CHAPTER 3

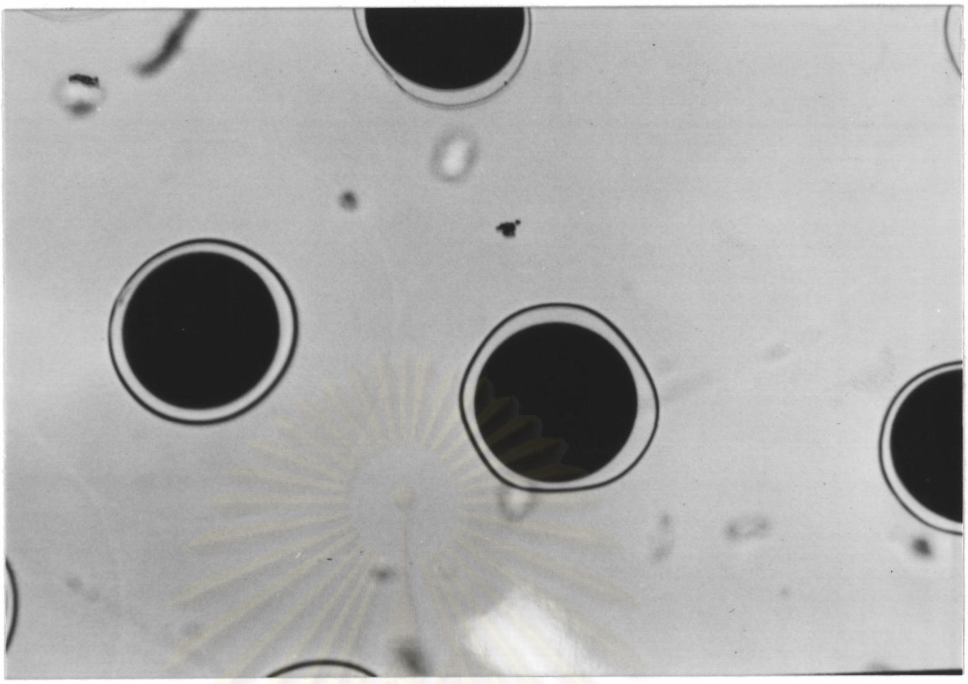
RESULTS

Experiment 1 : Embryonic Development, Larval Development and Early Growth of Hatchery-Produced Abalone Seed, *Haliotis ovina* Gmelin 1791.

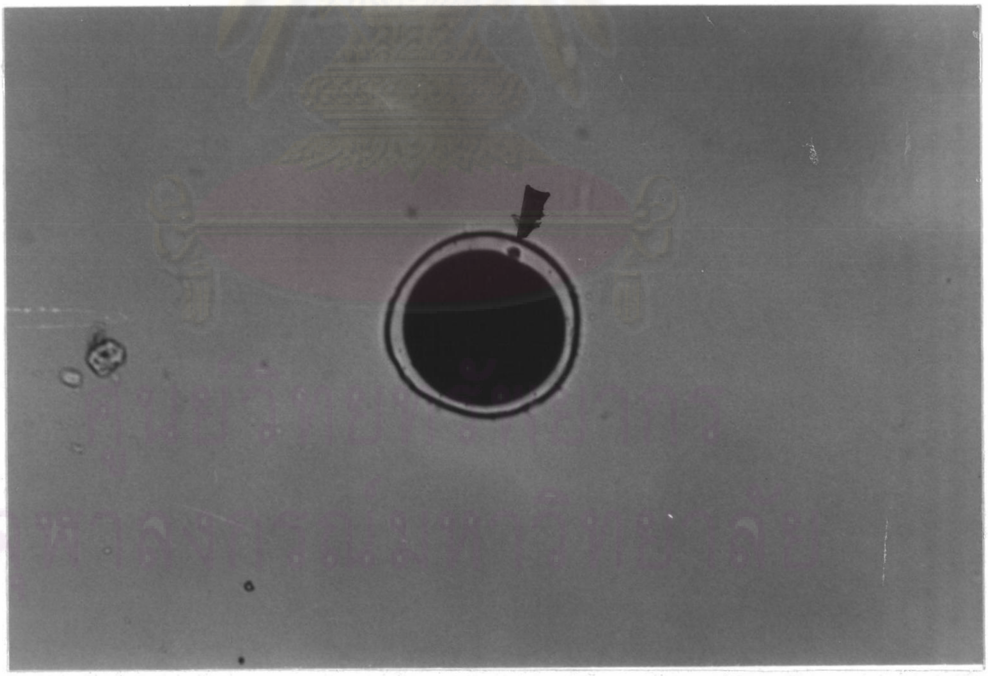
Seven males and fifteen females were chosen and put into separated spawning aquariums. Spawning began about 3 hour and 20 minutes after induced spawning (males spawn in prior to females) and lasted for 15-20 minutes. Number of spawning male and female were 7 and 8 respectively. First released eggs have many round-type shapes. These unfertilized eggs became round with egg membrane elevated over yolk portion. The egg diameter with and without egg membrane were 140 and 180 microns, respectively (Figure 10 a).

Ten minutes after insemination,(salinity 32 ppt. and temperature 30°C) first polar body became visible followed by second polar body (Figure 10 b and 11 a). First cleavage was observed at 20 minutes after fertilization followed by second cleavage (Figure 11 b and 12 a). Then these embryos developed from the first until they reached the sixth cleavage (Figure 12 b) and ready to hatch-out (Figure 13).

Hatching into swimming trochophore larvae with appearance of retractor muscle were seen within 6 to 7 hours after fertilization (Figure 14 a). Healthy larvae at this stage show clearly positive photo taxis behavior. They swam up



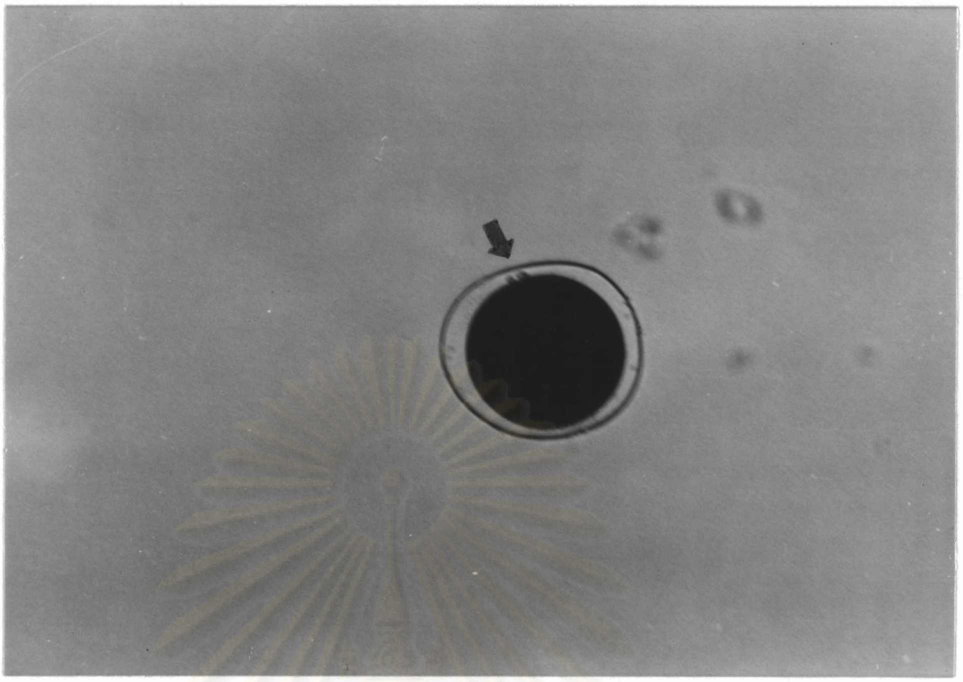
(a.)



