Status Report on Research with *Atya lanipes* and *A. scabra* in Puerto Rico

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RESUMEN

En Puerto Rico se están llevando a cabo investigaciones con los camarones de agua dulce *Atya lanipes* y *A. scabra* con el propósito de proveer información básica e útil para el cultivo larval en masa y la producción de juveniles para propósitos de acuicultura. Los estudios se conducen en torno a los factores ambientales que afectan el desarrollo y supervivencia larval, tales como salinidad, temperatura, y pH. También se están estudiando los parámetros reproductivos de número de huevos, eclosión, incubación , y la relación entre el peso del cuerpo de la hembra vs producción de huevos.

Se evaluó tolerancia media (TL50) para salinidad y temperatura en embriones y larvas recien nacidas durante pruebas estáticas de 96 horas. Se determinarón diariamente los parámetros de concentración total de nitrógeno en forma de amoníaco y la concentración total de nitrógeno en forma de nitritos. La tolerancia de larva a cambios en salinidad produjo más de 50% mortandad a 0 ppmil con un decenso en mortandad según aumenta la salinidad a 30 ppmil en ambas especies. La mejor eclosión ocurre en agua dulce mientras mejor supervivencia larval ocurre a una salinidad de 30 ppmil. El efecto de temperatura sobre la mortandad larval indica una tolerancia media de temperatura con intérvalo entre 20 y 32°C con ninguna diferencia significativa en la tasa de mortandad.

Las relaciones entre peso de la hembra vs producción de huevos demuestra una correlación significativa de ($r = 0.59$) para *A. scabra* y de ($r = 0.95$) para *A. lanipes*. Por cada gramo en peso de la hembra se estima una producción de 2,000 huevos en *A. scabra* (peso promedio de 4.45 gramos) y unos 1,600 huevos para *A. lanipes* (peso promedio de 3.66 gramos). Esto provee evidencia de apoyo para ambas especies como candidatas potenciales para la acuicultura.

INTRODUCTION

Freshwater shrimp of the family Atyidae have been reported from the Caribbean islands of Dominica (six species), Guadeloupe (seven species), Cuba (nine species), Jamaica (eight species), and Puerto Rico (seven species) (Fryer, 1977). Almost all of the species have been reported to live in swift flowing streams attached to rocky substrates, under vegetation and debris which provide adequate cover for them. Many small females become ovigerous, bearing several hundred eggs while small with approximately up to 4000 eggs in a

clutch (Hobbs and Hart, 1982). The eggs are retained beneath the abdomen of the female until they hatch and the escaping larvae must make their way to the mouth of the river where at higher salinities larvae develop and metamorphose into juveniles.

Nothing is known of the habits and factors which affect the larvae of these species except for Hunte's (1975) description of *Atya lanipes* first larval state. These detrital filter feeding species show little intraspecific aggressive or cannibalistic behavior, unlike the commonly reared *Macrobrachium* spp. Thus these atyid shrimp can be kept at high densities in the laboratory. Shrimp of the species *A. lanipes* and *A. scabra* are also fished for food in Puerto Rico which justify their potential for mass production in aquaculture.

Research in Puerto Rico has been conducted on *A. lanipes* and *A. scabra* since 1985 to provide basic information useful for mass larval culture and the production of juveniles for aquaculture purposes.

The primary objectives of this project have been:

1. To determine which salinities will give best egg hatch and larval survival.
2. To determine effects of variations in temperature on larval survival.
3. To evaluate the reproductive parameters of egg numbers, incubation, and eclosion.
4. To obtain procedures which may lead to mass larval culture and the production of juveniles for commercial purposes.

MATERIALS AND METHODS

Ovigerous females of *A. lanipes* and *A. scabra* were collected from neighboring rivers and streams in Ponce, Puerto Rico and maintained in Jewel Oceanic® 55 gallon aerated aquarium at 22°C containing aged tapwater. These females were fed with commercially prepared food (Zeigler®). In these gravid females, egg color changes, eye position in embryos, and egg counts were recorded. Eggs were stripped from females to determine egg mass weight and egg counts. Embryos were separated and counted from 14 females of each species during a six month period. The egg mass was weighed in an analytical balance and representative egg counts were determined per gram of female body weight. Female weight was determined prior to egg stripping. Incubation time was determined by egg color changes from orange to greyish green. Samples of each egg mass (with egg counts) were also mounted and preserved.

