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Evaluation of Male Reproductive Features in *Illex illecebrosus* for Maturity Staging

by

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INTRODUCTION

Basic information on the maturation process and size at maturity is still fragmentary for many cephalopods. In the particular case of the squid, Illex illecebrosus , whose fishery has become quite important, this constitutes an obstacle to the development of populational models for management purposes. A complete study of the reproductive cycle of the species over its range is needed. However, this presents great difficulties due to the still obscure migratory pattern of the species. The considerable amount of existing field data has provided information concerning only a part of the life cycle (late juveniles and adults). This information corresponds each year to three to six months which are not temporally and spatially consistent from year to year due to fluctuations in abundance and distribution.

Furthermore, Illex illecebrosus females and males present a marked dimorphism in relation to the maturity process. Females are still immature when squid are present inshore, before starting the migration offshore (Mercer 1973; Lange et al. 1981; Amaratunga 1982), but most maturity scales now in use report males as fully mature at this time. The simple presence of spermatophores in Needham's sac which is the usual criterion for full maturity is not adequate since laboratory observations indicate that mating behaviour, even in the presence of mature females, occurs only in males which have accumulated several hundred spermatophores (O'Dor et al. 1980; O'Dor 1983). The apparent early maturation of males may simply result from a truncated male maturity scale, which fails to distinguish final stages of male maturation. The use of such a truncated scale makes the comparison of populations in various areas difficult since all males are now considered fully mature for perhaps half of their lives.

Since the basic process of spermatogenesis is apparently continuous from an early stage, other reproductive features need to be examined. Three which have been suggested are spermatophore morphology (Voss 1983), spermatophore number (O'Dor et al. 1980) and degree of hectocotylization (Schuldt 1979). This report compares data on hectocotylization in natural and captive populations, presents a preliminary

description of the spermatophores and spermatozoa of Illex illecebrosus based on electron microscopy and discusses evidence for accidental release of spermatozoa and spermatophores.

MATERIAL AND METHODS

Squids were obtained from the inshore fishery at St.Margaret's Bay, Nova Scotia, and from the captive populations at the Pool Tank, Dalhousie University, during Summer 1982 and 1983 (Sample A). Sample B refers to unpublished results (Mallet 1983) on hectocotylus indices obtained with frozen animals captured off Newfoundland in September and October 1982; sample C consisted of squid preserved in formalin since July 1979 and captured in St. Margaret's Bay.

Hectotylus

Sample A - Recently dead animals were measured (ML), weighed and sexed according to standard procedures (Mercer 1973; Durward et al. 1978). The hectocotyli were fixed in seawater Bouin's solution.

Morphomertrics were done in 28 hectocotyli of Illex illecebrosus . The measurements done and the indices produced were according to standard methodology (Roper and Voss 1983); hectocotylized length (HcL) (Fig.1), hectocotylized arm (HcA) and corresponding indices, HcLI and HcAI, respectively. Hectocotylus tracings were drawn and computerized as an alternative way of expressing the hectocotylus size.

The following measurements were also taken from all fixed hectocotyli: number of nontransformed suckers, number of folds in the hectocotylized part, tracing of entire arm, tracing of the cross section at the base of the first sucker, diameter at the base of first sucker, and the diameter of the largest and smallest suckers. The ara of the arm tracings were computed using a 48K APPLE II with a Graphics Tablet.

Sample B - Mantle length and hectocotyli indices were obtained from an unpublished study (Mallet 1983).

Sample C - Mantle length, hectocotylus arm length and hectocotylized part length were taken from a collection of formalin fixed animals.

Spermatphores and spermatozoa

Occasionally, small samples of sperm were obtained from the surface of the water in the tanks used to transfer squid from the field to the Pool Tank (August/November 1983). Two similar samples were later obtained (14 and 20/Nov.) in the Pool Tank seawater. These pieces of whitish tissue were suspended in buffered (PO4) formaldehyde, washed in distilled water, stained with OsO4 and dehydrated in acetone with a POLARON E 3000. After critical-point drying they were affixed to alluminum stubs with silver paint and sputter coated with gold. Scanning micrographs were obtained with a BAUSCH and LOMB, ARL, NANOLAB 2000. Identically prepared tissues of testis, vas deferens and spermatophores from squids of sample A were used for comparative observation of spermatozoa.

RESULTS

Spermatophores and sperm release

The number, size, shape and amount of sperm of spermatophores in cephalopods have been largely used to separate maturity stages in males (Dragovich and Kelly 1962; Mangold 1963; Hafner 1964; Fields 1965; Hamabe and Shimigu 1966; Summers 1968; Hayashi 1970; Vovk 1972a; Mercer 1973; Araya and Ishi 1974; Zuev and Shevechenko 1973; Cohen 1976; Lipinski 1979). Spermatophores have also been suggested to represent to some extent clues to the degree of reproductive isolation (Voss 1977) and to be a more reliable taxonomic characteristic than other structures such as suckers, beaks, etc.. All these aspects emphasize the interest of adding information on the spermatophore characteristics.

In the Ommastrephids, spermatophores are generally transferred intact from male to female in an encapsuled form. Illex illecebrosus spermatophores have been observed in maturing and mature males (Fig. 2) and, after transfer, in a few mated females. In spite of the absence of any study on the spermatophores of this species, there are suggestions (Squires 1957) that they vary in size, probably according to the animal size as in other species (Mangold 1963). The most detailed information on spermatophores refers to the genus Loligo (Drew 1919) and to some Octopodidae (Franzen 1966), and it is primarily on its morphology. The formation of spermatophores, their storage and the possible relationship between these steps of the whole process and the peak of maturity, are still unclear.

Sexual maturity is usually considered to occur when spermatophores are found in the Needham's sac. However, indications exist that the first spermatophores manufactured may be incomplete and that the male makes spermatophores over a considerable period of time (Grieg and Beeman 1978; Coelho 1982; Hess, cited by Voss 1983). Against the exclusive use of the amount of sperm inside the spermatophore or the number of spermatophores in the Needham's sac for defining the male maturity of Illex illecebrosus , evidence is now presented that the male can expel sperm independently of the copulation. Sperm mass was obtained from the seawater during operations to capture live squid. Also, similar samples of sperm were obtained from the Pool Tank at Dalhousie. It appears that stressing conditions such as those related to fishing or to captivity explain the loss of sperm in male Illex illecebrosus . This extends the possibility of the occurrence of this phenomenon under other conditions (fighting for food or escape from predators) which obviously diminish the value of the criteria based on the amount of spermatophores in the Needham's sac to identify different stages of male maturity. Scanning micrographs of sperm were obtained to document this new information along with some aspects of the spermatophore and spermatozoa morphology.

The structure of spermatozoa in cephalopods has been rarely referred in the

literature describing spermatophores. However, the histochemistery of these cells has ben useful to develop interpretations based on their adaptive meaning. For example, the morphology and chemistry of spermatozoa appears to be related to the mode of fertilization (Mann et al. 1970). The observations on the available material for this study indicate distinct active biflagellate spermatozoa with a spheric to pearshape head about 3 n (Fig. 3). The samples from the field did not show individual intact cells (Fig. 3H). But those collected in the Pool Tank (Fig.3F) illustrate their structure which was compared to spermatozoa from other gonad tissues of mature squid (testis, vas deferens and spermatophores). It is interesting to note (Fig. 3A and 3B) the spiral structure in the external tunic of the spermatophore which show the way it packages the spermatozoa. The intensity of this spiralization reflects the density of spermatozoa inside the spermatophore.

Hectocotyli

The means and standard deviations of all measurements and indices according to data in Appendix 1,2 and 3 are presented in Tables 1 and 2. As regards to HcLI (relative hectocotylization, calculated as the ratio between hectocotylized part length and hectocotylized arm length x 100), the figures show ranges which do not fit a simple linear relashioship between this index and the maturity stages at length (Fig.4). Figures in Table 1 show that in sample A (Bouin's fixed animals) the HcLI declines from maturity stage 3 to 4; in sample B (frozen animals) the index increases from maturity 2 to maturity 4 but maturity stage 3 has the corresponding HcLI in average much smaller; in sample C (formalin fixed) there is an increase from stage 2 to 3 but a decrease to stage 4.

