Embryogenesis and Morphology of Larval Structures in *Chlamys hastata*, with an Examination of the Effect of Temperature on Larval Development and Factors Affecting Settlement and Metamorphosis

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ABSTRACT

The early life history of Chlamys hastata, the spiny scallop, from gamete release through metamorphosis to a benthic juvenile, was observed and described. Specimens at different stages of development were prepared for Scanning Electron Microscopy and histological examination to complement live observations. Study of embryology and ontogeny of larval structures revealed many structures previously undescribed for a pectinid species: 1) on two occasions, newly released oocytes were surrounded by a thick jelly coat, 2) elevation of fertilization envelope was very low and only observable in SEM preparations, 3) gastrulation occurred by both epiboly and invagination, 4) a hinge ligament was present in the larval stage, and 5) proviniculum length remained constant throughout larval development. Secretory cells among velar cilia and their probable function in particle collection were described.

Growth and survival of the larval stage was examined when reared at 12°, 16°, 19°, 24°C. Larvae reared at 19° and 24°C did not reach competency to metamorphose. Larvae reared at 12°C had 42 % survival by the end of the experiment whereas larvae reared at 16°C had 33 % survival. Larvae reared at 12°C reached a maximum valve length of 238.9 ± 0.93 um and were ready to metamorphose 42 days after

fertilization. Larvae reared at 16°C reached a maximum valve length of 231.0 ± 0.84 um and were ready to metamorphose 34 days after fertilization. Throughout development, valve length corresponded to valve height by a linear correlation ($r^2 = 0.87$) with a ratio of 1.1 : 1 for length to height. The observed inverse relationship between valve length and developmental time is probably a result of energy partitioning by larvae whereby larvae reared at higher temperatures require more energy to satisfy metabolic needs and so have less available for growth and accumulation of stores.

It was observed that C. hastata larvae did not metamorphose after a specified period time, but rather they required a stimulus before the process was initiated. Several types of substrates and chemicals, known to cause other invertebrate larvae to metamorphose, were tested for their effect on causing C. hastata larvae to metamorphose. Percent metamorphosis increased when a fouled surface and/or a water flow was introduced. Larvae appeared to settle and metamorphose preferentially along edges and corners of objects rather than on planar surfaces. They did not settle gregariously. C. hastata larvae could survive without metamorphosing for 103 days at 16°C and 130 days at 12°C. Age of larvae tested influenced the rate of settlement metamorphosis with a reduced response observed as maximum age was reached. A positive correlation between length of the precompetent period of larval life and competent period was observed.

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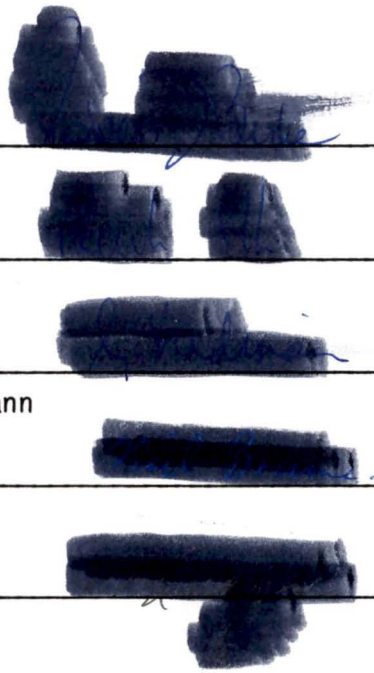

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Chlamys hastata (Sowerby)

INTRODUCTION

Documentation of the early life history of an organism may not have the same prominance in the literature today as it has had in the past. However, knowledge of an organism's life history is a basic requirement before experimental work on factors affecting development can be undertaken. With the advent of aquaculture an ever increasing number of marine invertebrate species are being evaluated for their potential in aquaculture and, although the adult stages may be understood, often little or nothing is known about their reproduction or early life history. But knowledge of early life stages as they apply to commercial culture is not the only facet that needs to be explored; with each study that examines embryogenesis and morphology of larval organs comes new information regarding the embryology of a species which may have broader implications, providing insight into embryology of species at a Class level, or even at a more general taxonomic level.

Ideally, an early life history study should incorporate details about seasonality of spawning, type of fertilization, morphogenesis from fertilization to metamorphosis, factors influencing development, mechanisms of feeding, factors affecting metamorphosis, and morphology of the post-larval stage. I chose to examine the early life

history of Chlamys hastata, the spiny scallop, for two reasons: 1) C. hastata is currently being examined for its potential as a culture species, and 2) no detailed examination of the embryonic and larval development, with emphasis on morphology of larval organs, of any member of the Family Pectinidae, has been published.

Chlamys hastata (Sowerby) is one of thirteen pectinid species endemic to eastern Pacific waters and occurs from the Gulf of Alaska (60°N) to southern California (33°N) at depths between 2 and 150 metres (Bernard, 1983). Adults attain a maximum valve height of 8 cm. and have 8 - 9 radiating ribs with small, arched spines on the left valve and about 9 - 11 pairs of narrow spiny ribs on the right valve (Grau, 1959). They generally occur in aggregates of 3 - 15 individuals / m^2 in areas where gravel and shell predominate (Kozloff, 1983) and in regions with strong current flow or tidal flushing. They rest on the right valve and sometimes are attached to the substrate by a weak byssus. The valves are frequently colonized by one of two species of Porifera, Myxilla incrustans or Mycale adhaerens (Kozloff, 1983).

C. hastata is primarily dioecious, but a low incidence of hermaphroditism occurs (about 1 %) (C. Cooke, unpub.). It is a free-spawning species and has a planktotrophic larval stage (this study). Adults are sexually mature by 2 years and generally only live

as long as 4.5 years (B. MacDonald, pers. comm.). Currently, C. hastata supports a small fishery in British Columbia. Approximately 28 tonnes were landed in 1985 and sold whole or shucked to local markets.

This study was undertaken to elucidate and describe larval development of the spiny scallop, C. hastata, and examine environmental requirements of the larval stages. In particular, the effect of temperature on growth and survival and factors affecting settlement and metamorphosis were examined. Information from this study will contribute toward a better understanding of the life history of this species and may prove useful in management of wild stocks and possible future culture of this species.

CHAPTER 1 - Embryonic and Larval Development of *C. hastata*

INTRODUCTION AND LITERATURE REVIEW

Reproduction in several pectinid species has been examined as a result of an increased interest to manage commercial scallop stocks and to develop techniques for culture operations. Information regarding time of spawning, duration of larval development, and growth rates of juvenile and adult stages for most commercially important species is available in the literature. However, no detailed study is available which describes larval development, from gamete release through to metamorphosis, or examines the morphology of larval organs in a pectinid species.

Most pectinids are dioecious, such as *Chlamys hastata* (this study), *C. gigantea* (Yonge, 1951), *Equichlamys bifrons* (Dix, 1976), *Patinopecten caurinus* (Hennick, 1970), *P. yessoensis* (Yamamoto, 1943), and *Placopecten magellanicus* (Naidu, 1970). Other pectinids are hermaphroditic, with simultaneous hermaphroditism the most common form. In this condition the gonad is divided into male and female parts and each follicle produces either eggs or sperm. *Pecten maximus* (Mason, 1958), *Placopecten meridionalis* (Dix and Sjardin, 1975), *Argopecten gibbus* (Costello, *et al.*, 1973) and *A. irradians* (Sastry, 1963) are

examples of this type of hermaphroditism.

For all pectinids investigated, fertilization is external and oocytes are 60 - 80 um in diameter (Table 3). Typically, eggs are fertilized before the formation of the first polar body, and the time between sperm penetration and the first meiotic division varies between 10 and 50 minutes (Wada, 1968; Longo, 1983; this study).

Embryology

Embryogenesis of a pectinid species has not been recorded in the literature but other pelecypod species, such as Ostrea edulis, have been studied extensively. Wada (1968), Sastry (1979), Andrews (1979), and Verdonk, et al. (1983) provide detailed reviews of embryogenesis in Molluscs. Below is a summary of events which take place during embryonic development in pelecypods.

Under laboratory conditions, embryonic development usually is complete in 2 days. All pelecypods exhibit a holoblastic, spiral mode of cleavage. The first three cleavage stages have polar lobe formation, resulting in a larger 1D blastomere. Gastrulation can occur by invagination (Verdonk and Biggelaar, 1983) or epiboly (Raven, 1958; Verdonk and Biggelaar, 1983). In O. edulis embryos, as with some other

lamellibranch species epiboly is followed by invagination (Raven, 1958; Sastry, 1979; Verdonk and Biggelaar, 1983). The micromeres divide rapidly and grow over the large endomeres followed by invagination of the endomeres to form the blastopore (Galtsoff, 1964; Raven, 1958). The blastopore is very wide initially but later deepens and narrows in most pelecypods, and is reduced to a longitudinal slit. The blastopore finally closes and the archenteron develops as a closed sac. The stomatoblasts lining the periphery of the blastopore invaginate to form the stomadaeum and later the mouth. In O. edulis, some authors contend that the blastopore remains open throughout embryonic development to later become the mouth (Horst, 1883; Fujita, 1934).

Following gastrulation, the blastopore, which is originally located in the center of the vegetal hemisphere, becomes displaced anteriorly and comes to lie on the ventral side. This displacement occurs because of considerable growth of the dorsal region to form the somatic plate. Not only is the blastopore ventrally displaced, but the pretrochal or anterior region is also shifted forward. Thus the main axis of the embryo from animal to vegetal pole becomes bent and the blastopore becomes situated anterior to the prototroch, a band of cilia girdling the embryo. During this time, the stomoblasts, cells lining the periphery of the blastopore, invaginate to form the stomadaeum.

The gastrula then develops into the trochophore larva. This stage is characterized by expansion of the apical ectoderm, differentiation of cilia, and formation of the stomadaeum and lumen of the gut. The prototroch girdles the trochophore and divides it into the upper pretrochal and lower postrochal regions. The pretrochal region bears a single large tuft of cilia, the apical tuft. Ectoderm of the apical region proliferates to form rudiments of the cephalic ganglion. The anterior part of the somatic plate forms the shell gland. The cells proliferate and then invaginate toward the blastocoel, some time after the invagination of the blastopore. The posterior part of the somatic plate extends toward the ventral side to be situated behind the mouth, forming the ventral plate. From the ventral plate develops the foot. In pelecypods, the foot undergoes practically no development during the trochophore stage. The mantle develops during the trochophore stage as an expansion of the ectoderm on the posterior surface of the larva.

About two days after fertilization embryonic development is complete and the planktotrophic larval stage is reached. Initially, larvae are D-stage larvae, characterized by the D-shape of the larval valves, a large velum, and a functional gut.

Not only does there not exist a study on the embryonic development of a pectinid species in the literature, but a detailed

examination of larval morphology in a pectinid species has not been published either. Most studies on pectinid species deal with duration of development, growth rates (usually increase in valve length over time) and size at time of metamorphosis. Only a handful of studies have examined specific organs in pectinid species. Cragg and Nott (1977) examined the ultrastructure of statocysts in P. maximus pediveligers and glands in the foot of P. maximus larvae were examined by Gruffydd, et al. (1975). As well, hinge morphology of Chlamys varia (LePennec, 1980), C. distorta, C. opercularis, and P. maximus (LePennec, 1978, from LePennec, 1980) has been examined. Morphology of larval structures has been examined in other lamellibranch species, such as O. edulis (Waller, 1981; Cranfield, 1973a, 1973b, 1973c; Hickman and Gruffydd, 1971), Crassostrea virginica (Elston, 1980), and Mytilus edulis (Lane and Nott, 1975; Bayne, 1971), and development in pectinids is believed to be similar as described for these species. Moor (1983) and Sastry (1979) provide summaries of available literature on larval morphology. Some of the structures which have been described in detail include the valves, velum, gut, foot, and gill rudiment.

Valves

The larval shell is first secreted by the shell gland in the trochophore stage. Only the cells at the margin of the shell field

secrete periostracum and formation of a hole in the center of the shell field is prevented by the margins being brought close together during the initial phase of secretion (Kniprath, 1979, 1981). With the evagination of the shell gland the shell field spreads over the dorsal epithelium of the larva. The shell field gradually develops into the mantle folds of the bivalve veliger (Waller, 1981) and continues secretion of the periostracum. O. edulis (Cranfield, 1974; Waller, 1981), C. virginica (Elston, 1980), and Venus striatula (Ansell, 1961) larvae have two mantle folds separated by the periostracal groove from which new periostracum emerges. Calcification of the shell occurs at an early stage of development soon after the shell gland evaginates (Erdmann, 1935, from Moor, 1983; LaBarbara, 1974). The calcium carbonate is aragonite (Stenzel, 1964; Carriker and Palmer, 1979) and is formed in a viscous matrix which underlies the periostracum (Cather, 1967).

Two types of larval shell, prodissoconch I and prodissoconch II, were first identified by Jackson (1888, from Moor, 1983). Prodissoconch I is the first larval shelled stage and is at least initially secreted by the shell gland. Prodissoconch I shell of C. virginica (Carriker and Palmer, 1979) and O. edulis (Waller, 1981) bear faint radial striae and have a pattern of shallow punctate depressions. Ansell (1961) also noted, but did not illustrate, small

punctate markings on prodissoconch I shell of V. striatula. The same authors also describe the prodissoconch II shell as generally less smooth than the prodissoconch I shell and bearing strong commarginal radial striae.

The prodissoconch I/II boundary traditionally has been described as the transition from shell secretion by the shell gland to shell secretion of the mantle folds (Wada, 1968). However, Waller (1981) argues that this assumption is based on a change in external sculpture rather than an actual observation of histological or shell ultrastructural changes. He believes that the prodissoconch I/II boundary represents nothing more than onset of valve closure and that change from shell gland to mantle folds is gradual and occurs well before the prodissoconch I/II boundary.

LePennec (1980) described hinge morphology of several larval bivalves, including four species of pectinids, C. opercularis, C. varia, C. distorta, and P. maximus. Among the pectinids described, generally all have three anterior and three posterior denticles symmetrically aranged in oval depressions at each end of a narrow central zone, the cardinal ridge, where the ligament is formed. Species can be distinguished by the changes in thickness of the cardinal ridge and general shape of the valves (LePennec, 1978, from LePennec, 1980).

Velum

The velum of the veliger larva develops from the prototroch of the trochophore. Waller (1981) describes the elaboration of the prototroch to a velum as involving five main events: 1) formation of a double row of cilia from the ring of trochoblasts, 2) elongation and clustering (compounding) of the cilia, 3) formation of the apical pit in the center of the apical disc, 4) enlargement of the tissue in the apical pit to produce an apical organ and eventually a cerebral ganglion, and 5) formation of velar retractor muscles. Shape of the velum is typically described as ovoid with ciliated bands around the outer margin of the velum. Past studies have differed on the number of ciliated bands observed. Erdmann (1935) noted only one ciliated band in newly released O. edulis larvae, but was able to distinguish three bands in O. edulis pediveligers. Prytherch (1934) described a single band of long cilia in C. virginica larvae whereas Galtsoff (1964) described two types of cilia on the velum, large cilia around the margin of the velum and small cilia covering the base of the velum. Bayne (1971) described two ciliated bands in O. edulis larvae, an inner band of long cilia and a peripheral band of shorter cilia. More recent observations have described four ciliated bands in O. edulis (Waller, 1981) and C. virginica (Elston, 1980); two preoral bands, one adoral, and one postoral band.

The inner preoral band (after Waller, 1981) lies immediately adjacent to the apical disc and is composed of 20 um long simple cilia. Elston (1980) did not suggest any function for the inner preoral band, or, as he described, the inner ciliary ring. Waller (1981) suggested that it may serve as an upcurrent tactile receptor.

The outer preoral band is the most conspicuous of the ciliated bands. Preoral cilia of O. edulis (Waller, 1981) are generally very long, 50-70 um, and are compound, arranged in clusters of 20 - 80 cilia. The outer preoral band is believed to provide the major locomotory force of the velum. Waller (1981) suggests that the effective stroke of cilia of the preoral band is downward, away from the apical disc, but slightly oblique to the plane of the velar margin. In O. edulis, each compound cilium is arranged in long orthoplectic and short diaplectic rows. Cilia arranged in long orthoplectic rows can flex more strongly (Knight-Jones, 1954) and thus provide a greater propulsive force than simple cilia. Erdmann (1935) described the outer preoral band as actually two rows or "ciliary clusters". Cells associated with the outer preoral band are packed with elongate mitochondria oriented longitudinally along deep cytoplasmic extensions which are continuous with the compound cilia (Elston, 1980).

The adoral and postoral bands are composed of relatively short

cilia, about 15 - 20 um long (Waller, 1981). Cilia of the postoral band are compound in O. edulis (Erdmann, 1935; Waller, 1981) consisting of a single orthoplectic row of four or five cilia. Elston (1980) described the margin of the velum as bilobed, with the outer preoral and postoral cilia situated at the apex of the upper and lower lobes and the adoral cilia situated in between.

The method by which the velum is believed to capture food particles is called the opposed band system (Strathmann, et al., 1972; Strathmann & Leise, 1979). Using high-speed cinefilms, Strathmann, et al. (1972) observed that cilia of the preoral and postoral bands in bdelloid rotifers beat toward each other. The opposed action of two parallel bands of cilia apparently allows the preoral cilia increased movement relative to the water during the latter part of the effective stroke and push particles toward the adoral cilia. The postoral band functions in retaining particles in the food groove as well as clearance and rejection. The adoral cilia beat toward the mouth region located at the ventral region of the velum. Yonge (1926) noted that food particles collected and carried to the adoral band were embedded in mucus and carried to the mouth. Waller (1981) suggested that the direction of currents generated by adoral cilia run from anterior to posterior on each side of the velum.

The velum is attached to the mantle by velar retractor muscles and a peripheral velar membrane (Elston, 1980). Two pairs of velar retractor muscles have been observed in Venerupis pullastra (Quayle, 1952) and V. striatula (Ansell, 1961) whereas three pairs have been observed in M. edulis (Bayne, 1971), Cardium edule (Creek, 1960), and C. virginica (Elston, 1980). Bayne (1971) described the velar retractors as having localized dorsal insertions on the shell and multiple insertions on the velum. Rather than having insertions on the shell, Elston (1980) observed that the velar retractors in C. virginica were actually inserted on the mantle immediately underlying the shell at the dorsal region.

The central portion of the velum, the velar disc or apical plate, originates from the pretrochal region of the trochophore (Moor, 1983). Hickman and Gruffydd (1971) noted short cilia covering the entire surface of the velar disc in O. edulis. However, Waller (1981) noted a non-ciliated microvillous surface in his examination of O. edulis. The apical organ, or apical pit, is situated centrally in the velar disc and is believed to serve some sensory function (Hickman and Gruffydd, 1971) since it directly overlies the cerebral ganglion. Cells of the apical pit are ciliated (Elston, 1980; Waller, 1981), dark staining (Bayne, 1971), and have been described as having a different cellular arrangement from the rest of the velar tissue (Hickman and Gruffydd, 1971).

Gut

The mouth, located at the ventral aspect of the velum, often has been described as a funnel-shaped structure leading into the esophagus (Creek, 1960; Hickman and Gruffydd, 1971; Elston, 1980). In both M. edulis (Bayne, 1971) and C. virginica (Elston, 1980) the walls of the esophagus are lined with cilia and have a simple cuboidal epithelium. The esophagus runs dorsally into the visceral mass and opens into the ventral region of the stomach. Elston (1980) noted a cellular constriction defining the junction between the esophagus and stomach in C. virginica larvae. He suggested that it serves as a control point for food particles entering the stomach as well as preventing dense mucous secretion and food particles in the stomach from passing to the esophagus.

The stomach lies in the dorsal region of the mantle cavity and connects with the digestive diverticula on either side and the style sac posteriorly. According to Elston (1980), the stomach wall is primarily a pseudostratified columnar ciliated epithelium interspersed with goblet cells. Ansell (1961) described two cell types in the stomach of V. striatula: columnar cells with cilia and cells of the gastric shield. The gastric shield covers the anterior and lateral luminal

aspect of the stomach wall. In thin sections, the gastric shield appears as long, intertwining microvilli embedded in a dense mucinous precipitation (Elston, 1980). The style sac consists of pseudostratified, densely ciliated cells with well-defined vacuoles (Bayne, 1971; Elston, 1980). Bayne (1971) observed that the style of M. edulis larvae rotated in a clockwise direction when viewed anteriorly. The intestine leaves the stomach posteriorly, loops anteriorly around the left side of the stomach and then passes ventrally to the anus located in the posterio-dorsal region adjacent to the posterior adductor muscle (Ansell, 1961; Bayne, 1971; Lane and Nott, 1975; Elston, 1980).

The digestive diverticula is generally described as a bilobed structure (Erdmann, 1935; Hickman and Gruffydd, 1971; Elston, 1980), although Bayne (1971) noted only a single diverticulum in M. edulis. Hickman and Gruffydd (1971) described the digestive diverticula of O. edulis as a mass of highly vacuolated cells with numerous intercellular spaces. Most authors agree that there are two cell types in the digestive diverticula but differ on their descriptions. One cell type described by most authors is the absorptive cell, a large cuboidal cell with large vacuoles. Elston (1980) noted that the vacuoles were suggestive of lipid. He also noted dense granular material surrounding each vacuole that was suggestive of glycogen. Ansell (1961) described

flagellated cells in V. striatula, triangular in shape, non-vacuolated and occurring in strips around the long axis of each tubule. Bayne (1971) noted dark staining cells similar in appearance to the flagellated cells, but did not observe flagellae or cilia on these cells. Elston (1980) observed secretory cells which stained intensely, similar to Bayne's observation. Elston (1980) further noted "undifferentiated cells", a third cell type in the digestive diverticula.

Foot

The foot remains as a small rudiment located between the mouth and anus in the posterio-ventral region of the mantle cavity during the larval stage until close to the pediveliger stage whereupon it develops rapidly (Sastry, 1979; Moor, 1983). In early O. edulis larvae (about 190 um in valve length) the metapodium, or heel of the foot, is first present as a small protuberance from the body wall (Waller, 1981). It is densely ciliated on the medial, ventral surface and bears a pair of ciliated duct openings on the ventral surface. The propodium, or toe, is first present in larvae about 250 um in valve length. Lane and Nott (1975) described the foot of M. edulis as a simple lobe-shape, with cilia at the tip of the lobe, which then underwent rapid development just prior to the pediveliger stage. In most bivalve larvae, the foot

is densely ciliated on the ventral surface (eg. Erdmann, 1935; Prytherch, 1934; Galtsoff, 1964; Lane and Nott, 1975; Gruffydd, et al., 1975; Cranfield, 1973a). A ciliated groove runs from the toe to heel in the middle of the ventral surface of the foot (Bayne, 1971; Cranfield, 1973a). Elston (1980) described the epithelium of the foot of C. virginica as a simple cuboidal epithelium with prominant nuclei. Microvilli have been noted on the cell surfaces of the foot of M. edulis (Lane and Nott, 1975) and O. edulis (Waller, 1981).

Pedal ganglia are situated dorsal to the base of the foot (Lane and Nott, 1975). Cranfield (1973a) noted three pairs of nerves leading from the ganglia in the foot of O. edulis. One pair, the anterio-ventral pedal nerves, runs toward the ciliated groove on the ventral surface of the foot. The second pair, the anterior pedal nerves, leads to the tip of the foot. The third pair, the posterior pedal nerves, runs posteriorly to the byssal duct.

The pedal musculature consists of two pairs of retractor muscles in M. edulis, the posterior and anterior pedal retractors (Bayne, 1971; Lane and Nott, 1975). Cranfield (1973a) noted a third pair, the cruciform retractor muscles, in O. edulis. Elston (1980) described only a single pair of pedal retractors in C. virginica.

Gland structures of the foot have been examined in larvae of O. edulis (Cranfield, 1973a, 1973b), M. edulis (Bayne, 1971; Lane and Nott, 1975), P. maximus (Gruffydd, et al., 1975) and C. virginica (Elston, 1980). Description of the glands varies between species and each author has ascribed different names to the various glands. Gruffydd, et al. (1975) endeavoured to group similar glands described in P. maximus with those described in M. edulis (Lane and Nott, 1975) and O. edulis (Cranfield, 1973a, 1973b). Elston (1980) failed to conform to the nomenclature of Gruffydd, et al. (1975) and developed his own to describe pedal glands in C. virginica. Despite the apparent confusion with nomenclature certain glands appear to be present in all the species described. (This study follows the nomenclature suggested by Gruffydd, et al. (1975)).

The largest and most conspicuous gland in the foot is the primary byssus gland located in the posterior region of the foot. Secretions of this gland contain highly sulphated acid mucopolysaccharides together with protein. Cranfield (1973b) suggested that this gland secreted the observed twin core of primary byssus thread in O. edulis. Ventral to the primary byssus gland is a pair of ciliated lateral pouches (incorrectly described as the byssus gland by Bayne (1971)) which receive the secretory products of the primary byssus gland. Leading from the lateral pouches is a ciliated duct,

called the byssal duct, which opens to the ventral surface of the foot near the heel (Gruffydd, et al., 1975; Lane and Nott, 1975; Cranfield, 1973a). Elston described a single lateral pouch leading to two ciliated ducts in C. virginica. Similarly, Prytherch (1934) noted two byssal ducts in C. virginica.

Other than the primary byssus gland, Gruffydd, et al. (1975) distinguished five groups of glands: the subsidiary byssus gland, secondary byssus gland, pedal mucus gland, tip attachment gland and phenolic glands. The subsidiary byssus gland is present in M. edulis and M. edulis but absent in P. maximus. The secondary byssus gland opens into the lower part of the byssal duct and contributes to the byssus. The pedal mucus gland has openings over the ventral sole of the foot and secretes mucus onto the sole of the foot to facilitate ciliary locomotion. The tip attachment gland consists of a group of cells lying in front of the pedal ganglia with ducts leading along the dorsal surface of the foot and opening at the tip of the foot. This gland was not observed in P. maximus larvae (Gruffydd, et al., 1975). Secretions of this gland probably facilitate temporary attachment of the tip of the foot to the substrate during contraction of the pedal retractors during forward movement (Cranfield, 1973b). The phenolic glands open into a duct located centrally on the ventral surface of the foot, anterior to the byssal duct and probably provide the cement for permanent attachment

in O. edulis (Cranfield, 1973b) and a holdfast between the byssus thread and the substratum in M. edulis (Lane and Nott, 1975) and P. maximus (Gruffydd, et al., 1975).

