Assiut Vet. Med. J. Vol. 39 No. 77, April 1998

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MORPHOLOGY OF OOSTEGITES AND BROODING HABITS IN THE MARINE AMPHIPOD *Parhyale hawaiensis* FROM RED SEA

(With 5 Tables and 2 Figures)

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(Received at 24/1/1998)

مورفولوجيا كيس الإحتضان وظاهرة الإحتضان فى نوع *باريال هوابنسس* (قشريات-مزدوجة الأرجل) من البحر الأحمر

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بينت الدراسه أن الأمهات تحتضن الأجنه داخل كيس الأحتضان و الذى يتكون من أربعة أزواج من صفائح تعرف بصفائح الأحتضان. يقع كيس الأحتضان على الناحيه البطنيه للصدر و تنشأ صفائح الأحتضان من الشدفه الحرقفيه للرجل التناسليه الثانيه و أرجل المشى من ١ إلى ٣. صفيحة الأحتضان متوسطة الحجم ومسجفه بالعديد من الشعيرات التى تتوزع على حافتها الخارجيه بكثافه عاليه. و يعتبر هذا التركيب هاما، حيث يعمل على تقليل الفاقد من البويضات و الأجنه و كذلك من أجل توفير حمايه أفضل. كذلك بينت الدراسه أن التكوين الجنينى مباشر و أن الأمهات تحتضن الأجنه حتى مرحله الفقس. ولقد بينت الدراسة أن العلاقه بين عدد البويضات و طول جسم الأنثى علاقه خطيه. ولقد تم تعيين الطول المثالى للأمهات والذى عنده تحتضن الأنثى اكبر عدد من الأجنه وهو ١٢,٢ و ١١,٤مم لعينتين جمعتا خلال عامى ١٩٩٢ و ١٩٩٣م.

SUMMARY

The marsupium of *Parhyale hawaiensis* consists of four pairs of coxal plates (brood plates or oostegites). The morphology and the size of brood plates, and type and quality of setae bordering the oostegites have been discussed. The marine amphipod *Parhyale hawaiensis* is a synchronous and short term brooder. Embryos, were in the same developmental stage and held together in the brood pouch. Brood size varied from 5 to 13 in specimens varying in body length from 5 to 10 mm. Brood size was found to be correlated linearly with female body length.

Key words: *Crustacea, Amphipoda, Red Sea, oostegites, brooding.*

INTRODUCTION

The brooding of embryos is a mode of parental care, which may extend beyond the brood protection and is common among small invertebrates. The mode of brood protection allows the animals to pass the pelagic development without great loss. Also, it offers protection for youngesters against the unfavourable conditions to decrease their mortality to a minimal level.

Starthmann & Starthmann (1982) pointed out that, as adult size increased, the capacity to produce young was greater than the capacity to brood them. Peters (1983) suggested that, the surface area of an organism increased with the square of body length, while the volume increased with the cube of length. This hypothesis is known as allometry of brooding and is based on data from the starfish *Asterina phylactica* (Starthmann et al., 1984). Also, allometry of brooding has been investigated in the marine crustacean amphipods *Bathyporeia pilosa* and *B. pelagica* (Fish, 1975), marine bivalves *Transenella tantella* and *Lasaea ruba* (Kabat, 1985; Aboul-Dahab, 1990), and the marine crustacean mysids *Tenagomysis tasmaniae*, *Anisomysis mixta australis* and *Paramesopodopsis rufa* (Fenton, 1994). Similar studies on some freshwater species of invertebrates were carried out by Aboul-Dahab and Obuid-Allah (1993), on the crustacean decapod *Caridina nilotica nilotica*; by Aboul-Dahab and El-Shimy (1993), on the leech *Helobdella conifera*, by Nishi and Nishihira (1993), on the serpulid polychaete *Salmacina dysteri*; and by Aboul-Dahab (1994), on the gastropod *Bellamya unicolor*.

The present investigation is focusing light on the morphology of the brood plates (oostegites) and the relationship between the brood size and the length of the marine amphipod *Parhyale hawaiensi*.