Fig. 5 A,B and C plot the HcLI with corresponding ML and maturities. They show that stage 2 has the highest variability of HcLI compared with stage 3 and 4 which suggests that it is at this stage where the modifications in the hectocotylized part are more pronounced. Two individuals stage 2 in sample C and 3 in sample B deviate considerably from the scattered points representing the whole sample. For these samples no histological data, data on weight or other hectocotylus morphometrics were available to allow any further comparisons. It appears that the hectocotylized part develops independently on maturity or that these animals belong to a different cohort of the main population.

Fig. 6 A,B and C plot the hectocotylus part against hectocotylized arm length. Again these points emerge from the scatter as distinct points. Excepting one point in plot B and one in plot C, all the others corresponding to stage 2 animals belong to the same range of hectocotylized arm length for very different hectocotylus part length.

Considering the limited information of sample B and C, a more detailed analysis on sample A follows. Unfortunately, this sample does not include stage 2

animals but it is still interesting to investigate how this index relates with stage 3 and 4 considering other variables of the hectocotylus. Table 2 summarizes the mean and standard deviations of animal weight, arm perimeter, section area at the base of first sucker, number of transformed suckers and diameter at the base of the hectocotylized part (SD) for sample A. According to Fig. 5A and Table 1 the HcLI is higher for stage 3 than for stage 4. Also, bor stage 3 there is a slight increase in HcLI with length but no increase considering both stages 3 and 4. If we relate HcLI with animal weight, it can be seen (Fig. 7) that this relationship distinguishes between stages 3 and 4. It seems that within stage 3 smaller animals have higher HcLI. However this relation is weak due to the small size of the samle.

Other variables related with the hectocotylus development, but not strictly related to its length, also fail to completly explain maturity variation. Fig. 8 illustrates the relationship between the number of transformed suckers and animal weight at maturity stage and makes evident a difference between stage 3 and 4. However, if we consider the figures concerning the number of transformed suckers and the respective hectocotylus length, no proportionality exists (Appendix 1). Similar relationships are drawn if we plot a thickness index constructed from the hectocotylizes arm perimeter and section area (multiplied) or the values of SD, against animal weight. Fig. 9 and 10 show how these variables change according to maturity stage and animal weight.

DISCUSSION

The main conclusion we can draw from this study is the unreliability of the relative hectocotylization to express maturity in Illex illecebrosus . The maturity staging based on hectocotylus length index should be viewed with reserve. It was made clear that the hectocotylus development is a complicated process with several morphological modifications besides hectocotylus length changes. Probably the whole process is closely related to the maturity process but not to the single linear process of hectocotylus length modifications. This goes with the loss of suckers, with sucker size variation, arm thickness and appearance of changed structures (folds). A combination of indices related to animal weight seems to improve the relationship with maturity stage. However this does not seem to meet the goal of finding an easier method for maturation staging in Illex illecebrosus

The reasons for the high variability in the HcLI relationships are not clear. Fixation techniques varied for different samples, but it seems unlikely that a fixative would differentially effect the hectocotylized part and the hectocotylized arm in animals in different maturity stages. While this can not be ruled out, there are at least two hypotheses about the nature of the populations being sampled which could explain why Illex illecebrosus failed to show the relation between hectocotylus length and maturity stage reported for I. argentinus by Schuldt (1979). First, samples B and C contain squid from different areas; the smaller

stage 4 animals could, for example, have been immigrants from a more southern stock exposed to higher temperatures and a different photoperiods. Alternatively, there may be varying maturation rates in animals from the same stock resulting from intrinsic or extrinsic factors.

If either of these hypotheses are correct the HcLI may provide interesting insights into the conditions that squid are exposed to in different areas and years, even if it can not be used as a maturity index.

Before considering the interpretations of these variations a brief review of the limited information on the factors controlling hectocotylus growth is needed. In Octopus vulgaris differentiation of the hectocotylus is genetically programmed in males and does not require gonadal hormones (Wells and Wells 1977); however, hectocotylized arms in maturing males with active optic glands regenerate at nearly three time the rate of other arms (O'Dor and Wells 1978). The fact that the optic gland gonadotrophin is required to drive spermatogenesis (Wells and Wells 1972) and also appears to drive hectocotylization is the major reason for expecting a close relationship between maturation stage and hectocotylus length. The precise environmental factors which activate the optic glands in Illex illecebrosus in nature are not known, but there is evidence from laboratory experiments indicating that both photoperiod changes (O'Dor et al. 1977) and fasting (Rowe and Mangold 1975) can be stimulatory.

These environmental cues could lead to maturity via two routes:

1) Seasonal triggered maturation in well fed populations producing a steady progression in both growth and maturation such that advanced maturity is associated with large size as in sample A. This was certainly the case in 1983 when squid were rare and came late to the Bay.

2) Early maturation in smaller males that competed unsuccessfully for food would produce small mature animals early in the season as seen in sample B and C. Squid populations were much larger in 1979 and 1982 than in 1983, so the likelihood of food limited situations arising was much greater. Thus the large variations in maturity pattern between samples need not reflect mixed populations.

Such a intermix of cues could also account for the variability of HcLI. If hectocotylization is stimulated at the same time as spermatogenesis between stage 2 and stage 3 it would explain why the variability in HcLI is highest at this stage. There is presumably a minimum efective length required for the hectocotylus to function effectively in spermatophore transfer (Callan 1974 cited in Wells and Wells 1977). The actual HcLI attained, however, may depend on the ratio growth rate during the period before optic gland activation. Thus a squid that matured during a fast would have a lower HcLI than one that was growing and maturing in the normal season.

The observations of the accidental loss of sperm and/or spermatophores suggest that spermatophore counts, are not an adequate index. Even in unmated males this criterion would be questionable, and males that had mated once would certainly have reduced spermatophore numbers. The examination of spermatophore and spermatozoa morphology is still incomplete, but has failed so far to show any difference which would be of value for distinguishing males with spermatophores from males ready to mate.

REFERENCES

AMARATUNGA, T. 1982. Population structure of the squid Illex illecebrosus. NAFO SCR Doc. 82/IX/104. Ser. no. N613.

ARAY, H. and ISHI 1974. Information on the fishery and ecology of the squid, Doryteuthis bleekeri in the waters of Hokkaido. Bull. Hokkaido Reg. Fish. Res. Lab. 40:1-13, Japan.

ARNOLD, J.M. and L. ARNOLD 1977. Cephalopoda: Decapoda, chapter 5 of íREPRODUCTION OF MARINE INVERTEBRATESí, vol 4. Giese and Pearse (Eds.), Academic Press, New York.

AUSTIN, C.R., G. LUTWAK and T.MANN 1964. Spermatophores and spermatozoa of the squid Loligo pealeii. Proc. Roy. Soc.,B,161:143-152, London.

COELHO, M.L., T. AMARATUNGA and H. DUPOUY 1982. Some histological observations on gonadal development of Illex illecebrosus (LeSueur). NAFO SCR Doc. 82/VI/30.

COHEN, A.C. 1976. The systsmatics and distribution of Loligo (Cephalopoda, Myopsida) in the western North Atlantic, with descriptions of two new species. Malacologia 15(2): 299-367.

DRAGOVICH,A. and J.A. KELLY 1962. A biological study and some economic aspects of squid in Tampa Bay, Florida. Procc. Gulf. Carib. Fish. Inst., 5 Ann Sess., 87-103.

DREW, G.A. 1919. Sexual activities of the squid, Loligo pealeii (Les.).II. The spermatophore, its structure, ejaculation and formation. J. Morph. 32:397-435.

DURWARD, R.D., T.AMARATUNGA and R.K.O'DOR 1978. Maturation index and fecundity of the female squid Illex illecebrosus. Fish. Mar. Ser. Tch. Rep. 833(24):1-10. Canada.

FRANZEN, A. 1966. Spermiogenesis and spermatozoa of the Cephalopoda. Arkiv for Zoologi band 19 no 16.

FIELDS, W.G. 1965. The structure, development, food relations, reproduction and life history of the squid Loligo opalescens. Fish.Bull. Calif. Dept. Fish. and Game 131:1-108.

GRIEB,T.M. and R.D. BEEMAN 1978. A study of the spermatogenesis in the spawning population of the squid, Loligo opalescens. Fish. Bull. Calif. Dept. Fish and Game 169:11-21.

