

Reproductive Biology of *Panulirus echinatus* (Crustacea: Palinuridae) from São Pedro and São Paulo Archipelago, Brazil

Allysson P. Pinheiro and Jorge E. Lins-Oliveira

(APP) Universidade Federal de São Carlos/DGEV. Rodovia Washington Luís, Km 235, 13565-905, Caixa Postal 676, São Carlos, SP, Brazil. Email: allyssonpp@yahoo.com.br

(JELO) Universidade Federal do Rio Grande do Norte/DOL, Laboratório de Biologia Pesqueira (LABIPE). Praia de Mãe Luiza s/n, Via Costeira, 59.014-100, Natal, RN, Brazil. Email: jorgelins@ufrnet.br

Abstract

P. echinatus is the only commercially captured species from northeastern Brazil that does not possess a specific regulation due the less information about its biology and ecology. Therefore, the objective of this work was to raise information on the reproduction of *Panulirus echinatus*. Specimens were captured using traps and free diving. The macro- and microscopic determinations of the existence of six gonadal development stages for females were possible. For males only tree stages of developmental stages could be determined by microscopy. The obtained result for L_{50} was approximately 13.5 cm of total length for females. The obtained results for *P. echinatus* point to a mean calculated fecundity equal to 56,160 eggs.

Key words: *Panulirus echinatus*, São Pedro and São Paulo Archipelago, reproduction, fecundity, L_{50} .

Introduction

One of the main economic resources in fisheries of northeastern Brazil is the capture of lobsters in depths that range from 10 to 60 meters with the aid of traps, nets, and by diving (Paiva, 1995). The latter method, not regulated by current legislation, is the most frequently used. The stock of lobsters in northeastern Brazil has decreased, mainly due to illegal and predatory fishing (Vasconcelos and Oliveira, 1996; Vasconcelos, *et al.* 1994; Oliveira, *et al.* 1993; Fonteles Filho, 1994).

The catches concentrate mainly on palinurid lobsters, specifically on *Panulirus argus* (Latreille, 1804), *Panulirus laevicauda* (Latreille, 1917), and *Panulirus echinatus* Smith, 1869. The two former species are the most intensively captured for commercial purposes in northeastern Brazil and under a specific fishing regulation with 13 and 11 cm of minimal tail length, respectively (Paiva, 1995).

The spatial distribution of lobster captures reflects the bioecological relationship of the species with the substrate as well as the interspecific variability. The larger Caribbean spiny lobster (*P. argus*) has a wider distribution and lives in greater depths, while the smaller Smooth tail spiny lobster (*P. laevicauda*) lives in more coastal shallower waters. Both inhabit bottoms with calcareous algae formations, clear, warm and well oxygenated water, occurring from the depth of 20 m to the edge of the continental shelf. The Brown spiny lobster (*P. echinatus*) prefers insular regions more distant from the coast and that have a rocky bottom (Paiva, 1995).

In northeastern Brazil *P. echinatus* predominates in areas such as the Rocas Atoll, the Fernando de Noronha Archipelago and the São Pedro and São Paulo Archipelago, clearly in contrast with the others that occur strictly along the coast. *P. echinatus* is the only one within the commercially captured species

that does not possess a specific regulation, due to the lack of information concerning biological aspects and also to the difficulty in performing studies in this species.

However, several studies have been focusing on reproductive characteristics of spiny lobsters. We can highlight the studies carried out by Mota-Alves and Tomé (1965; 1966), Mota-Alves and Paiva (1976), Ivo and Gesteria (1986), Plaut (1993), Ivo and Pereira (1996) Minagawa (1997), Soares and Peret (1998a,b), Soares *et al.* (1998) and Briones-Fourzán and Contreras-Ortiz (1999). For *P. echinatus* it can be underlined the works done by Pinheiro *et al.* (2003) that evaluated its population biology in São Pedro and São Paulo archipelago, northeastern Brazil, and by Barreto *et al.* (2003) that estimated the mean length at the first physiological maturity for males on coastal reefs off Tamandaré, Brazil.