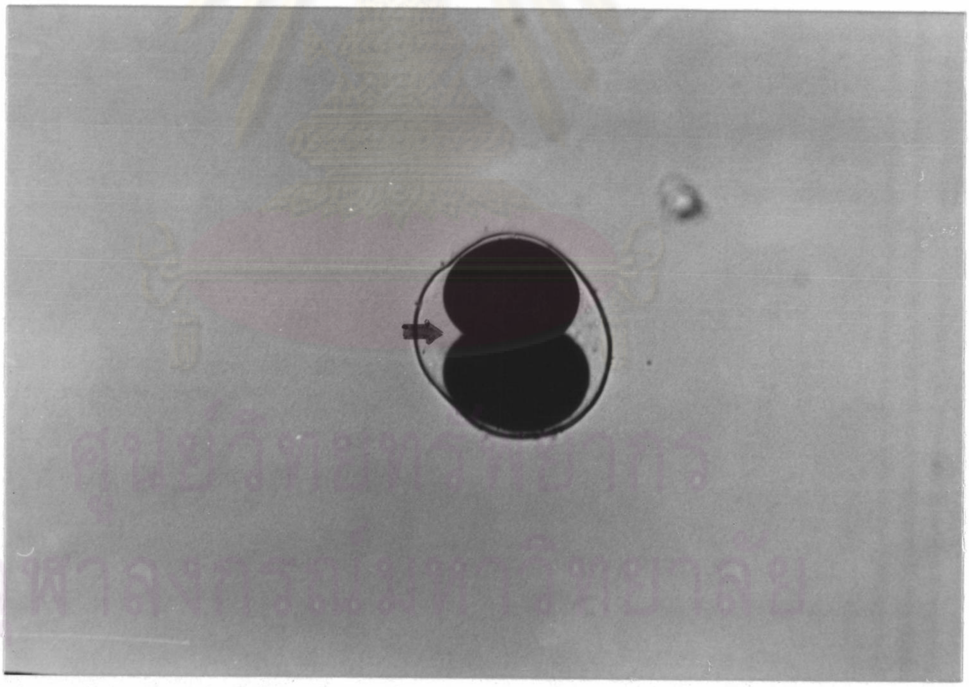
(b.)

Figure 10 a Unfertilized egg

b First polar body



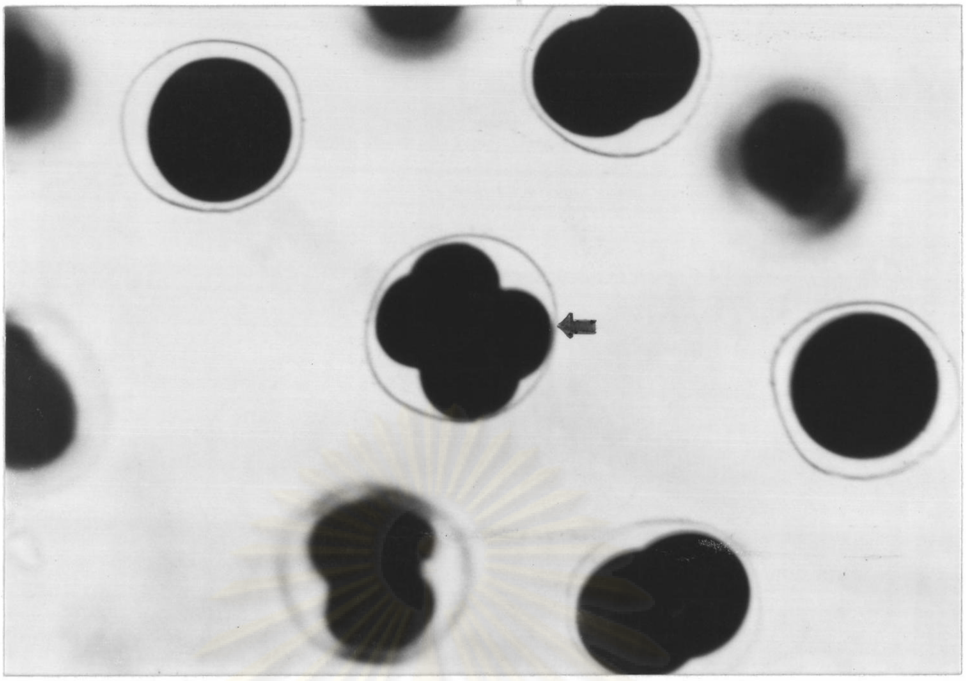
(a.)



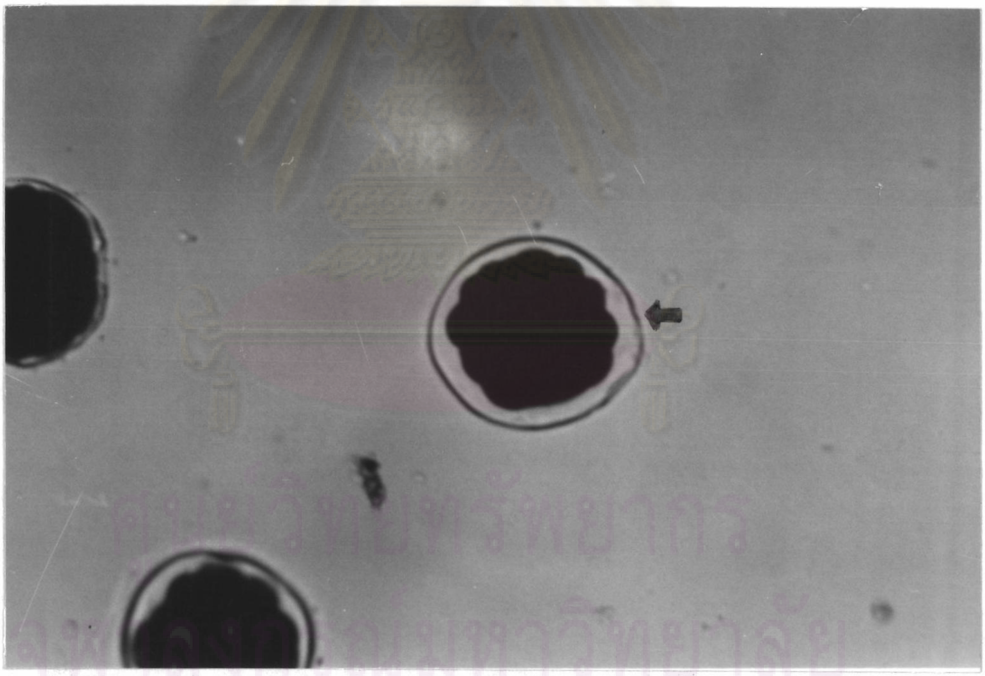
(b.)

