1994 - ANNUAL REPORT

SURVEY OF METAZOAN SYMBIONTS OF FIVE UNIONID SPECIES (UNIONIDAE) COLLECTED AT THE 88.1, 89.0, AND 197.6 MILE MARKERS OF THE TENNESSEE RIVER[1]

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28 October, 1994

[1] This report is the annual progress report for the Tennessee Wildlife Resources Agency contract No. 7A-4-11062-4-00 entitled, "Qualitative survey of metazoan inhabitants of freshwater mussels, TN River".

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Acknowledgements

We thank A. D. McKinney (TWRA) for overseeing the collection and shipping of mussels to S.C. for examination, Dr. J. N. Caira (The University of Connecticut) for facilities used throughout the laboratory analyses, and the Tennessee Aquarium for proposing the study, facilitating the contract and donating G.W.B.'s time on the project.

Abstract:

A total of 101 unionid mussels (Unionidae) comprising five species (*Amblema plicata*, *Fusconaia ebena*, *F. flava*, *Quadrula metanevra*, *Q. quadrula*) were examined for metazoan symbionts. The mussels were collected at the 88.1, 89.0 and 197.6 mile markers of the Tennessee River during May and July of 1994. A total of 538 symbionts representing five phyla, eight families, at least nine genera, and at least nine species were identified from the sampled mussels. Only three symbionts had prevalence levels consistently greater than 10 percent. The trematode *Aspidogaster conchicola* was the most common associate, representing 56.7 percent of all collected symbionts. Unionicolid gill mites were the next most abundant associates, accounting for 22.1 percent of all collected symbionts. Dorylaimid nematodes were the third most common associates, accounting for 18.2 percent of all associates collected. Although calculated prevalence and intensity values for the three most common associates were not statistically tested due to the preliminary nature of the data, prevalence values above 74 percent, and intensity values above 3.0 were not uncommonly associated with infection by *Aspidogaster conchicola* or unionicolid mites.

INTRODUCTION

The southeastern United States is the center of biodiversity for freshwater mussels of the family Unionidae (see Williams *et al.*, 1992; Neves, 1993). Currently this rich fauna is experiencing an unprecedented decline due to various environmental factors which are all ultimately associated with human activity (Williams *et al.*, 1992; Neves 1993). Because several unionid species are of considerable commercial importance (e.g., see McGregor and Gordon, 1992), and because preliminary studies show that some unionids are excellent indicator species regarding aquatic environmental health (e.g., see Jacobson *et al.*, 1993), studies are desirable that gather natural history data of potential use in mussel restoration, management, and aquaculture programs. In addition, studies concerning the natural history of American unionids are of considerable biological significance because this group of mussels is a fine example of a taxon which has radiated greatly in North America and no where else.

Parasites can play important roles in the natural history of free-living communities. While some parasites routinely act as agents of disease, most parasite species in natural settings coexist with their hosts by establishing population levels which do not overburden the host population. The transmission stage of a parasite's life cycle often represents the limiting phase that ultimately regulates parasite population levels. Environmental factors which increase the probability that parasite transmission will be successful can cause increases in parasite loads and may alter the overall pathogenicity of

parasites. However, even when parasite populations are stable in size the pathogenicity associated with them can vary. This variability can be caused by alterations in the overall energy budget of the host and may reflect natural host cycles associated with general health, ontogeny, seasonality, or with unnatural or catastrophic environmental events.

Although natural host populations can sometimes be detrimentally impacted by parasites, it is in semi-closed and closed-captive environments where parasites often are best known to cause undesirable levels of morbidity and mortality. The physical designs and high stocking densities of most closed aquaculture systems increase the probability of successful transmission for many parasite species (especially those with direct life cycles) and can ultimately overwhelm the capacity for hosts to support themselves under the pressures of parasitism.

Studies of the natural parasite burdens of unionid mussels are generally lacking. Current knowledge of the parasite fauna of freshwater mussels is in the form of geographically scattered parasite records associated with minimal taxonomic and temporal scope. Published records have typically documented the presence of parasites without data concerning the seasonal prevalence and intensity of infection or estimates of total parasite burden. Because comprehensive background data do not exist for healthy populations, the impact of parasitism on mussel populations is unknown, and the relationship of parasitic infection to sporadic "die-off" episodes within mussel populations is impossible to assess.

This report presents the first year's progress of a two season pilot study designed to survey the metazoan associates of several species of freshwater mussels within the Tennessee River system. In addition to symbiont identity, possible seasonal changes in total parasite burden, parasite prevalence, and parasite intensity were formulated to be of particular concern for this study in hopes of gathering baseline data which might be useful in managing both natural and cultured mussel populations.

