Kasetsart J. (Nat.Sci.) Vol. 24 : 529 - 539 (1990)

Preliminary Study on Reproductive Biology of the Abalone (*Haliotis varia*) at Phuket, Andaman Sea Coast of Thailand

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ABSTRACT

Reproductive biology of 214 individuals of abalone (*Haliotis varia*) was studied at Bon Island, Phuket in 1987. Mature gonads with apparently ripe eggs were found in January, March - April, June and August - October. Spawning, as indicated by gonad index and examination of ovaries, occurred in January - February, April - May, June - July, November - December. The smallest specimen with mature eggs measured was 17.3 mm. in length. Maximum fecundity was about 3.5 million eggs at the shell length of 41.8 mm. The sex ratio was not significantly different from 1 : 1 with respect to month and size class.

INTRODUCTION

Studies on reproduction are useful in formulating fisheries regulations for commercial species. Knowledge of breeding seasons can be essential for the estimation of growth rates, particularly of young stages, and the relationship between age, fecundity, and the minimum size at maturity must be taken into account when minimum takable size is determined.

Overseas, where *Haliotis* species have been fished commercially for many years, research on these aspects is extensive. Most work, however, has concentrated on the determination of spawning seasons, such as in Japan (Ino and Harada, 1961) and in California (Boolootian *et al.,* 1962) and in New Zealand (Poore, 1973). More comparative studies done in South Africa include estimations of fecundity (Newman, 1967).

Few studies have been done on tropical abalone species. Among ASEAN countries some biology and checklists have been reported from Philippines (Fuze, 1981), Indonesia (Robert *et al.,* 1982), Malaysia and Singapore (Purchon and Purchon, 1981). Also few studies have been undertaken in Thailand, where *Haliotis* is being occur both in the Gulf of Thailand and along the Andaman Sea coast. The first survey on abundance and distribution of *Haliotis* spp along the Andaman Sea coast of Thailand was carried out in 1985 - 1986 (Nateewathana and Bussarawit, 1988). A survey on Thai abalones around Phuket Island and a feasibility study of abalone culture in Thailand was reported by Nateewathana and Hylleberg (1986) and a survey on species and distribution of *Haliotis* spp. in Surat Thani, Nakhon Si Thammarat and Songkla in the Gulf of Thailand was reported in the same year (Tookvinas *et al.,* 1986).

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In this contribution aspects of the reproductive biology of *Haliotis* varia were examined by regular sampling in 1987 at Bon Island, Phuket province, southern Thailand.

MATERIALS AND METHODS

A. Study site.

Bon Island is situated on the southern part of Phuket Island near Phuket Marine Biological Center (PMBC) (Figure 1). Two species of *Haliotis* were found in the area, *H. varia* and *H. ovina,* the former being the most abundant (Nateewathna and Bussarawit, 1988).

B. Methods.

I. Gametogenesis and spawning seasons.

Haliotis varia were sampled by snorkling or SCUBA during January - December, 1987; approximately 30 individuals per month were taken from rocky crevices in the study area. Diving knife or forceps were used in collecting at 1 - 2 meter depth. The specimens were measured and weighed and later preserved in 10% buffered formalin.

The conical appendage, which consists of a gonad sheath over the conical hepatic gland, was removed by cutting through the stomach region. The gonad was sectioned at a point one third of the distance from the shell apex to its tip, and the exposed areas of gonad and hepatic gland were drawn with camera lucida on plain paper (Figure 2A, B). The paper areas were cut and weighed, either directly or after enlargement, and a gonad index was determined for each individual from the formula (Poore, 1973) :

$$\text{Gonad index} = \frac{\text{gonad area}}{\text{total cross sectional area of conical organ}} \times 100$$

Examinations of ovaries were made to measure actual gametogenic activity. Samples taken each month from a number of formalin hardened ovaries were dispersed in water with a mechanical agitator. The percentage of mature eggs was calculated after counting the numbers of eggs in each class in ten microscope fields. Developing oocytes are small round and later larger stalked shape, but as they become mature they first polygonal and then rounded shape. Because of the difficulty in removing all the smaller eggs (oocytes) from the gonad trabeculae these were probably underestimated. Notes were also made on the size and shape of the eggs.

