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Reproductive ecology of *Actinonaias ligamentina* (Bivalvia:Unionidae) in a regulated river

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Abstract. Factors affecting the reproductive success of freshwater mussels in lotic systems are poorly understood. Gravidity, fecundity, and fertilization success of *Actinonaias ligamentina* were examined at 4 sites along a 63-km reach of the Green River immediately below the Green River Dam, Kentucky. No gravid females were collected at the site closest to the dam, and the percentage of gravid females at downstream sites ranged from 20 to 36%. Not all females became gravid, despite the presence of early stages of ova in the gonadal fluid. This observation suggests that female *A. ligamentina* undergo a resting stage and, therefore, might not become gravid every year. Fecundity differed among sites and increased with distance from the dam. Fertilization rates ranged from 32 to 97% among sites and increased with distance from the dam. Fertilization rate was independent of local mussel density and position in the mussel bed. The high fertilization rates observed in the upstream portions of mussel beds indicate that freshwater mussel sperm have the ability to travel to distant females in lotic systems. Therefore, females are not necessarily dependent upon nearby males for fertilization. Successful fertilization of *A. ligamentina* at low mussel densities in the Green River suggests that natural recovery of rare endangered species might be possible if host fish and suitable conditions for juvenile survival and growth are present.

Key words: freshwater mussels, fertilization, gravidity, fecundity, dam.

Freshwater mussels have a unique and complex life cycle. Eggs are produced in the ovaries of the female. Prior to spawning, the eggs are moved to specialized chambers within the marsupial gills, and the gills become inflated. Males release sperm into the water column, and fertilization of eggs is achieved when females uptake sperm while siphoning. After fertilization, the embryos develop into larvae (glochidia) in the marsupium. During this brooding period, the female mussel is *gravid.* Once mature, the glochidia are expelled to infect fish. If a suitable host fish is encountered, the glochidium encysts on the fish and undergoes metamorphosis. Once mature, the newly metamorphosed juvenile excysts and settles to the substrate to continue the cycle if conditions are suitable. Disruption of any stage in the life cycle could result in recruitment failure.

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The greatest decline of mussel species in North America has occurred in the southeastern USA (Bogan 1993). At present, only 25% of the 269 species endemic to the southeastern USA are considered stable (Neves et al. 1997). Much attention has been given to the plight of mussels within the last 25 y, but some populations began declining nearly 150 y ago (Higgins 1858). These declines were perpetrated indirectly and, in some instances, directly by anthropogenic activities throughout the 20th century. The causes of the downward spiral in North American freshwater mussel populations include pollution, habitat destruction, introduction of exotic species, and dam construction (Strayer et al. 2004).

Construction and operation of dams have been particularly devastating to mussel populations. Upstream of dams, inundated mussels suffer from loss of riverine habitat and host fishes, increases in water depth and siltation, and anoxic conditions in the hypolimnion (Williams et al. 1992, Blalock and Sickel

1996). The habitat downstream of dams also differs substantially from a free-flowing river. Discharges of cold hypolimnetic water can alter dissolved O_2 concentrations and downstream thermal regimes (Layzer and Scott 2006). Cold-water releases can inhibit gametogenesis, thereby preventing reproduction of mussels (Layzer et al. 1993, Heinricher and Layzer 1999). Absence of minimum flows can cause prolonged dewatering of mussel beds (Layzer et al. 1993, Layzer and Scott 2006). Alteration of the flow regime during the critical life stages of spawning, glochidial release, and juvenile settlement probably has contributed to the decline of mussels in regulated rivers (Layzer and Madison 1995).

Dams often are a proximal factor in the decline of mussel populations, but the ultimate causes can be unclear. For instance, Hardison and Layzer (2001) found that mussel densities were negatively associated with shear stress below 3 dams regulated primarily for flood control. Hardison and Layzer (2001) hypothesized that increased discharge during spring and early summer resulted in higher shear forces that prevented settlement of newly metamorphosed juveniles. However, altered flow regimes also could affect mussel reproduction in other ways. During periods of low flow, fertilization might be reduced if flow were insufficient to transport sperm to distant females. During periods of high flow, fertilization might be reduced or impossible if sperm concentrations were severely diluted by high water volume. Impoundments might be a barrier to sperm transport. If female mussels are dependent for fertilization on sperm released by distant individuals upstream, fertilization success for females closest to a dam might be low because of the lack of available sperm. However, none of these hypothesized effects of dams on sperm transport and fertilization of freshwater mussels has been studied.

The effect of mussel density on fertilization success in lotic systems also is unknown. Successful fertilization has been shown to be dependent on mussel density in a lentic population of *Elliptio complanata* (Downing et al. 1993). Fertilization rates ranged from 100% at densities >45 ind./m^2 to 0% at densities <10 ind./m^2. Mussel densities typically are much lower in lotic than in lentic systems (Cochran and Layzer 1993, Richardson and Smith 1994, Ahlstedt and Tuberville 1997, Hardison and Layzer 2001, Villella and Smith 2005); thus, successful fertilization might occur at much lower densities in lotic than in lentic systems.