HAEFNER, P.A. 1964. Morphometry of the common Atlantic squid, Loligo pealei and the brief squid, Lolliguncula brevis in Delaware Bay. Cheasapeake Sci. 5(3):138-144.

HAMABE, M. and T.SHIMIZU 1966. Ecological studies on the commom squid, Todarodes pacificus Steenstrup, mainly in the southwestern waters of the Sea of Japan. Bull. Japan Sea Reg. Fish.Res.Lab. 16:13-55 (Fish. Mar. Serv. Trans. Ser. 812, Canada).

HAYASHI, Y. 1920. Studies on the maturity condition of the common squid.I. A method of expressing maturity condition by numerical values. Bull. Japan Soc. Sci.Fish. 36(10):995-999.

LANGE, A. MS 1981. Evidence of Summer spawning of Illex illecebrosus (LeSueur) off the Northeastern U.S.A. NAFO SCR Doc. 81/VI/33 Ser. no. N315, 15 pp.

LIPINSKI, M. MS 1979. Universal maturity scale for the commercially important squids. The results of maturity classification of the Illex illecebrosus population for the years 1973-1977. ICNAF Res.Doc. 79/2/38, ser. 5364, 40p.

KLYUCHNIK, T.S. and V.A.ZASYPKINA 1972. Some data on argentine squid Illex argentinus Castellanos, 1960. Tr. Atl. Nauchno. Issled. Inst. Rubn. Okenogr.42:190-192 (Fish.Mar.Serv.Trasn.Ser.3299, Canada).

MANGOLD, K. 1963. Biologie des Cephalopodes benthiques et nectoniques de la Mer Catalane. Vie et Milieu, 13:1-295.

MERCER, M.C. MS 1973. Sexual maturity and sex ratio of the ommastrephid squid, Illex illecebrosus, at Newfoundland (Subarea 3).ICNAF Res. Doc. 73/71, Ser. 3023: 14p.

MANN, T., MARTIN, A.W., and THIERSCH,J.B. 1970. Male reproductive tract, spermatophores and spermatophoric reaction in the giant octopus of the North Pacific, Octopus dofleini martini. Proc. R. Soc. London,Ser.B 175, 31-61.

O'DOR, R. K., R. D. DURWARD and N. BALCH. 1977. Maintenance and maturation of squid (Illex illecebrosus) in a 15 meter circular pool. Biol. Bull. 153: 322-335.

O'DOR, R. K. and M. J. WELLS. 1978. Reproduction versus somatic growth: hormonal control in Octopus vulgaris. J. Exp. Biol. 77: 15-31.

O'DOR, R. K., E. VESSEY and T. AMARATUNGA. MS 1980. Factors affecting fecundity and larval distribution in the squid, Illex illecebrosus. NAFO SCR Doc. 80/II/39, 9 pp.

O'DOR, R. K. 1983. Illex illecebrosus. In: P. Boyle (ed.), Cephalopod Life Cycles, Vol. I. Academic Press, New York.

ROPER, C. and G. VOSS 1983. Guidelines for taxonomic descriptions of cephalopod species. Memoirs of the National Museum Victoria, n˘44, 49-73.

ROWE, V. L. and K. MANGOLD. 1975. The effect of starvation on sexual maturation in Illex illecebrosus. J. Exp. Mar. Biol. Ecol. 17: 157-164.

SCHULDZT, M. 1979. Contribution al conocimiento del ciclo reproductor de Illex argentinus. Comision de investigaciones cientificas. Monogr.10. La Plata.

SUMMERS, W.C. 1968. The growth and size distribution of current year class Loligo pealei, Biol.Bull.135:366-377.

VOSS, G.L. 1977. Present status and new trends in cephalopod systematics. Symp. Zool. Soc. London no. 38, 49-60.

VOSS, G. 1983. A review of cephalopod fisheries biology. Memoirs of the National Museum Victoria, n˘44, 229-241.

VOVK, A.N. 1972. Method of determining maturing stages in gonads of the squid Loligo pealei. Zool.Zh. 51(1):127-132, (Fish. Mar. Serv. Trans. Ser. 2337,Canada).

WELLS, M. J. and J. WELLS. 1972. Optic glands and the state of the testis in Octopus. Mar. Behav. Physiol. 1: 71-83.

WELLS, M. and J.WELLS 1977. Cephalopoda: Octopoda, chapter 6 of REPRODUCTION OF MARINE INVERTEBRATES, vol 4. Giese and Pearse (Eds.).Academic Press, New York.

ZUYEV, G.V. and M.M. SHEVCHENKO 1973. The biology and distribution of the sea squid Ommastrephes pteropus (Steenstrup,1855) in the waters of the Tropical Atlantic. Gidrobiol.Zh. 6:15-21.

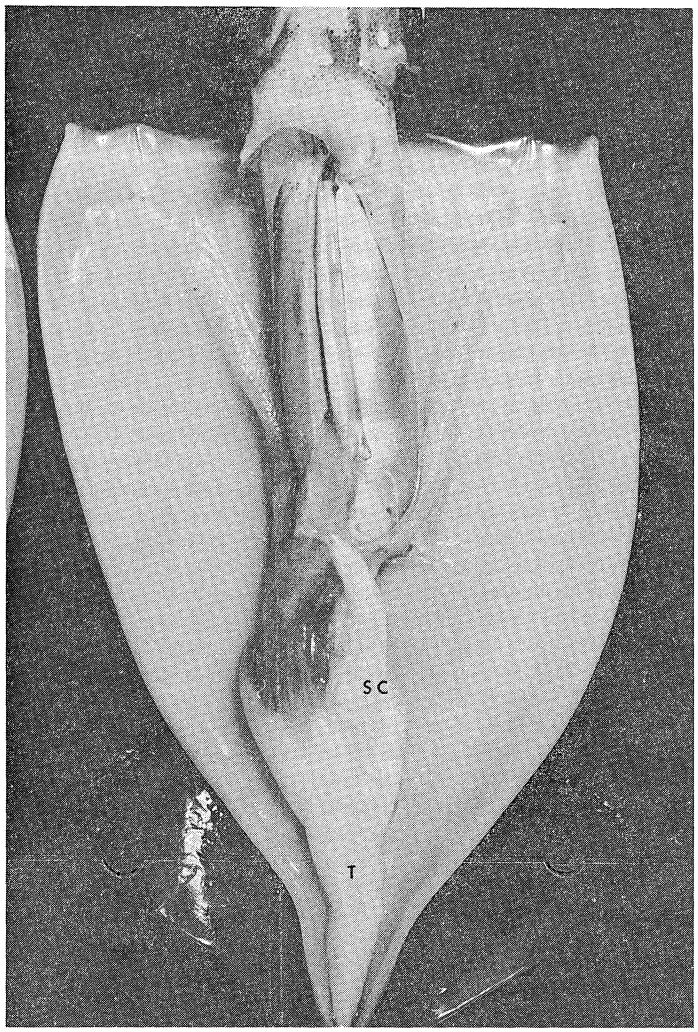

FIG.1- Testis and spermatophoric complex of a mature male Illex illecebrosus.

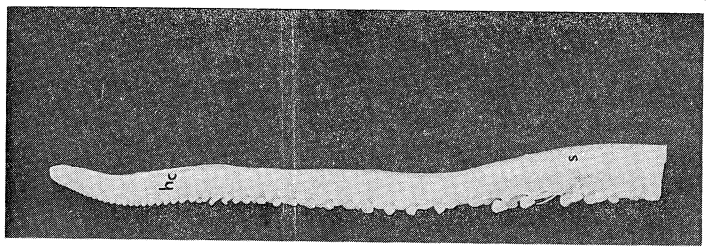

FIG.2- The hectocotylized part and spermatophores attached to the hectocotyl arm.