Figure 11 a Second polar body

b First cleavage



(a.)



(b.)

Figure 12 a. Second cleavage

b. Sixth cleavage

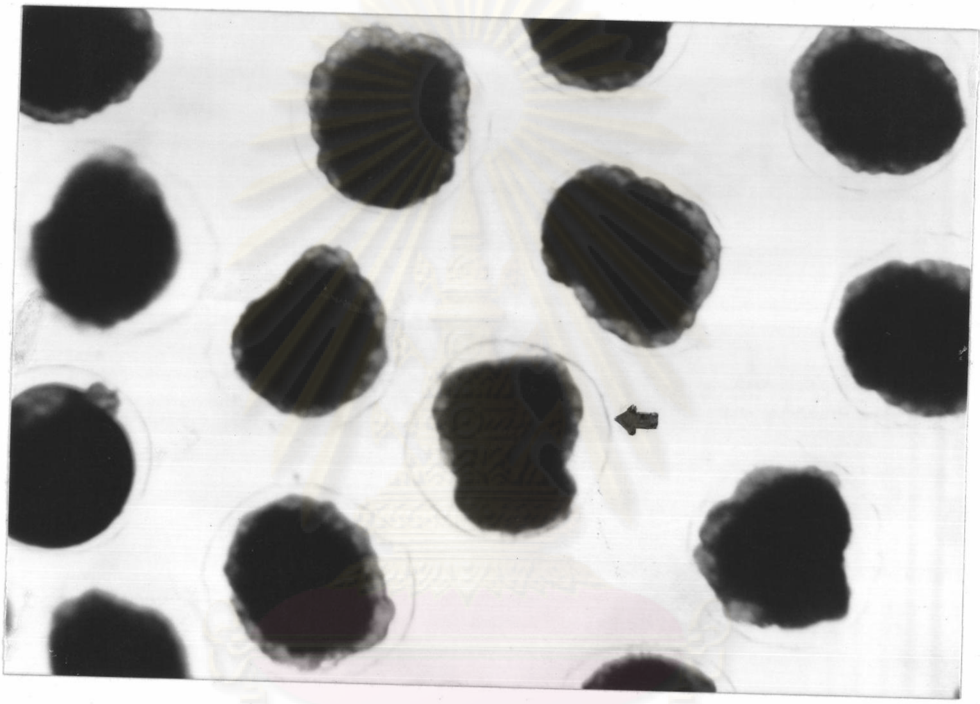
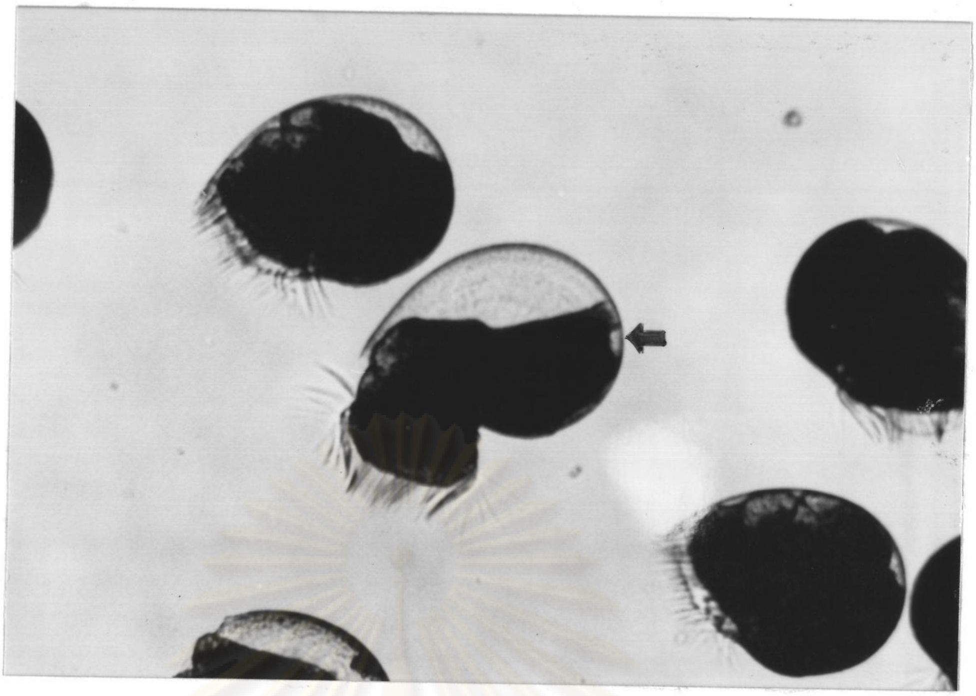


Figure 13. Fertilized egg which ready to hatch out



(a.)

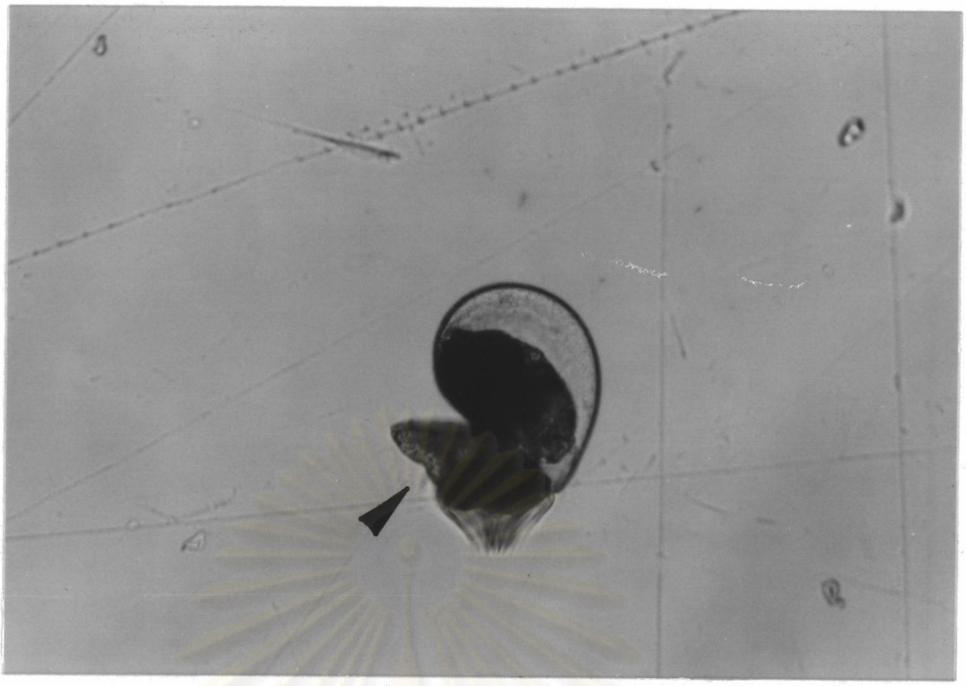
(b.)

