JOURNAL OF CRUSTACEAN BIOLOGY, 16(4): 684–688, 1996

VARIATION IN EGG SIZE OF THE FRESH-WATER PRAWN *MACROBRACHIUM AMAZONICUM* (DECAPODA: PALAEMONIDAE)

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ABSTRACT

Egg size of the fresh-water prawn *Macrobrachium amazonicum* varied among ecologically isolated or geographically distant populations of the Amazon basin. The mean volume of both recently spawned nonpigmented eggs and older pigmented-eyed eggs was smaller in prawns from the Tocantins River (0.14 and 0.20 mm^3, respectively) than in those from the middle Amazon (0.17 and 0.25 mm^3). The largest eggs (0.19 and 0.27 mm^3) were displayed by females from the Iquitos and Guaporé Rivers. Since the egg volume was independent of female body size, it was attributed to population-specific characteristics. The egg size increased with the distance of the sampling site from the ocean, suggesting a progressive divergence of this species from a typical littoral population to an inland form, in a still active fresh-waterization process similar to the pattern evolved among other species in the genus *Macrobrachium.*

RÉSUMÉ

La taille des oeufs de la crevette d'eau douce *Macrobrachium amazonicum* varie entre des populations écologiquement isolées ou géographiquement distantes dans le Bassin de l'Amazone. Le volume moyen, aussi bien des oeufs récemment émis et dépourvus d'oeil nauplien pigmenté, que celui des oeufs plus agés présentant un oeil nauplien bien pigmenté, est significativement plus petit chez les individus collectés dans le fleuve Tocantins (respectivement 0.14 et 0.20 mm^3) que chez des crevettes collectées dans le bassin moyen de l'Amazone (0.17–0.25 mm^3); les plus grands oeufs ont été observés chez des femelles provenant d'Iquitos et du Guaporé. Cette variation du volume des oeufs est indépendante de la taille de l'individu et des caractéristiques de l'environnement. Elle peut être reliée à la dispersion géographique des populations de *M. amazonicum* dans le Bassin Amazonien. Le volume des oeufs augmente avec la distance du site de collection de l'océan, suggérant une divergence progressive de cette espèce à partir de populations typiquement littorales vers des formes d'eau douce, dans un processus encore actif de continentalisation, similaire à celui qui caractérise l'ensemble des autres espèces du genre *Macrobrachium.*

The volume of the eggs produced by crustacean species is, in part, under genetic control (Raven, 1961). It shows a general pattern of interspecific variation, decreasing from colder to warmer waters over a north-south latitudinal gradient, and from deep-sea benthic to shallow coastal and epipelagic waters (e.g., Omori, 1974; Sastry, 1983). In fresh-water decapods, large eggs appear to be associated with landlocked species inhabitating plankton-poor, clear, and rather acid waters, and small eggs mostly to anadromous and brackish species (Shokita, 1979; Magalhães and Walker, 1988; Odinetz Collart and Magalhães, 1994; Jalihal *et al.,* 1993). The same continentalization pattern has been described in *Macrobrachium nipponense* (de Haan), where large eggs were found in fresh-water lake and river populations, small eggs in estuary populations, and intermediate-sized eggs in brackish-water populations. At times, the full size range is seen in a single water system (Chow *et al.,* 1988; Mashiko, 1990). Crossing experiments between individuals of *M. nipponense* from two adjacent groups of a single drainage system suggested that the egg-size differences among local, ecologically isolated populations are controlled as a quantitative genetic trait (Mashiko, 1992; Mashiko and Numachi, 1993).

The river prawn *Macrobrachium amazonicum* (Heller) occurs in South America throughout the basins of the Orinoco, Amazon, and Paraguay Rivers (Holthuis, 1952). It seems to be absent in estuaries despite successful laboratory larval culture in saline water (Vargas and Paternina, 1977; Guest, 1979; Guest and Durocher, 1979). In the Amazon basin, this species is very abundant in white waters (sensu Sioli, 1975), especially in floodplain lakes, and scarce in acid black waters of small highland forest streams. *Macrobrachium amazonicum* produces numerous small eggs which incubate for 15–17 days before hatching as a primitive free-swimming zoea, which completes a prolonged larval development with 10 or 11 larval stages in 28–33 days (Magalhães, 1985).

