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Embryonic development of *Metamysidopsis elongata* (Crustacea: Mysidae) in the Bay of Mazatlán, Sinaloa, Mexico

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Abstract. It is important to know the structure of the embryo when it is formed and developed. Therefore, the embryonic development, the morphology of four embryonic stages and a released fifth stage (juvenile) of the captured and cultivated wild mísidos (F1 and F2) of *Metamysidopsis elongata* were described. Monthly intertidal samples on foot at 0.5 and 1.0 m depth in the sandy area, were taken with a plankton net of a mesh size of 1 000 μm with a mouth opening of 50 cm in diameter from September 2010 to October 2011 in the Bay of Mazatlán, Sinaloa, Mexico. One thousand four hundred mysids were collected. The time from birth to the release of the first offspring of juveniles began at 26 days (F1, first generation), and at 28 days of F1 (F2, second generation). Sexual maturity occurred between 18 and 20 days after release in females (marsupium with eggs) and 12 days after release in males. The sexual rate showed predominance of females: in wild myids, 6.5: 1.0; in the generation F1, 2.3: 1.0; and in F2, 2.1: 1.0. The results indicate that if there are more females, there will be more descendants

Key words: Culturing, embryonic development, *Metamysidopsis elongata*, morphology, sex ratio.

Resumen: Desarrollo embrionario de *Metamysidopsis elongata* (Crustacea: Mysidae) en la Bahía de Mazatlán, Sinaloa, México. Es importante conocer la estructura del embrión cuando se forma y se desarrolla. Por lo tanto, se describió el desarrollo embrionario, la morfología de cuatro etapas embrionarias y una quinta etapa (juvenil) liberada de los mísidos silvestres capturados y cultivados (F1 y F2) de *Metamysidopsis elongata*. Se tomaron muestras intermareales mensuales a pie a 0.5 y 1.0 m de profundidad en el área arenosa, con una red de plancton de un tamaño de malla de 1 000 μm con una boca de 50 cm de diámetro desde septiembre de 2010 hasta octubre de 2011 en la Bahía de Mazatlán, Sinaloa, México. Mil cuatrocientos misidaceos fueron colectados. El tiempo desde el nacimiento hasta la liberación de la primera descendencia de juveniles comenzó a los 26 días (F1, primera generación) y a los 28 días de F1 (F2, segunda generación). La madurez sexual ocurrió entre 18 y 20 días después de la liberación en hembras (marsupium con huevos) y 12 días después de la liberación em machos. La tasa sexual mostró predominio de hembras: en los mísidos silvestres, 6.5: 1.0; en la generación F1, 2.3: 1.0; y en F2, 2.1: 1.0. Los resultados indican que si hay más hembras, habrá más descendientes

Palabras clave: Cultivo, desarrollo embrionario, *Metamysidopsis elongata*, morfología, proporción de sexos.

Introduction

In all species of the Mysida order, the embryos are transported in a ventral marsupium, when juveniles are released from the bag, they are morphologically similar to adults (Price 2004; Núñez-Lecuanda 2013). The moult of the mature female occurs during the night and lasts a few seconds (Mauchline 1980), followed by mating (Murano 1999). *Metamysidopsis elongata* W. Tattersall, 1932 has two currently recognized subspecies, the nominal subspecies *M. elongata elongata* from the Pacific Ocean and *M. elongate atlantica* B**ă**cescu 1968 from the Atlantic Ocean. *M. elongata* elongata (6-7 mm) is a neritic pelagic mysid which is found in the Pacific Ocean between about 40ºN and 40ºS; it is a free swimmer, and occurs in schools above fine sandy substrates and surf areas (Tattersal 1932, Clutter & Theilacker 1971).