This work aims to study the reproductive biology of *P. echinatus*, focusing on information on the average body size of the first gonadal maturity, fecundity and the macroscopic and microscopic stages of gonadal development characterization. The obtained data will be of importance for the government agencies responsible for the fishing sector, as a basis for elaboration of a rational exploitation program aiming sustainable development of lobster fishery.

Material and Methods

This study was developed from fifteen expeditions during June/1998 to September/1999 to the São Pedro and São Paulo Archipelago (Fig. 1). São Pedro and São Paulo Archipelago (29°20'44"W-00°55'01"N) in the unique group of Brazilian oceanic islands in the Northern Hemisphere. Of a volcanic origin, these islands are approximately 1000 km offshore of Natal, Rio Grande do Norte State, their nearest continental city.

Panulirus echinatus specimens were captured using traps and free diving. About four traps were set on a daily basis around the islands that make up the archipelago and in the small lagoon that is formed in between the islands during each expedition. Depths ranging from 10 to 50 m, were covered by the sampling to minimize the effects of a possible depth stratification.

Each captured specimen of lobster was identified according to Melo (1999) and sexed based on the analysis of external sexual characters (e.g., presence of pleopods on abdomen in females). Total length (TL) was measured using a pachymeter (0.01 mm). Gonads were removed through a rectangular opening in the posterior part of the cephalothorax and weighed in a digital scale with a precision of 0.001 g. Egg masses, when occurred, were removed from pleopods and stored in a 70% ethanol solution for a posterior study of fecundity.

Gonads were initially classified in five development stages (immature, initial maturation, final maturation, mature and spawned) based on the color scale proposed by Mota-Alves and Tomé (1965; 1966). A sixth stage (Soares and Peret, 1998) representing rematuration was considered. This stage corresponds to those individuals that are in the maturation process for, at least, the second time.

The determination of the gonadal maturation phases of animals is a routine activity that serves the purpose of describing reproductive cycles. The use of empiric macroscopic scales needs validation. A very useful technique is the histological technique, where the criteria for the determination of the gonadal maturation stages are based on sex cell diameter, cell affinity for determined stains, presence or absence of structures such as vitelline vesicles in the case of oocytes, among others (Aquilari and Malpica, 1993).

To confirm the macroscopic classification of the gonadal development stages, microscopical procedure was done. Gonadal samples were removed from some individuals in each developmental stage and submitted to dehydration, diaphanization, impregnation, embedding, microdissection, slide preparation and HE (hematoxylin/eosyn) staining using the techniques described by Michalany (1990). The microscopic classification was based on the methodology developed by Mota Alves and Tomé (1965; 1966), which determines the existence of three development stages for males (immature, in maturation and mature) and five for females (immature, initial maturation, final maturation, mature and spawned).

The average length of the first gonadal maturation (L_{50}) was calculated according to the graphic extrapolation method described by Fonteles Filho (1989), which consists in plotting the proportion

