Integrated analysis of sexual maturation through successive growth instars in the spider crab *Leurocyclus tuberculosus* (Decapoda: Majoidea)

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Running Head: Sexual maturation and growth in spider crabs

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Abstract An integrative analysis of sexual maturity associated with growth was developed for the "spider crab" *Leurocyclus tuberculosus*. Sexual maturity was characterized based on gonadal, morphological, morphometric and functional sexual maturity. Progress in sexual maturation was described through thirteen growth stages (instars) detected by the examination of size (CW) frequency distributions. Mature females displayed mature ovaries, developed vaginae, open gonopores, allometric changes in the abdomen and ovigerous stage on the transition from instars IX to X. Sexually mature males presented spermatophores in the distal vasa deferentia and allometric changes in several measurements of the right chela on the transition from instars X to XI. However, two pre-pubertal phases were recognized in both sexes separated from each other by a pre-puberal critical molt. Preceding the second critical molt, gonopores were sealed and vasa deferentia showed no spermatophores, and therefore neither sex was able to mate. The integrated analysis of size at maturity and size frequency distributions evidenced that in both sexes molt to gonadal, morphological, morphometric and functional sexual maturity occurred in advance of the terminal molt, in contrast with patterns observed in other Majoidea.

Keywords: Sexual maturity, stage of growth, Majoidea, *Leurocyclus tuberculosus,* spider crabs.

INTRODUCTION

The size and age at which individuals become sexually mature and the period of life over which they are sexually active define the ontogenetic timing of reproduction (Hartnoll 1978; Donaldson et al. 1981; González-Gurriarán et al. 1995; Gerhart and Bert 2008). Size at sexual maturity, defined as the size at which half of the individuals in a population are apt for reproduction, is one of the most important parameters of their life history (González-Gurriarán et al. 1995; Sainte-Marie et al. 1995; López Greco and Rodríguez 1999; Corgos and Freire 2006). Its estimation involves the choice of biological criteria used to define sexual maturity (López Greco and Rodríguez 1999; Corgos and Freire 2006).

In previous research on decapod crustaceans sexual maturity was identified by one or more criteria. 1)“gonad/gonadal maturity or physiological maturity”, defined as the physiological capacity to produce gametes, and detected by observation of fully developed gametes in the gonads, oocites in seminal receptacles or spermatozoids in vasa deferentia (Comeau and Conan 1992; González-Gurriarán et al. 1993; Sainte-Marie et al. 1995; Alunno-Bruscia and Sainte-Marie 1998; López Greco and Rodríguez 1999; Corgos and Freire 2006; Gerhart and Bert 2008). 2) “morphological maturity or morphometric maturity”, expressed as a change in the shape of different organs as an adaptation for mating or egg incubation, and identified as a shift in the growth rate of some of their dimensions relative to a standard measure of size (Hartnoll 1978; Somerton 1980; Sainte-Marie et al. 1995; Watters and Hobday 1998; Sampedro et al. 1999). 3) “functional maturity” is defined as the attainment of all the necessary functionalities, needed for effective reproduction, and is determined based on the presence of spermatophores in male´s vasa deferentia, female´s seminal receptacles, or eggs in the female´s pleopods

(Conan and Comeau 1986; López Greco and Rodríguez 1999; Corgos and Freire 2006; Gerhart and Bert 2008). 4) “behavioral maturity”, specified as the acquisition of ability to engage in mating and distinguished by direct observation of copula or the presence of mating marks (Orensanz et al. 2005, Gerhart and Bert 2008, Worton et al. 2010).

The sequence of events that characterizes the process of sexual maturation is not always the same in different species or even in different individuals from the same species (Fernandez-Vergaz et al. 2000; Flores et al. 2002; Gerhart and Bert 2008). In many brachyuran species, gonadal maturity precedes morphological maturity, which at the same time precedes behavioral maturity (Hartnoll 1963; Conan and Comeau 1986; Watters and Hobday 1998; Gerhart and Bert 2008). In others, both morphometrically immature and mature males can transfer viable sperm to females (Paul 1992; Sagi et al. 1994; Ahl and Laufer 1996). The first represent the lowest quality mating choice for females and frequently mate when sexual competition is relaxed (Sagi et al. 1994; Elner and Beninnger 1995; Ahl and Laufer 1996; Sainte-Marie et al. 1997; Sainte-Marie et al. 2008).

Individual growth is a discontinuous process in brachyura, whose rate depends on the size increment gained at ecdysis (molt) from one growth stage (namely “instar”) to the next, and on the frequency of molting events (Hartnoll 1978; Somerton 1980; Donaldson et al. 1981; González-Gurriarán et al. 1995). Although during this process the relationships between different body dimensions may fluctuate following simple allometric growth patterns, allometric growth rates can change at some point determining different “phases” in the life of individuals (Hartnoll 1978). Whether changes between phases are subtle or abrupt, transition coincides with a critical molt (Hartnoll 1978). Detected allometric growth discontinuities can be applied along with the size structure of populations to understand the maturation process, whose comprehension is essential for proper management of crab stocks (Hartnoll 1985; Enger and Saether 1994; González-Gurriarán et al. 1995; Sainte-Marie et al. 1995; Alunno-Bruscia and Sainte-Marie 1998).

Among the Eubrachyura, the superfamily Majoidea (i.e., “spider crabs”) contains important fisheries resources in several cold-temperate regions of the world (Orensanz and Jamieson 1998; Sainte-Marie et al. 2008), including members of the genus *Chionoecetes* and *Maja*, some of the most valued in the Northern Hemisphere (Sampedro et al. 1999; Sainte-Marie et al. 2008).