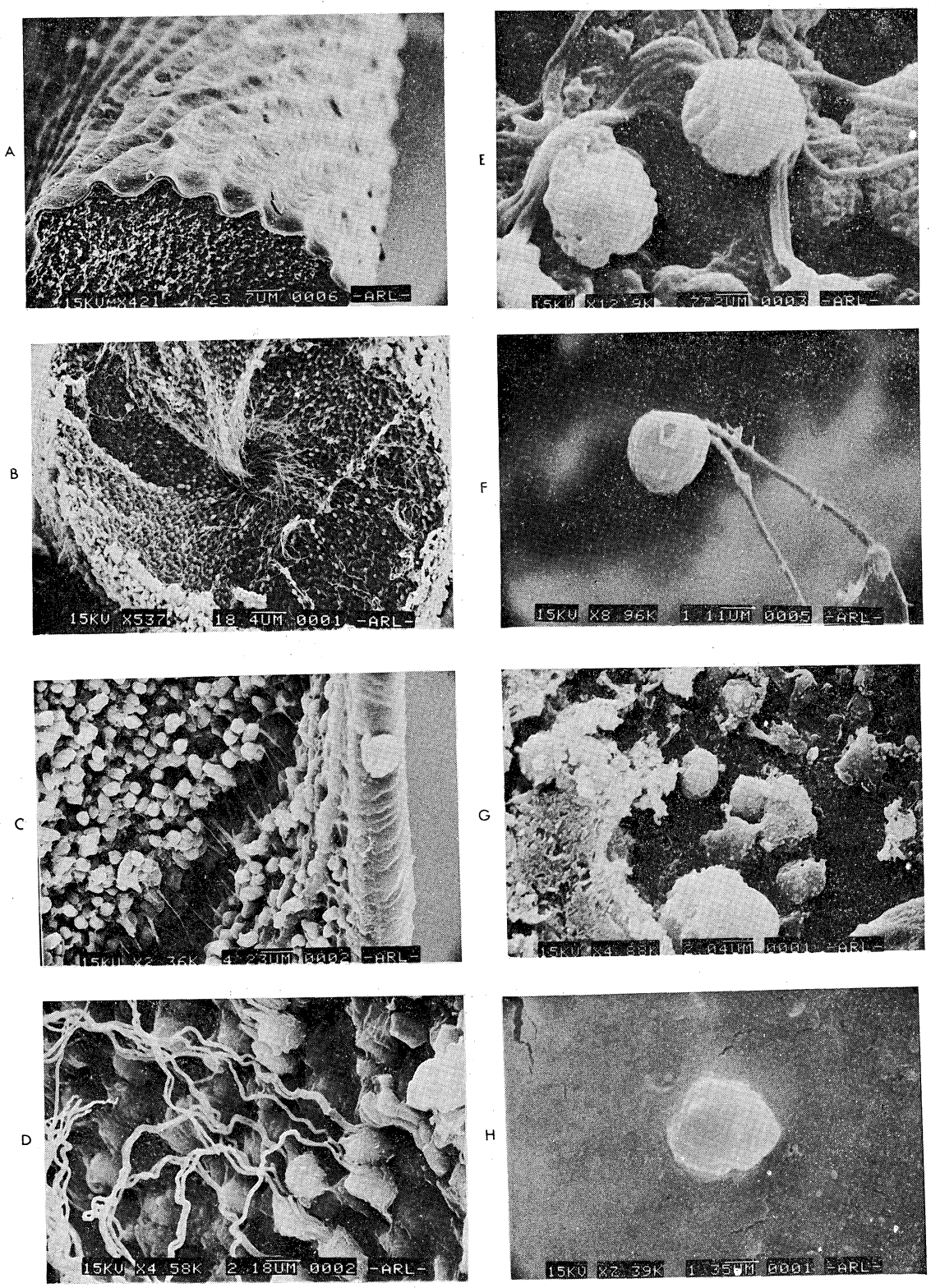

FIG.3- A to E- Spermatozoa in the spermatophore of a mated female; F- spermatozoa from sperm mass collected in the Pool Tank water; G and H- a similar sample obtained from the field (St. Margaret's Bay).

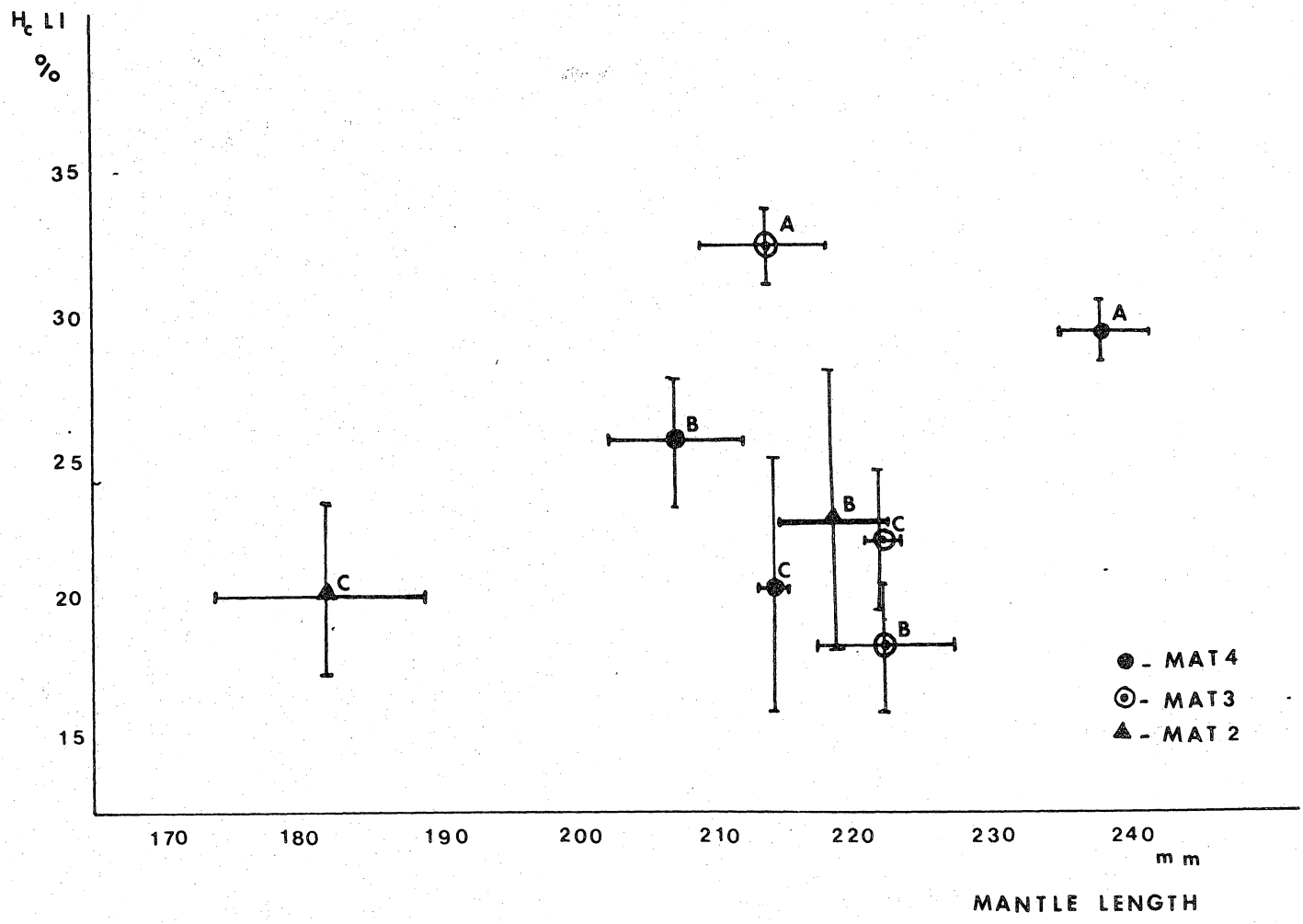

FIG.4- Graphic representation of the means and standard deviations of ML and HcLI referred in Table 1.

