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**EVALUATION OF FISH HOST SUITABILITY FOR THE ENDANGERED DWARF
WEDGEMUSSEL *ALASMIDONTA HETERODON***

A Thesis in
Wildlife and Fisheries Science
by
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ABSTRACT

The dwarf wedgemussel *Alasmidonta heterodon* is a federally endangered freshwater mussel native to the Atlantic Slope drainage. Like most unionids, *A. heterodon* glochidia (larvae) require a host fish to transform into juvenile mussels. Several fish hosts for *A. heterodon* have been identified at the southern and northern extents of its range, but it is uncertain if *A. heterodon* populations in the central portion of the range, namely in the Delaware River basin, use the same array of hosts. In spring and summer of 2006 and 2007, I used glochidia from mussels from Flat Brook (NJ) in the upper Delaware River basin to test suitability of a number of potential host fish species. I also collected fish from known *A. heterodon* sites in the upper Delaware River mainstem (PA/NY) during spring, when mussels are known to release glochidia in the wild, to identify any naturally infected fish. In addition, I examined population-level variation in fish host suitability of a known host, the tessellated darter, by comparing suitability of tessellated darters from five different locations in the Connecticut, Delaware and Susquehanna River basins, using glochidia of mussels from the upper Connecticut River (NH). I also conducted meristic and morphometric analysis of darters from different sources to identify any potential differences among populations that might correlate with differences in host suitability. Four previously identified hosts for *A. heterodon* were confirmed in this study: the slimy sculpin (*Cottus cognatus*), mottled sculpin (*Cottus bairdi*), Atlantic salmon (*Salmo salar*) and tessellated darter (*Etheostoma olmstedii*). In addition, four new potential hosts were identified: the shield darter (*Percina peltata*), striped bass (*Morone saxatilis*), banded killifish (*Fundulus diaphanus*) and brown trout (*Salmo trutta*). No fish collected from known *A. heterodon* locations in the upper Delaware River during spring 2006 were infected with glochidia of *A. heterodon* or any other mussel. Variation in fish host suitability of tessellated darters varied minimally among fish sources, but in general fish from the Upper Ammonoosuc River (NH) in closest proximity to the Connecticut River mussel source served as the most suitable hosts, producing the highest number of transformed juvenile *A. heterodon*, and host suitability of darters tended to decrease as degree of isolation from the mussel source increased. Tessellated darters from Pine Creek, located furthest from the mussel source in the upper Susquehanna River basin, produced fewest juvenile *A. heterodon*. Darters from the upper Delaware River mainstem, second

furthest from the mussel source, produced fewer transformed juvenile *A. heterodon* than darters from the Upper Ammonoosuc River, but more than those from Pine Creek. Meristic analysis of tessellated darters complement results from host suitability trials, showing that fish from each of the three major river basins were significantly different from one another. In particular, darters from the Upper Ammonoosuc River, located closest to the upper Connecticut River mussel source, were notably different from other populations.

TABLE OF CONTENTS

List of Figures	vi
List of Tables	vii
Acknowledgements.....	viii
INTRODUCTION	1
Overview.....	1
Unionid reproduction.....	1
Host specificity in mussels and immunity in fish.....	3
Dwarf wedgemussel (<i>Alasmidonta heterodon</i>)	4
GOALS AND OBJECTIVES.....	9
STUDY AREAS	10
Delaware River basin	10
Connecticut River basin.....	12
Susquehanna River basin.....	14
Mussel collection sites.....	16
Tessellated darter collection sites.....	17
Sites of collections to detect naturally-infected fish in the Delaware River mainstem.....	19
METHODS	20
Field collection procedures.....	20
Laboratory culture facility.....	24
Infection procedures	24
General approach.....	24
Tessellated darter host suitability: grouped fish.....	25
Tessellated darter host suitability: individual fish.....	29
Evaluation of other potential fish hosts.....	31
Observation of fish to detect natural infections.....	32
Disposition of adult and juvenile mussels.....	32
Morphometric and meristic analysis of tessellated darters.....	32
Data analysis.....	35
RESULTS	37
Host suitability of tessellated darters.....	37
Grouped fish.....	37
Individual fish.....	44
Evaluation of other potential fish hosts.....	49
Natural host identification.....	53
Morphometric and meristic analysis of tessellated darters.....	54
DISCUSSION	59
Tessellated darter host suitability.....	59
Other potential fish hosts for <i>A. heterodon</i> in the Delaware River basin.....	64
Concluding remarks.....	68
LITERATURE CITED.....	70
APPENDIX A: Tessellated darter field collections summary.....	75
APPENDIX B: Recorded and calculated values used in statistical analysis.....	77

LIST OF FIGURES

- Figure 1. Historic distribution of the dwarf wedgemussel (*Alasmidonta heterodon*)
- Figure 2. Delaware River basin
- Figure 3. Connecticut River basin
- Figure 4. Susquehanna River basin
- Figure 5. Schematic of aquarium setup for group-infected tessellated darters
- Figure 6. Boxplots of lengths of group-infected tessellated darters
- Figure 7. Boxplots of water temperatures for group-infected tessellated darters
- Figure 8. Boxplots of water temperatures for individually-monitored tessellated darters
- Figure 9. Numbers of transformed juveniles produced by group-infected tessellated darters
- Figure 10. Mean number (\pm 1 SD) of transformed juvenile *A. heterodon* per fish among group-infected tessellated darters.
- Figure 11. Numbers of shed glochidia and transformed juveniles produced by group-infected-tessellated darters.
- Figure 12. Transformation success of *A. heterodon* among group-infected tessellated darters.
- Figure 13. Total numbers of juvenile *A. heterodon* produced by individually-monitored tessellated darters.
- Figure 14. Transformation success of *A. heterodon* among individually-monitored tessellated darters.
- Figure 15. Scatterplot of numbers of juveniles produced per fish vs. individual fish lengths for individually-monitored tessellated darters.
- Figure 16. Plot of first principal component (meristic data) and second sheared principal component (morphometric data) for tessellated darters.

LIST OF TABLES

- Table 1. Names, sources and infection dates for fish species tested as potential hosts for *A. heterodon*
- Table 2. Experimental design to test host suitability of tessellated darters
- Table 3. Morphometric and meristic characters assessed in tessellated darter analysis
- Table 4. Summarized results for group-infected tessellated darters
- Table 5. (a) ANOVA and (b) least squares means comparisons of numbers of transformed juvenile mussels produced among group-infected tessellated darters
- Table 6. (a) ANOVA and (b) least squares means comparisons of numbers of transformed juveniles produced among group-infected tessellated darters, excluding previously infected fish
- Table 7. (a) ANOVA and (b) Wilcoxon ranked scores of transformation success for group-infected tessellated darters
- Table 8. ANOVA and Wilcoxon ranked scores among group-infected tessellated darters, excluding previously infected fish
- Table 9. Numbers of transformed juveniles and transformation success values for individually-monitored tessellated darters
- Table 10. (a) ANOVA and (b) least squares means comparison of numbers of transformed juveniles among individually-monitored fish
- Table 11. (a) ANOVA and (b) Wilcoxon ranked scores for transformation success among individually-monitored fish from the Delaware and Connecticut Rivers.
- Table 12. Results of screenings of potential fish host species for *A. heterodon* from the Flat Brook in the upper Delaware River basin.
- Table 13. Summary of fish collected during spring 2006 to detect natural *A. heterodon* infections
- Table 14. Summary of numbers of transformed juvenile mussels recovered from naturally infected tessellated darters collected from the Ashuelot River and Flat Brook
- Table 15. Results of MANOVA comparing (a) second sheared principal components (morphometric data) and (b) first principal components (meristic data) for tessellated darters
- Table 16. Values of morphometric measurements and meristic counts of tessellated darters
- Table 17. Results of Duncan's Multiple Range test comparing a) second sheared principal components (morphometric data) and b) first principal components (meristic data) for tessellated darters

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INTRODUCTION

Freshwater mussels (Bivalvia:Unionidae) are among the world's most imperiled fauna (Lydeard et al. 2004). Though historically more diverse in North America than in any other continent, approximately 70% of 300 known taxa in Canada and the United States are now threatened, endangered or extinct (Bogan 1993a; Master 2000). Freshwater mussels face many threats including water quality degradation, hydrological alteration and instability, habitat destruction (Haag and Warren 2005; Strayer et al. 2004), altered or reduced distribution of host fishes Haag and Warren 1998; Watters 1995), and introduction of exotic species (Strayer 1999a). Mussels play a complex and important role in the food web, serving as food items for fish and small mammals (Williams et al. 1993), and filtering phytoplankton, bacteria and particulate organic matter from the water column (Vaughn and Hakenkamp 2001). Native mussels also perform an important ecosystem service by processing and transferring the particulate matter they consume to the substrate in nutrient rich forms that can be utilized readily by other organisms (Howard and Cuffey 2006; Spooner and Vaughn 2006). Continued loss of freshwater mussel species is likely to lead to profound ecosystem level changes worldwide and particularly throughout North America.

Unionid reproduction

Unionid larvae, called glochidia, undergo a parasitic stage during which they must use a vertebrate host in order to transform into juvenile mussels. Glochidia may attach to the appropriate host, typically a fish, for a period of several days to a number of weeks before completing transformation (Kat 1084). Once transformed, juveniles settle to the substrate where they grow into filter feeding adults (Yeager and Neves 1994). Reproduction and dispersal of a certain mussel species may depend upon the availability and distribution of its host fish (McLain and Ross 2005; Smith 1985). Mussels exhibit relatively little partitioning of food, space, or habitat resources and rarely have specialized predator defenses. They do, however, exhibit strong partitioning of fish hosts as a resource and may employ various specialized means to infect these hosts with glochidia Haag and Warren 1998).

Mussels use a variety of methods to introduce glochidia to an appropriate host fish. Some unionid species such as *Elliptio complanata*, as well as *Anodonta* and *Pyganodon* species, release large numbers of glochidia directly into the water column, often in mucous webs, that encounter the host passively (Haag and Warren 1998; Matteson 1948). Other species use more specialized means. *Strophitus undulatus* releases packages of glochidia, or conglutinates, that may mimic the form of a fish food item, thereby attracting the host and increasing chances of infection (Van Snik Gray et al. 2002). Conglutinates of *S. undulatus* have even been observed to imitate movements of a live prey item (Watters 2002). The endangered *Ptychobranchus greeni* produces an intricate conglutinate that is similar in size, coloration and shape to chironomid larvae (Hartfield and Hartfield 1996). Other species, such those of the genus *Lampsilis* (Haag et al. 1999) and *Epioblasma* (Jones et al. 2004), are known to expose a highly modified portion of the mantle as a lure, mimicking both the shapes and movements of fish prey items to directly attract hosts.

Host use in unionids varies among taxa. Most mussels are host specialists, parasitizing only a limited number of fish species that are often taxonomically related (Zale and Neves 1982). Others are generalists, parasitizing as many as 25 fish species that are more taxonomically diverse (Lefevre and Curtis 1911; Van Snik Gray et al. 2002). Both host-specialists with fairly elaborate host-attracting mechanisms (such as *Lampsilis* and *Ligumia* species) and host-generalists (such as *Strophitus* species) have been shown to occupy headwater streams as well as large streams, while non-displaying host-specialists (e.g. *Elliptio* species) are often restricted to larger streams only (Haag and Warren 1998). It is presumed that non-displaying host specialists do not occur in headwater streams because in such streams fish hosts are often less diverse and their abundance is temporally variable. Very few unionid mussel species, such as the green floater *Lasmigona subviridis* and some *Strophitus* species, are able to produce transformed juveniles without a fish host (Barfield and Watters 1998; Lefevre and Curtis 1911; Lellis and King 1998). It is not well understood why certain relationships and various degrees of specificity occur among mussels and their hosts, but fish immunity to glochidia is presumed to be a determining factor (O'Connell and Neves 1999).

Host specificity in mussels and immunity in fish

Host specificity in mussels is a complex phenomenon thought to be regulated primarily by fish immune response to glochidiosis (Arey 1932; O'Connell and Neves 1999; Reuling 1919). Immune response may occur naturally among nonhost fish species that have or have not been exposed to glochidia. In contrast to this innate immunity, specific acquired immunity may also occur in host fish that are repeatedly exposed to glochidia of a particular mussel species (Reuling 1919; Rogers and Dimock 2003). Coevolutionary relationships between mussels and host fish may result in various degrees of specialization related to innate immune response. Fish often demonstrate resistance to glochidia of mussel species that are in the same subfamily or genus (Dodd et al. 2005). Likewise, mussels typically parasitize an array of host fish that are somewhat closely taxonomically related (Haag and Warren 1998).

It was once assumed that host specificity was defined by particular species-level associations between mussels and their fish hosts that normally involved parasitization of a single fish species by a particular mussel species (Isom and Hudson 1984). Recent studies, however, have revealed that host specificity may not only vary among closely related mussel species (Riusech and Barnhart 2000), but may also be related to variations in suitability among populations of host fish that are somewhat isolated from one another geographically (Rogers et al. 2001). It is now thought that coadaptation of sympatric mussels and fish hosts occurs so that fish located in closest proximity to the mussels that parasitize them serve as the most suitable hosts, and that as degree of isolation between fish and mussel sources increases, host suitability decreases (Bigham 2002; Rogers et al. 2001). Rogers et al. (2001) determined that numbers of transformed juvenile mussels of the tan riffleshell *Epioblasma florentina walkeri* were significantly higher on its host, the fantail darter (*Etheostoma flabellare*), in drainages where the mussel and its fish host co-occurred. In their study, fish sympatric with the mussel population produced the highest numbers of transformed juvenile mussels, fish from non-contiguous locations within the historic range of *E. f. walkeri* produced fewer transformed juveniles, and fish from outside the historic range of the mussel were capable of serving as hosts but produced the fewest juvenile mussels of all tested fish. The authors attribute this

variation in host suitability to variation in gene flow among fish populations that is related to fish immune response to glochidiosis (Rogers et al. 2001).

In comparing infection success of closely related mussel species, Riusech and Barnhart (2000) have demonstrated that the mussel *Venustaconcha pleasii* parasitizes sympatric rainbow darters (*Etheostoma caeruleum*) in the laboratory much more successfully than the closely related mussel *V. ellipsiformis* that occurs only allopatrically with the rainbow darter. Subsequently, Bigham (2002) observed that sympatric populations of *V. ellipsiformis* and its host the orangethroat darter (*Etheostoma spectabile*) served as a somewhat better host pair than allopatric populations.

Dwarf wedgemussel (*Alasmidonta heterodon*)

The dwarf wedgemussel (*Alasmidonta heterodon*) is a federally endangered freshwater mussel native to the Atlantic slope basin. It was once recorded at 70 localities in 15 major Atlantic slope drainages from New Brunswick to North Carolina, yet it was presumed to be extirpated from all but 20 of those original localities when it was listed as endangered in 1990 (USFWS 1993).

Alasmidonta heterodon may occur in smaller third or fourth order streams or in larger rivers (Strayer et al. 1996). It generally occupies patches of fine sediments (Michaelson and Neves 1995) in hydrologically stable stream reaches where disturbance by flood events may be minimized (Strayer 1999). Researchers have identified new *A. heterodon* populations, most in the Northeast (NH, PA/NY and NJ), since the adoption of Dwarf Wedge Mussel Recovery Plan in 1993. Now, a total of 70 sites in 15 major drainages are known to contain *A. heterodon*; however at 45 of these sites, located primarily in the southern portion of the range (MD, VA, NC), fewer than five individuals or only spent shells have been observed (USFWS 2007). Several potential fish hosts for newly identified populations in New Hampshire have been identified (Wicklow 1999; Wicklow 2004); however fish hosts for *A. heterodon* in the mid-Atlantic portion of its historic range have not been evaluated.

Like most unionids, *A. heterodon* exhibits a complex life cycle involving an obligate parasitic larval stage (Michaelson and Neves 1995). Life history of *A. heterodon* has been studied in North Carolina (Michaelson and Neves 1995), New Hampshire (Wicklow, 1999, 2004) and in Massachusetts (McLain and Ross 2005). All of these studies indicate *A. heterodon* may be capable

of parasitizing only a limited number of hosts, primarily darters and sculpins. Reliance upon these relatively sedentary fish hosts may limit the mussel's capacity for reproduction and dispersal, and may contribute to isolation of populations (McLain and Ross 2005). It is assumed that darter and sculpin species serve as hosts for *A. heterodon* throughout its range; however, no specific array of hosts has been identified for any other populations of this mussel, including those in the Delaware River basin in the mid-Atlantic portion of its range. As fish host suitability for other mussel species has been shown to vary geographically (Riusech and Barnhart 2000; Rogers et al. 2001), continued examination of host use by *A. heterodon* throughout its range will be important in developing and implementing effective management practices for distinct populations.

Tessellated darter populations that co-occur with four *A. heterodon* populations identified since the adoption of the Dwarf Wedge Mussel Recovery Plan in 1993 are evaluated in this study. One of these *A. heterodon* populations was identified in the Upper Delaware Scenic and Recreational River (PA/NY) in 2000 and a second population in the Flat Brook (NJ) within the Delaware Water Gap National Recreation Area in 2001, where the National Park Service is now developing programs for its conservation and management (Lellis 2001; Lellis 2002). The first population is distributed among three separate locations within a 30-km reach of the Delaware River mainstem between Hancock and Callicoon, NY (Lellis 2001). The second occurs in the Flat Brook, a tributary to the upper Delaware River in northwestern New Jersey (Lellis 2002). Potential hosts for these upper Delaware basin populations have not been evaluated. Two other relatively high density *A. heterodon* populations, identified in the 1990s, occur in the upper Connecticut River basin (NH). The first of these populations is found in the upper Connecticut River mainstem in northern New Hampshire near Lunenburg, VT (Nedeau 2002). The second is found in the Ashuelot River, a small tributary to the Connecticut River in southwestern New Hampshire (Nedeau 2004). Hosts for *A. heterodon* in the Connecticut River basin have been identified by Wicklow (1999, 2004); however no comparison of host suitability among populations, either within or among major river basins, has been completed to date.

To evaluate differences in host suitability among populations of a single fish host species co-occurring with *A. heterodon* populations throughout in the Northeast (Figure 1), both between major

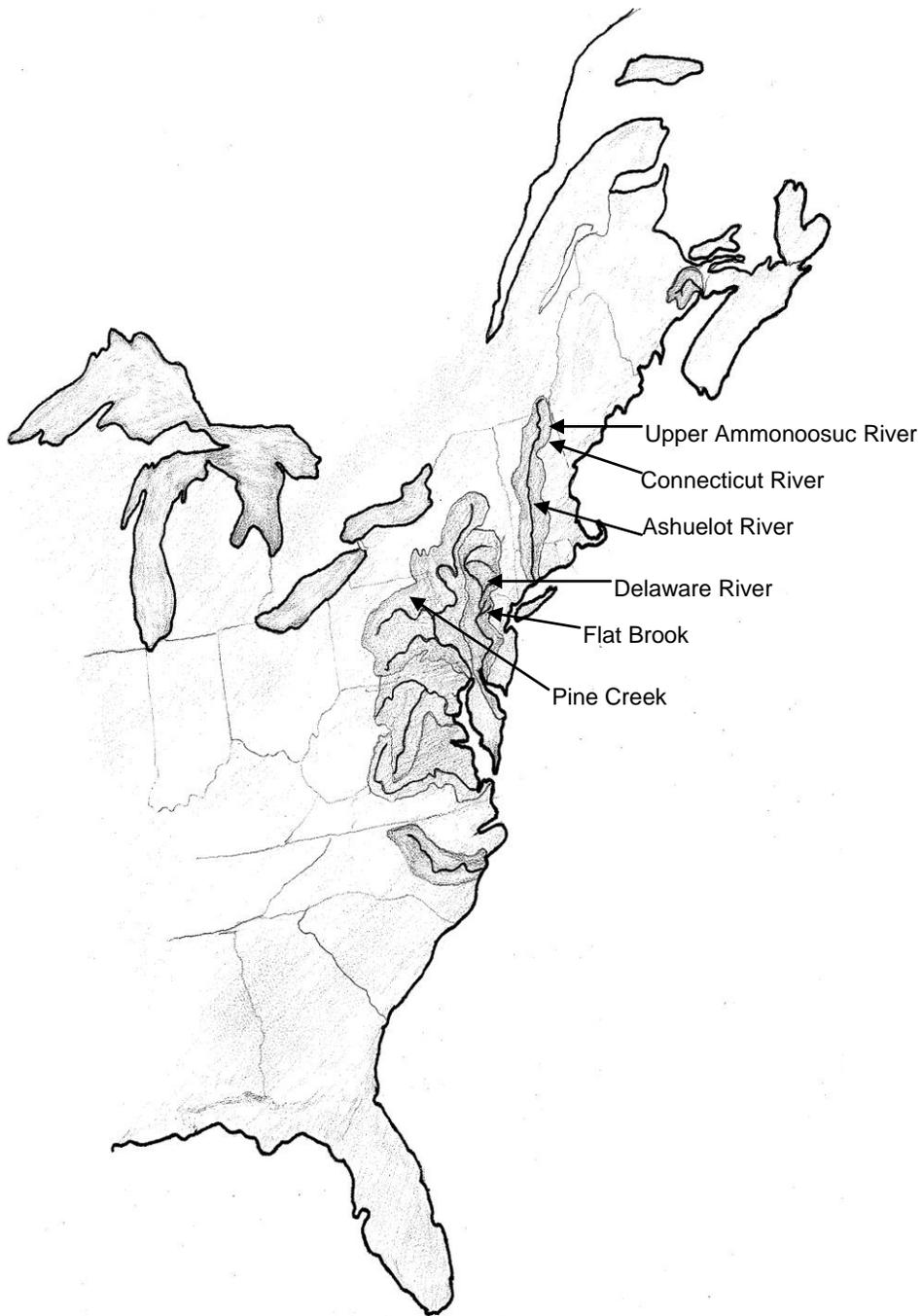


Figure 1. Major river basins in the Atlantic slope drainage with historic records of the dwarf wedgemussel (*Alasmidonta heterodon*). Arrows indicate approximate locations of study sites. *Alasmidonta heterodon* individuals for this study were collected from the upper Connecticut River and Flat Brook. Tessellated darters were collected from all study sites indicated except the upper Connecticut River mainstem.

basins (Delaware and Connecticut) and within each of these basins, I chose to test host suitability of the tessellated darter (*Etheostoma olmstedii*), a known host for *A. heterodon*. Three separate studies conducted in different portions of the *A. heterodon* range (Michaelson and Neves 1995, North Carolina; Wicklow 2004, New Hampshire; McLain and Ross 2005, Massachusetts) have identified the tessellated darter (*Etheostoma olmstedii*) to be a host. Knowledge of any variation in host suitability of this common darter species at locations where they co-occur with *A. heterodon* populations may be important, particularly if any future mussel relocations or translocations are to take place (Villevella et al. 1998) as proposed in the U. S. Fish and Wildlife Service Dwarf Wedge Mussel Recovery Plan (1993).

To compare suitability of tessellated darter hosts from different populations throughout the Northeast, I chose to use the upper Connecticut River *A. heterodon* population as a mussel source. This population is the northernmost known *A. heterodon* population, and I expected mussels at the limit of the range to exhibit the greatest variation in host use among fish from different locations. In addition, this population is thought to have the highest density and abundance of any extant *A. heterodon* population (USFWS 2007). Consequently, I was able to collect adequate numbers of gravid mussels and glochidia without negatively impacting the population. I tested suitability of tessellated darters from four populations that occur near the recently discovered *A. heterodon* populations described above. These included fish from two populations each in the Connecticut River basin (Upper Ammonoosuc River, located close to the upper Connecticut River mainstem site where *A. heterodon* were collected for tessellated darter host suitability trials, and Ashuelot River, known to support a second population of *A. heterodon*) and two in the Delaware River basin (Upper Delaware River mainstem and Flat Brook). I also tested suitability of tessellated darters from a fifth location, Pine Creek in the upper Susquehanna River basin, that has no historic record of *A. heterodon*, in order to evaluate suitability of potential host fish with no history of natural or acquired immune response to glochidia of *A. heterodon*. Of the five fish sources tested in this study, Pine Creek is also furthest isolated from the mussel source.

It is thought that both geographic distance (direct distance between two points) and extent of drainage interconnectedness (linear stream distance between two points) influence mussel distribution (Vaughn and Taylor 2000). Mussel distribution in turn is thought to be related to

distribution of host fish (Watters 1992). Rogers et al. (2001) concluded that host suitability of fish from a given population declines as the degree of isolation between that fish population and the mussels that parasitize it increases. Consequently, drainage interconnectedness may be particularly important in determining fish dispersal (and therefore mussel dispersal) as well as in facilitating or limiting genetic exchanges among both mussel and fish populations. Rogers et al. (2001) suggest that long-term isolation would likely lead to coadaptation between co-occurring mussel and fish populations that would result in increased compatibility between them.

