Endangered Species Final Report

Grant No. E-4-R-1

PROPAGATION OF ENDANGERED NATIVE MUSSELS FOR RESEARCH AND RECOVERY.

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INTRODUCTION

This report describes the third and final year of a 3-year project (E-4-R-1). Year 3 project period is October 1, 2008- September 30, 2009. The overall goal of the project is to recover endangered species of native freshwater mussels by developing our ability to propagate these species, determine their physical and biological requirements, and augment and restore wild populations. Specific objectives are 1) continue ongoing development and refinement of methods for the propagation and culture of federally endangered freshwater mussel species, including field enclosures, and 2) continue to propagate, grow out, release, and monitor federally endangered and candidate species at selected sites in Missouri.

Summary of project accomplishments

1. Developed continuous feeding system for laboratory culture of juveniles.
2. Developed prototype floating upweller systems (flupsys) for grow out of propagated juveniles and tested this approach at four pond sites.
3. Designed and built a large-scale flupsy at the Kansas City Zoo, and developed an ongoing collaboration with Zoo staff to grow-out propagated freshwater mussels.
4. Carried out a field experiment to investigate growth of caged juveniles in the James River in response to food availability at upstream versus downstream sites, and effect of access to deposited sediment versus water column contact only (Duzan 2008).
5. Collaborated with the USGS and USFWS to carry out a caging study of juvenile mussels on metal-contaminated sites in the Spring River.
6. Propagated and released of species of concern including pink mucket, scaleshell, Neosho mucket, black sandshell, and snuffbox (Barnhart 2007, 2008).
7. Produced and provided juvenile mussels to other agencies to promote research and conservation of endangered species. These agencies included USGS Columbia Environmental Research Center, USFWS Tulsa Field Office, Peoria Tribe of Oklahoma, North Carolina State University, Kansas Department of Wildlife and Parks, Virginia Aquatic Wildlife Conservation Center, and Illinois Natural History Survey.
8. Information on mussel research was disseminated through publications, websites, public programs and consultations, and presentations at local and national meetings. Conducted mussel workshops in each project year for MDC, Missouri Stream Team, and Kansas Wildlife and Parks.
9. Although Section 6 funding was discontinued in 2009, we will continue to grow out species of concern including pink mucket, scaleshell, Neosho mucket, black sandshell, and snuffbox in 2010.

Accomplishments in Year 3

1. Development and use of new compact floating upweller systems.

During 2009, we continued to use the large flupsys for grow-out and we developed and began using a new compact flupsy ("tub flupsy") (Figures 1, 2, 3). The tub flupsy fills the need for 1) a portable system that can be used to test water and food quality in ponds for mussel grow-out, and 2) a system suitable for short-term growth of young cohorts of juveniles that are too small to place directly in the large-scale unit.

The tub flupsy design utilizes a 40-g LDPE tub (Tuff Stuff® 40-gallon oval KMT-101). Four holes are cut in the bottom of the tub to closely fit the circumference of the bins that contain the mussels. Four bins with screen bottom and screen lids insert into the holes in the bottom of the tub and are secured with bungees attached to the bottom of the tub. Closed cell foam flotation (pool noodle) is attached to the perimeter inside the rim of the tub using cable ties looped through holes drilled in the tub wall.

A 1200 gallon per hour magnetic-drive pump (Danner® Model 12) is fastened to the floor of the tub at one end, with the pump outlet projecting through a closely-fit hole in the end wall. The pump ejects water from the tub, and water enters through the screen bottom of the bins. The tub fills with water but does not sink because of the flotation ring. Unlike our previous flupsy designs, there is no manifold or pipe attachment to the bins. Water enters the bins from underneath from the bottom and exits out the top of the bins, which have screen covers to prevent escapes. The bins are secured in the tub with bungees and can easily be removed for inspection and cleaning. A perforated cover of plastic peg-board (not shown) is secured with bungees on top of the float ring, to protect the bins.

Bins for the tub flupsy are constructed using 2-gallon HDPE buckets (Encore Plastics® #20256) (Figure 2). Each bin requires two buckets. The bottom is cut out from both buckets. Bucket #2 is cut in half and used to form the bottom and top closures of the bin. The lower half is press-fit over the bottom of bucket #1, holding a sheet of 2-mm mesh fiberglass window-screen across the opening. A disk of coarser (1/2 inch mesh) plastic screen is attached to the rim using cable ties, to protect and support the window screen within. The top half of bucket #2 was used to make the top closure for the bin. Window screen is attached using Plumbers Goop® adhesive. The top closure is inserted into the top of bucket #1 and held in place by hooking the handle of bucket #1 over the top. This bin design secures the animals with screen above and below. Mesh size as small as 1 mm can be used making it possible to place even very small

juveniles in pond culture. However, window screen (~2mm mesh) is more practical because finer mesh tends to clog within a few days and requires frequent cleaning.

2. Mussel culture in flupsys at the Kansas City Zoo

The flupsy installation is located in the Swope Park Lagoon at the Kansas City Zoo. The lagoon is a closed 25-acre water body that was excavated in 1908. The flupsys are in a protected area adjacent to the Boat House, where we have access to electrical power, running water, a concrete dock, and indoor working space for making measurements. The Zoo has provided access to these facilities free of charge. During 2009 the Zoo flupsys were inspected weekly by Mr. Tracy Divis, a Zoo employee. Each bin was examined and the screens were rinsed and cleaned as necessary using a power-washer. At monthly intervals, MSU faculty and students visited the Zoo to weigh, measure, and tag mussels and make any necessary adjustments. Animals were removed from the bins during these visits and the shells cleaned prior to measurements.

Mussels of different species and cohorts are maintained in separate flupsy bins. As mussels reach a suitable size, all individuals of species of concern, and selected subsets of other species cohorts, are tagged for identification. Hallprint® shellfish tags were affixed with Loctite Super Glue Control Gel®, a cyanoacrylate adhesive. Shells were scrubbed gently with a brush or scouring pad to remove biofilm. The shell surface was left damp. Adhesive was placed on the shell and the tag positioned and pressed to the shell using fine-point forceps. At least 5 minutes was allowed for the adhesive to polymerize before returning the tagged animals to water. Mussels were tagged on the right valve after they reached a size of approximately 25 mm.

Temperature was recorded at hourly intervals using IButton temperature digital loggers (Model DS1922L) that were submerged in watertight containers in the flupsys bins. Water samples were taken on each monthly visit for measurement of particulates (food) using a coulometric cell counter.

The major maintenance problem at the Zoo has been the removal of bryozoa from the flupsy screens and in some cases from the mussels themselves. Bryozoa are colonial filter-feeding organisms that attach to surfaces. Like mussels, bryozoa are suspension feeders and apparently find conditions in the flupsys to be ideal. The predominant bryozoa species in the lagoon grow as a dense network of tubular filaments that attach to surfaces and can block the flupsys screens within a few weeks during the summer if left unchecked. Therefore, weekly maintenance is necessary during the summer months.

The flupsys provide an efficient means for grow out of propagated juveniles to a size suitable for tagging. Our present approach is to propagate wild-collected glochidia on host fish and culture the newly transformed juveniles in bucket rearing systems to a size of several mm (Barnhart 2006, 2007). This laboratory culture step requires from 4-12 months depending on species. Juveniles larger than 3 mm can be moved to the 2-gallon bins in the tub flupsys at the zoo. As the cohort grows and requires more space it is moved to the 30-gallon bins in the main system. We are presently holding over 3000 mussels of 16 species in the Zoo flupsys (Table 1). These include propagated mussels up to 2 years of age, as well as wild subadults which are being monitored to study growth (see below).

3. Analysis of growth

Recording growth rate is important for judging the success of captive culture methods. Growth rate is also an important measure in toxicological and ecological studies. The flupsys provide an excellent opportunity for research on growth rates, including effects of season, species, and sexual maturation, and a major goal this year was year to gather data on growth. Growth can be recorded as change in length or as change in mass. Of these choices, mass is preferable, because mass represents the quantity of shell and living tissue that is present, while length does not provide this information directly. Individuals larger than about 1 cm can be weighed using laboratory or field-portable electronic balances with suitable precision. Measurement precision of about 1% is generally acceptable. Juvenile mussels 1-cm long have roughly 100 mg whole wet mass, so a balance with precision of 1 mg is adequate. 2.5 cm juveniles weigh roughly 1 gram, so that 10 mg precision is adequate. For mass measurements to be accurate, care should be taken to empty the mantle cavity of water.

Although mass is the preferred measure of size and growth, it is generally not practical to routinely weigh large numbers of mussels smaller than about 1 cm length, because of the limitations of balance precision & stability, and difficulties inherent in handling and drying very small juveniles. For juveniles less than about 1 gram, it is easier and more practical to routinely measure length from digital photographs, and then to infer mass from the species-specific relationships between length and mass. Length–mass equations for each species can be derived from precise measurements on a representative sample spanning the size range of interest.

Length-mass equations: Equations for predicting mass from length were derived for juveniles of seven species (Table 2). Individuals were selected to span size range from about 1 mg to several grams. Each

individual was weighed with a precision of at least 1% using precision electronic balances including a Cahn Electrobalance (1 microgram) and Sartorius analytical balance (0.1 mg). Whole wet mass, whole dry mass, and dry shell mass were determined for each individual. Before measuring whole wet mass, retention of water in the mantle cavity was minimized by placing live individuals ventral edge-down on damp absorbent toweling for 15-30 minutes. Change in mass before and after this treatment indicated that it was effective in wicking water out of the mantle cavity. To separate tissue and shell for measurement of dry mass, individuals larger than about 5 mm were heated briefly in water at 100C, which caused the shell to open and allowed removal of the intact body from the shell. The shell and the body were separated and dried to constant weight in a drying oven at 50C. Individuals smaller than about 5 mm were dried and weighed whole, after which the tissue was removed from the shell by retting in water, and the empty shell dried and weighed. Dry tissue mass was then estimated as the difference between whole dry mass and dry shell mass.

