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Najila Elias-Samlalsingh, Azad Mohammed and John B. R. Agard

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Metamysidopsis insularis (Crustacea: Mysidacea): The Life History of a Mysid Species Suitable for Toxicological Testing in the Tropical Americas

Najila Elias-Samlalsingh, Azad Mohammed and **John B. R. Agard**
Department of Life Sciences, The University of the West Indies
St. Augustine, Trinidad and Tobago
E-mail: mohc0134@yahoo.com

ABSTRACT
Mysid species are routinely used in many regions temperate for toxicological assessment of chemicals and industrial and municipal effluents. *Metamysidopsis insularis* has recently been used in Trinidad and Tobago for toxicity tests, however, not much is known about its life cycle. Newly hatched juveniles were maintained under laboratory conditions and sampled daily for a period of 26 days. The mean body length was determined and used to generate a growth curve for *M. insularis.* It was found that for *M. insularis,* the maximum growth phase occurred during the first sixteen days and organisms attained sexual maturity between 12 - 15 days. Eight moults in five growth phases were identified for this species. The main stages identified were, early juveniles (0 - 2 days), juveniles (3 - 6 days), late juveniles (7 - 12 days), early adults (13 - 15 days) and adults (>16 days). These growth phases (early juvenile, juvenile, late juvenile, early adult and adult) were similar to those identified for *Americamysis bahia,* however, the days at which these stages occurred were different from *M. insularis.*

INTRODUCTION

Mysids are small shrimp-like crustaceans which vary in size from 0.5 - 20 mm. They live in a variety of aquatic environments, including coastal and open sea waters, estuaries and other brackish water ecosystems. Mysid species are routinely used in many temperate regions for toxicological assessment of chemicals and industrial and municipal effluents. Investigators first began using the estuarine mysid species, *Americamysis bahia* (formerly *Mysidopsis bahia*) for toxicity tests, at the U.S. Environmental Protection Agency, Environmental Research Laboratory in Gulf Breeze, Florida, (Nimmo *et al.* 1977). Mysids have proven to be highly sensitive to a wide range of toxic substances (Nimmo and Hamaker 1982; Gentile *et al.* 1982; Gentile *et al.* 1983; ASTM 1987; Lussier *et al*. 1991; Suter and Rosen 1988; US EPA 1993; Buckler *et al.* 2003). Toxicity data presently exists for several temperate mysid species, *Americamysis bahia*, *Americamysis bigelowi* (formerly *Mysidopsis bigelowi*) and *Americamysis almyra* (formerly *Mysidopsis almyra*) (Buckler *et al.* 2003). However, the largest body of toxicological data (Ward 1984; Buckler *et al.* 2003) exist for *Americamysis bahia* which is a designate standard test species in programs such as the US EPA Ocean Disposal Permit Program and the National Pollutant Discharge Elimination System (NPDES), as well as other toxicological testing programs (Lussier *et al.* 1999; Kuhn *et al.* 2000).

Though information is readily available about the responses of these organisms to various toxicants, very little is available on their life cycle. McKenney (1998) investigating the effects of pesticide exposure on *Americamysis bahia*, identified five general phases throughout its life cycle (Early Juvenile [day 1], Juvenile [day 4], Advanced Juvenile [day 10], Young Adult [day 16] and Adults [day 20]). Lussier *et al*. (1988) indicated that the first moult for *Mysidopsis* species occurs within 24 h of release from the brood pouch. The US EPA (1993) also reported that *Americamysis bahia* reaches sexual maturity in 12 - 20 days, however they did not identify specific moults. Lussier *et al*. (1991) reported an average of six moults during the first 18 days, with 2 to 4 days intervals between moults for both *Americamysis bahia* and *Americamysis bigelowi*.

Metamysidopsis insularis, a tropical mysid species, is commonly found along the western coast of Trinidad, with large populations occurring in mangrove swamps (Caroni and Oropouche). This species is also reportedly found throughout the Caribbean (Lesser Antilles, Brattegard 1970) and Latin America (Quintero and Zoppi de Roa 1973). Mysids are pericarids with a well developed carapace. They typically occur in large swarms in the upper 30 cm of the water column. Though toxicity tests have been conducted using this species, (Elias-Samlalsingh 2000; Garcia 2001; Elias-Samlalsingh and Agard 2004; Mohammed 2005), little is known about its life cycle. Quintero and Zoppi de Roa (1973) identified five stages in the brood pouch of adult females but no investigation was done on development, post hatching.