Identification of the cause(s) of recruitment failure in specific mussel populations is difficult because factors associated with the reproductive success of mussels are largely unknown in streams. In addition, unknown effects of density on reproductive success can confound identification of dam-induced effects on reproductive success. We examined the effects of downstream distance from a dam and mussel density on gravidity rates, fecundity, and fertilization success in a widespread mussel species, the Mucket (*Actinonaias ligamentina*).

Study Area

Our study was conducted in the Green River, Kentucky. This river originates in Lincoln County, in south-central Kentucky, and it flows 645 km in a westerly direction to its confluence with the lower Ohio River. The Green River was once one of the most species-rich tributaries to the Ohio River and had at least 72 species of freshwater mussels (Cicerello 1999). Species diversity remains high in the Green River, and at least 53 species are currently documented. However, recent recruitment, particularly of *A. ligamentina*, has been limited in the upper Green River since completion of the Green River Dam at river kilometer (rkm) 492 in 1969 (Cicerello 1999). The dam is operated by the US Army Corps of Engineers for flood control and recreation. When discharge is <10 m^3/s, water is released from a multilevel bypass system consisting of 5 ports, which allow for temperature control (USACE 1977). The minimum flow released by the dam is ~1.4 m^3/s, which can exceed inflow to the reservoir during dry periods. Hardison and Layzer (2001) suggested that the limited recruitment was a consequence of the altered flow regime, especially the unseasonably high discharge during spring and summer.

Methods

Study species

Actinonaias ligamentina is a widespread interior-basin species that typically inhabits medium to large rivers from western New York to Minnesota south to Louisiana (Parmalee and Bogan 1998). *Actinonaias ligamentina* is a bradytictic species that spawns in late summer, broods the glochidia through the winter, and releases them in spring (Lefevre and Curtis 1910).

Timing of sample collections

Mussels were sampled qualitatively (by a tactile and visual search) during a 6-wk period beginning in early August to determine the spawning period and to track development of glochidia. Individuals were gently pried open and examined for the presence of inflated marsupial gills. All nongravid individuals were immediately returned to the substrate. A portion of

the gill contents of gravid *A. ligamentina* was extracted with a syringe and examined with a microscope (see *Laboratory methods*). Immediately following extraction of the gill contents, gravid females were returned to the substrate. Females with fully inflated gills were observed on 18 August, but fertilized eggs were not observed until 25 August. Development of embryos into glochidia occurred shortly thereafter, and viable glochidia were observed on 11 September. Therefore, mussel sampling was done in early October when fertilized and unfertilized eggs could best be differentiated.

Mussel sampling

At each site, a qualitative visual search was done to locate the approximate boundaries of the mussel bed and the area containing the highest density of individuals. An initial transect was centered over the area of highest density and additional transects were placed at 10-m intervals upstream and downstream of the initial transect. Transects were added until the upstream and downstream extents of the bed had been sampled. Transects were oriented perpendicular to flow and were delineated with 2 iron stakes driven into the substrate 10 m apart. Nylon string was stretched tight along the substrate and tied to each stake.

Mussel density was estimated by collecting 20 quadrat (0.25 m^2) samples along each transect. Quadrats were placed every 0.5 m along each transect in an alternating upstream and downstream pattern. The substrate within each quadrat was excavated to a depth of 10 cm using a hand trowel and placed into a 3-mm mesh bag (Dunn 2000). Contents of each bag were emptied into a 12.5-mm-mesh sieve to separate mussels from the sediment. All mussels were identified to species and measured to the nearest 1 mm along the longest axis parallel to the hinge, and all *A. ligamentina* were retained for analysis.

The number of *A. ligamentina* collected along the transect was increased by sampling the 0.25-m^2 area between quadrats qualitatively (by a tactile and visual search). All mussels encountered were removed and placed into 6-mm mesh bags. Mussels collected from quantitative and qualitative samples were kept separate. All mussels were identified to species and measured to the nearest 1 mm along the longest axis parallel to the hinge, and all *A. ligamentina* were retained for analysis.

All *A. ligamentina* collected from transects were gently pried open and examined for the presence of inflated gills. Individuals with inflated gills were classified as females. Gonadal fluid was extracted to determine the sex of nongravid *A. ligamentina* (Bauer 1987, Christian et al. 2000, Saha and Layzer 2008). Individuals <80 mm were considered to be sexually immature (KRM, unpublished data), and gonadal fluid was not extracted. A wooden wedge was inserted between the valves of sexually mature individuals to hold them open. Deionized water (0.3 mL) was injected into the gonad with a numbered syringe, and ~0.3 mL of gonadal fluid was withdrawn. The syringe was capped and placed on ice in a cooler. All nongravid individuals were returned to the substrate. Gravid individuals were preserved whole for fecundity and fertilization analysis. Each individual was placed into a polyethylene bag containing 70% ethyl alcohol and placed on ice in a cooler.

A visual search for additional gravid *A. ligamentina* was conducted in a 5-m-wide band upstream of each of 3 transects (the initial transect, the most upstream transect, and the most downstream transect) after all transects had been sampled. All visible *A. ligamentina* were removed from the substrate, gently pried open, and examined for inflated gills. A maximum of 10 gravid individuals per band were preserved whole (as described previously) for analysis. All other individuals were returned to the substrate.