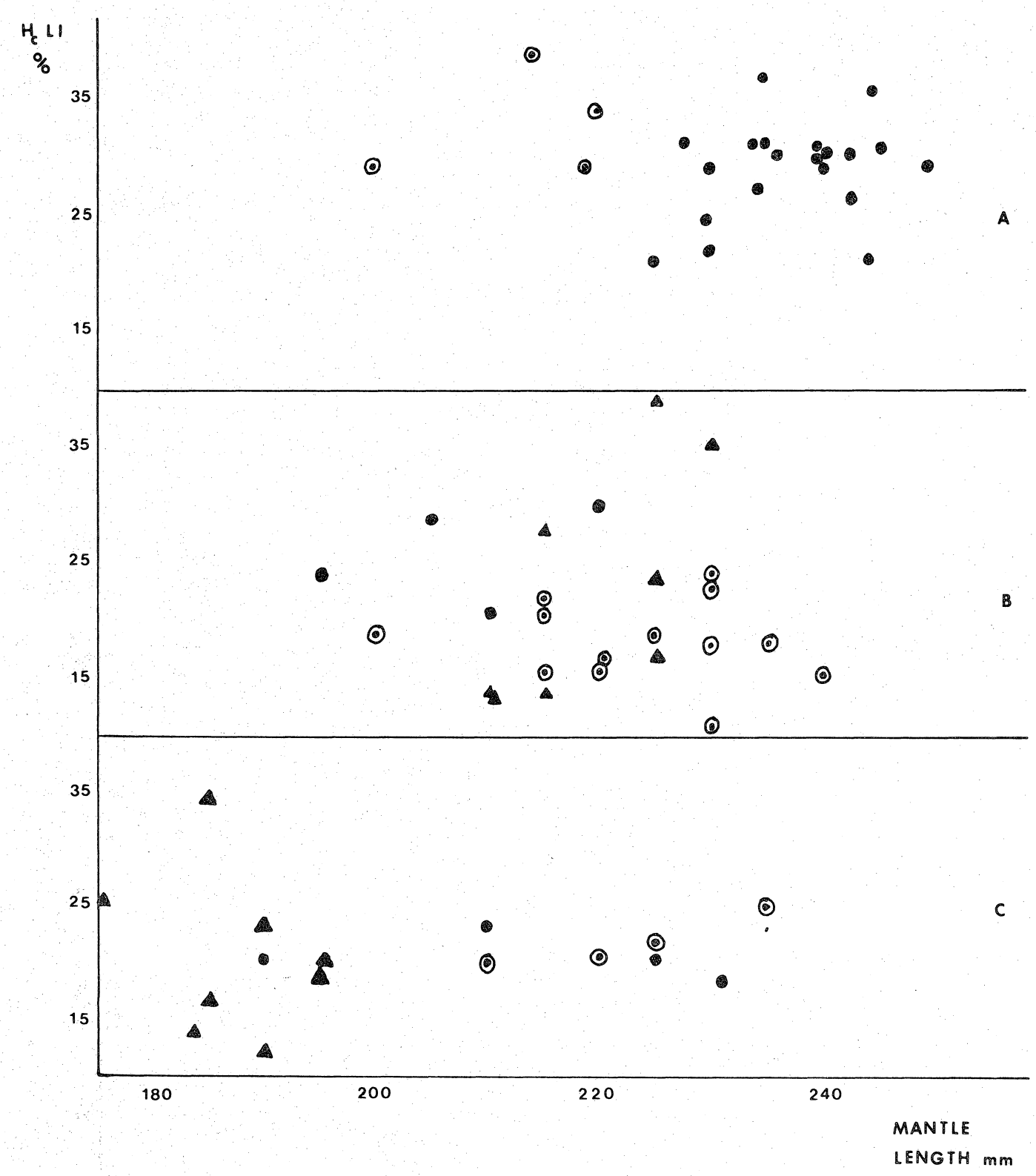

FIG.5- HcLI in relation to mantle length of samples A,B and C.

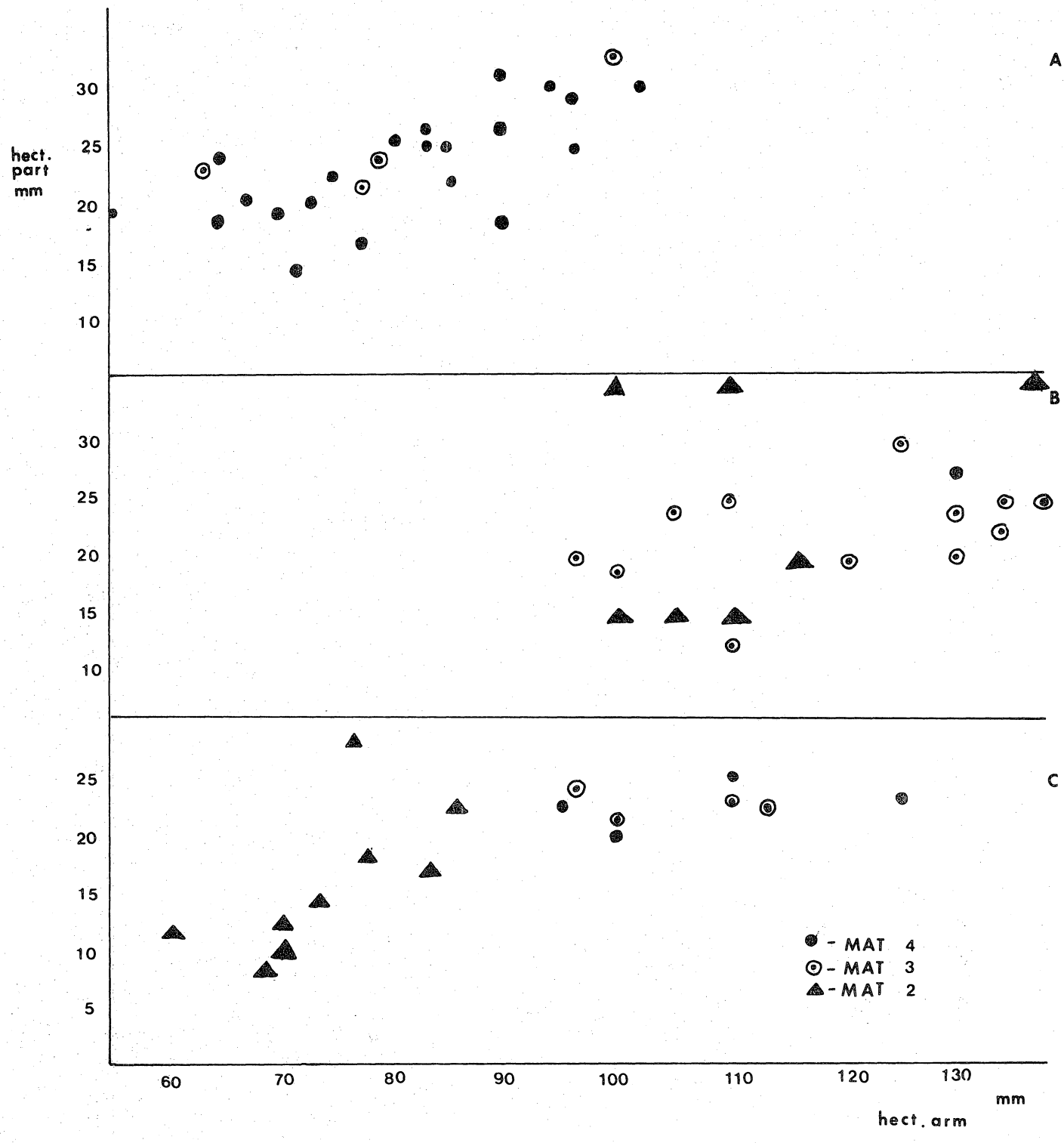

FIG.6- Relationship between hectocotyl part and hectocotyl arm length of maturity stages identified in samples A, B and C.