MATERIALS AND METHODS

A total of 117 unionid mussels comprising five species were collected by Tennessee Wildlife Resources Agency (TWRA) biologists from three locations along the Tennessee River (see Figure 1). Mussels collected at the 88.1 and 89.0 river mile sites were lumped for analysis in this report due to the small sample sizes of each of the collections, and also due to the close proximity of these collections relative to the 197.6 river mile collection site. A total of 78 individual mussels comprising four species were collected during May 1994 for the combined 88.1 and 89.0 river mile sample (Table 1). A total of 39 individual mussels comprising two species were collected during July 1994 for the 197.6 river mile sample (Table 1).

Tennessee Wildlife Resources Agency biologists identified the collected mussels and placed them in individual plastic bags along with data labels (species, date, location).

Bagged mussels were placed on ice in an insulated container and were shipped to the laboratory in Connecticut within two days of capture. Once received at the laboratory, mussels were stored in a refrigerator (6.5°C) and were examined within 14 days of their arrival.

In the laboratory, 101 mussels (see Table 1) were examined using the following procedure (see Figure 2 for an anatomical reference to major organs of unionids): water from the collection bag and the outside of the shell were examined for organisms using a dissection microscope. Next the shell was opened and the mantle liquid was drained into a petri dish and examined for organisms. Lastly, the soft tissues were examined under low power, dissected and reexamined under low power for symbionts. These tissues included the mantle, foot, gills, digestive gland, stomach, intestine, kidney, gonad, and pericardium. All discovered symbionts were collected and the exact location of each was recorded.

The following weights (g) were recorded for each examined mussel: total weight (*i.e.*, shell and soft tissues), soft weight (*i.e.*, total weight of all soft tissues including the retractor and adductor muscles), and shell weight (*i.e.*, weight of shell without retractor and adductor muscles). Mussel shells were individually marked and stored for later aging by TWRA biologists.

A total of six mussels comprising four species were selected for future histological examination (see Table 2). The examinations of these mussels differed from

those discussed above in that the organs mentioned above were excised using a scalpel and examined under low power prior to relaxation and fixation in bouin's fixative or 10 percent buffered formalin. Further processing of these samples has not as yet taken place.

Metazoan symbionts collected from the examined mussels were fixed, preserved, identified, and stored using standard laboratory techniques (e.g., see Pritchard and Kruse, 1982). While some symbionts were identified to the level of species, higher level taxa (e.g., genus, family) have been used to identify others and work continues to give a specific identity to as many symbionts as possible.

Symbiont prevalence and density statistics were calculated for each of the three most common groups of collected symbionts. Symbiont prevalence is defined as the percentage of individuals associated with the symbiont in a given host population, and was calculated as follows:

$$P_{yx_t} = \frac{C_{yx_t}}{N_{yx_t}} \times 100$$

where:

P_{yx_t} = the prevalence of symbiont species x in host species y at time t,

C_{yx_t} = the number of hosts of species y found associated with symbiont species x at time t,

and

N_{yx_t} = the total number of host species y examined for symbiont species x at time t.

Symbiont density (sometimes referred to as intensity) is defined as the mean number of symbionts associated with individuals in a given host population, and was calculated as follows:

$$D_{yx_t} = \frac{\sum_{1}^{N_y} A_{yx_t}}{N_{yx_t}}$$

where:

D_{yx_t} = the mean density of symbiont species x associated with host species y at time t,

$\sum_{1}^{N_y} A_{yx_t}$ = the sum of the total number of symbiont individuals of species x collected from each individual species y host examined at time t,

and

N_{yx_t} = the total number of host individuals of species y examined for association with species x at time t.

Other statistics used in this report were calculated according to Zar (1974).

RESULTS AND DISCUSSION

A total of 538 metazoan symbionts were found associating with the 101 totally examined mussels (Table 2). Together, these associates represented four phyla, eight families, at least nine genera, and at least nine species (Table 2). Arthropoda was the best represented phylum (six genera collected), while Platyhelminthes, Nematoda, and Tardigrada were each represented by a single genus. Of the 538 symbionts collected, only three taxa achieved prevalence values consistently greater than 10 percent (see Table 3). These most common associates included the trematode *Aspidogaster conchicola*, nematodes of the genus *Dorylaimus*, and unionicolid water mites. At this time, the dorylaimid nematodes and the unionicolid mites have not been identified to species, and they each have been treated as single taxa for the purposes of this report.