Laboratory procedures

Gonadal fluid samples.—The gonadal fluid stored in each syringe was flushed onto a standard microscope slide (76 × 25 × 1 mm). An approximately equal volume of 10% aqueous methylene blue solution was added to the gonadal fluid, and the slides were allowed to dry at room temperature for 24 h. Dried slides was examined with a compound microscope at 40×, 400×, and 1000× magnification to determine whether developing gametes were present (Saha and Layzer 2008). Individuals with mature or developing eggs were classified as females, and individuals with mature or developing sperm were classified as males. Individuals that did not contain gametes were recorded as unidentified. The presence of parasitic trematode cercariae in gonadal fluid samples was also noted.

Fecundity.—Gravid females were removed from the bags, and the contents of the bag were filtered and examined for expelled eggs and glochidia. The marsupial gills were excised from each female and stored in separate vials containing 70% ethyl alcohol. The gills were divided into 3 equal sections (i.e., anterior, medial, posterior) based on the number of inflated water tubes. The contents of each gill section were flushed into a square clear counting plate (100 × 100 × 15 mm) with a grid of 36 numbered 13 × 13 mm

cells (Beasley et al. 2003). The gill section was examined with a dissecting microscope to ensure the removal of all glochidia and eggs. The counting plate was shaken gently to distribute the contents evenly. The contents of 3 randomly chosen cells were removed with a pipette and placed in separate vials. The contents of each vial were flushed with water into a zooplankton counting wheel and placed under a dissecting microscope at 40× magnification, and all glochidia, fertilized eggs, and unfertilized eggs were counted. The presence of a fertilization membrane surrounding the egg was used to distinguish fertilized eggs from unfertilized eggs (Matteson 1948). Glochidia were easily identified by the presence of shell valves.

Fecundity was defined as the total number of unfertilized and fertilized eggs, and glochidia. The mean of the 3 counts per gill section was multiplied by 36 to estimate fecundity for the gill section. Fecundity estimates for all sections were combined to estimate total fecundity of a gill. Estimates of fecundity were similar for the left and right gills of the first 10 individuals (paired t-test, $t = 0.40$, $df = 9$, $p = 0.70$), so only the right gill was used to estimate fecundity for the remaining individuals.

Fertilization rate.—The contents of the left gills of all individuals used to determine fecundity (except the 10 used to compare fecundity between gills) were flushed in the counting plate. The contents of one randomly chosen cell were removed with a pipette and placed in a vial. The contents of the vial were flushed with water into a zooplankton counting wheel, and fertilized and unfertilized eggs and glochidia were counted until a combined total of 1000 units was reached. Fertilization rate was calculated by dividing the number of fertilized eggs plus glochidia by the total number of eggs plus glochidia in the section. Fertilization rate of the gill was calculated by dividing the number of fertilized eggs plus glochidia by the total number of eggs plus glochidia in the gill. Estimates of fertilization rates did not differ between the single sample from the left gill and the mean determined from the right gill of individuals used for determining fecundity (paired t-test, $t = 0.7572$, $df = 53$, $p = 0.45$), so fertilization rates were estimated from a single sample from the left gill for all other individuals not used to determine fecundity.

Age determination.—Age of individuals sacrificed for fecundity analysis was estimated by preparing thin sections of shells (Neves and Moyer 1988). The sections (~400 μm thick) were cut from the umbo to the shell margin using a Buehler Isomet low-speed saw with a diamond wafering blade (Buehler Ltd., Lake Bluff, Illinois). Thin sections were examined under a dissecting microscope using transmitted light to count opaque bands (annuli). The number of annuli missing because of erosion of the umbo region was estimated from the estimated total length of the individual at the first visible annulus. Growth arrests might not occur annually in some mussel populations (Kesler and Downing 1997). However, thin sections prepared from shells of live adult *A. ligamentina* notched on the ventral margin and returned to the Green River had 1 new annulus beyond the notch when recaptured 1 y later (JBL and D. J. Crawford, Tennessee Cooperative Fishery Research Unit, personal communication). Furthermore, data from an ongoing study of year-class strength show that growth arrests are annular for at least the first 5 y of life for *A. ligamentina* in the Green River (JBL, unpublished data).

Data analysis

Fertilization data were arcsine(x)-transformed to satisfy the assumption of normality. One-way analysis of variance (ANOVA) was used to test the effect of mussel density along the transect from which a female was collected on fertilization rate of the female. A separate 1-way ANOVA was used to compare fertilization rates among female positions (transect locations) within the mussel bed.

Results

Mussel density

Mussel density and species richness increased with distance from the Green River Dam. *Actinonaias ligamentina* was the most abundant species collected at each site (Table 1). Mean density of *A. ligamentina* ranged from 0.20 to 3.30 mussels/m^2 among sites. The largest and most dense mussel bed sampled was at site 4, where 440 *A. ligamentina* were collected from quadrats excavated along 14 transects. The smallest and sparest mussel bed occurred at site 2, where only 11 *A. ligamentina* were collected in excavated quadrats.