It is unclear precisely how different landscape characteristics or stream barriers may isolate tessellated darter and *A. heterodon* populations throughout the Northeast and potentially cause divergence among populations. Tessellated darter populations in the Connecticut, upper Delaware and upper Susquehanna drainages are presumed to be of the same subspecies (Cole 1967). Populations of *A. heterodon* are certainly less common and remain more isolated, perhaps because they are now presumed to rely on fish hosts, such as the tessellated darter, that individually undergo little dispersal (McLain and Ross 2005). *Alasmidonta heterodon* has been shown to exhibit notable intraspecific genetic variation, particularly among major river basins (King et al. 2004).

In this study, I aimed to determine whether different populations of even a relatively common host fish such as the tessellated darter might exhibit variation in host suitability, measured as differences in numbers of transformed juvenile mussels produced and transformation success (the proportion of attached glochidia that successfully transform into juvenile mussels). I expected that if any variation were to occur among populations, darters located closest to the upper Connecticut River mussel source (Upper Ammonoosuc River) would likely serve as the most suitable hosts, producing the greatest number of transformed juvenile mussels and generating the highest transformation success values. I expected that host suitability would decrease as stream distance and geographic distance of each population increased, with darters from the Ashuelot River being the second most suitable hosts. I expected fish from the Flat Brook and the Delaware River to be somewhat comparable in suitability but less suitable than fish from the Ashuelot River. Lastly I expected fish from Pine Creek, located furthest from the mussel source, to serve as least suitable hosts.

GOALS AND OBJECTIVES

The goal of this study was to evaluate fish host suitability for *A. heterodon*, and in particular, to identify an array of suitable fish hosts for this mussel in the Delaware River basin. My specific objectives include:

- 1) Examine geographic variation in fish host suitability for *A. heterodon* by testing the ability of tessellated darters from five different locations to serve as hosts: darters that co-occur with four different *A. heterodon* populations (two in the Delaware River basin and two in the Connecticut River basin) as well as darters from a fifth source outside the current *A. heterodon* range (Susquehanna River basin).
- 2) Identify other fish species that are capable of serving as hosts for *A. heterodon* from the Delaware River basin by conducting laboratory infection trials of a broad array of fish species that may currently serve or may have historically served as hosts.
- 3) Determine which fish species currently serve as hosts for *A. heterodon* at sites where they occur in the upper Delaware River mainstem by collecting fish at known mussel locations during spring to identify naturally-infected fish.

Delaware River *A. heterodon* populations are of particular interest in this study, as host suitability among fish populations may potentially vary, and no host suitability studies of any kind have been conducted for *A. heterodon* populations in the Delaware River basin to date. By comparing relative host suitability of tessellated darters that co-occur with *A. heterodon* populations in both the upper Connecticut and Delaware River basins, I also intend to gain additional information about any variation in fish host suitability that may occur among and within these two major river basins.

STUDY AREAS

Delaware River basin

The Delaware River basin occupies more than 35,000 square kilometers and drains 216 tributaries in New York, New Jersey and Pennsylvania (Featherstone 1996) (Figure 2). The mainstem, which stretches 533 kilometers from the confluence of the east and west branches at Hancock, NY, is the longest undammed river on the east coast, providing unique habitat for migratory fish species such as American eel (*Anguilla rostrata*) and American shad (*Alosa sapidissima*). Approximately 15 million people, primarily in the cities of New York and Philadelphia, rely on water resources from the upper Delaware River for drinking and industrial use (DRBC 2006). Reservoirs on three major tributaries to the upper Delaware River (the East Branch, West Branch and Neversink River) were constructed in the 1950s and 1960s to supply New York City with water (Weidner 1966). Releases from these reservoirs currently have important effects on aquatic habitat in the mainstem. The Delaware River Basin Commission (DRBC) is in the process of assessing potential ecological impacts of current release schedules and may reestablish minimum flow requirements in the near future. The Delaware River and many of its tributaries are currently known to support nine different mussel species, including three rare species of *Alasmidonta*: *A. undulata*, *A. varicosa*, and *A. heterodon*.

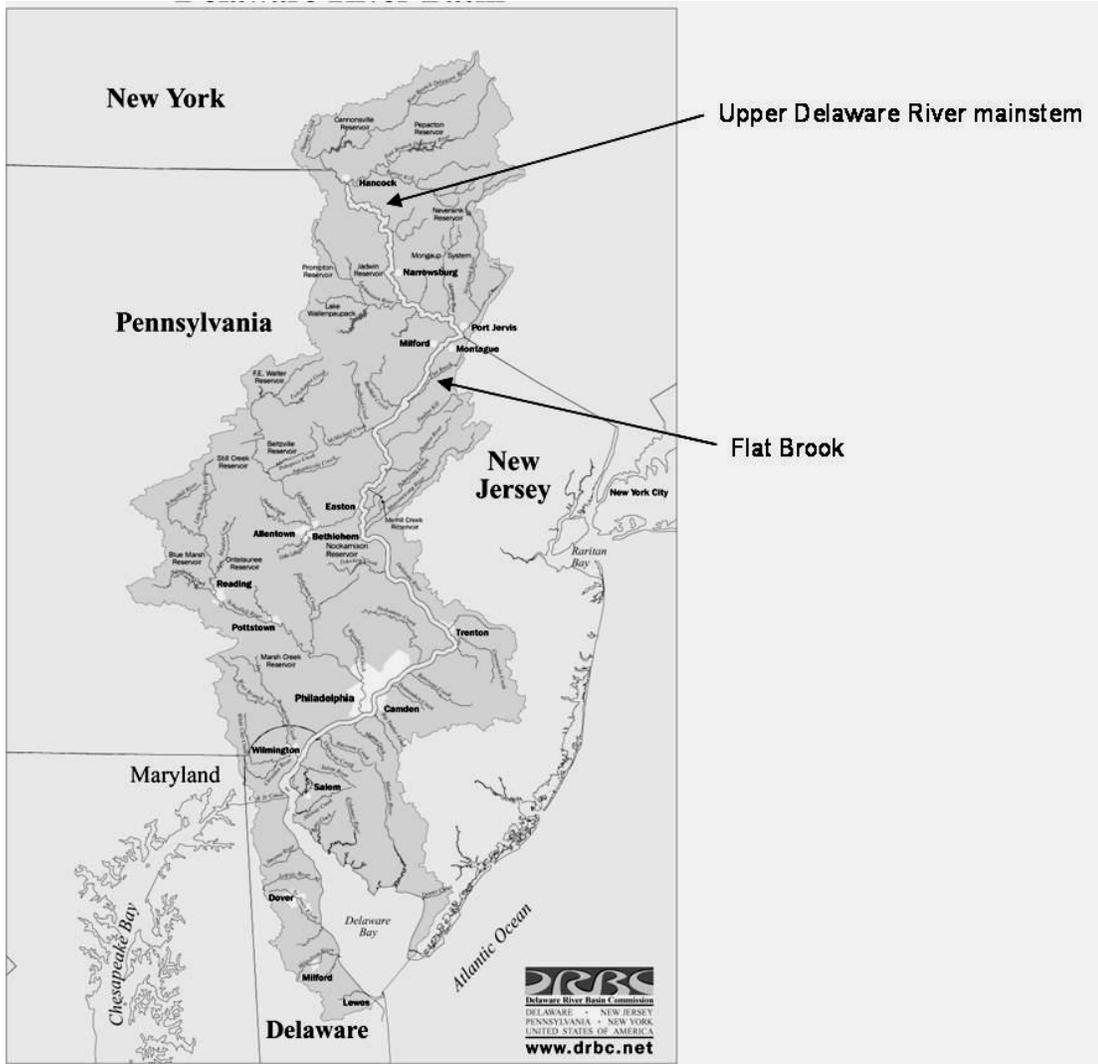


Figure 2. Map of the Delaware River basin (adapted from the Delaware River Basin Commission) indicating general locations of *Alasmidonta heterodon* and tessellated darter collection sites.

Connecticut River basin

The Connecticut River is the largest river system in New England, stretching 660 km from its source in Fourth Connecticut Lake near the Canadian border to its mouth at Long Island Sound at Old Saybrook, Connecticut. Its watershed constitutes more than 28,500 square kilometers and its tributaries include approximately 33,200 stream kilometers (USFWS 2007) (Figure 3). Prior to the Clean Water Act, the river was so heavily polluted that the majority of its waters were unsuitable for drinking, fishing or swimming. Improvements in water quality have occurred since the 1970s, and migratory fish, including American shad and Atlantic salmon that had been restricted from the Connecticut's waters, are now returning. Many new populations of the dwarf wedgemussel have been identified throughout the basin since the Dwarf Wedge Mussel Recovery Plan (1993) was adopted, and now hundreds of thousands of *A. heterodon* are estimated to occur within a 121-km stretch of the Connecticut River mainstem (USFWS 2007). A total of twelve mussel species, including the same three rare *Alasmidonta* species found in the Delaware River basin, occur throughout the Connecticut River basin.

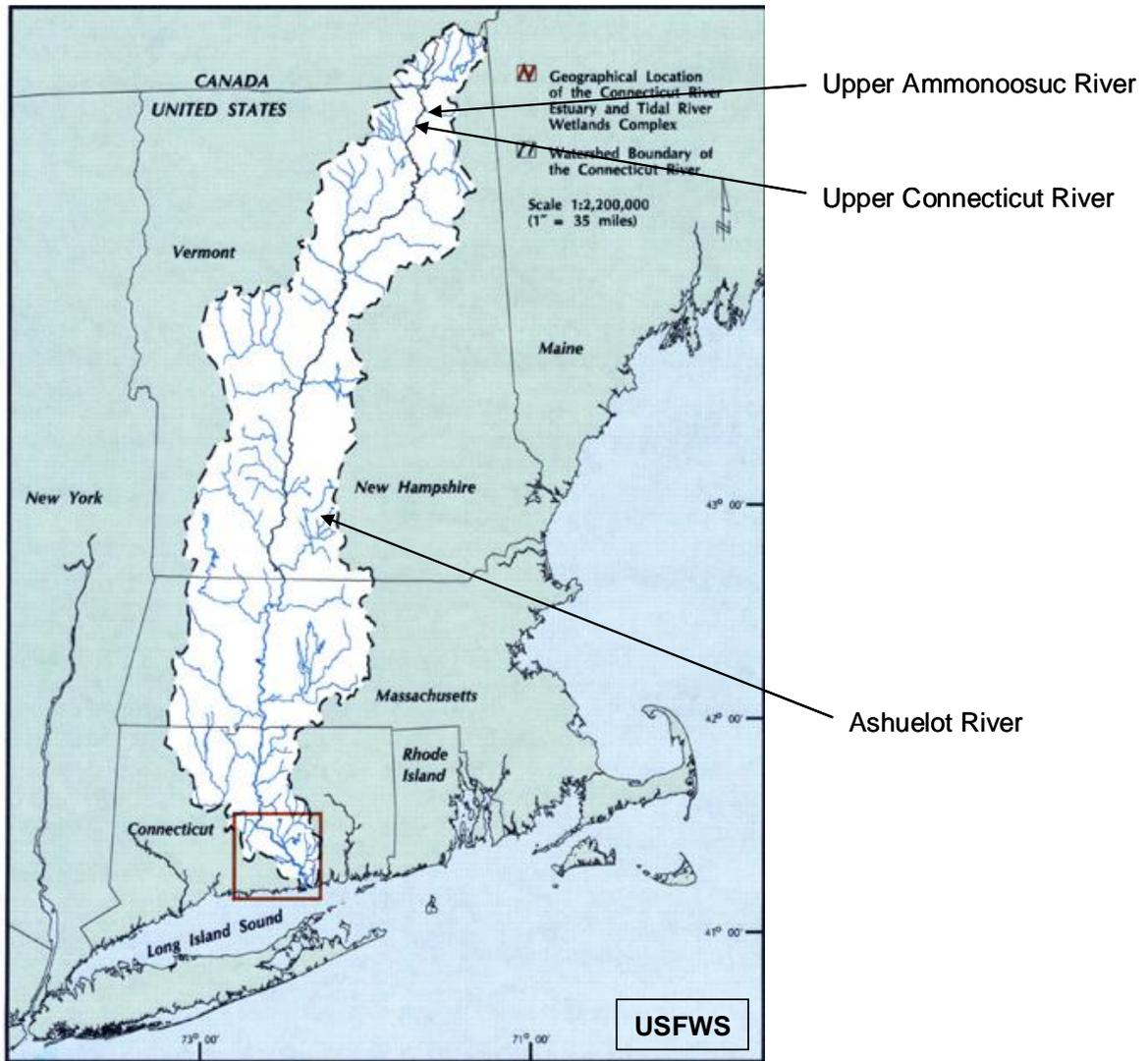


Figure 3. Map of the Connecticut River basin (adapted from the U.S. Fish and Wildlife Service) indicating general locations of *Alasmidonta heterodon* and tessellated darter collection sites.

Susquehanna River basin

The Susquehanna River basin is the largest river basin in the Atlantic Slope drainage. Its watershed encompasses approximately 71,250 square kilometers and the river provides half the supply of freshwater to the Chesapeake Bay (Figure 4). At least 12 freshwater mussel species are known to occur in the Susquehanna River basin (Bogan 1993; Strayer and Fetterman 1999), five of which do not occur in the Connecticut River or Delaware River basins. One historic record of *A. heterodon* exists in the lower Susquehanna River in Lancaster County, PA (USFWS 1993); however no *A. heterodon* have been documented in the basin in recent years. Other species of *Alasmidonta*, however, are found throughout the watershed: *A. undulata*, *A. varicosa* and *A. marginata*.

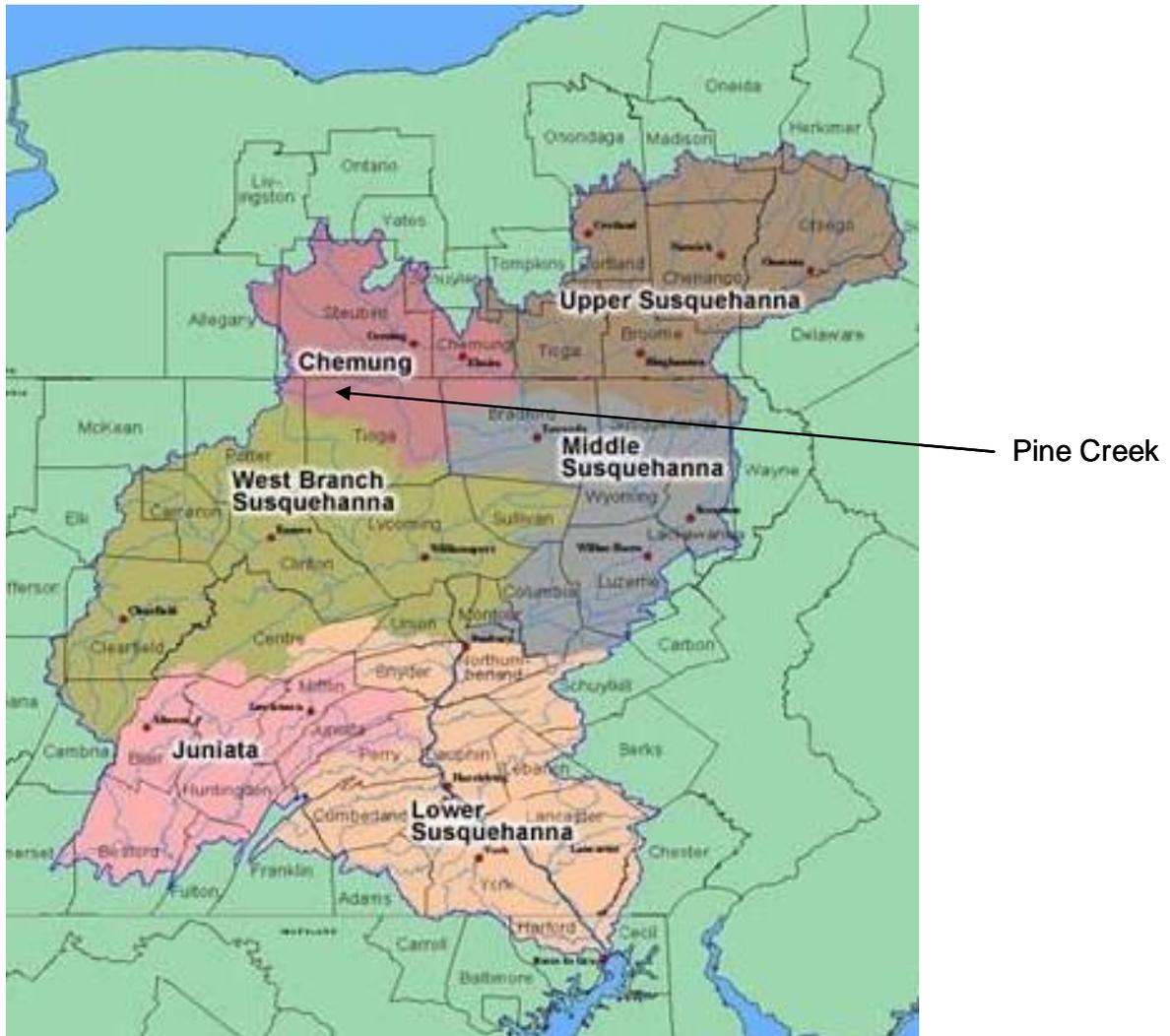


Figure 4. Map of the Susquehanna River basin (adapted from the Susquehanna River Basin Commission) indicating approximate location of Pine Creek, one of the tessellated darter collection sites.

Mussel collection sites

Upper Delaware River mainstem, NY/PA

Alasmidonta heterodon is known to occur at three separate locations in the upper Delaware River mainstem within a 30 km stretch of river between Hancock and Callicoon, NY (Lellis 2001). Mussels from two of these locations, where *A. heterodon* is known to occur at higher densities, were examined for gravidity as part of this study (Figure 2). All three sites fall within the Upper Delaware Scenic and Recreational River and are under jurisdiction of the National Park Service. At each site, mussels are found in pools or runs in areas of sand and gravel substrate at relatively shallow depths (0.1-1.0 m). Other unionid species, including *Elliptio complanata*, *Strophitus undulatus*, *Alasmidonta varicosa*, *A. undulata*, *Anodonta implicata*, and *Lampsilis cariosa* are also known to occur at these locations.

Flat Brook, NJ

The Flat Brook is a third-order tributary to the Delaware River in northwestern New Jersey and enters the Delaware at Walpack Bend north of the Delaware Water Gap (Figure 2). The Flat Brook is a stocked coldwater trout fishery, lies almost entirely within the Delaware Water Gap National Recreation Area and is primarily under jurisdiction of the National Park Service and High Point State Park of New Jersey. *Alasmidonta heterodon* is known to occur in the lower Flat Brook between the confluence of the Little Flat Brook and the Big Flat Brook and Walpack Center (Lellis 2002). Mussel species *E. complanata*, *A. undulata*, *A. varicosa* and *S. undulatus* also occur in the Flat Brook. Mussels were collected from a particular location that was observed in 2002 to contain notably higher densities of *A. heterodon* than other locations in the stream. As in the upper Delaware River, *A. heterodon* in the Flat Brook are found in shallow pools and runs in mixed sand and gravel substrate.

Upper Connecticut River mainstem, NH

Alasmidonta heterodon is known to occur at many locations throughout the upper Connecticut River, most of which have been identified since the adoption of the species recovery plan in 1990. One location in the upper Connecticut River mainstem near Lunenburg, VT is now presumed to contain higher densities and abundance of *A. heterodon* than any other location in the mussel's entire range (USFWS 2007) (Figure 3). As *A. heterodon* is now thought to be extirpated from the Petitcodiac River in New Brunswick (Hanson and Locke 2000), the upper Connecticut River is also presumed to support the northernmost extant population. Mussels were collected from a location on the Connecticut River mainstem near Lunenburg, VT along the Vermont shoreline within 25m of the bank, where a high density mussel bed occurs. Mussels at this location have been monitored for several years, following a stream bank stabilization project that was completed in 1997 (Nedeau 2002). Habitat for *A. heterodon* in the upper Connecticut River is very different from that of the Delaware basin. Mussels in the Connecticut mainstem occur along steep banks of mud and clay substrate that is up to 0.5m deep. They also occur in the main river channel approximately 15-20 m from the shoreline at depths up to 2-3 m, in gravel substrate. High abundances of other mussel species, including *E. complanata*, *S. undulatus*, and *A. undulata*, are also found at this site.

Collection sites for tessellated darter host suitability trials

Upper Ammonoosuc River, NH

Tessellated darters are known to occur in the Connecticut River mainstem at sites containing *A. heterodon*; however water 2-3m deep and muddy substrate 1-2 feet deep made collection of darters by backpack electrofishing unit or seine impracticable in the mainstem. Fish were collected instead from a nearby tributary, the Upper Ammonoosuc River (Figure 3). The Upper Ammonoosuc River is located approximately 27 river kilometers upstream from the location on the Connecticut River where gravid mussels were collected. Fish were collected approximately 2.1 river kilometers upstream of Groveton, NH at a location formerly within a reservoir that is now partially drained. Retention of water in this portion of the river may have reduced streamflow, perhaps providing suitable habitat for tessellated darters that may not be present elsewhere. Many attempts were

made to collect tessellated darters from tributaries closer to the mussel site, including the Israel River, which flows through Lancaster, NH, located 6.3 river kilometers upstream of the mussel site, and an unnamed tributary 6.9 river kilometers downstream of the mussel site. Even though the upper Connecticut River is a low gradient river, many of its tributaries, including the Israel River, are high gradient streams that probably support limited numbers of fish such as the tessellated darter that generally inhabit areas of more moderate flow velocities. Other fish species, in particular longnose dace (*Rhinichthys cataractae*), were observed in stream reaches where no darters could be found.

Ashuelot River

The Ashuelot River is a tributary to the Connecticut River located in southwestern New Hampshire known to support a population of *A. heterodon* (Figure 3). Tessellated darters were collected at a location 4.0 river kilometers upstream from Keene, NH and approximately 1100m downstream of the reservoir at the Surry Mountain Flood Control Dam.

Delaware River mainstem

Tessellated darters were collected 11.7 river kilometers upstream from the nearest known *A. heterodon* site in the upper Delaware River mainstem (Figure 2), approximately 0.64 river kilometers downstream of the confluence of the East and West Branches and 1.9 river kilometers downstream of Hancock, NY along the New York shoreline.

Flat Brook

Tessellated darters were collected from the Flat Brook (Figure 2) at a location 2.9 river kilometers upstream of the mussel collection site, near the confluence of the Little Flat Brook and Big Flat Brook. No mussels were observed to occur at this location in a 2001 survey or in snorkel searches conducted just prior to time of tessellated darter collection during spring 2006. Habitat characteristics in this section Flat Brook site are similar to those at the mussel collection site described above.

Pine Creek

Pine Creek is a tributary to the Susquehanna River in northern Pennsylvania (Figure 4). Tessellated darters were collected in upper Pine Creek in Tioga County, PA at the mouth of Phoenix Run near the town of Gaines. Pine Creek has no historic record of *A. heterodon*. Recent surveys of lower Pine Creek have identified other mussel species, including *Alasmidonta marginata*, *A. varicosa* and *A. undulata*; however no mussels of any species have been observed occur 4.5 kilometers upstream or downstream of the location from which tessellated darters were collected for this study.

Collections of fish to detect natural infections in the upper Delaware River

Delaware River at Frisbie Island (Site 1)

Fish were collected near one known *A. heterodon* location in the Delaware River mainstem near Frisbie Island less than 0.8 river kilometers upstream from Equinunk, PA (Figure 2). Mussels occur in the channel on the Pennsylvania side of the island, within a 200m stretch along the Pennsylvania shoreline (personal observation). Fish were collected from areas within 100m upstream and downstream of mussel beds along the Pennsylvania shoreline.

Delaware River near Hankins, NY (Site 2)

Fish were collected near known *A. heterodon* sites on the Pennsylvania shoreline approximately 1 river mile upstream of Hankins, NY (Figure 2). Mussels occur within a 400m stretch along the Pennsylvania shoreline (personal observation), and fish were collected within 100m upstream and downstream of areas where mussels were observed.

METHODS

Field collection procedures

Mussel collection

Five gravid *A. heterodon* females were collected from the Connecticut River during November 2005 for tessellated darter host suitability experiments. Five gravid females were collected from the Flat Brook during January 2006 and five more were collected in early April 2007 to conduct screenings each year of other potential fish hosts for *A. heterodon* in the Delaware River basin. Mussels were collected by snorkeling and searching with plexiglass-bottom buckets using standard methods (Nedeau and Victoria 2003; Strayer and Smith 2003). Snorkel and bucket searches are commonly used in *A. heterodon* collections and surveys to maximize their detection (Michaelson and Neves 1995; Smith et al. 2000; Strayer et al. 1996), as *A. heterodon* individuals are quite small and generally occur at low densities, making them relatively difficult to find by any other method. At each site, located specimens were opened gently and examined for gravidity under a field dissecting microscope. Female *A. heterodon* with swollen outer demibranchs were presumed to be gravid (McLain and Ross 2005; Michaelson and Neves 1995). Gravid mussels were transported in insulated coolers of chilled river water to the USGS Northern Appalachian Research Laboratory in Wellsboro, PA.

