Bull. Inst. Zool., Academia Sinica **26**(1): 9-17 (1987)

EARLY DEVELOPMENT, SURVIVAL, AND GROWTH OF THE ABALONE *HALIOTIS DIVERSICOLOR SUPERTEXTA* LISCHKE IN TAIWAN

LI-LIAN LIU and LI-SHING CHU

Institute of Marine Biology, Sun Yat-Sen University, Kaohsiung, Taiwan, R. O. C.

and

KUN-HSIUNG CHANG

Institute of Zoology, Academia Sinica, Nankang, Taipei, Taiwan, R. O. C.

(Accepted January 2, 1986)

Li-Lian Liu, Li-Shing Chu and Kun-Hsiung Chang (1987) Early development, surrival, and growth of the abalone *Haliotis diversicolor supertexta* Lischke in Taiwan. *Bull. Inst. Zool., Academia Sinica* **26**(1): 9-17. The development of the abalone *Haliotis diversicolor supertexta* Lischke was studied by scanning electronic microscope. Sensory tufts were found not only on the head of trochophores and veligers but also on the cephalic tentacles and epipodial tentacles of 21-day-old juveniles. Using seawater treated by UV-light, filtration (0.7 um) or antibiotics (dihydro-streptomycin 75ppm-225ppm) could increase the survival rate of larvae. There was no significant difference between the survival rate of larvae which reared in the density from 25 ind./100ml to 1600 ind./100ml. There was 95% of larvae settled in 48 hrs after fertilization at 25±1°C. If the temperature of culture water rised from 21°C to 25°C, the settling rate of larvae increased drastically.

When insulin and growth hormone were introduced to the tank where newly metamorphosed juveniles were kept, the growth rate of the juveniles had increased. The body length of juveniles were 30-50 um larger than control. These hormones also paced the growth of juveniles which in turn reduced the size difference within the brood.

The reproductive biology and artificial propagation of abalone. *Haliotis diversicolor supertexta* have been studied in recent years (Tzeng 1976, Tzeng and Lin 1976, Yang 1979). In spite of the techniques of inducing spawning and fertilization were well developed, the survival rate of brood was lower than 1% in Taiwan. As for *H. discus hanni* and *H. discus*, the survival rates were also in the range of 1% to 4.56% (Bang 1977, Pyen 1981).

Oba (1964), Lee *et al.* (1982) and Yang (1979) had studied on the development of the abalone, *H. diversicolor supertexta*, by light microscope. In 26.2-26.8°C, the eggs of the abalone had developed to trochophore stage through total, unequal and spiral call division 5 hours after fertilization. Trochophores hatched and began to swin 10-11 hour later. They transformed into veliger stage and grew

1. Paper No. 278 of the Journal Series of the Institute of Zoology Academia Sinica.

protoconch shell. After torsion, the veliger developed cephalic tentacles, eyes, foot and operculum. The veliger settled and metamorphosed to juvenile forming ciliary lobe, peristomal shell and radula after 65 hours. Finally, the respiratory pore formed after 23 days.

During the rearing period, there are many factors can result high mortality, such as: sperm and egg preservation time (Kikuchi and Uki 1974a), sperm density (Kikuchi and Uki 1974b, Vacquier *et al.* 1980), cultural temperature (Leighton 1972, Ebert 1974), cultural salinity (Yang 1979), dissolved oxygen and cultural density (Sagara 1971) and water quality (Tanaka 1969, Morse 1979) etc. In this study, the development of small abalone was examined by SEM. How to reduce the mortality of larvae and how to increase the growth rate of juveniles were also determined.

MATERIALS AND METHODS

Mature broodstocks of the abalone were collected from cultural ponds in Hua-lien. Spawning was induced by exposing mature abalones in the air about 40 minutes then adding UV-treated seawater. Water temperature increased from 21 to 26°C at the rate of 1°C/hr. Male and female abalones were held in separate containers during the above procedures. After spawning, the ova were collected by siphon and put into 2-liter breaker. Fertilization occurred within 30 minutes after spawning. Excess sperms were removed by gently washing the eggs six times. Following the last decantation, the cultures were refilled by seawater (0.7 μm filtered, UV-treated, 33‰) and ready for experimental uses.