Gill Rudiment

Rudiments of the adult gill lamellae first develop in the larval stages but are not functional until after metamorphosis. A single gill plate develops on each side at the posterior region of the mantle cavity as an ectodermal ridge of the mantle (Wada, 1968; Waller, 1981). In M. edulis, three gill primordia, or primary gill filaments, develop blocks which then elongate and project into the mantle cavity (Bayne, 1971). Waller (1981) noted that the cells of the gill rudiments in O. edulis have curved microvillous apical surfaces and bear only a few scattered cilia.

A connection between the two gill plates across the mantle cavity has been reported in O. edulis (Erdmann, 1935; Waller, 1980) and C. virginica (Elston, 1980). Waller observed a cross-contact, and possible across-fusion of cilia between the gill plates at the edge of the mantle, followed by a cross-fusion of epithelium in the pediveliger stage.

A pair of larval eyespots develop, one at the base of each first primary gill filament, adjacent to the foot. In O. edulis, the eye structure is evident in early D-stage larvae as a hemispherical, flower-like structure, approximately 6 um in diameter and covered with microvilli (Waller, 1981). Eyespots become pigmented in the pediveliger and are visible using bright field optics in the pediveliger. Bayne (1964) noted that the appearance of eyespots in M. edulis coincides with a change from negative to positive phototactic behaviour.

This chapter examines the embryonic and larval development of C. hastata, describing ontogeny, from gamete release through metamorphosis to the benthic juvenile. Morphology of several key larval organs, valves, velum, gut, foot, and gill rudiment, is emphasized.

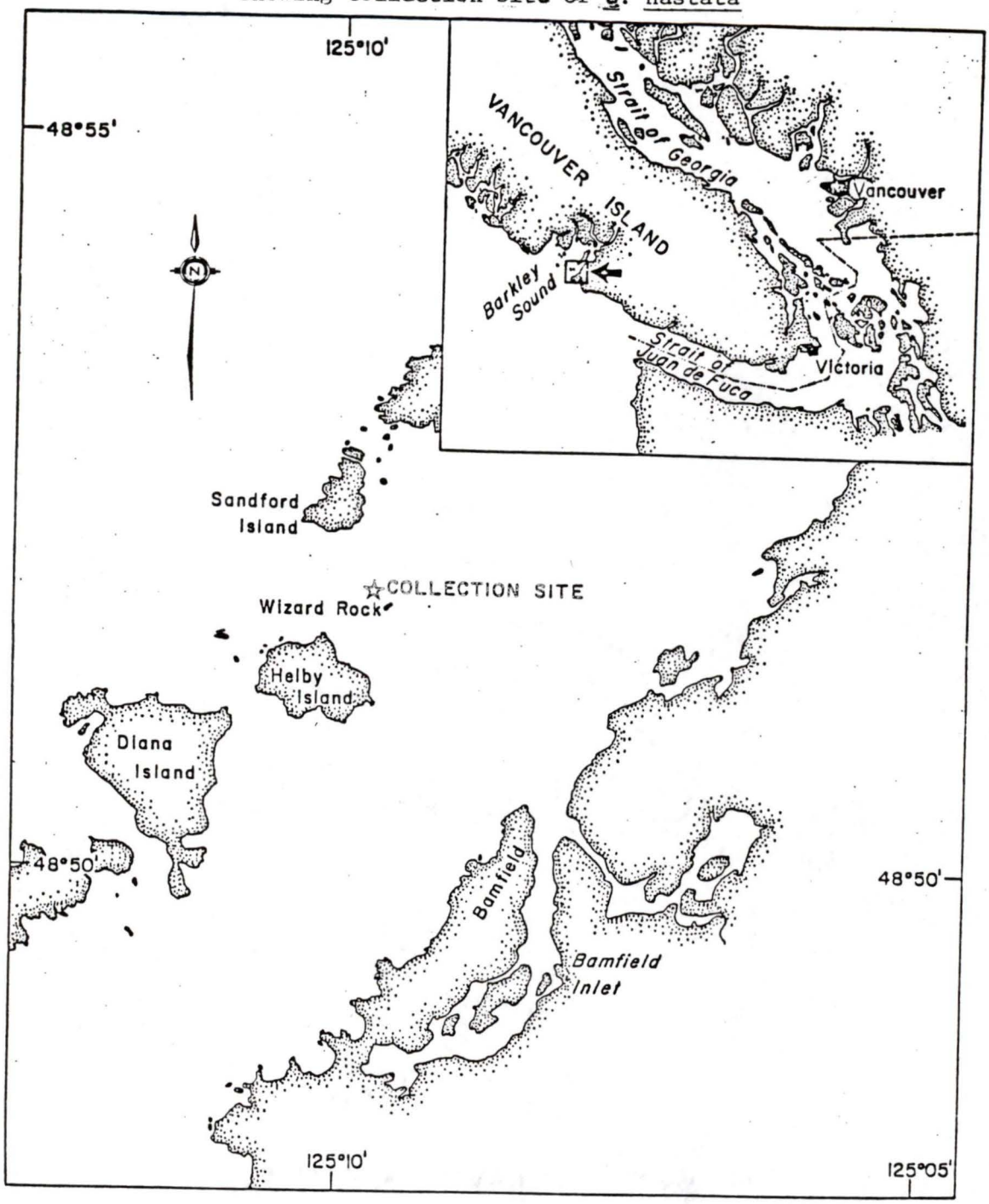
MATERIALS AND METHODS

Experimental work was done at Bamfield Marine Station, Bamfield, British Columbia during the summer and fall of 1982 and 1983. Adult C. hastata were collected by SCUBA near Wizard Rock in Barkley Sound from a depth of 20-30 metres (Figure 1). Animals were kept in a darkened, 20 litre tank for two to five weeks and supplied with a continuous flow of seawater at 14° - 16° C. Three times daily, the water was turned off for one hour and several litres of supplemental phytoplankton (2-3 x 10^6 cells/ml) were added.

Spawning and Larval Rearing

Spawning was induced by removing scallops from the holding tank, removing epifauna from the shells, and separating the sexes. Polyethelene spawning buckets were supplied with a slow, continuous flow of water and an air supply. Seawater, irradiated by ultraviolet light, was dripped into the buckets and the water warmed from 12°C to 16°C and occasionally 18°C over a period of one to two hours. Males generally spawned after 20 to 60 minutes whereas females took up to three hours. When one adult spawned, others in the bucket usually spawned as well.

Figure 1 - Map of Barkley Sound Area in British Columbia . Showing Collection Site of C. hastata



Gametes were collected and immediately washed with 0.8 um glass-filtered seawater. The oocyte suspension was passed through a 253 um nitex screen to remove debris, collected on a 20 um screen and rinsed carefully. The sperm suspension was passed through a 100 um screen and added to the oocytes and left for 6 - 10 minutes. Fertilized oocytes were collected on a 20 um screen, rinsed to remove excess sperm and transferred to a vessel containing 0.45 um membrane-filtered seawater (12°C). Water was decanted and replenished twice during embryonic development. Approximately 50 hours after fertilization, D-stage veliger larvae were transferred to clean 2.0 litre vessels containing 1.5 litres 0.45 um filtered seawater to which 50 mg/l streptomycin sulphate and 2 mg/l chloramphenicol had been added. Larvae were fed unicellular phytoplankton in concentrations of about 10 - 40 x 10^4 cells/ml. Phytoplankton species used in this study included Nannochloris atomus, Pavlova lutheri, Isochrysis galbana, Thalassiosira weissflogii and Dunaliella tertiolecta. Water was changed and larvae were transferred to clean vessels every two to three days. After metamorphosis, young juveniles, or postlarvae, were kept at 13°C in containers with nitex mesh bottoms and a slow (about 200 ml/min.), continuous flow of 1 um glass-filtered seawater was passed through the containers. Two or three times daily, the water was turned off for approximately one hour and phytoplankton added to the containers.

Phytoplankton used in this study was grown after the method of Guillard (1975). Five hundred ml Erlenmyer flasks were filled with 350 m. 0.8 um glass-filtered seawater enriched with 5 ml/l F/2 Guillard's nutrient formula and autoclaved for 20 minutes (6 kg., 123°C). Five to 10 ml of unialgal phytoplankton inoculum, obtained from Canadian Benthic Ltd., was added and the flasks were aerated and maintained at 20° - 23°C under constant illumination. A Clay-Adams Neubauer haemacytometer was used to determine algal cell densities.

The nutritional value of phytoplankton changes with age of the culture (Fogg, 1956, 1959; Guillard, 1975). During early logarithmic growth the phytoplankton is high in protein (Fogg, 1959), but changes to become high in lipid content as it approaches stationary growth. In order to supply food similar in nutritional content, phytoplankton were harvested in mid- or late-phase of logarithmic growth, or when cell densities were 3 - 4 x 10^6 cells/ml for P. lutheri, I. galbana, T. weissflogii, and D. tertiolecta and 10 - 12 x 10^6 cells/ml for N. atomus.

Morphology

Embryos and post-larvae were photographed while actively swimming or crawling, or while contained within a small piece of Nitex mesh placed on a microscope slide. Occasionally, larvae were narcotized with an iso-osmotic solution of MgCl to facilitate observation of larval

structures. Larvae were pipetted onto a depression slide, one-half the water drawn off with a small piece of bibulous paper, and replaced with 15% MgCl. Photomicrographs were taken with Kodak Plus-X film using a Leitz Ortholux compound microscope or a Wild dissecting microscope equipped with a 35 mm camera.

Scanning Electron Microscopy

To compliment live observations, whole larvae and disarticulated valves were examined using a scanning electron microscope (SEM). Structures that were examined included the foot, velum, gill rudiment, valve length, height, length of straight-hinge line, valve shape, and detail of the hinge area.

Valves of larvae and juveniles at different developmental stages were cleaned by placing specimens in distilled water for 30 minutes to remove soft parts (Calloway and Turner, 1978). Any larval tissue still adhering to the valves was removed by immersing the valves in a 6% solution of sodium hypochlorite. Cleaned valves were stored in 70% ethanol until required for SEM (Lutz, et al., 1982). Disarticulation of valves was facilitated by placing valves in a 6% sodium hypochlorite solution and shaking vigorously. Specimens were then rinsed in distilled water and mounted on double-sided sticky tape,

gold-coated, and viewed using a Jeol JSM-35 scanning electron microscope. Prior to photographing, each specimen was positioned so that four points along the shell margin, each at 90° intervals, were in exactly the same plane of focus to ensure accurate measurements (after Lutz, et al., 1982).

Larvae and postlarvae at different stages were relaxed using 1 : 1 15% solution of MgCl and seawater before fixation. Specimens were initially fixed in 2.5% gluteraldehyde, 0.2M Millonig's phosphate buffer (Millonig, 1961) and 0.14M NaCl (Cloney and Flory, 1968) for one hour at room temperature. Following primary fixation, specimens were rinsed in 0.2M Millonig's phosphate buffer containing 0.34M NaCl, post-fixed for one hour at 7°C in 2% OsO_4 and 0.2 M Millonig's phosphate buffer, and rinsed for 10 minutes twice in distilled water. Specimens were then dehydrated in a graded series of ethanol (30, 50, 70, and twice in 95%) for 10 minutes at each step. Finally, they were rinsed three times in 100% ethanol. Specimens were dried to critical point using CO_2 as a transitional fluid and then mounted on stubs and treated in the same manner as the disarticulated valves.

Histological Sections

Specimens were relaxed and fixed in the same manner as those

prepared for SEM. Following secondary fixation the specimens were decalcified in a 1 : 1 solution of 0.4% ascorbic acid and 0.34M NaCl for 24 - 48 hours (modified from Dietrich and Fontaine, 1975), rinsed in distilled water, dehydrated in a graded series of ethanol, and embedded in Epon (Luft, 1961).

Serial sections were cut with glass knives using a Sorval MT5000 ultramicrotome, mounted, and stained with 1% azure II and 1% methylene blue in 1% sodium borax solution (Richardson, et al., 1960).

RESULTS

Embryology

Oocytes of C. hastata are slightly compressed and irregular in shape upon release, but become round a few minutes after contact with water. Rounded oocytes are about 70 um in diameter. On two occasions, a 65 um thick, translucent jelly coat surrounded each oocyte, making the total diameter about 200 um (Figure 2a). The jelly coat was not evident using Koehler illumination but could be seen with phase contrast optics. Oocytes from most spawnings did not have a jelly coat (Figure 2b), and fertilization success and subsequent development of these gametes did not appear to be affected. Germinal vesicles were not distinctly visible in unfertilized oocytes but generally a less dense region could be noticed in most oocytes.

Sperm of C. hastata remain active for at least 2 hours after release. Head and midpiece of sperm is about 4 um long and tail is 40 um long (Figure 2c). Generally four mitochondrial bodies are present in the mid-piece. Height of the mid-piece is 0.75 um. The sperm head is arrowhead-shaped and is 1.25 um wide at the base and tapers to 1 um wide at the apex. The acrosome is triangular in shape and is 1.25 um long.

Figure 2 - Fertilization and Early Cleavage Stages of C. hastata Embryos

a) Primary oocytes with jelly coats (jc). Light micrograph (LM), phase contrast optics. Scale bar = 100 um.

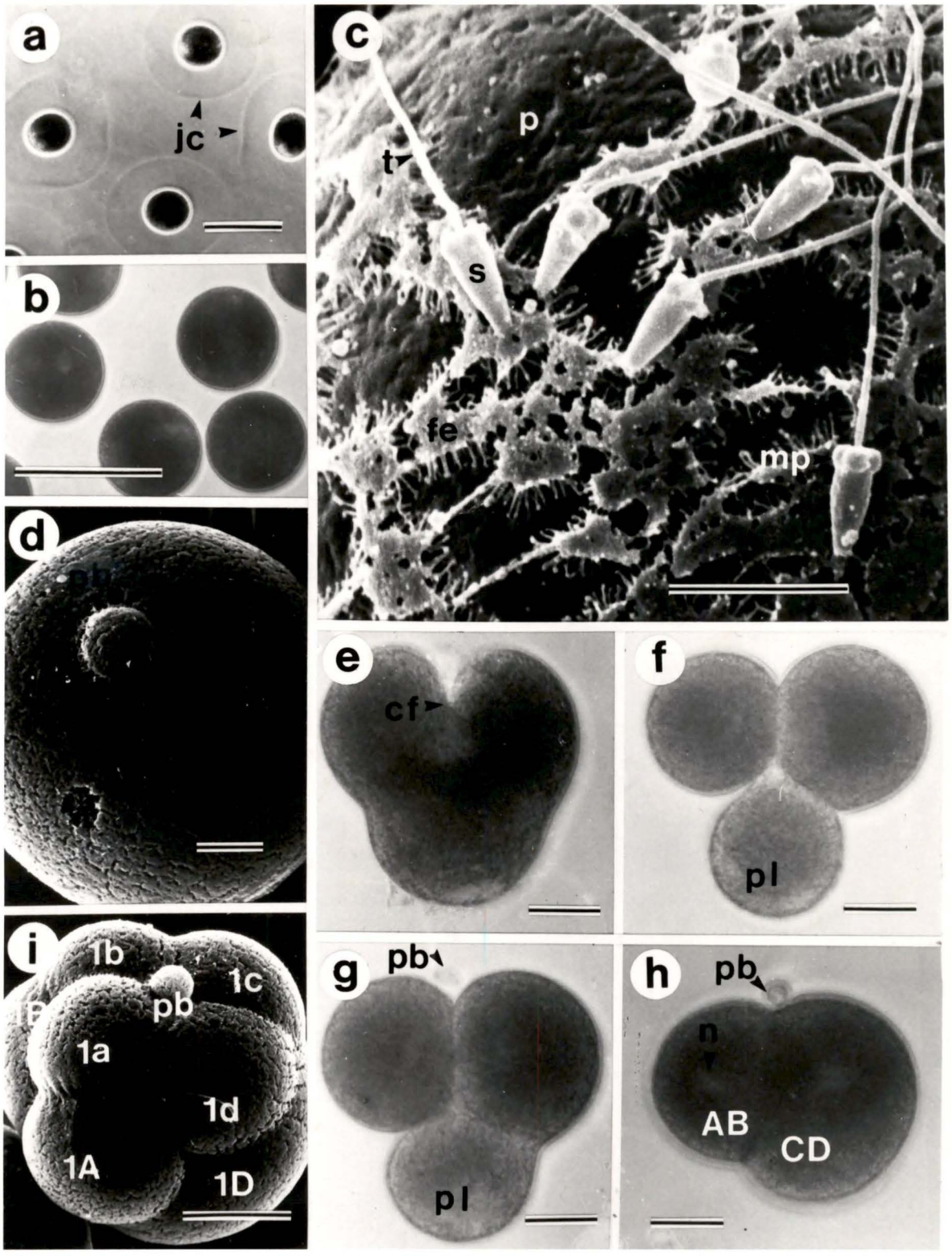
b) Primary oocytes lacking jelly coats. LM, bright field optics. Scale bar = 100 um.

c) Sperm cells (s) on surface of oocyte at time of fertilization. Scanning electron micrograph (SEM). mp, midpiece; p, plasmalemma; t, tail; fe, fertilization envelope. Scale bar = 5 um.

d) First polar body (pb) release. SEM. Scale bar = 10 um.

e) - h) First cleavage with polar lobe formation. Polar lobe (pl) forms at vegetal pole as cleavage furrow (cf) develops at animal pole. Polar lobe fuses with CD blastomere as cleavage becomes complete. LM, bright field optics. Scale bar = 20 um.

i) Third cleavage stage. Blastomeres labelled using nomenclature for spirally cleaving embryos. SEM. pb, polar body. Scale bar = 20 um.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Elevation of the fertilization envelope at the time of fertilization was not visible using light microscopy. Fertilization was recognized by germinal vesicle breakdown in oocytes and this was determined as 0 hours after fertilization (Table 1). Using the scanning electron microscope the fertilization envelope, vitelline space and underlying plasma membrane are apparent (Figure 2c). The fertilization envelope appears as a thin sheet supported from the surface of the plasma membrane by cytoplasmic extensions. The delicate nature of the fertilization envelope is evidenced by the disruption of the structure caused by shrinkage and handling during preparation. The vitelline space is 0.8 - 1.0 um thick. The underlying plasma membrane appears shiny smooth with pitted contours.

Polar body release begins about 20 minutes after fertilization (12°C) (Figure 2d). First cleavage is meridional, unequal and is initiated 3 hours after fertilization (Figure 2e - 2h). A deep cleavage furrow begins at the animal pole, the site of polar body release, and a large polar lobe forms at the vegetal pole (Figure 2e). The polar lobe fuses with the CD blastomere before first cleavage is complete (Figures 2f-2h). Second cleavage is also meridional, perpendicular to the first cleavage plane and is complete at 3.75 hours. A, B, and C blastomeres are of equivalent size and the D blastomere is slightly larger, indicating polar lobe formation during cleavage of the CD blastomere.

Table 1 - Chronology of Developmental Events for Chlamys hastata Embryos and Larvae

Developmental Event	Time after Fertilization (Valve length in parentheses)	
	16°C	12°C
Germinal vesicle breakdown		0 minutes
First polar body release		20 "
Trefoil stage		3.0 hours
First division		3.25 "
Second division		3.75 "
Third division		5.5 "
Gastrula		18.0 "
Hatched gastrula		21.0 "
Trochophore		30.0 "
Early veliger		45.0 "
D-stage veliger		50.0 " (105 um)
Umbo-shaped veliger, metapodium first evident	15 days (176 um)	18 days (170 um)
Eyespots evident	24 " (220 um)	29 " (222 um)
Gill rudiment evident	26 " (228 um)	
Propodium evident	28 " (228 um)	
Statocysts evident	30 " (230 um)	35 " (240 um)
Three primary filaments on gill bars	32 " (231 um)	38 " (240 um)
Pediveliger, foot functional	34 " (231 um)	42 " (240 um)
Maximum age of larva	103 "	130 "

Third cleavage is latitudinal and dexioplectic, indicating that the spiral pattern of cleavage is dextral (Figure 2i). Within 15 hours, the embryo has reached the morula stage, a non-motile ball of cells. Gastrulation occurs by epiboly and invagination and is complete by 18 hours (Figures 3a-3e). The micromeres extend over and enclose the macromeres and then sink into the blastoceol at the region of the vegetal pole. The blastopore is very wide and shallow initially (Figure 3a) but the opening becomes smaller as the invagination deepens (Figure 3d). A single large macromere is visible in the blastopore (Figure 3c). Eventually the opening narrows to a longitudinal slit (Figure 3e). On the perimeter of the blastopore are two groups of 2 - 4 cells bearing sparse cilia. As well, sparsely ciliated cells girdle the embryo between the animal and vegetal poles and these are probably the primary trochoblasts.

At 21 hours, gastrulae hatch from fertilization envelopes and by 30 hours trochophores swim near the surface of the water (Figure 4a). The prototroch girdles the midregion of the trochophore as a 24 um broad band of widely spaced simple cilia. The prototroch develops from the primary trochoblasts and consists of three rows of ciliated cells. The prototroch apparently provides the locomotory force for the trochophore which swims in a spiral path. The apical tuft, a group of 8 - 10 fused cilia, is situated centrally in the apical plate and projects

Figure 3 - Blastopore Formation in C. hastata Embryos

a) Blastopore (bl) is initially a wide, shallow depression at vegetal pole. Ventral view. SEM. c, cilia; mi, micromeres. Scale bar = 10 um.

b) Lateral view of blastopore formation. Invagination of ectodermal cells forming shell field (sf) on dorsal-lateral surface. SEM. Scale bar = 10 um.

c) Ventral view of blastopore. Invagination is deeper and perimeter of margin is narrower than in Figure 4a. A single macromere (ma) can be seen in blastopore. Two groups of ciliated cells are evident on perimeter of blastopore (t). SEM. Scale bar = 10 um.

d) Lateral view of blastopore. Opening becomes smaller by closing toward a longitudinal slit with primary trochoblasts (t) at either end. A third group of trochoblasts on dorso-lateral surface is visible in micrograph. SEM. Scale bar = 10 um.

e) Blastopore reduced to a narrow slit. Cells at periphery of blastopore invaginate to form the future stomadaeum. SEM. Scale bar = 10 um.

