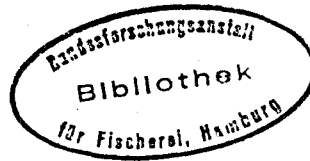


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Reproductive biology of the Subfamily ILLICINAE, *Illex coindetii*
and *Todaropsis eblanae* (Cephalopoda: Ommastrephidae Steenstrup, 1857)
off Northwest Africa

by

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ABSTRACT

This paper describes the investigations on the reproductive biology of two by-catch squid species, *Illex coindetii* and *Todaropsis eblanae* (Cephalopoda, Ommastrephidae) from the Spanish fishing trawl fleet off Northwest Africa (35° N - 4° N). Samples were taken between May 1989 and February 1992. Biological and fishery data were recorded for each individual: dorsal mantle length, total weight, sex and weight of the components of the reproductive system; fishing date, latitude, longitude, and depth of the trawl as well as others qualitative data (other species in the catches, type of bottom, etc).

Illex coindetii showed a year round spawning, although during spring it takes place with higher intensity. Male mature to a smaller size than female and, it was observed that the size of maturity decrease from north to south for both sexes. Males of *Todaropsis eblanae*, as it was observed for *I. coindetii*, mature to a smaller size than female. Results suggest that *T. eblanae* mate and spawns principally in April-June and October-January. A significative difference was found between the number of spermatophores by male of both species, being 1412 the highest number for *I. coindetii* and 269 for *T. eblanae*.

INTRODUCTION

Ommastrephid species are not object of an authentic fishing exploitation in the Central-eastern Atlantic, although occasionally large catches are obtained locally. Partly as a result of this, there are many aspects of the biology and life history of these species in this area of the Atlantic Ocean which remain unknown, being the reproductive biology one of them. Most of the works on reproduction are due to the Russian researches (see Laptikhovsky & Nigmatullin, 1993). In areas where there is a fishing tradition toward these squids, there is a larger knowledge of the reproduction after the works carried out by diverse researches (Durward *et al.* 1979; Sánchez, 1981; Osako & Murata, 1983; Nigmatullin, 1989;

Nigmatullin & Laptikovsky, 1990; Haimovici and Álvarez-Pérez, 1990; Brunetti, 1991; Laptikovsky & Nigmatullin, 1993; among others). And, they are still receiving special attention in those zones where they are not the target species (i.e. *Illex coindetii* (González, 1994; González *et al.*, 1994), and *Todaropsis eblanae* in Galician waters (Rasero, 1994; González *et al.*, 1994)).

It seemed necessary to study the reproductive biology of these squids to have an adequate knowledge of their biology and ecology. On the other hand, the results obtained could serve as a guide in posterior populational studies in this area of the Atlantic.

MATERIAL AND METHODS

This study is based on by-catches of squid taken in the Central-eastern Atlantic. A part of the sample given by the fishing vessels (trawlers) from the different zones off West Africa, between 36° N and 4° N, was used to study their reproductive biology.

A sample of 850 of *Illex coindetii* (448 females and 402 males) and 486 of *Todaropsis eblanae* (247 females and 239 males) were considered. Subsamples were taken for the analysis of other reproductive aspects, which are indicated in each case. After the morphometric and gravimetric study of each individual, the sex and maturity stage were determined (I, juvenile; II, immature; III, maturing; IV-V, mature; VI, spent). The last was assigned taking as a basis the appearance and relative size of their reproductive components (qualitative scale based on Lipinski's scale (1979, cited by Juanicó, 1983)). Data on reproductive components were collected by weighing (to 0.01 g) the female ovary, oviducts, oviducal glands and nidamental glands, male testis and the complex of Needham. The glands were separated from the ovary and from the oviducts, and the complex of Needham from the testis; trying to completely eliminate the vascular conduits and the membranes that cover each one of these elements. Separating the oviducts from the ovary without causing some loss of eggs in the mature females is a difficult task, due to the "fragility" of all the reproductive system. Because of that, often the oviducts were not separated from the ovary, so ovary and oviducts were weighted together. The weight of the testis and of the glands was determined after eliminating the excess of humidity placing them on filter paper. The weight of the spermatophoric sack was determined separately when it contained spermatophores. Paired organs (i.e. glands) were weighed together and the total weight recorded. These data were used to determine the maturity indices.

