Figure 4-1. Percent female Leptosynapta clarki from Grappler Inlet, Bamfield in size classes of A. Total weight (mg) and B. Length (mm). Point is plotted at upper limit of size class except for final category shown with (x); see details in text.

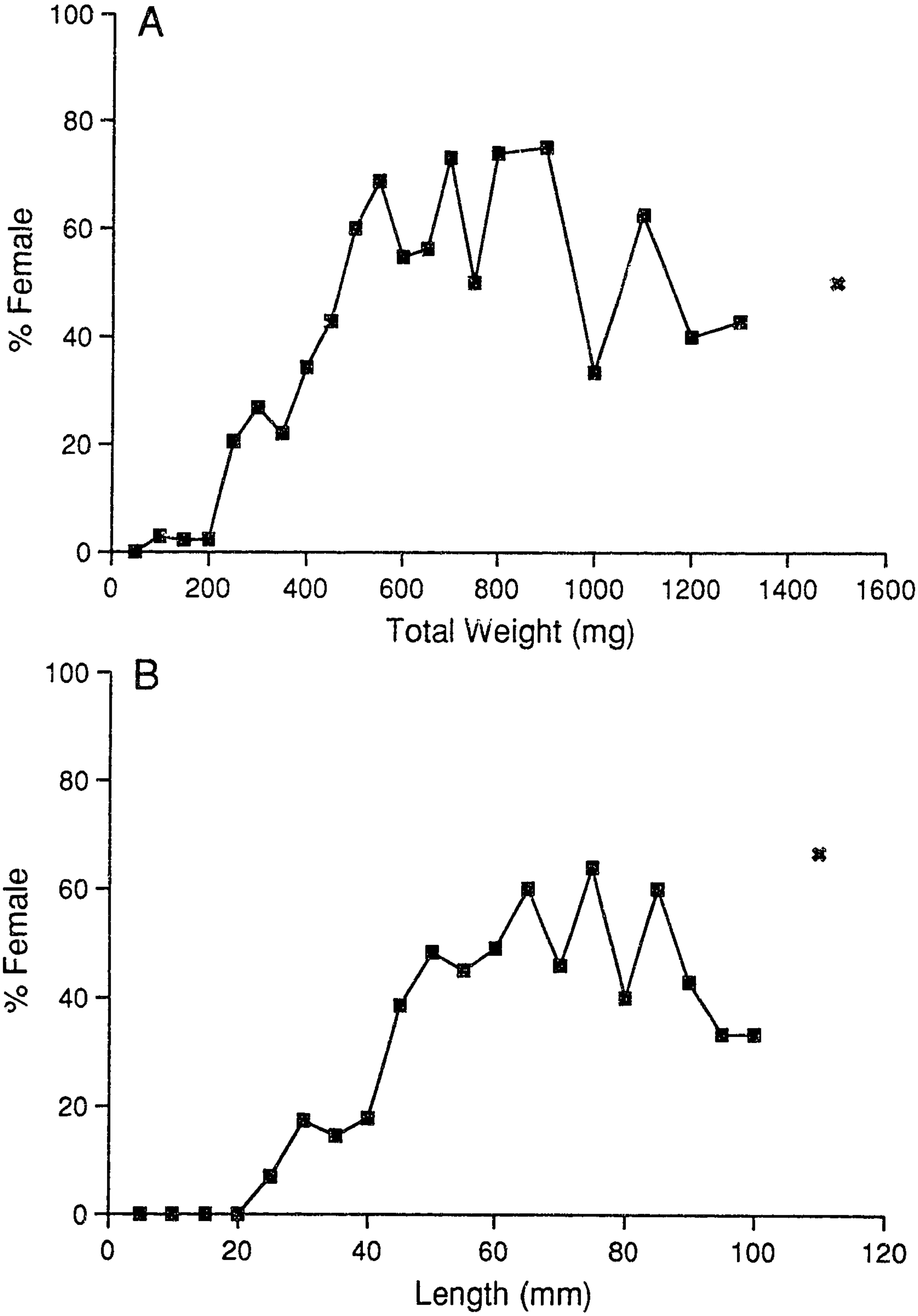

A
100
80
60
40
20
0
% Female
0
200
400
600
800
1000
1200
1400
1600
Total Weight (mg)
B
100
80
60
40
20
0
% Female
0
20
40
60
80
100
120
Length (mm)

Table 4-2. Breeding sex ratio of *Leptosynapta clarki* at Site 1, Grappler Inlet.

DATE	% MALE	SEX RATIO Male:Female	N
7 December 1990	70.5	2.4:1	112
7 February 1991	85.3	5.8:1	143
15 July 1991	85.9	6.1:1	128
12 August 1991	88.5	7.7:1	96
Mean	82.6		
Std. Deviation	8.2		

Figure 4-2. Number of transitional Leptosynapta clarki gonads found in each 50 mg total weight class during January and February 1990 and 1991. N= 75 sea cucumbers.

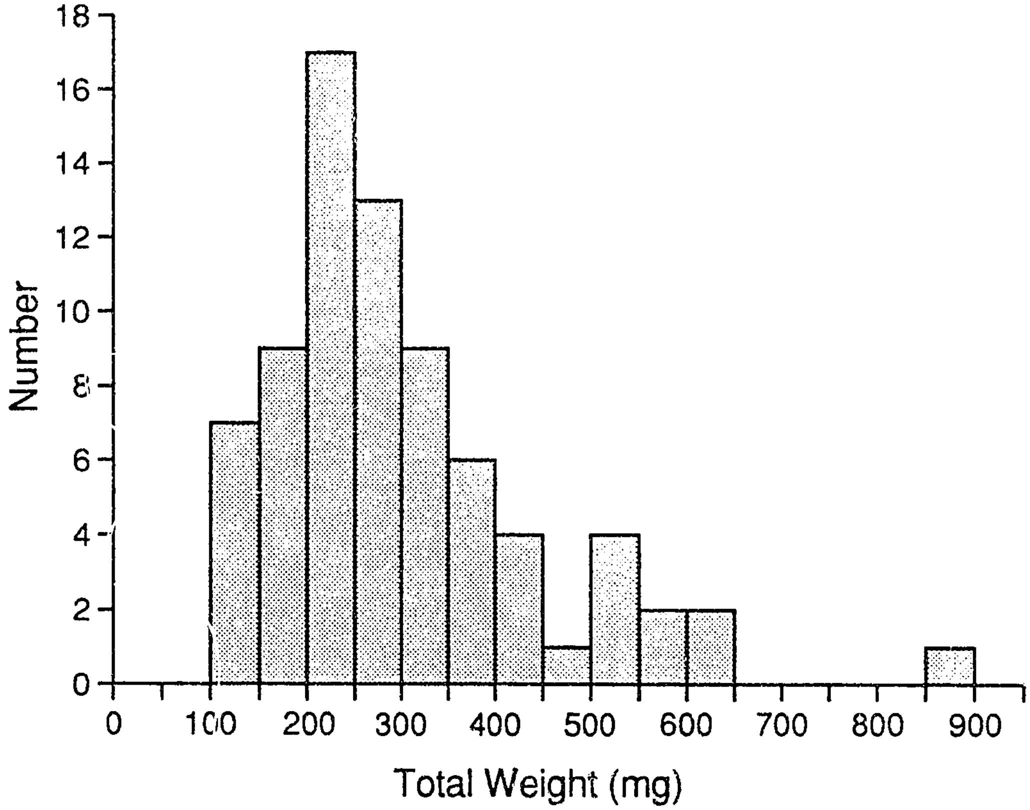

18
16
14
12
10
8
6
4
2
0
Number
0
100
200
300
400
500
600
700
800
900
Total Weight (mg)

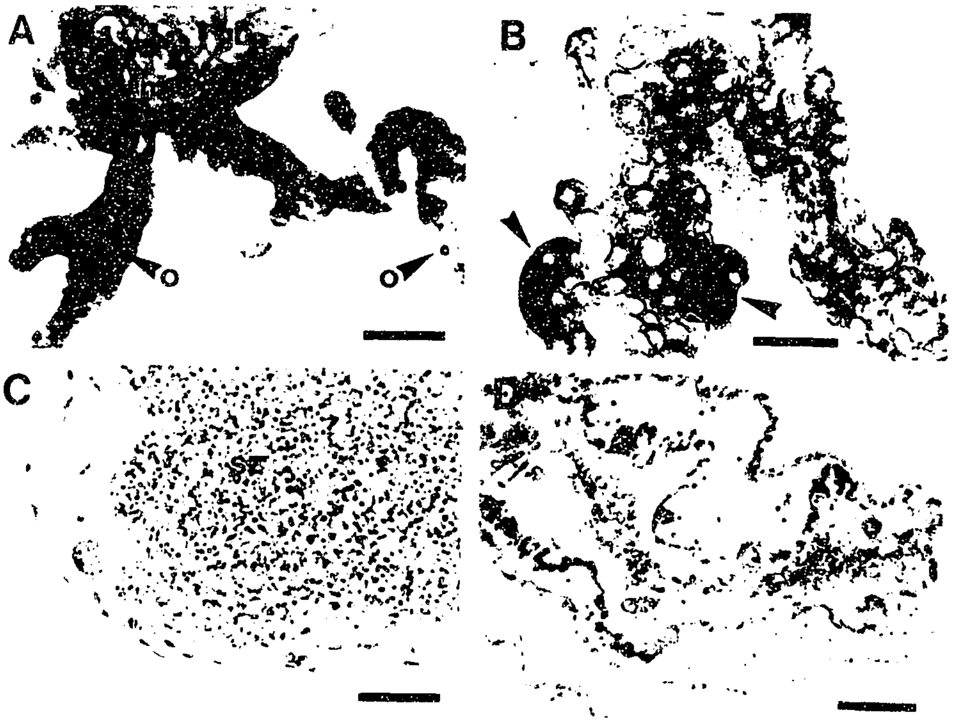
Figure 4-3. Transitional gonads in Leptosynapta clarki.

A. Photograph of small resorbing testis in process of sex change. Almost the entire gonad is shown. Note the swollen gonad basis (gb) with madreporite (m), and small oocytes (o) in the tubules. Bar= 500 µm.

B. Photograph of transitional testis. Tubule contains numerous oocytes and remnant packets of sperm (arrow). Some sperm has broken from tubule onto base of slide. Bar= 200 µm.

C. Histological section of mature testis with spermatozoa (sz) in the lumen and early oocytes forming along the tubule wall. Section stained with haematoxylin/eosin. Bar= 25 µm.

D. Histological section of post-spawned testis in transitional stage. Some remnant spermatozoa are seen in the lumen. Oocytes are present along the tubule wall. Bar= 50 µm.


A
o
o
B
C
SF
D

change sex must, therefore, be made prior to spawning in the current reproductive season.

3. Allometry of reproduction.

Reduced major axis (RMA) regressions were calculated between female size, number of eggs or pentactulae using raw and $\log_e$ transformed variables (Table 4-3). In the period prior to spawning, female sea cucumbers show a positive significant relationship between weight (TW or DW) and egg number (Fig. 4-4, Table 4-3). Regressions using natural log transformations of these variables are also significant, although there is a slight reduction of the r^2 in both cases (Table 4-3). The allometric exponent of the $\log_e$-transformed egg number/drained weight relationship is 1.46, with 95% confidence intervals being 1.3-1.65 (Table 4-3).

Lengths of the females used in the egg number/weight relationship were not recorded. These lengths were estimated using the relationship between total weight and length determined from the size/sex relationship described above using OLS regression (Length= 0.0595(TW) + 20.519, F= 2393.37, df = 1, 691, $p < 0.0001$, $r^2 = 0.776$). For this analysis OLS regression is appropriate as the value for length is being estimated from the measurement of another variable (LaBarbera 1989). A significant relationship was found between length and number of eggs in raw and natural log regressions, with an allometric exponent of 2.24 in the $\log_e$-transformation (Table 4-3).

Weak relationships were found between female size and the number of pentactulae brooded (Table 4-3). The only significant relationship was between drained weight and number of pentactulae in raw and $\log_e$-transformed data (Table 4-3). Both regressions have low r^2 values (< 0.4, Table 4-3). The allometric exponent of the $\log_e$-transformed regression was 1.84 (Table 4-3). This slope is significantly greater than 1 (T= 4.772, d.f.= 26, $p < 0.001$) indicating that fecundity is not proportionately smaller in larger sea cucumbers. In fact, larger females are producing disproportionately larger broods. This suggests that there are no scaling constraints on brood size in Leptosynapta clarki.

Fertilization success in female Leptosynapta clarki was variable. During the period sampled 22 of the 50 females (44%) were unfertilized. The remainder had percent fertilization ranging from 4.18-98.49% (mean ± SD = 47.74 ± 25.37%) with the mode at 40-50% fertilization (Fig. 4-5). There was no relationship between female weight and the percent fertilization (TW: F= 0.193, df = 1, 26, NS; DW: F= 0.986, df = 1, 26, NS).

Table 4-3. Scaling constants for relationships between Leptosynapta clarki size and number of eggs or pentactulae. Relationships are determined from an RMA regression on raw or $\log_e$ transformed data. Formula for standard error ($s^2\beta$) and confidence intervals (β lower, β upper) on the RMA slope from McArdle (1988). TW= total weight (mg), DW= drained weight (mg).

Regression	N	F	r^2	ln(a)	b	$s^2\beta$	β lower	β upper
TW vs # eggs	80	168.94^{***}	0.684	-12.300	3.637	0.038	3.206	4.127
DW vs # eggs	80	377.55^{***}	0.829	-237.726	6.348	0.071	5.785	6.968
Length vs # eggs	80	168.94^{***}	0.684	-1050.37	61.169	0.775	53.911	69.405
ln TW vs ln # eggs	80	161.69^{***}	0.675	-0.010	1.416	9.5×10^{-3}	1.246	1.610
ln DW vs ln # eggs	80	205.89^{***}	0.725	0.114	1.463	9.5×10^{-3}	1.300	1.646
ln Length vs ln # eggs	80	138.53^{***}	0.640	-0.589	2.465	0.011	2.154	2.821
TW vs # pentactulae	28	0.143 NS	0.005					
DW vs # pentactulae	28	15.590^{***}	0.375	44.960	1.795	0.055	1.312	2.456
Length vs # pentactulae	28	0.143 NS	0.005					
ln TW vs ln # pentactulae	28	0.184 NS	0.007					
ln DW vs ln # pentactulae	28	6.644^{**}	0.204	1.069	1.835	0.063	1.289	2.611
ln Length vs ln # pentactulae	28	0.122 NS	0.005					

*** $p < 0.001$; ** $p < 0.05$

Figure 4-4. Relationship between drained weight (mg) and number of eggs (filled squares) or pentactulae (open squares) in Leptosynapta clarki.
RMA regressions: Eggs: $y= 6.348x - 237.726$, $r^2= 0.829$, N=80.
Pentactulae: $y= 1.795x + 44.960$, $r^2= 0.375$, N= 28.