Though *Metamysidopsis insularis* has been proposed as a suitable toxicity test species for use in Trinidad and the wider Caribbean, nothing is known of its life cycle. This present study sought to investigate the life history of *Metamysidopsis insularis,* a tropical mysid species.

METHODS

Laboratory Culture

M. insularis was collected from the Caroni Swamp in Trinidad, and identified using taxonomic keys by Brattegard (1970). Culture methods generally followed those published by Nimmo *et al*. (1978), Reitsema and Neff (1980) and US EPA (1993). Animals were maintained in a re-circulating system which consisted of a large glass aquarium (76 L) with an undergravel filter (Ward 1984; Nimmo *et al*. 1991).

Culture tanks were maintained at a salinity of 25‰, a temperature of 25°±1° C, pH at 8.0 - 8.4 and dissolved oxygen at greater than 80% saturation. Cultures were illuminated on a 12 h light:12 h dark regime using "cool-white" fluorescent bulb. Animals were fed twice daily with *Artemia salina* (Bio-Marine, California) nauplii.

Culture Technique

Adult mysids were transferred from the holding tanks with an aquarium net (500 µm mesh) to the hatching assembly. The hatching assembly consisted of a spawning chamber made from a 2 L plastic container in which the base was covered with a 1mm mesh. This was then suspended in a 20 L glass aquarium fitted with an undergravel filter, fed with newly hatched *Artemia* nauplii and left overnight.

This hatching system proved quite efficient in achieving good separation of juveniles from adults, thus preventing predation of the young by adults and damage to juveniles by use of mechanical separation techniques. When the juveniles were released, they passed through the mesh and into the aquaria, while the adults were retained in the spawning chamber. The spawning chamber was subsequently removed and the adults transferred back to the holding tank. Juveniles were maintained in the spawning tank and fed ad libitum with newly hatched *Artemia* nauplii, which were found to be an appropriate size for young mysids.

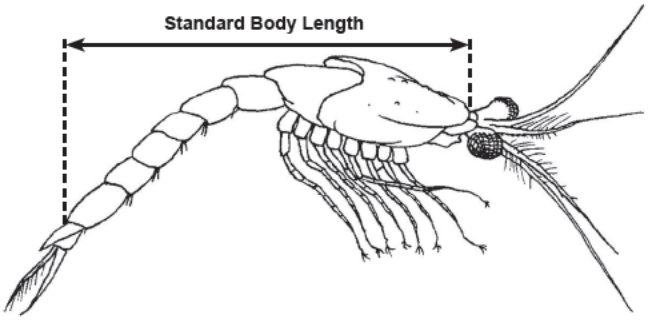

Fig. 1. Diagram showing the distance measured to determine the total standard body length (Modified from Quintero and Zoppi de Roa 1973).

Life History Assessment

A minimum of ten juveniles were sampled daily, preserved in Baker's formol calcium and stored at 4° C until required for analysis. Sampling was conducted over a 26 day period. The standard body length (base of eyestalk to anterior of the telson) (Fig. 1) was determined for each animal. The body length for each day was ranked and the upper and lower quantiles and median range determined. The lower quantile was determined as the $\frac{1}{4}(n+1)^{th}$ value and the upper quantile was the $\frac{3}{4}(n+1)^{th}$ value, where n = total number of animals. The mean length was calculated from the median range and used to generate a growth curve for *M. insularis*. Morphological characteristics such as changes in the telson and the appearance of male and female reproductive structures were also noted. Specific moults were identified from the growth curve, as these resulted in a significant change in average body length of the organism. ANOVA and TUKEY HSD analysis (SYSTAT Ver. 5.0) was used to determine whether the mean body lengths were significantly different between days.

RESULTS

Life History Studies

The growth cycle of *M. insularis* was determined from the time juveniles were release from the brood pouch (Day 0) until they became adults. Embryos hatch in the brood pouch of the females, where they remain until appendages develop. When released, juveniles are morphologically similar to the adults. Larval development in *M. insularis* can therefore be described as being epimorphic.

Adults (Fig. 1) range in length from 4 - 5 mm with females (4.0 - 4.9 mm) being slightly larger than males (3.0 - 4.3 mm). Adult males are easily distinguished from females by the presence of pleopods in the first five abdominal segments as well as male reproductive structures. Adult females have a well defined brood pouch.

Morphological Studies

The maximal growth period occurred during the first sixteen days, after which, no substantial increase in size (length) was apparent (Fig. 2). During this time, eight moults was evident (Fig. 2). Using the moults, it was possible to identify five distinct growth phases for this species (Table 1).

