

SAOWAPA SAWATPEERA

With compliments
of

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**STUDIES ON FEEDING AND GROWTH OF THE THAI
ABALONE *HALIOTIS ASININA* LINNEAUS**

Saowapa Sawatpeera

Ms. Saowapa Sawatpeera
Candidate

Suchart Upatham

Prof. Suchart Upatham, Ph.D.
Major-Advisor

M. Kruatrachue

Prof. Maleeya Kruatrachue, Ph.D.
Co-advisor

Yaowaluk Chitramvong

Assoc.Prof. Yaowaluk Chitramvong,
Ph.D.
Co-advisor

Lijet. Limlomwongse

Prof. Liangchai Limlomwongse,
Ph.D.
Dean
Faculty of Graduate Studies

P. Kittayapong

Assoc.Prof. Pattamaporn Kittayapong,
Ph.D.
Chairman
Doctor of Philosophy Programme
in Biology
Faculty of Science

Thesis
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**STUDIES ON FEEDING AND GROWTH OF THE THAI
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March 22, 1999

Saowapa Sawatpeera

Ms.Saowapa Sawatpeera
Candidate

M. Kruatrachue

Prof.Maleeya Kruatrachue, Ph.D.
Member

Suchart Upatham

Prof.Suchart Upatham, Ph.D.
Chairman

Yaowaluk Chitramvong

Assoc.Prof.Yaowaluk Chitramvong,
Ph.D.
Member

P. Jarayabhand

Assoc.Prof.Padermsak Jarayabhand,
Ph.D.
Member

Lizd. Limlomwongse

Prof.Liangchai Limlomwongse,
Ph.D.
Dean
Faculty of Graduate Studies
Mahidol University

P.M. Matangkasombut

Prof.Pornchai Matangkasombut,
Ph.D., M.D.
Dean
Faculty of Science
Mahidol University

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Samples of the abalone, *Haliotis asinina*, were collected from Samed Island, Rayong Province, Thailand by SCUBA diving every three months for a period of one year for determination of gut contents. The average shell length and total body weight for these samples were 68.3 ± 9.1 mm and 48.2 ± 11.4 g. The average volume of micro-contents (< 120 μ m) in gut contents was 80.3 % and that of macro-contents (> 120 μ m) was 19.7 %. Most micro-organisms were benthic diatoms (99.3 %) and the dominant genera were *Nitzschia*, *Amphora*, *Cocconeis*, *Navicula* and *Diploneis*. Macro-contents consisted mainly of sand granules and foraminiferans. Therefore, the main food of *H. asinina* along Samed Island appeared to be comprised of benthic diatoms which were the predominant floral elements in the local environment.

Larval development of *H. asinina* was observed from fertilization to the formation of the fourth tubules of cephalic tentacle under the water temperature 25, 28, 31 and 34 °C. After settlement, the development of the post-larvae through the formation of the first respiratory pore was observed under room temperature (28-35 °C). The average size of fertilized egg was 126.4 ± 17.1 μ m in width and 148.2 ± 10.4 μ m in length. The time periods of larval development were 65, 49, 41 and 41 hours at water temperatures of 25, 28, 31 and 34 °C, respectively. The biological zero point was 15.0 ± 0.3 °C. The effective accumulative temperatures (EAT) of complete larval development of *H. asinina* in water temperatures of 25, 28, 31 and 34 °C were 650, 637, 656 and 779 °C-hours, respectively. The size of larvae at the completion of development was 178.3 ± 5.2 μ m in width and 245.7 ± 4.6 μ m in length. The formation of the first respiratory pore began on day 24 to day 30 depending on the temperature, diatom type and density of abalone in settlement tank.

Experiments on natural food utilization by *H. asinina* were conducted over a period of 184 days using nine species of macroalgae, *Acanthophora spicifera*, *Gracilaria fisheri*, *G. salicornia*, *G. tenuistipitata*, *Enteromorpha intestinalis*, *Caulerpa racemosa*, *Dictyota dichotoma*, *Padina minor* and *Sargassum polycystum*. The highest growth rate (23.6 ± 2.4 mg/day in weight), survival rate (95.6 ± 1.6 %), and the best food conversion rate (FCR) (3.2 ± 0.5) were obtained with the red alga, *G. tenuistipitata*. The brown alga, *P. minor*, gave the lowest growth ($- 0.3 \pm 0.3$ mg/day in weight) and survival rate (7.8 ± 1.6 %). The proximate composition of these nine species of algae was determined to suggest correlation with growth rates. *G. tenuistipitata* was found to have the highest content of protein and carbohydrate.

Experiments of artificial food utilization by *H. asinina* were conducted over a period of 140 days using seven formulae of artificial diets and fresh *G. salicornia* as control. Protein sources of diet formulae I - IV were 30 % macroalgae (*A. spicifera*, *E. intestinalis*, *G. fisheri* and *G. salicornia*) and diet formulae V - VII were 30 % casein and 30 % macroalgae (*G. fisheri*, *G. salicornia* and *G. tenuistipitata*). The abalone fed fresh *G. salicornia* had a higher growth and survival rates than those fed artificial diets. Among artificial diets, the highest growth rate (6.4 ± 2.3 mg/day in weight) was obtained with the diet containing casein and *G. salicornia*; the best FCR (19.2 ± 4.7) with diet containing casein and *G. salicornia*; and the survival rate (78.3 ± 2.9 %) with diet containing *G. fisheri*.

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เลาภา สวัสดิ์พีระ : การศึกษาการกินอาหารและอัตราการเจริญเติบโตของหอยเป่าชื่อพันธุ์พื้นเมือง *Haliotis asinina* Lineaus (STUDY OF FEEDING AND GROWTH OF THE THAI ABALONE *HALIOTIS ASININA* LINNEAUS). คณะกรรมการควบคุมวิทยานิพนธ์ : สุชาติ อุปลัมภ์ Ph.D., มลียา เครือธราฐ Ph.D., ยศวัดกันต์ จิตราวงษ์ Ph.D., 143 หน้า. ISBN 974-662-085-1

ทำการศึกษาองค์ประกอบของอาหารในทางเดินอาหารของตัวอย่างหอยเป่าชื่อ *Haliotis asinina* จากเกาะเสม็ด จังหวัดระยอง ซึ่งเก็บด้วยการดำน้ำแบบใช้ SCUBA ทุกๆระยะเวลา 3 เดือนเป็นเวลาหนึ่งปี ตัวอย่างหอยเป่าชื่อมีความยาวเปลือกและน้ำหนักเฉลี่ยเท่ากับ 68.3 ± 9.1 มม. และ 48.2 ± 11.4 กรัม องค์ประกอบที่มีขนาดเล็ก (< 120 ไมโครเมตร) มีปริมาตร 80.3 % และขนาดใหญ่ (> 120 ไมโครเมตร) 19.7 % สิ่งมีชีวิตขนาดเล็กส่วนใหญ่เป็นกลุ่มไดอะตอม (99.3 %) กลุ่มที่พบมาก ได้แก่ *Nitzschia*, *Amphora*, *Cocconeis*, *Navicula* และ *Diploneis* องค์ประกอบขนาดใหญ่ส่วนใหญ่เป็นตะกอนดิน สิ่งมีชีวิตที่พบส่วนใหญ่เป็นกลุ่ม foraminiferans ดังนั้นอาหารส่วนใหญ่ของ *H. asinina* ในบริเวณเกาะเสม็ดเป็นพวกกลุ่มไดอะตอมที่อยู่ตามพื้นหินที่หอยเป่าชื่ออาศัยอยู่

ทำการศึกษาการพัฒนารูปร่างของตัวอ่อนของหอยเป่าชื่อ *H. asinina* ตั้งแต่ปฏิสนธิจนถึงระยะสุดท้ายของตัวอ่อนที่อุณหภูมิ 25, 28, 31 และ 34 °ซ และศึกษาการพัฒนาในระยะ post - larvae จนถึงระยะที่สร้างรูหายใจแรกในบ่ออนุบาลที่มีอุณหภูมิอยู่ระหว่าง 25 - 38 °ซ ไข่หอยเป่าชื่อมีความกว้างเฉลี่ย 126.4 ± 17.1 ไมโครเมตรและความยาวเฉลี่ย 148.2 ± 10.4 ไมโครเมตร ระยะเวลาในการพัฒนาจนถึงระยะสุดท้ายของตัวอ่อนที่อุณหภูมิ 25, 28, 31 และ 34 °ซ เท่ากับ 65, 49, 41 และ 41 ชั่วโมง ตามลำดับ อุณหภูมิต่ำสุดที่หอยสามารถพัฒนาได้อย่างสมบูรณ์ (biological zero point) เท่ากับ 15.0 ± 0.3 องศาเซลเซียส และอุณหภูมิสะสมของการพัฒนา (EAT) ของตัวอ่อนที่อุณหภูมิ 25, 28, 31 และ 34 °ซ เท่ากับ 650, 637, 656 และ 779 °ซ - ชั่วโมง ตามลำดับ ตัวอ่อนหอยเป่าชื่อที่มีการพัฒนาอย่างสมบูรณ์มีขนาดความกว้างเฉลี่ย 178.3 ± 5.2 ไมโครเมตร และความยาวเฉลี่ย 45.7 ± 4.6 ไมโครเมตร ลูกหอยเป่าชื่อเริ่มสร้างรูหายใจแรกเมื่ออายุได้ 24 - 30 วัน ขึ้นอยู่กับอุณหภูมิ ชนิดไดอะตอมที่เกิดอยู่บนแผ่นล่อ และ ความหนาแน่นของลูกหอยในถังเลี้ยง

ทำการทดลองเลี้ยงหอยเป่าชื่อ *H. asinina* ด้วยสาหร่ายใหญ่เก้าชนิด ได้แก่ *Acanthophora spicifera*, *Gracilaria fisheri*, *G. salicornia*, *G. tenuistipitata*, *Enteromorpha intestinalis*, *Caulerpa racemosa*, *Dictyota dichotoma*, *Padina minor* และ *Sargassum polycystum* เป็นระยะเวลา 184 วัน หอยเป่าชื่อที่เลี้ยงด้วยสาหร่ายสีแดง *G. tenuistipitata* มีอัตราการเจริญ (23.6 $\pm$ 2.4 มก. ต่อวัน) อัตราการรอด (95.6 $\pm$ 1.9 %) และอัตราการแลกเนื้อ (3.2 $\pm$ 0.5) ดีที่สุด ส่วนหอยเป่าชื่อที่เลี้ยงด้วยสาหร่ายสีน้ำตาล *P. minor* มีอัตราการเจริญ (- 0.3 $\pm$ 0.3 มก. ต่อวัน) และอัตราการรอด (7.8 $\pm$ 1.6 %) ต่ำที่สุด ปริมาณโปรตีนและคาร์โบไฮเดรตในสาหร่ายมีความสัมพันธ์กับอัตราการเจริญของหอยเป่าชื่อ โดยพบว่า *G. tenuistipitata* มีปริมาณโปรตีนและคาร์โบไฮเดรตสูงสุด และเป็นสาหร่ายที่ให้อัตราการเจริญเติบโตสูงที่สุด

ทำการทดลองเลี้ยงหอยเป่าชื่อ *H. asinina* ด้วยอาหารสำเร็จรูปเจ็ดสูตร โดยมีหอยที่เลี้ยงด้วยสาหร่ายสีแดง *G. salicornia* เป็นกลุ่มควบคุม เป็นระยะเวลา 140 วัน อาหารสำเร็จรูปสูตรที่ I - IV มีสาหร่าย (*A. spicifera*, *E. intestinalis*, *G. fisheri* และ *G. salicornia*) 30 % เป็นแหล่งโปรตีน และอาหารสำเร็จรูปสูตรที่ V - VII มีเคซีน 30 % และสาหร่าย (*G. fisheri*, *G. salicornia* และ *G. tenuistipitata*) 30 % เป็นแหล่งโปรตีน ผลการทดลองพบว่า หอยเป่าชื่อที่เลี้ยงด้วยสาหร่ายสด *G. salicornia* มีอัตราการเติบโตและการรอดสูงกว่าอาหารสำเร็จรูป ในพวกที่เลี้ยงด้วยอาหารสำเร็จรูปด้วยกันหอยเป่าชื่อที่เลี้ยงด้วยอาหารสำเร็จรูปสูตรที่ VII มีอัตราการเจริญสูงที่สุด (น้ำหนักเพิ่ม 6.4 ± 2.3 มก.ต่อวัน) หอยเป่าชื่อที่เลี้ยงด้วยอาหารสำเร็จรูปสูตรที่ VI มีอัตราการแลกเนื้อดีที่สุด (19.2 $\pm$ 4.7) หอยเป่าชื่อที่เลี้ยงด้วยอาหารสำเร็จรูปสูตรที่ V มีอัตราการรอดสูงที่สุด (78.3 $\pm$ 2.9 %) องค์ประกอบด้านโภชนาการของอาหารสำเร็จรูปทั้งเจ็ดมีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติ ซึ่งมีผลต่ออัตราการเจริญและอัตราการแลกเนื้อของหอยเป่าชื่อ

CONTENTS

	Page
ACKNOWLEDGEMENT	iii
ABSTRACT	iv
LIST OF TABLES	viii
LIST OF FIGURES	xi
CHAPTER	
I INTRODUCTION AND REVIEW LATERATURE	1
- Introduction	1
- Literature Review	2
- Ecology and Life-Cycle	3
- Larval Development	5
- Food and Feeding Habit	7
- Larval stage	8
- Post-settlement stage (spat)	8
- Weaning stage	9
- Grow-out stage	10
- Cultivation	10
- Broodstock	10
- Conditioning	11
- Induction of spawning	11
- Rearing of larvae	12

	Page
- Settlement	13
- Rearing of post-settlement larvae	13
- Weaning stage	14
- Grow-out stage	14
- Artificial Diet	15
- Composition of diets	15
- Diet preparation	16
- Stability of diet	16
- Nutritional requirement and growth	16
- Abalone and Culture Situation in Thailand	17
- Objectives	21
II MATERIALS AND METHODS	23
III RESULTS	33
IV DISCUSSION	42
V CONCLUSION	56
REFERENCES	62
BIOGRAPHY	143

LIST OF TABLES

Table	Page
1. External features and bionomy of <i>H. discus hannai</i> in early developmental stage.	88
2. Morphological changes and behavior of abalone larvae from pelagic stage to benthic stage.	89
3. Algal food of abalone species from different parts of the world.	90
4. Composition of artificial diet for abalone.	92
5. Different formulae of artificial diets using various species of macroalgae and casein as sources of protein.	93
6. The composition of vitamins in Cetavit 1,000 g from Eastern Marine Co., LTD. Thailand and minerals in Premix 1,000 g from Chanaphant Industry Co., LTD. Thailand.	93
7. The average sizes (mean $\pm$ S.D.) and percentage of visceral mass, foot and shell of fifteen <i>H. asinina</i> in each period of sampling.	94
8. The genera of micro-organisms (<120 μ m) in <i>H. asinina</i> gut.	95
9. The genera of micro-organisms (<120 μ m) in <i>H. asinina</i> gut.	96
10. The macro-organisms (> 120 μ m) in <i>H. asinina</i> gut.	98
11. The size of <i>H. asinina</i> larvae (μ m), from fertilization to formation of the first respiratory pore.	99

Table	Page
12. The rate of larval development (hours) of <i>H. asinina</i> at water temperatures of 25, 28, 31, 34 °C and room temperature (28-35°C).	100
13. Equations of relationship between water temperature and time inversion of beginning formation of the fifth larval developmental stage (1/time) and biological zero point of the important larval stages of <i>H. asinina</i> .	101
14. The effective accumulative temperature (EAT) of larval development of <i>H. asinina</i> at water temperatures of 25, 28, 31, 34 °C and room temperature (28-35°C).	102
15. Shell length (mm $\pm$ S.E.) of <i>H. asinina</i> fed nine species of macroalgae over a period of 184 days.	103
16. Percent increase of shell length of <i>H. asinina</i> fed nine species of macroalgae over a period of 184 days.	104
17. Body weight (g $\pm$ S.E.) of <i>H. asinina</i> fed nine species of macroalgae over a period of 184 days.	105
18. Percent increase of body weight of <i>H. asinina</i> fed nine species of macroalgae over a period of 184 days	106
19. Percent survival rate of <i>H. asinina</i> fed nine species of macroalgae over a period of 184 days.	107
20. Food conversion rate of <i>H. asinina</i> fed nine species of macroalgae over a period of 184 days.	108
21. Proximate composition of the nine species of macroalgae.	109

Table	Page
22. Shell length (mm $\pm$ S.E.) of <i>H. asinina</i> fed fresh <i>G. salicornia</i> and artificial diets formulae I-VII over a period of 140 days.	110
23. Percent increase of shell length of <i>H. asinina</i> fed fresh <i>G. salicornia</i> and artificial diets formulae I-VII over a period of 140 days.	111
24. Body weight (g $\pm$ S.E.) of <i>H. asinina</i> fed fresh <i>G. salicornia</i> and artificial diets formulae I-VII over a period of 140 days.	112
25. Percent increase of body weight of <i>H. asinina</i> fed fresh <i>G. salicornia</i> and artificial diets formulae I-VII over a period of 140 days.	113
26. Percent survival rate of <i>H. asinina</i> fed fresh <i>G. salicornia</i> and artificial diets formulae I-VII over a period of 140 days.	114
27. Food conversion rate of <i>H. asinina</i> fed fresh <i>G. salicornia</i> and artificial diets formulae I-VII over a period of 140 days.	115
28. Proximate composition of artificial diets formulae I-VII and <i>G. salicornia</i> over a period of 140 days.	116
29. The size of egg, trochophore and completion of larval shell of some <i>Haliotis</i> spp.	117
30. The biological zero point ($^{\circ}$ C) of some <i>Haliotis</i> spp.	117
31. The time of hatch out, 90 $^{\circ}$ torsion, first epipodium tentacle formation and fourth tubule on cephalic tentacle formation of several abalone species.	118
32. Growth rates reported for various <i>Haliotis</i> species fed different species of algae.	119

LIST OF FIGURES

Figure	Page
1. The life cycle of abalone larvae. Larvae are entrained near their natal reef by complex reef topography and stands of kelp.	120
2. Abalone seed production.	121
3. Settlement tanks and collectors.	122
4. (a) The abalone farm, Cayucos, CA. Partitioned concrete tanks used for grow-out of red abalone, <i>H. rufescens</i> .	123
(b) Red abalone being harvested from the growing tanks.	123
5. (a) Experimental cage used at S.R.L.	124
(b) Lobster pots modified for growing abalone.	124
(c) The plastic culture barrel at Abalone Farm Inc., California	124
6. The sampling area of <i>H. asinina</i> at Ao thein, Samed Island, Rayong Province, Thailand.	125
7. Gut dissection.	126
8. The experimental system of natural food utilization experiment.	127
9. The macroalgae used to feed <i>H. asinina</i> in the experiment of natural food utilization.	128
10. The experimental system of artificial diet utilization experiment.	129
11. The artificial diets used in the experiment of artificial food utilization.	130

Figure	Page
12. The micro-organisms (<120 μm) in <i>H. asinina</i> gut during different sampling periods.	131
13. The macro-organisms (>120 μm) in <i>H. asinina</i> gut during different sampling periods.	131
14. The development of <i>H. asinina</i> larvae from egg to the first respiratory pore formation stage.	132
15. The relationship between water temperature and time inversion (1/hours) of beginning formation of the fifth larval development stage (hatch out, larval retractor muscle, 90°C torsion, first epipodial tentacle and fourth tubule on cephalic tentacle) of <i>Haliotis asinina</i> .	136
16. The relationship between timing of larval stage development (hours) and effective accumulative temperature (EAT, °C - hours) in 25, 28, 31 and 34 °C	136
17. Shell length (mm) of <i>H. asinina</i> fed nine species of macroalgae.	137
18. Percent shell length increase of <i>H. asinina</i> fed nine species of macroalgae.	137
19. Body weight (g) of <i>H. asinina</i> fed nine species of macroalgae.	138
20. Percent weight increase of <i>H. asinina</i> fed nine species of macroalgae.	138
21. Percent survival of <i>H. asinina</i> fed nine species of macroalgae.	139
22. Food conversion rates of <i>H. asinina</i> fed nine species of macroalgae.	139

Figure	Page
23. Shell length (mm) of <i>H. asinina</i> fed fresh <i>G. salicornia</i> and artificial diet formula I-VII over a period of 140 days.	140
24. Percent shell length increase of <i>H. asinina</i> fed fresh <i>G. salicornia</i> and artificial diet formula I-VII over a period of 140 days.	140
25. Body weight (g) of <i>H. asinina</i> fed fresh <i>G. salicornia</i> and artificial diet formula I-VII over a period of 140 days.	141
26. Percent weight increase of <i>H. asinina</i> fed fresh <i>G. salicornia</i> and artificial diet formula I-VII over a period of 140 days.	141
27. Percent survival of <i>H. asinina</i> fed fresh <i>G. salicornia</i> and artificial diet formula I-VII over a period of 140 days.	142
28. Food conversion rate of <i>H. asinina</i> fed fresh <i>G. salicornia</i> and artificial diet formula I-VII over a period of 140 days.	142

CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Introduction

Abalone live on the coasts of every continent of the world and have been known as a food delicacy since historic times. The first fisheries were in China and Japan over 1,500 years ago, but it is only in the last 30 years that fisheries for abalone have burgeoned world-wide and become economically important in many countries (1).

In recent decades, exploitation has risen above the level at which abalone can maintain stocks by natural reproduction (2) and fisheries have collapsed (3). The landings in many abalone fisheries have declined following the development of major international markets (4). The total world abalone landings peaked in the 1970's and have declined steadily (5). Because of the over-harvesting and to protect their declining resources of abalone, many countries initiated restricted quotas or other management restrictions, for example, in Europe, the commercial harvest of the ormer *Haliotis tuberculata* Linnaeus was stopped or tightly restricted in the 1970's, in Canada its pinto abalone, *H. kamtschatkana* Jonas, fishery was closed in 1991 (5). The remedy for many countries is to use a combination of fishery management and out-planting to cultured abalone, to support existing stocks and to reintroduce abalone to areas where they have disappeared (5, 6, 7). Too often, management has been sadly

inadequate to meet the challenges posed by increasing fishing pressure, environmental problems and the economic needs of fishermen (1).

While many of the wild fisheries have collapsed, there is still a strong demand for abalone. Hence, aquaculture has been developed to provide the abalone to satisfy the increased demand. Japan has the most remarkable culture of abalone in the world, and now techniques are developed in many countries with local adaptations. It is predicted that the culture of abalone will expand to production levels that will eventually rival or exceed production from the wild stock (1). To develop the techniques of culture of abalone, it is necessary to understand about their ecology and life cycle, larval development, food and feeding habit and their artificial diets.

Literature Review

Abalone are marine seaweed-eating gastropods which inhabit open rocky coastlines in many parts of the world from tropical, subtropical and temperate regions. There are approximately 70 species worldwide (8). Some species attain a very large size, such as the Californian red abalone (*Haliotis rufescens* Swainson) which grows to a size of 260 mm in shell length, while some non-commercial species measure only a few centimeters (9). However, the maximum size for intensive cultivation would be 70 - 90 mm (10), as it would not probably be economically feasible to hold shellfish beyond this size (11). At present, there are about 25 species of abalone which are of commercial interests (12).

Abalone often feed by trapping drift seaweed under their muscular foot (3, 9, 13) and most of abalone species are associated with macroalgae or seaweed habitats. So, abalone farms are situated along the coastal region and

offshore island where giant kelp beds are extensive. The production of abalone relies on this seaweed which could be potentially limiting if abalone aquaculture expands several folds during the next decade. The industry's growth is now currently limited by the availability of natural feeds both in terms of location and amounts available (10, 14). An artificial diet could overcome this restriction. The understanding of abalone nutrition is very limited and only a few studies have been carried out (15). However, enough is known to construct a purified diet for abalone instead of natural feed (14).

Ecology and Life-Cycle

Abalone are found along the coasts of temperate and tropical regions in both hemispheres with the large species found in temperate regions and smaller species in tropical and Arctic regions (16). They are associated with rocks and boulder substrates (8). Most of the abalone species are associated with macroalgae habitats. Different species of abalone have evolved to match different parts of the world they live in, and so the fine-tuning of the trigger stimuli to which they responded may vary, but on the whole, most species of abalone respond to the same triggers (3).

Abalone are dioecious broadcast spawners and usually have a seasonal reproductive cycle with one annual spawning preceded by gametogenesis (17). The periodicity and duration of spawning can vary both intra- and inter-specifically (18, 19, 20). In the wild, most species of abalone come into breeding condition at restricted time of the year (21). Abalone are marine mollusks with external fertilization and planktonic larvae (8) and have aggregation behavior during the spawning period (22, 23). Aggregation is obviously advantageous to broadcast

spawners in that the synchrony of spawning is promoted and fertilization is enhanced (17). So that the stacking behavior of abalone may enhance fertilization success (24). It has been suggested that the removal of aggregation of abalone by fishermen could affect reproductive success and therefore recruitment to exploitable populations (2, 23, 25, 26)

As a rule, males begin spawning slightly earlier and require less stimulus to induce spawning than females (27), and then sperm will stimulate the female to release eggs. The spontaneous spawning in nature is not completely understood. The eggs of abalone are heavier than sea water and quickly sink after being released (27). The trochophore larvae swim toward the surface immediately after hatch out (28), later the veliger larvae swim downwards (17). The direction in which invertebrate larvae move is dictated by light or pressure receptors (29). The larval life-cycle of abalone is generalized and illustrated in Figure 1 (17).

Abalone larvae whose duration of planktonic life is estimated to vary from 3 to 10 days might not cross the oceans (30). It was postulated that in local dispersal of abalone larvae, by minimizing the duration of planktonic life (a few days) and movement, abalone larvae assume a demersal rather than a pelagic existence (25). The minimum duration of the larval life of abalone is about four days (31, 32), but the larval life would be expected to be much longer for most exploited species of abalone which inhabit cooler temperature water (33). Water movement over coastal reefs can be attenuated by seaweeds and local topography (20, 34, 35, 36) and the larvae of abalone can be retained in their parents' habitat. Indeed, the local reef topography and kelps attenuate near-bed currents are sufficient for larvae to stay on their natal reef for

the normal duration of the larval life (20, 36). Survival of abalone larvae would be enhanced if they could restrict or control their dispersal so that they remained near favorable settlement sites rather than being transported in the sea where the probability of encountering a suitable site for settlement is low (17). Also, the abalone larvae have a little movement that assists them to resist the water movement. Rather, larvae can be retained near their natal reef, particularly those reefs densely populated by large macroalgae (17). Therefore, the larvae of abalone are likely to settle successfully only within the same reef as the parent (25, 37). After settlement, the larvae retained in discrete areas by current eddies or by the protection of local reef topography could complete their larval life-cycle near their natal site (17, 37).