Mean tolerance (TL50) of salinity and temperature was determined on fertilized eggs and/or newly hatched larvae during the 96-hr static test as recommended in EPA Bioassays (1981). Duplicate glass jars filled with Millipore® (0.45 μ) filtered, unaerated seawater having 50 eggs or 100 larvae per jar were used in salinity and temperature bioassays. Total ammonia-nitrogen

and total nitrite-nitrogen were monitored according to Standard Methods APHA *et al.* (1980). Temperature (Digi-Sense Thermistor Thermometer model 8523-00 Cole Parmer), pH (Chemcadel pH/mv meter model 8350-95 Cole Parmer), and dissolved oxygen (Omega oxygen meter model 9070) were determined on a daily basis. Tests were conducted on eggs or larvae of each species during a 96-hour period in duplicate salinity concentrations of 0, 15, 20, 25, 30, and 35 ppt. Percent egg hatch and larval mortality were determined at intervals of 1.5, 3, 6, 12, 24, 36, 48, 60, 72, 84, and 96 hours (APHA *et al.*, 1980) . The TL50 (mean tolerance limit for salinity and for temperature) for each species was determined graphically from a plot of log TL50 vs log time. The salinity or temperature corresponding to the 50% mortality value is identified as the incipient TL50 (EPA, 1981).

Effect of Salinity on Hatch

Fifty eggs from females of each individual species were separately submitted to duplicate salinity trials at 0, 15, 20, 25, 30, and 35 ppt without aeration. The water quality parameters mentioned above were monitored initially and at 24-hour intervals. The trials were maintained at ambient temperature of 27°C, pH ranged from 7.8 to 8.5, dissolved oxygen was kept near saturation, total ammonia-nitrogen was below 1.0 mg/liter, and total nitrite-nitrogen was below 0.01mg/liter.

Effect of Salinity on Larval Mortality

One hundred two day old larvae of each species fed on Zeigler® AP-100 in solution were submitted to duplicate salinity trials of 0, 15, 20, 25, 30, and 35 ppt with filtered seawater and without aeration. During the 96-hour test, the temperature was maintained at ambient 28°C , pH ranged from 7.5 to 8.5, dissolved oxygen was kept near saturation, total ammonia-nitrogen was below 1.0 mg/liter, and total nitrite-nitrogen was below 0.01 mg/liter. Larvae submitted to test were fed before, but not during the 96 hour test.

Effect of Temperature

Fifty larvae of each species were separately submitted to filtered seawater of 30 ppt salinity in duplicate test temperatures of 20, 24, 28, and 32°C. They were maintained in precision low temperature incubators (Forma Scientific model 3980). The larvae were fed before, but not during the 96-hour static bioassays. The pH was maintained at 8.1 to 8.2, dissolved oxygen ranged from 5.5 to 7.6 ppm during 96-hour trials, total ammonia-nitrogen was below 0.03 mg/liter, and total nitrite-nitrogen was below 0.02 mg/liter.

RESULTS AND DISCUSSION

Eggs obtained from *A. scabra* produced 100% hatch within 3 hours at salinities of 0 and 15 ppt with a decrease in the number of eggs hatched as salinity increased to 35 ppt (Figure 1). Eggs obtained from *A. lanipes* also produced 100% hatch in less than three hours with a significant decrease in hatch as salinity increased to 35 ppt (Figure 2). Results indicate that best hatch occurs in freshwater rather than seawater.

Larval counts at intervals of 1.5, 3, 6, 12, 24, 36, 48, 60, 84, and 96 hours indicate 82% mortality within the first 6 hours at 0 ppt salinity for *A. scabra* and 100% mortality within 24 hours at 0 ppt for *A. lanipes* larvae. Mortality decreased significantly in both species as salinity increased to 35 ppt with 100% survival at a salinity of 30 ppt for both species during the 96 hr period (Figures 3 and 4). Therefore, survival was consistently increased by an increase in salinity. This compares favorably with studies on related species of *A. innocous* and *Micratya poeyi* (Hunte, 1977, 1979a, 1979b).

Percent larval survival fluctuated from 80% at 20°C to 94% at 32°C for *A. scabra* and from 84% at 20°C to 92% at 32°C for *A. lanipes* with no significant differences in mortality as temperature increased (Figures 5 and 6).