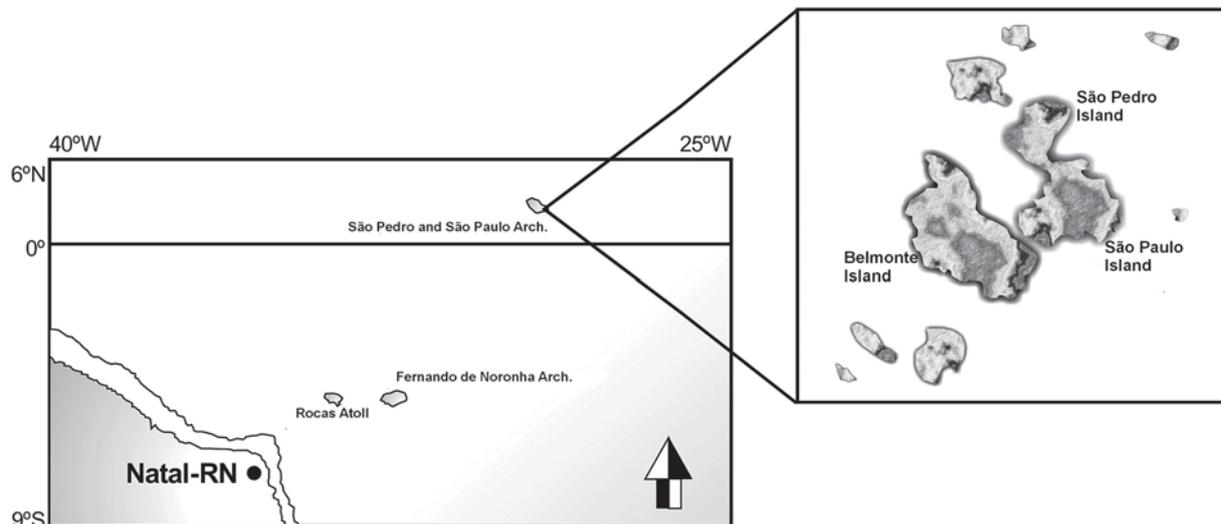


Figure 1. Localization of the São Pedro and São Paulo Archipelago, off coast of Brazil.

of adult females that were in a gonadal development stages different from I (immature) of each length class (1.5 cm interval). The L_{50} value is obtained when the cumulative proportion of these females reaches 50% in a class.

Absolute fecundity is a parameter that measures the potential reproductive capacity of the individuals, being defined as the number of ovules produced by the female during a spawning season. Fertility, a term generally used as a synonym, is the number of eggs (fertilized ovules) produced by a female. In species with external development but whose eggs remain attached to the individual (such as the *Panulirus* lobsters), or in species with internal fecundation and development, the calculation of fertility becomes much easier (Fonteles Filho, 1989).

According to the same author, most of the researchers that studied the fecundity/length relationship of *P. argus* and *P. laevicauda* lobsters of northeastern Brazil considered it to be a linear and positive relationship, with larger individuals presenting higher fecundities (Ivo and Pereira, 1996). To obtain the individual fecundity 15 females with egg mass were used. Eggs were removed from their eight pleopods, dried on a blotting paper for 1 min and weighed. A sub-sample was weighed, counted and the total number of eggs was obtained relating the sub-sample weight and the total egg mass weight. The relationship between fecundity and lobsters size body was considered positive and linear.

Results

One thousand four hundred and eighty-six (1,486) individuals of *P. echinatus* were collected.

The gonads of *Panulirus echinatus* are paired cylindrical structures in the H-shape that may extend from the anterior part of the cephalothorax to the anterior part of the abdomen in both sexes. Female gonads present differentiation in color, volume, size and aspect throughout the gonadal development process (Fig. 2). On the other hand, male gonads do not have significant macroscopic differentiations with the advance of the gonadal maturation process, and this prevented macroscopic classification in this study.

The macroscopic determination of six gonadal development stages for females of *P. echinatus* was possible by relating the observed aspects with studies performed with species of the same family (Table I, Fig. 3). This classification could be confirmed by microscopy. Male gonads presented little macroscopic differentiation and could not be classified. However, the microscopic characteristics of the male gonads permitted the identification of three development stages, but we could not obtain a good stage I picture (Table II, Fig. 4).

The obtained results for the average gonadal maturation length where 50 and 100% of the adult females of the population are undergoing the maturation process were approximately 13.5 and



Figure 2. Gonads of *P. ecbinatus* females in the diverse macroscopic stages of gonadal development: Virgin (I), Initial maturation (II), Final maturation (III), Mature (IV), Spawned (V) and Rematuration (II*).

Table I. Classification of the macro- and microscopic reproductive stages form females of *P. ecbinatus*.