This group represents an interesting study case due to particularities of the mating system and growth patterns of its members (Hartnoll 1963; Alunno-Bruscia and Sainte Marie 1998). The two more important fisheries resources: *Chionoecetes opilio* (O Fabricius 1780) and *Maja brachydactyla* (Balss 1922), have been characterized as having determinate growth with the pubertal molt or “morphometric maturity molt” coinciding with the terminal molt (Hartnoll 1963, 1978, 1985; Jones and Hartnoll 1997; González-Gurriarán et al. 1995; Corgos and Freire 1996; Alunno-Bruscia and Sainte-Marie 1998; Sampedro et al. 1999; Sainte-Marie et al. 2008). Although an alternative model has been proposed, where the attainment of morphometric maturity and terminal molt coincides in females but not always in males (Donaldson and Adams 1989; Dawe et al. 1991; Paul and Paul 1996; Guinot et al. 2013), the first is now widely accepted (Donaldson 1988; Donaldson and Johnson 1988; Conan et al. 1990; Dawe et al. 1991; Paul and Paul 1996; Tamone et al. 2007).

The goal of this study was to analyze the maturation process of *Leurocyclus tuberculosus* (H. Milne Edwards and Lucas 1842) (Decapoda: Majoidea), one of the largest

Majoidea in the Southern Hemisphere, integrating different criteria for maturity and growth patterns determination in females and males.

MATERIALS AND METHODS

Study Area and Sampling

Leurocyclus tuberculosus specimens were sampled on a monthly basis between 2007 and 2009 in several localities of the Northern Patagonian gulfs (Fig1). Crabs were collected with baited collapsible traps (diameter: 260mm; length: 500mm; mesh size 10mm) at depths ranging from 5 to 55m by SCUBA diving on subtidal bottoms (1 to 25m deep) and by manual picking on the intertidal. All specimens were transported to the laboratory and sex was determined by direct observation of the abdomen, broad and rounded in females, and triangular in males.

Criteria for Maturity

Sexual maturity was determined based on different criteria, including: “gonadal maturity”, defined as the capacity to produce fully developed gametes; “morphological maturity”, characterized by changes in the shape of the secondary sexual organs (i.e., gonopores and vaginae); “morphometric maturity”, resulting from the allometric changes in the growth of the chelipeds in males and the abdomen in females, and “functional maturity”, evidenced by the presence of spermatophores in male´s vasa deferentia, assuming that this reflects actual gamete evacuation, and of eggs in the female´s pleopods. Also, the existence of residual sperm contents in the seminal receptacles was considered an indication of functional maturity, assuming that this reflects previous spawning. For better interpretation, the results of the different analyses were presented in the following order:

morphometric, gonadal and functional maturity, followed by growth, separately for both sexes. Additionally, morphologic maturity organs were reported, but only for females.

Morphometrical Maturity

All crabs were measured using digital calipers to the nearest 0.01 mm. Recorded morphometric dimensions included: carapace width (CW) of individuals from both sexes; female's maximum abdomen width (AW); male's gonopod length (GpL), and length (ChL), height (ChH), width (ChW) and diagonal (ChD, distance between the dactyl (i.e., the moveable finger) insertion point to the base of the propodus (i.e., the fixed thumb) along a diagonal line of male's right chela (Fig2). Since the species is homochelous, dimensions of the left chela were registered when the right one was absent or regenerating.

Morphological Maturity in Females

The development of sexual organs was examined in the abdomen, gonopores and vaginae of all females. The following data were recorded: shape of the abdomen (flat or domed), presence/absence of extruded eggs or broken chorionic capsules on the pleopods, absence/formation/development of gonopores and vaginae. Gonopores and vaginae were dissected, and observed using a JEOL LSM-6460 LV scanning electron microscope. For this purpose, most of the reproductive system (part of the ovaries, along with seminal receptacles, vaginae and vulvae) was removed, cleaned with distilled water, and mounted with a double-sided adhesive tape on a labeled slide. Dry SEM samples without gold coating were viewed using the back-scattered electron technique at low vacuum (15–20 Pa).

Gonadal Maturity

All specimens were cold-anesthetized and dissected to determine gonad maturity stages. These were characterized based on macroscopic (color, consistency and relative size) and histological observations of the reproductive organs (Table 1). Thirty gonads from each stage of gonadal maturity (Table 1) were fixed in Bouin's solution for 4 h, and processed for routine histological analysis. Serial sections 5–6 µl thick were cut using a Carl Zeiss microtome, stained with hematoxylin-eosin, and examined and photographed with a Carl Zeiss Axioimager A1 light microscope.

Functional Maturity

Female' abdomens were examined for the presence of eggs. Presence/absence of spermatophores in female' seminal receptacles and male' distal vasa deferentia was determined by examining smears under microscope after dissection.

Analysis of Data

The size at morphometric maturity, CWmorph50% (i.e., CW at which 50% of individuals attain morphometric maturity), was determined by analysis of a discontinuity in the relationship ln(AW) on ln(CW) in females and ln(ChL), ln(ChH), ln(ChW) and ln(Chd) on ln(CW) in males, using the method developed by Somerton (1980). Also a breakpoint regression analysis was conducted for the relationship ln(AW) on ln(CW) on immature females using the Statistica 7.0, which is suitable to discern abrupt changes in morphometric relationships occurring at a single size value (Somerton 1980). Test of isometry were performed for all of the relationships analyzed in order to establish the type of allometry/isometry prevailing on each of the morphometrical maturation phases detected. Regression models fitted for all relationships were of the type $\ln(y) = \ln(a) + b \times$

ln(*CW*), where "*y*" is the dependent variable and "*a*" and "*b*" are parameters of the model (Hartnoll 1978).

CW at which 50% of individuals attained gonadal (CWgonad50%), lower genitalia full development (CWmorphol50%), and functional (CWfunc50%) maturity were estimated for each sex. Logistic curves of the form Pi = 1 / 1 + exp (*a* + *b* × Li), where Pi and Li are respectively the expected proportion of mature individuals and the central mark at size class "i". The parameters of the model ("*a*" and "*b*") were fitted to the proportions of mature individuals in 6-mm size classes using the maximum likelihood criterion. In both cases size maturity was estimated as the quotient a/b.

Growth stages

Growth stages (i.e., instars) were detected for each sex as modal components in frequency distributions through 1-mm size classes using the graphic method developed by Bhattacharya (1967) and included in FiSAT (FAO-ICLARM Stock Assessment Tools) (Gayanilo et al. 2003; Barriga et al. 2009). Mean, standard deviation, and proportion of individuals in each modal component were used as reference parameters to fit theoretical modal components to the size frequencies observed by nonlinear regression using the maximum likelihood criteria in Excel spreadsheets (Barriga et al. 2009). Modal components corresponding to individuals smaller than 11 mm were analyzed by pooling data from both sexes.