Phylum Platyhelminthes was represented by a single species, *Asidogaster conchicola* (Trematoda: Aspidogasteridae). This parasite (Figure 3) was found inhabiting the pericardial and renal cavities of mussels (Table 2, Figure 4). *Aspidogaster conchicola* was collected from four of the five examined unionid species (Tables 2 and 3). A total of 305 *A. conchicola* individuals were collected, representing 56.7 percent of all associates collected. Its high prevalence and density made *A. conchicola* the most abundant symbiont collected in this study (Table 2). Among the five species of mussels examined, the prevalence of infection ranged from 0 to 85 percent (Table 3). When present, *A. conchicola* ranged in abundance from 1 to 40 individuals, and its intensity of infection ranged from 1.89 ± 1.54 to 10.2 ± 9.94 individuals per host (Table 4).

The life cycle of *A. conchicola* (see Figure 5) may take place entirely in one host mussel, or it may involve infective larvae being liberated to the outside where they may infect other mussels (Olsen, 1962). Its presence has been shown to cause renal metaplasia in unionid hosts (Pauley and Becker, 1968). Given this species' malleable life cycle, its potential to cause disease, and its high prevalence and intensity as demonstrated by the present study, *A. conchicola* could have a detrimental impact on mussel populations. Furthermore, in a closed- or semi-closed aquaculture system, transmission of the highly motile first stage larvae of *A. conchicola* could be facilitated, and might result in abnormally high parasite burdens.

Phylum Nematoda was represented by at least one species belonging to the genus *Dorylaimus* (Adenophorea: Dorylaimidae). These nematodes were collected exclusively from the outside of unionid shells, and they were associated with all five mussel species examined (Table 2). All totaled, these nematodes accounted for 18.2 percent of the total associates collected in this study, and when present they ranged in abundance from 1 to 10 individuals per mussel (Table 4). Prevalences of infection for the five examined mussel species ranged from 21 to 65 percent (Table 3), and the intensity of these infections ranged from 1.0 ± 0.0 to 3.69 ± 2.95 individuals per host (Table 4). Considered temporally, 90.8 percent of the collected nematodes were taken in the May sample. Dorylaimids are commonly found free-living in moist soils and freshwater habitats where they prey on microbes (Poinar, 1991). Casual observations in this study

did not identify any mussel pathologies associated with nematode presence and we consider these symbionts to be commensals.

Phylum Tardigrada (the water bears) was represented by a single individual found on the external shell surface of *Fusconaia ebena* (Table 2). Tardigrades are an unusual group of small arthropod-like organisms within unknown phylogenetic origins (Nelson, 1991). The collected specimen probably represents an incidental association between the mussel and a tardigrade species which normally resides on the river substrate. Unfortunately, during its manipulation under the microscope, the collected tardigrade entered a latent state of encystment and was subsequently lost in transfer.

Phylum Arthropoda provided the most diversity of the four phyla collected in this study, accounting for 63 percent of the families and at least 66 percent of the species collected (Table 2). The most abundant arthropods collected were water mites belonging to the family Unionicolidae. One hundred and nineteen of these mites were found inhabiting the gills of four of the five species of mussels examined (Table 2), accounting for 22.1 percent of the total number of associates collected. Four mites of what appeared to be a separate unionicolid taxon were found in association with the external shell and internal fluid of *Quadrula quadrula* (see Table 2). The prevalence of infection by all mites ranged from 0 to 92 percent for the five examined mussel species (Table 3). When present, mites ranged in abundance from 1 to 12 individuals per host, with intensity of infection values ranging from 1.0 ± 0.0 to 4.67 ± 2.15 individuals per mussel (Table 4).

It is notable that the larval stages of some unionicolid mites are parasitic on chironomid larvae (Jones, 1978). In the present study, chionomid and other aquatic insect larvae were collected from the shells of several mussels (see Table 2). Certainly a parasite life cycle that utilizes an intermediate host which can be found in close physical association with the definitive host will be very effective, and this may be the case for some mite species.

Unionicolids have been shown to cause disease in unionid mussels (e.g., see Baker, 1976, 1977). Attached to the gills of mussels, unionicolids can cause tissue damage resulting in edema and inflammation, and histological examinations have revealed that mites feed on mussel haemocytes (Baker, loc. cit.). Additional damage to mussel hosts can be caused by oviposition by mites (Mitchell, 1965). In the present study, localized areas of inflammation were noticed on the gills of some mussels infected with mites.