Figure 14 a. 5-6 hours old larvae (appearance of retractor muscle)

b. 10-12 hours larvae (rotation of foot mass) rapidly to the water surface following the torch light. At early veliger stage rotation of foot mass was seen within 10 - 12 hours after insemination (Figure 14 b). Appearance of cephalic tentacle and formation of first epipodial tentacle were seen at 26 and 30 hours after insemination respectively (Figure 15 a and b)

Creeping stage was obtained 36 hours after insemination (Figure 16 a) at this stage larvae were ready to settle on diatom plates (appearance of fourth tubule on cephalic tentacle). Settled juvenile (40 hours-old) was shown in Figure 16 b Larvae metamorphosis into juvenile reached within 3-4 days after fertilization (Figure 17a) First respiratory pore stage was seen 20 to 24 days after insemination the average size (measured from shell length) of animals was 1.375 $\pm$ 0.306 mm. (mean $\pm$ standard variation, n=4). At this stage, they could seen by naked eyes (Figure 17 b).

Two months after insemination, the average size of 4 juveniles that randomly collected from diatom plates were measured for their shell length. The shell length was 6.653$\pm$0.557 mm.(Figure 18). Three months after insemination, 99 young abalones were obtained and most of them fell down to the bottom of the tank and began to feed on macroalgae. Shell length of young abalones ranged from 11.00 to 22.90 millimeters (16.523$\pm$2.533 millimeters), shell width ranged from 8.40 to 18.00 millimeters (12.887$\pm$1.973 millimeters) and weight ranges from 0.130 grams to 1.380 grams (0.526$\pm$0.242 grams). Growth in shell length of juvenile during the first 3 months is shown in Figure 19. A summary of embryonic development, larval development and early growth of *H. ovina* is shown in Table 6.

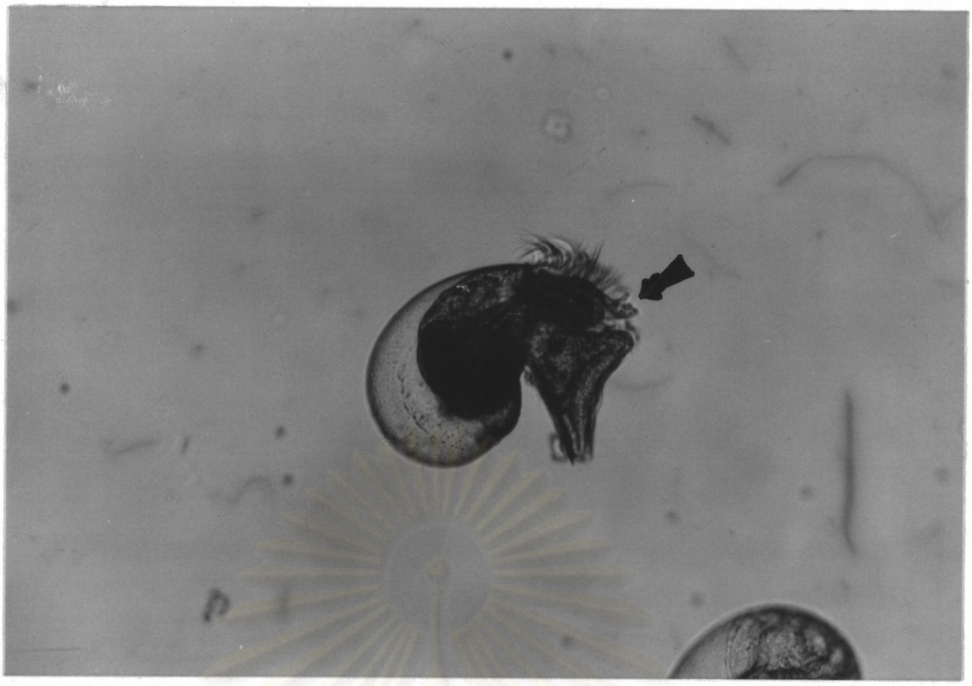


(a.)

(b.)

Figure 15 a 26 hours old larvae (appearance of cephalic tentacle)

b 30 hours old larvae (formation of epipodial tentacle)



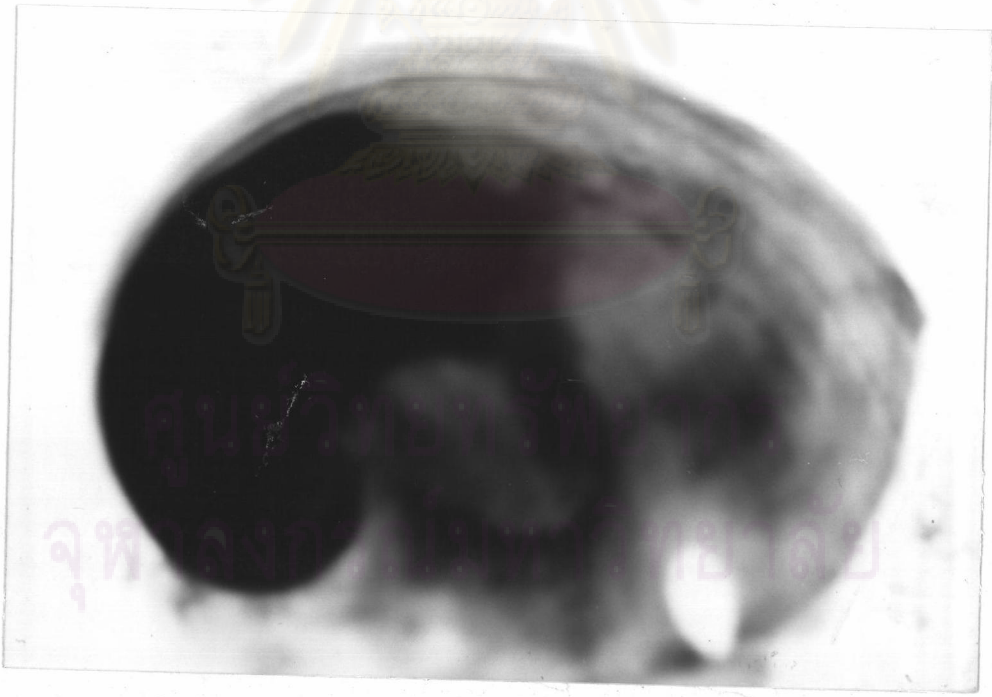
(a.)

(b.)

Figure 16 a 36 hours old larvae (appearance of fourth tubule on cephalic tentacle) at this stage larvae was ready to settled.

b 40 hours old larvae(settled larvae).

(a.)



(b.)

