Assessment of Current Information Available for Detection, Sampling, Necropsy, and Diagnosis of Diseased Mussels

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Summary

Trematodes and mites are common in freshwater mussels (order Unionoida), and other metazoan organisms reported in unionids include oligochaetes, copepods, and chironomids. The only well-documented protists in unionids are ciliates, but these include *Conchophthirus* spp., which are among the most common inhabitants of unionids. The effects of all of these eukaryotic organisms on the health of their hosts have not been adequately evaluated, but in some circumstances, these parasites cause focal lesions or energy depletion in unionids. Some of the digenetic trematodes cause gonadal injury that reduces fecundity and may also decrease the ability of freshwater mussels to survive low concentrations of oxygen and elevated temperature. The only common copepod parasite of unionids occurs in Europe and has not been reported in North America. Zebra mussels encrusting unionids can interfere with water flow and valve movements by the unionid, in addition to competing for food. Bitterlings are cyprinid fish that deposit their eggs in the gills of unionids; these eggs impair water flow in the unionid. Except for zebra mussels, none of the eukaryotic organisms living in or on unionids seem likely to cause lethal injury to their host expect perhaps during unusual circumstances. However, some of these eukaryotic organisms probably have the potential to decrease the fitness of the host, and there has not been adequate evaluation of potentially greater harm during periods of suboptimal environmental conditions or in captive unionids.

Several species of potentially pathogenic bacteria have been isolated from unionids, but their role in unionid diseases has not been determined. No viruses have been discovered in North American unionids, but there are reports of a viral disease in a Chinese species. The inadequacies of currently available cell culture methods for isolation of viruses are a serious obstacle for virology of unionids. Many of the studies of unionid diseases have relied primarily on methods suitable for isolation of fish or human pathogens; additional emphasis is needed on histopathology, electron microscopy, and other approaches that might reveal the presence of other types of pathogens.

For unionids, there is no information about some groups of pathogens that cause important diseases in marine bivalves. Additional research is needed to determine whether these pathogens have been overlooked or are not present in freshwater bivalves. There is also a need for studies of the diseases of glochidia and newly transformed juvenile unionids. Controlled experiments to determine the effects of potential pathogens on unionids are needed to facilitate evaluation of parasitological and bacteriological findings obtained from necropsies.

Introduction

Unionids

The freshwater mussels of North America, also called unionids, clams, naiades, or pearly mussels, include two families: Margaritiferidae and Unionidae (class Bivalvia, subclass Palaeoheterodonta; order Unionoida; superfamily Unionoidea). These families have a worldwide distribution, with most species found only in North America, and there are additional families of the order Unionoida that are not native to North America. There are additional species of freshwater bivalve mollusks in North America—some native and others introduced. The most common introduced species are the Asian clam *Corbicula fluminea* (Sphaeriidae) and the zebra mussel *Dreissena polymorpha* (Dreissenidae). This review includes information about all freshwater bivalves, with an emphasis on those found in North America (Table 1). Names of mussels in our review follow Turgeon et al. (1998).

The characteristics that set the superfamily Unionoidea apart from other bivalves is restriction to freshwater, parental care of offspring until they are released as larvae, and parasitic larvae (Kat 1984). Adults are relatively sedentary, but the larvae (glochidia) are parasitic on fish or amphibians (Watters 1997), which provides a mechanism for dispersal.

The conservation status and threats to unionids have been reviewed (Bogan 1993; Williams et al. 1993; Neves et al. 1997; Bogan 1998; Neves 1999; Garner et al. 2004a, 2004b; Lydeard et al. 2004; Strayer et al. 2004). There are about 300 recent species of freshwater mussels in North America, but many are extinct or imperiled. For unionids in the U.S., 37 species are presumed to be extinct or are possibly extinct, and another 115 species are imperiled or critically imperiled (Master et al. 2000). As of February 2007, the U.S. Fish and Wildlife Service listed 72 species or subspecies of unionids as threatened or endangered (http://ecos.fws.gov/tess_public/SpeciesReport.do?groups=F&listingType=L). Alabama alone had 175 species or subspecies of unionids, but 27 of these are possibly extinct and an additional 90 are considered imperiled or have been extirpated from Alabama (Garner et al. 2004b). Alabama has 47 species on the federally protected list, the most of any state. Although diseases are mentioned in some reviews of threats to unionids, infectious diseases have not generally been considered a factor in the decline of wild populations.

The introduction of new species to North America threatens unionids. The best-documented threat to unionids by an exotic species is the zebra mussel, which was introduced into North America (Lake St. Clair) about 1985 (Hebert et al. 1989). The Asian clam is also a competitor to some unionid populations (Clarke 1988). More difficult to determine is the harm to unionids by introduced species of pathogens. There are no pathogens of unionids in North America that are known to have been introduced from other continents, but interbasin transfers within North America seem likely. The potential for introduction of pathogens should be a consideration when animals are transported from one watershed to another. There are several examples of health problems in mollusks following the introduction of a pathogen (Burreson et al. 2000; Naylor et al. 2001; Friedman and Finley 2003; Ruesink et al. 2005).

Concern about the well being of mussel populations has stimulated interest in the propagation of some species (Ellis 1929; Biggins and Butler 2000; Hanlon 2003), but as for all cultured animals, there are numerous factors that affect the health of mussels reared in captivity (Jones et al. 2005). The potential for pathogens to kill or have sublethal effects on mussels in culture conditions has generally not been adequately evaluated.

Table 1. Freshwater bivalve mollusks of North America (Parmalee and Bogan 1998; Turgeon et al. 1998; Roe and Hartfield 2005).

Taxa	Common name or comments
Class Bivalvia	
Subclass Palaeoheterodonta	
Order Unionoida	freshwater mussels
Superfamily Unionoidea	
Family Margaritiferidae	
Margaritifera	
Cumberlandia	
Family Unionidae	50 genera
Subclass Heterodonta	
Order Veneroida	
Superfamily Corbiculoidea	
Family Corbiculidae	
Corbicula fluminea	Asian clam [I]
Family Sphaeriidae	
Eupera	fingernailclams
Musculium	fingernailclams
Pisidium	peaclams
Sphaerium	fingernailclams
Superfamily Dreissenoidea	
Family Dreissenidae	
Dreissena polymorpha	zebra mussel [I]
Dreissena bugensis	quagga mussel [I]
Mytilopsis leucophaeata	dark falsemussel

[I] = introduced

Disease

Disease is a negative deviation from normal health and is indicated by functional impairment, structural changes, or both. Functional impairment is potentially linked to energy procurement, ability to escape predation, competitiveness, reproduction, growth rate, and survival. The concept of disease is broad and includes infectious diseases, which are caused by pathogens, and noninfectious diseases caused by environmental agents (chemical or physical), nutritional insufficiencies, or genetic defects. Infectious diseases are the primary focus of this review.

Pathogens are causative agents of infectious diseases and include a wide range of organisms: viruses, prokaryotes (bacteria), and eukaryotes. Eukaryotic (protozoan and metazoan) pathogens have traditionally been called parasites (an organism that lives in or on another living organism [the host] and at the expense of the host), but there is nothing fundamentally different between the relationship between a parasitic eukaryote and its host and that of a pathogenic bacterial organism and its host. We will also use a broad definition for the term symbiosis to indicate a

relationship of two dissimilar organisms living together with no implication about whether the relationship is beneficial or harmful to either organism.

There are several reviews of diseases affecting bivalve mollusks (Lauckner 1983; Sparks 1985; Fisher 1988; Gibbons and Blogoslawski 1989; Sindermann 1990; Bower 1992; Perkins 1993; Cheng 1993; Bower et al. 1994; Ford and Tripp 1996; Elston 1999; McGladdery 1999; Ford 2001; Gosling 2003; AFS-FHS 2005; Bower 2006; McGladdery et al. 2006). These reviews generally consider only marine bivalves, and most of the diseases in these reviews, especially the diseases considered most serious, have not been reported in freshwater mussels. Levine et al. (2006) provide an excellent review of issues related to the health of bivalves and diagnosis of diseases. Procedures for investigating die-offs of freshwater mussels were described by Southwick and Loftus (2003). A brief review of the diseases of freshwater mussels was presented by Fuller (1974), and cursory overviews are presented in several general references about unionids or freshwater bivalves (e.g., Oesch 1984; McMahon and Bogan 2001; Smith 2001).

Information about diseases of freshwater mussels and other freshwater bivalves was obtained from published and unpublished sources. In our review, most of this information is categorized by taxonomic groups of pathogens, followed by sections of general information (Table 2).

Table 2. Topics reviewed.

Viruses
Bacteria
Protists
Aspidogastrea
Digenea
Cestoda
Nematoda
Bryozoa
Oligochaeta
Leeches
Zebra Mussel Infestation on Unionids
Mites
Copepoda
Chironomidae
Bitterling Eggs
Interactions Between Environmental Conditions and Infectious Diseases
Diagnostic Methods
Recommendations for Future Research

The information in this review may be useful for investigations of die-offs or the decline of unionid populations, either wild or captive. Because of the use of unionids as sentinels for environmental perturbations, information about the interaction between environmental conditions and infectious diseases is important but is generally lacking. In addition, this review could be useful in future considerations of avoiding transfer of pathogens during relocation of unionids (Villella et al. 1998). Because of the presently inadequate state of knowledge related to unionid diseases, there is a need for additional information in all areas of unionid pathology before any of these goals can be reached.

Viruses

Viruses in Unionids

The only unionid that is known to be affected by viral diseases is a Chinese pearl mussel, *Hyriopsis cumingii*, and viral diseases of this species have been reported only in China. One virus from this mussel has single-stranded RNA and has been reported as the cause of *Hyriopsis cumingii* "plague" (Zhang et al. 1986; Shao et al. 1993). This cytoplasmic virus was usually 80-100 nm in diameter, although larger and smaller virus-like particles were also observed. The envelope of the virus had club-shaped projections about 12 nm in length and was considered to resemble viruses in the family *Arenaviridae*. Lesions were mainly in the alimentary tract and digestive gland where infected cells had acidophilic, cytoplasmic inclusion bodies and eventually lysed (Shao et al. 1995). This disease was experimentally transmitted to *Hyriopsis cumingii* by injection of filtered homogenate and may be species specific because injection of this homogenate did not kill the unionid species *Cristaria plicata* (Zhang et al. 1986).

Liu et al. (1993) also reported a virus from diseased *Hyriopsis cumingii*. Virus-like particles resembling herpesvirus were icosahedral, enveloped, 80-120 nm in diameter, and located in the nuclei of cells of the gonad and digestive gland. Mussels from a healthy population were injected with two types of bacteria-free homogenate of organs from diseased mussels: filtered through a 0.45-µm membrane or treated with antibiotics. Mussels injected with either type of homogenate developed signs resembling those of the naturally infected mussels, and the disease was passed seven times. The relationships between this virus, the RNA virus also found in *Hyriopsis cumingii*, and the disease attributed to the RNA virus are uncertain (Zhang et al. 2005).

In a review of unionid diseases, Fuller (1974) attributed one disease to a virus. This disease had been described by Pauley (1968) and called spongy disease. All of the *Margaritifera margaritifera* collected from the Ozette River, Washington, were found lying on top of the substrate rather then in the normal partially buried position. Of the 123 *M. margaritifera* necropsied, 61% had grossly visible lesions described as "polypoid growths, ulcers, nodular wounds, and watery cysts." All of these lesions were in the foot, and these lesions were considered to be different stages of spongy disease, except for the polypoid growths, which were hyperplastic glands found in only eight mussels. The watery cysts varied in size from 3 x 3 x 3 mm to 27 x 14 x 4 mm, and some mussels had multiple lesions of this type. Histologically, the spongy lesions consisted of edematous connective tissue replacing degenerating muscle and gland cells. Later, hemocytes infiltrated the lesion, and the epithelium over the lesion became squamous or necrotic. At this stage and in more advanced lesions, some cells contained one or more cytoplasmic inclusions having clear halos. As the disease progressed, granulation tissue formed and epithelium regenerated on the lesion. Pauley (1968) considered "amoeboid inclusion cells" observed in the diseased mussels to be the cause of the lesions, and Fuller (1974)

considered these inclusions to be an indication of a viral disease. However, there is no evidence that a viral agent caused this disease.

Virus-like particles resembling picornavirus were observed in a parasite infesting the unionid *Elliptio complanata* (Ip and Desser 1984). In electron micrographs of the aspidogastrid trematode *Cotylogaster occidentalis*, the virus-like particles were 23 nm in diameter and formed paracrystalline arrays in the cytoplasm. Intracellular inclusions formed by these particles were visible with light microscopy and were found in all 48 *C. occidentalis* examined from a river in Ontario. There was no indication that the trematode was harmed by these virus-like particles.

The low number of viral diseases in unionids is probably the result of limitations in methods available for detection of viruses in mollusks and the lack of effort in freshwater bivalve virology. Another factor that may have hindered advances in knowledge of unionid viruses is an apparent over-estimation of our ability to detect viruses. For example, Newton et al. (2001) claimed that the unionids they used in experiments "were certified free of bacterial and viral agents." For viruses this was clearly impossible with methods available and led to the apparent presumption that the cause of mortality in subsequent experiments was not viral.

Concern that bivalves could be reservoirs of viruses that are human (Shumway 1992; Potasman et al. 2002) or fish (Meyers 1984) pathogens has prompted much of the research related to viruses in bivalves. Some bivalve species can concentrate certain viruses from the water (Mitchell et al. 1966; Gerba and Goyal 1978), and most studies and reviews of viruses in bivalves assume that this is generally true for most viruses and bivalves. However, Giray et al. (2006) reported that *Mytilus edulis* did not serve as a reservoir for infectious salmon anemia virus, but rather reduced the viability of the virus beyond that resulting from incubation of the virus in seawater. There is little information about the ability of unionids to concentrate viruses from the water (Donnison and Ross 1999).

Viral Pathogens of Marine Bivalves

In contrast to the scarcity of evidence for viruses in freshwater bivalves, marine bivalves seem to be virus factories. This difference is undoubtedly because of the greater study of marine bivalve diseases. Although many of the reports of viruses in marine bivalves do not include conclusive evidence that the presumptive virus is the cause of a disease, there are some marine bivalve diseases that have well-established viral causes. There are several reviews of viral diseases of marine bivalves (Elston 1997, 2000; McGladdery 1999; Renault and Novoa 2004; Munn 2006). The following are examples of virus families reported from marine bivalves. The basic characteristics mentioned for these families are based on van Regenmortel et al. (2000).

Birnaviridae.—Viruses in this family have double-stranded RNA, are not enveloped, and are round. Birnaviruses have been found in several bivalve species, but the only report from a freshwater bivalve is an uncharacterized virus mentioned by Reno (1998) from the Asian clam. *Birnaviridae* includes infectious pancreatic necrosis virus (IPNV), a well-studied pathogen of fish (Reno 1998). Viruses related to IPNV have been found in marine bivalves, including the great scallop *Pecten maximus* (Mortensen et al. 1992), edible oyster *Ostrea edulis*, Pacific oyster *Crassostrea gigas*, Mediterranean mussel *Mytilus galloprovincialis* (Rivas et al. 1993), and common periwinkle *Littorina littorea* (Cutrin et al. 2000); however, it appears that in some cases the virus was sequestered in the bivalve and was not necessarily replicating. Some birnaviruses, such as $13p_2$ isolated from eastern oysters *Crassostrea virginica* (Meyers 1979; Meyers and Hirai 1980), have characteristics that distinguish them from IPNV, including serological

differences. The birnaviruses from marine bivalves and certain marine fish species have been called marine birnaviruses (MABV) because they are genetically distinct from IPNV (Suzuki and Nojima 1999; Isshiki et al. 2004). However, the genetic grouping of IPNV with some viral isolates from bivalves (Cutrín et al. 2004) indicates that the creation of new species for isolates from bivalves may not be justified. Some birnaviruses have low virulence in mollusks (Meyers 1980; Kitamura et al. 2000), but high mortality has also been reported (Lo et al. 1988).

Herpesviridae.—This family includes DNA viruses that have an envelope and are assembled in the nucleus of the host cell. Ostreid herpesvirus (OsHV-1) is the only herpesvirus from bivalves that has been well characterized (Davison et al. 2005). A similar virus has been found in North America, but in a limited geographic area (Friedman et al. 2005). Genomic characterization supports the inclusion of OsHV-1 in *Herpesviridae*, although in a different category than the herpesviruses of vertebrates (Davison et al. 2005). The polymerase chain reaction (PCR) has been used to detect OsHV-1 (Renault et al. 2000; Renault and Arzul 2001; Vigneron et al. 2004).

Virus-like particles resembling herpesviruses have been observed in electron micrographs of several species of marine bivalves, including eastern oyster *Crassostrea virginica* (Farley et al. 1972), Pacific oysters *Crassostrea gigas* (Hine et al. 1992), edible oyster *Ostrea edulis* (Comps and Cochennec 1993), flat oysters *Ostrea angasi* (Hine and Thorne 1997), carpet shell clam *Ruditapes decussatus* (Renault and Arzul 2001) and great scallop *Pecten maximus* (Arzul et al. 2001). A disease that appears to be caused by a herpes-like virus occurs in several species of adult oysters (Vásquez-Yeomans et al. 2004) and also kills larval oysters (Hine et al. 1998).

Iridoviridae.—These viruses have DNA, are assembled in the cytoplasm, and are icosahedral in shape. An iridovirus has been suggested as the cause of a disease in oysters that is characterized by hypertrophied, polymorphic cells up to 30 µm in diameter, necrosis of gills and labial palps, and hemocytic infiltration into the affected area (Comps 1988). Electron microscopy revealed cytoplasmic virus-like particles that were icosahedral, with a capsid formed by two layers and a diameter of 380 nm. DNA was demonstrated in inclusion bodies formed by this virus-like agent. A similar agent affects oyster veligers (Elston 1979; Elston and Wilkinson 1985).

Papovaviridae.—Viruses in this family have DNA, have no envelope, are 40-55 nm in diameter, and are round. Virus particles resembling papovavirus were observed in golden-lipped pearl oysters *Pinctada maxima* from Australia (Norton et al. 1993). Lesions of the labial palps had hypertrophied cells containing viral inclusions in enlarged nuclei, and virus-like particles were visible with electron microscopy.

Dong et al. (2004) observed papovavirus-like particles in gonads of Pacific oyster *Crassostrea gigas*. These virus-like particles were non-enveloped, icosahedral, 40-45 nm in diameter, and formed basophilic bodies that were 15 to 60 µm long. Basophilic bodies were seen in 3.3 to 7.1% of the oysters examined by light microscopy, and this disease could potentially reduce reproductive capability. Similar lesions, but with hypertrophied cells up to 500 µm and virus 50-55 nm in diameter, occur in eastern oyster *Crassostrea virginica* and other marine bivalves (Farley 1976, 1985; Winstead and Courtney 2003).

Picornaviridae.—Viruses in this family have single-stranded RNA, are 30 nm or smaller in diameter, and are not enveloped. Jones et al. (1996) reported virus-like particles resembling picornavirus (this resemblance was suggested by Elston 1997) in green-lipped mussel *Perna canaliculus* in New Zealand. Mortality of spat was 50 to 100% during summer and autumn, and mortality of adult mussels was 2 to 5%. Cellular inclusions were not visible with light microscopy, but with electron microscopy there were virus-like particles ranging from 25 to 45 nm in diameter and without an envelope. Similar lesions and virus-like particles were also seen in *Mytilus galloprovincialis*. Rasmussen (1986) examined *Mytilus edulis* with granulocytomas in the digestive gland and mantle; electron microscopy revealed picornavirus-like particles in the cytoplasm of the granulocytes in the lesions.

Retroviridae.—These single-stranded RNA viruses are enveloped, 80-100 nm in diameter, and pleomorphic. Retrovirus is potentially the cause of disseminated neoplasia (also called hemic neoplasia), which is a leukemia-like disease reported in several species of marine bivalves. This disease can have substantial impacts on natural populations of bivalves (Farley 1969a, 1969b; Peters 1988; Elston et al. 1992). The neoplastic cells are large, mitotically active, anaplastic, and appear to have a hematocytoblast origin. This disease can be diagnosed by histopathology or by examining a sample of hemolymph for neoplastic cells, which have a prominent nucleolus and a large nucleus in proportion to the cell size. These abnormal cells do not form the pseudopods and aggregates typical of normal hemocytes from bivalves.

Virus particles can be isolated by density gradient centrifugation of hemocytes or homogenates of softshell clam *Mya arenaria* that have disseminated neoplasia (Oprandy et al. 1981). Healthy softshell clams injected with these virus particles developed disseminated neoplasia. Electron microscopy of the concentrated virus revealed two types of virus particles; one was enveloped, had an eccentrically located nucleoid, and averaged 120 nm in diameter; and the other type of particle was 80 nm in diameter with a centrally located nucleoid and could be an immature stage in the formation of the larger particles.

Exposure of softshell clams to 5-bromodeoxyuridine resulted in an apparent activation of a retrovirus and development of hematopoietic neoplasia (Oprandy and Chang 983). Reverse transcriptase activity has been demonstrated in bivalves with this type of neoplasia, and this is further evidence that a retrovirus is the cause of this disease (Medina et al. 1993; House et al. 1998). In other species of bivalves, this disease has been transmitted by cell-free homogenates (Collins and Mulcahy 2003). Taken together, there is strong evidence that a virus causes this disease, although further characterization of the virus is needed. However, the etiologic agent may vary in different species that are susceptible to this disease, and the activity of the etiologic agent may be influenced by environmental factors (Barber 2004).