Lengths of individuals smaller than about 1 cm were measured from digital photographs. Photos were taken using a Canon EOS 20D camera and a 60 mm macro lens mounted on a stand. The set-up was tested for edge distortion by photographing graph paper. Suitable scale references were included in each photograph. Maximum linear dimension (=length) of each shell was determined using ImageJ software (US NIH) using the feret diameter function and the particle analysis or the line functions in ImageJ. The precision of these measurements depended on the image magnification, which was adjusted to provide precision of at least 1% of length. Individuals larger than about 1 cm were measured to within 0.1 mm using digital calipers (Mitutoyo model CD-6PSX).

The relationship between length and mass was described by regression according to an exponential model (Table 2). Relationships between log mass and log length were linear and closely correlated; values of R^2 generally exceeded 0.98. The relationship between length and mass varied among species as was expected from differences in shell shape and thickness. For example, black sandshell are relatively long and thin compared to pink mucket, and are correspondingly lower in mass at a give length. Shell mass as a proportion of whole mass also varied among species (Table 3). For example, plain pocketbook had shell mass of only 28% of whole wet mass, while shell mass of butterfly was over 66% of whole wet mass. These differences probably relate both to shell thickness and to shell shape.

Allometry in fatmucket: Changes in shape with change in size (allometry) during 1 year of growth was analyzed in a cohort of 95 *Lampsilis siliquoidea*. These mussels were propagated in early January 2008. They were grown in bucket culture for 7 months, and caged in mussel silos during August-September

2008. Having reached a mean length of 25 mm, they were tagged and placed in the KC Zoo flupsy in late October 2008 and were measured and weighed approximately monthly thereafter. Mean individual mass in this cohort increased from 1.5 g to 36.4 g from 10/23/09 to 11/12/09. Essentially all of the growth occurred in 8 months from March-November (Table 4, Figure 4).

The length-mass curves of this cohort show an interesting pattern over time (Table 4, Figure 4). Slope remained relatively constant but intercepts increased, showing higher mass relative to length in older animals. This pattern appears to result from change in shape as the mussel ages. Younger animals were relatively compressed, with the ratio of shell width to height being smaller than in older animals (Figures 5, 6). The pattern appears to be related to age more than to body mass, because the slopes of the length-mass relationship were relatively invariant among measurement periods (Figure 4, Table 4).

Sexual dimorphism and maturation in 2-y old fatmucket: Sexual dimorphism became evident in this cohort as it approached 2-years of age. In November 2009, 57/95 individuals (60%) showed inflated posterior shell shape and pigmented outer gill margins and were thereby identifiable as females. Because these animals were tagged as 10-month olds, it was possible to compare the size of males and females throughout the second year of growth. Females were significantly longer and heavier than males throughout the second year (Figure 7). Interestingly, the difference increased during the late summer, when this species normally spawns. The ratio of female/male mass approximately doubled in August of 2009, from about 7% to about 14% (Figure 8). Hypothetically, the increase in mass difference could reflect the diversion of resources from growth to gamete production in males, which may mature sooner than females. The observation can be taken as evidence that the male fatmucket were sexually mature at the end of the second year of growth. No egg production (brooding) was observed in females. The hypothesis of male maturation can be tested next year by bringing a second-year cohort into the lab and observing spermatozeugmata release.

Interestingly, individuals later identified as female tended to be larger even in the first measurement at 11 months of age. The mechanism of sex determination in Unionoid mussels is not known. The correlation between size and sex could be conceivably reflect the result or the cause of sex determination. If sex determination is not genetic, fast growing individuals might tend to become female. If sex determination is genetic, female individuals might tend to grow faster. In future measurements, it will be interesting to see if sex ratio is labile and perhaps correlated with growth rate.

Growth rates: Subsets of each mussel cohort were measured at monthly intervals to track patterns of growth. Individuals larger than about 2 cm shell length were usually weighed directly to determine mass. The mass of individuals smaller than about 2 cm was usually inferred from measurements of length using a length-mass regression equation determined for that species and size range.

Growth rates can be compared as the slope of log of mass versus time. This approach allows meaningful comparisons of growth rate to be made between different sizes of individuals. The slope of log(mass) vs time is related to doubling time (Td) according to the following equation: $Td = \log(2)/(\Delta mass/\Delta Time)$. Growth rates were related to species, size class, and date.

Over time growth was sigmoidal, with virtually no growth over the winter months, rapid growth in the spring and summer as water temperatures rose (Figure 9), and declining growth in the fall (Figures 10, 11, 12). However, this pattern was complicated by differences in growth rate related to size. Smaller, younger mussels had much faster relative growth rates than larger, older animals. For example, black sandshell of about 10 mg in mass doubled their body mass in approximately 1 week during September, while 10 gram mussels had a doubling time of about 2 months, and 100 gram mussels more than 9 months (Figure 10). It will be very interesting to explain these remarkable differences. Previous work has shown that small juveniles have much higher mass-specific filtration rates, which presumably support the acquisition of food to fuel higher growth rates.

Growth rates also differed among species (Figures 11, 12). Remarkably, certain lampsiline species appear to grow more than 4 times faster than other mussels at similar size. Fatmucket and pink heelsplitter are particularly fast (Figure 11), while heavy-shelled taxa such as *Quadrula* and *Ellipsaria* appear to be relatively slow growers (Table 3, Figure 12). Part of the apparent difference in growth rates may be an artifact of comparing mussels of similar whole mass. Species with heavy shells have less metabolically active tissue at a given whole mass, and might therefore be expected to grow at a lower absolute rate. However, it appears certain that some species are much more efficient at growth than others.

The question of whether normal growth rates are obtained in pond culture can be answered only by comparisons with mussels in natural environments. We are gathering data on growth rates of subadult mussels in the Sac River, where a recruitment event in 2005-2006 produced a large multispecies juvenile cohort. Individuals were collected and measured this year, and these measurements will be compared to same-age animals in the flupsys. Hopefully further collecting next year will expand this dataset.

4. Propagation of species of concern

This year we propagated glochidia of scaleshell, pink mucket, snuffbox, and black sandshell. We were unable to obtain Neosho mucket glochidia due to high water during the brooding season. We are currently holding 6 female pink mucket and 2 black sandshell females collected by USFWS from the lower Osage River in October 2009, for propagation this winter. We did not release any juveniles this year. We are presently holding most of this year's and last year's production of species of concern for release at larger size (see table 1).

5. Juveniles provided to other researchers.

We continued to collaborate with other researchers and we provided glochidia and juveniles of several species for use in toxicology studies (Table 5). Provision of mussel larvae and juveniles to other non-profit educational and government entities by MSU is permitted under a Memorandum of Understanding between MSU and MDC.

6. Related publications, theses and meeting presentations during Year 3.

Publications:

Pandolfo, Tamara J., W. Gregory Cope, Consuelo Arellano, Robert B. Bringolf, M. Christopher Barnhart, Edward Hammer. Beating the heat: upper thermal tolerances of early life stages of freshwater mussels. Submitted to Ecological Applications.

Wang, N., C.A. Mebane, J.L. Kunz, C.G. Ingersoll, T.W. May, W.R. Arnold, R.C. Santore, T. Augspurger, F.J. Dwyer, and M.C. Barnhart. 2009. Evaluation of acute copper toxicity to juvenile freshwater mussels (fatmucket, *Lampsilis siliquoidea*) in natural and reconstituted waters. Environmental Toxicology and Chemistry. 28(11): 2367–2377. doi:10.1897/08-655.1.

Theses:

Crownhart, A. K. 2009. Factors affecting metamorphosis success of larval freshwater mussels (Unionidae). MS thesis, Missouri State University.

Pillow, M. J. 2009. Acute toxicity of ammonia and copper to glochidia of two unionid mussel species inside and outside of conglutinates. MS thesis, Missouri State University.

Presentations:

Barnhart, M.C. 2009. Mussel conservation in Missouri. US Fish and Wildlife Service Region 3 Endangered Species Coordinators Meeting, Camdenton, MO 10/8/09.

Barnhart, M.C. 2009. Seduction and reproduction in the freshwater pearly mussels, Unionoida. Auburn University, September 4, 2009.

Barnhart, M. C. 2009. Basic mussel biology and reproduction. Stream Team Freshwater Workshop. Missouri Department of Conservation, Sedalia. July 18-19, 2009.

Barnhart, M. C. 2009. Mussel reproductive behavior- the video records. Stream Team Freshwater Mussel Workshop. Missouri Department of Conservation, Sedalia. July 18-19, 2009.

Barnhart, M. C. 2009. Taxonomy and diversity. Freshwater Mussel Conservation & Identification Workshop. Missouri Department of Conservation, Missouri State University. July 22 – 24, 2009.

Barnhart, M. C. 2009. Biology and reproduction. Freshwater Mussel Conservation & Identification Workshop. Missouri Department of Conservation, Missouri State University. July 22 – 24, 2009.

Miao, JingJing and M. C. Barnhart. 2009. Review of gene expression biomarkers in bivalve toxicology. International Symposium of the Freshwater Mollusc Conservation Society. Baltimore MD

Hazelton, P. D. , W. G. Cope, M. C. Barnhart, and R. B. Bringolf. 2009. Evaluating the effects of emerging contaminants on reproduction in fatmucket (*Lampsilis siliquoidea*). International Symposium of the Freshwater Mollusc Conservation Society. Baltimore MD

Pandolfo, T.J., W. G. Cope, R. B. Bringolf, and M. C. Barnhart. 2009. Beating the heat: upper thermal tolerances of early life stages of freshwater mussels. International Symposium of the Freshwater Mollusc Conservation Society. Baltimore MD

Crownhart, Andrea K., Michael J. Pillow, Jingjing Miao, and M. Christopher Barnhart. 2009. Analyzing variation in metamorphosis success. International Symposium of the Freshwater Mollusc Conservation Society. Baltimore MD.