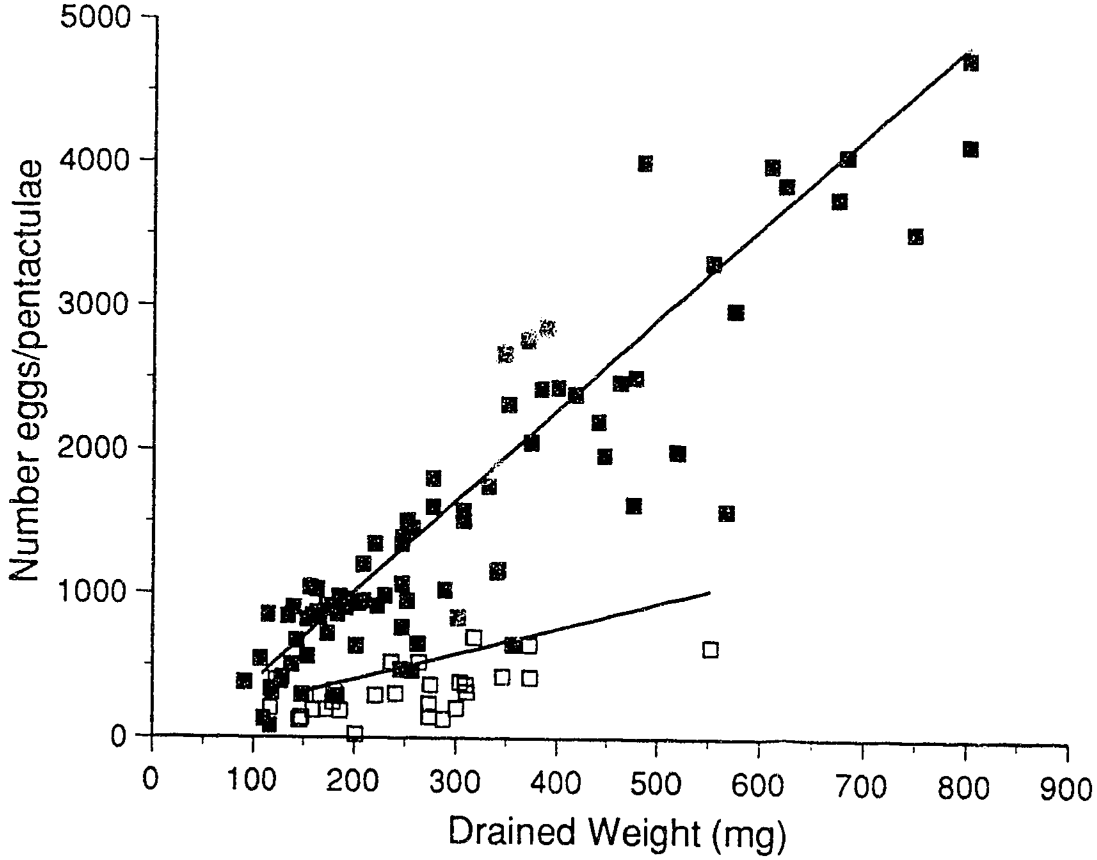
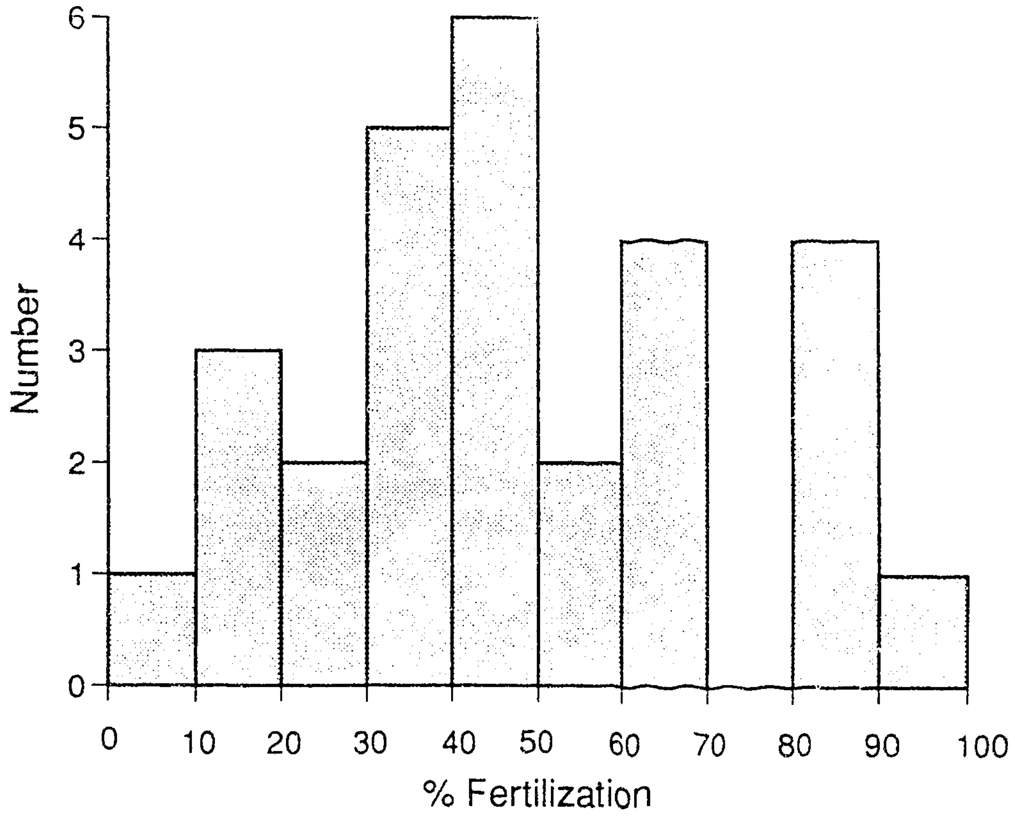

5000
4000
3000
2000
1000
0
Number eggs/pentactulae
0
100
200
300
400
500
600
700
800
900
Drained Weight (mg)

Figure 4-5. Number of female Leptosynapta clarki in each percent fertilization class during November and December 1989 and 1990. N= 28 females.


Number
0
1
2
3
4
5
6
0
10
20
30
40
50
60
70
80
90
100
% Fertilization

D. Discussion.

1. Protandry in Leptosynapta clarki.

The histological and population information presented here confirms that Leptosynapta clarki is a protandric hermaphrodite and provides the first direct evidence of sex change within the Class Holothuroidea. Two other holothurians in the Order Apodida have been suggested to be protandric, though the evidence in both cases is somewhat circumstantial. Leptosynapta inhaerens was believed to be protandric as in 80 spawning specimens only 4 shed eggs (Runnström 1927, cited in Hyman 1955), while the lack of males in a sampled population of Labidoplax media led Gotto and Gotto (1972) to suggest that only very small and young specimens produce sperm.

There are few reports of sex change in the five remaining holothurian orders. There are some anecdotal reports of protandry among dendrochirote holothurians (Smiley et al. 1991), but protandry has not been clearly demonstrated in any species. In aspidochirote holothurians, protandry is suggested in Holothuria atra based on a change in the sex ratio with increased size (Harriott 1982). However, sex change in H. atra is complicated because of asexual reproduction by binary fission (Harriott 1982). In addition, evidence for sex change in H. atra may be difficult to obtain as gonad regression after each spawning makes determination of the previous sex impossible (Harriott 1982).

Sex allocation theory predicts that sex change should be an "all or nothing" response, with the animal or plant producing gametes of one sex until it is more profitable to produce gametes of the other sex, and then switching entirely to the latter (Charnov 1982). Research on marine invertebrates has, however, shown a number of species that do not show an "all or nothing" response but have a 1:1 sex ratio above a certain size (Patella argenvillei Branch 1981, Lottia gigantea Wright 1989, Patella kermadecensis Creese et al. 1990, present study), or a wide size range or time over which sex change occurs (Fromia ghardaqana Achituv and Delavault 1972, Ophionereis olivacea Byrne 1991, Coralliophila violacea Soong and Chen 1991, Patiriella exigua Byrne 1992). The debate continues as to whether this is the result of a genetically programmed sex change, environmental sex determination, or both.

One explanation applied in a number of marine invertebrates is that there are two sexual races within a species. Bacci (1951), who proposed this terminology in Asterina gibbosa, described one race whose individuals change sex at a specific time in their existence (= balanced hermaphrodites). In the other race, sex change occurs earlier or later depending on the individual (= unbalanced hermaphrodites), or an individual may remain male or female for its entire lifespan (= true males or females, Bacci 1951). In the latter race, the presence of "true" males or females suggests a genetic predetermination for that sex. Evidence for this is seen in the echiuran Bonellia viridis where 10% of the larvae develop as males even if the "male determining factors" of a female proboscis are absent (Leutert 1975). Similarly, some individuals of Patella vulgata may never change sex (Orton 1928, Ballantine 1961) and in Lottia gigantea about 15% of the population change sex annually regardless of environmental setting or age (Lindberg and Wright 1985).

In Leptosynapta clarki, as all individuals start their reproductive life as males (Sewell 1991, Chapter 2, Chapter 6), there can only be "true" males in the population that do not change sex during their lifetime. Division of the remainder of the population must be into those who change sex at some specific time, and those that change sex under the influence of some genetic or environmental factor. Male Leptosynapta clarki may either delay sex change as shown by the large size of some transitional males (Fig. 4-2) or have a labile sexuality which alternates between male and female. There is some circumstantial evidence that sex can be reversed from female to male (Chapter 2), and it is conceivable that sex may change more than once in an holothurian's lifetime (Everingham 1961, Harriott 1982).

The presence of transitional Leptosynapta clarki gonads containing mature sperm and previtellogenic eggs during the reproductive season (Fig. 4-3) and the continued growth of oocytes within the same gonad tubule after spawning of the sperm (Chapter 2) makes it difficult to envisage how a genetic factor is involved in the control of sex change. The ability to maintain gametes from two sexes, selectively resorb the remnant sperm, and continue to grow oocytes suggests some form of complex control. As little is known of sex determination in echinoderms (Delavault 1966) and especially unknown in holothurians (Smiley et al. 1991) this could be a fruitful area for further research.

The size-sex relationship presented here is a static data set which combines sea cucumbers of many ages within a size class (Fig. 4-1). Juveniles released from the mother in May 1990 were maintained in the laboratory for 1 year and used for measurements of growth (Chapter 6). These individuals reproduced as males in November 1990 (Chapter 6). When the growth experiment finished in May 1991 the surviving sea cucumbers were kept in the laboratory until the gonads were grown. In July 1991, eight animals of the surviving 78 had changed sex (unpub. data). These protandric sea cucumbers were above 185 mg TW (unpub. data) which is approximately the critical size of 200-250 mg determined from field collections. The other 70 individuals, while of the same age, were male or of undetermined sex (unpub. data). Seven of these 70 sea cucumbers were above 185 mg TW but had not changed sex (unpub. data). These results suggest, therefore, that size in combination with some environmental or genetic factor may be more important than age per se to the timing of sex change in Leptosynapta clarki.

2. Brooding and small body size.

Brooding is size-dependent in many marine invertebrates, with species with smaller adults providing protection to more advanced stages of embryonic development (see review by Strathmann 1990). This trend has been noted in two families of holothurians, the Cucumaridae (Menge 1975) and the Synaptidae (Strathmann 1990). Leptosynapta clarki conforms to these trends in being small (adult size max. at Bamfield 113 mm), and brooding pentactulae in the ovary (Everingham 1961, McEuen 1986, Chapter 2).

A test of the allometry hypothesis to explain the association of small adult size with brooding (Strathmann and Strathmann 1982) in Leptosynapta clarki did not show proportionately smaller broods in larger sea cucumbers. The slope of the relationship between $\log_e$ drained weight and $\log_e$ number of pentactulae was not less than one, indicating no spatial constraints on brood size in larger Leptosynapta clarki.

Constraints on brood size can also occur if the larger broods from larger adults develop more slowly or suffer higher mortality (Strathmann and Strathmann 1982). In Leptosynapta clarki while there is a trend for larger females to have a higher number of pentactulae, larger females or females with a high number of pentactulae do not show a higher degree of mortality (Chapter 5). These results suggest that brood size is not constrained in larger Leptosynapta clarki by either brood space or differential embryonic mortality.

While the allometry hypothesis is intuitively appealing (Hess 1993), the evidence for scaling constraints on brood size in marine invertebrates is remarkably poor. Studies that suggest that brood size is unconstrained by brood space include species of polychaetes (Daly 1972, Gremare and Olive 1986, Hess 1993), bivalves (Ockelmann and Muus 1978, Kabat 1985, McGrath and ÓFoighil 1986, Russell and Huelsenbeck 1989, Brey and Hain 1992), asteroids (Menge 1974), ophiuroids (Rumrill 1982, Byrne 1991), holothuroids (Rutherford 1973) and pinnothurid crabs (Hines 1992). In fact, the study on Asterina phylactica which provided the first empirical evidence for the hypothesis (Strathmann et al. 1984) when reanalysed with RMA regression does not indicate any scaling constraints on brooding (Hess 1993).

One species that might show allometric constraints on brooding is the hermaphroditic bursal brooding Axiognathus squamata (Rumrill 1982). In this species the relationship between brood number and parental body size is significantly different from zero, but with a low slope (untransformed data b=0.57, Rumrill 1982). This indicates that smaller sized adults brood nearly equivalent numbers of embryos as larger adults (Rumrill 1982). However, in Axiognathus squamata staggered oocyte maturation and brood release ensures that the production of oocytes never exceeds the capacity of the adult to brood the developing embryos and thus clutch size depends more upon the rate of oocyte maturation than body size per se (Rumrill 1982).

Fertilization in brooding marine invertebrates is by three methods: self-fertilization, some form of intromittant device is employed, or sperm are shed externally into sea water and are attracted towards the eggs retained by the other parent (Heath 1977). The latter method will encourage the maximum production of male gametes since large numbers of ova may be available for fertilization (Heath 1977). Poor relationships between the number of embryos brooded and parental size seen in internal (e.g. Axiognathus squamata Rumrill 1982, Leptosynapta clarki present study) or external brooders (Engstrom 1982) could result from problems associated with fertilization in all females regardless of size.

Increased fertilization success in large sea cucumbers might be observed if there is an advantage in large size in competition for breeding sites or mates, or intense sperm competition (Conover 1984). Little is known about spawning behaviour in Leptosynapta clarki (Chapter 2). Although Everingham (1961) and McEuen (1986) reported seeing individuals lying close together or intertwined on the mud surface during the spawning period, this behaviour was not observed at Bamfield (Chapter 2). As neither of these authors observed spawning or found spermatozoa in the depressions formed by intertwined sea cucumbers, it is unknown whether these groups are in fact formed by spawning individuals (Chapter 2). Until the spawning behaviour of Leptosynapta clarki is determined it is assumed that there is no competition for breeding sites or mates in this species. Sperm competition, however, might be intense.