Evaluation of Virology Methods

Viruses are fundamentally different from other pathogens because a virus is dependent on an infected cell to provide enzymes and organelles required for replication of the virus. The nucleic acid and proteins of the virus are synthesized by the host cell and then assembled inside the infected cell to form new virus particles. For viruses of vertebrate hosts, the most common method of virus isolation involves cultures of cells, and the most convenient cell cultures are cell lines that are capable of living in culture indefinitely. The study of viruses causing disease in mollusks is hindered by a shortage of cell lines derived from mollusks (Elston 2000; Villena 2003).

No permanent cell lines derived from bivalves are available for isolation of viruses (McGladdery et al. 2006), and the only permanent cell line from any mollusk is from the snail, *Biomphalaria glabrata* (Hansen 1976; American Type Culture Collection number CRL-1494). Methods for primary culture of bivalve cells have been described, and short-term cultures from several organs have been reported (Brewster and Nicholson 1979; Mulcahy 2000).

A shortage of continuous cell lines derived from mollusks has limited cell culture isolation of viruses to those that can replicate in fish cells. Some viruses of bivalves can replicate in fish cell lines; the most commonly reported are birnaviruses. However, there have been other types of viruses isolated from bivalve mollusks by the use of fish cell lines (Miyazaki et al. 1999). This does not lessen the need for other methods for virus detection because it is likely that most viruses causing disease in mollusks will not replicate in cell lines derived from vertebrates.

Most reports of viruses in mollusks are based on electron microscopy. Although electron microscopy is a useful tool for studying viral diseases, this method does not provide sufficient information for a definitive identification of the virus. With currently available methods for electron microscopy, this technique is also not suitable for screening large numbers of samples, which is necessary to determine that a population is likely to be free of specific viruses.

Molecular methods, including the sequencing of nucleic acids and PCR, are important for viral identification. These methods are available for some viruses of mollusks (e.g., OsHV-1), but are currently limited by problems with viral isolation. As additional information becomes available about the viruses causing disease in freshwater bivalves, molecular methods will become more useful. However, until progress is made in isolation of viruses, molecular methods will be of limited use for virology of mollusks.

If a cell culture system suitable for viral isolation is not available, physical isolation methods are potentially useful. Virus particles can be isolated by high-speed centrifugation in a density gradient, and this method has been used to isolate viruses of bivalves (Oprandy et al. 1981; Zhang et al. 1986; Shen et al. 1986). Viruses can also be physically isolated by precipitation with polyethylene glycol (Shao et al. 1993; Lewis et al. 1996). These methods can provide a highly purified sample of virus for research applications and for development of molecular diagnostic methods.

Bacteria

Bacteria are considered a likely cause of disease in unionids (Ellis 1929; Starliper et al. 2007), but obtaining evidence for the pathogenicity of bacteria in unionids has been difficult. Problems with linking bacteria to disease include the normal finding of bacteria in healthy mussels and the rapid change in the bacterial composition of unionids after death. Considering the diversity of bacteria that cause disease in marine bivalves (McGladdery 1999), it seems likely that some major groups of pathogenic bacteria (e.g., Rickettsiales, Chlamydiales, and Mycoplasmatales) have been overlooked in unionids.

Concentration of Bacteria from Water

Actively feeding bivalves acquire bacteria from the water, accumulate them, and in some cases digest and assimilate them as nutrients. However, various species differ in the efficiency with which they filter bacteria from water. Silverman et al. (1995) found that zebra mussels removed bacteria from water 30 times more quickly than did Asian clams and 100 times faster

than the unionid *Toxolasma texasiensis* (= *Carunculina texasensis*). The difference in efficiency of removal of bacteria was attributed to the structure of the laterofrontal cirrus, which was more complex and more densely ciliated in zebra mussels. The speed at which bacteria are filtered from water also varies among unionids collected from different aquatic environments (Silverman et al. 1997). Clearance of bacteria by three unionid species that were considered lentic was slower by a factor of 10 when compared with clearance by six lotic species of unionids. The structure of the cirrus was also different in the two groups, with lentic unionids having about half the number of cilia per cirrus and half the cirral area per mg of dry tissue compared with lotic species.

Freshwater bivalves are able to discriminate among bacteria and other microscopic particles they acquire from water. It is not clear whether their initial uptake of bacteria is selective; but once filtered from the water, microscopic particles can be sorted according to preference and the undesirable material is expelled. Frischer et al. (2000) reported that the rate of clearance of bacteria by zebra mussels was affected by the size of the bacterial cells. Larger bacteria were cleared more quickly than smaller cells. On the other hand, Baker and Levinton (2003) found that three unionids (*Margaritifera margaritifera*, *Amblema plicata*, and *Pyganodon cataracta*) filtered particles of different sizes and types with equal efficiency. All three mussel species sorted the particles for ingestion or rejection once they were removed from water; two of the species preferentially ingested *Microcystis* spp. (unicellular cyanobacteria) over almost all other particles, and strongly rejected large-celled phytoplankton. In contrast, *Amblema plicata* strongly rejected *Microcystis* in preference to the diatom *Cyclotella*. These unionid species also differed in their response to mixtures of particles. The unionids and zebra mussels preferred similar food types.

Unionids acquire toxic cyanobacteria from water, but the tendency to accumulate cyanobacterial toxins varies among mussel species. Yokoyama and Park (2002) measured the cyanobacterial toxin microcystin in the digestive gland of *Anodonta woodiana*, *Cristaria plicata,* and *Unio douglasiae* collected from Lake Suwa in central Honshu, Japan. The amount of microcystin accumulated by *A. woodiana* was 20-fold less than that found in the other two unionids. The relationship between the presence of toxic cyanobacteria in the water and the presence of toxin in tissues was not clear-cut—it differed among these three species found in the same lake. The authors concluded that the toxin was acquired via ingestion of toxin-containing cyanobacteria, rather than from direct absorption of toxin molecules across epithelial tissues. The study did not report effects of microcystin on the condition or health of the mussels themselves.

Some freshwater bivalves are able to distinguish highly toxic strains of cyanobacteria from less-toxic ones and selectively expel the highly toxic cells (Juhel et al. 2006). Zebra mussels were fed two strains of toxic cyanobacteria *Microcystis aeruginosa* plus the non-toxic diatom *Asterionella formosa*. The mussels could distinguish the two strains of cyanobacteria from each other and also from the non-toxic diatoms. They preferentially rejected the highly toxic cyanobacteria, expelling them as large quantities of "pseudofeces" through their incurrent and excurrent siphons. In the case of the highly toxic cells, the quantity of pseudofeces was much greater than normal and it contained more mucus than usual. The mussels also expelled highly toxic *Microcystis* cells through the pedal gape, using a hydraulic process rather than the normal ciliary activity. Juhel et al. (2006) used the term "pseudodiarrhea" for this atypical reaction to ingested material.

Bacteria as Nutrients

Bacteria that are ingested and retained by freshwater bivalves can be digested and assimilated as nutrients. Silverman et al. (1995) demonstrated that the bacteria taken up by zebra mussels, Asian clams, and the unionid *Toxolasma texasiensis* could be digested and incorporated into these mollusks. When the bivalves were suspended in water containing ^{35}S-labeled *Escherichia coli* bacteria, they removed the bacteria from the water, and the radioactivity was found in the hemolymph of all three species 48 hours later. Tissues from the zebra mussels were analyzed with polyacrylamide gel electrophoresis, and the radioactivity that had been in the bacteria was in proteins of the mussels.

Bacteria may even be necessary for the survival of juvenile mussels. In a study of factors affecting survival and growth of juvenile unionids, Jones et al. (2005) found that survival improved significantly when juvenile mussels were cultured in a recirculating system that contained fine sediment rather than sand or no substrate. These authors speculated that the juvenile mussels might use microbial flora in the sediment as a source of nutrients or as a means of digesting algae.

Resident Bacteria Versus Transient Bacteria

Bacteria can be easily cultured from bivalves, but the nature of the relationship between bivalves and their microbial flora is still unclear. The main dispute is whether the bacteria are residents (endosymbiotic) or transients (acquired incidentally as a consequence of siphoning activity). Garland et al. (1982) reported that microorganisms were not attached to or physically associated with epithelial surfaces in healthy adult Pacific oyster *Crassostrea gigas*. Actively feeding oysters were collected and transported to the laboratory, where some were immediately processed and examined by scanning electron microscopy (SEM). Others were kept for up to 24 hours in seawater to which various amounts of marine bacteria (*Vibrio anguillarum*, *Vibrio alginolyticus*, *Pseudomonas marina*, or *Alteromonas macleodii*) had been added. In addition to examination by SEM, tissues were homogenized and cultured on a variety of media to detect aquatic bacteria. Electron microscopy revealed that a sheet of mucus covered part of the surface of all organs of the oysters except the mid-gut. Surface-associated microorganisms were rare—they were only found on the external shell surface. The epithelial surfaces of all mantle and digestive tissues were mostly covered with cilia. No microorganisms were attached even where mucus was absent, and they were never seen on or within epithelium. Even when oysters were fed bacterial cultures in the laboratory, there were no bacteria associated with mucus or attached to the epithelium. Bacteria were readily detected in the midgut and cecum of fed oysters but they were not present in the mucus sheet over those tissues. Only when oysters were removed from water and allowed to spoil by storage (10 to 13 days at 10°C or 7 to 10 days at 20°C) was a surface microflora present. Even though a variety of media and culture conditions were used to permit growth of a wide range of organisms, bacterial counts were always less than 10^7/g of tissue (wet weight). Garland et al. (1982) speculated that the nearly uniform presence of cilia on epithelial surfaces of oysters probably swept the surfaces free of bacteria. Only when the oysters died were their tissues invaded by bacteria. Garland et al. (1982) concluded that bivalves did not have endosymbiotic bacteria or normal flora—the bacteria that were present were transient.

A similar study with the unionids *Lampsilis cardium* (= *ventricosa*), *Lampsilis siliquoidea*, and *Ptychobranchus fasciolaris* also did not find surface-associated microorganisms (Nichols et al. 2001). Foot, gills, labial palps, siphons from the mantle cavity, stomach, digestive gland, style sac, style, and various portions of the intestinal tract were examined by SEM. A thick layer

of mucus was present on epithelial surfaces in March and August. No bacteria or other microorganisms were found attached to the epithelial surface of any organs except in one animal. Every unionid examined had round structures (possibly spores) attached to the intestinal tract below the mucous layer and between enterocytes. The spore-like structures were 1 μm in diameter and were attached by a stalk to the cells but could not be identified. Organ contents and water used to rinse the mantle cavity were inoculated into enrichment broths to detect bacteria. All of the unionids had bacterial growth from organ contents at various times. The presence of microorganisms varied by collection locality, type of culture medium, and season; but not by unionid species, organ source, or aerobic/anaerobic culture conditions. The culture media used in this study were enrichment broths containing different sources of carbohydrate: cellulose (carboxymethylcellulose), chitin, and cellobiose. No attempt was made to isolate or identify the bacteria in the cultures. Nichols et al. (2001) concluded that the absence of microorganisms attached to or within mussel tissues meant there were no true symbiotic (endemic) microorganisms in the unionid mussels they examined. They concluded that the cellulolytic and chitinolytic microbes they did find were apparently transient, because their presence varied by season. Microorganisms that were able to degrade cellobiose were present throughout the year but were still not considered endosymbionts because they were not attached to mussel tissues.

Even though bacteria may not be physically attached to epithelial surfaces of healthy unionids, they can be difficult to eliminate from tissues. Gardiner et al. (1991) studied methods to improve the success of maintaining unionid gill explants in culture. They noted that one of the reasons why freshwater mussel tissues have not been cultured successfully was the inability to rid the cultures of bacterial and fungal contamination. Serotonin was used in their study to relax the musculature of the gill and increase ciliary activity of the gill epithelial cells. This improvement moved contaminants out of the channels of the gill. Without serotonin, the gill explants maintained function for several days but ultimately succumbed to contamination. Gardiner et al. (1991) concluded that the gill was harboring microorganisms within the branchial channels.

Fecal Bacteria

Because of their ability to accumulate bacteria to concentrations several-fold higher than in surrounding water, unionids have been used to monitor water quality and detect fecal contamination. Al-Jebouri and Trollope (1984) reported that all of the *Anodonta cygnea* collected from a highly contaminated urban lake contained coliforms, *Pseudomonas* spp., *E. coli*, and *Clostridium perfringens*; and 80% contained fecal streptococci. In contrast, *A. cygnea* collected from a less contaminated rural lake were less likely to contain some types of fecal bacteria: all contained coliform bacteria; but only 75% had *Pseudomonas* spp., 25% had *E. coli*, 50% had fecal streptococci, and none had *C. perfringens*. In addition, the actual concentration of bacteria in mussels was much lower in the mussels from the rural lake than from the urban lake. Bacterial content of the mussels from the urban lake could be decreased by holding them in containers in the rural lake for 24 to 48 hours.

Turick et al. (1988) used the unionid *Elliptio complanata* to test whether mussels take up and concentrate *E. coli* from water. The mussels were able to accumulate the bacteria to a concentration 5-fold greater than in the water after as little as 5 hours of exposure. After 28 to 50 hours of exposure, the concentration of bacteria in the mussels was 15 times higher than in the water, although concentrations in both water and mussels were very low by those later times. Turick et al. (1988) also evaluated the relation between temperature and bacterial content and

concluded that *E. coli* did not replicate within mussels at temperatures below 30°C. The bacterial density in mussel viscera was maximal within 48 hours of exposure, and bacteria remained in the tissue for several hours after the density in the surrounding water declined. The authors proposed that freshwater mussels could serve as a record of recent episodes of fecal pollution in water, because of their ability to concentrate and retain bacteria from their aquatic environment.

The indicator bacterium *E. coli* was isolated from all of the New Zealand freshwater mussels *Hydridella menziesi* tested, even those from a forested control site (Donnison and Ross 1999). However, the human pathogens targeted in this study (*Campylobacter jejuni, Campylobacter coli, Salmonella typhimurium,* and *Yersinia enterocolitica*) were found only in mussels exposed to water receiving treated sewage, treated meat-processing waste, or run-off from dairy farms.

Zebra mussels also concentrate *E. coli* from the water, and the maximum concentration is reached within a few hours of exposure (Selegean et al. 2001). The high number of bacteria in zebra mussels is retained for a few days, which provides a potential monitoring method for the detection of bacterial contamination in water.

Depuration

Although bivalves take up bacteria from the water, these bacteria do not necessarily have a long-term relationship with the mollusk. Transient bacteria can be eliminated by holding the bivalves in clean water to remove the bacteria (depuration). Removal of bacteria by depuration has been especially important in marine bivalves that are raised or harvested for human consumption (Son and Fleet 1980). Fecal coliforms can be recovered from bivalves living in contaminated water, and it is generally assumed that fecal bacteria are present in bivalves only as a consequence of their presence in an aquatic habitat contaminated by municipal wastewater or other sources of fecal bacteria. High numbers of fecal coliforms may be found in the lumen of the digestive tract of bivalves (Al-Jebouri and Trollope 1981), but they are not thought to replicate there, except perhaps at elevated temperatures. Depuration of unionids may also be important to avoid the transfer of pathogenic bacteria to hatcheries or other facilities when unionids are introduced (Starliper 2001, 2005).

Bacteria Cultured from Healthy Freshwater Bivalves

Unionids randomly collected from apparently healthy populations contain a diverse assemblage of bacteria. Motile *Aeromonas* spp. and *Pseudomonas* spp. were the predominate groups of bacteria isolated from apparently healthy unionids collected from the Ohio River (Starliper and Morrison 2000). Although the total number of bacteria in apparently healthy unionids is rather stable, the bacterial species in unionids vary depending on the bacterial species that are in the water (Starliper et al. 1998; Starliper 2001, 2005).

Bacteria were isolated from 80 of the 90 unionids sampled by Sparks et al. (1990). Bacterial isolates were obtained from 54 mussels that were considered healthy and 43 of these were sampled immediately after collection from the Illinois and Mississippi rivers rather than after confinement at a hatchery. Locations in the mussels that were sampled were stomach, hemolymph, midgut, gill, mouth, and mantle; bacteria were isolated from all of these samples. *Aeromonas hydrophila* was the most commonly isolated bacterial species from both healthy and dying mussels, and of the 37 bacterial taxa, seven were found only in healthy mussels.

In a study of the unionid *Elliptio complanata*, Chittick et al. (2001) cultured only the digestive gland and isolated bacteria from 19 of 20 mussels. There were 18 bacterial species

identified, and the most common species was *Aeromonas hydrophila*, which was found in 11 mussels.

Nichols et al. (2001) cultured the contents and rinse water from the mantle cavity, stomach, digestive gland, style sac, and the fore-, mid-, and hindgut of three unionid species. Bacteria were isolated from all of the mussels sampled. Three types of enrichment broths were used: cellulose (carboxymethylcellulose), chitin, or cellobiose. Mussels from river and lake habitats had bacteria that grew in the medium containing cellobiose, but only the mussels collected from the river had bacteria that grew in the other types of media. There were no differences in bacteria isolated from the various organs.

Zebra mussels collected from three sites in the Great Lakes region had three predominant bacterial genera: *Pseudomonas*, *Aeromonas*, and *Bacillus* (Toews et al. 1993). The same genera were found in living and dead mussels and in lake water. Gu and Mitchell (2002) listed 17 species of bacteria isolated from zebra mussels collected from Lake Erie. At least some of the zebra mussels had been held in aquaria with artificial lake water (10% Instant Ocean in distilled water) before isolation of the bacteria. *Pseudomonas* spp. were dominant under natural conditions, and *Aeromonas* spp. and *Shewanella* spp. became more prevalent under conditions of crowding, temperature elevation, or starvation.

Bacteria Associated with Disease in Freshwater Bivalves

Compared with marine bivalves, relatively little is known about bacterial diseases of unionids. The importance of bacteria as the cause of some diseases in marine bivalves did not become apparent until they were intensively cultured for commercial purposes, and the increasing interest in keeping unionids in captivity may lead to a greater awareness of bacterial diseases in freshwater bivalves. An important compilation of information about potential causes of die-offs of wild unionids resulted from a 1986 workshop (Neves 1987a). Concern about massive, widespread die-offs of unionids in the U.S. during the 1970's and 1980's, especially in the Mississippi River and some of its tributaries, led to this workshop about the causes of these die-offs, including the potential of bacterial diseases.

Jenkinson and Ahlstedt (1987) studied die-offs of unionids downstream from the Pickwick Landing Dam on the Tennessee River during 1985 and 1986. Large numbers of bacteria were observed in connective tissue and in the digestive gland of affected mussels. The bacteria were located outside the mussels' cells—few were inside hemocytes. Several species of bacteria were isolated from dead and dying mussels, but none could be correlated with death of the mussels. Large numbers of some bacterial species were isolated from some of the dead or dying mussels.

The mussel die-off that occurred downstream from Pickwick Landing Dam in 1985 and 1986 was also investigated by Scholla et al. (1987). Mussels that appeared to be healthy were compared with unhealthy mussels, which were defined as those whose valves did not close completely or did not remain closed after manual stimulation. The number of coliform bacteria did not differ significantly between healthy and sick mussels, but there was a 10-fold greater number of total bacteria in the sick mussels than in the healthy ones (5.15×10^5 versus 5.46×10^4 colony forming units [CFU]/g). A Gram-negative bacillus that produced characteristic yellow colonies and "copious extracellular polysaccharide" on plate count agar was prevalent among the isolates. Although the relative percentage of the yellow-pigmented bacteria was greater in apparently healthy mussels than in sick ones (4.0% of total colonies versus 2.9%), tissue from the sick mussels contained 10-fold more of the yellow-pigmented bacteria than was found in the healthy mussel tissue (1.49×10^4 versus 1.48×10^3 CFU). To determine whether the yellow-

pigmented bacteria could cause illness in mussels, sick mussels were co-cultured with healthy ones. The previously healthy mussels underwent an increase in the total number of bacteria, as well as in the number of yellow-pigmented bacteria, after co-culture with sick mussels. However, the number of animals used in this part of the study was small and was not sufficient to draw conclusions about pathogenicity.

Thiel (1987) described several die-offs of unionids in the upper Mississippi River. Although various types of bacteria, including columnaris-like bacteria, were found in affected mussels during these die-offs, there was no conclusive evidence that bacteria had caused any of these events.

Bacteria were isolated from 80 of 93 unionids from the Illinois River and the upper Mississippi River, and the isolated bacteria were grouped into 37 taxa (Sparks et al. 1990). Of the mussels from which bacteria were isolated, 26 were moribund but still had actively beating cilia on the gills. There were seven taxa of bacteria isolated only from healthy mussels, and 10 types of bacteria were isolated only from moribund mussels. However, none of the bacterial species that were found only in moribund mussels were isolated from more than two specimens.

Experimental Studies Demonstrating Lethal Effects of Bacteria on Freshwater Bivalves

Distinguishing between normal flora and pathogens has been difficult in bivalves. Because most of the bacterial species found in dying unionids are also found in apparently healthy mussels, bacteria isolated from diseased unionid have been considered by some investigators to be facultative invaders rather than primary causes of disease. However, there have been few experimental studies with unionids, and most of the information about this topic is for zebra mussels.