In the lecithotrophic species such as abalone, a successful settlement, therefore, depends on competent larvae making contact with suitable substrata before they starve to death (17). However, the settlement of abalone larvae can be delayed for only a few days before their yolk reserves are exhausted (31, 38). In natural environment, the abalone larvae are reported to settle extensively on crustose coralline algae (39, 40, 41), such as *Haliotis rubra* Leach (42). Coralline algae provide good substrata for settlement of benthic larvae because concavities in the coralline algae provide shelter for settling abalone larvae (17) and post-settlement abalone may depend on the cuticle and epithelial contents grazed from coralline algae (43). However, the settlement of larvae in aquaria is enhanced if the diatom films have been previously grazed by conspecifics (44, 45).

Once settled, the abalone eat and grow by feeding on marine algae. The adults feed on loose pieces of algae drifting with the surge of the current.

Most abalone species become mature between the first and third year and can live longer than a decade (3)

Larval Development

The life-cycle of abalone is composed of larva, post-larva, juvenile and adult. The larval stage starts from fertilization through the formation of the fourth tubule cephalic tentacle. Then, the larvae begin to settle and undergo metamorphosis. The deposition of peristomal shell marks the transition from larval to post-larval stage. The post-larval period continues until the formation of the first respiratory pore (notch stage). From notch stage until sexual maturity, the abalone is called a juvenile (32).

Larval development is a gradual process that does not occur in discrete stepwise stages. However, various stages can be recognized during larval and post-larval development (28). In *Haliotis discus hannai* Ino, there are 41 distinct larval stages with recognizable external features, from fertilization until initiation of metamorphosis (Table 1) (46). These morphological features and the order of their appearances in larval developmental stage are the guide-lines for studies of larval development of other abalone species.

After the larvae settle, there are externally recognized 11 features that appear following the appearance of the cilia in the mantle cavity. These are confirmed to be the characteristics occurring before metamorphosis (Table 2) (47).

Larval development rate is measured by the time required for larvae to exhibit features distinctive to each stage (28). Temperature is the factor that affects the larval development rate. However, it is not twice as fast when double the water

temperature (28). The accumulative temperature during larval development and the minimum water temperature that allows larvae to complete development are important in hatchery management. The water temperature at which larval development is possible but would require infinity to complete development is called biological zero point temperature (46). Once the biological zero point is known, larval development can be quantified by calculating the hourly exposure to water temperatures above that point (28). The water temperature difference between the water temperature and biological zero point is called the effective temperature, and summation of this value during larval development is called the effective accumulative temperature (EAT) (28).

Information on the biological zero point is necessary for convenient handling of fertilized eggs and larvae in abalone seed production. If the precise EAT can be determined, the exact day and hour of each larval stage can be planned in advance and require maintenance (28). In addition, the EAT also provides a mean of obtaining synchronously developed larvae at repeatable stages (between experiments) for developmental, physiological, ecological and biochemical studies on larval development in Haliotidae (46). Therefore, the exact day and hour of each larval stage can be planned in advance and required maintenance jobs (e.g., water changes, induction of settlement) can be scheduled at the culturist's convenience (28).

At present, abalone seed production remains in a primitive and small scale stage for a long period. This was partly because of the difficulty in planning spawning and the high mortality in the early developmental stage (47). The studies of developmental morphology, behavior and biological requirement of abalone larvae

during settlement and metamorphosis can be applied to improve the technology for the abalone seed production system (47). Hence, the understanding and knowledge of larval development, biological zero point and EAT can lead to the improved techniques and management in abalone hatchery system.

Food and Feeding Habit

Abalone are herbivores and primitive gastropods. They exhibit rather primitive feeding methods such as browsing and grazing on algae (48) with nocturnal feeding behavior (49). They change their feeding habit along their development from larvae through adults.

Larval stage

Abalone larvae do not feed and lack a digestive system (49, 50). Thus, they cannot feed on particulate food during early development. Their larvae are considered to be lecithotrophic (feeding on stored nutrients), with sufficient yolk provided in the egg, so that larvae do not need to feed between hatching and metamorphosis (51, 45). However, it was found that lecithotrophic larvae might derive some nutrients from seawater. This was considered to be larval feeding (52). Jaeckle and Manahan (53) reared two batches of abalone, from eggs through settlement, in two different systems, i.e., sand filtered and non-sand filtered seawater systems. They found that the larvae in sand filtered seawater showed a continuous decrease in dry organic weight relative to eggs, while larvae cultured in non-sand filtered seawater had a net increase in biomass and subsequently only a small loss of biomass was observed. From this study, it was suggested that abalone larvae were feeding and the source of exogenous food was in the dissolved form. Thus, the

alteration of organic chemistry of seawater can affect the metabolism and development of abalone larvae (50).

Post-settlement stage (spat)

Once the abalone leave the larval stage and settle, they are called post-settlement abalone or spat. In the wild, abalone spat generally settle on coralline red algae that provide a good substrate for settlement as well as food for abalone. The crustose red algae normally have microbial biota in the cuticle surface (43). The biota consisted almost entirely of bacteria; mostly small cocco-bacilli of genus *Moraxella* were dominant in different seasons and different geographical locations. It was also observed that abalone grazed and presumably utilized material from the surface of crustose coralline algae. So, the natural food of post-settlement abalone larvae should be the microbial biota on the coralline red algae.

In the hatchery situation, diatoms are the principal food source for newly settled juveniles and are needed at sufficient levels until the individuals are 7 to 10 mm, when they switch to eating macroalgae (54). Most culture facilities rely on naturally occurring diatom species to supply the food for juveniles (45, 55).

Weaning stage

In weaning stage, abalone begin to change the feeding habit. The radular teeth grow bigger and further apart. Eventually, they become so big that microalgae are not trapped but slipped between the teeth and are damaged. Moreover, the larger size of radula and teeth means that they are strong enough to grind larger seaweeds or artificial diets. This change of feeding habit varies among species. However,

it generally begins at the shell size approximately 3 to 10 mm and at the age of 50 to 180 days (56, 57, 58, 59, 60).

In the wild, the readiness of abalone to take adult food depends on various factors. If food offered is not suitable, the juvenile abalone may be reluctant to eat which results in a temporary cessation in growth and increased mortality (45). Generally, juvenile abalone prefer soft algae (61) such as red algae and sea lettuce (*Ulva* spp.). In California, kelps are used as weaning diet for *H. rufescens* (58, 59). Many species of seaweeds could be used as food, but only a few species have been employed (45, 56, 61, 60).

In the farm situation, juvenile abalone are usually fed with chopped seaweed, especially tough seaweed such as kelps which are ground into small pieces that are more easily processed by abalone (3). Initially the juveniles are fed these seaweeds every 2 to 3 days in addition to diatoms (62). At this stage, juveniles can feed on macroalgae, but show a strong preference for diatoms. So, a major impediment in abalone culture, however, is an inadequate supply of diatoms for weaning phase (45). Artificial diets are more efficient than some natural foods and are now widely used in Japan (63), China (64) and elsewhere.

Grow-out stage

After abalone pass the weaning stage, they are ready to feed on adult food. In the wild, adult abalone normally feed on macroalgae such as brown, green and red seaweeds. The feeding habits of different abalone species vary throughout the world as shown in Table 3. Some abalone species prefer brown algae (54), and some species prefer red algae (65, 66, 67, 68, 69) depending upon the location and

season (55). Thus, the feeding preference of these different species may simply reflect differences in the relative abundance of algae in their habitats (70).

Cultivation

The Japanese pioneered the use of hatchery-reared juvenile abalone to enhance native stocks (71). At present, juvenile abalone are also produced in the farm. The steps of cultivation of abalone are acquiring broodstock, conditioning broodstock, inducing spawning, larval rearing, settlement of larvae, weaning spat and grow-out or seed ranching (Figure 2) (3, 57, 72).

Broodstock

Abalone broodstock can be obtained from the wild (60, 73, 74), captive animals (57) or existing farms (3, 73). Wild animals are often more vigorous than their domesticated counterparts and are not so likely to be subject to inbreeding and consequent loss of resilience (3). Moreover, the difficulty in finding enough mature wild adults for a large-scale production led to the maturation of adults in culture (64). In the countries like Japan, Taiwan and the United States of America, abalone farming is well developed and the broodstock is commonly obtained from an existing farm (3). These domestic abalone broodstocks have been specifically selected by farmers, for example, Abalone Farm Inc. in California has been running a broodstock program for red abalone (*H. rufescens*) since the late 1970's (59).

Conditioning

Conditioning is the process used to induce the gonad ripeness and spawning ability (usually out of the normal spawning season) in adult animals (75).

This requires controlling the environment, e.g., temperature, photoperiod and food, to induce a state of readiness (45, 60, 76, 77). The most common conditioning method consists of giving adults good living conditions (tailored to the ecology of the particular species) and *ad libitum* feeding (75). The water temperature is believed to be the main exogenous factor regulating reproductive cycles in marine animals. Also in abalone, the water temperature controls gonad maturation but the effect of the water temperature was not simply additive (78). The ripe broodstock gonad tissue with gametes completely surrounds the conical appendage and the sex of abalone can be easily determined (45).

Induction of spawning

In the wild, spawning might be caused by a sudden change in water temperature (increase or decrease), exposure to air during low tide, photoperiod, lunar cycle, release of gametes from other individuals in the population or some combination of these or others (79, 80, 81, 82, 83). There are many techniques for stimulating spawning such as temperature shock, ultraviolet light, hydrogen peroxide, other abalone spawning and pH shock (57, 61, 75, 83).

Rearing of larvae

It is a distinct advantage for cultivation that the larval development of abalone is completely supported by nutrients from the egg yolk (84). In abalone hatchery, immaculate cleanliness must be maintained at all times and all possible sources of contamination must be eliminated during the larval development period. By controlling the time allowed for fertilization, it is possible to synchronize the larval

development which only makes culture procedures easier and also results in a higher larval survival rate (57).

A successful culture and high yield are assured by changing the rearing conditions at three important stages during larval development: hatch out, development of larval shell and settlement of larvae (28).

1. Hatch out. After the trochophore larvae hatch out, the rearing container contains normally developed trochophore larvae and also egg membranes, unfertilized eggs and abnormally developed larvae, which can cause contamination of larval-rearing container. Therefore, the trochophore larvae are decanted as soon as possible from the empty egg cases and unfertilized egg. This can reduce the bacterial contamination from decomposition of egg membranes and unfertilized eggs (28).

2. Development of larval shell. After the larval shell develops, the water in the rearing container is changed three times a day (28).

3. Settlement of larvae. The water is changed three times a day until larval development is complete and the larvae are ready for settlement. The larvae are usually introduced into the settlement tanks after they have developed the third tubercle on the cephalic tentacle (28).

The above-mentioned abalone culture is call a static system. But in some places such as New Zealand, the larvae are reared using a flow-through system.

Settlement

After the larvae show signs of settling, they are moved to the nursery tanks for settlement. These tanks often contain transparent PVC plates or collectors (Figure 3) (60, 72).

In Japan, the substrata used for settlement are translucent, corrugated, hard plastic sheets. Clean plates are placed in a plastic-coated metal rack and placed in the raft tank to accumulate a film of diatoms. Juvenile abalone (> 10 mm) are allowed to graze on the plates. During grazing, they also cover the plate with mucus. This mucus is a factor necessary for inducing settlement and metamorphosis of abalone larvae (85). Besides juvenile mucus, the biotic materials including crustose coralline red algae (39, 84, 86), various microalgae and bacteria (86) and gamma amino-butyric acid (GABA) (84, 86) can also induce settlement of abalone larvae.

Rearing of post-settlement larvae

After the larvae settle and undergo metamorphosis, they are called post-settlement larvae. At this stage, diatoms are the principal food (60, 87). After the larvae settle for about two weeks, the racks with abalone larvae are moved to the raft tanks (85). To maintain an optimum density of benthic diatoms, light level must be controlled and food level can also be enhanced by further dosing of the tanks with cultured diatoms (60). In Japan, diatom growth is stimulated by adding inorganic nutrients to the water in the tank (85).

Weaning stage

After the post-settlement larvae reach 3 to 5 mm in shell length, they are weaned on to macroalgae (60). In some places, after 10-20 % of abalone reach the size of 6 mm, they start to cull these abalone of these sizes to grow-out containers or they are shipped out to the other growers who contract for the seed. Abalone smaller than 6 mm are returned to the hatchery tanks (73). In some places, diatom diet is supplemented with macroalgae (kelps) for abalone with 5 to 12 mm size range and

only kelps are provided thereafter (88). In Japan, artificial diets are gradually becoming popular and are fed together with algae (15).

Grow-out stage

Usually, juvenile abalone are transferred for grow-out after being weaned to the seaweed diet. The grow-out system can be categorized into land-based system and sea-based system where abalone are grown in either cages or barrels that are on or off the sea bottom.

1. Land-based system. Land-based abalone grow-out farms have been established in the USA (89), New Zealand (60) and Chile (90). They usually employ shore-based concrete tanks in which abalone are grown to a size of 80 mm or 3.5 inches (10, 59, 89, 91) (Figure 4). This system is appropriate for areas where the coastline is too exposed for off-shore growth facilities.

2. Sea-based system. Abalone are cultured in the cages, that are hung on the rafts, long-lines or wharf just off the bottom or rest on the sea floor (64, 89, 92). Cages are made of plastic barrels (11), modified lobster pots (12), polyethylene tubes and cylindrical cylinders made of net (64) (Figure 5).

Artificial Diet

Seaweeds are the main food of commercial abalone farming, but there are many problems such as natural seaweeds are limited by seasons and sources (15). It is impractical to harvest large quantities of seaweeds for an abalone farm (14, 93). Furthermore, some types of seaweeds are economically important for their extracts

such as agar, alginic acid and carrageenan (94) which are used in food and pharmaceutical products (95, 96) and cosmetics (97). An artificial diet could overcome these restrictions and conflictions in use.

An artificial food solves several of the biggest problems involved with producing large numbers of juvenile abalone. Moreover, artificial food is easy to store, less expensive than macroalgae (either dried or frozen), produce better growth in body weight and length (54). The artificial diets are still of high cost and there are problems with decomposition and digestion (14, 57). However, many abalone farmers believe that the future of the industry lies in the development of cheap and cost-effective diets (3).

Composition of diets

Generally, the artificial diet of aquatic animals is composed of protein, carbohydrate, lipid, minerals and vitamins. There are many artificial diets which have been developed and used for abalone feed. The summation of artificial composition of these diets is shown in Table 4 (15, 62, 98, 99, 100). They are the main compositions that are usually found in artificial diets for aquatic animals. However, the diets contain other supplements such as algal powder (dried *Undaria*, dried *Laminaria* etc.), amino acids (lysine, DL-methionine, arginine, L-threonine, etc.), essential fatty acid (n3, n6), vitamins and minerals (100).

Diet preparation

The diet preparation technique almost follows that of Uki *et al.* (101). All ingredients are mixed until they are completely homogenized. Water is added (60 - 150 %), which results in the mixture forming into a paste. The mixture is shaped

into a cylinder 80 mm long and 10 mm in diameter. Cylinders are dipped in an aqueous solution of CaCl_2 (5 % W/V) for one minute. This treatment converts sodium alginate to an insoluble calcium alginate in which the nutrients are bound. The diet can be kept and stored at $-40\text{ }^\circ\text{C}$ (15, 102, 103).

However, the techniques of preparation may be different in each place. Viana *et al.* (1993) produced pellets by flattening the mixture to form a product of 2 mm in thickness (99). The pellets of 2 cm^3 were cut, and dried at $60\text{ }^\circ\text{C}$ for 30 hours. In South Africa, an extruded diet is produced for feeding experiments. The pellet was a dark, flat, extruded, ribbon-like which was stable in water for up to 48 hours (93).

Stability of diet

The stability of diet in water depends on the binder used in the diet. These are agar, starch (93), alginate (99). and gelatin (98). The artificial diet used for feeding abalone should be stable for at least 24 hours in water.

Nutritional requirement and growth

Normally, animals require different levels of nutrition for growth. Also in abalone, they require various levels of nutrient to maintain and support their lives.

Protein is a very important constituent of diet, both qualitatively and quantitatively, as it is a building material for the growing animals and organisms, and is also important for the production of enzymes (104). Protein has been given the most attention by researchers as it is the most expensive nutrient to supply in an artificial diet and is essential for soft tissue growth (100).

There are various sources of protein for artificial diet of abalone such as casein, fish meal, wheat, soybean. Casein is suitable as a semipurified test diet for

abalone (15), but it is not suitable for commercial diet, because it is too expensive. The protein level above 30 % is of no significance for the growth of abalone (101).

Abalone and Culture Situation in Thailand

In Thailand, there are three species of abalone, *Haliotis asinina* Linnaeus, *H. ovina* Gmelin and *H. varia* Linnaeus. They inhabit in coral reefs in the depth range 1 - 8 m (105, 106, 107, 108, 109). On the basis of their size and meat textures, there is a potential to culture all three species of the Thai abalone for the market of cocktail-sized abalone (110). *H. asinina* was the first species that experimental breeding has been accomplished in the laboratory (111, 112). There were some experiments on the biology and culture of *H. ovina* (113, 114, 115), while there are very few experiments on *H. varia* except for the distribution aspects (106, 107).

Among these three species, *H. asinina* is the largest. It is noted for its high percentage of edible parts and is commonly distributed in Thai waters. *H. asinina* has a very unusual, thin, long and narrow shell. The shell is small and covers a relatively small portion of the body (16). *H. asinina* is widely distributed in the southern Pacific Ocean in sub-tropical and tropical regions at the depths around 3 - 7 m, for example, they are found in Thailand, Australia, Japan and the Philippines (16, 106, 107, 108, 109). During the day, they are usually found clinging to the undersides of rocks and dead plate corals or within crevices of the rocks or dead head corals. They are active at night as are most abalone species and venture out of hiding to feed after dark (116).

H. ovina are usually found in the same area with *H. asinina* and they are widely distributed than *H. asinina* (117). *H. ovina* prefer the depths between 1.5 - 4 m, select rock crevices rather than coral substrate and the preferred substrate size is

larger than 0.1 m² (118). Light does not affect *H. ovina* as it does to *H. asinina*. So, during the day, *H. ovina* are usually found clinging in the crevices of rocks or dead corals on the upper surface, while *H. asinina* hide under the rocks or dead plate corals (117).

Predatory prosobranch gastropods are among the diverse and abundant groups of invertebrates in the coral reef faunas (119). The feeding habits of many species from hard substrate habitats have been investigated in detail, especially members of the families Conidae and Muricidae (120, 121, 122, 123, 124, 125). *H. asinina* also occupies hard substrate in the coral reef environment but its feeding habits have been little studied. It was speculated that *H. asinina* fed on the organisms that are attached on the hard substrate such as rocks or dead corals. Typically feeding in abalone is by non-selective browsing or selective feeding on specific algae (65).

Abalone are herbivorous gastropods that often feed by trapping drift seaweed under their muscular foot (3, 9, 13), although they may also occasionally forage or graze on attached plants (116). In temperate or sub-tropical regions, abalone usually feed on seaweed or macroalgae that are most abundant in their habitats (8, 116). However, *H. asinina* are inhabitants in coral reef communities where there frequently are few or no macroalgae or seaweeds (126). So, they should feed on the organisms that attach on the surface of substrata in their habitat.

In the case of abalone culture in Thailand, it is at the very early stages of basic and applied research conducted in small-scale operations. There have been several reports concerned with the abalone culture in Thailand. Because of the high percentage of edible part, *H. asinina* has a high market value and is the most

promising species of abalone for culture in Thailand. The Eastern Marine Fisheries Development Center was the first place that is successful in breeding and larval rearing of *H. asinina* in Thailand (127).

The techniques in abalone culture were introduced and applied from Japanese techniques (128). The broodstocks of abalone were natural conditioning and they could spawn throughout the year. The obvious high peak of spawning activity was in October (129). The broodstocks were spawned and fertilized in the conditioned culture tank at night. The hatched-out trochophores were collected in the morning and incubated in 30 liters nursing tank until they reach creeping stage. The abalone larvae were transferred to 1-ton spat collection tanks. This tank had culture plates that already propagated diatoms 4 - 5 days before being introduced to the culture tank. The post-larvae abalone were cultured in the collection tanks for about five months. At this time, most of them reached 1 cm in shell length and began to feed on macroalgae and benthic microalgae. After the abalone reached 2 cm in shell length, they actively fed on seaweed. Then, they were transferred to 2.5 ton concrete tanks and fed with macroalgae *Gracilaria salicornia* until they reached maturity (111, 112). The average growth of shell length of *H. asinina* was 116, 98, 21 and 14 μ m per day when fed with *G. salicornia*, *Laurencia* sp. *G. fisheri* and *Eucheuma* sp. (130, 131). Sex maturation of *H. asinina* can be observed when they reach 7.5 months and they can spawn when they reach 8.5 months (130). The culture system was the main factor that affected the growth of abalone. *H. asinina* reared in the flow-through system had a higher growth rate than those cultured in the static system. It was found that the growth of *H. asinina* reared in a dark culture system did not differ significantly from those reared in the

natural photoperiod culture system (132). The optimum density of *H. asinina* when cultured in 17-l tanks with the 30 x 30 cm shelter plates was 50 - 100 individuals (133) and the optimum salinity was 32.5 ppt (134). Because of the limitation of macroalgae in the Thai waters, some experiments were performed to use other vegetables and artificial diets in abalone culture. It was found that the vegetables could not substitute macroalgae (135). The artificial diets are possible, but they still are in the early stage of studies (112).

In Thailand, studies of abalone are still in the beginning phase. So, food and feeding of both natural and artificial diets, larval development, settlement and metamorphosis are all important basic knowledge for improvement and management of abalone culture in Thailand.

Objectives

The objectives of this investigation were as follows.

1. To study the gut contents of *H. asinina* from the natural habitat.
2. To study the larval development, biological zero point and effective accumulative temperature (EAT) of *H. asinina* in the laboratory.
3. To study the effects of natural food (macroalgae) on growth, survival and food conversion rate of *H. asinina*.
4. To study the effects of artificial diets on growth, survival and food conversion rate of *H. asinina*.

CHAPTER II

MATERIALS AND METHODS

Determination of Gut Contents

Collection of *H. asinina*

Abalone, *H. asinina*, were collected from Ao Thein, Samed Island, Rayong Province, Thailand (Figure 6) by SCUBA diving every three months (October 1995; January, April and July 1996) throughout a period of one year. For each sampling, twenty-five abalone were collected and five of them were immediately fixed in 10 % formalin and brought back to the laboratory of the Eastern Marine Fisheries Development Center, Rayong Province.

Dissection of *H. asinina* digestive system

In the laboratory, the total body weight, shell width and shell length of the abalone were measured before dissection. Then, the guts were dissected out and the gut contents were separated for determination of organisms (Figure 7).

Preparation and determination of gut contents

The gut contents were separated and put in a 5 % household Chlorox solution and placed through a 120 μm -mesh sieve. They were divided into two groups: those which were smaller than 120 μm (micro-contents) and those larger than 120 μm (macro-contents). Volumes of macro-contents and micro-contents were obtained by sedimentation in graduated tubes. The micro-contents were identified to genus of organisms. Their frequencies of occurrence were estimated using a

Sedgwick-rafter counting chamber under the compound light microscope. The macro-contents were examined, sorted by group or species and total counts made under the dissecting microscope. The volumes of sand granules, foraminiferans, algal pieces and the remains of organisms were measured by sedimentation in graduated tubes.

Statistical analysis

The mean and standard deviation were used to calculate the average size of abalone in each duration of sampling. The quantities of macro-contents and micro-contents and the numbers of organisms in the gut were figured in percentages. The difference of size distribution in each sampling period and organism occurrence in gut among sampling period were determined by one-way analysis of variance (ANOVA). The significant differences among the period of sampling were further determined by the multiple comparison, Duncan's multiple range test. SPSS for Windows, Version 6.0 was the statistical software for all statistical analyses, at $P < 0.05$.

Larval Development

Conditioning of broodstocks

The broodstocks of abalone *H. asinina* were natural conditioned in 10 tons concrete race-way tanks at the Coastal Aquaculture Development Center, Prachuap Khiri Khan Province. The flow-through system with the flow rate of 2 - 3 liters per minute was employed. Broodstocks were fed with macroalgae such as *Gracilaria fisheri*, *G. tenuistipitata* and *Acanthophora* spp. The abalone were

examined for gonad maturation every month. Gonad maturation can be examined by size and color according to Ebert and Houk (45).

When gonad maturation reaches stages 2 - 3 (45), the abalone were transferred to the spawning room for induced spawning.

Induced spawning

Males and females were separated in 500-l rectangular flow-through system tank with the flow rate of 2 - 2.5 liters per minute. Each tank contains 50 - 60 abalone. The spawning room was set up with the photoperiod as follows: light (06:00 p.m. to 06:00 a.m.) and dark (06:00 a.m. to 06:00 p.m.). The reversal of photoperiod would affect the timing of spawning. So, *H. asinina* spawned during the day instead of at night. The males released sperm during 00:15 p.m. to 01:30 p.m. and the females laid egg during 00:30 p.m. to 01:30 p.m. The eggs and sperms were collected separately in a 5-l container. The fertilization period was 30 minutes after spawning. The ratio of egg : sperm was 1 : 100,000.

Observation of larval development stage

The observation of larval development of *H. asinina*, from fertilization to initiation of settlement, follows 41 stages of larval development of Seki, Kan-no (46) and Seki (47.) (Table 1).

The experiments were done in 5-l container until the larvae reach the last stage of larval development. Immediately after fertilization, the eggs were placed in the experimental container. Each container contained 5 eggs per ml. Then they were placed under four temperature conditions: 25 and 28 °C (in controlled room

temperature), 31 and 34 °C (in water bath) and at ambient temperatures of 28 - 35 °C which served as control. Each experiment was performed in triplicate.

The observation of larval development started immediately after fertilization and then for every five minutes until the embryos reached stage 10 or gastrula stage. After gastrula stage, larval development was observed for every 30 minutes until the embryos reached stage 41 which was the last stage of larval development. The development of larvae was continued observation once a day after settlement until the abalone produced the first respiratory pore. This observation was done in the 250-l settlement and hatchery tank. The developmental stages after settlement through the beginning of juvenile stage followed those of Seki, Kan-no (46) and Seki (47) (Table 2).