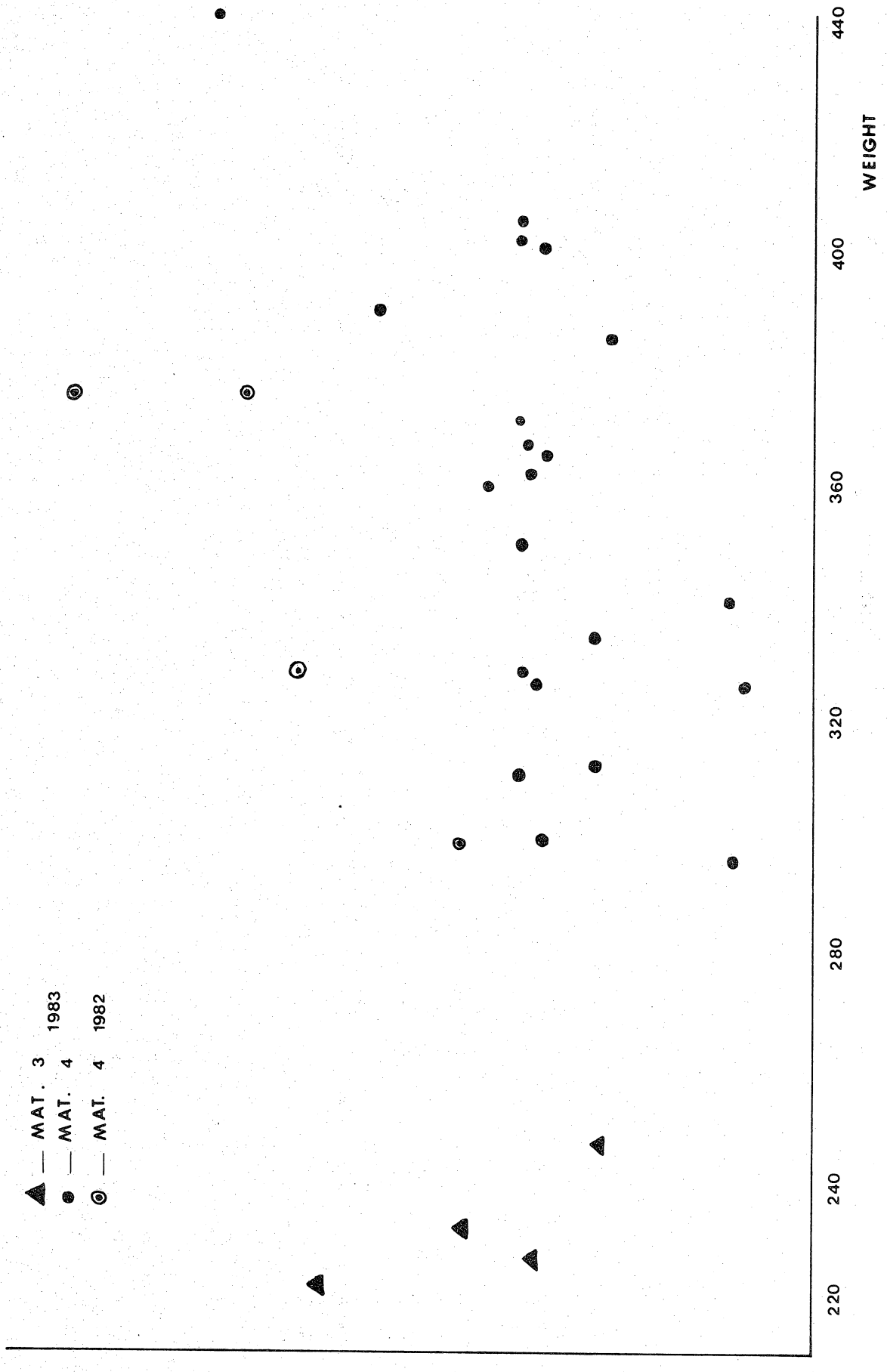

FIG.7- HcLI in relation to body weight and maturity stages of sample A.

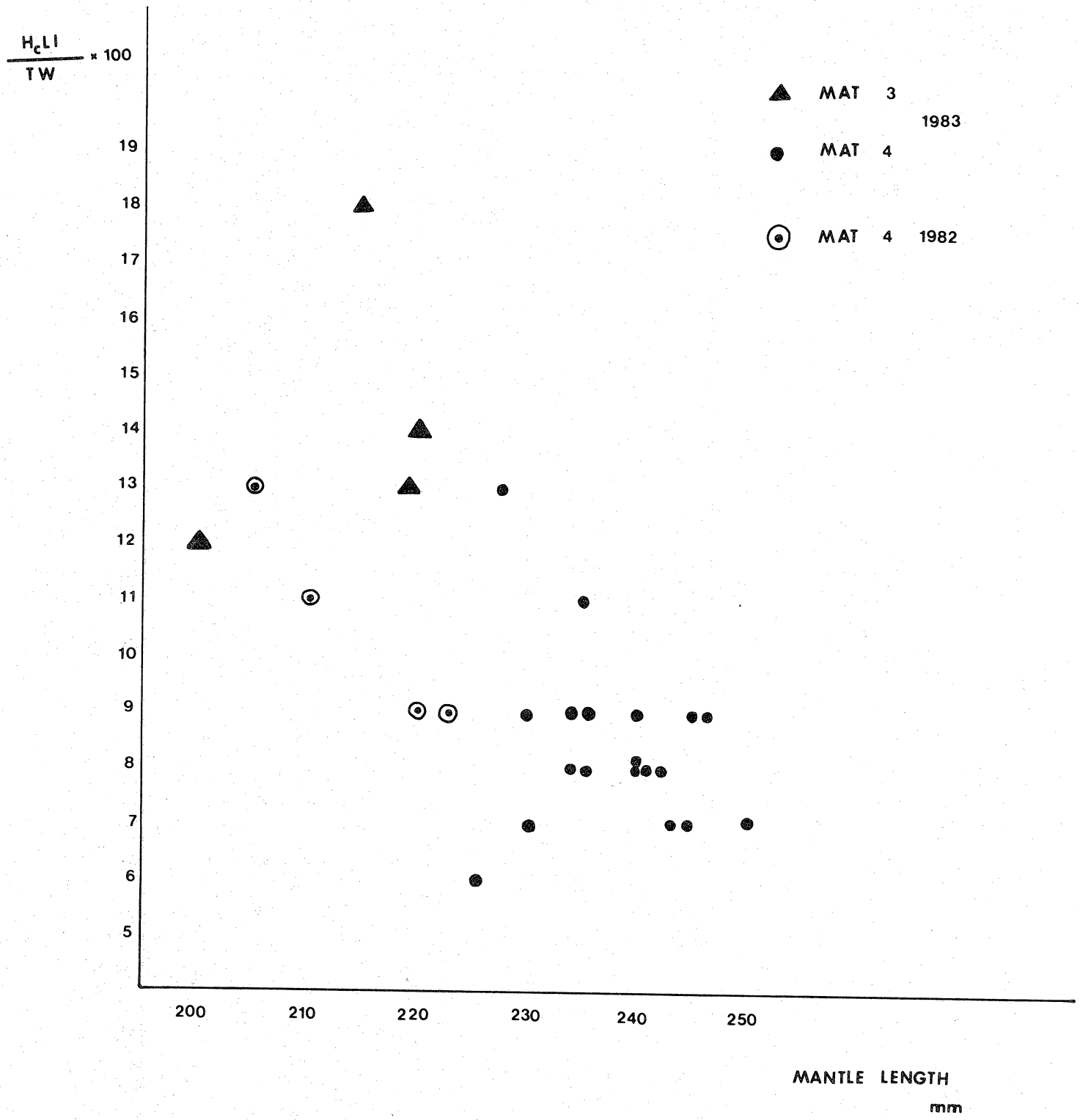

FIG.8- Relationship between HcLI/TW x 100 and ML of sample A.