Unionids were exposed to bacteria isolated from fish, including the fish pathogens *Aeromonas salmonicida* and *Renibacterium salmoninarum* (Starliper and Morrison 2000). The bacteria were added to the water, and after a 24-hour static exposure, water flow was resumed. None of the challenged mussels died during the following three weeks.

Because of concern about the harm caused by zebra mussels, there has been research directed toward discovery of bacteria for the control of zebra mussels. Some of these bacteria, including *Bacillus* spp., can be used to kill the target animal via a water-borne toxin rather than by bacterial infection (Genthner et al. 1997; Singer et al. 1997). A *Pseudomonas fluorescens* isolate (ATCC 55799) that produces toxin will kill 92-100% of dreissenids exposed to 42 mg of bacterial dry mass/liter of water (Molloy 2001).

Genthner et al. (1997) exposed adult zebra mussels to suspensions of *Bacillus alvei* 2271, which had previously been shown to have toxic activity toward zebra mussels. A titration was performed to determine the volume of a standard suspension of the bacteria (3×10^8 CFU/ml) that would kill zebra mussels after various exposure periods. The 50% lethal concentration after a 3-day exposure was 0.81% v/v; after a 6-day exposure it was 0.21% v/v. Histopathological examination was performed on mussels exposed to a 1.0% suspension of the bacteria for 48 hours. None had died by that time, and there was no histological evidence of bacterial infection in the gut, gills, or gonads. Abnormalities were seen only in digestive tissues. The epithelium of the digestive tubules was extensively vacuolated at 24 hours. At 36 hours there was atrophy of digestive epithelial cells, disruption of apical cytoplasm, and sloughing of epithelial cells with pyknotic nuclei into the tubular lumen. The cause was thought to be a bacterial toxin, since there was no indication of active infection of the mussel tissues.

Toews et al. (1993) inoculated broth cultures of *Serratia liquefaciens* and *Escherichia coli* at two concentrations (10^6 CFU/ml and 10^4 CFU/ml) into water containing zebra mussels. Mussels suspended in water containing both concentrations of *S. liquefaciens* filtered the bacteria from the water but then became detached and lay moribund at the bottom. Mussels in containers with *E. coli* also removed the bacteria from the water but were not adversely affected.

Three species of *Aeromonas* isolated from dead zebra mussels (*Aeromonas jandaei, Aeromonas veronii,* and *Aeromonas media*) plus additional type cultures (*Aeromonas salmonicida salmonicida* and *Aeromonas hydrophila*) were tested for pathogenicity in laboratory experiments (Maki et al. 1998). Bacteria (10^6 CFU/mussel) were inoculated into zebra mussels "by inserting a narrow-gauge needle dorsally just between the valves and injecting the cell suspensions." All of the bacterial species tested were pathogenic, and the test bacteria were reisolated from the exposed mussels.

Gu and Mitchell (2001, 2002) concluded that *Aeromonas media*, *A. salmonicida*, *A. veronii*, and *Shewanella putrefaciens* are virulent pathogens in zebra mussels. A fractional chemical analysis was done in an attempt to discover the components of bacteria that were lethal to zebra mussels. The size of the bacterial population and water temperature were important variables related to the effects of bacteria on zebra mussels.

Bacterial Diseases of Marine Bivalves

Because of their economic importance, numerous studies have been conducted to identify bacterial causes of disease in marine bivalves, and many of the serious diseases of marine bivalves affect the larval or juvenile stages (McGladdery 1999; Paillard et al. 2004). In some cases, catastrophic losses have occurred, and specific bacterial pathogens have been identified. A bacterial genus that is commonly implicated as a cause of disease in marine bivalves is *Vibrio*.

Bacteria of the genus *Vibrio* were identified as a cause of bacillary necrosis of larval and juvenile, hatchery-reared northern quahog *Mercenaria mercenaria* and eastern oyster *Crassostrea virginica* (Tubiash et al. 1965, 1970). Results of experimental challenge studies implicated a Gram-negative bacillus with characteristics of *Aeromonas* sp. or *Vibrio* sp. as the etiologic agent (Tubiash et al. 1965). Subsequent work indicated that the most likely identity of the bacterial isolates was *V. alginolyticus*, *V. anguillarum*, other *Vibrio* sp., or a mixture of these *Vibrio* spp. The etiological agent(s) of bacillary necrosis appear to be normally present as saprophytes or symbionts in the marine environment.

Tubiash (1971) injected five strains of *Vibrio* spp. (three strains of *Vibrio anguillarum*, one strain of *Vibrio alginolyticus*, and a *Vibrio* sp.) by various routes into softshell clams *Mya arenaria*. All the isolates caused death in some animals but none was 100% lethal by any of the routes. The route of administration significantly affected the mortality rate; cardiac injection caused the highest mortality (mean 64%) except with *V. alginolyticus*. Injection into siphon tissue caused the second-highest mortality (mean 38%) except, again, with *V. alginolyticus*. Paradoxically, injection through the lumen of the excurrent siphon was more likely to kill the clams than injection through the lumen of the incurrent siphon. Three other bacteria were also tested (*E. coli*, *Serratia marcescens*, and *Aeromonas salmonicida*), and none was lethal. Heat-killed and filter-sterilized preparations of the *Vibrio* spp. were tested, and none caused death among the clams. There was a wide variation in susceptibility to the pathogenic bacteria among different animals.

A strain of *Vibrio anguillarum* was found to be responsible for mortality in larval Pacific oysters in a shellfish hatchery in coastal California (DiSalvo et al. 1978). The fortuitous

observation that penicillin rescued the larval oysters led investigators to suspect a bacterial etiology. Yellow-pigmented bacterial colonies were isolated on TCBS agar (thiosulfate, bile salts, sucrose), and were eventually identified as *V. anguillarum*. The authors noted that bacteria were not considered initially to be a cause of the problem because oysters could be cultured successfully despite the presence of high numbers of bacteria.

Vibrio alginolyticus, with or without *Vibrio splendidus*, was causally associated with disease in carpet shell clams *Ruditapes decussatus* (Gómez-León et al. 2005). These *Vibrio* spp. were isolated as the predominant microorganisms from moribund larval carpet shell clams. In experimental challenge studies, both species were able to cause significant mortality in carpet shell clam spat. The authors reported that intravalvar injection was more effective than immersion in establishing the experimental infections.

Persistent morbidity and mortality that involved extrapallial abscesses in juvenile Pacific oysters was described by Elston et al. (1999). The abscesses occurred locally between the inner shell surface and the mantle, and contained a mixture of bacteria and dying host cells, including hemocytes. Bacteria associated with the abscesses were usually rod-shaped, and some had been phagocytized by hemocytes, but the causative bacteria were not identified. The disease process was thought to be chronic, a feature that distinguished it from acute bacterial infections of the extrapallial space and mantle. Acute infections of the extrapallial space usually had a rapid course and ended with overwhelming bacterial infection of soft tissue. This disease appears different from brown ring disease, caused by *Vibrio tapetis*, because it affects only juvenile oysters.

Dungan et al. (1989) investigated a disease of juvenile Pacific oysters that principally affected the hinge ligament. The bacterial genus found most often in the hinge ligaments of juvenile Pacific oysters was *Pseudomonas*, but the presence of these bacteria was not associated with disease in the laboratory-confined animals. Half the groups of oysters yielded *Vibrio* spp. in small percentages, but those groups had no laboratory-associated mortality either. In contrast, one group that experienced 62% mortality in the laboratory had a cytophaga-like bacterium associated with degenerative lesions in the hinge ligament.

Colwell and Sparks (1967) isolated bacteria from healthy and dead or dying Pacific oysters from Washington. Two of the isolates were characterized more completely and were determined to be *Pseudomonas enalia*. Experimental challenge of oysters by injection of a broth culture of *P. enalia* killed all injected animals within 6 weeks.

Roseovarius crassostreae was reported as the apparent cause of juvenile oyster disease in cultured eastern oysters (Boettcher et al. 2005). The etiologic agent belongs to the marine α-proteobacteria in the *Roseobacter* clade. First isolated in 1997, *Roseovarius crassostreae* is a Gram-negative, aerobic bacillus that is strictly marine.

Problems with Investigation of Disease Outbreaks in Unionids

Often, the earliest observations of a particular disease outbreak among unionids were made by commercial harvesters or employees of commercial shell companies (Ballenger 1987; Scholla et al. 1987; Zale and Suttles 1987). Those “clammers” or “shellers” were also the most readily available source of information about the nature and extent of the disease problems. Even with commercial and non-commercial interests periodically monitoring mussel beds, disease problems are not easy to detect because the evidence is underwater (Jenkinson and Ahlstedt 1987; Thiel 1987). Often, the first indication of a problem in unionids was a report of the discovery of thousands of empty shells (Havlik 1987; Neves 1987b). In some studies, empty shells with

white nacre that was not discolored, and whose valves were still firmly attached to each other, were classified as "freshly dead." Obviously, the absence of soft tissue made it extremely unlikely that an infectious etiology could be identified from such samples. It was not clear how much time had elapsed between the death of the mussels and the discovery of the "fresh-dead" shells.

The widespread die-offs of freshwater mussels in the U.S. in the 1980's apparently were especially noteworthy because of the finding of large numbers of mussel meats floating on the surface of the water (Buchanan 1987; Havlik 1987; Neves 1987b; Thiel 1987). Havlik (1987) considered the phenomenon of floating mussel meats to be highly unusual. She argued that postmortem decomposition in unionids usually began with the gills, viscera, and foot; and the adductor muscles were generally the last to deteriorate. Thus, it was typical for the visceral mass of a dead mussel to decompose while remaining within, and attached to, the gaping shell. The finding of large numbers of floating meats meant the visceral mass was being released from the shell before it had decomposed. This phenomenon required that deterioration of the adductor muscle precede the decay of the soft tissues. Nevertheless, the floating meats were in varying degrees of decomposition and were unsuitable for histological or microbiological analysis.

Even when soft tissues were present, it was difficult to establish the extent of disease and even the difference between "healthy" and "diseased" animals. The criterion used to distinguish a "healthy" mussel from a "diseased/dying" mussel is often the ability of the animal to close its valves when prodded or disturbed. Mussels that do not close their valves completely or whose valves do not remain closed (i.e., are weak) are considered abnormal. Unfortunately, it is probably simplistic to use valve closure as the single indicator of mussel health.

Methods for the Study of Bacterial Diseases of Unionids

The number and types of bacteria isolated from either healthy or diseased mussels depend on the organ sampled. A common procedure for culturing bacteria from bivalves involves removing and homogenizing the entire visceral mass. The lack of attention to the location where bacteria are located within bivalves probably reflects a general lack of interest in bacteria that might cause disease in mollusks; the priority has often been on overall bacterial content or the number of human pathogens. For the diagnosis of disease in unionids, lesions or likely sites of infection should be sampled individually, preferably without contamination from other organs or the environment. Histopathology or in some cases even a careful gross examination could provide clues about the most important locations for bacteriological sampling within the unionid.

The media inoculated and the incubation temperature will also dictate the results of bacterial isolation. Starliper and Morrison (2001) used a wide variety of media, including media that are selective for likely pathogens, in a survey of potential bacterial pathogens in unionids. This approach will also be useful for diagnosing bacterial diseases of unionids. In another survey of bacteria in unionids, 18 of 46 isolates grew at 20°C but not at 35°C (Chittick et al. 2001). Selection of both a suitable culture medium and temperature for isolation of bacteria causing disease in unionids requires a different approach than used in many earlier studies that employed methods more suitable for detection of environmental bacteria or human pathogens. The development of new media for use in diagnosis of unionid diseases could aid in isolation and culture of unionid bacteria that do not grow well on existing media (Scholla et al. 1987).

The prompt examination of diseased unionids after collection is important. Scholla et al. (1987) found that the number of coliform bacteria recovered from unionids was 10-fold lower for specimens that had been refrigerated overnight. A delay in necropsy can also provide an

opportunity for growth of environmental isolates, which would complicate detection and identification of pathogenic bacteria.

The detection and identification of bacteria isolated from water or bivalves can be challenging. Bacteria that grow slowly in culture can be overlooked because of other faster growing species (Murchelano and Bishop 1969), and most bacteria cannot be cultured with present methods (Riesenfeld et al. 2004). The use of selective media can be useful for detection of some types of bacteria (Starliper and Morrison 2001), but this requires knowledge of the bacteria likely to be causing disease—at present, this knowledge does not exist for unionids. Identification of some bacterial species, e.g., *Aeromonas* spp., is difficult, and some of the existing methods are not completely reliable (Martínez-Murcia et al. 2005; Ørmen et al. 2005). The use of genomic methods provides more reliable identification of bacterial species, and improvements in these methods may allow their routine use for identification or confirmation of bacterial species. However, after the bacterial species is identified it is important to realize that virulence can be dramatically different for bacterial isolates that are given the same species name (Olivier 1990; Han et al. 2006). Published conclusions about the lack of differences in the bacteria isolated from healthy and diseased unionids have not adequately considered the possibility that bacterial virulence could be different.

Protists

Protists Other Than Ciliates

Marine bivalve mollusks have a wide variety of protist parasites from several phyla (Bower 2006); however, all of the protists commonly found in unionids and other freshwater bivalves are in the phylum Ciliophora. Some of the most serious diseases of marine bivalves are caused by protists that are not ciliates; examples include *Perkinsus* spp., *Bonamia ostreae*, *Haplosporidium nelsoni*, *Mikrocytos mackini,* and *Marteilia* spp. Because unionids are phylogenetically and environmentally separated from the marine bivalves that are susceptible to these pathogens, it is possible that protists similar to those of marine bivalves do not occur in unionids. It is also possible that some protists have been over looked in freshwater bivalves.

Some diseases of marine bivalves that have historically been considered "fungal diseases" are caused by organisms that are not true fungi. An example is larval mycosis of eastern oysters *Crassostrea virginica* and northern quahog *Mercenaria mercenaria* caused by *Sirolpidium zoophthorum*. This pathogen is an oomycete, currently considered to be Peronosporomycetes and thus unrelated to organisms in the kingdom Fungi (Adl et al. 2005). Another example is the protist organism commonly called quahog parasite X (QPX), which is the cause of a disease in northern quahog (Whyte et al. 1994). This organism is in the order Labyrinthulida, family Thraustochytriidae (Gast et al. 2006), which in spite of the "chytrid" appearing name for this family is not related to the chytrids that are included in the kingdom Fungi.

"Fungal hyphae" were mentioned by Pekkarinen (1993) in *Unio* spp. affected by "pustular disease." The cause of this disease is unknown, and the hyphae were observed in marsupia containing dead unionid embryos. It is likely that these "fungi" were saprophytic oomycetes.

Numerous studies of protists in bivalves are related to the sequestering of human pathogens in species consumed by humans or in species that have potential as bioindicators (e.g., Graczyk et al. 2004; Miller et al. 2005; Gómez-Couso et al. 2005). *Cryptosporidium parvum* oocysts in water are concentrated by zebra mussels, which provides a potential means of detecting low

concentrations of this human pathogen in water supplies (Graczyk et al. 2001). Because the *C. parvum* oocysts that are sequestered by bivalves are viable (Freire-Santos et al. 2001), humans could be infected by handling or consuming contaminated mollusks.

Ciliates

Conchophthirus.—The most common protozoans in unionids are *Conchophthirus* spp. (family Conchophthiridae). Some authors have spelled this genus "*Conchophthirius*" (Kidder 1934) or "*Conchopthirius*" (Penn 1958), but these spellings are not considered correct by more recent authors. Species in this genus are only found in freshwater bivalves and are among the most common organisms living in unionids. The body of these ciliates is laterally flattened, elliptical in profile, and with the mouth near the middle of the body (Fenchel 1965; Antipa and Small 1971a). They have dense cilia over their entire surface, and some of these cilia are thigmotactic (capable of moving independently of the other cilia and become stationary and stiff when in contact with a substrate). The average length of most species is about 100 µm. *Conchophthirus* spp. move within the mantle cavity and are not firmly attached to the host. If removed from their host, these organisms usually die within 24 hours (Kidder 1934).

Clark and Wilson (1912) considered *Conchophthirus* spp. to have "universal occurrence" in unionids and thus did not specifically mention this genus in their survey of unionid parasites. They stated that there were several species of *Conchophthirus* that were parasitic on freshwater mussels and mentioned *Conchophthirus curtus* and *Conchophthirus anodontae.* Kelly (1899) reported *Conchophthirus anodontae* and *Conchophthirus hirtus* [sic] (probably intended *C. curtus*) in 30 of the 44 species of unionids examined from Illinois, Iowa, and Pennsylvania. These protozoan species were not separated in the results presented by Kelly (1899), and 68% of the individual mussels were infested with at least one of these species.

Some species of *Conchophthirus* appear to prefer certain host species. *Conchophthirus anodontae* was most commonly found adhering to the nonciliated surfaces of the palps of the unionid *Elliptio complanata*, and in natural conditions was not found on other hosts except occasionally on *Alasmidonta* (= *Anodonta*) *marginata* (Kidder 1934). Even more specific, *Conchophthirus magna* was found only in *Elliptio complanata*. A similar level of host specificity was not characteristic of *Conchophthirus curtus*, which was found in *A. marginata*, *Anodonta implicata, Pyganodon* (= *Anodonta*) *cataracta, Lampsilis radiata, Lampsilis cariosa, Alasmidonta undulata,* and *Elliptio complanata*. *Conchophthirus curtus* was rarely found on *E. complanata* in natural conditions, and when *C. curtus* was found in *E. complanata*, *Conchophthirus anodontae* was also present. However, when various hosts were confined in aquaria for a week, cross infections occurred so that about equal numbers of *C. anodontae* and *C. curtus* were found on all of the exposed mussels species (Kidder 1934).

Conchophthirus curtus is the most commonly studied member of this genus in North American unionids. Length range for living specimens is 62-140 µm (Antipa and Small 1971a). This protozoan was observed in the fluid of the mantle cavity and "creeping" on the gills and palps of several unionid species; prevalence in *A. marginata* was 100% (Kidder 1934). Antipa (1977) reported that all of the *Lampsilis cardium* (= *ventricosa*) and *Pyganodon* (= *Anodonta*) *grandis* collected from one location were infested. In collections of *Elliptio complanatus* in North Carolina over a 7-month period, only one of 77 specimens did not have *C. curtus*, and the maximum number per host was over 1000 (Beers 1962). Penn (1958) found *C. curtus* in two species of unionids, *P. grandis* and *Lampsilis siliquoidea*, and all of the mussels examined were

infested. Antipa and Small (1971b) found *C. curtus* in 12 of 16 species of mussels examined in Illinois, and some hosts contained over 1000 protozoans.

Food vacuoles of *Conchophthirus curtus* contain algae and "sloughed-off" epithelial cells (Kidder 1934). Antipa and Small (1971a) used electron microscopy to further support the conclusion that *C. curtus* feeds on its host. Almost all of the food vacuoles of this protozoan contained host cell components, with host gill cilia the most common item. Bacteria were the only other recognizable material in these food vacuoles. The cells consumed by *C. curtus* could have been sloughed by the host before consumption by the protozoan, but conclusive support for this has not been presented. Antipa and Small (1971a) did not observe any adverse effects, even on hosts with hundreds of this protozoan.

Conchophthirus anodontae has also been reported as a common inhabitant of the mantle cavity of unionids (Kelly 1899; Clark and Wilson 1912). Fenchel (1965) considered *Conchophthirus anodontae* to be a junior synonym of *Conchophthirus raabei* and reported this species in the unionid *Anodonta cygnea*. The average size of *C. anodontae* measured by Kidder (1934) was 103 µm long and 69 µm wide. All of the *Elliptio complanata* examined by Kidder (1934) were infested, and palps removed from heavily infested hosts appeared mottled with *C. anodontae*. Kidder (1934) concluded that the food vacuoles of this protozoan contained algae, bacteria, and sloughed-off epithelial cells, but he did not present evidence that the epithelial cells had been sloughed before being consumed.

Conchophthirus magna is larger than other species in this genus, averaging 180 µm long and 95 µm wide (Kidder 1934). In natural conditions, this protozoan was found in the mantle cavity of about 25% of the *Elliptio complanata*. The number per host was 10 to 20, and food vacuoles contained only epithelial cells. Kidder (1934) considered the possibility that the ingested cells might not have been sloughed because they were regular in outline and stained well.

Zebra mussels harbor at least two additional species of *Conchophthirus*: *Conchophthirus acuminatus* and *Conchophthirus klimentinus* (Fenchel 1965; Molloy et al. 1997; Karatayev et al. 2003a, 2003b). *Conchophthirus acuminatus* is host-specific for dreissenids and is perhaps the most common symbiont of zebra mussels in Europe (Burlakova et al. 1998; Laruelle et al. 1999; Karatayev et al. 2000a). This protozoan occurs in quagga mussels *Dreissena bugensis* but at a low prevalence and intensity compared with the infestation of zebra mussels (Karatayev et al. 2000b). All of the zebra mussels in some European samples had this ciliate, and the intensity of infestation was high (Burlakova et al. 1998). The number of *C. acuminatus* per zebra mussel was positively correlated with mussel length and often exceeded 500. Karatayev et al. (2000a) reported maximum intensities of over 10,000. The level of infestation varied seasonally (Karatayev et al. 2000b). When a zebra mussel dies, the *C. acuminatus* rapidly leave the dying host (Burlakova et al. 1998), but emergence also occurs from healthy mussels (Karatayev et al. 2003b). *Conchophthirus acuminatus* and the other species of protozoans specific for D*reissena* spp. have not yet been found in North America.