II. Fecundity and minimum size of maturity.

Females from each relevant centimeter size class were observed for fecundity estimates. The ovary was carefully dissected from the hepatic gland and stomach and weighed to the nearest 0.1 g. A weighed fragment was dispersed in water and a subsamples of eggs counted under a binocular microscope. Fecundity, here defined as the number of mature eggs present in the ovary prior to spawning, was calculated by multiplying by the appropriate factor.

During spawning season gonad index was plotted as a function of shell length to determine minimum size of maturity.

III. Sex ratio.

Sexing of mature abalones can generally be done by visual inspection if the foot and mantle are forced away from the right side of the shell to expose the horn - shaped conical appendage (Figure 3). Sex ratio was

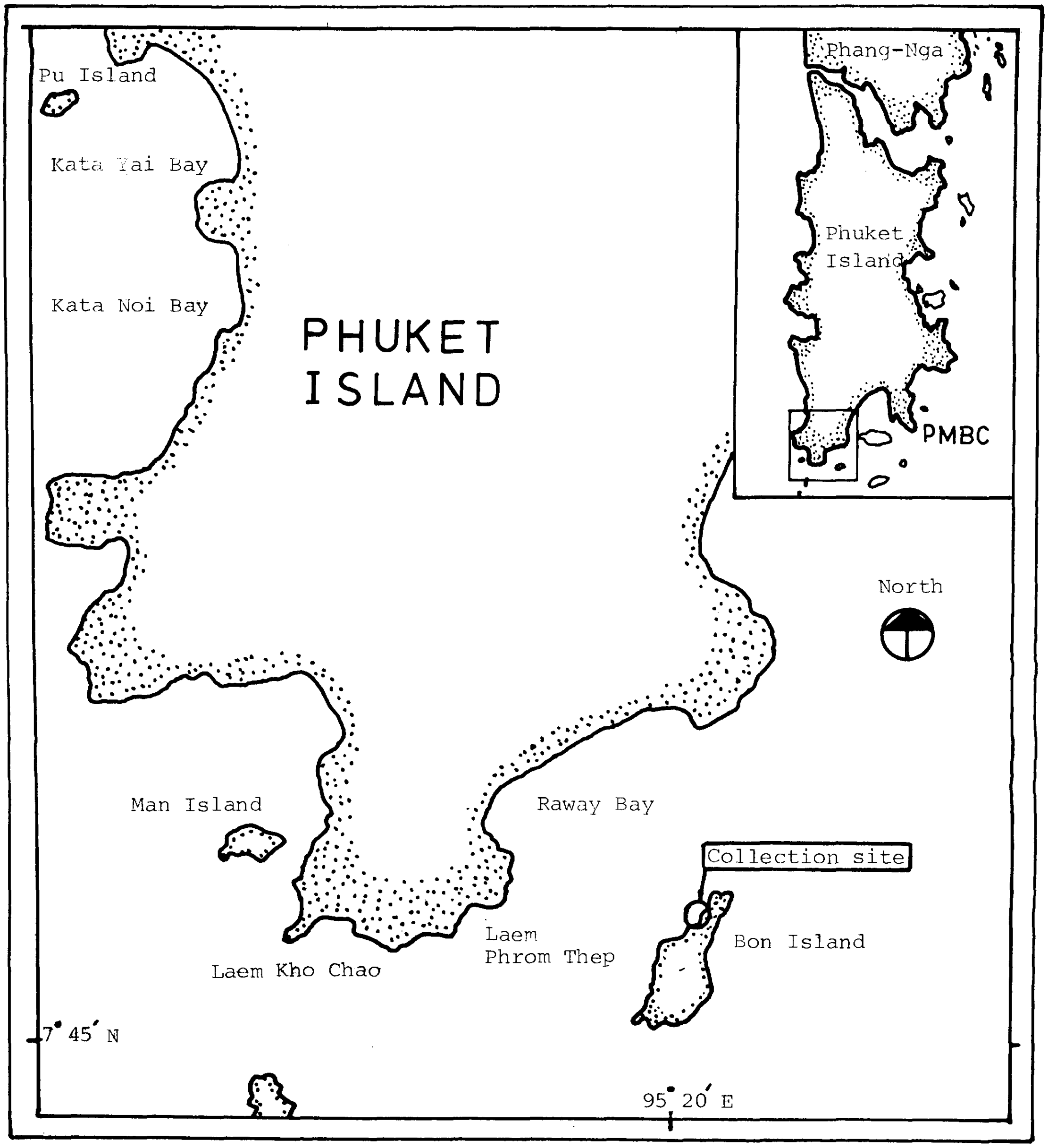

Figure 1. Location of Bon Island, monthly sampling of *Haliotis varia* for reproductive biology study.