MATERIAL and METHODS

For investigating the allometry of brooding, several specimens were collected from the Red Sea at Sharm El-Nagha site during December 1992 and December 1993. All specimens were preserved in 70% alcohol. Embryos were incubated in the marsupium (brood pouch) from the spawning of ova to hatching of juveniles. In the laboratory, the total body length of females were measured from the frontal tip of the body to the base of the posterior margin of the telson (Fish, 1975). Also, the number of embryos for each female was counted under a binocular dissecting microscope and related to the females body length. The percentage of females carrying embryos was estimated. The number of embryos per brood female (brood size, NB) is regressed to the body length (L) of the female. Statistical analysis of the data was performed using computer program. The exponential equation Y = AX was used to examine the relationship between brood size (NB) and body length (L), where the exponent (b) is derived from the logarithmic equation: Lin Y = Lin a + b Lin X; where (Y) is the dependent variable, represents the brood size (NB), and (X) is the independent variable, represents the total body length of ovigerous female (L). From the exponential equation, if the exponent (b) is equal to one, brood size scales to the adult body length (L). If (b) equals two, then brood size scales to the square of the adult body length (area relationship), while if (b) equals three, then brood size scales to the cube of adult body length (volume relationship). The value of (b) was tested using the following equation:

$$t = \frac{b}{s.e..of..b}$$

Other forms of regression equations were used during the study as :

Y = a + bX (Linear).

Y = a + bX + cX (Quadratic).

The quadratic equation was used to calculate the optimal level for both body length (L) and the number of brooded embryos (NB) of the females.

RESULTS

Morphology of oostegites (brooding plates): (Fig. 1)

The oostegites are delicate structures projecting from the coxal plates of the female pereiopods from 2 to 5 which are arranged with each other, in an overlapping manner, forming the brood pouch. The brood pouch opens only at its posterior end facing the pleopods. In the present species, oostegites are formed in the beginning as soft and short plates. When the brood pouch contains early embryos, the oostegites are not fully expanded. As embryos increase in size, the oostegites are forced to extend ventrally. The size of the first three pairs of oostegites are similar and suboval in shape, measuring about 1.15, 1.0, and 0.96 mm in length and 0.55, 0.64, and 0.43 mm in maximum width, respectively. The fourth pair of oostegites is quite smaller and irregular in shape and measures about 0.84 mm in length and 0.33 mm in maximum width. The first three pairs of oostegites carry many of curl-tipped setae, while the fourth pair carries a few number of these setae.

The curl-tipped setae range from 0.18 to 0.27 mm in length. The setae on the corresponding plates of each pair of oostegites are interlaced with each other and serve for preventing the escape of embryos or eggs from the brood pouch.

Brood size:

The females brood their embryos on the ventral surface of the thorax in the brood pouch located between the thoracic appendages. In live females, the embryos were observed releasing from the oviducts to the brood pouch in two groups, one from each oviduct, and then they were mixed with each other by the beating action of the gnathopod 2. Careful examination of eggs, that were collected from ovaries of large numbers of females, shows that they were not fertilized. So, fertilization has occurred inside the brood pouch. The females brood their embryos from zygote stage until they hatch as juveniles (miniature-like adult). So, the species has direct development. Careful examination of embryos, of any female, shows that the embryos are always in the same stage of development. Some females, however, had eggs at zygote stage, while others had same stages of cleavage or gastrulation. Other females had pre-hatching embryos with the same stage of development containing eye pigments. This suggests that, all ova are fertilized at the same time and the species is termed as "synchronous brooder".

The Relationship between brood size and body length:

In a collected sample during December 1992, the maximum number of brooding embryos was 12 recorded from a female at body length of 7 mm, while the minimum number was 5 from a female at body length of 6 mm, with a mean value of 8.36 (S.D.=1.99). The largest specimen carrying embryos was 10 mm in body length, while the smallest one measured 6 mm (Table 1). In another sample, collected during December 1993, the maximum number of brooding embryos was 13 recorded from a specimen of 9 mm body length, while the minimum number was 5 from a specimen of 6 mm body length with a mean value of 9.18 (S.D.=1.76). The largest specimen carrying embryos was 10 mm in body length, while the smallest one measured 5 mm.

The percentage of brooding females during December 1992 was 93%, while that of December 1993 was 88%. The Ordinary Least Square (OLS) was used to examine the relationship between the body length (L) and number of brooded embryos (NB).