Calculation of the biological zero point

The biological zero point is the critical water temperature that the abalone larvae can develop completely until settlement. It was calculated from the development rate of some developmental stages such as hatch-out, formation of larval retractor muscle, 90° torsion, formation of epipodial tentacle and formation of the fourth tubule on cephalic tentacle (46).

The times of occurrence of hatch-out, formation of larval retractor muscle, 90° torsion, formation of epipodial tentacle and the fourth tubule on cephalic tentacle in each temperature were recorded for the calculation of biological zero point. The biological zero point was calculated from the line across the x-axis of the linear relationship line of the graph. This graph was plotting between water temperature and the conversion of time (1/time) in each developmental stage or calculate from the

equation of relationship between water temperature at beginning (1/time) of the important larval stage as follows: (28, 45)

$$1/t = bT - a$$

Where t = Timing of larval development at each stage.

T = Water temperature of each experimental treatment.

a = The constant value of the point of linear line across the y-axis.

b = The constant value of slop.

Theoretically, there should be a linear relationship between water temperature and 1/time for each stage, and, more importantly, all the lines should cross the x-axis at the same point (46).

Calculation of the effective accumulative temperature (EAT)

The effective temperature is the difference between the water temperature and biological zero point and the quantity above the biological zero point has an additive effect toward larval development. The summation of effective temperature value during larval development is called the effective accumulative temperature (EAT) which is calculated from the equation as follows (46):

$$Y_i = \sum_{n=1}^i (T_i - \theta)$$

Where Y_i (°C - hr) = EAT

T_i (°C) = Water temperature in larval-rearing container

θ (°C) = Biological zero point for larval development.

Natural Food Utilization

Abalone sample

Juvenile *H. asinina* (age 5 months) were obtained from the Coastal Aquaculture Development Center, Prachuap Khiri Khan Province. They were fed *G. salicornia* prior to the experiment and acclimated to experimental conditions for two weeks before collection of initial data.

Culture system

The feeding experiments with monospecific algal diets were conducted in plastic aquaria (40 x 25 x 30 cm). The animals were confined in a basket which was placed in plastic aquaria. Flow-through seawater was supplied at ambient temperature which ranged from 20 °C in winter to 30 °C in summer. Water flowed in at approximately 200 to 250 ml per minute. Each aquarium was continually aerated (Figure 8).

Natural food utilization experiment

Nine species of macroalgae were selected consisting of the red algae *Acanthophora spicifera*, *Gracilaria fisheri*, *G. salicornia* and *G. tenuistipitata*, the green algae *Enteromorpha intestinalis* and *Caulerpa racemosa*, and the brown algae *Dictyota dichotoma*, *Padina minor* and *Sargassum polycystum* (Figure 9).

Algae were weighed and provided in excess every three day. On the third day, the remaining fragments of algae were collected and weighed, then dry matter

content was determined by drying in a hot air oven at 80 °C until a constant weight was reached. Juvenile abalone (13.29 ± 1.36 mm in shell length) were used in the experiments. Treatments were performed in triplicate. Thirty abalone were used for each replicate. Shell length, wet weight of each abalone, and mortality were recorded every two weeks throughout the course of six months (184 days). Increases in shell length and body weight of abalone fed on various species of algae were determined fortnightly. Growth rates of abalone were expressed in terms of shell length ($\mu\text{m}/\text{day}$) and body weight (mg/day).

Food conversion rate

The food conversion rates (FCR) for *H. asinina* fed nine species of tested algae were calculated on the basis of the total dry weight gain of abalone and the dry weight of algae consumed as follows:

$$\text{FCR} = \frac{\text{Dry weight algae consumed (g)}}{\text{Dry weight gain (g)}}$$

[Algal Value Quotient, Leighton and Boolootian (65)]

At the beginning and the end of experiments (184 days), the total dry weight of abalone of each tested group was determined. Twenty animals were weighed and dried at 85 °C until a constant weight was reached.

Samples of the nine species of algae tested were analyzed for crude protein (136), crude fat (137), crude carbohydrate, ash and moisture (138).

Statistical analysis

Growth and survival rate, FCR and nutritional value were determined in different treatments by one-way analysis of variance (ANOVA). The multiple comparison, Duncan's multiple range test was further used to determine the significant differences between each treatment. SPSS for Windows, Version 6.0 was statistical software for all statistical analyses, at $P < 0.05$.

Artificial Food Utilization

Abalone sample

Six-month-old abalone, *H. asinina*, were obtained from the Coastal Aquaculture Development Center, Prachuap Khiri Khan Province. They were fed *G. salicornia* prior to the experiment and acclimated to experimental conditions for two weeks before collection of initial data.

Culture system

The experiments were performed in 2.5 tons concrete tank with flow-through system that had the flow rate of 25 - 30 liters per minute. The abalone were placed in the 45 x 30 x 20 cm baskets and floated in the tanks (Figure 10).

Composition of artificial diets

The composition of artificial diets was applied from that of Uki *et al.* (103, 159). In the formulae I - IV of macroalgae, *A. spicifera*, *E. intestinalis*, *G. fisheri* and *G. salicornia*, were protein sources (Tables 5 - 6). From the study of natural food utilization, *G. tenuistipitata* rendered the base results in terms of growth and survival. However, fresh *G. tenuistipitata* could not be found during this

experiment. In the formulae V - VII, 30 % casein was added. The macroalgae used were *G. fisheri*, *G. salicornia* and *G. tenuistipitata* (dry) (Tables 5 - 6). Agar was used as a binder in these diets.

Preparation of artificial diets

All dry ingredients were mixed well together and then the oil was added. The agar was boiled in 60 - 80 ml distilled water at 60 - 80 °C until it was melted. It was poured into the mixed ingredients and kneaded immediately. After all ingredients and agar were mixed well, the mixture was flattened (0.5 cm thick) and kept in the freezer. It was cut into pieces (1.5 x 1.5 cm) before feeding (Figure 11).

Artificial food utilization experiment

Abalone fed *G. salicornia* were used as control. The artificial diets and algae were weighed equal to the total body weight of abalone samples in each tank and they were fed to abalone in the evening every day. In the morning, the remaining diets were collected and weighed. The dry matter content of artificial diets and algae was determined by drying in a hot air oven at 80 °C until a constant weight was reached. Juvenile abalone (13.29 ± 0.03 mm in shell length) were used in the experiments. Treatments were performed in triplicate. Twenty abalone were used for each replicate. Shell length, wet weight of each abalone, and mortality were recorded every two weeks throughout the course of 140 days. Increases in shell length and body weight of abalone fed *G. salicornia* and artificial diets formulae I - VII were determined for each two weeks period. Growth rates of abalone were expressed in terms of shell length ($\mu\text{m/day}$) and body weight (mg/day).

At the beginning and the end of experiments (140 days), the total dry weight of abalone of each tested group was determined. Fifteen animals were weighed and dried at 85 °C until a constant weight was reached.

Samples of the artificial diets and *G. salicornia* were analyzed for crude protein (136), crude fat (137), crude carbohydrate, ash and moisture (138).

Food conversion rate (FCR) and statistical analyses were determined similar to those described in the natural food utilization.



CHAPTER III

RESULTS

Determination of Gut Contents

Size of sample

The average sizes (shell length, shell width and total body weight) of *H. asinina* were similar in each period of sampling with no significant difference ($P < 0.05$). They were 68.3 ± 9.1 mm in shell length, 32.1 ± 3.5 mm in shell width and 48.2 ± 11.4 g in total body weight (Table 7). The percentages of visceral mass, foot and shell in total body weight were 23.8 %, 61.6 % and 14.5 %, respectively (Table 7).

Gut contents

The average percentages of volume of micro-contents and macro-contents for all samples throughout the year were 80.3 % and 19.7 %, respectively. The macro-content portion was composed of sand granules (86.8 %), foraminiferans (5.6 %), algal pieces (4.6 %) and other organisms (3.0 %). The micro-organisms were divided into eight groups: benthic diatoms (99.3 %), crustaceans (0.2 %), protozoa (0.2 %), eggs of unknown organisms (0.1 %), blue-green algae (0.1 %) and bivalves (0.1 %) (Table 8). Benthic diatoms were the dominant group in *H. asinina* gut (Figure 12) represented by about 36 genera (Table 9). The principal benthic diatoms were *Nitzschia* spp., *Amphora* spp., *Navicula* spp., *Cocconeis* spp., *Plagiogramma* spp., *Diploneis* spp., *Fragilaria* spp., *Coscinodiscus* spp. and

Surirella spp. (Table 8). The relative frequencies of these micro-organisms in *H. asinina* gut content were not significantly different ($P > 0.05$) in different sampling periods (Figure 12, Table 9).

The macro-organisms found in samples of *H. asinina* gut content can be divided into five groups: foraminiferans (71.4 %), crustaceans (10.8 %), annelids (9.7 %), large benthic diatoms (4.6 %) and mollusks (3.5 %) (Table 10). The relative frequencies for groups of macro-organisms are shown in Figure 13. They were not significantly different between sampling periods ($P > 0.05$). Most of macro-contents in the *H. asinina* gut were sand granules. Foraminiferans were dominant macro-organisms in the gut contents.

Larval Development

Spawning

The reversal of photoperiod affected the spawning time of *H. asinina*. Therefore, *H. asinina* spawned during 0:00 to 1:30 p.m. instead of 0:00 to 0:100 a.m. The males spawned during 0:15 to 1:30 p.m. and the females spawned during 0:30 to 1:30 p.m.

Larval size

The eggs of *H. asinina* were dark green in color. The dark green pigments were concentrated at the vegetal pole, while the animal pole was transparent yellowish. The average size of unfertilized eggs was $88.0 \pm 8.0 \mu\text{m}$ in width, $102.4 \pm 3.6 \mu\text{m}$ in length and the nuclear size of eggs was $70.4 \pm 6.7 \mu\text{m}$. After fertilization, the egg size was increased; their width, length and nuclear size were

126.4 ± 17.1, 148.2 ± 10.4 and 99.5 ± 16.3 µm, respectively (Table 11). The egg size did not change until hatch-out stage. The width and length of *H. asinina* trochophore larvae were 105.3 ± 4.1 and 130.7 ± 4.8 µm, respectively (Table 11). After the larvae developed to completion of larval shell, the size of larval shell did not change until they started to secrete the juvenile shell. They started to build the first respiratory pore after they reached the size of 1142.4 ± 94.6 x 1530.2 ± 140.7 µm (Table 11).

Larval development rate

The larvae of *H. asinina* had 42 stages of development (Table 12, Figure 14). The total time of larval development depended on the water temperature in the larval tank (Table 12). They were 65 hours at 25 °C, 49 hours at 28 °C and 41 hours at 31 °C and 34 °C. In controlled group (28 - 35 °C), the total time of development was 40 - 41 hours.

After 44 hours or when the larvae already formed the fourth tubule on cephalic tentacle, they were placed in the settlement tank. On the first day of settlement, the larvae were still in the exploration and inspection stage. They were repeatedly swimming with the velum and temporarily crawling over the surface of diatom plate. On the second day, most larvae were shedding the velum and the mantle membrane was moved to the shell edge. Some of them already secreted the new shell. The settled larvae already formed the fifth and sixth tubules on the cephalic tentacle and ciliated process was already formed near the right cephalic tentacle. All of them were crawling on the plate. In the mantle cavity, the cilia on the roof of mantle were beating to circulate the water and functioned as a ctenidium. However, some larvae still had the velum and were still in the exploration and inspection stage.

On the third day of settlement, the juvenile shell was increased in length and the digestive organ was developed. The mouth was completely formed and they began to form the radula. The post-larvae began to feed on diatoms that were smaller than 10 μm . On the fourth day, the heart began to form and was beating. On the roof of mantle was a prominent structure which was formed into the ctenidium. The juvenile shell increased more in length. On the eighth day, the second pair of epipodium began to form and the number of tubules on cephalic tentacle was increased. The eye stalks were completely developed. From day 9 to day 24, the post-larvae had increased the number of ctenidium and epipodium. The juvenile shell was increased in size until the first respiratory pore began to form. The first respiratory pore began to form on day 24 to day 30 depending on the temperature, diatom type and density of abalone in the settlement tank.

Biological zero point

The biological zero point was calculated from the developmental rate of hatch-out, formation of larval retractor muscle, 90° torsion, formation of epipodial tentacle and the fourth tubule on cephalic tentacle stage of *H. asinina* at temperatures of 25, 28, 31 and 34 °C. In this experiment, the biological zero point of the larval development rate at 34 °C was not calculated because it was close to that of the development at 31 °C. The average biological zero point of *H. asinina* was 15.0 ± 0.3 °C (Figure 15, Table 13).

Effects of water temperature on larval development

The important stages of larvae in the aspect of abalone aquaculture management are hatch-out, completion of larval shell, formation of the first epipodial

tentacle and fourth tubule on cephalic tentacles. The effective accumulative temperatures at water temperatures of 25, 28, 31 and 34 °C were 95, 98, 96 and 114 °C-hour, respectively, in the hatch-out stage; 165, 176, 176 and 209 °C-hours, respectively, in the completion of the larval shell stage; and 650, 637, 656 and 779.0 °C-hours, respectively, in the formation of the fourth tubule on cephalic tentacles stage (Figure 16 and Table 14).

Natural Food Utilization

Growth rates

The results obtained for each algal diet are shown in Tables 15 - 18 and Figures 17 - 20. Growth rates (shell length and body weight increase per day) of *H. asinina* were significantly different ($P < 0.05$) among five groups of feed treatments, i.e., (1) *G. tenuistipitata*, (2) *A. spicifera*, (3) *G. salicornia*, (4) *G. fisheri* and *E. intestinalis* and (5) *P. minor*. No data were obtained for *H. asinina* fed the remaining algal species (*C. racemosa*, *D. dichotoma* and *S. polycystum*) because all died before the end of experiment (Figures 16 - 20, Tables 15 - 18), presumably through starvation.

Shell length

The maximum cumulative increase in shell length was 97.4 ± 4.2 % for abalone fed the red alga, *G. tenuistipitata* (Figures 17, 18 and Tables 15, 16). This equated to a growth rate of 70.3 ± 3.4 µm/day (Table 20). The intermediate cumulative increases of the shell length were obtained with abalone fed *A. spicifera* (86.2 ± 1.8 %), *G. salicornia* (82.2 ± 4.6 %), *E. intestinalis* (75.1 ± 5.4 %) and

G. fisheri (66.0 ± 0.9 %) (Figure 18 and Table 16). These gains corresponded to growth rates of 62.2 ± 1.4 $\mu\text{m/day}$, 59.4 ± 3.2 $\mu\text{m/day}$, 54.3 ± 3.8 $\mu\text{m/day}$ and 47.6 ± 0.6 $\mu\text{m/day}$, respectively (Table 20). The lowest cumulative increase in shell length was 2.1 ± 0.8 % for abalone fed *P. minor* with a corresponding growth rate of 2.1 ± 3.0 $\mu\text{m/day}$ (Figure 18 and Table 20).

Body weight

The maximum rate of cumulative weight increase was found in abalone fed *G. tenuistipitata* (1056 ± 118 % cumulative weight increase, 23.6 ± 2.4 mg/day) (Figures 19, 20 and Tables 17, 18). Abalone fed *A. spicifera*, *G. salicornia*, *G. fisheri* and *E. intestinalis* resulted in intermediate cumulative increases in weight (726 ± 5 %, 610 ± 94 %, 508 ± 43 % and 478 ± 68 %, respectively) (Figure 20 and Table 18). These equated to growth rates of 16.6 ± 0.6 mg/day (*A. spicifera*), 13.8 ± 2 mg/day (*G. salicornia*), 11.0 ± 0.5 mg/day (*G. fisheri*) and 10.9 ± 1.7 mg/day (*E. intestinalis*) (Table 20). The smallest increase in weight was found in abalone fed *P. minor* (-16.4 ± 0.1 % weight increase, 0.3 ± 0.3 mg/day) (Figure 20, Table 20).

Survival rate

The survival rates of *H. asinina* fed the various species of algae were significantly different ($P < 0.05$) among four groups of feed treatments (Figure 21 and Table 19), i.e., (1) *G. tenuistipitata* (95.6 ± 1.6 %), *A. spicifera* (94.4 ± 4.2 %) and *E. intestinalis* (87.8 ± 9.6 %), (2) *G. fisheri* (76.7 ± 7.2 %), (3) *G. salicornia* (55.6 ± 8.3 %) and (4) *P. minor* (7.8 ± 1.6 %). Abalone fed *C. racemosa*, *D. dichotoma* and *S. polycystum* all had died by the 93th, 150th and 162th days of the experiment, respectively (Figure 21 and Table 19).

Food conversion rate

The dry weight food conversion rates were significantly different ($P < 0.05$) among three groups of feed treatments (Figure 22, Table 20), i.e., (1) *G. tenuistipitata* (3.3 ± 0.6), *A. spicifera* (5.7 ± 0.2) and *G. fisheri* (7.4 ± 1.0) (2) *A. spicifera* (5.7 ± 0.2), *G. fisheri* (7.4 ± 1.0) and *E. intestinalis* (9.8 ± 2.7) and (3) *G. salicornia* (15.5 ± 4.0). The lowest or best food conversion rate was obtained on a diet of *G. tenuistipitata*, *A. spicifera* and *G. fisheri* while *G. salicornia* produced the highest food conversion rate. The food conversion rate for *P. minor* could not be calculated due to dry weight loss.

Proximate compositions

Table 21 shows the analysis of proximate composition of the nine species of algae with respect to protein, fat, carbohydrate, ash and moisture. *G. tenuistipitata* showed significantly higher ($P < 0.05$) content of protein (24.7 %) than those of the remaining algae.

Artificial diets utilization

Growth rates

The results obtained for each artificial diet and *G. salicornia* are shown in Tables 22 - 25 and Figures 23 - 26. Growth rates (shell length and body weight increase per day) of *H. asinina* were significantly different ($P < 0.05$) among eight groups of feed treatments, i.e., (1) fresh *G. salicornia* or control, (2) artificial diets formulae V, VI and VII and (3) artificial diets formulae I - IV (Tables 22 - 25).

Shell length

The maximum cumulative increase in shell length was 57.7 ± 1.0 % for abalone fed *G. salicornia*, (control) (Figures 23, 24 and Tables 22,23). This equated to a growth rate of 54.7 ± 3.7 $\mu\text{m/day}$ (Table 27). The intermediate cumulative increases of the shell length were obtained with abalone fed artificial diets formula VII (37.8 ± 3.2 %), V (37.4 ± 4.0 %) and VI (30.7 ± 3.2 %), (Figure 24 and Table 23). These gains corresponded to growth rates of 35.8 ± 0.3 $\mu\text{m/day}$, 35.6 ± 6.5 $\mu\text{m/day}$ and 29.1 ± 1.1 $\mu\text{m/day}$, respectively (Table 27). The lowest cumulative increase in shell length was obtained with abalone fed artificial diets formula I (8.7 ± 0.8 %), IV (7.9 ± 3.2 %), II (5.3 ± 1.2 %), and III (2.5 ± 1.4 %) (Figure 24 and Table 23). These gains corresponded to growth rates of 8.3 ± 1.3 $\mu\text{m/day}$, 7.6 ± 3.4 $\mu\text{m/day}$, 5.1 ± 2.0 $\mu\text{m/day}$ and 2.4 ± 1.5 $\mu\text{m/day}$, respectively (Table 27).

Body weight

The maximum rate of cumulative weight increase was found in abalone fed *G. salicornia* (control) (316.1 ± 66.9 % cumulative weight increase, 9.4 ± 1.9 mg/day) (Figures 25, 26 and Tables 24, 25). Abalone fed artificial diets formulae VII, V and VI resulted in intermediate cumulative increases in weight (219.5 ± 13.6 %, 199.58 ± 24.0 % and 179.4 ± 13.6 %, respectively) (Figure 26 and Table 25). These equated to growth rates of 6.4 ± 2.3 mg/day (VII), 6.0 ± 2.3 mg/day (V) and 5.8 ± 1.3 mg/day (VI) (Table 27). The smallest increase in weight was found in abalone fed artificial diet formula III (86.3 ± 11.2 % weight increase, 2.5 ± 0.2 mg/day), IV (81.4 ± 9.1 % weight increase, 2.4 ± 0.4 mg/day),

I (65.1 ± 1.4 % weight increase, 1.9 ± 0.6 mg/day) and II (57.2 ± 7.9 % weight increase, 1.7 ± 0.1 mg/day) (Figure 26, Tables 25, 27).

Survival rate

The survival rates of *H. asinina* fed the seven formulae artificial diets and *G. salicornia* (control) were significantly different ($P < 0.05$) among three groups of feed treatments (Figure 27 and Table 26), i.e., (1) *G. salicornia* (96.7 ± 5.8 %), artificial diets formula IV (95.0 ± 5.0 %) and II (88.3 ± 7.6 %), (2) artificial diets formula III (80.0 ± 18.0 %), V (78.3 ± 2.9 %), VI (76.7 ± 2.9 %) and I (76.7 ± 7.6 %) and (3) artificial diet formula VII (71.7 ± 5.8 %) (Figure 27 and Table 26).

Food conversion rate

The dry weight food conversion rates were significantly different ($P < 0.05$) among four groups of feed treatments (Figure 28, Table 27), i.e., (1) *G. salicornia* (control) (14.6 ± 0.3) and artificial diets formula VI (19.2 ± 4.7), (2) artificial diet formula VII (27.5 ± 10.3) and artificial diet formula III (28.0 ± 1.1), (3) artificial diets formula V (29.8 ± 1.1) and IV (36.2 ± 2.6), and I (40.1 ± 5.1) (4) artificial diets formula II (53.2 ± 1.6).

Proximate compositions

Table 28 shows the analysis of proximate compositions of the seven formulae of artificial diets and *G. salicornia* (control) with respect to protein, fat, carbohydrate, ash and moisture. Artificial diets formulae VII, V and VI showed a significantly higher ($P < 0.05$) content of protein (37.0 ± 1.9 , 35.4 ± 2.5 and 34.7 ± 2.3 %, respectively) than those of *G. salicornia* and the remaining artificial diets.

CHAPTER IV

DISCUSSION

Determination of Gut Contents

Among the three abalone species in Thailand, *H. asinina*, *H. ovina* and *H. varia*, *H. asinina* seem to be the most suitable for cultivation due to the highest percentage of edible part (112). They were reported to have the approximate proportion of flesh of 85 %. *H. asinina* from Samed Island in the present study also showed a relatively similar percentage of flesh in total body weight (85.4 %).

The habitat of *H. asinina* in Thai waters and elsewhere within its range is different from those of most abalone in other parts of the world. They inhabit the coral reef environment (106, 107, 108, 109, 117). On Samed Island, there are no macroalgae in their habitat, only the tuft algae are found. These algae are *Cladophora* spp., *Centroceras* spp. and *Ectocarpus* spp. *H. asinina* appears to feed almost exclusively on benthic diatoms. Benthic diatoms were dominant in the gut contents of *H. asinina* in the present study. Where abundant, *H. asinina* may have a dramatic effect on benthic algal populations and communities similar to the algal denudation commonly observed as sea urchins become dominant (140, 141).

The black abalone, *Haliotis cracherodii* Leach that are common in the littoral of rocky southern California shorelines, was formerly believed to feed principally on diatoms (142, 143, 144). This concept was changed after the study of Leighton and Boolootian (65), which concluded that *H. cracherodii* fed as adults on macroalgae

such as *Egregia laevigata*, *Pelvetia fastigiata*, *Gigartina canaliculata* and *G. spinosa* that were often abundant in the habitat of this abalone species. In their gut contents were fragments of drift algae, filamentous algae and diatoms. Fecal pellets often contained fragments of coralline algae, surf grass, *Phyllospadix* sp. and diatoms (65). Some diatoms in feces were observed to possess cytoplasmic and nuclear material and were therefore assumed not to have been completely digested. In contrast, the benthic diatoms in *H. asinina* gut contents in the present study appeared to be completely digested except for the siliceous shells. Many researchers have reported that diatoms were the principal diet of very small abalone. Newly settled juveniles typically graze on diatoms from rock surfaces for the first few months of development (38, 145). Diatoms were secondary or supplementary to the harvesting of macroalgae by adult *H. cracherodii* (65). However, this is not the case in *H. asinina* on Samed Island. The present study clearly shows that *H. asinina* on Samed Island feeds on benthic diatoms throughout its life, especially species of the genera *Nitzschia*, *Amphora*, *Navicula* and *Cocconeis*. These diatoms were consistently found in high percentages in each period of sampling. Although the main organisms in *H. asinina* gut content were benthic diatoms, the behavior of grazing apparently caused them to ingest other organisms that were also attached on the substrate such as foraminiferans, pieces of tuft algae, crustaceans, annelids and mollusks. Some abalone species, during grazing, were found to ingest hydrozoans, copepods, small gastropods, ascidians, bryozoans, foraminiferans and even sponges (65, 68, 146).