Ideally, gravid females to be used in screenings of potential hosts for Delaware River *A. heterodon* would have been collected from the Delaware River mainstem. However, repeated searches were conducted in 2005 and 2007 at all three known *A. heterodon* locations in the upper Delaware River and no gravid females could be found. A total of 21 mussels were examined for gravidity in the field during 2005: 8 from Hankins and 4 from Frisbie Island in April and 9 from Hankins in November. None were gravid. One female was located in the Delaware River mainstem during searches conducted in March and April 2007, but was not gravid.

Tessellated darter collection for laboratory infection trials

Tessellated darters were collected from areas upstream or downstream of *A. heterodon* locations to ensure that mussels and habitat would not be disturbed. Fish were collected using

seines and backpack electrofishing gear at relatively low voltage to prevent injury to darters (Weddle and Kessler 1993). Darters were also collected from Pine Creek in the upper Susquehanna River basin (PA). Fish were inspected visually in the field for signs of disease, and only those that appeared disease free were retained. All darters were transported in oxygenated bags of water in coolers to the USGS Northern Appalachian Research Laboratory in Wellsboro, PA. A summary of dates of collection activities and numbers tessellated darters collected at each site is included in Appendix A.

Whenever possible, I collected fish only from locations known to contain no mussels or very low densities of mussels in order to reduce the probability that they had been previously exposed to glochidia of *A. heterodon* or any other mussel species. Fish were collected from locations in the Upper Ammonoosuc River, Delaware River mainstem and Pine Creek where no mussels were observed. Prior to collecting fish at each location, I searched for mussels for 1-2 search-hours with a mask and snorkel or plexiglass-bottomed bucket 100m upstream and downstream of each collection location, and found none. Once transported to the laboratory, fish from these locations were held in aquaria for at least one month prior to infection trials. Aquaria were siphoned periodically to detect any glochidia or transformed juveniles that might have infected fish in the field. No glochidia or juveniles were observed.

In the Flat Brook and the Ashuelot River, however, I was unable obtain fish that had not been previously infected. I collected fish from the Flat Brook in areas where no mussels were observed in a 2001 survey or in snorkel searches conducted prior to fish collection. In the Ashuelot River, I searched in many locations for tessellated darters but could find none in areas that did not contain mussels. Furthermore, fish from both of these locations proved difficult to maintain in the laboratory. Although I attempted to collect fish during fall and to hold them in the laboratory over the winter in preparation for spring infections, very few survived, requiring me to collect fish again in the spring when risk of infection was high.

Fish collections for screenings of additional potential hosts

Fish used in screenings of potential hosts for *A. heterodon* were collected from the wild in locations known to contain no mussels or were obtained from fish hatcheries in Pennsylvania to minimize any risk of prior glochidial exposure (Arey 1932). Fish were collected in the wild either by electrofishing or seining. A list of fish species selected for screenings and respective sources for each are included in Table 1.

Fish collections to detect natural infection

During spring 2006, fish were collected from *A. heterodon* locations on the upper Delaware River in order to detect any natural glochidial infections. I collected fish from two of three *A. heterodon* sites where mussels are known to occur at higher densities, as I presumed incidence of infection in fish would be greatest at these sites. *Alasmidonta heterodon* is known to release glochidia at water temperatures near 0°C in New Hampshire (B. Wicklow, Saint Anselm College, personal communication), presumably in winter or very early spring, and in May and June in Massachusetts (McLain and Ross 2005). In 2005, I observed *A. heterodon* releasing large numbers of glochidia in the laboratory at 15°C. Given this information, I collected fish from *A. heterodon* locations in the Delaware River twice during early spring, in March (water temperatures ranging 3-10°C), and twice later in the spring, in May (water temperatures ranging 16-23°C), in order to detect natural infections. In pilot infection trials conducted in 2005 for this study, *A. heterodon* glochidia metamorphosed within 30 days at temperatures above 14°C. I assumed that duration of metamorphosis for *A. heterodon* glochidia in the Delaware River during spring 2006 would be at least 30 days (temperatures in the upper Delaware River during March and April rarely exceed 14°C), and collected fish four times during spring at *A. heterodon* sites over a broad range of temperatures. Fish were collected by seining or using backpack electrofishing gear for 1.5 – 2 hours at low voltage to limit risk of injury to darters (Weddle and Kessler 1993). Captured fish were transported to the laboratory in oxygenated bags of chilled river water.

Table 1. Common and scientific names, sources and dates of infection for fish species selected for screenings of potential hosts for *A. heterodon*. Introduced fish species not native to the Delaware River basin are indicated with an asterisk.

Common name	Scientific name	Source	Date infected
sea lamprey ammocoetes	<i>Petromyzon marinus</i>	Delaware River mainstem (PA/NY)	5/19/06
American eel	<i>Anguilla rostrata</i>	Delaware River mainstem (PA/NY)	7/11/06, 8/6/07
brook trout	<i>Salvelinus fontinalis</i>	Lamar National Fish Hatchery (PA)	7/11/06
Atlantic salmon parr	<i>Salmo salar</i>	Lamar National Fish Hatchery (PA)	7/11/06
brown trout*	<i>Salmo trutta</i>	Rainbow Paradise Trout Farm (PA)	7/11/06
rainbow trout*	<i>Oncorhynchus mykiss</i>	Lamar National Fish Hatchery (PA)	7/11/06
golden shiner	<i>Notemigonus crysoleucas</i>	Zetts Fish Hatchery (PA)	5/18/07
central stoneroller	<i>Campostoma anomalum</i>	Pine Creek (PA)	8/6/07
cutlips minnow	<i>Exoglossum maxillingua</i>	Pine Creek (PA)	5/19/06
blacknose dace	<i>Rhinichthys atratulus</i>	Pine Creek (PA)	2/2/06
longnose dace	<i>Rhinichthys cataractae</i>	Pine Creek (PA)	2/2/06
common shiner	<i>Luxilus cornutus</i>	Pine Creek (PA)	8/6/07
spotfin shiner	<i>Cyprionella spiloptera</i>	Delaware River mainstem (PA/NY)	2/2/06
bluntnose minnow	<i>Pimephales notatus</i>	Delaware River mainstem (PA/NY)	7/11/06
white sucker	<i>Catostomus commersoni</i>	Delaware River mainstem (PA/NY), Neversink River (NY)	1/18/06
northern hog sucker	<i>Hypentelium nigricans</i>	Zetts Fish Hatchery (PA)	5/19/06
channel catfish*	<i>Ictalurus punctatus</i>	Zetts Fish Hatchery (PA)	5/18/07
marginated madtom	<i>Noturus insignis</i>	Delaware River mainstem (PA/NY)	8/6/07
banded killifish	<i>Fundulus diaphanous</i>	Delaware River mainstem (PA/NY)	5/19/06
slimy sculpin	<i>Cottus cognatus</i>	Pine Creek (PA)	2/2/06
mottled sculpin	<i>Cottus bairdi</i>	Pine Creek (PA)	5/23/07
striped bass	<i>Morone saxatilis</i>	Lamar National Fish Hatchery (PA)	8/6/07
white crappie*	<i>Poxomis annularis</i>	Zetts Fish Hatchery (PA)	5/18/07
rock bass*	<i>Ambloplites rupestris</i>	Delaware River mainstem (PA/NY)	7/11/06
largemouth bass*	<i>Microperus salmoides</i>	Zetts Fish Hatchery (PA)	5/18/07
smallmouth bass*	<i>Micropterus dolmieu</i>	Pine Creek (PA)	8/6/07
bluegill sunfish*	<i>Lepomis macrochirus</i>	Pine Creek (PA)	5/18/07
pumpkinseed	<i>Lepomis gibbosus</i>	Shavers Creek (PA)	8/6/07
yellow perch	<i>Perca flavescens</i>	Zetts Fish Hatchery (PA)	5/18/07
shield darter	<i>Percina peltata</i>	Delaware River mainstem (PA/NY); Neversink River (NY)	1/18/06
tessellated darter	<i>Etheostoma olmstedii</i>	Pine Creek (PA), Delaware River mainstem (PA/NY), Upper Ammonoosuc River (NH)	5/23/07
banded darter*	<i>Etheostoma zonale</i>	Pine Creek (PA)	8/6/07

Laboratory culture facility

In the laboratory, *A. heterodon* individuals were held separately by location in flow-through glass aquaria. In each aquarium, mussels were kept in trays containing approximately 10cm of sand (Flat Brook mussels) or mud (Connecticut River mussels) substrate to mimic natural substrate conditions at each of these locations. Refrigeration units connected to stainless steel heat exchangers submerged in each aquarium maintained water temperatures at 6-7°C to delay release of glochidia by *A. heterodon*. Mussels were fed daily with cultured algae, primarily *Neochloris oleoabundans* and *Bracteacoccus grandis* (White Sulfur Springs National Fish Hatchery, White Sulfur Springs, West Virginia) (Van Snik Gray et al. 2002). Fish were separated by species and location and held in flow-through, 38-L or 76-L glass aquaria. Fish were fed frozen bloodworms and brine shrimp once every 1-2 days.

Infection procedures

General approach

As viability of unionid glochidia may vary over the course of the year, I conducted all infections of tessellated darters (both grouped and individual fish) in which I used glochidia of *A. heterodon* from the upper Connecticut River on the same day (June 21, 2006) to maximize the likelihood that all test fish would be exposed to glochidia of equal viability. In separate host suitability trials the following year (May 23, 2007), I also tested host suitability of individual tessellated darters for *A. heterodon* from the Flat Brook (NJ). For all tessellated darter infections I standardized the numbers of fish exposed to glochidia, the numbers of glochidia used in exposures, the means and ranges of fish lengths, and water temperatures. As my goal was to assess differences in numbers of glochidia and juveniles among different tessellated darter sources, minimizing variability among aquaria was particularly important.

Screenings of other potential fish host species were conducted at various times between January and August of 2006 and 2007, when both glochidia and test fish were available to be infected (Table 1). In testing multiple fish species as potential hosts, direct standardization of numbers and lengths of fish was somewhat impractical. The significance of the effects of these

variables on host suitability is likely to be associated with other variables I could not control in my experiments that might vary among fish species used for infections, such as morphology or life history stage. In the screenings, I intended to assess 1) whether any fish of each species were potential hosts (e.g., capable of producing at least one juvenile *A. heterodon*) and 2) whether those fish that produced juveniles were either decidedly suitable hosts (producing large numbers of juvenile mussels or having high transformation success) or marginal hosts (producing relatively few juvenile mussels or having low transformation success). I did not aim to quantitatively assess host suitability as with the tessellated darter infections. For this reason, I recorded numbers of fish infected, numbers of glochidia used in infections, and numbers of rejected glochidia and transformed juvenile mussels recovered during the experiment, depending up the numbers of glochidia available at the time of infection; however I did not attempt to standardize these values among species or to conduct all infections at one time.

Tessellated darter host suitability: grouped fish

Methods for grouped tessellated darter infections were similar to those of Rogers et al. (2001). Prior to conducting infection trials of grouped darters, I designated three banks, each containing five 38-L aquaria, to hold infected fish. Three replicate aquaria (each to contain 9 fish) were designated for each fish source location, and one each of these three replicate aquaria was randomly designated within each bank (Figure 5; Table 2). The aquaria contained no substrate so that rejected or transformed glochidia could be easily collected by siphoning. In addition, false bottoms were installed in each aquarium so that fish could not eat glochidia or transformed juveniles that settled at the bottom of the aquarium during the experiment. A PVC pipe half was placed in each tank to provide some cover for fish. Drain standpipes in each aquarium were covered by 100 μ m screening to ensure that no glochidia were lost.

Delaware (1)	Upper Ammonoosuc (1)	Ashuelot (1)	Flat Brook (1)	Pine Creek (1)
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Pine Creek (2)	Upper Ammonoosuc (2)	Delaware (2)	Flat Brook (2)	Ashuelot (2)
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Delaware (3)	Pine Creek (3)	Upper Ammonoosuc (3)	Flat Brook (3)	Ashuelot (3)
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Figure 5. Schematic of aquaria set up for grouped tessellated darter infections. One replicate each of the five tessellated darter sources was designated randomly within each of three banks of aquaria.

In addition, I distributed tessellated darters among aquaria so that sizes of fish did not vary significantly among them, thereby potentially influencing total numbers of transformed juvenile mussels or transformation success values among aquaria and fish sources. Lengths of tessellated darters used in grouped fish infections ranged overall from 35-61mm, and mean lengths of grouped tessellated darters per aquarium ranged from 41-49 mm (Figure 6). Most fish (75.3%) were 40-50mm in length.

Table 2. Experimental design to test host suitability of tessellated darters from different locations as a host fish for *A. heterodon*.

Tessellated darter source	No. fish	No. replicates	Total no. fish per source
Upper Ammonoosuc River	9	3	27
Ashuelot River	9	3	27
Delaware River	9	3	27
Flat Brook	9	3	27
Pine Creek	9	3	27
Total			135

To conduct infections of grouped tessellated darters, glochidia were first extracted from five female *A. heterodon* from the Connecticut River. To obtain glochidia, I used a 26-gauge hypodermic needle to perforate the marsupia of each parent mussel and gently flushed out glochidia with deionized water from a 10-cc syringe (Michaelson and Neves 1995). Glochidia from all five mussels were pooled together so that fish would be equally likely to be exposed to glochidia from all mussels. This approach prevented any effects of variability in numbers or viability of glochidia among individual parent mussels. Once glochidia were pooled, three separate subsamples were exposed to salt to test for viability. Glochidia that snapped shut in response to salt were presumed viable (Zale and Neves 1982).

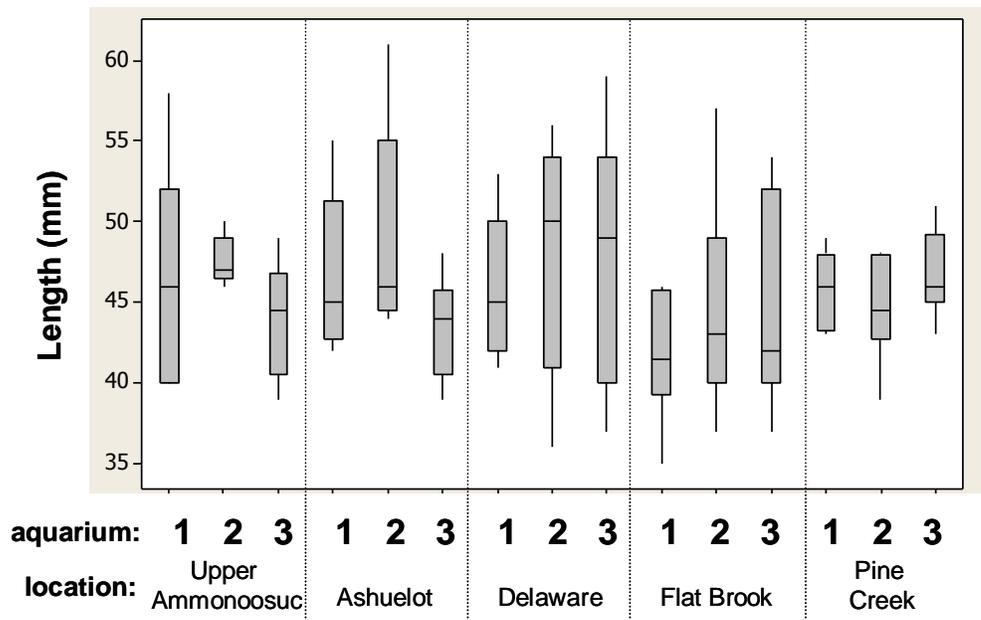


Figure 6. Boxplots (middle line indicating mean, boxes indicating a 50% interquartile range) of lengths of tessellated darters from each of three replicate tanks per fish source for grouped tessellated darter infections. Mean lengths in each aquarium ranged from 41.75 -49.00mm.

Pooled glochidia were then used to infect tessellated darters from each of the five test locations. A total of 27 darters from each test location (9 individuals per replicate) were used in the experiment, for a total of 135 darters (Table 2). A total of 350 glochidia were introduced into each of fifteen one-liter beakers (3 beakers, or replicates, per test location) containing 500ml of water. Groups of 9 darters were then placed into each beaker, where they remained for 45-55 minutes at 17.9-19.2°C. Beakers were swirled gently at intervals to induce fish to swim and facilitate their coming into contact with glochidia. Shortly after the exposures began, glochidia were observed to attach readily to the fins of darters.

Once fish were infected, contents of each beaker (fish with attached glochidia and remaining unattached glochidia) were transferred to a 10L aquarium. Each aquarium was then immediately siphoned to remove any glochidia that had not successfully attached to the fish. Numbers of unattached glochidia were counted and recorded.

Water temperatures in each aquarium were measured with a digital thermometer prior to each siphoning. Temperatures were summarized and evaluated to ensure no significant variation in temperature occurred over the course of the experiment or among aquaria. Temperatures remained relatively constant among aquaria over the course of the experiment (41 days), with all aquaria being within 1°C of one another on a given day, and ranging from 17.9-19.0°C over the course of the experiment (Figure 7).

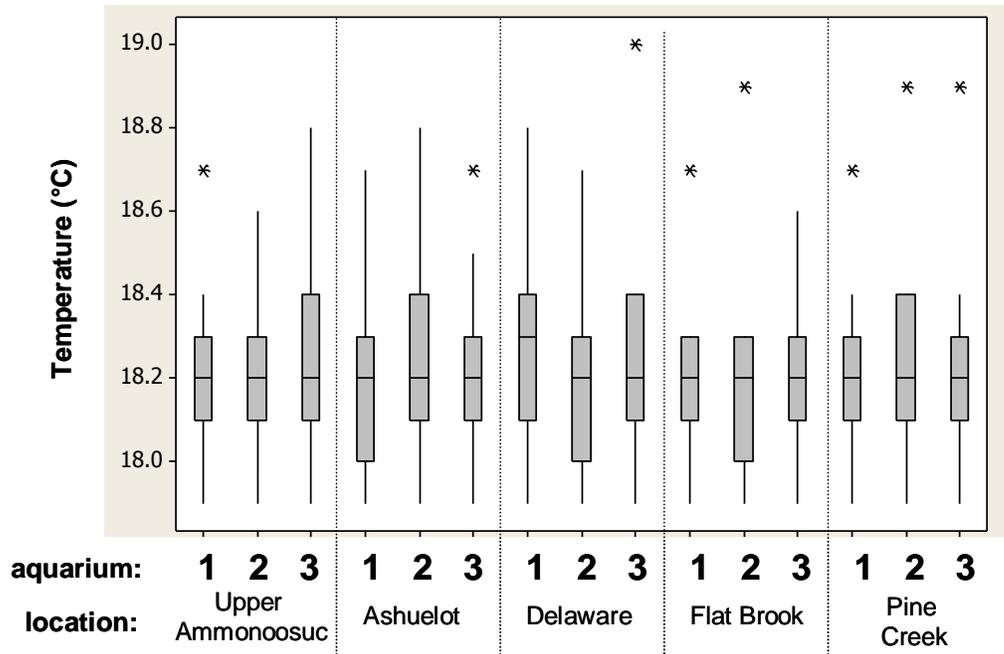


Figure 7. Boxplots (middle line indicating mean temperature, boxes indicating a 50% interquartile range, stars indicating outliers) of water temperatures in each aquarium measured before each siphoning in grouped tessellated darter infections over the course of the experiment (41 days; June 21 - July 31, 2006). Mean temperatures were typically 18.2°C and at least half of daily temperature measurements were between 18.0°C and 18.4°C.

Tessellated darter host suitability: individually-monitored fish infected with glochidia from Connecticut River A. heterodon

In addition to infecting tessellated darters by group, I infected and monitored some fish individually to evaluate any variation in host suitability that might occur among these individuals. In 2006, I infected darters from the Delaware and Upper Ammonoosuc Rivers with glochidia from upper Connecticut River mussels and monitored them separately. In infections of individual fish, a total of approximately 300 glochidia were introduced into each of two one-liter beakers. Seven darters from the Delaware River were introduced to one beaker and seven from the Upper Ammonoosuc River were introduced to the second beaker. Fish remained in the beakers for approximately 1 hour at 18.7-19.1°C. Each individual fish was then transferred to a separate 38-L aquarium where it would remain for the duration of the experiment. Numbers of glochidia remaining in each beaker were counted and recorded. Water temperature was monitored as described above and a summary of these data is presented in Figure 8.

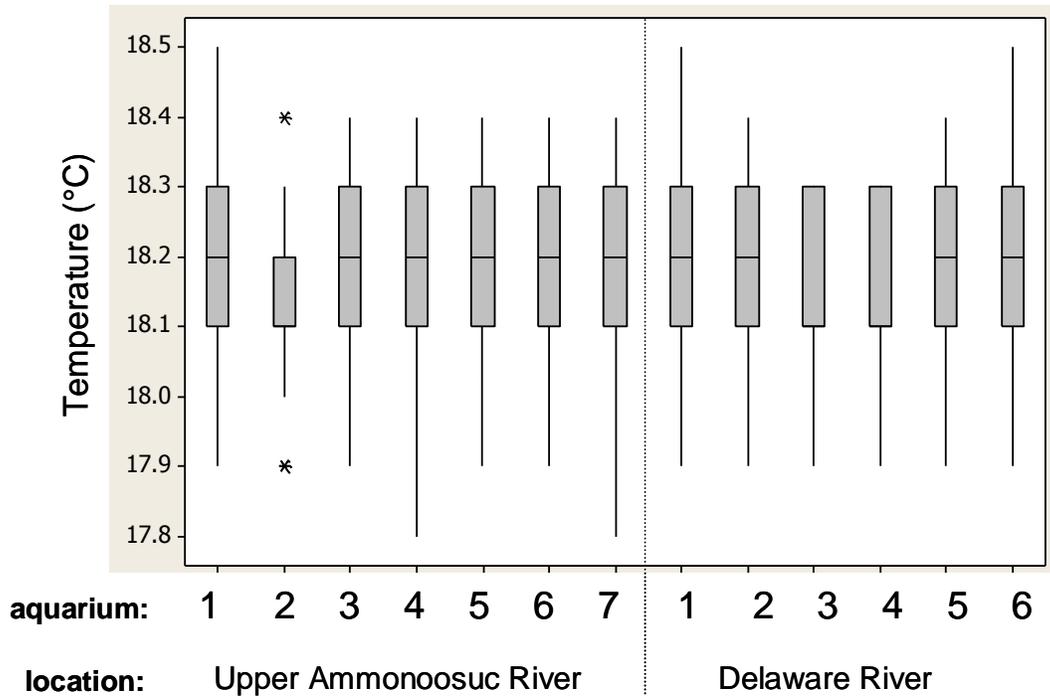


Figure 8. Boxplots (middle line indicating mean temperature, boxes indicating a 50% interquartile range, stars indicating outliers) of water temperatures in each aquarium measured before each siphoning in grouped tessellated darter infections over the course of the experiment (40 days; June 21 – July 31, 2006). Mean temperatures were typically 18.2°C and at least half of daily temperature measurements were between 18.0°C and 18.3°C.

Tessellated darter host suitability: individually monitored fish infected with glochidia from Flat Brook

A. heterodon

To evaluate host suitability of individual tessellated darter from the Upper Ammonoosuc River, Delaware River mainstem and Pine Creek for *A. heterodon* from the Flat Brook, I infected 9 darters from each of these three locations with Flat Brook *A. heterodon* glochidia. Methods for infection and monitoring were similar to those used in infections trials of individual darters using glochidia from Connecticut River *A. heterodon*, as described above. A total of 250 Flat Brook *A. heterodon* glochidia were introduced into each of three one-liter beakers. Nine tessellated darters from each location were then introduced to each of the three beakers. Fish remained in the beakers for 60-65 minutes at 19.5 – 20.0°C. Once infected, each of the nine fish from each location was transferred to a separate aquarium where it would remain for the duration of the experiment.

Evaluation of other potential fish hosts

Screenings of other potential fish hosts were conducted using *A. heterodon* glochidia from the Flat Brook. Procedures were much the same as those used for infections of tessellated darters described above. Glochidia were extracted from female mussels by flushing the marsupia of each individual using a 26-gauge hypodermic needle and deionized water from a 10-cc syringe (Michaelson and Neves 1995). Numbers of glochidia used in exposures varied depending upon numbers and sizes of the fish to be tested as well as the number of glochidia available to be used in infections. Tested fish were exposed to 100-250 glochidia in 500 mL of water in 1-L beakers for 45-60 minutes at 17-19°C. Larger fish were infected in 18.9 L buckets rather than in 1-L beakers. Rainbow trout were too large, very active and risked sustaining injury if infected in buckets. Instead, I infected them by depositing approximately 25 glochidia directly onto the left gill of each fish using a pipette. Fewer glochidia were used by this technique than in a bath because large numbers of glochidia applied directly to the fish's gills might potentially limit its ability to breathe. Following infections, all fish were placed in 38-L (for smaller fish) or 76-L (for larger or more numerous fish) glass aquaria where they remained for the duration of the experiment.