RESULTS

In total, 1741 specimens of *Leurocyclus tuberculosus* were collected during the study period, from of which 1202 were females (2.2-78.79 mm CW) and 539 were males (3.13-82.56 mm CW).

Maturity and Growth of Females

Morphometrical Maturity of Females

Throughout the ontogeny of females, discontinuities in the relationship ln(AW) on ln(CW) revealed three allometric growth phases corresponding to immature (i.e., without secondary sexual organs differentiated), juvenile (i.e., morphometrically immature with developed secondary sexual organs) and adult (i.e., morphometrically mature) individuals (Fig3A). The transition from immatures to juveniles was delimited by a breakpoint at 17 mm CW (Fig3A). Juvenile and adult phases overlapped over a size range at their transition: the smallest adult measured 40.3 mm CW and the largest juvenile was 48.4 mm in CW (Fig3A). Estimated CWmorph50% was 42 mm (Fig3A). The allometric relationship of AW on CW shifted from positive in the juvenile to negative in the adult phases (Table 2).

Morphological Maturity of Females

The changes in the secondary sexual organs agreed with the patterns detected by morphometric analysis. In females smaller than 6 mm CW, the reproductive tracts, gonopores or vaginae were not detectable (Fig3B). At 9 mm CW 50% of the individuals displayed gonopores in formation without vagina (Fig3B; Fig4 first column). At 14 mm CW, 50% of the individuals presented still closed gonopores and vaginae in formation (Fig3B; Fig4 second column). The juvenile phase ranged approximately between 14 mm and 42 mm CW. CWmorphol50% was attained by the female population at 42 mm, individuals in the adult phase presenting open gonopores and domed abdomens (Fig3B; Fig4 third column).

Gonadal Maturity of Females

Visual and histological inspection of the reproductive system of females allowed observing changes in the gonadal condition through different size ranges. Ovaries could not be detected in individuals smaller than 31 mm CW (OV0, Table1, Fig3C). In females smaller than 38 mm ovaries did not show any degree of maturation (Fig3C). Between this size and 48 mm CW individuals showed maturing ovaries (OV2,3 Table1, Fig3C). Starting from 41 mm CW specimens showed fully mature ovaries (OV3, Table1, Fig3C) or a condition representative of subsequent stages of the reproductive cycle (OV4 and OV5, Table1).

Functional Maturity of Females

Females smaller than 38 mm CW had empty seminal receptacles and none was found ovigerous, whereas some individuals between 38 and 53 mm CW were inseminated and sometimes ovigerous (Fig3D,E). The smallest and largest ovigerous females were 40.3 mm and 78.7 mm in CW respectively (Fig3E).

Females smaller than 38 mm CW did not show contents in their seminal receptacles, or developing embryos attached to their pleopods. Instead, a proportion of the population between this size and 53 mm CW presented either one of these conditions. Estimated CWfunc50% was achieved at 48 mm CW

Growth Stages of Females

Discrimination of modal components in females' SFD (Table3; Fig3E) allowed detecting eight instars for individuals larger than 11 mm in CW. Taking all together, 50% of the *L. tuberculosus* female population reached gonadal, morphological, morphometric, and functional maturity within 42-46 mm CW in the transition from instar IX to X (Fig3).

Maturity and Growth of Males

Morphometrical Maturity of Males

A variable proportion of morphometrically immature/juvenile and adult male *Leurocyclus tuberculosus* was observed within the range of 48–69 mm CW (Fig5A). Depending on the morphometric relationship analyzed (i.e., those of different dimensions of chelae on CW), the estimated size at which 50% of for males reached morphometric maturity (CWmorph50%) varies between 58 and 60 mm CW (Fig5A,B). The most noticeable change between the immature and mature phases was detected in the ln(ChW) on ln(CW) relationship (Fig5A). Growth of different dimensions of chelae relative to CW during the immature/juvenile and adult phases showed specific patterns for each variable analyzed (Table2). No morphometrical discontinuity was observed in the length of gonopods (Table2).

Gonadal Maturity of Males

Macroscopic inspection of dissected males and histological analysis of their reproductive tracts resulted in the detection of different phases of maturation during growth. Individuals smaller than 19 mm CW did not present discernible testes (M0, Table1). Between 19 and 61 mm CW a proportion of specimens showed formed testes (M1, Table1), 50% of the male population being in this condition at approximately 36 mm CW (Fig5C). No specimen smaller than 48 mm CW showed seminal contents had (i.e., free spermatozoa or spermatophores) in its vasa deferentia. Between this size and 68 mm CW a proportion of males showed mature testes and vasa deferentia with sperm contents, CWgonad50% of the individuals reaching this condition at 53 mm CW (Fig5C).

Functional Maturity of Males

Spermatophores were detected in distal vasa deferentia at 54 mm CWfunc50% and in all males larger than 58 mm CW (Fig5D).

Growth stages of Males

Analysis of male´s SFD allowed to detect eight modal components between 11 mm CW and the largest sizes observed (Fig5E). Overall, *L. tuberculosus* males achieved gonadal maturity at instars VIII and IX, and both morphometrical and functional maturity in the transition from instar X to XI (Fig5E).

Growth stages of individual smaller than 11 mm CW

Inspection of the size frequency distributions (SFD) of individuals smaller than 11 mm, both sexes pooled together, revealed the existence of five instars between 2.2 mm and 11.0 mm CW (Table3; Fig6).

DISCUSSION

Integration of several aspects of sexual maturation in the context of individual growth, as implemented in this study, provide a useful approach to gain broad understanding on the processes determining the life history of brachyurans (Stearns 1992; Conan et al. 1992; Gerhart and Bert 2008).

Discrimination of modal components in size (CW) frequency distributions of both sexes of *Leurocyclus tuberculosus* allowed to detect thirteen growth stages (instars), and to establish the maturity condition in each of them. In females, 50% of the *L. tuberculosus* population reached gonadal, morphological, morphometric and functional maturity within 42-46 mm CW, in the transition from instar IX to X. In males, gonadal maturity was achieved at instars VIII and IX, and both morphometrical and functional maturity in the transition from instar X to XI.