Development stages	Macroscopic characteristics	Microscopic characteristics
Immature	Ovaries with a brownish-white color, smooth surface and with small weight, volume and size. Characterizes the group of immature individuals (Fig. 2(I)).	The tissue of irregular form is characterized by the presence of only undifferentiated cells (oogonia) that are disposed along the narrow conjunctive lamellae (Fig. 3A).
In Maturation	Ovaries with a light-yellow color, very similar to the previous stages, however with larger weight, volume and size. Characterizes the group of individuals that is entering the reproductive cycle for the first time (Fig. 2(II)). Ovaries with a rough surface, orange color and with much larger volume, width and weight (Fig. 2(III)). The size is almost the same to the previous stage.	Initial maturation: Relating with the previous stages, cells undergoing the differentiation process may be observed in this stage, although undifferentiated cells predominate. The conjunctive lamellae are thicker (Fig. 3B). Final Maturation: In this development stage, a predominancy of cells with a superior volume than the previous stages is observed, containing a well defined nucleus that tends to migrate to one of the poles. Also, the presence of oogonia (cells of the initial reproduction phase) can be observed. The former are disposed bordering the cells characteristic of this stages (Fig. 3C).
Mature	Very volumous ovaries with rough surfaces and ovules visible by transparency. The whole set is red in color, with weight, volume and size very superior than those of the previous stages. Characterizes the group of adult individuals prepared for mating or already mated (Fig. 2(IV)).	In this phase, the cells have reached the peak of their development and, consequently, their greatest volume. Besides having the approximate shape of a polyhedron, their nucleus could not be seeing. These characteristics give the tissue a uniform and extremely organized aspect (Fig. 3D).
Spawned	Ovaries with flaccid tissues, of brownish-gray color, similar in size to the previous stages but with much lower weight and volume. Characterizes the group of adult individuals that have already reproduced, indicating the end of the reproductive cycle (Fig. 2(V)).	The determinant characteristic of this stage is the presence of empty follicles delimited by the egg lamelles of the cells eliminated in the spawning process. Only mature cells remain in the ovary. The tissue presents a chaotic and hollow aspect (Fig. 3E).
Rematuration	Ovary tissues similar to the previous stages, however with lower weight, volume and size. Characterizes the group of individuals that have already reproduced at least once (Fig. 2(II*)).	The marking characteristics of this stage are cell disorganization, presence of cells undergoing a reabsorption process (atresia) and presence of all the stages of cell development previously described. Undifferentiated cells and/or in initial development predominate. This stage characterizes the restart of the reproductive process (Fig. 3F).