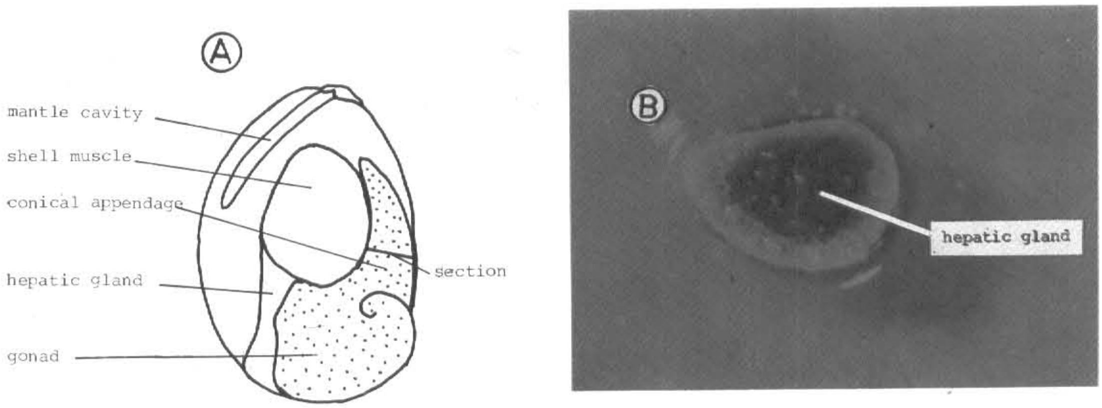

Figure 2. **A. *Haliotis varia* with shell removed showing position of section through conical appendage, B. section of conical appendage showing exposed areas of hepatic gland and gonad as used in calculating the gonad index.**

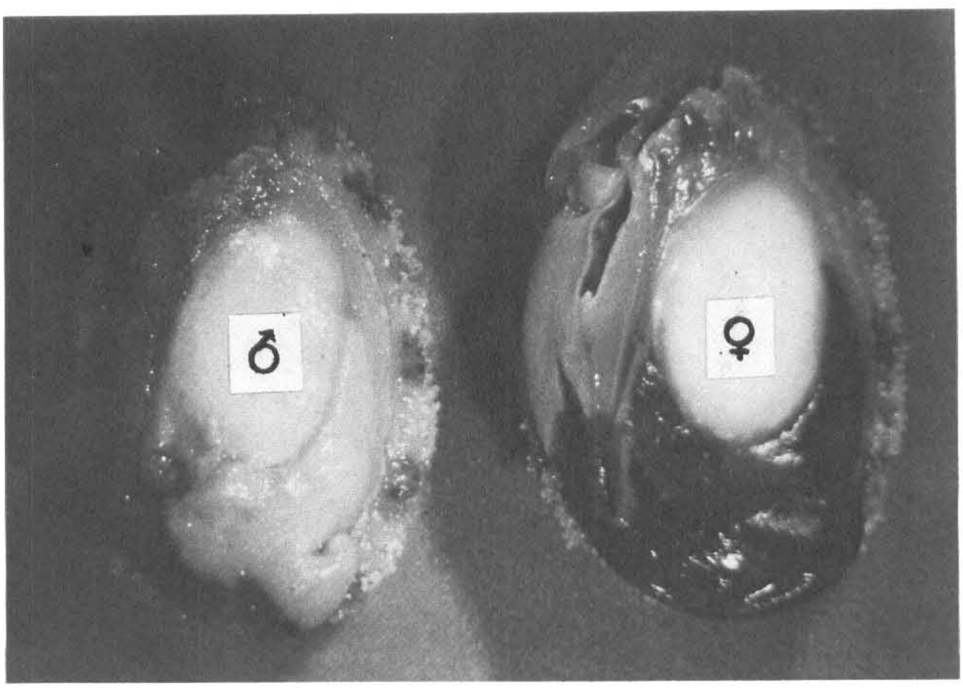

Figure 3. **Shows different colour of male and female gonads of *Haliotis varia*. Dorsal view (without shell).**