Gonadal fluid samples

Gonadal fluid samples were extracted from 577 *A. ligamentina*. The sex of 567 nongravid individuals from all sites was determined from the presence of gametes in the gonadal fluid; gonadal fluid from 10 individuals from site 4 did not contain any visible gametes, and sex could not be determined (Table 2). Sex ratios at sites 1, 2, and 3 were not significantly different from 1:1 (χ^2 tests, $p \geq 0.522$; Table 2); however, significantly more males than females were collected at site 4 ($\chi^2 = 24.195$, $df = 1$, $p < 0.0001$).

Gonadal fluid samples from 16 of the 577 individuals sampled contained cercariae of bucephalid trematodes. Only the cercariae stage was observed.

TABLE 1. Species richness, density, sample size (n), % gravid, and mean (±1 SE) fertilization rates of *Actinonaias ligamentina* at 4 sites in the upper Green River, Kentucky.

			Density (no./m^2)			
Site	Distance from dam (km)	Species richness	All species	*Actinonaias ligamentina*	% gravid (n)	Fertilization rate (n)
1	2.8	6	1.03	0.57	0.0 (18)	(0)
2	6.7	5	0.60	0.20	20.0 (5)	32.5 ± 1.7 (4)
3	36.3	11	2.83	1.36	36.1 (47)	88.1 ± 4.9 (21)
4	62.5	20	6.64	3.30	31.7 (164)	96.9 ± 0.9 (76)

Cercariae were present only in samples from individuals >100 mm in length. Ten samples that contained cercariae also contained gametes; 6 infected individuals were male and 4 were female. Six samples that contained cercariae did not contain any visible gametes and were obtained from individuals ≥129 mm long.

The percentage of gravid females varied among sites and generally increased with distance from the dam (Table 1). Gravid *A. ligamentina* were collected from all sites except site 1, the site closest to the dam. One of 5 females collected from excavated quadrats and 3 of 71 females collected from the three 5-m bands at site 2 were gravid. The percentage of females that were gravid did not differ between sites 3 (36.1%) and 4 (31.7%) ($\chi^2 = 0.058$, df = 1, $p = 0.810$).

Fecundity

Fecundity was estimated for 64 individuals: all gravid individuals from sites 2 ($n = 4$) and 3 ($n = 21$) and 39 of the 76 gravid individuals from site 4. Fecundity varied among sites and among individuals within sites and ranged from 80,616 to 1,561,224 eggs and glochidia (Fig. 1).

At site 2, fecundity was not related to age ($r^2 = 0.061$, df = 3, $p = 0.217$) or shell length ($r^2 = 0.003$, df = 3, $p = 0.943$). At site 3, shell length was strongly related to age ($r^2 = 0.94$, df = 20, $p < 0.0001$), but neither age ($r^2 = 0.004$, df = 20, $p = 0.929$) nor shell length ($r^2 = 0.001$, df = 20, $p = 0.852$) was related to fecundity. At site 4, shell length was strongly related to age ($r^2 = 0.90$, df = 38, $p < 0.0001$), and fecundity was significantly related to both age ($r^2 = 0.25$, df = 38, $p = 0.0012$; Fig. 2A) and shell length ($r^2 = 0.37$, df = 38, $p < 0.0001$; Fig. 2B).

Inspection of the raw fecundity data for site 4 suggests that fecundity varied spatially within the bed. Therefore, separate regressions of fecundity on shell length were calculated for individuals from transects upstream of the initial transect ($r^2 = 0.58$, df = 16, $p = 0.0004$) and for individuals from transects downstream of the initial transect ($r^2 = 0.64$, df = 21, $p < 0.0001$) (Fig. 3). The slopes of the 2 regression lines were similar (ANCOVA, $F_{1,35} = 0.04$, $p = 0.85$); however, the adjusted means differed (ANCOVA, $F_{1,36} = 0.17$, $p < 0.0001$). Individuals from the upstream portion of the bed were less fecund than individuals from the downstream region.

Fertilization rates

A total of 101 individuals was examined to determine fertilization rates. Fertilization rates did not differ between gills within a female (paired t-test, $t = 0.7572$, df = 53, $p = 0.45$) or among sections within a gill (ANOVA, $F_{2,87} = 0.45$, $p = 0.64$).

Mean fertilization rates increased with distance from the dam (Table 1). No gravid individuals were collect from site 1. Fertilization rates were low (mean = 32.5%) at site 2 and markedly higher at sites 3 (88.1%) and 4 (96.9%).

Fertilization rate was not a function of total density or male density at site 3 (ANOVA, total density: $F_{4,7} = 0.17$, $p = 0.95$; male density: $F_{4,7} = 0.17$, $p = 0.95$) or site 4 (ANOVA, total density: $F_{11,37} = 1.07$, $p = 0.41$; male density: $F_{9,39} = 1.25$, $p = 0.29$). Fertilization rate also

TABLE 2. Sex, sex ratio, χ^2 value, and associated p value for all adult *Actinonaias ligamentina* collected at 4 sites in the upper Green River, Kentucky.