Simultaneously, other qualitative and quantitative data were taken, i.e. those data related to the presence or absence of spermatangues in the females, their position and the number of groups of spermatangues in *Illex coindetii*. A subsample of spermatangues groups was taken. After weighing the complex of Needham and the spermatophoric sack, this was fixed and preserved in alcohol (70%). Spermatophores were counted from the spermatophoric sacks.

Study of the maturity and period of spawning

In order to know the size or sizes of maturity, the percentage of mature specimens for each class of size was determined, assembling them in sizes classes of 1 cm and representing them under the central mark of the size class.

To find out the time of spawning, the mean of the maturity index for each month was calculated.

Maturity scales based on the relationship among weights: indices of Maturity

Once taken the weights of the components of the reproductive system, it was possible to calculate the indices which show moment of the maturation process in each animal

through the evolution of their values. Moreover, it would allow us to check that the qualitative scale differentiated the stages of sexual maturity on adjusting each one of the degrees to an interval of the values of the indices. In order to carry out this confirmation, the series of data were analyzed using Kruskal-Wallis' statistical test (Siegel, 1990) and doing for each one all the possible comparisons among the groups of data corresponding to each stage, which were differentiated by means of a code. As null hypothesis (H_0) it was established that there is no difference among the values of F for each one of the maturity stages (I to VI). The alternative hypothesis (H_1) stated that the degrees do not possess the same average value, which would indicate that there are some differences among the maturity stages. The α fixed was 0.05. The following indices were calculated:

a) **Gonadosomatic Index (IG)**: this index expresses the relationship between the body weight (BW) of each individual (excepting the weight of the stomach and intestines) and the reproductive system weight.

$$IG = \text{Reproductive System Weight} / (\text{BW} - \text{weight of the stomach})$$

b) **Maturity M Index**: it is based only in the relationship among the weight of the different components of the reproductive system.

$$M (\text{female}) = (\text{OgW} + \text{NgW}) / \text{Reproductive System Weight}$$

For the male the index of maturity utilized M was:

$$M (\text{male}) = (\text{ScW} + \text{SsW}) / \text{Reproductive System Weight}$$

where *OgW* = Oviductal glands weight; *NgW* = Nidamental glands weight; *ScW* = Spermatophoric complex weight; *SsW* = Spermatophoric sac weight. The reproductive system weight include the ovary and oviduct weight or the testis weight too.

RESULTS

Illex coindetii

Males (mean ML = 144 mm, range 55-214 mm, N = 708) were smaller on average than females (mean ML = 178 mm, range 79-304 mm, N = 721). No totally spent individuals were obtained, but some male with the testis near to exhaustion were found. Since results of the statistical analysis of the size distribution (paper in preparation) was considered the study of the reproductive aspects of this species by zones (North Zone 36°-20° N; Central Zone 19°59'-8°N; South Zone 7°59' N-6° S. Figure 1 and 2 show the weights of the components of the reproductive system for female and male respectively, and for the North and South Zones. The difference in weight of each one of the components (ovary+oviduct, nidamental gland, etc) in both zones is clear. This is in agreement with the differences observed in the size ranges of this species depending on the latitude (paper in preparation). These data seem to indicate that in the South Zone (Gulf of Guinea) specimens reach sexual maturity at a lower size.

Sexual maturity in females (stages IV and V) occurred at 131-304 mm (Figure 3), whereas in males it was evident over a shorter size range (97-214 mm, Figure 3).

Previous to the study of the monthly variation of the sexual maturity, was analyzed the correspondence between the values of the maturity indices utilized (IG, M) and the stages of maturity of each individual over the scale utilized. A sample of 107 females and 95 males was considered. The index IG represents the different phases or stages considered better (all comparisons showed significant differences, Kruskal-Wallis; $p < 0.0001$; Table 1); however, the index of maturity M did not prove to be a useful index for females, since it just allowed to distinguish between juvenile individuals and non juvenile ($H(4, N = 107) = 30.22623$, $p < 0.0001$) (Table 2). Both indices are adequate for males.

As a result of this, it was considered more adequate, at least for females, to study the maturity based on the IG index. In general, it was observed that mature females could be found all year round ($IG > 0.1600$). Ovaries whose weight mean more than 8 % of the body

weight were observed during the annual cycle; but, when just those values above the mean were considered, they were found in spring and summer only. This coincides with the annual distribution of females with spermatangues. Thus, "receptive" females could be found all year round, although it was in spring when the females of shorter size with spermatangues inside were observed. These spermatangues were stuck thanks to their disk on the region of the base of the gills, on both sides, although they were found most frequently on the right side.