No significant increase in size ($P>0.05$) occurring between day 0 - 2 (Early Juvenile Phase). The average length increased from 1.120 ± 0.009 mm (day 0) to 1.350 ± 0.006 mm (day 2). Day 0 animals ranged in length from 1.04 - 1.14 mm, after day one the length ranged from 1.23 - 1.35 mm. The first moult of the early juvenile phase was identified on day two (Fig 2). Juvenile organisms showed no distinct morphological features that could

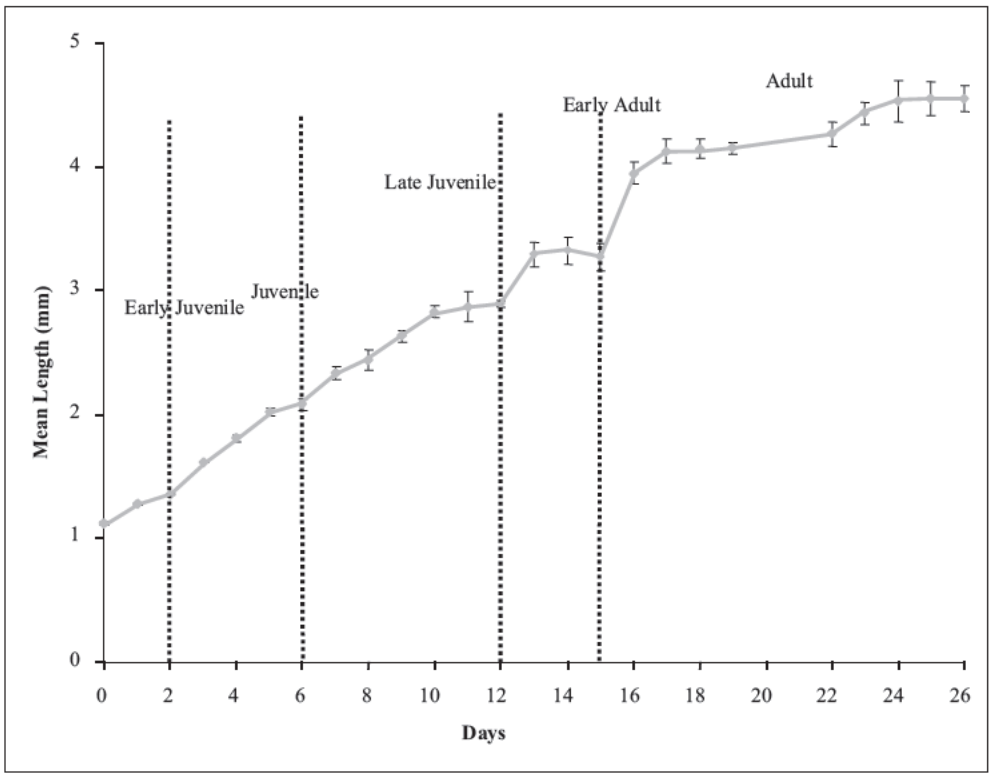

Fig. 2. Growth curve for *Metamysidopsis insularis*.

be used to differentiate males and females. The telson generally appeared flattened, with two centrally positioned spines (Fig. 3a). This growth phase (between 0 - 2 days) represents the early juvenile phase at the end of which (on day 2) the organism moults and enters the juvenile phase.

The juvenile phase extends from day 3 to 6, during which three moults were identified (Table 1). The organisms in this phase show significant ($P<0.05$) increases in body length when compared with the early juveniles. The average body length increased from 1.35 ± 0.006 mm (day 2) to 1.61 ± 0.004 mm (day 3). At the end of the juvenile phase, on day 6, the average body length was 2.08 ± 0.05 mm with moults on day three, four and six during this phase (Table 1, Fig 2). It was found that variation in

Table 1. Growth pattern of *Metamysidopsis insularis* in the laboratory and general characteristics of each growth phase.