In Leptosynapta clarki the males release sperm externally which enters the ovary through an as yet unknown means (Everingham 1961, McEuen 1987, Hess et al. 1988, Chapter 2), but probably via the gonopore. Sperm availability during the spawning season is maximised in two ways: by early reproduction which skews the sex ratio dramatically towards males (Table 4-2), and the production of large quantities of sperm by each male (Chapter 2). While the males invest a similar proportion of energy in reproduction (ca. 5% in all sized males, Chapter 2), larger males will have larger volumes of sperm which might result in higher fertilization success. However, even though sperm does not appear to be limiting because of high density populations (mean 169 per m^2, Chapter 6), a skewed sex ratio, and large amounts of sperm per male, in some females fertilization is low or zero. There is also no evidence to suggest that larger females have a higher fertilization success. If fertilization is a constraint for Leptosynapta clarki, or other internal brooders, then limitations on brood size due to space or embryonic requirements for dissolved materials (Strathmann and Strathmann 1982) may never be reached.

In brooding species where fertilization success is high (e.g. Rutherford 1973, Ophioplocus esmarki Rumrill 1982, Byrne 1991) allometric constraints may be avoided by brooding embryos to a limited stage of development (Daly 1972, Ockelmann and Muus 1978), three-dimensional packing of embryos in the brood space (Kabat 1985), sequential brooding throughout the reproductive season (Kabat 1985, Hess 1993), or brooding within a distensible structure (Hines 1992, present study). In the latter case, the ability for the brood structure to expand to accommodate the number of fertilized eggs might ensure that even when fertilization success is high all the embryos can be brooded.

The alternative hypotheses on the association of small size and brooding such as lower energetic reserves (Chia 1974), dispersal or recruitment (Strathmann and Strathmann 1982) cannot be considered in relation to brooding in Leptosynapta clarki until there is comparative information on other sea cucumber species. Hess (1993) has recently suggested that the slower developmental rate of embryos in large brooders may be a factor in the evolution of brooding. This time constraint hypothesis suggests that longer developmental times may be disadvantageous because the stage specific mortality rate is experienced over a longer period of time, and the number of broods per unit time is reduced. Hess (1993) assumes in the development of this hypothesis that embryonic mortality may be low and relatively unimportant in brooders, which may not be a valid assumption in all brooding species (Chapter 5).

While the time constraint hypothesis may be applicable to species that spawn more than once per season, alternative hypotheses will be needed to explain the association of small size and brooding in semelparous brooders or individuals that produce only one brood per season (Hess 1993). As the latter category includes many echinoderms in which the association of small size and brooding is particularly pronounced (see Strathmann 1990), this emphasises that no single hypothesis will be applicable in all taxa (Strathmann and Strathmann 1982).

3. Brooding and hermaphroditism.

The association between brooding and hermaphroditism has been noted in a variety of marine invertebrates (Ghiselin 1969, 1974, Charnov 1982, Strathmann and Strathmann 1982), including asteroid, ophiuroid and holothuroid echinoderms (Ghiselin 1969, 1974). The fairly high incidence of protandry in ophiuroids and holothuroids was believed by Ghiselin (1969) to favour the gene dispersal model for hermaphroditism.

In the gene dispersal model, sequential hermaphroditism can act to prevent inbreeding due to fertilization between siblings, especially in forms with restricted gene flow (Ghiselin 1969, 1974). For example, if all members of a clutch are males at the onset of sexual maturity, and simultaneously change into females then they are never able to mate with each other (Ghiselin 1974). The observation that there is a higher incidence of sequential hermaphroditism in forms with restricted gene flow (species with no larvae or brooders) provides some evidence for the hypothesis (Ghiselin 1974) though no direct evidence for reduced levels of inbreeding in sequential hermaphrodites is available. Studies on the scale of genetic differentiation in Leptosynapta clarki (Hess et al. 1988) and the confirmation of protandry in the present study may provide direct evidence for this hypothesis.

In an electrophoretic study of three geographically separate Leptosynapta clarki populations on San Juan Island, Washington, Hess et al. (1988) found no evidence for inbreeding. The two polymorphic loci studied did not show the reduced numbers of heterozygotes expected in inbred populations (Hess et al. 1988). Hess et al. (1988) concluded that there was enough movement of either individuals or gametes within each population to allow random mating and maintain heterozygosity.

Leptosynapta clarki juveniles released from the mother rapidly form shallow burrows and although dispersal is rare it might occur by rafting, floating, swimming or incidental dispersal as a result of water movement (Chapter 6). The combination of occasional dispersal and sequential hermaphroditism might reduce levels of inbreeding in a Leptosynapta clarki population. However, as growth rates in juvenile Leptosynapta clarki are extremely variable (Chapter 6), and protandry appears to be dependent upon the combined effects of size and environmental/genetic effects it is unlikely that all siblings undergo a simultaneous sex change. While Leptosynapta clarki does show some evidence for the gene-dispersal model for sequential hermaphroditism further clarification is required to determine how far siblings disperse from one another, if all or only some individuals in a clutch change sex at one point in time, and if there is a reduction in inbreeding in the Bamfield population.

If we assume that sequential hermaphroditism is an advantageous strategy for Leptosynapta clarki, the question to be answered is: why is Leptosynapta clarki protandric and not protogynic?

Juvenile sea cucumbers are released from the mother into the adult habitat in April or May (Chapter 2, Chapter 6). The transitional gonads with new eggs are, however, seen in sex-changing animals in January while these pentactulae were still being brooded. Therefore, if the sea cucumbers are to be reproductively active as females in their first year, they would need to start egg development while being brooded within the female. In addition, based on the regression line for drained weight versus number of eggs (Table 4-3, Fig. 4-4) to produce any eggs at all a sea cucumber must weigh over 37 mg DW during late October. While growth to sizes at which eggs could be produced is possible based on laboratory measurements (Chapter 6), the time required to produce mature eggs (10 months, Jan-Nov., Chapter 2) or the energy to support oogenesis (Lawrence 1987) might be a constraint to early reproduction as a female. If energy is limited, there may be a size threshold below which it is uneconomical to produce the energetically more expensive eggs (Branch 1981) but where the energy is adequate to produce a functional number of smaller sperm (Lawrence 1987).

In terms of the size advantage hypothesis, while there is clearly an increase in the number of eggs produced per female with size (Fig. 4-4), the potential for low fertilization (as number of eggs fertilized) in all females may reduce any advantage in large females. In addition, there is a strong relationship between testis weight and male size (unpub. data) so larger males are producing larger volumes of sperm. Until the mechanism of fertilization in Leptosynapta clarki is determined, it is not known whether male size is important to an individual's fertilization success.

4. Future research.

The genus Leptosynapta provides an ideal group in which to conduct further research on the association of small size, hermaphroditism and brooding in marine invertebrates. Sea cucumbers within this genus are of small to moderate size (Hyman 1955; 12-30 cm maximum adult length, except for L. minuta 1 cm, McEuen 1986), are characterised by burrowing habits (Hyman 1955), and exhibit a wide range of reproductive strategies from lecithotrophic broadcast spawners (L. inhaerens, L. galliennei, L. girardii, L. tenuis), to coelomic (L. minuta), and ovarian brooders (=viviparity: L. clarki, L. transgressor; for references see Smiley et al. 1991). Of these species L. clarki and probably L. inhaerens are protandric, while L. minuta is reported to be hermaphroditic (Smiley et al. 1991). Further comparative work on this genus would therefore be of considerable interest to address life history questions relating small size, brooding, and hermaphroditism without phylogenetic constraints.

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CHAPTER 5

Mortality of pentactulae during intraovarian brooding in the apodid sea cucumber Leptosynapta clarki.

A. Introduction.

In 1950, the Danish marine biologist Gunnar Thorson published a review that provided the basis for much of our theory in marine invertebrate larval ecology (Roughgarden 1989, Young 1990). Because of the low number of eggs produced by species with non-feeding larvae or brooders, Thorson suggested that "... embryos developing into non-pelagic young are free from the huge waste found among larvae with a pelagic stage" (Thorson 1950, p.10). He also believed that the best protection for embryos during development was given by species with viviparity or with highly organised brood protection (Thorson 1950).

The assumption that mortality during brooding is lower than in planktotrophic species has been generally accepted when discussing life history theory (e.g. Mileikovsky 1971, Vance 1973a, 1973b, Chia 1974, Strathmann 1985, Emlet et al. 1987). Chia (1974) further postulated that larval mortality increased with the number of eggs produced, and in non-feeding forms increased with the distance of larval development from the female (viviparity → brooded → benthic → pelagic).

Determination of natural mortality rates in marine invertebrate larvae has presented a formidable challenge to scientists for many years (Rumrill 1990). Although there are numerous estimates of larval mortality in planktonic species (see reviews: Chaffee and Strathmann 1984, Strathmann 1985, Hines 1986, Rumrill 1990) there are few studies that focus on mortality during encapsulation or brooding (see Rumrill 1990, Rawlings 1990). These and other authors have noted the need for estimates of mortality in non-pelagic species, and the importance of estimating "the relationship between the energy put into brood protection and the reduction in mortality which results" (Grant 1983, p. 554).

There is some evidence of mortality during brooding in asteroids (Menge 1974, Hendler and Franz 1982, Strathmann et al. 1984) and ophiuroids (Rumrill 1982), and prior to hatching in holothuroids (Rutherford 1973). However, these studies make assumptions about fertilization success which may be inappropriate and result in inaccurate estimates of mortality. Menge (1974) based his initial embryo number on the regression between egg number and female body size, assuming 100% fertilization. This assumption may not be valid in broadcast spawning species (e.g., Pennington 1985, Levitan et al. 1992, Sewell and Levitan 1992 and references therein) and is generally unknown in brooders. Fertilization rates of less than 100% will result in under-estimates of survival and over-estimate instantaneous mortality (Rumrill 1990). The remaining studies on mortality assume that there is the same degree of fertilization in each female, and estimate mortality based on the difference in the number of early and late stage embryos in different females (Hendler and Franz 1982, Strathmann et al. 1984, Rumrill 1982) or consider only pre-hatching mortality (Rutherford 1973).

Accurate estimates of mortality during brooding can only be obtained if the initial number of embryos can be determined. A unique situation for measuring embryo mortality was found in the intraovarian brooding sea cucumber Leptosynapta clarki. This species broods pentactulae in the ovarian tubules (= viviparity, Komatsu et al. 1990, Wourms and Callard 1992) from November to April or May (Everingham 1961, Chapter 2). During the pentactula stage a calcareous ring is present at the base of the tentacles (Everingham 1961, Chapter 2). While death of the pentactula results in the resorption of the embryonic tissues, the calcareous ring remains visible in the ovarian tubule. Counts can thus be made of the numbers of living and dead pentactulae and the mortality rate determined for each female. This chapter reports mortality in Leptosynapta clarki pentactulae throughout the brooding period and suggests that mortality in a viviparous species can be extremely high.

B. Materials and Methods.

Adult Leptosynapta clarki were collected at two sites in Grappler Inlet, Bamfield, British Columbia (Site 1: 48°49'57" N, 125°06'45" W; Site 2: 48°50' N, 125°06'36" W). Site 1 is located on the northern side of Barge Bay, has a short intertidal area (ca. 20 m) and a high proportion of gravel in the substrate. Site 2 is a gentler sloping mudflat (intertidal area ca. 60 m) at the opposite end of the channel separating "No-name" Island from Vancouver Island proper (see Chapter 2, Fig. 2-1). This site has a finer sediment composition with little gravel and contains Leptosynapta clarki of a larger size than Site 1 (Mean total weight ± SD: Site1: 307.8 ± 153.99 mg, N=720; Site 2: 490.4 ± 268.80 mg, N=660, Chapter 2). Both sites have extensive Zostera sp. beds in the lower intertidal that extend into the shallow subtidal.

Leptosynapta clarki were collected from the mid-intertidal at each site, transported in buckets of mud to the laboratory and kept overnight in running sea water. Sea cucumbers were removed from the sediment using a 0.85 mm sieve and placed in a bowl of sea water. Female sea cucumbers that had not autotomized their posterior ends were chosen at random from the bowl and relaxed in 2.5% $MgCl_2$ in sea water (w/v) for 10-15 minutes. After relaxation the sea cucumber was placed on a tissue to remove external water and weighed on a fine-scale balance (= total weight in mg). A longitudinal incision was then made from the base of the calcareous ring to the posterior, the coelomic water drained from the body cavity and the sea cucumber reweighed (= drained weight).

The ovary of Leptosynapta clarki is located in the anterior coelom and consists of two gonad tubules, one to each side of the dorsal mesentery (Chapter 2). The two gonad tubules were dissected from the coelom of each female under a binocular microscope, placed on a glass slide (38 x 75 mm) with a small amount of sea water and temporarily mounted with a coverslip (35 x 50 x 0.02 mm). The weight of the coverslip revealed the pentactulae within the ovary without damaging the tubule. The slide was placed under a dissecting microscope and the number of living pentactulae, dead pentactulae or calcareous rings were recorded for each female. A conservative attitude was adopted in counting a pentactula as dead; in tubules where there was extensive resorption of the dead pentactulae the number of calcareous rings were counted. Photographs of ovaries with dead pentactulae were taken on a Leitz compound microscope immediately after counting was completed.

Samples of female Leptosynapta clarki were taken at Sites 1 and 2 at monthly intervals throughout the brooding period (Nov.-May, Chapter 2). Ten females were dissected from each site at each time interval. However, not all females were brooding pentactulae so the number of females on which mortality is based varied between 1 and 10 at each site. In February 1991, mortality estimates are also included from females brooding pentactulae in gonad index dissections (Chapter 2).

Relationships between features of the mother (female size, number of pentactulae brooded) and percent mortality were tested by combining data from Sites 1 and 2 for each month. As additional pentactulae mortality might occur after sampling, the data for each month were not pooled for analysis. Ordinary Least Squares (OLS) regressions were calculated using Microsoft Excel Version 3.0.

C. Results.

1. Mortality estimates.

Embryonic development in Leptosynapta clarki has been described by Everingham (1961) at False Bay, San Juan Island, Washington. Hatching occurs after the prepentactula stage to form a 5-tentacled pentactula which is able to move freely in the ovarian tubule (Everingham 1961, Chapter 2). Development to the pentactula stage occurs in approximately 1-2 weeks (Chapter 2), so the majority of the brooding period is spent as a pentactula (Chapter 2).

During dissections, dead pentactulae were observed in the ovarian tubules. Dead pentactulae had a discoloured body wall due to early resorption (Fig. 5-1A), closed tentacles and an incompletely connected gut (Fig. 5-1B), or the pentactula was in a contorted position. In more advanced stages of resorption the body of the pentactula was a "ghost" around the calcareous ring (Fig. 5-1C), or the calcareous ring was the only structure remaining in the ovarian tubule (Fig. 5-1D). By totalling the number of living pentactulae, dead pentactulae and calcareous rings the initial number of brooded pentactulae could be determined and mortality calculated.