Monitoring of infected fish and transformed juvenile mussels

Aquaria containing infected tessellated darters were maintained at 17.9-19.0°C throughout the course of the 41-day experiment. As water temperature is known to affect duration and success of transformation in glochidia of other unionid species (Watters and O'Dee 1999), all aquaria were monitored to ensure minimal variation in temperature occurred among them. Other infected potential host fish species were maintained at 17.3 – 20.6 °C. Aquaria were siphoned at least three times per week until at least six days after the last juvenile was found to ensure that no glochidia or juveniles went undetected. At each siphoning, siphoned material was collected in 100µm sieves and transferred to a glass Petri dish where contents were observed under a dissecting scope. A polarizing lens was used with the dissecting scope during observation so that each glochidium or juvenile mussel could be more readily identified (Johnson 1995). Numbers of

both non-transformed and transformed glochidia were recorded. Transformed juveniles were identified by presence of a foot (Michaelson and Neves 1995).

Observation of fish to detect natural infections

Fish collected from the Delaware River mussel sites to detect natural infections were transported to the laboratory and placed in separate aquaria by species. Each aquarium contained a false bottom to prevent fish from eating shed glochidia or juvenile mussels. Aquaria were siphoned weekly for approximately one month so that any rejected glochidia or transformed juveniles from natural infections could be collected. As mentioned previously, tessellated darters collected from other locations for host suitability evaluations were also held in the laboratory and siphoned to detect natural infections. Any transformed juvenile mussels or non-transformed glochidia recovered from naturally infected fish were identified using reference glochidia from *A. heterodon* in the laboratory, photographs and descriptions of other glochidia from previous host identification studies conducted at the USGS Northern Appalachian Research Laboratory, and descriptions of *Alasmidonta* glochidia provided by Clarke (1981).

Disposition of adult and juvenile mussels

Adult *A. heterodon* were returned to the locations from which they were originally collected each year after infections were complete, first in July 2006 and again in August 2007. Juvenile mussels from infection trials were transferred to aquaria where they were held in sieves for observation. They were fed cultured algae daily and their growth and behavior was observed regularly. While many individuals continued to feed, remain active, and grew from approximately 300µm in width to 600µm within one month after they dropped off their fish host, no juvenile *A. heterodon* survived beyond 5 weeks.

Morphometric and meristic analysis of tessellated darters

In order to further assess potential differences among tessellated darters that might be related to any differences in host suitability among fish source populations, I compared

morphometric and meristic characters for fish from each of three locations: Upper Ammonoosuc River (Connecticut River basin, NH), the upper Delaware River (Delaware River basin, PA/NY), and Pine Creek (Susquehanna River basin, PA). Darters were collected by seining, fixed in 10% formalin and preserved in 70% ethanol. Morphometric measurements and meristic counts followed Stauffer and Konings (2006) and descriptions and abbreviations of measurements and counts used are included in Table 3.

Table 3. Morphometric characters, or lengths (mm), and meristic characters, or counts, assessed in analysis of tessellated darters from three locations: Upper Ammonoosuc River (Connecticut River basin, NH), Delaware River (Delaware River basin, PA/NY) and Pine Creek (Susquehanna River basin, PA)

Character description	Abbreviation
<i>Measurements (mm)</i>	
Standard length	SL
Head length	HL
Snout length	SNL
Postorbital head length	POHL
Horizontal eye diameter	HED
Vertical eye diameter	VED
Preorbital head length	PRE
Cheek depth	CD
Lower jaw length	LJL
Head depth	HD
Body depth	BD
Snout to dorsal fin origin	SNDOR
Snout to pelvic fin insertion	SNPEL
First dorsal fin base length	DFBL1
Distance from posterior of first dorsal fin to anterior of second dorsal fin	BTDF
Second dorsal fin base length	DFBL2
Distance from anterior of first dorsal fin to posterior of second dorsal fin	TDFBL
Distance from anterior of first dorsal fin to anterior of anal fin	AD1AA
Distance from anterior of second dorsal fin to anterior of anal fin	AD2AA
Distance from anterior of first dorsal fin to posterior of anal fin	AD1PA
Distance from anterior of second dorsal fin to posterior of anal fin	AD2PA
Distance from posterior of first dorsal fin to anterior of anal fin	PD1AA
Distance from posterior of second dorsal fin to anterior of anal fin	PD2AA
Distance from posterior of first dorsal fin to posterior of anal fin	PD1PA
Distance from posterior of second dorsal fin to posterior of anal fin	PD2PA
Distance from posterior of first dorsal fin to ventral point of least caudal peduncle	PD1VC
Distance from posterior of second dorsal fin to ventral point of least caudal peduncle	PD2VC
Distance from posterior of anal fin to dorsal point of least caudal peduncle	PADC
Distance from anterior of first dorsal fin to pelvic fin insertion	AD1PL
Distance from anterior of second dorsal fin to pelvic fin insertion	AD2PL
Distance from posterior of first dorsal fin to pelvic fin insertion	PD1PL
Distance from posterior of second dorsal fin to pelvic fin insertion	PD2PL
Caudal peduncle length	CPL
Least caudal peduncle depth	LCPD
<i>Meristics</i>	
Number of dorsal fin spines (first dorsal fin)	DSPINES
Number of dorsal fin rays (second dorsal fin)	DRAYS
Number of anal fin rays	ARAYS
Number of pelvic fin rays	PLRAYS
Number of pectoral fin rays	PCRAYS
Number of lateral line scales	LLS
Number of gill rakers	GR

Data Analysis

Tessellated darter host suitability

Univariate analysis of variance (ANOVA, SAS 9.1, Proc GLM) and comparisons of least squares means were used to compare total numbers of transformed juvenile *A. heterodon* produced throughout the experiment by tessellated darters from different locations. As darter mortalities occurred periodically throughout infection trials, equal numbers of fish were not present in all tanks on each day. To standardize total numbers of juvenile mussels for each aquarium, total numbers of juveniles that transformed in each tank on each day were divided by the total number of fish present in that tank on that day. Numbers of juveniles per fish each day were summed over the course of the experiment to generate a total number of juveniles produced per fish for each tank for the entire experiment. These totals (numbers of juveniles/fish/day) were used in statistical comparisons of numbers of juvenile mussels produced by tessellated darters from each of the test locations.

In addition to total numbers of juveniles per fish produced, I compared transformation success among the different locations. Transformation success was defined as the proportion of attached glochidia that successfully transformed into juvenile mussels (Khym and Layzer 2000; Riusech and Barnhart 2000; Van Snik Gray et al. 2002). I calculated this value at the end of the experiment by dividing total numbers of juvenile mussels per fish in each aquarium by the sum of the total number of shed glochidia and transformed juveniles per fish in that aquarium that were generated over the course of the entire 41-day infection period. Transformation success ratios were arcsine transformed and compared nonparametrically using Wilcoxon ranked scores and the Mann-Whitney test statistic (Zar 1999).

Screenings of additional potential hosts

Even when certain fish prove capable of acting as hosts in the laboratory, they may not be equally capable of acting as natural hosts in the wild. Incidence of natural infection is often low, and numbers of glochidia that survive release from the parent mussel, successfully attach to the appropriate host and transform into juvenile mussels may be few. Consequently, some studies

have designated hosts identified in the laboratory as either "suitable" or "marginal". Suitable hosts may be designated as such when they produce sufficient numbers of glochidia to potentially serve as hosts in the wild, while "marginal hosts" may only generate a small number of transformed mussels in the laboratory and may be unlikely to serve as natural hosts (Haag 2002). Van Snik Gray et al. (2002) determined fish hosts of *S. undulatus* with transformation success of 10% or more to be suitable hosts while those less than 10% were deemed marginal. In other studies, particularly those which identified only hosts that produced large numbers of transformed juveniles, investigators have found it unnecessary to make such designations (Michaelson and Neves 1995). In this study, I designated all hosts that produced at least one juvenile *A. heterodon* to be potential hosts, but presumed fish that generally produced low numbers of transformed juveniles or low transformation success values might not be capable of serving as hosts in the wild. I hypothesize that hosts with transformation success values comparable to those of tessellated darters, a known host (e.g. greater than 0.20) could serve as suitable hosts in the wild; however, without further investigation of host use by *A. heterodon* in the field, including measures of glochidial attachment and transformation success, it is not possible to know if transformation success observed in the laboratory is the best measure of host suitability or which fish may be most suitable natural hosts.

Morphometric and meristic analysis of tessellated darters

Principal components analysis (PCA) was used to analyze meristic data and sheared principal components analysis (SPCA) was used in analysis of morphometric data. Sheared second principal components (morphometric data) were then plotted against the first principal components (meristic data) to illustrate differences among individual fish by source group, and minimum polygon clusters for each group were identified (Stauffer and Konings 2006). Both MANOVA and Duncan's multiple range test were used to analyze differences among fish from each location following the methods of Stauffer et al. (1997).

RESULTS

Host suitability of tessellated darters

Infections of grouped fish

Tessellated darter source had a statistically significant effect ($p < 0.05$, ANOVA, $F = 4.69$) on numbers of juvenile *A. heterodon* produced per fish in host suitability trials when all five fish source locations were compared. In particular, tessellated darters from the Upper Ammonoosuc River produced significantly higher mean numbers of transformed juveniles per fish (10.45) than those produced by fish from the Flat Brook (3.74), Ashuelot River (4.61) and Pine Creek (4.14), but not from the Delaware River (6.85) (pairwise least squares means comparisons; Tables 4 & 5). Mean numbers of juveniles per fish produced by darters from non-Upper Ammonoosuc River locations did not differ significantly from one another.

While highest compatibility was observed between mussels from the upper Connecticut River and host fish from the nearby Upper Ammonoosuc River, compatibilities of tessellated darter hosts from non-Upper Ammonoosuc River locations did not decrease successively overall as degree of isolation of each fish source from the upper Connecticut River mussel location increased. Rather, darters from the Upper Ammonoosuc River produced the highest numbers of juveniles per fish and darters from the Delaware River produced the second highest number. Numbers of juveniles produced per fish among remaining locations were all lower than both the Upper Ammonoosuc and Delaware rivers and were quite comparable to one another (Figures 9 and 10). Timing and duration of transformations was somewhat similar among all five locations, with the first transformations occurring at 15-17 days post-infection and the last occurring at days 28-35 post-infection (Figure 11; Table 4).

I performed a second ANOVA in which I removed from the model fish source locations that included previously infected fish (Ashuelot River and Flat Brook) in case immune response to glochidiosis in these fish affected their host suitability. I only compared fish that were not previously infected (from the Upper Ammonoosuc River, Delaware River and Pine Creek). Darters from the Upper Ammonoosuc River produced the highest mean number of juveniles per fish (10.45),

Table 4. Numbers of transformed juvenile *Alasmidonta heterodon* (number of juveniles produced per fish each day, summed over course of the experiment), transformation success values (numbers of attached glochidia that transformed) and duration of transformations for grouped tessellated darters from each of the five test locations. All grouped tessellated darters were infected on June 21, 2006 and the experiment lasted a total of 41 days. Summaries of values used in calculations of numbers of juveniles produced per fish for each aquarium are included in Appendix B.

Location	Aquarium replicate	Numbers of juvenile mussels per fish	Transformation success	Days to first transformation	Days to peak number of transformations	Days to last transformation
Upper Ammonoosuc	1	7.42	0.45	18	19	27
	2	14.64	0.63	14	19	27
	3	9.30	0.43	18	18	27
<i>mean</i>		<i>10.45</i>	<i>0.50</i>	<i>16.7</i>	<i>18.7</i>	<i>27.0</i>
Ashuelot	1	5.58	0.48	18	18	34
	2	3.53	0.25	16	19	34
	3	4.71	0.37	16	20	31
<i>mean</i>		<i>4.61</i>	<i>0.37</i>	<i>16.7</i>	<i>19.0</i>	<i>33.0</i>
Delaware	1	5.58	0.35	18	19	29
	2	6.47	0.48	14	20	31
	3	8.50	0.41	16	22	29
<i>mean</i>		<i>6.85</i>	<i>0.41</i>	<i>16</i>	<i>20.3</i>	<i>29.7</i>
Flat Brook	1	4.46	0.28	18	19	31
	2	1.29	0.09	18	20	23
	3	5.46	0.43	16	18	31
<i>mean</i>		<i>3.74</i>	<i>0.27</i>	<i>17.3</i>	<i>19.0</i>	<i>28.3</i>
Pine Creek	1	6.06	0.56	14	19	23
	2	3.00	0.26	18	19	22
	3	3.37	0.20	18	19	25
<i>mean</i>		<i>4.14</i>	<i>0.34</i>	<i>16.7</i>	<i>19.0</i>	<i>23.3</i>

Table 5. (a) ANOVA and (b) least squares means comparisons of numbers of transformed juvenile *Alasmidonta heterodon* (number of juveniles/fish/day summed over course of experiment) among group-infected tessellated darters from all five test locations. Significant values ($p \leq 0.05$) are highlighted in bold.

a)

Source	DF	SS	MS	F	P
Model	4	91.76231	22.94058	4.69	0.0216
Error	10	48.90667	4.890667		
Corrected Total	14	140.669			

b)

Location	Location	P
Upper Ammonoosuc River	Flat Brook	0.0040
Upper Ammonoosuc River	Pine Creek	0.0065
Upper Ammonoosuc River	Ashuelot River	0.0089
Upper Ammonoosuc River	Delaware River	0.0739
Delaware River	Flat Brook	0.1154
Delaware River	Pine Creek	0.1845
Delaware River	Ashuelot River	0.2424
Flat Brook	Ashuelot River	0.6403
Flat Brook	Pine Creek	0.7710
Ashuelot River	Pine Creek	0.8585

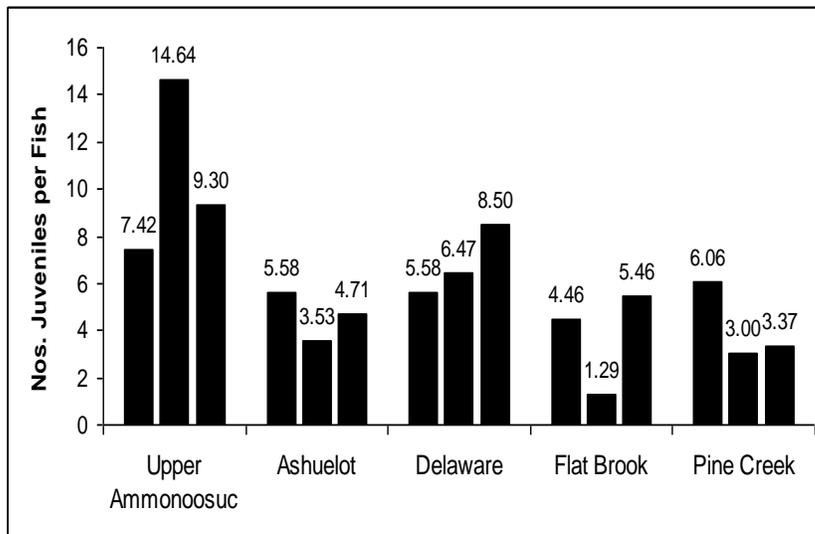


Figure 9. Numbers of transformed juveniles/fish (summed over the course of the experiment) among group-infected tessellated from all five test locations.

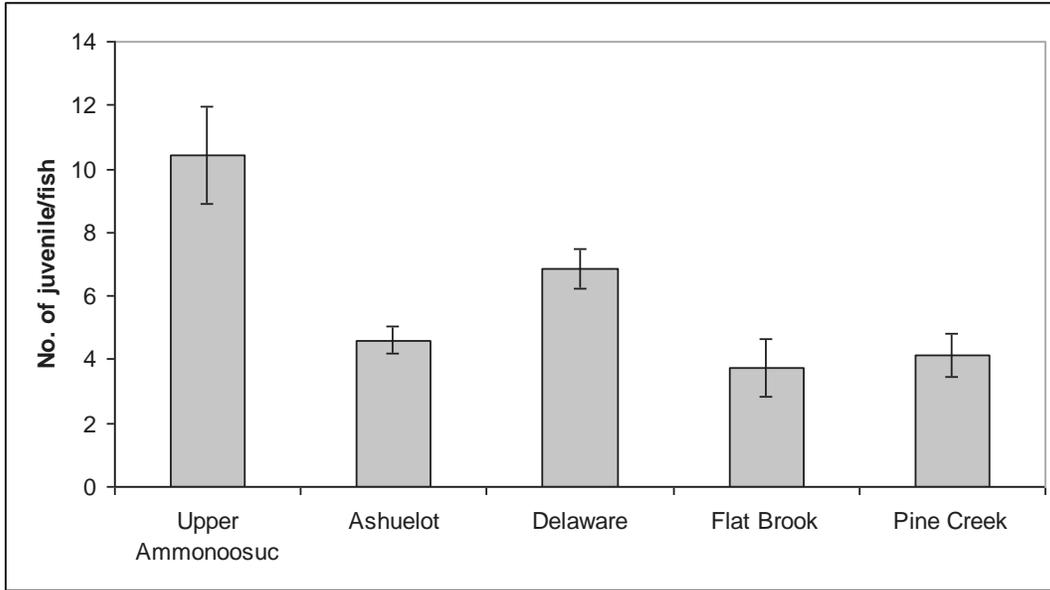


Figure 10. Mean number (± 1 SD) of transformed juvenile *A. heterodon* per fish among group-infected tessellated from all five test locations.

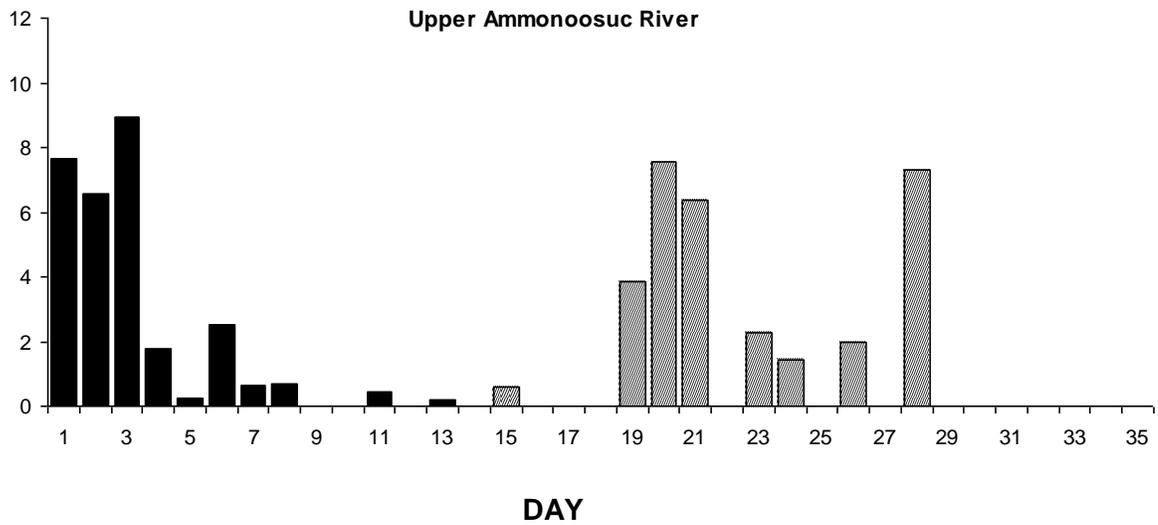


Figure 11. Numbers of shed glochidia (black) and transformed juvenile *Alasmidonta heterodon* produced per fish (black and white stripes) among tessellated darters from all five test locations observed at each siphoning over the course of the experiment. Continued on following page.

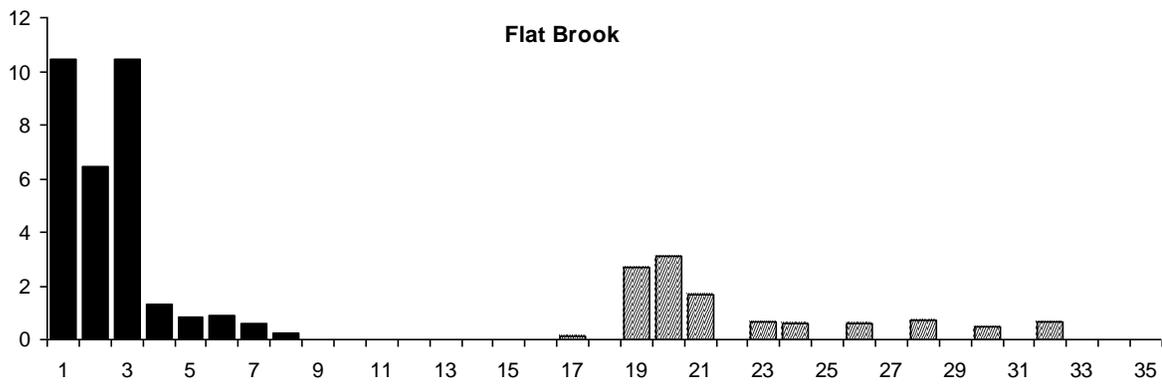
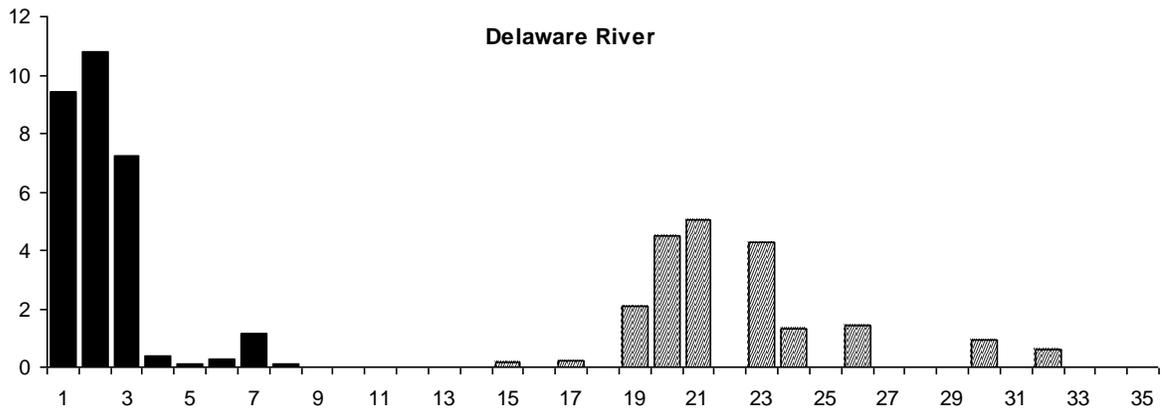
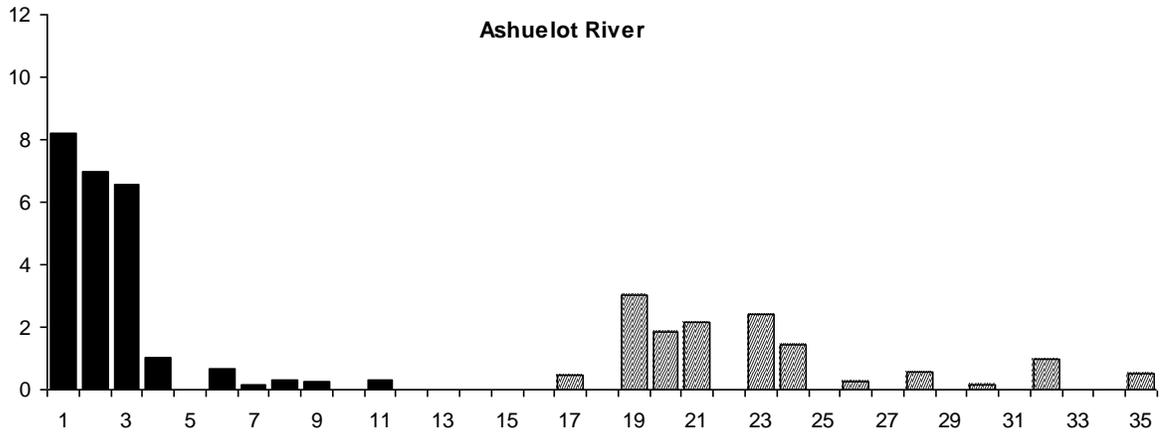


Figure 11 continued.