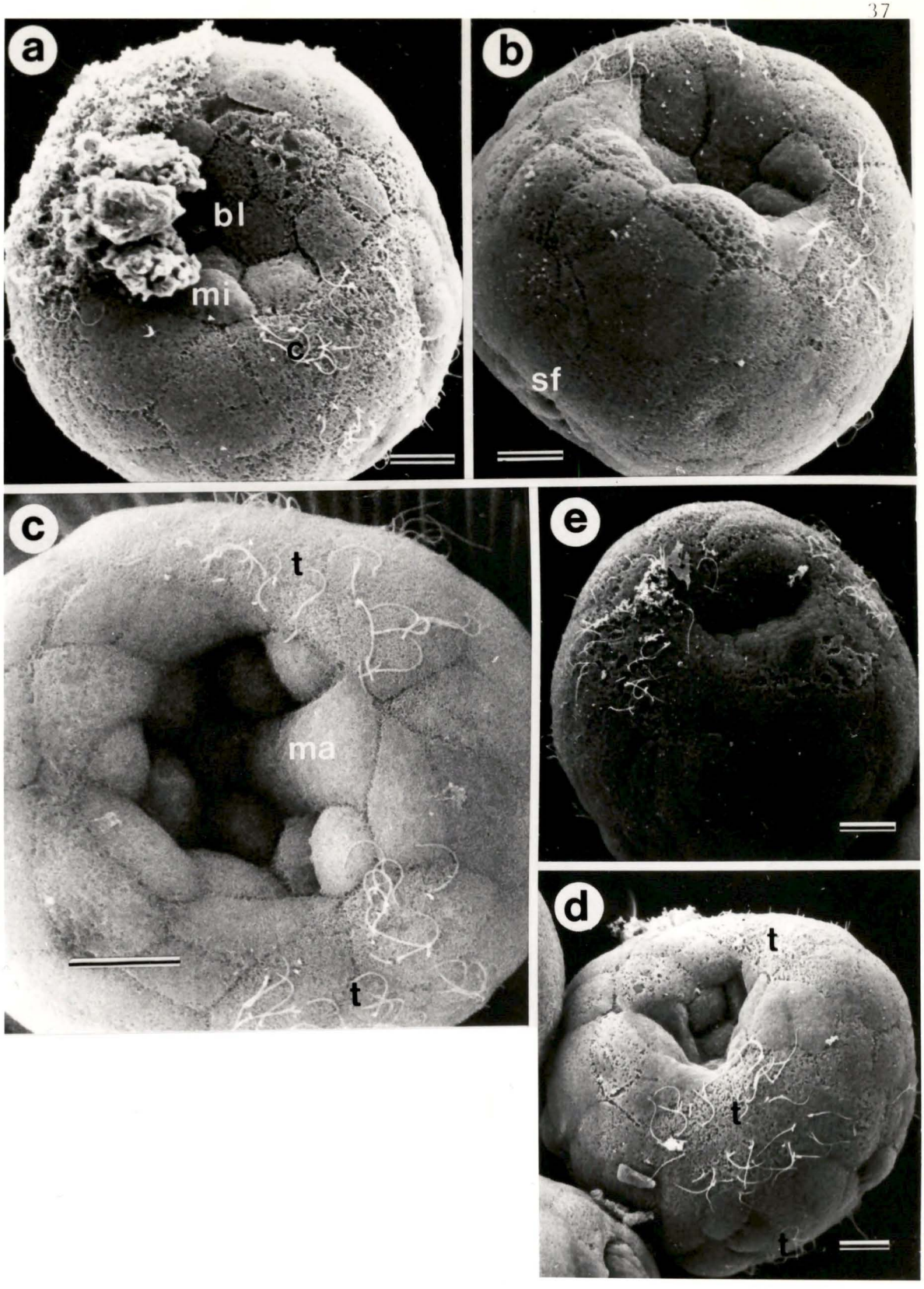
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Figure 4 - Trochophore and Early Veliger Stages in C. hastata Larvae

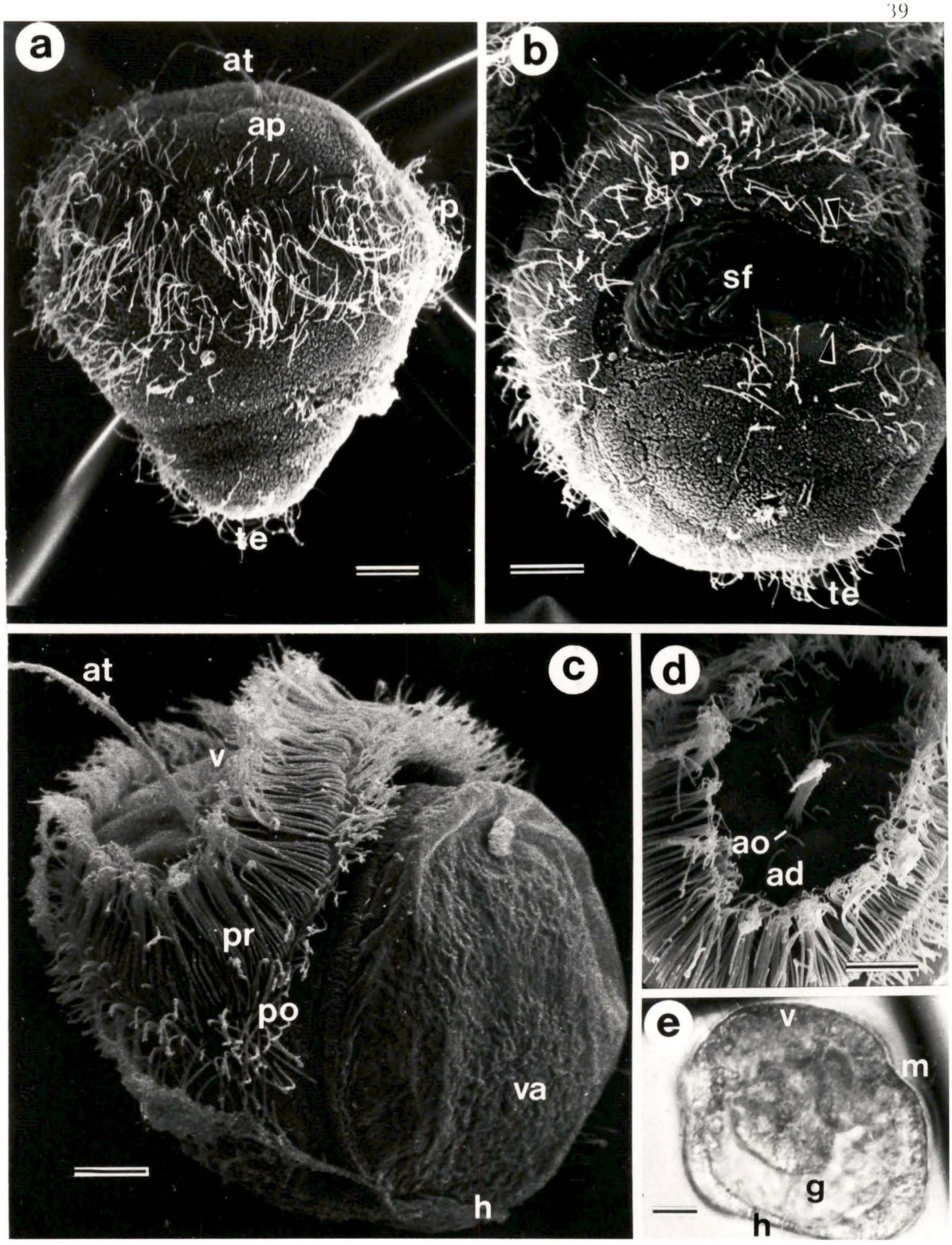
a) Trochophore larva. SEM. at, apical tuft; p, prototroch; te, telotroch, ap, apical plate. Scale bar = 10 um.

b) Trochophore larva with developing shell field on dorsal surface. Shell secretion is initiated in two regions. Arrows point to region of future hinge line. SEM. p, prototroch; sf, shell field; te, telotroch. Scale bar = 10 um.

c) Early D-stage larva, anterior view. Velum has two ciliated bands on outer margin. SEM. at, apical tuft; h, hinge region; pr, preoral band; po, postoral band; v, velum; va, valve. Scale bar = 10 um.

d) Detail of velar disc from Figure 4c. SEM. ao, apical organ; ad, apical disc. Scale bar = 10 um.

e) D-stage larva trapped in Nitex screen. LM, bright field optics. g, gut; h, hinge line; m, mouth region; v, velum. Scale bar = 20 um.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forward 10 - 15 um. The ventral apex bears a small tuft of cilia, the telotroch, and the rest of the body wall is sparsely ciliated.

The shell field, located on the dorsal surface of the trochophore (Figure 4b), begins secretion of the prodissoconch I shell in two regions. These two regions make contact in a straight line, forming the hinge line. Larval valves are first apparent using Koehler illumination in larvae 45 hours old. Using cross-polarization of two polarizing filters, no birefringence was observed, indicating that the valves are not calcified at this time. By 50 hours the embryo has developed into a D-stage veliger (Figure 4c, 4e). The valves are capable of closing and surrounding the entire larva. At this stage valve length is about 105 um and valve height is 82 um (Figure 4e). The prototroch transforms into the velum of the veliger larva with two distinct rows of cilia, a row of 20 um long compound cilia, the preoral band, and a row of shorter, simple cilia, the postoral band. The gut is a simple U-shape in D-stage larvae and is functional in 50 hour old larvae, as indicated by the presence of food particles in the stomach and intestine.

Valves

The shell gland of the trochophore transforms into the mantle

of the D-stage larva and resumes secretion of the valves (Figure 4d). Two distinct types of shell are secreted, the prodissoconch I and prodissoconch II shell (Figure 5a - 5c). The prodissoconch I shell is rougher in surface appearance and is uniformly dimpled with shallow pits over the surface (Figure 5c). Carriker and Palmer (1979) referred to this pattern as the punctate-stellate pattern. The prodissoconch II shell is smoother and is distinctly comarginally striate (after Waller, 1981). Between the prodissoconch I and II shell is a zone approximately 12 - 14 um broad. This region is sharply convex and is radially striate. Waller (1981) refers to this region as the stellate-radial zone.

The original D-shape of the early veliger valves is lost as the umbones become more rounded and obscure the hinge region. In later stage veligers, the valves are almost spherical with the anterior end slightly pointed (Figure 6a).

The hinge region, or proviniculum, of 15 day old larvae consists of a narrow, smooth ridge along the hinge line with 3 - 4 small denticles at either end in a narrow, triangular depression (Figure 5e). The denticles in early veligers are stout. Development of the denticles at both ends is symmetrical. Hinge length is 75.3 um and total proviniculum length is 100.5 um in 15 day old larvae (Table 2).

Figure 5 - Valve and Hinge Structures of C. hastata Larvae

a) Anterio-dorsal view of 15 day old larva. SEM. h, hinge line; b, prodissoconch I/II boundary; p1, prodissoconch I shell; p2, prodissoconch II shell. Scale bar = 10 um.

b) External surface of right valve of 15 day old larva. SEM. b, prodissoconch I/II boundary; p1, prodissoconch I shell; p2, prodissoconch II shell; v, velar cilia. Scale bar = 20 um.

c) Detail of Figure 5b. SEM. ps, punctate-stellate region; sr, stellate-radial region. Scale bar = 10 um.

d) Internal surface of left valve of 15 day old larva. SEM. p, proviniculum. Scale bar = 20 um.

e) Detail of Figure 5d. SEM. d, denticles; c, cardinal region; h, hinge line. Scale bar = 10 um.

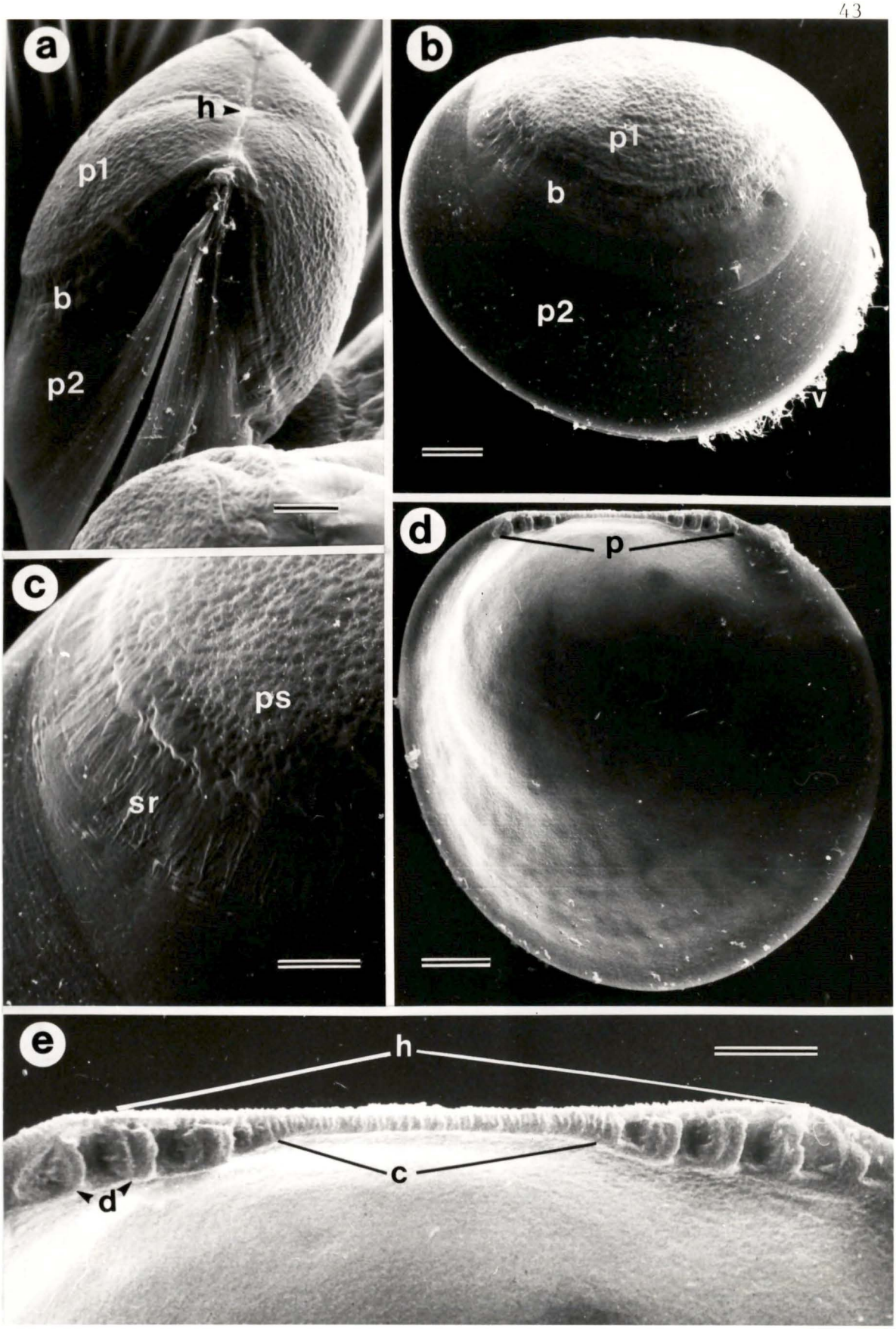
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Figure 6 - Valve and Hinge Structures of C. hastata Larvae and Juveniles

a) Inside view of right valve of 39 day old larva. SEM. Scale bar = 50 um.

b) Hinge region of 39 day old larva. SEM. d, denticles; l, ligament; lp, ligament pit. Scale bar = 10 um.

c) Proviniculum of 39 day old larva. SEM. d, denticles; h, hinge line; lp, ligament pit. Scale bar = 10 um.

d) Proviniculum of 39 day old larva. SEM. Scale bar = 10 um.

e) Proviniculum of 39 day old larva. Note the crown of each denticle bears a groove (gc) and the sides bear transverse grooves (gs). SEM. lp, ligament pit. Scale bar = 10 um.

f) Proviniculum of 12 day old juvenile. Note the enlarged ligament scar (ls). SEM. Scale bar = 10 um.

g) External view of postlarva, 2 days after metamorphosis, viewed from posterior region. Note dissoconch shell (d) on left valve. SEM. p2, prodissoconch shell. Scale bar = 50 um.

h) Inside view of left valve of 12 day old juvenile. Note developing anterior auricle. SEM. a, auricle; d, dissoconch shell; h, hinge region; p2, prodissoconch II shell. Scale bar = 50 um.

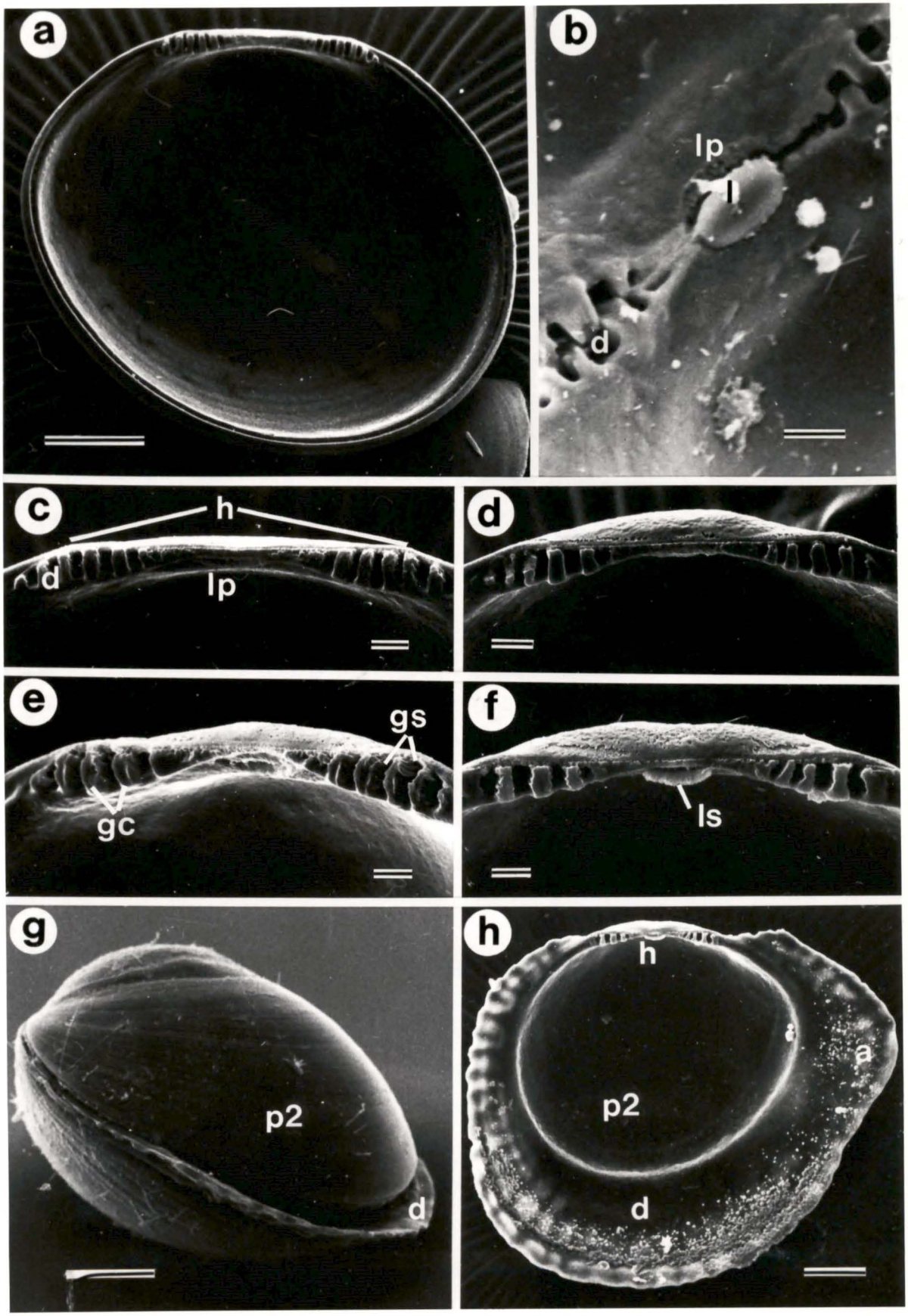
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Table 2 - Proviniculum Measurements of C. hastata Larvae, 15 and 39 Days After Fertilization (Standard Deviation in Paraentheses)

Day	Temp. (°C)	Sample Size (N)	Mean Valve Length (um)	Mean Valve Height (um)	Proviniculum Length (um)	Hinge Line Length (um)	Distance Between Denticles (um)	Resilium Length (um)
15	12	4	217.6 (28.44)	143.9 (6.1)	100.5 (9.5)	75.3 (6.8)	41.3 (5.5)	-
39	12	9	235.9 (12.2)	216.0 (13.6)	100.3 (6.0)	75.9 (3.6)	27.5 (3.3)	15.6 (0.7)
39	16	5	221.6 (8.6)	210.8 (2.9)	101.7 (4.0)	78.2 (5.4)	28.1 (5.5)	14.3 (1.4)

In pediveliger larvae, the smooth ridge along the hinge line is thicker and 5 - 6 denticles are present at either end (Figure 6c - 6e). Proviniculum length, or distance betwen outer margins of outside denticles, remains constant whereas distance between the inner margins of the inside denticles is less in 39 day old larvae (Table 2), indicating that the 2 - 3 denticles added during larval development are added to the inside of the initial 3 denticles. In pediveliger larvae, denticles are more pronounced and slightly longer than those of younger veligers and have a columnar appearance. The crest of each denticle is slightly indented along the long axis such that there are two small ridges perpendicular to the hinge (Figure 6e). The anterior and posterior sides of the denticles bear curved ridges which are concave toward the hinge line (Figure 6c, 6e).

A ligament pit is evident in veligers approximately 210 um in shell length (Figure 6b - 6e). The ligament pit develops as a shallow depression immediately below the hinge line in the center. The ligament is broad, about 15 um (Table 2) and takes up almost one-half of the area between denticles (Figure 6b).

Once metamorphosis is complete the mantle folds begin secretion of the dissoconch shell. Secretion of the left valve is initiated first and is visible in juveniles two days after metamorphosis

(Figure 6g). The left valve has strong radial striations and formation of the anterior auricle is evident in early juveniles (Figure 7a - 7d). The right valve is smooth, similar to the prodissoconch II shell, and a byssal notch is evident in juveniles 3 - 4 days after metamorphosis (Figure 7c, 7d). The dissoconch shell is secreted with little convexity (Figure 6h, 7a, 7c). Shell secretion is about 8.0 um / day (n=5) after metamorphosis compared with 4.5 um / day among larvae. Growth proceeds more rapidly in the direction of shell height than shell length.

The proviniculum is thicker and the ligament pit is larger (19 ± 1.8 um, n=6) and more pronounced in 14 day old juveniles (Figure 6f). No further denticles are added after metamorphosis and larval denticles are gradually lost by overgrowth of the juvenile shell. A ligament scar is evident at the region of the ligament pit (Figure 6f), providing an increased surface area to which the ligament may attach.

Velum

The velum is the locomotory and feeding organ of the larva (Figure 8a, 8c). Very sparsely ciliated mantle tissue connects the velum to the rest of the larval body (Figure 9d). The shape of the velum is oval throughout larval life with ciliated bands on the outer margin. When extended, the velum drapes over the external surface of

Figure 7 - Valve Structures of C. hastata Juveniles

a) External view of left valve of 12 day old juvenile. SEM. a, anterior auricle; d, dissoconch shell; pd, prodissoconch II/dissoconch boundary; p2, prodissoconch II shell; u, umbo region. Scale bar = 50 um.

b) Detail of Figure 7a. SEM. d, dissoconch shell; pd, prodissoconch II/dissoconch boundary; p2, prodissoconch II shell. Scale bar = 20 um.

c) External view of right valve of 12 day old juvenile. Right valve is overlapped by larger left valve. Note distinct byssal notch (bn). SEM. a, anterior auricle; d, dissoconch shell; p2, prodissoconch II shell. Scale bar = 50 um.

d) Anterior view of valve margins of 4 day old juvenile. Note ciliated gill filaments (g) in mantle cavity. SEM. bn, byssal notch; lv, left valve; rv, right valve. Scale bar = 50 um.

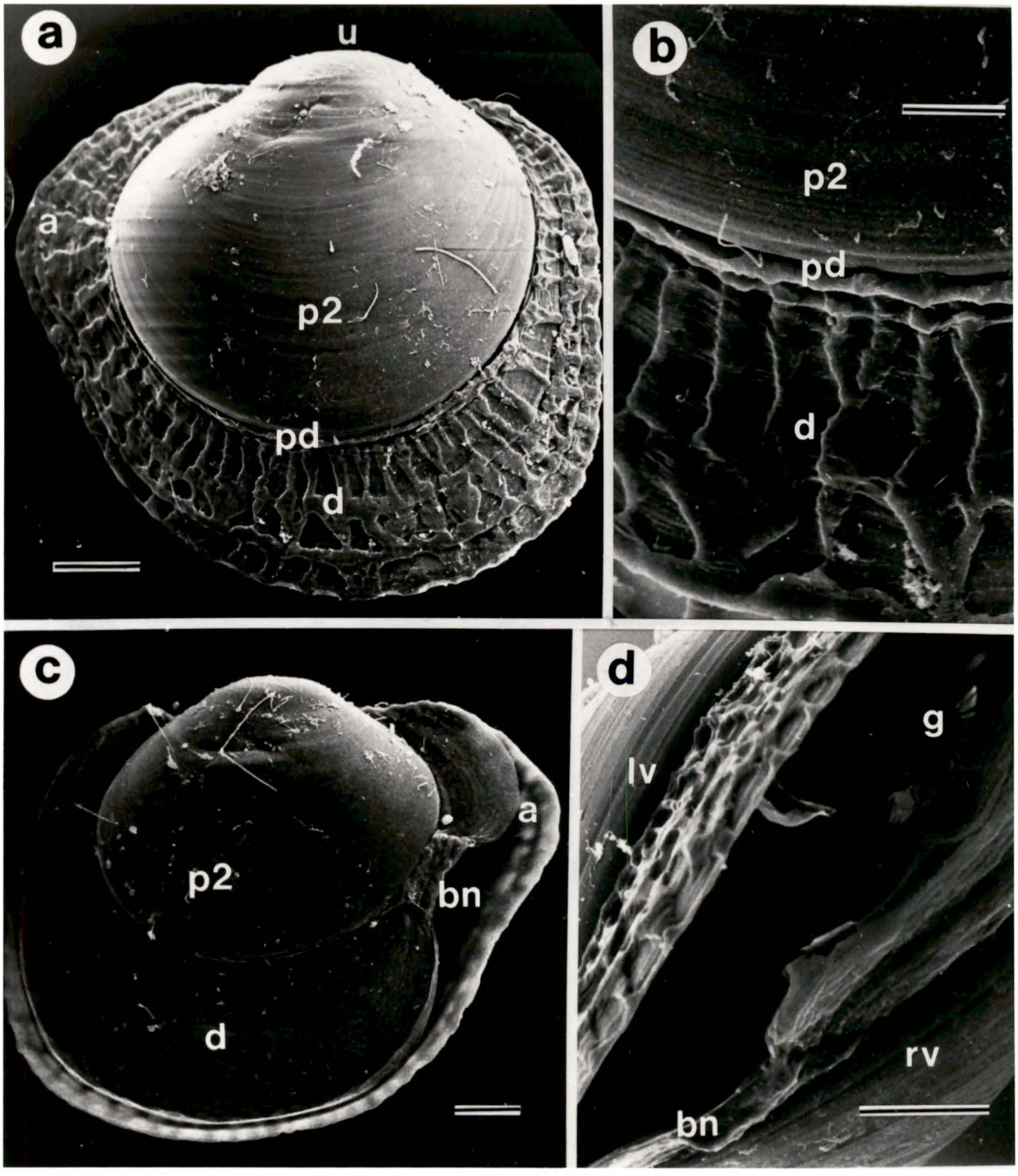
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Figure 8 - Velar Structures in C. hastata Larvae

a) Pediveliger larva with opened velum. SEM. ad, apical disc; ao, apical organ; lv, left valve; rv, right valve; vc, velar cilia. Scale bar = 50 um.

b) Detail of Figure 8a. Note apical cilia (ac) emerging from apical organ. SEM. Scale bar = 10 um.

c) Swimming pediveliger larva with foot (f) extended. LM, phase contrast optics. e, eyespot; d, right digestive diverticulum; u, umbo; v, velum. Scale bar = 50 um.

d) Velar disc epithelium. Note striated muscle fiber (m) of velar retractor muscle with A, H, and Z zones (a, h, and z, respectively). TEM. e, epithelial cell; g, glycocalyx; m, mitochondria; mv, microvilli. Scale bar = 10 um.

e) Velar retractor muscle fibers (m) leading to velum. Each individual muscle fiber has a separate insertion on velar epithelium. Histological section. c, cerebral ganglion. Scale bar = 10 um.

f) Ventral view of larva, 20 days after fertilization, with velum (v) withdrawn into mantle cavity. LM, bright field optics. f, foot; l, left valve; r, right valve; m, mouth region. Scale bar = 50 um.