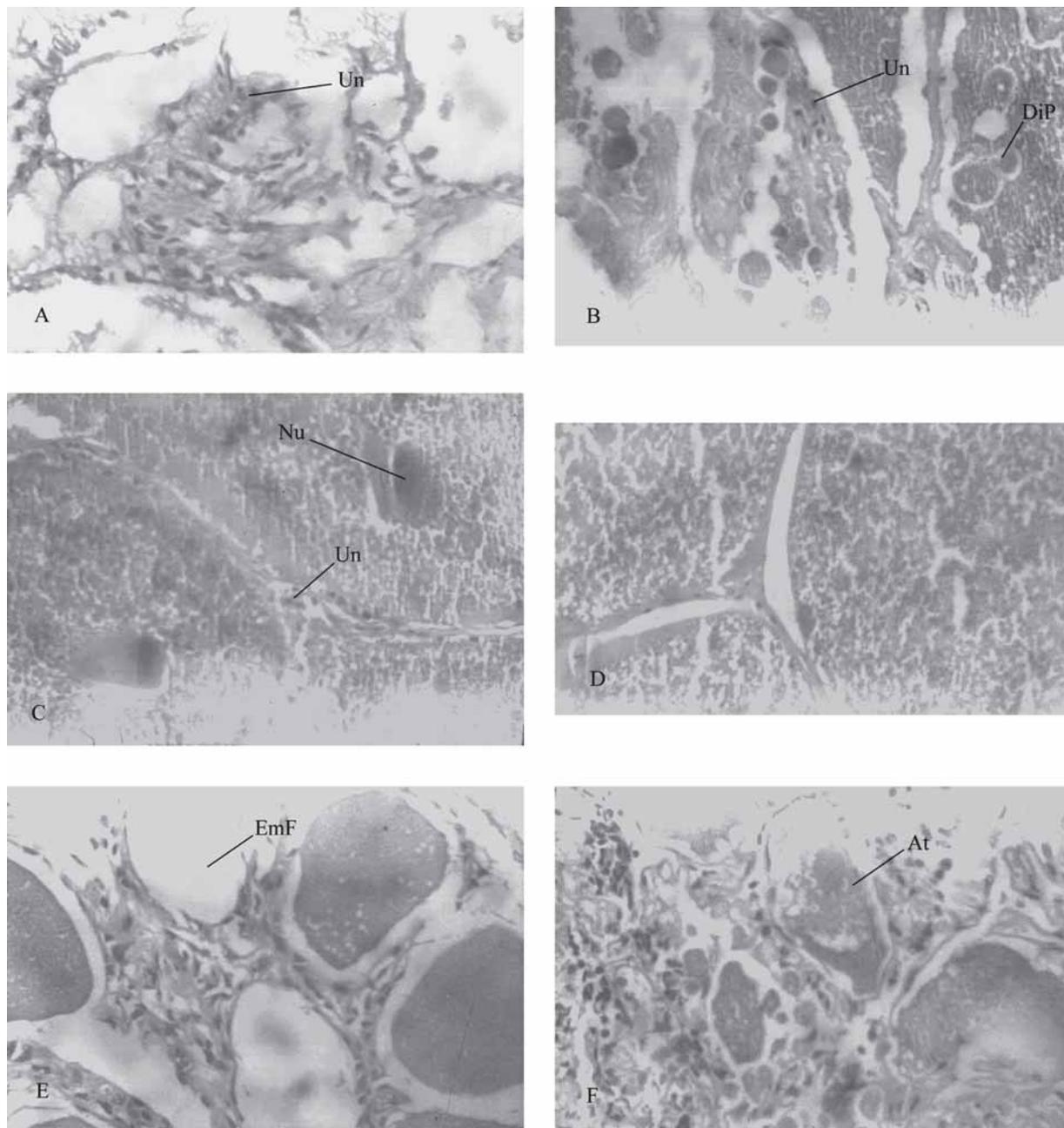


Figure 3. Transversal cut of *P. ecbinatus* ovaries showing the characteristics of the immature (A), initial maturation (B), final maturation (C), mature (D), spawned (E) and in rematuration (F) stages, 40x increase. The follow structures are able to see: undifferentiated cells (Un), cells in process of differentiation (DiP), Nucleus (Nu), empty follicles (EmF) and atresic cell (At).

16.5 cm of total length, respectively (Fig. 5). This analysis could not be performed for males.

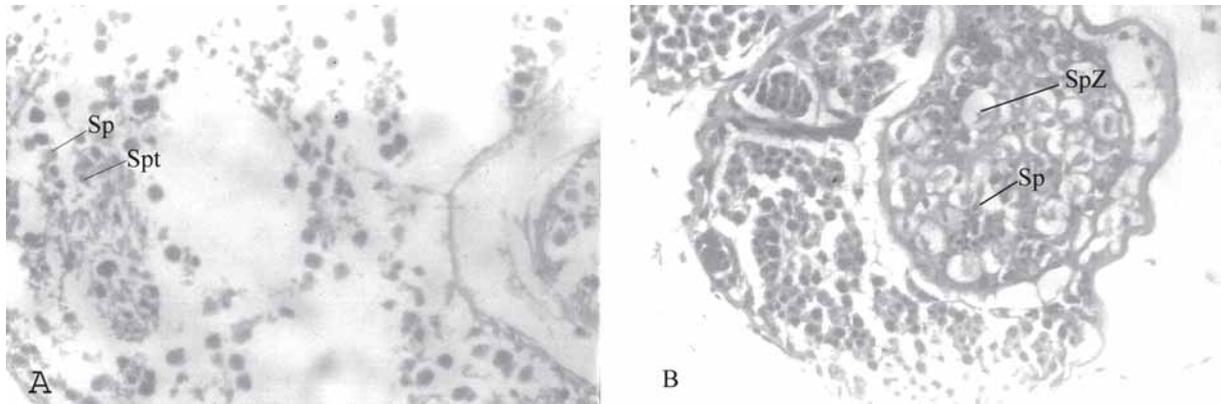
Considering L_{50} as the length where half of the individuals of a population are adults undergoing the process of reproduction and that the L_{50} value obtained for females could be replaced for males, it is verified that only 4% of the sampled individuals were imature. The average capture length found was 17.0 cm (TL) for females, and

18.4 cm (TL) for males, therefore being larger than the values found for L_{50} (Fig. 5).