Figure 17 a 5 days old juvenile

b juvenile with first respiratory pore

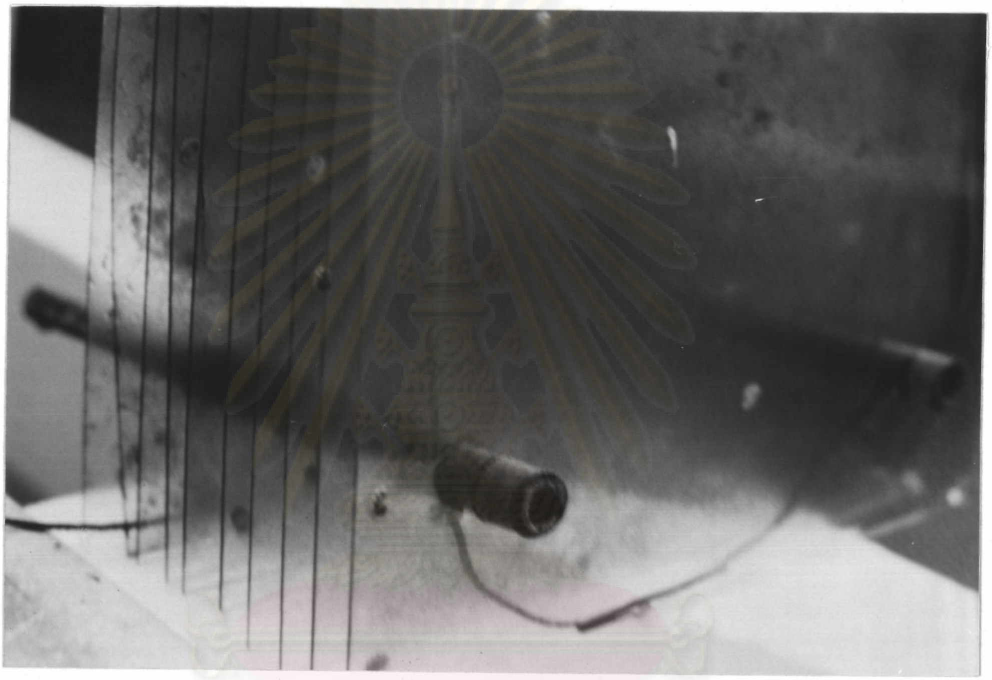


Figure 18 Two months old juvenile on diatom plate bundle

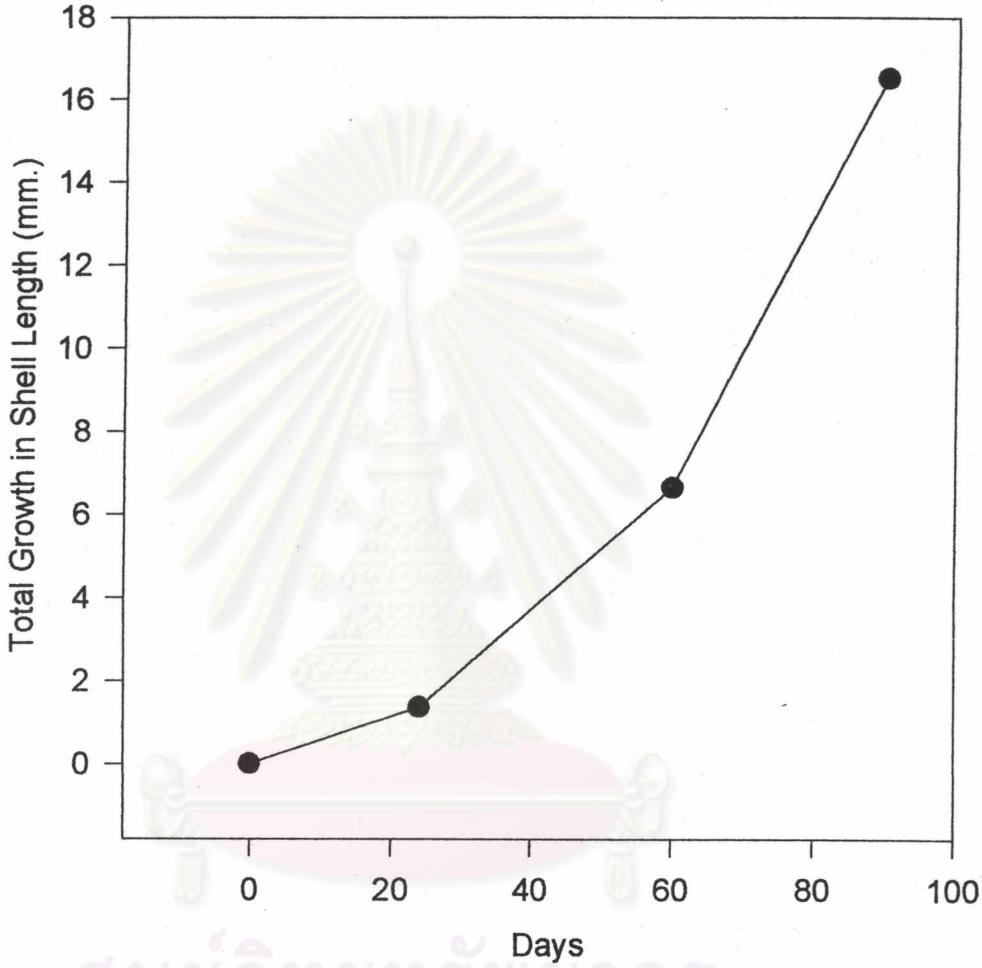


Figure 19. Growth in shell length juvenile hatchery produced abalone *Haliotis ovina*, Gmelin, 1791. during the first three months

Table 6. Embryonic development, larval development stages of *H. ovina* including early growth rate.

Time	Stage	Size
0 minute	unfertilized egg	180 microns
10 minutes	first polar body	180 microns
15 minutes	second polar body	180 microns
20 minutes	first cleavage	180 microns
30 minutes	second polar body	180 microns
2-4 hours	third-sixth cleavage	180 microns
5-6 hours	rotating trochophore	180 microns
7-8 hours	hatch out	180 microns
10-12 hours	early veliger	220 microns
18-22 hours	late veliger	250 microns
36-40 hours	creeping larvae*	340 microns
12-15 days	juvenile (young shell)	0.5-0.8 mm.
20-24 days	first respiratory pore	1.375 ± 0.306 mm.
60 days	juvenile	6.653 ± 0.557mm.
3 months	juvenile	16.523 ± 2.533 mm. (shell length)

Experiment 2: Effect of Different Macroalgal Diets on Growth of Juveniles Abalone, *Haliotis ovina*

Experiment I

The experiment has a serious problem, some kind of toxic chemical substance was leak into culture system caused from human error after started experiment for 1 month, more than 50% of juveniles were died. It was necessary to finished this experiment because of amount of animals were insufficient for statistical analysis. Data of this experiment which reported in final size and daily rate increase in shell length(µm/day), shell width (µm/day) and specific growth rate (%/day) after finished experiment in 1 month was shown in Table 7 and 8.

Juveniles fed with *Gracilaria changii* (GC) showned the highest growth in all parameters (81.67±3.69 µm/day in daily rate increase in shell length, 67.00±1.67 µm/day in daily rate increase in shell width and specific growth rate = 1.89±0.28 %/day). Result in this treatment was similarly to growth of juveniles fed mixed kind of macroalgaes (*G. changii* and *Enteromorpha intestinalis*) while juveniles fed with *E. intestinalis* had a poor growth compared with other treatments.