The time from birth to release of a first clutch of offspring in *M. elongata* (22 ± 1°C) was observed at 26 days in the F1 (first generation) and 28 in the F2 (second generation). Many mysids are easy to culture, such as *M. californica* on a large scale in the laboratory as they are highly adaptive, and can tolerate a wide range of conditions (Ortega-Salas *et al*. 2008). Despite low fecundity, these species have a short reproductive cycle which means they can quickly reproduce in vast numbers, and are a potentially useful food source for both wild and cultured organisms.

Among the main studies on the marsupial development of the mysids are those performed by Wittmann (1981) who studied *Leptomysis* and other Mediterranean mysids and Wortham & Price (2002) who studied *Americamysis bahia* of the Gulf of Mexico in terms of reproduction. In Mazatlán Bay Clutter & Theilacker (1971) found that *M. elongata* had first reproduction at 38 days age. Also mentioned that in *M. elongata* the females differed sexually at 4.0 mm while males at 3.7 mm, at a temperature of 14-20°C. The Brazilian mysid, *Metamysidopsis elongata atlantica* obtained the sexual differentiation at 14 days, in a temperature range of 19-21°C (Gama *et al*. 2002). Ovigerous females of *Metamysidopsis neritica* Bond-Buckup & Tavares 1992 occurred throughout the year, with a greater proportion in winter (Calil & Borzone 2008). Its use has been slowed down due to the lack of knowledge about its biology (mainly reproductive aspects). Although Núñez-Lecuanda (2013) offers an overview of *M. elongata*, there is little information in the external literature about embryo development, so the present research is a first attempt to describe the embryonic development, the morphology of the embryos and the sex ratio.

Materials and methods

Intertidal monthly samples were collected on foot at 0.5 and 1.0 m depth in the sandy zone using a plankton net towed manually (1,000 µm mesh size) and mouth opening of 50 cm in diameter. The sampling area was located between 23º11.6'10.44 " N and 106º25'20.2 " O from September 2010 to October 2011 in the Bay of Mazatlán, Sinaloa, Mexico; A population of 1 400 mysids of *M. elongata* were collected. The organisms were transported to the Plankton Laboratory of Mazatlán Academic Unit of the Institute of Marine Sciences and Limnology, UNAM; mysids were separated under a stereoscopic microscope (Carl Zeiss) and / or with the naked eye aided by an illuminated magnifier, they were transferred to 4 L transparent bottles in the lab and acclimatized to the water from sampling site for 3 days. They were fed with freshly hatched *Artemia* nauplii *ad libitum.*

The surface temperature was 14.3-31.2ºC (avg. 25ºC SD ± 5.51) and salinity was 33.5-34.7 (avg. 34.1 ± 0.37) when collected the mysids. The brooding females were removed from the incidental fauna and placed in containers until the next morning, when the juveniles were born, laboratory experiments began in semi-controlled conditions. To obtain the F1 and F2 generations, three 20 L aquariums were maintained with 100 newly released juveniles (density of 5 org L-1). The F1 juveniles were obtained from the reproduction of wild progenitors after 26 days, and these produced a second generation F2 after 28 days. With the purpose of obtaining samples to estimate the beginning of the sexual maturity and first release of juveniles, on the same day another 100 L aquarium was placed with 1,000 freshly released juveniles (10 org L^{-1}) (F1), which was maintained with the same aeration conditions, temperature of 22 ± 1°C, salinity of 32 ± 1, feed *ad libitum* with *Artemia* nauplii of 18-48 h of age and average size of 480 µm and daily cleaning of aquaria through siphoning. This procedure is repeated with youngsters born to evaluate the same aspects for juveniles of the F2 generation for 30 days. It was observed the onset of sexual maturity in females by the presence of a large brood bag among the thoracopods and in males by the presence of long antennas and a fourth elongated pleopod. The aquarium was checked daily to detect the beginnings of sexual maturity and the first release of juveniles.

Live wild-caught and cultured gravid females (F1 and F2) were observed for marsupial stages and the frequency of their four developmental stages within the marsupium.