In addition to water mites, Arthropoda was also represented by class Insecta. Commensal larval stages of two orders of insects were collected from the external shells of mussels, with four of the five examined mussel species supporting at least one of these two insects orders (Table 2). Order Trichoptera (the caddis flies) was represented by only two individuals of separate species. Order Diptera (the true flies) was represented by a more diverse assemblage of larvae, including eight individuals of the family Chironomidae (the midges), and a single individual of the family Ceratopogonidae (the biting midges). Chironomidae is the largest family of aquatic insects in North America, with over 2500 nominal species inhabiting a broad range of ecological habitats

(Hilsenhoff, 1991). Ceratopogonidae is represented by many riparian zone species but also includes species that occupy a range of aquatic habitats (Hilsenhoff, 1991). Unfortunately, it is currently difficult if not impossible to identify larval aquatic insects below to level of family without completing their life cycles, as identification keys are based on adult characteristics.

Because of the small sample size available for analysis, statistics were not used to test calculated prevalence and intensity values for significant differences. Certainly these calculated values differed among hosts, associates and sample locations/dates (see Tables 3 and 4). A general trend seems to possibly exist between infection prevalence and intensity levels for two of the associate taxa, appearing as a positive correlation regarding infection with *Aspidogaster conchicola* and also with unionicolid mites (see Tables 3 and 4). It is also interesting that *Fusconaia flava* was not infected with either *Aspidogaster conchicola* or unionicolid mites even thought this species was collected at a location where other mussel species were infected with these parasites (see Table 2). This is particularly interesting given the high prevalence of *Aspidogaster conchicola* at this location and this species' low level of host specificity which seems to allow it to infect many species of unionids.

Values describing the percent association between all possible combinations of the three most common associates of sampled mussels (see Table 5) were not tested for statistical significance due to the preliminary nature of these data. Casual inspection of percent association values (see Table 5) revealed no readily apparent trends in symbiont

association other than the already noted (see Tables 2-4) lack of symbionts associated with Fusconaia flava.

SUMMARY

Examination of 101 unioinid mussels representing five species collected from the 88.1, 89.0, and 197.6 mile markers of the Tennessee River during May and July of 1994 revealed:

1) sampled mussels to be associated with four phlya, eight families, at least nine genera, and at least nine species of symbiotic associates all totaling 538 individuals.
2) only three associate taxa had prevalence values consistently greater than 10 percent, namely the trematode *Aspidogaster conchicola*, unionicolid mites, and nematodes of the genus *Dorylaimus*.
3) of all the associates collected, only *Aspidogaster conchicola* and the unionicolid mites are known to be parasites of mussels.
4) the prevalence of *Aspidogaster conchicola* infection in three samples was greater than 74 percent, with a maximum of 40 worms in one mussel.
5) the prevalence of unionicolid infection in two samples was 90 percent or greater, with a maximum of 12 mites in one mussel.