Leurocyclus tuberculosus females showed three maturity phases (immature, juvenile and adult), revealed by morphometric discontinuities as well as changes in the development of secondary sexual organs. The “first critical molt”, from the immature to juvenile phases, occurs in individuals passing from instars V to VI, while gonopore and vagina attain full development but the gonopore is still sealed. The transition from the juvenile to adult phases occurs during the “second critical molt”, while individuals pass from instars IX to X, their abdomen changes (flat to domed) and gonopores open, enabling the acquisition of functional maturity observed in individual larger than 40.3 mm CW.

The northern Patagonian female population showed a CWmorph50% of 42 mm CW in this study, and 47.9 mm CW in that of Barón et al. (2009), contrasting with estimations reported for populations of the same species inhabiting at lower latitudes: 17.1-29.4 mm CW and 30 mm CW respectively for the coasts of San Pablo and Rio de Janeiro States (Brazil) (Enger and Saether 1994; Almeida et al. 2007; Stauffer et al. 2011). Thus, marked latitudinal variation in reproductive population parameters seem to occur in *Leurocyclus tuberculosus* as reported for other crab species (Ruffino et al. 1994).

Males of *Leurocyclus tuberculosus* also showed three maturity phases (immature, juvenile and adult) based on gonadal development. Male gonads mature in the transition from instars VIII to IX, from the immature to juvenile phases. Juvenile males show sperm in the testes but not sperm is detected in the vasa deferentia. The “second critical molt” occurs from instars X to XI, when males present spermatophores in the distal portion of the vasa deferentia, achieving their functional maturity in coincidence with their morphometrical maturity. Therefore, our results suggest that “morphometrically immature males that can transfer viable sperm to females" are not present in the patagonian population of *L. tuberculosus*.

Molt to morphometric maturity can occur within a wide size range (48-69 mm CW) in male *Leurocylus tuberculosus*. This is not unusual in other eubrachyuran females, and in both sexes of other Majoidea such as *Chionoecetes bairdi* (M.J. Rathbun, 1925) (Somerton 1980), *C. opilio* (Comeau and Conan 1992) and *Leucippa pentagona* (H. Miles Edwards, 1833) (Varisco and Vinuesa 2011; Figueroa 2013). In this study the morphometric analysis showed that in male *Leurocyclus tuberculosus* the relationship of ln(ChW) on ln(CW) was the most effective in revealing the transition from "juvenile" to "adult" shapes of chelae trough the size range in which molt to maturity occurs. This relationship reflects the acquisition of robust chelae in adult males, which can be related to reproductive behavior such as competition for receptive female during mating (Christy 1987; González-Pisani 2011; Figueroa 2013). In contrast, the relationship of ln(ChL) on ln(CW), which is generally the reference size variable in most analyses of morphometric maturity, poorly represents the transition from "juveniles" to "adults".

Leurocyclus tuberculosus increments in size (CW) through molting events in specimens smaller than approximately 17 mm CW vary between 45 and 55%, similarly to what has been reported for *Chionoecetes opilio* (Donaldson et al. 1981; Sainte-Marie et al. 1995). For larger specimens, CW increments steadily decrease in the transition to successive instars, showing their lowest values from instars IX to XI in females, and from instars VIII to IX (gonad formation), and XI to XII (pubertal molt) in males, evidencing the allocation of energy from growth to reproductive development (Wolff and Soto 1992; Alunno-Bruscia and Sainte-Marie 1998).

As mentioned above, Majoidea has been characterized as having determinate growth with the pubertal molt ("morphometric maturity molt" according to the criterium of this study) coinciding with the terminal molt (Hartnoll 1963, 1978, 1985; Jones and Hartnoll 1997; Alunno-Bruscia and Sainte Marie 1998; Sainte-Marie et al. 2008). Size frequency distributions, and size at molt to morphometric maturity reveal that in both sexes of *Leurocyclus tuberculosus* there is more than one instar after the puberty molt. Additionally, for the largest immature individuals to reach the size of the largest mature individual observed in the population, they should increase their size (CW) by more than 60% during the molt to morphometric maturity. This is highly unlikely since size increments at the pubertal molt, and even through previous molting events, do not exceed 40% for any of both sexes in the Eubrachyura (Paul and Paul 1996; Hartnoll and Bryant 2001; Hébert et al. 2002). In conclusion; the size gap between the largest morphometrically immature and the largest morphometrically mature individuals is too large to be bridged in one molt, thus relatively large morphometrically mature individuals must be able to molt.

However, since the maturation schedule can be density/temperature dependent in the Majoidea, with successive generations and successive year-classes or pseudo-cohorts undergoing terminal molt to different sizes and in different instars, this could also be interpreted to be a deterministic effect of recruitment cycles and/or density-dependence (Orensanz et al. 2007; Sainte-Marie et al. 2008; Burmeister and Sainte-Marie 2010). Therefore, although this study is based on data collected through three consecutive years, "pseudo-cohorts" could have been present in the population of *L. tuberculosus*, resulting in that the pubertal molt may have occurred between one or more consecutive instars in different years depending on environmental conditions (Ernst et al. 2005; Orensanz et al.

2007; Burmeister and Sainte-Marie 2010). Thus, considering that cold temperate populations of Majoidea may have sporadic recruitment (Burmeister and Sainte-Marie 2010), further analysis is necessary to evaluate the time required for *L. tuberculosus* to grow from instars I to XIII. Moreover, in view of our result, it could be useful to apply the technique of titration of circulating ecdysteroids (Tamone et al. 2007) to confirm that the molt to morphometric maturity is not the terminal molt.

