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Study of size relationships and relative growth of *Cestopagurus timidus* (Roux). A method for separating groups

(Crustacea, Decapoda, Anomura)

By **M. E. Manjón-Cabeza & J. E. García Raso**

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In the relative growth study of the small hermit crab *Cestopagurus timidus* a preliminary classification method, based on those of Lefkovitch (1976) and Noy-Meir (1973), was designed for separing juveniles and adults. This method is interesting when the size-overlap zone of the relative-growth curves of the two phases is large, because there are much specimens (the ones found in the overlap zone) which anatomically are very difficult to know whether are small adults or large juveniles. This method also allows as to recognize the most appropriate structure to differentiate juveniles from adults.

Structures studied, in both sexes, were: the carapace shield, the cheliped, and the male sexual tube. The male cheliped revealed a change of growth rhythm. The growth of all three structures in adults was isometric or slightly allometric, the slope being in females lower than in males.

In males, the puberty size is slightly bigger than in females. It has been established at 1.67 mm of big cheliped length or, about, 1.6 mm of cephalothoracic shield length.

M. E. Manjón-Cabeza & J. E. García Raso, Dept. Biología Animal, Fac. Ciencias, Univ. Málaga, 29071-Málaga, Spain.

Introduction

Ideally, growth studies of any species should define and analyze the juvenile and adult phases and show the size or age at which the individuals reach sexual maturity, because growth patterns and puberty sizes of different species differ considerably according to the species life history (Hartnoll 1985, Hartnoll & Gould 1988). It is particularly important to know puberty sizes when studying commercial fisheries.

Most growth studies of Crustacea Decapoda are based on brachyuran species (Hartnoll 1974, 1978, 1982, Mori 1994), but there appears to be little information about Anomura Paguroidea (Bush 1930, Blackstone 1986a, b). This group is difficult to study (Lancaster 1988) because the slightly calcified body and the different sizes and shapes of shells available to, or chosen by, individuals appear to affect their growth and, consequently, the usual measurements of structures like cephalothorax and abdomen do not serve to represent the growth.

The present work reports a study of a population of the small hermit crab *Cestopagurus timidus*, in a *Posidonia oceanica* meadow off southeast Spain where it was the dominant species (García Raso 1990).

Material and methods

The specimens of the small hermit crab *Cestopagurus timidus* (Roux, 1930) came from a well-established *Posidonia oceanica* meadow growing on a sandy bottom with a few rocks off Genoveses beach in the Cabo de Gata-Nijar Natural Park, Almería (S.E. Spain).

Samples were collected by SCUBA divers every two months over two years (1986 and 1987). Depths sampled ranged between three and seven metres. Sample areas were always larger than 900 cm^2 and the rhizome heights were approximately 20 cm (García Raso 1990). In the laboratory the leaves and rhizomes were separated and washed over a three-sieve column; the smallest mesh was 0.5 mm.

The different anatomical structures were analysed by a VID V computer program that processed stereoscopic microscope images of the samples recorded by video camera, measurement error was ± 0.0001 mm. Five parameters were measured: cephalothoracic shield length (CL); cephalothoracic shield width (CW); propodus length of the first ambulatoty leg (cheliped length) (QL); propodus width of the first ambulatory leg (cheliped width) (QW); and the length of the right male sexual tube (TL).

Of the many ways for separing juvenile and adult phases and to define puberty size, the best method uses histological techniques to determine gonad-development states, degrees of maturity and the presence of spermatozoa in the vasa deferentia, but they require either fresh or appropriately-fixed specimens and they have the disadvantage of being destructive.

In the present work the specimens came from collections in which the preservative is alcohol and, also, they must not be destroyed. Thus, other methods had to be used.

For separing females, the simplest was to consider the smallest-sized ovigerous female found as the representative of the first mature size, but this would have been misleading because some eggless females could well be mature and as the presence of ovigerous females in samples would depend more on the time in the annual reproductive cycle at which sampling was carried out. More appropiate is the study of the population during the reproductive period and to consider as the first-mature-size the size at which 50 % of the females examined were ovigerous.