I. Observation of the development

Fertilized eggs were incubated in waters of 24±1°C to observe the development and samples were taken for scanning electron microscopy (SEM). Veligers were relaxed for about 5 minutes in saturated Chlorobutanol before fixation. Specimens were fixed in 2.5% glutaraldehyde in seawater for 1 h and post-fixed at room temperature in 2% OsO_4 and 1.2% $NaHCO_3$ (pH=7.2) for 2 h. Followed the fixation procedures, specimens were dehydrated through a graded series of alcohols, then critical-point dried with CO_2 directly from 100% alcohol, coated with gold and observed with a Cambridge Stereoscan S-4 scanning electron microscope.

II. Treatment for reducing the mortality of larvae

The effects of water quality and antibiotics (dihydro-stryptomycin) on the mortality of larvae were investigated in waters of 25± 1°C. Water quality were divided into untreated, UV-treated, UV-treated plus 0.7 μm filtered seawater and treated with different contration or antibiotics (0, 75, 150, 225 ppm). Each treatment filled with 100 ml treated seawater in acrylic container ($6\times9\times2.5\ cm^3$) and had 2 duplicates. Finally, fertilized eggs (110±10) were added into each container. The culture water were changed daily with fresh seawater during experimental period. The survival rates were recorded after nine days. The Factorial experiment of hierarchical classification test (Li 1964) and χ^2-independence test (Yeh 1977) were used to analyze the data.

The effects of cultural density on survival rate were also examined. Fertilized eggs were grouped into 25, 50, 100, 200, 800 and 1600 individuals per container (6, 9, $2.5\ cm^3$) with seawater of 75 ppm antibiotics. Other conditions were the same the same as previously mentioned. One-way ANOVA (Yeh 1977) were used to analyze the data.

The effect of temperature on settling rate were studied with early veligers which were taken from cultures incubated in water of 23±1°C for 11 hours. Water conditions were the same as above. Experimental temperature were 21, 23 and 25°C. Each container added 206±6 early veliger to assess the settlement from 12 to 48 hours on 6 hour intervals. The data were analyzed by χ^2-independence test.

III. Acceleration of on growth rate

Juveniles (nonfeeding group: 270±30 μm each, feeding group: 260±20 μm each) were produced and cultivated at 23±1°C. Each container added 100±20 ind. Treatments were insulin (1 ppm) and growth hormone (1 ppm) in the presence or absence of mixed diatoms and other microalgae. After three days, samples were examined and measured with microscopy. Student *t*-test was used to compare the results (Yeh 1977).

RESULTS AND DISCUSSION

I. Observation of the development

The sperm of *H. diversicolor supertexta* is about 22 μm in length which divides into three parts: head, midpiece and tail (Fig. 1). It is a type of primitive archaeogastropod. The head is bullet-shaped with acrosome and nucleus. Midpiece composes with five mitochondria. As for the development of fertilized egg, the third cleavage is completed about 2.5 h after fertilization and giving rise to an 8-cell stage (Fig. 2). The trochophore escapes from egg membrane 10.5 h after fertilization. It has prototroph girdle with cilia and blastopore on lateral surface (Fig. 3). In the upper surface, it has rounded terminal sensory (apical) tufts about 10 μm in length (Figs. 4,

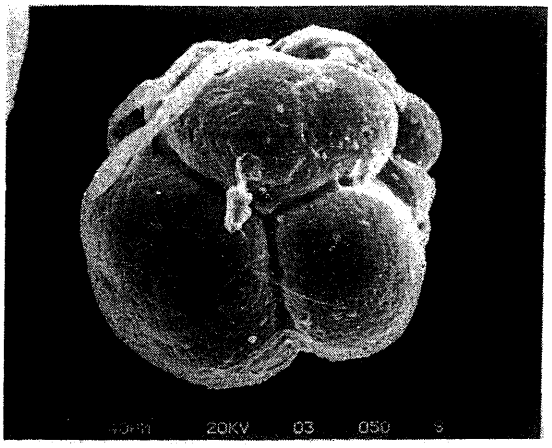

Fig. 2. 8-cell stage at 2.5h after fertilization. Note the smaller cells at the animal pole.