Before started this experiment, there was no significantly different ($p<0.05$) between treatment in initial shell length, initial shell width and initial body weight. After stopped experiment, juvenile fed with *Gracilaria changii* (GC) and both *G. changii* and *Enteromorpha intestinalis* (MI) exhibited significantly different ($p<0.05$) daily rate increased in shell length and daily rate increase in shell width with

Table 7 Final size (mean±standard deviation) of juveniles abalone *H. ovina* fed for 1 month on *Gracilaria changii*, *Enteromorpha intestinalis* and mixed of the two macroalgaes (MI).

Treatment	Length (mm)	Width (mm)	Weight (g)
Gracilaria changii(n=7)	19.42±2.462	15.16±1.954	0.95±0.285
Enteromorpha intestinalis(n=10)	17.29±2.298	13.16±1.699	0.63±0.204
MI (n=9)	18.65±2.109	14.64±1.891	0.84±0.232

Table 8 Growth rates of juvenile abalone *H. ovina* fed for 1 month on *Gracilaria changii*, *Enteromorpha intestinalis* and mixed of the two kinds of macroalgaes (MI). Standard errors are indicted in brackets.

Treatment	DILt (µm/day)	DIWd (µm/day)	SGR (%/day)
Gracilaria changii(n=7)	81.67(3.69)^{a}	67.00(1.67)^{a}	1.89(0.28)^{a}
Enteromorpha intestinalis(n=10)	57.00(4.32)^{b}	38.00(1.03)^{b}	1.43(0.27)^{a}
MI (n=9)	76.00(2.28)^{a}	64.33(1.14)^{c}	1.55(0.31)^{a}

DILt = Daily rate increase in shell length (µm/day)

DIWd = Daily rate increase in shell width (µm/day)

SGR = Specific growth rate (%/day)

a, b and c denoted significant different in growth rate ($p<0.05$)

juvenile fed with *E. intestinalis* (ES). In contrast, there was no significantly different between treatments (p<0.05) in specific growth rate.

Experiment II

In this experiment juveniles fed with *Gracilaria changii* shown the highest growth in daily rate increased in shell length (14.2±3.1 μm/day) and specific growth rate (0.20 %/day). Juveniles fed with *Enteromorpha intestinalis* had very similarly growth in daliy rate increase in shell width compared with juveniles fed with *G. changii* (8.6±1.8 and 8.3±1.7μm/day respectively). Juveniles fed with *Euchema sp.* showned clearly the lowest growth in all paramaters (7.4±2.5 μm/day in daliy rate increase in shell length, 6.0±1.4 μm/day in daliy rate increase in shell width and specific growth rate=0.04±0.01 %/day). At the end of the experiment, abalones fed with *Euchema sp.* (EU) had high mortality (50% of juvenile were died, n=15) while juvenile fed with *E. intestinalis* (ES) had low mortality (n=28) and juvenile fed with *G. changii* (GC) juveniles did not died in this experiment. Survival rate and growth of all parameters are summarized in Table 9 and 10.

Growth rate of juveniles fed with EI during 0 to 30 days were higher than other treatments except specific growth rate which similarly to juveniles fed with GC. In this period juveniles fed with EI and EU had mortality (2 and 7 amimals respectively). During 31 to 60 days daily rate increase in shell length and specific growth rate of juveniles fed with GC were clearly increased while specific growth rate and survival rate of juveniles fed with EU were lowest During 61 to 90 days specific growth rate in treatment EI were cleraly declined (from 0.27 %/day to 0.03 %/day).

Table 9. Suvival rate (%) of juvenile abalone fed with various kinds of macroalgae in experiment II

Treatment	30 days	60 days	90 days
Enteromorpha intestinalis	92.4	92.4	92.4
Euchema sp.	75.9	50	50
Gracilaria changii	100	100	100

Table 10 Growth rates of juvenile abalone *H. ovina* fed for 3 months. Standard errors are indicted in brackets.

Treatment	Days	DILt (μm/day)	DIWd (μm/day)	SGR (%/day)
Enteromorpha intestinalis	0 - 30	14.4 (5.1)	12.8 (5.0)	0.13 (0.02)
	31 - 60	10.7 (2.3)	6.7 (1.9)	0.27 (0.03)^{a}
n = 28	61 - 90	12.1 (3.4)^{a}	6.0 (2.4)	0.03 (0.01)^{b}
Average growth	0 - 90	12.4 (2.3)	8.6 (1.8)	0.14 (0.01)^{a}
Euchema sp.	0 - 30	7.5 (2.6)	9.1(4.1)	0.09 (0.05)
	31 - 60	8.1 (4.7)	2.6(1.0)	0.01 (0.06)^{b}
n = 15	61 - 90	3.5 (1.1)^{b}	4.4(2.5)	0.03 (0.02)^{b}
Average growth	0 - 90	7.4 (2.5)	6.0(1.4)	0.04 (0.01)^{b}
Gracilaria changii	0 - 30	5.4 (2.0)	10.2(4.0)	0.14 (0.02)
	31 - 60	18.3 (0.56)	7.8 (2.2)	0.30 (0.03)^{a}
n = 30	61 - 90	19.0 (3.9)^{a}	7.0 (2.7)	0.20 (0.02)^{a}
Avg. growth	0 - 90	14.2 (3.1)	8.3 (1.7)	0.20 (0.02)^{a}

DILt = Daily rate increase in shell length (μm/day)

DIWd = Daily rate increase in shell width (μm/day)

SGR = Specific growth rate (%/day)

Avg. growth = Growth rate from 0 to 90 days.

a and b denoted significant different ($p<0.05$), tested at the same time period.

However, it could be mentioned that initial shell length, shell width and body weight among treatments in this experiment are different but analysis of variance revealed that no significantly difference for all three parameters.

The analysis of variance revealed that during 0 to 30 days there was no significantly different in daily rate of increased in shell length, shell width and specific growth rate (SGR) among treatments. During 30 to 60 days SGR of juvenile fed with EI and GC were significantly different higher than SGR of juvenile fed with EU (p=0.000). During 61 to 90 days daily rate increase in shell length of juveniles fed with EI and GC were significantly different higher than juveniles fed with EU whereas SGR in juvenile fed with GC was significantly different higher than juveniles fed with EI and EU (Figure 20 to 22).

As regards with the results during 0 to 90 days, analysis of covariance reveal that initial shell length and initial body weight had an effect on growth of juveniles in daily rate increase in shell length and SGR. There were no significant difference in daily rate increase in shell length and shell width among treatments. SGR in juvenile fed with GC was significantly higher than juveniles fed with EI and EU and SGR in juvenile fed with EI was significantly higher than juvenile fed with EU.