Another method analyses relative growth (Huxley 1924, 1932, 1950, Teissier 1935, 1960, Mayrat 1967, Hartnoll 1974, 1978, 1982; Laird 1965, Laird, Tyler & Barton 1965, Laird, Barton & Tyler 1968, Barton & Laird 1969), especially of sexually dimorphic structures. This method represents the relationships between the chosen parameters, either by the exponential $y = a\ x^b$, or by its logarithmic transformation, Lg y = Lg a + b Lg x; in which the value of b defines the type of relative growth (b<1 is negative allometry, b=1 is isometry, b>1 is positive allometry). However, there is now criticism of logarithmic transformation (Lovett & Felder 1989).

The relative growth method determines puberty-size indirectly because maturing gonads, ovaries and androgenic glands, begin to secrete hormones that stimulate development of the adult secondary sexual characters that modify the growth curves of their associated parameters, either by making the "moult-discontinuity" more marked, or by changing the slope of the relative growth equation (regression line). The point at which the slope becomes discontinuous or changes direction marks the transition from the juvenile to the adult phase. This method has a five-fold advantage: it can be used for both males and females; the material need not be "fresh"; no special fixing process is required; the specimens need not be from the reproductive period; and, because it can reveal changes in the growth rhythms of the different anatomical structures, it greatly helps the interpretation of behaviour strategies.

The transition step from immaturity to maturity does not take place at the same time in all individuals of a population, that is to say, it may occur at an earlier or later instar. Specimens with the same age may have different sizes and this gives rise to considerable scatter around the mean puberty size; this is the overlap zone in which it is practically impossible to know (using anatomical external feactures) which are small adults or large juveniles. Somerton (1980) suggests a method to identify specimens in the overlap zone that is adopted by other authors like Gaertner & Laloé (1986), but it requires the prior analysis of juveniles or adults outside the overlap.

The classification study reported in this paper is based mainly on that of Noy-Meir (1973) and of Lefkovitch (1976). Both these authors use principal component analysis (PCA) to fit the scattered points of two associated variables. The classification of the variables is carried out subsequently with the help of dendrograms that take into account the locations of the points in the different quadrants and their distance from the Principal Axis 1 (Pielou 1984).

To classify juveniles and adults in this present work we used the point that marks a change in the

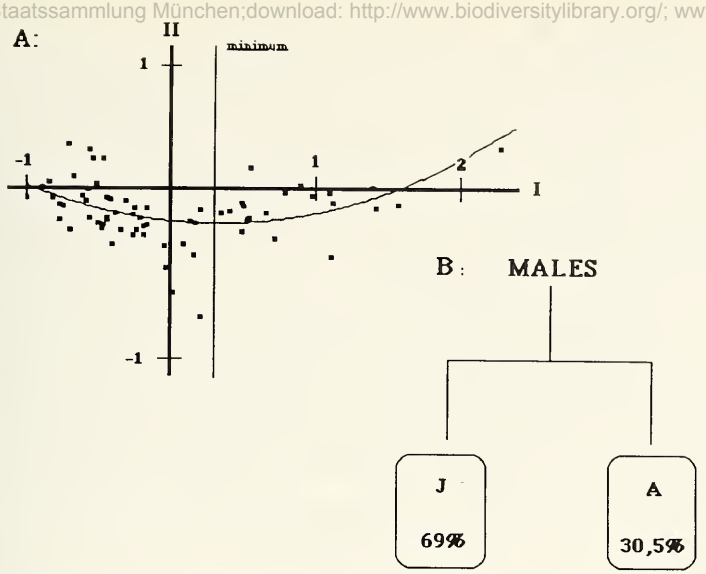

Fig. 1. A. Principal Component Analysis and Polynomial Curve fit in males of *Cestopagurus timidus*. B. Dendrogram resulted of the classification with the minimum.

resultant curve after using principal component analysis, with untransformed data. This point is the maximum or minimum of the curve fitted to the scattered PCA points, but only when the curve is significant. A non-significant curve implies that there is no change of slope of the relative growth of the parameters being tested and that this parameter would be unsuitable to differentiate between juvenile and adult phases.