Relation between body weight and number of eggs

Oviparous crustaceans carry their developing eggs until hatching which results in greater egg survival. Among the many factors that regulate the number of eggs carried by a female, the size of the female appears to be important. In several marine crustaceans, the number of eggs carried has been found to have a linear relationship to the length of the female. Jensen (1958) concluded that the "absolute number" of eggs (the total number of eggs carried in all the broods of an individual) was determined by environmental factors. However, the "relative number" of eggs (total number of eggs carried in a single brood at any one time) exhibits a linear relationship to the volume of the mother's body. This relationship has been reported for other freshwater shrimp species by Shakuntala (1977), Ching and Velez (1985), and others. The weight range for female *A. scabra* was 2.58 to 6.1 grams with clutch sizes ranging from 3,114 to 15,513 for a mean female body weight of 4.45 grams and a mean clutch size of 7,008 (Figure 7). The weight range for female *A. lanipes* was 1.2 to 9.4 grams and clutch sizes ranged from 1,340 to 15,058 with a mean female body weight of 3.66 grams and mean clutch size of 5,315 (Figure 8). Incubation time varied from 21 to 28 days. At the beginning of incubation, eggs were orange in color and became greyish green before eclosion.

The linear regression analysis indicated a significant correlation between body weight and number of eggs (clutch size) for *A. scabra* ($r = 0.59$) and for *A. lanipes* ($r = 0.95$). There are approximately 2,000 and 1,600 eggs produced for each gram of body weight for *A. scabra* and *A. lanipes*, respectively. The

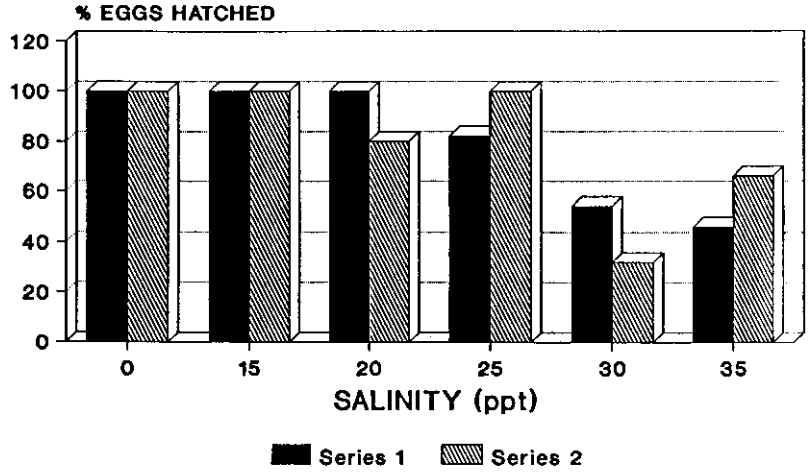

Figure 1. Salinity vs. egg hatch for *A. scabra* with series 1 and 2 representing simultaneous replicates.

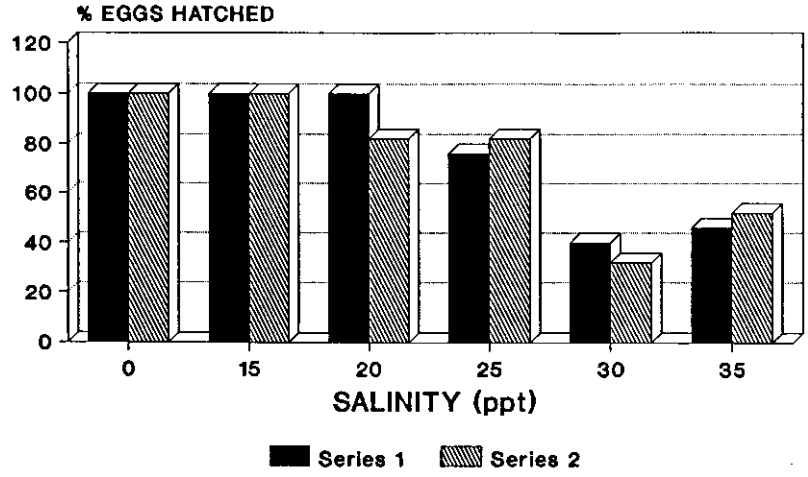

Figure 2. Salinity vs. egg hatch for *A. lanipes* with series 1 and 2 representing simultaneous replicates.