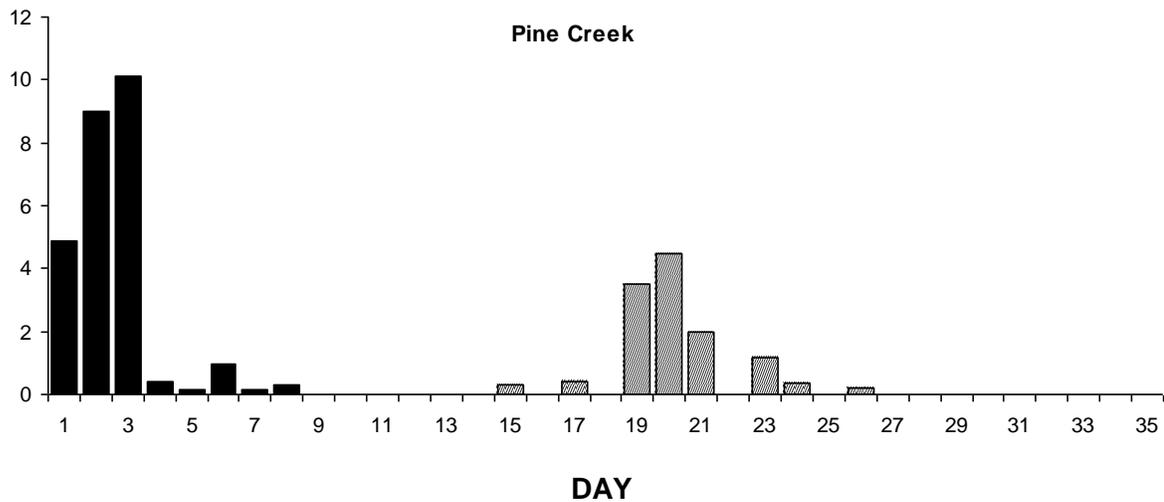


Figure 11 continued.

followed by the Delaware (6.85), and Pine Creek (4.14). Clearly, among fish from these three locations, numbers of transformed juvenile mussels decreased successively as the degree of each fish source from the mussel source increased. Nonetheless, fish source did not have a statistically significant effect overall on the numbers of juveniles produced per fish ($p = 0.06$, ANOVA, $F = 4.64$) (Table 6a). Ability to detect significant differences may have been reduced due to small sample sizes (three aquaria per fish source). However, one significant pairwise difference was observed between the Upper Ammonoosuc River, nearest to the mussel location, and Pine Creek, furthest isolated from the Connecticut River mussel source ($p < 0.05$, ANOVA)(Table 6b). Pine Creek also has no historic record of *A. heterodon*.

Transformation success values varied little among the five tessellated darter sources (Figure 12), and the effect of location on transformation success (proportion of attached glochidia that transformed into juvenile mussels) was not significant, whether or not previously infected fish were included in the model (Tables 7 and 8). Although ability to detect any significant differences may have been reduced because sample sizes were small, relative differences in transformation success among locations were not as notable as differences in numbers of juveniles produced per fish among locations.

Table 6. (a) ANOVA and (b) least squares means comparisons of numbers of transformed juvenile *Alasmidonta heterodon* (juveniles/fish/day summed over course of experiment) among group-infected tessellated darters, excluding fish that were previously infected in the wild (Ashuelot River and Flat Brook). Statistically significant values are highlighted in bold.

a)

Source	DF	SS	MS	F	P
Model	2	57.75726667	28.87863	4.64	0.0604
Error	6	37.31013333	6.218356		
Corrected Total	8	95.0674			

b)

Location 1	Location 2	P
Upper Ammonoosuc River	Pine Creek	0.0230
Upper Ammonoosuc River	Delaware River	0.1272
Delaware River	Pine Creek	0.2532

Table 7. (a) ANOVA and (b) Wilcoxon ranked scores of transformation success (proportion of attached *Alasmidonta heterodon* glochidia that transformed) for group-infected tessellated darters from all five test locations.

a)

Source	DF	SS	MS	F	P
Among	4	0.105227	0.026307	1.5976	0.2493
Within	10	0.164667	0.016467		

b)

Wilcoxon Scores (Rank Sums)					
Location	N	Sum of Scores	Expected Under Ho	Std Dev Under Ho	Mean Score
Pine Creek	3	20.0	24.0	6.928203	6.666667
Flat Brook	3	15.0	24.0	6.928203	5.000000
Ashuelot	3	23.0	24.0	6.928203	7.666667
Connecticut	3	36.0	24.0	6.928203	12.000000
Delaware	3	26.0	24.0	6.928203	8.666667

Table 8. (a) ANOVA and (b) Wilcoxon ranked scores transformation success (proportion of attached glochidia that transformed) for group-infected tessellated darters, excluding fish that were previously infected (Ashuelot River and Flat Brook)

a)

Source	DF	SS	MS	F	P
Among	2	0.046956	0.023478	1.2947	0.3408
Within	6	0.108800	0.018138		

b)

Wilcoxon Scores (Rank Sums)					
Location	N	Sum of Scores	Expected Under Ho	Std Dev Under Ho	Mean Score
Connecticut	3	20.0	15.0	3.872983	6.666667
Delaware River	3	14.0	15.0	3.872983	4.666667
Pine Creek	3	11.0	15.0	3.872983	3.666667

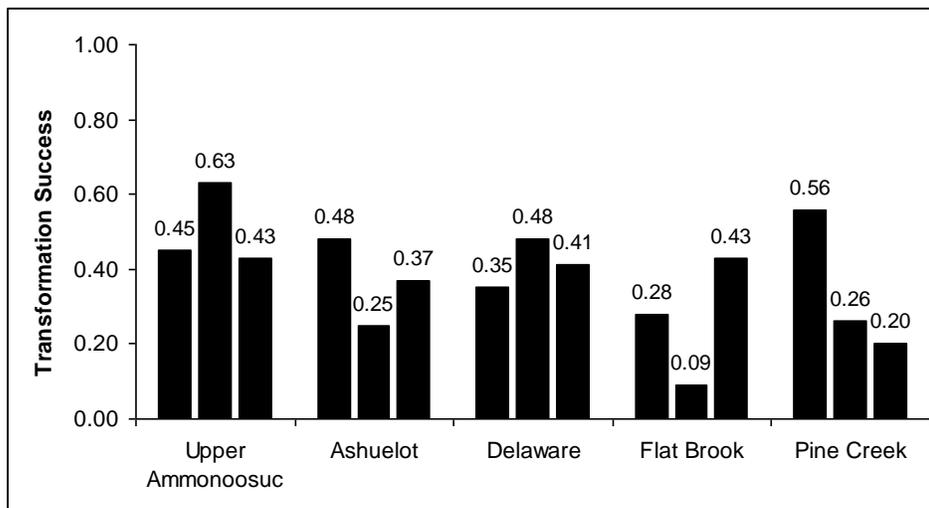


Figure 12. Transformation success (proportion of attached glochidia that transformed) of *A. heterodon* among group-infected tessellated darters from the five test locations

Infections of individually-monitored fish

Neither numbers of transformations nor transformation success values among individual fish from the Delaware and Upper Ammonoosuc Rivers differed significantly (Tables 9, 10, 11).

This relative similarity in numbers of transformed juveniles produced between fish from the

Connecticut and Delaware Rivers is consistent with findings from the grouped darter infections, where pairwise comparisons showed that of all five locations, the Connecticut and the Delaware rivers were the least different from one another. While overall mean values of transformations of individually infected fish by location were quite similar, both numbers of transformations and transformation success values were quite variable among individuals within each location group (Figures 13 & 14). Numbers of juveniles that transformed on individual fish from both locations varied from 2 to 11, and transformation success values varied from 0.20 to 1.00 (Table 9a). Among individually infected darters (lengths ranging 56-74mm), lengths of individual fish were not correlated with numbers of juveniles produced per fish (Figure 15).

Tessellated darters infected with glochidia from Flat Brook *A. heterodon* produced very few transformed juvenile mussels overall. Darters from the Delaware River mainstem, located closest to the mussel source, generated 0-3 juveniles per fish, darters from the Upper Ammonoosuc River produced 0-2 juveniles per fish, and darters from Pine Creek produced 0-1 juveniles per fish. Numbers of transformed juveniles were too few to conduct statistical analysis; however a summary of total numbers of juveniles produced and mean transformation success values per location shows that darters from the Delaware River, located closest to the mussel source, produced more juveniles and higher transformation success values than fish from the Upper Ammonoosuc River or Pine Creek (Table 9b).

Table 9. Numbers of transformed juveniles produced per fish and transformation success values (proportion of attached glochidia that transformed) for tessellated darters that were infected and then individually monitored (a) from the Delaware and Upper Ammonoosuc Rivers infected with glochidia of Connecticut River *A. heterodon* and (b) from the Upper Ammonoosuc River, Delaware River mainstem and Pine Creek infected with glochidia of Flat Brook *A. heterodon*

(a)

Fish	Delaware River mainstem			Upper Ammonoosuc River		
	No. shed glochidia	No. transformed juveniles	Transformation success	No. shed glochidia	No. transformed juveniles	Transformation success
1	3	3	0.50	1	11	0.92
2	8	2	0.20	6	2	0.25
3	2	11	0.85	1	4	0.80
4	2	7	0.78	2	5	0.71
5	0	5	1.00	1	8	0.89
6	2	3	0.60	1	9	0.90
				5	3	0.38
Mean	17	31	0.66	17	42	0.69
Total	2.83	5.17	--	2.43	6.00	--

(b)

Fish	Upper Ammonoosuc River			Delaware River mainstem			Pine Creek		
	No. shed glochidia	No. transformed juveniles	Transformation success	No. shed glochidia	No. transformed juveniles	Transformation success	No. shed glochidia	No. transformed juveniles	Transformation success
1	1	2	1	0	0	0	0	0	0
2	3	2	0	0	2	0.67	0	1	1
3	2	2	0	1	0	0.50	1	1	0.50
4	1	0	1	0	3	0	3	0	0
5	1	0	0.75	1	3	0	4	0	0
6	1	1	0.67	1	2	0.50	2	1	0.67
7	0	0	1	0	1	0	1	0	0
8	1	2	1	0	2	0.67	4	0	0
9	0	0	0	1	0	0	0	0	0
mean	1.11	1.00	0.6	0.44	1.44	0.26	1.67	0.33	0.24
total	10	9	--	4	13	--	15	3	--

Table 10. (a) ANOVA and (b) least squares means comparison of numbers of transformed juvenile *Alasmidonta heterodon* produced per fish among individually-monitored tessellated darters from the Delaware and Connecticut Rivers.

(a)

	DF	SS	MS	F	P
Model	1	2.2435897	2.24359	0.2	0.6652
Error	11	124.8333333	11.34848		
Corrected total	12	127.0769231			

(b)

Location	Least Square Mean	P
Connecticut	6.00000000	0.6652
Delaware	5.16666667	

Table 11. (a) ANOVA and (b) Wilcoxon ranked scores comparing transformation success (proportion of attached *Alasmidonta heterodon* glochidia that transformed) for individually-monitored tessellated darters from the Delaware and Connecticut Rivers.

a)

Source	DF	SS	MS	F	P
Among	1	0.000495	0.000495	0.0043	0.9491
Within	11	1.279105	0.116282		

b)

Location	N	Sum of Scores	Expected Under Ho	Std Dev Under Ho	Mean Score
Connecticut	7	52.0	49.0	7.0	7.428571
Delaware	6	39.0	42.0	7.0	6.500000

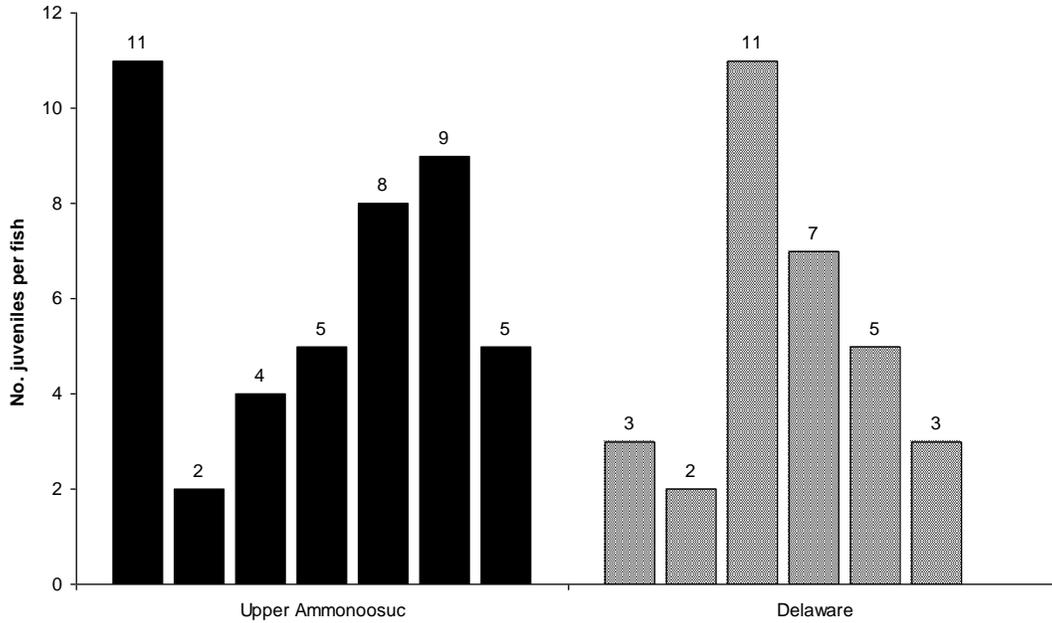


Figure 13. Total numbers of juvenile *A. heterodon* produced by individually-monitored tessellated darters from the Delaware and Upper Ammonoosuc rivers.

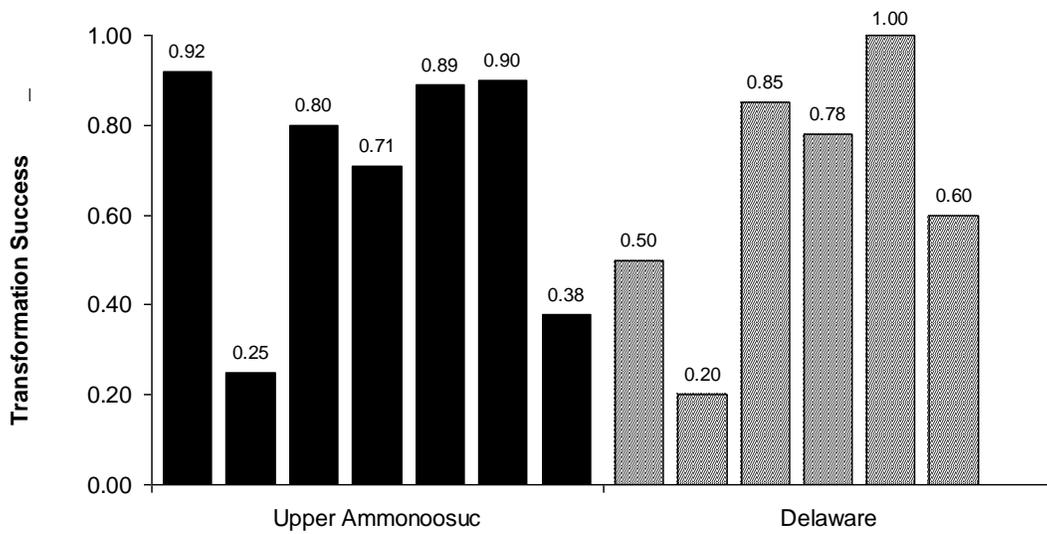


Figure 14. Transformation success (proportion of attached glochidia that transformed) of *A. heterodon* on individually-monitored tessellated darters from the Delaware and Upper Ammonoosuc rivers.

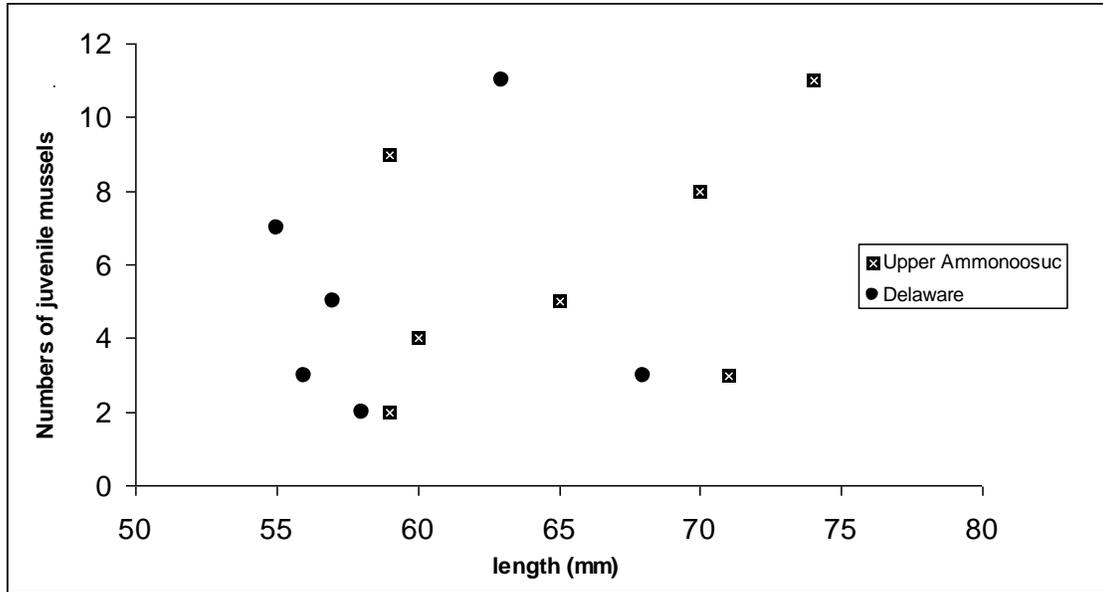


Figure 15. Scatterplot of numbers of juveniles produced per fish vs. individual fish lengths for individually-monitored tessellated darters

Evaluations of other potential fish hosts

Confirmed hosts

In laboratory screenings of additional potential fish host species, I confirmed four hosts for *A. heterodon* that had been identified in previous studies: tessellated darter (McLain and Ross 2005; Michaelson and Neves 1995; Wicklow 1999), mottled sculpin (*Cottus bairdi*) (Michaelson and Neves 1995), slimy sculpin (*Cottus cognatus*) and Atlantic salmon parr (*Salmo salar*) (Wicklow 1999) (Table 12). In this study, tessellated darters showed a transformation success value (proportion of attached glochidia that successfully transformed) of 0.44, mottled sculpins 0.41 and slimy sculpins an even higher value of 0.90. Transformation success for of Flat Brook *A. heterodon* on Atlantic salmon was 0.23.

New hosts

I identified four new potential hosts for *A. heterodon*: the shield darter (*Percina peltata*), the banded killifish (*Fundulus diaphanous*), brown trout (*Salmo trutta*) and striped bass (*Morone saxatilis*) (Table 12). I did not conduct direct quantitative comparisons of numbers of juveniles

produced and transformation success values for these species. It may be worthwhile to note, however, that shield darters tested in the laboratory produced higher mean numbers of transformed juveniles per fish (2.05) than those produced by tessellated darters (0.85), a known natural host, although they showed a lower transformation success rate (0.16 as opposed to 0.44 shown by tessellated darters). Although transformation success values were somewhat low for shield darters, these results indicate that fish of this species may serve as suitable hosts for *A. heterodon* in the wild. Only one of the three striped bass I attempted to infect survived the full duration of the experiment (two individuals died on the day of exposures, perhaps due to stress from changes in salinity incurred prior to infection). This single fish produced 23 transformed juvenile *A. heterodon* with a transformation success of 0.79, as only 6 non-transformed glochidia were recovered from its aquarium. This indicates striped bass, where it co-occurs with *A. heterodon*, may potentially serve as a very good host. Banded killifish produced only 2 transformed juveniles, yielding a transformation success value of only 0.07 and indicating banded killifish are not likely to be suitable hosts in the wild. Brown trout produced only 3 transformed juveniles and had a transformation success value of 0.16; however a relatively large number of brown trout (30 fish) were exposed to approximately 200 glochidia and only 16 glochidia successfully attached. Only 3 of these attached glochidia transformed into juvenile mussels. Such a low incidence of attachment, despite a moderate transformation success value, indicates that brown trout may not necessarily be a viable natural host.

Table 12. Results of screenings of multiple potential fish hosts for *A. heterodon* from the Flat Brook in the upper Delaware River basin. Fish that tested positive as hosts are highlighted in bold and marked with an asterisk.

Common name	Scientific name	Date infected	Number of fish exposed	Approximate number glochidia used in infection	Number shed glochidia	Number transformed juvenile mussels	Transformation success	Water temperature range (°C)
sea lamprey ammocoetes	<i>Petromyzon marinus</i>	5/19/06	30	150	60	0	-	17.3 – 17.9
American eel (adult)	<i>Anguilla rostrata</i>	7/11/06	1	200	11	0	-	17.8 – 20.1
American eel (elver)	<i>Anguilla rostrata</i>	7/11/06	1	200	19	0	-	17.8 – 20.4
American eel (glass)	<i>Anguilla rostrata</i>	8/6/07	20	200	134	0	-	19.6 – 20.6
brook trout	<i>Salvelinus fontinalis</i>	7/11/06	17	150	58	0	-	17.9 – 18.6
Atlantic salmon parr*	<i>Salmo salar</i>	7/11/06	2	150	36	11	0.23	17.8 – 18.4
brown trout*	<i>Salmo trutta</i>	7/11/06	30	200	19	3	0.14	17.9 – 18.6
rainbow trout	<i>Oncorhynchus mykiss</i>	7/11/06	4	30	26	0	-	16.4 – 20.0
golden shiner	<i>Notemigonus crysoleucas</i>	5/18/07	4	100	81	0	-	18.6 – 20.2
central stoneroller	<i>Campostoma anomalum</i>	8/6/07	4	230	94	0	-	19.8 – 20.6
cutlips minnow	<i>Exoglossum maxillingua</i>	5/19/06	5	150	91	0	-	17.3 – 17.9
blacknose dace	<i>Rhinichthys atratulus</i>	2/2/06	10	150	12	0	-	17.6 – 18.3
longnose dace	<i>Rhinichthys cataractae</i>	2/2/06	6	150	103	0	-	17.6 – 18.3
common shiner	<i>Luxilis cornutus</i>	8/6/07	3	250	33	0	-	19.7 – 20.6
spotfin shiner	<i>Cyprionella spiloptera</i>	2/2/06	9	150	44	0	-	17.6 – 18.3
bluntnose minnow	<i>Pimephales notatus</i>	7/11/06	15	125	23	0	-	17.8 – 18.3
white sucker	<i>Catostomus commersoni</i>	1/18/06	19	450	48	0	-	17.6 – 18.4
northern hog sucker	<i>Hypentelium nigricans</i>	5/19/06	3	150	29	0	-	17.3 – 17.9
channel catfish	<i>Ictalurus punctatus</i>	5/18/07	4	100	30	0	-	18.6 – 19.3
margined madtom	<i>Noturus insignis</i>	8/6/07	12	200	66	0	-	17.6 – 19.7
banded killifish*	<i>Fundulus diaphanus</i>	5/19/06	6	150	24	2	0.08	17.3 – 17.9
slimy sculpin*	<i>Cottus cognatus</i>	2/2/06	6	150	9	80	0.90	17.6 – 18.3
mottled sculpin*	<i>Cottus bairdi</i>	5/23/07	4	200	22	9	0.41	17.8 – 19.3
striped bass*	<i>Morone saxatilis</i>	8/6/07	3	230	6	23	0.79	18.6 – 19.3

Table 12 continued.

Common name	Scientific name	Date infected	Number of fish exposed	Number glochidia used in infections	Number shed glochidia	Number transformed juvenile mussels	Transformation success	Water temperature range (°C)
white crappie	<i>Poxomis annularis</i>	5/18/07	5	100	60	0	-	18.8 – 19.5
rock bass	<i>Ambloplites rupestris</i>	7/11/06	5	200	63	0	-	17.8 – 18.3
largemouth bass	<i>Micropterus salmoides</i>	5/18/07	5	100	53	0	-	18.9 – 19.5
smallmouth bass	<i>Micropterus dolomieu</i>	8/6/07	1	200	8	0	-	18.5 – 19.7
bluegill sunfish	<i>Lepomis macrochirus</i>	5/18/07	5	100	64	0	-	18.9 – 19.4
pumpkinseed	<i>Lepomis gibbosus</i>	8/6/07	4	230	46	0	-	18.6 – 20.3
yellow perch	<i>Perca flavescens</i>	5/18/07	4	100	46	0	-	18.8 – 19.5
shield darter*	<i>Percina peltata</i>	1/18/06	20	600	213	41	0.16	17.6 – 18.4
tessellated darter*	<i>Etheostoma olmstedi</i>	5/23/07	27	750	29	23	0.44	17.1 – 19.5
banded darter	<i>Etheostoma zonale</i>	8/6/07	5	230	16	0	-	17.5 – 19.5
control (no fish)	-	2/2/06	0	150	110	0	-	17.6 – 18.3

Non-host fish

I tested a number of fishes that did not produce juvenile *A. heterodon* (Table 12). Among them were longnose dace (*Rhinichthys cataractae*), blacknose dace (*Rhinichthys atratulus*), pumpkinseed sunfish (*Lepomis gibbosus*), yellow perch (*Perca flavens*), golden shiner (*Notemigonus crysoleucas*) and the margined madtom (*Noturus insignis*), which have been shown to act as hosts for the mussel *Alasmidonta varicosa* that is of the same genus and often co-occurs with *A. heterodon* (Wicklow 2005). The northern hog sucker (*Hypentelium nigricans*) and white sucker (*Catostomus commersoni*), known hosts for the mussel *A. marginata* (Clarke 1981), also tested negative. The banded darter (*Etheostoma zonale*), although it is included in the same genus *Etheostoma* as the known *A. heterodon* hosts tessellated darter and johnny darters, did not produce any juvenile mussels. Additional species found regularly at Delaware River *A. heterodon* sites in this study, including sea lamprey ammocoetes, American eels, smallmouth bass, bluegill sunfish, bluntnose minnows, and cutlip minnows, also tested negative as hosts. Although Atlantic salmon parr and brown trout tested positive as hosts (these two particular species are most closely related of salmonids tested in this study, both being of the genus *Salmo*), rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*) did not produce any transformed juvenile *A. heterodon*.