After this preliminary classification study, we continued to identify the morphological parameter that permitted the best differentiation of juveniles and adults with the greatest certainty, which will be most useful for subsequent growth studies of this species. The best parameter was determined by testing each one against the Principal Axis I and then analysing the resultant correlations and distributions of the scatters in relation with two new theoretical axes. These are defined for each case by taking the minimum value of the fitted curves of the PCA and the value of the variable (parameter) obtained by substituting the minimum value in the equation of the correlations described above.

The distributions of the different scatters are then analysed taking into account their position in the new positive or negative quadrants and this will reveal the morphological parameter that classifies with greatest certainty.

The validity of this method for separing groups was checked by applying it to an analysis of data from previous studies of males of *Alpheus dentipes* Guerin, 1832, in monthly samples of the calcareous seaweed *Mesophyllum lichenoides*. The puberty size found coincided with that given by data from a previous work (Fernández Muñoz & García Raso 1987).

Results

Preliminary classification study

In males (Fig. 1) the polynomial curve that fitted the scatter following principal components analysis of cephalothoracic shield length (CL) against cheliped length (QL) had a 99 % significance. The equation of the resulting curve is $y=0.15x^2-0.10x-0.073$, and the minimum that served as the criterion for classification (Fig. 1B) revealed that 69.5 % of the males were juveniles, while 30.5 % were adults. When the QL and CL variables were analysed with the principal axis I (Figs 2A, 3A), the significant

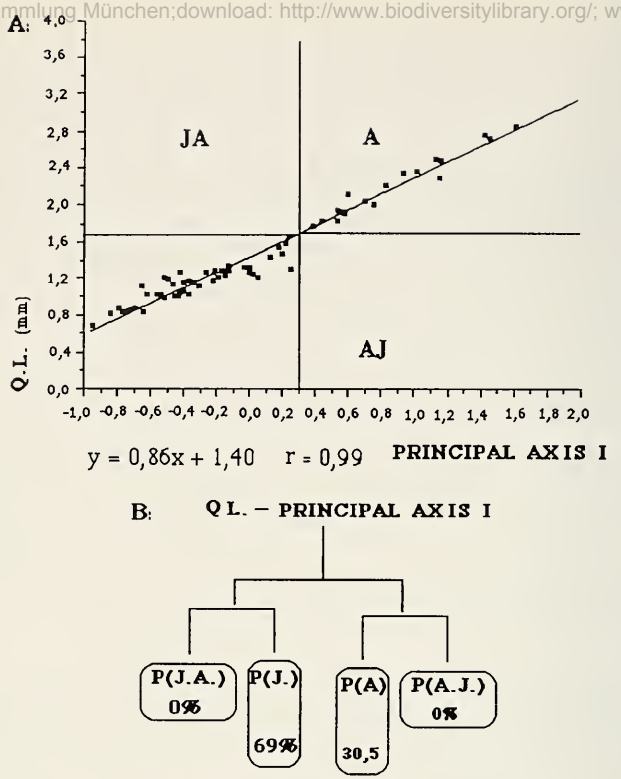

Fig. 2. A. Representation of propodus cheliped length (Q.L.) versus Principal Axis 1 in *Cestopagurus timidus*. B. Dendrogram obtained by propodus cheliped length (Q.L.). A: Adults, J: Juveniles, A.J.: Adults classificated as juveniles, J.A.: Juveniles classificated as adults.

correlations had a 99 % significance. The above data shows that the error produced by an attempt to classify juveniles and adults by cheliped length (QL) was cancelled (Fig. 2B). However,when cephalothoracic shield length was chosen, the error was greater because 12.4 % of specimens are indeterminate (11 % of juveniles could be classified as adults and 1.4 % of adults as juveniles) (Fig. 3B).