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TABLES

Table 1. Scale of gonadal maturity developed for female and male of *Leurocyclus tuberculosus*

Stage	**Macroscopic characteristics**	**Histological characteristics**
Range of Size (CW – mm)	***Females***	
OV0: Undifferenciation (2.2-31 mm)	Reproductive organs cannot be differentiated to the naked eye.	
OV1: Immaturity (31-48 mm)	Whitish or pale-orange ovaries, tubular and slender, connect directly to the vaginal channels with no discernible presence of seminal receptacles.	Oogonia with reduced cytoplasm are the predominant cell type in ovary sections, but also some oocytes in primary vitellogenesis are present.
OV2: Incipient maturity (38-48 mm)	Orange ovaries, slender and firm, connect to collapse seminal receptacles.	Clusters of oogonia are surrounded by oocytes, mostly in primary but some in secondary vitellogenesis. Follicular cells distribute among oogonia and oocytes. Seminal receptacles show no spermatic contents.
OV3: Full maturity (41-78.79 mm)	Voluminous intense-red-colored ovaries, tubular and turgid, connect to well-defined seminal receptacles with variable fullness condition.	Many oocytes in secondary vitellogenesis are surrounded by flattened follicular cells. Seminal receptacles are full of spermatic contents.
OV4: Spawning (41-78.79 mm)	Pale-orange voluminous and flaccid ovaries connect to seminal receptacles with variable fullness condition.	Atretic oocytes and empty follicles are present along with few oocytes in secondary vitellogenesis and oogonia. Seminal receptacles have little spermatic contents.
OV5: Re-maturation (41-78.79 mm)	Orange, average turgid and tubular ovaries connect to seminal receptacles with variable fullness condition.	Clusters of oogonia are surrounded by similar proportions of oocytes in primary and secondary vitellogenesis. Follicular cells distribute among oogonia. Seminal receptacles have spermatic contents.
	Males	
M0: Undifferenciation (3.13-19 mm)	Reproductive organs cannot be differentiated to the naked eye.	
M1: Incipient maturity (19-61mm)	Whitish slender and tubular testes and vasa deferentia show firm aspect.	Spermatogonia, spermatocytes and spermatozoa present in sections of the testicular lobes. Vasa deferentia show no spermatic content.
M2: Full maturity (49-82.56 mm)	Intense-white voluminous tubular and highly folded testes connect to well developed, thick and also highly folded vasa deferentia with discernible anterior, medial and posterior regions.	Spermatogonia, spermatocytes and spermatozoa present in sections of the testicular lobes. Vasa deferentia contain spermatozoa and spermatophores.

Table 2. Isometry/allometry tests for morphometric relationships of selected dimensions of female's abdomen and male's chelas and gonopods on the standar measure of size (CW) of *Leurocyclus tuberculosus* in different phases of maturity.

Sex	Relationship	Immature phase	Pre-pubertal phase	Mature phase
Females	ln AW on ln CW	+	+	-
	Range of Size (CW – mm)	(2.2 – 16.78)	(17.12 – 46.99)	(40.45 – 68.72
Males	ln ChL on ln CW		0-	-
	ln ChD on ln CW		0-	0
	ln ChW on ln CW		0+	+
	ln ChH on ln CW		0+	0
	ln GpL on ln CW		-	-
	Range of Size (CW – mm)		(7.51 – 44.15)	(29.48 – 78.42)

Note: 0: isometric (b=1); 0-: slight negative allometry (b=0.9 to 0.99) ; 0+: slight positive allometry (b=1.01. to 1.1); -: negative allometry (b<0.9); +: positive allometry (b>1.1);. *AW: abdomen width; ChL: Chela length; ChD: Chela diagonal; ChH: Chela height; ChW: Chela Width; GpL: CW: carapace width; Gonopod length.*

Table 3. Parameters of normal distribution probability models fitted to modal components detected in size (CW) frequency distributions of female and male *Leurocyclus tuberculosus* and percent growth increment between means of normal distributions corresponding to successive modal components.

Instar	Mean (sd) CW mm	Size increment (%)
I	2.2	---
II	3.4 (0.4)	55
III	5.3 (0.9)	55
IV	8.2 (0.8)	54
V	12.2 (0.9)	48

	Females		**Males**	
	Mean (sd) CW mm	**Size increment (%)**	**Mean (sd) CW mm**	**Size increment (%)**
VI	16.4 (2.1)	34	16.4 (1.7)	34
VII	22.7 (2.4)	38.6	22.9 (2.6)	39.5
VIII	30.7 (2.6)	34.7	30.9 (2.4)	35.1
IX	39.9 (3.4)	30.2	38.0 (3.1)	22.9
X	50.7 (3.6)	26.9	48.3 (3.9)	27.1
XI	57.0 (4.0)	12.4	58.5 (3.8)	20.9
XII	68.2 (2.4)	19.6	68.2 (2.7)	16.5
XIII	74.5 (1.5)	9.1	74.5 (1.4)	9.2

FIGURE CAPTIONS

Fig.1 Location of crab collection sites in the northern Patagonian gulfs. *GNG: Golfo Nuevo gulf; SJG: San José gulf; SMG: San Matías gulf.*

Fig.2 Body dimensions of *Leurocyclus tuberculosus*. A- Maximum carapace width; B- Maximum abdomen width (female); C- Dimensions of the right chela (male). *AW: abdomen width; CW: carapace width; ChD: chela diagonal; ChH: chela height; ChL: chela length; ChW: chela width.*

Fig.3 Relationship between morphometric, morphological, gonadal and functional expressions of sexual maturation and growth instars of female *Leurocyclus tuberculosus*. A- Discontinuities in the relationship of ln(AW) on ln(CW) revealed by regression models. *AW, abdomen width; CW, carapace width*; B- Ogives fitted to the proportion of individuals in different stages of morphological maturity: gonopore in formation/without vagina (thin gray line, lower left); gonopore formed-still closed/vagina in formation (black line-lower left); juvenile abdomen morphometry (dark grey line, lower left) and adult abdomen morphometry (upper right), at different size classes; C- Gonadal maturity ogives fitted to the proportions of individuals in stages OV1 (left), OV2 (center) and OV3-5 (right) at different size classes; D- Functional maturity ogives fitted to the proportions of females with oocites in their seminal receptacles or egg masses in their abdomens at different size classes; E- Normal probability distribution functions fitted to size frequency distributions of females with CW > 11 mm (upper) and size frequency distributions of ovigerous females (lower). *Grey bars: morphometrically immature females; light grey bars: morphometrically mature females; dark bars: ovigerous females. Roman numbers indicate the growth instar number. Dotted line represents the aggretation of all normal distribution* *functions fitted to modal components in size frequency distributions. Marks in the ogives represent sizes at which 50% of individuals in a size class show the characteristic denoted by the ogive.*

Fig.4 Sexual organs developing at succesive growth instars in female *Leurocyclus tuberculosus*. First column: Flat abdomen (100 mm), Gonopore in formation (200 µm); Vagina ausent (100 µm); Second column: Flat abdomen (100 mm), Seal Gonopore (500 µm), Vagina without seminal receptacle (1 mm); Third column: Domed abdomen (100 mm); Open Gonopore (500 µm), Open Gonopore and vagina with seminal receptacle (1 mm).