Natural host identification

A total of 498 individuals representing 13 fish species were collected from *A. heterodon* sites on the Delaware River in March and May 2006 (Table 13), and none were infected with glochidia of *A. heterodon* or any other mussel species. At these sites, *A. heterodon* and other mussels, with the exception of the common *Elliptio complanata*, occur at low densities (W. Lellis, USGS, personal communication) and incidence of natural infection by these mussels might have been too low to detect.

Table 13. Summary of fish collected during March and May 2006 to detect natural infections of fish by *Alasmidonta heterodon* at two locations in the upper Delaware River mainstem.

Date	3/11	3/24	5/10	5/29	TOTAL
Location	Site 2	Site 2	Site 2	Site 1	
sea lamprey ammocoetes	--	37	23	--	60
American eel	--	--	--	5	5
blacknose dace	15	--	--	6	21
longnose dace	10	--	--	--	10
cutlip minnow	8	--	4	19	31
bluntnose minnow	17	21	--	--	38
white sucker	--	--	15	--	15
marginated madtom	16	--	14	15	45
banded killifish	--	10	--	--	10
rock bass	9	--	6	--	15
Pumpkinseed	--	--	3	--	3
tessellated darter	14	32	28	33	107
shield darter	25	22	16	41	104
YOY cyprinids	12	22	--	--	34

Naturally infected tessellated darters were found in the Flat Brook in mid-April 2006 and in the Ashuelot River during early May 2006. Incidence of infection among Ashuelot River fish was notably higher than that of the Flat Brook. Darters from both locations were infected primarily with glochidia of *A. heterodon*. A total of 70 tessellated darters collected from the Flat Brook in mid-April yielded 16 *A. heterodon* juvenile mussels. A total of 65 darters collected from the Ashuelot River in early May were heavily infected, yielding a total of 254 transformed *A. heterodon* juvenile mussels and 3 *Strophitus undulatus* juveniles (Table 14). In both the Ashuelot River and the Flat Brook, *A. heterodon* is the predominant mussel species occurring that is known to infect darters. *Elliptio complanata* is quite common in both systems but has never been shown to parasitize tessellated darters (Lellis et al. 2001; Matteson 1948).

Morphometric and meristic analysis of tessellated darters

In plots of sheared second principal components of morphometric data versus the first principal components of meristic data for tessellated darters, the minimum polygon cluster for darters from the Upper Ammonoosuc River did not overlap with those of Upper Delaware River and Pine

Table 14. Summary of numbers of transformed juvenile *Alasmidonta heterodon* recovered from naturally infected tessellated darters collected from the Ashuelot River (NH) and Flat Brook (NJ) during spring 2006. All were *A. heterodon* glochidia with the exception of 3 individuals from the Flat Brook, identified as *Strophitus undulatus*.

Date siphoned	No. juvenile mussels by location	
	Ashuelot River	Flat Brook
5/15/06	--	4
5/17/06	--	4
5/18/06	--	3
5/20/06	72	2
5/23/06	--	--
5/24/06	121(3)**	--
5/26/06	5*	--
5/28/06	4*	--
5/30/06	55	1
6/1/06	0	2
Total	257	16
*denotes transformed juveniles found attached to gills of deceased fish. **3 of 121 glochidia recovered were <i>Strophitus undulatus</i> rather than <i>A. heterodon</i>		

Creek. Clusters for the Upper Delaware River and Pine Creek, however, did overlap considerably (Figure 16). This indicates that the Upper Ammonoosuc River tessellated darter population is somewhat different from both the Upper Delaware and Pine Creek populations, but that fish from the Upper Delaware and Pine Creek are not different from one another. Overall differences in morphometric measurements among fish from the three locations was not statistically different ($p > 0.05$, MANOVA)(Table 15a); however meristic data did vary significantly among the three populations ($p < 0.05$, MANOVA)(Table 15b). Mean values and ranges of all morphometric measurements and meristic counts conducted are summarized in Table 16. Results from Duncan's multiple range test indicate that meristic data are significantly different among darters from each of the three locations (Table 17). These findings, in particular the difference indicated by the distinct separation between minimum polygon clusters between the Upper Ammonoosuc River and those of the other two locations, indicate that tessellated darters from the Upper Ammonoosuc may be phenotypically different from other populations. These results complement findings from tessellated darter host suitability tests that indicate darters from Upper Ammonoosuc River are different in that they serve as somewhat better hosts for *A. heterodon* than fish from the upper Delaware River or Pine Creek.

Table 15. Results of MANOVA comparing a) second sheared principal components (morphometric data) and b) first principal components (meristic data) for tessellated darters from the Upper Ammonoosuc, upper Delaware River and Pine Creek. Statistically significant values are highlighted in bold.

a)

Source	DF	SS	MS	F	P
Model	2	0.43504046	0.21752023	1.97	0.1512
Error	47	5.19730077	0.11058087		
Corrected Total	49	5.63234123			

b)

Source	DF	SS	MS	F	P
Model	2	34.88636654	17.44318327	58.09	<0.0001
Error	47	14.11355007	0.30028830		
Corrected Total	49	48.99991661			

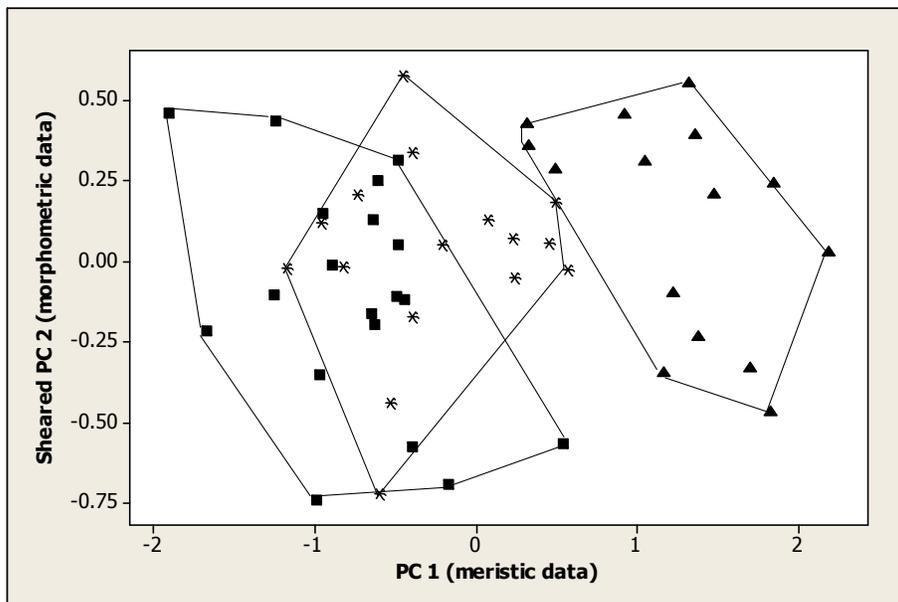


Figure 16. Plot of first principal component (meristic data) and second sheared principal component (morphometric data) for tessellated darters from Pine Creek in the Susquehanna River basin (squares), the upper Delaware River mainstem (stars) and the Upper Ammonoosuc River in the upper Connecticut River basin (triangles).

Table 16. Values of morphometric measurements and meristic counts of tessellated darters from the Upper Ammonoosuc River (Upper Connecticut River basin), upper Delaware River (Delaware River basin) and Pine Creek (Susquehanna River basin). Abbreviation descriptions are included in Table 3.

Character	Upper Ammonoosuc River <i>n</i> = 15		Upper Delaware River <i>N</i> = 16		Pine Creek <i>n</i> = 19	
	Mean(mm)	Range (mm)	Mean (mm)	Range (mm)	Mean (mm)	Range (mm)
SL	45.72	37.20 – 52.02	43.46	34.14 – 53.29	42.91	34.01 – 52.73
HL	12.99	10.36 – 14.96	12.69	9.94 – 15.08	12.40	9.74 – 14.66
SNL	5.90	4.47 – 7.09	5.72	4.53 – 6.66	6.03	4.62 – 7.04
POHL	7.40	5.89 – 8.88	7.09	5.69 – 7.71	6.94	5.42 – 8.92
HED	2.84	2.46 – 3.37	2.94	2.53 – 3.30	2.86	2.32 – 3.42
VED	2.62	2.15 – 3.28	2.71	2.30 – 2.99	2.62	2.07 – 3.09
PRE	3.29	2.58 – 4.15	3.59	2.68 – 4.31	3.62	2.49 – 4.73
CD	1.18	0.91 – 1.50	1.32	1.05 – 1.67	1.17	0.51 – 1.98
LJL	2.83	2.19 – 3.56	2.85	2.19 – 3.28	2.72	1.71 – 3.42
HD	6.01	4.85 – 7.29	5.92	4.48 – 6.71	6.13	4.41 – 7.84
BD	8.74	6.59 – 11.02	8.12	7.16 – 9.62	7.03	4.88 – 8.87
SNDOR	15.84	12.93 – 18.18	15.41	12.72 – 17.89	14.84	11.39 – 17.72
SNPEL	13.51	10.93 – 15.37	13.12	12.72 – 15.43	13.41	10.28 – 16.79
DFBL1	10.69	8.01 – 12.88	9.94	7.71 – 12.80	10.22	7.89 – 12.77
BTDF	1.24	0.64 – 1.94	1.09	0.00 – 1.70	0.81	0.00 – 1.47
DFBL2	12.63	9.63 – 15.12	11.51	8.91 – 14.23	11.96	8.78 – 16.73
TDFBL	24.67	18.85 – 28.32	22.51	17.38 – 27.42	23.34	18.61 – 29.19
AD1AA	15.65	11.05 – 18.70	15.65	11.09 – 18.66	14.79	11.13 – 18.71
AD2AA	7.13	5.29 – 9.05	7.25	4.35 – 9.15	6.52	4.18 – 8.72
AD1PA	24.22	18.20 – 27.94	21.54	17.55 – 24.94	22.09	16.56 – 28.22
AD2PA	12.98	10.21 – 15.23	11.19	8.47 – 14.63	11.46	7.70 – 14.95
PD1AA	7.57	5.67 – 9.84	7.52	4.81 – 9.13	6.74	4.60 – 8.72
PD2AA	12.25	8.84 – 14.39	10.74	8.63 – 14.56	11.74	8.42 – 15.76
PD1PA	13.99	10.85 – 15.83	12.17	9.67 – 13.92	12.23	8.63 – 15.49
PD2PA	4.41	3.36 – 5.49	5.03	3.02 – 7.58	5.02	3.72 – 7.29
PD1VC	20.43	16.52 – 23.48	19.07	14.73 – 22.74	18.58	14.52 – 22.92
PD2VC	6.16	4.24 – 7.49	6.60	3.79 – 8.71	5.43	3.91 – 6.79
PADC	8.22	6.89 – 9.73	8.65	5.93 – 12.09	8.03	6.05 – 10.81
AD1PL	8.67	5.81 – 10.83	7.87	4.73 – 9.66	6.89	5.18 – 9.39
AD2PL	15.22	11.20 – 18.55	14.77	9.56 – 23.77	13.07	10.01 – 16.23
PD1PL	14.15	10.11 – 17.29	13.04	8.67 – 16.64	12.08	9.60 – 15.21
PD2PL	26.15	21.11 – 29.81	23.83	17.08 – 27.11	23.68	17.43 – 28.82
CPL	7.65	5.59 – 8.79	7.57	5.39 – 10.71	7.29	6.18 – 10.35
LCPD	3.62	2.78 – 3.92	3.76	2.31 – 4.93	3.83	2.53 – 5.07

Meristics	Mean	Range	Mean	Range	Mean	Range
DSPINES	9	8-10	9	8-9	9	8 – 10
DRAYS	14	13 - 15	14	13-15	15	13 – 16
ARAYS	9	9 - 10	10	8-11	10	9 – 11
PLRAYS	7	6 - 7	6	6-7	6	6
PCRAYS	13	12 - 13	13	12-13	13	12 – 14
LLS	48	42 - 53	49	43-54	48	45 – 53
GR	7	4 - 8	6	5-7	6	5 – 6

Table 17. Results of Duncan's Multiple Range test comparing a) second sheared principal components (morphometric data) and b) first principal components (meristic data) for tessellated darters from the Upper Ammonoosuc, upper Delaware River and Pine Creek.

a)

Duncan grouping	Mean	N	Darter source
A	0.1174	15	Upper Ammonoosuc River
A	0.0185	16	Upper Delaware River
A	-0.1083	19	Pine Creek

b)

Duncan grouping	Mean	N	Darter source
A	1.2372	15	Upper Ammonoosuc River
B	-0.2640	16	Upper Delaware River
C	-0.7544	19	Pine Creek

DISCUSSION

Host suitability of tessellated darters

Fish host specificity is known to vary among mussel species, with some mussels being highly host-specific (e.g., *Alasmidonta atropurpurea* (Gordon and Layzer 1993) is presumed to parasitize only the northern hog sucker) and others being host generalists (e.g., *Strophitus undulatus* (Van Snik Gray et al. 2002) and *Pyganodon grandis* (Hoggarth 1992) are each known to parasitize a rather taxonomically diverse array of more than 20 fish species throughout their ranges). Until recently, however, it had been presumed that known fish hosts for a certain mussel are likely to act as hosts for that mussel wherever the two co-occur. Neither host specificity in mussels nor host suitability in fish had been shown to vary among subspecies or populations. Recent studies have demonstrated that closely related fish species or populations of the same species may show variability in their suitability as hosts for a given mussel.

Riusech and Barnhart (2000) determined that two closely related mussel species from the Ozark Plateaus, *Venustaconcha pleasii* and *Venustaconcha ellipsiformis*, exhibit different levels transformation success when introduced to rainbow darters (a darter species known to co-occur only with *V. pleasii* but not *V. ellipsiformis*). *Venustaconcha pleasii* was observed to infect sympatric rainbow darters in the laboratory much more successfully (transformation success values of 31%) than two distinct populations of *V. ellipsiformis* (with transformation success values of 3% and 6%).

Rogers et al. (2001) were the first researchers to evaluate population-level differences in host suitability, determining that fantail darters from four different populations exhibited different levels of host suitability for the mussel *Epioblasma florentina walkeri* in the Clinch River system in Virginia. Fantail darters that occurred sympatrically with *E. f. walkeri* (Indian River, VA) produced significantly more transformed juveniles per fish in laboratory infection trials than darters from a drainage that is geographically isolated from the Indian River (Roanoke River, VA). Fantail darters from non-contiguous drainages not entirely isolated from the Indian River (where *E. f. walkeri* occurs) produced fewer juveniles per fish than those from the Indian River, but more than those

from the Roanoke River, furthest isolated from the mussel source. The authors suggest that coadaptation between highly sympatric mussels and fish has led to the greatest degree of compatibility between them and that increased isolation of a fish host population from the mussel source results in its reduced host suitability.

Based on findings of Rogers et al. (2001) and Riusech and Barnhart (2000) that sympatric mussel and fish host pairs are more compatible than those populations which are isolated from one another, I expected that tessellated darters from the Upper Ammonoosuc River, near the upper Connecticut River *A. heterodon* source, would serve as more suitable hosts, as measured by numbers of transformed juvenile mussels produced and transformation success, than darters from other locations. I also expected that host suitability of tessellated darters would decrease successively as degree of isolation of each fish source from the mussel source increased. I observed patterns in numbers of juveniles produced per fish by location that were somewhat consistent with this expectation; however my results were not entirely conclusive.

Darters from the Upper Ammonoosuc River, located closest to the mussel source, did produce the highest numbers of transformed juveniles per fish of any from the five test locations. In comparing darters that were not previously infected in the wild (from three locations in three separate major river basins: Upper Ammonoosuc River in the Connecticut basin, Delaware River in the Delaware basin and Pine Creek in the Susquehanna basin), I observed that host suitability of tessellated darters (measured as numbers of juvenile mussels produced per fish) decreased as the relative degree of isolation of each darter source from the mussel source increased. While numbers of juvenile mussels produced per fish among these three locations were not significantly different overall, pairwise comparisons showed that numbers of juvenile mussels produced by darters from the Upper Ammonoosuc River, closest to the mussel source, were significantly greater than those produced by darters from Pine Creek, located furthest from the mussel source.

Other findings, however, were not consistent with my expectation that increased isolation from the mussel source would result in reduced host suitability. Fish from all four non-Upper Ammonoosuc River locations exhibited very little difference in the numbers of juvenile mussels they produced despite their varying relative distances and degrees of isolation from the upper

Connecticut River mussel source. Of these four fish sources, I expected that darters from the Ashuelot River, located within the same major river basin as the upper Connecticut River mussel source, would be more suitable hosts than fish from any locations in the Delaware and Susquehanna River basins, but this was not the case. Admittedly, prior exposure to *A. heterodon* glochidia may have reduced host suitability of Ashuelot River darters (Rogers and Dimock 2003).

It is possible that relative similarity in fish host suitability of tessellated darters among at least four of the five test locations (excluding the Upper Ammonoosuc River) may be related to the general lack of phylogenetic variation (and therefore immune response to glochidiosis) among populations of the tessellated darter in the Northeast. Rogers et al (2001) observed variation in host suitability among fantail darter populations for the mussel *E. f. walkeri*; however the fantail darter is more rare than the tessellated darter. Tessellated darters are highly abundant and ubiquitous throughout their range, making potential for gene flow among populations quite high. Populations of fantail darters tested by Rogers et al. (2001), presumably having been somewhat isolated from one another over time, were likely more genetically distinct from one another than tessellated darter populations tested in this study.

Given that variation in host suitability may be related to phylogenetic relationships among fish, it may be important to further examine potential differences among populations of hosts, such as the tessellated darter, throughout the *A. heterodon* range. Cole (1967) identified four subspecies of the tessellated darter to occur in the Atlantic slope drainage: *Etheostoma olmstedi olmstedi*, *E. o. atromaculatum*, *E. o. vexillare*, and *E. o. maculaticeps*. All but *E. o. maculaticeps* occur at some location within the current range of *A. heterodon* and may act as hosts, and the subspecies *E. o. olmstedi* predominates in the Connecticut, Susquehanna and upper Delaware River basins. It is unknown precisely how tessellated darters of any subspecies may exhibit specific immune response for glochidia of *A. heterodon*; however it is reasonable to assume that most darters collected for this study, with the possible exception of the Upper Ammonoosuc River (as Cole did not survey darters from the upper Connecticut River basin) would likely be of the subspecies *E. o. olmstedi*, according to the Cole's (1967) criteria. Darters of the same subspecies

would be more likely to exhibit similar innate immune response to glochidial infections by *A. heterodon*.

Results of morphometric and meristic analyses of tessellated darter populations in this study (from the Upper Ammonoosuc River, upper Delaware River, and Pine Creek) indicate that these populations, particularly the Upper Ammonoosuc River, may be phenotypically distinct from one another. Whether any phylogenetic difference occurs among these populations or at what level such differentiation might occur (e.g. among subspecies, populations or some other phylogenetic unit) remains unclear; however, it may be important to note that in both the laboratory host suitability trials and morphometric and meristic analysis of these three tessellated darter populations, fish from the Upper Ammonoosuc River appears to be somewhat distinct from the other populations. Further study of tessellated darter populations in the Northeast may be warranted.

As host suitability may vary among fish populations, host specificity in mussels may also be expected to vary by population. Such variation among *A. heterodon* populations may be important, as it has recently been determined that *A. heterodon* from the upper Connecticut River are somewhat genetically distinct from other populations throughout Mid-Atlantic and southern portions its range (King et al. 2004). *Alasmidonta heterodon* in the upper Connecticut River mainstem also occupy habitat that is markedly different from that of *A. heterodon* in the Delaware River basin (personal observation). In Delaware River basin sites, *A. heterodon* generally occurs at depths of less than 1m in sand and gravel substrate, mussels in the upper Connecticut occur in 0.5m-thick muddy substrate along steep banks as well as greater depths of as much as 3m in coarse gravel substrate. To what degree any differences among these populations may be reflected in tessellated darter host use by the upper Connecticut mussel population is unknown, but could potentially be related to more successful use of sympatric tessellated darter hosts from a nearby source (Upper Ammonoosuc River) by Connecticut River *A. heterodon*.

It has been shown that certain patterns in distribution of mussels can be attributed, at a basin-level geographic scale, to distribution of host fish (Haag and Warren 1998; Watters 1992) and that geographic distance and degree of isolation in the form of drainage interconnectedness have

been shown to relate to structure of mussel and fish host communities (Vaughn and Taylor 2000). Differences in compatibility between tessellate darter and *A. heterodon* populations among major drainage basins evaluated in this study could reasonably be attributed to their long term isolation since the last glaciation. Beyond these major drainage divisions, it is difficult to determine exactly to what degree populations may have been historically isolated from one another. First, effects of geographic distance on relationships between populations may vary depending on landscape characteristics and the extent of geographic barriers (Vaughn and Taylor 2000). Second, it is likely that though they are linked in a linear fashion within in the same drainage system, populations of *A. heterodon* and other rare mussels may still remain isolated when dispersal is low (Strayer et al. 1996). It is uncertain, for example, whether *A. heterodon* at their three known locations in the Delaware River mainstem should be considered three separate populations or one single population. It is also unclear to what degree mussels in the mainstem may have been isolated from those in the Flat Brook. Despite these uncertainties, it seems that *A. heterodon* from the genetically distinct upper Connecticut River population, which is located at the limit of the *A. heterodon* range, are capable of parasitizing darters from a number of locations in three major river basins, and that functional compatibility of distinct mussel populations and their hosts has not been affected significantly by isolation of fish or mussel populations.

Although immune resistance is thought to be the principal factor controlling host use in mussels, it may also be important to consider how certain environmental variables might affect fish host suitability. Despite genetic similarity among fish populations, phenotypic plasticity in fish related to various environmental variables may affect their capacity for resistance, and therefore, host suitability. For example, resistance of freshwater snails to trematode parasites has been shown to be related to diet, host morphology and growth, all of which may be controlled by various environmental factors (Sandlord and Minchella 2004). The authors suggest that the snails alter other life history traits to overcome reductions in fitness that might result from infection. No such evaluation of such effects on mussel-fish host relationships has been completed to date; however, similar environmentally based changes in fish could potentially affect their host suitability. If environmental conditions such as predation pressure or availability of resources vary among fish

populations, behavioral or physiological responses of fish to these conditions might affect their suitability as hosts without directly changing the genetic basis for immune resistance. In such a case, patterns in host suitability among various fish populations would be difficult to characterize, particularly by the traditional laboratory methods used in this study to evaluate host suitability, and it is possible that patterns of host suitability observed among fish populations in laboratory experiments might not provide a complete picture of host-parasite interactions as they occur in the wild.

Other potential fish hosts for *A. heterodon* in the Delaware River basin

My findings indicate that *A. heterodon* are capable of parasitizing a broader range of fish hosts than was once thought, although not all of these potential hosts may act as natural hosts in the wild. Tessellated and shield darters appear to be the most likely natural hosts for *A. heterodon*, particularly in the Delaware basin. Both darter species are documented throughout the basin (Mihursky 1962), and I have observed them to occur at all Delaware basin *A. heterodon* sites I have surveyed during the last several years (e.g., Delaware River main stem, Flat Brook and Neversink River). While slimy and mottled sculpins and juvenile Atlantic salmon may serve as excellent hosts in the laboratory, they rarely co-occur with *A. heterodon* in the wild (in particular in the Delaware basin) (personal observation), and therefore, are unlikely to be natural hosts. The Flat Brook is one exception where mottled sculpins have been observed to occur near *A. heterodon* locations; however, tessellated darters seem to be much more common. Atlantic salmon and striped bass were once present in the Delaware basin (Mihursky 1962) and may historically have served as hosts. If striped bass populations in the upper Delaware continue to recover, it is possible that at some point they might serve as hosts; however, no striped bass have been observed to date at any *A. heterodon* sites in the upper Delaware River basin (personal observation). Other species I tested did not yield sufficient numbers of juveniles to be considered viable natural hosts (e.g., brown trout and banded killifish), although both are known to co-occur with *A. heterodon*. The johnny darter (*Etheostoma nigrum*), previously identified as a host by Michaelson and Neves (1995) in North Carolina, does not occur in the upper Delaware mainstem. Although this species would quite

possibly have served as a host for *A. heterodon* in the laboratory had we tested it, it would not be capable of serving as a host for this mussel in the upper Delaware River.