Laruelle et al. (1999) used histological examination of zebra mussels to evaluate the relation between ciliated protozoans and their host. *Conchophthirus acuminatus* were most commonly found on the outer gill surfaces and less often on the epithelium covering the visceral mass. In addition, *C. acuminatus* was discovered histologically in locations where this species had not been found previously: within the gill water tubes and within suprabranchial cavities. Epithelial tissue in contact with *C. acuminatus* appeared to be normal histologically.

Karatayev et al. (2003a) found three species of ciliated protozoans in zebra mussels from Belarus: *Conchophthirus acuminatus, Ophryoglena* sp., and *Ancistumina limnica*. The most

common of these protozoans was *C. acuminatus*, which had a prevalence of 98.7 to 100% and mean number per host of 348 to 1672. The number of protozoans was highest during summer.

***Heterocinetopsis unionidarum*.**—Antipa and Small (1971b) found the ciliate *Heterocinetopsis unionidarum* (family Ancistrocomidae) in 2 of the 4 species of mussels examined in the one locality where it occurred. Infested unionids were *Pyganodon* (=*Anodonta*) *grandis* and *Lasmigona complanata*. There were no obvious detrimental effects from this protozoan, which was attached to the gills and palps but not on other surfaces within the mantle cavity. Attachment is by a "sucker or tentacle." The inferior surface of *H. unionidarum* is concave and has nine or ten rows of cilia. The superior surface is convex and non-ciliated. The average length of silver-stained specimens was 40.5 µm. *Heterocinetopsis unionidarum* was also reported by Chatton and Lwoff (1950). Antipa (1977) found this protozoan on all of the *P. grandis* examined from one location, but it was not on *Lampsilis cardium* (= *ventricosa*) from the same location.

***Trichodina*.—**The genus *Trichodina* and related genera (Peritrichia: Trichodinidae) include numerous species that are mostly parasites of fish, but a few species are found in bivalve mollusks including unionids. These protozoans are found in both freshwater and seawater habitats and have a distinctive flattened body with internal denticles forming a circular pattern. *Trichodina unionis* is found in the mantle cavity of *Anodonta cygnea* and *Unio* spp. in Europe (Fenchel 1965). Prevalence approaches 100% in some populations but with only about 10 per host. Diameter of *T. unionis* is about 70 to 100 µm (Raabe and Raabe 1961; Fenchel 1965). The most common location of this organism is on the labial palps, and less often on gills (Raabe and Raabe 1961). *Trichodina* sp. was observed in unionids collected in Illinois (Antipa and Small 1971b) and North Carolina (Chittick et al. 2001). Histological examination did not reveal lesions associated with *Trichodina* sp. (Chittick et al. 2001).

Other ciliates of unionids.—Chittick et al. (2001) found the scyphidiid peritrich *Mantoscyphidia* sp., and low numbers of a scuticociliatid ciliate on the gills of the unionid *Elliptio complanata* in North Carolina. In addition, a *Trichodina* sp. (mentioned above) was present on the gills. *Mantoscyphidia* sp. had a diameter of 11 to 29 µm, a broad scopula, and a compact C-shaped macronucleus. There was no indication of damage to the mussel by these protozoans. Additional ciliates reported in the mantle cavity of unionids are *Tetrahymena* sp. and a colonial contractile peritrich (Antipa and Small 1971b).

Other ciliates of zebra mussels.—*Ophryoglena hemophaga* (family Ophryoglenidae) is a common protozoan of zebra mussels in Europe (Molloy et al. 1997; Karatayev et al. 2002; Molloy et al. 2005). *Ophryoglena hemophaga* trophonts are found only in the lumen of the digestive gland and feeds, at least in part, on hemocytes of zebra mussels (Molloy et al. 2005). Trophonts of this species are ovoid to elongate, with a length after staining of 96 to 288 µm. The prevalence of this protozoan and the number per host varies seasonally (Karatayev et al. 2002), but in a lake in the Netherlands about 95% of the individual zebra mussels were infested and most mussels had dozens of *O. hemophaga* (Molloy et al. 2005). In another survey of zebra mussels, prevalence of *Ophryoglena* sp. ranged from 43.3 to 100%, and the mean number ranged from 1.4 to 65.8 per host (Karatayev et al. 2003a). As is typical for the family Ophryoglenidae, trophonts living in a host form protomonts that leave the host and encyst to form tomonts. The

tomonts undergo mitosis to produce theronts that re-enter the host and form parasitic trophonts. A protozoan reported as *Ophryoglena* sp. was found in zebra mussels from lakes Erie and St. Clair, and the protozoan was more abundant in moribund zebra mussels than in apparently healthy individuals (Toews et al. 1993).

Ancistrumina limnica (family Ancistridae), which is found on zebra mussels in Europe, is a ciliated protozoan often found in the gill water tubes, less often on the gill surface, and rarely in the suprabranchial cavities (Laruelle et al. 1999). *Ancistumina limnica* has a highly variable prevalence ranging from 0 to 93.3% (Karatayev et al. 2003a) and the maximum number in a single zebra mussel was 299 (Karatayev et al. 2000a). No lesions were observed in association with this protozoan (Laruelle et al. 1999).

Sphenophrya dreissenae, which is specific for zebra mussels, is found within gill water tubes and on the epithelium covering the mantle cavity, visceral mass, and outer gill surfaces. Less often this protozoan is in the suprabranchial cavities (Laruelle et al. 1999). *Sphenophrya dreissenae* is in the family Sphenophryidae, which is characterized by a lack of cilia and mouth in the adult stage and has a large, irregular-shaped macronucleus. They attach to the host epithelium and several layers of this protozoan can cover the epithelium of the visceral mass or gills of an infested host (Laruelle et al. 1999). Epithelial vacuolization, hyperplasia, and hypertrophy occurred under foci of high numbers of this pathogen and resulted in cellular protrusion into the water tubes. *Sphenophrya naumiana* also occurs on zebra mussels in Europe (Molloy et al. 1997).

Hypocomagalma dreissenae (family Ancistrocomidae) is a protozoan specific for zebra mussels and is most often found on gills (Laruelle et al. 1999). Additional locations for this organism are on the visceral mass, the mantle cavity epithelium, in gill water tubes, on labial palps, and within the suprabranchial cavities. *Hypocomagalma* spp. have a suctorial tentacle that is used for attachment to the host. This parasite feeds on epithelial cells, but lesions were not evident, perhaps because only low numbers of this parasite were found.

A peritrich protozoan was found attached to the epithelium of the visceral mass in zebra mussels in Europe (Laruelle et al. 1999). This organism had an elongated, coiled macronucleus and was also found on the external surface of zebra mussel shells. Lesions were not observed in association with this protozoan.

Aspidogastrea

The Aspidogastrea, also known as Aspidobothrea or Aspidocotylea (Schmidt and Roberts 2000; Zamparo and Brooks 2003) is one of two subclasses of Trematoda (phylum Platyhelminthes). This is a relatively small group, including only about 80 species (Rohde 2001) compared with about 18,000 nominal species and 150 families in Digenea, the other subclass of Trematoda (Cribb et al. 2001b).

Unlike the Digenea (discussed below), some aspidogastrid species complete their life cycle within a mollusk. Of the four families in this subclass, complete life cycles are known only for the Aspidogastridae (other families are Multicalycidae, Rugogastridae, and Stichocotylidae), which is also the largest of these families and includes all of the aspidogastrids that parasitize freshwater mollusks. Some aspidogastrids develop to sexual maturity in a mollusk, but if the final host is a vertebrate, infestation of the vertebrate host is by ingestion of the mollusk. Vertebrate hosts are either fish or turtles (Fulhage 1954; Rohde 2002). Some of the life cycles in

this family involve the release of eggs via feces of a vertebrate host, and either the egg or a ciliated cotylocidium, which hatches from the egg, enter a mollusk (Ferguson et al. 1999). The location of infestation in the mollusk depends on the species of the parasite; common locations are the mantle cavity, pericardial cavity, kidney, intestine, and inside gill filaments.

The most distinctive morphological feature of aspidogastrids is the large adhesive disk located on the ventral, posterior portion of the body. This holdfast organ is often the widest part of the body. In addition to its use for attachment to the host, the holdfast organ may also have a sensory function or be used for external digestion (Fredericksen 1980). For the family Aspidogastridae, the adhesive disk has multiple rows of alveoli.

Only four species of aspidogastrids have been reported in unionids in North America: *Aspidogaster conchicola, Cotylaspis insignis, Cotylogaster* (= *Cotylogasteroides*) *occidentalis,* and *Lophotaspis interiora*. Two of these species, *Aspidogaster conchicola* and *Cotylaspis insignis,* are among the most common symbionts of unionids, are widely distributed, and are found in several hosts (Huehner 1984; Hendrix et al. 1985). There are a few additional species of aspidogastrids that infest bivalves. *Aspidogaster antipai* has been reported in unionids but not in North America (Rohde 1972), *Aspidogaster indicum* infests Indian bivalves (*Indonaia caerulea*, *Corbicula striatella*, and *Lamellidens corrianus*) after experimental exposure (Rai 1964), and *Aspidogaster limacoides* has been reported in zebra mussels in Russia but not from North America (Kuperman et al. 1994; Molloy et al. 1997; Laruelle et al. 2002). *Lobatostoma ringens* infests marine bivalves, *Donax* spp. (Hendrix and Overstreet 1977), but has not been reported in freshwater.

Aspidogaster conchicola

Aspidogaster conchicola is a common parasite of freshwater mussels, and is usually located in the kidney or pericardial cavity of the mussel host (Kelly 1899; Hendrix and Short 1965; Huehner and Etges 1981; Duobinis-Gray et al. 1991). Kelly (1899) considered the pericardium to be the primary location for this parasite, with the nephridial cavity invaded in hosts having a large number of *A. conchicola*. Adult trematodes were found only in the pericardium by Bakker and Davids (1973), but Benz and Curran (1997) found adults in both the kidney and pericardial cavity. In the unionid *Gonidea angulata*, *A. conchicola* also occurs in muscle, connective tissue, hemolymph vessels, and digestive gland (Pauley and Becker 1968).

Adult *A. conchicola* are 2.5 to 2.7 mm long and have four rows of alveoli in the holdfast organ (Williams 1942). *Aspidogaster conchicola* has no eye spots (Leidy 1858), which aids in distinguishing it from *Cotylaspis insignis*, another common aspidogastrid in unionids.

This species is widely distributed and infests numerous unionid species (Kelly 1899; Vidrine and Causey 1975; Hendrix and Short 1965; Hendrix et al. 1985). This parasite is also found in the intestine of fish and turtles, typically in species that eat infested mussels (Faust 1922; Gao et al. 2003), but infestation of a vertebrate is not required for completion of the life cycle (Williams 1942; Huehner and Etges 1977). In the Tennessee River, 14 of 16 unionid species were infested in one study (Hendrix 1968) and all eight species of unionids examined were infested in a later study (Benz and Curran 1997). In addition to unionids, other mollusks parasitized by *A. conchicola* include Dreissenidae (Molloy et al. 1997), Mutelidae, Sphaeriidae, Corbiculidae, and certain gastropods (Michelson 1970; Rohde 1972; Huehner and Etges 1977). Zebra mussels from lakes Erie and St. Clair have a low prevalence of *A. conchicola* (Toews et al. 1993). *Aspidogaster conchicola* is the only parasite species found in Eurasian dreissenids that is also native to North America (Molloy et al. 1997).

Large numbers of *A. conchicola* have been reported in a single unionid host. Curry and Vidrine (1976) found 229 *A. conchicola* in one *Potamilus purpuratus* (=*Proptera purpurata*), which also hosted 57 mites (*Unionicola* spp.) and an unspecified number of leeches (*Placobdella montifera*). Nelson et al. (1975) found a maximum of 1,545 *A. conchicola* in a *P. purpuratus* and 413 in a *Lampsilis ovata*.

Prevalence of infestation by *A. conchicola* in a population of unionids is often over 50% (Bakker and Davids 1973; Williams 1978; Huehner 1984; Benz and Curran 1997). Of the 33 species of unionids with 14 or more individuals examined by Kelly (1899), 41% of the individual mussels had this parasite.

Aspidogaster conchicola appear to feed on their host. Gentner (1971) found that the gut contents of *A. conchicola* resembled host hemocytes, and Bakker and Davids (1973) concluded that hemocytes and hemolymph of the host are consumed. Huehner et al. (1989) provided evidence that *A. conchicola* feeds on host epithelium.

The holdfast organ on the ventral surface of aspidogastrids has glands that are called marginal organs and secrete esterases (Huehner et al. 1989). Morphological evidence also indicates that the holdfast organ produces digestive enzymes (Bakker and Diegenbach 1974). These observations, along with histological evidence of physical distortion of host epithelium underlying the holdfast organ, suggests that aspidogastrids use the holdfast organ for external digestion to aid in consumption of host cells.

Morphological injury to hosts infested by *A. conchicola* appears to be variable. There have been reports of no grossly visible lesions in unionids infested with *A. conchicola* (Williams 1978), and sometimes lesions are not apparent histologically (Benz and Curran 1997). However, based on previously published observations of high intensity of infestations and in some cases lesions, Benz and Curran (1997) concluded that *A. conchicola* has the potential to adversely affect mussels.

A large number of *A. conchicola* in a host does not seem to be required for host injury. Although the largest number of *A. conchicola* found in a single unionid was only nine (four adult worms in the pericardium and five juvenile worms in the kidney), Bakker and Davids (1973) found epithelial hyperplasia and absence of cilia at the site of parasite attachment to the pericardial and kidney epithelium. In addition, the epithelial cells under the attachment site were flattened.

Seven of the 13 unionid species examined by Huehner and Etges (1981) were infested with *A. conchicola*, and in six species, capsules sometimes formed around the eggs and body of *A. conchicola* (for the seventh infested species, only 1 of 2 individuals examined was infested). The percentage of hosts with encapsulation ranged from 5.8 to 23.6% for the six species that formed capsules. The capsule that forms around the parasites has two layers, each composed mostly of fibroblasts. Fibroblasts of the inner layer of the capsule have basophilic cytoplasm, and those of the outer layer have eosinophilic cytoplasm. Hemocytes are present between the two layers of the capsule or within the inner layer. The collagen in the capsule did not stain with Mallory's stain but was silver positive, indicating the presence of reticulum fibers. Erosion of the epithelium lining the digestive gland of the host was also noted by Huehner and Etges (1981).

In the unionid *Gonidea angulata*, a thin, fibrous capsule formed around the *A. conchicola* that were in locations other than the pericardial and renal cavities (Pauley and Becker 1968). Hemocytes were in the capsules, and in some instances there was hemocytic infiltration around the cyst. In the kidney of *Anodonta californiensis* and *Anodonta oregonensis*, but not *G.*

angulata, there was distortion and distention of the renal cavities and metaplasia of the normally ciliated columnar epithelium to squamous or cuboidal without cilia. There was also fibrosis of the renal connective tissue. Severity of the metaplasia and fibrosis increased with the number of *A. conchicola* present in the kidney. The absence of renal metaplasia in *G. angulata* may have been because of the lighter infestation in this host (Pauley and Becker 1968). Flook and Ubelaker (1972) only examined infested unionids grossly and not surprisingly did not find the histological lesions described by Pauley and Becker (1968). Several types of histological lesions of the pericardial epithelium were associated with *A. conchicola* in the unionid *Anodonta cellensis* (Wasielewski and Drozdowski 1995).

In zebra mussels collected in Europe, Laruelle et al. (2002) found hemorrhage in association with an *Aspidogaster* sp. in the stomach; because the parasite was only observed histologically, it is uncertain whether it was *A. conchicola* or *A. limacoides*. In another zebra mussel, a single *Aspidogaster* sp. had been encapsulated in the digestive gland.

Cotylaspis insignis

Cotylaspis insignis is usually located in the branchial region of its mussel host, including the junction of the foot and inner gills, within gills, and in suprabranchial cavities (Kelly 1899; Hendrix and Short 1965). Turtles can also be infested (Fulhage 1954) but are not required for completion of the life cycle (Huehner 1984). *Platyaspis anodontae* and *Cotylaspis reelfootensis* are junior synonyms of this species (Kofoid 1899; Hendrix and Short 1965).

Adult *C. insignis* are 1.0 to 1.6 mm long and have an adhesive disk with three rows of alveoli (Hendrix and Short 1965). The worm is translucent white or pink and has two eyes (Leidy 1858), which are not present in other genera of aspidogastrids. This is the only aspidogastrid that is commonly found in the mantle cavity of North American unionids rather than inside organs.

Cotylaspis insignis is widely distributed geographically and infests numerous unionid species (Vidrine and Causey 1975; Hendrix et al. 1985). Kelly (1899) found *C. insignis* in 24 of the 44 species of unionids examined, and Huehner (1984) found this parasite in 16 of 32 unionid species. However, in some studies, *C. insignis* is notable by its absence (e.g., not found in eight species of unionids examined from Kentucky Lake by Benz and Curran [1997]). In another study of unionids of Kentucky Lake, only one species (*Anodonta suborbiculata*) of the 10 species examined was infested with *C. insignis* (Duobins-Gray et al. 1991). Of 16 species of unionids examined from the Tennessee River in Hardin County, Tennessee, only *Plethobasus cypheus* was infested (Hendrix 1968).

There have been reports of differences in host preference by *C. insignis* (Osborn 1903; Stromberg 1970), but the variation in infestation of different unionid species may be more closely linked to host habitat rather than species. Huehner (1984) found higher prevalence and intensity of infestation by *C. insignis* in unionids living in habitats with low water velocity compared with higher velocity.

In most studies, the number of *C. insignis* per host is low (Danford and Joy 1984), at least in comparison with *A. conchicola*, but as many as 212 *C. insignis* has been reported from a single host (Najarian 1955). Other reports of relatively high numbers of *C. insignis* per host include 92 by Kelly (1899) and 83 by Nelson et al. (1975).

The prevalence of *C. insignis* is highly variable. Kelly (1899) reported that for 33 species of unionids with 14 or more individuals examined, 18% of the individual mussels were infested.

Prevalence of more than 50% for a sample of a unionid species has been reported (Najarian 1955; Nelson et al. 1975).

In spite of the external location of *C. insignis* on unionids, Gentner (1971) concluded that this trematode consumes host hemocytes. Morphological lesions resulting from infestation by *C. insignis* have not been reported.

Gangloff (2003) collected aspidogastrid trematodes from the unionid *Pyganodon grandis*, and although the trematodes were not identified, they were collected by flushing the mantle cavity. The location within the host indicates that these aspidogastrids were probably *C. insignis*. All of the mussels examined were infested with both aspidogastrids and unidentified mites. The mean number of aspidogastrids per host was 41.3 (range, 6-86), and there was a significant negative correlation between trematode density (number of parasites per gram of host) and the glycogen concentration in the mantle of the host.

Cotylogaster occidentalis

Cotylogaster occidentalis parasitizes the intestine of unionids (Kelly 1926; Fredericksen 1972; Ip et al. 1982; Ip and Desser 1984; Benz and Curran 1997) and the intestine of freshwater drum *Aplodinotus grunniens* (Nickerson 1902; Sogandares-Bernal 1955; Fredericksen 1972). There are also reports of *C. occidentalis* in snails (Dickerman 1948; Whittaker and Kozel 1975). Adults of *C. occidentalis* occur in both mollusks and fish, but a vertebrate host is not required for completion of the life cycle (Dickerman 1948). Fredericksen (1980) found adult *C. occidentalis* in the intestine of the unionid *Ligumia nasuta*, but the youngest juveniles of this parasite were in the mouth and esophagus and older juveniles were in the stomach. *Cotylogaster occidentalis* did not reach sexual maturity until reaching the intestine.

Cotylogaster occidentalis has been considered to be in the genus *Cotylogasteroides* by some authors, but Fredericksen (1972) concluded that *Cotylogasteroides* should be considered a junior synonym of *Cotylogaster*. Fredericksen (1972) considered *Cotylogasteroides barrowi*, described by Huehner and Etges (1972), to be a synonym of *C. occidentalis.*

Cotylogaster occidentalis are reddish and in unionids are reported as 3.5 to 6.0 mm long (Fredericksen 1972) or 2.4 to 4.3 mm long (Huehner 1972). *Cotylogaster occidentalis* grows to larger size (up to 18 mm long) in the freshwater drum than in unionids. The holdfast organ covers the entire ventral body surface except for the neck. Alveoli are arranged in a continuous line around the margin of the holdfast organ, with additional transversely elongate alveoli in a median row.

Generally *C. occidentalis* is less common and found in fewer species of unionids than *Aspidogaster conchicola* and *Cotylaspis insignis* (Hendrix et al. 1985). Huehner (1984) found *C. occidentalis* in 3 of 32 species of unionids examined from Missouri, and Benz and Curran (1997) found this parasite in 2 of 8 species of unionids examined from Tennessee. A prevalence of 93% was reported for *Cotylogaster occidentalis* in *Ligumia nasuta* but there was "little if any pathological effect" (Fredericksen 1980).

Lophotaspis interiora

Only immature *Lophotaspis interiora* have been found in unionids (Hendrix and Short 1972), and the only adult known was in an alligator snapping turtle (Ward and Hopkins 1931), which suggests that this parasite has a two-host life cycle (Hendrix et al. 1985). In unionids, *L. interiora* are in the pericardial cavity, kidney (Hendrix and Short 1972), and beneath the skin covering the foot (Vidrine 1980). The original discovery of this species in unionids was in six

species from northwestern Florida (Hendrix and Short 1972). The only additional report of this parasite was also in Florida (Vidrine 1980). Other species of *Lophotaspis* occur in bivalves but have not been reported in North America.