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Figures

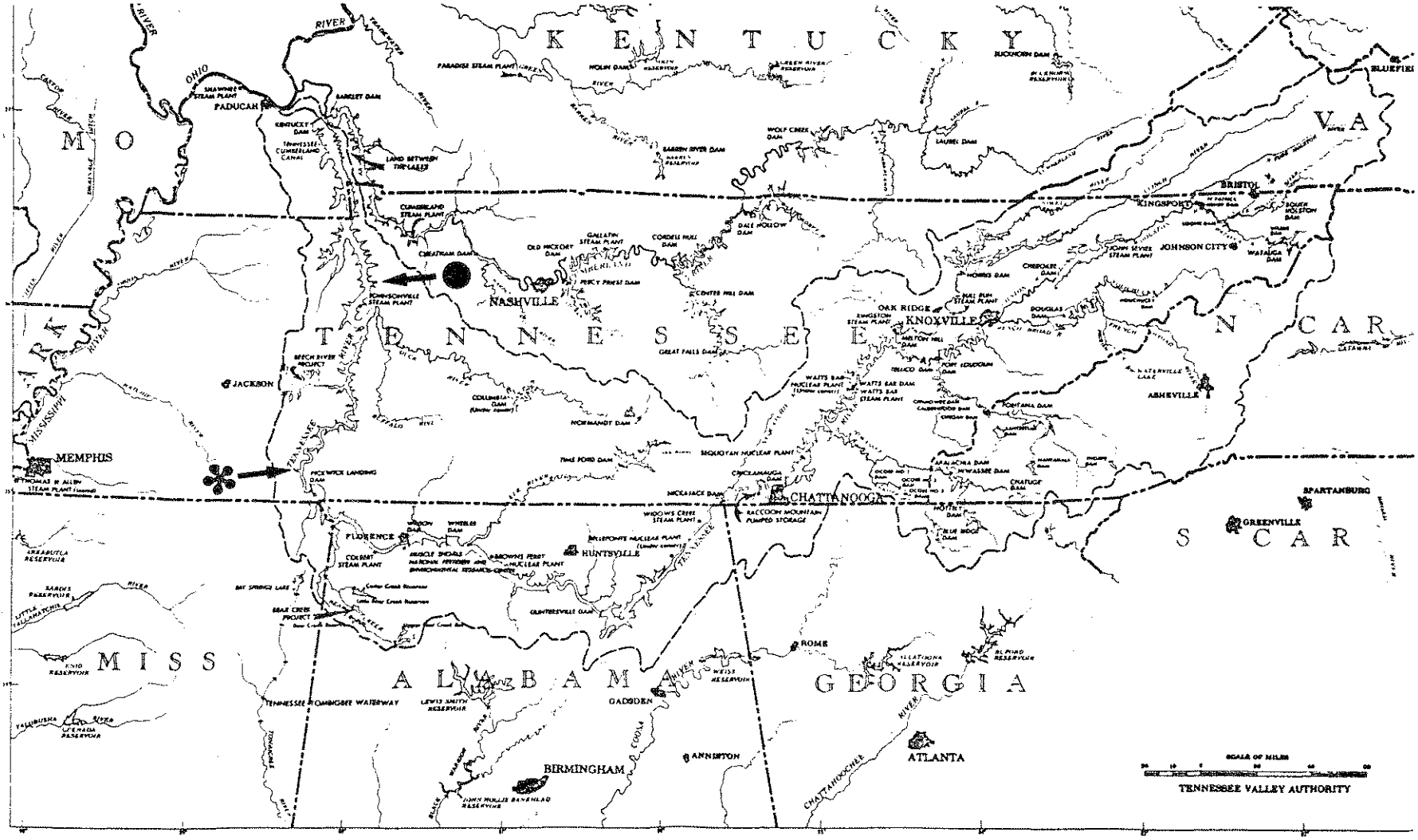

Figure 1. Map of Tennessee region showing the location of the three sampling sites where mussels were collected. Solid dot and arrow indicate the 88.1 and 89.0 river mile locations, asterisk and arrow indicate the 197.6 river mile location.