While capture and identification of naturally infected hosts in the upper Delaware River mainstem might have elicited more information about relative host suitability of tessellated darters and other potential hosts at these sites, I was unable to capture any fish from these locations that were naturally infected with glochidia of *A. heterodon* or any other mussel species. As the common and abundant mussel species *Elliptio complanata* co-occurs with *A. heterodon* at these sites, I expected that known hosts of *E. complanata*, yellow perch (Matteson 1948) and American eel (Lellis et al. 2001), at these sites might be naturally infected with *E. complanata* glochidia; however no yellow perch were found and only 4 American eels were collected, none of which were infected with glochidia of any mussel species. *Elliptio complanata* is known to spawn and release glochidia at temperatures above 15°C (W. Lellis, USGS, personal communication), and so it is unlikely that any fish, even those such as the American eel capable of acting as hosts for this common mussel, would be infected with glochidia between March and May, when collections were conducted for this study to detect natural *A. heterodon* infections.

It is likely that low mussel densities at sites in the upper Delaware River, as well as relative absence of gravid female mussels, result in low levels of infection that made naturally infected fish difficult to detect. Tessellated darters that I collected from other locations where *A. heterodon* occurs at higher densities (Ashuelot River and Flat Brook) during spring 2006 were infected with *A. heterodon*. McLain and Ross (2005) found that incidence of infection at *A. heterodon* sites on the Mill River in Massachusetts was highly correlated with proportions of gravid females and overall mussel abundances at each site, with incidence of infection being quite low at locations where abundance was reduced and few gravid mussels could be found. To evaluate if such correlations might occur among *A. heterodon* populations in this study and to potentially account for the lack of infected fish and gravid females in the Delaware River mainstem, I compared both qualitative catch per unit effort (CPUE) data and quantitative data available from the Ashuelot River, Flat Brook and upper Delaware River mainstem.

Quantitative mussel surveys conducted in the Ashuelot River in 2003 (Nedeau 2004) and the Delaware River in 2000 and 2002 (W. Lellis, USGS, personal communication) predict much higher abundances in the Ashuelot than in the Delaware River. In a 2004 survey, abundance estimates for *A. heterodon* in two 50m reaches of the Ashuelot River were 989 (90% CI: 713,1366) and 464 (90% CI: 295,730) respectively. Abundance values generated in a 2002 survey of two sections of the highest known density *A. heterodon* site on the upper Delaware were comparable in value (1095 (90% CI: 466, 2407) and 597 (90% CI: 295, 1210)); however, two 200m reaches were sampled in the Delaware survey rather than two 50m reaches surveyed in the Ashuelot. The Delaware is also a much larger river than the Ashuelot in general, with widths of at least 100m near the mussel site, while the Ashuelot is only 20m wide. This indicates *A. heterodon* density in these two Ashuelot sites may be at least 4 times greater than at sites surveyed in the Delaware.

Extensive quantitative surveys such as those conducted in the Ashuelot and Delaware have not been completed in the Flat Brook; however qualitative surveys conducted during summer 2006 (W. Lellis, USGS, personal communication) throughout the Flat Brook generated CPUE values ranging from 0.0 to a very high 13.0 mussels/search hour. No mussels were observed in the furthest upstream reaches of the Flat Brook where fish were collected for this study; however, one single *A. heterodon* was found approximately 300m downstream of this fish collection site, resulting in a CPUE of 0.95 mussels/search hour in that particular reach (155m in length). Within 1.3km downstream of the site, a CPUE of 4.57 mussels/search hour was measured, and in areas within 1.5-2km downstream of the fish collection site, catch per unit effort was measured from 7.33-13.00 mussels/search hour. Qualitative snorkel searches conducted in the Ashuelot River near the fish collection site as part of this study in 2005 yielded CPUE values of approximately 2.5 mussels/search hour. In recent qualitative searches conducted repeatedly at the highest density *A. heterodon* site on the Delaware River at Hankins, however, CPUE has never exceeded 1.16 mussels/search hour. At the Frisbie Island *A. heterodon* site in the upper Delaware, CPUE measured as part of snorkel searches for gravid mussels in this study was only 0.5 mussels/search hour. Consequently, densities of *A. heterodon* in the Delaware River may be low enough in general

to make natural infections in fish hosts very difficult to detect, particularly when low incidences of gravidity occur, as I also observed during this study.

It is possible that incidence of gravid mussels and infected fishes is low at these Delaware River sites overall, or perhaps that mussels only reproduce once every several years and did not reproduce and infect fish in 2006. Michaelson and Neves (1995) observed annual variation in gravidity of *A. heterodon* in North Carolina, where gravid mussels and infected fish were found at certain sites during one year but could not be found the following year. Furthermore, it has been shown that in some mussel species, in years when reproduction does occur, only a proportion of females become gravid. Only 64% of *Margaritifera margaritifera* females, for example, have been shown to become gravid in a given season (Bauer 1987). In any case, if reproduction in Delaware River *A. heterodon* may be limited generally or may occur only interannually, it may be important to monitor them regularly for gravidity, glochidial release, and infection success. Annual searches for gravid mussels and infected fish (darter species in particular) may indicate when detectable levels of reproduction and glochidia infection actually occur in the Delaware mainstem, and could be an important management tactic in long term monitoring and protection of Delaware River *A. heterodon* populations.

Observations of naturally infected tessellated darters collected during spring 2006 from the Ashuelot River and Flat Brook indicate that mechanisms for infection used in the wild by *A. heterodon* may be very different than those typically employed to infect fish in laboratory host infection trials. For this study, I infected all fish in baths of glochidia and observed during the experiment that they were infected primarily on their pectoral and pelvic fins, but rarely on the gills. In contrast, I observed that naturally infected fish I collected from the wild (Flat Brook and Ashuelot Rivers) were almost always infected on the gills. One fish I examined from the Ashuelot River carried a total of 26 glochidia on its gills but none on its fins. In the past, it has been assumed that *A. heterodon* attached primarily to the fin margins of their host fish and that these mussels were broadcast spawners, releasing glochidia somewhat indiscriminately which would then come into contact with hosts in a passive manner (McLain and Ross 2005; Michaelson and Neves 1995). This may be the case under some circumstances, as McLain and Ross (2005) observed female *A.*

heterodon releasing glochidia directly into the water column, even when no host fish were present. My observations suggest, however, that mechanisms of infection by *A. heterodon* in the wild may be different than the passive exposures I attempted to simulate in laboratory infections. Wicklow (1999) documented *A. heterodon* individuals in the laboratory displaying and undulating a small modified portion of their mantle flap in the presence of a host fish, presumably as a lure. High incidence of infection on the gills of naturally-infected fish observed in this study indicates that use of such a lure may be common among *A. heterodon*, at least in the Ashuelot River and the Flat Brook. It may be that *A. heterodon* are capable of infecting host fish more successfully and with greater intensity than was previously thought.

Concluding remarks

This study confirms that variation in host suitability among fish populations may be important in evaluating fish host use among mussel populations. While I observed a somewhat marginal degree of variation in host suitability among tessellated darter populations, fairly distinct differences observed in morphometric and meristic analysis as well as in host suitability of tessellated darters from one location, the Upper Ammonoosuc River, indicate that darters from this particular population may in fact be different from others in its range. The successive reduction in host suitability observed among fish from the Upper Ammonoosuc, Delaware River and Pine Creek as distance of each of these locations from the mussel source increased suggests that even among populations of an abundant and ubiquitous fish host such as the tessellated darter, fish host suitability may vary. It is less certain to what degree this variability among fish populations in the laboratory may take effect in the wild. Further field studies examining glochidial release, incidence of natural infection, and perhaps reproductive traits of adult mussel such as incidence of gravidity or fecundity may be important. Comparison of such characteristics among *A. heterodon* populations throughout its range may be useful in understanding mussel-fish host interactions as they occur in the wild.

My results also suggest that it also is important to examine differences in host use among populations of mussels such as *A. heterodon* throughout its range, as mussels from the Flat Brook

successfully parasitized a total of eight relatively taxonomically diverse fish species in this study, several of them new hosts. Although these results indicate that *A. heterodon* is less of a host specialist than was once thought, functional hosts in the wild may still be limited to just two of these eight species, the shield darter and tessellated darter, as they are the only fish which are likely to co-occur with *A. heterodon* at its known locations in the Delaware River basin in sufficient numbers to serve as suitable natural hosts.

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Appendix A

Field collection dates and numbers of fish collected for tessellated darter host suitability trials and morphometric and meristic analysis

Table A.1. Collection dates and numbers of fish collected from each of the five test locations for tessellated darter host suitability trials

Tessellated darter source	Collection date	Number tessellated darters collected
Upper Ammonoosuc River	5/20/05	30
	8/9/05	100
	11/26/05	0
	11/27/05	0
	11/28/05	0
	4/1/06	15
	4/2/06	23
	5/6/06	81
Ashuelot River	5/21/05	5
	8/10/05	36
	11/29/05	0
	3/31/06	0
	4/1/06	35
	4/2/06	30
	5/5/06	30
	5/6/06	35
Delaware River mainstem	4/17/05	20
	4/18/05	55
	3/11/06	50
Flat Brook	6/8/05	60
	11/23/05	0
	4/15/06	75
Pine Creek	11/20/05	5
	1/11/06	72

Table A.2. Summary of dates tessellated darters were collected for morphometrics and meristics analysis

Tessellated darter source	Collection date	Number tessellated darters collected
Upper Ammonoosuc River	5/9/07	25
Delaware River mainstem	5/22/07	22
Pine Creek	5/23/07	24

Appendix B

Summary of recorded and calculated values used in analysis of tessellated darter host suitability

Table B.1. Summary of recorded and calculated values for Upper Ammonoosuc River, Aquarium 1 in tessellated darter host suitability trials. Daily totals of shed *Alasmidonta heterodon* glochidia and fish remaining in each tank were used to generate numbers of shed glochidia generated per fish per day. Daily totals of transformed juvenile *A. heterodon* and remaining fish in each tank were used to generate numbers of transformed juveniles produced per fish per day.

<i>Upper Ammonoosuc River (Aquarium 1)</i>							
Date	Day of experiment	Shed glochidia	Trans-formed juveniles	Fish mortalities	Fish remaining	Mean shed glochidia per fish	Mean juveniles per fish
6/21/06	1	26	0	0	9	2.89	0
6/22/06	2	26	0	0	9	2.89	0
6/23/06	3	14	0	0	9	1.56	0
6/24/06	4	4	0	0	9	0.44	0
6/25/06	5	1	0	1	9	0.11	0
6/26/06	6	2	0	0	8	0.25	0
6/27/06	7	3	0	0	8	0.38	0
6/28/06	8	3	0	0	8	0.38	0
6/29/06	9	0	0	0	8	0	0
6/30/06	10	--	--	0	8	--	--
7/1/06	11	0	0	1	8	0	0
7/2/06	12	--	--	0	7	--	--
7/3/06	13	0	0	1	7	0	0
7/4/07	14	--	--	0	6	--	--
7/5/06	15	0	0	0	6	0	0
7/6/07	16	--	--	0	6	--	--
7/7/06	17	0	0	0	6	0	0
7/8/06	18	--	--	2	6	--	--
7/9/06	19	0	5	0	4	0	1.25
7/10/06	20	0	6	0	4	0	1.50
7/11/06	21	0	4	0	4	0	1.00
7/12/07	22	--	--	0	4	--	--
7/13/06	23	0	4	1	4	0	1.00
7/14/06	24	0	3	0	3	0	1.00
7/15/07	25	--	--	0	3	--	--
7/16/06	26	0	4	0	3	0	1.33
7/17/07	27	--	--	0	3	--	--
7/18/06	28	0	1	0	3	0	0.33
7/19/07	29	--	--	0	3	--	--
7/20/06	30	0	0	0	3	0	0
7/21/07	31	--	--	0	3	0	0
7/22/06	32	0	0	1	3	0	0
7/23/07	33	--	--	0	3	--	--
7/24/07	34	--	--	0	3	--	--
7/25/06	35	0	0	0	2	0	0
7/26/06	36	0	0	1	2	0	0
7/27/06	37	--	--	0	2	--	--
7/28/06	38	0	0	0	1	0	0
7/29/06	39	--	--	0	1	--	--
7/30/06	40	0	0	0	1	0	0
7/31/06	41	0	0	1	1	0	0
TOTAL						8.89	7.42

Table B.2. Summary of recorded and calculated values for Upper Ammonoosuc River, Aquarium 2 in tessellated darter host suitability trials. Daily totals of shed *Alasmidonta heterodon* glochidia and fish remaining in each tank were used to generate numbers of shed glochidia generated per fish per day. Daily totals of transformed juvenile *A. heterodon* and remaining fish in each tank were used to generate numbers of transformed juveniles produced per fish per day.

<i>Upper Ammonoosuc River (Aquarium 2)</i>							
Date	Day of experiment	Shed glochidia	Trans-formed juveniles	Fish mortalities	Fish remaining	Mean shed glochidia per fish	Mean juveniles per fish
6/21/06	1	19	0	0	9	2.11	0
6/22/06	2	7	0	1	9	0.78	0
6/23/06	3	32	0	0	8	4.00	0
6/24/06	4	6	0	0	8	0.75	0
6/25/06	5	0	0	0	8	0	0
6/26/06	6	2	0	0	8	0.25	0
6/27/06	7	2	0	1	8	0.25	0
6/28/06	8	0	0	0	7	0	0
6/29/06	9	0	0	0	7	0	0
6/30/06	10	--	--	0	7	--	--
7/1/06	11	3	0	0	7	0.43	0
7/2/06	12	--	--	0	7	--	--
7/3/06	13	0	0	0	7	0	0
7/4/07	14	--	--	--	--	--	--
7/5/06	15	0	4	2	7	0	0.57
7/6/07	16	--	--	--	--	--	--
7/7/06	17	0	0	0	5	0	0
7/8/06	18	--	--	0	5	--	--
7/9/06	19	0	4	0	5	0	0.80
7/10/06	20	0	24	0	5	0	4.80
7/11/06	21	0	18	0	5	0	3.60
7/12/07	22	--	--	0	5	--	--
7/13/06	23	0	5	0	5	0	1.00
7/14/06	24	0	1	2	5	0	0.20
7/15/07	25	--	--	0	3	--	--
7/16/06	26	0	2	2	3	0	0.67
7/17/07	27	--	--	0	1	--	--
7/18/06	28	0	3	1	1	0	3.00
7/19/07	29	--	--	0	0	--	--
7/20/06	30	0	0	0	0	0	0
7/21/07	31	--	--	0	0	--	--
7/22/06	32	0	0	0	0	0	0
7/23/07	33	--	--	0	0	--	--
7/24/07	34	--	--	0	0	--	--
7/25/06	35	0	0	0	0	0	0
7/26/06	36	0	0	0	0	0	0
7/27/06	37	--	--	0	0	--	--
7/28/06	38	0	0	0	0	0	0
7/27/06	39	--	--	0	0	--	--
7/30/06	40	0	0	0	0	0	0
7/31/06	41	0	0	0	0	0	0
TOTAL						8.57	14.64

Table B.3. Summary of recorded and calculated values for Upper Ammonoosuc River, Aquarium 3 in tessellated darter host suitability trials. Daily totals of shed *Alasmidonta heterodon* glochidia and fish remaining in each tank were used to generate numbers of shed glochidia generated per fish per day. Daily totals of transformed juvenile *A. heterodon* and remaining fish in each tank were used to generate numbers of transformed juveniles produced per fish per day.

Upper Ammonoosuc River (Aquarium 3)							
Date	Day of experiment	Shed glochidia	Trans-formed juveniles	Fish mortalities	Fish remaining	Mean shed glochidia per fish	Mean juveniles per fish
6/21/06	1	24	0	0	9	2.67	0
6/22/06	2	26	0	1	9	2.89	0
6/23/06	3	27	0	1	8	3.38	0
6/24/06	4	4	0	0	7	0.57	0
6/25/06	5	1	0	0	7	0.14	0
6/26/06	6	14	0	0	7	2.00	0
6/27/06	7	0	0	1	7	0	0
6/28/06	8	2	0	0	6	0.33	
6/29/06	9	0	0	0	6	0	0
6/30/06	10	--	--	0	6	--	--
7/1/06	11	0	0	1	6	0	0
7/2/06	12	--	--	0	5	--	--
7/3/06	13	1	0	0	5	0.20	0
7/4/07	14	--	--	0	5	--	--
7/5/06	15	0	0	0	5	0	0
7/6/07	16	--	--	0	5	--	--
7/7/06	17	0	0	0	5	0	0
7/8/06	18	--	--	0	5	--	--
7/9/06	19	0	9	1	5	0	1.80
7/10/06	20	0	5	0	4	0	1.25
7/11/06	21	0	7	0	4	0	1.75
7/12/07	22	--	--	0	4	--	--
7/13/06	23	0	1	0	4	0	0.25
7/14/06	24	0	1	2	4	0	0.25
7/15/07	25	--	--	0	2	--	--
7/16/06	26	0	0	1	2	0	0
7/17/07	27	--	--	0	1	--	--
7/18/06	28	0	4	1	1	0	4.00
7/19/07	29	--	--	0	0	--	--
7/20/06	30	0	0	0	0	0	0
7/21/07	31	--	--	0	0	--	--
7/22/06	32	0	0	0	0	0	0
7/23/07	33	--	--	0	0	--	--
7/24/07	34	--	--	0	0	--	--
7/25/06	35	0	0	0	0	0	0
7/26/06	36	0	0	0	0	0	0
7/27/06	37	--	--	0	0	--	--
7/28/06	38	0	0	0	0	0	0
7/29/06	39	--	--	0	0	--	--
7/30/06	40	0	0	0	0	0	0
7/31/06	41	0	0	0	0	0	0
TOTAL						12.18	9.30

Table B.4. Summary of recorded and calculated values for Ashuelot River, Aquarium 1 in tessellated darter host suitability trials. Daily totals of shed *Alasmidonta heterodon* glochidia and fish remaining in each tank were used to generate numbers of shed glochidia generated per fish per day. Daily totals of transformed juvenile *A. heterodon* and remaining fish in each tank were used to generate numbers of transformed juveniles produced per fish per day.

Ashuelot River (Aquarium 1)							
Date	Day of experiment	Shed glochidia	Trans-formed juveniles	Fish mortalities	Fish remaining	Mean shed glochidia per fish	Mean juveniles per fish
6/21/06	1	6	0	0	9	0.67	0
6/22/06	2	12	0	0	9	1.33	0
6/23/06	3	26	0	1	8	3.25	0
6/24/06	4	3	0	0	8	0.38	0
6/25/06	5	0	0	0	8	0	0
6/26/06	6	1	0	2	6	0.17	0
6/27/06	7	1	0	0	6	0.17	0
6/28/06	8	0	0	1	5	0	0
6/29/06	9	0	0	1	4	0	0
6/30/06	10	--	--	0	4	--	--
7/1/06	11	0	0	0	4	0	0
7/2/06	12	--	--	0	4	--	--
7/3/06	13	0	0	0	4	0	0
7/4/07	14	--	--	0	4	--	--
7/5/06	15	0	0	0	4	0	0
7/6/07	16	--	--	0	4	--	--
7/7/06	17	0	0	0	4	0	0
7/8/06	18	--	--	0	4	--	--
7/9/06	19	0	8	0	4	0	2.00
7/10/06	20	0	0	0	4	0	0
7/11/06	21	0	2	0	4	0	0.50
7/12/07	22	--	--	0	4	--	--
7/13/06	23	0	5	0	4	0	1.25
7/14/06	24	0	2	0	4	0	0.50
7/15/07	25	--	--	0	4	--	--
7/16/06	26	0	1	0	4	0	0.25
7/17/07	27	--	--	0	4	--	--
7/18/06	28	0	1	0	4	0	0.25
7/19/07	29	--	--	0	4	--	--
7/20/06	30	0	0	0	4	0	0
7/21/07	31	--	--	0	4	--	--
7/22/06	32	0	2	0	4	0	0.50
7/23/07	33	--	--	1	4	--	--
7/24/07	34	--	--	0	3	--	--
7/25/06	35	0	1	0	3	0	0.33
7/26/06	36	0	0	0	3	0	0
7/27/06	37	--	--	0	3	--	--
7/28/06	38	0	0	0	3	0	0
7/29/06	39	--	--	0	3	--	--
7/30/06	40	0	0	0	3	0	0
7/31/06	41	0	0	0	3	0	0
TOTAL						5.96	5.58

Table B.5. Summary of recorded and calculated values for Ashuelot River, Aquarium 2 in tessellated darter host suitability trials. Daily totals of shed *Alasmidonta heterodon* glochidia and fish remaining in each tank were used to generate numbers of shed glochidia generated per fish per day. Daily totals of transformed juvenile *A. heterodon* and remaining fish in each tank were used to generate numbers of transformed juveniles produced per fish per day.

Ashuelot River (Aquarium 2)							
Date	Day of experiment	Shed glochidia	Trans-formed juveniles	Fish mortalities	Fish remaining	Mean shed glochidia per fish	Mean juveniles per fish
6/21/06	1	54	0	0	9	6.00	0
6/22/06	2	15	0	0	9	1.67	0
6/23/06	3	17	0	1	9	1.89	0
6/24/06	4	2	0	0	8	0.25	0
6/25/06	5	0	0	0	8	0	0
6/26/06	6	4	0	0	8	0.50	0
6/27/06	7	0	0	0	8	0	0
6/28/06	8	0	0	0	8	0	0
6/29/06	9	2	0	0	8	0.25	0
6/30/06	10	--	--	0	8	--	--
7/1/06	11	0	0	2	8	0	0
7/2/06	12	--	--	0	6	--	--
7/3/06	13	0	0	0	6	0	0
7/4/07	14	--	--	0	6	--	--
7/5/06	15	0	0	0	6	0	0
7/6/07	16	--	--	0	6	--	--
7/7/06	17	0	2	0	6	0	0.33
7/8/06	18	--	--	0	6	--	--
7/9/06	19	0	2	0	6	0	0.33
7/10/06	20	0	6	0	6	0	1.00
7/11/06	21	0	4	0	6	0	0.67
7/12/07	22	--	--	0	6	--	--
7/13/06	23	0	1	0	6	0	0.16
7/14/06	24	0	3	0	6	0	0.50
7/15/07	25	--	--	0	6	--	--
7/16/06	26	0	0	0	6	0	0
7/17/07	27	--	--	0	6	--	--
7/18/06	28	0	1	0	6	0	0.17
7/19/07	29	--	--	0	6	--	--
7/20/06	30	0	0	0	6	0	0
7/21/07	31	--	--	0	6	--	--
7/22/06	32	0	1	1	6	0	0.17
7/23/07	33	--	--	0	5	--	--
7/24/07	34	--	--	0	5	--	--
7/25/06	35	0	1	0	5	0	0.20
7/26/06	36	0	0	0	5	0	0
7/27/06	37	--	--	0	5	--	--
7/28/06	38	0	0	0	5	0	0
7/29/06	39	--	--	0	5	--	--
7/30/06	40	0	0	0	5	0	0
7/31/06	41	0	0	0	5	0	0
TOTAL						10.56	3.53

Table B.6. Summary of recorded and calculated values for Ashuelot River, Aquarium 3 in tessellated darter host suitability trials. Daily totals of shed *Alasmidonta heterodon* glochidia and fish remaining in each tank were used to generate numbers of shed glochidia generated per fish per day. Daily totals of transformed juvenile *A. heterodon* and remaining fish in each tank were used to generate numbers of transformed juveniles produced per fish per day.

Ashuelot River (Aquarium 3)							
Date	Day of experiment	Shed glochidia	Trans-formed juveniles	Fish mortalities	Fish remaining	Mean shed glochidia per fish	Mean juveniles per fish
6/21/06	1	14	0	0	9	1.56	0
6/22/06	2	32	0	1	8	4.00	0
6/23/06	3	10	0	1	7	1.42	0
6/24/06	4	3	0	0	7	0.43	0
6/25/06	5	0	0	0	7	0	0
6/26/06	6	0	0	0	7	0	0
6/27/06	7	0	0	0	7	0	0
6/28/06	8	2	0	0	7	0.29	0
6/29/06	9	0	0	0	7	0	0
6/30/06	10	--	--	0	7	--	--
7/1/06	11	2	0	0	7	0.29	0
7/2/06	12	--	--	0	7	--	--
7/3/06	13	0	0	0	7	0	0
7/4/07	14	--	--	0	7	--	--
7/5/06	15	0	0	0	7	0	0
7/6/07	16	--	--	0	7	--	--
7/7/06	17	0	1	0	7	0	0.14
7/8/06	18	--	--	0	7	--	--
7/9/06	19	0	5	0	7	0	0.71
7/10/06	20	0	6	0	7	0	0.86
7/11/06	21	0	7	0	7	0	1.00
7/12/07	22	--	--	0	7	--	--
7/13/06	23	0	7	0	7	0	1.00
7/14/06	24	0	3	0	7	0	0.43
7/15/07	25	--	--	0	7	--	--
7/16/06	26	0	0	0	7	0	0
7/17/07	27	--	--	0	7	--	--
7/18/06	28	0	1	0	7	0	0.14
7/19/07	29	--	--	0	7	--	--
7/20/06	30	0	1	0	7	0	0.14
7/21/07	31	--	--	0	7	--	--
7/22/06	32	0	2	0	7	0	0.29
7/23/07	33	--	--	0	7	--	--
7/24/07	34	--	--	0	7	--	--
7/25/06	35	0	0	0	7	0	0
7/26/06	36	0	0	0	7	0	0
7/27/06	37	--	--	1	7	--	--
7/28/06	38	0	0	0	6	0	0
7/29/06	39	--	--	0	6	--	--
7/30/06	40	0	0	0	6	0	0
7/31/06	41	0	0	0	6	0	0
TOTAL						7.98	4.71

Table B.7. Summary of recorded and calculated values for Delaware River mainstem, Aquarium 1 in tessellated darter host suitability trials. Daily totals of shed *Alasmidonta heterodon* glochidia and fish remaining in each tank were used to generate numbers of shed glochidia generated per fish per day. Daily totals of transformed juvenile *A. heterodon* and remaining fish in each tank were used to generate numbers of transformed juveniles produced per fish per day.