Digenea

Digenea is a subclass of Trematoda, and almost all digeneans use mollusks as a primary intermediate host and use vertebrates as final hosts, although there is great diversity in life cycles (Gibson 2002). Several groups of Digenea infest bivalve mollusks, but there is more information about Digenea in snails because most Digenea (Cribb et al. 2001a, 2003), especially those that infest humans (Lockyer et al. 2004), have snails as intermediate hosts.

Digenean life cycles are complex and vary among groups. Adult digeneans that are relevant to this review live in the gut or urinary tract of vertebrates. The adult digeneans shed eggs that are passed with feces or urine into the water where they hatch and release miracidia, a free-swimming ciliated stage. The miracidia penetrate a mollusk that becomes the first intermediate host. The species of mollusk depends on the species of digenean—some digeneans being highly specific. In the mollusk, the miracidia transform into sporocysts, and this stage is often the most obvious cause of damage in mussels. Depending on the family of digenean, sporocysts generally give rise to daughter sporocysts or directly produce rediae. If daughter sporocysts are formed, they may produce rediae. Sporocysts do not have a digestive system and absorb nutrients from the host.

Depending on the species of digenean, cercariae are produced from either rediae or sporocysts. For most digeneans, the cercaria is a tailed stage that leaves the first intermediate host and swims through the water until a suitable fish host is located. The fish is penetrated by the cercaria, and the digenean then becomes a metacercaria. Less commonly, as in the Gorgoderidae described later in this review, the metacercariae are formed in sporocysts, which are within the first intermediate host rather than in a second intermediate host. After metacercariae are ingested by the final host, often by consuming the second intermediate host, the adult digenean begins producing eggs.

Digenea in bivalve mollusks are immature stages, which have traditionally been difficult to identify to species because the morphological features used for species identification are often not developed. However, molecular techniques, such as DNA sequencing and PCR, offer improved capabilities for identification of digenean species in mussels (Nolan and Cribb 2005). A current limiting factor is the availability of genetic sequences for different species of digeneans, but as more information becomes available, molecular methods will allow more reliable identification of digeneans. Because of the problems with morphological identification, many of the studies of digeneans in bivalves are based on identification of the parasite to genus or family.

Bucephalidae

This family has been reported from 15 superfamilies of mollusks, the greatest number for any family of digeneans (Cribbs et al. 2001a), and some species in this family use unionids as the first intermediate host. Distinctive features of bucephalids include a gut that opens midventrally and the presence of a specialized attachment organ called a rhynchus (Overstreet and Curran 2002). The sporocysts of bucephalids penetrate the liver, gills, and gonads of mollusks and cause reduced fecundity (Baturo 1977; Molloy et al 1996; Jokela et al. 2005).

Adult bucephalids are found in the intestine of teleost fish and shed eggs that are passed from the fish (Cribb et al. 2001a; Overstreet and Curran 2002). The miracidium that hatches from these eggs infests a mollusk where cercariae are produced. The cercariae leave the mollusk, penetrate a fish, and encyst in the flesh of this second intermediate host. The adult stage then occurs in another fish that consumes the second intermediate host. The worm reaches sexual maturity in the intestine of the final fish host.

Bucephalus polymorphus is a digenetic trematode that occurs as sporocysts in zebra mussels in Eurasia (Baturo 1977; Molloy et al. 1997; Laruelle et al. 2002). Hoffman (1999) considered *B. polymorphus* to be a European species and did not list any records from North America. There is some evidence that this trematode is specific for *Dreissena* spp. and that reports of *B. polymorphus* in unionids is doubtful because of confusion with a different genus of trematode, *Rhipidocotyle.* Baturo (1977) found that sporocysts of *B. polymorphus* were found in zebra mussels but not in *Unio pictorum* and *Anodonta* sp. collected from the same lakes. The sporocysts from *U. pictorum* developed into *Rhipidocotyle campanula* (= *Rhipidocotyle illense*) when experimentally introduced into the final host, and the sporocysts from zebra mussels developed into *B. polymorphus*. Laruelle et al. (2002) examined zebra mussels from Europe and North America and found *B. polymorphus* only in the European specimens. *Bucephalus polymorphus* sporocysts are located in intercellular spaces of connective tissue, gonad, and sometimes digestive gland; the germinal tissue in gonads is destroyed in advanced cases (Laruelle et al. 2002).

Bucephalid trematodes that are found in unionids are probably *Rhipidocotyle* spp., although they have until recently been called *Bucephalus polymorphus* (Kelly 1899; Yanovich and Stadnchenko 1997). Baturo (1977) found that the sporocysts from *Unio pictorum* developed into *Rhipidocotyle campanula* (= *Rhipidocotyle illense*) when experimentally introduced into the final hosts and provided a detailed description of the developmental stages of this parasite. In Europe there are two species of *Rhipidocotyle* in the unionid *Anodonta anatina*: *Rhipidocotyle campanula* and *Rhipidocotyle fennica* (Gibson et al. 1992). In North America the *Rhipidocotyle* spp. known to infest unionids are *Rhipidocotyle septpapillata* (Kniskern 1952) and *Rhipidocotyle papillosa* (Woodhead 1929, 1936).

The most serious effect of bucephalid trematodes is host sterility (Kelly 1899; Kniskern 1952; Taskinen et al. 1997; Yanovich and Stadnichenko 1997). The normal gonadal tissues are replaced by sporocysts and fibrosis. Additional lesions sometimes occur in the kidney, which appears swollen.

Stadnichenko et al. (1994) studied the effect of "*Bucephalus polymorphus"* (probably a *Rhipidocotyle* sp.) on the heart rate of unionids in Russia. Moderate infestations caused an increase in heart rate by a factor of 1.2 to 1.3, and a decrease in the duration of heart contraction by 23 to 31%. The heart rate was more severely affected as the level of infestation increased.

During a 3-week exposure to elevated water temperature (26°C compared with 10°C for the collection location), about 15% of the *Anodonta piscinalis* died, and all of the dead mussels were infested with *Rhipidocotyle fennica*, *Rhipidocotyle campanula*, or both species (Saarinen and Taskinen 2005a). The overall prevalence of infestation in this population of mussels was not reported, but in earlier collections of this mussel from similar habitats, the prevalence of infestation by one or both of these parasites was less than 50% (Taskinen and Valtonen 1995; Taskinen 1998). The presence of digeneans in all of the mussels dying at the elevated temperature suggests that the infested mussels were more susceptible to the lethal effects of high temperature.

In a lake in Finland, *Rhipidocotyle fennica* was present in *Anodonta piscinalis* with an overall prevalence of 32.3% (Taskinen and Valtonen 1995). Mussels collected from the littoral zone had a higher prevalence of infestation (46.6%) than for those from the sublittoral zone (19.6%). Only mature mussels were infested, and the prevalence was higher in females than in males. There was reduced fecundity of infested mussels.

Taskinen (1998) found that *Anodonta piscinalis* under field conditions had a reduced rate of growth if infested with *Rhipidocotyle fennica*. The growth suppression increased with the number of parasites present. Mussels infested by both *R. fennica* and *Rhipidocotyle campanula* also had a reduced rate of growth, similar to that of mussels with a heavy infestation of only *R. fennica*.

Jokela et al. (2005) examined *Anodonta piscinalis* infested with *Rhipidocotyle fennica* or *Rhipidocotyle campanula*. Prevalence of *R. campanula* was less than 5%, and this parasite destroyed an average of 90% of the host gonad. *Rhipidocotyle fennica* was more common (prevalence of 20-60%) and destroyed an average of 30% of the gonad. During anoxia, mortality of infested mussels was higher than for uninfested mussels, and the effect of the parasite was greater for *R. campanula* than for *R. fennica*. Similarly during periods of starvation, mortality of infested mussels was higher than for uninfested mussels.

Bucephalus elegans uses the unionid *Villosa* (= *Eurynia*) *iris* as the first intermediate host with the sporocysts located in the gonad, and the final host is rock bass *Ambloplites rupestris* (Woodhead 1930). In some mussels, all of the germinal tissue of the gonad is replaced by sporocysts of *B. elegans*. Anatomical details about the cercaria of *B. elegans* and other bucephalid cercariae of unionids were described by Woodhead (1936). *Bucephalus elegans* was found by Flook and Ubelaker (1972) in the unionids *Potamilus purpuratus* (= *Proptera purpurata*), *Lampsilis hydiana* (= *Lampsilis radiata hydiana*), and *Lampsilis teres* (= *anodontoides*). Sporocysts were located in the gonad, gill, and digestive gland. Gonads were nonfunctional in infected hosts, and damage to the gonad of some hosts was so severe that gender could not be determined.

Allocreadiidae

Depending on the species, the first intermediate host for this family is a unionid (Seitner 1951), a sphaeriid clam, or a snail (Cribb et al. 2001a). The second intermediate host is most commonly a crustacean or aquatic insect, and the final host is generally a freshwater teleost, where the adult worm is located in the intestine. Genera in this family include *Bunodera, Bunoderina, Crepidostomum,* and *Polylekithum*.

Seitner (1951) found that all of the unionids examined from the Tippecanoe River, Indiana, and most from the Wabash River, Indiana, were infested with metacercariae of *Polylekithum* (= *Allocreadium*) *ictaluri*. The metacercarial cysts were in the anterior portion of the mantle, with a maximum number of more than 500 cysts per host. Cyst diameter ranged from 3.3 mm to 9.9 mm and some cysts had a light orange color. Each cyst was enclosed in a dense, fibrous capsule formed by the host. Cable and Peters (1986) reported that the cercariae develop in the gastropod *Laevapex fuscus* (Ancylidae) and experimentally would encyst in the unionid *Lampsilis* sp. The adult parasite is in the intestine of catfish.

Gentner and Hopkins (1966) found *Polylekithum ictaluri* metacercariae encysted in the mantle or foot of unionids in Texas. All of the *Amblema plicata* (= *perplicata*) examined were infested, and a lower prevalence was found in eight additional unionid species. The 81 *A. plicata* examined had a total of 12,366 metacercariae, and 24 of these unionids contained 121 pearls.

Dissolving small pearls in acetic acid revealed that they were formed around cysts presumed to be *P. ictaluri* (Hopkins 1934). The prevalence of this parasite was lower after a severe drought (Gentner and Hopkins 1966).

Ginetsinskaya (1968) noted that infestation of mollusks by digenetic trematodes can be localized within a body of water because of the biotic variables associated with the probability of infestation. For *Bunodera luciopercae* parasitic in the greater European peaclam *Pisidium amnicum* (Sphaeriidae) near Rybinsk Reservoir in Russia, prevalence was 38% in one location but only 0.3% in nearby locations. This clam species has been introduced into North America (Turgeon et al. 1998).

Heinonen et al. (1999, 2003) studied greater European peaclams in Finland. The population of clams used for these experiments was heavily parasitized by digenetic trematodes, primarily *Bunodera luciopercae*, and the metabolic rate as measured by heat output was lower in clams with trematodes. The proportion of the total clam soft tissue mass composed of *B. luciopercae* was up to 14-19% in summer and 5-10% in winter (Heinonen et al. 1999, 2000). There was a positive correlation between the concentration of pentachlorophenol in the clams and the duration of valve closure. Unexpectedly, Heinonen et al. (2001) found that the clams with digenetic trematodes were more tolerant of the pollutant pentachlorophenol during summer than were the uninfested clams.

In Finland, the population dynamics of the greater European peaclam is regulated by the gonadal damage caused by digeneans, primarily *Bunodera luciopercae* (Holopainen et al. 1997; Rantanen et al. 1998). There were two other digenean species (*Palaeorchis crassus* and *Phyllodistomum elongatum*) infesting this clam, but *B. luciopercae* was most common, and less than 1.5% of the clams were infested by multiple parasite species. The sporocysts of *B. luciopercae* are usually located in the gonads but also occur in the digestive gland. The intensity of infestation increases with increasing size of the clam; no parasites were found in clams less than 4 mm long, and by the time a length of 7 mm is reached, about 70% are infested. The number of clam embryos produced by this clam population was about half of its potential because of the total effect of all species of Digenea. Holopainen et al. (1997) concluded that there was high mortality of the large parasitized clams. Other reports of *B. luciopercae* in Sphaeriidae include Cannon (1971) and Schell (1970).

Wenke (1965) found adult *Crepidostomum ictaluri* and *Crepidostomum cooperi* in several fish species in Pool 19 of the Mississippi River. The first intermediate host for these species was the sphaeriid *Musculium* (= *Sphaerium*) *transversum*, and in this host the miracidium develops directly into a redia without a sporocyst stage. Cercariae emerge from the clam and infest mayflies of the genus *Hexagenia*. Adult *Crepidostomum cornutum* were found in bowfin *Amia calva* during this survey, and the second intermediate host was crayfish. *Crepidostomum illinoiense* was also found during this survey, but the life cycle is unknown. During examination of *M. transversum* from Pool 19, the *Crepidostomum* species were not differentiated and the genus was uncertain for some immature Digenea. The prevalence of Digenea in clams ranged from 0 to 38.7% on various collection dates over an 18-month period, and the highest prevalence was during summer. *Sphaerium striatinum* collected from the same location were not infested with digeneans. In the infested clams, rediae were located in the digestive gland with up to 100 in a single host, and the parasites were also in the gonads and gills. One infested clam was examined histologically, and the digestive gland had some tubules slightly displaced where parasites were most numerous. The absence of embryos in infected clams was attributed to gonadal damage. Because of the low overall prevalence (6% average during the 18-month

study) in the clam population, Wenke (1965) concluded that sterility caused by *Crepidostomum* spp. would not have a significant effect on the clam population. However, this conclusion was based on the overall prevalence, which was strongly influenced by collecting clams during times of the year when the parasites were absent. The maximum prevalence of *Crepidostomum* spp. infestation reached 38.7%, and if the fecundity of this percentage of the population was impaired, a population-level effect seems possible.

Musculium (= Sphaerium) *transversum* in some samples from Pool 19 of the Mississippi River were heavily infested with trematode cercariae and sometimes with rediae (Gale 1973). Although the trematodes were not identified by Gale (1973), *Crepidostomum* spp. had been the dominant parasite in this population in earlier surveys (Wenke 1965). Cercariae were abundant above the foot and near the digestive gland, and the whole visceral mass of some clams was infested. One clam contained 2,184 cercariae and several others contained over 1,000. Heavily parasitized clams contained no living embryos, but some had marsupial sacs with decaying embryos. All unparasitized or lightly parasitized clams in the same sample were gravid. Similar observations were made for *M. transversum* collected in Briar Creek, Oklahoma, and from a borrow pit near Jackson, Mississippi. Gale (1973) concluded that these trematode cercariae killed most of the large clams at one of the collecting locations in the Mississippi River during late July and August 1967.

Cheng and James (1960) concluded that *Crepidostomum cornutum* ingested liver cells of *Sphaerium striatinum*, which resulted in death of the host. However, their report of differences in parasitism of "male" and "female" *S. striatinum* is unlikely to be correct because sphaeriids are hermaphroditic. Their observation of differences in reproduction related to parasitism could indicate that the cercariae affected gonadal function.

Gorgoderidae

For this family, *Phyllodistomum* is the only genus found in fresh water in North America (Hoffman 1999), but immature stages in this family are difficult to identify and have often been assigned only to the family Gorgoderidae. Karyotype and molecular data indicate that species identification of immature stages of *Phyllodistomum* based on morphological features is not reliable (Petkevičiūtiė et al. 2004). Species in the genus *Phyllodistomum* have cercariae that do not leave the first intermediate host but transform to metacercariae within the sporocyst. Sporocysts in bivalve mollusks are usually located in gills, and the fish final host is infected by consuming sporocysts shed from the first intermediate host. Adult worms are usually in the urinary system of fish.

Sporocysts of an unidentified digenean (family Gorgoderidae) were found in the gonad, digestive gland, and kidney of the unionid *Amblema plicata* (Flook and Ubelaker 1972). Prevalence of this digenean was 5.5% in the location sampled in Texas. This parasite caused gonadal damage similar to that caused by bucephalid trematodes. Gentner and Hopkins (1966) found two species of larval gorgoderids in unionids in Texas and considered these digeneans to be *Phyllodistomum* spp. Benz and Curran (1997) found larval gorgoderids in three unionid species (*Amblema plicata, Quadrula metanevra*, and *Quadrula pustulosa*) in Tennessee. This parasite was present as cercariae within sporocysts located in the digestive gland, gonad, and viscera surrounding the foot of the host. Larval Gorgoderidae were also reported in unionids by Fischthal (1951) and Coil (1954).

The sphaeriid clam *Pisidium amnicum* can be infested by larval *Phyllodistomum elongatum*, which causes castration of the host (Rantanen et al. 1998). Another European gorgoderian,

Phyllodistomum simile, is found in the urinary bladder of brown trout as an adult, and sporocysts are found in the epibranchial cavity and between the gill lamellae of *Sphaerium corneum* (Thomas 1956).

Zebra mussels serve as the first intermediate host of *Phyllodistomum folium* **(**Davids and Kraak 1993). The sporocysts are grossly visible as yellowish stripes in the gills. The sporocysts contain metacercariae with a mean length of 3.6 mm (range 2.0 - 6.1 mm) and mean width of 0.7 mm (range 0.59-0.92 mm). Each sporocyst contains eight metacercariae, and the sporocysts are released spontaneously from mussels kept in tanks. Common carp *Cyprinus carpio* fed metacercariae developed adult worms in the urinary ducts. Body weight and fecundity of infested zebra mussels was reduced, and the concentration of metals (i.e., zinc, copper, cadmium, and lead) was higher in infested than in uninfested zebra mussels. Based on histological examination, Laruelle et al. (2002) found *P. folium* in the zebra mussels from Europe but not from U.S. samples. Sporocysts were in the spaces between the inner and outer epithelium of the gill lamellae, and this parasite caused the gills to be highly deformed in advanced cases.

Echinostomatidae

Zebra mussels are often a second intermediate hosts to trematodes in the family Echinostomatidae (Molloy et al. 1997). Snails and tadpoles can also be second intermediate hosts. The first intermediate hosts for this family are snails, and the final hosts are birds (often ducks) or mammals. Members of this family, even as immature stages, can be recognized by a collar of spines around the anterior end (Noble et al. 1989). Morphological features of the Echinostomatidae that occur in zebra mussels are not useful for assigning these digeneans to a genus.

Laruelle et al. (2002) found an unidentified species of Echinostomatidae in zebra mussels. These parasites were only found in zebra mussels from Europe and were not found in U.S. samples. The unidentified Echinostomatidae was in cysts embedded in epithelium of the mantle cavity including gill surfaces or in the connective tissue under epithelium and near gonads and the digestive gland. Cysts were also occasionally in the kidney, hemolymph sinuses, pericardial cavity, suprabranchial cavity, intestinal lumen, and gonads.

Apocreadiidae

This family includes the genera *Homalometron* and *Microcreadium*, which have also be in the families Homalometridae and Lepocreadiidae. *Homalometron armatum* (= *Distomum isoporum var. armatum, Anallocreadium armatum,* and *Anallocreadium pearsei*) use unionids and sphaeriids as the first intermediate host, and was redescribed by Miller (1959). Final hosts are fish, including freshwater drum and *Lepomis* spp.

Chittick et al. (2001) examined the unionid *Elliptio complanata* from two locations; *Homalometron armatum* was found in 9 of 10 mussels at one site but was not found at the second site. The infested mussels had 4 to 20 encysted *H. armatum* per histological section, and the mussels with the most cysts had this trematode in the mantle, gonad, digestive gland, and kidney. The cyst wall was 12 µm thick and consisted of material from both parasite and host. Little to no inflammation was evident around the cysts.

Gustafson et al. (2005b) found that 39% of the unionid *Elliptio complanata* were infested with a larval trematode, possibly *Homalometron armatum*, and parasitized mussels were more common in locations having low mussel abundance or diversity. This digenean was usually in the foot and mantle but was also in the gill, gonad, and digestive gland. Both sporocysts and

metacercarial stages were present. The host gonad was injured by the parasites, and parasitized mussels were less likely to be gravid than mussels that were not parasitized. There was minimal inflammation associated with the parasites, but infested mussels had an elevated hemocyte count. Gustafson et al. (2005b) concluded that the infested mussels were well adapted to the parasite.

Homalometron armatum metacercariae were also found in six species of unionids and in the sphaeriid *Musculium ferrissi* during a survey in Texas (Gentner and Hopkins 1966). The unionid *Lampsilis teres* (= *anodontoides*) contained the greatest number of metacercariae: 467 metacercariae in 15 infested mussels. Another digenean in this family, *Microcreadium parvum*, was found in *M. ferrissi* and in about 90% of the *L. teres* in this Texas survey (Gentner and Hopkins 1966).

Macroderoididae

This family typically uses snails as the first intermediate host and arthropods as the second intermediate host before becoming adults in fish or other vertebrates. Some species in the genus *Alloglossidium* have an abbreviated life cycle and become adults in leeches or crustaceans (Carney and Brooks 1991). *Alloglossidium corti* occurs in the sphaeriid *Musculium ferrissi* (Gentner and Hopkins 1966), which serves as the first intermediate host. Effects on mollusks have not been determined.

