Histological and ultrastructural studies of larval and juvenile Lampsilis (Bivalvia) from the Upper Mississippi River

by

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metallothionein-like low molecular weight proteins (Accomando et al. 1990) and immobilization in phagolysosomes (Coombs and George 1978).

With continuous prolonged metal exposures nonspecific binding at susceptible sites may occur, possibly producing lesions. Reported histopathological effects of contaminants on adult bivalves include: 1) for gills, fusion of filaments, mucous hypersecretion, degeneration of collagenous connective tissue, lesions, hyperplasia, detachment of abfrontal cells from chitinous rods, increased vacuolization and loss of cilia (Sunila 1986, 1988; Marshall and Talbot 1979); (2) for digestive glands, changes in epithelial structure, vacuolization and accumulation of lipids in digestive and basophil cells (Lowe and Clarke 1989); (3) for the kidneys, ultrastructural changes including swollen mitochondria and reduced glycogen reserves (Hemelraad et al. 1990); (4) neoplasia (Mix 1983; Gardner and Yevich 1988) and (5) acute and chronic inflammatory responses (Sunila 1988; Farley 1988). There are apparently no histopathological studies dealing with effects of metal contaminants on juvenile unionids.

Study Objectives

Objectives of this study were to: 1) describe the ultrastructure of the larval and post-larval shell of L. ventricosa and P. cordatum; 2) document the post-larval development of the digestive organs, gills, foot and nervous system of L. ventricosa reared in the laboratory; 3) determine acute toxicities of cadmium to larval and juvenile stages of L. ventricosa and 4) evaluate growth and histopathological effects of subchronic cadmium exposures on juvenile L. ventricosa.

Explanation of Dissertation Format

The dissertation uses alternate format and is presented in three sections. Each section is a separate manuscript that will be submitted for publication in a professional journal. Each section contains its own abstract, introduction, materials and methods, results, discussion and literature cited. An appendix follows section 1 and section 2. The figures included in appendices will not be used in publications. A general summary follows section 3. Literature cited in the general introduction follows the general summary.

Chapter 1 describes larval shell ultrastructure and early juvenile shell growth of L. ventricosa and observations of P. cordatum shell ultrastructure.

GENERAL SUMMARY

Specific reasons for recent declines in mussel populations in the Upper Mississippi River (UMR) are currently unknown. Overharvesting, impoundments, dredging, siltation and pollution have been implicated as contributing factors for the declining mussel populations. Water quality has improved in the UMR in recent years; however, sediments remain sufficiently contaminated with metal pollutants at levels that potentially could be a threat to aquatic biota. Benthic filter-feeding bivalves may be especially vulnerable. Very little is known about the effects of habitat alterations on the life stages of freshwater unionids.

There is little information on early growth, development or habitat requirements of juvenile unionid mussels. New information is critically needed in order to develop recovery plans that will adequately protect threatened and endangered mussel species, as well as commercially and ecologically important species. The early shell development of juvenile Lampsilis ventricosa and Pleurobema cordatum, reared in the laboratory, was described using scanning electron microscopy. There was limited shell growth during encystment. The glochidial (larval) shell exhibited numerous pores which extended through the calcareous shell layer but did not penetrate the outer shell cuticle. The postmetamorphic (juvenile) shell was added to the peripheral edges of the larval shell. Shell deposition into larval shell pores coincided with early formation of the juvenile shell. This pore-filling had not previously been observed. Growth of the juvenile shell was rapid for L. ventricosa, and by 21 days, concentric growth rings were evident.

Light microscopy and electron microscopy were used to describe post-larval development of internal organs of L. ventricosa reared in the laboratory for up to 56 days. Glochidia underwent metamorphosis while encysted on the gills of fish hosts. Prior to drop off, presumptive digestive organs, three pairs of rudimentary gill filaments, mantle, a protractile foot and a well developed nervous system were present. Digestive glands contained large numbers of lipid bodies. At the time of release, juveniles exhibited ontogenetic changes required to ingest and process phytoplankton. Most notably, the stomach had differentiated into an anterior gastric portion and a posterior style sac. The style sac contained a translucent crystalline style and dense short cilia. Subsequent developments in the digestive system consisted of elongation of the intestine and further elaboration of ciliary sorting areas and linings.

The postmetamorphic alignment of the alimentary system was not altered until 56 days, when the style sac shifted ventrally. By 2 weeks, lipid bodies were absent from digestive glands. This loss of lipids correlated to increases in mortality of laboratory-reared juveniles. At 3 weeks, digestive glands had become diverticulated, and by 8 weeks, numerous diverticula surrounded the stomach and projected ventrally into the foot. Growth of gill filaments, by papillary outgrowths, began approximately 3 weeks following drop off. Frontal gill surfaces were covered with distinct lateral, frontal and laterofrontal ciliary tracts, which are characteristic for gills of adult lamellibranchs. The juvenile foot was well developed at release, and surfaces were morphologically distinct. Lateral dorsal surfaces were covered with a microvillar epithelium; whereas, ventral surfaces were covered with long dense cilia. By 8 weeks, the byssus complex had formed. Byssus glands were diverticulated, and a ciliated groove (the gland opening) had formed along the ventral length of the foot. A byssus thread was not elaborated by any of the juveniles examined.

When establishing acceptable metal exposure limits for unionid bivalves, the sensitivities of all life stages need to be taken into account. The juvenile stage represents the first free-living stage and, therefore, may be the stage most vulnerable to metal exposures. In the present study, cadmium was used to determine the relative sensitivities of larval and juvenile stages of L. ventricosa to metal exposure. Acute toxicity (48-h) tests were performed on L. ventricosa glochidia and juveniles aged 0- (0-12 hours post-drop off), 7- and 14-d old. Based on LC50's and EC50's (derived from number of stressed and dead juveniles), young juveniles (0- and 7-d old) were more sensitive to cadmium than 14-d old juveniles. Glochidia were very tolerant to cadmium; the LC50 was approximately three times greater than that of any of the juvenile stages.

The effects of metals on growth and development of juveniles apparently has not been evaluated. A 7 day static renewal test was used to determine growth and histopathological effects of cadmium on 0-d old juveniles. Morphometric analysis of external shell parameters revealed significant reductions in anterior shell length following exposure to 10 µg Cd/L. Histopathological alterations (atrophy, increased vacuolization and tissue separations) appeared more severe for the mantle, ganglia and digestive gland tissues than for foot and muscle tissues. Exposure to 30-100 µg Cd/L

resulted in dissolution of the crystalline style in several juveniles. Histological examinations also indicated that juvenile feeding was reduced, lipid catabolism was altered, and mucous hypersecretion may have occurred.

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