At different marsupial stages, the length was measured as follows: a dilute solution of menthol was added to slowly anesthetize the mysids in about 10-15 min, then they were fixed in 4% formaldehyde before measurement under a stereoscopic microscope. The total body lengths of mature and juvenile individuals released were measured along the midline of the body from the base of the ocular peduncles (or onset of the face) to the end of the telson and uropods, excluding the spines. The diameter of the embryo (Stage I) was measured along the maximum axis. The embryos of stages II, III and IV were measured along the distance between the anterior end (ventral side) and the posterior end of the uropod without stretching the body. Observations and measurements were made using a Carl Zeiss Discovery V12 binocular microscope with a Canon A620 camera adapted to an ocular micrometer and an automatic measurement program.

During each month of sampling the wild population, 200 wild mysids were randomly selected for determination of the reproductive activity and monthly frequency of adult mysid stages (Figure 1) under a stereoscope microscope. Wild gravid females measured 4.24-6.98 mm (avg.5.67 mm DS±0.54). Males measured 4.17-5.23 mm (avg.4.49 mmDS±0.21).Temperature measured with a bucket thermometer (Brannan; 76 mm immersion, -20° to 110°C); >25ºC y <25°C.Salinity measured with a hand refractometer (A. O. Corp. 10419 was32 ± 1). Aeration was constant, and mysids were fed *ad libitum* with freshly hatched *Artemia* nauplii. The water was partially replaced every two days with sea water from the original place.

Frequencies of embryonic stages were determined for the wild and cultured mysids (F1 and F2). Four embryonic stages and a fifth stage released (juvenile) were found and described. The main morphological features in the embryonic stages and phases were identified according to the criteria of Wortham-Neal & Price (2002) with *Americamysis bahia*. For the F1 and for the F2 generations, 100 newly released juveniles were maintained in each of 20 L aquaria (5 org · L^{-1}) to calculate survival.

Results

Sexual maturity occurred at 18-20 days after release in females and 12 days after release in males (22±1ºC and 32±1‰). The time from birth to release of a first clutch of off spring of juveniles began at 26 days in the F1 and at 28 days in the F2 at 22±1ºC. All embryos from the same pouch were at the same stage of development and the presence of the five stages remained constant over the sampling cycle and at the end of the culture experiments. The duration of each embryo stage was about 5-6 day and the F1 survival was 78% and F2 was 77% in 30 days at 21-23°C. The respective marsupial fecundity was recorded in terms of number of embryos, and all marsupial stages were observed in natural and laboratory populations (Table I). Figure 1 Shows ovigerous females were present (70%) all the year round few with empty pouch and males 15%.

Embryonic stage: Stage I began when eggs were fertilized inside the pouch and ended with the moult of the egg membrane. In contrast to unfertilized eggs, fertilized eggs were brightly colored along the flattened ventral region of the embryo, the germinal disc. Thoracic appendices were faintly visible through the egg membrane. The yolk was not dispersed into the abdominal rudiment (Figure 2a). Length at stage I: 0.28-0.41 (avg. 0.35±0.04) mm.

*Nauplioid early phase:*Stage II began with the release of the new embryo from the egg membrane and ended with the appearance of thoracic chromatophores. In Stage II the embryo showed flexion due to the transfer of the anterodorsal yolk mass to the abdominal rudiment; it was dorsally concave, with the body full of yolk and abdominal rudiments dorsally flexed; it had cephalic appendages; and there was slight segmentation of the thorax (Figure2b).Length at stage II: 0.53-0.71 (avg. 0.62±0.03) mm.

Nauplioid late stage: Stage III began with the appearance of eye pigment and ended with the moult of the naupliar cuticle. The optic lobes became bulbous and contained eye pigment, eyestalks began to form, birramous antennae appeared, and the abdomen, telson and uropods were fully developed (Figure 2c) Length at stage III: 0.70-0.95 (avg. 0.73±0.04) mm.