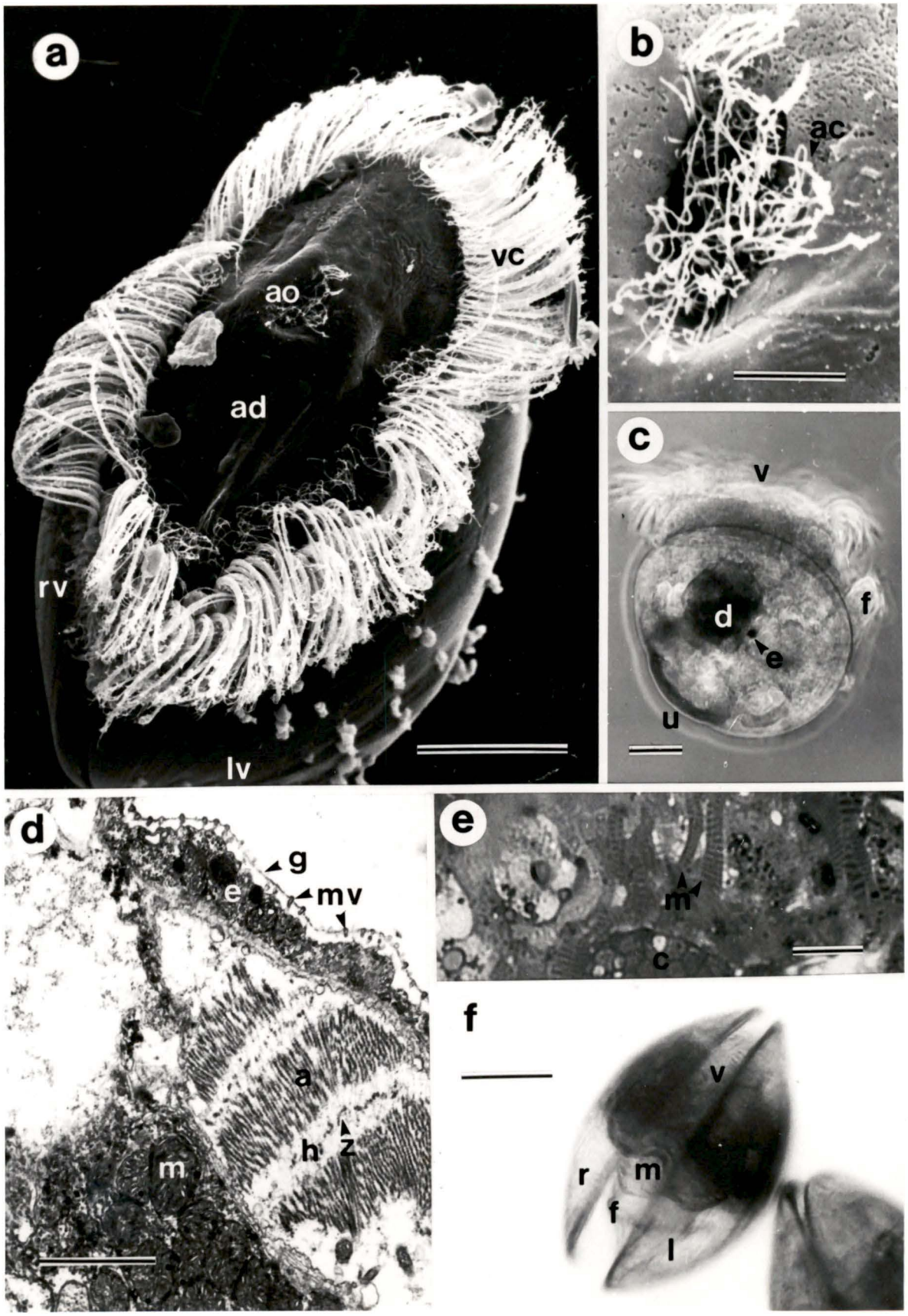
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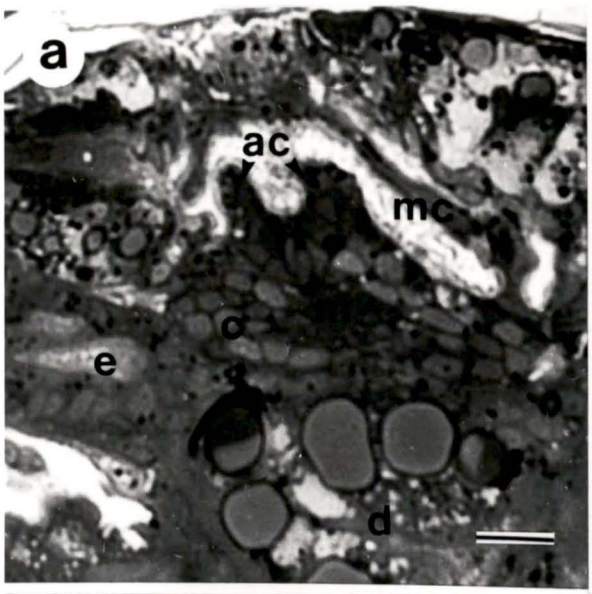
Figure 9 - Velar Structures in C. hastata Larvae

a, b, and c) Histological sections of apical organ and cerebral ganglion in pediveliger larva with partially retracted velum. ac, apical cells; cg, cerebral ganglion; c, cortex; m, medula; p, apical pit; d, digestive diverticulum; ma, mantle tissue; mc, mantle cavity; e, esophagus. Scale bar = 10 um.

d) Cross-sectional view of ciliated bands on velar margin. SEM. ac, adoral cilia; g, food groove; m, mantle tissue; pr, preoral cilia; po, postoral cilia; s, shell. Scale bar = 10 um.

e) Compound cilia of preoral band showing long orthoplectic rows (or) and short diaplectic rows (dr). SEM. Scale bar = 2 um.

f) Cross-section of a single compound cilium of preoral band showing arrangement of cilia (c) within a compound cilium. TEM. dr, diaplectic row. Scale bar = 0.5 um.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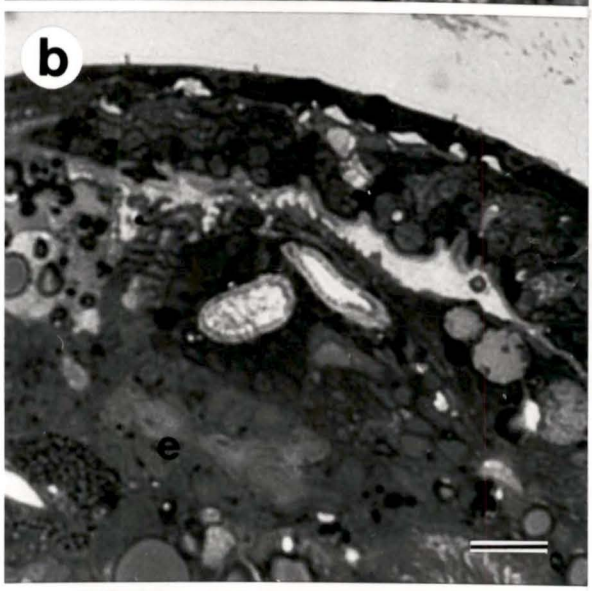
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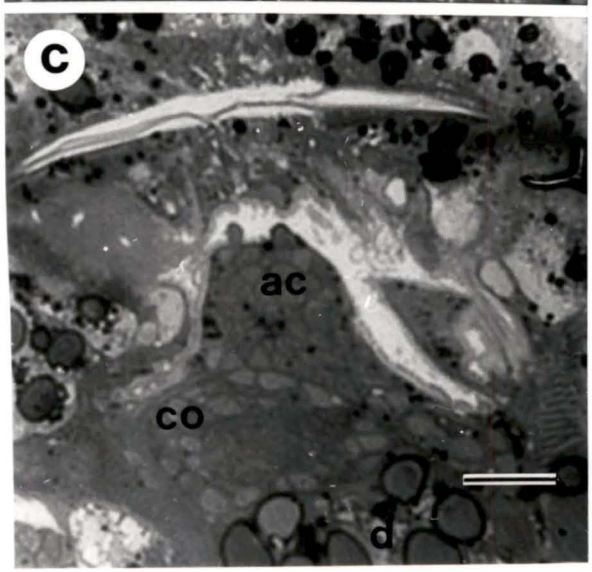
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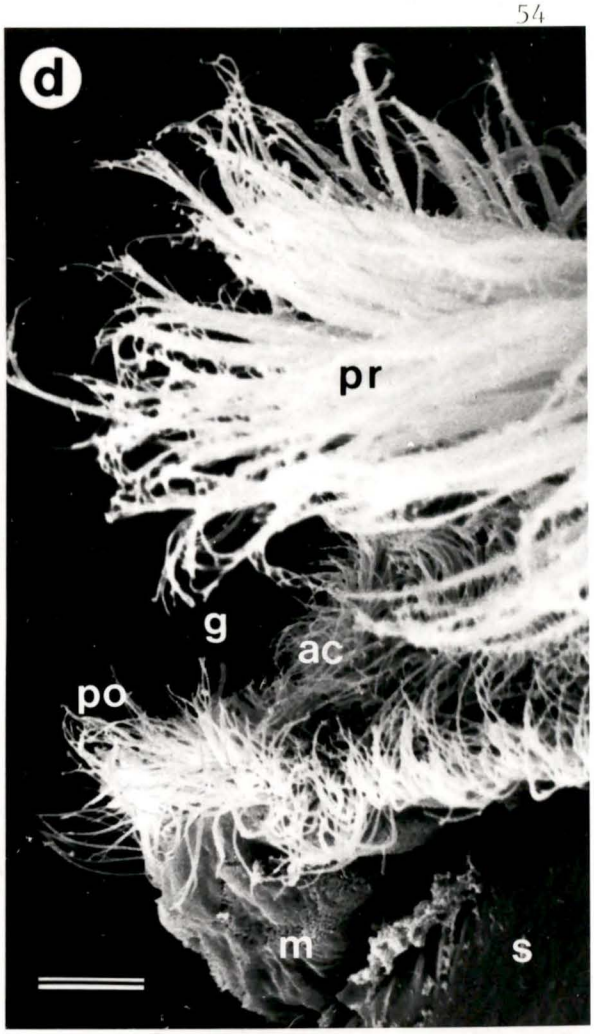
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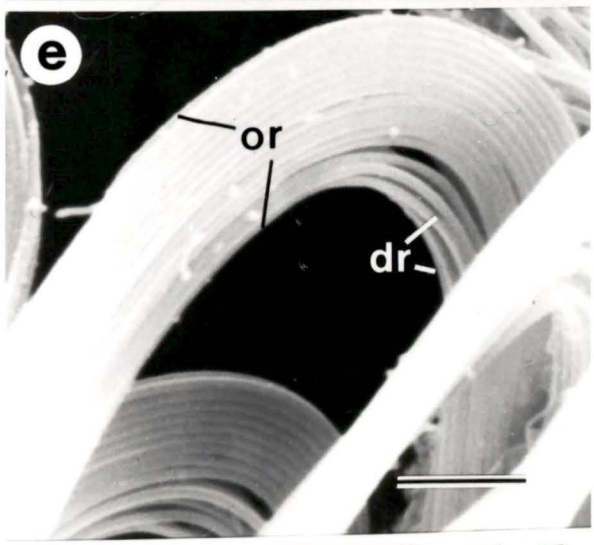
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the valves such that the mantle tissue lies directly on the valves and the velum's outer margin is extended furthest along the valve surface (Figure 8a, 9d). Two pairs of velar retractor muscles, the anterior and posterior retractor muscles, have several insertions at the epithelium of the velum along the anterio-posterior axis. Each muscle fiber is striated (Figure 8e) and the A, Z, and H bands are easily distinguished in Transmission Electron micrographs (Figure 8d). When retracted, the velum is folded anterio-posteriorly and occupies the anterior half of the mantle cavity (Figure 8f).

The apical plate of the trochophore remains as the central portion of the velum, or the apical disc (Figure 4c). The apical pit is a small, deep invagination located in the center of the apical disc (Figure 4d). The apical tuft of the trochophore originates from the apical tip and persists for 2 - 3 days in D-stage veligers and then is lost. In older larvae the apical pit is oval, approximately 6 - 8 um wide, 20 um long, and 10 um deep (Figure 8b). Numerous fine, simple cilia originate from the apical pit and barely project beyond the rim of the pit. Epithelium of the velar disc is a simple squamous to cuboidal epithelium with a thick glycocalyx supported by microvilli (Figure 8d). Cells of the apical pit are high columnar, approximately 7 um high, with nuclei at the apical aspects of the cells. Directly underlying these cells is the cerebral ganglion (Figure 9a - 9c). Surrounding the

central core of the cerebral ganglion is a medulla of large cuboidal cells, a single cell layer thick. Within the medulla lie cells of the cerebral ganglion, some of which express the typical owl's eye appearance of nerve cells with the nucleus, surrounded by a large nucleolus, situated centrally in the cell (Figure 9c).

On the outer margin of the velum of a D-stage veliger, compound cilia of the preoral band are arranged in a single row with each compound cilium arranged in 2 - 3 orthoplectic rows (Figure 4d). In a later stage larva the preoral band consists of two rows of compound cilia that are parallel to the plane of beat (Figure 10c, 10d). Each compound cilium is arranged in 6 - 15 orthoplectic rows and 2 - 6 diaplectic rows (Figure 9e, 9f). Cilia comprising each compound cilium fit closely together along most of their length except at the tips where they tend to splay apart (Figure 9d). Cilia of the postoral band are simple and short (6-8 um and are arranged in one or two rows situated closely together (Figure 10d). A third band of cilia develops between these two bands, called the adoral band, and forms the food groove (Figure 9d, 10d). The adoral band is approximately 24 um wide and cilia are 8 um long. A fourth poorly defined band of 17 um long simple cilia is situated immediately adjacent to the apical region (Figure 8a).

On the ventral surface of the velum the postoral band extends

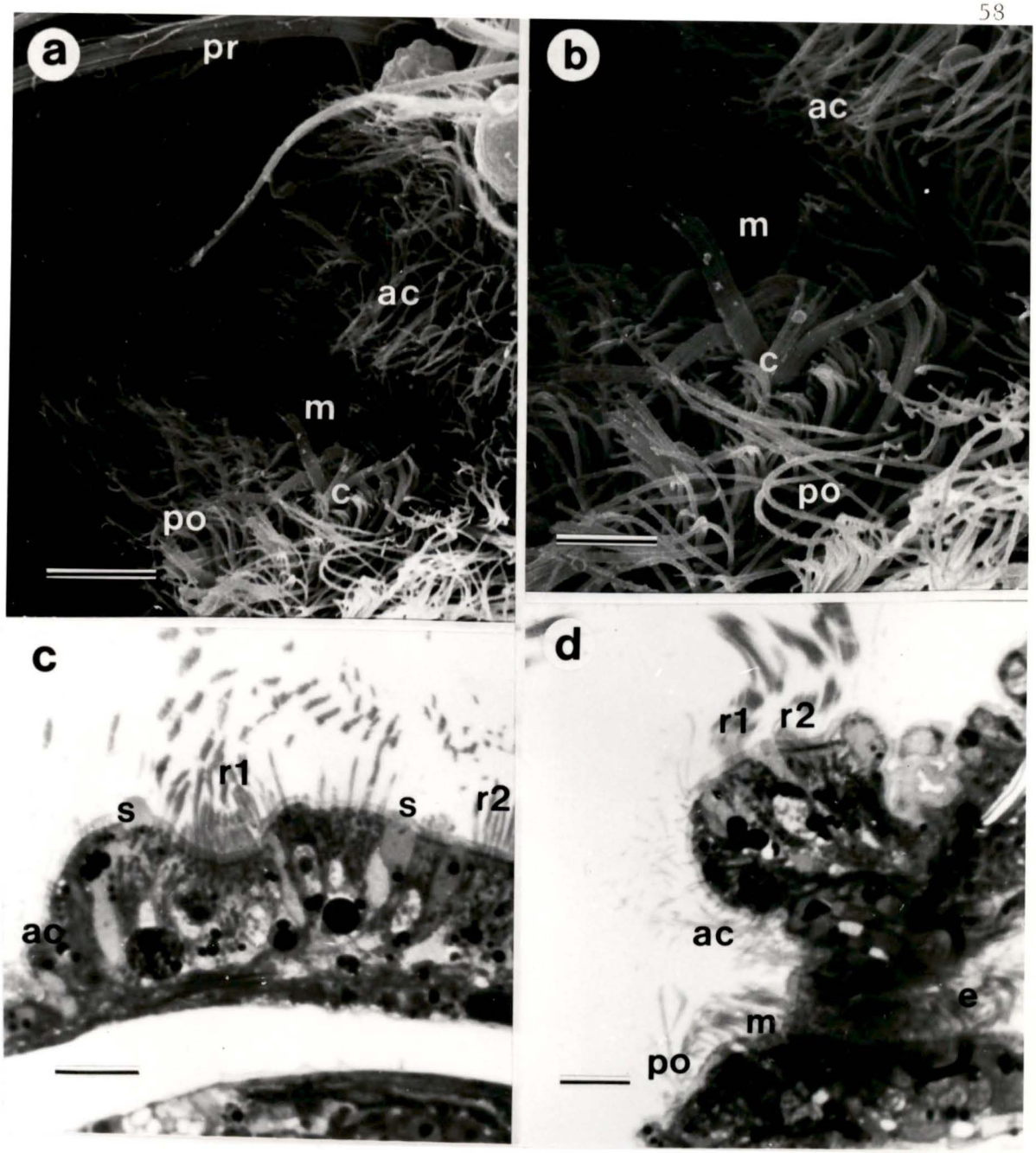
Figure 10 - Velar Structures in C. hastata Larvae

a) Mouth region at posterior edge of velar margin. The mouth (m) is situated between the adoral (ac) and postoral (po) ciliary bands. Several short, compound cilia (c) are located just ventral to mouth. SEM. pr, preoral cilia. Scale bar = 10 um.

b) Detail of Figure 10a. SEM. ac, adoral cilia; c, compound cilia; m, mouth; po, postoral cilia. Scale bar = 5 um.

c) Tangential histological section of preoral band of velar margin. The preoral band consists of two rows of compound cilia (r1, r2) with secretory cells (s) situated between the two rows and between the preoral and adoral (ac) ciliary bands. Scale bar = 10 um.

d) Cross-section through ciliated bands on velar margin at the mouth region (m). Histological section. ac, adoral cilia; e, esophagus; po, postoral cilia; r1, r2, double row of compound cilia of preoral band. Scale bar = 10 um.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posteriorly and forms a V-shaped ventral lip, called the postoral tuft by Waller (1981) (Figure 10a). The mouth is situated anterior to the ventral lip, between the postoral and adoral cilia (Figure 10a). Several short, broad compound cilia are located immediately ventral to the mouth (Figure 10b). Each cilium consists of 7 - 9 cilia arranged in a single row and are at least 8 um in length. They are interspersed among, and appear to be continuous with, the simple cilia of the postoral band.

The outer margin of the velum is bilobed with the preoral and postoral bands at the apex of either lobe and the adoral band in a shallow trough between them (Figure 10d). Cells of the ciliated bands are specialized epithelial cells and are more columnar than other epithelial cells (Figure 10c, 10d). Cells of the preoral band have large vacuoles at the basal aspect of each cell. Between the two rows of preoral cilia, and between the preoral and adoral bands, are several secretory cells with large secretion granules extruding from or at the apical aspect (Figure 10c, 10d).

Gut

The larval mouth, situated between the postoral and adoral bands at the ventral aspect of the velum, passes into a densely ciliated

esophagus (Figure 10d, 11a). The esophagus is shaped like a straight tube, approximately 8 um in diameter and 40 um long, and runs dorsally, passing directly under the cerebral ganglion. Cells of the esophagus are a simple cuboidal epithelia with prominant nuclei and have a thick glycocalyx on the apical surface (Figure 11b). The esophagus-stomach junction appears to be a cellular constriction with no muscle cells evident (Figure 11a). The stomach lies in the dorsal region of the larva, near the hinge region. It extends posteriorly into a deep, narrow U-shape to form the style sac (Figure 10b, 10c).

Two cell types are present in the stomach epithelium. The anterior portion of the stomach has densely ciliated cells with 7 um long cilia. Epithelium in this region is simple or pseudostratified and in early umbone larvae, the cells have large vacuoles filled with lipid or yolk granules (Figure 11b). In pediveliger larvae the epithelial cells possess largely empty vacuoles and prominent nuclei with large nucleoli (Figure 11c). Girdling the midportion of the stomach is the gastric shield. Epithelial cells of this region stain darker than the ciliated epithelial cells and are not vacuolated in the pediveliger stage (Figure 11b,11c).

Epithelium of the style sac possess a 5 um thick densely ciliated mat extending into the lumen (Figure 11b, 11c). The dense

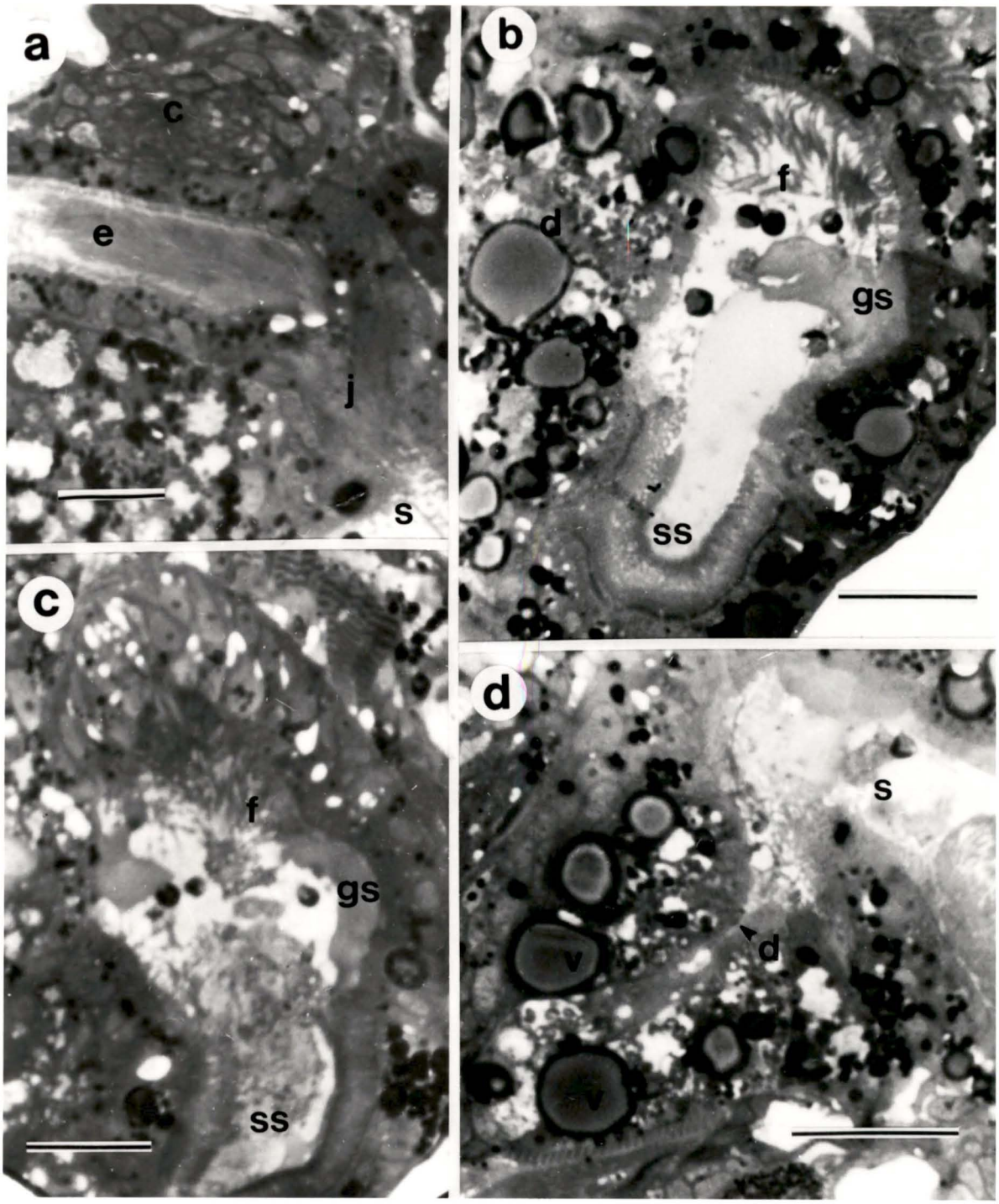
Figure 11 - Gut Structures in C. hastata Larvae

a) Longitudinal section of densely ciliated esophagus (e) and junction (j) between esophagus and stomach (s). Histological section. c, cerebral ganglion. Scale bar = 10 um.

b) Histological section of stomach region in 6 day old larva. Lumen of the anterior region of stomach, foregut (f), bears dense, long cilia. Gastric shield (gs) covers the mid-portion of the stomach. d, digestive diverticulum; ss, style sac. Scale bar = 10 um.

c) Histological section of stomach region in 33 day old larva. gs, gastric shield; f, foregut; ss, style sac. Scale bar = 10 um.

d) Histological section of left digestive diverticulum with ducts (d) leading from left-ventral region of stomach (s). v, lipid-filled vacuoles. Scale bar = 10 um.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ciliation of the style sac ends abruptly at the stomach-style sac junction.

At the midportion of either side of the ventro-lateral aspect of the stomach are tubules leading to the left and right digestive diverticula forming an H-shape (Figure 11d). As larvae approach the pediveliger stage, increasing amounts of glycogen and lipid are stored in the digestive diverticula and the organ becomes very dark (Figure 8c, 13a).