Macrobrachium amazonicum shows different reproductive patterns depending on the locality sampled (Odinetz Collart and Magalhães, 1994). In the Lower Tocantins, this species displays a typical reproductive pattern of anadromous littoral palaemonids with mass spawning during falling waters (Odinetz Collart, 1987, 1991a). In central Amazonia, lacustrine populations breed all year long; however, a significantly higher number of eggs is released during rising waters (Odinetz Collart, 1991b, 1992).

The present study, focused on intraspecific variations of egg size, discusses the fresh-waterization process of distant populations, considering the strength of geographic barriers and species-specific dispersal abilities.

Materials and Methods

Ovigerous females were collected from the lower Tocantins, close to Cametá on the Tocantins River (Site 1), in the Tucurui reservoir (Site 2), in the Amazon River, close to Manaus, Lago do Rei (Site 3), Lago de Tefé, Tefé district (Site 4), Japurá River, Japurá district (Site 5), Ucayali River, Iquitos district (Site 6), and from the Guaporé River (Site 7) close to the Guaporé Indian reserve (Fig. 1). The distances of the sampling sites (1–7) to the estuary of the Amazon River were, respectively: 120, 330, 1,225, 1,750, 2,200, 3,410, and 2,940 km from the ocean. Sampling was conducted using traps or hand nets during the breeding season. At every site, females with both pigmented-eyed and nonpigmented eggs were collected. Recently spawned eggs do not show eye pigmentation in the embryo. During incubation, the egg volume gradually increases and the embryonic eye pigmentation appears a few days before hatching (Magalhães, 1985; Mashiko, 1982). All samples were preserved in 70% alcohol.

Prawn size was estimated using carapace length, measured from the posterior margin of the orbital cavity to the posterior edge of the cephalothorax. Ten eggs were removed from each female and measured in the laboratory to the nearest 0.01 mm with a binocular micrometer. The egg volume was calculated from the formula $v = \pi l h^2/6$, where l and h are, respectively, the long and short axes of the ellipsoidal egg.

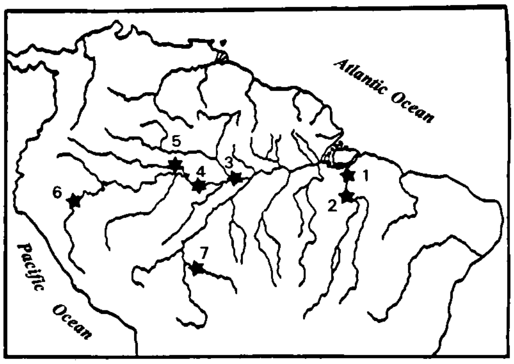

Fig. 1. Collecting sites for *Macrobrachium amazonicum*, with their distance (D) from the Amazon River estuary: (1) Tocantins River, Cameta district, D = 120 km; (2) Tucurui reservoir, D = 330 km; (3) Amazon River, Manaus, Lago do Rei, D = 1,225 km; (4) Lago de Tefé, Tefé district, D = 1,750 km; (5) Japurá River, Japurá district, D = 2,200 km; (6) Ucayali River, D = 2,940 km; (7) Guaporé River, Indian Reserve, D = 3,410 km.

Results

When all 75 ovigerous prawns were considered, the mean volume of nonpigmented eggs varied from 0.1207 mm^3 in a female collected in Cametá, lower Tocantins, to 0.2188 mm^3 in a prawn from Tefé. In eggs with pigmented eyes, the mean volume changed from 0.1648 mm^3 in Cametá to 0.3272 mm^3 in the Guaporé River. Egg size within the same clutch had a mean coefficient of variation (SD/mean) of 8.72%. There was no significant difference in variation of egg volume within a single clutch between the two groups of females, with pigmented and nonpigmented eggs, nor between the six prawn populations ($P > 0.05$; two-way ANOVA). When the seven populations were considered, the mean volume increased 33.42% ± 8.6% from the nonpigmented to pigmented-eyed egg stage.