The puberty size in males is reached at 1.67 mm of big cheliped length or, about 1.6 mm of cephalothoracic shield length.

In females (Fig. 4) the curve that fitted the scatter of principal components analysis of cephalothoracic shield length (CL) against cheliped length (QL) is $y=0.22x^2+0.176x-0.0186$, but as r=0.26, and n=40, it was not significant (P> 0.001). Consequently, there was no significant change in the slope of these two parameters to differentiate between juveniles and adults.

Relative growth

Figs. 5-8 give the equations for the relative growth of the parameters analysed.

1. Relationship between cephalothoracic shield length (CL) and cheliped propodus length (QL).

For males, the slopes of the relative growth curves of juveniles and adults were significantly different (Fig. 5A); confidence limits for juveniles were $b=0.52 \pm 0.007$, and those of adults were $b=1.01 \pm 0.10$. The coefficient for juveniles revealed negative allometry, while that for adults was isometric.

For females, growth was almost isometric (Fig. 5B). The slope value was $b=0.86 \pm 0.30$, which is intermediate between those of juvenile and adult males.

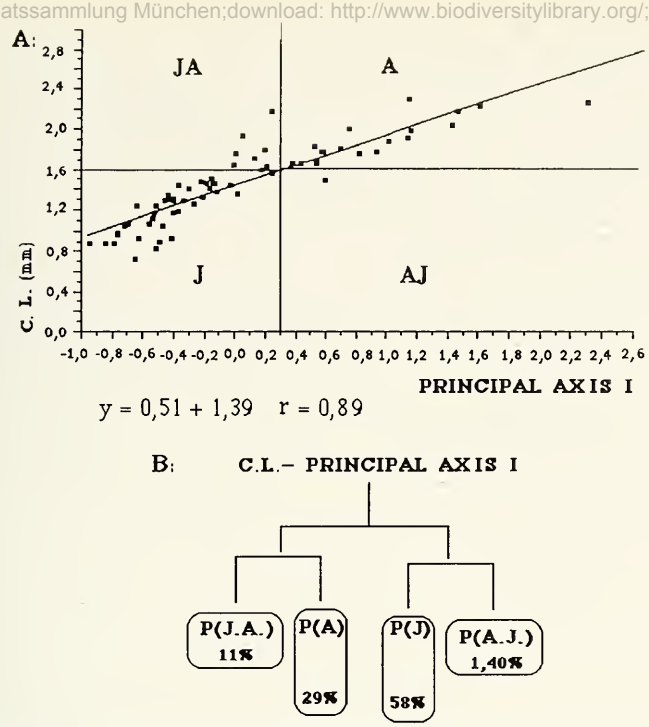

Fig. 3. A. Representation of the cephalothoracic shield length (C.L.) versus Principal Axis I in *Cestopagurus timidus*. B. Dendrogram obtained by cephalothoracic shield length (C.L.). A: Adults, J: Juveniles, A.J.: Adults classificated as juveniles, J.A.: Juveniles classificated as adults.

2. Relationship between propodus length (QL) and propodus width (QW).

For males (Fig. 6A), the slopes of the relative growth curves of juveniles and adults were significantly different, the juveniles had slightly positive allometry (b=1.14±0.51), while the adults had almost isometric relative growth (b=0.88±0.09).