Lissorchiidae

This family has also been known as Monorchiidae. Infestation by *Palaeorchis crassus* caused castration of the sphaeriid clam *Pisidium amnicum* (Holopainen et al. 1997; Rantanen et al. 1998).

Unidentified Digenea

Kelly (1899) found four forms of "distomum" in unionids, but the identity of these is uncertain. One form called "free distomata" was found in loose, salmon-colored masses either upon or slightly within the mantle. The position of this parasite was usually marked by rusty staining of the nacre, by malformation of the shell or of the hinge teeth, and not infrequently by a number of dark, poorly formed pearls. The "free distomata" were found in 14 of the 44 unionid species examined, and the other three forms of "distomum" were rare.

Digenea may also be the cause of malformed shells of *Lampsilis* spp. having pits near the margin of the shell, sometimes containing a pearl, or with the anterio-ventral portion of the shell thickened or with a gape from the midline so that the valves do not close tightly (Clark and Wilson 1912). These authors called the parasite associated with these malformed shells the "marginal-cyst distomid" and considered them to be a common cause of pearl formation. They also observed the "distomid of Osborn" in the outer surface of the mantle of *Anodonta* spp. This parasite apparently caused the nacre to form raised blisters or white areas covering sporocysts. After release from the sporocyst, the parasite moves to the umbonal region of the mussel where it affects the nacre, staining it a salmon color. Clark and Wilson (1912) considered this parasite to somewhat resemble *Distomum duplicatum*, but with some important differences. Pearl formation in unionids has also been attributed to digeneans by Utterback (1916) and Oesch (1984).

Cestoda

Cestodes are a strictly parasitic class in the phylum Platyhelminthes. No reports were found for cestodes parasitizing freshwater bivalves. Larval cestodes occur in marine bivalves, although there are few publications related to this topic. Sparks and Chew (1966) found a large number of Pacific littleneck clams *Protothaca* (= *Venerupis*) *staminea* that were exposed on gravel beds rather than buried as normal, and these moribund clams were heavily infested with larval cestodes in the genus *Echeneibothrium*. These cestodes were encysted in the mantle and foot and were the apparent cause of the emergence and morbidity of these clams. Each cestode was surrounded by a thick cyst wall of fibrous connective tissue and hemocytes.

Nematoda

This phylum contains both parasitic and free-living species. Nematodes have been mentioned as intestinal inhabitants of unionids, but details were not presented (Clark and Wilson 1912; Coker et al. 1921). Benz and Curran (1997) reported the nematode *Dorylaimus* sp. on the external shell surface of five of eight unionid species.

There are also reports of nematodes in zebra mussels and quagga mussels. Conn et al. (1994) found at least four species of nematodes in zebra mussels and quagga mussels from the St. Lawrence River. One of the nematodes was in the genus *Mononchus* and another was a dorylaimid. The prevalence of nematodes in one collection was 40% in zebra mussels and the mean number of nematodes per infested mussel was 2.8 (range, 1-10). Nematodes were also found in a small sample of quagga mussels. A high prevalence of nematodes has also been reported for zebra mussels from Belarus (Karatayev et al. 2000a).

Karatayev et al. (2003a) found that the nematodes in zebra mussels examined from Belarus were free-living, benthic species. The most common nematode was *Chromadorina bioculata*, and other species were rare. The mean number of nematodes in infested zebra mussels was negatively correlated with temperature.

Bryozoa

Bryozoans may be a cause of erosion of the periostracum on the outside of unionid shells (Williams 1969). This erosion is usually in the umbonal region. Curry et al. (1981) found that bryozoans were usually present on unionids having any growth on the exterior of the shell, and that *Pottsiella erecta* was the most common bryozoan species.

Oligochaeta

Oligochaetes are members of the phylum Annelida and are highly diverse, including both terrestrial and aquatic species. However, *Chaetogaster limnaei* (family Naididae) is the only oligochaete commonly found in unionids and other freshwater bivalves. *Chaetogaster limnaei* are located in the mantle cavity, on the gills, and in the kidney (Kelly 1899; Sickel and Lyles 1981).

Kelly (1899) reported *C. limnaei* in five of the 44 species of unionids examined, but *Utterbackia imbecillis* (= *Anodonta imbecilis*) was the only species in which *C. limnaei* was common (33 of 46 were infested). Anderson and Holm (1987) found *C. limnaei* in unionids and Asian clams from the Mississippi River. *Chaetogaster limnaei* was found in the mantle cavity of five of the eight species of unionids collected, but only one or two specimens were examined for the species not found to be infested. The heaviest infestation in unionids was 124 oligochaetes in a *Leptodea fragilis*. Prevalence of infestation in unionids (all species combined) was 53% during November through January and then declined to 16% during February through April. During November through January, all of the Asian clams were infested with *C. limnaei* with 3 to 85 worms/host. After January the density of Asian clams decreased dramatically and none were collected in April. Other reports of *C. limnaei* in unionids include Coker et al. (1921) and Antipa and Small (1971b).

In Asian clams in California, the prevalence of *C. limnaei* was 87% during March through May, but decreased to less than 3% during other months (Eng 1976). These worms were usually on the gill, and there was no grossly visible damage. Sickel and Lyles (1981) found large numbers of *C. limnaei* in moribund Asian clams in Kentucky. Clams collected during June often had 40 to 50 worms/host, with a maximum of 167 worms/host, and clams with higher levels of infestation were in the poorest condition. There were calcareous deposits with a chalky appearance on the inner surface of the shell of heavily infested clams. During August, more dead and moribund clams were observed, but oligochaetes were not found in live or dead clams.

Chaetogaster limnaei also infest sphaeriid clams. In Lake Ontario and Cayuga Lake, New York, Barbour (1977) found *C. limnaei* in three species of Sphaeriidae: *Sphaerium corneum, Sphaerium striatinum*, and *Musculium* (= *Sphaerium*) *partumeium.* This worm was not found in *Sphaerium* spp. smaller than 4 mm and was not found in *Pisidium* spp. The number of worms per host was usually two to 15, but one individual had 30. In clams from the Mississippi River, Gale (1973) found *C. limnaei* in the mantle cavity of *Musculium* (= *Sphaerium*) *transversum, Musculium* (= *Sphaerium*) *securis,* and *Sphaerium striatinum*. At one station, 95% of the clams over 4.5 mm long were infested in July but prevalence of this parasite was "near zero" during other months. At other locations, prevalence of infestation was "moderate" during winter and spring and did not increase dramatically in July. Although there was no grossly visible damage caused by the *C. limnaei*, Gale (1973) concluded that the decrease in prevalence of infestation from July to August was because of the death of many of the larger, heavily infested clams. In laboratory experiments, this parasite entered the host by attaching to the foot of *M. transversum* and was then carried into the shell when the clam retracted its foot. Gale (1973) found a different species of *Chaetogaster* inside the mottled fingernailclam *Eupera cubensis* (= *singleyi*) in Briar Creek, Oklahoma.

Zebra mussels and quagga mussels in the St. Lawrence River were infested with *C. limnaei* (Conn et al. 1994, 1996). Prevalence varied from 1% to 80% depending on collection site and date, and the number of this parasite per infested host ranged from 1 to 18. Gross lesions were not observed in the mantle cavity, but histopathology revealed erosion of the gill and mantle epithelia in areas adjacent to parasites. Some of the *C. limnaei* penetrated the siphon, and one *C. limnaei* was found inside the ovary of its host and had consumed some of the mussel's oocytes. Other species of oligochaetes were also present but were uncommon; *Ophidonais serpentine* was the only additional species of oligochaete identified. Unionids examined from one location in the St. Lawrence River were not infested with *C. limnaei*.

Chaetogaster limnaei also infests snails. In snails they reportedly enter the kidney and feed on host cells, but when in the mantle cavity, they feed primarily on plankton (Gruffydd 1965).

The potential of *C. limnaei* to harm populations of freshwater bivalves has not been adequately evaluated, especially for unionids. Even the prevalence and intensity data in some studies may not be correct because it is not clear that hosts were examined internally (e.g., kidney and gonads). In addition, these internal locations have not been adequately examined for lesions. Dreissenid mussels with no grossly visible lesions had histological lesions, which indicates that there should be skepticism about other reports of no host damage based on only gross examination.

Leeches

Leeches are annelids in Hirudinea (variously considered a rank ranging from class to order) and have been reported as inhabitants of the mantle chamber of unionids (Kelly 1899; Clark and Wilson 1912; Coker et al. 1921). The leech *Placobdella montifera* (family Glossiphoniidae) was found in the mantle cavity of several species of unionids (Fuller 1974; Curry and Vidrine 1976, 1978; Curry 1977). The leeches associated with unionids were morphologically indistinguishable from free-living leeches of the same species. Lengths of the leeches ranged from 1 mm to 20 mm. Fuller (1974) also mentioned the rare occurrence of *Placobdella parasitica* in the mantle cavity of unionids.

Gale (1973) considered leeches to be predators of the sphaeriid clam *Musculium transversum*. Two species of leeches, *Glossiphonia complanata* and *Helobdella stagnalis*, that had not been fed for 2 weeks attacked *M. transversum*. The leech *Erpobdella punctata* did not attack clams under the same circumstances. At some locations in the Mississippi River, the three leech species mentioned above were abundant; frequently leech density was over 1,000/m^3 and at one station was 68,000/m^3.

In zebra mussels, three species of leeches have been reported: *Caspiobdella fadejewi*, *Helobdella stagnalis*, and *Erpobdella octoculata* (Kuperman et al. 1994; Karatayev et al. 2000a). These leeches have not been reported from zebra mussels collected in North America.

Zebra Mussel Infestation on Unionids

Several studies support the conclusion that zebra mussels attached to the outside of unionid valves interfere with feeding and normal valve movement of the unionid; however, there are also studies reporting that unionids are not affected by attached zebra mussels (Lewandowski 1976). The effects of zebra mussel attached to valves probably depend on the species of unionid and the number of zebra mussels. Mortality was higher for zebra mussel encrusted *Lampsilis radiata* than for *Amblema plicata* (Haag et al. 1993). Glycogen concentrations in *Elliptio complanata* did not decrease as zebra mussel density on the unionid increased, but there was a significant decrease in glycogen concentrations in *L. radiata* (Hallac and Marsden 2000). If *E. complanata* is relatively resistance to the adverse effects of encrustment by zebra mussels, this would explain the lack of change in hemolymph pH, osmolality, total carbon dioxide, or partial pressure of oxygen or carbon dioxide in this species with a maximum of 80 attached zebra mussels (Byrne et al. 1995).

Zebra mussel infested *Actinonaias ligamentina* and *Amblema plicata* have seasonally dependent changes in ammonia excretion, oxygen uptake, and grazing rates (Baker and Hornbach 1997). The effects on *Actinonaias ligamentina* were more pronounced than for *Amblema plicata*. In addition, *A. plicata* infested with zebra mussels had significantly decreased whole-body concentrations of protein and carbohydrate (Baker and Hornbach 2000). This suggests that the death of infested unionids could be related to starvation resulting from reduced food intake, increased metabolic costs, or both.

Zebra mussels tended to settle near or moved towards the siphons of *Anodonta cygnea* rather than being randomly distributed on the host valves, and the incurrent siphons of the zebra mussels were directed towards the unionid's siphons (Hörmann and Maier 2006). With 10 to 30 zebra mussels per unionid, the growth of zebra mussels attached to *Anodonta cygnea* was generally greater than for those attached to stones, perhaps because the zebra mussels were able to obtain food from the siphon current of the unionid.

Schloesser and Kovalak (1991) observed zebra mussels attached to three species of unionids in Lake Erie and concluded that the zebra mussels smothered siphons and prevented valve closure and opening of the unionids. These authors listed the following potential adverse effects of zebra mussels on unionids: (1) impair normal locomotion and burrowing, (2) prevent valve closure, (3) prevent valve opening, (4) smother siphons, (5) eliminate food from the water, (6) cause shell deformities, (7) generate metabolic wastes, and (8) add weight to the unionid shell, which could cause the host to sink into soft sediments. Zebra mussels appear to be the cause of extirpation of unionids from some locations (Schloesser et al. 2006).

Mites

Mites are arachnids, and the freshwater mites (variously called Hydracarina, Hydrachnidia, or Hydrachnida) are highly diverse, with thousands of species (Smith 2001; Smith et al. 2001). Most species of freshwater mites have a larval stage that is parasitic on insects (e.g., chironomids), but are otherwise free living. However, two genera of mites include parasites of unionids: *Unionicola* (family Unionicolidae) and *Najadicola* (family Pionidae). The genus *Unionicola* has more than 200 described species, but only one species of *Najadicola* has been described (Simmons and Smith 1984; Gledhill and Vidrine 2002). Some mites that are parasitic on mollusks were formerly in other genera (*Vietsatax*, *Atacella*, *Unionicolopsis,* and *Heteratax*) but were considered to be subgenera of *Unionicola* by Vidrine (1985; 1986; 1996c). In older literature, all of the mites parasitic on unionids were assigned to the genus *Atax*. Important sources of information about *Unionicola* and *Najadicola* are summaries published by Vidrine (1996a, 1996b, 1996c, 1996d, 1996e).

Not all members of the genus *Unionicola* parasitize unionids during the deutonymph and adult stages. Some mites are associated with other bivalves (e.g., Sphaeriidae [Wolcott 1899; Gale 1973]), snails (Gledhill 1985), or sponges (Crowell and Davids 1979; Davids et al. 1985; Proctor and Pritchard 1990). Mites and mite eggs have been found in zebra mussels, but the mite species and effects on the zebra mussels have not been determined (Kuperman et al. 1994; Molloy et al. 1997; Karatayev et al. 2000a).

In addition to freshwater mites, there are also families of mites that are generally marine, and some of these mites can infest marine bivalve mollusks. Cáceres-Martínez et al. (2000) found two species of mites, *Hyadesia* sp. (Hyadesiidae) and *Copidognathus* sp. (Halacaridae), in

the marine mussel *Mytilus galloprovincialis* in Mexico. *Hyadesia* sp. was located in the gut and caused lesions, which were not adequately described to evaluate the severity. *Copidognathus* sp. was found on the mantle and gills, and lesions were not found in infested mussels. For both species, only the adult stage was found in the mussels examined, and these species were also found living free in the sediment.

Najadicola ingens

This species has been reported from only North America and southeast Asia but infests several unionid species (Vidrine 1980; Vidrine 1996a). The body of this mite is cream-colored and relatively large, with males up to 2.5 mm in length and gravid females reaching 5 mm long (Simmons and Smith 1984). *Najadicola ingens* does not leave the mussel host except during the larval period (Simmon and Smith 1984), and the number of *N. ingens* per host is often low, with only one male and one female mite often found in some host species. The highest number in a single host reported by Humes and Jamnback (1950) was 12.

***Unionicola* spp.**

The genus *Unionicola* has a worldwide distribution in fresh water, but some subgenera have distributions restricted to certain continents and are not currently known from North America (Vidrine 1993, 1994). Vidrine (1996b) listed 69 species of *Unionicola* in North America. Body length of most *Unionicola* spp. is 0.5 to 1.6 mm (Wolcott 1899; Vidrine 1980).

Abundance of Mites in Unionids

Mites are one of the two most commonly reported groups of organisms living in unionids (the other being aspidogastrid trematodes). In some populations of unionids, the prevalence of mites exceeds 90% (Mitchell 1955; Najarian 1955; Flook and Ubelaker 1972; Vidrine and Clark 1993; Benz and Curran 1997; Gangloff 2003). *Najadicola ingens* was found in 87.3% of the *Pyganodon* (= *Anodonta*) *cataracta* in a collection in New Hampshire (Humes and Jamnback 1950). For the overall prevalence of mites infesting all species of a mussel community, Vidrine and Clark (1993) considered 50.9% to be low. Other populations of unionids (Kelly 1899; Benz and Curran 1997) or sometimes all of the unionids in a particular location (George and Vidrine 1993) are devoid of mites.

The number of mites in each infested host is highly variable, with the mite species being an important factor. For some mite species, the number of mites in each host is kept low by intraspecific competition. The males of *Unionicola ypsilophora* and *Unionicola formosa* are territorial and fight with other males of their species (Dimock 1983; Davids et al. 1988). For these species, most hosts have only one male mite, but there are often several females in a host with one male. Of the 360 *Utterbackia* (= *Anodonta*) *imbecillis* examined by Dimock (1983), all contained female *U. formosa* with an average of 30.8 per host. One or more male mites were present in 91.5% of the hosts, and 86% contained only one male. Edwards et al. (2004) noted that for *Unionicola foili* in *U. imbecillis*, there was one male per mussel and a mean of 12 females per mussel.

Many of the mite species that inhabit gills (e.g., *Unionicola fossulata*) or labial palps (e.g., *Unionicola serrata*) typically have only one female between either pair of demibranchs or on a pair of labial palps (Mitchell 1965; Vidrine and Clark 1993). *Unionicola intermedia*, which does not have territorial males, is excluded from the unionid *Anodonta cygnea* by the mite *Unionicola ypsilophora* and is restricted to *Anodonta anatina* by competitive exclusion (Davids et al. 1988).

For *Unionicola formosa* in *Utterbackia imbecillis* from a North Carolina pond, 86% of hosts had one male mite per host, and the number of female mites per host was positively correlated with host size (Dimock 1985). All mussels longer than 35 mm harbored mites. Edwards and Dimock (1988) examined *Pyganodon cataracta* and *U. imbecillis* from a North Carolina pond and found that all mussels were infested with female *U. formosa* (*Utterbackia imbecillis* were probably infested with *U. foili* rather than *U. formosa* [Edwards and Vidrine 1994]). There was generally only one male mite per host.

The relation between host size and the number of mites probably depends on both the mite and host species. Humes and Jamnback (1950) reported an inverse relation between prevalence of *Najadicola ingens* and size of *Elliptio complanata* and *Pyganodon* (= *Anodonta*) *cataracta*. A negative correlation between mite density and the size of *Pyganodon grandis* was also reported by Gangloff (2003), but the species of mites were not identified. Joy and Hively (1990) found that the number of mites per host was positively correlated with host shell length of the mussel in one pond but there was no correlation in a second pond. They offered no explanation for this contradiction. Gordon et al. (1979) and Dimock (1985) found positive correlations between host size and the presence of *U. formosa*. Abundance of *Unionicola arcuata* was positively correlated with host size but was not related to gender of the host (Wen et al. 2006). Abundance and prevalence of *Unionicola formosa* increased with size of host up to a length of 5 cm and then remained stable with a prevalence of about 52% (Gordon et al 1979).

Identification of Mite Species

The mites found in unionids can be identified to species with keys (Vidrine 1996b, 1996e), but identification of some species is facilitated by knowledge of host specificity. This is especially true for mite species (e.g., *Unionicola poundsi* and *Unionicola lasallei*) that appear similar except for morphological features that are variable (Downes 1990). Although *U. poundsi* and *U. lasallei* are difficult to distinguish based on morphological features, these species are genetically distinct (Edwards and Labhart 2000). Differences in DNA sequences facilitate identification of morphologically similar mite species and offer hope of improved methods for identification of species in the future (Ernsting et al. 2006).

New species of mites from unionids have been recently described (John and Inasu 2005; Vidrine et al. 2005, 2006), and there are undoubtedly many more undescribed species. Existing keys for identification of mites should be used with an awareness that undescribed species and the description of new species after the key was prepared limit their usefulness (Vidrine 1996e).

Mite Location on Host

Najadicola ingens inhabit the suprabrachial chambers, gills, and pericardial region of unionids (Humes and Jamnback 1950; Baker 1982). Some species of *Unionicola* tend to prefer gills and other species are usually on other body surfaces. The unionid *Lampsilis siliquoidea* at one collection station had five different species of mites, but the mite species were spatiotemporally separated with different seasonal cycles and variation in the preferred site for oviposition within the mussel (Mitchell 1955). Kelly (1899) reported mites on the body surface between the gills or between the gills and abdomen, between the labial palps, or among the papillae fringing the mantle edges at the incurrent siphon. Their eggs are laid either in the body wall, the gills, or the mantle. The most common locations for *Unionicola arcuata* in a Chinese unionid were the inner and outer gills (Wen et al. 2006).

Life Cycle

The typical life cycle of water mites has seven stages, and the species that inhabit unionids appear to fit this pattern (Table 3). *Unionicola* that deposit eggs in mollusks have modified genital plates to insert eggs into the host, but *Najadicola* do not deposit eggs in host tissue (Wolcott 1899; Mitchell 1955). Larval mites typically leave the mussel and attach to aquatic insects such as chironomids for a few days (Jones 1965, 1978; Smith and Oliver 1986; Edwards and Dimock 1995a, b; Edwards and Smith 2003). The larval mites then return to a mussel to complete their life cycle. Some species of mites that inhabit unionids can leave their host during the deutonymph and adult stages. Mites that spend most of the deutonymph and adult periods in mussels are considered "resident" species while those that reside in mussels primarily only to lay eggs and during transformation stages (protonymph and tritonymph) are considered "transient" species (Baker 1987, 1991). Compared with transient species of mites, the resident species have shorter legs and are weak swimmers (Mitchell 1955).

Table 3. Typical periods in the life cycle of mites inhabiting freshwater mussels (Böttger 1977; Walter and Proctor 1999; Smith et al. 2001).