Fig.5 Relationship between gonadal, morphometric and functional expressions of sexual maturation and growth instars of male *Leurocyclus tuberculosus*. A- Regression models fitted to ln(ChL) on ln(CW); ln(Chd) on ln(CW); ln(ChW) on ln(CW), and ln(ChH) on ln(CW). *ChL: Chela length; ChD: Chela diagonal; ChH: Chela height; ChW: Chela Width; CW, carapace width*; B- Maturity ogives fitted to the proportion of morphometrically mature individuals in different size classes, as revealed by discontinuities in the relationships of ln(ChL; dashed line), ln(ChD; continuous light grey line), ln(ChW; continuous dark grey line), ln(ChH; dotted line) on ln(CW) (ogives of lnChW and lnChH are equal); C- Gonadal maturity ogives fitted to the proportions of individuals in stages M1 (left) and M2 (right); D- Functional maturity ogives fitted to the proportions of males with spermathophores in the distal vasa deferentia; E- Normal probability distribution functions fitted to size frequency distributions of males with CW > 11 mm. Dark/light bars indicate morphometrically immature/mature males. Roman numbers indicate the growth instar. Dotted line represents the aggretation of all single normal distribution functions fitted to modal components in size frequency distributions. Marks in the ogives represent sizes at which 50% of individuals in a size class show the characteristic denoted by the ogive.

Fig.6 Size frequency distributions of *Leurocyclus tuberculosus*with CW < 11 mm. Roman numbers indicate the growth instars.

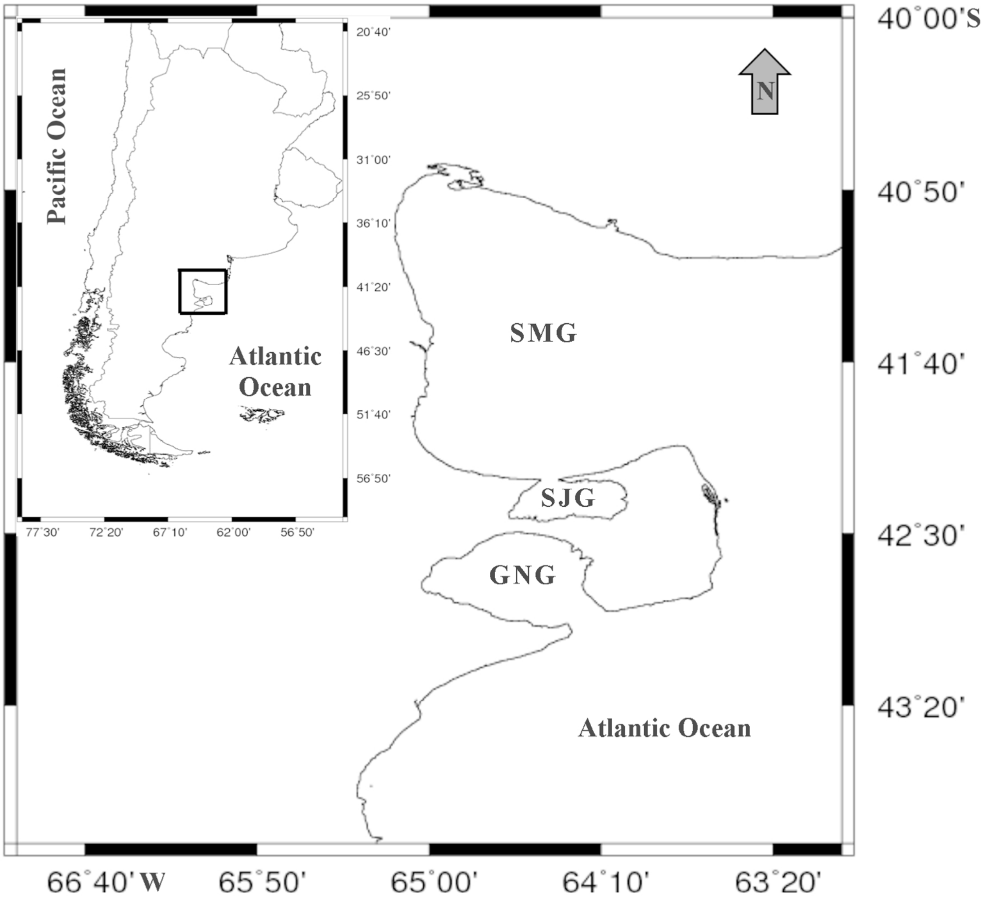

Fig.1 Location of crab collection sites in the northern Patagonian gulfs. GNG: Golfo Nuevo gulf; SJG: San José gulf; SMG: San Matías gulf.

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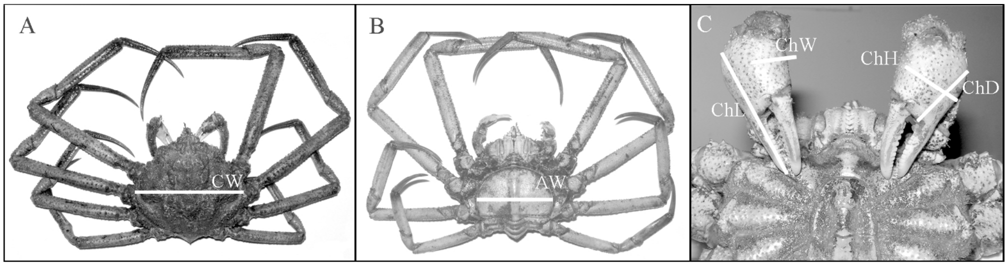

Fig.2 Body dimensions of Leurocyclus tuberculosus. A- Maximum carapace width; B- Maximum abdomen width (female); C- Dimensions of the right chela (male). AW: abdomen width; CW: carapace width; ChD: chela diagonal; ChH: chela height; ChL: chela length; ChW: chela width.