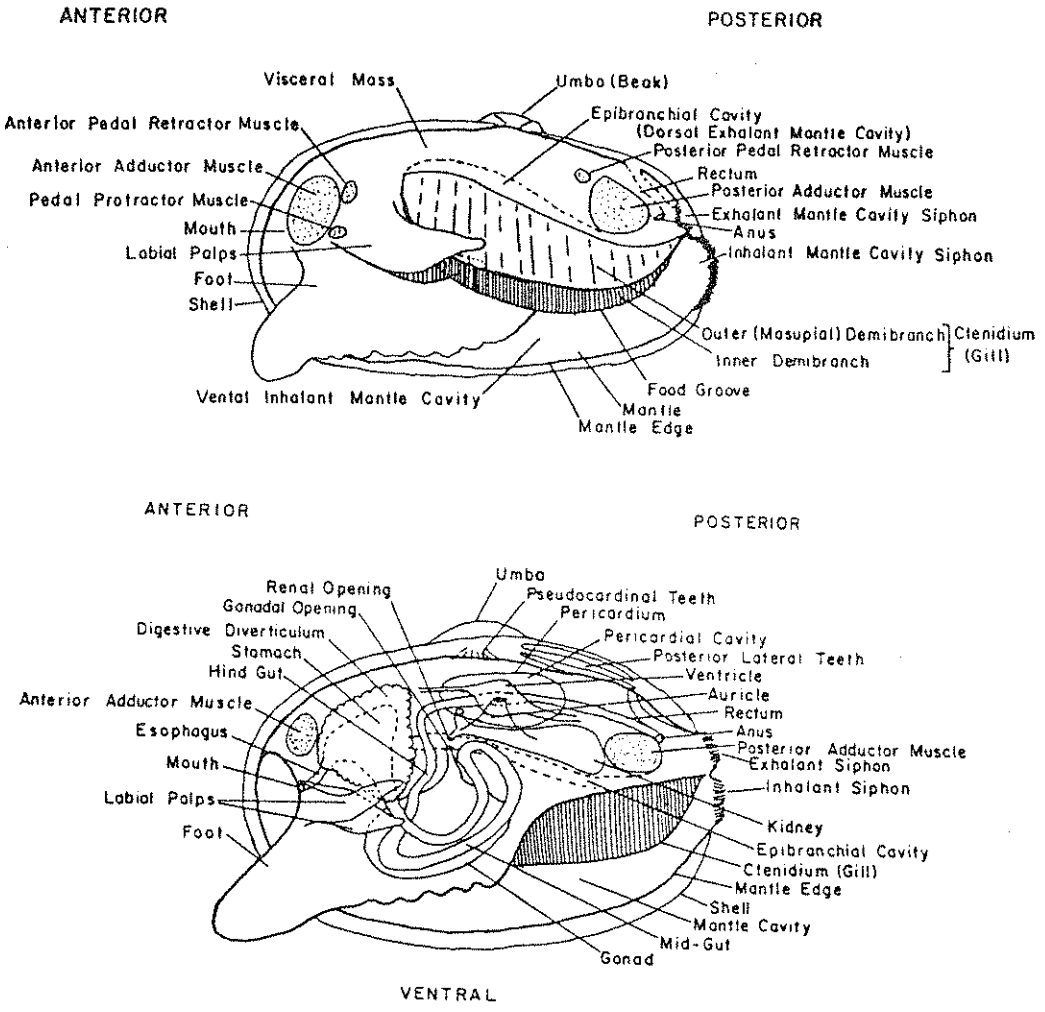

Figure 2. General external (top) and internal (bottom) anatomy of a unionid bivalve. (modified from McMahon, 1991)

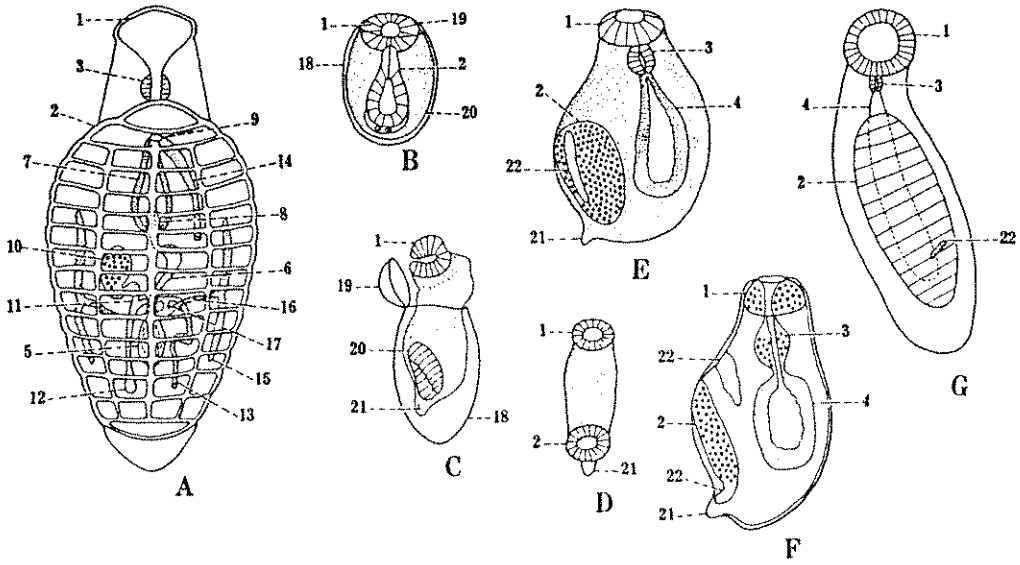

Figure 3. *Aspidogaster conchicola*. A. Adult fluke. B. Embryonated egg. C. Egg in process of hatching with first stage larva escaping. D. Recently hatched first stage larva. E. Second stage larva. F. Third stage larva. G. Fourth stage larva. Key to numbers: 1. mouth sucker. 2. ventral sucker (divided into alveoli in adult and late fourth stage larva but not in first, second and third stage larvae). 3. pharynx. 4. intestine. 5. testis. 6. vas efferens. 7. cirrus pouch. 8. cirrus papilla. 9. common genital pore. 10. ovary. 11. oviduct. 12. Laurer's canal. 13. descending limb of uterus. 14. terminal portion of uterus. 15. vitelline gland. 16. transverse vitelline duct. 17. common vitelline duct. 18. egg shell. 19. operculum. 20. first stage larva. 21. tail. 22. groove in ventral sucker. (figure modified from Olsen, 1962)