Several abalone species that occur in open areas have been described as opportunistic graspers or trappers, e.g., *Haliotis laevigata* Donovan, *H. iris* Gmelin,

H. rufescens, *H. roei* Gray (13, 68, 147, 148, 149). Some abalone have been reported to starve rather than move to attached vegetation when drift components are not available as may occur when sea urchin overgrazing attached seaweeds from the substrate (150). In common with most abalone species, *H. rubra* Leach, *H. cyclobates* Péron and Lesueur, *H. cracherodii*, *H. midae* Linnaeus and *H. iris* are reported to be both grazers and trappers depending on environmental conditions (68, 116, 147, 148). *H. asinina* in the present study exhibited similar behavior. They too can be both grazers and trappers. In their natural habitat at Samed Island, they probably graze as juveniles and adults on benthic diatoms attached on the surface of substrate. However, they can be reared in the laboratory and fed with different species of macroalgae such as *G. salicornia* (112), *G. tenuistipitata*, *G. fisheri*, *Enteromorpha intestinalis* and *Acanthophora spicifera* (151). The possibility exists that macroalgae, if present, could be important to their nutrition. McNamara and Johnson (152) studied the growth of *H. asinina* on Heron reef, tropical eastern Australia, by feeding them various species of macroalgae which occurred in this habitat, such as *Hypnea pannosa*, *Laurencia* sp., *Lobophora* sp. They reported the highest growth rates for any species of abalone. However, in the coral reef environment at Samed Island, there are no macroalgae present, only some tuft algae and abundant benthic diatoms (126). It is possible that macroalgae are consumed by other grazers such as sea urchin and *Trochus* spp. that commonly found in coral reef area. These animals are continuous feeder, while *H. asinina* are nocturnal feeders. These may be a competition for macroalgae between *H. asinina* and other grazer. Hence, feeding experiments of the Thai abalone,

H. asinina with these species of tuft algae and benthic diatoms should be further investigated.

Larval Development

The pigments of abalone eggs were derived from maternal yolk and they were retained through the trochophore and veliger larvae. The ovarian tissue of *H. asinina* was dark green similar to those of *H. rufescens*, *H. cracherodii*, *H. walallensis* Stearns, *H. asimilis* Dall and *H. kamtschatkana* Jonas (28). The colors of abalone eggs and larvae vary among species. For example, the eggs and larvae were dark green in *H. asinina*, brown in *H. fulgens* Philippi, beige in *H. sorenseni* Bartsch, olive in *H. corrugata* Wood (28), violet in *H. coccinea canariensis* Nordsieck (153) and green or blue-green in *H. cyclobates*, *H. laevigata*, *H. scalaris* Leach and *H. roei* (18). In some abalone species, the color of eggs vary in each stage of development, such as the eggs of *H. rubra*. In spent or developing stages, the ovaries are gray-blue or brown and when they approach maturity, they are first gray-green and finally olive-green (18). The pigments in the eggs of *H. asinina* are concentrated at the vegetal pole, while the animal pole is transparent yellowish. This feature is in contrast with other abalone species whose pigmentation of the eggs is dark at the animal pole and light at the vegetal pole (28). In *H. coccinea canariensis*, the animal pole is violet and the vegetal pole is slightly yellowish (153). The pigments in *H. asinina* also appear in the visceral mass, so that the visceral mass of larvae is dark green, while the foot, velum and cephalic parts are transparent yellowish. *H. sorenseni* larvae are beige in color, while the velar margin is yellowish (28). In some abalone species, the pigments seem to be concentrated at the velar, for example, *H. fulgens* larvae are generally

brown with green velar margins, *H. corrugata* larvae are light yellow-green with velar fringe of a darker shade of green (31), *H. coccinea canariensis* orange in visceral mass and violet in foot, velum and cephalic mass (153).

The size of fertilized egg of abalone also varies from species to species. The diameter of fertilized egg of *H. asinina* was 125.4 x 148.2 μm . In the other abalone species, they are 103 μm in *H. coccinea canariensis* (153), 150 - 225 μm in *H. cyclobates* (18), 200 - 250 μm in *H. laevigata* (18), 200 μm in *H. sorenseni* (31) and *H. rubra* (154), 210 μm in *H. tuberculata* (155), 230 μm in *H. discus* Reeve (38) and *H. iris* (154), 270 μm in *H. gigantea* Gmelin (38) and 280 μm in *H. sieboldii* Reeve (31) (Table 29). The sizes of trochophore and veliger also vary depending on species (18, 38, 45, 153) (Table 29).

The stage of larval development of *H. asinina* are mostly similar to those of *H. discus hannai* except that there are 42 stages in *H. asinina* while only 41 stages were reported in *H. discus hannai* (46). In *H. discus hannai*, the fourth cleavage produced 16 cells while 12 cells were observed in the fourth cleavage and 16 cells were observed in the fifth cleavage of *H. asinina*. *H. asinina* had both long and short cilia on the prototrochal cell while *H. discus hannai* had only long cilia. In addition, *H. asinina* trochophore appeared to lack the apical tuft cilia as these were reported in the trochophore of *H. discus hannai*. The other differences of larval development between *H. asinina* and *H. discus hannai* were observed in the sequence of feature appearance as follow:

1. In *H. asinina*, the spine at the end of propodium was very fine and very difficult to observe. It could be seen after the formation of operculum.

In *H. discus hannai*, the spine was found at the end of propodium before the formation of operculum.

2. In *H. asinina*, the cilia in the mantle cavity up to the anterior edge of the velum were seen after the formation of the first epipodial tentacle. In *H. discus hannai*, the formation of the first epipodial tentacle occurred before the appearance of apophysis and cilia in the mantle cavity.

3. In *H. asinina*, the ciliary process on the roof of the mantle cavity was formed after the formation of the third tubule on cephalic tentacle. In *H. discus hannai*, the third tubule on cephalic tentacle was seen before the formation of ciliary process on the roof of the mantle cavity.

The duration of pelagic stage in gastropods is variable, and the larvae may spend from 2 to 14 days in the water column (32). Also in abalone, their larvae are pelagic and the duration of development varies from species to species. In general, tropical abalone species usually have a short larval development, such as *H. asinina* and *H. diversicolor supertexta* Lischke. In *H. asinina*, the time durations for hatch-out, torsion and formation of the first epipodium tentacle were 6.0, 7.5 and 11.5 hours, respectively (at 28 °C) and 7.5, 14.0 and 36.0 hours, respectively (at 34 °C) (Table 31). These durations are quite similar to those in *H. diversicolor supertexta*, i.e. 6.0, 13.0 and 38.0 hours for hatch-out, torsion and formation of the first epipodium tentacle, respectively (at 26.2 °C) (156) (Table 31). The first epipodial tentacle of *H. asinina* appeared before the formation of apophysis on propodium, and the larvae started to crawl on the bottom after the formation of apophysis. In *H. discus hannai* larvae, the apophysis on propodium was formed before the appearance of the

first epipodial tentacle and the larvae started to crawl on the bottom when the first epipodial tentacle was formed (46).

The induction of settlement could be done in *H. asinina* after the formation of apophysis on propodium. However, the larvae were not able to metamorphose at this stage until they reached the last stage of development. The abalone larvae are competent to metamorphose when they have four branches on the cephalic tentacles, and the fully developed foot is able to pull the larvae upright and they can move by ciliary action (45, 46). The duration of complete larval development of *H. asinina* was 41.0 to 65.0 hours or only 1.7 to 2.7 days (Table 31), while the duration of larval development of temperate abalone species was from 99.0 to 360.0 hours or 4.1 to 15 days depending on the species and water temperature (28, 31, 32, 38, 45, 46, 153, 155, 156, 157) (Table 31). The duration of larval development in *H. asinina* at water temperatures of 31 and 34 °C were identical. However, the water temperature at 34 °C is rather high and may cause abnormal development and high mortality of larvae. The optimum water temperature of larval development in *H. asinina* is 28 to 31 °C.

Temperature is an important factor in many stages of development such as gonad maturation, spawning (16, 54) and larval development (28, 46). Normally, the animals grow faster in higher temperature. Larval development in *Haliotis* spp. also occurs at a faster rate at higher water temperature (28). The development of *H. asinina* larvae at water temperature of 31 °C (41 hours) was faster than that at 25 °C (65 hours). In *H. rufescens*, the hatching time in water temperature of 17 - 18 °C was faster than that at 15 °C (28, 32, 45, 157) (Table 31).

A successful culture and high yield are assured by changing the rearing conditions at three important stages during larval development (hatch-out, development of larval shell, and settlement) (28). So the information on biological zero point and effective accumulative temperature (EAT) are necessary for hatchery management. The biological zero point varies among abalone species, depending on water temperature in each location. The ambient water temperature is always above the biological zero point (28). Sometimes, the biological zero point is lower than the ambient water temperature. For example, the biological zero point of *H. rufescens* is 0.9 °C and it is below the ambient water temperature (28). In *H. asinina*, their biological zero point (15.0 °C) is also below the ambient water temperature (20 °C).

Biological zero point is one of the values used to calculate the EAT. The average EAT of larval development of *H. asinina* is lower than those of temperate abalone species such as *H. discus hannai* and *H. rufescens* (Table 30). The important EATs value in hatchery are during hatch-out, completion of larval shell formation and competency of settlement stage. After hatch-out, the healthy trochophore larvae will swim toward the surface immediately. They can be collected near the water surface and transferred to the other tank (158). The trochophore larvae of *H. asinina* should be decanted as soon as possible after they reach the EAT 101 °C-hours, in order to reduce bacterial contamination from decomposition of egg membranes and unfertilized eggs. Moreover, the enzymes secreted by the larvae to facilitate hatching may contribute to the lowering of water quality (28). During trochophore stage to completion of larval shell stage, the larvae are very fragile, because they do not have the protection organ. The water in the tank should be changed after the formation of

larval shell or the completion of larval shell or when the EAT of larvae reaches 181.4 °C-hours. The EAT of settlement stage of *H. asinina* larvae was 680.5 °C-hours when the larvae reached the formation of the third tubercle on the cephalic tentacle. Settlement occurs rapidly on wavy-plate substrata if the larvae are healthy and competent to settle and metamorphose (28)

Natural Food Utilization

The results of the study of natural food utilization by the Thai abalone, *H. asinina*, when provided nine species of algae showed that the red alga, *G. tenuistipitata* was the most readily consumed species. It yielded the greatest growth and survival rates. The other three species of red algae (*A. spicifera*, *G. salicornia* and *G. fisheri*) also showed high utilization and provided relatively high growth rates. In contrast, the brown alga *P. minor* gave the lowest growth and survival rates while the green alga, *C. racemosa*, was the least effective species; the abalone did not survive beyond the 93rd day of the experiment.

There are numerous reports on algal foods of abalone species from different parts of the world (70). Except for abalone species in the Southwest Pacific and Australasia, most abalone species prefer brown algae, especially phenolic-poor species (70). In Australasia, the abalone species *H. roei*, *H. laevigata*, *H. rubra* and *H. iris* seemed to prefer red algae such as *Gracilaria*, *Jeannerettia*, *Pterocladia* and *Asparagopsis* (13, 68, 70, 159, 160, 161, 162). Reports on food preferences of *H. asinina* have indicated that red algae such as *Gracilaria* and *Laurencia* are preferred (111, 131, 135). Hence, the present study is in good agreement with earlier studies on *H. asinina* and several other *Haliotis* species of Australasia. In the tropics,

red algae may predominate by number of species in some areas and in terms of biomass, red algae may be more abundant than brown and green algae.

Food preferences appear to be correlated with the results of growth studies. Generally, *Haliotis* species in temperate zones have shown better growth rates when fed brown algae than red algae (45, 131, 135, 161, 162, 163, 164, 165, 166) (Table 32). However, the Australasian species showed better growth rates when fed red algae (45, 131, 135, 161, 162, 163, 164, 165, 166) (Table 32). In New Zealand, Stuart and Brown (161) reported the best growth (11.6 mg/day in weight, 57.2 $\mu\text{m/day}$ in shell length) in *H. iris* fed mixed algae (*Gracilaria chilensis*, *Ulva lactuca* and *Macrocystis pyrifera*). In Australia, *H. rubra* fed the red alga *Jeannerettia lobata* exhibited a very high growth rate (51.0 mg/day) (162).

In Thailand, *H. asinina* fed red algae also showed better growth than those fed on either green or brown algae. Singhagraiwan (135) reported very high growth rates for body weight (51.1 mg/day) and shell length (51.6 $\mu\text{m/day}$) when *H. asinina* was fed *G. salicornia*. Kunavongdate *et al.* (131) reported that *H. asinina* fed *Laurencia* sp. showed very high growth rates (24 mg/day in weight, 98 $\mu\text{m/day}$ in shell length). In the present study, *H. asinina* fed *G. tenuistipitata* showed the highest growth rates (23.6 mg/day in weight, 70.3 $\mu\text{m/day}$ in shell length). These results on growth rates are similar to the study of Kunavongdate *et al.* (131). The difference in growth rates (body weight) of *H. asinina* fed *G. salicornia* in the present study (23.6 mg/day) and the study of Singhagraiwan (51.1 mg/day) (135) can be explained that this study used *H. asinina* of small size that they did not completely adapt to the changes feed macroalgae as well as those of larger size used by Singhagraiwan (135).

In addition, the survival rates of *H. asinina* fed over a period of 184 days, *G. salicornia* (100 %), *Laurencia* sp. (100 %) and *G. tenuistipitata* (95.6 %) were not significantly different (131, 135). Singhagraiwan (135) reported that the FCR of *H. asinina* fed *G. salicornia* was about 20.4 and Kunavongdated *et al.* (131) reported that the FCR of *H. asinina* fed *Laurencia* sp. was about 98.5. These authors calculated their FCRs based on the wet weight of algae and abalone. The FCR of *H. asinina* fed *G. salicornia* fed *G. tenuistipitata* in this study calculated on the basis of wet weight was about 27.2. This FCR is relatively similar to that of Singhagraiwan (135).

Shepherd and Steinberg (70) proposed three factors which may affect the acceptability of food algae in nature. These include (a) the presence of chemical metabolites in algae, (b) algal morphology (toughness) and (c) nutritional value. Among the three species of *Gracilaria* tested (*G. salicornia*, *G. fisheri* and *G. tenuistipitata*), the *G. tenuistipitata* thallus is soft and palatable. That alga has a high protein content compared with the other algae which may have contributed to the relatively high growth rates obtained on this seaweed.

Artificial Food Utilization

Seaweeds are the main food of commercial abalone farming in many parts of the world. But seaweeds are limited by season (15) and some types of seaweeds are economically important (94, 95, 96, 97). Artificial diet is the choice for abalone culture in the future. In the natural food utilization study, the body weight increase of *H. asinina* fed red algae, *G. tenuistipitata* (1057 %), *A. spicifera* (725 %), *G. salicornia* (612 %) and *G. fisheri* (505 %), was higher than those fed green algae, *E. intestinalis* (486 %), while body weight of abalone fed brown algae was not

increased (-16 %) (151). However, these seaweeds do not occur all year round. In the present study, several formulae of artificial diets with seaweeds (*A. spicifera*, *G. fisheri*, *G. salicornia*, *E. intestinalis*) as protein sources showed that the abalone fed with these diets containing seaweeds only had a rather low growth rate. The protein contents of these diets were analyzed to be 4.7 % (with *A. spicifera*), 4.9 % (with *G. fisheri*), 5.1 % (with *G. salicornia*) and 5.8 % (with *E. intestinalis*). It is apparent that the low protein content in diet results in low growth rate. However, these percent protein seem to can maintenance the metabolism in their body. Although the growth rate is low but the animals still fresh when compare with the abalone fed with brown algae in the natural feed utilization experiment. In that experiment the weigh of abalone was decrease and extremely die. When 30 % casein was added as an additional protein source, these new formulae of artificial diets were analyzed to contain protein contents of about 6 - 8 times higher than those formulae with seaweeds alone (33 - 36 %). The abalone fed with these new diet formulae also had significantly higher growth rates (29 - 35 $\mu\text{m/day}$ in shell length, 5.8 - 6.4 mg/day in weight). Hence, higher protein content results in higher growth rates. These results are similar to the study of Uki *et al* (1986). They reported that, over 50 days of experiment, *H. discus hannai* fed diets containing low protein levels (4.7 - 5.8 %) produced low growth (0.38g or 11.0 % body weight gain). When protein content was increased to 37.5 %, the growth of abalone was increased (1.3 g or 37.0 % body weight increase) (102). Also in *H. midae*, over 95 days of experiment, specific growth rate was positively related to the level of dietary protein, increasing from 0.94 to 1.11 % of body weight per day between 27 and 47 % protein contents (167).

Uki and Watanabe (1992) investigated the dietary values of casein and fish meal diet compared to that of live algae *E. bicyclis*, which has a high dietary value. The dietary value of casein diet was comparable or superior to that of *E. bicyclis* (15). This is similar to the result of Viana *et al* (1993). They found that growth rates of *H. fulgens* fed with artificial diets were containing fish meal and casein as protein source 2.6 and 3.7 times superior to that of natural foods (99). Nie (1992) also reported that growth rate of *H. discus hannai* fed artificial diet was faster than those fed *Laminaria* sp. (64). These results are in contrast with the present study. The growth rates of *H. asinina* fed artificial diets containing 33.6 - 36.3 % protein (179 - 220 % body weight increase) was lower than those fed live alga, *G. salicornia* (345 % body weight increase). Because the use of artificial diet on abalone is still in the early stage, these artificial diet formulae or protein sources may not be suitable. In addition, the leaching of trace nutrients from the artificial diet may cause low growth rates in abalone. Biologically significant leaching may occur without a measurable change in feed weight (100). Therefore, the artificial diets for *H. asinina* must be improved until the suitable diets are found.

Abalone are aquatic animals that feed in water and they are slow feeder, and so the diets must be stable in the water during feeding times. An additional requirement of the feed by a slow feeder is that the water-soluble nutrients remain in the feed and the food particles remain bound together so that pellets stay intact for at least two days in water (100). Therefore, the binder agents are important factors in abalone diets. Some ingredients such as algae, dextrin and fish meal also have the binding property. Balance against the requirement for the feed to be water-stable is the

need for the pellets to be of suitable toughness. But, increasing the toughness of an artificial feed by adding more binder (2 % to 8 % agar) resulted in decreased intakes (168). In *H. discus hannai*, their intake decreased as the sodium alginate content of the feed was increased from 30 to 50 % (101). The present study used 5 % agar as a binder. However, 30 % algae in diets also increased the binding property of diets. So, these diets were stable in water for at least 48 hours.

During the experimental period, the water qualities in the experimental tanks were checked weekly. It was found that the levels of nitrite and ammonia in both *G. salicornia* and artificial diet treatments were quite low. It appears that although some leaching might occur in the artificial diet not affect the survival of abalone samples since the flow rate of water was very high. The survival rates of abalone feed artificial diet were not much lower than that of control.

Not only protein level (167, 169) and protein sources (170, 171) in artificial diets are important for growth of abalone, the other factors such as lipid (172, 173), amino acid (174, 175, 176), fatty acid (103, 177), feeding attractant (178, 179, 180, 181, 182), binding agent (98), preparation technique (183), minerals (15), etc. are also important factors that affect the growth of abalone and these informations are still in the very early stage of study.

CHAPTER V

CONCLUSION

Determination of Gut Contents

Most of the gut contents of *Haliotis asinina* were micro-contents, $< 120 \mu\text{m}$ (80.3 %), while macro-contents, $> 120 \mu\text{m}$ were present in small amount (19.7 %). The dominant organisms in micro-contents were benthic diatoms (99.3 %). They were represented by about 36 genera and the principal benthic diatoms were *Nitzschia* spp., *Amphora* spp., *Navicula* spp., *Cocconeis* spp., *Plagiogramma* spp., *Diploneis* spp., *Fragilaria* spp., *Coscinodiscus* spp. and *Surirella* spp., respectively. The other micro-organism groups were crustaceans (0.2 %), protozoa (0.2 %), eggs of unknown organisms (0.1 %), blue-green algae (0.1 %) and bivalves (0.1 %). The main macro-contents in *H. asinina* gut were sand granule (86.7 %), while the remaining were organisms (13.3 %). The dominant macro-organisms were foraminiferans (71.4 % of macro-organisms). The other groups such as crustaceans, annelids, large benthic diatoms and mollusks constituted about 10.8, 9.7, 4.6 and 3.5 % of macro-organisms, respectively.

This result indicated that *H. asinina* around Samed island fed mainly on benthic organisms especially diatoms.

Larval Development

The larvae of *H. asinina* had 42 stages of development. The trochophore of *H. asinina* appeared to lack the apical tuft cilia that found in *H. discus hannai*

trochophore. In addition, *H. asinina* larvae had both long and short cilia on the prototrochal cell of trochophore larvae.

The main larval stages that are important in abalone hatchery are fertilization, trochophore, complete larval shell formation and formation of third tubule on cephalic tentacle. The formation of fourth tubule on cephalic tentacle is a completion of larval development indicating that abalone larvae can undergo settlement and metamorphosis. The period of larval development of *H. asinina* depended on the water temperature. They were 65, 49, 41 and 41 hours at water temperature of 25, 28, 31 and 34 °C, respectively.

The average biological zero point of *H. asinina* was 15.0 ± 0.3 °C. The effective accumulative temperature, EAT, of four important development stages of *H. asinina* larvae were 100.6, 181.4, 608.8 and 680.5 °C-hours at hatch-out, complete of larval shell formation, formation of third tubule on cephalic tentacle and formation of fourth tubule on cephalic tentacle, respectively. Therefore, the induction of settlement should begin after larvae reached the formation of third tubule on cephalic tentacle stage i.e., 36 - 37 hours after fertilization, or at the EAT of 609 °C-hours.

After settlement, *H. asinina* larvae were in exploration and inspection stage. Most larvae developed to post-larval stage on the second day. They were shedding the velum; the mantle membrane was moved to the shell edge and began to secrete a new shell. Besides the cilia in mantle cavity, ciliated process was performed near the right cephalic tentacle and the beating of cilia increased the circulation of water. Mouth, radula and digestive organs were developed on the third day of settlement. Some larvae started to feed on diatoms. The heart was seen on the fourth day of

settlement. The prominent structure on the roof of mantle cavity was the formation of ctenidium. The second pair of epipodium tentacle began to form and the eyes stalks were completely developed on the eighth day of settlement. After day 9 to day 24, post-larvae increase in shell size, number of epipodia, number of tubules on cephalic tentacle and ctenidium structure was more developed. The first respiratory pore began to form on day 24 to day 30 depending on the temperature, diatom type and density of abalone in the settlement tank.

Natural Food Utilization

The abalone, *H. asinina*, were fed nine species of macroalgae which consisted of the red algae *Acanthophora spicifera*, *Gracilaria fisheri*, *G. salicornia* and *G. tenuistipitata*, the green algae *Enteromorpha intestinalis* and *Caulerpa racemosa*, and the brown algae *Dictyota dichotoma*, *Padina minor* and *Sargassum polycystum*, over 180 days of experiment.

Growth rate: The growth rates of *H. asinina* fed various macroalgae species were significantly different ($P < 0.05$) and could be divided into five groups: (1) *G. tenuistipitata* (23.6 ± 2.4 mg/day), (2) *A. spicifera* (16.6 ± 0.6 mg/day), (3) *G. salicornia* (13.8 ± 2.0 mg/day), (4) *G. fisheri* (11.0 ± 0.5 mg/day) and *E. intestinalis* (10.9 ± 1.7 mg/day) and (5) *P. minor* (-0.3 ± 0.3 mg/day). The *C. racemosa*, *D. dichotoma* and *S. polycystum* all died before the end of the experiment, therefore their growth rates could not be calculated.

Shell length: The cumulative increase of shell length of *H. asinina* fed various macroalgae species corresponded to the growth rate, which could be divided into five groups: (1) *G. tenuistipitata* (97.4 ± 3.9 %), (2) *A. spicifera* (86.2 ± 2.6 %),

(3) *G. salicornia* (82.2 ± 4.2 %), *E. intestinalis* (75.1 ± 5.4 %), (4) *G. fisheri* (66.0 ± 0.9 %) and (5) *P. minor* (2.3 ± 0.8 %).

Body weight: The cumulative increase of body weight of *H. asinina* fed various macroalgae species corresponded to the growth rate and cumulative shell length increase. They could be divide into five groups: (1) *G. tenuistipitata* (1056 ± 32 %), (2) *A. spicifera* (725 ± 29 %), *G. salicornia* (610 ± 18 %), *E. intestinalis* (75.1 ± 5.4 %) and *G. fisheri* (66.0 ± 0.9 %) and (3) *P. minor* (2.1 ± 0.8 %).

Survival rate: The survival rates of *H. asinina* fed various species of macroalgae were significantly different ($P < 0.05$). They could be divided into five groups: (1) *G. tenuistipitata* (95.6 ± 1.6 %), *A. spicifera* (94.4 ± 4.2 %) and *E. intestinalis* (87.8 ± 9.6 %), (2) *G. fisheri* (76.7 ± 7.2 %), (3) *G. salicornia* (55.6 ± 8.3 %), (4) *P. minor* (7.8 ± 1.6 %) and (5) with *C. racemosa*, *D. dichotoma* and *S. polycystum* (all of them had died before the end of experiment).

Food conversion rate: The food conversion rates (FCR), of *H. asinina* fed various macroalgae species were significantly different ($P < 0.05$). They could be divided into three groups: (1) *G. tenuistipitata* (3.3 ± 0.6), *A. spicifera* (5.7 ± 0.2) and *G. fisheri* (7.4 ± 1.0) (2) *A. spicifera* (5.7 ± 0.2), *G. fisheri* (7.4 ± 1.0) and *E. intestinalis* (9.8 ± 2.7) and (3) *G. salicornia* (15.5 ± 4.0). The FCR of *P. minor* treatment could not be calculated due to dry weight loss.

Proximate composition: The proximate compositions of nine species of macroalgae were significantly different ($P < 0.05$). The protein contents was highest in *G. tenuistipitata* that produced the highest growth rate in *H. asinina*.

Artificial Diet Utilization

The seven formulae of artificial diets were different in protein source. In diets number I - IV, protein sources were 30 % macroalgae: *A. spicifera*, *E. intestinalis*, *G. fisheri* and *G. salicornia*. Diets number V - VII, protein sources were 30 % casein and 30 % macroalgae, *G. fisheri*, *G. salicornia* and *G. tenuistipitata* (dry). *H. asinina* were fed seven formulae of artificial diets and fresh *G. salicornia* (control group) over 140 days of experiment.

Growth rate: The growth rates of *H. asinina* fed seven formulae of artificial diets and *G. salicornia* were significantly different ($P < 0.05$) and could be divided into three groups: (1) abalone fed fresh *G. salicornia* (9.8 ± 2.1 mg/day) (2) abalone fed diets number V (6.0 ± 2.3 mg/day), VI (5.8 ± 1.3 mg/day) and VII (6.4 ± 2.3 mg/day) and (3) abalone fed diets number I (1.9 ± 0.6 mg/day), II (1.7 ± 0.1 mg/day), III (2.5 ± 0.2 mg/day) and IV (2.4 ± 0.4 mg/day).

Shell length: The cumulative increase of shell length of *H. asinina* was significantly different among fresh *G. salicornia* and seven formulae of artificial diets. They could be divided into five groups: (1) abalone fed fresh *G. salicornia* (56.9 ± 3.8 %), (2) abalone fed diets number V (37.1 ± 4.0 %) and VII (30.7 ± 1.3 %), (3) abalone fed diet number VI (30.7 ± 1.3 %), (4) diets number I (8.7 ± 0.8 %) and IV (7.9 ± 3.2 %), II (5.3 ± 1.2 %) and (5) diet number II (5.3 ± 1.2 %) and III (2.5 ± 1.4 %).