Pillow, Michael J., Crownhart, Andrea K., Brondel, Rebecca L., M. C. Barnhart, and Ning Wang. 2009. Acute toxicity of copper and ammonia to glochidia inside and outside of conglutinates. International Symposium of the Freshwater Mollusc Conservation Society. Baltimore MD.

Barnhart, M.C., A.R. Roberts, S. McMurray and S. Faiman. 2009. Use of floating upweller systems for culture of freshwater mussels. International Symposium of the Freshwater Mollusc Conservation Society. Baltimore MD.

McMurray, S., M.C. Barnhart, A.R. Roberts, and S. Faiman. 2009. Use of floating upweller systems for culture of freshwater mussels. Annual Meeting of the North American Benthological Society, Grand Rapids, MI.

Pillow, Michael J., Crownhart, Andrea K., Brondel, Rebecca L., and M.C. Barnhart. 2009. Understanding the role of conglutinates in the survival of glochidia larvae exposed to dissolved copper. Missouri Natural Resources Conference, Lake Ozark, MO. Best Student Poster Award, Society for Conservation Biology.

Crownhart, Andrea K., Michael J. Pillow and M. Christopher Barnhart. 2009. Transformation variation of freshwater mussel glochidia attached to fins versus gills. Missouri Natural Resources Conference, Lake Ozark, MO

Duzan, J., M. Duzan and M.C. Barnhart. 2009. Factors affecting feeding and growth of juvenile freshwater mussels. Missouri Natural Resources Conference, Lake Ozark, MO

Brondel, R, D Packwood, and M. C. Barnhart, 2009. Size-dependent predation by crayfish (Orconectes neglectus) on juvenile freshwater mussels (Lampsilis siliquoidea). Missouri Natural Resources Conference, Lake Ozark, MO.

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Barnhart, M. C. 2006. Propagation and restoration of mussel species of concern. Final Report, Endangered Species Grant E-1-42. Missouri Department of Conservation. 42 pages.

Barnhart, M. C. 2007. Endangered Species Grant Interim Report, Grant No. E-1-46, Propagation of endangered native mussels for research and recovery. Missouri Department of Conservation. 10 p.

Barnhart, M C. 2008. Endangered Species Grant Interim Report, Grant no. E-1-46. Propagation of endangered native mussels for research and recovery. Missouri Department of Conservation. 48 p.

Duzan, Jennifer 2008. Factors affecting feeding and growth of juvenile freshwater mussels (Unionidae). Master of Science, Missouri State University.

TABLES AND FIGURES

Table 1. Mussels currently being held in floating upweller systems at the Kansas City Zoo. These include mussels propagated from glochidia and also Sac River subadults from a recruitment event in 2005, which being monitored for growth in captive conditions and comparisons with growth of this cohort in natural conditions. Ebonyshell adults are being held for host work proposed by MDC.

Age	Species		Locality	Number
		Propagated		
1-y	*Ellipsaria lineolata*	butterfly	Sac River	400
1-y	*Lampsilis abrupta*	pink mucket	Meramec River	335
1-y	*Lampsilis rafinesqueana*	Neosho mucket	Spring River KS	550
1-y	*Lampsilis siliquoidea*	fatmucket	Lake Springfield	710
0.5-y	*Lampsilis siliquoidea*	fatmucket	Silver Fork	160
2-y	*Lampsilis siliquoidea*	fatmucket	Silver Fork	95
0.5-y	*Leptodea leptodon*	scaleshell	Meramec River	100
1-y	*Ligumia recta*	black sandshell	Meramec River	78
0.5-y	*Ligumia recta*	black sandshell	Sac River	200
1-y	*Megalonaias nervosa*	washboard	Sac River	6
1-y	*Quadrula cylindrica*	rabbitsfoot	Spring River KS	220
		Other		
4-y	*Amblema plicata*	three-ridge	Sac River	68
4-y	*Elliptio dilatata*	spike	Sac River	2
adult	*Fusconaia ebena*	ebonyshell	Mississippi River	22
4-y	*Fusconaia flava*	Wabash pigtoe	Sac River	3
4-y	*Ligumia recta*	black sandshell	Sac River	11
4-y	*Obliquaria reflexa*	three-horn wartyback	Sac River	4
adult	*Obliquaria reflexa*	three-horn wartyback	Sac River	9
4-y	*Potamilus alatus*	pink heelsplitter	Sac River	2
4-y	*Quadrula pustulosa*	pimpleback	Sac River	30
4-y	*Truncilla donaciformis*	fawnsfoot	Sac River	3
4-y	*Truncilla truncata*	deertoe	Sac River	22
			total	3030

Table 2. Regressions for predicting whole wet mass (grams) of juvenile mussels from shell length (mm) according to the following model: Whole mass = A * length^B

These regressions were derived from measurements on individuals from 3-30 mm shell length.

Species		A	B	R^2
Lampsilis cardium	plain pocketbook	2.7456E-04	2.7527	0.9909
Lampsilis rafinesqueana	Neosho mucket	1.1860E-04	2.8668	0.9956
Ligumia recta	black sandshell	1.0013E-04	2.8254	0.9814
Lampsilis siliquoidea	fatmucket	8.1470E-05	3.0366	0.996
Lampsilis abrupta	pink mucket	5.7510E-05	3.3445	0.9929
Quadrula cylindrica	rabbitsfoot	3.5960E-04	2.6569	0.9988
Ellipsaria lineolata	butterfly	4.5080E-04	2.5732	0.986

Table 3. Shell mass of seven species of juvenile mussels (~3-30 mm shell length). The species are arranged in order of increasing shell mass.

Species		Shell fraction of whole wet mass	Shell fraction of whole dry mass
Lampsilis cardium	plain pocketbook	0.2808 ± 0.0102	0.8654 ± 0.0291
Lampsilis rafinesqueana	Neosho mucket	0.4229 ± 0.0181	0.8959 ± 0.0059
Ligumia recta	black sandshell	0.4348 ± 0.0100	0.9020 ± 0.0056
Lampsilis siliquoidea	fatmucket	0.4542 ± 0.0139	0.9076 ± 0.0084
Lampsilis abrupta	pink mucket	0.5437 ± 0.0772	0.9162 ± 0.0053
Quadrula cylindrica	rabbitsfoot	0.6624 ± 0.0143	0.9505 ± 0.0039
Ellipsaria lineolata	butterfly	0.6657 ± 0.0097	0.9497 ± 0.0027

Table 4. Regression equations relating whole mass (grams) to length (mm) of 1-2 year old fatmucket, *Lampsilis siliquoidea*. The data are from a cohort of 95 individuals reared in a flupsys at the Kansas City Zoo. Length and mass data are given as means ± 95% confidence intervals from measurements on each date. Using the individual measurements on each date, regression was used to derived equations relating length and mass according to the following model: Whole mass = A * length^B Coefficient A increased with time, indicating that older animals were heavier than younger animals at fixed length, which is related to change in shape as the animals become more inflated (i.e. wider relative to height). Exponent B (the slope of a log-log plot) increased slightly but was relatively constant, indicating that the shape change may be more closely related to age than to size. See Figures 4, 5, 6.

date	Length (mm)	Whole mass (g)	A	B	R^2
10/23/2008	25.04 ± 0.537	1.50 ± 0.090	1.4920E-04	2.85151	0.957
6/20/2009	40.88 ± 0.645	7.80 ± 0.348	3.0860E-04	2.72755	0.914
7/20/2009	47.60 ± 0.703	13.17 ± 0.548	3.4560E-04	2.72694	0.9
8/19/2009	54.81 ± 0.860	21.62 ± 0.905	4.8940E-04	2.66756	0.895
9/17/2009	60.01 ± 0.943	29.91 ± 1.247	5.1580E-04	2.67482	0.869
10/15/2009	63.40 ± 1.009	35.62 ± 1.422	6.7110E-04	2.61834	0.883
11/12/2009	63.58 ± 1.007	36.44 ± 1.452	7.3400E-04	2.60058	0.893

Table 5. Mussel shipments in 2009. These larval and juvenile mussels were supplied to other researchers for toxicology studies under a Memorandum of Understanding with the Missouri Department of Conservation.