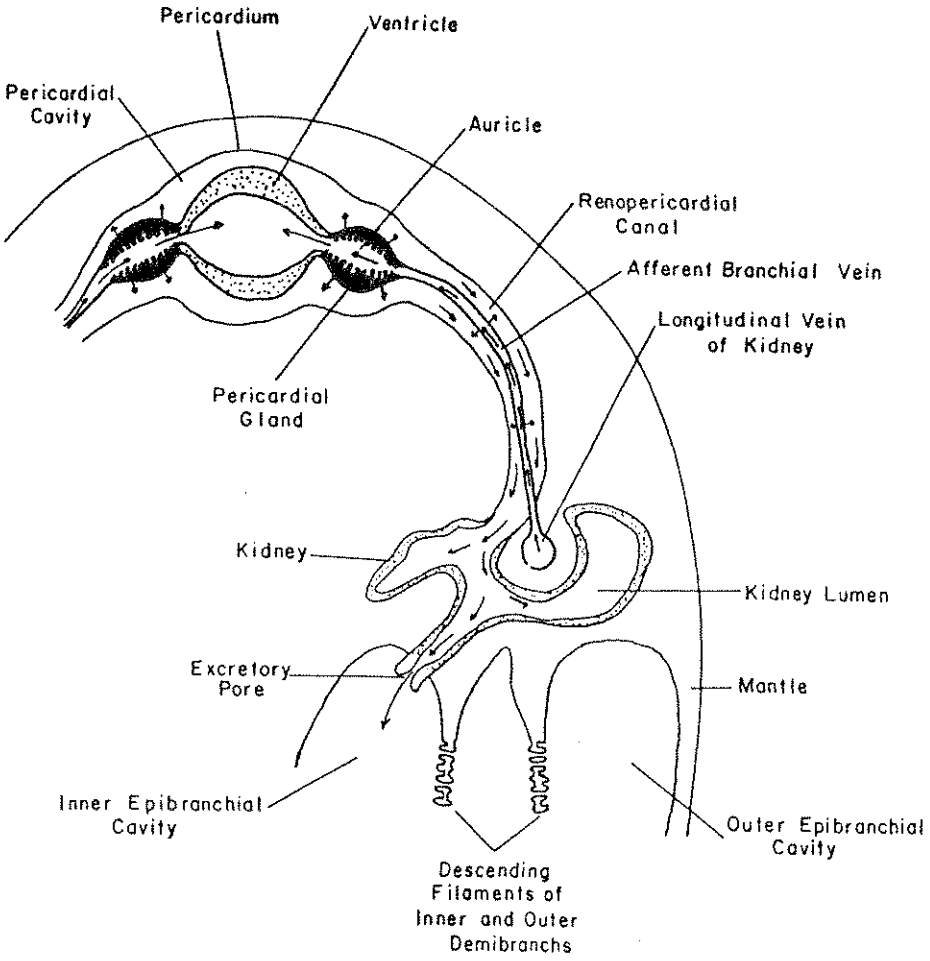

Figure 4. Diagram of anatomical features of the excretory system of a typical freshwater bivalve. In this study, the pericardial cavity and the lumen of the kidney represent the two regions where the trematode *Aspidogaster conchicola* was collected. Arrows indicate water excretion pathways. (figure modified from McMahon, 1991)