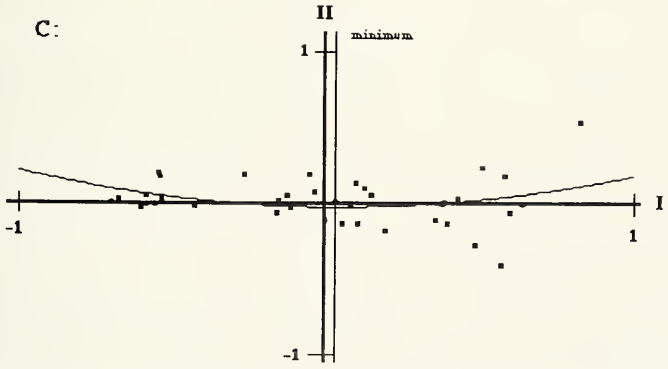

Fig. 4. Principal Component Analysis and Polynomial Curve fit in females of *Cestopagurus timidus*.

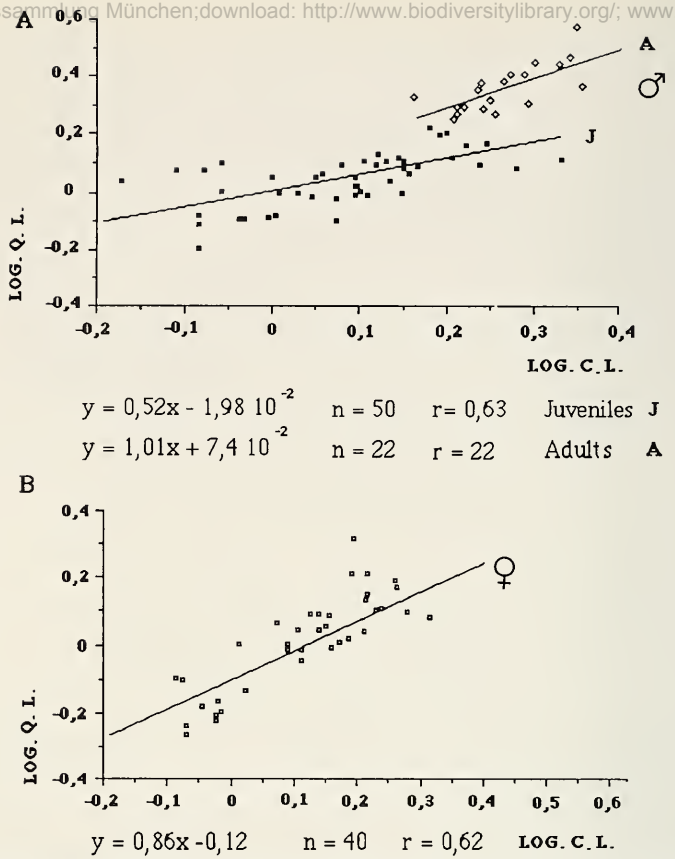

Fig. 5. Logarithmic relationship between cephalothoracic shield length (C.L.) and propodus cheliped length (Q.L.) of *Cestopagurus timidus*. A: Males. B: Females.

For females (Fig. 6B), the value was (b=0.75±0.61) being the slope not significantly different to one of adult males.

3. Relationship between cephalothoracic shield width (CW) and cephalothoracic shield length (CL).
No significant differences were found between the growth levels of juveniles and adults (Figs. 7A, 7b). The growth of both sexes was isometric and this indicates that both parameters increase at the same rate. The allometry coefficient for males was b=1.01±0.15 and for female was b=1.11±0.17.

4. Cephalothoracic shield length (CL) against sexual tube length (TL) (Fig. 8B).
Relative growth was isometric (Fig 8A); b=1.07±0.75.

Discussion

Somerton (1980) classified those juvenile or adult specimens located within the overlap zone, but in that method, one must first analyse the two non-overlap zones. This is difficult when the overlap is large and the relative growth curves of the clearly-defined juvenile and/or adult phases are short or badly defined. This could be because the size range of the population sample only represents a fraction of the whole population (because of high mortality, adult migration, etc.).