Postnauplioid early stage: Stage IV started after the moult of the naupliar cuticle inside the pouch and ended with yolk resorption in the thoracic region. After the molt, the transparent embryo resembled a juvenile, except that the eyes were directed dorsally rather than laterally and there was

Table I. Marsupial fecundity shows the number of embryos, in different size classes of M. elongata during the sampling months.

	MONTHS													
	2010					2011								
Class size (mm)	S	O	N	D	J	F	M	A	M	J	J	A	S	O
4.00-4.50	0	0	0	0	0	19	0	0	0	0	32	15	50	12
4.51-5.00	8	0	0	0	97	87	100	0	74	63	70	67	94	70
5.01-5.50	548	14	23	42	356	290	249	242	165	179	173	126	166	173
5.51-6.00	404	194	131	113	236	138	261	493	295	270	150	95	110	93
6.01-6.50	10	284	264	317	0	88	0	10	35	47	110	95	96	154
6.51-7.00	0	181	182	247	0	0	0	0	0	6	35	10	16	18

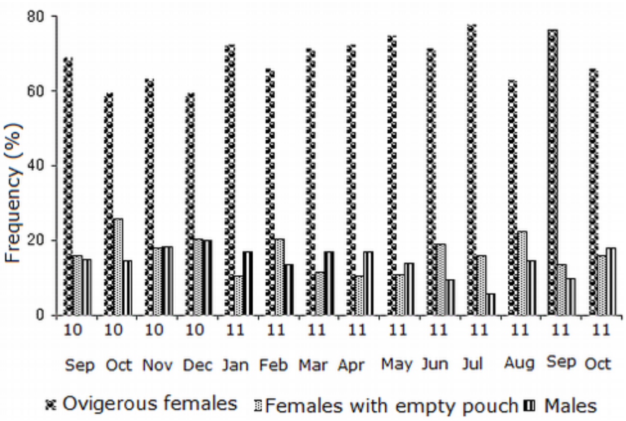

Figure 1. Monthly frequencies of adult mysid stages of *M. elongata* in the wild, throughout the sampling months (September 2010 to October 2011) in the Bay of Mazatlán, Sinaloa, Mexico.

elongation of the mandible; the cuticle was well developed in the posterior region of the thorax and yolk resorption began (Figure2d). Length at stage IV: 0.92-1.24 (avg. 1.10±0.08) mm.

Postnauplioid late stage: Stage V was identified as the newly released juvenile. It started with the complete absorption of the yolk inside the cuticle and ended with a moult leading to the release of free-living juveniles from the marsupium. The final release of juveniles was indicated by a bulge in the abdomen at the posterior end of the oostegites (Figure 2e). Length at stage V: 1.14-1.30 (avg. 1.24±0.03) mm.

In the wild population the monthly female: male ratios ranged from 4:1 to 15.6:1. In the laboratory, the ratio ranged from 1.3:1 to 2.8:1 (Table II). The average sex ratios how dominance of females in the wild mysids, 6.5:1.0; in the F1, 12.3:1.0; and in the F2, 2.1:1.0. Through the sampling cycle (*n*= 2 653), the average frequencies in wild as in F2 and F2 of the four stages of marsupial development were as follows: Stage I 26%, Stage II 25%, Stage III 23% and Stage IV 26%. The average sex ratios between wild population and from the lab could be due to the variety of natural food in the wild population have compared with just new born *Artemia* food we offer them.

The highest percentage of ovigerous females in the sampling cycle and laboratory were in the early post nauploid stage (Stage IV) the stage before the juveniles are released.