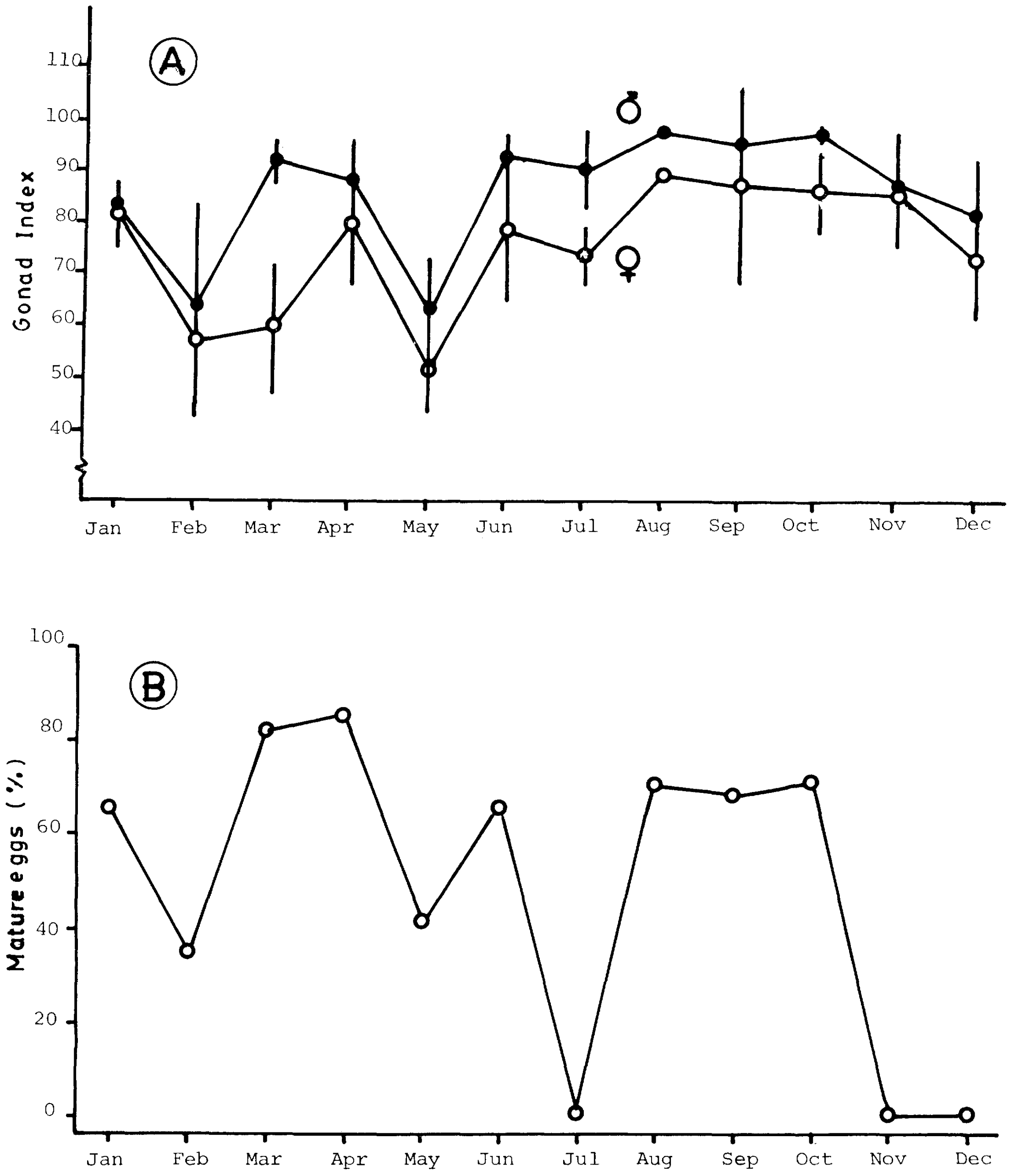

Figure 4. **A.** Monthly gonad indices (mean and 95% confidence limits) for *Haliotis valia* males (closed circles) and females (open circles).
B. percentage mature eggs.

calculated for each month and size class and later tested for significance by Chi - square test (Shepherd and Laws, 1974).