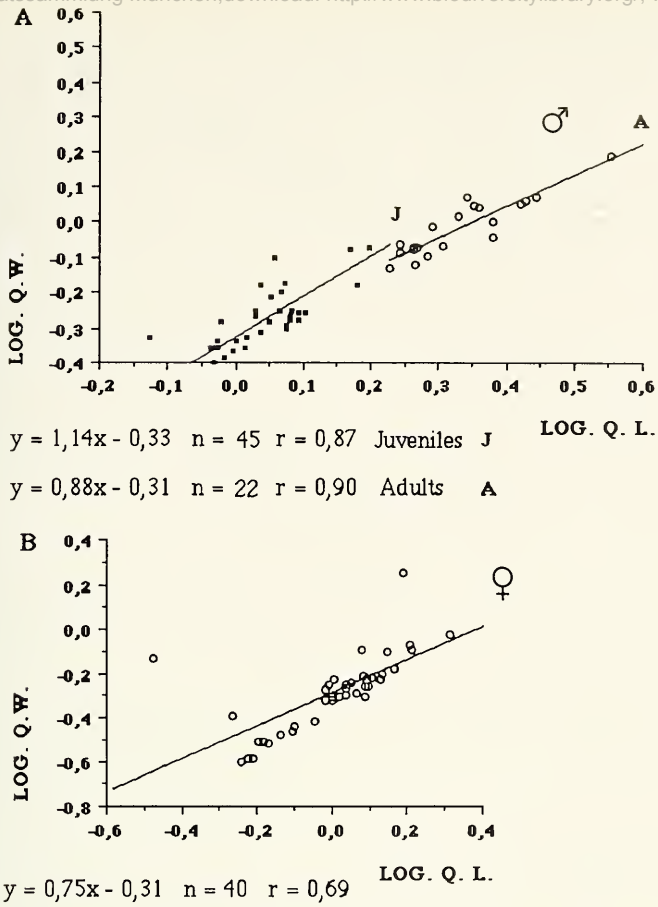

Fig. 6. Logarithmic relationship between propodus length (Q.L.) and propodus cheliped width (Q.W.) of *Cestopagurus timidus*. A: Males. B: Females.

In these cases, it is necessary to carry out a preliminary classification of all the specimens before analysing relative growth because a wrong classification of different specimens, or an insufficient number of them, could greatly distort the slope values.

Connan, Comeau & Moriyasu (1985) and Connan & Comeau (1986) also carried out a principal component analysis, but only as a selection tool to determine the morphological variables (parameters) that best define the changes of their relative growth, particularly of the secondary sexual characters. This method has the advantage of avoiding the arbitraty use of an "a priori" independent variable. Freire and González Gurriarán (1992), just as the latter authors, show that principal component analysis (with a correlation matrix of logarithmically transformed data) permits the analysis of the relations between variables, the changes of relative growths of both sexes, and also helps to define the variables that determine the allometric changes that may be associated with sexual maturity.

Our method is different because it uses PCA analysis only as a preliminary process, for reorientating spatially the scattered distributions, to better identify the minima and maxima (inflexion point) of the polynomial curve derived from analysis of the groups of scatter points to separate clearly juvenile and adult specimens, according to the classical relative growth theory.