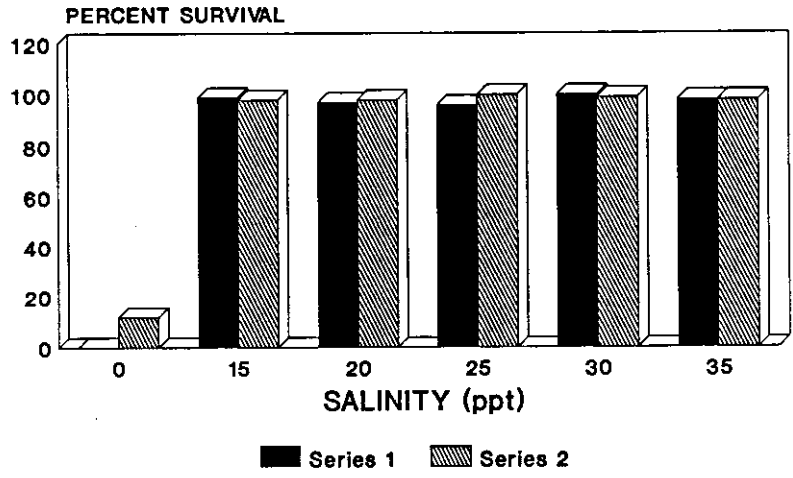

Figure 3. Salinity vs. larval survival of *A. scabra* with series 1 and 2 representing simultaneous replicates.

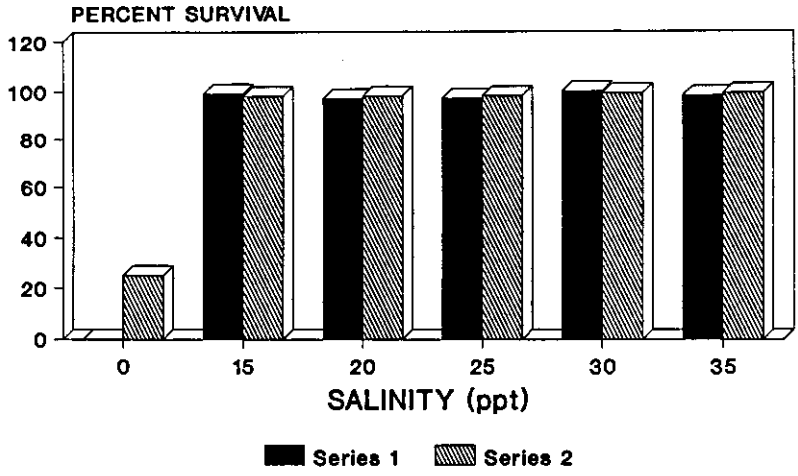

Figure 4. Salinity vs. larval survival of *A. lanipes* with series 1 and 2 representing simultaneous replicates.

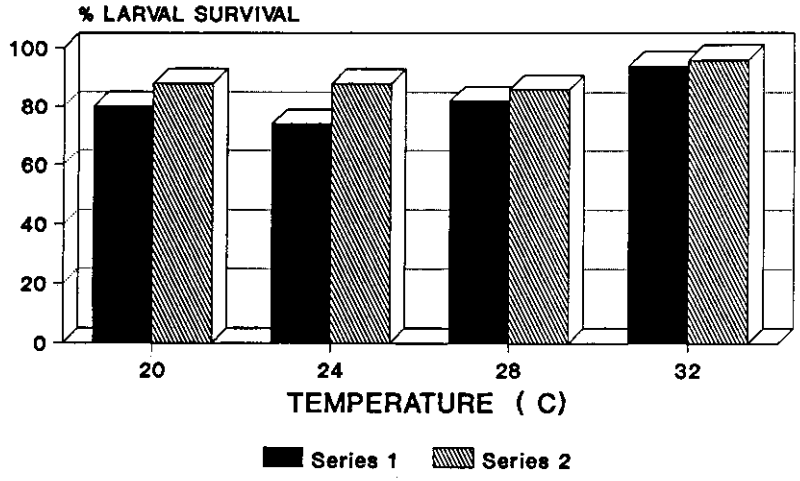

Figure 5. Temperature vs. larval survival of *A. scabra* with series 1 and 2 representing simultaneous replicates.