Date	Destination	Species	Source river	number	age
1/20/09	NCSU	*L. siliquoidea*	Silver Fork	40,000	glochidia
1/27/09	NCSU	*L. siliquoidea*	Silver Fork	40,000	glochidia
2/2/09	NCSU	*L. siliquoidea*	Silver Fork	40,000	glochidia
2/12/09	NCSU	*L. siliquoidea*	Silver Fork	400	0 mo
3/2/09	NCSU	*L. siliquoidea*	Silver Fork	40,000	glochidia
3/19/09	NCSU	*L. siliquoidea*	Silver Fork	400	0 mo
3/26/09	NCSU	*L. siliquoidea*	Silver Fork	400	0 mo
4/9/09	NCSU	*L. siliquoidea*	Silver Fork	200	0 mo
4/30/09	NCSU	*L. siliquoidea*	Silver Fork	400	0 mo
5/14/09	NCSU	*L. siliquoidea*	Silver Fork	500	0 mo
5/21/09	NCSU	*L. siliquoidea*	Silver Fork	500	0 mo
1/20/09	NCSU	*L. recta*	Sac	50,000	glochidia
1/27/09	NCSU	*L. recta*	Sac	40,000	glochidia
2/2/09	NCSU	*L. recta*	Sac	40,000	glochidia
2/12/09	NCSU	*L. recta*	Sac	400	0 mo
3/2/09	NCSU	*L. recta*	Sac	40,000	glochidia
3/19/09	NCSU	*L. recta*	Sac	400	0 mo
3/26/09	NCSU	*L. recta*	Sac	400	0 mo
5/14/09	NCSU	*L. recta*	Sac	500	0 mo
5/28/09	NCSU	*L. recta*	Sac	500	0 mo
6/11/09	NCSU	*L. recta*	Sac	500	0 mo
2/2/09	TWRA	*L. siliquoidea*	Silver Fork	547	0 mo
5/26/09	TWRA	*L. siliquoidea*	Silver Fork	400	0 mo
3/9/09	CERC	*L. siliquoidea*	Silver Fork	1,000	0 mo
3/9/09	CERC	*L. siliquoidea*	Silver Fork	5,000	0 mo
4/13/09	CERC	*L. siliquoidea*	Silver Fork	1,000	0 mo
4/13/09	CERC	*L. siliquoidea*	Silver Fork	6,000	0 mo
6/9/09	CERC	*L. siliquoidea*	Silver Fork	11,000	0 mo
6/1/09	INHS	*L. siliquoidea*	Silver Fork	500	0 mo

NCSU = North Carolina State University (Dr. Greg Cope)
TWRA = Tennessee Wildlife Resources Agency (Dave McKinney)
CERC = USGS Columbia Environmental Research Center (Dr. Chris Ingersoll)
INHS = Illinois Natural History Survey (Dr. David Soucek)

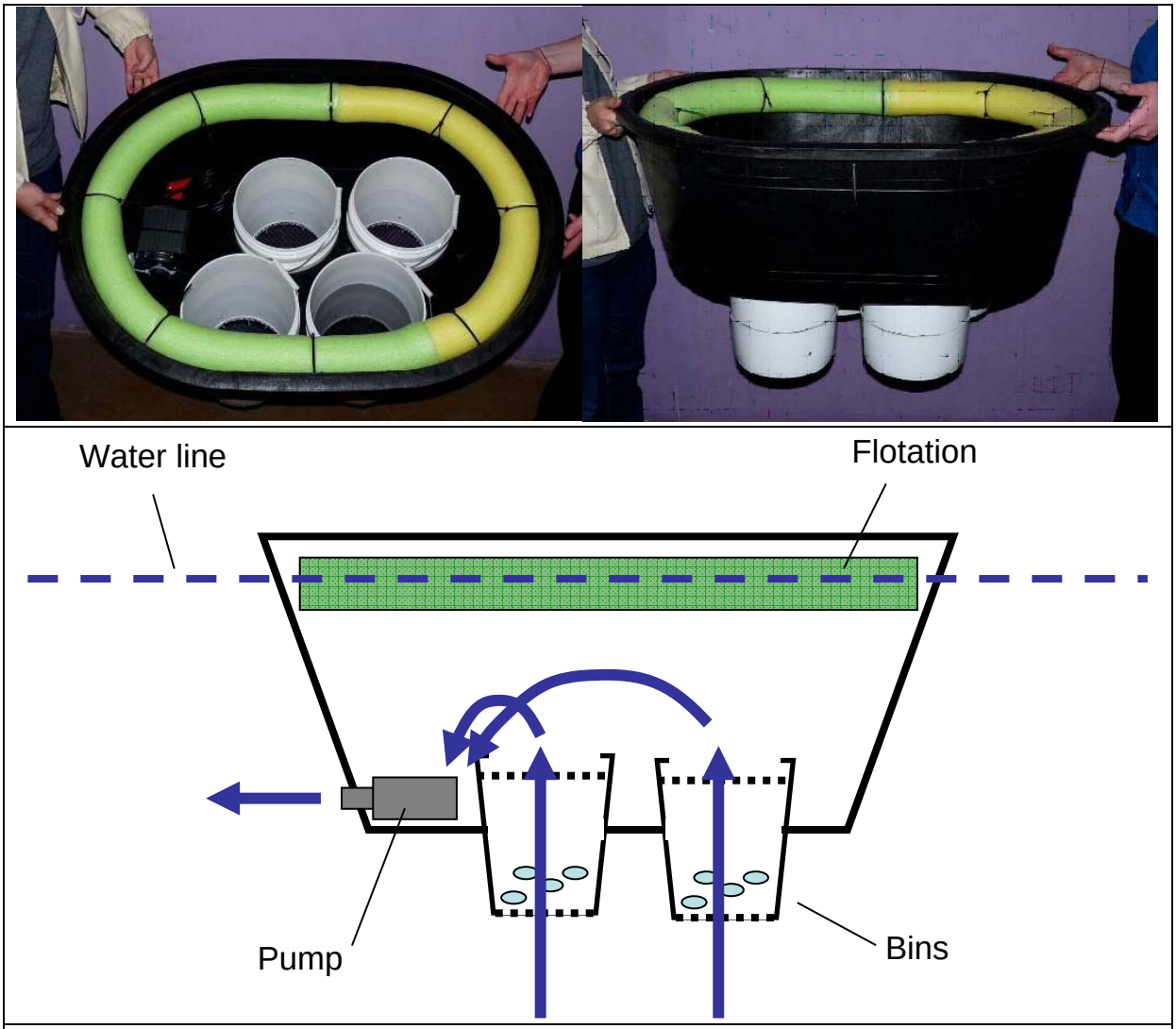

Figure 1. Top panels: top and side views of tub flupsy (floating upweller system). See text for description. Lower panel: diagram showing tub flupsy components and water flow through the system.

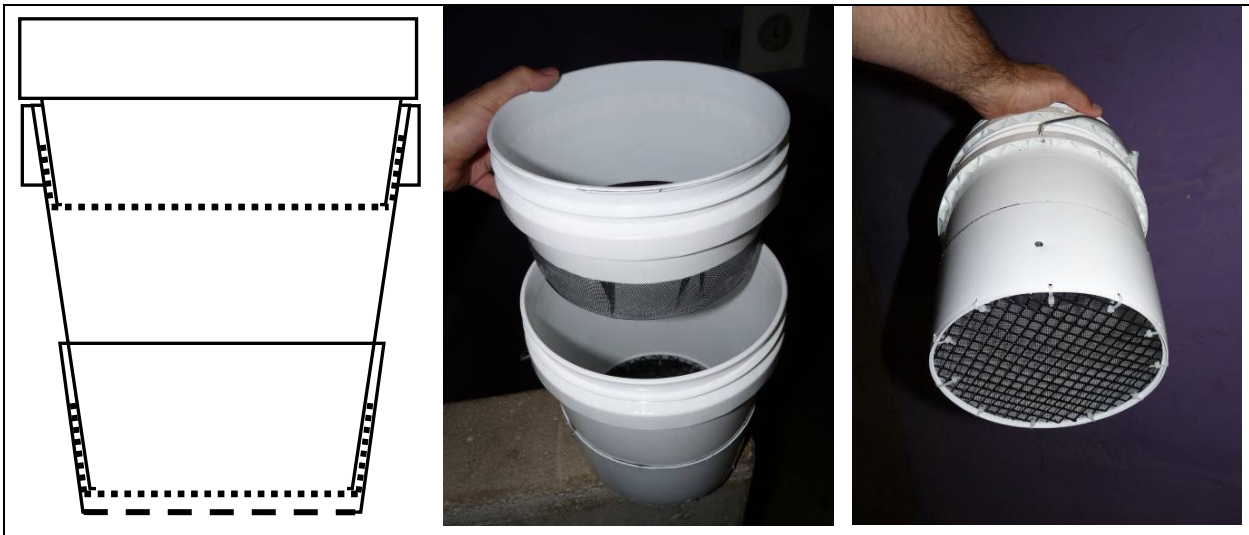

Figure 2. Bin design for tub flupsy. Each bin requires two buckets. One bucket is cut in half and used to form screen closures for the top and bottom of the other bucket. See text for details.

Figure 3. Left panel: tub flupsy in the water at Chesapeake Hatchery. Note elastic strap across bin at upper left. Right panel shows 1-year old Neosho mucket in a tub flupsy bin at the Kansas City Zoo.