RESULTS

I. Gametogenesis and spawning seasons.

Two resonably distinct classes of eggs were found : oocytes (50 - 100 μm); with cleary visible nucleus in the pale cytoplasm, isodiametric when small but becoming stalked when larger (200 - 400 μm); and mature eggs in which the nucleus is not obvious, they become upto 200 - 250 μm when polygonal, and 250 μm when round. The two classes were less distinct in the post spawning period, when the whole range of gametogenetic stages was present.

Short breeding periods were apparent manisfesting itself as a sudden drop in gonad index and in the percentage mature eggs (Figure 4 A, B). This suggests that spawning occurred during January - February, April - May, June - July and November - December. In July, November - December, there was a marked reduction in the fraction of mature eggs and an increase in the fraction of younger egg stages.

II. Fecundity and minimum size of maturity.

Figure 5. illustrates the relationship between body weight and the fecundity in 50 females. Overall fecundities was related to body weight by the equation : F = 0.215W - 0.228 (r = 0.771), where F is the fecundity in million eggs and W is the body weight in grams. There were poor relations to length and width (r = 0.628 and r = 0.613, respectively). The number of mature eggs calculated per individual ranged from 96,747 at 17.3 mm to 3,505,230 at 41.8 mm.

The minimum size of maturity of *Haliotis varia* was indicated by the length of the smallest individual containing mature eggs, which in this study was 17.3 mm. (Figure 6).

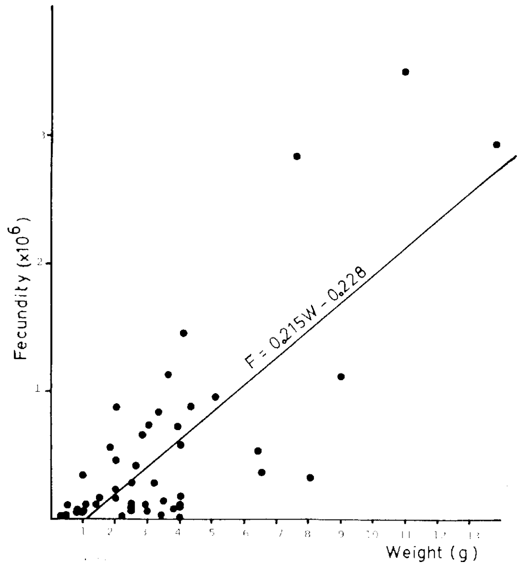

Figure 5. Relationship of fecundity and weight of 50 females, *Haliotis varia*, from Bon Island during study period.

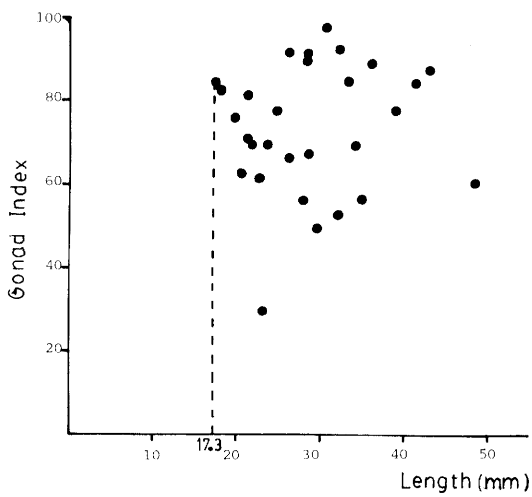

Figure 6. Shows gonad index of mature eggs prior to spawning at different length of females (*H. varia*).

III. Sex ratio.

The colour of the gonads is lighter in males than in females. Male gonads are creamy white while dark green in females. The sex ratio was not significantly different from 1:1 in any month ($P < 0.05$), except August where only one female was obtained (Table 1, 2). Also, sex ratio did not change with size (Table 3).

Table 1 Sex, mean size (Lenght, Width), mean weight (Weight), gonad index (G.I.), fecundity (F) and percentage of mature eggs of *Haliotis varia* from each monthly samples.