Mortality of pentactulae during brooding varied between females, ranging from 0-100% (Table 5-1, Fig. 5-2). During the early part of the brooding period, the percent mortality was generally low but it increased over time (Table 5-1, Fig. 5-2). Differences in mortality were also seen between sites and years. Site 2 had a higher mean percent mortality in all samples in both years (Table 5-1), and the mean percent mortality was higher in each month during 1990-1991 than in 1989-1990 (Table 5-1). The number of females that had lost the entire brood was also higher in the 1990-1991 reproductive season (1989-1990: 1 female; 1990-1991: 8 females; Fig. 5-2). This reproductive season was characterised by its unusually cold conditions in December and January (Days with temperatures below 0°C in Dec.and Jan.: 1989-1990= 9 d; 1990-1991= 25 d, P. Janitas unpub. data) and on many days the intertidal was overlain with discontinuous ice sheets (pers. obs.).

Females that had 100% mortality ranged in size from 141-671 mg total weight (mean ± SD: 372 ± 180 mg, N= 9)and are within the size range of females with <100% mortality (82-1161 mg total weight, mean ± SD: 368 ± 184 mg, N=146). However, females with 100% mortality were brooding a lower number of pentactulae (mean ± SD: 30.7 ± 34.7 pentactulae, range 1-99, N= 9) than females with <100% mortality (mean ± SD: 173.5 ± 172.9 pentactulae, range 1- 839, N=146). Two females contained only a single dead pentactula.

2. Instantaneous mortality.

Estimates of instantaneous mortality (day^{-1}) were calculated for pentactulae brooded in female Leptosynapta clarki in March and April (1990, 1991) in the period

Figure 5-1. Photomicrographs of Leptosynapta clarki ovarian tubules during the brooding period.

A. Dead pentactula with discoloured body wall. Bar= 500 µm.

B. Anterior end of dead pentactula showing calcareous ring (cr) at base of tentacles and resorbing intestine (i). Bar= 500 µm.

C. Dead pentactula with extensive resorption of the soft body tissues. Calcareous ring is visible within "ghost" of body. Bar= 500 µm.

D. Complete resorption of dead pentactula. Calcareous ring is the only remaining structure. Bar= 200 µm.

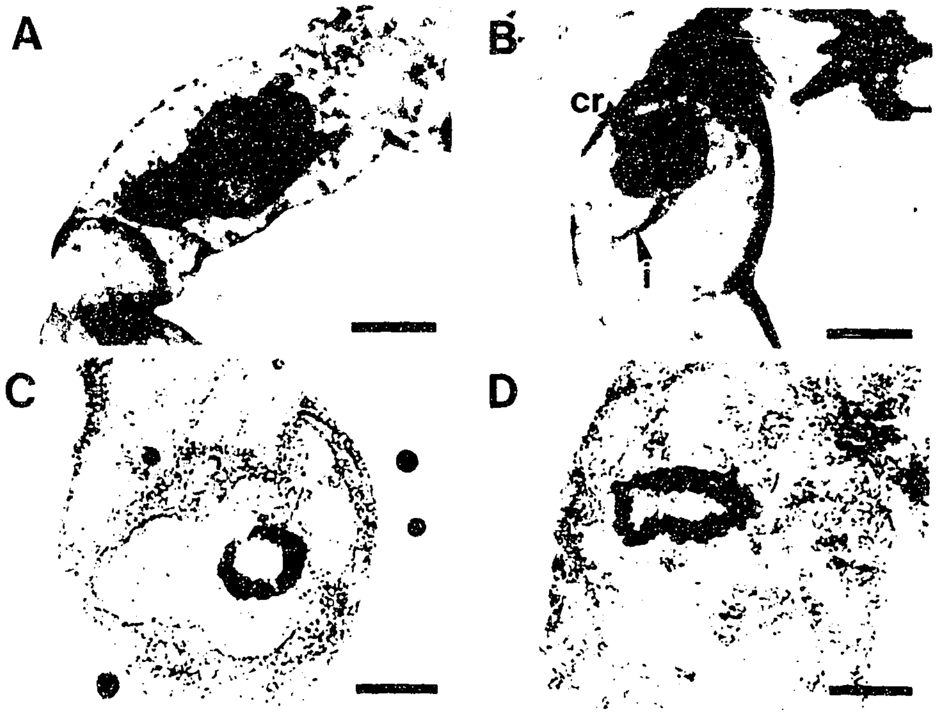

A
B
cr
i
C
D

Table 5-1. Mean percent mortality of brooded pentactulae in female Leptosynapta clarki. Samples were taken at Site 1 and Site 2 in the 1989/1990 and 1990/1991 seasons. Sample size (N) refers to the number of females in which pentactulae were present.

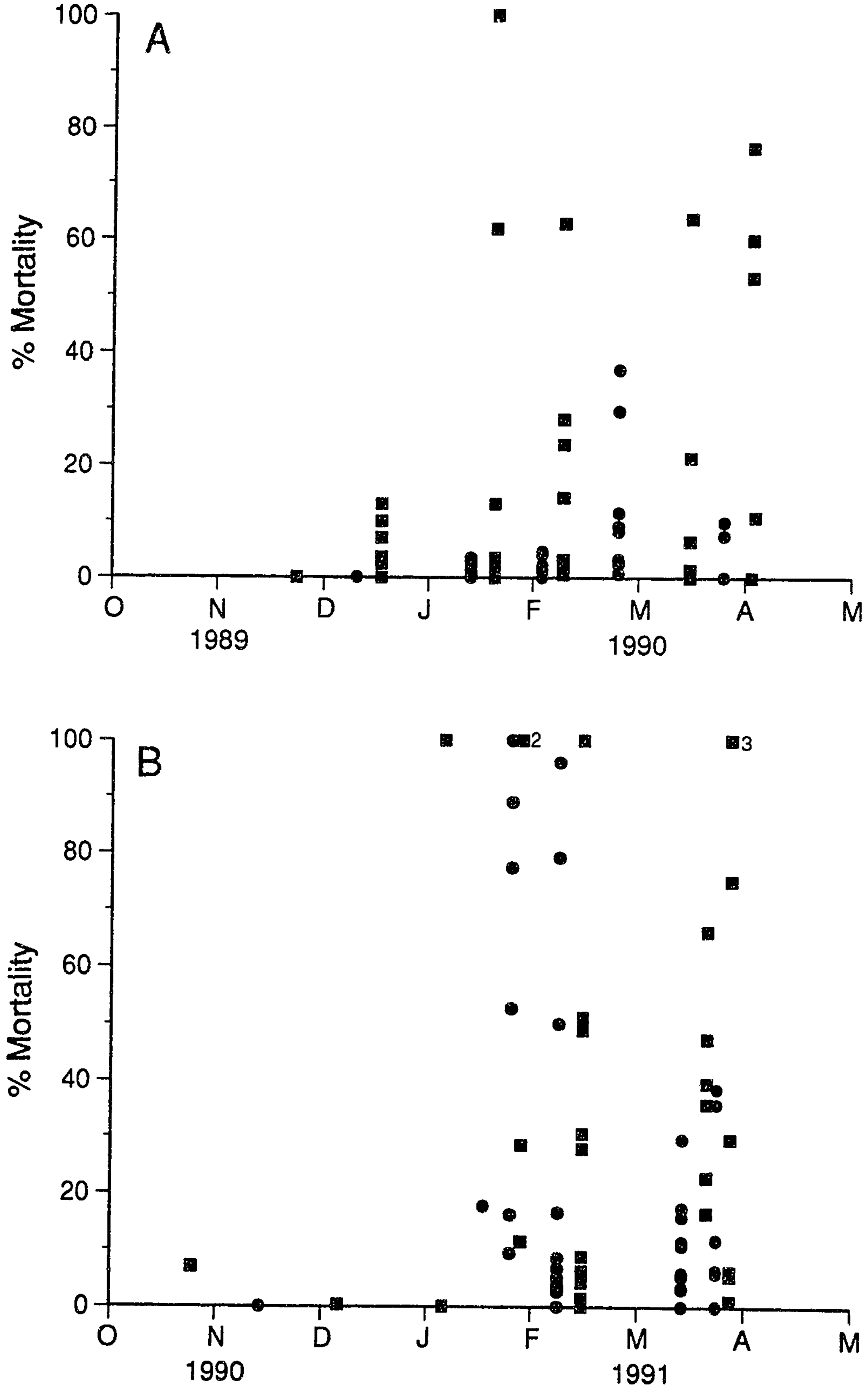
Site 1:

DATE	MEAN	SD	N	RANGE
16 December 1989	0	0	4	
19 January 1990	1.27	1.10	10	0 - 2.70
9 February 1990	1.59	1.76	9	0 - 4.62
3 March 1990	10.54	12.72	10	0.65 - 37.04
3 April 1990	5.77	5.12	3	0 - 9.8
18 November 1990	0	-	1	
24 January 1991	17.56	-	1	
1 February 1991	57.37	38.06	6	9.35 - 100
25 February 1991	22.93	33.23	12	0 - 96.08
23 March 1991	9.53	8.61	11	0 - 29.41
2 April 1991	12.24	15.88	8	0 - 38.46

Site 2:

DATE	MEAN	SD	N	RANGE
23 November 1989	0	0	5	
18 December 1989	4.31	4.72	9	0 - 13.04
21 January 1990	25.85	39.46	7	0 - 100
10 February 1990	15.14	20.83	9	0.51 - 62.86
4 March 1990	11.61	22.28	8	0 - 63.64
6 April 1990	40.10	33.04	5	0 - 76.47
24 October 1990	6.9	-	1	
6 December 1990	0.31	-	1	
17 January 1991	50.00	70.71	2	0 - 100
30 January 1991	59.92	46.80	4	11.32 - 100
27 February 1991	20.66	28.93	14	0 - 100
25 March 1991	37.95	17.83	6	16.42 - 66.15
1 April 1991	46.98	45.74	9	0.93 - 100

Figure 5-2. Percent mortality of pentactulae during brooding in Leptosynapta clarki at Site 1 (circles) and Site 2 (squares). A. Mortality in the 1989-1990 reproductive season. B. Mortality in the 1990-1991 reproductive season. Points for Site 1 are offset by five days to avoid overlapping points. In B, numbers adjacent to values of 100% mortality describe the number of observations with that value.


A
% Mortality
100
80
60
40
20
0
O
N
D
J
F
M
A
M
1989
1990
B
% Mortality
100
80
60
40
20
0
2
3
O
N
D
J
F
M
A
M
1990
1991

prior to pentactulae release (Everingham 1961, Chapter 2). The formula used was as defined in Strathmann (1985) and Rumrill (1990):

$$M = \ln (N_0 / N_t) / -t$$

assuming that fertilization occurred on December 1st of each year. This date was chosen as it is approximately the centre of the spawning period in Leptosynapta clarki at Bamfield (Chapter 2).

Values for instantaneous mortality in Leptosynapta clarki are low (Table 5-2) primarily because of the long brooding period (t = 93-128 days). Ten females had zero mortality (M=0). Three females had lost their entire brood (i.e. $N_0 / N_t = 0$) and the value of M could not be calculated. For comparison to other species the grand mean for females with some living pentactulae is -0.0021 day^{-1} (SD= 0.0030, N=57).

3. Mortality in relation to features of the mother.

No clear relationships were seen between the percent mortality and features of the mother such as size (drained weight) or number of pentactulae brooded (Table 5-3). The relationship between percent mortality and the size of the female was non-significant for each sampling date except March 1991 (Table 5-3). At this time the largest females had the highest percent mortality (Fig. 5-3).

The relationship between percent mortality and the total number of pentactulae brooded was similarly significant in January 1990 and February 1991 only (Table 5-3, Fig. 5-4). In both cases the r^2 for the OLS regression is less than 0.3 (Table 5-3) and there is considerable scatter in the relationship (Fig. 5-4). In these months there is a trend for the highest percent mortality in those females brooding the fewest pentactulae (Fig. 5-4).

D. Discussion.

The retention of the calcareous ring of dead pentactulae in the ovarian tubules Leptosynapta clarki has provided a unique system for studying mortality in a viviparous marine invertebrate as the number of pentactulae beginning the brooding period can be determined. As the calcareous rings or pentactulae are retained within a discrete structure, there is no error due to the loss of embryos during sampling (as in Leptasterias tenera, Hendler and Franz 1982). Additionally, as the pentactulae are released from the ovary at the same point in time (Chapter 2) errors associated with the release (Rumrill 1982) or migration of early maturing juveniles (Hendler and Franz 1982) are avoided.

Table 5-2. Instantaneous mortality rates (M, day^{-1}) for pentactulae brooded in female Leptosynapta clarki in March and April 1990, 1991. Formula as defined in text and assuming the beginning of the brooding period as December 1st.

Date	mean M	SD	minimum M	maximum M	N
March 1990	-0.0015	0.0027	-0.0108	0	13
April 1990	-0.0034	0.0043	-0.0114	0	6
March 1991	-0.0022	0.0025	-0.0094	0	17
April 1991	-0.0018	0.0031	-0.0114	0	14 *

* Three females had no living embryos (i.e. 100% mortality). Values for M could not be calculated as $(N_0 / N_t) = 0$ and are omitted from the mean value for M.

Table 5-3. Ordinary Least Squares regressions between female size (drained weight), total pentactulae number and observed mortality (%). Data points from Sites 1 and 2 are combined for each sampling period in 1990 and 1991. Values given are F-statistic (F) and r^2 for regression. Significance of regression: NS = nonsignificant, * $p < 0.05$, ** $p < 0.01$.

	Drained Weight (mg)		Total Pentactulae Number		
Date	F	r^2	F	r^2	N
January 1990	0.016^{NS}	0.001	4.851^{*}	0.244	17
February 1990	0.094^{NS}	0.006	2.889^{NS}	0.152	18
March 1990	0.0004^{NS}	2.3×10^{-5}	0.297^{NS}	0.018	18
April 1990	2.140^{NS}	0.263	0.761^{NS}	0.113	8
January 1991	2.659^{NS}	0.347	0.165^{NS}	0.032	7
February 1991	1.231^{NS}	0.039	9.268^{**}	0.236	32
March 1991	5.325^{*}	0.262	2.117^{NS}	0.124	17
April 1991	0.030^{NS}	0.002	2.164^{NS}	0.126	17

Figure 5-3. Relationship between drained weight and percent mortality of pentactulae brooded in female Leptosynapta clarki in March 1991.