	Sex					
Site	Undetermined	Males	Females	Sex ratio (M:F)	χ^2	p
1	0	20	18	1.1:1	0.105	0.746
2	0	6	5	1.2:1	0.091	0.763
3	0	41	47	0.9:1	0.409	0.522
4	10	266	164	1.6:1	24.195	<0.0001

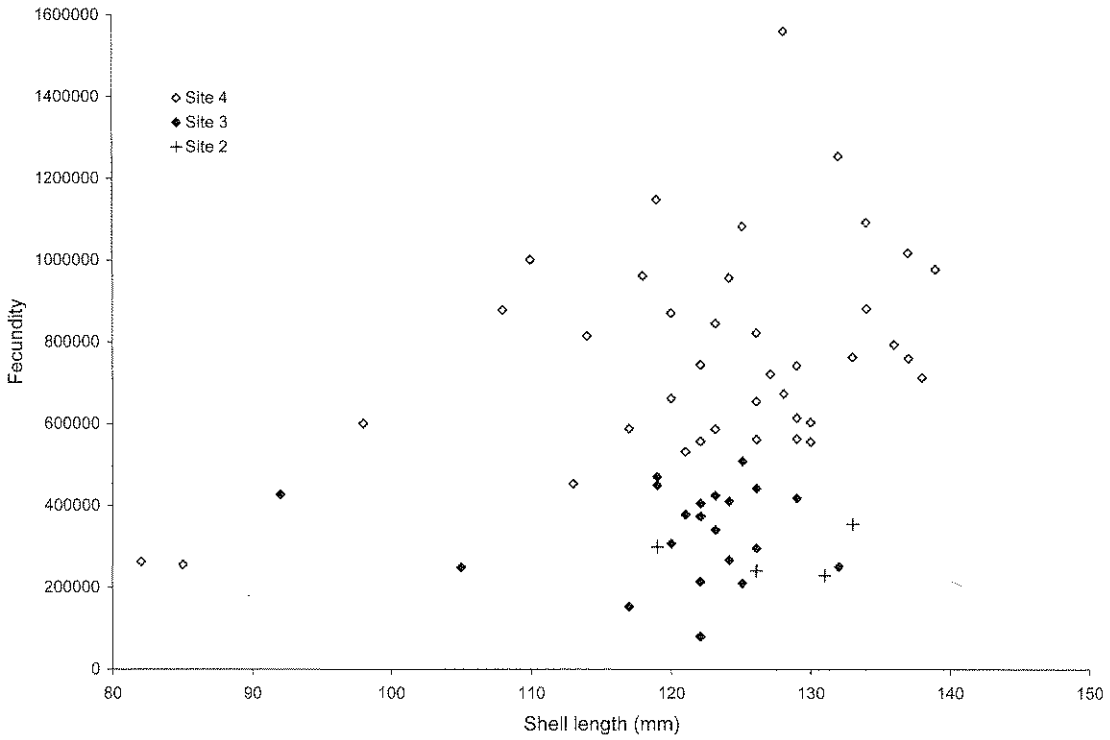

FIG. 1. Scatter plot showing fecundity (sum of unfertilized and fertilized eggs and glochidia) and total length of *Actinonaias ligamentina* at 3 sites in the upper Green River, Kentucky.

was independent of the location of a female within the mussel bed. Fertilization was similar among transects at site 3 (ANOVA, $F_{4,9} = 0.14$, $p = 0.874$) and site 4 ($F_{12,36} = 1.08$, $p = 0.41$) (Fig. 4).

Discussion

Gravidity rates

The highest gravidity rates observed in the Green River (~31–36%; Table 1) are low compared to rates in studies of other species, in which >85% of the females reproduce in a given year (Yokley 1972, Trdan 1981, Jansen and Hanson 1991, Bruenderman and Neves 1993, Haggerty and Garner 2000, Haag and Staton 2003). High trematode infection rates can render mussels sterile (Zale and Neves 1982, Jokela et al. 1993, Haag and Staton 2003), but the incidence of trematodes was similar for gravid (1.4%) and nongravid (1.2%) female *A. ligamentina*. The presence of early developmental stages of ova in the gonadal fluid of nearly all nongravid females indicates they were not sterile; at most, only 6 of the 577 individuals examined might have been sterilized by trematodes.

Gravidity rates of *A. ligamentina* in locations further downstream from the Green River Dam than our study sites and in a population in the Clinch River, Tennessee (JBL, unpublished data) are similar to those in our study. Thus, gravidity rates ≤36% seem to be characteristic of *A. ligamentina*. Bauer (1987) found that an average of 64% of female *Margaritifera margaritifera* became gravid in a given year; he considered the remaining females to be *pausing* or in a resting stage. Pausing females might be replenishing energy reserves depleted during production of gametes in preceding years. Saha and Layzer (2008) reported evidence of pausing in a captive population of *A. ligamentina*, and most nongravid females in our study appeared to be in a natural pausing phase. However, we think it unlikely that all females at site 1 were pausing and more likely that the absence of gravid individuals at site 1 is related to operation of the Green River Dam 2.8 km upstream of site 1.