Month	Sex (ind.)	L (cm)	Wi (cm)	W (g)	G.I.	F	% mature eggs
Jan.	♀ (7)	3.4	2.3	4.61	81.36 ± 6.68	302460	65.16
	♂ (5)	3.2	2.2	3.80	82.73 ± 3.64		
Feb.	♀ (10)	2.4	1.6	1.40	56.91 ± 14.99	258235	34.71
	♂ (7)	2.7	1.8	1.90	63.66 ± 19.57		
Mar.	♀ (7)	2.6	1.7	1.80	59.40 ± 12.64	92947	81.81
	♂ (12)	3.0	2.1	4.13	91.72 ± 4.66		
Apr.	♀ (13)	2.6	2.1	1.54	79.96 ± 12.31	437800	85.24
	♂ (16)	2.2	1.5	1.60	87.59 ± 8.89		
May.	♀ (20)	2.4	1.6	1.79	50.81 ± 7.30	67945	41.12
	♂ (20)	2.6	1.8	2.34	62.54 ± 10.56		
Jun.	♀ (6)	3.4	2.3	5.3	78.70 ± 13.45	1172682	65.44
	♂ (9)	5.9	2.1	3.8	92.58 ± 4.87		
Jul.	♀ (10)	3.3	2.2	4.40	73.20 ± 6.07	no data	immature
	♂ (6)	3.3	2.2	4.40	90.08 ± 8.27		
Aug.	♀ (1)	2.7	1.8	2.24	89.50 ± 0.00	16640	70.12
	♂ (7)	3.5	2.4	5.8	97.34 ± 0.75		
Sep.	♀ (8)	3.4	2.3	5.11	87.32 ± 19.30	678649	68.10
	♂ (12)	3.5	2.3	5.36	94.94 ± 8.65		
Oct.	♀ (5)	2.6	1.8	2.91	86.14 ± 8.22	293228	71.20
	♂ (8)	2.9	2.0	3.63	97.83 ± 1.52		
Nov.	♀ (3)	2.6	1.8	2.07	85.20 ± 8.51	no data	immature
	♂ (4)	2.4	1.6	1.71	88.09 ± 9.67		
Dec.	♀ (5)	3.0	2.1	3.26	72.52 ± 10.35	no data	immature
	♂ (13)	2.8	3.9	2.72	82.03 ± 10.76		

Table 2 Monthly distribution of sexes of *Haliotis varia* during 1987 from Bon Island samples.

Month (1987)	Male No.	Male %	Female No.	Female %	Total No.	Ratio M : F	Chi - Square
January	5	41.7	7	58.3	12	0.71	0.33
February	7	41.1	10	58.8	17	0.70	0.53
March	12	63.1	7	36.8	19	1.71	1.32
April	16	55.2	13	44.8	29	1.23	0.31
May	20	50	20	50	40	1	0
June	9	60	6	40	15	1.50	0.06
July	6	37.5	10	62.5	16	0.60	1.0
August	7	87.5	1	12.5	8	7	4.5
September	12	60	8	40	20	1.50	0.8
October	8	61.5	5	38.5	13	1.60	0.69
November	4	57.1	3	42.9	7	1.33	0.14
December	13	72.2	5	27.8	18	2.60	3.56
Total	119	57.3	95	42.7	214	1.25:1	2.69

Table 3 Sex ratio of *Haliotis varia* with all size classes sample.

Length (cm)	Male No.	Male %	Female No.	Female %	Total No.	Ratio M : F	Chi - Square
< 2	13	50	13	50	26	1	0
2 - 3	61	56	48	44	109	1.27	1.55
3 - 4	37	57	28	43	65	1.32	1.25
> 4	8	57	6	43	14	1.33	0.28

DISCUSSION

I. Gametogenesis and spawning seasons.

During this one year study on gametogenesis and spawning seasons of *H. varia,* it was found that the development of gametes and spawning occured simultanaeusly throughout the year, with a few exception such as males in April and females in October. A rapid post - spawning recovery in the present study suggests that there is a greater gametogenetic activity in tropical than in temperate species (Poore, 1973).

In this study spawning seasons were deduced from gonad index, as has been widely used in several species (Ino and Harada, 1961; Boolootian *et al.*, 1962; Newman, 1967). Some workers have used direct observations of spawning or fertilization in the laboratory. Of these methods, histological examinations and gonad indices are the most reliable. Few direct observations of spawning in the field have also been done (Poore, 1973).