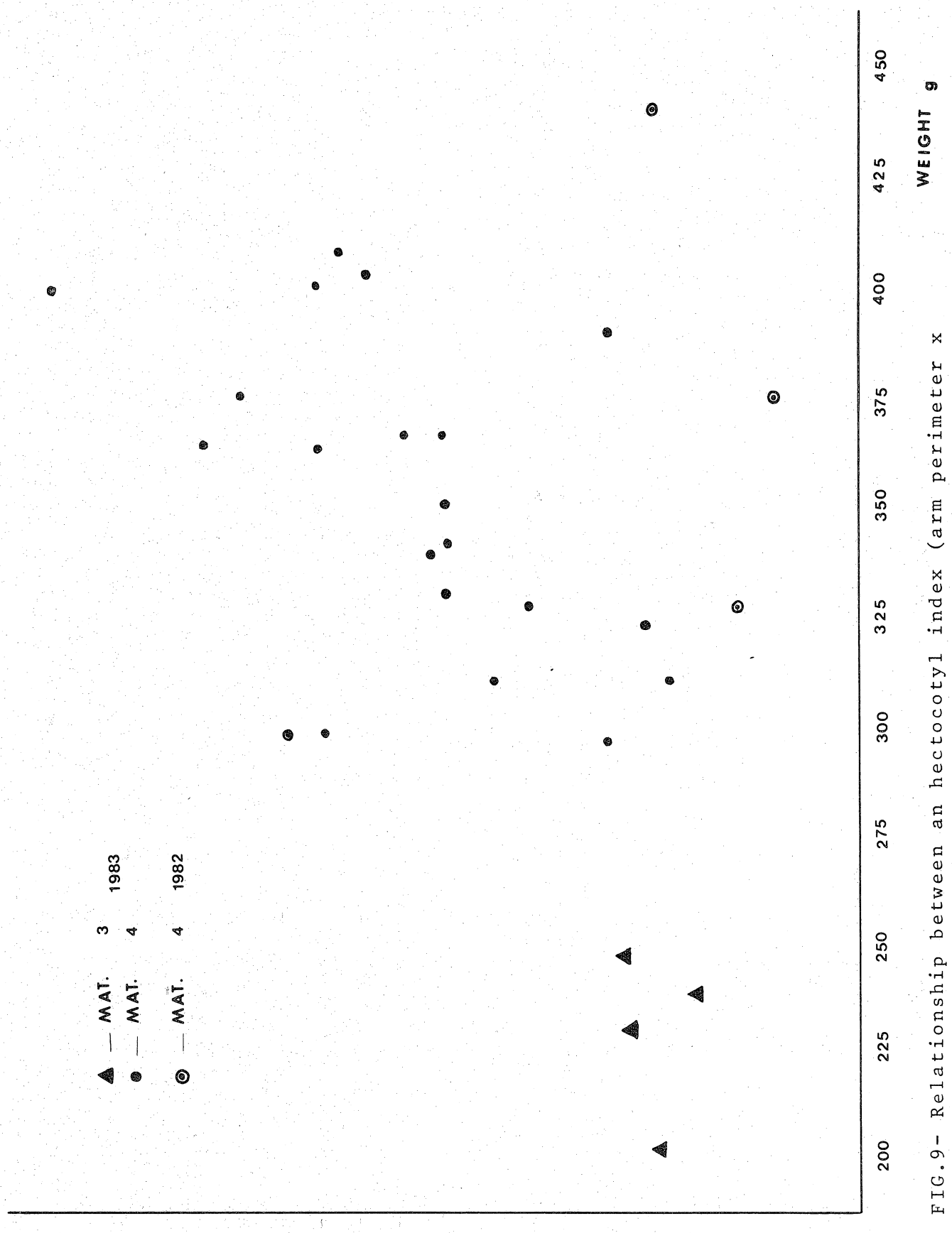

FIG.9- Relationship between an hectocotyl index (arm perimeter x x arm section area/100) and body weight (sample A).

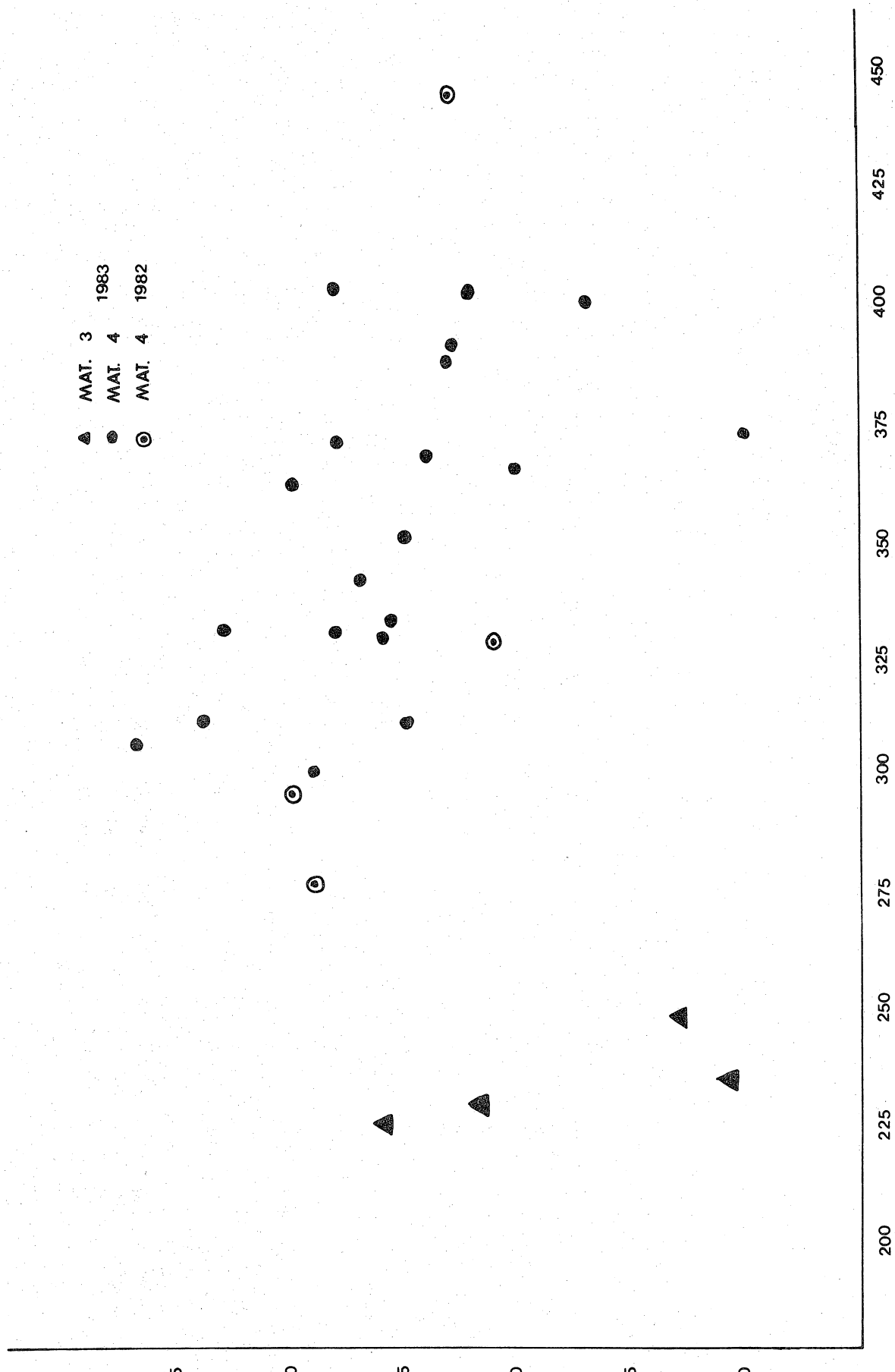

FIG.10-Relationship between nº of transformed suckers of the hectocotyl and the body weight (sample A).