Correlation between initial body weight and SGR was found in treatment EI and GC (r^2=0.361 and 0.384 respectively). The linear equation for these relationship were as follow :

$SGR = 0.309 - 0.035(\ln W_0)$; n=28, r^2=0.361, p-value=0.007 for EI[a]

$SGR = 0.123 - 0.015(\ln W_0)$; n=15, r^2=0.257, p-value=0.054 for EU[b]

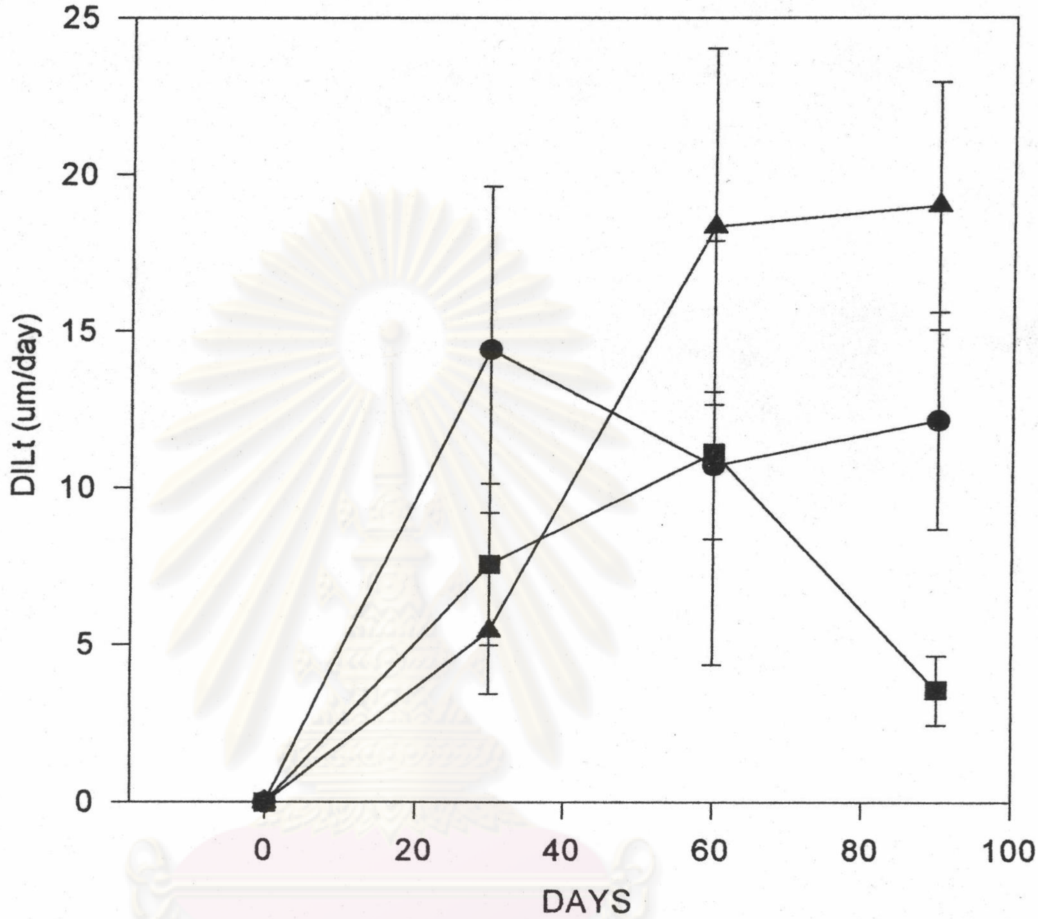


Figure 20. Total growth in daily rate increase in shell length of juvenile abalone, *Haliotis ovina*, fed with *Enteromorpha intestinalis* (EI), *Euchema* sp.(EU) and *Gracilaria changii* (GC) for 3 months

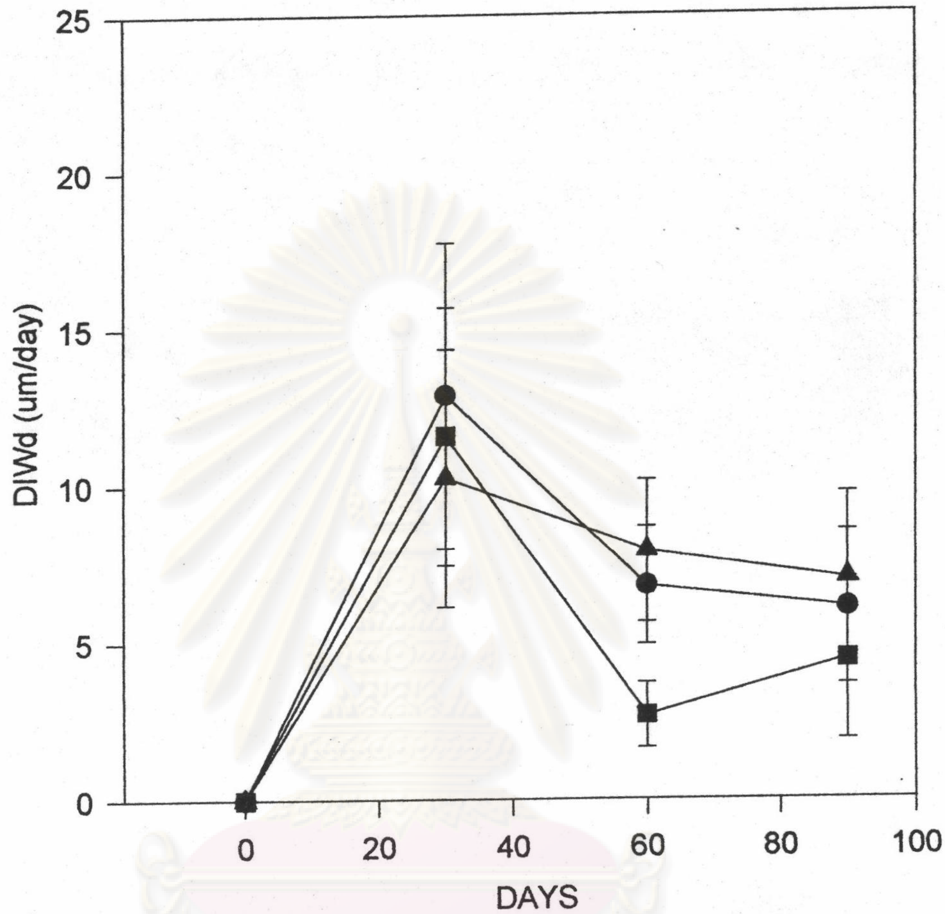


Figure 21. Total growth in daily rate increase in shell width of juvenile abalone, *Haliotis ovina*,Gmelin, 1791 fed with *Enteromorpha intestinalis* (EI), *Euchema* sp. (EU) and *Gracilaria changii* (GC) for 3 months