Fecundity

In some species of freshwater mussels, fecundity is a function of size and age (Downing et al. 1993, Haag and Staton 2003). Fecundity of *A. ligamentina* individuals was positively related to both length and age at site 4 (Fig. 2A, B), but not at sites 2 and 3. Fecundity of bivalves also is closely associated with food and nutrient availability (Beekey and Karlson 2003), and the difference in relationships among sites might indicate that resources are limited at sites 2 and 3. Typically, the proportion of assimilated energy allocated

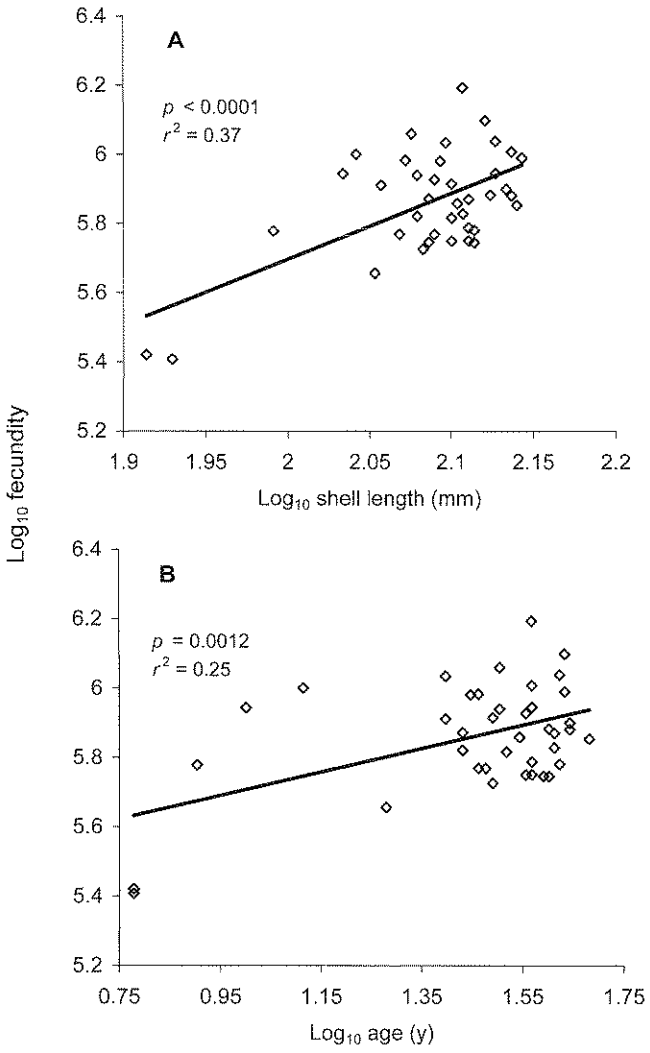

FIG. 2. Regression of fecundity (sum of unfertilized and fertilized eggs and glochidia) as a function of age (A) and shell length (B) of *Actinonaias ligamentina* collected from site 4 in the upper Green River, Kentucky.

to reproduction is relatively high in bivalves (Bayne et al. 1983, Sprung 1991, Jokela et al. 1993). Bauer (1998) found that fecundity of *M. margaritifera* in nutrient-poor streams was better predicted by surplus assimilated resources than by age or length. As mussels become larger, energy requirements for maintenance of base metabolism increase (Jokela et al. 1993), with the result that surplus resources for reproduction decrease with age and size. This shift in resource allocation might explain why the smallest individuals were as fecund as larger individuals at sites 2 and 3. The relatively low fecundity of larger females at sites 2 and 3 compared to site 4 suggests a food deficiency at sites 2 and 3.

The reproductive success of *A. ligamentina* in the Green River increased with distance from the Green River Dam. Impoundment can cause critical changes in the river food web by reducing nutrients and food in the water (Petts 1984, Thornton and Kimmel 1990, Stanley and Doyle 2002). Moreover, some physiochemical conditions in dam tailwaters resemble conditions in lower-order streams (Ward and Stanford 1983, Stevens et al. 1997). Thus, mussels downstream of the Green River Dam might be dependent on tributary inflow for food. Tributary inflow to the river is almost nonexistent in the reach of river between the dam and site 3, and mussels at sites 1, 2, and 3 could be limited by the amount of particulate organic matter discharged by the dam. Russell Creek, a major tributary, enters the Green River between sites 3 and 4 and might contribute a substantial amount of food to mussels at site 4.

The difference in fecundity between upstream and downstream areas from site 4 (Fig. 3) was unexpected and indicates that factors affecting reproductive success of mussels are more complex than generally is recognized. If food availability were limiting reproduction at site 4, then fecundity should be higher in the upstream compared to downstream portion of the bed because upstream mussels siphon food from the water column before it can reach downstream mussels. At site 4, fecundity was lower in the upstream portion of the bed than in the downstream portion. This result provides support for the conclusion that food availability does not limit reproduction at site 4.

Another factor that might have influenced fecundity patterns at site 4 is habitat suitability. Mussel beds exist in areas of stable substrate (Strayer 1999). The upstream periphery of a mussel bed might be less stable and more prone to scour than the center or bottom of the bed. Thus, mussels along the upper end of a bed might expend more energy maintaining position during high flows and have less energy available for gamete production.