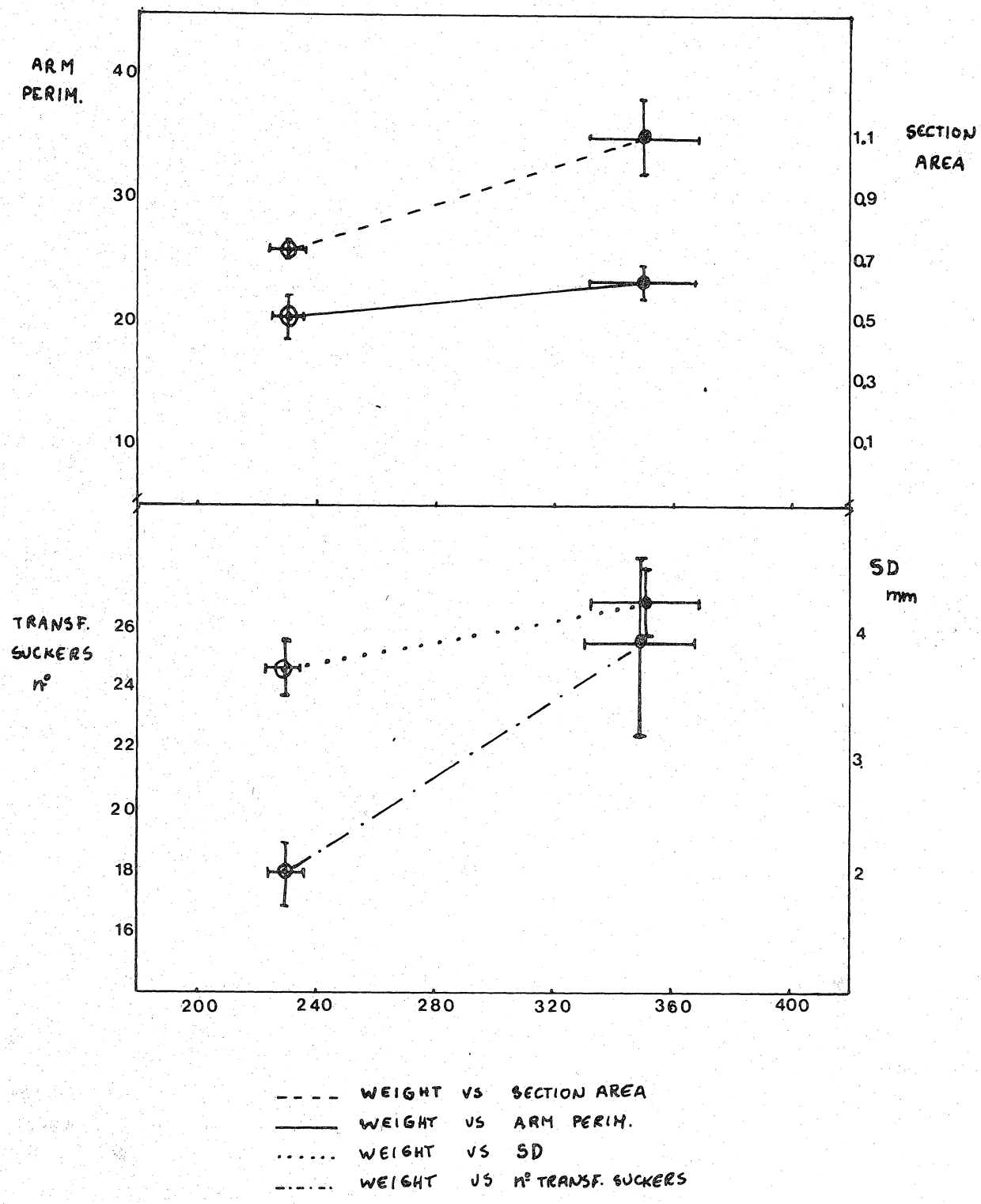

FIG.11-Graphic representation of the means and standard deviations of the hectocotyl measurements referred in Table 2.

TABLE 1- means and standard deviations of ML and H_c LI at mat. 2-4

sample ref. / Mat. stage	A		B		C	
	ML	H_c LI	ML	H_c LI	ML	H_c LI
2	- -	- -	219 7.59	23.2 10.6	182 16.9	20.8 6.9
3	213 9.02	32.5 4.73	223 10.55	18.4 3.6	223 10.4	22.6 2.2
4	238 6.99	29.2 4.20	208 10.4	26.0 4.2	214 18.6	20.8 2.1

TABLE 2 – means and standard deviations of hectocotylus measur. .

SAMPLE / PAR / DATE / mat.	A					
	weight g	arm per.	section area	transf. suck. n°	SD	N
1983 3	231.3 10.7	20.3 2.3	0.71 0.02	18.0 7.16	3.70 0.48	4
1983 4	351.2 34.9	23.4 2.6	1.10 0.25	25.75 6.00	4.21 0.68	20
1982 4	359.5 62.6	19.8 6.4	0.80 0.26			4

Appendix 1. Measurements in sample A (Boujn's)

DATE	LENGTH mm	WEIGHT g	hect. arm	hect. part	H_c %	nontransf. suckers n°	transf. suckers n°	total n°	MAT.	sucker diam. 1 larger	sucker diam. 2 smal.	1-2	SD	arm perim. ¤	arm section area ¤
OCTOB-1983	200	246	77	22	29	40	13	53	3	2.5	1.0	1.5	3.0	22.3	0.7
°	214	221	62	24	39	38	26	64	3	2.3	1.0	1.3	4.0	19.7	0.7
°	230	294	77	17	22	51	29	80	4	1.0	0.8	0.2	3.0	19.2	0.9
°	230	311	70	20	29	48	25	73	4	3.0	0.7	2.3	4.3	20.2	0.7
°	235	328	65	24	37	46	26	72	4	3.0	1.0	2.0	5.0	21.1	1.0
°	235	371	83	26	31	58	10	68	4	3.0	0.3	2.7	4.0	23.3	1.4
°	240	402	85	26	31	50	22	72	4	3.0	0.9	2.1	5.0	23.5	1.2
NOVEM-1983	220	231	100	33	33	27	11	38	3	3.0	1.2	1.8	3.8	17.3	0.7
°	218	227	79	23	29	48	22	70	3	2.7	0.5	2.2	4.0	21.8	0.7
°	225	340	71	15	21	52	27	79	4	3.0	0.9	2.1	3.5	22.5	1.1
°	227	310	75	23	31	43	34	77	4	2.9	1.0	1.9	4.2	22.5	1.0
°	230	299	70	20	29	50	37	87	4	3.0	1.0	2.0	4.0	22.0	1.4
°	234	360	81	26	32	45	30	75	4	3.0	1.0	2.0	5.2	23.8	1.0
°	234	334	97	26	27	53	26	79	4	2.5	0.5	2.0	4.0	25.1	1.0
°	236	328	90	27	30	44	33	77	4	2.4	1.0	1.4	3.2	25.0	1.0
°	240	367	97	29	30	54	24	78	4	3.0	0.5	2.5	4.6	28.7	1.3
°	240	365	72	21	29	33	20	53	4	3.3	1.0	2.3	3.5	23.3	1.2
°	242	405	68	21	31	50	28	78	4	3.0	1.0	2.0	5.0	22.9	1.3
°	243	361	102	31	30	46	28	74	4	3.5	1.0	2.5	5.0	27.9	1.1
°	243	385	86	22	26	49	23	72	4	2.8	0.7	2.1	4.0	22.1	0.7
°	244	323	90	19	21	44	28	72	4	2.7	0.7	2.0	3.3	22.3	0.7
°	245	390	90	32	36	35	23	58	4	4.0	1.0	3.0	-	29.1	1.6
°	246	350	83	26	31	47	25	72	4	3.0	1.0	2.0	4.6	22.9	1.1
°	250	400	65	19	29	51	17	68	4	3.0	0.9	2.1	4.5	21.4	1.4
NOVEM-1982	210	297	94	31	33	40	30	70	4	3.2	1.0	2.2	5.0	28.8	1.1 °
°	205	375	48	24	50	35	29	64	4	1.9	0.5	1.4	4.6	14.5	0.6
°	220	326	120	50	42	33	21	54	4	2.0	1.5	0.5	5.2	18.3	0.6
°	223	440	50	20	40	29	23	52	4	3.0	1.0	2.0	4.0	16.5	0.9

¤ - standardised units

° fresh

Appendix 2. Hectocotyl measurements in sample B (frozen - Oct. 1982).

ML mm	hect. arm mm	hect. part mm	H_c LI %	MAT. STAGE	
210	110	15	13.6	2	
225	100	40	40.0	2	
215	115	15	13.0	2	
230	110	40	36.0	2	
210	110	15	13.6	2	
225	115	20	17.4	2	
223	75.	15	24.0	2	
215	145	40	27.6	2	
240	130	20	15.4	3	
230	125	30	24.0	3	
230	110	12	11.0	3	
225	135	25	18.5	3	
220	135	22	16.3	3	
220	120	20	16.6	3	
215	120	20	16.7	3	
215	110	25	22.7	3	
215	95	20	21.0	3	
230	130	23	17.7	3	
200	100	18	18.0	3	
230	105	24	22.9	3	
235	140	25	17.8	3	
220	165	50	30.0	4	
195	125	30	24.0	4	
205	75.	22	29.0	4	.fixed
210	130	27	21.1	4	

Appendix 3. Hectocotyl measurements in sample C (formalin - July 1979).

ML mm	hect. arm mm	hect. part mm	H_c LI %	MAT. STAGE
210	110	25	23	4
195	72	14	18	2
190	68	8	12	2
183	70	10	14	2
195	83	17	20	2
185	70	12	17	2
175	85	22	26	2
185	77	27	35	2
190	78	18	23	2
225	100	22	22	3
235	96	24	25	3
225	110	23	21	4
220	110	23	21	3
190	105	22	21	4
232	110	20	18	4
140	60	13	22	2
210	112	22	20	3
215	125	23	18	4