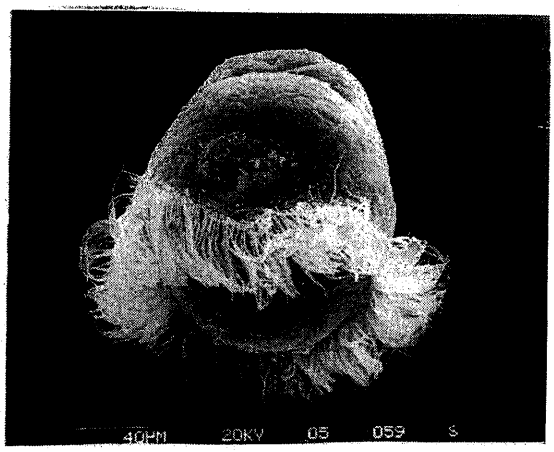

Fig. 3. The lateral surface of a trochophore b: blastopore; p: prototroph.

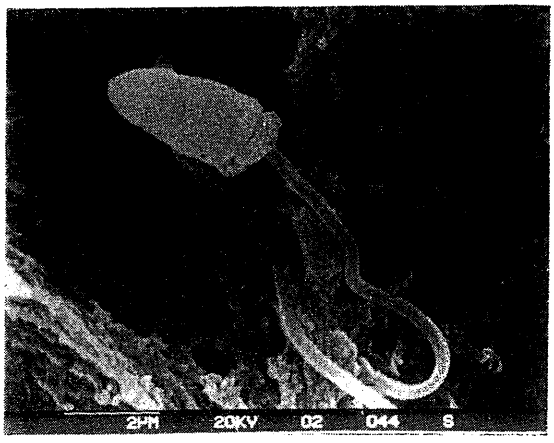

Fig. 1. A sperm. a: acrosome, n, nucleus, m: mitochondria and t: tail.

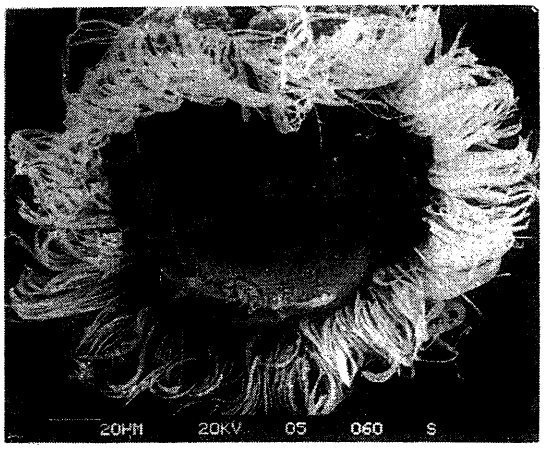

Fig. 4. A trochophore showing the sensory tufts (st) on the upper surface.

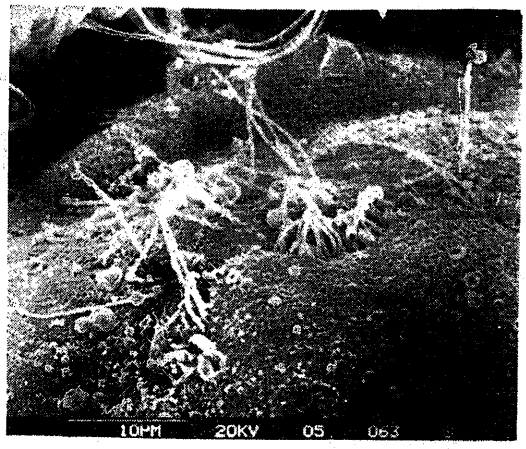

Fig. 5. A trochophore showing the sensory tufts (st) on the upper surface.

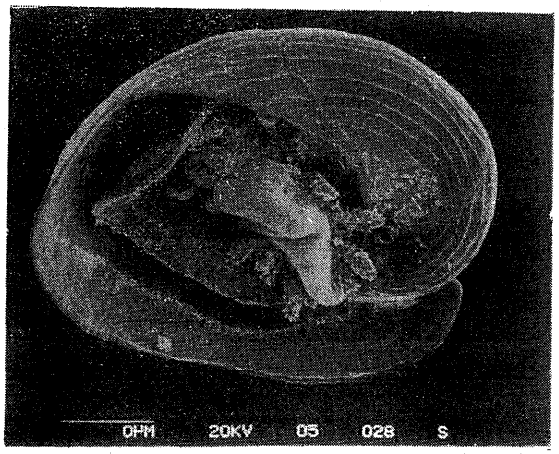

Fig. 6. A 21-day old juvenile. ct: cephalic tentacle; et: epipodial tentacles; rs; radula shealth; m: mantle.