Data from samples collected during December 1992 and 1993 (Fig. 2 A, B) show that, the brood size increases with the increasing of body length and the relationship between the body length and brood size can be represented by three models; linear, logarithmic and quadratic (Tables 2 & 3). The regression coefficient (b) of the linear equation suggests that for every 1 mm increase in adult length, there is an increase in brood size of 1.46 and 1.19 for the two samples, respectively (Tables 2 & 3). The exponential equation is derived from the logarithmic one. Therefore, the values of (b) for the exponential equations equal those of the logarithmic ones, which are 1.36 and 1.07 for the two samples, respectively. The equations show that, the brood size proportionally increases with the body length which is a linear relationship and this is consistent with the actual data shown in Fig. 2 A & B.

The values of (R^2) of the linear equations (Tables 2 & 3) show that the variation in body length of ovigerous females is responsible for 57 to 63% of the total variations in number of the brooded embryos for the samples collected during December 1992 and 1993, respectively.

The correlations between the body length and brood size for the two samples are 0.76 and 0.79 (Tables 2 & 3), which indicates that the degree of association between the brood size and body length is about 76% and 79% for the two samples, respectively.

From the quadratic equations (Tables 2 & 3), it is possible to calculate the optimal level for both length (L) and number of brooded embryos (NB) by taking the partial differentiation of the equation as to equal zero.

$$\frac{NB}{L} = 0 \quad \text{at maximum level of L}$$

$$\text{Then, } \frac{\partial NB}{\partial L} = 0 + 3.9 - 2\,(0.16)\,L \qquad \text{(Table 2)}$$

$$0 = 3.9 - 0.32\,L$$

$$L = \frac{3.9}{0.32}$$

$$L = 12.2 \text{ mm}$$

By substituting (L = 12.2 mm) in the quadratic equation, we obtain (NB) at the turning point.

$NB = - 11.5 + 3.9\,(12.2) - 0.16\,(12.2)$

$NB = 12.26$

$$\text{Then, } \frac{\partial NB}{\partial L} = 0 + 3.9 - 2\,(0.17)\,L \text{ (Table 3)}$$

$$0 = 3.9 - 0.34\,L$$

$$L = \frac{3.9}{0.34}$$

$$L = 11.4 \text{ mm}$$

By substituting (L = 11.4 mm) in the quadratic equation, we obtain (NB) at the turning point, the point at which brood size decreases.

NB = - 10.3 + 3.9 (11.4) - 0.17 (11.4)

NB = 12.06

These calculations, which are using data for samples collected during December 1992 and 1993, show that, individuals with body lengths of 12.2 and 11.4 mm, contain the optimal number of brooded embryos, which are 12.26 and 12.06, respectively. At greater body lengths, brood size decreases suggesting that some of the oldest individuals have less fecundity (Fig. 2 C, D). It is possible to predict the number of brooded embryos (NB) as a function of the body length (L) using the linear and quadratic equations. Tables (4 and 5), give such prediction using a range of true lengths and a range of hypothetical lengths.

DISCUSSION

In amphipods, the morphology, especially the size of brood plates, the type and number of setae bordering the plates are good factors preventing the escape of embryos from the brood pouch, where, the wide plates of the brood pouch providemore protection than the narrow ones. Also, the medium curl-tipped, regularly distributed and tightly interlaced setae give more protection than the few, smooth, irregularly distributed and loosely interlaced ones. This view is supported by Leite et al., (1986) who reported that, the flexibility of the oostegites and interlacing of the setae are important factors for the protection of embryos during their development, in which the volume of the eggs is gradually increasing to utilize the total free space of the marsupium. They also, suggested that the shape of brood plates of a single species may be similar or different from each other with various degrees, or is completely different.

Parhyale hawaiensis lives in a high level of intertidal region underneath the stones, in a harsh condition as desiccation, and exhibiting some jumping in its movement. So, the curl-tipped, medium sized setae of brood plates of *Parhyale hawaiensis* are firmly closing the marsupium to provide more protection and security for the eggs during its movement or hard conditions. Also, this type of oostegites provides a uniform distribution of the eggs inside the brood pouch and diminishes the chance for accidental egg losses.