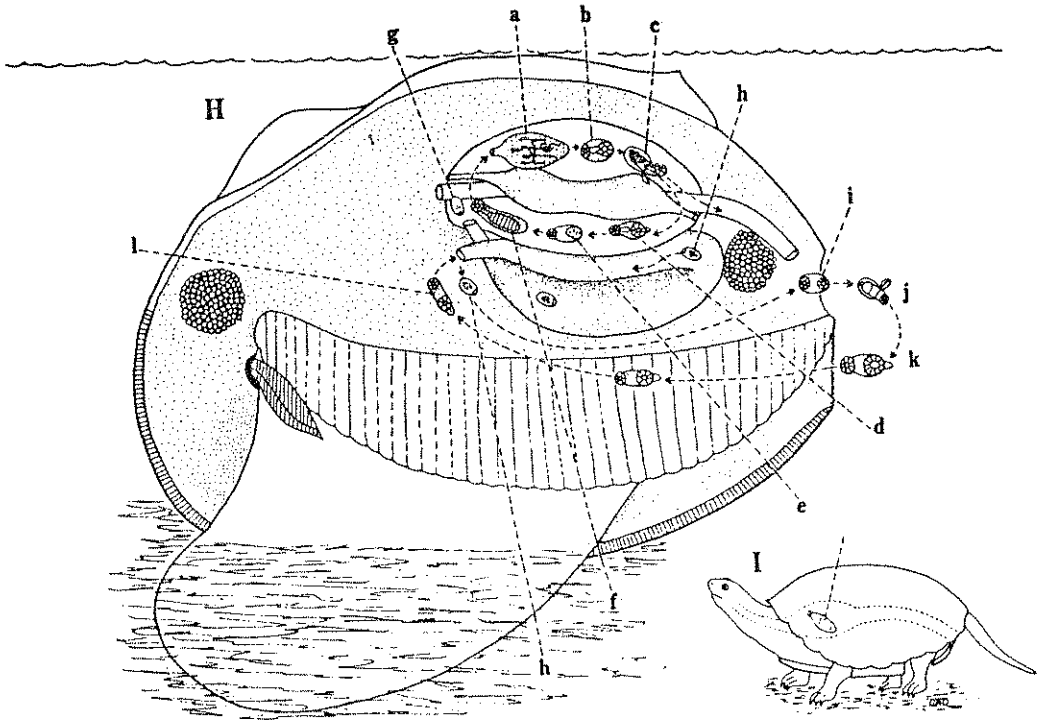

Figure 5. Life cycle pathways of *Aspidogaster conchicola.* Two possible pathways are presented. The first pathway (shown as a-f) is as follows: adult fluke in pericardial chamber (a) of mussel lays embryonated egg (b) which hatches, liberating the first stage larva (c) which then matures into a second stage larva (d), a third stage larva (e), a fourth stage larva (f), and then an adult (a). All of these stages reside in the pericardial chamber of the mussel. Via this pathway, infected mussels infect themselves with future generations of flukes (i.e., autoinfection). The second possible life cycle pathway (shown as g-l) is as follows: embryonated egg passes into kidney (g) from pericardial chamber via renopericardial canal (see Figure 4), egg passes through kidney (h) and out excretory pore (see Figure 4) into suprabranchial cavity, egg is carried from branchial cavity through excurrent siphon (i), egg hatches in water releasing first stage larva (j), first stage larva is drawn into branchial cavity via incurrent pore (k), first stage larva enters kidney (l) via excretory pore and migrates to pericardial chamber via renopericardial canal. As the larva migrates it molts through second, third, and fourth larval stages to eventually assume form as an adult. Via this pathway, parasites can be transmitted between mussels and hence can increase in prevalence throughout mussel populations. Diagram of turtle denotes that if infected mussels are eaten and digested by turtles, *A. conchicola* will be liberated in the gut where they may survive. (figure modified from Olsen, 1962)

Tables

Table 4. Intensity of infection and range of infection of predominant taxa found in association with unionid mussels collected from three locations on the Tennessee River in May and July of 1994

sample location (river mile marker)	unionid sp.	*N*	intensity of infection ± 1SD (range of infection)		
			Aspidogaster conchicola	*Dorylaimus* sp.	unionicolids
88.1 and 89.0					
	Amblema plicata	13	2.4 ± 0.84 (1-4)	2.16 ± 1.47 (1-4)	4.67 ± 2.15 (2-8)
	Fusconaia ebena	24	3.06 ± 3.31 (1-3)	1.89 ± 1.62 (1-6)	1.0 ± 0.0 (1)
	Fusconaia flava	14	0.0 ± 0.0 (0)	1.38 ± 0.74 (1-3)	0.0 ± 0.0 (0)
	Quadrula metanevra	0	-	-	-
	Quadrula quadrula	20	10.2 ± 9.94 (1-40)	3.69 ± 2.95 (1-10)	3.22 ± 2.46 (1-12)
197.6					
	Amblema plicata	0	-	-	-
	Fusconaia ebena	14	4.0 ± 3.61 (1-13)	1.0 ± 0.0 (1)	0.0 ± 0.0 (0)
	Fusconaia flava	0	-	-	-
	Quadrula metanevra	14	1.89 ± 1.54 (1-5)	1.67 ± 0.58 (1-2)	1.0 ± 0.0 (1)
	Quadrula quadrula	0	-	-	-

Table 5. Percent association among the major taxa found symbiotic with five species of unionid mussels collected from the 88.1, 89.0, and 197.6 mile markers of the Tennessee River during May and June of 1994 [1]

unionid species	*N*	associate taxon	percent association with			
			Aspidogaster conchicola	*Dorylaimus* sp.	unionicolid water mites	*Aspidogaster conchicola* and *Dorylaimus* sp.
Amblema plicata	13	*Aspidogaster conchicola*	0	-	-	-
		Dorylaimus sp.	0	8	-	-
		unionicolid water mites	38	0	15	38
Fusconaia ebena	38	*Aspidogaster conchicola*	42	-	-	-
		Dorylaimus sp.	26.3	7.9	-	-
		unionicolid water mites	2.6	0	0	0
Fusconaia flava	14	*Aspidogaster conchicola*	0	-	-	-
		Dorylaimus sp.	0	57.1	-	-
		unionicolid water mites	0	0	0	0
Quadrula metanevra	14	*Aspidogaster conchicola*	42.9	-	-	-
		Dorymaimus sp.	7.1	7.1	-	-
		unionicolid water mites	14.3	7.1	7.1	0
Quadrula quadrula	18	*Aspidogaster conchicola*	0	-	-	-
		Dorylaimus sp.	5.5	0	-	-
		unionicolid water mites	33.3	5.5	5.5	50.0

[1] percent association is defined as the percent of individuals in an associate taxon that were found in association with associates of another taxon. In this table, values for redundant association groupings have not been tabled and instead are signified by a dash mark.