The lowest observed fecundity was of approximately 44,000 eggs from a specimen that presented a total length of 16.5 cm, while the largest observed fecundity was of 97,120 eggs from a specimen with a total length of 19.4 cm. The dispersion of the empiric points of the individual fecundity *vs.* total length relationship reveals a linear

Table II. Microscopic classification of the reproductive stages for males of *P. echinatus*.

Stages	Microscopic Classification
Immature	Characterizes the group of individuals of the population that have not yet reproduced. The sole presence of spermatogonia disposed around the border of the seminiferous tubules is the diagnostic characteristic of this stage.
In maturation	Characterized by the presence of spermatocytes I and II as well as spermatogonia in the border of the cells. No mature elements are found in the lumina of the seminiferous tubules and most of the time even the lumina are invisible (Fig. 4A).
Mature	In this stage, the mass of spermatids and spermatozoa are observed in the lumina of the seminiferous tubules and in the collector tubules. Spermatogonia are still found in the tubules (Fig. 4B).

**Figure 4.** Transversal cut of *P. echinatus* testicles showing the characteristics of the maturation (A) and mature (B) stages, 40x increase. The follow structures are able to see: spermatocytes (Sp), spermatogonia (Spt), spermatids (St) and spermatozoa (SpZ).

equation expressed as $F = 13.06 TI - 164.86$ (Fig. 6). The average fecundity was calculated for the species from the equation, equaling 56,160 eggs.

Discussion

According to Soares and Peret (1998), the knowledge of reproduction of species with high commercial value is very important to fisheries to give a basis for a good management.

Mota-Alves and Tomé (1965 and 1966) described five macro- and microscopic development stages in females of *P. argus* and *P. laevicauda*, and three exclusively microscopic stages in males. On the other hand, Soares *et al.*, (1998) for *P. laevicauda* in the state of Ceará – Brazil, and Soares and Peret (1998) for *P. argus* in the Northeastern Brazil, described six macroscopic development stages in females. *P. echinatus* showed six stages of gonad development for females and tree stages for males. Although there was not a macroscopical classification of the males due to the same difficulties found

in this study and by Mota-Alves and Tomé (1965 and 1966). In this way, the results obtained for *P. echinatus* are similar to those ones found for *P. argus* and *P. laevicauda* in the literature and seems to be a generic pattern for maturation process in Palinurid lobsters.

According to Soares and Peret (1998a), the average size of the first gonadal maturity (L_{50}) is a populational parameter considered as the indicator of when the individuals have reached the adult phase. For males of *P. echinatus* from northeastern coast of Brazil-Tamandaré, Barreto *et al.*, (2003) obtained L_{50} value of 3.7 cm of cephalothorax length. Using the equations described by Pinheiro *et al.*, (2003) in L_{50} value obtained for Barreto *et al.* (2003) we obtain 7.6 and 4.8 cm of total and abdomen length, respectively. This difference between the L_{50} values found in the present study and that one obtained by the author cited above may be due to spatial differences. Little and Watson (2005) presented results that show differences in the maturation size of *Homarus americanus* throughout the range of its offshore distribution. Juinio (1987)

stated that these differences could be found comparing populations from diverse areas, due to differences in the growth rates.