Fertilization rate

Fertilization of *A. ligamentina* occurred successfully at mussel densities far lower than minimum densities required for successful fertilization in a lentic population of *E. complanata* (Downing et al. 1993). Most (92%) gravid *A. ligamentina* collected from our study sites showed high (>90%) fertilization rates (Table 1). Moreover, fertilization rates were similar in low- and high-density areas within a bed. Some threshold of male density must have to be exceeded to achieve high fertilization rates, but the threshold density probably is much lower than the density of *A. ligamentina* at site 3. Fertilization rates of 2 female *Lampsilis fasciola* and 3 female *Lampsilis cardium* (also bradytictic species)

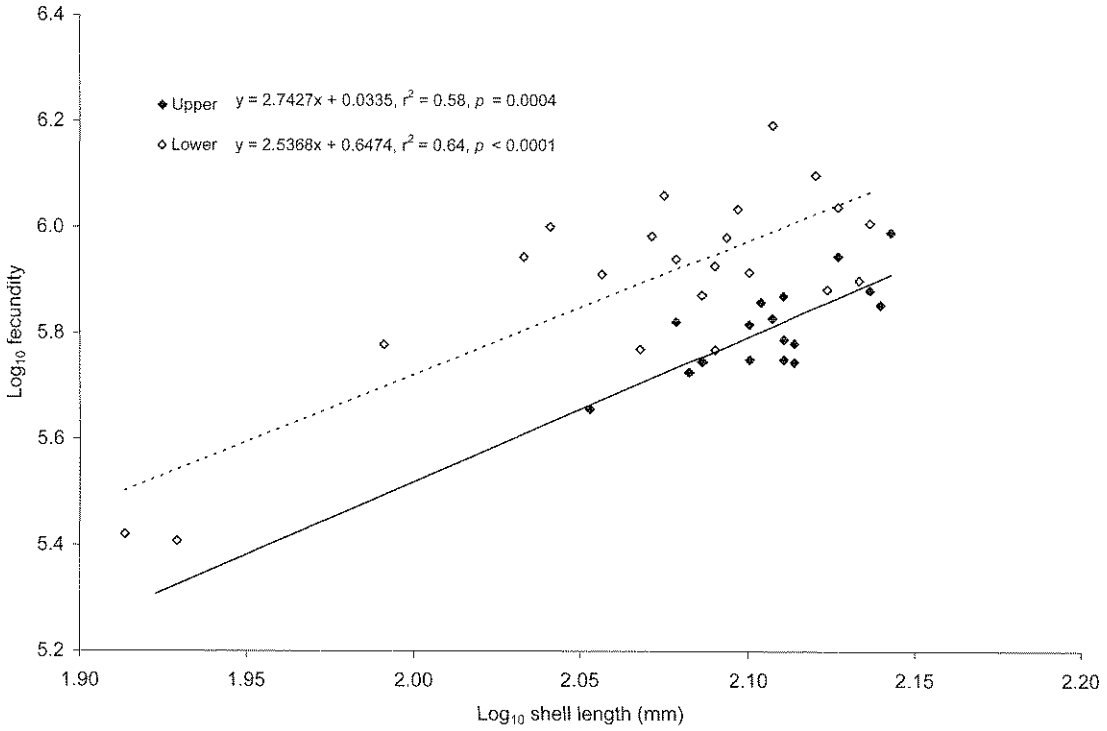

FIG. 3. Length–fecundity relationships for *Actinonaias ligamentina* in the upper and lower portions of the mussel bed at site 4 in the upper Green River, Kentucky. Fecundity is the sum of unfertilized and fertilized eggs and glochidia.