The number of groups of spermatangues oscillated between one and three, to except for one case in which they were four. At times some very big cluster were found, these probably came from several sexual intercourses, since you could see in them fractions or areas in which the spermatangues looked "wornout", as well as a great disk that seemed to be actually formed by several disks partially leaning one against the others. Among the 16 females whose spermatangues were analyzed, one of them had 1996 spermatangues, 450 units above the maximal number of spermatophores found in the male with the highest number. The average number of spermatangues per female was 484 ($N = 16$, Range = 150-1996, Standard Deviation = 432); although, individual clusters with 8-25 spermatangues were found in females showing several clusters. Moreover, the groups of spermatangues in the same female showed a difference of about 7 mm. On the other hand, some females carried groups of "fresh" spermatangues while other clusters barely contained the disk. The mean size of the spermatangues was 12.66 mm ($N = 14$, Range = 6.10-15.00 mm, SD = 1.77). In conclusion, it can be deduced that females can receive the spermatophores from more than one male and carry out partial spawnings.

As for the males, IG values above 0.04 were found all year round. Likewise, the M index showed values higher than 0.4 during the annual cycle. Mean values for both indices remained very near to the values corresponding to degree of maturity IV.

For the estimation of the number of spermatophore produced by male, in 41 individuals carrying spermatophores into the sack, the number of those was determined and in 37 of these a sample constituted by 10 spermatophores was measured in each one. The mean number was 465 ($N = 41$, Range = 25-1412, SD = 340). It was observed that the number of spermatophores increase according to the size of the animal, but the definition of this increase was not clear, as it is shown by the correlation coefficient ($\beta = 0.24905$). The mean sizes of the spermatophores in each one of the animals under consideration oscillated between 18.74-34.83 mm; but, these showed a considerable homogeneity in size in each individual ($N = 37$, SD = 0.38-5.15 mm). Only in two cases, the deviations were above to 3.50 mm. Therefore, there are very appreciable differences among the mean sizes of the spermatophores of several individuals, but this is not the case between the spermatophores of a same animal.

Todaropsis eblanae

Males (mean ML = 107.3 mm, range 43-164 mm, $N = 347$) were smaller on average size than females (mean ML = 126.2 mm, range 44-215 mm, $N = 357$). After the results obtained in relation to the statistic analysis for this species depending on the latitude (paper in preparation), it seemed unnecessary to carry out the analysis of the reproductive aspects making a distinction according to the zones. As it was observed for *Illex coindetii*, spent individuals were not found. On the other hand, it is remarkable that even on examining the samples, we could see that the proportion of males with spermatophores in the spermatophoric sack (degree IV and V) and of females with spermatangues stored in buccal receptacles were very low.

Sexual maturity in females begins at a larger size than in males. This is in agreement with the differential size (sexual dimorphism) between both sexes. The nidamental gland reaches very high weights, as much as 14.33% (maximal value observed) of the total weight (excluding the weight of the stomach); value slightly lower than the weight of the ovary and oviduct together, 17.41%.

Todaropsis eblanae mature females are distributed into a wide range of sizes, between 128-212 mm. It can be appreciated, even though the sample consist of a small number of cases, that there is a low percentage of mature females of small and of large size. Although this is a mere reflection of the distribution of sizes in the sample, it is not in contradiction with the fact that the proportion of mature individuals increases with the size, which suggests a sole size of maturity. The size of maturity was 168 mm. It was calculated like that one in which 50% of the individuals reached this stage.

The situation was similar for males. The size range for mature males was between 109-164 mm. Low percentages of mature male were also observed for small and great size animals. Consequently, it was considered that males, like females, show a sole maturity size: 130 mm.

Therefore, males mature at a smaller size than females, being the difference of about 19 mm among the smallest mature males and females and of 38 mm among the sizes of first maturity of both sexes.