Foot

The foot is first apparent in 15 day old larvae (16°C) as a small ciliated rudiment in the posterio-ventral region between the mouth and anus (Figure 8f). This rudiment ultimately forms the metapodium of the foot. Little development occurs until about 28 days after fertilization when the propodium develops rapidly. By 34 days (16°C) the foot is functional and larvae are often observed crawling on the substrate or swimming while the foot is extended (Figure 8c). The foot is bilaterally symmetrical, about 110 um long and 53 um wide with a distinct toe and heel (Figure 12a, 12b). Lateral and dorsal surfaces of the foot are sparsely ciliated and the ventral surface is covered with long (8-24 um), simple cilia (Figure 12a, 12b). A byssal groove, 48 um

Figure 12 - Foot Structures of C. hastata Larvae

a) Lateral view of foot of pediveliger. Note dense cilia on sole of foot and only sparse cilia on lateral surface. SEM. gr, gill rudiment; h, heel; s, shell; t, toe. Scale bar = 10 um.

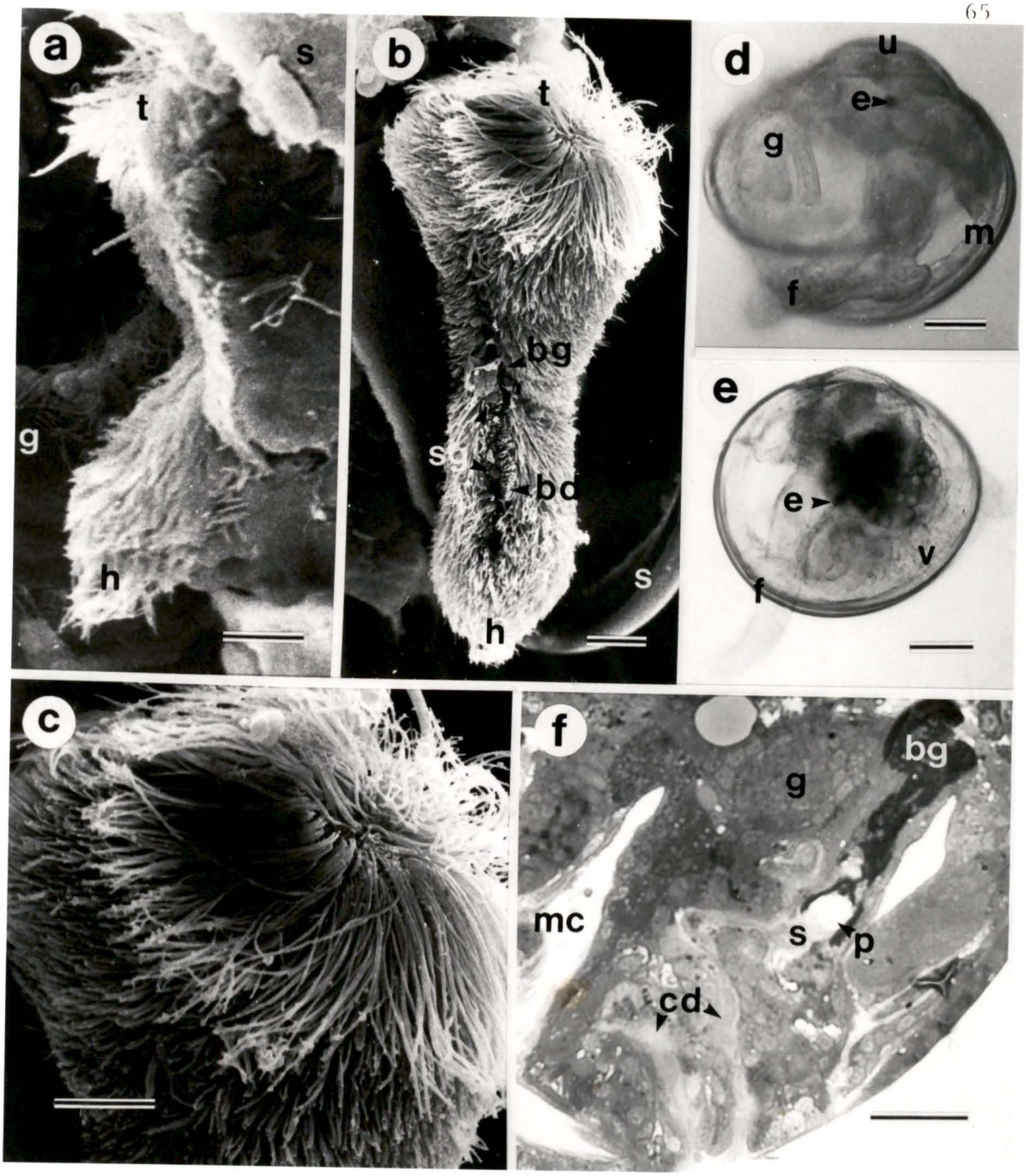
b) Ventral view of foot. Note dense ciliation on sole of foot with the byssal groove (bg) running medially from toe (t) to heel (h). SEM. bd, region of byssal duct; s, shell; sg, secretion granules. Scale bar = 10 um.

c) Detail of Figure 12b, showing long cilia at tip of foot. SEM. Scale bar = 10 um.

d) Light micrograph of postlarva, 1 day after metamorphosis. Foot (f) is oriented ventrally and is very flexible (toe of foot is actually pointing posteriorly). Gill lamellae (g) occupy the posterior half of mantle cavity. LM, phase contrast optics. e, eyespot; m, mantle; u, umbo. Scale bar = 50 um.

e) Light micrograph of pediveliger, 40 days after fertilization, with toe of foot (f) extended. LM, bright field optics. e, eyespot; d, digestive diverticulum; v, velum. Scale bar = 50 um.

f) Histological section of foot region. Secretions (s) of primary byssus gland (bg) are deposited into the lateral pouch (p) which leads into two ciliated ducts (cd). g, pedal ganglion; m, mantle cavity. Scale bar = 10 um.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long, extends along the midline of the longitudinal axis of the sole of the foot. At the posterior-most region of the heel is a tuft of cilia (Figure 12b).

When crawling, both ciliary and muscular action aid in forward movement of the foot along the substrate. The remainder of the larval body is dragged behind with the hinge region posterior-most, and is periodically "caught up" to the foot by sharp contraction of the pedal retractor muscles. The tip of the foot occasionally lifts off the substrate or moves laterally in a swaying motion during forward movement, giving the impression of a sensory function. The velum is extended while crawling and probably aids in the forward movement of the larva. The shell is dragged along the substrate, behind the foot. Periodically, the larva will lift off the substrate and swim, leaving the foot extended (Figure 8c).

The primary byssus gland stains dark blue with Richardson's stain and is situated in the posterio-dorsal region of the foot, immediately posterior to the pedal ganglion (Figure 12f). The ventral aspect of the primary byssus gland surrounds an open, ciliated region, the lateral pouch, and two byssal ducts pass ventrally from the lateral pouch to the sole of the foot. (Figure 12f). The entire length of each duct is densely ciliated and lined with several secretory cells which

probably also pass secretion granules into the duct. The ducts open close to each other at the posterior of the byssal groove on the sole of the foot (Figure 12b).

Gill Rudiment

Gill rudiments are first visible in larvae about 26 days old (16°C). On either side of the foot, a ridge of tissue, the gill plate, extends from the mantle tissue into the mantle cavity (Figure 13a, 13d). From each gill plate three small lobes of tissue develop, called the primary gill filaments (Figure 13a, 13d). Primary filaments develop almost simultaneously and are all about 22 um in height. By 32 days each rudiment is sparsely ciliated with a single row of simple cilia along the apical margin which beat in an anteriorly directed metachronal wave (Figure 13d, 13e).

The primary filaments consist of a simple cuboidal epithelium with large vacuoles filled with granules (Figure 13b, 13c). Located centrally in each filament is a narrow ciliated lumen which appears to have no connection to each other (Figure 13b, 13c). No cellular or ciliary connection between gill filaments on opposing sides of the mantle cavity was observed among larvae.

Figure 13 - Gill structures in C. hastata Larvae and Juveniles

a) Light micrograph of swimming pediveliger. Bright field optics. e, eyespot; d, right digestive diverticulum; f, foot; g, gill rudiment; m, mouth; u, umbo; v, velum. Scale bar = 50 um.

b) Longitudinal histological section through primary gill filaments. c, cilia; l, lumen; p, gill plate. Scale bar = 10 um.

c) Cross-section of primary gill filaments. Histological section. c, cilia; l, lumen; mc, mantle cavity; m, mantle; e, eyespot. Scale bar = 5 um.

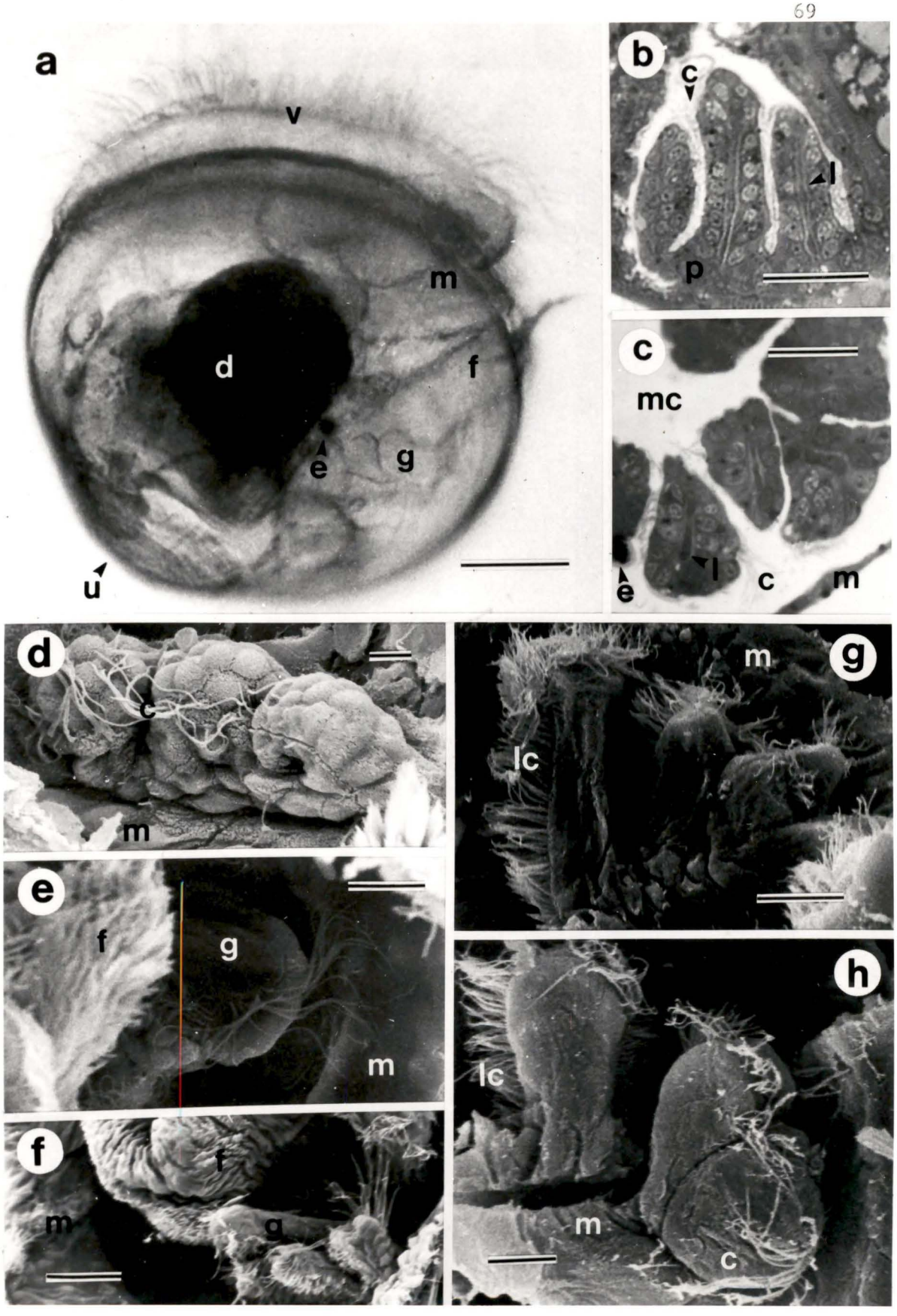
d) Three primary gill filaments with a single row of cilia (c). SEM. m, mantle tissue. Scale bar = 10 um.

e) Anterior-most primary gill filament (g) from right gill plate of a pediveliger larva. Note the single row of cilia. SEM. f, foot; m, mantle. Scale bar = 10 um.

f) Gill lamellae (g) of 12 day old juvenile. SEM. m, mantle; f, foot. Scale bar = 20 um.

g) Proximal view of left gill lamellae of 4 day old juvenile. Note dense row of lateral cilia (lc) on 2 of the gill filaments. SEM. m, mantle tissue. Scale bar = 10 um.

h) Proximal view of left gill lamellae of 3 day old juvenile. Note long cilia (c) at distal end of posterior-most gill filament. SEM. lc, lateral cilia; m, mantle tissue. Scale bar = 10 um.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The larval eyes or pigment cells are first visible in larvae about 24 days old (16°C) (Figure 13a). Located anterior to the gill bar, each eye consists of a cell with darkly staining granules arranged in a cup shape oriented anteriorly and another cell situated within and anterior to the cup shape (Figure 14c, 14d).

During metamorphosis, the primary filaments lengthen, more are added, and occupy the posterior half of the mantle cavity (Figure 12d). By 2 - 3 days after metamorphosis, the single row of simple cilia present in the larva becomes densely ciliated and form the lateral ciliated band of the adult gill lamellae (Figure 13g, 13h). Length of gill lamellae is about 28 um at this time. Feeding is taken over by the gill lamellae, from the velum, within 2 days after metamorphosis, as indicated by the presence of food particles in the gut.

Another band of cilia, the frontal cilia is just beginning to develop by 2 - 3 days (Figure 14a) and is clearly visible in 12 day old juveniles (Figure 14b). There is no evidence of a tissue connection between the left and right gill filaments in juveniles, however there is a ciliary connection. Long cilia at the apex of the posterior-most, or third filaments of early juveniles, extend toward mantle tissue surrounding the posterior adductor and across to the opposing filament (Figure 13f, 13h, 7d). These cilia are longer (20 um) than other cilia

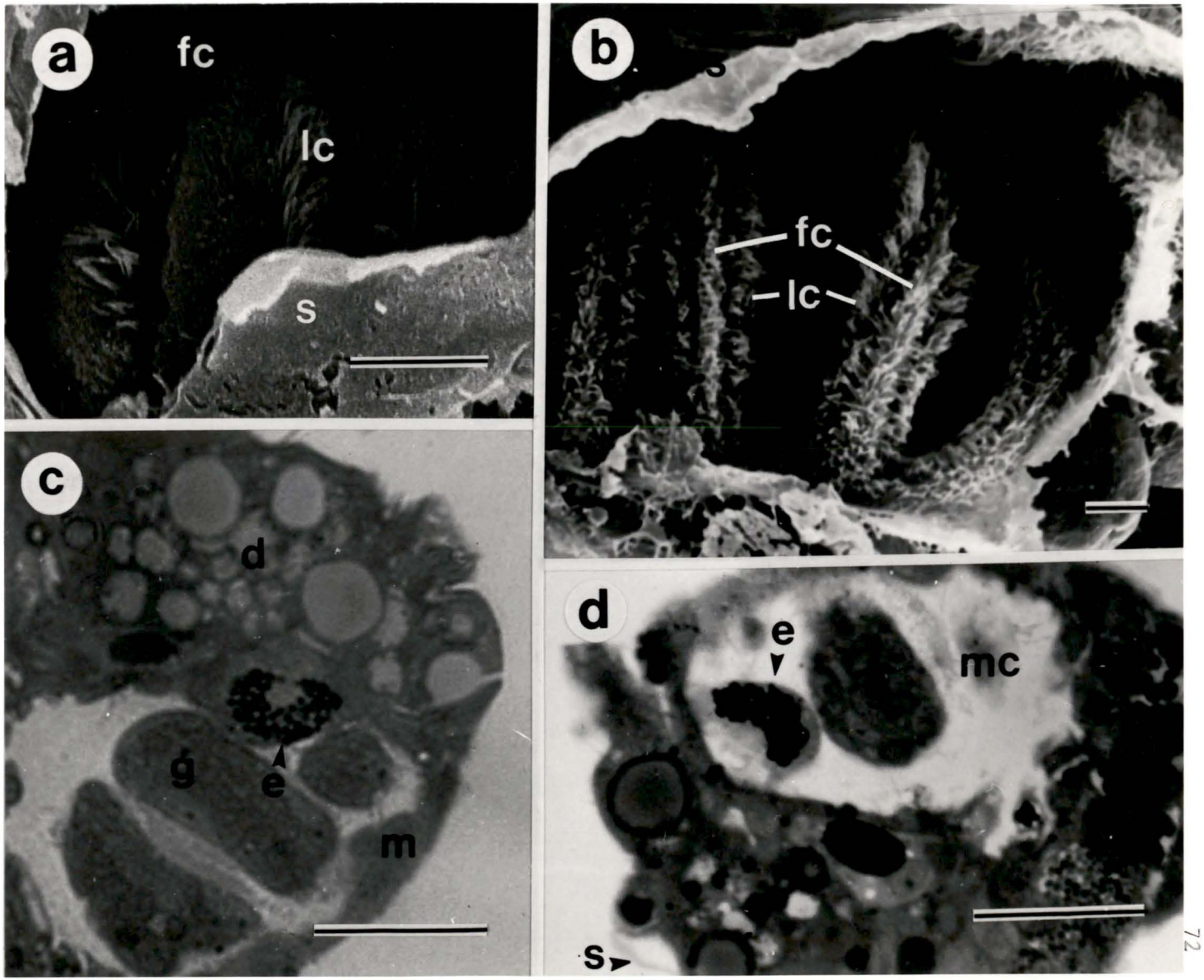
Figure 14 - Gill Structures and Eyespots in C. hastata Larvae and Juveniles

a) Distal view of gill lamellae of 3 day old juvenile. Shell (s) was broken away to facilitate view. Frontal ciliary band (fc) is just beginning to develop on distal region of gill lamellae. SEM. lc, lateral cilia. Scale bar = 10 um.

b) Distal view of gill lamellae of 12 day old juvenile. SEM. fc, frontal cilia; lc, lateral cilia; s, shell. Scale bar = 10 um.

c) Histological section through primary gill filaments (g) and eyespot (e) of 33 day old larva. Dense, pigmented granules are deposited in the shape of a cup within a single cell. d, digestive diverticula; m, mantle. Scale bar = 10 um.

d) Histological section through one primary gill filament (g) and eyespot (e). mc, mantle cavity; s, shell matrix. Scale bar = 10 um.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of the lateral band (Figure 13h).

The primary filaments form the descending limb of the inner demibranch gill of the adult. In juveniles about 3 weeks after metamorphosis, each filament is reflected upward to form the ascending portion of the gill filament.

DISCUSSION

As a Family, pectinids show little variation in embryonic development (Table 3). With the exception of E. bifrons (Dix, 1976) all species have a small egg (60 - 80 um) and a planktotrophic larval stage. Duration of larval development varies with temperature, and diet, but generally larvae reared at 15° - 18°C metamorphose 20 - 35 days after fertilization.

A jelly coat surrounding newly released oocytes has not been reported in pectinid species other than C. hastata and unpublished observations by the author of jelly coats surrounding eggs of P. yessoensis and C. gigantea. The presence of the jelly coat may be common among pectinid species but since it is translucent and difficult to observe using light optics, it is possible that it has not been noticed by other authors. Since gametes studied are generally released by stress spawning adults in the laboratory, it is also possible that the lack of a jelly coat surrounding most oocytes is an artifact of the spawning technique.

Elevation of the fertilization envelope is generally very low among pectinids (Wada, 1968) and, in the case of C. hastata, is not visible using light optics. Allen (1953) noted that no sudden

Table 3 - Summary of Larval Development in Species of the Family Pectinidae

Species	Egg Diameter (um)	Valve Length at Metamorphosis (um)	Duration (days)	Temperature (°C)	Reference
Pecten maximus	60-80	225	33-38	16	Gruffydd and Beaumont (1972)
		245-250	26-33	18	Buestel, et al. (1982)
P. meridionalis	70	230	31	13-15	Dix and Sjardin (1975)
Argopecten gibbus	60	235-270	16	23	Costello, et al. (1973)
A. irradians	62	190	13	24	Sastry (1965)
Placopecten magellanicus	64	280	35	15	Culliney (1974)
	80-90				Naidu (1970)
Equichlamys bifrons	120	200	17-20	12-18	Dix (1976)
Patinopecten yessoensis	72	265	28	14	Bourne (unpub.)
Chlamys islandica	70	305	70	6-8	Gruffydd (1975)
C. purpuratus	64	232	21	21	Disalvo, et al. (1984)
C. asperrimus	61	190-220	20-22	17-18	Rose and Dix (1984)
C. gigantea	65	220	22	15	Bourne (unpub.)
C. hastata	71-74	240	42	12	
		231	34	16	

observable change in the nature of Spisula solidissima eggs occurs at the time of fertilization except that the space between the plasma membrane and fertilization envelope may become a little wider.

Among echinoderms, the fertilization envelope of Strongylocentrotus purpuratus embryos is supported by microvilli, projections of the plasma membrane (Epel, 1978). In Figure 2c, the support structures of the fertilization envelope appear as extensions of the plasma membrane. Elevation of the fertilization envelope in echinoids is caused by the release of products of cortical granules located just below the plasma membrane (Epel, 1978). One of the proteins released from the cortical granules is a vitellin protein which is incorporated into the elevating vitelline layer. Possibly, the proteinaceous extensions observed below the fertilization envelope are made up of this vitellin protein.

Evidence from this study suggests that gastrulation occurs by both epiboly and invagination (Figure 3a - 3e). Micrographs of blastopore formation show micromeres completely surrounding the embryo with the blastopore beginning as a wide, shallow depression at the vegetal pole. This agrees with observations by Raven (1958) of O. edulis embryos. Other lamellibranch species in which this form of gastrulation has been observed include Ensis, M. edulis, and Teredo

(Verdonk and Biggelaar, 1983).

The two groups of cells located near the perimeter of the blastopore are probably two groups of the quartet of ciliated cells called the primary trochoblasts (Figure 3a - 3e). Ultimately, they form the prototroch of the trochophore and ciliated bands of the velum in the veliger stage. These are generally described as originating from four points around the equatorial region of the embryo whereas the blastopore invaginates at the vegetal pole. However, the blastopore is displaced ventrally during development and the pretrochal region is shifted forward, owing to considerable growth of the dorsal region, and the main axis of the embryo becomes bent and the blastopore becomes situated in front of the prototroch (Verdonk and Biggelaar, 1983). Since the mouth is situated just below the prototroch, the location of the primary trochoblasts near the blastopore provides further evidence that the region of the blastopore becomes the future mouth.

The prodissoconch I shell of C. hastata bears the punctate-stellate pattern as first described by Ansell (1961). In published micrographs of C. virginica (Carriker and Palmer, 1979), the punctate-stellate region is about 30 um in diameter and in O. edulis (Waller, 1981) the region is ovoid, about 30 x 50 um. Carriker and

Palmer (1979) suggested that this region probably overlies the embryonic shell gland. However, in C. hastata the punctate-stellate pattern covers an area of about 40 x 80 um (Figure 5b), about twice the size of the region of O. edulis or C. virginica. If indeed the punctate-stellate region represented the area of the embryonic shell gland, then the shell gland of C. hastata should be twice the size of shell glands of O. edulis and C. virginica.

The stellate-radial zone presumably represents the region where shell secretion is taken over by the mantle folds (Carriker and Palmer, 1979; Waller, 1981). Waller (1981) described the transfer of secretion to be a gradual process. Waller (1981) argued that the transition from shell gland secretion to mantle secretion occured long before the prodissoconch I/II boundary in O. edulis. In his micrographs, it is apparent that the stellate-radial zone ends long before the prodissoconch I/II boundary and therefore his argument for the prodissoconch I/II boundary simply representing the first time of closure deserves consideration. However, in C. hastata the prodissoconch I/II boundary occurs at the periphery of the stellate-radial zone. In C. hastata then, it appears that the prodissoconch I/II boundary represents a change in secretion, in support of past studies (eg. Wada, 1968). It may also represent the first time of valve closure, to support Waller's view, but this study presents no

evidence to suppose that valve closure occurs at that point.

Other pectinids examined for larval hinge morphology are P. maximus, C. varia, C. distorta, and C. opercularis (LePennec, 1980). Shell shape and hinge morphology of C. hastata larvae is similar to these species. All pectinids have hinge teeth which are symmetrical at each end with a thin cardinal ridge between which lacks cardinal teeth. C. hastata may have up to 5 - 6 denticles at each end by the time of metamorphosis whereas LePennec (1980) noted only three denticles in other pectinids. No further denticles were added after metamorphosis in C. hastata, which is similar to observations in other pectinids, except in C. opercularis which add 1 - 2 more denticles after metamorphosis.

Among C. hastata larvae proviniculum length remains constant throughout larval life. This contradicts the findings of Lutz and Hidu (1979) who examined proviniculum lengths of M. edulis and Modiolus modiolus valves. They found that proviniculum length increased with valve length and valve height by a linear relationship. The characteristic of a constant proviniculum length, combined with hinge structure and valve dimensions, may be of assistance in distinguishing C. hastata larvae from other closely related species.

A common characteristic of bivalve larval denticles is the

transverse ridges on the sides. O. edulis (Waller, 1981), C. virginica (Carriker and Palmer, 1979), C. gigas (Waller, 1981), M. edulis, and M. modiolus (Lutz and Hidu, 1979), as well as C. hastata, have transverse ridges. Lutz and Hidu (1979) suggested the ridges serve to reduce shear between the valves. A groove on the crown of each denticle of C. hastata corresponds to a small rise or bump located between each denticle of the opposing valve. No mention of these structures has been made in previous literature and of the published scanning electron micrographs of larval valves in the literature, only M. edulis (Lutz and Hidu, 1979; see plate II, Figure A) has a groove on the crown of each denticle. This crown and groove feature may further assist in the interlocking nature between opposing valves (Figure 6b).

