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HAPTOBENTHOS ON SHELLS OF LIVING FRESHWATER CLAMS IN LOUISIANA

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Abstract.—Haptobenthos reported from the shells of living freshwater unionid clams in Louisiana included unidentified blue-green algae; green algae of the genera *Basicladia*, *Cladophora*, *Oedogonium* and *Spirogyra*; the mosses *Fissidens fontanus* (B.-Pyl.) Steud. and *Leptodictyum riparium* (Hedw.) Warnst.; the sponge *Trochospongilla leidyi* (Bowerbank); the colonial hydroid *Cordylophora lacustris* Allman; the entoproct *Urnatella gracilis* Leidy; the ectoprocts *Fredericella sultana* (Blumenbach), *Pectinatella magnifica* Leidy, *Plumatella repens* Linnaeus and *Pottsiella erecta* (Potts); unidentified sessile rotifers; and cases of chironomid larvae and caddisfly larvae. A total of 26 species of clams was examined. Within most species of clams, the size of the clams was approximately proportional to the presence and/or the amount of haptobenthic growth on the valves. On most clams bearing haptobenthic growth, a bryozoan, usually *P. erecta*, was present, regardless of the presence or absence of any other epizoont.

Introduction

Records of epizoonts on the shells of living or dead freshwater clams are relatively scarce; an excellent review is given by Fuller (1974). Additional records include Leidy (1884), Rao (1929), Seshaiya (1947), R. Moore (1953), Vinyard (1953), Sinclair and Isom (1961 and 1963), Weise (1961), Bushnell (1966), Lewandowski (1976) and Neumann and Vidrine (1978).

There are reports on the ecology and distribution of freshwater bryozoans (Everitt, 1972 and 1975; Poirrier and Johnson, 1970), clams (Vidrine, 1974; Stern, 1976), colonial hydroids (Poirrier and Denoux, 1973), and sponges (W. Moore, 1953; Poirrier, 1969) in Louisiana. These authors, however, reported no association in which a clam served as a substrate for the other invertebrates.

Substrate preference and avoidance are topics of current interest in the study of freshwater ecology. Fuller (1974) stated that "mussel symbioses and other biotic relationships are critically important ingredients of their normal and pollution ecologies—and of any attempt to understand the value of mussels as indicator organisms." The diversity of freshwater clams in Louisiana and the abundance of bryozoans, colonial hydroids, and sponges stimulated the initial interest in this investigation. The purposes of this study were to determine the extent to which Louisiana unionaceans serve as substrates for haptobenthos and to observe any preference or avoidance of any epizoont for any species of clam. Throughout this study, the terms "haptobenthos" and "epizoonts" are used synonymously for those organisms growing on the shells of the living clams. However, haptobenthos, in the more strict use of the term, refers to "forms adnate to solid surfaces" (Hutchinson, 1967).

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Materials and Methods

Clams were collected from 1974 through 1979 by hand from eleven localities in seven freshwater streams in central and southwestern Louisiana: Anacoco Bayou, Vernon Parish; Bayou Cocodrie, Evangeline and Rapids parishes; Bayou des Cannes, Acadia Parish; Bayou Maringouin, Iberville Parish; Bayou Peyronnet, St. Landry and St. Martin parishes; Bayou Pierre, De Soto and Red River parishes; Ramah Borrow Canal, Iberville Parish.

The purpose of the 1974–1976 collection was to determine the clam fauna of each of several localities, therefore, although some attempt was made to collect samples which represented the relative abundance of clams, individuals with less growth on their shells were collected preferentially over individuals with abundant growth. Clams were transported to the laboratory in burlap sacks and were preserved in 70% ethanol.

The 1979 sampling of two of the streams sampled previously was random and was only for the purpose of obtaining data for this haptobenthic survey. Because the examination of the clams' surfaces was conducted in the field, the sampling included small clams and clams with luxuriant haptobenthic growth.