The correspondence between the values of the maturity indices used (IG, M) was analyzed as well as the qualitative degree of maturity of each individual as regards the appreciative scale. To analyse them, a sample constituted by 167 females was considered and 157 males for the M index. In the study of the IG index, 104 females and 96 males were considered. M index did not prove a useful index for the discrimination between the stages of maturity in females, just allowing to differentiate immature individuals from mature ones. On the other hand, although the IG index represents satisfactorily the different qualitative phases considered, the difference between the levels IV and V is not significant ($H(1, N=24) = 2.090020$; $p = 0.1483$).

About males, the M index allows to distinguish the qualitative levels of maturity better. In addition, the distinction by means of the IG index between the three first levels was satisfactory ($p < 0.0001$). No significant differences were obtained in the comparison of the levels III to V; so, the comparison between III-IV led to a value with probability above 0.05 ($H(1, N=23) = 2.660822$, $p = 0.1029$).

Consequently, the study for females was analyzed taking as a basis the IG index. The period of higher reproductive activity can be divided into two phases, spring, concretely the months of March and April ($IG > 0.1660$); and autumn, although this phase would be prolonged until the winter. It coincides with female evidencing mating. The number of spermatangues per females was very variable, being the size of these approximately 5 mm, with a thin part larger than 1 mm, thicker at the base and thinner at the end.

The males showed the highest values of the indices in spring and autumn, clear in the case of the M index. Therefore, there is coincidence between the maturity periods of both sexes.

DISCUSSION

It is necessary to bear in mind that the results come from the combination of data compiled during several years, so there is a certain effect over the interannual variations.

If the size range of the mature individuals is considered, it is clearly observed that this is of great amplitude for males and females of each one of the two species. Besides, only one size of maturity was observed. Female size of first maturity estimated (208 mm) is above to the size of 184 mm estimated by González (1994) for the Galician waters; but, it is intermediate between the values corresponding to the sizes of maturity (195 and 241 mm) found by Brunetti *et al.* (1991) for two spawning subpopulations of *I. argentinus*.

Male first maturity size is clearly larger than the size 128 mm observed by González (1994). On the other hand, and although we have determined only one size of maturity against the two sizes observed by Brunetti *et al.* (1991) for the *I. argentinus* males, that value of 160 mm is between those considered for this last species (141 and 202 mm).

Although there is a sole size of first maturity for the species *Illex coindetii*, male and

females show a very wide range of sizes in which the individuals are mature. Therefore, there is great individual variability. Difference exists between male and female, since males mature at a smaller size than females. This coincides with the statements of Durward *et al.*, (1979) for *I. illecebrosus* and Sánchez (1981) and González (1994) for *I. coindetii*. If we considered the results in Galician waters and the ones on this paper for the Central-eastern Atlantic, even though the matter should be analyzed more deeply, these seem to indicate a variation on the reproductive aspects (maturity sizes) as the latitude goes from 30° to 35° N toward the north and south of their limit of distribution, regardless the maximal size reached by the species.

As far as males are concerned, the two indices used (IG and M) proved adequate to differentiate the stages of maturity established. This was not the case for females, for whom M index did not allow to distinguish the stages, being useful only the IG index. The fact that M index was unuseful could be due to the stability in the proportions which show each one of the elements in the reproductive system of females during the first phases of their development and to the fact that they have a fixed number of oocytes from the beginning of its vitellogenesis to spawning (Laptikhovsky & Nigmatullin, 1992; cited by Laptikhovsky & Nigmatullin, 1993).

The analysis of the monthly variation of the maturity for *Illex coindetii* showed that mature individuals of both sexes were present all through the sampling period. Moreover, females with clusters of spermatangues were caught all year round. These data suggest that spawning takes place year round, although the reproductive activity is more intense in spring and summer. For this species in the Mediterranean Sea, Sánchez (1981) observed the same situation, though she found maximum activity in autumn. González (1994) obtained similar results, spawning presented maximum activity at the end of the spring-summer period.

The number of groups of spermatangues contained by females oscillated between 1 and 3, but the discovery of very big clusters which in fact seemed to consist of small ones, proved that the maximal number could actually oscillate between 4 and 6. Therefore, these females would have carried out diverse sexual intercourses. The same conclusions was suggested by other authors for different species of the genus *Illex* (Nigmatullin, 1989; González, 1994). To support this, it can be cited the case of one of the 16 females in which 1996 spermatangues were counted, a number larger than the maximal number of spermatophores found in the male with the most elevated number. Other elements that sustain the thesis of multiple mating are for example the presence of groups of spermatangues with different sizes and non "wornout" or the presence of fresh and wornout groups in the same female, which implies that these females had carried out at least a partial spawning. Nigmatullin & Laptikhovsky (1990), and Laptikhovsky & Nigmatullin (1993) proposed as reproductive model for genus *Illex* the existence of multiple spawning, falling in intensity up to the death of the animal. The observations described adjust, therefore, to this model.