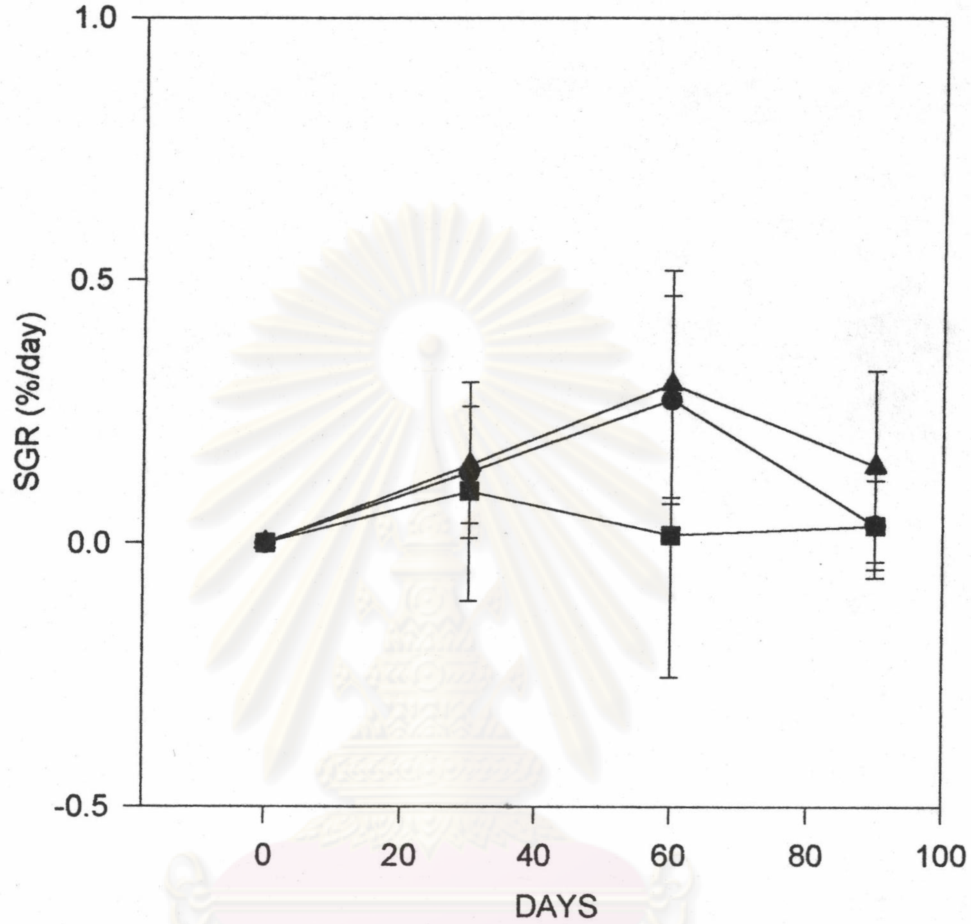


Figure 22. Total growth in spcific growth rate of juvenile abalone, *Haliotis ovina*, fed with *Enteromorpha intestinalis* (EI), *Euchema* sp. (EU) and *Gracilaria changii* (GC) for 3 months.

$$SGR = 0.440 - 0.043(\ln W_0);\ n=30,\ r^2=0.384,\ p\text{-value}=0.000 \text{ for GC}^{a}$$

Where SGR = specific growth rate

$\ln W_0$= natural logarithm value of initial weight

a and b denoted significant different ($p<0.05$)

From these linear equations, it could predict that in treatment EI and GC small size juvenile(in shell length and body weight) grew faster in the term of SGR than big size juvenile in the same condition in this experiment. Expected line from these equations were shown in Figure 23.

Analysis of covariance was performed to test these three correlations. It was found that slope of EU and GC were significant different from EU. There was no significantly differece between slope of EU and GC.

Proximate analysis of three kinds of macroalgal diets in this experiment and body contents of juveniles were shown in Table 11 and Table 12. The highest percent of protein was in GC (2.45 %) and fat content in ES being 0.2 % higher than that of EU and GC. After finish the experiment , body contents of juveniles fed with GC had the highest protein and fat content (64.06% and 0.56 % respectively).

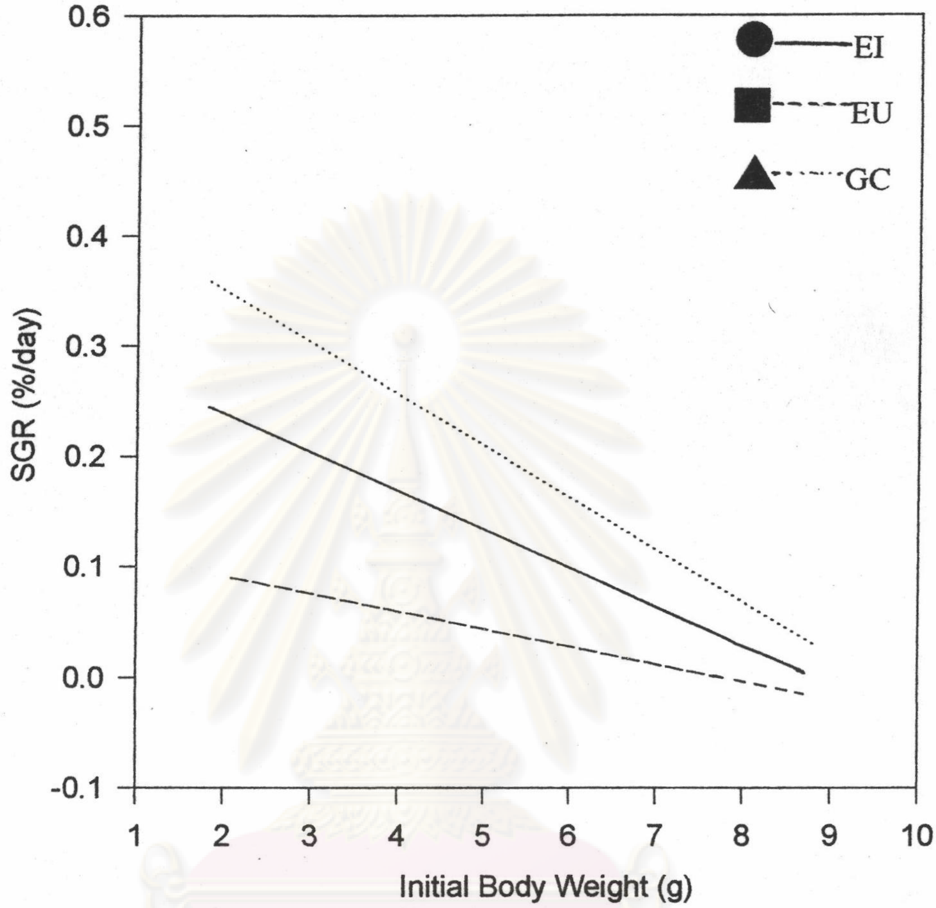


Figure 23. Expected line of relation between initial body weight and specific growth rate (SGR) from 0 to 90 days in this experiment.

Table 11. Proximate analysis of the 3 kinds of macroalgae in this experiment.

Macroalgae	%crude protein	%total lipid	%moisture	% ash
Enteromorpha intestinalis	1.96	0.39	85.8	2.0
Euchema sp.	1.44	0.15	90.5	3.9
Gracilaria changii	2.45	0.17	88.6	2.7

Table 12. Proximate analysis of body contents of juvenile abalone, *H.ovina* (Gmelin, 1791) in this experiment. Treatment EI fed with *Enteromorpha intestinalis*, EU fed with *Euchema* sp. and GC fed with *Gracilaria changii*.

Treatment	% crude protein	% total lipid
1. *Enteromorpha intestinalis*	45.53	0.27
2. *Euchema* sp.	25.06	0.44
3. *Gracilaria changii*	64.06	0.56