Neither the larval ligament nor the ligament pit was observed in other larval pectinids investigated (LePennec, 1980). Lutz and Hidu (1979) suggested the ligament is a post-larval feature, ie. observed only after metamorphosis has been initiated. Larval valves of C. hastata larvae which were capable of metamorphosing (39 days old at 16°C) had a ligament pit, yet had not metamorphosed. The ligament pit may appear among larvae which are ready to metamorphose and not necessarily only among post-larvae.

Dissoconch shell of C. hastata is visibly different from, and

is secreted at a faster rate than, prodissoconch II shell. These differences probably reflect the differences in the composition of the two types of shell. Prodissoconch I shell is entirely aragonitic (Stenzel, 1964) whereas dissoconch shell is entirely calcitic (Taylor, et al., 1969).

Among C. hastata post-larvae, the left valve is always slightly larger than the right valve. Even at 12 days after metamorphosis, the right valve is about 12 um shorter than the left valve. Secretion of the dissoconch left valve is initiated before secretion of the right valve and this probably leads to the difference in size of the valves. This overlap of the left valve over the right valve has not been reported in previous literature.

The bilobe-shaped configuration of the outer margin of the velum was first noted by Elston (1980) in C. virginica larvae. Bivalve larvae probably feed by the opposed ciliated band method (Strathmann, et al., 1972; Strathmann and Leise, 1979) and this configuration probably assists in keeping particles in the food groove. The adoral cilia are probably responsible for transporting collected particles toward the mouth region, and the trough shape of the velar margin may assist in retaining particles by forming a sunken channel for the particles. As well, once particles are retained in the recessed area, they may not be

as disturbed by the ciliary currents created by the preoral and postoral bands than if the food groove was not trough-shaped.

Secretory cells among the velar cilia previously have not been described although other authors have either observed or assumed mucus was involved in collection of particles (Yonge, 1926; Erdmann, 1935; Waller, 1981; Strathmann, et al., 1972). The mechanism by which veliger larvae are believed to concentrate food particles is direct interception of particles by the preoral cilia (Rubenstein and Koehl, 1977; Strathmann and Leise, 1979). Strathmann and Leise (1979) proposed that preoral cilia overtake particles in the latter part of the effective stroke and weakly adhere to them, pushing them faster than water. Secretion granules released from the secretory cells, located between the double row of compound cilia of the preoral band and between the preoral and adoral bands, possibly enhance adhesion between the cilia and the particles and thus aid in particle collection.

In C. hastata, the postoral band is comprised of simple cilia rather than compound cilia as observed in O. edulis (Erdmann, 1935; Waller, 1981). Since postoral cilia have been examined in these two species only, it is difficult to make any sort of statement of the general form of the postoral band among bivalve veligers. Whether the cilia are compound or simple may reflect on their role or efficiency in

particle capture.

Compound cilia situated at the mouth region have not been previously described. Owing to their shape and location within the postoral band, I suggest their effective stroke is upward, toward the mouth, and therefore they are arranged in a single diaplectic row. Compound cilia arranged in a row perpendicular to the plane of beat can flex more strongly than simple cilia and are capable of a greater tip velocity (Knight-Jones, 1954). These oral compound cilia probably aid in forcing food particles toward the mouth region as they travel posteriorly along the food groove. Waller (1981) noted a postoral tuft in O. edulis which he described as rigid simple cilia, distinct from postoral cilia. He suggested the postoral tuft is sensory and may facilitate streaming of excess mucus and food posteriorly. Oral compound cilia described above are located between the postoral tuft and the mouth of C. hastata. Possibly these oral compound cilia serve a sensory function in selecting or rejecting food particles before they enter the mouth.

In the foot of C. hastata larvae, secretions of the primary byssus gland are deposited into a single lateral pouch which then leads to two byssal ducts. Some confusion exists in the literature regarding the number of byssal ducts and lateral pouches in other species. For

O. edulis (Cranfield, 1973a), P. maximus (Gruffydd, et al., 1975), and M. edulis (Lane and Nott, 1975), two lateral pouches were noted in the foot leading into a single byssal duct. Elston (1980) observed only a single lateral pouch leading into a single byssal duct in C. virginica. However, Prytherch (1934) probably observed two byssal ducts in C. virginica. He described two long tapering, ciliated ducts leading down into the foot, emptying secretions just anterior to the heel. Waller (1981) noted two byssal duct openings in early O. edulis larvae and, noting Cranfield's conclusions, suggested that the double opening may represent an early stage in the development of the byssal complex before a common duct is formed. In addition to C. hastata, other pectinid species examined, P. yessoensis (S. Bower, pers. comm., 1985) and P. maximus (Gruffydd, et al., 1975), each have only a single byssal duct. Little can be concluded about the structures of the foot between similar species until further ultrastructural examination is complete.

Luminal spaces within each primary gill filament also have been observed in C. virginica (Galtsoff, 1964; Elston, 1980) and M. edulis (Bayne, 1971). However, the authors were unable to observe any connection of these spaces to each other or with other vascular tissue. Elston (1980) noted that the spaces within the gill filaments, as well as other vascular tissues, may contain wandering ameoboid cells, suggesting a possible connection between luminal spaces. Prytherch

(1934) observed blood cells circulating to the base of the gill filaments.

The number of primary gill filaments present in C. hastata larvae was three on each gill plate; on occasion a fourth filament was observed in older larvae (over 40 days after fertilization). Prytherch (1934) observed up to eight papillae on each gill plate in C. virginica but generally authors reported three or four filaments in other bivalve larvae (eg. Bayne, 1971; Waller, 1981; Galtsoff, 1964).

No connection between opposing primary gill filaments in the form of a gill bridge, as seen in O. edulis and C. virginica, was observed in C. hastata larvae. A cluster of distinctly longer cilia was observed on the posterior-most primary gill filaments, and in 3 day old juveniles these were overlapping (Figure 7d), but no cross-fusion of cilia was observed. The gill bridge may be a feature unique to oyster species only.

Structure of the larval eyespots in C. hastata is similar to that described for M. edulis (Bayne 1971), a cup-shaped group of small pigment granules deposited in a single cell. Galtsoff (1964) described the eyespots of O. edulis as consisting of several pigmented cells arranged in a circle around a transparent lens. Hickman and

Gruffydd (1971) observed several pigment cells surrounding a lens, similar to Galtsoff, but noted that the granules were in the shape of a cup. There is little doubt that the eyespots are functional sensory organs in the larval stage; Galtsoff (1964) described nerve tracts leading to the eyespots of O. edulis, and Bayne (1964) demonstrated that the appearance of the eyespots coincided with a change in phototactic behaviour in M. edulis larvae.

CHAPTER 2 - Effect of Temperature on Growth and Survival in C. hastata Larvae

INTRODUCTION AND LITERATURE REVIEW

For species which have a planktotrophic larval stage, success and rate of larval development may be affected by physical or endogenous parameters. Endogenous factors which have been examined in other bivalve species include genetic variation between adults (Chanley, 1955), the effects of self-fertilization or inbreeding of close relatives (Beaumont and Budd, 1982; Beattie, et al., 1984), and the relationship of egg size with larval survival (Kraeuter, et al., 1982). Physical parameters which have been investigated include diet (Davis, 1953; Davis and Guillard, 1958; Walne, 1963; Loosanoff and Davis, 1963; Bayne, 1965; Jespersen and Olsen, 1982), pH (Calabrese and Davis, 1966; Calabrese, 1970), salinity (Loosanoff, 1952; Davis, 1958; Davis and Ansell, 1962), and combined effects of temperature and salinity (Loosanoff, 1948, 1952; Davis and Calabrese, 1964; Calabrese, 1969; Brenko and Calabrese, 1969; Cain, 1973; Lough and Gonor, 1973; Lough, 1975; Tettelbach, 1979; Falmagne, 1984). Among physical parameters which could affect larval growth and survival, temperature probably has been investigated most frequently. Generally temperature is one of the

first parameters examined when first investigating the larval development of a species because of its profound effect on growth and survival of larvae. Since the larval stages of C. hastata previously have not been examined, I also chose to study the effects of temperature on larval growth and survival.

Growth, and therefore length of the larval period, is affected by temperature, with more rapid growth generally occurring at higher temperatures, up to an optimum, and then declining with further temperature increase (Bayne, 1983). Larvae of Venus mercenaria had faster growth rates at higher temperatures, between 18° and 30°C, but developed abnormally at 33°C. Similarly, V. striatula larvae had an increasingly faster growth rate with increasing temperature between 5° and 16°C, but at 20°C growth rate was the same as at 16°C, and growth was retarded at temperatures above 20°C (Ansell, 1961). The larval life of M. edulis was 34 - 38 days when reared at 11°C and 16 - 20 days at 16°C (Bayne, 1965). Growth rate increased with increases in temperature, but an increase from 13° to 16°C produced a smaller increment in growth than an increase from 10° to 13°C. Loosanoff, et al. (1951) suggested that, although rate of growth was generally more rapid at higher temperatures, small temperature differences of 1 or 2°C did not have a measurable effect on growth.

Growth curves of bivalve larvae have been described as linear for Venus striatula (Ansell, 1961), Siliqua patula (Breese and Robinson, 1981), Chlamys purpurata (Disalvo, et al., 1984), and Arctica islandica (Lutz, et al., 1982), sigmoidal for V. mercenaria (Loosanoff, et al., 1951) and Crassostrea gigas (Gerdes, 1983), and exponential for Chlamys asperimus (Rose and Dix, 1984). M. edulis has been described as having both a linear (Jespersen and Olsen, 1982; Sprung, 1984a) and a sigmoidal (Bayne, 1965) growth curve and Ostrea edulis a linear (Walne, 1965), sigmoidal (Walne, 1956), and exponential (Walne, 1966) growth curve. Bayne (1983) reviewed the different types of growth curves reported in the literature and suggested that when laboratory rearing conditions are optimal, growth may be linear, or even exponential, but sigmoidal when conditions are less ideal.

Loosanoff and Davis (1963) found that C. virginica larvae survived at 10°C but did not grow. They suggested that the larvae were unable to grow at lower temperatures because certain enzymes were inactivated; larvae held at 10°C were able to ingest phytoplankton cells but were unable to digest them. Feeding activity, or grazing rate, of M. edulis larvae increased with increasing temperature (Wilson, 1980), and hence influenced growth rate.

Survival of embryonic and larval stages under various

temperature regimes has been examined for several bivalve species and larval stages tend to be more tolerant of temperature extremes than embryos (Loosanoff and Davis, 1963). M. edulis larvae can survive for at least two months at 5°C with no significant affect on mortality (Beaumont and Budd, 1982). Although larvae did not feed and therefore did not grow at this temperature, feeding and growth were resumed when larvae were returned to 17°C water.

MATERIALS AND METHODS

C. hastata larvae were reared from fertilization to metamorphosis as described in Description of Development, Materials and Methods, at 12°, 16°, 19°, and 24°C. At each temperature, four, 1500 ml vessels were maintained, each containing about 3500 - 4000 larvae in 750 ml of 0.45 um filtered seawater (31 - 33 parts per thousand). Experiments were started using D-stage veligers, three days after fertilization. Valve length and height of about 70 larvae from each of three vessels at each temperature were measured every six days. All measurements were made to the nearest 4.5 um with a compound microscope fitted with an ocular micrometer. The fourth vessel held at each temperature was sampled to estimate larval survival during development. Each vessel was agitated with a perforated plunger to ensure even distribution of larvae in the water. Twenty, 5 ml samples were collected and an average value for larval density was calculated. Larval densities were measured every six days.

RESULTS

For temperature-growth experiments, more variance was found between treatment than between replicates (Table 4) and therefore data were pooled for analysis. Initial valve length at the start of the experiment was 112.0 ± 0.85 um (Table 5, Figure 15) for all treatments. By Day 9, valve lengths between temperatures were significantly different (Duncan's Multiple Range Test, $P>0.05$; Table 6). Larvae reared at 12°C had a valve length of 238.9 ± 0.93 um by Day 39, a growth increment of 4.6 um/day between Days 3 and 31. No significant increase in valve length was noted after Day 31, yet larvae did not metamorphose until Day 42, 11 days later.

At 16°C larvae reached maximum valve length, 231.0 ± 0.84 um by Day 27, a growth rate of 4.9 um/day. Metamorphosis did not occur until Day 34, 7 days after maximum size was reached. At both temperatures, growth rate was constant during the first few weeks of development and then tapered off as maximum size was reached (Figure 16). Larvae reared at 19°C developed at a slower rate, an average of 2.9 um/day, reaching a maximum valve length of 182.2 ± 2.4 um at Day 27. Growth rate of these larvae declined over time; between Days 3 and 15 larvae grew at a rate of 3.7 um/day whereas between Days 21 and 27 growth rate was only 1.9 um/day. Length was not recorded after Day 27

Table 4 - Valve Lengths of C. hastata Larvae Reared at Different Temperatures

Day	Temperature (°C)	Replicate Number	Sample Size	Mean Valve Length (um)	Standard Deviation	Minimum Valve Length (um)	Maximum Valve Length (um)
3	all	all	70	112.0	3.6	102.1	119.8
9	12	1	70	134.1	3.7	128.8	142.1
		2	10	129.6	5.8	119.9	137.6
		3	70	132.2	3.5	124.3	142.1
	16	1	49	142.5	6.0	124.3	150.9
		2	53	141.2	6.2	128.8	151.0
		3	63	141.2	5.8	124.3	151.0
	19	1	70	141.4	6.1	124.3	155.4
		2	70	137.5	6.2	124.3	151.0
		3	70	134.7	5.9	120.0	146.5
	24	1	17	122.8	2.7	119.9	128.8
		2	12	123.9	3.5	115.4	128.8
		3	8	123.2	5.7	115.4	133.2
15	12	1	70	163.6	6.0	151.0	177.6
		2	70	158.4	5.4	146.5	173.2
		3	70	160.0	5.8	146.5	168.7
	16	1	70	175.2	7.6	159.8	195.4
		2	70	176.0	6.8	159.8	186.5
		3	70	177.3	7.3	164.3	195.4
	19	1	70	155.3	8.9	137.6	177.6
		2	70	157.0	9.3	137.6	177.6
		3	70	157.3	10.4	142.1	186.5
21	12	1	70	191.4	10.8	168.7	217.6
		2	70	187.2	9.4	168.7	204.2
		3	70	186.2	7.9	168.7	208.7
	16	1	70	208.5	12.3	182.0	230.9
		2	70	211.0	11.9	190.9	235.3
		3	70	215.0	10.8	182.0	235.2

Table 4 - (continued)

Day	Temperature (°C)	Replicate Number	Sample Size	Mean Valve Length (um)	Standard Deviation	Minimum Valve Length (um)	Maximum Valve Length (um)
21	19	1	70	171.7	11.5	151.0	204.2
		2	70	172.1	13.5	146.5	199.8
		3	70	167.7	9.9	142.1	186.6
27	12	1	70	221.1	15.2	177.6	248.6
		2	70	220.9	10.7	190.9	239.8
		3	70	218.1	10.6	186.5	235.3
	16	1	70	228.2	6.9	208.7	239.8
		2	70	226.1	8.8	190.9	248.6
		3	70	230.3	7.1	208.7	244.2
	19	1	70	182.9	16.9	151.0	226.4
		2	70	181.5	13.5	155.4	222.0
		3	70	182.0	14.7	159.8	217.6
31	12	1	70	232.9	11.7	195.4	253.1
		2	70	237.9	8.0	217.6	257.5
		3	70	235.1	8.5	213.1	253.1
	16	1	70	229.6	6.4	213.1	244.2
		2	70	231.3	6.0	222.0	244.2
		3	70	232.2	6.0	217.6	244.2
39	12	1	70	239.1	5.9	226.4	253.1
		2	70	240.3	7.7	222.0	257.5
		3	70	237.3	6.7	222.0	248.6
	16	1	70	230.0	5.0	217.6	239.8
		2	70	230.8	6.3	217.6	244.2
44	12	1	70	239.6	6.4	222.0	253.1
		2	70	239.8	6.8	222.0	253.8
		3	70	240.3	6.6	226.4	253.1
	16	1	70	230.5	6.3	213.1	244.2
		2	70	231.7	5.6	222.0	248.6
		3	70	231.5	6.0	222.0	244.2

Table 5 - Valve Lengths of C. hastata Larvae Reared at Different Temperatures, Pooled Replicates

Day	Temperature (°C)	Sample Size	Mean Valve Length (um)	Standard Deviation	Minimum Valve Length (um)	Maximum Valve Length (um)	95% Confidence Interval
3	all	70	112.0	3.6	102.1	119.8	0.85
9	12	150	133.2	3.9	119.9	142.1	0.63
	16	165	141.5	6.0	124.3	150.9	0.92
	19	165	137.9	6.6	119.8	155.4	0.90
	24	37	123.2	3.5	115.4	133.2	1.14
15	12	210	160.7	6.0	146.5	177.6	0.82
	16	210	176.2	7.3	159.8	195.4	0.98
	19	210	156.6	9.5	137.6	186.5	1.29
21	12	210	188.3	9.7	168.7	217.6	1.31
	16	210	211.5	11.9	182.0	235.3	1.61
	19	210	170.5	11.8	142.1	204.2	1.60
27	12	210	220.0	12.4	177.6	248.6	1.67
	16	210	228.2	7.8	190.9	248.6	1.06
	19	210	182.2	15.1	151.0	226.4	2.04
31	12	210	235.3	9.7	195.4	257.5	1.31
	16	210	231.0	6.2	213.1	244.2	0.84
39	12	210	238.9	6.9	222.0	257.5	0.93
	16	140	230.4	5.7	217.6	244.2	0.77
44	12	210	239.9	6.6	222.0	253.1	0.89
	16	210	231.2	5.9	213.1	248.6	0.81

Figure 15 - Valve lengths of C. hastata larvae reared at different temperatures

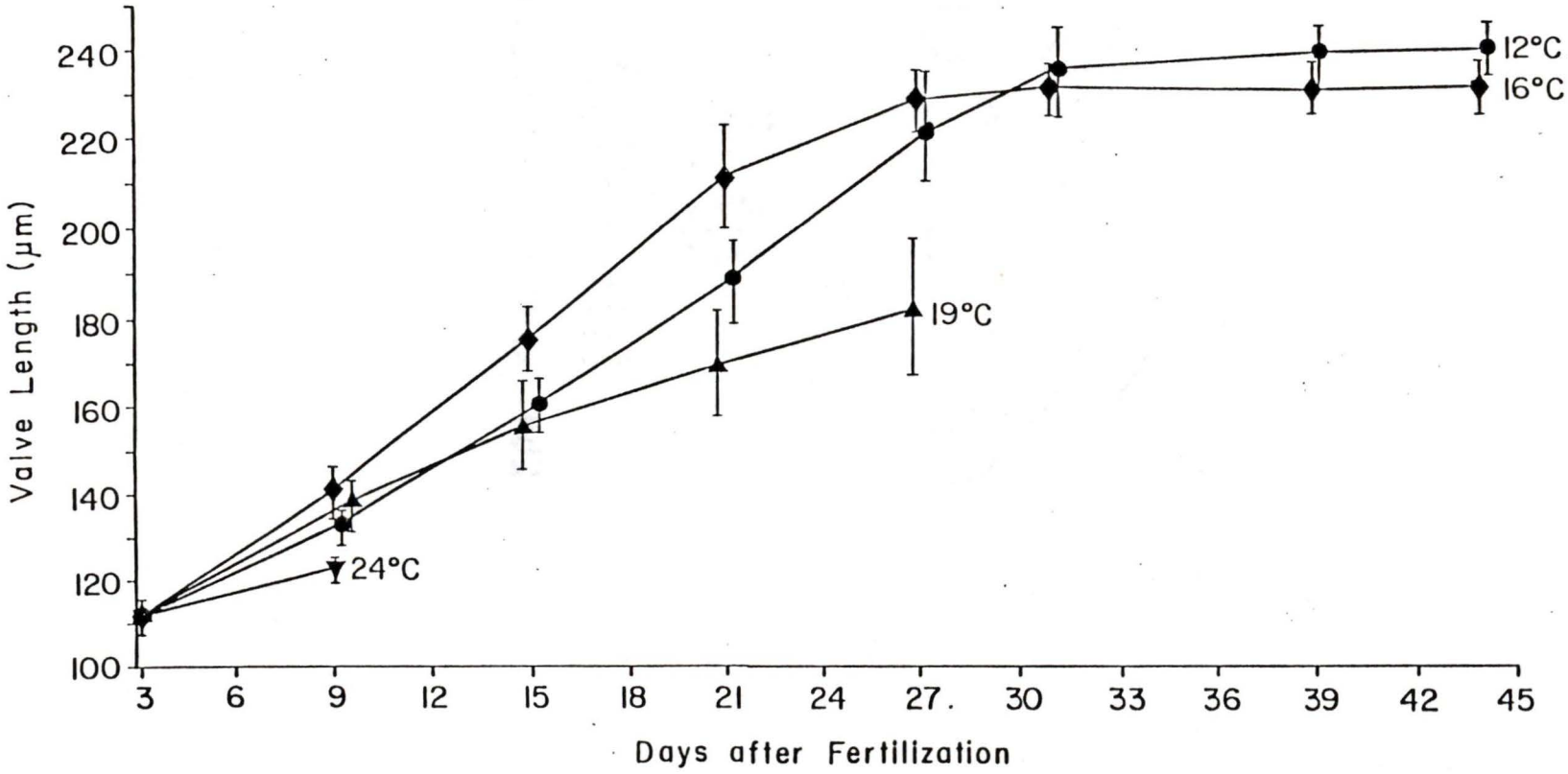
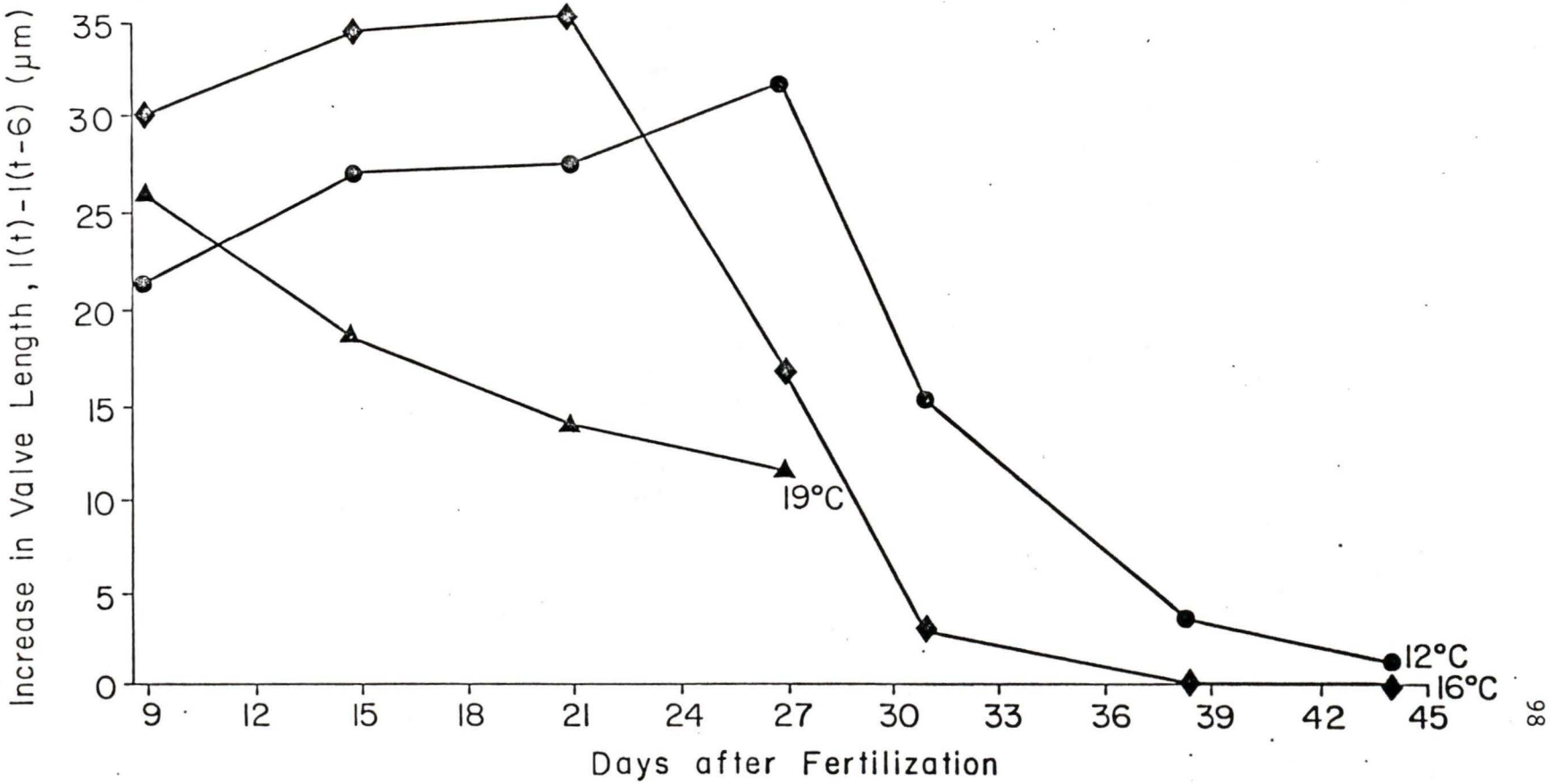


Table 6 - Results of Duncan's Multiple Range Test for Valve Lengths of C. hastata Larvae 9 Days After Fertilization, Reared at 4 Temperatures

Duncan Grouping	Temperature (°C)	Mean Valve Length (um)	Sample Size (n)
A	16	141.6	165
B	19	137.9	210
C	12	133.2	150
D	24	123.2	37

Values with different Duncan Groupings are significantly different ($\alpha = 0.05$).

Figure 16 - Instantaneous Growth Rates of C. hastata Larvae Reared at Different Temperatures



because of low larval numbers. Larvae reared at 24°C did not develop beyond the D-stage.