Mature males were found along the year. Similar results were obtained by Sánchez (1981) and González (1994) for the males of this species in the Mediterranean and Galician waters respectively.

The results obtained for *Todaropsis eblanae* are close to those observed by other authors in two different areas of the North Atlantic (González *et al.*, 1994; Hastie *et al.*, 1994). They observed for this species mature individuals of both sexes to a minor size in the North Sea. These authors found mature females being their size 110 mm, as well as mature males to a size of 92 mm. It is necessary consider that the maximal sizes observed at that latitude were lower. So, the range of size for male was 40-141 mm (Hastie *et al.*, 1994). Both values, number and size of the spermatophores are larger than those observed by Hastie *et al.*, (1994) for this species taken in Scottish coastal waters. So, the maximal number observed was 130 spermatophores and their maximal size was 18.1 mm. These lower values could be related with the shorter size.

It is remarkable the fact of the low proportion of individuals in the level III. It suggests that the maturity process is very fast, being therefore very brief the period of time

between the phase of immature juvenile and mature individuals. This coincides with results obtained Durward *et al.*, (1979) for *Illex illecebrosus*. They observed that the process in females was about one month. The same observation, low proportion of individuals in III degree of maturity have been described by González (1994) for *I. coindetii*.

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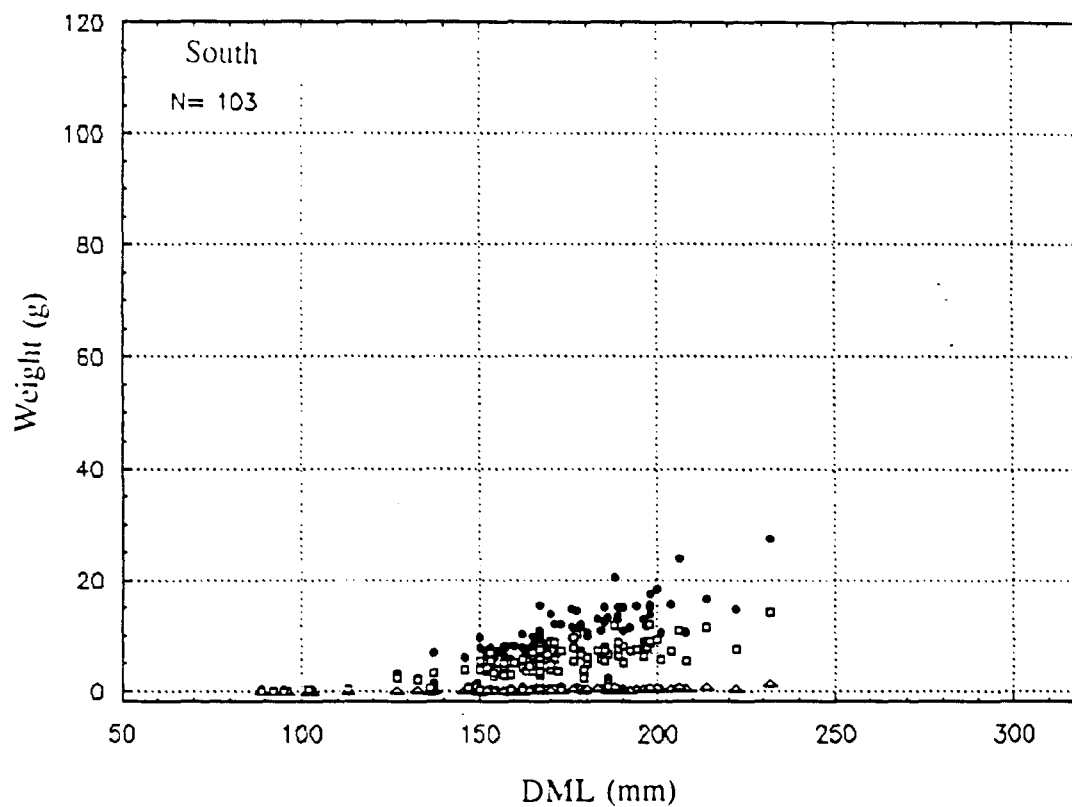
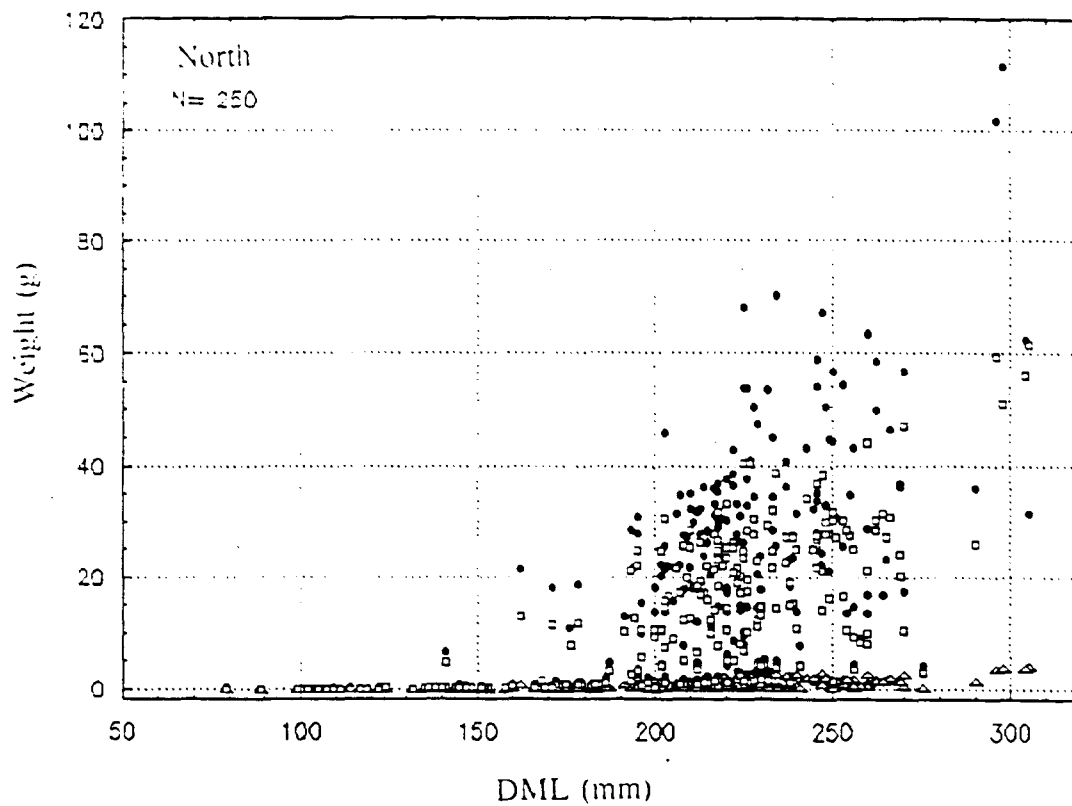


Figure 1. Representation of the weight of the reproductive system against dorsal mantle length in females of *Illex coindetii*. (•) weight of ovary plus oviduct, (□) weight of the nidamental gland, (△) weight of the oviductal gland.