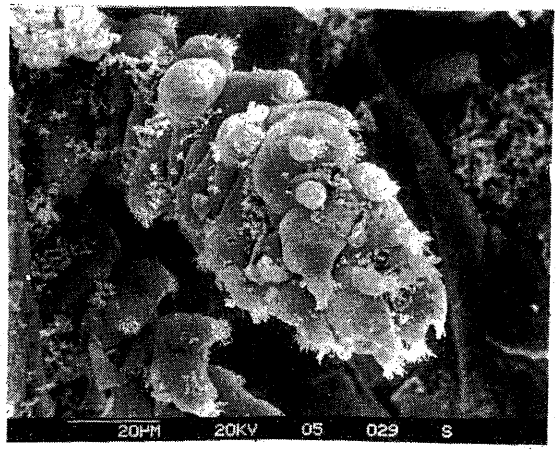

Fig. 7. A portion of a epipodial tentacle showing sensory tufts.

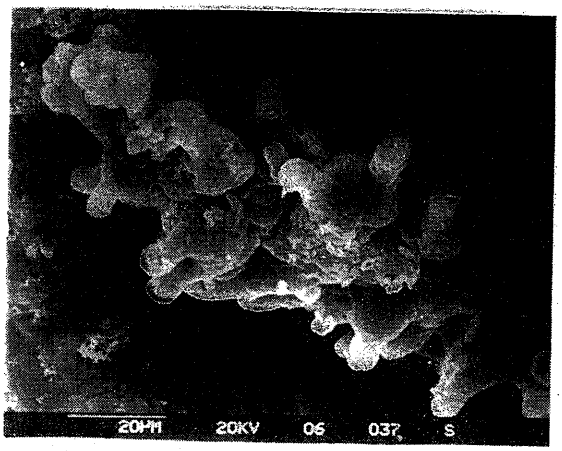

Fig. 8. A portion of a 28-day juvenile showing sensory tufts appearance in epipodial tentacles.

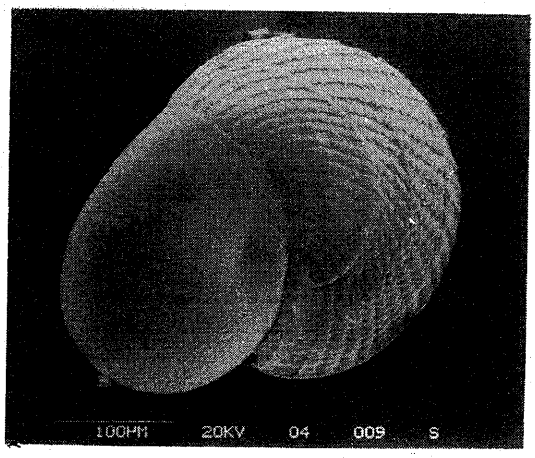

Fig. 9. A juvenile shell at 7 days. p: protoconch; ps: peristomal shell.

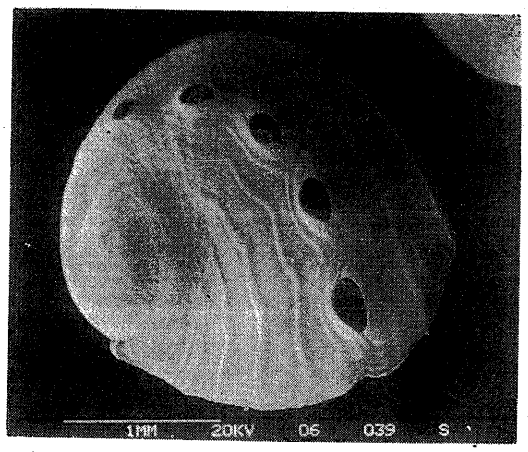

Fig. 10. A 45-day juvenile showing five respiratory pores. rp: respiratory pore.