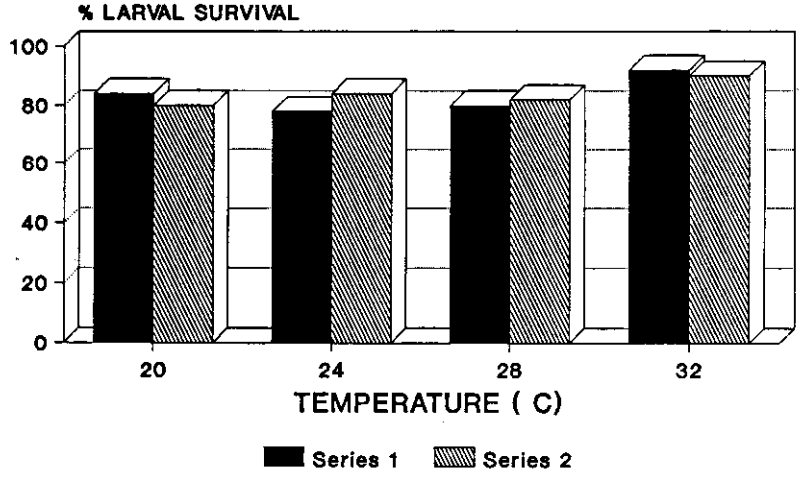

Figure 6. Temperature vs. larval survival of *A. lanipes* with series 1 and 2 representing simultaneous replicates.

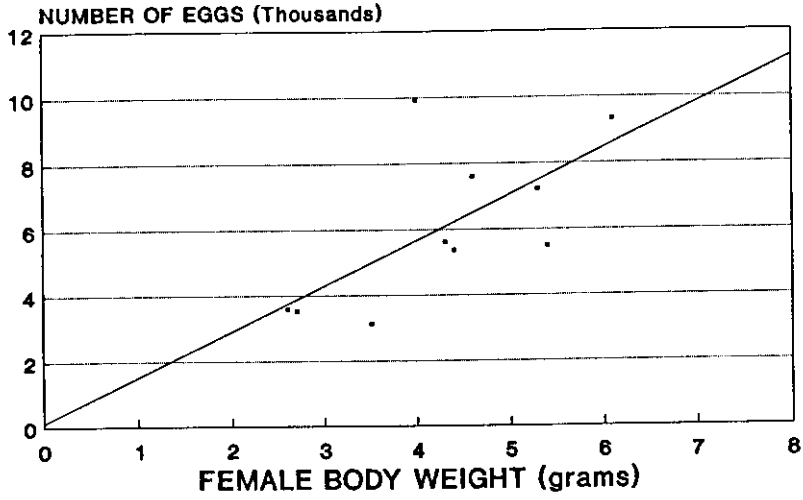

Figure 7. Female body weight vs. egg number of *A. scabra*.

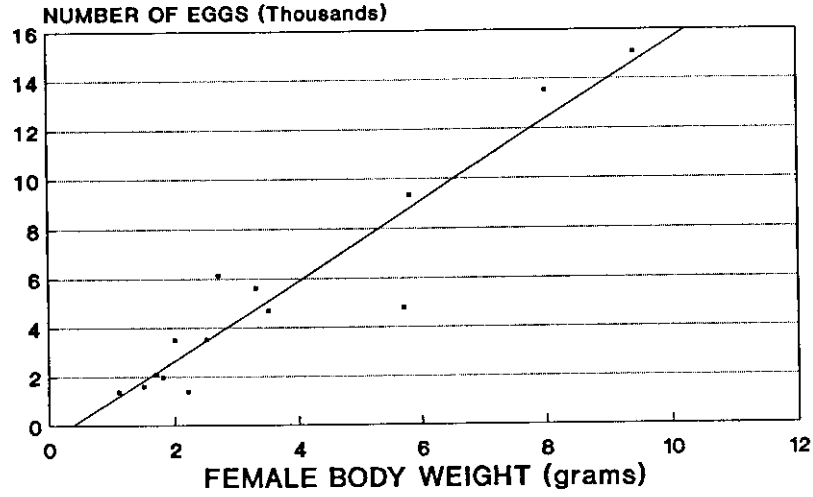

Figure 8. Female body weight vs. egg number of *A. lanipes*.

present work corroborates findings which indicate that variation in embryo numbers are dependent on gravid female size for other freshwater shrimp. Therefore an increase in female body weight increases clutch size and provides support that these species may well serve as potential candidates for aquaculture.

ACKNOWLEDGEMENTS

We would like to express our gratitude to the following laboratory technicians for their most valuable assistance in the investigation: Mr. Francisco Hernandez, Mr. Manuel Llegus, and Mr. Carlos Rodriguez and specially to Dr. Andrew McGinty for his collaboration.

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