Body weight: The cumulative increase of body weight was corresponded to the growth rate. They could be divided into three groups: (1) fresh *G. salicornia* (330.3 ± 58.4 %), (2) diets number VII (219.5 ± 10.0 %), V (199.5 ± 24.0 %) and

VI (179.4 ± 16.8 %) and (3) diets number III (86.3 ± 11.2 %), IV (81.4 ± 9.1 %), I (65.1 ± 1.4 %) and II (57.2 ± 7.9 %).

Survival rate: The survival rates of *H. asinina* were significantly different among *G. salicornia* and seven formulae of artificial diets ($P < 0.05$). They could be divided into three groups: (1) fresh *G. salicornia* (96.7 ± 4.7 %), diets number IV (95.0 ± 50 %) and II (88.3 ± 7.6 %) (2) diets number III (80.0 ± 8.0 %), V (78.3 ± 2.9 %), I (76.7 ± 7.6 %) and VI (76.7 ± 2.9 %) and (3) diet number VII (71.7 ± 5.8 %).

Food conversion rate: The food conversion rates (FCR), of *H. asinina* were significantly different among eight feed treatments ($P < 0.05$). They could be divided into four groups: (1) fresh *G. salicornia* (15.0 ± 2.9) and diet number VI (19.2 ± 4.7), (2) diets number VII (27.5 ± 10.3), III (28.0 ± 1.1) and V (29.8 ± 12.1), (3) diets number IV (36.2 ± 2.6) and I (40.1 ± 5.1) and (4) diet number II (53.2 ± 1.6).

Proximate composition: The proximate compositions of *G. salicornia* and seven formulae of artificial diets were significantly different ($P < 0.05$). The protein content was highest in artificial diets number VII (36.3 ± 1.1 %), VI (33.8 ± 1.2) and V (33.6 ± 1.4 %), (2) fresh *G. salicornia* (16.5 ± 0.9 %) and (3) artificial diets number II (5.5 ± 0.2 %), IV (4.9 ± 0.2 %), VII (4.8 ± 0.1) and I (4.5 ± 0.1).

REFERENCES

1. Shepherd SA, Tegner MJ, Guzman del Proo SA, editors. Abalone of the World, Biology, Fisheries and Culture. Oxford: Fishing News Books; 1992. IX-X.
2. Sluczanowski PR. A management oriented model of an abalone fishery whose substocks are subject to pulse fishing. Can. J. Fish Aquat. Sci. 1984; 41:1008-14.
3. Fallu R. Abalone farming. Oxford: Fishing News Book; 1991.
4. Shepherd SA, Brown LD. What is an abalone stock: Implications for the role of refugia in conservation. Can. J. Fish. Aquat. Sci. 1993;50:2001-9.
5. Anon. Farmed abalone is needed to fill supply gap. Fish Farming International 1992;21:11.
6. Saito K. Ocean ranching of abalones and scallops in northern Japan. Aquaculture 1984;39:361-73.
7. Tong LJ, Moss GA, Sllingworth J. Enhancement of a natural population of the abalone, *Haliotis iris*, using cultured larvae. Aquaculture. 1987;62:67-72.
8. Linberg DR. Evolution, distribution and systematics of Haliotidae. In: Shepherd SA, Tegner MJ, Guzman del Proo SA, editors. Abalone of the World, Biology, Fisheries and Culture. Oxford: Fishing News Books; 1992. p. 3-18.



9. LaTouche B, Moylan K, Twomey W. Abalone on-growing manual. Dublin: 1993.
Aquaculture explained. no.14.
10. Wray T. Red abalone by the million. Fish Farming International 1989a;16:10-1.
11. LaTouche B, Moylan K. Abalone. Aquaculture Ireland 1986;28:12-13.
12. Hensey J. Abalone. Aquaculture Ireland 1990;45:40-1.
13. Wells FE, Keesing JK. Reproduction and feeding in the abalone *Haliotis roei*
Gray. Aust. J. Mar. Freshwat. Res. 1989;40:187-97.
14. Norman-Boudreau K. Abalone nutrition and the potential role of purified diets.
J. Shellfish Res. 1988;7:564.
15. Uki N, Watanabe T. Review of the nutritional requirements of abalone
(*Haliotis* spp.) and development of more efficient artificial diets.
In: Shepherd SA, Tegner MJ, Guzman del Proo SA, editors. Abalone of
the World, Biology, Fisheries and Culture. Oxford: Fishing News Books;
1992. p. 504-17.
16. Hahn KO. Survey of the commercially important abalone species in the world.
In: Hahn KO, editor. Handbook of Culture of Abalone and Other Marine
Gastropods. Boca Raton; 1989a. p. 4-11.
17. McShane PE. Early life history of abalone: a review. In: Shepherd SA, Tegner
MJ, Guzman del Proo SA, editors. Abalone of the World, Biology,
Fisheries and Culture. Oxford: Fishing News Books; 1992. p. 120-38.
18. Shepherd SA, Laws HM. Studies on southern Australian abalone (genus
Haliotis): II. Reproduction of five species. Aust. J. Mar. Freshwater Res.
1974;25:49-62.

19. Tutschulte T, Connell FJ. Reproductive biology of three species of abalones (*Haliotis*) in southern California. *Veliger* 1981;23:195-206.
20. McShane PE, Black KP, Smith MG. Recruitment processes in *Haliotis rubra* (Mollusca: Gastropoda) and regional hydrodynamics in southeastern Australia imply localized dispersal of larvae. *J. Exp. Mar. Biol. Ecol.* 1988;124:175-203.
21. Hayashi I. The reproductive biology of the ormer, *Haliotis tuberculata*. *J. Mar. Biol. Ass. U.K.* 1980;60:415-30.
22. Breen PA, Adkins BE. Spawn in a British Columbia population of northern abalone, *Haliotis kamtschatkana*. *Veliger* 1980;23:177-9.
23. Shepherd SA. Studies on southern Australian abalone (genus *Haliotis*): VII. Aggregative behaviour of *H. laevigata* in relation to spawning. *Mar. Biol.* 1986;90:231-6.
24. Douros WJ. Stacking behaviour of an intertidal abalone: an adaptive response or a consequence of space limitation? *J. Exp. Mar. Biol. Ecol.* 1987; 108:1-14.
25. Prince JD, Seller TL, Ford WB, Talbot SR Confirmation of relationship between the localized abundance of breeding stock and recruitment for *Haliotis rubra* Leach (Mollusca: Gastropod). *J. Exp. Mar. Ecol.* 1987;106:243-63.
26. McShane PE, Smith MG. Direct measurement of fishing mortality in abalone (*Haliotis rubra* Leach) off southern Australia. *Fish. Res.* 1989;8:93-102.

27. Hahn KO. Artificial induction of spawning and fertilization. In: Hahn KO, editor. Handbook of Culture of Abalone and Other Marine Gastropods. Boca Raton: 1989b. p. 53-70.
28. Hahn KO. Larval development of abalone. In: Hahn KO, editor. Handbook of Culture of Abalone and Other Marine Gastropods. Boca Raton; 1989c p. 71-98.
29. Yano I, Ogawa Y. Effects of light, temperature and hydrostatic pressure on vertical distribution of abalone larvae, *Haliotis gigantea*. Bull. Tokai Reg. Res. Lab. 1977;91:19-27.
30. Foster GR, Potts GW, Swinfen R. Changes in the ormer populations of Guernsey and Jersey. J. Mar. Biol. Assoc. UK. 1982;62:717-27.
31. Leighton DL. Laboratory observation on the early growth of the abalone, *Haliotis sorenseni*, and the effect of temperature on larval development and settling success. Fish. Bull. 1972;70:373-81.
32. Leighton DL. The influence of temperature on larval and juvenile growth in three species of southern California abalones. Fish. Bull. 1974;72:1137-45.
33. Cox KW. California abalone, family Haliotidae. Calif. Fish Game, Fish. Bull. 1962:118.
34. Fretter V, Manly R. Algal associations of *Tricolia pullus*, *Lacuna vincta* and *Cerithiopsis tubercularis* (Gastropoda) with special reference to the settlement of their larvae. J. Mar. Biol. Assoc. UK 1977;57:999-1017.
35. Bernstein BB, Jung N. Selective processes and co-evolution in a kelp canopy community in southern California. Ecol. Monogr. 1979;49:335-55.

36. Eckman JE, Duggins DO, Sewell AT. Ecology of understory kelp environments. I. Effects of kelps on flow and particle transport near the bottom. J. Exp. Mar. Biol. Ecol. 1989;129:173-87.
37. Prince JD, Sellers TK, Ford WB, Talbot SR. Confirmation of a relationship between the localised abundance of breeding stock and recruitment for *Haliotis ruber* Leach (mollusca: Gastropoda). J. Exp. Mar. Biol. Ecol. 1988;122:91-104.
38. Ino T. Biological studies on the propagation of the Japanese abalone (genus *Haliotis*). Bull. Tokai Reg. Fish. Res. Lab. 1952;5:1-102.
39. Morse ANC, Morse DE. Recruitment and metamorphosis of *Haliotis* larvae induced by molecules uniquely available at the surface of crustose red algae. J. Exp. Mar. Biol. Ecol. 1984; ;75:191-215.
40. Shepherd SA, Turner JA. Studies on southern Australian abalone (Genus *Haliotis*): VI. Habitat preference, abundance and predators of juveniles. J. Exp. Mar. Biol. Ecol. 1985;93:285-98.
41. Clavier J, Richard O. Growth of juvenile *Haliotis tuberculata* (Mollusca: Gastropoda) in their natural environment. J. Mar. Biol. Ass. U.K. 1986; 66:497-503.
42. McShane PE, Smith MG. Measuring recruitment of abalone *Haliotis rubra* Leach (Gastropoda: Haliotidae) - comparison of a novel method with two other methods. Aust. J. Mar. Freshwater Res. 1988;39:331-6.

43. Garland CD, Cooke SL, Grant JF, Meekin TA. Ingestion of the bacteria on the cuticle of crustose (non-articulated) coralline algae by post-larval and juvenile abalone (*Haliotis rubra* Leach) from Tasmanian waters. J. Exp. Mar. Biol. Ecol. 1985;91:137-49.
44. Seki T, Kan-no H. Induced settlement of the Japanese abalone, *Haliotis discus hannai*, veliger by the mucous trails of the juvenile and adult abalones. Bull Tohoku Reg. Fish. Res. Lab. 1981a;43:29-36.
45. Ebert EE, Houk JL. Elements and innovations in the cultivations of red abalone, *Haliotis rufescens*. Aquaculture 1984;39:375-92.
46. Seki T, Kan-no H. Synchronized control of early life in the abalone, *Haliotis discus hannai* Ino, Haliotidae, Gastropoda. Bull. Tohoku Reg. Fish. Res. Lab. 1977;38:143-53.
47. Seki T. Biological studies on the seed production of the northern Japanese abalone *Haliotis discus hannai* Ino. Bull. Tohoku Natl. Fish. Res. Inst. 1997;59:1-71.
48. Pérchon RD. The Biology of the Mollusca. 2nd ed. Oxford: Pergamon Press, 1977:41-6.
49. Godoy C, Jerez T, Ponce F. The introduction of abalone into Chile. In: Shepherd SA, Tegner MJ, Guzman del Proo SA, editors. Abalone of the World, Biology, Fisheries and Culture. Oxford: Fishing News Books; 1992. p. 485-88.

50. Manahan DT, Jaeckle WB. Implications of dissolved organic material in seawater for the energetics of abalone larvae *Haliotis rufescens*: a review. In: Shepherd SA, Tegner MJ, Guzman del Proo SA, editors. Abalone of the World, Biology, Fisheries and Culture. Oxford: Fishing News Books; 1992. p. 95-106.
51. Kohn AJ. Feeding biology of gastropods. In: Saleuddin ASM, Wilber KM, editors. The Mollusca Vol.5: Physiology, part 2. New York: Academic Press; 1983. p. 1-63.
52. Jaeckle WB, Manahan DT. Feeding by a “nonfeeding” larva: Uptake of dissolved amino acids from seawater by lecithotrophic larvae of the gastropod *Haliotis rufescens*. Mar. Biol. 1989;103:87-94.
53. Jaeckle WB, Manahan DT. Experimental manipulations of the organic chemistry of seawater: implications for studies of energy budgets in marine invertebrate larvae (Echinodermata, Mollusca). J. Exp. Mar. Biol. Ecol. 1992;156:273-84.
54. Hahn KO. Nutrition and growth of abalone. In: Hahn KO, editor. Handbook of Culture of Abalone and Other Marine Gastropods. Boca Raton; 1989d. p. 135-56.
55. Hooker H, Morse DE. Abalone: The emerging development of commercial cultivation in the United States. In: Huner JV, Brown EE, editors. Crustacean and Mollusk Aquaculture in the United States. Westport: AVI Publishing Co.; 1985. p. 365-413.

56. Leighton DL, Byhower MJ, Kelly JC, Hokker GN, Morse DE. Acceleration of development and growth in young green abalone (*Haliotis fulgens*) using warmed effluent seawater. J. World Maricul. Soc. 1981;12:170-80.
57. McCormick TB, Hahn KO. Japanese abalone practices and estimated costs of juvenile production in the USA. J. World Maricul. Soc. 1983;14:149-61.
58. Svenson G. California shore farm profits from abalone. Fish Farming International 1988;15:30-1.
59. Wray T. Highly quality abalone from Point Estero. Fish Farming International 1989b;16:14-6.
60. Tong LJ, Moss GA. The New Zealand culture systems from abalone. In: Shepherd SA, Tegner MJ, Guzman del Proo SA, editors. Abalone of the World, Biology, Fisheries and Culture. Oxford: Fishing News Books; 1992. p. 583-91.
61. Chen HC. Recent innovations in cultivation of edible molluscs in Taiwan, clam *Meretrix lusonia*. Aqauculture 1984;39:11-27.
62. Bardach, JE, Ryther, JH, Mclarney WO. Culture of marine gastropods, Especially abalone. In: Bardach JE, Ryther JH, Mclarney WO, editors. Aquaculture: The farming and husbandry of freshwater and marine organisms. USA: John Wiley & Sons, 1972. p. 777-85.
63. Anon. Abalone race to markets. Fish Farming International. 1988;15:27.
64. Nie ZQ. A review of abalone culture in China. In: Shepherd SA, Tegner MJ, Guzman del Proo SA, editors. Abalone of the World, Biology, Fisheries and Culture. Oxford: Fishing News Books; 1992. p. 592-602.

65. Leighton DL, Boolootian RA. Diet and growth in the black abalone *Haliotis cracherodii*. Ecology 1963;44:227-38.
66. Leighton DL. Studies on food preference in algivorous invertebrates of southern California kelp beds. Pac. Sci. 1966;20:104-13.
67. Poore GCB. Ecology of New Zealand abalones *Haliotis* species (Mollusca: Gastropoda): IV. Reproduction. NZ J. Mar. Freshwater. Res. 1972a; 7:67-84.
68. Shepherd SA. Studies on southern Australian abalone (Genus *Haliotis*): I. Ecology of five sympatric species. Aust. J. Mar. Freshwat. Res. 1973;24:217-57.
69. Steinberg PD. Biogeographical variation in brown algal polyphenolics and other secondary metabolites, comparison between temperate Australasia and North America. Oecologia 1989;78:373-82.
70. Shepherd SA, Steinberg PD. Food preferences of the three Australian abalone species with a review of the algal food of abalone. In: Shepherd SA, Tegner MJ, Guzman del Proo SA, editors. Abalone of the World, Biology, Fisheries and Culture. Oxford: Fishing News Books; 1992. p. 169-81.
71. Tegner MJ, Butler RA. Abalone seeding. In: Hahn KO, editor. Handbook of Culture of Abalone and Other Marine Gastropods. Boca Raton; 1989. p. 157-82.

72. Kufuku T, Ikenoue H. Abalone (*Haliotis* (Nordotis) *discus*) culture. In: Kufuku T, Ikenoue H, editors. Modern method of aquaculture in Japan. Japan: Elsevier; 1983. p. 172-83.
73. McMullen J, Thompson T. Abalone culture in urban environment. In: Hahn KO, editor. Handbook of Culture of Abalone and Other Marine Gastropods. Boca Raton; 1989. p. 277-83.
74. Leighton DK. A floating laboratory applied to culture of abalone and rock scallops in Misson Bay, California. Proc. World Maricul. Soc. 1979;10:349-65.
75. Hahn KO. Artificial induction of conditioning (gonad maturation). In: Hahn KO, editor. Handbook of Culture of Abalone and Other Marine Gastropods. Boca Raton; 1989e. p. 41-51.
76. Uki N, Kikuchi S. Regulation of maturation and spawning of an abalone, *Haliotis* (Gastropoda) by external environmental factors. Aquaculture 1984; 39:247-61.
77. Doumenge F. Aquaculture in Japan. In: Barnabe G, editor. Aquaculture Vol.2. England: Ellis Horwood Limited; 1990. p. 930-2.
78. Kikuchi S, Uki N. Technical study on artificial spawning of abalone, genus *Haliotis*: VIII. Duration of fertility related to temperature. Bull. Tohoku Reg. Fish. Res. Lab. 1974;34:73-5.
79. Boolootian RA, Farman-farmaian A, Giese AC. On the reproductive cycle and breeding habits of two western species of *Haliotis*. Biol. Bull. 1962;122:183-93.

80. Webber HH and Giese AC. Reproductive cycle and gametogenesis in the Black abalone *Haliotis cracherodii* (Gastropoda: Prosobranchiata). Mar. Biol., 1969;4:152-9.
81. Young JS, DeMartini JD. The reproductive cycle and gonadal histology and gametogenesis of the red abalone *Haliotis rufescens* (Swainson). Calif. Fish Game 1970;56:298-309.
82. Giorgi AE, Demartini JD. A study of the reproductive biology of the red abalone, *Haliotis rufescens* Swainson, near Mendocino, California. Calif. Fish Game 1977;63:80-94.
83. Morse DE, Duncan H, Hooker N, Morse A. Hydrogen peroxide induces spawning in mollusks, with activation of prostaglandin endoperoxide synthetase. Science 1977;196:298-300.
84. Morse DE, Hooker N, Duncan H and Jensen L. γ -Aminobutyric acid, aneurotransmitter, induces planktonic abalone larvae to settle and begin metamorphosis. Science 1979a;204:407-10.
85. Hahn KO. Japanese abalone culture techniques of the Oyster Research Institute. In: Hahn KO, editor. Handbook of Culture of Abalone and Other Marine Gastropods. Boca Raton; 1989f. p. 195-227.
86. Morse DE, Hooker N, Jensen L, Duncan H. Induction of larval abalone settling and metamorphosis by γ -aminobutyric acid and its congeners from crustose red algae: II. Applications to cultivation, seed-production and bioassay; principal cause of mortality and interference. Pro. World Maricul. Soc. 1979b;10:81-91.

87. Ski T, Kan-no H. Observations of the settlement and metamorphosis of the veliger of Japanese abalone, *Haliotis discus hannai* Ino, Haliotidae, Gastropoda. Bull. Tohoku Reg. Fish. Res. Lab. 1981b;42:31-9.
88. Ebert EE, Houk JL. Abalone cultivation methods used at the California Department of Fish and Game's Marine Resources Laboratory. In: Hahn KO, editor. Handbook of Culture of Abalone and Other Marine Gastropods. Boca Raton; 1989. p. 239-54.
89. Ebert EE. Abalone aquaculture: a North America regional review. In: Shepherd SA, Tegner MJ, Guzman del Proo SA, editors. Abalone of the World, Biology, Fisheries and Culture. Oxford: Fishing News Books; 1992. p. 570-82.
90. Wray T. Chilean get help to farm the red abalone. Fish Farming International 1994;21:8-9.
91. Wray T. Abalone Unlimited get green light. Fish Farming International 1989c;16:14-6.
92. Anon. South American farms buying California seed. Fish Farming International 1994;21:11.
93. McVeigh S. South African develop new diet for abalone. Fish Farming International 1994;21:15
94. Trainor LJ, Moss GA, Sllingworth J. Enhancement of natural population of the abalone, *Haliotis iris*, using culture larvae. Aquaculture 1978;62:67-72.

95. Indergaard M, Minsaas J. Animal and human nutrition. In: Guiry M, Blunden G, editors. Seaweed resources in Europe: Use and potential. London: John Wiley and Sons; 1991. p. 21-64.
96. Indergaard LM, Stagaard K. Polysaccharides for food and pharmaceutical uses. In: Guiry M, Blunden G, editors. Seaweed resources in Europe: Used and potential. London: John Wiley and Sons; 1991. p. 169-83.
97. Roeck-Holtzhaer VD. Use of seaweeds in cosmetics. In: Guiry M, Blunden G, editors. Seaweed resources in Europe: Use and potential. London: John Wiley and Sons; 1991. p. 83-94.
98. Knauer J, Britz PJ, Hecht T. The effect of seven binding agents on 24-hour water stability of an artificial weaning diet for the South African abalone, *Haliotis midae* (Haliotidae, Gastropoda). Aquaculture 1993;115:327-34.
99. Viana MT, Lopez LM, Salas A. Diet development for juvenile abalone *Haliotis fulgens*: Evaluation of two artificial diets and macroalgae. Aquaculture 1993;117:146-56.
100. Fleming AE, Van Barneveld RJ, Hone PW. The development of artificial diet for abalone: a review and future directions. Aquaculture 1996;140:5-53.
101. Uki N, Kemuyana A, Watanabe T. Development of semipurified test diets for abalone. Bull. Japan Soc. Sci. Fish. 1985a;51:1825-33.
102. Uki N, Kemuyama A, Watanabe T. Optimum protein level in diets for abalone. Bull. Japan. Soc. Sci. Fish. 1986a;52:1005-12.

103. Uki N, Sugiura M, Watanabe T. Requirement of essential fatty acids in the abalone, *Haliotis discus hannai*. Bull. Japan. Soc. Sci. Fish. 1986b;52:1013-23.
104. Steffens W. Principles on fish nutrition. Uk: Ellis Horwood Limited. 1989: 66-171.
105. Tantanasiwong S. An illustrated checklist of marine shelled gastropods from Phuket Island, adjacent mainland and offshore island, western peninsular Thailand. Phuket Mar. Biol. Center Res. Bull. 1978;15:1-65.
106. Nateewathana A, Hylleberg J. A survey on Thai abalones around Phuket Island and feasibility study of abalone culture in Thailand. Thai Fish. Gazette 1986;39:177-190.
107. Tookvinart S, Leknim W, Donyadol Y, Preeda-Lampabutra Y, Perngmak P. Survey on species and distribution of abalone in Surattani, Nakornsrihamarat and Songkhla Provinces. Songkhla: National Institute of Coastal Aquaculture, Department of Fisheries, 1986. Technical Paper No.:1/2529. (in Thai, with English abstract)
108. Nateewathana A, Bussawarit S. Abundance and distribution of abalones along the Andaman Sea coast of Thailand. Kasetsart Gazette (Science) 1988;22:8-15. (in Thai, with English abstract).
109. Sungthong S, Ingsrisawang V, Fujiwara S. Study on the relative growth of abalone, *Haliotis asinina* Linné, off Samet Island. Thai. Mar. Fish. Res. Bull. 1991;2:15-20.

110. Jarayabhand P, Paphavasit N. A review of the culture of tropical abalone with special reference to Thailand. *Aquaculture* 1996;140:159-68.
111. Singhagraiwan T, Sasaki M. Breeding and early development of the donkey's ear abalone, *Haliotis asinina* Linné. *Thai. Mar. Fish. Res. Bull.* 1991; 2:95-100.
112. Singhagraiwan T, Doi M. Seed production and culture of a tropical abalone, *Haliotis asinina* Linné. Bangkok: Department of Fisheries, Ministry of Agriculture and Cooperatives; 1993. The Research Project of Fishery Resource Development in the Kingdom of Thailand.
113. Jarayabhand P, Jew N, Choonhabandit S. Gametogenic cycle of abalone, *H. ovina* (Gmelin, 1791), at Khangkao Island, Chon Buri Province. Proceeding of the eighteenth Congress on Science and Technology of Thailand; 1992 Oct 27-29; Bangkok; 1992. p. 340-41. (in Thai with English abstract)
114. Kesthong W, Jarayabhand P. Study on fecundity in abalone, *Haliotis ovina* (Gmelin, 1791) along the eastern coast of Thailand. Proceeding of the nineteenth Congress on Science and Technology of Thailand; 1993 Oct 27-29, Bangkok; 1993. p. 472-3. (in Thai with English abstract)
115. Jarayabhand P, Iojima H, Menasveta P. Embryonic and larval development and early growth of hatchery-produced abalone (*Haliotis ovina* Gmelin, 1791) seed. *Thai J. Aqua. Sci.* 1995;1:194-202.

116. Wood AD, Buxton CD, Aspects of biology of the abalone *Haliotis midae* (Linné, 1758) on the east coast of South Africa: 1. Feeding biology. S. Afr. J. Mar. Sci. 1996;17:61-8.
117. Kakhai N, Petjamrat K. Survey on species and broodstock collection of abalone (*Haliotis* spp.) in Chon Buri, Rayong and Trad Provinces. Rayong: Rayong Coastal Aquaculture Station, Department of fisheries, Ministry of Agriculture and Cooperative, 1992 Technical Paper No.:6/2535. (in Thai with English abstract)
118. Ngow O, Jarayabhand P. Study on fecundity in abalone, *Haliotis ovina* (Gmelin, 1791) along the eastern coast of Thailand. Proceeding of the nineteen Congress on Science and Technology of Thailand; 1993 Oct 27-29, Bangkok; 1993. p. 472-3. (in Thai with English abstract)
119. Taylor JD. Diets of sand-living predatory gastropods at Piti Bay, Guam. Asian Marine Biology 1986;3:47-58.
120. Kohn AJ. The ecology of *Comus* in Hawaii. Ecol. Monogr. 1959;29:47-90.
121. Kohn AJ. Microhabitats, abundance and food of *Comus* on atoll reefs in Maldiva and Chagos Islands. Ecology 1968;49:1046-62.
122. Kohn AJ, Nybakken JW. Ecology of *Comus* on eastern Indian Ocean fringing reefs: diversity of species and resource utilization. Marine Biology 1975;29:211-34.
123. Leviten PJ. Resource partitioning by predatory gastropods of the genus *Comus* on subtidal Indo-Pacific coral reefs: the significance of prey size. Ecology 1978;59:614-31.