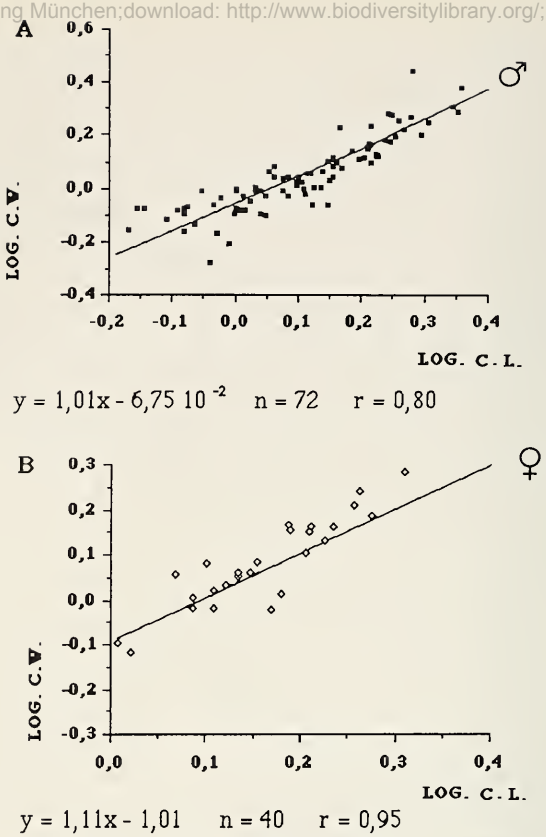

Fig. 7. Logarithmic relationship between cephalothoracic shield length (C.L.) and cephalothoracic shield width (C.W.) of *Cestopagurus timidus*. A: Males. B: Females.

The second step of our method allows us to analyse the different anatomical parameters to determine the most suitable parameter to discriminate between juveniles and adults. On the other hand, it is possible to know the errors that may be generated by this separation. So, in this present work, cheliped length was the best criterion to determine puberty size of males of *Cestopagurus timidus* and it permitted juveniles and adults to be separated without error.

Sexual dimorphism occurs in the the propodus cheliped of many hermit crabs (Bouvier 1940, Zariquiey 1968, etc.) and sometimes this is revealed by the existence of different relative growths (Gherardi 1991). Sexual dimorphism exists in *Cestopagurus timidus*, but, in addition, in adult males the growth rhythm of the cheliped length increases. This has biological significance, probably because it is used in reproduction. A similar pattern is seen in Brachyura (Hartnoll 1982). In females there is no change in the growth rhythm of the cheliped throughout life probably because it is not used specially in reproduction.

The puberty size in females of *C. timidus* were established at 1.25-1.49 mm of cephalothoracic shield length (Manjón-Cabeza & García Raso 1994, Pessani & Premoli 1993), the present study shows that it is bigger in males.

It is particularly interesting that no change was found in the growth rhythm of the length of the male sexual tube, which appears to be unrelated to sexual maturity unlike the relative growths of the sexual pleopods of Brachyura and Caridea (Teissier 1935; Mayrat 1967, Noel 1976, Hartnoll 1985, Fernández Muñoz & García Raso 1987). How does this longcurved "sexual" tube could be used, is unknown.

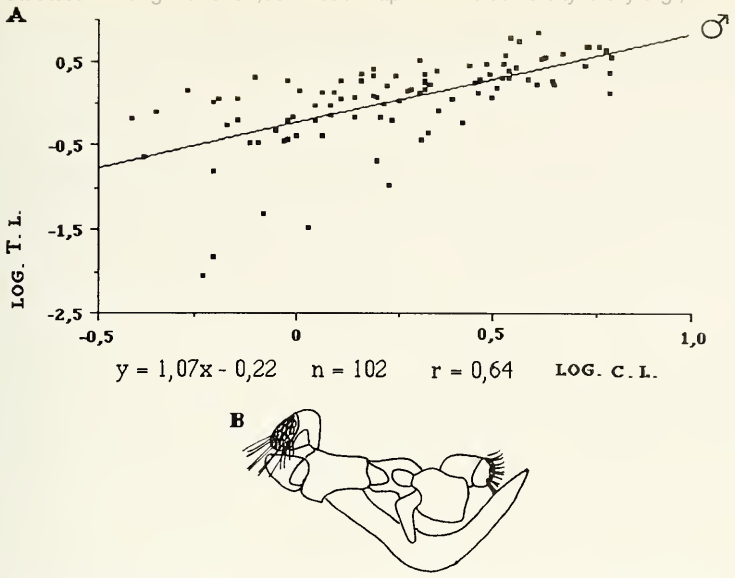

Fig. 8. A. Logarithmic relationship between cephalothoracic shield length (C.L.) and sexual tube length (T.L.) in males of *Cestopagurus timidus*. B. Right sexual male tube of *C. timidus*.

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