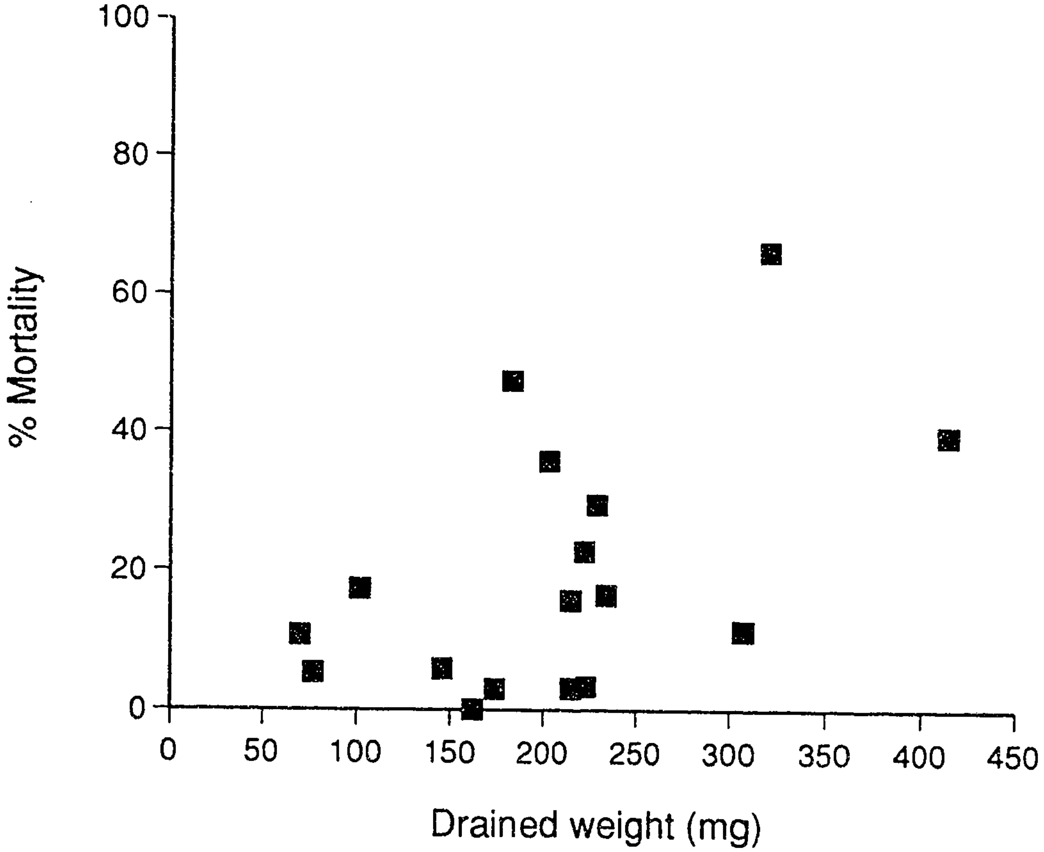
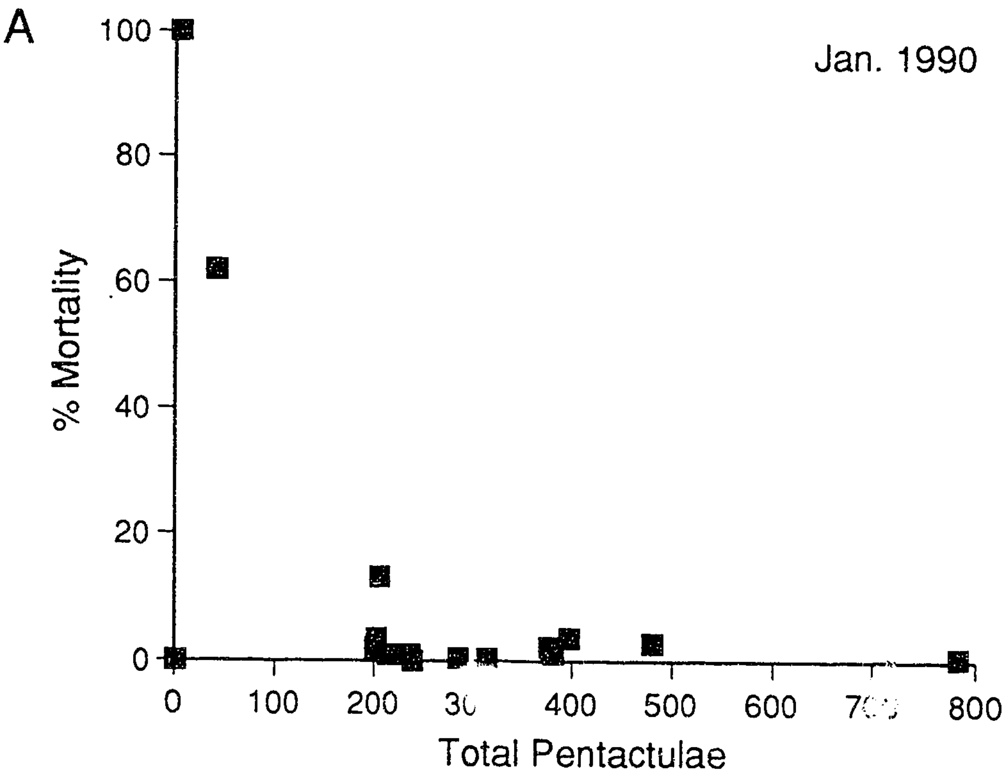
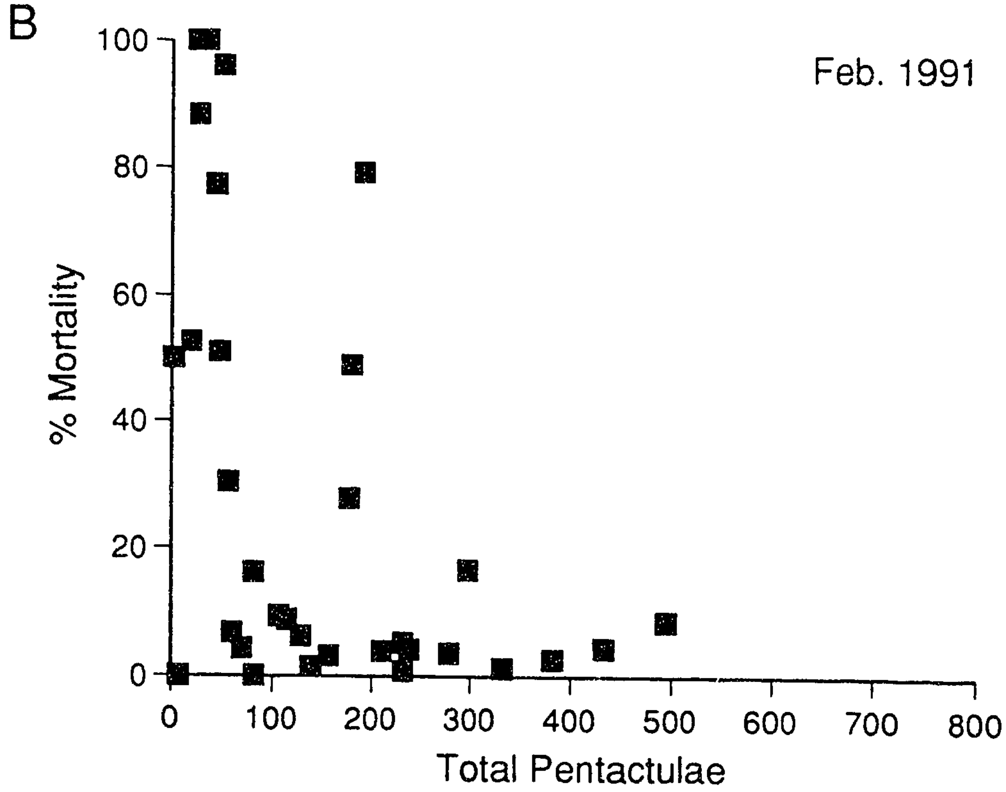

100
80
60
40
20
0
% Mortality
0
50
100
150
200
250
300
350
400
450
Drained weight (mg)

Figure 5-4. Relationship between total pentactulae number and percent mortality of pentactulae brooded in female Leptosynapta clarki in January 1990 and February 1991.


A
Jan. 1990
% Mortality
0
20
40
60
80
100
Total Pentactulae
0
100
200
30
400
500
600
7
800


B
Feb. 1991
% Mortality
0
20
40
60
80
100
Total Pentactulae
0
100
200
300
400
500
600
700
800

Two assumptions have, however, been made in the present study: a) that there is negligible mortality in the period prior to hatching of the pentactulae. In Leptosynapta clarki this is probably a valid assumption as the pentactulae hatch from the follicular membrane 1-2 weeks after fertilization (Chapter 2). Embryos, therefore, have a calcareous ring for ca. 93% of the 30 week brooding period (Chapter 2). b) This method assumes that there is limited resorption of calcareous rings within the ovarian tubule. There is currently no evidence to suggest that the calcareous rings are resorbed. In some dead pentactulae the connective tissue holding the pieces of the ring was resorbed but in all cases the calcareous pieces of the ring remained in the tubule.

By studying embryonic mortality in a brooding species we are able to examine mortality within a single female. While planktonic mortality rates by other authors (see reviews: Chaffee and Strathmann 1984, Strathmann 1985, Hines 1986, Rumrill 1990) are taken on a mixture of offspring from any number of females, in brooders the characteristics of the mother, such as size, may be directly related to the reproductive success of a specific female (number of embryos successfully brooded).

However, my data suggest no clear relationship between maternal features and reproductive success. Strathmann and Strathmann (1982) suggested that larger broods from larger adults might develop more slowly or suffer higher mortality during brooding. In Leptosynapta clarki while there is a trend for larger females to have a higher number of pentactulae, this relationship is weak and probably a consequence of problems with internal fertilization regardless of female size (Chapter 4). Although sample sizes are small, there is no consistent trend for a higher degree of mortality in large females or females with the largest number of pentactulae. In fact in the two samples with significant relationships, the highest percent mortality was observed in females brooding the fewest pentactulae.

While the data from Leptosynapta clarki suggest that mortality in a viviparous species can be extremely high, the reasons for death of pentactulae within the ovary are at present unknown. Pentactulae might die because of insufficient supplies of food or oxygen, genetic abnormalities, or the buildup of waste products within the ovary. Once dead, however, resorption of the dead pentactulae may be important in providing nutrition for the remaining pentactulae. Initially, nutrition for the pentactulae is provided from the breakdown of unfertilized eggs (Everingham 1961, McEuen 1986, Chapter 2). Later in the brooding period there are no discrete droplets visible in the tubules and the pentactulae feed on the fluid itself (Everingham 1961, Chapter 2). Resorption of the dead pentactulae may be important in providing additional organic material to the ovarian fluid.

While loss of the entire brood has been observed in other brooding echinoderms (Engstrom 1982, Strathmann et al. 1984), observations of non-brooding females might alternatively result from unsuccessful fertilization. In contrast, although eggs of some female Leptosynapta clarki are not fertilized during the spawning period (Chapter 2), the presence of calcareous rings in the tubules confirms that some female Leptosynapta clarki that were fertilized lose their entire brood.

Leptosynapta clarki shows values of instantaneous mortality of a similar order of magnitude to other marine invertebrates with benthic development (0.0046-0.0734 d^{-1} Rumrill 1990). However, as noted earlier, these comparative data need to be viewed with some caution because of assumptions about fertilization and the possibility of embryo release during sampling. Additionally, instantaneous mortality rates are probably not constant over the interval of observation in these studies (Chaffee and Strathmann 1984). Seasonal changes in mortality have been observed in Leptasterias tenera which broods slow-developing abortive embryos late in the season (Hendler and Franz 1982) and in the present study.

An additional source of mortality in brooding marine invertebrates not considered in a measure of instantaneous mortality results from mortality of the mother during the brooding period. For example, Asterina phylactica are consumed while brooding by the larger Asterina gibbosa (Emson and Crump 1976), presumably resulting in death of the brood. Female Leptasterias hexactis dislodged by water movement may also lose their entire clutch (Menge 1974), though this might not result in death of the female. In Leptosynapta clarki during periods of high rainfall and low intertidal salinity adult sea cucumbers were found dead on the sediment surface (unpub. obs.). Unless the pentactulae are released beforehand, death of the female will result in death of her brood as well.

The presence of an embryonic structure that is retained by the parent provides a method to study both fertilization and mortality in internal brooders. Preliminary data collected in Leptosynapta clarki suggests that mortality in a viviparous species can be extremely high, and questions the assumption that the lowest mortality will be found in species with the most advanced brood protection (Thorson 1950). The information available from this species is, however, insufficient to test Chia's (1974) hypothesis of decreasing mortality with increased brood protection.

Brooding sea cucumbers may provide better estimates of embryonic mortality than previous studies of brooding echinoderms (Menge 1974, Hendler and Franz 1982, Rumrill 1982, Strathmann et al. 1984) and allow a direct test of Chia's (1974) hypothesis. At least 41 species of holothurians brood their young, with 26 species brooding externally or in pouches, 11 species brooding internally in the coelom and 4 species in the ovary or ovotestis (Smiley et al. 1991, Materia et al. 1991). Species chosen for such a comparative test should brood within a structure (pouch, ovary) or discrete area (e.g. under the female) that will retain the calcareous rings of any dead pentactulae. Comparing mortality in external brooders to coelomic and ovarian brooders such as Leptosynapta clarki could provide the basis for testing Chia's (1974) hypothesis in brooding sea cucumbers.

It is also possible that the present method may be applicable to measuring mortality in internal brooders such as gill-brooding bivalves and in the 13 other intraovarian brooding echinoderms (see Chapter 1). Messing (1984), for example, observed a sphere with the intraovarian embryos of the crinoid Comatilia iridometriformis that might represent an embryo undergoing resorption. Degenerating embryos observed fused to the internal surface of adult Ophionotus hexactis (Morison 1979) may have been too large to exit the bursae (Turner and Dearborn 1979), or alternatively may have died earlier in development. Careful dissections in such species to look for remains of embryonic structures might provide further estimates of mortality during brooding.

Because most species probably experience highest mortality during their early developmental period, Vance (1973a) concluded that "those forces affecting larval mortality probably play a very important role in the evolution of the general pattern of reproduction" (p. 339). Thus we should continue to collect information on larval mortality in species with a wide range of reproductive patterns. By focusing attention on encapsulating and brooding species we may be able to understand the importance of larval mortality in the evolution of life history strategy.

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CHAPTER 6

Birth, recruitment and juvenile growth in the intraovarian brooding sea cucumber Leptosynapta clarki.

A. Introduction.

The majority of sea cucumbers are broadcast spawners, with external fertilization and planktonic development. However, at least 41 species of holothurians brood their young under the sole, in the tentacles, in brood pouches or pockets, or internally in the coelom or ovary (Smiley et al. 1991). Although there is some information on life history characters and embryonic development in brooding species (Smiley et al. 1991), there is limited information on the events occurring immediately after juvenile release. For internal brooders, only one study has observed juveniles in the benthic habitat soon after release, and gives information on early growth (Engstrom 1980).