124. Taylor JD. Habitats and diet of predatory gastropods at Addu Atoll, Maldives. *J. Exp. Mar. Biol. Ecol.* 1978;31:83-103.
125. Taylor JD. A partial food web involving predatory gastropods on a Pacific fringing reef. *J. Exp. Mar. Biol. Ecol.* 1984;74:273-90.
126. Anunpongsuk S, Sawatpeera S. Sessile organism. Bangkok: Southeast Asian Fisheries Development Center; 1991. TD/RES/29-49-60.
127. Singhagraiwan T. The experiment on breeding and nursing of Donkey's Ear abalone (*Haliotis asinina* Linné). Rayong: Eastern Marine Fisheries Development Center, Department of Fisheries, Ministry of Agriculture and Cooperative; 1989a. Extension Paper No.:21/2532. (in Thai, with English abstract).
128. Singhagraiwan T. Some technique for donkey's ear abalone culture (*Haliotis asinina* Linné). Rayong: Eastern Marine Fisheries Development Center, Department of Fisheries, Ministry of Agriculture and Cooperative; 1989b. Extension Paper No.:1/2532. (in Thai, with English abstract).
129. Singhagraiwan T, Doi M. Spawning pattern and fecundity of the donkey's ear abalone, observed in captivity. *Thai. Mar. Fish. Res. Bull.* 1992;3:61-9.
130. Singhagraiwan T. Study on growth rate of donkey's ear abalone (*Haliotis asinina* Linné). Rayong: Eastern Marine Fisheries Development Center, Department of Fisheries, Ministry of Agriculture and Cooperative. 1991a. Technical Paper No.:28/2534. (in Thai, with English abstract).

131. Kunavongdate, P., W. Sakares & S. Muangsakorn. 1995. Experimental rearing on abalone, *Haliotis asinina* Linné with three species of seaweed. Chantaburi: Coastal Aquaculture Division, Department of Fisheries, Ministry of Agriculture and Cooperative; 1995. Technical Paper No.:39/2538. (in Thai, with English abstract).
132. Singhagraiwan T. Comparative study on the growth and survival of juvenile donkey's ear abalone (*Haliotis asinina*) in different rearing system. Rayong: Eastern Marine Fisheries Development Center, Department of Fisheries, Ministry of Agriculture and Cooperative; 1993. Technical Paper No.:37/2536. (in Thai, with English abstract).
133. Singhagraiwan T. Study on the optimum rearing density of juvenile Donkey's Ear abalone (*Haliotis asinina* Linné). Rayong: Eastern Marine Fisheries Development Center, Department of Fisheries, Ministry of Agriculture and Cooperative; 1992. Technical Paper No.:36/2535. (in Thai, with English abstract).
134. Singhagraiwan, T., M. Doi & M. Sasaki. Salinity tolerance of juvenile donkey's ear abalone *Haliotis asinina* Linné. Thai. Mar. Fish. Res. Bull. 1992; 3:1-7.
135. Singhagraiwan T. An experiment on feeding of donkey's ear abalone (*Haliotis asinina* Linné). Rayong: Eastern Marine Fisheries Development Center, Department of Fisheries, Ministry of Agriculture and Cooperative; 1991b. Technical Paper No.:20/2534. (in Thai, with English abstract).

136. Horwitz W. Official Methods of Analysis of the Association of Official Analytical Chemists. 13th ed. Washington D.C.: Association of Official Analytical Chemists; 1980.
137. Pearson D. The Chemical Analysis of Foods. 7th ed. New York: Churchill Livingstone; 1976.
138. Kangsadalampai K, Sungpuag P. Laboratory Manual for Food Analysis. Bangkok: Institute of Nutrition, Mahidol University; 1984.
139. Uki N, Kemuyama A, Watanabe T. Nutritional evaluation of several protein sources in diet for abalone. Bull. Japan. Soc. Sci. Fish. 1985b;51:1835-9.
140. Paine RT, Vadas RL. The effects of grazing by sea urchins, *Strongylocentrotus* spp. on benthic algal populations. Limnol. Oceanogr. 1969;14:710-9.
141. Sousa WP. Experimental investigations of disturbance and ecological succession in a rocky intertidal algal community. Ecol. Monogr. 1979;49:227-54.
142. Bonnot P. Abalones in California. California Fish and Game 1930;16:15-23.
143. Bonnot P. California abalones. California Fish and Game 1940;26:200-11.
144. Bonnot P. The abalones of California. California Fish and Game 1948; 34:141-68.
145. Tomita K, Tazawa N. On the stomach contents of young abalone, *Haliotis discus hannai* Ino, in Rebun Island, Hokkaido. Scient. Rep. Hokkaido Fish. Expl. Stn. 1971;13:31-8.
146. Ueda S, Okada Y. Studies on the food of useful Gastropoda in Japan. I. Two species of Japanese abalone, *Haliotis gigantea* Gmelin and *H. kamtschatkana* Jonas. Bull. Japan. Soc. Sci. Fish. 1939;8:51-7.

147. Olsen DA. Banding patterns in *Haliotis*. II. Some behavioral considerations and the effects of diet on shell coloration for *Haliotis rufescens*, *Haliotis corrugata*, *Haliotis sorenseni* and *Haliotis asisimilis*. Veliger 1968;11:135-9.
148. Poore GCB. Ecology of New Zealand abalones, *Haliotis* species (Mollusca: Gastropoda) I. Feeding. N Z J. Mar. Freshwat. Res. 1972b; 6:11-22.
149. Barkai R, Griffiths CL. Diet of the South African abalone *Haliotis midae*. S. Afr. J. Mar. Sci. 1986;4:37-44.
150. MacGinitie N, MacGinitie GE. Starved abalones. Veliger 1966;8:313.
151. McNamara DC, Johnson CR. Growth of the ass's ear abalone (*Haliotis asinina* Linné) on Heron Reef, tropical eastern Australia. Mar. Freshwater Res. 1995;46:571-4.
152. Pena JB. Preliminary study on the induction of artificial spawning in *Haliotis coccinea canariensis* Nordsieck (1975). Aquaculture 1986;52:35-41.
153. Harrison AJ, Grant JF. Progress in abalone research. Tasmanian Fish. Res. 1971; 5:1-10.
154. Koike Y. Biological and ecological studies on the propagation of the ormer, *Haliotis tuberculata* Linnaeus. I. Larval development and growth of juveniles. La Mer 1978;16:124-36.
155. Oba T. Studies on the propagation of the abalone *Haliotis diversicolor supertexta* Lischke. II. On the development. Bull. Jpn. Soc. Sci. Fish. 1964;30:809-19.

156. Owen B, Disalvo LH, Ebert EE, Fonck E. Culture of the California red abalone *Haliotis rufescens* Swainson (1922) in Chile. *Veliger* 1984;101-5.
157. Hayashi I. Larval shell morphology of some Japanese haliotids for the identification of their veliger larvae and early juveniles. *Venus* 1983; 42:49-51.
158. Shepherd SA. Distribution, habitat and feeding habits of abalone. *Aust. Fish.* 1975; 34:1-4.
159. Prince JD. The fisheries biology of *Haliotis rubra* in Tasmanian waters [Ph.D. Thesis in Biology]. Tasmanian: University of Tasmania. 1989.
160. Stuart MD, Brown MT. Growth and diet of cultivated black-footed abalone, *Haliotis iris* (Martyn). *Aquaculture* 1994; 127:329-37.
161. Fleming AE. Growth, intake, feed conversion efficiency and chemosensory preference of the Australian abalone, *Haliotis rubra*. *Aquaculture* 1995; 132:297-311.
162. Uki N. Feeding behavior of experimental populations of the abalone, *Haliotis discus hannai*. *Bull. Tohoku Reg. Fish. Res. Lab.* 1981;43:53-8.
163. Morrison J, Whittington J. Development of an artificial feed for abalone. *Austasia Aquaculture* 1991;5:53-4.
164. Gorfine H, King R. New food for abalone. *Austasia Aquaculture* 1991;5:40.
165. Koike Y, Flassch J, Mazurier J. Biological and ecological studies on the propagation of the ormer *Haliotis tuberculata* Linnaeus: II. Influence of food and density on the growth of juveniles. *La Mer* 1979;17:43-5.

166. Upatham ES, Sawatpeera S, Kruatrachue M, Chitramvong YP, Singhagraiwan T, Pumthong T, Jarayabhand P. Food utilization by *Haliotis asinina* Linnaeus. J. Shellfish Res. Inpress.
167. Britz PJ. Effect of dietary protein level on growth performance of South African abalone, *Haliotis midae* fed fishmeal-based semipurified diets. Aquaculture 1996a;140:55-61.
168. Gorfine HK. An artificial diet for hatchery-reared abalone *Haliotis rubra*. Marine Science Laboratory, Interna Rep., Queenscliff, Australia, 1991 No.:190.
169. Britz PJ, Hecht T. Effect of dietary protein and energy level on growth and body composition of South African abalone, *Haliotis midae*. Aquaculture 1997;156:195-210.
170. Britz PJ. The suitability of selected protein sources for inclusion in formulated diets for the South African abalone, *Haliotis midae*. Aquaculture 1996b;140:63-73.
171. Viana MT, Lopez LM, Garcia-Esquivel Z, Mendez E. The use of silage made from fish and abalone viscera as an ingredient in abalone feed. Aquaculture 1996;140:87-98.
172. Mai K, Mercer JP, Donlon J. Comparative studies on the nutrition of two species of abalone, *Haliotis tuberculata* L. and *Haliotis discus hannai* Ino: III Response of abalone to various levels of dietary lipid. Aquaculture 1996a;134:65-80.
173. Dustan GA, Baillie HJ, Barrett SM, Volkman JK. Effect of diet on the lipid composition of wild and cultured abalone. Aquaculture 1996;140:115-27.

174. Mai K, Mercer JP, Donlon J. Comparative studies on the nutrition of two species of abalone, *Haliotis tuberculata* L. and *Haliotis discus hannai* Ino: II. Amino acid composition of abalone and six species of amcroalgae with an assessment of their nutritional value. Aquaculture 1994;128:115-30.
175. Knauer J, Brady D, Duncan JR, Hecht T. Amino acid, fatty acid and mineral element profile of juvenile South african abalone, *Haliotis midae*. aquacult. res. 1995;26:283-8.
176. King RH, Rayner CJ, Derr M, Gorfine HK, McShane PE. The composition and amino acid balance of abalone (*Haliotis rubra*) tissue. aquaculture 1996;140:109-13.
177. Mai K, Mercer JP, Donlon J. Comparative studies on the nutrition of two species of abalone, *Haliotis tuberculata* L. and *Haliotis discus hannai* Ino: V. The role of polyunsaturated fatty acids of macroalgae in abalone nutrition. Aquaculture 1996b;134:65-80.
178. Harada K, Kawasaki O. The attractive effect of seaweeds based on the behavioral responses of young herbivorous abalone, *Haliotis discus*. Bull. Jpn. Soc. Sci. Fish. 1982;48:617-21.
179. Harada K, Maruyama S, Nakano K. Feeding attractants in chemical constituents of brown alga for young abalone. Bull. Jpn. Soc. Sci. Fish. 1984;1541-4.
180. Harada K. Feeding attraction activities of nucleic acid-related compounds for abalone, Oriental weather fish and yellowtail. Bull. Jap. Soc. Sci. Fish. 1986;52:1961-8.

181. Viana MT, Cervantes-Trujano M, Solana-Sansores R. Attraction and palatability activities in juvenile abalone (*Haliotis fulgens*): nine ingredients used in artificial diets. *Aquaculture* 1994;127:19-28.
182. Harada K, Miyasaki T, Kawashima S, Shiota H. Studies on the feeding attractants for fishes and shellfishes: XXVI. Probable feeding attractants in allspice *Pimenta officinalis* for black abalone *Haliotis discus*. *Aquaculture* 1996;140:99-108.
183. Uki N, Watanabe T. Effect of heat-treatment of dietary protein sources on their protein quality for abalone. *Bull. Japan. Soc. Sci. Fish.* 1986c; 52:1199-204.
184. Paul AJ, Paul JM, Hood DW, Neve RA. Observations on food preference, daily ration requirements and growth of *Haliotis kamtschatkana* Jonas in captivity. *Veliger* 1977;19:303-9.
185. Tutschulte TC, Connell JH. Feeding behaviour and algal food of three species of abalones (*Haliotis*) in southern California. *Mar. Ecol. Prog. Ser.* 1988;49:57-64.
186. Stein JL. Subtidal gastropods consume sulphur-oxidizing bacteria: evidence from coastal hydrothermal vents. *Science* 1984;223:696-8.
187. Crofts DR. *Haliotis*. Liverpool Marine Biology Committee Memoirs. 1929; 29:1-74.
188. Culley MB, Peck LS. The feeding preferences of the ormer *Haliotis tuberculata* (L). *Kieler Meeresforsch. Sonderh.* 1981;5:570-2.

189. Sakai S. Ecological studies on the abalone, *Haliotis discus hannai* Ino I. Experimental studies on the food habit. Bull. Jap. Soc. Sci. Fish. 1962;28:776. (in Japanese with English abstract)
190. Uki N, Sugiura M, Watanabe T. Dietary value of seaweeds occurring on the Pacific coast of Tohoku for growth of the abalone *Haliotis discus hannai*. Bull. Jap. Soc. Sci. Fish. 1986d;52:257-66.
191. Nie Z, Yan J. Studies on food habit of adult abalone *Haliotis discus hannai* Ino. J. Fish. China 1985;9:19-27.
192. Kikuchi W, Sakurai Y, Sasaki M, Ino T. Food values of certain marine algae for the growth of the young abalone *Haliotis discus hannai*. Bull. Tohoku Reg. Fish. Res Lab. 1967;27:93-100.
193. Tomita K. Experiments on the food selectivity and digestion of abalone seed *Haliotis discus hannai*. J. Hokkaido Fish. Scient. Inst. 1972;29:17-23.
194. Sinclair M. Studies on the paua, *Haliotis iris* Martyn in the Wellington District 1945-46. Wellington: Zool. Publ. of Victoria University of Wellington; 1963. No.: 35.
195. Dutton S, Tong LJ. Food preferences of paua. Catch 81. 15 March 1981. n.p.; 1981.
196. Barratt L, Ormond RFG, Wrathall TJ. Ecological studies of southern Oman kelp communities Part I. New York: Tropical Marine Research Unit, University of York; 1986.
197. Newman GG. Distribution of the abalone *Haliotis midae* and the effect of sea temperature on productivity. Invest. Rep. Div. Sea. Fish. S. Afr. 1969;74.

198. Pena JB. Preliminary study on the induction of artificial spawning in *Haliotis coccinea canariensis* Nordsieek (1975). *Aquaculture* 1986;52:35-41.
199. Harrison AJ. Gastropod fisheries of the Pacific with particular reference to Australian abalone. *Can. Spec. Publ. Fish. Aquat. Sci.* 1986:14-22.



Table 1 External features and bionomy of *H. discus hannai* in early developmental stage (modified from Seki, 1997) (47).

Sequence	Stage	External features and bionomy
1	egg	fertilization
2		discharge of first polar body
3		discharge of second polar body
4		first cleavage
5		second cleavage
6		third cleavage
7		fourth cleavage
8	morula	
9	blastula	
10	gastrula	
11		appearance of prototrochal girdle
12	trochophore	appearance of stomodeum (rotation)
13		completion of prototrochal girdle
14		bursting of egg membrane (hatch out)
15		beginning of larval shell formation
16	veliger	completion of velum
17		appearance of larval retractor muscle
18		appearance of integumental attachment
19		development of foot mass
20		completion of larval shell
21		cephalo-pedal mass 90° torsion
22		foot muscle 180° torsion
23		appearance of spine on metapodium end
24		operculum (velum withdrawable into shell)
25		appearance of cilia on foot sole
26		vertical groove formation in velum
27		appearance of eye spot
28		appearance of propodium
29		appearance of cephalic tentacles
30		propodium cilia growing
31		appearance of cilia in mantle cavity
32		apophysis on propodium
33		first epipodial tentacle (able to crawl)
34		appearance of otolith
35		appearance of spine on cephalic tentacle
36		snout protrusion
37		appearance of two tubules on cephalic tentacles
38		ciliary process on roof of mantle cavity
39		third tubule appearance on cephalic tentacles
40		development of mantle cavity
41		fourth tubule appearance on cephalic tentacles

Table 2 Morphological changes and behavior of abalone larvae from pelagic stage to benthic stage (modified from Seki, 1997) (47).

Classification by life-cycle	Behavior	Characteristic action	Morphological changes
Pediveliger	Exploration	Repeatedly swimming and temporarily crawling over a wide surface area	None
	Inspection	Continuously crawling within a narrow area with intermittent movement	None
	Orientation	Cease crawling at a spot, change heading temporarily, elevate shell posteriorly, touching anterior part of cephalic tentacles	None
Plantigrade	Metamorphosis	Standstill	Sequence Velar cell abscission
		Restart crawling	1 Right ciliary lobe
			2 Mantle develops to shell edge
			3 Peristomal shell secretion
			4 Buccal process anterior to snout protrusion
			5 Functional radula
			6 Fifth tubule on cephalic tentacle
			7 Development of intestine
			8 Mucus gland
			9 Heart beat starts
	Feeding	Ingestion of small sized microalgae	10 Functional snout
		Grazing of diatoms	11 Development of mouth opening (30 μ m)

Table 3 Algal food of abalone species from different parts of the world: an asterisk indicates a preferred species as determined by feeding trials or inferred from field data (70).

Species and references to feeding studies	Habitat and algal dominants	Phenolic-poor brown algae	Algal food Phenolic-rich brown algae	Other algae
Northeast Pacific				
<i>Haliotis kamtschatkana</i> Paul <i>et al.</i> , 1977 (184); Ueda & Okada 1939 (146)	Shallow subtidal (to 5 m) <i>Laminaria</i> spp. <i>Alaria</i> <i>Agarum</i>	<i>Laminaria</i> spp.* <i>Alaria</i> *		Diatoms Red algae* <i>Ulva</i> * <i>Enteromorpha</i> *
<i>Haliotis rufescens</i> Cox, 1962 (33) Leighton, 1966 (66) Olsen, 1968 (147)	Depth range 0-40 m <i>Macrocystis</i> <i>Nereocystis</i> <i>Laminaria</i> <i>Agarum</i> <i>Pelagophycus</i>	<i>Macrocystis</i> * <i>Egregia</i> * <i>Pealgophycus</i> <i>Laminaria</i> spp.* <i>Nereocystis</i>		Red algae
<i>Haliotis corrugata</i> <i>Haliotis fulgens</i> Leighton, 1966 (66) Tutschulte & Connel, 1988 (185)	Depth range 5-40 m <i>Macrocystis</i> <i>Laminaria</i> spp. <i>Agarum</i>	<i>Macrocystis</i> * <i>Eisenia</i> * <i>Egregia</i>		Red algae*
<i>Haliotis cracherodii</i> Leighton & Boolootian, 1963 (63) Stein, 1984 (186)	Intertidal and shallow subtidal <i>Egregia</i> , <i>Eisenia</i> <i>Macrocystis</i> <i>Pelvetia</i>	<i>Egregia</i> <i>Eisenia</i> * <i>Macrocystis</i> *	<i>Pelvetia</i> <i>fastigiata</i>	Sulphur bacteria
Northeast Atlantic				
<i>Haliotis tuberculata</i> Crofts, 1929 (187) Culley & Peek, 1981 (188)	Depth range 0-10 m Commonly intertidal <i>Laminaria</i> spp.			<i>Ulva</i> * <i>Palmaria</i> * <i>Enteromorpha</i> Red algae*
Northwest Pacific				
<i>Haliotis discus hannai</i> Sakai, 1962 (189) Uki <i>et al.</i> , 1986 (190) Nie & Yan, 1985 (191) Kikuchi <i>et al.</i> 1967 (192) Tomita, 1972 (193)	Depth range 0-10 m	<i>Undaria</i> * <i>Laminaria</i> spp.		<i>Ulva</i> * <i>Enteromorpha</i>

Table 3 Continued

Species and references to feeding studies	Habitat and algal dominants	Phenolic-poor brown algae	Algal food Phenolic-rich brown algae	Other algae
Southwest Pacific				
<i>Haliotis iris</i> Sinclair, 1963 (194) Poore, 1972b (148) Dutton & Tong, 1981 (195)	Depth range 0-10 m <i>Eckloma</i> <i>Carpophyllum</i> <i>Macrocystis</i>	<i>Macrocystis</i> <i>Lessonia</i>	<i>Ecklonia</i> <i>Carpophyllum</i> <i>Blossevillea</i>	Red algae*
Northwest Indian				
<i>Haliotis mariae</i> Barratt <i>et al.</i> , 1986 (196)	Depth range 0-10 m <i>Ecklonia radiata</i> <i>Sargassopsis</i> <i>Sargassum</i>	<i>Ecklonia</i> * <i>Sargassopsis</i>		Red algae Green algae
Southern Australia				
<i>Haliotis roei</i> Shepherd, 1973 (68) Wells & Keesing, 1989 (13) Shepherd & Stienberg, 1992 (70)	Intertidal, shallow subtidal <i>Sargassum</i> <i>Ecklonia</i> <i>Cystophora</i> spp.		<i>Ecklonia</i> <i>Sargassum</i> spp.	Red algae*
<i>Haliotis laevigata</i> Shepherd, 1973 (68) Shepherd & Stienberg, 1992 (70)	Depth range 5-40 m <i>Ecklonia</i> <i>Cystophora</i> spp. <i>Sargassum</i> spp.	<i>Lobospira</i> <i>Pachydictyon</i>		Red algae*
<i>Haliotis rubra</i> Shepherd, 1973 (68), 1975 (157); Shepherd & Stienberg, 1992 (70) Prince, 1989 (159)	Depth range 1-30 m <i>Ecklonia</i> <i>Macrocystis</i> <i>Phyllospora</i>	<i>Macrocystis</i> <i>Phyllospora</i>		Red algae*
Southwest Pacific				
<i>Haliotis midae</i> Newman, 1969 (197) Barkai & Griffiths, 1986 (149)	Depth range 0-10 m <i>Ecklonia maxima</i> <i>Laminaria pallida</i>	<i>Laminaria</i>	<i>Ecklonia</i> *	Red algae*

Table 4 Composition of artificial diet for abalone (values expressed as percentage) [modified from Fleming *et al.* (diets no. 1-12) (100), Uki and Watanabe (diet no. 13-15) (15), Viana *et al.* (16-17) (99), Bardach *et al.* (diet no. 18) (62), Knauer *et al.* (diet no. 19) (98)].

Nutrient	Diet number																		
	1 ^a	2	3	4	5	6 ^a	7 ^a	8	9	10	11	12	13	14	15	16	17	18	19
Protein source																			
Fish meal	15	0	32	0	55	2-3	0	0	9	17	12	25	0	0	47	51.4	0	40	14.7
Soybean meal/flour	20	0	32	+	0	0	0	17.3	40	10	16	15	0	0	0	0	0	0	0
Casein	0	20-30	0	0	0	42	0	13	8	0	0	0	30	30	0	0	38.2	0	18.6
Lacto serum	0	0	0	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Whey	0	0	0	0	0	0	0	0	0	0	7	0	0	0	0	0	0	0	0
<i>Spirulina</i> sp.	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	14.3
Bloodmeal	0	0	0	0	0	0	0	4.3	0	0	0	0	0	0	0	0	0	0	0
Abalone viscera silage	0	0	0	0	0	0	0	0	0	20	0	0	0	0	0	0	0	0	0
Shrimp meal	0	0	0	0	0	0	0	0	0	0	20	0	0	0	0	0	0	0	0
Carbohydrate / binder source																			
Wheat / flour / products	+	0	0	+	0	0	+	50.7	0	0	16	10	0	0	0	0	0	0	0
Sodium alginate	0	20	0	0	0	0	0	0	0.8	10	4	20	20	20	20	23.4	25.5	45	0
Dextrin	0	34	0	0	0	0	0	0	0	0	0	0	34	31	21	0	0	0	41.9
Starch	0	+	0	0	35	+	0	0	10	0	0	0	0	0	0	0	0	5	0
Bran	0	0	0	0	0	+	0	0	0	0	0	0	0	0	0	0	0	0	0
Taramel A40	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0
Corn flour / meal	0	0	0	0	0	0	0	0	0	10	0	0	0	0	0	4.7	6.4	0	0
Gelatin	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0
Semolina	0	0	0	0	0	0	+	0	32	0	0	0	0	0	0	0	0	0	0
Cellulose	0	3	0	2	0	0	0	0	0	0	0	0	5	5	5	0	6.4	0	0
Lipid	1.5	5	5	3.5	+	5.3	+	3.2	3	>5	4.2	3	5	5	1	6.5	6.4	5	2.5
Mineral mixture	4	4	+	+	4	+	+	+	4	5	+	4	4	4	4	4.7	5.1	0	4
Vitamin mixture	1.5	1.5	1.5	+	1	+	+	+	0.5	2	0.8	2	1.5	1.5	1.5	1.9	1.9	0	1.5

1 Japan: Nihon Nosan Kogyo K.K.; 2 Japan: Uki's test diet; 3 China: Nie's diet; 4 France: Argenton Experimental Station; 5 South Africa: Rhodes University and Sea Plant Production Ltd.; 6 New Zealand: Pronak Technology Ltd; 7 Australia: Deakin University; 8 Australia: MSL and Sou'west Seafoods Pty. Ltd.; 9 Australia: FRDC/CRC Cooperative Research Program; 10 Mexico: University Autónoma de Baja California; 11 USA: Terminal Island Aquaculture; 12. Korea: National Fisheries Research and Development Agency; 13 - 15 from Uki and Watanabe (15); 16 - 17 from Viana *et al.* (99); 18 from Bardach *et al.* (62); 19 from Knauer *et al.* (98); ^a Commercial available diet; + unknown inclusion levels.



Table 5 Different formulae of artificial diets using various species of macroalgae and casein as sources of protein.

Ingredients	Formulae						
	I	II	III	IV	V	VI	VII
<i>A. spicifera</i>	30	-	-	-	-	-	-
<i>E. intestinalis</i>	-	30	-	-	-	-	-
<i>G. fisheri</i>	-	-	30	-	30	-	-
<i>G. salicornia</i>	-	-	-	30	-	30	-
<i>G. tenuistipitata</i> (dry)	-	-	-	-	-	-	30
Casein	-	-	-	-	30	30	30
Dextrin	25	25	25	25	10	10	10
Cellulose	25	25	25	25	10	10	10
Vitamin mixture [#]	5	5	5	5	5	5	5
Mineral mixture [@]	5	5	5	5	5	5	5
Cod liver oil	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Soybean oil	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Agar	5	5	5	5	5	5	5

[#] Cetavit, see also Table 6 [@] Premix, see also Table 6

Table 6 The composition of vitamins in Cetavit 1,000 g from Eastern Marine Co., LTD. Thailand and minerals in Premix 1,000 g from Chanaphant Industry Co., LTD. Thailand.