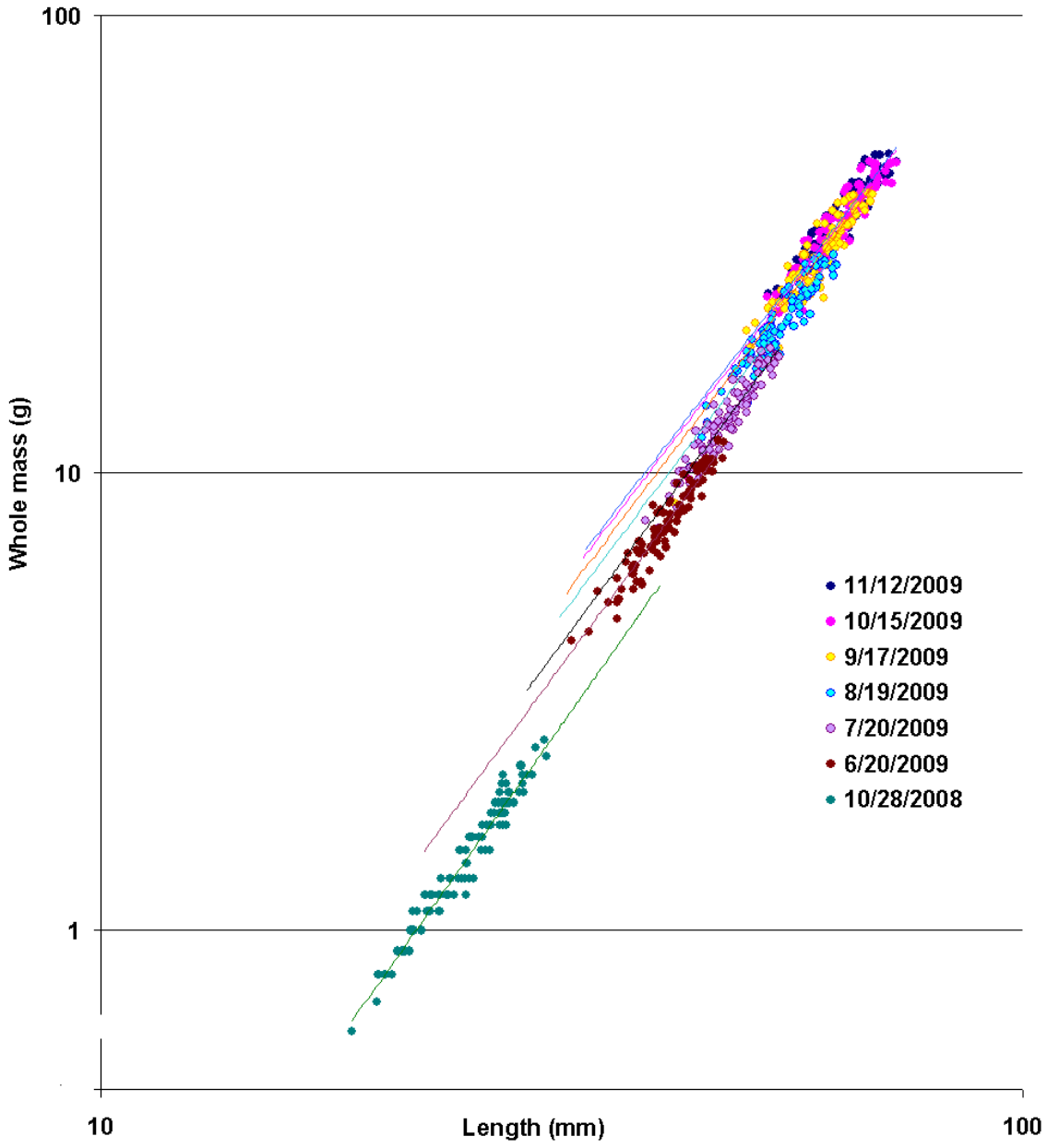

Figure 4. Allometry of whole mass vs. length in a cohort of 95 2-y old fatmucket, *Lampsilis siliquoidea.* Each point is an individual measured on the dates indicated. Colors indicate dates. Note log scales. Lines are regressions (Table 4) on data from each date. Slope remained constant but intercepts increased, showing higher mass relative to length in older animals. This pattern is explained by change in shape with age (see Figures 5, 6)

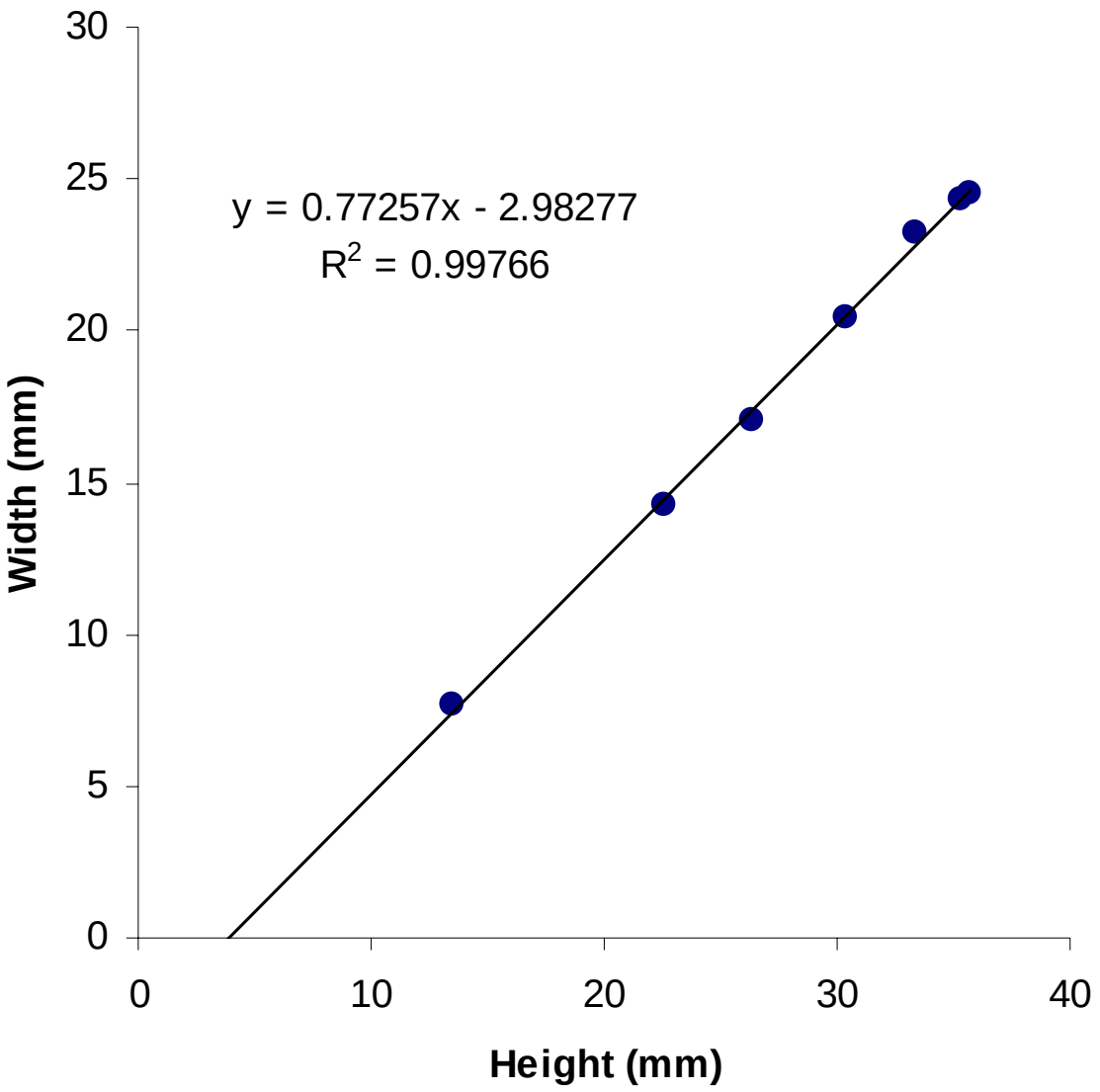

Figure 5. Shell width vs. shell height of a cohort of fatmucket, *Lampsilis siliquoidea*, during their second year of growth Each point is a mean of the 95 individuals measured on a particular date. The intercept of this relationship was significantly different from zero, indicating that width was not a constant proportion of height

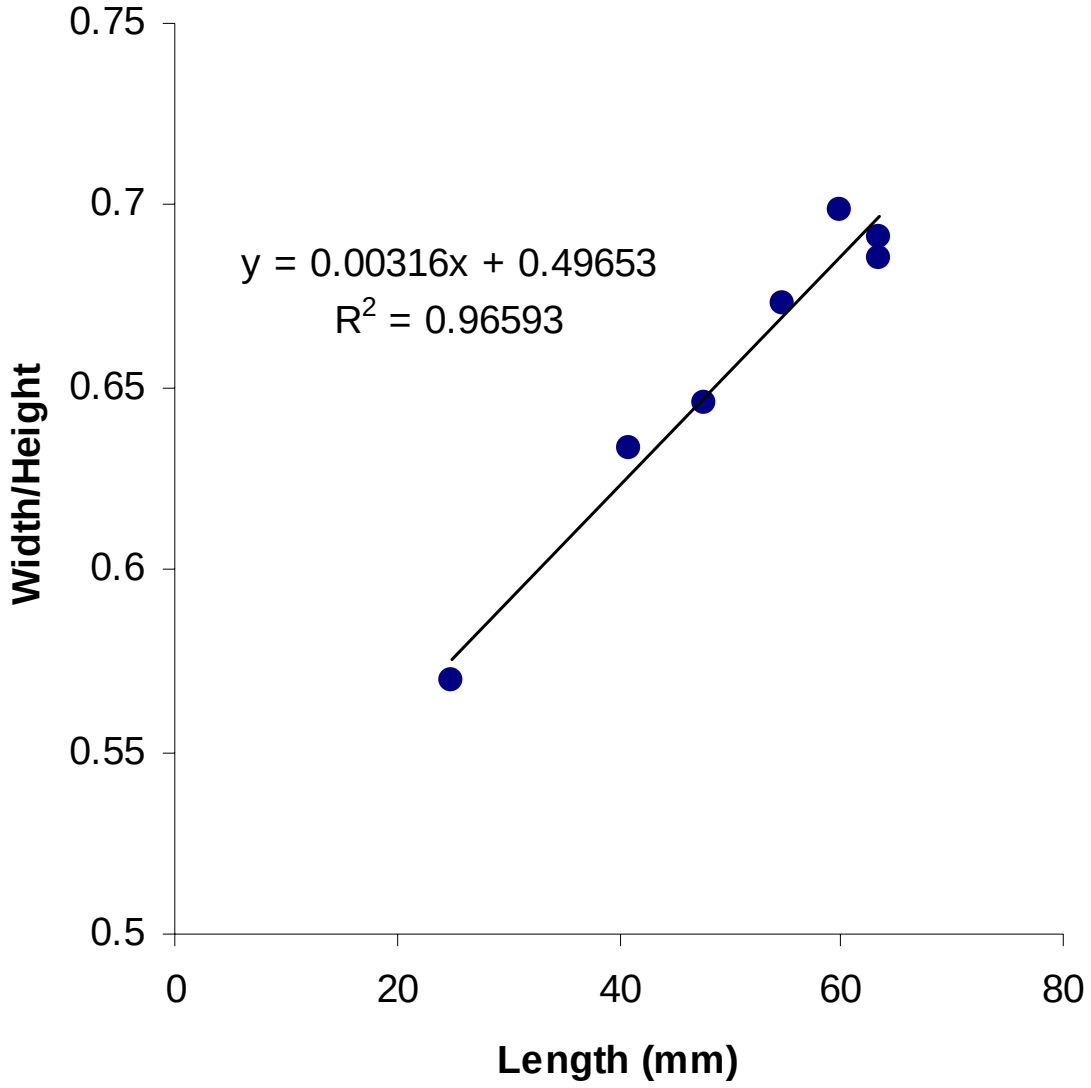

Figure 6. The ratio width/height vs. length of a cohort of fatmucket, *Lampsilis siliquoidea*, during their second year of growth. Each point is a mean of the 95 individuals measured on a particular date. If shape were constant, this ratio would be constant. The ratio increases with age and shell length, showing that that shape becomes less compressed and more inflated as the animals age and grow.