5). The trochophore transformes into veliger and cephalic tentacles, eyes, foot and operculum appeares. On trochophore the sensory tufts grow already and still remain on veliger. In other abalones, *H. gigantea* and *H. rufescens*, also have similar structure (Carlisle 1962, Maruyama 1935). At this veliger stage, larvae swims vertically to water surface or to the bottom and using its head to hit substrate. It is proposed that the sensory tufts fuction to search substrate to attach.

After settling, the veliger metamorphoses to juvenile, discards the operculum, grows radula, epipodial tentacles, cephalical tentacles and mental (Fig. 6). There are many sensory tufts on cephalical and epipodial tentacles in 21 days' juvenile (Fig. 7). After 28 days, the sensory tufts on epipodial tentacles disappear (Fig. 8). Like other structures, the protoconch and teleoconch shell form gradually (Fig. 9). This processes is similar to *H. discus hannai* Ino (Iwata 1980) and the respiratory pores form after the biomineralization of teleoconch shell (Fig. 10).

II. Treatment for reducing the mortality of larvae

High survival rate of cultural broods were dependent on good control of water quality and microbial growth (Morse 1979). Treated good with water quality and antibiotics, the mortality of the abalone was reduced, especially on the group of UV-treated and 0.7 μm filtered seawater (Table 1). When comparing the results, it was found that the survival rate were strongly influenced by water quality ($F=289.61$, $p<0.01$) than by antibiotics. The survival rates were no difference on antibiotics from 75 to 225 ppm when UV-treated and 0.7 μm filtered seawater was used (Table 3, $p<0.01$).

The survival rates of cultural density were indicated on Table 4. There were no significant difference from 25 ind./100 ml to

TABLE 1
Compairsm of the survival rate of the abalones as observed in the 9th days after ferterligation, under the treatment of different water quality

Water quality	Dihydrostryptomycin (ppm)	Initial ferte. eggs (No.)	Survial rate	
			No.	%
Untreated seawater	0	104	0	0
		103	0	0
	75	110	0	0
		105	0	0
	150	108	0	0
		102	0	0
	225	106	0	0
		116	0	0
UV-treated seawater	0	110	0	0
		121	0	0
	75	109	10	9.2
		114	2	1.8
	150	107	18	16.8
	225	105	22	21.0
UV-treated & 0.7 um filtered seawater	0	115	20	17.4
		120	29	24.2
	75	105	45	42.9
	150	113	40	35.4
		104	43	41.5
	225	104	46	46.2
		105	52	49.5

TABLE 2
Results of hierarchical classification factorial test to determine the signifi cance of differences between water quality and antibiotics concentration on small abalone survival rate

Resource	SS	df	MS	F	$F_{0.01}$
Water quality	5288.22	2	2644.11	289.61*	8.0215
Antibiotics	1143.44	9	127.05	13.92*	
Error	82.15	9	9.13		
Total	6513.81	20			

*: $p<0.01$

TABLE 3
X^2-independence test to compare the effect of antibiotics on small abalone survival rate

Resource antibiotics con. (ppm)	UV-treated seawater	UV-treated & 0.7 um filtered seawater	Resource antibiotics con. (ppm)	UV-treated seawater	UV-treated & 0.7 um filtered seawater
0- 75	10.76*	16.49*	75-150	9.67*	0.45
0-150	37.78*	15.69*	75-225	2897.49*	0.37
0-225	48.42*	32.70*	150-225	0.36	2.90

*: $p<0.01$

TABLE 4
Survial rate in different cultural density as observed in the 5th, 6th and 9th days after, fertilization. 5th and 6th day in metamorphic stage, 9th in stage

Density	Survial rate			
	5th day (%)	6th day (%)	9th days (%)	
26	0	0	7	26.9
27	0	0	8	29.6
50	0	0	13	26.0
54	0	1	15	27.8
105	1	5	23	21.9
102	0	0	41	40.2
203	0	1	89	43.8
203	2	2	66	32.5
801	69	97	176	22.0
800	96	108	210	26.3
1608	92	112	433	26.9
1606	142	109	377	23.5

1600 ind/100 ml when caculated with one-way ANOVA ($F=1.248$, $p<0.01$).