Results and Discussion

More than 1,100 unionid clams, representing 26 species in 21 genera, were examined: *Amblema plicata* Say, *Anodonta grandis* Say, *A. imbecillis* Say, *Arcidens confragosus* (Say), *Carunculina parva* (Barnes), *Fusconaia undata* (Barnes), *Glebula rotundata* (Lamarck), *Lampsilis hydiana* (Lea), *L. satura* (Lea), *L. teres* (Rafinesque), *Leptodea fragilis* Rafinesque, *Ligumia subrostrata* (Say), *Megalonaias gigantea* (Barnes), *Obliquaria reflexa* Rafinesque, *Obovaria castanea* (Lea), *Plectomerus dombeyanus* (Valenciennes), *Pleurobema cordatum* (Rafinesque), *Proptera purpurata* (Lamarck), *Quadrula apiculata* (Say), *Q. nodulata* Rafinesque, *Q. pustulosa* (Lea), *Strophitus subvexus* (Conrad), *Truncilla truncata* Rafinesque, *Tritogonia verrucosa* (Rafinesque), *Uniomerus tetralasmus* (Say), and *Villosa lienosa* (Conrad). The following organisms were attached to the shells of one or more of those species: unidentified blue-green algae; green algae of the genera *Basicladia*, *Cladophora*, *Oedogonium* and *Spirogyra*; the mosses *Fissidens fontanus* (B.-Pyl.) Steud. and *Leptodictyum riparium* (Hedw.)

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Warnst.; the sponge *Trochospongilla leidyi* (Bowerbank); the colonial hydroid *Cordylophora lacustris* Allman; the entoproct *Urnatella gracilis* Leidy; the ectoprocts *Fredericella sultana* (Blumenbach), *Pectinatella magnifica* Leidy, *Plumatella repens* Linnaeus and *Pottsiella erecta* (Potts); unidentified sessile rotifers; and cases of chironomid larvae and caddisfly larvae.

Blue-green and green filamentous algae occurred on a moderate number of clams and were well represented on limbs, bottles and cans from the same collecting sites. Because most clams examined were preserved in the field, no attempt was made to identify the algae to species or to correlate the algae with their respective clam substrates. However, green filamentous algae, primarily *Cladophora* spp., occurred on the following clams: *Amblema plicata, Anodonta grandis, Arcidens confragosus, Fusconaia undata, Glebula rotundata, Lampsilis hydiana, L. satura, L. teres, Megalonaias gigantea, Obliquaria reflexa, Plectomerus dombeyanus, Proptera purpurata, Quadrula apiculata, Q. nodulata, Q. pustulosa, Strophitus subvexus* and *Tritogonia verrucosa*.

Previous reports of *Basicladia, Oedogonium* and *Spirogyra* attached to clam shells are unknown to the authors, although *Basicladia* has been reported on turtles (Vinyard, 1953). Clark and Wilson (1912) reported "filamentous algae, probably *Cladophora*," occasionally growing on shells of mussels. Vinyard (1953) suggested that some algal species "may be restricted to mollusks, or even to individual genera or species as seems to be the case in certain algal-turtle relationships." There is a paucity of information on this subject in the literature.

The moss *Fissidens fontanus* was collected on the shells of living *Quadrula pustulosa* and *Tritogonia verrucosa*. The moss-clam association was rare in the habitats surveyed. This dearth of moss growing on clams may be the result of reduced light penetration due to the turbidity of the water. *Fissidens fontanus* is common in lakes, streams and swamps in Louisiana (Neumann and Vidrine, 1978).

Fissidens fontanus was reported for the first time from live North American freshwater clams (*Quadrula pustulosa, Tritogonia verrucosa* and *Lampsilis straminea* Conrad) by Neumann and Vidrine (1978). Those authors mentioned Scandinavian reports (Luther, 1951; Lohammer, 1954) of *F. fontanus* on empty clam shells and on living *Anodonta* and *Margaritana* (=*Margaritifera*) species. Neumann and Vidrine (1978) also reported the moss *Leptodictyum riparium*, which is "not generally considered aquatic," associated with *F. fontanus* on *L. straminea*. However, according to a review of the literature by Neumann and Vidrine (1978), *L. riparium* is commonly found in aquatic situations.

The sponge *Trochospongilla leidyi* and several unidentified sponges were encountered on the shells of living *Quadrula apiculata, Lampsilis hydiana, Glebula rotundata* and *Anodonta grandis*. The sponges often co-occurred with one or more of the following species: unidentified filamentous green algae, *Cordylophora lacustris, Plumatella repens, Pottsiella erecta* and *Urnatella gracilis*.