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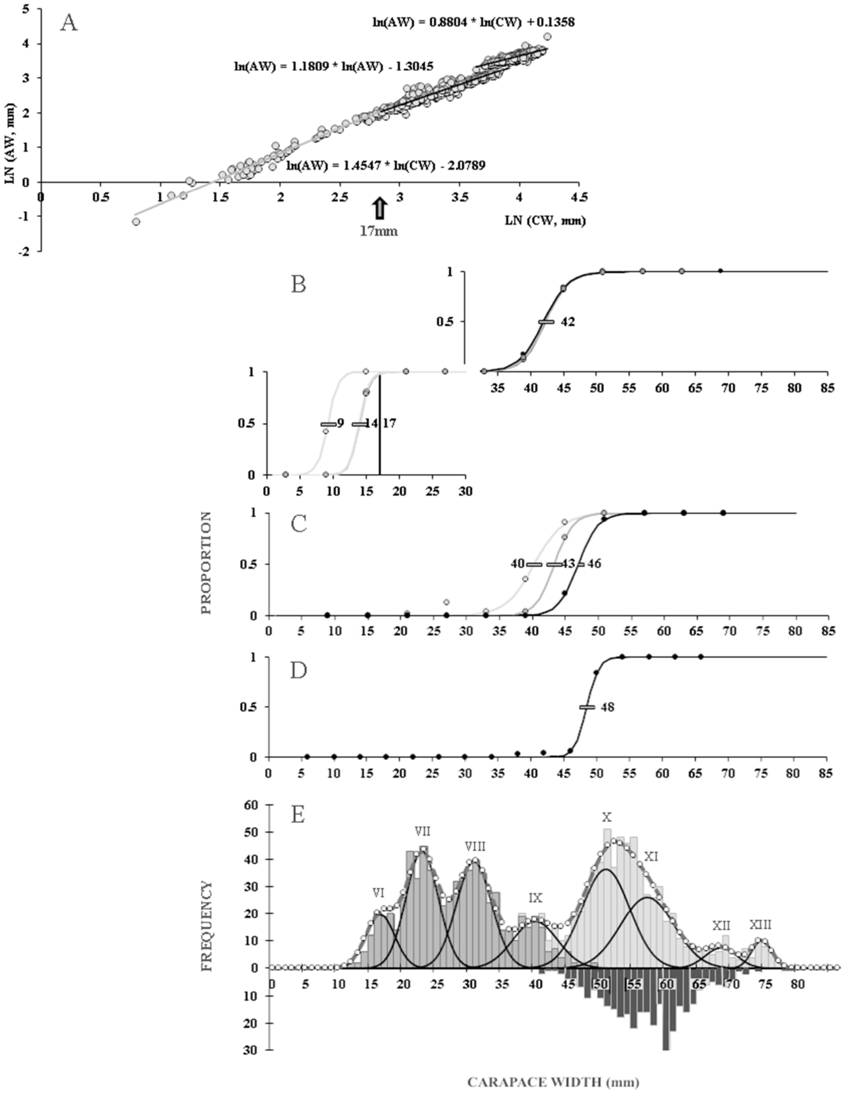

Fig.3 Relationship between morphometric, morphological, gonadal and functional expressions of sexual maturation and growth instars of female Leurocyclus tuberculosus. A- Discontinuities in the relationship of ln(AW) on ln(CW) revealed by regression models. AW, abdomen width; CW, carapace width; B- Ogives fitted to the proportion of individuals in different stages of morphological maturity: gonopore in formation/without vagina (thin gray line, lower left); gonopore formed-still closed/vagina in formation (black line-lower left); juvenile abdomen morphometry (dark grey line, lower left) and adult abdomen morphometry (upper right), at different size classes; C- Gonadal maturity ogives fitted to the proportions of individuals in stages OV1 (left), OV2 (center) and OV3-5 (right) at different size classes; D- Functional maturity ogives fitted to the proportions of females with oocites in their seminal receptacles or egg masses in their abdomens at different size classes; E- Normal probability distribution functions fitted to size frequency distributions of females with CW > 11 mm (upper) and size frequency distributions of ovigerous females (lower). Grey bars: morphometrically immature females; light grey bars: morphometrically mature females; dark bars: ovigerous females. Roman numbers indicate the growth instar number. Dotted line represents the

aggretation of all normal distribution functions fitted to modal components in size frequency distributions. Marks in the ogives represent sizes at which 50% of individuals in a size class show the characteristic denoted by the ogive.

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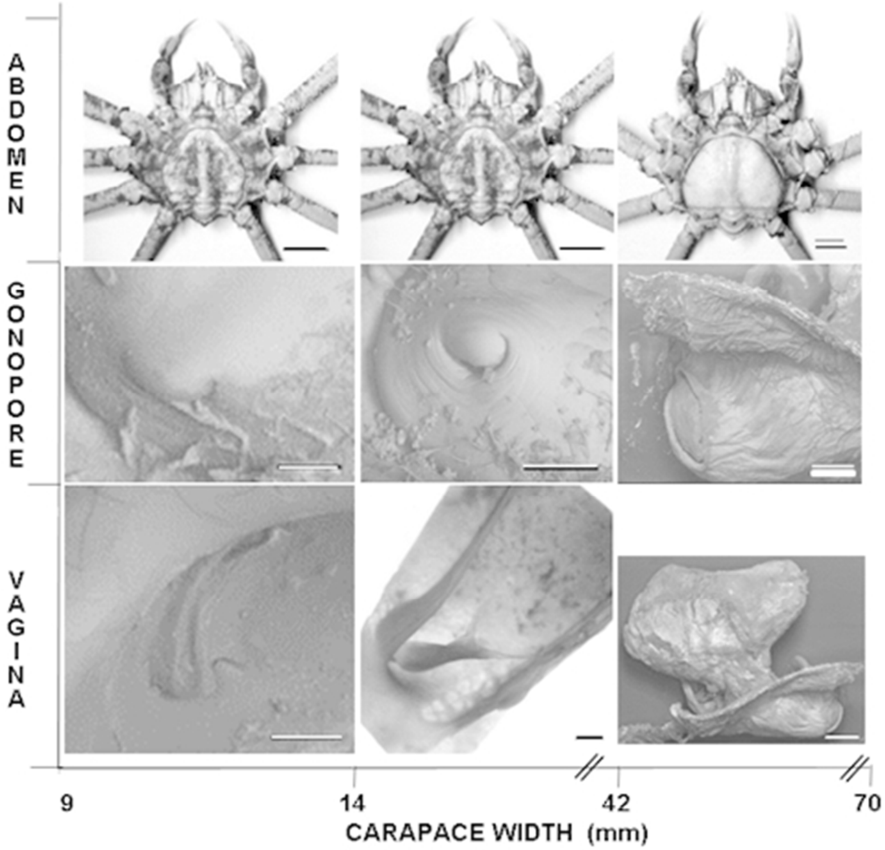