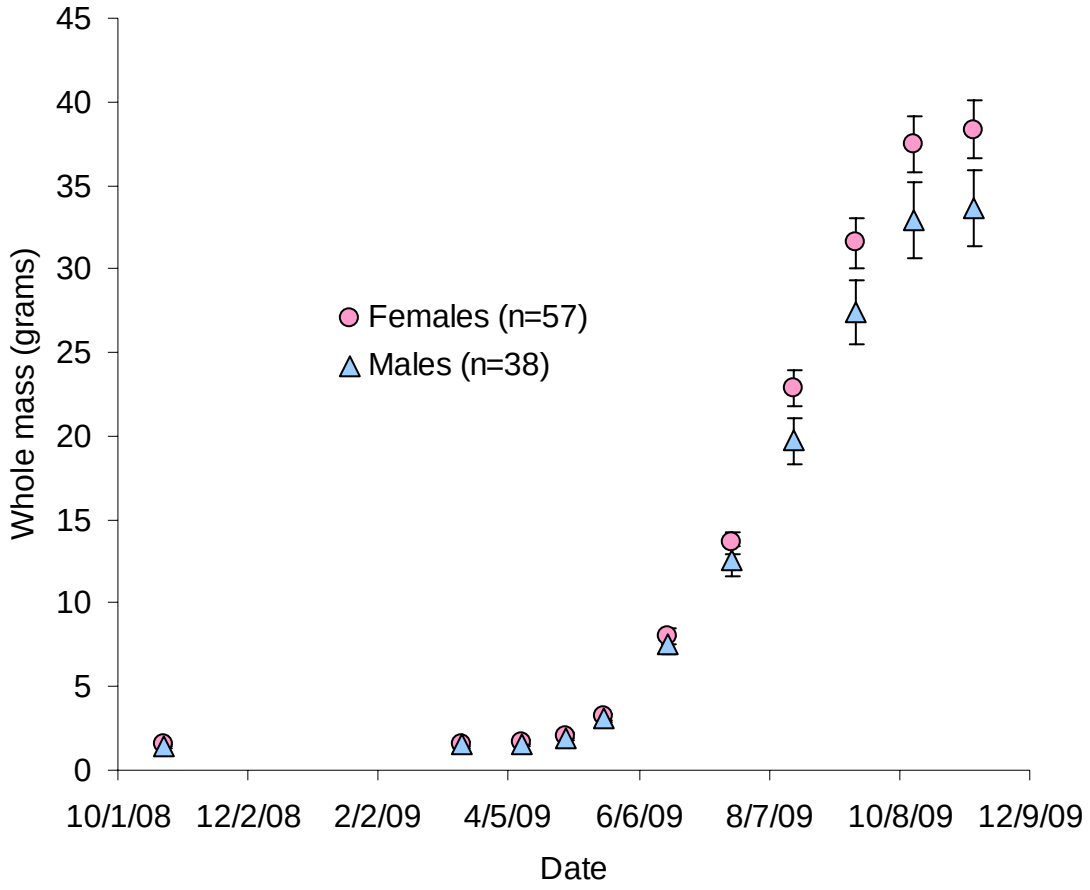

Figure 7. Growth in mass and the onset of sexual dimorphism in fatmucket (*Lampsilis siliquoidea*) reared in a flupsys at the Kansas City Zoo. Females were identified by shell shape and by pigmentation of the ventral margin of the outer ctenidia that was evident by the end of 2009. Females averaged 14% heavier than males in the last measurement (November 12), when the mussels were 2 years old. No brooding was observed. Data are means and 95% confidence intervals. The relative difference in size of males and females doubled abruptly between the July 20 and August 10 measurements (see Figure 8)

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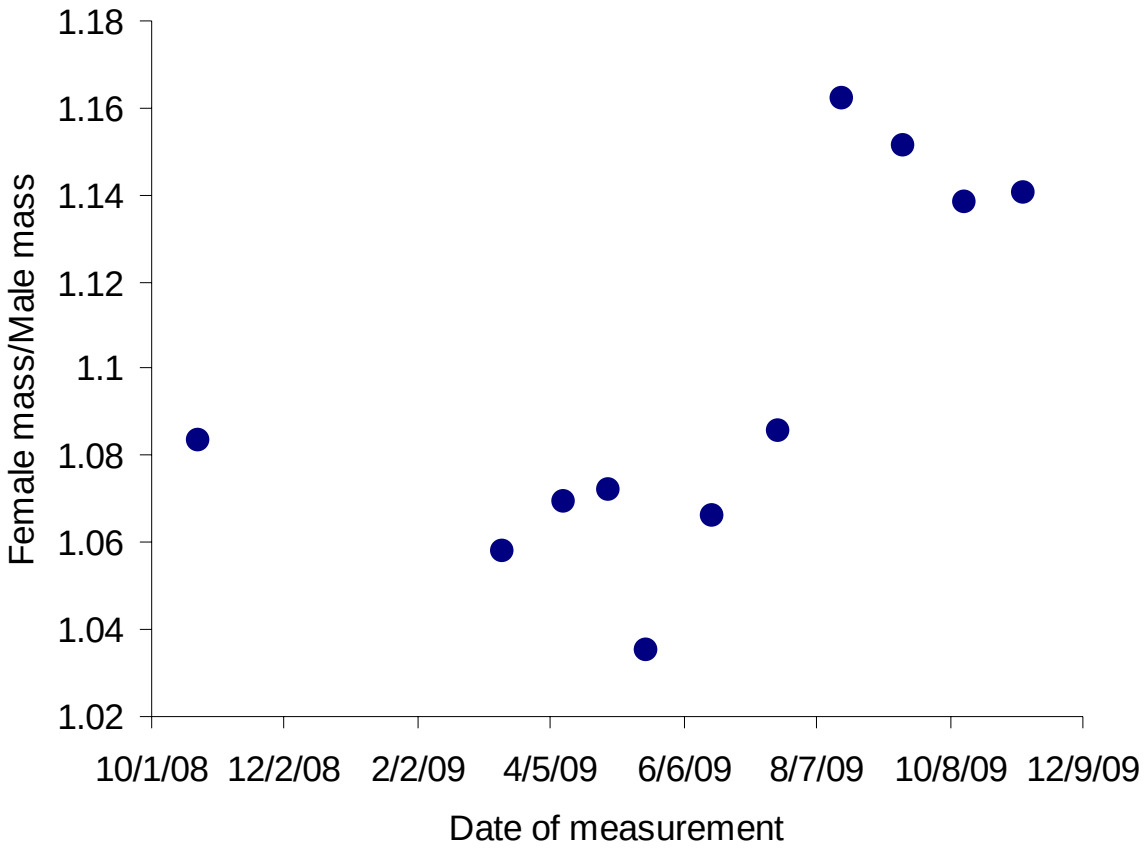

Figure 8. Ratio of mean female mass (n=57) to mean male mass (n=38) vs. date in a cohort of maturing fatmucket (*Lampsilis siliquoidea*) reared in a flupsys at the Kansas City Zoo. Females were identified by shell shape and by pigmentation of the ventral margin of the outer ctenidia that was evident by the end of 2009. Because they were tagged the previous year, measurements predating sexual maturation could be compared. Relative difference in mass was evident in 1-y animals (10/23/08). However, the difference but approximately doubled between the July 20 and August 10 measurements in 2009. This change suggests that 2-y males may have diverted resources from growth to sperm production. No egg production (brooding) was observed.