But in the groups of high cultural density (fertil. eggs 800 and 1600), metamorphosis was faster than that of the low density ones. The factor which accelerates the metamorphosis is uncertain. To compare the temperature effect, the settling rate of small abalone

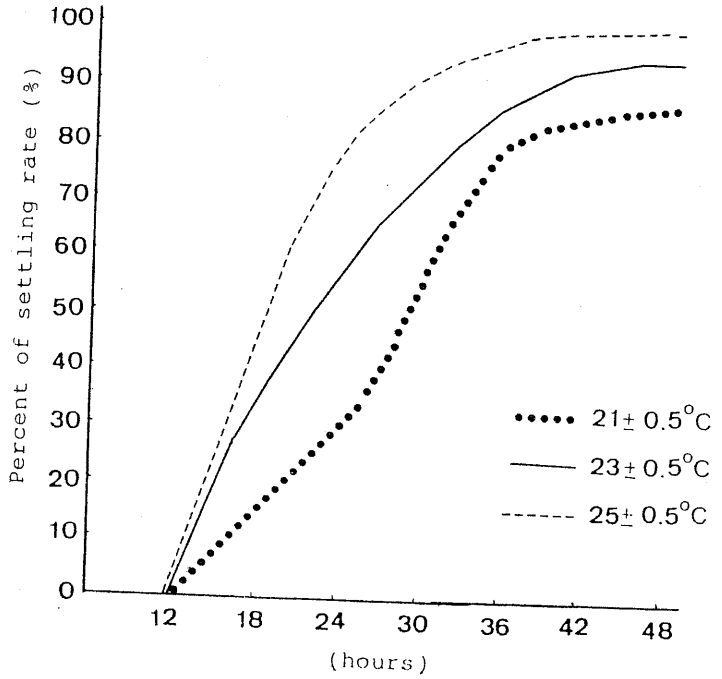

Fig. 11. Effects of temperature on mean settling rate of small abalone larvae.

TABLE 5
Comparion of growth rate of the abalone under different treatment and food contition

Food condition	Hormone treated	The numbers of individual	Body length (um)	*t*-value
Without food	insulin	211	362.0±27.27	13.53*
	growth hormone	198	341.4±33.20	7.37
	control	217	312.0±46.36	
With food	insulin	117	344.1±27.20	9.00*
	growth hormone	193	343.2±28.48	10.00*
	control	163	309.7±34.23	

*: $p<0.01$

had the trend of 25°C>23°C>21°C (Fig. 11).

III. Acceleration of growth rate

Morse (1980) suggested that externally provided hormonal control may be useful for accelerating early growth and reducing growth-rate variability in red abalone. In our study, siblings' acceleration growth was examined in the prensence or absence of hormones and with or without exogeneous food. Differences between treated and untreated hormone groups are significant (Table 5, $p<0.01$). In addition, groups treated with hormones also decreased the variation of individual size and paced the growth of small abalone.

Acknowledgements: This research was sponsored by the National Science Council, Republic of China. Special thanks to Mr. S. I. Lin, Hua-Lian Fishery Company for generously providing facilities in support of this study.

REFERENCES

BANG S. B. (1977) Studies of the seed production of abalone, *Haliotis discus* Reeve. *Bull. Fish. Res. Dev. Agency* **17**: 7-16.

CARLISLE J. G. (1962) Spawning and early life history of *Haliotis rufescens* Swainson. *Nautilus* **76**: 44-49.

EBERT E. E. (1974) Red abalone temperature tolerance study. *Mar. Res. Admin. report* **74**: 75-87.

IWATA K. (1980) Minerization and architecture of the larval shell of *H. discus hannai* Ino, (Archaeogastropoda). *J. Fac. Sci. Hokkaido Univ.* **19**: 305-320.

KIKUCHI and UKI (1974) Technical studies on artificial spawning of abalone, genus *Haliotis*. III. Resonal sperm density for fertilization. *Bull. Tohoku Reg. Fish. Lab.* **34**: 67-71.

KIKUCHI and UKI (1974) Technical studies on artificial spawning of abalone, genus *Haliotis*. IV. Duration of fertility related to temperature. *Bull. Tohoku Reg. Fish. Res. Lab.* **34**: 72-78.