The embryos of *Parhyale hawaiensis*, within any one individual, are at the same developmental stage, which indicates that the species is a synchronous brooder. Similar results, were recorded by Sheader & Chia (1970) for the intertidal *amphipod Marinogammarus obtusatus*, Fish (1975) for the amphipods *Bathyporeia pilosa* and *B. pelagica*, and by Aboul-Dahab & Obuid-Allah (1993) for the fresh water decapod *Caridina nilotica nilotica*.

Interestingly, the pleopods of the present species help for preventing the escape or re-entry of embryos from / to the brood pouch. Also, the juveniles hatch inside the brood pouch and wait for a part of time within the pouch, before their releasing into the exterior. Therefore, the embryos and the juveniles benefit from the parental care and brood protection of the adult females before and after their hatching. Similar findings were reported by Croker (1968) for the amphipod *Neohaustorius schmitzi*, Sheader & Chia (1970) for the amphipod *Marinogammarus obtusatus*, and Fish (1975) for the amphipods *Bathyporeia pilosa* and *Bathyporeia pelagica*. They concluded that the time spent by the juveniles within the brood pouch varies from one species to another.

In the present species, females less than 5 mm in length did not carry embryos. In the marine bivalve *Lasaea rubra* (Aboul-Dahab, 1990) and in the fresh water decapod *Caridina nilotica nilotica* (Aboul-Dahab & Obuid-Allah, 1993), the small classes of their populations did not carry embryos. The previous authors concluded that the gonads of individuals with small body length, may not reach to full maturation. In the present study, brood size was linearly correlated with the female body length. In exponential equations, the exponent values (1.36 and 1.07) imply that, the reproductive output of *Parhyale hawaiensis* is proportional to the adult length (Tables 2 & 3), which is a linear relationship (Fig. 2 A, B). A linear relationship between brood size and female body length in some amphipods was reported by Fish (1975) and Sheader & Chia (1970), while, an exponential one was recorded by Powell (1992), who explained that, this relationship may be a resultant of senility and variation in size at first breeding of females, which are combined with the production of a minimum number of embryos. He suggested that, the linear relationship between brood size and body length of females would only be true for females in the middle of the size classes.

LIST OF FIGURES

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Table (1) The maximum and minimum values of brood size and body length for females collected during December 1992 and December 1993.

Date	Females No.	Length (L)				No. of embryos (NB)				Brooding %
		Max.	Min.	Mean	SD	Max.	Min.	Mean	SD	
Dec., 1992	50	10	5	7.3	1.04	12	5	8.36	1.99	93
Dec., 1993	50	10	6	8.02	1.26	13	5	9.18	1.76	88

Table (2): Regression models for the brood size (NB) and adult body length (L) for data of *Parhyale hawaiensis* (N = 50, December 1992).

Equation	Type	b	Corr.	R^2	F	P	df
$NB=-2.29+1.46L$	Lin.	1.46		56.3	64.1		
$NB=-0.16+1.36L$	Log.	1.36		55.3	61.7		
$NB=0.356L^{1.36}$	Expo.	1.36	0.76	55.3	61.7	0.000	49
$NB=-11.5+3.9L-0.16L^2$	Quad.	3.9 0.16		56.6	33		

Table (3): Regression models of the brood size (NB) and adult body length (L) for data of *Parhyale hawaiensis* (N = 50, December 1993).

Equation	Type	b	Corr.	R^2	F	P	df
$NB=0.05+1.19L$	Lin.	1.19		62.4	82.5		
$NB=0.02+1.07L$	Log.	1.07		60.8	77.2		
$NB=1.044L^{1.07}$	Expo.	1.07	0.79	60.8	77.2	0.000	49
$NB=-10.3+3.9L-0.17L^2$	Quad.	3.9 0.17		63.7	44.1		

Lin = linear equation
Log = logarithmic equation
Expo = exponential equation
Quad = quadratic equation

Table (4): Predicted brood size for different body lengths for *Parhyale hawaiensis* using regression models (Dec., 1992).