Vitamin mixture		Mineral mixture	
Compositions	Weight	Composition	Weight
Vitamin A	15,000,000 units	Manganese	5.400 g
Vitamin D ₃	3,000,000 units	Iron	14.200 g
Vitamin C	83.00 g	Copper	1.000 g
Vitamin E	27.50 g	Zinc	2.900 g
Vitamin K	4.67 g	Sodium	3.300 g
Vitamin B ₁	25.00 g	Iodine	0.019 mg
Vitamin B ₂	25.00 g	Potassium	0.900 g
Vitamin B ₆	5.00 g	Cobalt	1.100 g
Vitamin B ₁₂	0.05 g	Medium	971.181 g
Nicotinamide	20.00 g		
Calcium D-Panthenate	5.00 g		
Folic acid	0.40 g		

Table 7 The average sizes (mean $\pm$ S.D.) and percentage of visceral mass, foot and shell of fifteen *H. asinina* in each period of sampling.

	Average size (mean $\pm$ S.D.)				
	October	January	April	July	Average
Shell length (mm)	63.8 $\pm$ 12.8	70.1 $\pm$ 6.6	69.1 $\pm$ 9.2	69.8 $\pm$ 6.4	68.3 $\pm$ 9.1
Total body weight (g)	48.5 $\pm$ 10.1	48.4 $\pm$ 14.3	49.5 $\pm$ 8.7	46.3 $\pm$ 12.8	48.2 $\pm$ 11.4
Visceral mass (g)	12.1 $\pm$ 2.8 (24.9)*	11.4 $\pm$ 3.9 (23.5)*	11.8 $\pm$ 3.9 (23.9)*	10.7 $\pm$ 3.2 (23.0)*	11.5 $\pm$ 3.8 (23.8)*
Foot (g)	29.7 $\pm$ 6.5 (61.4)*	29.8 $\pm$ 8.8 (61.5)*	30.5 $\pm$ 5.0 (61.6)*	28.5 $\pm$ 7.6 (61.5)*	29.7 $\pm$ 6.8 (61.6)*
Shell (g)	6.7 $\pm$ 1.8 (13.8)*	7.3 $\pm$ 1.9 (15.0)*	7.2 $\pm$ 1.4 (14.5)*	7.2 $\pm$ 2.4 (15.4)*	7.1 $\pm$ 1.9 (14.7)*

* = % of total weight

Table 8 The micro-organism groups (<120 μm) in *H. asinina* gut.

Organism	Relative frequency (%)*				
	October	January	April	July	Average
Benthic diatoms	99.3	98.6	99.7	99.7	99.3
Other organisms	0.7	1.4	0.3	0.3	0.7
Crustaceans	0.2	0.4	0.1	0.1	0.2
Protozoa	0.2	0.2	0.1	0.2	0.2
Unidentified eggs	0.0	0.6	0.0	0.0	0.1
Blue green algae	0.2	0.0	0.1	0.0	0.1
Bivalves	0.1	0.0	0.0	0.0	0.1
Flagellated algae	0.0	0.2	0.4	0.1	0.2
Annelids	0.0	0.1	0.0	0.0	0.0

* = Relative abundance in Sedgwick-rafter counting chamber subsample (1 ml) from the group considered in detail in Table 9.

Table 9 The genera of micro-organisms (<120 μm) in *H. asinina* gut.

Organism	Relative frequency (%)*				
	October	January	April	July	Average
Benthic diatoms	99.3	98.6	99.7	99.7	99.3
<i>Nitzschia</i>	13.3	13.1	16.0	36.1	19.6
<i>Amphora</i>	22.9	11.8	16.0	18.2	17.2
<i>Navicula</i>	4.2	4.5	8.0	11.0	6.9
<i>Cocconeis</i>	6.2	9.4	10.8	9.7	9.0
<i>Plagiogramma</i>	6.7	15.7	0.5	5.0	7.0
<i>Diploneis</i>	7.5	1.5	4.5	3.2	4.2
<i>Fragilaria</i>	5.6	21.0	5.2	3.2	8.7
<i>Coscinodiscus</i>	3.7	1.1	2.7	2.2	2.4
<i>Surirella</i>	4.0	1.6	10.5	1.7	4.4
<i>Thalassionema</i>	3.1	0.0	1.8	1.5	1.6
<i>Asteromphalus</i>	0.5	0.1	0.1	1.3	0.5
<i>Synedra</i>	5.2	4.0	6.2	0.7	4.0
<i>Grammatophora</i>	3.2	2.1	2.3	0.6	2.0
<i>Melosira</i>	3.1	1.4	0.5	0.6	1.4
<i>Achnanthes</i>	0.7	2.5	1.6	0.6	1.3
<i>Bacteriastrum</i>	0.2	0.1	0.2	0.6	0.3
<i>Paralia</i>	2.7	0.1	2.3	0.5	1.4
<i>Bidduphia</i>	1.4	0.1	1.2	0.4	0.8
<i>Licmophora</i>	1.2	5.4	2.4	0.4	2.4
<i>Cyclotella</i>	0.4	0.6	0.5	0.4	0.5
<i>Odontella</i>	0.4	0.2	0.2	0.4	0.3
<i>Rhizosolenia</i>	0.0	0.1	0.2	0.4	0.2
<i>Pluerosigma</i>	1.0	0.3	1.1	0.2	0.7
<i>Rhabdonema</i>	0.7	0.0	1.5	0.2	0.6
<i>Podocystis</i>	0.6	0.7	0.9	0.2	0.6

Table 9 (Continued)

Organism	Relative frequency (%)*				
	October	January	April	July	Average
<i>Chaetoceros</i>	0.0	0.2	0.1	0.2	0.1
<i>Campylodiscus</i>	0.1	0.0	0.4	0.1	0.2
<i>Triceratium</i>	0.0	0.0	0.3	0.1	0.1
<i>Isthmia</i>	0.4	0.3	0.6	0.0	0.3
<i>Peridinium</i>	0.0	0.1	0.1	0.0	0.1
<i>Actinoptycus</i>	0.1	0.1	0.1	0.0	0.1
<i>Pinnularis</i>	0.2	0.1	0.1	0.0	0.1
<i>Asterionella</i>	0.0	0.0	0.7	0.0	0.2
<i>Auliscus</i>	0.0	0.1	0.0	0.0	0.0
<i>Hyalotheca</i>	0.0	0.3	0.0	0.0	0.1
<i>Palmeria</i>	0.0	0.0	0.1	0.0	0.0
Blue-green algae	0.2	0.0	0.1	0.0	0.1
<i>Oscillatoria</i>	0.1	0.0	0.1	0.0	0.1
<i>Spirulina</i>	0.1	0.0	0.0	0.0	0.0
Flagellate algae	0.0	0.1	0.0	0.0	0.0
<i>Tetraselmis</i>	0.00	0.1	0.0	0.0	0.0
Protozoa	0.2	0.2	0.1	0.2	0.2
<i>Dictyocha</i>	0.2	0.1	0.0	0.0	0.1
Foraminiferan	0.0	0.1	0.1	0.2	0.1
Crustaceans	0.2	0.4	0.1	0.1	0.2
Annelids	0.0	0.1	0.0	0.0	0.0
Bivalves	0.1	0.0	0.0	0.0	0.1
Unidentified egg	0.0	0.6	0.0	0.0	0.1

* = Percent presence in Sedgwick-rafter counting chamber subsampling (1 ml)

Table 10 The macro-organisms (> 120 μm) in *H. asinina* gut.

Organism	Frequency percentage (total count)				
	October	January	April	July	Average
Foraminiferans	87.4	71.5	70.9	55.8	71.4
Crustaceans	6.9	14.1	12.1	9.9	10.7
Cypris	3.0	3.5	5.0	3.5	3.7
Harpacticoids	1.4	3.5	2.3	1.0	2.0
Copepods	2.1	3.5	1.2	0.6	1.9
Crustaceans	0.0	2.6	2.0	2.3	1.7
Gnathopods	0.4	0.0	1.1	2.1	0.9
Other crustaceans ^c	0.0	1.0	0.5	0.4	0.5
Annelids	2.3	8.5	8.4	19.8	9.7
Mollusks	2.7	4.5	3.4	3.1	3.5
Gastropods	1.8	2.0	2.1	1.6	1.9
Bivalves	0.9	2.5	1.3	1.5	1.6
Benthic diatoms	0.7	1.4	5.2	11.4	4.7
<i>Fragillaria</i>	0.0	0.8	0.7	6.9	2.1
<i>Triceratium</i>	0.2	0.0	1.8	2.3	1.1
<i>Campylodiscus</i>	0.3	0.0	1.0	1.3	0.7
<i>Rhabdonema</i>	0.1	0.6	1.4	0.0	0.5
<i>Coscinodiscus</i>	0.1	0.0	0.1	0.7	0.2
Others ^d	0.0	0.0	0.2	0.2	0.1

^c Composed of nauplius, isopods, cumaceans shrimp and amphipods.

^d Composed of *Pleurosigma*, *Navicula* and *Cocconeis*.

Table 11 The size of *H. asinina* larvae (μm), from fertilization to formation of the first respiratory pore.

Stage	Size (μm)	
	Width	Length
Unfertilized eggs	88.0 ± 8.0	102.4 ± 3.6
Fertilized eggs to hatch out	126.4 ± 17.1	148.2 ± 10.4
Trochophore larvae	105.3 ± 4.1	130.7 ± 4.8
Completion of velum	108 ± 3.6	135.3 ± 2.9
Larval retractor muscle	110.7 ± 4.1	139.5 ± 4.2
Completion of larval shell	174.5 ± 6.2	242.0 ± 6.4
Formation of operculum	175.5 ± 6.3	239.8 ± 5.9
Fourth tubule on cephalic tentacle	178.3 ± 5.2	245.7 ± 4.6
First day of settlement	178.8 ± 5.6	247.7 ± 2.5
Beginning of first respiratory pore formation	1142.4 ± 94.6	1530.2 ± 140.7

Table 12 The rate of larval development (hours) of *H. asinina* at water temperatures of 25, 28, 31, 34 °C and room temperature (28-35°C).

Sequence	Larval development stage	Time period of larval development (hour)				
		25°C	28°C	31°C	34°C	28-35°C
1	Fertilization	0.00	0.00	0.00	0.00	0.00
2	First polar body	0.25	0.25	0.25	0.25	0.25-0.33
3	Second polar body	0.33	0.33	0.42	0.33	0.25-0.42
4	First cleavage (2 cells)	0.50	0.50	0.42	0.50	0.33-0.58
5	Second cleavage (4 cells)	1.00	0.92	0.83	0.92	0.75-1.00
6	Third cleavage (8 cells)	1.33	1.25	1.00	1.17	1.00-1.17
7	Fourth cleavage (12 cells)	1.42	1.33	1.25	1.25	1.17-1.33
8	Fifth cleavage (16 cells)	1.50	1.42	1.33	1.33	1.33-1.50
9	Morula (32 cells)	2.83	2.42	1.83	2.00	2.00-2.50
11	Blastula	3.83	3.17	2.83	3.00	2.50-3.00
11	Gastrula	5.50	4.00	3.50	3.50	3.00-4.00
12	Prototrochal cilia	6.00	5.00	4.17	4.00	4.50-5.00
13	Stomodeum	7.00	5.50	4.50	4.50	5.00-5.50
14	Prototrochal girdle	8.00	3.50	5.00	5.00	5.00-5.50
15*	Hatch out	9.50	7.50	6.00	6.00	5.50-7.00
16	Beginning of larval shell	10.50	8.00	6.50	7.00	6.00-7.00
17	Completion of velum	12.00	9.50	8.00	8.00	7.50-8.00
18*	Larval retractor muscle	13.50	11.00	9.00	9.00	10.00-11.00
19	Integumental attachment	14.50	11.50	10.50	10.50	10.50-12.00
20	Protrusion of foot mass	16.00	12.50	10.50	10.50	11.00-12.50
21	Completion of larval shell	16.50	13.50	11.00	11.00	11.00-13.00
22*	Torsion (90° twisting)	18.00	14.00	11.50	11.50	12.00-14.00
23	180° rotation of foot mass	21.00	18.00	13.50	13.50	13.00-15.00
24	Operculum	25.00	22.50	16.00	16.00	16.00-16.50
25	Long spines on end of metapodium	29.00	23.00	18.50	17.00	18.00-19.00
26	Fine cilia on foot	30.50	25.00	20.50	20.50	19.00-20.00
27	Vertical groove in velum	35.00	27.00	21.00	21.00	21.00-22.50
28	Eye spot	40.00	28.00	21.00	21.00	21.00-23.00
29	Propodium	41.00	29.50	24.00	24.00	24.00-24.50
30	Cephalic tentacle	43.50	30.50	25.00	24.00	24.00-25.50
31	Cilia on propodium	45.00	32.00	26.50	26.00	25.00-27.00
32*	First epipodial tentacle	45.50	36.00	28.00	28.00	27.00-29.00
33	Cilia in mantle cavity	48.00	36.00	30.00	29.00	31.00-32.00
34	Apophysis on propodium	51.00	39.00	30.00	30.00	32.00-33.00
35	Otolith	55.00	41.00	34.00	33.00	32.00-33.00
36	Short spine on cephalic tentacle	57.00	42.00	34.00	34.00	33.00-34.00
37	Two tubules on cephalic tentacle	59.00	42.00	35.00	35.00	34.00-35.00
38	Third tubule on cephalic tentacle	60.00	43.00	37.00	36.00	36.00-37.00
39	Snout protrusion	62.00	45.00	39.00	38.50	38.00-39.00
40	Ciliary process in mantle cavity	64.00	46.00	40.00	38.50	39.00-41.00
41	Retractor muscle drawn in mantle cavity	64.00	47.00	40.00	40.00	40.00-42.00
42*	Fourth tubule on cephalic tentacle	65.00	49.00	41.00	41.00	42.00-44.00

* the stage used for calculating the biological zero point.

Table 13 Equations of relationship between water temperature and time inversion of beginning formation of the fifth larval developmental stage (1/time) and biological zero point of the important larval stages of *H. asinina*.

Larval developmental stage	Equation of relationship	Biological zero point
Hatch out	$1/t^a = 0.0102T^b - 0.1515$	14.9
Larval retractor muscle	$1/t = 0.0073T - 0.1091$	14.9
Torsion (90° twisting)	$1/t = 0.0052T - 0.0768$	14.8
First epipodial tentacle	$1/t = 0.0023T - 0.0356$	15.5
Fourth tubule on cephalic tentacle	$1/t = 0.0015T - 0.0220$	14.7
Average		15.0 ± 0.3

^a t = hours, ^b T = °C

Table 14 The effective accumulative temperature (EAT) of larval development of *H. asinina* at water temperatures of 25, 28, 31, 34 °C and room temperature (28-35°C).

Sequence	Larval development stage	EAT (°C - hour)				
		25°C	28°C	31°C	34°C	average
1	Fertilization	0.0	0.0	0.0	0.0	0.0
2	First polar body	2.5	3.3	4.0	4.8	3.6
3	Second polar body	3.3	4.3	6.7	6.3	5.1
4	First cleavage (2 cells)	5.0	6.5	6.7	9.5	6.9
5	Second cleavage (4 cells)	10.0	12.0	13.3	17.5	13.2
6	Third cleavage (8 cells)	13.3	16.3	16.0	22.2	16.9
7	Fourth cleavage (12 cells)	14.2	17.3	20.0	23.8	18.81
8	Fourth - fifth cleavage (12-16 cells)	15.0	18.5	21.3	25.3	20.0
9	Morula (32 cells)	28.3	31.5	29.3	38.0	31.8
10	Blastula	38.3	41.2	45.3	57.0	45.4
11	Gastrula	55.0	52.0	56.0	66.5	57.4
12	Prototrochal cilia	60.0	65.0	66.7	76.0	66.9
13	Stomodeum	70.0	71.5	72.0	85.5	74.8
14	Prototrochal girdle	80.0	45.5	80.0	95.0	75.1
15	Hatch out	95.0	97.5	96.0	114.0	100.6
16	Beginning of larval shell	105.0	104.0	104.0	133.0	111.5
17	Completion of velum	120.0	123.5	128.0	152.0	130.9
18	Larval retractor muscle	135.0	143.0	144.0	171.0	148.3
19	Integumental attachment	145.0	149.5	168.0	199.5	165.5
20	Protrusion of foot mass	160.0	162.5	168.0	199.5	172.5
21	Completion of larval shell	165.0	175.5	176.0	209.0	181.4
22	Torsion (90° twisting)	180.0	182.0	184.0	218.5	191.1
23	180° rotation of foot mass	210.0	234.0	216.0	256.5	229.1
24	Operculum	250.0	292.5	256.0	304.0	275.6
25	Long spines on end of metapodium	290.0	299.0	296.0	323.0	302.0
26	Fine cilia on foot	305.0	325.0	328.0	389.5	336.9
27	Vertical groove in velum	350.0	351.0	336.0	399.0	359.0
28	Eye spot	400.0	364.0	336.0	399.0	374.8
29	Propodium	410.0	383.5	384.0	456.0	408.4
30	Cephalic tentacle	435.0	396.5	400.0	456.0	421.9
31	Cilia on propodium	450.0	416.0	424.0	494.0	446.0
32	First epipodial tentacle	455.0	468.0	448.0	532.0	475.8
33	Cilia in mantle cavity	480.0	468.0	480.0	551.0	494.8
34	Apophysis on propodium	510.0	507.0	480.0	570.0	516.8
35	Otolith	550.0	533.0	544.0	627.0	563.5
36	Short spine on cephalic tentacle	570.0	546.0	544.0	646.0	576.5
37	Two tubules on cephalic tentacle	590.0	546.0	560.0	665.0	590.3
38	Third tubule on cephalic tentacle	600.0	559.0	592.0	684.0	608.8
39	Snout protrusion	620.0	585.0	624.0	731.5	640.1
40	Ciliary process in mantle cavity	640.0	598.0	640.0	731.5	652.4
41	Retractor muscle drawn in mantle cavity	640.0	611.0	640.0	760.0	662.8
42	Fourth tubule on cephalic tentacle	650.0	637.0	656.0	779.0	680.5

Table 15 Shell length (mm ± S.E.) of *H. asinina* fed nine species of macroalgae over a period of 184 days.

Days	Red algae				Green algae		Brown algae		
	<i>A. spicifera</i>	<i>G. fisheri</i>	<i>G. salicornia</i>	<i>G. tenuistipitata</i>	<i>E. intestinalis</i>	<i>C. racemosa</i>	<i>D. dichotoma</i>	<i>P. minor</i>	<i>S. polycystum</i>
0	13.3 ± 1.3	13.3 ± 1.4	13.3 ± 1.4	13.3 ± 1.4	13.3 ± 1.4	13.3 ± 1.3	13.3 ± 1.2	13.3 ± 1.4	13.3 ± 1.4
16	15.0 ± 1.6	14.3 ± 2.7	13.9 ± 1.6	15.0 ± 1.6	14.8 ± 1.5	13.4 ± 1.6	13.5 ± 1.3	13.5 ± 1.4	13.9 ± 1.4
30	15.9 ± 1.8	15.0 ± 1.7	14.8 ± 1.9	16.3 ± 1.8	16.2 ± 1.7	13.9 ± 1.1	13.5 ± 1.2	13.5 ± 1.4	13.9 ± 1.4
44	16.7 ± 0.9	15.8 ± 1.8	15.7 ± 2.1	17.5 ± 2.0	17.8 ± 1.9	14.5 ± 0.9	13.8 ± 1.2	13.5 ± 1.4	13.9 ± 1.5
58	17.2 ± 2.1	16.4 ± 2.0	16.6 ± 2.3	18.5 ± 2.2	18.6 ± 2.2	14.6 ± 0.9	13.8 ± 1.2	13.4 ± 1.4	13.8 ± 1.4
72	18.0 ± 2.3	17.7 ± 2.2	17.6 ± 2.5	20.6 ± 2.6	19.9 ± 2.4	15.4 ± 0.2	14.4 ± 1.0	13.5 ± 1.4	14.1 ± 1.5
86	18.2 ± 2.4	18.1 ± 2.4	18.0 ± 2.6	21.5 ± 2.7	20.2 ± 2.5	15.4 ± 0.3	14.3 ± 1.2	13.5 ± 1.5	14.2 ± 1.5
100	19.0 ± 2.7	18.9 ± 2.5	19.0 ± 2.7	22.6 ± 2.7	20.9 ± 2.6	15.7	14.1 ± 1.2	13.5 ± 1.6	14.4 ± 1.4
114	19.4 ± 2.9	19.6 ± 2.8	19.8 ± 2.7	23.3 ± 2.9	21.7 ± 2.8	–	14.1 ± 1.2	13.5 ± 1.5	14.6 ± 1.4
128	20.2 ± 3.0	20.2 ± 3.0	20.2 ± 3.0	23.9 ± 3.0	22.0 ± 2.9	–	14.1 ± 1.2	13.7 ± 1.3	15.1 ± 1.2
142	21.1 ± 3.1	20.7 ± 3.0	20.9 ± 3.1	24.4 ± 3.1	22.4 ± 3.0	–	15.2 ± 0.7	13.6 ± 1.3	15.2 ± 1.4
156	22.3 ± 3.2	21.2 ± 3.1	22.0 ± 3.0	25.0 ± 3.3	22.7 ± 3.1	–	–	13.6 ± 1.2	15.7 ± 1.6
170	23.1 ± 3.3	21.3 ± 3.1	22.7 ± 2.9	25.3 ± 3.4	23.0 ± 2.9	–	–	13.6 ± 1.4	–
184	24.7 ± 3.4 ^b	22.0 ± 3.2 ^d	24.3 ± 2.7 ^b	26.2 ± 3.5 ^a	23.4 ± 2.9 ^c	–	–	13.6 ± 1.4 ^e	–

Means and standard deviations are presented. Analysis of variance and Duncan's multiple range test were performed on the means of shell length increases, the same letter identifies the values that are not significantly different (P<0.05).

Table 16 Percent increase of shell length of *H. asinina* fed nine species of macroalgae over a period of 184 days.

Days	Red algae				Green algae		Brown algae		
	<i>A. spicifera</i>	<i>G. fisheri</i>	<i>G. salicornia</i>	<i>G. tenuistipitata</i>	<i>E. intestinalis</i>	<i>C. racemosa</i>	<i>D. dichotoma</i>	<i>P. minor</i>	<i>S. polycystum</i>
0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
16	12.8 ± 0.3	7.9 ± 0.6	4.8 ± 0.1	12.8 ± 0.5	11.1 ± 0.2	0.7 ± 0.0	0.9 ± 0.0	1.4 ± 0.1	4.5 ± 0.3
30	19.7 ± 0.6	13.2 ± 0.4	10.9 ± 0.5	22.7 ± 1.6	21.9 ± 0.4	5.2 ± 0.2	1.4 ± 0.1	1.1 ± 0.1	4.3 ± 0.2
44	25.8 ± 0.8	19.1 ± 1.3	17.9 ± 0.5	31.5 ± 0.9	33.4 ± 1.3	9.2 ± 0.2	3.2 ± 0.1	1.6 ± 0.0	4.6 ± 0.1
58	29.3 ± 1.8	23.8 ± 1.4	24.4 ± 1.5	39.6 ± 0.8	39.7 ± 2.4	9.8 ± 0.5	3.6 ± 0.1	1.0 ± 0.0	3.8 ± 0.2
72	35.9 ± 1.1	33.7 ± 0.7	32.6 ± 0.7	55.1 ± 3.3	49.5 ± 1.5	15.9 ± 0.8	8.2 ± 0.4	1.8 ± 0.1	5.8 ± 0.2
86	37.4 ± 1.5	36.1 ± 1.4	35.2 ± 2.5	62.1 ± 3.7	51.4 ± 1.0	16.5	7.1 ± 0.3	1.6 ± 0.1	7.3 ± 0.1
100	43.2 ± 2.6	42.1 ± 2.1	43.1 ± 2.6	69.8 ± 4.2	56.9 ± 2.3	—	5.9 ± 0.2	1.7 ± 0.1	8.4 ± 0.3
114	46.4 ± 1.9	47.8 ± 1.4	48.5 ± 2.9	75.2 ± 3.0	62.7 ± 3.1	—	6.1 ± 0.4	1.7 ± 0.1	9.6 ± 0.6
128	52.4 ± 2.6	51.9 ± 2.1	51.6 ± 3.1	79.7 ± 4.0	65.2 ± 1.3	—	5.9 ± 0.4	2.9 ± 0.1	14.0 ± 0.8
142	58.7 ± 4.1	55.7 ± 3.3	57.4 ± 2.3	83.5 ± 2.5	68.5 ± 2.1	—	13.8 ± 0.8	2.4 ± 0.1	14.1 ± 0.7
156	68.2 ± 2.0	59.8 ± 1.8	65.4 ± 4.6	88.5 ± 5.3	70.7 ± 1.4	—	—	2.6 ± 0.1	18.0 ± 0.7
170	74.4 ± 3.7	60.3 ± 1.8	70.8 ± 5.0	90.9 ± 3.6	72.8 ± 3.6	—	—	2.2 ± 0.2	—
184	86.2 ± 2.6 ^b	66.0 ± 0.9 ^d	82.2 ± 4.2 ^b	97.4 ± 3.9 ^a	75.1 ± 5.4 ^c	—	—	2.3 ± 0.8 ^e	—

Means and standard deviations are presented. Analysis of variance and Duncan's multiple range test were performed on the means of percent increase of shell length, the same letter identifies the values that are not significantly different ($P < 0.05$).

Table 17 Body weight (g ± S.E.) of *H. asinina* fed nine species of macroalgae over a period of 184 days.