Discussion

Both males and females of *Rhopalophthalmus indicus* attain sexual maturity at a length of 8.4 mm (Biju *et al*. 2010) while in *M. elongata* sexual maturity began in males at 12 days after being released at a length of 4.6 and 4.3 mm; in females occurred at 18 and 20 days to a length of 5.35 (F1) and 5.31 mm (F2).The age at first reproduction of *M. elongata* was 18-20 days at21-23°Cinthe present study, and 38 days at14-20°C in a study by Clutter & Theilacker (1971). It is assumed that the development is completed with the energy supplement of the embryo. The initial size of the embryo (Stage I) indicates the energy that is constantly transferred to the remaining stages (Huck*etal*.2007). Marsupial development time is inversely related to temperature (Wortham-Neal & Price 2002), as are the beginning of first reproduction and the start of the release of juveniles in mysids (McKenney 1996, Sudo 2003). The time from birth to release of a first clutch of offspring in *M. elongata* (22 ± 1°C) was observed at 26 days in the F1 and 28 in the F2, whereas in *Mysidopsis bahia* at 28°C this occurs in 16 days (McKenney 1996). An F1 of *M. californica* at 20.5-21.2°C released the first batch at 19-24 days, and the F2 at 21.6-23.3°C released the first batch at 18 days (Ortega-Salas *et al*. 2008). The ovigerous females were present (70%) throughout the year, few with the empty bag and males 15%, this means that all year they reproduce.