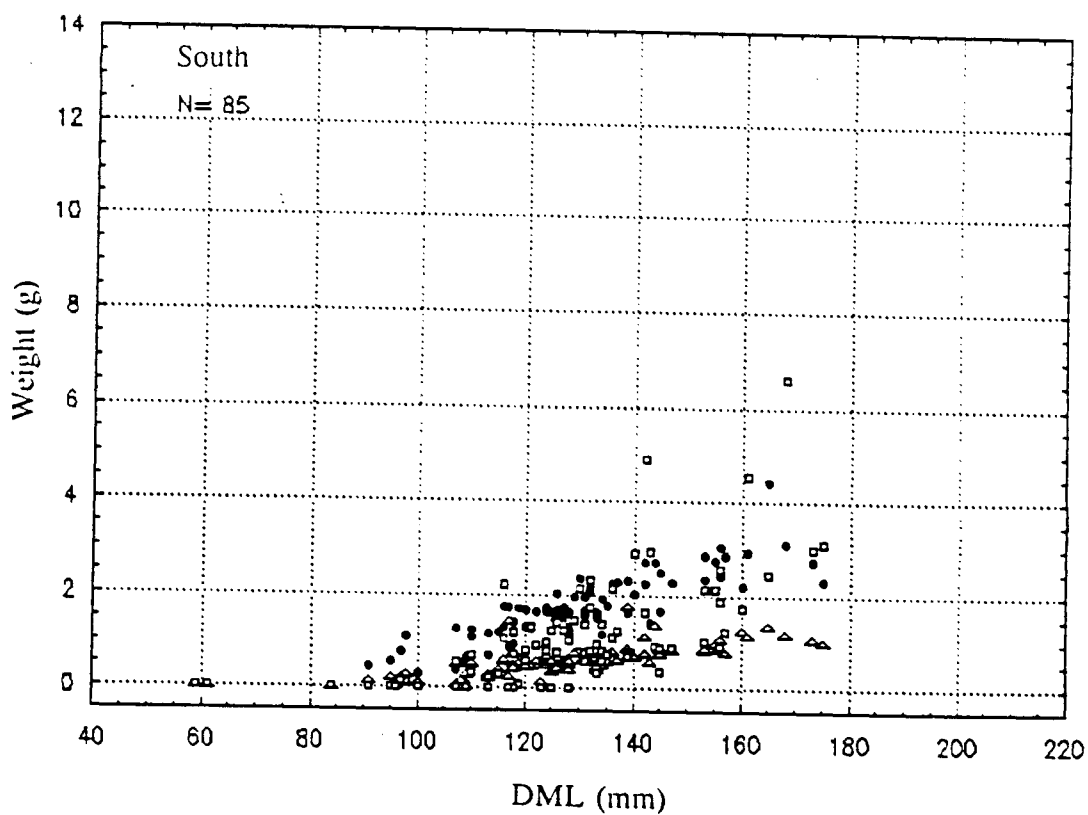
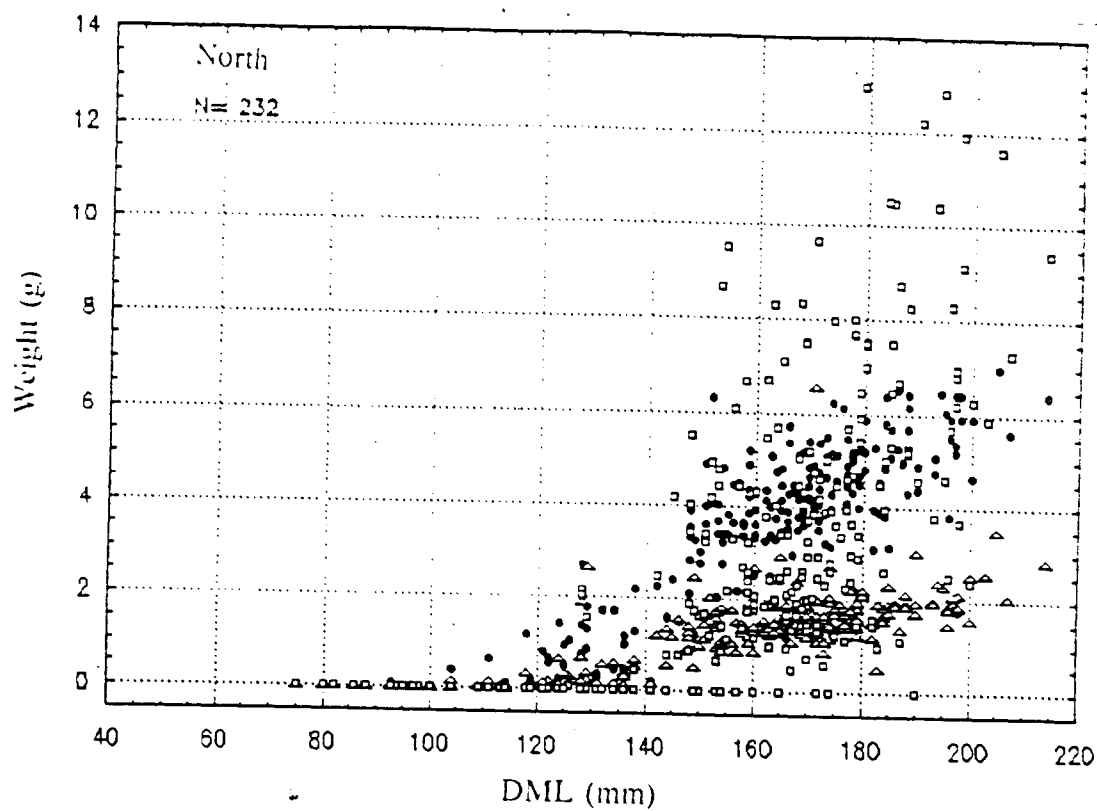


Figure 2. Representation of the weight of the reproductive system against dorsal mantle length in males of *Illex coindetii*. (•) weight of the testis, (□) weight of the spermatophoric sack, (△) weight of the Needham gland.