Stage	Characteristics	Host	Notes
Egg		Mussel	Location in host depends on mite species
Prelarva	Inactive	Mussel	
Larva	Active; 6 legs	Chironomids	Leaves mussel to find chironomid
Protonymph[1]	Inactive	Mussel	Attached or embedded in host
Deutonymph[2]	Active; similar to adult but sexually immature	Mussel	Some species can leave the mussel
Tritonymph[3]	Inactive	Mussel	Attached or embedded in host
Adult	Active; 8 legs; sexually mature	Mussel	Some species can leave the mussel

[1] also known as postlarval resting stage I or nymphochrysalis.
[2] also known as nymph
[3] also known as postlarval resting stage II, imagochrysalis, or teleiochrysalis.

Parasitism of Larval *Unionicola* on Chironomids

Jones (1965) provided the first report of larval mites of the genus *Unionicola* parasitizing chironomids, which is typical of water mites in other genera. He found larval mites of the species *Unionicola intermedia* and *Unionicola aculeata* on *Chironomus plumosus* with 64 of 82 infested. Additional evidence for the association of larval *Unionicola* with chironomids was provided by Jones (1978) and Booth and Learner (1978). Edwards and Dimock (1995b) described the parasitism of a chironomid (*Chironomus tentans*) by larval *Unionicola formosa* and *Unionicola foili*. The larval mites attach only on the ventral surface of the chironomids, and the maximum number of larval mites on a single chironomid was nine. The maximum duration of the larval mite attachment was 8 days, but most stayed attached for 2 or 3 days. The weight of the larval mite more than doubled during the attachment to a chironomid, indicating that the mite feeds on the chironomid. Baker (1991) experimentally exposed chironomid larvae to larval mites and found that *Unionicola ypsilophora* and *Unionicola aculeata*, but not *Unionicola*

intermedia, attached to chironomids. Larval *Unionicola* sp. have been reported embedded in the wall of the esophagus and stomach of mountain whitefish *Prosopium williamsoni*, possibly the result of fish consuming chironomids infested with mite larvae (Ching and Parker 1983). Infestation with larval mites (*Unionicola ypsilophora*) enhanced the mating success of male chironomids *Paratrichocladius rufventris*; however, the reason for this paradoxical result is unknown (McLachlan 1999).

Some authors have stated that some species of *Unionicola* may not have an obligatory larval association with chironomids. Paterson and MacLeod (1979) reported that larval *Unionicola formosa* transform to nymphs without a chironomid host, and Baker (1988) speculated that *Unionicola intermedia* might not require a host for the larval stage. Although it is possible that some species of *Unionicola* do not have a parasitic larval stage, current evidence for this is weak.

Variation in the Association Between Mites and Hosts

Many of the species in the subgenera *Unionicola* and *Pentatax* use mussels as egg-laying sites and as sites for metamorphosis (protonymphs and tritonymphs), while larvae, deutonymphs, and adults are found outside of the mussel host (Vidrine 1980; Vidrine et al. 1986). Other species in these subgenera use sponges for similar purposes. A lack of specificity for any particular species of host is typical of mites with this life history. Vidrine (1989) used three categories for residency; two of his categories were based on the resident and transient categories of Baker (1987). These three categories were (1) transient [deutonymphs and adults are not in the host for the entire stage], (2) resident [deutonymphs and adults are permanent residents in the host], and (3) vagrant [mites found in hosts other than the preferred host]. Resident species often have a strong preference for a single species or group of related species.

An example of a transient species is *Unionicola aculeata* in the unionid *Lampsilis siliquoidea*. Eggs of this mite are usually laid in the connective tissue beneath the epithelial lining of the incurrent siphon (Mitchell 1955). Larvae leave the host but then return to the unionid to transform to protonymphs, a quiescent stage embedded in the gills. Upon completion of this stage, the deutonymph leaves the host to become a free-living predator. Near the end of the deutonymph stage, the mites again return to a unionid host to become tritonymphs, another quiescent stage embedded in the gills or excurrent siphon. Immediately after emergence, the adult mites leave the host and can be collected from open water.

Host Specificity

Some species of mites, such as *Unionicola aculeata*, *Unionicola serrata*, and *Najadicola ingens*, are not considered host specific for hosts within Unionidae (Vidrine 1977, 1978), but these species of mites do not infest all unionid species (Mitchell and Pitchford 1953; Baker 1982) and they may have preferences for certain host species (Downes 1986). Some mite species infest a wide range of mussel species but apparently a narrow range of gastropods; *Unionicola aculeata* has been found in 73 species of unionids but only two species of gastropods (Gledhill and Vidrine 2002). Other mites are typically found on several related species of mussels, e.g., *Unionicola hoesei* on unionids of the tribe Lampsilini (Vidrine 1990). Although some species of mites are not considered to be host specific for mussels in the family Unionidae, this lack of host specificity does not extend to mussels in the family Margaritiferidae (genera *Margaritifera* and *Cumberlandia*) even though Margaritiferidae and Unionidae are both in the superfamily Unionoidea. Mites have not been reported from the genera *Margaritifera* and

Cumberlandia, although they do occur on gastropods and non-unionid bivalves such as *Sphaerium simile* (Gledhill and Vidrine 2002).

In contrast to the mite species that have little specificity for any particular unionid species, most mites that parasitize mussels are highly host specific (Mitchell and Pitchford 1953; Edwards and Vidrine 2006). Many species of mites are usually found on only one or two species of unionid, and if two or more host species are used as hosts, the hosts are typically in the same genus. Mites that are found on gills of unionids tend to be more host specific than mites found on the mantle. It is noteworthy that while some species of mites appear to be specific for certain unionids, the "host-specific" mite species can also be found on gastropods. *Unionicola campelomaicola* and *Unionicola scutigera* have both been reported in only two species each of unionids and one species each of gastropod (Gledhill and Vidrine 2002). Even more host specific are *Unionicola dobsoni* and *Unionicola viviparaicola*, which have both been found in one species each of unionid and gastropod (Gledhill and Vidrine 2002).

Unionicola formosa and *Unionicola foili* are closely related species but parasitize different species of mussels, *Pyganodon cataracta* and *Utterbackia imbecillis*, respectively (Edwards and Vidrine 1994; Edwards and Dimock 1997; Ernsting et al. 2006). Both larvae and adults have behavioral preference for the host species (LaRochelle and Dimock 1981; Edwards and Dimock 1995a).

Downes (1989) divided mites that are associated with mussels into two groups based on host specificity (Table 4). Mites with relatively low host specificity tend to be small, non-territorial species that have good dispersal ability, and the mites that are more specific for hosts tend to be larger, territorial mites that are relatively poor dispersers.

Table 4. Categories of mite species related to host specificity (Downes 1989).

Mite characteristic	Low host specificity	More specific for host
Body size	small	large
Dispersal (swimming) capability	good	poor
Adults change host	yes	no
Methods of searching for hosts	general	specific
Territorial	no	yes
Number of mites/host	varies	set numbers of one or both sexes
Subgenera (information is not available for all species in these subgenera)	*Polyatax, Anodontinatax, Atacella, Unionicolides, Pentatax*	*Unionicolides, Parasitatax, Berezatax*

Seasonal Variation in Life Cycle

Seasonality of mite life cycles depends on both the mite species and host species. In the unionid *Utterbackia imbecillis*, female *Unionicola formosa* are most abundant during the fall (Edwards and Dimock 1988) or winter (Dimock 1985), but this mite species does not appear to have a seasonal pattern in *Pyganodon cataracta* (Edwards and Dimock 1988). Deutonymphs were present all year in both host species but were most abundant during the summer. Mite eggs

were also present during all months, and larval mites emerged from mussels during May through September.

Baker et al. (1992) determined seasonal changes in a Yorkshire population of *Anodonta anatina* infested with *Unionicola intermedia*. Of 186 mussels examined, 171 were infested. Overall, 84% of the adult mites ($N = 2,896$) were females (there were 465 males in the 171 infested hosts). There were 3,785 deutonymphs in 166 mussels. Males were least common from January to April (43 males in 70 mussels, with 71% containing no males), and were most common from June to August when all mussels had at least one male. The overall mean was 2.5 male mites/mussel compared with 13.1 female mites/mussel. Six mussels had 12 or more male mites. Deutonymphs were present 11 months of the year, with an overall mean of 20.3/mussel, but were most common from November to February.

The mite *Unionicola arcuata* was investigated in a Chinese unionid, *Cristaria plicata*, during an annual cycle (Wen et al. 2006). The overall prevalence was 68% with an abundance of 20 ± 38 (mean ± SD) for all samples pooled. Prevalence was highest in February (100%) and during August-November (100% in October) with minimum prevalence in June. Abundance was highest during February and October (66 in October) and was lowest in December-January and in April-July (minimum was 0.27 in June).

Gordon et al. (1979) did not find a seasonal change in adult *Unionicola formosa* in the unionid species *Pyganodon* (= *Anodonta*) *cataracta*. Only one male mite was found in each host, and there was no difference in prevalence and abundance of mites in male and female hosts. Eggs were laid from late May to early June in the mantle of hosts and hatch in June of the following year.

Injury Caused by Mites

There are some reports of no injuries in unionids infested by mites (Mitchell 1955, 1965; Benz and Curran 1997). Mitchell (1955) states that none of the stages of *Unionicola aculeata*, a transient species inhabiting the unionid *Lampsilis siliquoidea*, causes any noticeable host response or host pigmentation. In contrast, *Unionicola abnormipes* eggs in the same host appeared to cause mild hyperplasia. Mitchell (1965) observed that *Unionicola fossulata* were attached to gills of *L. siliquoidea* and presumed that they "feed on the host by tearing and eating the gill." However, later in this same paper he states that *Unionicola* species "do not obviously damage host tissues."

Eggs of *Unionicola* spp. are inserted into the host by use of modified genital plates. Mitchell (1965) compared the genital fields of four species of mites and suggested that host injury occurs during oviposition by mites. Hyperplasia of host tissue occurs near mite eggs in some species (Mitchell 1955; Flook and Ubelaker 1972), and similar hyperplasia has been mentioned as a response to tritonymphs (Flook and Ubelaker 1972). Mite eggs are a potential cause of pearl formation in unionids (Clark and Wilson 1912; Utterback 1916; Coker et al. 1921; Oesch 1984).

At least some species of mites encyst in the mantle and foot of their host. Deutonymphs, tritonymphs, and adults of *Unionicola abnormipes* in the unionids *Lampsilis radiata* (= *luteola*) and *Lampsilis siliquoidea* sometime burrow beneath the epidermis and become surrounded by a cyst wall (Faust 1918; Mitchell 1955). Lesions were not evident other than for the formation of the cyst. Mitchell (1955) concluded that the mite species studied by Faust (1918) was *Unionicola abnormipes* rather than *Unionicola aculeata*.

Perhaps the most obvious lesions caused by mites are papillae that develop in the suprabranchial chambers of unionids infested by *Najadicola ingens* (Humes and Jamnback 1950). There were up to 40 papillae in a single chamber, and the largest papillae are 3 mm in diameter and 5 mm long. Less often, papillae were also located on the side of the visceral mass. These authors speculated that the papillae prevent the mite eggs from being carried out of the gill chambers. Humes and Jamnback (1950) and Humes and Russell (1951) concluded that this species of mite interferes with the use of gills as marsupia for the developing unionids. Mitchell (1965) observed that this mite tears host epithelium and may destroy the inner gill supports.

Davids (1973) concluded that different species of mites vary in their interaction with mussels at different stages of mite development. He considered the mites *Unionicola aculeata* and *Unionicola bonzi* inhabiting the mussels *Anodonta anatina* and *Unio pictorum* to be using the host only as a "shelter for their transformation stages" in contrast with *Unionicola intermedia* and *Unionicola ypsilophora* that he considered to be "parasitic on the gills" of *Anodonta anatina* and *Anodonta cygnea*, respectively. A large number ("dozens") of tritonymphs of *Unionicola aculeata* were found on or near the incurrent siphon and the posterior portion of the gills in *Anodonta anatina* collected during July. Smaller numbers of mites were found on *Anodonta cygnea*. In these mussel species, the tritonymphs were attached by their chelicerae, rather than embedded in the host as reported by Mitchell (1955) for *Lampsilis siliquoidea*. *Unionicola ypsilophora* females have territories in the mussel while *Unionicola intermedia* females occur together. Although detailed descriptions of lesions were not presented, Davids (1973) concluded that large numbers of *Unionicola ypsilophora* cause the mussel gills to be less firm and covered with slimy threads.

Experiments by LaRochelle (1979) indicated that mites that were probably *Unionicola foili* feed on their host *Utterbackia imbecillis* (=*Anodonta imbecilis*). LaRochelle (1979) used the name *Unionicola formosa* for the mites used in these experiments, but information about host specificity of *Unionicola formosa* indicates that mites on *Utterbackia imbecillis* are more likely *Unionicola foili* (Edwards and Vidrine 1994). LaRochelle (1979) did not present conclusive evidence that the mites fed on the mussels because the contents of the gut of the mite were not identified as originating from the host, and only one mite had an engorged gut after feeding on the host. However, the mites penetrated the gill epithelium of the mussel with their pedipalps so that the chelicerae contacted the host. This attachment of the mite to the mussel was similar to that described in *Anodonta anatina* (Baker 1976).

Fisher et al. (2000) found that *Unionicola formosa* consumes mucus and also gill or hemolymph from their unionid host, *Pyganodon cataracta*. However, the mites do not consume the particulate matter being trapped in the mucous strand of the mussel as the host feeds.

Lesions caused by the mite *Unionicola intermedia* attached to the unionid *Anodonta anatina* was described by Baker (1976, 1977). Similar lesions were caused by the deutonymph and adult stages of the mite. Although extracellular enzymes were absent, the lumen and cells of the midgut of the mite contained host material. The mite uses its pedipalps to attach to the host, and these attachment structures penetrate into the connective tissue of the gill. Displacement and erosion of the gill epithelium occurs at this site of attachment. Swelling of the gills was a result of inflammation at the attachment site, including inflammatory edema and a localized increase in hemocytes. These hemocytes were consumed by the mite along with mucus (Baker 1977). Swelling resulted in the gills projecting distally beyond the normal gill surface. There was also epithelial hyperplasia towards the base of individual gill filaments, forming a mass of elongated cells.

Gangloff (2003) examined 29 *Pyganodon grandis* collected during February, and 22 of these unionids contained glochidia. All of the mussels were infested with both mites and aspidogastrid trematodes. The mites were not identified, but the total number per host was inversely correlated to host size. The glycogen concentration in a sample of mantle, the number of glochidia, and the number of parasites was determined for each mussel, and there was a significant negative correlation between the density of mites (mites per gram of mussel) and glochidia abundance. There are 19 species of mites known to infest *P. grandis* (Vidrine 1996b,1996e); therefore, there could have been a mixture of species that varied among the mussels examined.

Copepoda

Copepods are crustaceans and most species of copepods are free living. However, several different families of copepods are parasitic, and at least one species, *Paraergasilus rylovi* (family Ergasilidae), is found on the gills of unionids. *Paraergasilus rylovi* has been found in several European locations (Chernysheva and Purasjoki 1991; Saarinen and Taskinen 2004) but has not been reported in North America. Adult *P. rylovi* have a body that averages 738 µm (range 712-765 µm) in length (Chernysheva and Purasjoki 1991). Mussel hosts are *Anodonta piscinalis* (main host) and *Pseudanodonta complanata*, while *Unio pictorum* and *Unio tumidus* from the same habitats are not infested (Saarinen and Taskinen 2004). *Anodonta anatina* has also been reported as a host for this parasite (Pekkarinen 1993). In addition to unionids, *Paraergasilus rylovi* infests the nostrils of fish (Chernysheva and Purasjoki 1991), which is typical of other species of *Paraergasilus*. During larval stages and while maturing, *P. rylovi* is planktonic, and adults are also found in plankton (Chernysheva and Purasjoki 1991). There are at least 14 species of *Paraergasilus*, all occurring on fish hosts, but only *P. rylovi* has been reported on unionids. El-Rashidy and Boxshall (2001) provide a key to the genus. *Paraergasilus markevichi* was reported from European unionids but is considered a junior synonym of *P. rylovi* (Chernysheva and Purasjoki 1991).

In Finland, the prevalence of *Paraergasilus rylovi* in some populations of the unionid *Anodonta piscinalis* is 90 to 100% with a mean parasite abundance of 16.3 to 28.8 (Taskinen and Saarinen 1999), while in other lakes this parasite was not found (Saarinen and Taskinen 2004; Taskinen and Saarinen 2006). Female hosts brooding glochidia had twice the number of *P. rylovi* found on female hosts that did not have glochidia (Taskinen and Saarinen 1999). The authors suggested that the greater abundance of *P. rylovi* on reproducing female mussels could result from greater filtration activity of brooding hosts or reproduction could decrease the energy available for host immunologic defense.

The abundance of *Paraergasilus rylovi* on *Anodonta piscinalis* decreased when the water temperature increased from 18°C to 26°C or dissolved oxygen decreased to less than 2 mg/L (Saarinen and Taskinen 2003). Maintaining 26°C for 3 weeks removed all *P. rylovi* from *A. piscinalis* (Saarinen and Taskinen 2005a). *Anodonta piscinalis* kept in water with a low concentration of dissolved oxygen for 25 days and then exposed to natural infestation 11 months later had a greater number of *P. rylovi* per host, as well as lower growth rate, lower reproduction, and lower survival, than the control mussels (Saarinen and Taskinen 2005b). Concurrent infestation by the digenean *Rhipidocotyle fennica* did not affect the intensity of the copepod infestation (Saarinen and Taskinen 2004).

Paraergasilus rylovi abundance was positively correlated with burrowing depth for the unionid *Anodonta piscinalis* (Taskinen and Saarinen 2006). In this study the percentage of the mussel shell length buried in the substrate was determined and compared with the number of *P. rylovi* on each host. The authors concluded that burrowing deeper increased the level of infestation rather than the infestation changing the burrowing behavior of the mussels; however, the reason for increased parasitism in mussels that had burrowed deeper was not determined.

The larval stage of *P. rylovi* probably enters a unionid host through the incurrent siphon (Taskinen and Saarinen 2006). The attachment site in unionids is the gill, but injury to the host has not been determined.

Chironomidae

Chironomids are insects in the order Diptera and have been found in the mantle cavity or on the gills of unionids and other freshwater bivalves. Some of these chironomids are common benthic species and their occurrence in bivalves is probably accidental. However, some species of chironomids appear to be obligatory symbionts of mollusks. Other chironomids occasionally colonize the outside of the shell of bivalves, but these seem unlikely to have an effect on the bivalve and are not considered in this review.

Immature stages of the chironomid *Ablabesmyia janta* infest the gills of at least 15 species of North American unionids (Roback et al. 1980). This chironomid occurs as at least two physiological races: living on the gills of mussels and free living (Roback 1982, 1985). The adults of these races fit the description of *Ablabesmyia janta,* but there are morphological differences between the free-living larvae and those living in mussels. *Ablabesmyia janta* and the species of *Unionicola* mites that live on gills were not found between the same pair of demibranchs, which suggests that these two types of organisms have a mutually exclusive relationship (Roback et al. 1980; Vidrine 1990). No lesions have been reported in mussels infested by this chironomid, which was classified as a commensal species by Tokeshi (1993). However, there is no evidence to support conclusions related to the type of relationship between *A. janta* and its host.

A chironomid species that is probably in the genus *Acamptocladius* infests several unionid species in North America. This chironomid was called "near *Phycoidella*" when discovered (Gordon et al. 1978; Roback 1979) and was considered *Acamptocladius* sp. by Cranston and Saether (1982). The stage infesting mussels is about 1 mm long and is probably the first instar (Gordon et al. 1978; Cranston and Saether 1982). This chironomid occurs on the gills with up to 25 larvae per demibranch pair (Roback 1979). Gordon et al. (1978) stated that this chironomid does not cause any obvious damage to its host, but there have been no studies of the relation between this chironomid and its host.

Gordon et al. (1978) found the chironomid *Baeoctenus bicolar* attached to the anteriodorsal surface of the gills near the labial palps of the unionids *Pyganodon* (= *Anodonta*) *cataracta* and *Anodonta implicata* collected in Canada. The instar III larvae of this chironomid invade the host and remain through pupation. There appeared to be some degree of host specificity because two other unionids, *Elliptio complanata* and *Leptodea* (= *Lampsilis*) *ochracea* from the same location were not infested. The larvae and pupae inhabit tubes constructed of particulate organic material, and there were as many as three larvae attached to the gills on one side of a mussel.

The larvae feed on the host gill tissue with most of the damage surrounding the site of attachment. As much as 50% of the host gill was absent when two or more larvae were present.

Beedham (1971) reported larval *Glyptotendipes* (possibly *Glyptotendipes paripes*) between the mantle and shell (extrapallial cavity) of *Anodonta cygnea*. The chironomid stimulated the aggregation of hemocytes and caused the "breakdown" of the outer mantle epithelium. It appeared that the chironomid fed on the hemocytes and other cells of the mantle. The shell of the infested mussels had been experimentally injured several months before the chironomids were discovered and the injury could have provided a route for invasion by the chironomid.

Third- and fourth-instar larvae of a chironomid *Paratanytarus* sp. were found in the mantle cavity of three species of unionids, and also in zebra mussels and quagga mussels collected from the St. Lawrence River (Ricciardi 1994). The percentage of zebra mussels harboring this chironomid ranged from 38% (2 July) to 0% (26 August). All of the unionid species examined (*Elliptio complanata, Pyganodon cataracta*, and *Lampsilis radiata*) were infested with this chironomid and 90% of the chironomids in unionids were in silk tubes. The mean number of chironomids in infested zebra mussels was only 1.5 compared with 13.4 in unionids. Most infested zebra mussels had only a single chironomid. There was no indication of injury to the host in either unionids or dreissenids. Conn et al. (1994) also reported *Paratanytarus* sp. in zebra mussels from the St. Lawrence River.