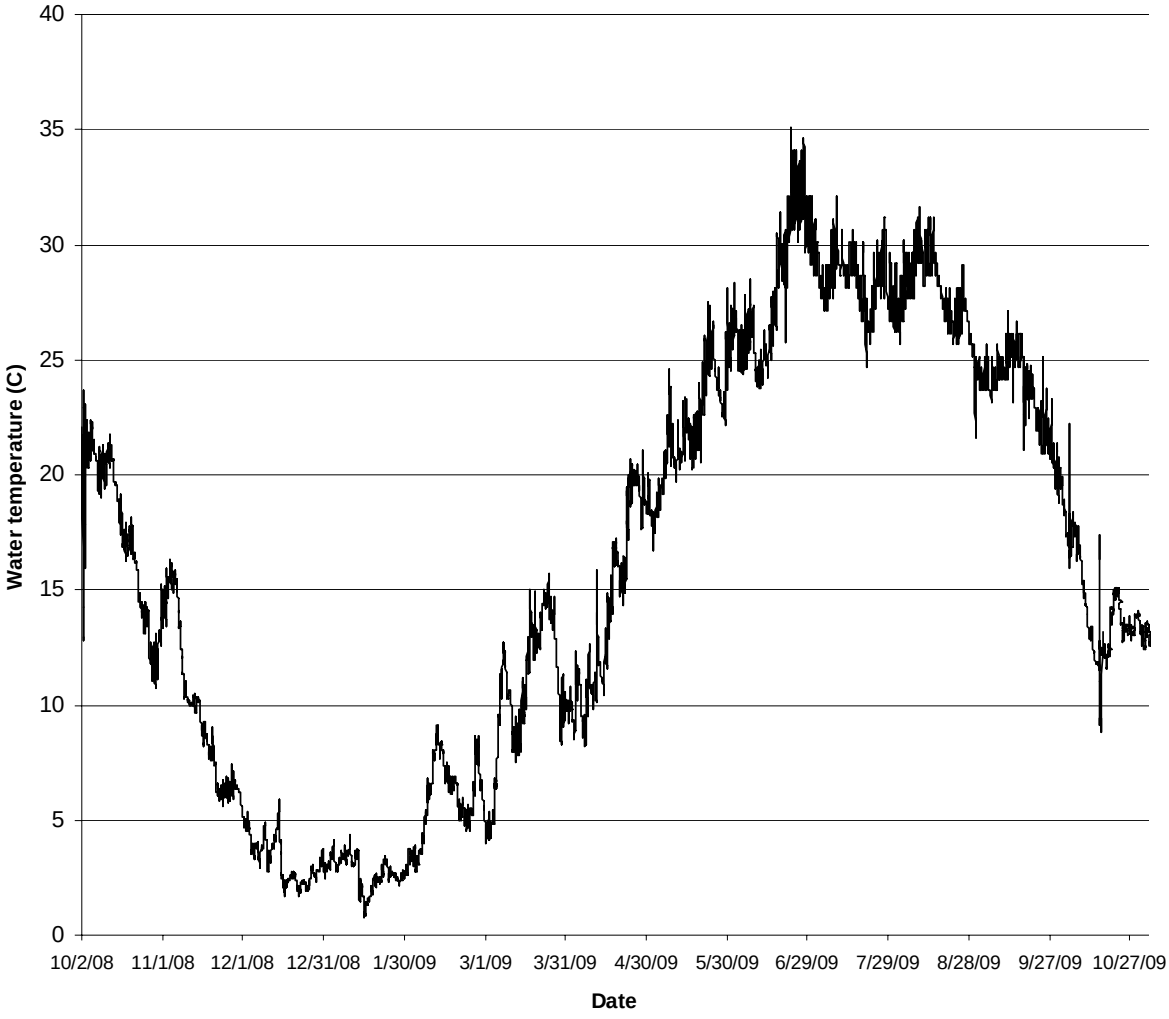

Figure 9. Water temperature recorded hourly from 10/2008 through 10/2009 in the flupsys at the Kansas City Zoo.

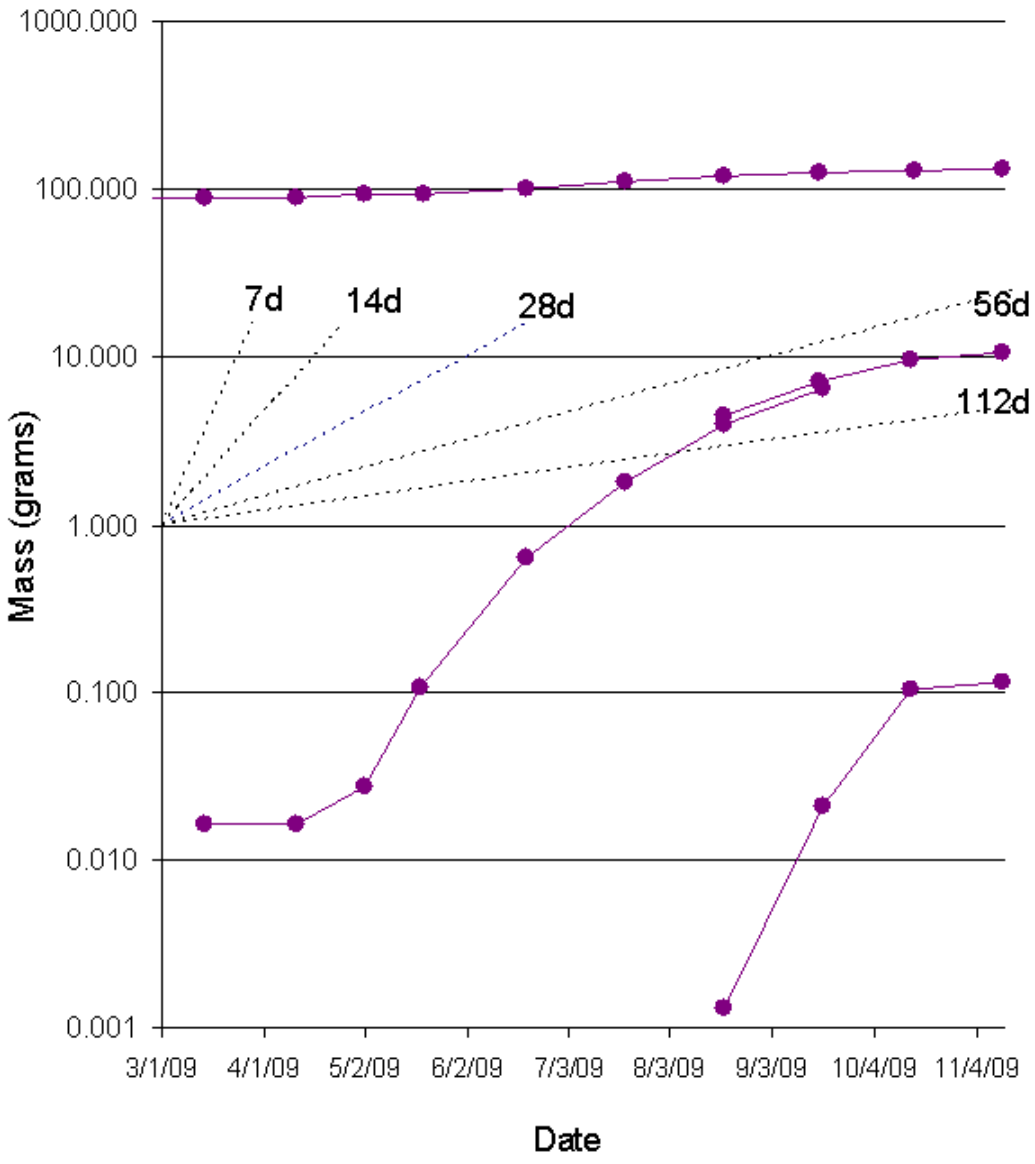

Figure 10. Effect of season and body size on growth rate of black sandshell, (*Ligumia recta*). Growth of three cohorts is shown as whole mass (log scale) versus time. Data are means. The dotted lines and numbers indicate slopes that correspond with doubling times of 7-days, 14-days, etc. One cycle on the Y-axis indicates a 10-fold change in mass. The uppermost curve is a group of 13 Sac River specimens that appear to have recruited in 2005 and are presumably in their 4th year of growth. The middle curve is a group of animals propagated in winter 2008 and in their first year. The lower curve is a group that were propagated in May of 2009 and moved to the flupsy at the end of August. Note the much faster relative growth rates of smaller animals.