Delaware River mainstem (Aquarium 1)							
Date	Day of experiment	Shed glochidia	Trans-formed juveniles	Fish mortalities	Fish remaining	Mean shed glochidia per fish	Mean juveniles per fish
6/21/06	1	25	0	0	9	2.78	0
6/22/06	2	45	0	3	9	5.00	0
6/23/06	3	12	0	0	6	2.00	0
6/24/06	4	0	0	0	6	0	0
6/25/06	5	0	0	1	6	0	0
6/26/06	6	0	0	0	5	0	0
6/27/06	7	1	0	0	5	0.20	0
6/28/06	8	0	0	0	5	0	0
6/29/06	9	0	0	0	5	0	0
6/30/06	10	--	--	0	5	--	--
7/1/06	11	0	0	0	5	0	0
7/2/06	12	--	--	0	5	--	--
7/3/06	13	0	0	0	5	0	0
7/4/07	14	--	--	0	5	--	--
7/5/06	15	0	0	1	5	0	0
7/6/07	16	--	--	0	5	--	--
7/7/06	17	0	0	0	4	0	0
7/8/06	18	--	--	0	4	--	--
7/9/06	19	0	3	0	4	0	0.75
7/10/06	20	1	8	0	4	0.25	2.00
7/11/06	21	0	5	0	4	0	1.25
7/12/07	22	--	--	0	4	--	--
7/13/06	23	0	3	1	4	0	0.75
7/14/06	24	0	1	1	3	0	0.33
7/15/07	25	--	--	0	3	--	--
7/16/06	26	0	0	0	2	0	0
7/17/07	27	--	--	0	2	--	--
7/18/06	28	0	0	0	2	0	0
7/19/07	29	--	--	0	2	--	--
7/20/06	30	0	1	0	2	0	0.50
7/21/07	31	--	--	0	2	--	--
7/22/06	32	0	0	0	2	0	0
7/23/07	33	--	--	0	2	--	--
7/24/07	34	--	--	0	2	--	--
7/25/06	35	0	0	0	2	0	0
7/26/06	36	0	0	1	2	0	0
7/27/06	37	--	--	0	1	--	--
7/28/06	38	0	0	0	1	0	0
7/29/06	39	--	--	0	1	--	--
7/30/06	40	0	0	0	1	0	0
7/31/06	41	0	0	0	1	0	0
TOTAL						10.23	5.58

Table B.8. Summary of recorded and calculated values for Delaware River mainstem, Aquarium 2 in tessellated darter host suitability trials. Daily totals of shed *Alasmidonta heterodon* glochidia and fish remaining in each tank were used to generate numbers of shed glochidia generated per fish per day. Daily totals of transformed juvenile *A. heterodon* and remaining fish in each tank were used to generate numbers of transformed juveniles produced per fish per day.

<i>Delaware River mainstem (Aquarium 2)</i>							
Date	Day of experiment	Shed glochidia	Trans-formed juveniles	Fish mortalities	Fish remaining	Mean shed glochidia per fish	Mean juveniles per fish
6/21/06	1	18	0	0	9	2.00	0
6/22/06	2	13	0	1	9	1.44	0
6/23/06	3	22	0	0	8	2.75	0
6/24/06	4	3	0	1	8	0.38	0
6/25/06	5	0	0	0	7	0	0
6/26/06	6	1	0	0	7	0.14	0
6/27/06	7	3	0	0	7	0.43	0
6/28/06	8	0	0	0	7	0	0
6/29/06	9	0	0	0	7	0	0
6/30/06	10	--	--	0	7	--	--
7/1/06	11	0	0	0	7	0	0
7/2/06	12	--	--	0	7	--	--
7/3/06	13	0	0	1	7	0	0
7/4/07	14	--	--	0	6	--	--
7/5/06	15	0	1	0	6	0	0.17
7/6/07	16	--	--	0	6	--	--
7/7/06	17	0	0	0	6	0	0
7/8/06	18	--	--	0	6	--	--
7/9/06	19	0	2	0	6	0	0.33
7/10/06	20	0	3	0	6	0	0.50
7/11/06	21	0	13	0	6	0	2.17
7/12/07	22	--	--	0	6	--	--
7/13/06	23	0	8	0	6	0	1.33
7/14/06	24	0	3	0	6	0	0.5
7/15/07	25	--	--	0	6	--	--
7/16/06	26	0	4	1	6	0	0.67
7/17/07	27	--	--	0	5	--	--
7/18/06	28	0	0	0	5	0	0
7/19/07	29	--	--	0	5	--	--
7/20/06	30	0	1	0	5	0	0.20
7/21/07	31	--	--	0	5	--	--
7/22/06	32	0	3	1	5	0	0.60
7/23/07	33	--	--	0	4	--	--
7/24/07	34	--	--	0	4	--	--
7/25/06	35	0	0	0	4	0	0
7/26/06	36	0	0	0	4	0	0
7/27/06	37	--	--	0	4	--	--
7/28/06	38	0	0	0	4	0	0
7/29/06	39	--	--	0	4	--	--
7/30/06	40	0	0	0	4	0	0
7/31/06	41	0	0	0	4	0	0
TOTAL						7.14	6.47

Table B.9. Summary of recorded and calculated values for Delaware River mainstem, Aquarium 3 in tessellated darter host suitability trials. Daily totals of shed *Alasmidonta heterodon* glochidia and fish remaining in each tank were used to generate numbers of shed glochidia generated per fish per day. Daily totals of transformed juvenile *A. heterodon* and remaining fish in each tank were used to generate numbers of transformed juveniles produced per fish per day.

Delaware River mainstem (Aquarium 3)							
Date	Day of experiment	Shed glochidia	Trans-formed juveniles	Fish mortalities	Fish remaining	Mean shed glochidia per fish	Mean juveniles per fish
6/21/06	1	42	0	0	9	4.67	0
6/22/06	2	39	0	1	9	4.33	0
6/23/06	3	20	0	0	8	2.50	0
6/24/06	4	0	0	0	8	0	0
6/25/06	5	1	0	0	8	0.12	0
6/26/06	6	1	0	0	8	0.13	0
6/27/06	7	4	0	0	8	0.50	0
6/28/06	8	1	0	0	8	0.13	0
6/29/06	9	0	0	0	8	0	0
6/30/06	10	--	--	2	8	--	--
7/1/06	11	0	0	0	6	0	0
7/2/06	12	--	--	0	6	--	--
7/3/06	13	0	0	1	6	0	0
7/4/07	14	--	--	0	5	--	--
7/5/06	15	0	0	0	5	0	0
7/6/07	16	--	--	0	5	--	--
7/7/06	17	0	1	0	5	0	0.20
7/8/06	18	--	--	0	5	--	--
7/9/06	19	0	5	0	5	0	1.00
7/10/06	20	0	10	0	5	0	2.00
7/11/06	21	0	8	0	5	0	1.60
7/12/07	22	--	--	0	5	--	--
7/13/06	23	0	11	1	5	0	2.20
7/14/06	24	0	2	0	4	0	0.50
7/15/07	25	--	--	0	4	--	--
7/16/06	26	0	3	0	4	0	0.75
7/17/07	27	--	--	0	4	--	--
7/18/06	28	0	0	0	4	0	0
7/19/07	29	--	--	0	4	--	--
7/20/06	30	0	1	0	4	0	0.25
7/21/07	31	--	--	0	4	--	--
7/22/06	32	0	0	0	4	0	0
7/23/07	33	--	--	0	4	--	--
7/24/07	34	--	--	0	4	--	--
7/25/06	35	0	0	0	4	0	0
7/26/06	36	0	0	0	4	0	0
7/27/06	37	--	--	0	4	--	--
7/28/06	38	0	0	0	4	0	0
7/29/06	39	--	--	0	4	--	--
7/30/06	40	0	0	0	4	0	0
7/31/06	41	0	0	0	4	0	0
TOTAL						12.38	8.50

Table B.10. Summary of recorded and calculated values for Flat Brook, Aquarium 1 in tessellated darter host suitability trials. Daily totals of shed *Alasmidonta heterodon* glochidia and fish remaining in each tank were used to generate numbers of shed glochidia generated per fish per day. Daily totals of transformed juvenile *A. heterodon* and remaining fish in each tank were used to generate numbers of transformed juveniles produced per fish per day.

Flat Brook (Aquarium 1)							
Date	Day of experiment	Shed glochidia	Trans-formed juveniles	Fish mortalities	Fish remaining	Mean shed glochidia per fish	Mean juveniles per fish
6/21/06	1	23	0	0	9	2.56	0
6/22/06	2	36	0	0	9	4.00	0
6/23/06	3	29	0	0	9	3.22	0
6/24/06	4	6	0	1	9	0.67	0
6/25/06	5	2	0	0	8	0.25	0
6/26/06	6	1	0	1	8	0.12	0
6/27/06	7	4	0	0	7	0.57	0
6/28/06	8	0	0	0	7	0	0
6/29/06	9	0	0	0	7	0	0
6/30/06	10	--	--	0	7	--	--
7/1/06	11	0	0	0	7	0	0
7/2/06	12	--	--	0	7	--	--
7/3/06	13	0	0	1	7	0	0
7/4/07	14	--	--	0	6	--	--
7/5/06	15	0	0	0	6	0	0
7/6/07	16	--	--	0	6	--	--
7/7/06	17	0	0	0	6	0	0
7/8/06	18	--	--	0	6	--	--
7/9/06	19	0	6	0	6	0	1.00
7/10/06	20	0	10	0	6	0	1.67
7/11/06	21	0	2	0	6	0	0.33
7/12/07	22	--	--	0	6	--	--
7/13/06	23	0	3	0	6	0	0.50
7/14/06	24	0	1	0	6	0	0.17
7/15/07	25	--	--	0	6	--	--
7/16/06	26	0	2	0	6	0	0.33
7/17/07	27	--	--	0	6	--	--
7/18/06	28	0	1	0	6	0	0.16
7/19/07	29	--	--	0	6	--	--
7/20/06	30	0	0	0	6	0	0
7/21/07	31	--	--	0	6	--	--
7/22/06	32	0	2	0	6	0	0.33
7/23/07	33	--	--	0	6	--	--
7/24/07	34	--	--	0	6	--	--
7/25/06	35	0	0	0	6	0	0
7/26/06	36	0	0	0	6	0	0
7/27/06	37	--	--	0	6	--	--
7/28/06	38	0	0	0	6	0	0
7/29/06	39	--	--	0	6	--	--
7/30/06	40	0	0	0	6	0	0
7/31/06	41	0	0	1	6	0	0
TOTAL						11.39	4.46

Table B.11. Summary of recorded and calculated values for Flat Brook, Aquarium 2 in tessellated darter host suitability trials. Daily totals of shed *Alasmidonta heterodon* glochidia and fish remaining in each tank were used to generate numbers of shed glochidia generated per fish per day. Daily totals of transformed juvenile *A. heterodon* and remaining fish in each tank were used to generate numbers of transformed juveniles produced per fish per day.

Flat Brook (Aquarium 2)							
Date	Day of experiment	Shed glochidia	Trans-formed juveniles	Fish mortalities	Fish remaining	Mean shed glochidia per fish	Mean juveniles per fish
6/21/06	1	49	0	0	9	5.44	0
6/22/06	2	10	0	0	9	1.11	0
6/23/06	3	48	0	0	9	5.33	0
6/24/06	4	3	0	0	9	0.33	0
6/25/06	5	1	0	1	9	0.11	0
6/26/06	6	1	0	0	8	0.13	0
6/27/06	7	0	0	0	8	0	0
6/28/06	8	0	0	0	8	0	0
6/29/06	9	0	0	0	8	0	0
6/30/06	10	--	--	0	8	--	--
7/1/06	11	0	0	0	8	0	0
7/2/06	12	--	--	1	8	--	--
7/3/06	13	0	0	0	7	0	0
7/4/07	14	--	--	0	7	--	--
7/5/06	15	0	0	0	7	0	0
7/6/07	16	--	--	0	7	--	--
7/7/06	17	0	0	0	7	0	0
7/8/06	18	--	--	0	7	--	--
7/9/06	19	0	2	0	7	0	0.29
7/10/06	20	0	2	0	7	0	0.29
7/11/06	21	0	4	0	7	0	0.57
7/12/07	22	--	--	0	7	--	--
7/13/06	23	0	0	0	7	0	0
7/14/06	24	0	1	1	7	0	0.14
7/15/07	25	--	--	0	7	--	--
7/16/06	26	0	0	1	6	0	0
7/17/07	27	--	--	0	6	--	--
7/18/06	28	0	0	2	5	0	0
7/19/07	29	--	--	0	3	--	--
7/20/06	30	0	0	1	3	0	0
7/21/07	31	--	--	0	2	--	--
7/22/06	32	0	0	0	2	0	0
7/23/07	33	--	--	0	2	--	--
7/24/07	34	--	--	0	2	--	--
7/25/06	35	0	0	0	2	0	0
7/26/06	36	0	0	0	2	0	0
7/27/06	37	--	--	0	2	--	--
7/28/06	38	0	0	0	2	0	0
7/29/06	39	--	--	0	2	--	--
7/30/06	40	0	0	0	2	0	0
7/31/06	41	0	0	0	2	0	0
TOTAL						12.45	1.29

Table B.12. Summary of recorded and calculated values for Flat Brook, Aquarium 3 in tessellated darter host suitability trials. Daily totals of shed *Alasmidonta heterodon* glochidia and fish remaining in each tank were used to generate numbers of shed glochidia generated per fish per day. Daily totals of transformed juvenile *A. heterodon* and remaining fish in each tank were used to generate numbers of transformed juveniles produced per fish per day.

Flat Brook (Aquarium 3)							
Date	Day of experiment	Shed glochidia	Trans-formed juveniles	Fish mortalities	Fish remaining	Mean shed glochidia per fish	Mean juveniles per fish
6/21/06	1	22	0	0	9	2.44	0
6/22/06	2	12	0	0	9	1.33	0
6/23/06	3	17	0	0	9	1.89	0
6/24/06	4	3	0	0	9	0.33	0
6/25/06	5	4	0	0	9	0.44	0
6/26/06	6	6	0	0	9	0.67	0
6/27/06	7	0	0	0	9	0	0
6/28/06	8	2	0	0	9	0.21	0
6/29/06	9	0	0	0	9	0	0
6/30/06	10	--	--	0	9	--	--
7/1/06	11	0	0	0	9	0	0
7/2/06	12	--	--	0	9	--	--
7/3/06	13	0	0	0	9	0	0
7/4/07	14	--	--	0	9	--	--
7/5/06	15	0	0	0	9	0	0
7/6/07	16	--	--	0	9	--	--
7/7/06	17	0	1	0	9	0	0.11
7/8/06	18	--	--	1	9	--	--
7/9/06	19	0	11	0	8	0	1.37
7/10/06	20	0	9	0	8	0	1.12
7/11/06	21	0	6	0	8	0	0.75
7/12/07	22	--	--	0	8	--	--
7/13/06	23	0	1	1	8	0	0.13
7/14/06	24	0	2	0	7	0	0.29
7/15/07	25	--	--	0	7	--	--
7/16/06	26	0	2	0	7	0	0.29
7/17/07	27	--	--	0	7	--	--
7/18/06	28	0	4	1	7	0	0.57
7/19/07	29	--	--	0	6	--	--
7/20/06	30	0	3	0	6	0	0.50
7/21/07	31	--	--	0	6	--	--
7/22/06	32	0	2	0	6	0	0.33
7/23/07	33	--	--	0	6	--	--
7/24/07	34	--	--	0	6	--	--
7/25/06	35	0	0	0	6	0	0
7/26/06	36	0	0	0	6	0	0
7/27/06	37	--	--	0	6	--	--
7/28/06	38	0	0	0	6	0	0
7/29/06	39	--	--	0	6	--	--
7/30/06	40	0	0	0	6	0	0
7/31/06	41	0	0	0	6	0	0
TOTAL						7.33	5.46

Table B.13. Summary of recorded and calculated values for Pine Creek, Aquarium 1 in tessellated darter host suitability trials. Daily totals of shed *Alasmidonta heterodon* glochidia and fish remaining in each tank were used to generate numbers of shed glochidia generated per fish per day. Daily totals of transformed juvenile *A. heterodon* and remaining fish in each tank were used to generate numbers of transformed juveniles produced per fish per day.

Pine Creek (Aquarium 1)							
Date	Day of experiment	Shed glochidia	Trans-formed juveniles	Fish mortalities	Fish remaining	Mean shed glochidia per fish	Mean juveniles per fish
6/21/06	1	14	0	0	9	1.56	0
6/22/06	2	15	0	0	9	1.67	0
6/23/06	3	10	0	0	9	1.11	0
6/24/06	4	0	0	1	9	0	0
6/25/06	5	0	0	1	8	0	0
6/26/06	6	1	0	0	7	0.14	0
6/27/06	7	0	0	0	7	0	0
6/28/06	8	1	0	0	7	0.14	0
6/29/06	9	0	0	0	7	0	0
6/30/06	10	--	--	0	7	--	--
7/1/06	11	0	0	0	7	0	0
7/2/06	12	--	--	0	7	--	--
7/3/06	13	0	0	0	7	0	0
7/4/07	14	--	--	0	7	--	--
7/5/06	15	0	2	0	7	0	0.29
7/6/07	16	--	--	0	7	--	--
7/7/06	17	0	3	0	7	0	0.42
7/8/06	18	--	--	0	7	--	--
7/9/06	19	0	13	0	7	0	1.86
7/10/06	20	0	16	0	7	0	2.29
7/11/06	21	0	5	0	7	0	0.71
7/12/07	22	--	--	0	7	--	--
7/13/06	23	0	2	2	7	0	0.29
7/14/06	24	0	1	0	5	0	0.20
7/15/07	25	--	--	0	5	--	--
7/16/06	26	1	0	0	5	0.20	0
7/17/07	27	--	--	0	5	--	--
7/18/06	28	0	0	0	5	0	0
7/19/07	29	--	--	0	5	--	--
7/20/06	30	0	0	0	5	0	0
7/21/07	31	--	--	0	5	--	--
7/22/06	32	0	0	0	5	0	0
7/23/07	33	--	--	0	5	--	--
7/24/07	34	--	--	0	5	--	--
7/25/06	35	0	0	0	5	0	0
7/26/06	36	0	0	0	5	0	0
7/27/06	37	--	--	0	5	--	--
7/28/06	38	0	0	0	5	0	0
7/29/06	39	--	--	0	5	--	--
7/30/06	40	0	0	0	5	0	0
7/31/06	41	0	0	1	5	0	0
TOTAL						4.82	6.06

Table B.14. Summary of recorded and calculated values for Pine Creek, Aquarium 2 in tessellated darter host suitability trials. Daily totals of shed *Alasmidonta heterodon* glochidia and fish remaining in each tank were used to generate numbers of shed glochidia generated per fish per day. Daily totals of transformed juvenile *A. heterodon* and remaining fish in each tank were used to generate numbers of transformed juveniles produced per fish per day.

Pine Creek (Aquarium 2)							
Date	Day of experiment	Shed glochidia	Trans-formed juveniles	Fish mortalities	Fish remaining	Mean shed glochidia per fish	Mean juveniles per fish
6/21/06	1	19	0	0	9	2.11	0
6/22/06	2	22	0	1	9	2.44	0
6/23/06	3	22	0	0	8	2.75	0
6/24/06	4	1	0	0	8	0.13	0
6/25/06	5	1	0	1	8	0.13	0
6/26/06	6	1	0	0	7	0.14	0
6/27/06	7	1	0	1	7	0.14	0
6/28/06	8	1	0	0	6	0.17	0
6/29/06	9	0	0	0	6	0	0
6/30/06	10	--	--	0	6	--	--
7/1/06	11	0	0	1	6	0	0
7/2/06	12	--	--	0	5	--	--
7/3/06	13	0	0	0	5	0	0
7/4/07	14	--	--	0	5	--	--
7/5/06	15	0	0	0	5	0	0
7/6/07	16	--	--	0	5	--	--
7/7/06	17	2	0	0	5	0.40	0
7/8/06	18	--	--	0	5	--	--
7/9/06	19	0	5	0	5	0	1.00
7/10/06	20	0	6	0	5	0	1.20
7/11/06	21	0	3	0	5	0	0.60
7/12/07	22	--	--	0	5	--	--
7/13/06	23	0	1	0	5	0	0.20
7/14/06	24	0	0	0	5	0	0
7/15/07	25	--	--	0	5	--	--
7/16/06	26	0	0	0	5	0	0
7/17/07	27	--	--	0	5	--	--
7/18/06	28	0	0	0	5	0	0
7/19/07	29	--	--	0	5	--	--
7/20/06	30	0	0	1	5	0	0
7/21/07	31	--	--	0	4	--	--
7/22/06	32	0	0	0	4	0	0
7/23/07	33	--	--	0	4	--	--
7/24/07	34	--	--	0	4	--	--
7/25/06	35	0	0	0	4	0	0
7/26/06	36	0	0	0	4	0	0
7/27/06	37	--	--	0	4	--	--
7/28/06	38	0	0	0	4	0	0
7/29/06	39	--	--	0	4	--	--
7/30/06	40	0	0	0	4	0	0
7/31/06	41	0	0	0	4	0	0
TOTAL						8.41	3.00

Table B.15. Summary of recorded and calculated values for Pine Creek, Aquarium 3 in tessellated darter host suitability trials. Daily totals of shed *Alasmidonta heterodon* glochidia and fish remaining in each tank were used to generate numbers of shed glochidia generated per fish per day. Daily totals of transformed juvenile *A. heterodon* and remaining fish in each tank were used to generate numbers of transformed juveniles produced per fish per day.

Pine Creek (Aquarium 3)							
Date	Day of experiment	Shed glochidia	Trans-formed juveniles	Fish mortalities	Fish remaining	Mean shed glochidia per fish	Mean juveniles per fish
6/21/06	1	11	0	0	9	1.22	0
6/22/06	2	44	0	1	9	4.89	0
6/23/06	3	50	0	1	8	6.25	0
6/24/06	4	2	0	0	7	0.28	0
6/25/06	5	0	0	1	7	0	0
6/26/06	6	4	0	0	6	0.67	0
6/27/06	7	0	0	0	6	0	0
6/28/06	8	0	0	0	6	0	0
6/29/06	9	0	0	0	6	0	0
6/30/06	10	--	--	0	6	--	--
7/1/06	11	0	0	0	6	0	0
7/2/06	12	--	--	0	6	--	--
7/3/06	13	0	0	0	6	0	0
7/4/07	14	--	--	0	6	--	--
7/5/06	15	0	0	0	6	0	0
7/6/07	16	--	--	0	6	--	--
7/7/06	17	0	0	0	6	0	0
7/8/06	18	--	--	0	6	--	--
7/9/06	19	0	4	0	6	0	0.67
7/10/06	20	0	6	0	6	0	1.00
7/11/06	21	0	4	0	6	0	0.67
7/12/07	22	--	--	0	6	--	--
7/13/06	23	0	4	0	6	0	0.67
7/14/06	24	0	1	1	6	0	0.16
7/15/07	25	--	--	0	5	--	--
7/16/06	26	0	1	1	5	0	0.20
7/17/07	27	--	--	0	4	--	--
7/18/06	28	0	0	0	4	0	0
7/19/07	29	--	--	0	4	--	--
7/20/06	30	0	0	0	4	0	0
7/21/07	31	--	--	0	4	--	--
7/22/06	32	0	0	0	4	0	0
7/23/07	33	--	--	0	4	--	--
7/24/07	34	--	--	0	4	--	--
7/25/06	35	0	0	0	4	0	0
7/26/06	36	0	0	0	4	0	0
7/27/06	37	--	--	0	4	--	--
7/28/06	38	0	0	0	4	0	0
7/29/06	39	--	--	0	4	--	--
7/30/06	40	0	0	0	4	0	0
7/31/06	41	0	0	0	4	0	0
TOTAL						13.31	3.37