Reproductive cycles in marine invertebrates are often correlated with sea temperature (Orton, 1920; Giese, 1959). Unfortunately, in this study we did not measure sea tempearature at the collection site but temperature variation is small (27 - 29°C) in this region (Khokiattiwong pers. comm.). Some works have also reported that in *Haliotis* the correlation to temperature is slight, and for example in temperate species autumn spawning often occur 1 or 2 months after the maximum temperature. Although temperature changes can stimulate spawning of New Zealand abalone in the laboratory, and has been used to obtain gametes by Oba (1964) and Ino (1952), the same phenomenon has not been demonstrated in the field. Wide and rapid temperature changes, such as are used in laboratory stimulations are rare naturally. Fretter and Graham (1964) discussed the effect of environment on breeding in molluscs, and noted that maturation of gammetes is controlled by annual temperature fluctuations, whereas a combination of other factors may trigger spawning. This difference between maturation and spawning stimuli was also noted by Giese (1959). Spawning stimuli may include mechanical disturbance (Cox, 1962) or, for females the presence of sperm in the water (Murayama, 1935).

II. Fecundity and minimum size of maturity.

In its most restricted sense, fecundity refers to total egg production. As used here fecundity is the total number of mature eggs present in the ovary prior to spawning; very rarely are all these eggs shed in the short spawning season. However, spent gonads were found abundantly in July, November and December. Generally, however gonads were only partially spawn.

Fecundity of *H. varia* was estimated and ranged from 96,747 to 3,505,230 mature eggs in ovary of the length 17.3 to 41.8 mm. respectively. Fecundity has been estimated in few other *Haliotis* species, eg. about 1,286 eggs at 68 mm. to 11,253,000 eggs at 155 mm. in *H. iris* (Poore, 1973); about 46,000 eggs at 62 mm. to 2,910,000 eggs at 91 mm. in *H. australis* (Poore, 1973); about 140,000 eggs in *H. sieboldii* (Ino, 1952); about 10,000 eggs in *H. tuberculata* (Fretterand Graham, 1964). This *H. varia* showed a similar range in fecundity as other bigger size species.

In this study the minimum age at maturity could not be deduced from the minimum size of maturity (17.3 mm.) due to lack of information on its growth rate. However, minimum size and age at maturity have been reported in three other species; *H. cracherrodii,* 8 - 9 cm long (Palmer, 1907); *H. discus hannai* 8 cm long (Ino and Harada, 1961), about 4 years old (Sakai, 1962); *H. tuberculata* 5 cm long or 3 years old (Crofts, 1937). It should be noticed that all those species are bigger than *H. varia.*

III. Sex ratio.

Of the total of 214 individuals of *H. varia,* 119 were males and 95 were females. The mean sex ratio of male to female was 1.25:1. However, a Chi - square test showed no significant difference in the relative numbers of each sex over the year. This result agrees with findings in others species eg. *Haliotis midae, H. cracherodii, H. rufescens* and *H. tuberculata* which all near 1 : 1 sex ratio (Newman, 1967, Boolootian *et al.*, 1962; Crofts, 1937), although a preponderance of males has been recorded in the last species (Forster, 1962; Stephenson, 1924). However, different sex ratios has been reported in *Haliotis* species at various sites. Bolognari (1954) reported 43.10% of a sample of 2,237 *H. lamellosa* to be females; this result was highly significantly different from equal representation of sexes.

Over the year, sexes of *H. varia* are distinguished by gonad colour; creamy white in males and dark green in females. Similar colour differences occur in most other species of *Haliotis* (Boolootian *et al*, 1962; Newman, 1967).

ACKNOWLEDGEMENTS

We wish to thank Mr. Boonlert Phasuk, former Director of PMBC who initiated the abalone survey project and also supported this study. Thanks to Mrs. Nipavan Bussarawit for her suggestion, and to Mr. Sahet Ussaha for his assistance with field work. Drs. Jorgen Hylleberg and Thomas Kiorboe are acknowledged for correcting and commencing the manuscript. This study was presented at the Fifth International Congress of Invertebrate Reproduction in Nagoya in 1989. The first author would like to thanks the Organizing Committee and Thai - Danish Foundation for financial support.

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