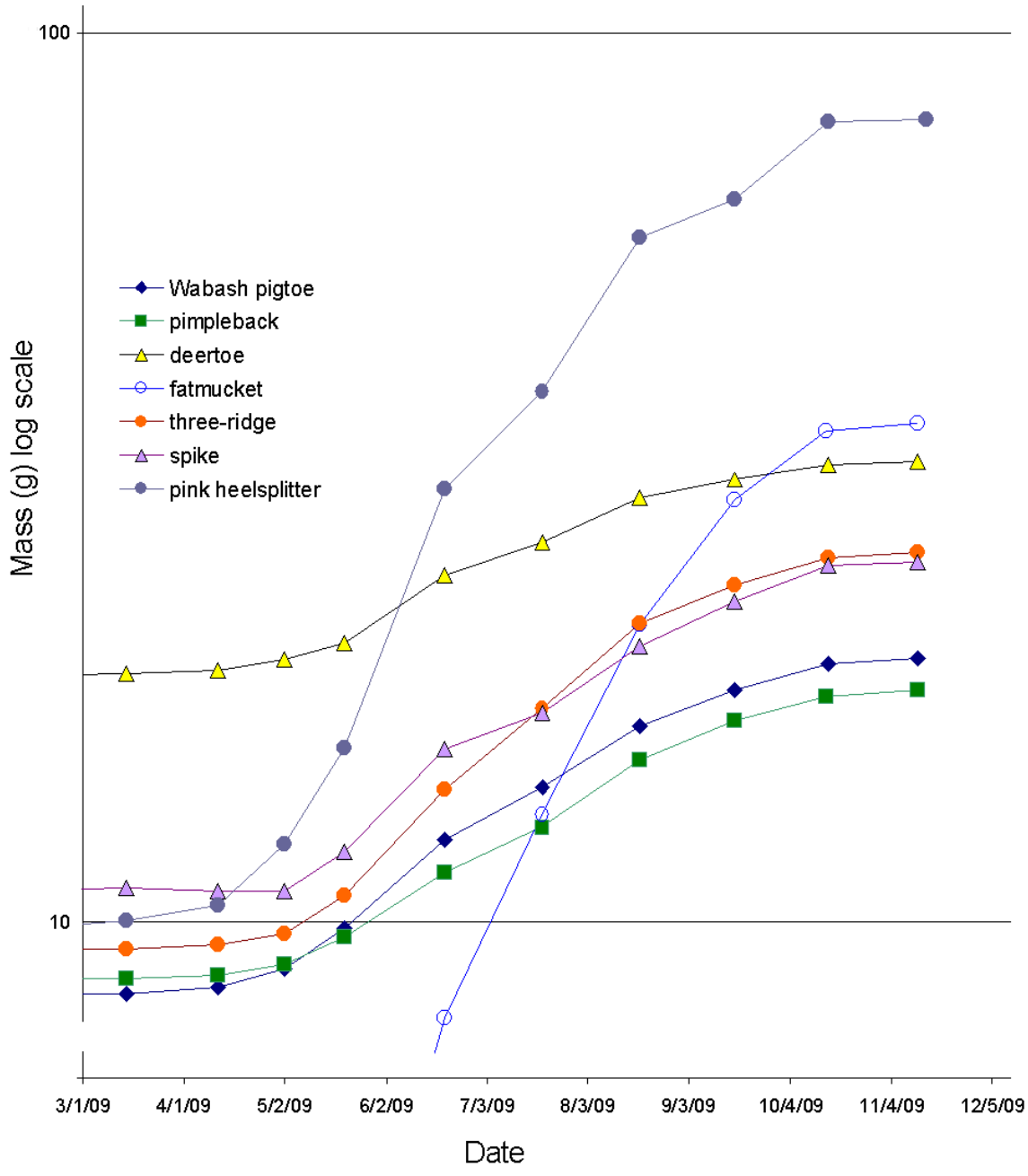

Figure 11. Growth curves of several species of subadult mussels held in the Kansas City Zoo flupsy in 2009. Data are means of cohorts of individuals of similar age and size. Fatmucket were propagated mussels in their second year of growth. The other species were subadults collected from the Sac River and placed in the flupsy in Oct 2008. Growth rates (slope) of fatmucket and *alatus* were 2-4 times higher than the other species when compared at similar mass and over the same growth periods.

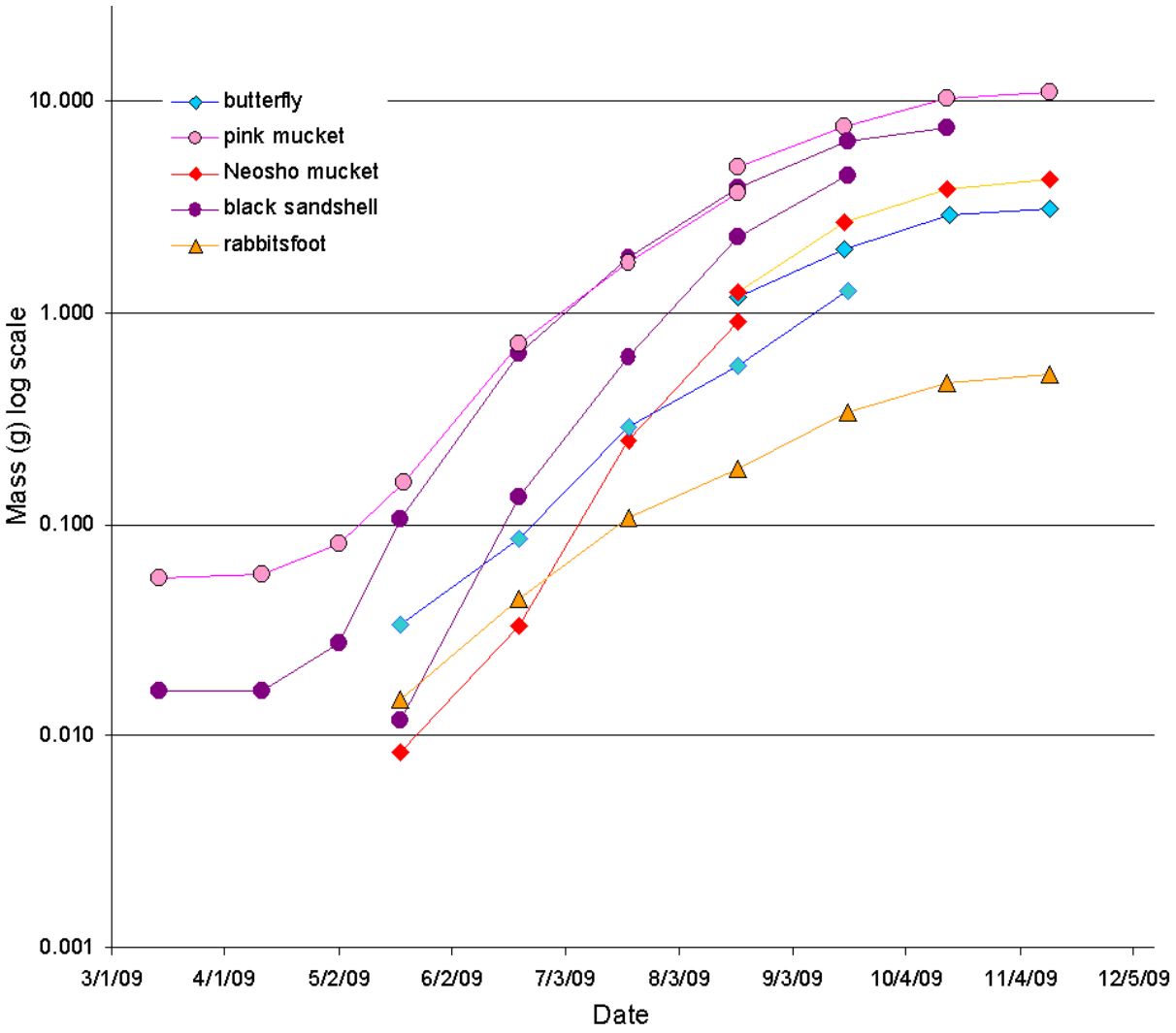

Figure 12. Growth curves of small juveniles (0.5–1 year old) of several species of propagated mussels held in the Kansas City Zoo flupsy in 2009. Data are means of groups of cohorts of similar age and size individuals. Mass of individuals less than about 1 gram was estimated from length measurements and length-mass relationships (Table 2). Separations in species lines occur when a subset of animals in that cohort was tagged and subsequently weighed directly. Each cycle on the Y axis (log scale) indicates a 10-fold change in mass. Note the relatively slow growth of rabbitsfoot and butterfly compared to Neosho mucket and black sandshell. Pink mucket grew at rates comparable to similar-size black sandshell in late summer.

FACTORS AFFECTING METAMORPHOSIS SUCCESS OF LARVAL FRESHWATER MUSSELS (UNIONIDAE)

Biology

Missouri State University, August 2009

Master of Science

Andrea K. Crownhart

ABSTRACT

Larval parasitism of fish is a critical stage in the lifecycle of freshwater mussels (Unionoida). Most fish species are innately immune to most mussel species, and each mussel is able to utilize only particular host species. The evolutionary origin and mechanisms of host specificity are not well understood. Metamorphosis success (%M = percent of attached glochidia that successfully metamorphose) was analyzed for 25 mussel species and 41 mussel-host species pairs. Mean %M of 11 mussels with large carnivorous hosts was 79% (range=57-92%). However, among individual fish, %M varied from near zero to 100%, with mean CV=0.16. Mussels with small lures or conglutinates and insectivorous hosts (n=19) had lower %M with mean=40% (range=2-71%) and greater variation among individual fish (CV=0.49). The %M of five mussel species that attach to fins as well as gills was also low with mean %M= 24% (range 19-42%). However, in simultaneous infections %M was higher on fins than gills (p=<0.001). These results support hypotheses that mussel host infection strategies influence the evolution of host specificity. Individual differences in %M among fish were tested by inoculating freshwater drum simultaneously with *Ellipsaria lineolata* and *Potamilus alatus*. Metamorphosis success of both mussels was strongly correlated among individual hosts (R^2=86%). Differences in %M among individual fish were not species-specific and might result from a different mechanism than host-specificity.

KEYWORDS: Unionoida, glochidia, parasitism, specificity, captive culture

This abstract is approved as to form and content

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Chairperson, Advisory Committee
Missouri State University

ACUTE TOXICITY OF AMMONIA AND COPPER TO GLOCHIDIA OF TWO UNIONID MUSSEL SPECIES INSIDE AND OUTSIDE OF CONGLUTINATES

Biology

Missouri State University, June 2009

Master of Science

Michael John Pillow

ABSTRACT

Regulations regarding the allowable limits of water pollution are often based on the levels of those pollutants that are toxic to the most sensitive freshwater organisms. Freshwater mussels (Unionoida) are unusually sensitive to certain pollutants and therefore have potential to drive water quality standards. However, data must be based on the normal routes and duration of exposure of each life stage to pollutants. Larval mussels (glochidia) are parasites on the gills or skin of fish. Glochidia of many species are released from the female mussel within aggregates of eggs (conglutinates) that act to attract a host. The host frees the glochidia and becomes infected when attempting to feed on conglutinates. Time spent within conglutinates may far exceed the time spent free in the water. This study compares the toxicity of ammonia and copper to glochidia inside and outside of conglutinates. Two species were investigated, one with cohesive conglutinates (*Pleurobema sintoxia*) and one with sheathed conglutinates (*Ptychobranchus occidentalis*). Glochidia of both species remained viable longer within conglutinates than in water, both in the presence and absence of toxicants. *P. occidentalis* glochidia survived longer than those of *P. sintoxia*, but the different conglutinate structures of these species appeared to confer similar protection against the toxicants. This information aids in understanding the ecological exposure of glochidia to dissolved aquatic pollutants and in determining protective water quality criteria.

KEYWORDS: (Unionoida, toxicity, water quality, conglutinates, glochidia, ammonia, copper)

This abstract is approved as to form and content

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