Another explanation could be attributed to differential reproductive strategies between the

sexes, as suggested by Pinheiro *et al.*, (2003) in order to explain the different lengths reached by each sex in many egg-laying crustacean groups. Therefore, juvenile males could have some advantage in competition for females if they reach the maturation

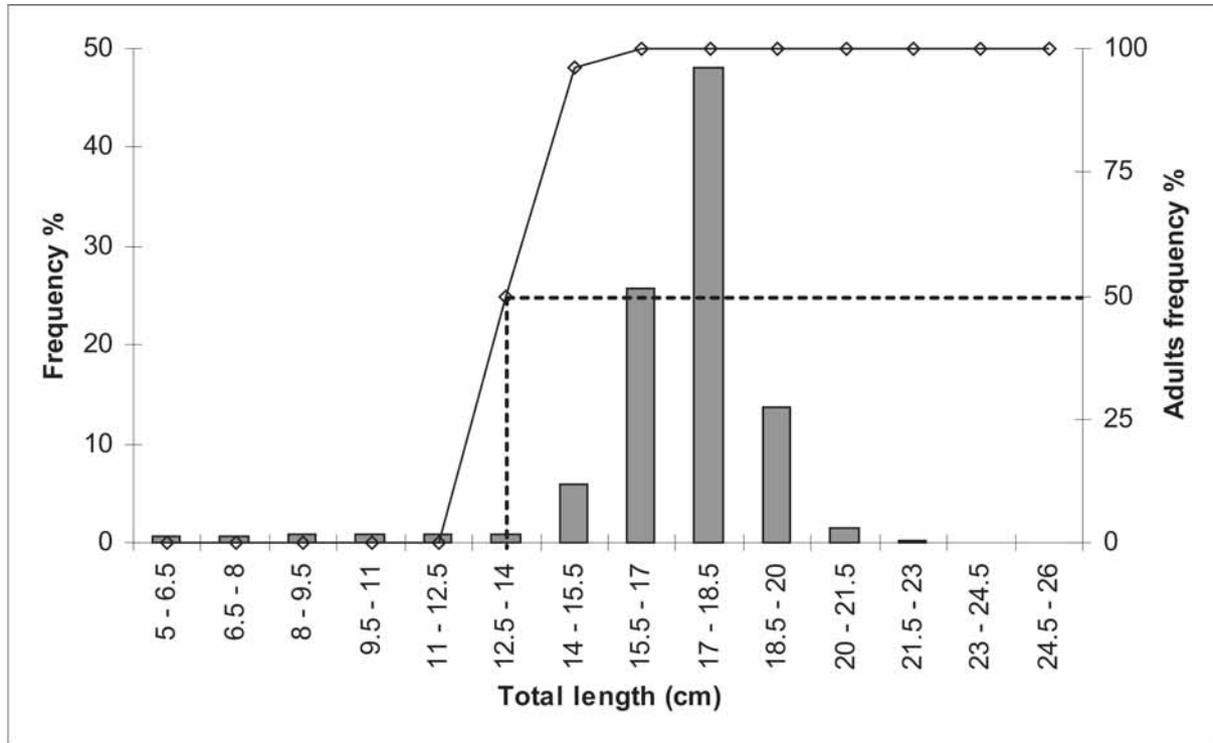


Figure 5. Total length class frequency distribution of females (n=654) and the average length of sexual maturation.