Leptosynapta clarki is an intraovarian brooding apodid holothurian found on intertidal mudflats on the west coast of Vancouver Island, Canada. During brooding, the fertilized eggs develop rapidly to the pentactula stage (1-2 weeks, Chapter 2) and remain in the ovary for 5-6 months (Everingham 1961, Chapter 2). Although the embryology of brooding in Leptosynapta clarki has been described (Everingham 1961) the subsequent fate of the juveniles after release has not been determined. In this study a recruitment pulse of newly released juveniles was observed on the mudflats in the late spring (April/May). Growth of the juveniles could be followed in the field until the end of the summer, when rapid growth made them indistinguishable from sea cucumbers of age > 1 year. Hereafter, laboratory growth experiments allowed growth to be measured until the sea cucumbers were 1 year old. This chapter provides the first detailed field study of growth of an apodid sea cucumber from the point of juvenile release, and represents a first step in our understanding of the early life history in Leptosynapta clarki after release of pentactulae from the ovary.

B. Materials and Methods.

1. Measurement of size.

Measurements of length (in mm) were made after relaxation of the sea cucumbers in 2.5% $MgCl_2$ in sea water (w/v) in a 90 mm petri dish. During relaxation the sea cucumbers would often retract or withdraw the tentacles. To standardise this measurement, length was measured from the anterior end of the calcareous ring to the posterior while in the $MgCl_2$ relaxation solution. Animals less than 20 mm were measured on a dissecting microscope using an eyepiece graticule calibrated against a stage micrometer. Larger sea cucumbers were gently held straight with tweezers and length measured to the nearest millimetre with reference to a small plastic ruler under the petri dish.

After measurement of length, the sea cucumber was placed on a tissue to remove external water, and weighed on a fine scale balance to the nearest mg (total weight). There are some errors in this measure as the weight includes sediment and/or water in the alimentary canal. However, sea cucumbers left in sea water for removal of sediment tended to autotomize the posterior end, which led to greater inaccuracies in measurement of size.

2. Size of pentactulae prior to release.

Female Leptosynapta clarki were collected from Barge Bay, Grappler Inlet, Bamfield, British Columbia (48°49'57" N, 125°06'45" W) on 13 April 1990 (N=6) and during late March and early April 1991 (23 to 30-03-91 and 8-04-91, N=29). Females were relaxed in 2.5% $MgCl_2$ and the gonad dissected into a petri dish. Pentactulae were removed from the gonad tubules by gentle teasing with fine needles and size was measured as described above. In 1990 the pentactulae from four females were used to set up subcontainers 1-6 as a pilot study for the growth experiments (N= 38 pentactulae; see details below). Pentactulae from the remaining two females were used in pilot studies to test for recovery after relaxation in the $MgCl_2$ solution.

3. Recruitment and growth in the field.

Recruitment in Leptosynapta clarki was monitored in sediment cores from the mid-intertidal mudflat in Barge Bay (Site 1: 48°49'57" N, 125°06'45" W). Haphazard samples were taken using a perspex core (cross-sectional area= 78.54 cm^2) to a depth of ca. 10 cm (N=10 cores; Total area sampled = 785.4 cm^2). Core samples were combined in buckets and returned to the laboratory. Pilot studies found no sea cucumbers below 10 cm because of the shallow anoxic layer (unpub. data). Samples were taken every two months from May 1990 until August 1991.

In the laboratory, the sediment was sieved through a 2 mm or 0.85 mm sieve to remove adults, and the remaining fraction sieved through a 0.25 mm sieve to remove juveniles. Animals were kept in sea water for a maximum of 4 hours before measurement (length and weight). From May until August 1990, only juvenile sea cucumbers were measured. Juveniles < 1 year old could be distinguished from older individuals as they were unpigmented. However, after August there was no clear distinction between age classes and measurements were made on all sea cucumbers in the sample. Weight of newly released juveniles in May 1991 was determined by regression (length vs log weight for animals 0-10 mm in length from the 15 July 1991 sample: log weight= 0.114(length) - 0.0197; $r^2 = 0.793$, N= 33).

4. Laboratory growth of juveniles.

Growth experiments were conducted in two flat plastic trays (41.7 x 35.6 x 11.5 cm, L x W x D), subdivided into six approximately equal divisions by wooden partitions sealed at the edges with silicon sealant (i.e. each subcontainer ca. 12.5 x 15.5 cm). Each subcontainer was provided with air from an airstone glued to the base. Airstones were constructed from a 7.5 cm length of 1 mm borosilicate disposable pipette with three small holes on the upper surface. The pipettes were covered with 64 μm Nitex screening which provided slow diffuse aeration throughout each subcontainer.

Each container was filled to a depth of 7-10 cm with sediment collected from Barge Bay. This sediment was sieved through a 0.85 mm sieve to remove any sea cucumbers before placing in the experiment. However, when mud was added to the container to replace sediment lost as a result of aeration, additional juvenile sea cucumbers were sometimes introduced. These animals could be identified because of their small size and were ignored in the size-frequency analyses. The containers were immersed in a 1.7 x 0.72 x 0.22 m sea water tray with flow-through sea water. Water temperature throughout the experiment ranged from ca. 8-11°C.

Juvenile sea cucumbers used in the growth experiment were obtained by dissection of the mother (subcontainers 1-6) as described above, or from field collections (subcontainers 7-12). Sediment was collected from Barge Bay on 14 May 1990, sieved through a 2 mm sieve to remove the adults, and then the remaining fraction spread in a shallow layer in trays and allowed to settle. Under bright light, reflection of the calcareous ring made juvenile Leptosynapta clarki visible on, or near the surface in shallow burrows. Juveniles were removed from the trays using a wide-bore pipette. Length was measured after relaxation in the $MgCl_2$ solution and the sea cucumbers placed in the sediment of the subcontainers.

Repeated measurements of size were made at 2-monthly intervals from May 1990 until May 1991. At such times sea cucumbers were removed from the sediment in each subcontainer using a 0.85 mm sieve, measured and returned to their respective subcontainers. The data presented are the pooled results of the two containers from 24

July 1990 until 12 May 1991. Although the experiment began in May 1990, the early results could not be used because of contamination of subcontainers 1-6 by additional juvenile sea cucumbers. In subsequent additions of sediment, special care was made to remove all sea cucumbers. The initial number of sea cucumbers in subcontainers 1-12 on 24 July 1990 was 245.

C. Results.

1. Field collections.

Pentactulae removed from the ovarian tubules in April 1990 ranged from 0.46 to 6.67 mm (mean ± SD= 2.00 ± 1.04 mm, N= 100). Pentactulae in most ovaries were less than 4 mm in length, with a modal size of 1-2 mm (Fig. 6-1). A similar size-frequency distribution was observed in late March 1991 (Fig. 6-2), although the sample was taken earlier and the mean length was lower at 1.24 mm (SD = 0.463 mm, N= 1735, range = 0.32-3.4 mm).

Birth of the pentactulae was never observed in nature, although in the laboratory normal handling of the female sea cucumbers during April and May would often result in the release of juveniles. Prior to release of the pentactulae from the coelom a break point occurs in the area between the brooding portion of the ovary and the basal region of renewed growth (Chapter 2). The posterior distal part of the ovarian tubule then becomes loose in the coelom (Chapter 2). During handling, the end of this tubule would often protrude out the body wall in the region of the anus. It is uncertain whether the tubule exited through the anus itself or through the body wall adjacent to the anus. Movement of the female resulted in the breaking of the thin ovarian wall and release of the juveniles. Female sea cucumbers were also very prone to autotomise during this period. Autotomy of the posterior end of the animal similarly exposed the tubules to the exterior and resulted in juvenile release. If sediment was provided, the juveniles rapidly formed shallow burrows.

After release from the mother at a length of 1-2 mm (Fig. 6-1, 6-2), rapid growth was observed in the field to a maximum of 5.00 mm in mid-May 1990 (Fig. 6-1) and 9.13 mm in late May 1991 (Fig. 6-2). By July, the modal length was 5-6 mm, with a maximum of 13.78 mm in 1990 (Fig. 6-1), and 14.77 mm in 1991 (Fig. 6-2). There is considerable variability in growth during the late summer, so that by August the juveniles are generally 6-16 mm in length (Fig. 6-1, 6-2).

Release of juveniles into the sediment could be observed as a recruitment pulse into the adult population in Barge Bay (Fig. 6-3). The juveniles released in April/May

Figure 6-1. Size-frequency distributions of Leptosynapta clarki pentactulae prior to release (grey bars) and during growth in sediment (black bars) in 1990.

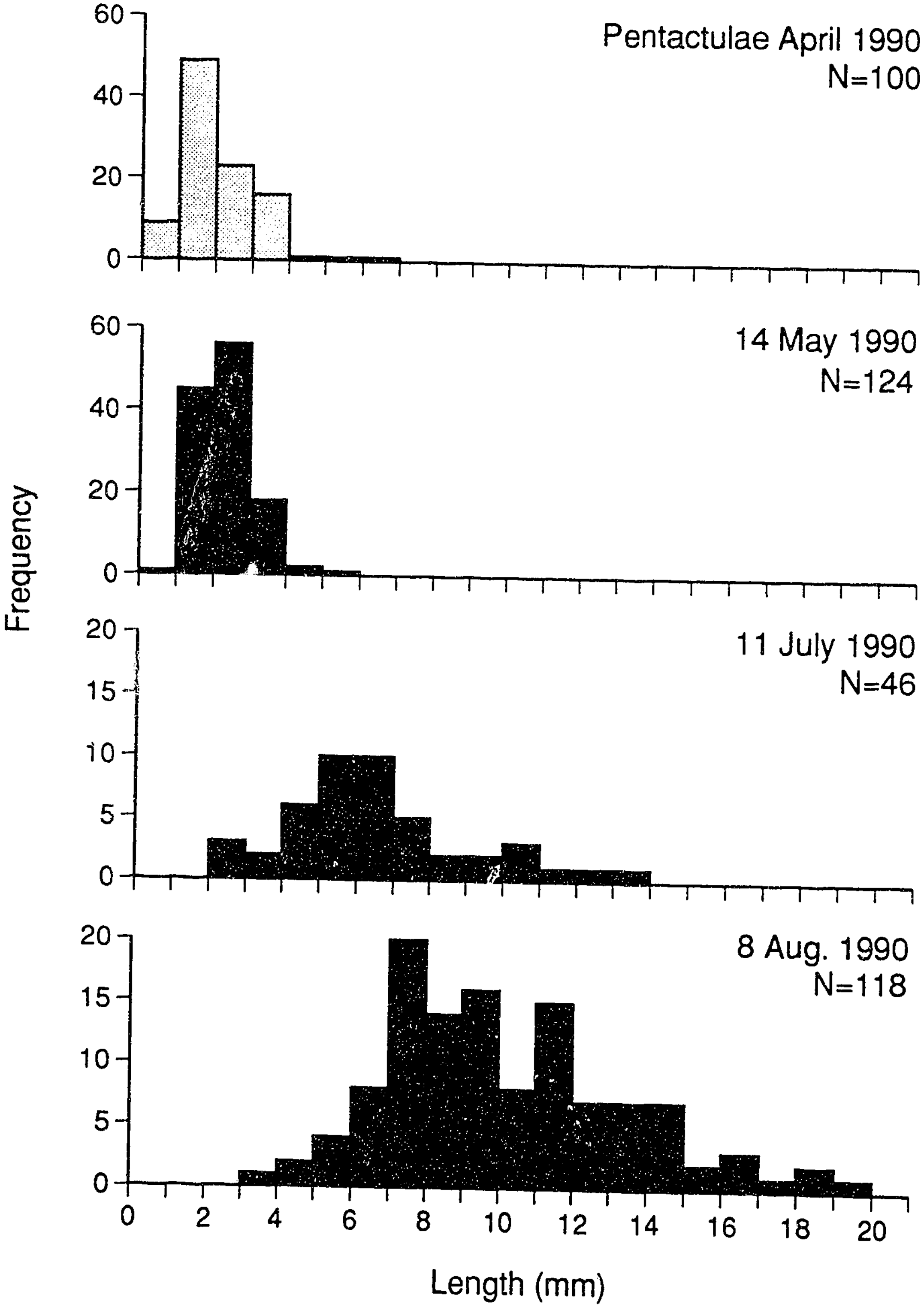

Pentactulae April 1990
N=100
14 May 1990
N=124
11 July 1990
N=46
8 Aug. 1990
N=118
Frequency
60
40
20
0
20
15
10
5
0
2
4
6
8
10
12
14
16
18
20
Length (mm)

Figure 6-2. Size-frequency distributions of Leptosynapta clarki pentactulae prior to release (grey bars) and during growth in sediment (black bars) in 1991.