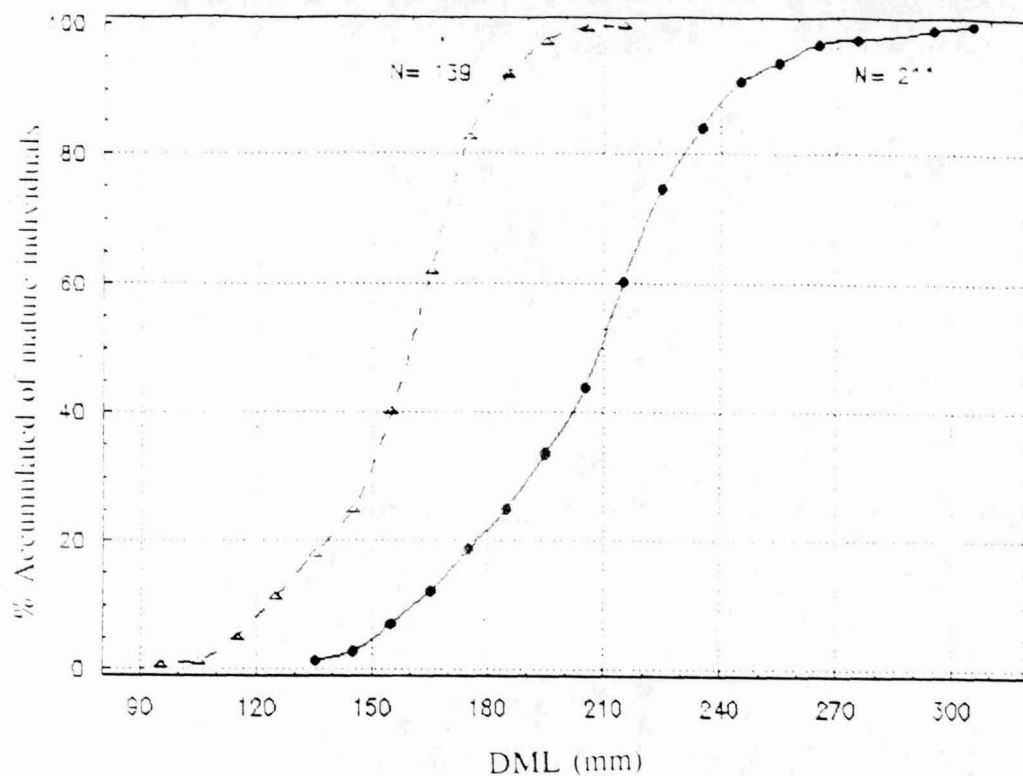


Figure 3. Accumulated percentage of mature individuals of *Illex coindetii* in the Central-east Atlantic. (•) females. (□) males.

STAGE	N	\bar{x}	SD	I-II-III-IV-V	II-III-IV-V	III-IV-V	IV-V
I	19	.0005	.0022	$p < .0001$			
II	16	.0206	.0156		$p < .0001$		
III	12	.1050	.0323			$p < .0001$	
IV	11	.1600	.0219				$p = .0004$
V	49	.1971	.0368				

Table 1. Statistical parameters of the IG index and probabilities from the Kruskal-Wallis test in females of *Illex coindetii*.

STAGE	N	\bar{x}	SD	I-II-III-IV-V	II-III-IV-V	III-IV-V	IV-V
I	18	.2205	.1496	$p < .0001$			
II	17	.3629	.0686		$p = .4594$		
III	11	.3981	.0362			$p = .6689$	
IV	15	.3953	.0299				$p = .5014$
V	46	.3926	.0459				

Table 2. Statistical parameters of the M index and probabilities from the Kruskal-Wallis test in females of *Illex coindetii*.