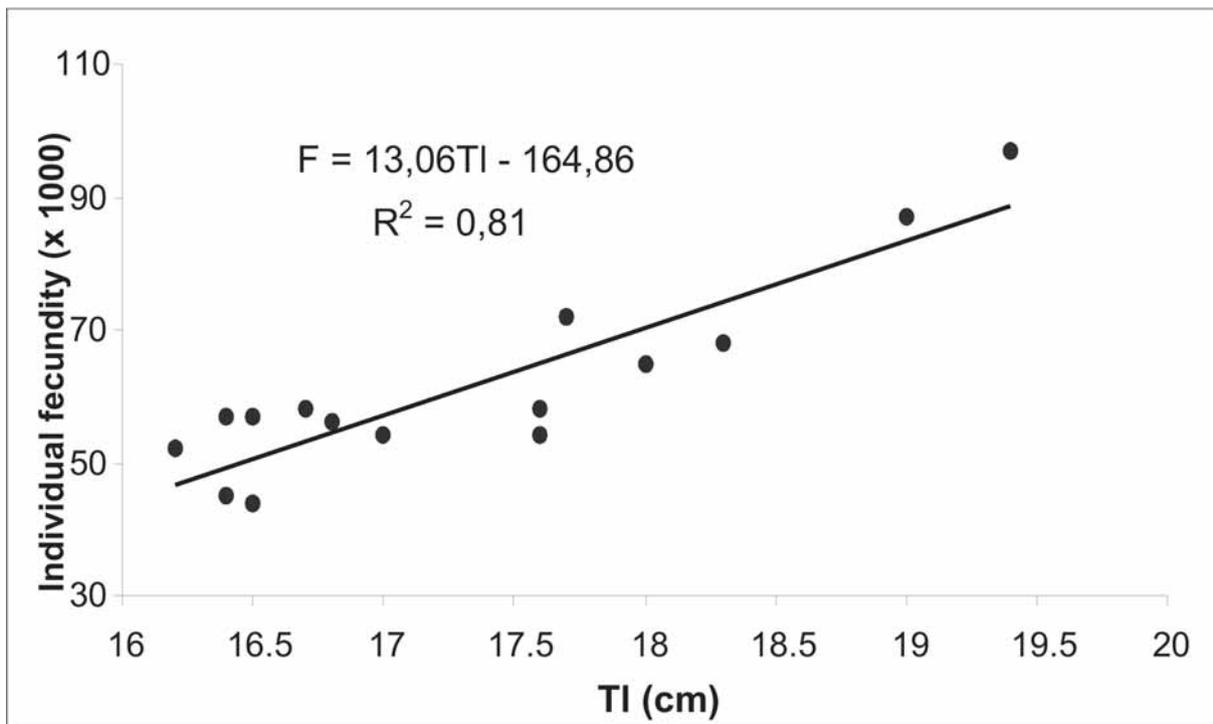


Figure 6. Relationship between individual fecundity and total length (cm).

tion length earlier. On the other hand, females, apparently, do not have any advantage to reach the maturation length soon.

According Vazzoler (1982) differences in L_{50} index could be attributed to the percentage of immature individuals sampled. These differential percentages could result in sub estimation or over estimation in L_{50} .

Comparing the L_{50} value found for *P. echinatus* and the minimum capture sizes of the congeneric (*P. argus* and *P. laeviscauda*) of northeastern Brazil, Barreto *et al.* (2003) asserted that *P. echinatus* is the species that reaches maturity with the smallest sizes. Associated with the growing demand of external market for reduced size lobsters (Vasconcelos, personal communication), this information may be of great value regarding the management of the activity, due to its reduced size, *P. echinatus* could supply this growing sector of the market without inflicting major damages to the lobster stocks. However, additional studies should be carried out for a good management of this stock.

Comparing the values of the average capture length and the average gonadal maturation length (L_{50}), it was observed that the L_{50} found was lower than the average capture length (17.0 cm for females and 18.4 cm for males). Considering that Henriques (1999) states that the relationship between the breeder/recruitment stocks will be kept in balance if, among other populational parameters, the average capture length is approximately equal to the average length of the first gonadal maturation, the information above indicates an apparent equilibrium of the stock in question.

Soares *et al.*, (1998) suggest that *P. argus* possesses a parcelled spawn, preventing calculation of fecundity based on the egg mass adhered to the pleopods. On the other hand, Ivo and Pereira (1996) state that the different authors always used the same methodology (gravimetric method) to calculate the individual fecundity of lobsters in northeastern Brazil as well as in Cuba, using the egg mass adhered to the pleopods, only varying the weight of the mass used for counting. In relation to the mean fecundity, Ivo and Pereria (*op. cit.*) further state that independently of the geographic position and collection season, *P. argus* and *P. laeviscauda* lobsters of northeastern Brazil pre-

sented mean fecundities of 367,169 and 196,880 eggs, respectively. The obtained results for *P. echinatus* point to a mean calculated fecundity equal to 56,160 eggs, indicating that among the three most commercially captured species of northeastern Brazil, it is the one that possesses the lower reproductive potential. This result may be in function of the reduced size of *P. echinatus* when compared with the other two species (Pinheiro *et al.*, 2003).

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