Growth Phase	Growth Period (days)	No. of Moults	Standard body length (mm) for each growth phase	Recognised Characteristics
Early juvenile	0 2	1	1.12 1.35 (*n*=31)*	Eyes of moderate size. Apex of telson appears flattened with two centrally positioned spines of equal length. No morphological differences between males and females.
Juvenile	3 6	3	1.61 2.08 (*n*=31)*	Apex of telson appears rounded with two centrally positioned spines still present up to day four. At day five approximately seven spines were present, with the central two being the longest. No morphological differences between males and females were evident.
Late juvenile	7 12	3	2.33 2.89 (*n*=54)*	The apex of the telson appears rounded with at least twelve spines of which the central two are the longest. Between days 8 9, developing biramous pleopods and gonads were visible in males. Female brood pouch was visible (8 10 days).
Early adult	13 15	1	3.27 3.32 (*n*=78)*	Apex of telson rounded with no further increase in the number of spines. Well developed biramous pleopods and fully developed gonads in males were evident. Female brood pouch shows increase vascularisation through this phase.
Adult	> 16	Infrequent moults	3.95 4.55 (*n*=72)*	Apex of telson rounded with a regular row of approximately twenty four blunt spines of which the central two are the longest. Eggs become apparent in the now mature brood pouch. No significant changes were observed in male reproductive structures.

**n* = sample size

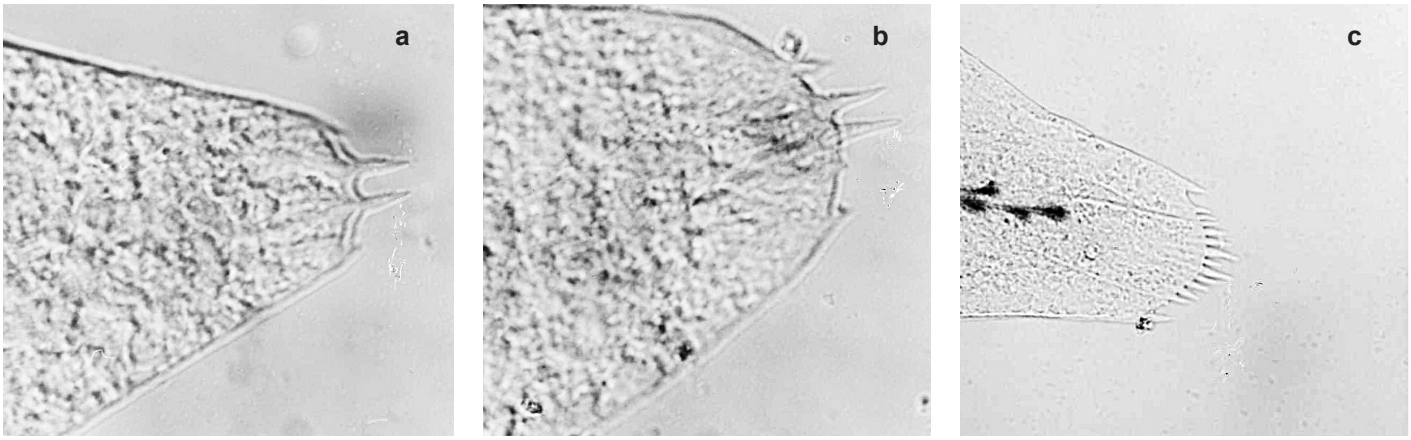

Fig. 3. Morphological changes in the telson at different growth stages, (a) 2-day olds, (b) 4-day olds and (c) 7-day olds.

body length became increasingly distinct from this stage onwards. At day four, the telson appeared rounded with two spines (Fig. 3b). However, seven spines were evident in 5-day old animals.

The late juvenile phase occurred between days 7 to 12, during which three moults (Table 1, Fig. 2) were identified. The average length of animals increased from 2.08 ± 0.05 mm on day six to 2.33 ± 0.05 mm on day seven, the beginning of the late juvenile phase (Table 1). The telson of seven day old animals had about twelve spines (Fig. 3c). However, no increase in the number of telson spines was observed following subsequent moults within this phase. During the latter part of this growth phase (days 10 - 12), reproductive structures became evident. In the females, early brood pouches appeared by day 9 (Fig. 4a) and by day 10, blood vessels became evident. Male gonads appear at about day 9 (Fig. 4b) and pleopods were apparent at about day 7.