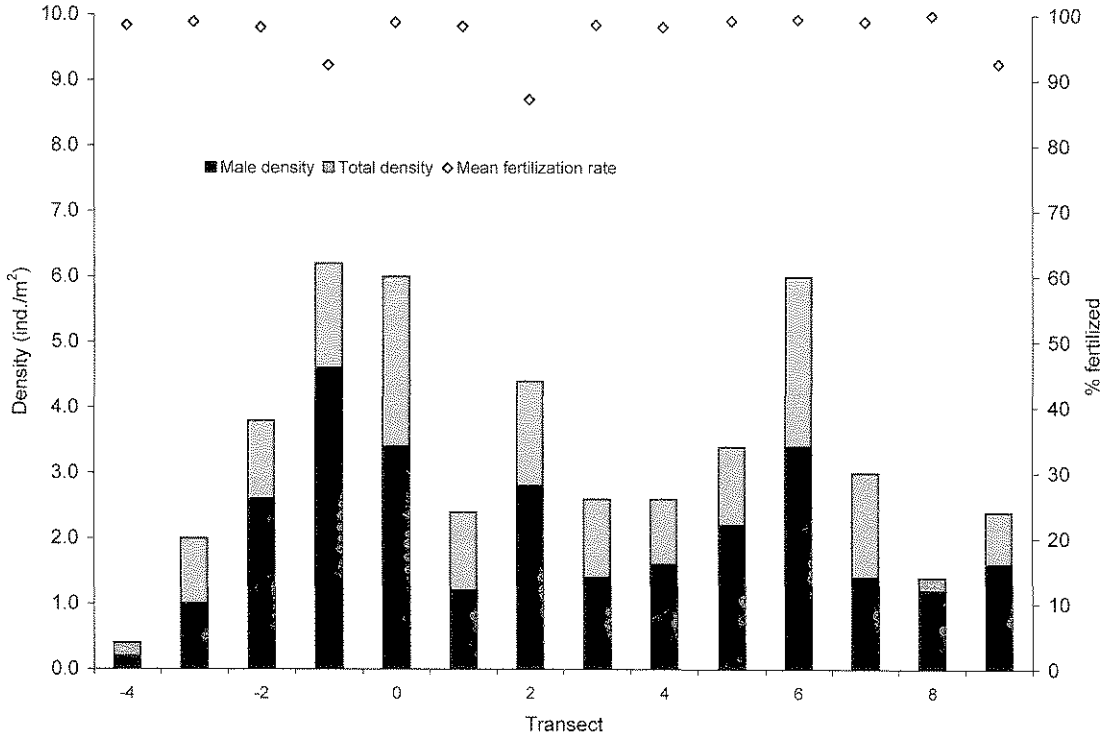

FIG. 4. Total density, male density, and mean fertilization rates of *Actinonaias ligamentina* for each transect at site 4 in the upper Green River, Kentucky. Transect 0 is the initial transect. Negative numbers designate upstream transects, and positive numbers designate downstream transects.

collected at site 4 during our study were >90%, yet the total densities of these species were ≤0.07 ind./m^2. Successful fertilization at these low densities suggests that natural recovery of rare endangered species might be possible if host fish and suitable conditions for juvenile survival and growth are present.

Fertilization rates did not differ between upstream and downstream portions of beds. However, densities tended to be lower at upstream than at downstream transects, and few or no mussels were found immediately upstream of beds. Thus, the supply of sperm from nearby males was limited in upstream portions of beds. Distant males appear to be an important source of sperm for females in the upstream areas of a bed. This result also suggests that gene flow between beds must occur frequently.

The mechanisms through which successful fertilization is achieved in sparse populations are not fully understood. Males release sperm balls (spermatozeugmata) that consist of a spherical colorless body in which the heads of thousands of spermatozoa are embedded (Edgar 1965, Lynn 1994, Waller and Lasee 1997, Ishibashi et al. 2000). The beating tails of embedded sperm move spermatozeugmata through the water (Edgar 1965, Ishibashi et al. 2000) and might help to keep the spermatozeugmata suspended in the water column. Free spermatozoa are viable for only a few minutes when exposed to water, but spermatozoa embedded in a spermatozeugmatum remain viable for 48 to 72 h (Ishibashi et al. 2000). These characteristics would allow sperm from distant males to fertilize females and might be the mechanism that ensures successful fertilization at densities much lower than the minimum density needed for successful fertilization in a lentic system.

The low fertilization success of *A. ligamentina* at site 2 probably was the result of several factors. First, density of *A. ligamentina* at the site was low (0.2/m^2). Second, few *A. ligamentina* individuals occur upstream of site 2. Only a few individuals were found at site 1, and no known individuals occur between sites 1 and 2. Third, gametogenesis was low in females at site 1 and also might have been low in males. The combination of fewer males contributing sperm and potentially fewer sperm per male at site 1 and a probable increase in sperm concentration as water moves across downstream beds and accumulates spermatozeugmata might have created an upstream–downstream gradient of sperm density in the water column.

Effects of dams on mussel reproductive success

Dams have had major effects on mussel populations. Many of these effects, such as streambed dewatering, extirpation of host fishes, and coldwater discharges that inhibit gametogenesis have long been recognized. Dams might also have more subtle effects on mussel reproduction by disrupting the hydrologic connectivity in lotic systems. These effects might be more insidious because they are not as readily apparent as those previously identified. We see 2 critical gradients below the Green River Dam that might have produced the spatial patterns of fecundity and fertilization observed in our study. First, food availability probably increased in the downstream direction. Second, sperm availability probably increased in the downstream direction.

Energy requirements for gametogenesis can be substantial. The increases in gravidity and fecundity with distance from the Green River Dam suggest a gradient in food availability. Recovery in fecundity occurred only after major tributary inflow and 64 km of instream production. We would expect the distance for recovery of fecundity in other streams to be influenced by a multitude of factors, including geology, watershed size and development, mode of dam operation, and water retention time.

Fertilization rates are probably related to sperm density in the water column. Because sperm originating from distant males seemed to be as important for fertilization as sperm originating from local males, unrestricted sperm transport is critical for successful fertilization. The low fertilization rate close to the Green River Dam probably was caused by an absence of sperm originating from males upstream of the impoundment and the low density of mussels in the upper reach of our study area.

The distance the river traveled below the Green River Dam before full reproductive recovery of *A. ligamentina* occurred seems ominous for mussels in other southeastern US rivers. Many of these rivers have multiple dams with limited lotic habitat between impoundments. Potentially, recovery zones in these streams might be too short or nonexistent, and mussel reproduction could be severely impaired.

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