Yang and Wang (1994) reported that the larvae of two species of chironomid, *Glyptotendipes pallens* and *Chironomus dorsalis*, killed young *Hyriopsis cumingii*, an economically important pearl-producing unionid in China.

The chironomid *Xenochironomus canterburyensis* lives in the mantle cavity or the incurrent siphon groove of the New Zealand freshwater mussel *Hyridella menziesi* (Forsyth and McCallum 1978). Larval chironomids were present in mussels all year, with the highest prevalence (84%) in November. There was only one chironomid per valve, and no injury to the host was mentioned.

Unidentified chironomid larvae were found in some of the *Anodonta anatina* that had a "pustular disease" in Finland (Pekkarinen 1993). The cause of this disease is unknown, and chironomids were not found in all of the diseased mussels.

Other species of chironomids that have been found in unionids are probably free-living species that only occasionally invade a host. Gordon et al. (1978) reported *Pseudochironomus, Phaeopsectra, Polypedilum* (two species), *Dicrotendipes,* and *Micropsectra* in *Pyganodon* (= *Anodonta*) *cataracta* and *Elliptio complanata*. Roback et al. (1980) considered the following chironomid larvae to be accidental inhabitants of bivalves: *Orthocladius dorenus* (in *Actinonaias ligamentina* [= *carinata*]), *Psectrocladius* sp. (in *Lampsilis hydiana*), Pseudochironomus sp. (in *Quadrula houstonensis* and *Amblema plicata*), and *Glyptotendipes* sp. (in *Obliquaria reflexa*). Epler (2001) mentions the chironomid genus *Trichochilus* as living within unionids.

Bitterling Eggs

The European bitterling *Rhodeus amarus* (this species has been previously considered a subspecies of *Rhodeus sericeus* [Bohlen et al. 2006]) is a fish in the family Cyprinidae. This species is native to Europe and Asia and has been introduced to the United States, where it spawns in *Pyganodon cataracta* and *Elliptio complanata* (Smith et al. 2004). A female bitterling

deposits eggs in the gills of unionids (Unionidae and Margaritiferidae) by inserting her ovipositor through the excurrent siphon (Smith et al. 2004). The eggs are fertilized by sperm drawn into the mussel through the inhalant siphon, and multiple spawns can result in a single mussel containing over 100 eggs. The young fish hatch after about a month and exit the mussel via the siphon.

The ventilation rates of *Unio pictorum* and *Anodonta anatina* were lower for mussels with bitterling eggs than for mussels without eggs, and the decrease in ventilation was linearly related to the number of bitterling eggs (Mills et al. 2005). The decreased ventilation may be caused by impairment of water flow through the gills and could be detrimental for filter feeding. The growth rate of *Unio pictorum* harboring a large number of bitterling eggs (mean 38.3) was lower than for mussels with a mean of only 3.3 eggs (Reichard et al. 2006). Smith et al. (2004) reviewed earlier reports of gill injury, impaired water flow through the gills, and increased oxygen consumption in unionids used as spawning sites for bitterling eggs. Although there is evidence that mussels are harmed by the presence of bitterling eggs, there is no evidence that long-term or fatal injuries occur.

Interactions Between Environmental Conditions and Infectious Diseases

Although there is a dogma that "stress" is often the predisposing factor leading to infectious disease, the interactions between environmental conditions and pathogens is complex and, in some cases, unexpected. Many types of suboptimal conditions are likely to increase the probability of infectious disease. An increased intensity of infestation by the copepod *Paraergasilus rylovi* in the unionid *Anodonta piscinalis* was attributed to a 25-day exposure to low concentrations of dissolved oxygen (means < 0.3 mg/L) that occurred 11 months prior to exposure to the parasite (Saarinen and Taskinen 2005b). However, during the laboratory exposure to low concentrations of oxygen in a small volume of static water, the mussels were starved and probably exposed to high concentrations of ammonia and perhaps other toxicants.

For other adverse environmental conditions, some pathogens may be more susceptible than the mollusk. For example, the ciliated protozoans *Conchophthirus curtus* and *Heterocinetopsis unionidarum* were lost from *Lampsilis cardium* and *Pyganodon grandis* after these unionids were transferred to locations below a municipal wastewater treatment plant (Antipa 1977). Similarly, the high vulnerability of free-living stages of Digenea (Morley et al. 2003) indicates that digenetic trematodes might be less common if environmental conditions are unfavorable for their survival. For polluted environments where the pathogens are more susceptible than the host, a decrease in infectious disease would be expected.

In addition to toxicants altering the susceptibility to pathogens, there can also be changes in susceptibility to toxicants because of infectious diseases. Susceptibility of bivalves to the toxicity of metals can be enhanced by digenean inhibition of metallothionein biosynthesis, which normally provides some protection against metal toxicity (Baudrimont et al. 2003). An unexpected finding was that sphaeriid clams with Digenea were more tolerant of the pollutant pentachlorophenol than were uninfested clams, perhaps because the high concentration of lipid in the parasites altered the distribution of the toxicant in the clam (Heinonen et al. 2001).

Diagnostic Methods

Mussel Anatomy

Knowledge of normal anatomy is an important aspect of disease diagnosis. Gross anatomy of unionids is described by McMahon and Bogan (2001) and Smith (2001), and information about the anatomy of marine bivalves is available from many sources (e.g., Pearse et al. 1987; Gosling 2003; Levine et al. 2006). Although there are few sources of general information about unionid histology and ultrastructure, there are excellent sources of information about other bivalves (Eble and Scro 1996; Morse and Zardus 1997; Elston 1999; Eble 2001). Lasee (1991) described the early life stages of the unionid *Lampsilis ventricosa* at the light and electron microscope levels.

Selection of Mussels for Detection of Pathogens

In some cases it is important to determine that certain pathogens are not present in a population. For example, if unionids are transported to a different watershed or to a hatchery, the transfer of pathogens that are not present in the new location should be avoided. The number of mussels sampled should be sufficient to provide a high probability that pathogens are detected if present (Thrusfield 1995). A sample size of 60 is often used in such cases to obtain a 95% probability of selecting at least one positive individual from a large population having a 5% prevalence of positive individuals. If the likely prevalence is lower than 5% or if a greater certainty of finding a diseased individual is desired, a larger sample size is required. If the pathogen is likely to be in a large proportion of the population, the sample size can be smaller—if the prevalence is 50%, only six mussels are needed have a 95% probability of a positive sample. A small sample can also be used if the presence of pathogens is indicated by lesions or abnormal behavior—this allows the section of the affected animals rather than random sampling.

Selection of Mussels for Disease Diagnosis

If an infectious disease is present in a population and the affected individuals can be identified by readily apparent lesions or by changes in their behavior, a small number of individuals can be examined. Those sampled should include all of the species affected and cover any variation in disease manifestation. However, the most important aspect of sampling a diseased population is to select the diseased individuals rather than random samples. For diagnosing a disease, one diseased mussel is more valuable than an infinite number of healthy individuals.

Recognition of grossly visible lesions requires a familiarity with the appearance of normal organs. Although the references mentioned above are important sources of information about anatomy, first hand experience with normal anatomy, including the normal variation among species and related to seasons, gender, and nutritional status, is helpful to avoid overlooking lesions or mistaking normal variation for an abnormality (Sparks 1985). It is also important to realize that some diseases may not always result in grossly visible lesions (Conn et al. 1996; Hine and Wesney 1997).

The mussels collected for disease diagnosis must not have postmortem changes. The collection of dead mussels is not likely to provide specimens suitable for necropsy because of the inevitable autolysis and invasion by saprophytic bacteria. Rather than collecting dead animals, which may be easy to identify, extra effort should be exerted to identify the mussels that are diseased but have not died.

Behavioral Indications of Disease

Unless the outer surface of the shell is abnormal, examination of a bivalve for lesions requires opening the valves. To reduce the likelihood of examining healthy individuals from a diseased population, observations of abnormal behavior can be an aid in the selection of diseased mussels for morphological examination. Examples of behavioral changes and other externally visible indications of disease are presented in the following paragraphs. Some of these examples are not directly applicable to the identification of diseased bivalves in field situations, but rather indicate possible approaches for laboratory monitoring or for the development of instrumentation for detection of impaired mussels.

Unionids normally burrow into the substrate with only the posterior shell protruding above the substrate. When dislodged from the substrate, the behavior needed to reestablish the normal position in the substrate is to turn the shell upright, to move across the substrate to locate a suitable location for burrowing, and to burrow by use of the foot (Waller et al. 1999). Obvious changes in diseased mussels could include lying on top of the substrate or not responding to physical stimulation (Berg et al. 1995). Pauley (1968) observed diseased *Margaritifera margaritifera* lying on top of the substrate rather than partially buried. A similar behavioral changed was observed for Pacific littleneck clams *Protothaca* (= *Venerupis*) *staminea* infested with larval cestodes (Sparks and Chew 1966).

Healthy juvenile mussels normally have externally visible activities such as extruding the foot and movement of the valves (Jacobson et al. 1993). In addition, the translucent valves of juvenile mussels allow observation of some internal activities, such as ciliary movement (Lasee 1991) and heart contractions (Keller and Zam 1991). To rapidly assess whether individual juvenile mussels are alive, a neutral red vital stain can be used (Jacobson et al. 1993). This dye is absorbed only by live cells, and in addition to serving as an aid to distinguish between live and dead mussels, reduced staining can indicate an abnormal individual. The use of a vital stain would only be useful for mussels young enough to have translucent valves. Similarly, the lack of foot movement, but continuing ciliary movement could indicate that a juvenile mussel is impaired (Lasee 1991).

Bivalves alter their valve movement patterns when exposed to suboptimal environmental conditions or certain toxicants. Changes in valve movement could also be an indication of infectious disease, but valve movements are influenced by numerous factors. The openness of unionid valves, the frequency of valve movements, and variability of movements are related to diurnal rhythms and can be influenced by whether the mussel is in a lake or river and by confinement in a cage (Englund and Heino 1996). An example of an environmental factor affecting valve gape of unionids is pulses of elevated water velocity, which caused a partial closure of the valves of *Amblema plicata* in laboratory conditions (Miller et al. 1999). None of the tested mussels completely closed their valves, and they recovered in less than 20 min. In a field study, boat traffic had little effect on mussel valve gape. There was a tendency for unionids to have valves open more widely in response to water conditions such as a higher concentration of algae, although laboratory conditions may have affected results (Shaffer et al. 1999). European fingernailclams *Sphaerium corneum* tended to close their valves in hypoxic water (less than 4 mg/L), and uptake of 2,4,5-trichlorophenol was reduced in hypoxic conditions, presumably because of the valve closure (Heinonen et al. 1997).

Salánki et al. (1991) measured the water flow from mussels by positioning a sensor 0.5 cm from the mussel siphon. *Anodonta* and *Unio* species were exposed to metals, and both valve movements and pumping of water through the siphons changed in response to metal exposure.

The change in water flow varied for the various metals used in this experiment but generally there was a reduction of water flow through the siphons. Changes in water flow could be detected within a few seconds after exposure.

Automated systems have been developed to monitor valve movements of zebra mussels (Borcherding and Wolf 2001; Borcherding 2006). Zebra mussels were fitted with a sensor glued to the valves, thus allowing continuous monitoring of valve movements. A 30-minute exposure of the zebra mussels to the toxicants 2-chloro-4-nitro-aniline, cadmium, or pentachlorophenol elicited an increase in the percentage of the population with closed valves and generally stimulated an increase in the number of valve movements. The addition of suspended solids (540 mg/L) did not result in a change in valve movements or valve closure. This system is used for routine monitoring of water toxicity in German rivers (Borcherding 2006).

Clinical Samples

Hemolymph.—Gustafson et al. (2005a) found that collection of hemolymph from the anterior adductor muscle sinus of the unionid *Elliptio complanata* did not impair growth or survival. Hemolymph is potentially useful as a diagnostic sample and has been used in several types of physiologic studies of unionids (Dietz 1974; Pynnönen 1994; Pekkarinen 1997; McMahon and Bogan 2001), and may also be useful as a bacteriological sample (Sparks et al. 1990). Normal concentrations for electrolytes are similar in related species of mussels (Dietz 1974; Pynnonen 1994; Gustafson et al. 2005b), but there are hematologic responses to season (Pekkarinen 1997), dehydration, anoxia (Dietz 1974; McMahon and Bogan 2001), transportation (Pekkarinen and Suoranta 1995), and acidity (Pynnonen 1994). Malagoli and Ottaviani (2005) described an assay for cytotoxicity of the hemolymph from the marine bivalve *Mytilus galloprovincialis*; they concluded that reduced cytotoxicity was an indication of impaired health status because of the importance of hemolymph cytotoxicity in defense against infectious agents.

Other nonlethal samples.—Biopsies for determination of glycogen concentration, isolation of bacteria, or other evaluations can be obtained from the mantle (Berg et al. 1995; Patterson et al. 1999) and foot (Naimo et al. 1998). Glycogen was relatively evenly distributed in the foot of unionids, but varied by a factor of two in the mantle (Naimo and Monroe 1999). Twenty-four months after *Amblema plicata* were relocated from their native environment to a pond, the relocated mussels had significantly less glycogen and this change was greater in the mantle than in the foot. Patterson et al. (1997, 1999) reported the concentrations of glycogen in mantles of mussels during starvation. The mantle glycogen concentration can be useful to determine adverse effects of disease, although habitat alteration or other factors affecting food availability or quality should be considered. Gangloff (2003) successfully used the glycogen concentration in the mantle of a unionid to demonstrate the harmful effects of aspidogastrid trematodes.

A nonlethal sample of the foot can be obtained by forcing open the valves approximately 5 mm (Naimo et al. 1998). Reverse pliers are useful for opening the valves, and a 5 to 10 mg sample from the ventral margin of the foot can be removed with a biopsy needle. Survival of biopsied and control mussels was not significantly different during a 581-day observation period.

A mantle biopsy can be collected by forcing open the valves approximately 10 mm, inserting a wooden wedge between the valves to keep them open, and cutting a sample from the margin of the mantle (Berg et al. 1995). Sampling this site avoids damage to the siphon or other structures. After the collection of this type of sample, survival of *Quadrula quadrula* and *Actinonaias ligamentina* was not significantly different from the survival of controls.

Diagnosing Diseases

The information needed to reach a diagnosis varies depending on the disease, but important information can often be gained from a thorough case history including detailed information about environmental conditions and diet, a complete examination for gross and histological lesions, and detection (ideally quantification) of infectious agents and toxicants. The identification of potential pathogens without examination for morphological, physiological, or behavioral indications of disease severely limits the usefulness of a necropsy because at present there is inadequate information about the virulence of most infectious agents in mussels. Although histopathology has often been used as a component of the examination of mussels (Williams 1890; Baker 1976; Chittick et al. 2001), many studies of unionids harboring potential pathogens could have been improved by a greater attention to host injury. Histological techniques for bivalves were described in detail by Howard et al. (2004).

Recommendations for Future Research

A difficult aspect of investigating the death of unionids and other bivalves is obtaining specimens suitable for examination. Frequently, the animals have been selected randomly and could have included individuals that were not affected by the disease. In other cases, the "fresh dead" individuals examined were not suitable for necropsy because of postmortem change and contamination by saprophytic organisms. In some cases, observing bivalves for subtle changes in the appearance of the outer surface of the valve or changes in valve movements or other behavior could provide an indication of which animals would be most valuable for necropsy. Additional investigation is needed to evaluate underwater monitoring methods to develop practical methods for selecting diseased mussels for examination.

A conclusion that unionids do not have lesions or pathogens should be supported by observations with appropriate methods and based on a thorough examination of all organs. For light microscopy, the failure to observe indications of viruses does not indicate that viruses are not present. The use of fish cell lines for attempted isolation of viruses from mollusks also provides no evidence about viruses unless a virus is isolated—negative results with these methods do not indicate that viruses are not present. Similarly, the lack of an obvious change from normal in the bacterial growth on culture plates does not indicate that a population of unionids is not affected by a newly acquired bacterial pathogen; perhaps the pathogen will not grow in the culture conditions used. Even some studies of grossly visible parasites have involved only the examination of the gills and mantle cavity and did not include an examination of internal organs.

An important need in future investigations of mussel die-offs is a more complete search for pathogens that are not easily detected. For example, there should be additional emphasis on detection of fastidious bacteria, including anaerobic species, which require special methods for isolation. There should also be searches for viruses that will not replicate in cell lines derived from fish. There are methods currently available for more thorough searches for pathogens, but there is also the possibility that new media or culture techniques will be required to isolate microbes that cause mussel die-offs. There should also be additional emphasis placed on histopathology, which could reveal evidence of pathogenic microbes that are difficult to culture.

Existing methods for detecting viruses that will not grow in currently available cell lines are ultrastructural examination of diseased mussels by electron microscopy and the physical isolation of viruses. Virus particles can be concentrated from infected cells by high-speed centrifugation in a density gradient or by precipitation methods. After the virus is physically isolated, it can be visualized by electron microscopy and if the virus was not damaged during isolation it can be used to infect healthy animals to determine its virulence. Perhaps most importantly, the nucleic acid of the isolated virus can be sequenced, which provides the information needed to develop molecular diagnostic tools. Although these methods are challenging, they have been used successfully and there should be additional effort to discover viruses that are potentially the cause of mussel diseases.

Cell lines derived from bivalves are not readily available, and this is an important limiting factor for the discovery of viruses. Although some viruses of bivalves will replicate in fish cell lines, it seems likely that this will not be true for most viruses of invertebrates (most of these viruses have not yet been discovered). The development of permanent cell lines is an important need in bivalve virology, but based on the failure of previous efforts, it may be difficult to make progress in this area.

An alternate approach is to use primary cultures of cells for isolation of viruses. Typically cell cultures of this type persist for only a few days or weeks after they are established, and having primary cell cultures available when needed for the investigation of a mussel die-off requires considerable expense. However, improvement in the methods for establishing primary cell cultures could increase the suitability of this approach for isolating viruses. An advantage of primary cell cultures is the potential to use the species that is diseased as the source of cells for *in vitro* culture. Establishing primary cultures of cells from the affected species could allow isolation of highly specific viruses.

Viruses can also be detected by exposing test animals under controlled conditions to the filtered homogenate of cells from a diseased individual. This approach is limited by difficulties in the use of mussels as experimental animals, including the availability of stocks that are known to be free of the presumptive pathogen. However, when cell culture assays are not available, passage in test animals is the only technique available to demonstrate that a virulent virus is present. If facilities for experiments with live mussels are available, this method may be the most practical for current investigations of mussel die-offs. Unlike methods relying on physical isolation of virus or on visualization of virus-like particles by electron microscopy, the passage of presumptive virus to test animals provides evidence about pathogenicity during the initial phase of the study.

Identification of bacterial diseases of unionids is complicated by the difficulty of finding moribund mussels for examination and by the bacteria present in healthy individuals . The use of dead mussels for isolation of bacteria should be avoided because of the rapid proliferation of saprophytic organisms. It also seems advisable to put greater emphasis on attempting to isolate bacteria from locations, such as the kidney or hemolymph, that are least likely to contain nonpathogenic, environmental bacteria. The stomach, intestine, and digestive gland are likely to contain bacteria filtered from the water, making recognition of pathogens difficult. Selective media should be used for isolation of bacteria from the gills and other external surfaces because of the presence of environmental bacteria. The lack of information about the types of bacteria capable of causing disease in unionids makes it difficult to select the most suitable selective media, but a medium useful for isolation of columnaris-like bacteria (Decostere et al. 1997)

should be a priority. *Flavobacterium columnare* has been isolated from unionids (Starliper et al. 1998) but is likely to be rare unless it is causing disease.

Microscopic observation of gill cilia movement can be used to avoid the use of dead bivalves for attempted isolation of bacterial pathogens (Sparks et al. 1990). Mussels without actively beating cilia should not be used because this would indicate that the mussel is dead and that saprophytic bacteria could have invaded the mussel. The time required for this examination would be trivial compared with the time required to identify the extraneous, saprophytic bacteria that contaminate dead bivalves. This microscopic observation would also provide an opportunity to examine the gill for protozoans and columnaris-like bacteria.

The incubation temperature can affect which bacteria are isolated from unionids. The use of a single temperature can prevent the growth of some bacteria present in unionids (Chittick et al. 2001), although it seems unlikely that bacteria that will not grow at a temperature that is typical of the environment where the diseased unionids were collected would be relevant to diagnosis of the disease. If it is not feasible to incubate the inoculated medium at multiple temperatures, the temperature selected should be near the water temperature where the unionid was collected.

For many of the eukaryotic and bacterial agents known to occur in freshwater bivalves, there is a need for controlled experiments to determine the effects of these potential pathogens. This is perhaps most important for mussels that are likely to be kept in captivity. The confinement of unionids in either extensive or intensive conditions is likely to increase their susceptibility to infectious diseases. Organisms that have not been considered serious pathogens in wild populations could cause problems in captive population of unionids.

This is also a need for studies of the diseases of glochidia and newly transformed juvenile unionids. These early life history stages have generally not been considered in studies of potential pathogens. The examination of young unionids for protozoan, bacterial, and viral agents is especially important.

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