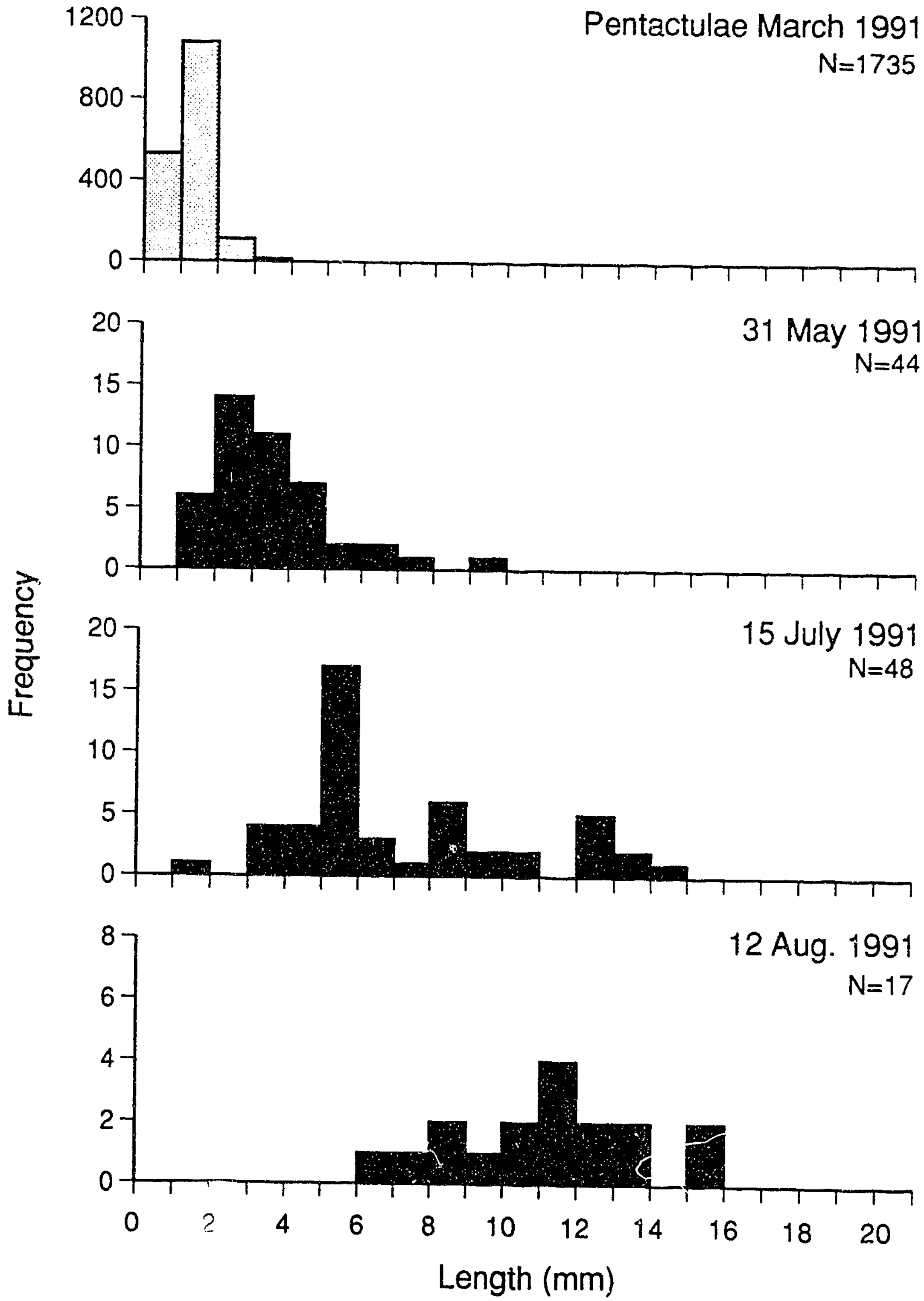
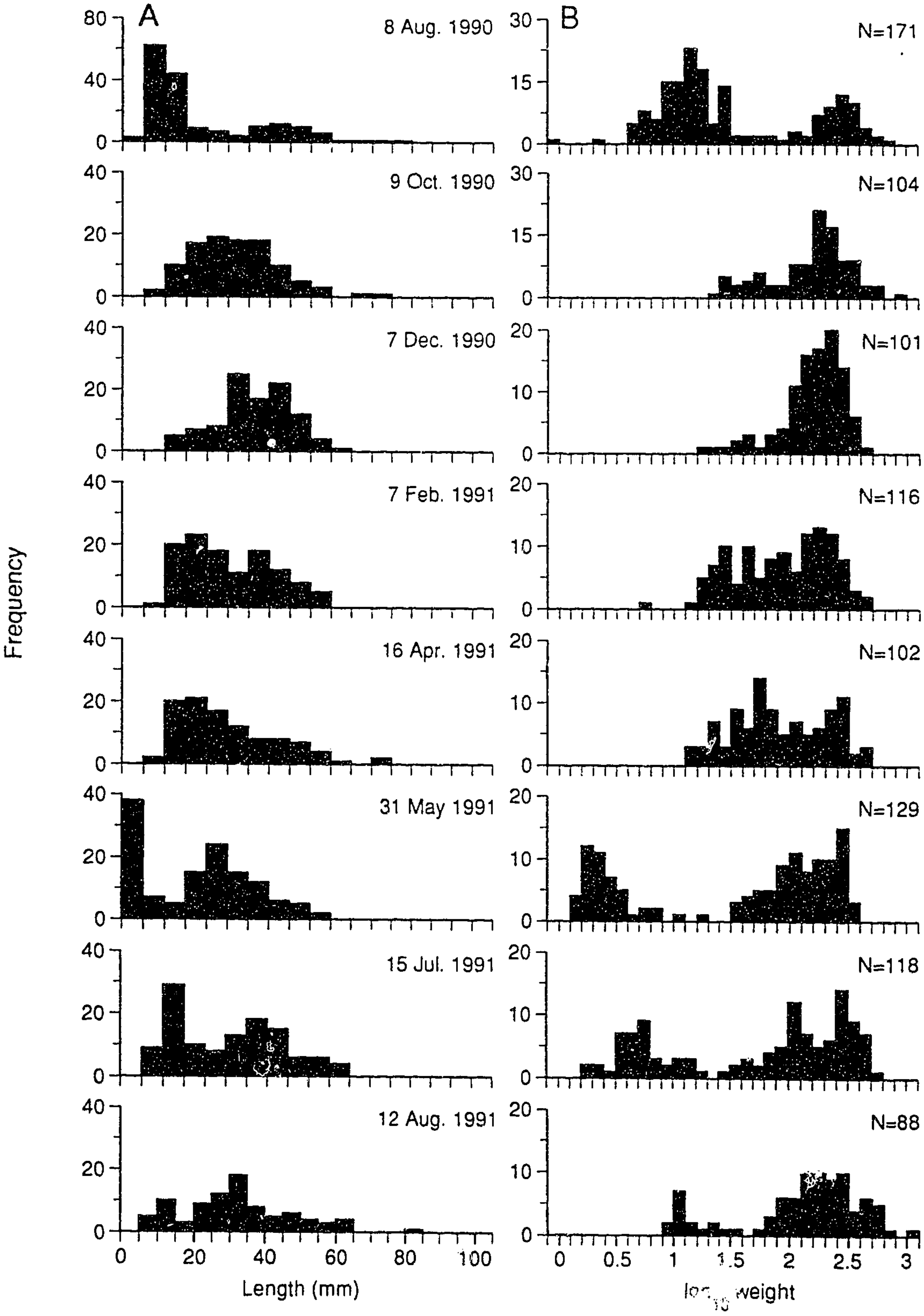

Pentactulae March 1991
N=1735
1200
800
400
0
31 May 1991
N=44
20
15
10
5
0
15 July 1991
N=48
20
15
10
5
0
12 Aug. 1991
N=17
8
6
4
2
0
Frequency
0
2
4
6
8
10
12
14
16
18
20
Length (mm)

Figure 6-3. Size-frequency distributions of Leptosynapta clarki in Barge Bay from August 1990 to August 1991. A. Size measured as length (mm). B. Size measured as $\log_{10}$-weight (original data in mg). Dates of collection are shown in A; number of sea cucumbers in sample in B.


A
B
Frequency
8 Aug. 1990
N=171
9 Oct. 1990
N=104
7 Dec. 1990
N=101
7 Feb. 1991
N=116
16 Apr. 1991
N=102
31 May 1991
N=129
15 Jul. 1991
N=118
12 Aug. 1991
N=88
0
20
40
60
80
100
Length (mm)
0
0.5
1
1.5
2
2.5
3
log10 weight

1990, were still visible as a distinct cohort in both length and weight in August (Fig. 6-3). By October, the juvenile cohort was indistinguishable from the adult population in length (Fig. 6-3A), and remained so throughout the autumn and winter. There were, however, a number of indistinct modes in weight (Fig. 6-3B). Newly released juveniles are clearly visible as a recruitment pulse in May 1991 (Fig. 6-3). This new cohort showed rapid growth through the summer (Fig. 6-2), and was still visible as a distinct cohort in both length and weight in August 1991 (Fig. 6-3).

The density of Leptosynapta clarki in the Barge Bay population was fairly constant throughout the period of sampling (mean ± SD: 168.8 ± 34.6 m^{-2}, N= 8). No consistent changes in density were noted throughout the year as both the lowest density (124.8 m^{-2}) and the highest density were found in August (244.5 m^{-2}). Density estimates of Leptosynapta clarki in Barge Bay may, however, be affected by the patchy distribution of juveniles (unpub. obs.).

The largest individual observed at this site was 82 mm in length and 1060 mg in weight. Specimens of Leptosynapta clarki do, however, attain larger sizes at other sites in Grappler Inlet. The largest specimen found at Bamfield had a length of 113 mm and weighed 1291 mg (Chapter 4).

2. Laboratory growth of juveniles.

Growth of Leptosynapta clarki in the laboratory was similar to that observed in the field collections (Fig. 6-4, Table 6-1), but growth could be monitored after August when the Year 1 juveniles were indistinguishable from adults in field samples. In late July 1990, juveniles in the laboratory showed a mode at 6-7 mm (Fig. 6-4), which was similar to that in the field (Fig. 6-1), although with a larger variability. Juveniles continued to grow rapidly until they reached a maximum of 30 mm by September (Fig. 6-4), and a mean weight of 23 milligrams (Table 6-1). No laboratory measurements were made in August to compare with the field collections, but the mean for the August field sample (mean ± SD: 10.22 ± 3.20 mm, N= 118) is intermediate between the laboratory means for July and September (Table 6-1). Based on the 1990 field collections, it appears that juvenile growth estimates from the laboratory are in the same range as those found in natural populations.

At the time of spawning of Leptosynapta clarki in November/December (Chapter 2), the maximum length of young of the year was 35.5 mm (Fig. 6-4, Sewell 1991). This particular individual was 200 mg total weight. In many of these Year 1 sea cucumbers white testes were observed through the body wall (10 Dec 1990 sample, 65 of 120 were identified as males). Dissections of sea cucumbers in this size range confirmed that

Figure 6-4. Laboratory growth in Leptosynapta clarki until an age of approximately 1 year. Size-frequency distributions in length (mm) are pooled results from subcontainers 1-12.

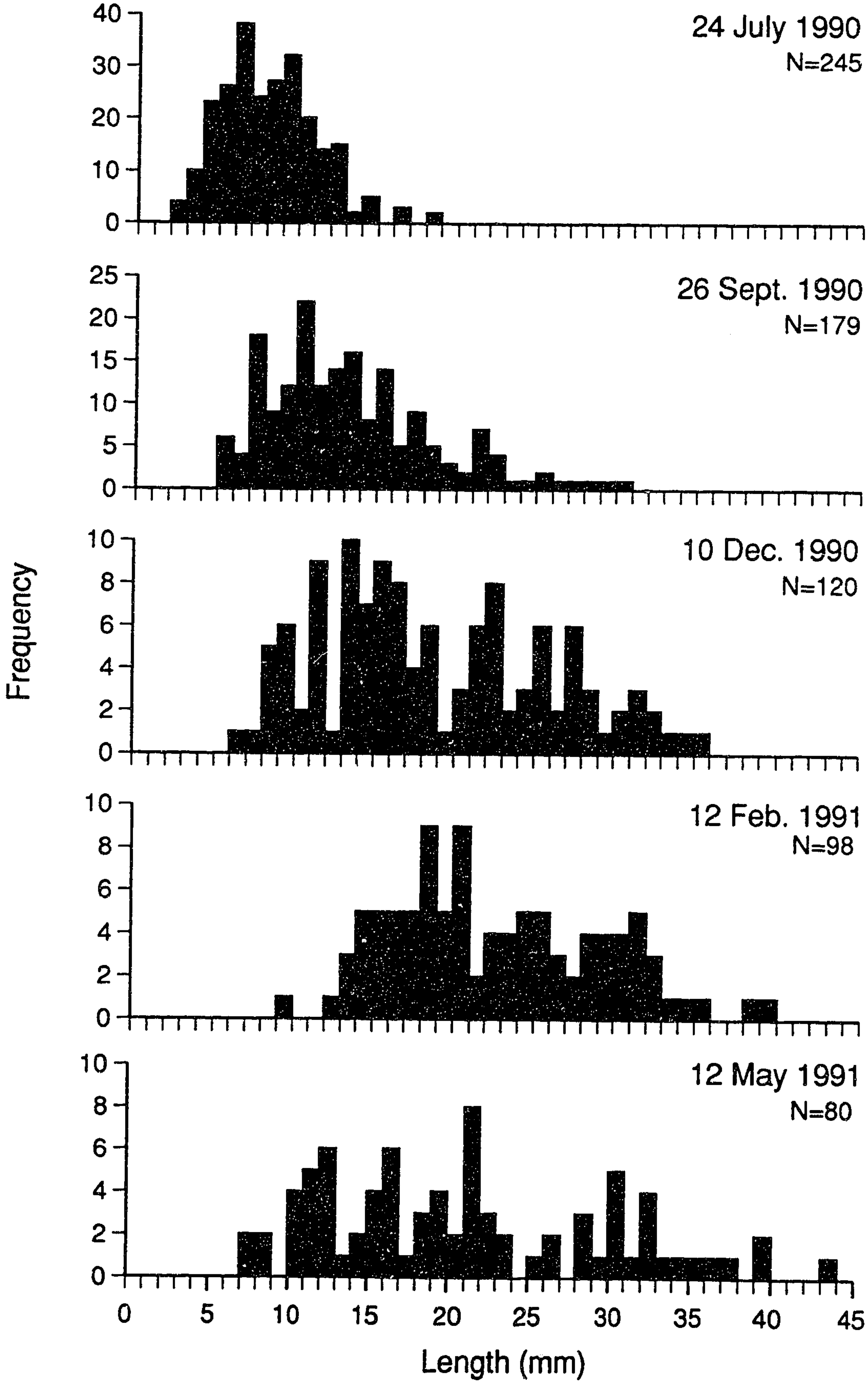

24 July 1990
N=245
26 Sept. 1990
N=179
10 Dec. 1990
N=120
12 Feb. 1991
N=98
12 May 1991
N=80
Frequency
Length (mm)
0
5
10
15
20
25
30
35
40
45

Table 6-1. Laboratory growth in *Leptosynapta clarki*. No weight measurements were made at the start of the experiment due to small size. Size-frequency data for this experiment are shown in Fig. 6-4.

	Length (mm)		Weight (mg)		
DATE	Mean	S.D.	Mean	S.D.	N
24 July 1990	8.19	3.04	-	-	245
26 Sept. 1990	13.37	5.13	23.0	18.6	179
10 Dec. 1990	18.73	6.95	53.4	46.3	120
12 Feb. 1991	19.61	6.27	50.2	31.1	98
12 May 1991	21.08	8.72	74.7	63.1	80

Leptosynapta clarki have active sperm and are reproductively active as males for their first reproductive season.

By the end of the first year, the mean size of juveniles was 21.1 mm length and 74.7 mg weight (Table 6-1). The largest juvenile was 44 mm in length (Fig. 6-2), and 261 mg in weight.

There is considerable variability in growth rate which is partly due to food availability, but is also related to unintentional damage during measurement. The small size and delicate nature of the body wall made it difficult to remove the animals after sieving. Juveniles also autotomize the posterior end which decreases the length and weight. Damage, poor conditions, and loss of some animals in measurement lead to a gradual decline in the number of sea cucumbers. Numbers were reduced from 245 to 80 (Table 6-1); an overall recovery of 33%.

D. Discussion.

Two principal methods have been described for the release of embryos from the coelom of internally brooding holothurians: (a) By rupture of the body wall (Leptosynapta clarki, Everingham 1961, present study; Staurothyone inconspicua, Materia et al. 1991; unidentified cucumariid, O'Loughlin 1991) or (b) by escaping through the anus, either by rupturing the rectal wall (Synapta vivipara, Clark 1898; Pachythyone rubra, Chaffee 1982; Lissothuria antillensis, Miller 1985) or via anal pores (Leptosynapta clarki, Brooks 1973). Embryos are also suggested to escape through rectal or abdominal pores in Leptosynapta minuta (Becher 1912, in Ohshima 1916) and Synaptula hydriformis (Turner 1973), and may escape through the anus in Neoamphicyclus lividus (Materia et al. 1991). Opportunistic release of embryos can also occur during autotomy (Brooks 1973, present study), evisceration (Chaffee 1982) or handling (Materia et al. 1991).