Using valve height as an indicator of growth produces curves similar to those with valve length (Table 7; Figure 17). Initial valve height at Day 3 was 85.9 ± 0.75 um for all treatments. At 12°C larvae reached a maximum height of 215.7 ± 3.1 um by Day 39, a growth rate of 3.6 um/day. Valve heights at Day 39 and 44 were not significantly different (Student's T-test, $P>0.05$). At 16°C larvae attained a maximum height of 209.9 ± 2.2 um by Day 31, a growth rate of 4.4 um/day. Again, valve height was not significantly different between Days 31 and 44.

Valve length corresponds to height by a linear correlation (Figure 18, $r^2 = 0.87$) with a ratio of 1.1 : 1 for length vs. height. If values are separated by temperature, both regressions have similar slopes, 0.95 for 12°C and 0.99 for 16°C (Figure 19, 20).

During embryonic development, most cultures were maintained at 12° to 14°C and reached the trochophore stage by 30 hours. To determine if embryos could tolerate a slightly elevated temperature, approximately 100,000 fertilized oocytes were held at 16°C. These reached early gastrula stage within 17 hours but all died before they reached the trochophore stage.

Table 7 - Valve Heights of *C. hastata* Larvae Reared at Different Temperatures

Day	Temperature (°C)	Sample Size	Mean Valve Height (um)	Standard Deviation	Minimum Valve Height (um)	Maximum Valve Height (um)	95% Confidence Interval
3	all	70	85.9	3.1	79.9	93.2	0.75
9	12	150	110.3	4.7	98.6	119.9	0.75
	16	13	116.1	4.0	106.6	120.0	2.41
	19	30	117.2	6.1	97.7	128.7	2.29
15	12	30	139.7	6.5	128.8	151.0	2.41
	16	40	145.6	11.7	106.6	159.8	3.77
	19	30	144.4	16.6	120.0	177.6	6.21
21	12	30	172.6	12.4	146.5	195.4	4.84
	16	20	166.5	23.1	133.2	199.8	10.79
	19	20	180.0	27.7	142.1	217.6	12.98
27	12	35	202.6	11.0	177.6	222.0	3.81
	16	35	190.3	26.2	137.6	217.6	9.01
	19	35	173.9	28.8	137.6	217.6	9.94
31	12	35	209.6	9.8	186.5	226.4	3.38
	16	35	209.9	6.2	199.8	226.4	2.15
39	12	35	215.7	8.9	195.4	230.9	3.07
	16	35	209.2	6.8	190.9	226.4	2.34
44	12	35	214.6	7.8	195.4	217.6	2.68
	16	25	206.2	6.0	195.4	217.6	2.49

Figure 17 - Valve heights of C. hastata larvae reared at different temperatures

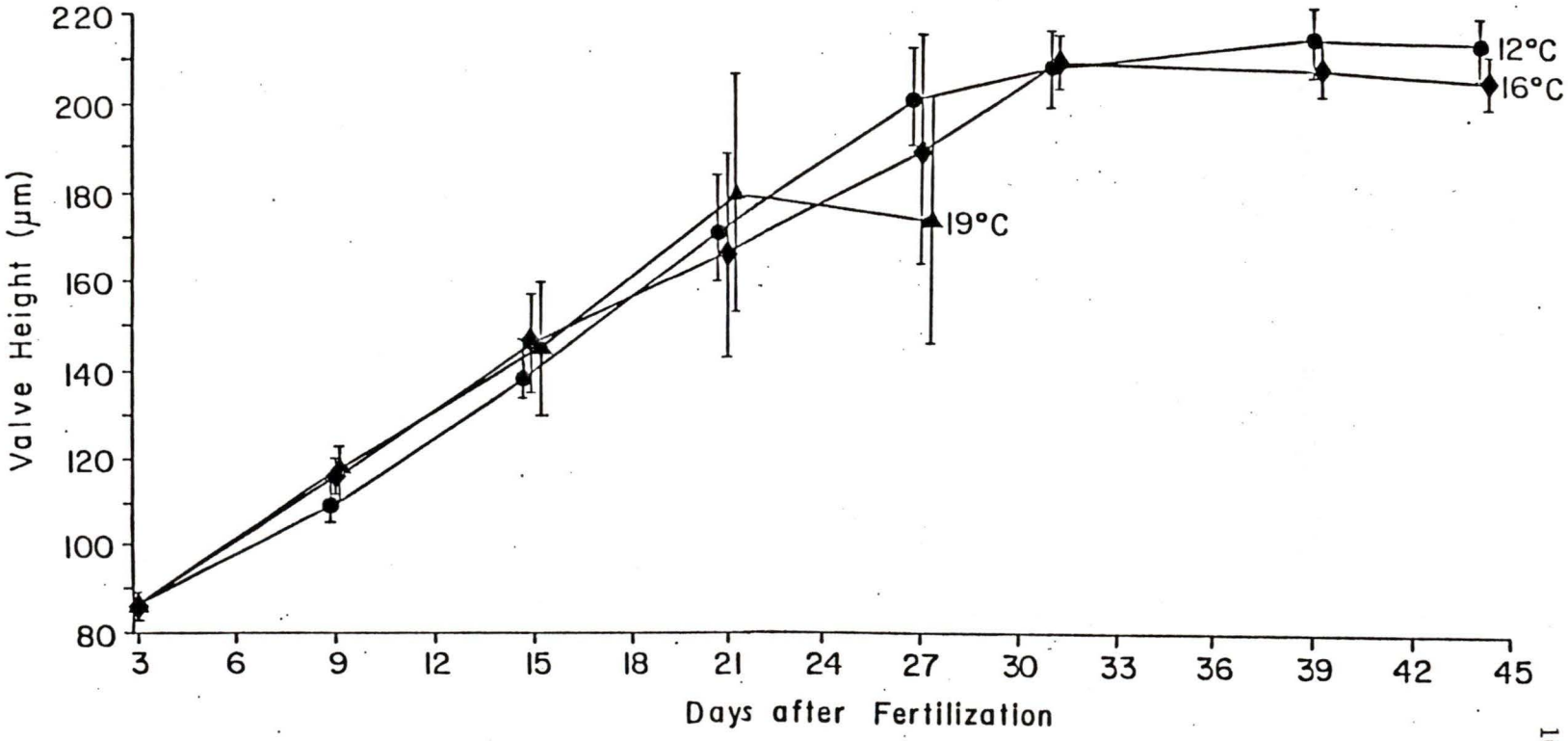


Figure 18 - Relationship between valve length and valve height for C. hastata larvae

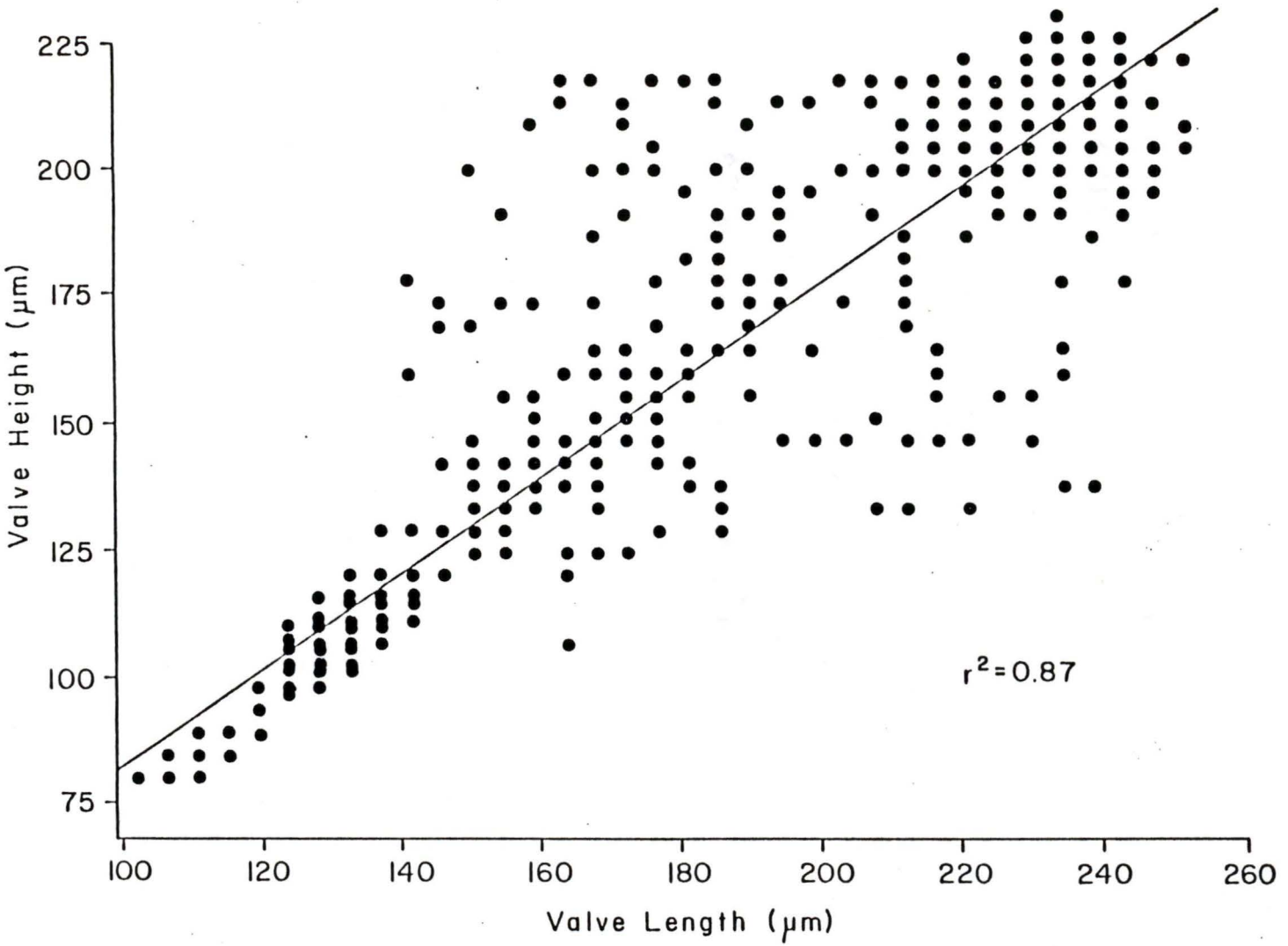


Figure 19 - Relationship between valve length and valve height for C. hastata larvae reared at 12°C

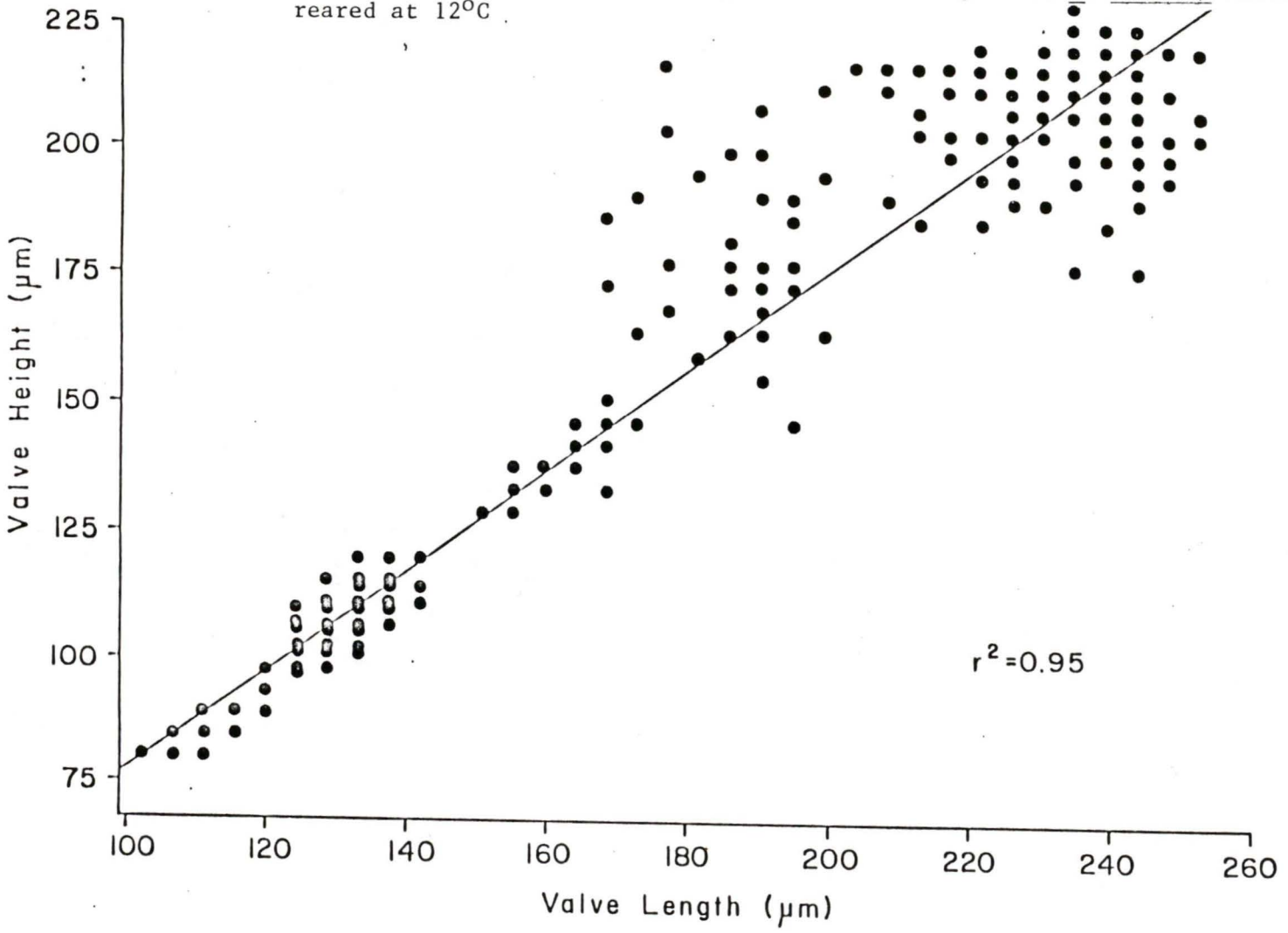
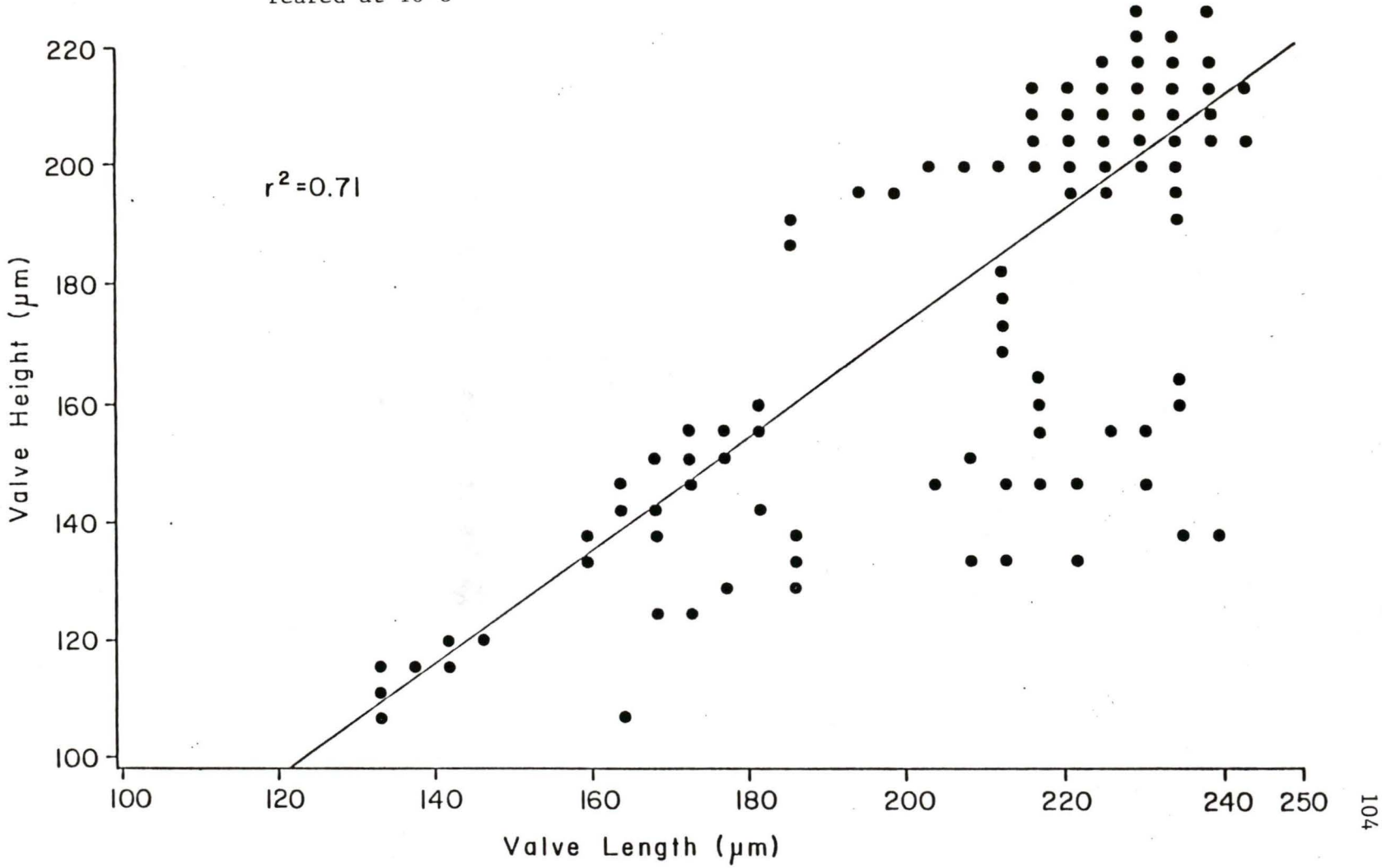


Figure 20 - Relationship between valve length and valve height for C. hastata larvae reared at 16°C

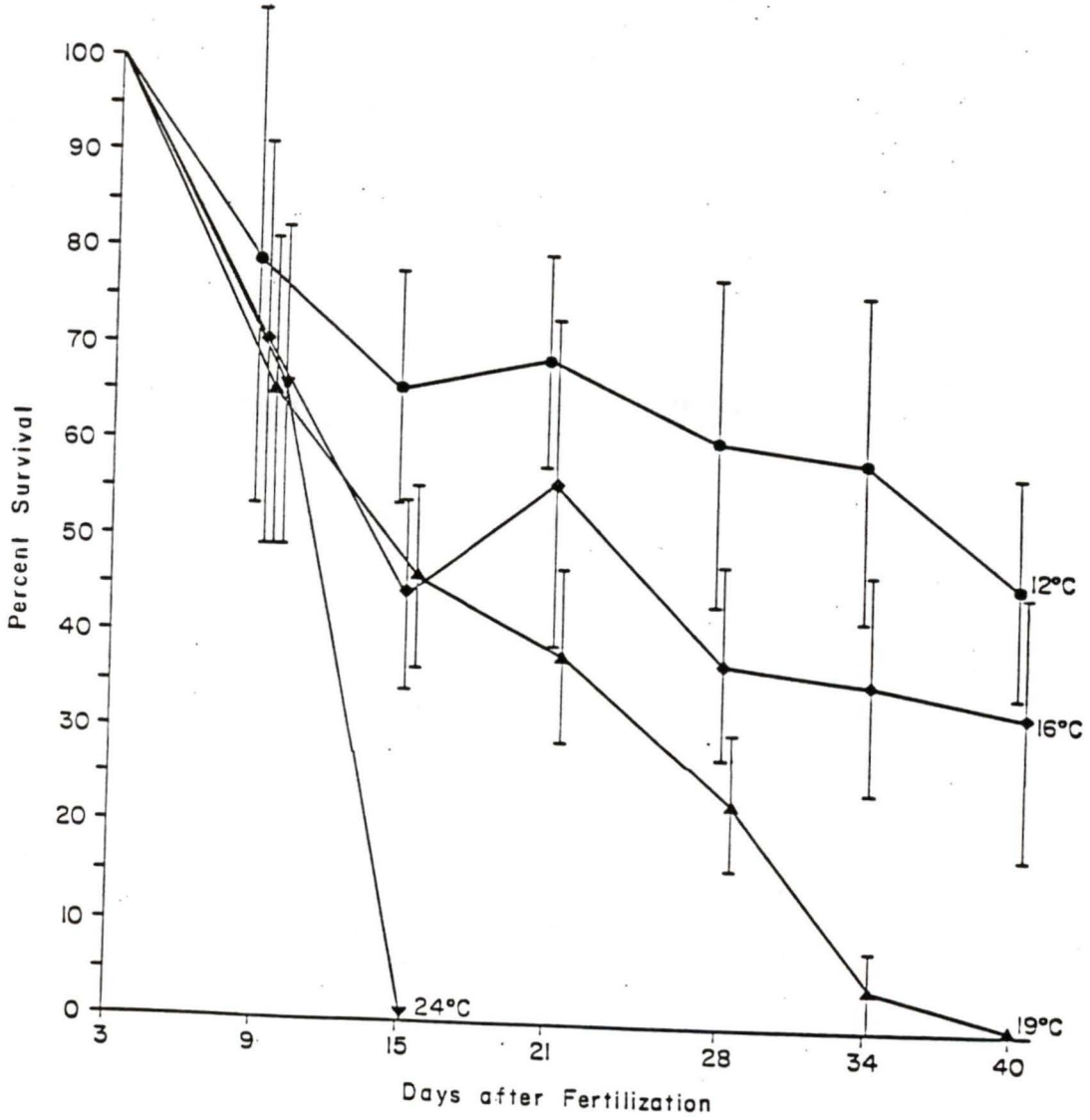


Larval survival was also influenced by temperature (Table 8; Figure 21). Initial larval density was 5 - 6 larvae/ml. This value was designated as 100 % survival in Figure 21 and all other estimates were recorded as proportions of the original density. At all temperatures, percent survival dropped sharply during the first two weeks, and then leveled off to a slower mortality rate. At 12°C, survival dropped to 60 % (3.5 larvae/ml) by Day 15, but from Day 15 to Day 40 larval density remained fairly constant, with 42 % survival by Day 40. At 16°C, survival dropped to 44 % (2.3 larvae/ml) by Day 15, which then declined to 33 % by Day 40. Larvae reared at 16°C had a lower survival than those reared at 12°C (Students' T-test, $P>0.001$). At 19°C, 41 % of the larvae survived to Day 15. Survival then continued to decline until all larvae were dead by Day 38. Larvae reared at 24°C did not survive beyond Day 15.

Table 8 - Density of C. hastata Larvae Reared at Different Temperatures (number of larvae per ml.)

Day	Temperature (°C)	Sample Size	Mean Density (larvae/ml.)	Standard Deviation	Minimum Density	Maximum Density	95% Confidence Interval
3	12	9	5.78	1.21	3.4	7.8	0.91
	16	10	5.28	0.77	4.0	6.4	0.54
	19	18	6.09	0.90	4.2	7.6	0.45
	24	10	4.48	0.81	3.4	6.0	0.56
9	12	20	4.15	1.40	2.0	7.0	0.65
	16	20	3.59	1.35	1.8	6.4	0.63
	19	20	3.36	1.11	1.0	6.0	0.52
	24	20	2.96	0.83	1.6	4.2	0.39
15	12	20	3.50	0.66	2.6	5.0	0.31
	16	20	2.34	0.52	1.6	3.4	0.24
	19	20	2.45	0.52	1.2	3.0	0.24
	24	20	0.00	-	-	-	-
21	12	20	3.62	0.59	2.6	4.6	0.27
	16	20	2.98	0.92	1.8	4.4	0.43
	19	20	2.02	0.49	1.4	3.0	0.23
28	12	20	3.21	0.92	1.8	4.8	0.43
	16	20	2.00	0.55	1.2	2.8	0.25
	19	20	1.22	0.39	0.4	1.8	0.18
34	12	20	3.12	0.93	1.4	5.2	0.43
	16	20	1.88	0.61	1.0	3.0	0.28
	19	20	0.25	0.25	0.0	1.0	0.12
40	12	20	2.43	0.63	1.6	3.6	0.29
	16	20	1.73	0.68	0.8	3.4	0.31
	19	20	0.00	-	-	-	-

Figure 21 - Percent survival of C. hastata larvae reared at different temperatures



DISCUSSION

Embryonic development in C. hastata is more sensitive to changes in temperature than larval stages. Although larvae held at 16°C had the fastest growth rate, embryos reared at 16°C all died. Tettelbach (1979) noted that embryonic stages of A. irradians were more sensitive to temperature and salinity than larval stages and could only survive within a limited range. Generally, embryos reared at higher temperatures reached ciliated blastula and sometimes early gastrula but development beyond this was abnormal.

Development of C. hastata larvae followed a linear growth pattern during the first 20 - 25 days (12° or 16°C) but then leveled off just prior to the pediveliger stage. Bayne (1965) and Gerdes (1983) suggested that the decline in growth rate as the pediveliger stage is reached is caused by a decline in feeding rate owing to the degeneration or decrease in size of the velum. However, the velum of C. hastata does not begin to show visible signs of degeneration until about 95 days after fertilization, well after growth has ceased (see Figure 8a) and so there appears to be no relationship between the decline in rate of increase of valve length and the ability to feed in C. hastata larvae. Sprung (1984c) suggested that growth may be affected by a proportionally larger amount of energy being utilized for swimming in larger larvae.

This may account for the gradual decline in growth rate noted between Day 20 - 25 and Day 30 but does not entirely explain why an increase in valve length ceases altogether after Day 30. Even larvae which survived as long as 100 days had the same valve length as larvae only 30 days old. It appears that a maximum size is reached and no further energy is expended in shell secretion.