Fig.4 Sexual organs developing at succesive growth instars in female Leurocyclus tuberculosus. First column: Flat abdomen (100 mm), Gonopore in formation (200 µm); Vagina ausent (100 µm); Second column: Flat abdomen (100 mm), Seal Gonopore (500 µm), Vagina without seminal receptacle (1 mm); Third column: Domed abdomen (100 mm); Open Gonopore (500 µm), Open Gonopore and vagina with seminal receptacle (1 mm).

45x42mm (300 x 300 DPI)

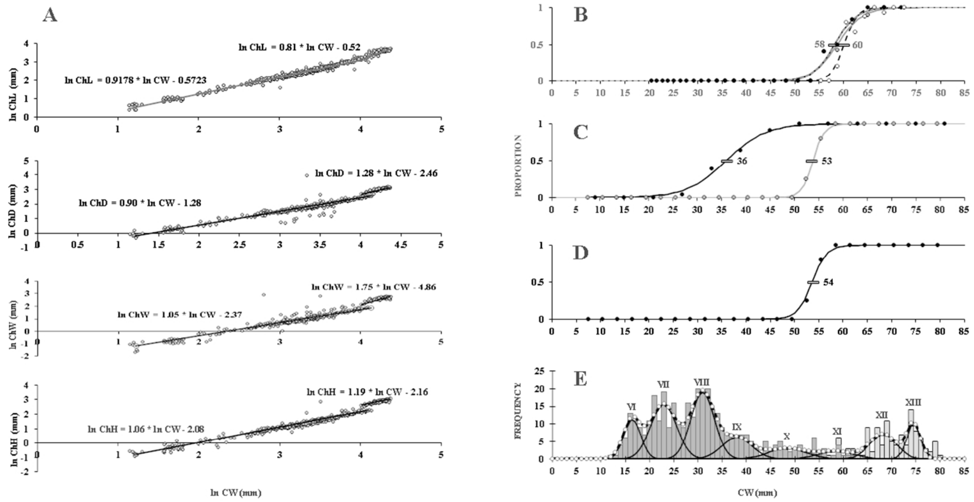

Fig.5 Relationship between gonadal, morphometric and functional expressions of sexual maturation and growth instars of male Leurocyclus tuberculosus. A- Regression models fitted to ln(ChL) on ln(CW); ln(Chd) on ln(CW); ln(ChW) on ln(CW), and ln(ChH) on ln(CW). ChL: Chela length; ChD: Chela diagonal; ChH: Chela height; ChW: Chela Width; CW, carapace width; B- Maturity ogives fitted to the proportion of morphometrically mature individuals in different size classes, as revealed by discontinuities in the relationships of ln(ChL; dashed line), ln(ChD; continuous light grey line), ln(ChW; continuous dark grey line), ln(ChH; dotted line) on ln(CW) (ogives of lnChW and lnChH are equal); C- Gonadal maturity ogives fitted to the proportions of individuals in stages M1 (left) and M2 (right); D- Functional maturity ogives fitted to the proportions of males with spermathophores in the distal vasa deferentia; E- Normal probability distribution functions fitted to size frequency distributions of males with CW > 11 mm. Dark/light bars indicate morphometrically immature/mature males. Roman numbers indicate the growth instar. Dotted line represents the aggretation of all single normal distribution functions fitted to modal components in size frequency distributions. Marks in the ogives represent sizes at which 50% of individuals in a size class show the characteristic denoted by the ogive.

114x62mm (300 x 300 DPI)

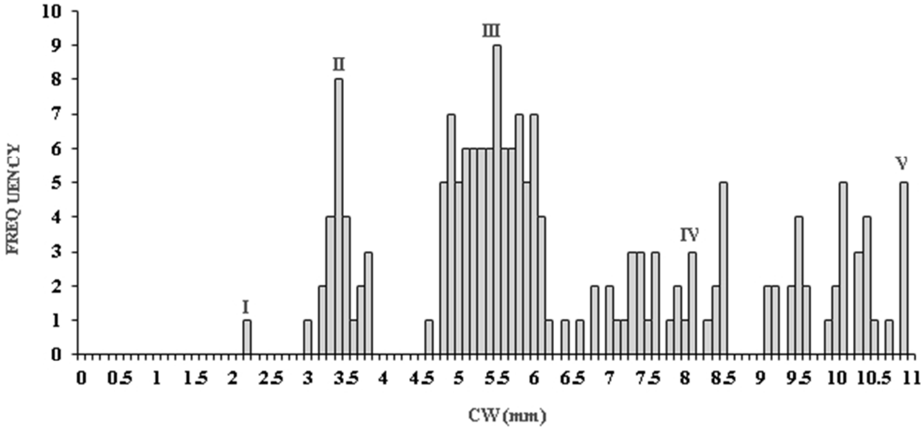

Fig.6 Size frequency distributions of Leurocyclus tuberculosuswith CW < 11 mm. Roman numbers indicate the growth instars.

57x28mm (300 x 300 DPI)