Once juvenile Leptosynapta clarki are released from the mother they burrow into the sediment immediately and are visible as a distinct recruitment pulse in the adult habitat. However, while not directly examined in this study there is anecdotal evidence for subsequent dispersal of juveniles from the site of release. This dispersal may occur by rafting, floating, swimming, or incidental dispersal as a result of currents or wave action.

Rafting has been observed in a single Leptosynapta clarki in Mitchell Bay, San Juan Island, Washington on an Ulva-Monostroma algal mat (Highsmith 1985). During the spring and summer Leptosynapta clarki at Barge Bay are also found within filamentous Enteromorpha spp. mats on the surface of the mudflat. Outgoing tides from Barge Bay include clumps of this alga (pers. obs.) and therefore algal rafting might provide a method for dispersal in both juvenile and adult L. clarki (Highsmith 1985).

More passive dispersal might occur if the juveniles can float suspended on the water surface (sensu Highsmith 1985) as noted in juvenile synaptids (Clark 1907). Juveniles of Leptosynapta clarki were often caught in the surface tension in the laboratory, and if present on the mud surface during outgoing tides could float away from the natal site. The finding of a juvenile Leptosynapta clarki in a sediment collector in Barge Bay 10 cm above the surface and another in an oblique plankton tow in the mouth of Grappler Inlet (unpub. obs.) suggests that there is some potential for dispersal. However, the frequency and importance of floating for dispersal in this species is as yet undetermined.

Additionally, many apodids are observed to be nocturnal swimmers at restricted times of the year (Leptosynapta inhaerens, Costello 1946; Labidoplax dubia, Hoshiai 1963; Leptosynapta albicans, Glynn 1965). In all cases the sea cucumbers had empty or reduced amounts of sediment in the gut during swimming (Costello 1946, Hoshiai 1963, Glynn 1965). While the velocity of swimming is slow (5-6 cm/min in juveniles, Costello 1946; ca 1 m/min in adults, Glynn 1965) if aided by water currents the sea cucumbers might be able to disperse over a reasonable distance.

Finally, incidental dispersal of juveniles and adults may occur due to currents or wave action. Engstrom (1980) noted that Chirodota rotifera if starved or in foul water would empty the gut of sediment and move actively on the substrate surface. This behaviour made the animals nearly neutrally buoyant and Engstrom (1980) suggested they could be easily transported by tidal currents or mild wave action. Wave action may also sweep juveniles of brooding intertidal species into deeper water (Pseudocucumis africanus, Ohshima 1916; Cucumaria pseudocurata, Rutherford 1973, Martel 1990) or lift burrowing species to the surface during severe storms (e.g. adult Leptosynapta inhaerens, Fish 1925).

Dispersal of either juvenile or adult Leptosynapta clarki may be important in assuring random mating within and between populations. Electrophoretic studies of Leptosynapta clarki on San Juan Island, Washington, by Hess et al. (1988) found there was enough movement of either individuals or gametes within False Bay to allow random mating and maintain heterozygosity within populations. However, differences in the genetic composition between geographic locations suggests that although gene flow between L. clarki populations is probably rare, it does occasionally occur (Hess et al. 1988). Gene flow could result from the dispersal of juveniles which introduces new males into a population, or as adults. In the latter case, dispersal of brooding female L. clarki might introduce large numbers of individuals into a population allowing genetic mixing or colonization of suitable habitat.

While the potential for dispersal in brooding holothurians deserves more attention the fate of the juveniles once they reach the benthic habitat is virtually unknown. Although recruitment has been studied in holothurians with planktonic larvae (see Ebert 1983, Muscat 1983, Cameron and Fankboner 1989), there are only a few studies that report finding juvenile apodids (Menker 1970, Rao 1973, Engstrom 1980) and only one study of recruitment in a brooding sea cucumber (Cucumaria pseudocurata, Rutherford 1973). The latter study, although only one month in duration, provides the only directly comparable study of recruitment in a brooding sea cucumber.

In brooding species because the juveniles are usually released over a short period of time and the numbers of recruits are not subject to the vagaries of planktonic transport we might expect a pronounced increase in the number of juveniles during the period of release. This pattern has been observed in Cucumaria pseudocurata after juveniles crawl from under the mother (Rutherford 1973) and in Leptosynapta clarki after pentactulae release. In the present study there was, however, no dramatic decrease in numbers of juvenile L. clarki in the first month as observed in Cucumaria pseudocurata (Rutherford 1973). While there must be some juvenile mortality in L. clarki, in the present study the juvenile cohort could be followed for approximately 6 months until it was incorporated into the adult population. This, therefore, provides the first long-term study of recruitment in a brooding holothurian and in any apodid sea cucumber.

Comparative information on growth rates in brooding sea cucumbers is similarly limited. Growth estimates are available for only two species: the external brooder Cucumaria pseudocurata (Rutherford 1973) and the coelomic brooder Chirodota rotifera (Engstrom 1980). There is, however, some qualitative evidence for rapid growth after release in three other apodids: Synapta vivipara Clark (1898), Labidoplax buskii (Nyholm 1951), and Rhabdomolgus ruber Menker (1970).

Once in the sediment juvenile Leptosynapta clarki grow very rapidly from a length of 1-2 mm to a mean length of 21 mm in their first year. In L. clarki, as in juvenile Cucumaria pseudocurata (Rutherford 1973), this rapid growth occurs during the summer months. The rate of growth observed in L. clarki is comparable to that seen over a shorter period of time in the apodid Chirodota rotifera by Engstrom (1980). Four C. rotifera juveniles collected in sand from Key Biscayne, Florida, and maintained in laboratory culture for 3 months grew from 1.5 mm to 10 mm in length before death (Engstrom 1980). As the largest pentactula found in C. rotifera from Jamaica was 3 mm

(Clark 1910), this suggests that Engstrom (1980) cultured recently released juveniles. If this rate of growth had continued these C. rotifera would probably have attained a similar size to Leptosynapta clarki in their first year.

By the age of one year, Leptosynapta clarki have already reproduced as males in the early winter (Nov./Dec., Chapter 2). Two other apodids show rapid growth and early sexual maturity. The burrowing Labidoplax buskii is sexually mature after one year (Nyholm 1951) and the interstitial Rhabdomolgus ruber has gonads within 4-5 months of settlement (Menker 1970). In the latter species it is, however, unclear whether these individuals reproduce (Menker 1970).

By regular field sampling the population dynamics of Leptosynapta clarki could be followed in Barge Bay from the point of juvenile release. The use of two measures of size (length and $\log_{10}$weight) has allowed the juvenile cohort to be distinguished from adult sea cucumbers in field collcctions for appoximately 6 months after release. However, in the absence of distinct year classes in adults and the inability to determine the age of individuals, the rate at which L. clarki individuals move through the population from recruitment to death is unknown.

The method developed herein where sea cucumbers could be maintained in the laboratory, with growth rates approximating those in natural conditions, will be a useful tool for future population studies in small burrowing sea cucumbers. For those species in which tagging or marking is inappropriate because of size, a combination of laboratory experiments and field recruitment data may give us a greater insight into the early life history of many smaller sea cucumber species.

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CHAPTER 7

General Discussion.

The reproductive biology of the viviparous sea cucumber Leptosynapta clarki has been examined in Bamfield, British Columbia. While in general terms reproduction is similar to other annually reproducing holothurians, the reproductive cycle in Leptosynapta clarki is complicated by two features that may be related to its small adult size: brooding and protandry.

The most profound difference in reproduction results from the retention of the gonad tubules after spawning. This results in part of the previously fecund tubule remaining for the following reproductive season. In fertilized female Leptosynapta clarki the ovarian tubules are filled with pentactulae and remain at a large size until the breaking of the distal portion of the ovary in pentactulae release. The basal end regrows oocytes for the coming reproductive season. Similarly, in male L. clarki the previously fecund tubule is rarely completely resorbed after spawning and the regressed bud regrows new spermatogenic tissue. Most dramatically, however, is the retention of the post-spawned transitional testis where active oogenesis gradually fills the testis lumen with oocytes and the sea cucumber reproduces in the following season as a female.

The original questions posed in the introduction (Chapter 1) have been addressed in the various chapters of the thesis resulting in a better understanding of the reproductive biology of Leptosynapta clarki. In terms of reproduction, the only unanswered question in this study and the most important area for future research is the biology of spawning and fertilization.

The spawning behaviour of Leptosynapta clarki is still unclear. Aggregations of sea cucumbers, such as those observed at low tide during the spawning period of L. clarki at False Bay (Everingham 1961, McEuen 1988) were not observed at Bamfield. Furthermore, these authors were unable to provide evidence that these groups are in fact formed by spawning individuals. As natural spawning in two other apodid sea cucumbers occurs when water covers the burrows it is possible that intertidal L. clarki will spawn at periods other than low tide. By keeping L. clarki in the laboratory in approximately natural conditions observations on spawning might be made.

Everingham (1961) suggested that self-fertilization might occur in Leptosynapta clarki. The present study has clearly determined that the timing of fertilization (Nov.-Dec.) and the period of protandric sex change (Jan.-May) do not overlap. This means that mature eggs and sperm are not present in the same gonad at one point in time, and self-fertilization is unlikely. Genetic evidence from *Leptosynapta clarki* on San Juan Island also found no evidence for self fertilization or inbreeding within the studied populations (Hess et al. 1988).

Leptosynapta clarki might be suitable for studying fertilization success in a brooding species . Preliminary results suggest that fertilization is variable with some females having a poor percent fertilization, while in others almost all the eggs are fertilized. Little is known of natural rates of fertilization in marine invertebrates (see Sewell and Levitan 1992), but especially in brooding species. Quantification of fertilization success in such brooding species would allow testing of assumptions such as those of Chia (1974) that problems with fertilization are reduced in brooding and viviparous species. *Leptosynapta clarki* is ideal for such a study as fertilized and unfertilized eggs are retained in the ovary, and the percent fertilization can be readily determined. These values of percent fertilization could then be compared to results from other brooding species.

Once the mechanism of fertilization in *Leptosynapta clarki* is understood we may be better able to determine the role size plays in reproduction in this species. The size-advantage hypothesis predicts that sequential hermaphroditism will occur where an individual reproduces most efficiently as a member of one sex when small or young, but as a member of the other sex when it gets older and larger (Ghiselin 1969, Charnov 1982). The 1:1 sex ratio seen above a weight of 500 mg in *L. clarki* is contrary to sex allocation theory but has been seen in three limpets (Branch 1981, Wright 1989, Creese et al. 1990). This pattern might be more explicable if the reproductive success of *Leptosynapta clarki* of a range of sizes could be determined. Preliminary evidence in Chapter 4 suggests that there is no relationship between female size and fertilization success but there is presently no method whereby the fertilization success of male *Leptosynapta clarki* can be determined. If spawning could be induced in the laboratory this would provide an interesting area for further experimentation.

Leptosynapta clarki shows the association of brooding and hermaphroditism as seen in the reproductive patterns of many small marine invertebrates. The allometry hypothesis for the association of brooding and small adult size predicts that larger females can successfully brood a smaller number of embryos because of space limitations (Strathmann and Strathmann 1982). A test of this hypothesis in female *Leptosynapta clarki* did not, however, indicate either spatial constraints on brood size or differential embryonic mortality in larger females. This might result from problems in fertilization regardless of size, or because brooding occurs in a distensible structure that can expand to accommodate large numbers of pentactulae.

A second important area for further research is the mechanism of sex determination in Leptosynapta clarki. Growth studies in the laboratory and field recruitment studies have shown that all Year 1 individuals reproduce as males approximately 6 months after their birth. The sex of that individual in subsequent years is not simply a function of the size or age of the individual but seems to depend on the interaction of additional factors. If there are two sexual races within a population the genetic predetermination for one sex or the other may be also influenced by environmental factors as yet unmeasured.

With respect to protandry, two questions remain: 1) do some individuals remain as males for their reproductive life?, i.e. never change sex and 2) is it possible for females to reverse the direction of sex change and return to reproduction as males? Answering these questions will probably be difficult. Firstly, while sex can be determined in the autumn without dissection, by visualising the gonad through the semi-transparent body wall, a method would need to be developed to individually mark sea cucumbers over 2 to 3 years to determine the sequence of sex in each year's reproduction. Tagging of large sea cucumbers has been successful in two temperate species (Muscat 1983, Da Silva et al. 1986) but not in seven tropical species (Conand 1991) and may be impossible in smaller sea cucumbers. Alternatively, adult sea cucumbers could be maintained over their reproductive life in individual containers or cages in the laboratory or field. However, there are two potential problems with this approach. Firstly, adult Leptosynapta clarki maintained in the laboratory shrink in size (unpub. data) which would confound the test as size is important to reproducing as a female. Secondly, the ability of adult L. clarki to escape through Nitex screen as small as 560 μm would make construction of cages that do not affect environmental conditions extremely difficult.

The general structure of the Leptosynapta clarki ovary is similar to that descibed in other holothurians. The structural changes in the ovarian wall that occur prior to fertilization are, to my knowledge, undescribed in echinoderms. Electron microscopy has shown that these changes in texture and wall thickness are not the result of any additional cell types but result from changes in the size and shape of existing cells of the ovarian wall. The functional significance of this structural change is as yet undetermined. Until comparative studies are conducted on broadcast spawning and viviparous sea cucumbers we will not be able to determine if these changes are related to the additional function of the ovary as a brood structure.

A significant finding in this study was that there may be extensive mortality of pentactulae during the brooding period. The retention of the calcareous ring after pentactulae death allowed accurate counts to be made of dead pentactulae and provided the first estimate of embryonic mortality in a viviparous marine invertebrate. The finding of high embryonic mortality in some female Leptosynapta clarki questions the assumption that there is low mortality in viviparous species. The generality of this finding, however, awaits further research on other brooding species.

For the first time in an apodid sea cucumber we have been able to measure growth after release of pentactulae from the mother and recruitment to the adult habitat. In combination with laboratory experiments this has allowed a detailed study of the age and size of sexual maturity and some information about the timing of sex change. The absence of distinct modes in the size-frequency distributions, except immediately after juvenile release, has however prevented estimates of growth in older animals.

Based on the information presented throughout the thesis we are able to estimate longevity of individual Leptosynapta clarki. As all Year 1 individuals are male, some males undergo protandric sex change after their first year, and there is evidence that females reproduce again as females, then individual Leptosynapta clarki probably live at least 3 years. However, as most juveniles in the laboratory were male for a second reproductive period, individual longevity may be 4 or 5 years. Leptosynapta clarki therefore confirms to the suite of life history traits associated with small adult size (Strathmann 1985), i.e., lower fecundity (max. 4739 eggs), early sexual maturity (6 months), lower adult longevity (ca. 5 years) and shorter generation time.

The present study has addressed questions on viviparity in Leptosynapta clarki in terms of life history theory. Information about brooding, protandry and embryonic mortality have been considered in light of appropriate theory. By taking this approach the information presented has applicability to other viviparous species rather than just to Leptosynapta clarki itself. As little information is available on viviparous species it is hoped that the present study acts as a catalyst to future research.

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