Body size	5	7	8	9	10	11	12	13	14	15	17	19	Equ.
Embryos	5	7.9	9.4	10.9	12.3	13.8	15.2	16.7	17.9	19.6	22.5	25.5	Lin.
no.	4	8	9.5	10.6	11.5	12	12.3	12.2	11.7	11	8.6	5.8	Quad

Table (5): Predicted brood size for different body lengths for *Parhyale hawaiensis* using regression models (Dec., 1993).

Body size	5	7	8	9	10	11	12	13	14	15	17	19	Equ.
Embryos	6	8.4	9.6	10.8	11.9	13.1	14.3	15.5	16.7	17.9	20.3	22.7	Lin.
no.	4	8.7	11.1	11.7	12.1	12	11.7	10.9	10.9	9.9	6.9	2.4	Quad

Fig. 1

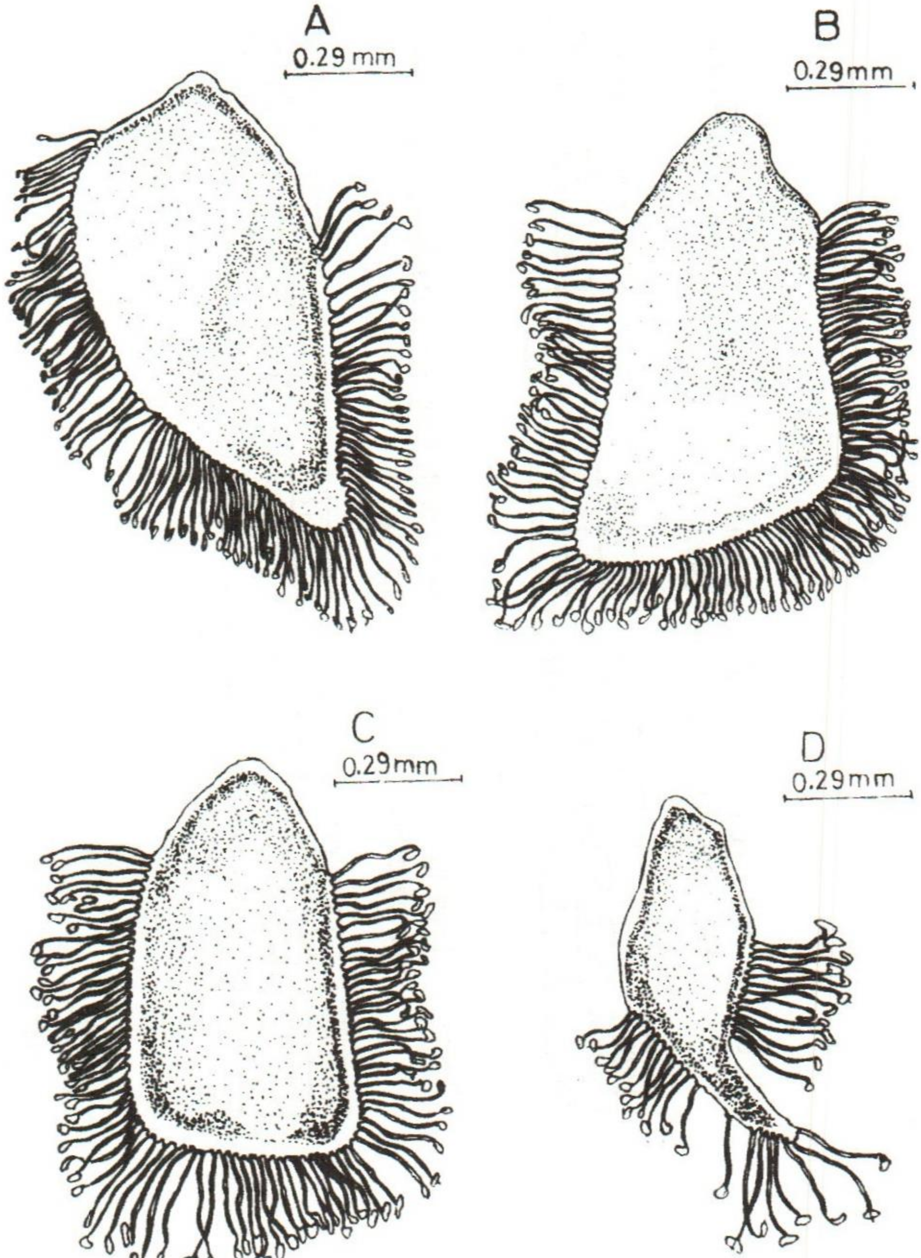
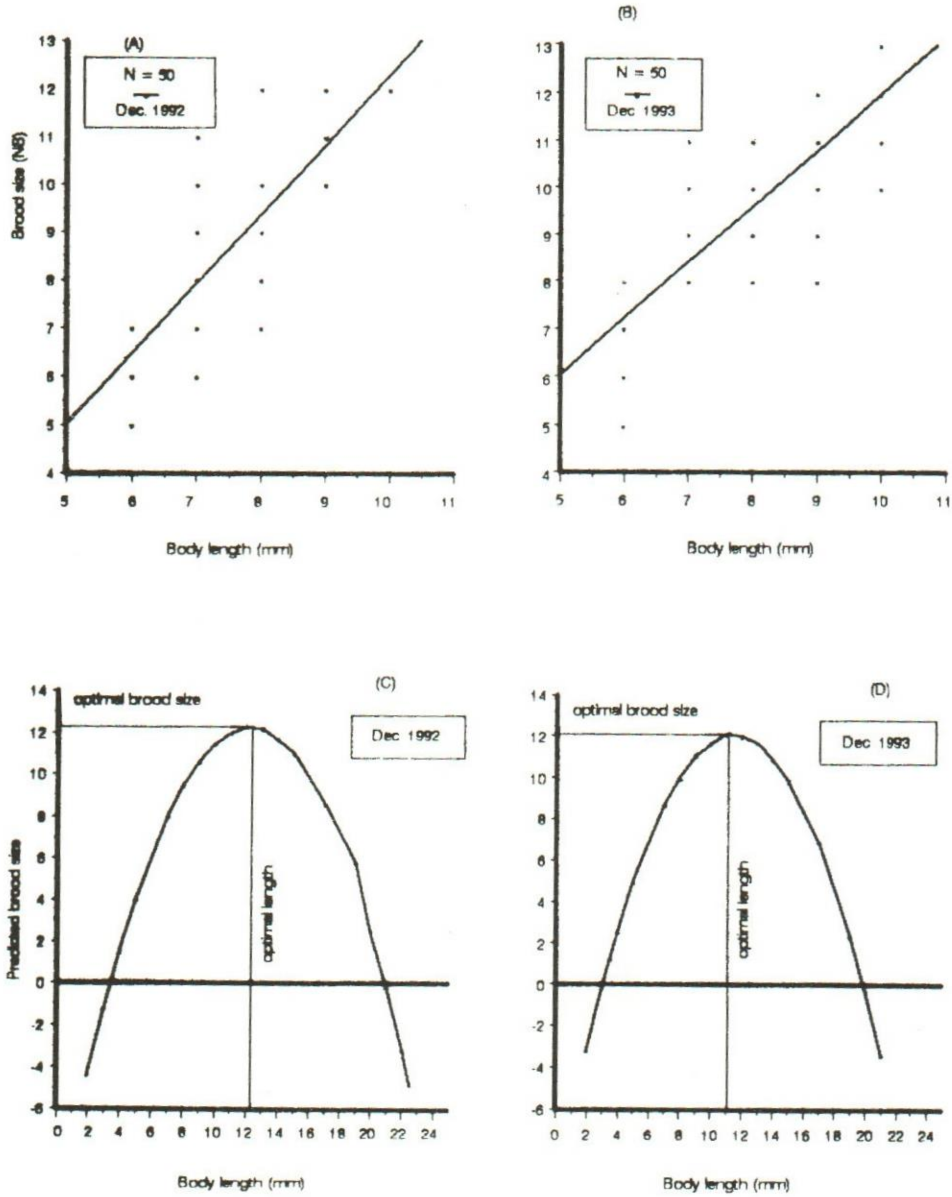


Fig. 2


(A)
N = 50
Dec. 1992
Brood size (NB)
Body length (mm)
(B)
N = 50
Dec 1993
Body length (mm)
(C)
optimal brood size
Dec 1992
optimal length
Predicted brood size
Body length (mm)
(D)
optimal brood size
Dec 1993
optimal length
Body length (mm)