In Fig. 2, mean egg volumes are plotted against parental cephalothorax length for each female with pigmented or nonpigmented eggs. Mean egg volume was independent of prawn size in both nonpigmented and eyed eggs ($r = -0.06$ and $r = -0.17$, respectively; $P > 0.05$).

Developmental stage and sampling site significantly affected the mean egg volume when all the prawns collected were considered. The higher variance was associated with developmental stage. The interaction between these two factors was not signifi-

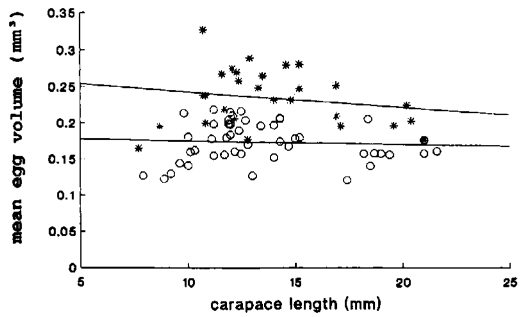

Fig. 2. Relationship in the female prawn (*Macrobrachium amazonicum*) between carapace length (mm) and the mean egg volume (mm^3), considering individuals with nonpigmented eggs (o) and eyed eggs (*).

Table 1. Two-way ANOVA analyzing the effect of incubation stage (N=2) and sampling site (N=7) on mean egg volume of the prawn *Macrobrachium amazonicum*

Source	*d.f.*	MS	*F*	*P*
Ontogenesis	1	0.550934	93.621	0.0001
Distance	6	0.0062011	10.538	0.0001
Interactions	6	0.000681189	1.158	0.3408

cant (two-way ANOVA, Table 1). The multiple range analysis for ANOVA denoted a statistically significant difference in egg size between both Tocantins samples and Guaporé populations, and considered the populations from near Manaus, Tefé, and Japurá to be homogeneous (Bonferroni range test; $P < 0.05$).

The mean egg volume in each population was calculated separately for females with nonpigmented and pigmented-eyed embryos. For both groups, the prawns from the Tocantins River (Site 1 and Site 2) had the smallest eggs, and those from the Guaporé (Site 7) the largest eggs. For both incubation stages, the mean egg volume increased with the distance of the sampling site from the estuary (Fig. 3). Both regression lines showed a statistically significant correlation between the two parameters ($P < 0.01$), following the equations: $V(mm^3) = 0.1532 + 1.379\ D\ (km)$; $r = 0.834$ for the nonpigmented samples, and $V(mm^3) = 0.196 + 2.296\ D\ (km)$; $r = 0.933$ for the the eyed samples.

Discussion

Rodriguez (1981) separated South American palaemonids into three groups based on marine affinity: Atlantic and Pacific littoral species which depend on brackish water for their survival, and typically continental species. In an analysis of larval development, Magalhães and Walker (1988) regrouped Amazonian prawns into: (1) coastal species which show complete development of numerous small-sized eggs, (2) typically continental species characterized by abreviated metamorphosis of a few large eggs, and (3) widely distributed species with an intermediate type of development. They considered this pattern as an adaptative response to the selection pressure of plankton-poor inland waters.

Crustacean species display a general pattern of variation in egg size described as a geographical cline following water temperature (e.g., Patel and Crisp, 1960; Nishino, 1990). Mashiko (1990) recognized variation in egg size in populations of *Macrobrachium nipponense* depending on the hydrogeographic features of their habitat. Large eggs were found in fresh-water inland populations and small eggs in estuarine prawns, supporting the hypothesis of an interspecific continental pattern. However, no mating behavioral incompatibility nor fertilization failure in laboratory hybrids have been noted between groups with different egg sizes, suggesting that geographic distance is the main mechanism of reproductive isolation for *M. nipponense* in a particular water system (Mashiko, 1992). However, opposite results have been de-