Although valve length may be a useful tool to observe a relative increase in size, it is not an exact measurement of increase in biomass. Increase in valve length, or height, traditionally has been the method used to measure larval growth (see Loosanoff and Davis, 1963; Bayne, 1983) and is a convenient measurement to make. Tissue dry weight (W) of bivalve larvae can be expressed as a power function of valve length (L), where $W = a \cdot L^b$ (Riisgard, et al., 1980; Nasciomento, 1980; Jespersen and Olsen, 1982; Sprung, 1984a). However, differences in tissue weight of larvae with the same valve length may exist. When starved for 48 hours, 4 and 8 day old O. edulis larvae lost 17.5 % and 13.8 % of total organic weight respectively, yet valve length remained constant or increased slightly (Holland and Spencer, 1973). Mya arenaria larvae reared in the presence of chloramphenicol, an antibiotic, grew from 75 um to 220 um valve length in 12 days but did not show and increase in body mass (Gustafson, 1980, from Bayne, 1983). Valve length probably provides a reasonable estimate of growth and can

be a useful tool to monitor growth in bivalve larvae as long as its limitations are recognized.

Larvae reared at 12°C developed slower and reached a significantly larger maximum size than those reared at 16°C (Student's T-test, $P>0.001$; Figure 16). An inverse relationship between valve length and developmental time also has been observed in larvae of M. edulis (Bayne, 1965), C. fornicata (Pechenik and Lima, 1984) and C. plana (Lima and Pechenik, 1985). Bayne (1965) suggested that the time available for feeding among larvae reared at higher temperatures was not sufficient despite the faster rate of growth and therefore a smaller maximum valve length is reached. However, as mentioned above, maximum valve length is reached long before larvae are no longer able to feed, and so it is unlikely that the smaller size is a result of dietary constraints. Rather, the increased respiration of larvae reared at higher temperature may not be adequately compensated by increased ingestion rate (Sprung, 1984b). Thus, a greater portion of energy assimilated is used to satisfy metabolic needs rather than growth and accumulation or reserves.

Initial drop in density observed in all cultures may have been due to overcrowding and hence was independent of the temperature at which larvae were reared. Initial densities were 5 - 6 larvae/ml and,

although other larvae can survive at those densities (eg. Jespersen and Olsen, 1982; Disalvo, et al., 1984), it appears that C. hastata larvae may have better survival if reared at densities no more than 3 - 4 larvae/ml. Density- dependent mortality has been observed with other bivalve larvae (Loosanoff and Davis, 1963; Gruffydd and Beaumont, 1972).

Increase in valve length corresponds to a proportional increase in valve height (1.1 : 1). This relationship is independent of temperature even though growth rates and maximum valve length vary. Knowledge of this relationship, combined with detailed examination of hinge morphology, may be a useful tool for identifying C. hastata larvae from plankton samples.

CHAPTER 3 - Factors Affecting Settlement and Metamorphosis in *C. hastata* Larvae

INTRODUCTION AND LITERATURE REVIEW

Upon completion of larval development, invertebrate larvae undergo metamorphosis into a juvenile. A lengthy literature describes how settlement and metamorphosis in benthic invertebrate larvae are affected by a variety of stimuli, such as surface texture and contour, chemical inducers, bacterial films, and adult prey species (Meadows and Campbell, 1972; Crisp, 1974). Metamorphosis is a dramatic morphological and physiological reorganization and usually marks a complete change in the lifestyle of the organism. Often the larval stage is the only motile stage for benthic marine invertebrates, which then spend the remainder of their lives where they settled and metamorphosed as larvae. It is generally agreed that larvae settle and metamorphose nonrandomly and usually in areas suitable for adult life (Meadows and Campbell, 1972; Crisp, 1974; Burke, 1983). If a site for settlement and metamorphosis is not located, larvae of many invertebrate species may delay metamorphosis until conditions are more suitable for the completion of this important process (Thorson, 1946; Wilson, 1960; Crisp, 1974).

One factor which has been shown to influence the rate of settlement and metamorphosis in invertebrate larvae is a film of micro-organisms on a surface. Larvae of O. edulis (Cole and Knight-Jones, 1949; Walne, 1974), Spirorbis borealis (Knight-Jones, 1951; Meadows and Williams, 1963) and Watersipora cucullata (Wisely, 1958) will settle and metamorphose more frequently on a surface that has a bacterial film than on cleaned surfaces. A bacterial or primary film probably is formed by three processes: 1) the attachment of micro-organisms, 2) production by these micro-organisms of extra-cellular metabolites and slimes which adsorb to the surface, and 3) adsorption of organic materials from seawater (Meadows, 1964). Within minutes of being placed in natural seawater inert surfaces are likely to become colonized by micro-organisms (ZoBell, 1939; Crisp, 1974).

Water movement has been demonstrated to influence settlement behaviour in some marine invertebrates. Relative movement of water over a surface stimulated cyprid larvae of Balanus balanoides to settle a substrate and begin exploration (Crisp and Meadows, 1962). Crisp (1955) suggested that cyprid larvae swim along the surface against the water current and attachment occurs at or below velocity gradients where they are able to maintain position over a surface. When there was no relative movement of water, a lower frequency of settlement was observed (Crisp, 1955).

Contours of the substrate also influence settlement and metamorphosis. For example, larvae of B. balanoides preferred to settle and metamorphose in pitted or grooved depressions, or on rough surfaces (Crisp and Barnes, 1954). Among pectinids, most studies have dealt with substrate preferences of larvae during settlement and metamorphosis. Monofilament gill-netting is used primarily for the commercial collection of P. yessoensis spat, but palm bark, scallop shells, and polyethylene film are also used (Motoda, 1977). Larvae of C. opercularis settled on a wide range of natural and artificial material, provided they were silt-free (Brand, et al., 1980). In a survey of waters near the Isle of Man, Brand, et al. (1980) found most C. opercularis spat attached to bryozoans of the genus Cellaria. They did not determine whether a chemical from Cellaria sp. induced settlement and metamorphosis of the scallop. In the laboratory, P. magellanicus larvae settled on the underside of shell fragments or small pebbles (Culliney, 1974). A. gibbus larvae settled on unlaid polyethylene line and filamentous hydroids (Allen, 1979). P. maximus larvae settled and metamorphosed on filamentous red algae (Gruffydd and Beaumont, 1972).

Invertebrate larvae develop until competent to metamorphose but then may prolong larval life until a proper stimulus or stimuli for

metamorphosis is encountered (Crisp, 1974; Bayne, 1965; Sastry, 1979). Culliney (1974) noted that P. magellanicus did not metamorphose unless shell fragments or small pebbles were introduced into the experimental vessels. In some species, if the larva does not encounter the proper stimulus over a long period of time, its "selectivity" declines and it will eventually metamorphose presumably in the absence of a stimulus (see Crisp, 1974). Larvae of other species will metamorphose only if the proper cue is encountered and will die as larvae if the cue is not received (Bayne, 1965). In the laboratory, M. edulis larvae did not metamorphose unless a filamentous substrate was introduced to the experimental vessels (Bayne, 1965). Bayne described three morphological stages in M. edulis larvae that were denied a suitable substrate. In the first stage, the pediveliger was uninhibited in its feeding and swimming behaviour. Over time, however, the velum gradually degenerated, proceeding from the posterior edge of the velum forward. The grazing rate declined owing to gradual degeneration of the velum. As well, the pediveliger was only able to swim weakly, and primarily swim near the bottom. The foot increased in length and moved anteriorly. Ultimately, in the third stage, the velum was reduced to a small mass of ciliated cells lying under the anterior adductor muscle. The pediveliger was unable to swim or feed. A pediveliger at any of these stages was capable of settling and metamorphosing if a filamentous substrate was introduced. M. edulis larvae were able to survive as

pediveligers for up to six weeks before dying. This ability to remain physiologically ready to metamorphose for a long period of time may enhance the chance of larvae encountering a suitable substrate for metamorphosis (Bayne, 1965).

This study was undertaken to determine what factors affected rate of settlement and metamorphosis in C. hastata larvae. In particular, physical parameters examined included water flow, microbiol films on surfaces, and contours of the surface. As well, observations were made of larvae that were capable of metamorphosing but did not.

MATERIALS AND METHODS

C. hastata larvae were reared from fertilization to metamorphosis as described in Description of Development, Materials and Methods. Experiments were carried out in 100 x 50 mm Pyrex dishes containing 100 ml of 0.45 um filtered seawater (16°C) using 50 larvae about 40 days old. Only larvae reared at 16°C were used unless otherwise stated. Metamorphosis was estimated by counting the number of larvae firmly attached to a surface 24 hours after onset of the experiment and this estimate was confirmed by counting the number of metamorphosed individuals, or postlarvae, 2 days after the experiment.

A selection of substrates, pieces of adult shell, a 'fouled substrate', and polypropylene line, and chemicals, GABA and L-Dopamine, were tested. Pieces of adult shell were scrubbed and autoclaved prior to use. Pieces of polypropylene line were frayed and added to test dishes. Pieces of glass slide or paraffin plastic (Parafilm) were placed in running seawater for approximately 10 days to allow colonization of micro-organisms. These were used to test the influence of a fouled surface on metamorphosis. The Wilcoxan matched-pair sample test was applied to test for significant differences between test and control (clean glass slide) containers. Concentrations of GABA (10^{-3}, 10^{-4}, 10^{-5}M) and L-Dopamine (10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}M) were added to test

dishes. Adult extract was prepared by grinding and centrifuging pieces of adult tissue in a small quantity of filtered seawater. Small aliquots (5 - 10 ml) of the resulting supernatant were added to experimental dishes. Control test dishes contained 50 larvae in 0.45 um filtered seawater only.

To determine the effect of flowing seawater on metamorphosis, groups of 50 larvae were placed in nitex cups (250 ml plastic beakers with the bottom removed and replaced with 102 um nitex screen) through which a slow, continuous flow of 1 um glass-filtered seawater passed. Control larvae were placed in nitex cups set in Pyrex dishes filled with filtered seawater. Water in the control containers was changed at least once a day. The temperature for all containers was 12°C. Metamorphosis was estimated as described above and tested using the Wilcoxon matched-pair sample test.

The influence of a combination of fouled surface and water flow on metamorphosis was tested using a 2-factor analysis design. Groups of 50 larvae were placed in Nitex cups and provided with a flow of seawater, a fouled surface (glass slide with a flora of micro-organisms), or both. Larvae placed in nitex cups set in Pyrex containers served as controls.

The time when C. hastata larvae are first able to metamorphose was determined by placing groups of 50 larvae of a known age with a stimulus or stimuli that was shown to induce metamorphosis by the above experiments. Larvae reared at 12°C and 16°C were used and ranged in age from 22 days to 45 days after fertilization. Metamorphosis was estimated as described above.

Larvae of different ages, between the time when they are able to metamorphose and maximum age, were tested to see if the ability to metamorphose was affected by the age of the larva. Larvae reared at 12°C and 16°C were used and metamorphosis was estimated as described above.

RESULTS

Settlement and metamorphosis was only observed on a fouled surface (Table 9). In addition to a fouled surface, a flow of water also influenced settlement and metamorphosis (Table 10; $P>0.0001$) and a combination of flow and fouled surface was most effective in inducing larvae to settle and metamorphose (Table 11; $P>0.01$).

GABA and L-Dopamine, two neurotransmitters which have been shown to induce metamorphosis in other invertebrate larvae (Morse, et al., 1976; Cooper, 1981), were not effective in causing C. hastata to metamorphose and when higher concentrations of the chemicals were tested (10^{-4} and 10^{-5}M), they were found to be toxic to larvae. No larvae metamorphosed when placed in containers with pieces of adult shell, adult extract, or strands of polypropylene line (Table 9).

Larvae settled on surfaces in a non-random distribution such that more larvae settled along edges and corners than on planar surfaces; in the nitex cups, larvae tended to settle in the corners rather than on the screen or walls of the cup, and on slides larvae attached along edges rather than on planar surfaces. In one experiment, less than 2 % of the larvae settled and metamorphosed on surfaces other than the edge of the slide, indicating a non-random settling pattern

Table 9 - Metamorphosis of C. hastata Pediveligers when Different Chemicals or Substrates are Used

Chemical or Substrate	Number of Trials (50 larvae per trial)	Percent Metamorphosis
Control	2	0
GABA, 10^{-5}M	3	0
10^{-4}M	3	0
10^{-3}M	3	0
L-Dopa, 10^{-6}M	1	0
10^{-5}M	1	0
10^{-4}M	1	0
10^{-3}M	1	0
Adult Shell	2	0
Adult "Extract"	2	0
Polypropylene Line	3	0
Fouled Surfaces	4	5

Table 10- Results of Wilcoxon Paired-Sample Test for the Effect of Fouled Surface or Flowing Seawater on Percent Metamorphosis of C. hastata Pediveligers

Percent metamorphosis in prescence or absence of fouled surfaces	
Present	Absent
53.2	26.0
14.0	2.0
10.0	0.0
30.0	11.4
12.5	6.0
2.0	2.0
2.0	0.0
4.4	0.0
2.0	0.0
9.1	0.0
13.0	0.0
59.0	5.7
40.5	6.6

N = 13

H_o: Percent metamorphosis is independent of substrate

Reject H_o P>0.0001

Percent metamorphosis in prescence or absence of flowing seawater	
Present	Absent
45.2	2.0
26.0	6.0
33.3	2.0
5.7	0.0
40.5	0.0
6.6	0.0
29.0	0.0
11.4	0.0
53.2	12.5
14.0	2.0
10.0	0.0
20.6	0.0
43.0	0.0
30.0	4.4

N = 14

H_o: Percent metamorphosis is independent of flow

Reject H_o P>0.001

Table 11 - Effect of Flowing Seawater and Fouled Surface on Percent Metamorphosis of *C. hastata* Pediveligers

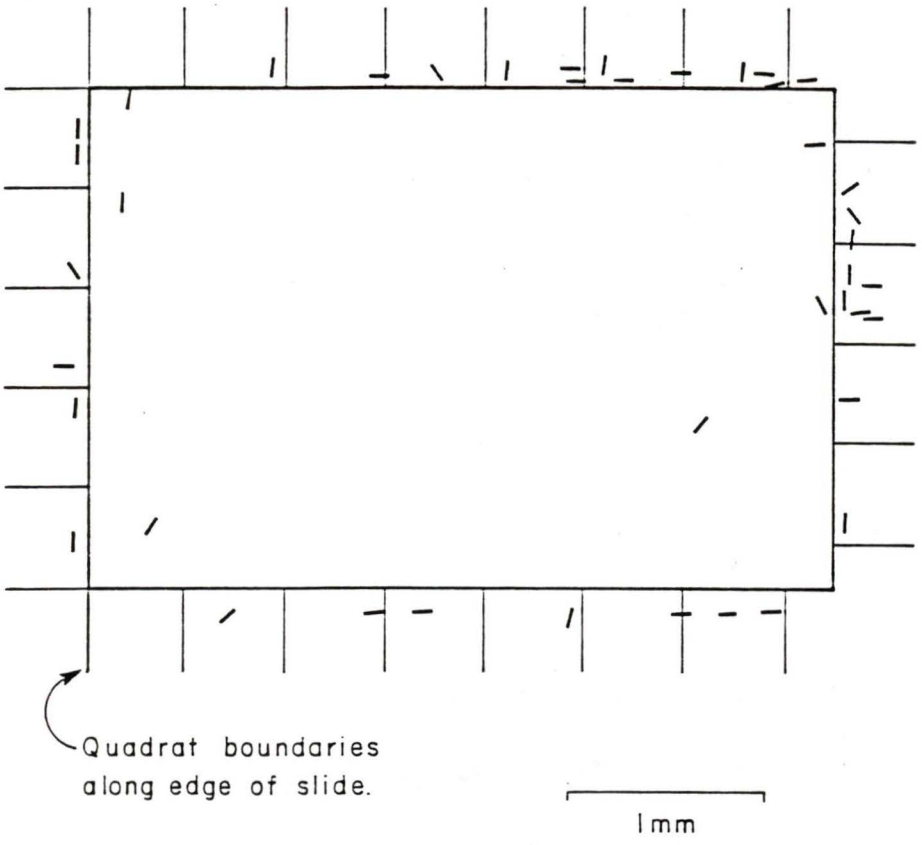
Source	F Value	P>F
Flow	18.1	0.0006
Surface	11.3	0.0039
Flow*Surface	8.3	0.0109

Two-way Analysis of Variance, unequal n

(Figure 22). The area along the edge of the slide was divided into quadrats and the resulting frequency distribution indicated a random distribution of larvae (Figure 23; $P>0.05$).

Success of settlement and metamorphosis was influenced by age of larvae tested. Larvae reared at 16°C were competent to metamorphose 34 days after fertilization whereas those reared at 12°C were competent to metamorphose at 42 days. A decline in percent metamorphosis toward the limit of maximum age of larvae was observed for larvae reared at both temperatures.

Figure 22 - Distribution of Attached and Metamorphosed C. hastata Postlarvae on Planar Surface and Edges of Glass Slide. Area Along Edges of Slide was Divided into 25 0.5mm Quadrats. For Explanation, see Figure 24.

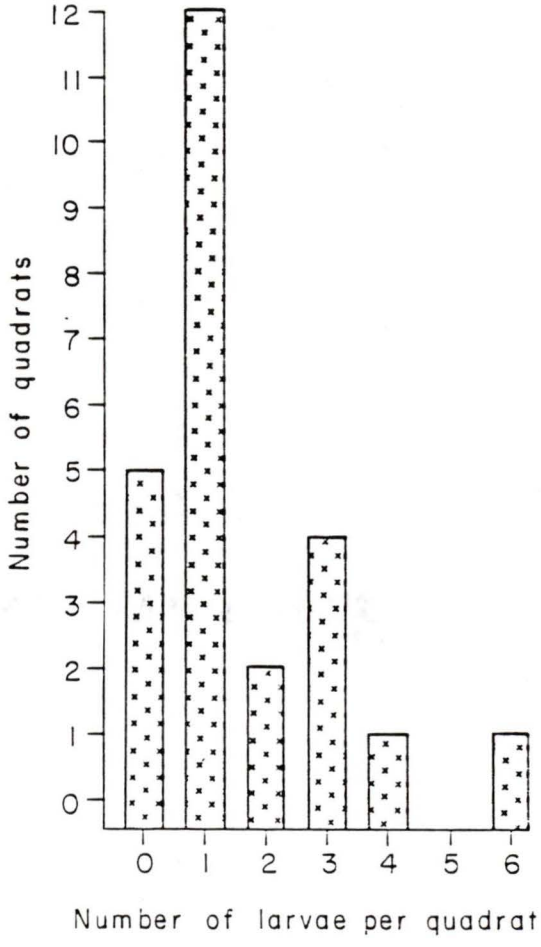


-- - Denotes position and orientation of postlarva on slide

Figure 23 - Frequency Distribution of C. hastata Postlarvae Along Edges of Glass Slide (shown in Figure 23). Area along edges of slide was divided into 25 0.5mm quadrats and number of postlarvae per quadrat was tested for agreement with a random distribution (Poisson series) using X^2 test.

n = 31

P < 0.05, Distribution is random



DISCUSSION

Settlement and metamorphosis in C. hastata probably does not occur spontaneously; rather, events are initiated by a stimulus or combination of stimuli. This hypothesis is supported by observations that show metamorphosis could be delayed for an extended period of time and larvae would die without metamorphosing. As well, settlement and metamorphosis were influenced by the presence or absence of two physical factors tested in this study: water flow and fouled surfaces.

The observation that C. hastata larvae settle and metamorphose in response to either a fouled surface or a flow of seawater suggests that a combination of stimuli induce settlement and metamorphosis and not just a single, specific cue. Perhaps a series of stimuli must be present before settlement and metamorphosis is initiated. The relatively low numbers of metamorphosed individuals in the experiments, even when both factors were present ($\bar{X}$ = 31.6 %, n = 12) suggests that settlement and metamorphosis may be effected by some other factor or factors not considered in this study.

A physical movement of water past a larva influenced success of settlement and metamorphosis, suggesting that physical factors of the environment play a part in settlement and metamorphosis. No attempt was

made to alter the type or velocity of flow and there was undoubtably considerable turbulence within the nitex cup. The effect of flow on settlement has been examined in B. balanoides. Movement of water over a surface induces swimming cyprids to attach to a substrate and begin exploration (Crisp and Meadows, 1962). Perhaps a similar pattern of events occurs in C. hastata whereby a flow induces larvae to begin crawling along a substrate and then metamorphosis is induced by the microbiol layer adhering to the substrate. Adults generally occur in areas with flow, and so it is possible that a feature of the adult habitat (flow) is one of the stimuli that induces settlement and metamorphosis.

Postlarvae were not randomly distributed over the surfaces (Figure 22). Apparently, some feature of edges and corners corresponded to a favourable site. Settlement along the edges of a slide followed a random distribution (Figure 23), suggesting that larvae do not settle gregariously.

Metamorphosis is not a developmental event which follows larval development within a specific time interval. C. hastata larvae were capable of metamorphosing 34 days after fertilization when reared at 16°C (or 42 days at 12°C) yet metamorphosed between 34 and 103 days. Larval life can be divided into two periods: the precompetent period

where larval development proceeds up to the point where a larva is capable of metamorphosis, and the competent period where a larva which is capable of metamorphosis continues to swim and feed in the water column until a suitable site for settlement is encountered. Larvae are believed to settle nonrandomly and preferably in areas with the most favourable environmental conditions. If these areas are not located as soon as a larva reaches competency, then presumably metamorphosis is delayed. The ability to withold the process of metamorphosis until a suitable site is found possibly enhances the larva's chance of survival. The length of time that a larva can delay metmorphosis, ie. remain competent, is related to the length of the precompetent period. C. hastata larvae reared at 12°C had a longer precompetent period (42 days) and a longer competent period (88 days) than larvae reared at 16°C. Crepidula fornicata, Mopalia muscosa (Pechenik, 1980) and C. plana (Lima and Pechenik, 1982) also have longer competent periods associated with longer precompetent periods. Jackson and Strathmann (1981) proposed a model in which the competent period increases with the precompetent period and the competent period is at least as long as the precompetent period. They suggested that since pelagic larvae with a longer precompetent period are in the water column longer, they probably move farther from suitable substrata and a longer competent period is required for them to encounter a suitable settling site.

The way by which length of larval life, including both precompetent and competent periods, is determined is probably by the rate at which development proceeds toward a fixed endpoint (Pechenik, 1980). If the precompetent period proceeds rapidly, then the length of the competent period will be shorter. During the competent period, a larva is already "equipped" to proceed through metamorphosis. Tissue development appears to go into a holding pattern waiting for metamorphosis to be initiated. However, if metamorphosis is delayed for a substantial length of time, then certain adult rudiments present in the larva continue to develop slowly. The most obvious example of this is the gill rudiment; as larval life is prolonged the three primary filaments on either side of the mantle cavity become longer and sometimes a fourth filament develops at the posterior end of the rudiment. Besides the development of adult rudiments, larval organs which are histolysed at metamorphosis begin to show signs of senescence. For example, the velum begins to deteriorate in larvae about 95 days old (16°C). Since this is the larval locomotory and feeding organ the larva soon begins to show signs of starvation, and becomes pale in colour, indicating that it is not feeding. As well, large lipid droplets stored in the digestive gland, presumably a food store to provide energy during metamorphosis, gradually become fewer in number. Once all available food stores are depleted, the larva dies (103 days at 16°C, 130 days at 12°C). Even though metamorphosis was not

initiated, these processes occur and ultimately the larva dies without metamorphosing. These observations suggest that a larva cannot exist indefinitely. Metamorphosis must be initiated within a certain time period before larval tissues begin to deteriorate and energy stores are depleted.

Observations reported here were made in a laboratory setting and thus artifacts created in the laboratory must be considered. Generally larvae are observed in a laboratory setting rather than in their natural habitat because of the complexity associated with larval studies done in the field. Although delay of metamorphosis and cues for settlement and metamorphosis were identified in this study it would be difficult to extrapolate to the natural setting without first sampling larvae in their habitat.

SUMMARY

A study of this scope, that provides detailed information on embryonic and larval development of a pectinid species, has never before been published. Combined with the information regarding the effect of temperature on larval development and factors affecting settlement and metamorphosis, this study has elucidated certain aspects about early development in pectinids in general, and C. hastata in particular.

Features described in this study which have never before been described for any bivalve larva include 1) the interlocking crown and groove feature on the larval denticles, 2) location and probable function of secretory cells on the outer margin of the velum, and 3) possible function of the bilobe configuration of the outer margin of the velum and how this may tie in with existing theories on particle capture by the velum. The following features were observed and described for the first time in a pectinid species in this study: 1) newly released oocytes may be surrounded by a thick jelly coat, 2) gastrulation probably occurs by both epiboly and invagination, 3) the two groups of ciliated cells surrounding the blastopore during embryogenesis are probably primary trochoblasts, 4) the ligament may be present in the larval stage, and 5) specialized compound cilia at the mouth region are probably involved in collection and sorting of food particles. As well,

this study provided information on C. hastata larvae which may assist in identification of C. hastata larvae in plankton samples: size of the punctate-stellate region on the prodissoconch I shell, proviniculum length, number of larval denticles, maximum valve length observed, and ratio of valve length to valve height.

Larvae reared at 12°C developed slower, reached larger maximum size, had better survival, and a longer larval life than larvae reared at 16°C. The observed inverse relationship between valve length and development time between larvae reared at different temperatures may be a result of energy partitioning by larvae, whereby larvae reared at a higher temperature require more energy to satisfy metabolic needs and so have less available for growth and accumulation of stores.

This study concluded that more C. hastata larvae will metamorphose if a fouled substrate or a water flow is introduced. It seemed that they did not metamorphose after a specified period of time, but could survive as larvae for up to 130 days (12°C). Age of larvae tested influenced the rate of settlement and metamorphosis observed with a decline in response among larvae near the maximum age. A positive correlation between the length of larval development, the precompetent period, and the length of time that a larvae that is capable of metamorphosing can survive without metamorphsing, the competent period, was observed.

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Title of Thesis

Embryogenesis and Morphology of Larval Structures in Chlamys hastata, with an Examination of the Effect of Temperature on Larval Development and Factors Affecting Settlement and Metamorphosis

Author [redacted]

CHRISTINE A. COOKE

MARCH, 1986