had been raised on zooplankton were thin-walled, with large fluid-filled lumina traversed by thin lamellae (Fig. 3A). The digestive glands of squids raised on DOM (Fig. 3B) appeared to be much more robust and well developed, with thick tissue and many small tubules.

Numerous small-to-large vacuoles were found in even the smallest of the field-collected *L. pealei* (Fig. 4A). Digestive-gland tissue was thick, although a large central lumen was present. The internal yolk sac remained in one squid of 2.0 mm dorsal mantle length (DML). In larger *L. pealei* (Fig. 4B, C), vacuoles were numerous and of various sizes but the digestive-gland tissue varied in thickness. In the largest squids examined, tissue growth had filled most of the lumen so that it was characterized by many smaller tubules, similar to those in Figure 4C. These larger paralarvae had few vacuoles but those present were large.

Discussion

Whereas *L. forbesi* has large hatchlings that can be reared in the laboratory (Hanlon et al. 1985), paralarval *L. forbesi* are seldom collected

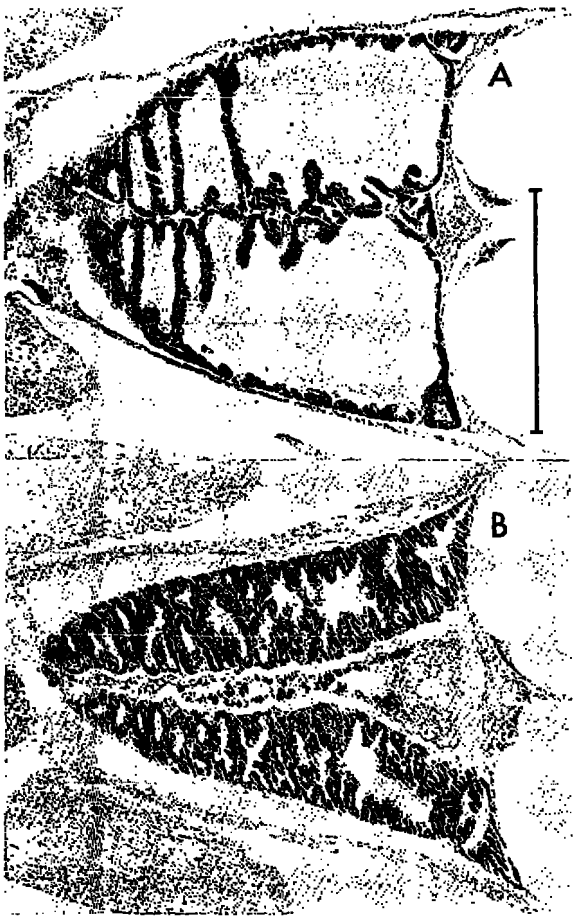

FIGURE 3.—Digestive glands of laboratory-reared *Loligo forbesi*, > 1 week old: A, 4.0 mm DML, from a container with zooplankton food organisms present; B, 4.2 mm DML, from a container with elevated concentrations of dissolved organic matter. Scale bar = 500 μm.

FIGURE 4.—Field-collected *Loligo pealei* paralarvae: A, 2.0 mm dorsal mantle length (DML); B, 3.0 mm DML; C, 3.4 mm DML. Scale bar = 100 μm.

in plankton samples (Holme 1974). *Loligo pealei*, however, is commonly collected in the field (Vecchione 1981), but hatches at a much smaller size and is difficult to rear in the laboratory, suffering at least 99% mortality in the first few days, presumably from starvation (Hanlon et al. 1987). The former species, therefore, was used for laboratory studies and the latter for field observations, even though extrapolation from one species to another must be approached with caution.

The *L. forbesi* hatchlings (< 1 d old) that had not been offered food had unquestionably not fed, but their digestive glands contained many mature cells with conspicuous apical vacuoles. Therefore, the presence or absence of vacuoles is not an adequate indicator of successful first-feeding in this species. Even in *Sepia*, there were indications from tissue-culture experiments that digestive-gland cells may mature slowly in unfed hatchlings (Boucher-Rodoni et al. 1987). Similarly, the large vacuoles cannot be taken as an indication of having fed because the unfed hatchlings had large vacuoles as did the older *L. forbesi* that had been fed and had survived for more than 1 week.

Hatchlings <1 d old that had been offered food may or may not have fed. However, unfed hatchlings typically die, presumably from starvation because the internal yolk sac has been absorbed, within 5 days after hatching. Therefore, the older *L. forbesi* (>1 wk old) that had been offered food probably had fed, although no observations were available to indicate the number of hours or days between their final meal and their time of death and fixation.

Culturing experiments have indicated that elevated concentrations of DOM enhance survival of paralarval *L. forbesi* (P. G. Lee[1]). Thus, the squids from the DOM experiment, while not having fed in the typical sense, had grown and, therefore, were not necessarily starving. The DOM added was a complete diet formulation and would have provided all necessary acids. Approximately 50% of the paralarvae in the DOM experiment survived for 10 days after hatching. Ten of 184 paralarvae from the DOM experiment lived for 12 days, before the experiment was terminated because of an intense bacterial bloom (P. G. Lee[2]). Throughout that period, mortality was higher for paralarvae that had been offered food than for those cultured on elevated DOM.

Digestive-gland structure in the older *L. forbesi* that had been fed was distinctly different from those raised on DOM. Whereas the fed squids had a few very large vacuoles, the DOM squids had very many smaller vacuoles. As noted above, the large vacuoles cannot be taken as an indication of having fed because the unfed hatchlings also had similarly large vacuoles. Furthermore, digestive-gland tissue was very thin on the fed squids compared with those from either the DOM experiment or the field-collected *L. pealei*. It is possible that the fed paralarvae had fed enough to survive beyond yolk absorption but not enough to be completely healthy.

Alternatively, it is possible that the distended lumina and the large vacuoles of the older, fed squids may have been caused by alimentary fluid reaching the digestive gland and entering the cell by phagocytosis for intracellular digestion. If this was the case, the squids raised on DOM simply would have retained immature digestive gland morphology and histology. However, because the field-collected squids, especially the larger ones, were similar in digestive gland structure to those raised on DOM, it seems likely that this is the normal, well-nourished condition. Furthermore, the digestive glands of the field-collected squids and those raised on DOM are both similar in structure to the adult condition (c.f. Boucher-Rodoni and Boucaud-Camou, in press). In the largest field-collected squids, which undoubtedly had passed first feeding successfully, the lumen was largely filled by tissue growth, transforming it into a series of many small tubules. If thin walls of the digestive gland are indicative of poor feeding, none of the field-collected squids showed this indication.

In conclusion, we are not certain which condition (thin tissue with a few large vacuoles or thicker tissue with many smaller vacuoles) is the healthier state. However, we believe that it is likely that the condition of thick tissue with many small vacuoles and reduced lumina, found in the DOM and field-collected squids and similar to the adult condition, represents the healthy, well-nourished condition. The differences in gross morphology between these two conditions are obvious even in cursory examination of sections of the digestive glands of paralarvae. This characteristic may therefore be useful for deter-

[1]P. G. Lee, University of Texas Marine Biomedical Institute, Galveston, TX, pers. commun. 1986.

[2]P. G. Lee, University of Texas Marine Biomedical Institute, Galveston, TX, pers. commun. 1987.

mining the nutritional condition of paralarval loliginids.

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