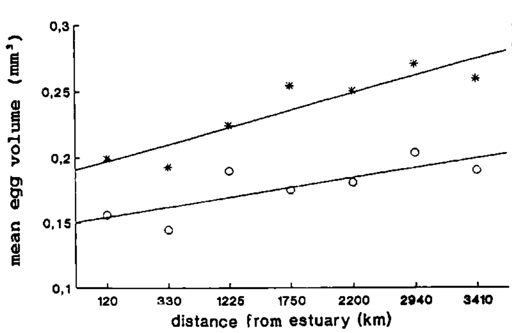

Fig. 3. Relationship between the mean egg size in six populations of *Macrobrachium amazonicum* with both nonpigmented (o) and eyed eggs (*), and the distance from the Amazon River estuary to their collecting sites. The regression lines follow the respective equations: $V(mm^3) = 0.1532 + 1.379\ D\ (km)$, $r = 0.834$ for nonpigmented eggs; $V(mm^3) = 0.196 + 2.296\ D\ (km)$, $r = 0.933$ for eyed eggs.

scribed in *Palaemon paucidens* de Haan, with spawning of many small eggs by small individuals upstream in standing waters, and laying of fewer but larger eggs in colder running waters of the middle and lower course of the river (Nishino, 1990; Mashiko, 1982).

Two distinct categories of eggs were identified in *Macrobrachium amazonicum* along the Amazon Basin: spawning small eggs in the Tocantins River (0.14–0.20 mm^3 in mean volume), and laying large eggs in the upper course (0.19–0.26 mm^3) and in the Guaporé River (0.20–0.27 mm^3). Intermediate-sized eggs (0.17–0.25 mm^3) were collected in the middle course of the Amazon River. The egg volume was independent of female carapace length in each population ($P < 0.01$). Body size, growth rate, and reproductive pattern are usually affected by environmental factors such as temperature, current velocity, food availability, and population density. Kamita (1970) found that the body size of *Palaemon paucidens* inhabiting rivers is greater than in those living in lakes and swamps, though he did not report egg sizes. In the Tocantins basin, large prawns (mean carapace length = 19.5 mm), collected in running waters, and smaller individuals (mean carapace length = 9.9 mm), sampled in a recently impounded reservoir, had eggs of about the same volume. Thus, the difference in egg size cannot be attributed to temporary environmental differences, but may be a population-specific character in each locality of the Amazon Basin with the mean egg volume increasing with the distance of the sampling site from the ocean. Differences in egg size were not significant between populations inhabiting the same water system (the Tocantins River or the Middle Amazon floodplain) even over a wide geographical range, as from Manaus (Site 3) to Tefé (Site 4), Japurá (Site 5), and Iquitos (Site 6), suggesting the presence of gene flow between these four groups. In contrast, the Tocantins River is not considered to be part of the Amazon hydrological system, and Site 1 exhibits water-current inversions following tidal changes. The prawn populations collected in the Tocantins were statistically significantly smaller than those of the other groups. This seems to be a marine adaptive trait. Guaporé prawns are regionally isolated at ecologically different habitats from other middle Amazon populations, separated by a series of waterfalls on the Madeira River. Their large eggs appear to be a typical inland trait.

In heterogeneous or geographically isolated environments, a single species may present genetically diversified populations due to the depression of gene flow between them. The difference in egg size between the seven groups of *M. amazonicum* in the Amazon Basin suggests a progressive divergence of this species from a typical littoral population to an inland form in a still active adaptive process. These results are consistent with the general patterns evolved in the genus *Macrobrachium*: an increase in egg size with proportionate reduction in the number of eggs, reduction in both the number of larval stages and the duration of larval period, and increased larval survival in populations from interior continental waters (Jalihal *et al.*, 1993).

ACKNOWLEDGEMENTS

This research was undertaken jointly with the Instituto Nacional de Pesquisas da Amazônia (INPA) within the ORSTOM/CNPq–INPA convention, with the financial support of the CNPq through a student grant. The authors are grateful to Angela Pimentel and Adriana Enriconi for technical assistance, Dr. Bruce Nelson for corrections of the English, and Mario Barletta for valuable suggestions.

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RECEIVED: 26 June 1995.
ACCEPTED: 3 May 1996.

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