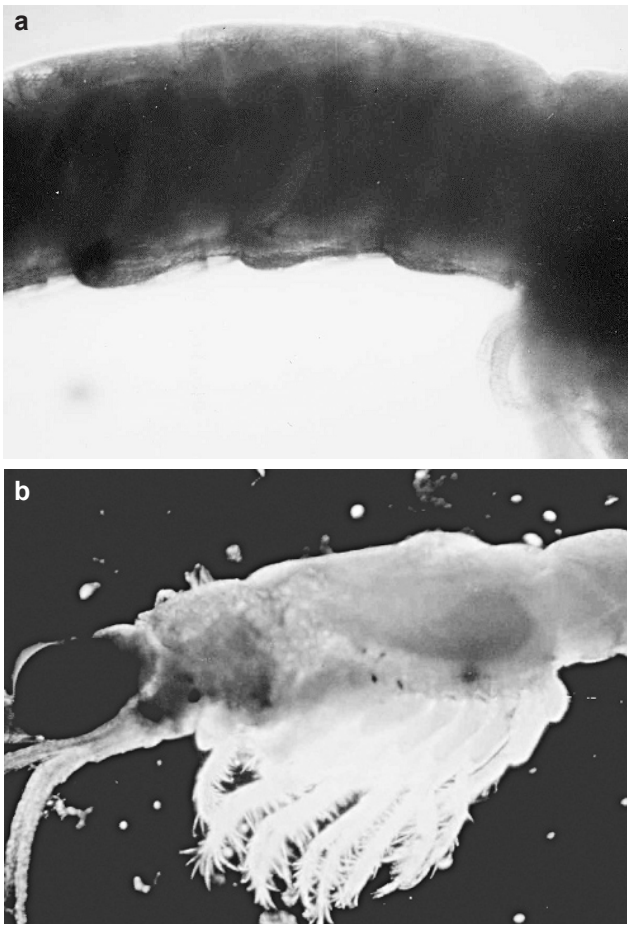

Fig. 4. Appearance of sexual characteristics in (a) females and (b) males.

On day 12, the organism moulted and entered the early adult phase which extended from day 13 - 15. During this phase they attained sexual maturity, with both males and females having fully developed reproductive structures. Only one moult occurred at the end of this phase. The average length of animals showed a significant increase from 2.89 ± 0.03 mm at the end of the late juvenile phase to 3.27 ± 0.11 mm at the start of the early adult phase ($P<0.05$). Eggs become apparent in the brood pouch which also showed increased vascularisation, and male reproductive organs appear fully developed. No additional changes in the structure of the telson became evident during this phase. Moults did not occur as frequently as in the earlier growth phases.

The adult stage began after day 16 (Table 1) with few moults evident. Average body lengths of 16 day old animals were 3.95 ± 0.09 mm. A large number (approximately 12 pairs) of very closely set apical spines were apparent on the telson. After day 18, moulting can be described as sporadic and no substantial increase in size was evident.

DISCUSSION

M. insularis has been maintained successfully in the laboratory using a re-circulating system with an undergravel filter. This method is quite similar to those described by other authors working on temperate species such as *Americamysis bigelowi* (Lussier *et al.* 1988). The methods also proved adequate for successful spawning

of the mysids under laboratory conditions. Spawning was best achieved by a small increase in salinity (<10‰) above or below that of the holding water. It was observed that salinity increases greater than this often resulted in high adult mortality. Generally, *Metamysidopsis insularis* were maintained in the laboratory for approximately three months. Lussier *et al*. (1988) reported that the normal life span of mysids varied, but *Americamysis bahia* and *Americamysis bigelowi* in laboratory cultures had a maximum life span of 3 - 5 months at a temperature of 25°C and 30‰ salinity.

The maximum growth period for *Metamysidopsis insularis* occurred between days 0 and 16 and was characterised by five distinct growth phases (Table 1) with eight moults. Each growth phase was defined by a significant increase in body length after moulting. These growth phases (early juvenile, juvenile, late juvenile, early adult and adult) were similar to those identified by McKenney (1998). However, the days at which McKenney (1998) identified these stages (Early Juvenile [day 1], Juvenile [day 4], Advanced Juvenile [day 10], Young Adult [day 16] and Adults [day 20]) for *Americamysis bahia*, were different from *M. insularis*. For *M. insularis,* the stages were; Early Juvenile [day 0 - 2], Juvenile [day 3 - 6], Late Juvenile [day 7 - 12], Early Adult [day 13 - 15] and Adult [> day 16]. Lussier *et al*. (1988) identified nine moults in the first eighteen days for *Americamysis bahia* cultured at 25°C and 30‰ in the laboratory. For *Americamysis bigelowi*, the first moult occurred on day four followed by six successive moults up to day 12. At sexual maturity, moults were generally less frequent, averaging one every three days (Lussier *et al*. 1991). The first moult for *Metamysidopsis insularis* occurred two days after release from the brood pouch. Moulting occurs frequently and at regular intervals, until sexual maturity (day 13 - 15), after which it becomes less frequent. Laboratory cultures of *Americamysis bahia* reached sexual maturity in 12 to 20 days, depending on the culture conditions of water temperature and diet (Nimmo *et al.* 1977).

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