Trochospongilla spp. and other spongillids are common in Louisiana and occur on substrates other than clams (Poirrier, 1969). Reports of sponges growing on the shells of living clams are unknown. From Ohio, Clark and Wilson (1912) reported "a species of fresh water sponge . . . frequently coating the inside of dead valves with expanded thick feltlike covering." They also reported that sponges were "rather common on rocks and shells."

The colonial hydroid *Cordylophora lacustris* on living clams usually was associated with one or more species of bryozoans. The hydroid occurred with filamentous green algae on the shells of living *Amblema plicata* and *Proptera purpurata*; with *Pottsiella erecta* and sometimes with *Urnatella gracilis* on *Glebula rotundata*; and with *P. erecta, U. gracilis* and an unidentified sponge on *Lampsilis hydiana*. We know of no previous records of *C. lacustris* on the shells of living clams. However, the occurrence of *C. lacustris* and bryozoans on substrates other than the shells of living clams was reported by Everitt (1972).

All species of freshwater bryozoans previously reported from Louisiana (Poirrier and Johnson, 1970; Everitt, 1972 and 1975) occurred on the clams in this study. Most clams with any haptobenthos supported the growth of at least one species of bryozoan, usually *Pottsiella erecta*. Many "positive" clams bore *P. erecta* and *Urnatella gracilis*. On several occasions, *Anodonta grandis, Glebula rotundata, Quadrula apiculata* and *Villosa lienosa* supported the growth of *Plumatella repens, Pottsiella erecta* and *Urnatella gracilis* or *P. erecta, U. gracilis* and plumatellid sessoblasts.

Pectinatella magnifica (a colony 6 cm in diameter) was found on only one clam, a specimen of *Anodonta grandis* (18 cm long) and was also uncommon on non-clam substrates. Everitt (1975) reported that this ectoproct was abundant in some Louisiana habitats in a 6.8–7.1 pH range and a specific conductance range of 82–330 micromhos/cm. In the habitats included in the present study, perhaps some of the physicochemical parameters are outside the tolerance range of *P. magnifica*.

Other reports of ectoprocts on clams include *Pottsiella erecta* on a relic valve of *Fusconaia undata* (Sinclair and Isom, 1961 and 1963), *Plumatella* sp. on *Unio* sp. (R. Moore, 1953), and *Plumatella polymorpha* on *Quadrula plicata* (=*Amblema plicata*) (Wilson and Clark, 1912). Bushnell (1966) reported *Fredericella sultana* on *Lampsilis ventricosa* (Barnes) (=*L. ovata*), *Plumatella emarginata* on *Lasmigana complanata* (Barnes) and several bryozoans on snails.

The entoproct *Urnatella gracilis* occurred relatively frequently on living *Anodonta grandis, Glebula rotundata, Lampsilis hydiana, L. teres, Plec-*

tomerus dombeyanus, *Quadrula apiculata* and *Villosa lienosa*. The growth was often moderately dense to luxuriant. This entoproct has been reported on *Unio* sp., *Unio complanatus* [=*Elliptio complanata* (Lightfoot)] and *Melania virginica* (Leidy, 1884), *Amblema* sp. and *Pleurocera canaliculatum* (Weise, 1961), and *Elliptio complanata* (Fuller, 1974). Seshaiya (1947) reported *Urnatella indica* on *Paludomus* sp., *Potomida* sp. and *Lamellidens* sp. in India, and Sinclair and Isom (1963) reported *U. gracilis* attached to rocks and common on mussel shells in Tennessee. Eng (1978) collected *U. gracilis* encrusting living *Corbicula manilensis* (Philippi) [=*C. fluminea* (Mueller)] and various other substrates including empty *Corbicula* shells in an irrigation canal in California. Eng, however, did not find other bryozoans (*Fredericella* and *Plumatella*) present in the canal on *C. manilensis* (letter dated 22 July 1980 from Dr. Larry L. Eng, California Department of Fish and Game, Sacramento, California).