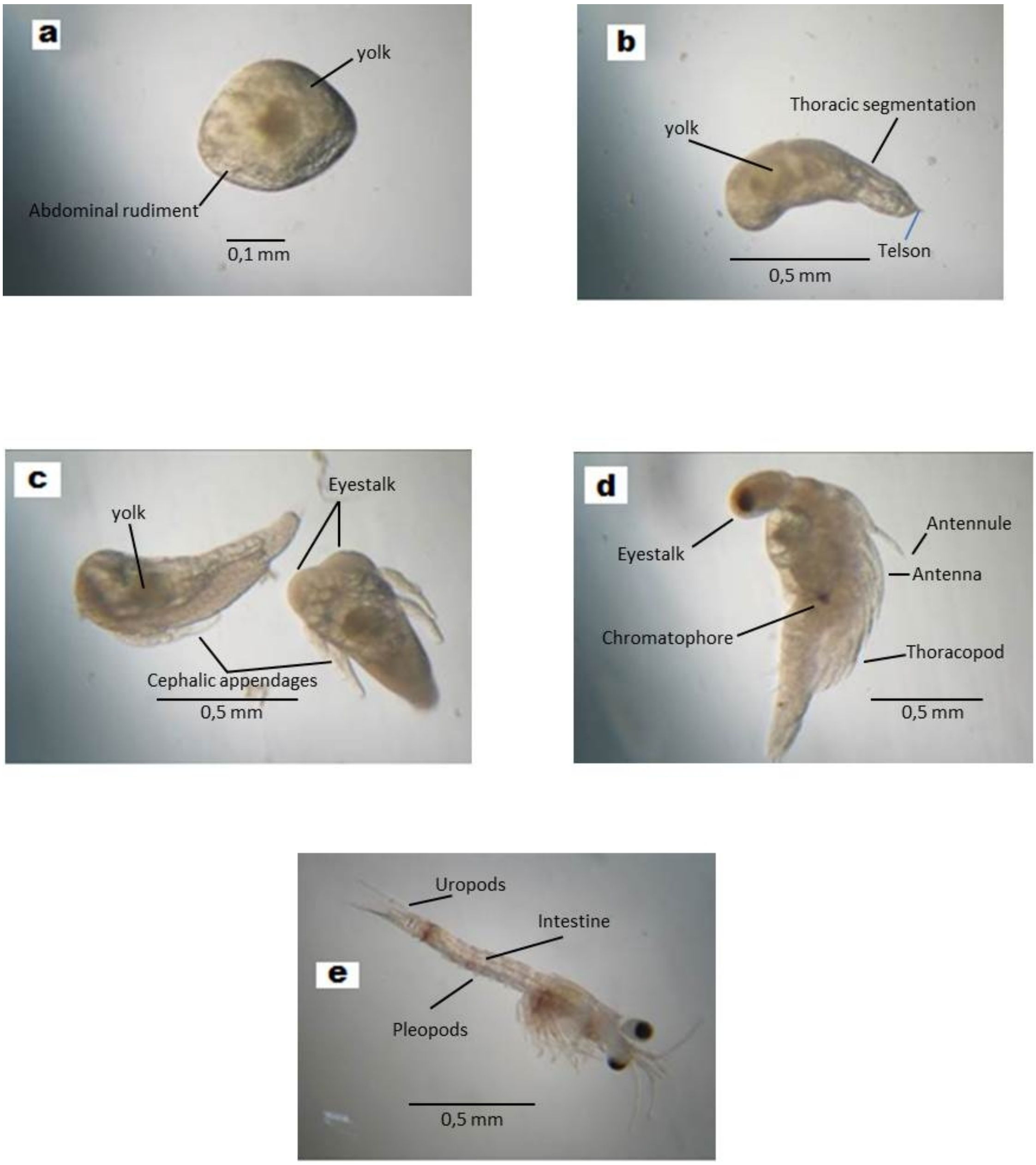

Figure 2. The four early intra-marsupial stages from female *M. elongata*: Embryonic stage, b: Nauplioid, early stage, c: Nauplioid late stage, d: Postnauplioid, early stage, and the fifth phasee: Postnauplioid, late stage (juvenile) (September 2010 to October 2011 in the Bay of Mazatlán, Sinaloa, Mexico).

Table II. Sexual ratio found in wild mysids, in lab F1 and F2 (September 2010 to October 2011)

	Sexual ratio	
	Mean	**Ratio f : m**
Wild (n=200)	6.5:1.0	4.0:1.0 - 15.6:1.0
F1 (n=172)	2.3:1.0	1.3:1.0 - 1.5:1.0
F2 (n=193)	2.1:1.0	1.8:1.0 - 2.8:1.0

Three larval stages were found in the marsupium of brooding females *M. neritica* (Calil & Borzone 2008).The embryonic development at different species was as shown in Table III, which are related with temperature and the latitudinal gradients.

The average sex ratios showed dominance of females to males in *M. elongata was* 6.5:1.0; in *M. californica* ranged from 2.5:1 to 3.0:1 (Ortega-Salas *et al*. 2008); this ratio was also high in the estuarine mysid *Acanthomysis thailandica*, ranging from

Table III. The embryonic development at different species in literature and present work.

Species	Temperature (°C)	Embryonic development (days)	Author
Metamysidopsis elongata (F1)	21-23	8	Present paper
F2	21-23	8	“
Mesopodopsis slabberi	15	13	Greenwood *et al.* (1989)
“	15	9-19	Biju & Panampunnayil (2011)
Mesopodopsis orientalis	25-29	4	“
Mysis relicta		270	Lasenby & Langford (1972)
Schistomysis spiritus	12.5	21	Mauchline (1973)
Acanthomysis sculpta	12	5	“
Americamysis bahia	16	12.7	Wortham-Neal & Price (2002)
“	20	9.4	“
“	29	3.7	“

0.62:1 to 3.86:1 (Ramarn*etal.*2012) and in *Mesopodopsis orientalis* with an average of 1.4:1 (Allen 1982). The sex ratio of *Amathimysis trigibba*, another mysid from Mazatlán, has shown considerable variation with an average of 3.8:1 (Rendón-Valdez 2013). However, Rappé *et al.* (2011) recorded a balance between the number of female and male in all the populations of mysids in their study, and no segregation was found governed by the salinity between the different stages of life of each population of mysids. This paper shows that more females represent more offspring.

Studies on fertility, sex ratio, survival and growth will provide knowledge about their biology, which will allow to know their potential to be cultivated on a large scale.

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