LEE J. J. and LEE C. K. (1982) Gametogenesis, reproductive cycle and inducing spawning of the abalone, *Sulculus diversicolor aquatilis* Reeve. *Bull. Mar. Resou. Res. Inst. Jeju Univ.* **6**: 9-25.

LEIGHTON D. L. (1972) Laboratory observation on the early growth of the abalone, *Haliotis sorenseni*, and the effects of temperature on larval development and settling success. *Fish. Bull.* **70**(2): 373-381.

LI JEROME C. R. (1964) Statistical Inference I. Edwards Brothers, Inc., Ann Arbor, Michigan.

MARUYAMA S. (1935) On the development of the Japanese abalone, *Haliotis gigantea*. *J. Coll. Agricult.* **8**(3): 228-233.

MORSE D. E. (1979) *r*-aminobutyric acid, a neurotrasmitter, induce plantonic abalone larvae and begin metamorphosis. *Science* **204**: 407-410.

MORSE D. E. (1980) Biochemical and genetic control of critical physiological processes in molluscan life-cycles: Basic mechanisms, water quality requirements and sensitivities to pollutants. A Report on the California Sea Grant College Program for 1978-1980.

OBA T. (1964) Studies on the progation of an abalone, *Haliotis diversicolor supertexta* Lischke. II. On the development. *Bull. Jap. Soc. Sci. Fish.* **30**(10): 809-818.

PYEN C. K., J. Y. JO, K. N. JANG and K. H. YANG (1981) The induced spawning and the rearing of the early stage of the abalone, *Haliotis discus hannai*. *Bull. Fish. Res. Dev. Agency* **26**: 37-49.

SAGARA and AROCHI (1971) Oxygen consumption of abalone in early development stage and juvenile. *Bull. Tokai Reg. Fish. Res. Lab.* **65**: 766-779.

TANAKA Y. (1969) Studies on the reducing motality of larvae and juveniles in the course of the mass-production of seed abalone I. Satisfastory result with streptomycin to reduce intensive motality. *Bull. Tokai. Reg. Fish. Res. Lab.* **58**: 155-161.

TZENG W. N. (1976) The biology of reproduction in the abalone, *H. diversicolor supertexta* Lischke, in the northeastern Taiwan. *J. Fish. Soc. Taiwan* **5**(1): 24-32.

TZENG W. N. and F. Y. LIN (1976) Histological studies on the gonadal maturation of the abalone, *H. diversicolor supertexta* Lischke. *Bull. Malacol. Soc. China* **3**: 35-45.

VAQUIER V. D., D. L. LEIGHTON and C. A. LEWIS (1980) Assessment of sperm-egg interactions during fertilization and hybrid formation of California abalones. Calif. Sea Grant Coll. Program Biennial Report: 89-91.

YANG H. S. (1979) Studies on the artificial propagation of the small abalone (*H. diversicolor supertexta* Lischke). M. S. thesis. Inst. of Oceanograph, The College of Chinese Culture. 37pp.

YEH S. F. (1977) Experimental design. Part I. Biostatistics. Nat. Taiwan Univ., Taiwan.

九孔 (*Haliotis diversicolor supertexta*) 之初期發生及成長之研究

劉莉蓮　朱麗馨　張崑雄

本文係利用掃描式電子顯微鏡研究本省產九孔 (*Haliotis diversicolor supertexta*) 之發育情形。結果發現九孔之感覺毛束不僅在其擔輪子與覆面幼蟲期之頭部有同時在它達 21 天的稚貝的頭部觸手及上肢觸手上均有出現。

此外經過紫外線照射，過濾 (0.7 μm) 或是施以抗生素 (dihydro-streptomycin 75–225 ppm) 之海水均能提高九孔幼苗的存活率。至於飼養密度在從每 100 cc 25 個個體到 1600 個體由對於九孔幼苗存活的比率却無明顯的差異。在 25±1°C 情況下受精後之九孔幼苗在 48 小內會有 95%成功地附著，如果養殖水溫從 21°C 提昇到 25°C 時，稚貝之附著率亦會顯著地提昇。

如果在甫行變態的稚貝的水缸中加入胰島素和生長荷爾豪時，九孔稚貝之成長率會增加。其體長較對照組增加 30–50 μm。這些荷爾蒙同時也會調整稚貝之成長使得同胎內稚貝大小間之差異減小。