Clams bearing epizoonts were not preferentially collected in the field and in many early collections were avoided. Therefore, the percent of clams without haptobenthic growth was not significant. For most species of clams, the presence and/or the amount of haptobenthic growth on the valves was approximately proportional to the size of the clams. On most clams bearing haptobenthic growth, a bryozoan, usually *Pottsiella erecta*, was present, regardless of the presence or absence of any other epizoont.

Although the phylactolaemate bryozoans produce statoblasts, many of which float, the asexually produced resistant structures of the gymnolaemate *Pottsiella erecta*, hibernacula (or lasting buds), are sessile. Perhaps most of the *P. erecta* colonies on the clams and other substrates resulted from the settlement of larvae. *Pottsiella erecta* reproduces sexually in Louisiana (Everitt's observations), but we know of no published studies of settlement behavior of *P. erecta* larvae. Hubschman (1970) showed that *Pectinatella magnifica* larvae preferred larger substrates to particles no larger than sand grains and that the larvae preferred limestone to plastic and aluminum. That a bryozoan larva would be attracted to a clam shell, a large, firm structure composed of calcium salts, seems logical. The apparent lack of preference for clam shells over other substrates in this preliminary study, however, suggests that in a natural system bryozoan larvae select substrates not simply by the type of substrate but by the biological and chemical composition of the surface of the substrates. The influence of surface chemistry and surface microflora on the settlement of marine bryozoan larvae has been studied extensively (Dexter *et al.*, 1975; Driscoll *et al.*, 1971; Wilson, 1970).

Field observations of two ecologically similar streams (Bayou Peyronnet and Ramah Borrow Canal) in southwestern Louisiana suggested that the composition of macroinvertebrate assemblages on the valves of living freshwater clams closely resembles that occurring on sticks and limbs, the natural substrates usually studied. However, more extensive investigations of localities ecologically different from these two streams may reveal that haptobenthic assemblages do indeed vary among substrates. In addition, careful observation of clams as substrates for macroinvertebrates will increase our knowledge of the biology of all of these organisms and render our understanding of freshwater Aufwuchs communities more nearly complete.

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USE OF THE ENZYME-LINKED IMMUNOSORBENT ASSAY TO MEASURE INFLUENZA ANTIBODIES IN HUMAN SERUM[1]

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Abstract.—Human influenza antibodies were measured using the ELISA and the results compared to hemagglutination-inhibition (HI) titers. ELISA titers averaged 10 times higher than HI titers.

Due to its simplicity and sensitivity, the enzyme-linked immunosorbent assay (ELISA) is increasingly being used to detect antigens and antibodies (Engvall and Pesce, 1978). Antibodies to influenza virus are conventionally detected using hemagglutination-inhibition (HI) and complement-fixation tests (CF) (Dowdle, 1976). However, these tests have the respective disadvantages of nonspecific inhibitors (HI) and complexity in performance (CF) (Dowdle, 1976). In order to evaluate the effectiveness of the ELISA in detecting low levels of anti-influenza antibodies, 12 pairs of sera were obtained from individuals prior to immunization (pre-immunization) and after immunization (post-immunization) with swine influenza virus (A/New Jersey/8/76 (Hsw1N1)), through the courtesy of Dr. Alfred Evans of Yale University School of Medicine. These sera were titrated in his laboratory using the HI technique (Dowdle, 1976) and in our laboratory using the ELISA technique (Voller *et al.*, 1976). This paper describes the results of this comparative study.

Briefly, the principle of the ELISA is that by conjugating an enzyme to an antibody, the extent of binding of the antibody to its homologous antigen can be detected by adding the substrate for that enzyme. The amount of substrate converted to product is proportional to the amount of enzyme-labeled antibody present, which in turn is proportional to the amount of antibody bound to the antigen. The ELISA has been shown to be a reproducible assay, with the same range of sensitivity as radioimmunoassay (Voller *et al.*, 1976).

The ELISA was carred out on a micro-scale, using techniques and reagents previously described (Voller *et al.*, 1976). Two influenza antigens were used for the titrations: the immunizing virus, swine influenza virus vaccine (A/New Jersey/8/76(Hsw1N1), HA antigen, Lot 40030002, Flow Laboratories, Inc., Rockville, Maryland 20852); and another influenza

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