Days	Red algae				Green algae		Brown algae		
	<i>A. spicifera</i>	<i>G. fisheri</i>	<i>G. salicornia</i>	<i>G. tenuistipitata</i>	<i>E. intestinalis</i>	<i>C. racemosa</i>	<i>D. dichotoma</i>	<i>P. minor</i>	<i>S. polycystum</i>
0	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1
16	0.6 ± 0.2	0.6 ± 0.5	0.5 ± 0.2	0.7 ± 0.2	0.6 ± 0.2	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.4 ± 0.1
30	0.8 ± 0.3	0.7 ± 0.2	0.6 ± 0.3	0.9 ± 0.3	1.0 ± 0.6	0.3 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.2
44	0.9 ± 0.3	0.8 ± 0.3	0.8 ± 0.3	1.1 ± 0.4	1.3 ± 0.4	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.2
58	1.0 ± 0.4	0.9 ± 0.4	0.9 ± 0.4	1.6 ± 0.6	1.4 ± 0.5	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.1
72	1.2 ± 0.5	1.1 ± 0.5	1.1 ± 0.5	2.2 ± 0.8	1.7 ± 0.6	0.4 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.4 ± 0.2
86	1.3 ± 0.5	1.2 ± 0.5	1.1 ± 0.5	2.5 ± 0.9	1.9 ± 0.7	0.4 ± 0.0	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.2
100	1.4 ± 0.6	1.4 ± 0.6	1.3 ± 0.6	2.5 ± 1.0	1.9 ± 0.7	0.4	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1
114	1.5 ± 0.7	1.6 ± 0.8	1.4 ± 0.7	2.8 ± 1.1	2.2 ± 0.9	–	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1
128	1.7 ± 0.8	1.7 ± 0.8	1.4 ± 0.7	3.0 ± 1.2	2.2 ± 0.9	–	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.1
142	1.9 ± 0.9	1.8 ± 0.9	1.9 ± 1.6	3.3 ± 1.3	2.4 ± 1.0	–	0.4 ± 0.1	0.3 ± 0.1	0.4 ± 0.2
156	2.5 ± 1.2	2.0 ± 1.0	2.2 ± 1.0	3.9 ± 1.6	2.4 ± 1.1	–	–	0.3 ± 0.1	0.5 ± 0.1
170	2.8 ± 1.3	2.1 ± 1.1	2.5 ± 1.1	4.1 ± 1.7	2.5 ± 1.0	–	–	0.4 ± 0.1	–
184	3.5 ± 1.3 ^b	2.4 ± 1.2 ^d	3.0 ± 1.3 ^c	4.8 ± 2.0 ^a	2.5 ± 1.0 ^d	–	–	0.4 ± 0.1 ^e	–

Means and standard deviations are presented. Analysis of variance and Duncan's multiple range test were performed on the means of body weight increases, the same letter identifies the values that are not significantly different (P<0.05).

Table 18 Percent increase of body weight of *H. asimina* fed nine species of macroalgae over a period of 184 days.

Days	Red algae				Green algae			Brown algae		
	<i>A. spicifera</i>	<i>G. fisheri</i>	<i>G. salicornia</i>	<i>G. tenuistipitata</i>	<i>E. intestinalis</i>	<i>C. racemosa</i>	<i>D. dichotoma</i>	<i>P. minor</i>	<i>S. polycystum</i>	
0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	
16	48.8 ± 1.5	37.0 ± 1.5	18.4 ± 1.3	66.0 ± 2.0	52.29 ± 1.6	-25.49 ± 1.0	-15.62 ± 0.5	-12.0 ± 0.6	4.0 ± 0.2	
30	84.4 ± 5.9	68.3 ± 2.0	50.8 ± 1.0	113.5 ± 4.5	134.13 ± 4.0	-22.09 ± 1.1	-11.42 ± 0.3	-14.0 ± 0.4	-0.0 ± 0.0	
44	113.5 ± 2.3	89.9 ± 6.3	83.0 ± 2.5	173.2 ± 5.2	206.57 ± 8.3	-20.45 ± 1.0	-15.67 ± 0.9	-25.0 ± 1.3	-2.6 ± 0.2	
58	143.2 ± 5.7	135.0 ± 4.1	114.4 ± 5.7	279.2 ± 14.0	238.03 ± 7.1	-16.04 ± 0.6	-16.88 ± 0.5	-22.7 ± 0.7	-4.3 ± 0.3	
72	180.3 ± 5.4	182.1 ± 10.9	157.6 ± 7.9	424.0 ± 25.4	313.84 ± 22.0	-10.41 ± 0.3	-11.35 ± 0.8	-24.5 ± 1.2	-7.1 ± 0.4	
86	198.0 ± 13.9	202.7 ± 6.1	175.3 ± 5.3	497.0 ± 29.8	344.29 ± 24.1	2.04	-4.02 ± 0.2	-13.6 ± 0.8	6.9 ± 0.3	
100	231.4 ± 11.6	236.2 ± 11.8	218.0 ± 13.1	516.1 ± 36.1	354.60 ± 7.1	-	-9.49 ± 0.6	-13.3 ± 0.7	1.4 ± 0.0	
114	258.4 ± 10.3	293.1 ± 8.8	247.8 ± 9.9	572.4 ± 34.3	418.12 ± 20.9	-	2.05 ± 0.1	-16.2 ± 0.6	-2.0 ± 0.1	
128	302.3 ± 21.2	319.8 ± 19.2	246.2 ± 9.8	617.1 ± 30.9	414.10 ± 8.3	-	-16.64 ± 0.5	-20.8 ± 1.3	-3.5 ± 0.2	
142	357.8 ± 21.5	354.8 ± 14.2	366.1 ± 22.0	704.3 ± 42.3	469.67 ± 32.9	-	-3.16 ± 0.2	-19.1 ± 0.6	-2.7 ± 0.1	
156	500.2 ± 10.0	400.8 ± 16.0	420.3 ± 16.8	849.3 ± 34.0	482.38 ± 14.5	-	-	-17.6 ± 0.5	12.5 ± 0.6	
170	575.0 ± 34.5	428.0 ± 17.1	500.9 ± 35.1	889.5 ± 35.6	492.82 ± 24.6	-	-	-13.2 ± 0.3	-	
184	725.1 ± 29.0 ^b	508.4 ± 35.4 ^{c,d}	610.2 ± 18.3 ^{b,c}	1056 ± 31.7 ^a	478.35 ± 19.5 ^d	-	-	-16.1 ± 0.1 ^e	-	

Means and standard deviations are presented. Analysis of variance and Duncan's multiple range test were performed on the means of percent increase of body weight, the same letter identifies the values that are not significantly different (P<0.05).

Table 19 Percent survival rate of *H. asinina* fed nine species of macroalgae over a period of 184 days.

Days	Red algae				Green algae		Brown algae		
	<i>A. spicifera</i>	<i>G. fisheri</i>	<i>G. salicornia</i>	<i>G. tenuisipitata</i>	<i>E. intestinalis</i>	<i>C. racemosa</i>	<i>D. dichotoma</i>	<i>P. minor</i>	<i>S. polycystum</i>
0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
16	98.9 ± 1.9	100.0 ± 0.0	100.0 ± 0.0	98.9 ± 1.9	96.7 ± 0.0	98.9 ± 1.9	93.3 ± 0.0	95.6 ± 5.1	96.7 ± 3.3
30	98.9 ± 1.9	100.0 ± 0.0	100.0 ± 0.0	98.9 ± 1.9	96.7 ± 1.9	62.2 ± 6.9	85.6 ± 6.9	86.7 ± 8.8	94.4 ± 5.1
44	98.9 ± 1.9	100.0 ± 0.0	98.9 ± 1.9	98.9 ± 0.0	95.6 ± 1.9	27.8 ± 63.3	70.0 ± 6.7	81.1 ± 7.7	84.4 ± 1.9
58	98.9 ± 1.9	100.0 ± 0.0	98.9 ± 1.9	98.9 ± 0.0	95.6 ± 1.9	13.3 ± 0.0	56.7 ± 3.3	62.2 ± 5.1	77.8 ± 8.4
72	97.8 ± 1.9	100.0 ± 0.0	98.9 ± 0.0	98.9 ± 0.0	95.6 ± 1.9	6.7 ± 0.0	34.4 ± 1.9	47.8 ± 8.4	61.1 ± 1.9
86	97.8 ± 1.9	100.0 ± 0.0	98.9 ± 0.0	98.9 ± 0.0	95.6 ± 3.3	3.3 ± 1.9	22.2 ± 1.9	34.4 ± 10.2	45.6 ± 6.9
100	97.8 ± 1.9	100.0 ± 0.0	97.8 ± 1.9	98.9 ± 0.0	95.6 ± 3.3	–	13.3 ± 5.8	30.0 ± 3.3	40.0 ± 3.3
114	97.8 ± 1.9	100.0 ± 0.0	93.3 ± 3.3	98.9 ± 0.0	95.6 ± 1.9	–	8.9 ± 15.4	30.0 ± 8.8	33.3 ± 8.8
128	96.7 ± 3.3	98.9 ± 1.9	86.7 ± 8.8	98.9 ± 0.0	95.6 ± 5.1	–	8.9 ± 15.4	20.0 ± 5.8	11.1 ± 10.7
142	96.7 ± 3.3	98.9 ± 1.9	74.4 ± 3.8	98.9 ± 0.0	95.6 ± 5.1	–	4.4 ± 7.7	18.9 ± 6.9	6.7 ± 8.8
156	96.7 ± 3.3	95.6 ± 5.1	63.3 ± 3.3	97.8 ± 1.9	93.3 ± 6.7	–	–	13.3 ± 10.0	3.3 ± 5.8
170	95.6 ± 5.1 ^a	86.7 ± 8.8	58.9 ± 6.9	97.8 ± 1.9	87.8 ± 11.7	–	–	10.0 ± 8.8	–
184	94.4 ± 5.1 ^a	76.7 ± 8.8 ^b	55.6 ± 10.2 ^c	95.6 ± 1.9 ^a	87.8 ± 11.7 ^a	–	–	7.8 ± 10.7 ^d	–

Means and standard deviations are presented. Analysis of variance and Duncan's multiple range test were performed on the means of survival rate, the same letter identifies the values that are not significantly different (P<0.05).

Table 20 Food conversion rate of *H. asinina* fed nine species of macroalgae over a period of 184 days.

Species of algae	Number and total weight of abalone*				Total wet weight gain (g)	Total dry weight gain (g)	Dry weight intake of algae (g)	Food Conversion Rate ¹	Growth rate	
	Initial		Final						Length (µm/day)	Weight (mg/day)
	Number	Weight (g)	Number	Weight (g)						
Red algae										
<i>A. spicifera</i>	90	37.8	85	294.3	85.5 ± 2.2 ^b	32.5 ± 0.8 ^b	186.5 ± 0.3	5.7 ± 0.2 ^{a,b}	62.2 ± 1.4 ^b	16.6 ± 0.6 ^b
<i>G. fisheri</i>	90	36.1	69	203.3	43.9 ± 5.4 ^c	16.7 ± 2.1 ^c	122.0 ± 1.7	7.4 ± 1.0 ^{a,b}	47.6 ± 0.6 ^d	11.0 ± 0.5 ^d
<i>G. salicornia</i>	90	37.5	50	148.4	36.9 ± 10.7 ^c	14.0 ± 4.1 ^c	201.2 ± 1.4	15.5 ± 4.0 ^c	59.4 ± 3.2 ^b	13.8 ± 2.0 ^c
<i>G. tenuistipitata</i>	90	36.6	86	410.3	124.4 ± 14.8 ^a	47.3 ± 5.6 ^a	151.6 ± 7.1	3.3 ± 0.6 ^a	70.3 ± 3.4 ^a	23.6 ± 2.4 ^a
Green algae										
<i>C. racemosa</i>	90	37.3	0	0	—	—	25.9 ± 0.4	—	—	—
<i>E. intestinalis</i>	90	37.7	79	194.1	52.1 ± 15.5 ^c	19.8 ± 5.9 ^c	178.3 ± 1.0	9.8 ± 2.7 ^b	54.3 ± 3.8 ^c	10.9 ± 1.7 ^d
Brown algae										
<i>D. dichotoma</i>	90	36.7	0	0	—	—	21.2 ± 1.8	—	—	—
<i>P. minor</i>	90	38.2	1	2.0	—	-4.5 ± 0.06 ^d	36.8 ± 7.2	—	2.1 ± 3.0 ^e	-0.3 ± 0.3 ^e
<i>S. polycystum</i>	90	37.1	0	0	—	—	34.5 ± 4.6	—	—	—

Means and standard deviations are presented. Analysis of variance and Duncan's multiple range test were performed on the means of growth rate, the same letter identifies the values that are not significantly different (P<0.05); 1. FCR = grams of algae to produce 1.0 gram of abalone weight gain; * from three replication.

Table 21 Proximate composition of the nine species of macroalgae.

Species of algae	Composition (% dry weight)				Moisture (%)
	Protein	Fat	Carbohydrate	Ash	
Red algae					
<i>A. spicifera</i>	15.4 ± 2.0 ^{c, d}	1.3 ± 1.0 ^{d, e}	34.6 ± 4.2 ^{d, e, f}	48.7 ± 6.8 ^{a, b, c}	93.2 ± 12.9 ^a
<i>G. fisheri</i>	16.8 ± 2.5 ^{b, c}	1.1 ± 0.1 ^{e, f}	57.2 ± 7.0 ^{a, b}	24.9 ± 2.5 ^d	81.2 ± 6.7 ^a
<i>G. salicornia</i>	16.9 ± 1.8 ^{b, c}	1.3 ± 0.1 ^{d, e}	31.1 ± 4.6 ^{e, f}	50.7 ± 3.3 ^{a, b}	90.3 ± 13.4 ^a
<i>G. temistipitata</i>	24.7 ± 2.1 ^a	0.7 ± 0.0 ^g	52.0 ± 6.7 ^a	22.7 ± 1.5 ^d	84.9 ± 5.0 ^a
Green algae					
<i>C. racemosa</i>	19.4 ± 1.5 ^b	3.2 ± 0.2 ^b	27.4 ± 3.9 ^{f, g}	50.0 ± 6.2 ^{a, b}	93.8 ± 13.2 ^a
<i>E. intestinalis</i>	18.9 ± 2.5 ^b	3.8 ± 0.4 ^a	20.8 ± 2.0 ^g	56.6 ± 3.5 ^a	94.9 ± 6.7 ^a
Brown algae					
<i>D. dichotoma</i>	14.0 ± 0.7 ^{c, d}	2.8 ± 0.3 ^c	38.3 ± 3.9 ^{c, d, e}	44.9 ± 5.5 ^{b, c}	89.6 ± 10.0 ^a
<i>P. minor</i>	12.3 ± 1.4 ^d	0.8 ± 0.0 ^{f, g}	46.2 ± 2.4 ^{b, c}	40.8 ± 4.7 ^c	87.0 ± 5.0 ^a
<i>S. polycystum</i>	9.2 ± 1.1 ^e	1.5 ± 0.2 ^d	41.2 ± 3.2 ^{c, d}	48.1 ± 6.7 ^{a, b, c}	87.4 ± 12.9 ^a

Means and standard deviations are presented. Analysis of variance and Duncan's multiple range test were performed on the means of each composition, the same letter identifies the values that are not significantly different (P<0.05).

Table 22 Shell length (mm ± S.E.) of *H. asinina* fed fresh *G. salicornia* and artificial diets formulae I-VII over a period of 140 days.

Days	<i>G. salicornia</i>	Formulae						
	(Control)	I	II	III	IV	V	VI	VII
0	13.3 ± 0.7	13.3 ± 0.8	13.3 ± 0.7	13.3 ± 0.8	13.3 ± 0.7	13.3 ± 1.0	13.3 ± 1.0	13.3 ± 1.2
14	13.9 ± 0.8	13.8 ± 0.8	13.8 ± 0.8	13.5 ± 0.8	13.8 ± 0.7	13.7 ± 1.6	13.6 ± 1.1	14.8 ± 1.5
28	14.8 ± 0.9	14.0 ± 0.8	13.8 ± 0.8	13.5 ± 0.8	13.8 ± 0.7	14.0 ± 1.4	13.7 ± 1.1	15.4 ± 1.3
42	15.7 ± 0.9	14.0 ± 0.8	13.8 ± 0.8	13.5 ± 0.8	13.8 ± 0.7	14.2 ± 1.5	14.3 ± 1.2	15.8 ± 1.3
56	16.6 ± 1.0	14.1 ± 0.9	13.9 ± 0.8	13.6 ± 0.8	13.8 ± 0.7	14.7 ± 1.4	15.1 ± 1.2	16.2 ± 1.4
70	17.6 ± 1.1	14.1 ± 0.9	13.9 ± 0.8	13.6 ± 0.9	13.8 ± 0.7	15.1 ± 1.4	15.8 ± 1.3	16.9 ± 1.6
84	18.0 ± 1.2	14.2 ± 0.8	13.9 ± 0.8	13.6 ± 0.8	13.9 ± 0.8	15.3 ± 1.5	16.0 ± 1.3	17.1 ± 1.5
98	19.0 ± 1.4	14.2 ± 0.8	13.9 ± 0.8	13.6 ± 0.9	14.2 ± 0.7	16.2 ± 1.5	16.3 ± 1.3	17.6 ± 1.5
112	19.8 ± 1.4	14.3 ± 0.8	14.0 ± 1.0	13.6 ± 0.8	14.3 ± 1.1	16.7 ± 1.6	16.5 ± 1.3	17.7 ± 1.6
126	20.2 ± 1.4	14.3 ± 0.9	14.0 ± 1.1	13.6 ± 1.0	14.4 ± 1.2	17.2 ± 1.7	17.3 ± 1.4	18.1 ± 1.7
140	20.9 ± 1.2 ^a	14.5 ± 1.0 ^c	14.0 ± 0.9 ^c	13.6 ± 1.0 ^c	14.4 ± 1.2 ^c	18.3 ± 1.9 ^b	17.4 ± 1.2 ^b	18.3 ± 1.3 ^b

Means and standard deviations are presented. Analysis of variance and Duncan's multiple range test were performed on the means of shell length increases, the same letter identifies the values that are not significantly different (P<0.05).

Table 23 Percent increase of shell length of *H. asinina* fed fresh *G. salicornia* and artificial diets formulae I-VII over a period of 140 days.

Days	<i>G. salicornia</i> (Control)	Formulae						
		I	II	III	IV	V	VI	VII
0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
14	5.0 ± 0.6	3.6 ± 0.2	3.5 ± 0.2	2.0 ± 0.2	3.7 ± 0.1	3.0 ± 3.9	2.2 ± 0.1	11.5 ± 0.6
28	11.1 ± 1.1	4.9 ± 0.2	3.9 ± 0.2	2.0 ± 0.1	3.7 ± 0.1	5.3 ± 2.6	2.5 ± 0.5	15.5 ± 1.2
42	18.2 ± 1.1	5.1 ± 0.1	3.9 ± 0.5	2.0 ± 0.2	3.9 ± 0.2	6.8 ± 3.1	7.1 ± 0.5	18.8 ± 1.4
56	24.6 ± 1.5	5.5 ± 0.0	4.1 ± 0.5	2.1 ± 0.3	4.0 ± 0.1	11.0 ± 2.4	13.0 ± 0.3	21.7 ± 0.5
70	32.8 ± 1.4	5.8 ± 0.1	4.1 ± 0.2	2.1 ± 0.8	4.1 ± 0.1	13.6 ± 1.8	18.0 ± 0.6	27.0 ± 0.0
84	35.4 ± 2.0	6.1 ± 0.5	4.2 ± 0.3	2.2 ± 0.3	4.2 ± 1.0	15.0 ± 2.4	19.8 ± 0.5	28.7 ± 0.7
98	43.4 ± 2.9	6.2 ± 0.4	4.3 ± 0.3	2.2 ± 0.4	6.8 ± 0.0	21.9 ± 2.1	21.9 ± 0.5	31.9 ± 0.6
112	48.8 ± 2.7	7.1 ± 0.5	4.9 ± 1.8	2.5 ± 0.3	7.7 ± 2.5	25.4 ± 2.7	23.7 ± 0.4	32.9 ± 0.1
126	51.8 ± 3.2	7.4 ± 0.1	4.9 ± 3.0	2.5 ± 1.4	7.9 ± 3.4	29.2 ± 3.0	29.8 ± 0.6	35.7 ± 0.1
140	57.7 ± 1.0 ^a	8.7 ± 0.8 ^d	5.3 ± 1.2 ^{d,e}	2.5 ± 1.4 ^e	7.9 ± 3.2 ^d	37.4 ± 4.0 ^b	30.7 ± 1.3 ^c	37.8 ± 3.2 ^b

Means and standard deviations are presented. Analysis of variance and Duncan's multiple range test were performed on the means of percent increase of shell length, the same letter identifies the values that are not significantly different (P<0.05).

Table 24 Body weight (g ± S.E.) of *H. asinina* fed fresh *G. salicornia* and artificial diets formulae I-VII over a period of 140 days.

Days	<i>G. salicornia</i> (Control)	Formulae						
		I	II	III	IV	V	VI	VII
0	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.40 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.4 ± 0.1
14	0.5 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.2	0.5 ± 0.1	0.4 ± 0.2
28	0.6 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.5 ± 0.2	0.6 ± 0.2	0.5 ± 0.2
42	0.8 ± 0.1	0.47 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.6 ± 0.2	0.8 ± 0.2	0.6 ± 0.2
56	0.9 ± 0.2	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.7 ± 0.3	0.8 ± 0.2	0.6 ± 0.2
70	1.1 ± 0.2	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	0.8 ± 0.2	0.8 ± 0.2	0.8 ± 0.2
84	1.1 ± 0.2	0.6 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	1.0 ± 0.3	1.0 ± 0.2	0.9 ± 0.3
98	1.3 ± 0.3	0.6 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	1.0 ± 0.3	1.0 ± 0.3	1.0 ± 0.3
112	1.4 ± 0.2	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	1.1 ± 0.3	1.2 ± 0.3	1.1 ± 0.3
126	1.5 ± 0.3	0.7 ± 0.2	0.6 ± 0.1	0.7 ± 0.2	0.7 ± 0.2	1.2 ± 0.4	1.3 ± 0.3	1.3 ± 0.4
140	1.7 ± 0.3 ^a	0.7 ± 0.2 ^c	0.7 ± 0.1 ^c	0.7 ± 0.1 ^c	0.8 ± 0.1 ^c	1.3 ± 0.5 ^b	1.3 ± 0.3 ^b	1.3 ± 0.4 ^b

Means and standard deviations are presented. Analysis of variance and Duncan's multiple range test were performed on the means of body weight, the same letter identifies the values that are not significantly different (P<0.05).

Table 25 Percent increase of body weight of *H. asinina* fed fresh *G. salicornia* and artificial diets formulae I-VII over a period of 140 days.

Days	<i>G. salicornia</i> (Control)	Formulae						
		I	II	III	IV	V	VI	VII
0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
14	18.1 ± 17.4	1.6 ± 1.4	4.0 ± 3.1	4.3 ± 3.1	5.1 ± 1.1	11.9 ± 2.2	19.7 ± 8.4	0.0 ± 0.0
28	50.3 ± 25.9	10.6 ± 3.6	5.7 ± 1.1	8.4 ± 2.2	10.7 ± 5.6	21.2 ± 9.7	35.3 ± 6.8	31.6 ± 4.0
42	82.4 ± 27.1	13.7 ± 4.7	7.8 ± 3.2	15.8 ± 4.6	22.2 ± 3.7	47.5 ± 7.4	68.8 ± 3.4	38.9 ± 2.8
56	113.6 ± 38.1	16.2 ± 4.8	10.3 ± 6.6	17.2 ± 3.5	24.5 ± 5.0	56.8 ± 15.7	77.4 ± 10.7	43.9 ± 1.4
70	157.2 ± 26.8	31.0 ± 13.3	22.0 ± 7.3	34.5 ± 1.3	38.2 ± 4.2	92.6 ± 11.6	86.4 ± 7.5	90.2 ± 0.0
84	174.5 ± 40.7	34.3 ± 15.4	24.4 ± 2.5	36.1 ± 5.5	51.0 ± 3.0	128.3 ± 16.0	111.0 ± 4.2	107.1 ± 6.2
98	217.1 ± 48.8	41.6 ± 9.4	30.5 ± 2.6	45.6 ± 7.3	53.3 ± 3.1	132.9 ± 21.0	126.4 ± 9.1	148.3 ± 15.0
112	245.5 ± 40.5	48.9 ± 9.4	36.8 ± 4.1	55.7 ± 7.8	55.6 ± 3.1	166.1 ± 26.7	173.0 ± 11.0	175.0 ± 19.3
126	268.6 ± 46.9	65.1 ± 2.7	51.2 ± 4.5	78.7 ± 5.4	72.5 ± 8.7	175.8 ± 19.4	177.2 ± 16.7	206.8 ± 15.4
140	316.1 ± 66.9 ^a	65.1 ± 1.4 ^c	57.2 ± 7.9 ^e	86.3 ± 11.2 ^e	81.4 ± 9.1 ^e	199.5 ± 24.0 ^b	179.4 ± 16.8 ^b	219.5 ± 0.0 ^b

Means and standard deviations are presented. Analysis of variance and Duncan's multiple range test were performed on the means of percent increase of body weight, the same letter identifies the values that are not significantly different ($P < 0.05$).

Table 26 Percent survival rate of *H. asinina* fed fresh *G. salicornia* and artificial diets formulae I-VII over a period of 140 days.

Days	<i>G. salicornia</i>	Formulae						
	(Control)	I	II	III	IV	V	VI	VII
0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
14	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
28	98.3 ± 2.9	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	86.7 ± 5.8	80.0 ± 5.0	81.7 ± 5.8
42	98.3 ± 2.9	100.0 ± 0.0	98.3 ± 2.9	90.0 ± 13.2	98.3 ± 2.9	86.7 ± 5.8	80.0 ± 5.0	76.7 ± 7.6
56	98.3 ± 2.9	96.7 ± 2.9	96.7 ± 2.9	90.0 ± 13.2	98.3 ± 2.9	85.0 ± 5.0	80.0 ± 5.0	76.7 ± 7.6
70	98.3 ± 2.9	96.7 ± 2.9	96.7 ± 2.9	90.0 ± 13.2	96.7 ± 5.8	85.0 ± 5.0	78.3 ± 2.9	76.7 ± 7.6
84	98.3 ± 2.9	76.7 ± 7.6	90.0 ± 5.0	80.0 ± 18.0	95.0 ± 5.0	83.3 ± 2.9	78.3 ± 2.9	71.7 ± 5.8
98	98.3 ± 2.9	76.7 ± 7.6	90.0 ± 5.0	80.0 ± 18.0	95.0 ± 5.0	83.3 ± 2.9	76.7 ± 2.9	71.7 ± 5.8
112	98.3 ± 2.9	76.7 ± 7.6	90.0 ± 5.0	80.0 ± 18.0	95.0 ± 5.0	83.3 ± 2.9	76.7 ± 2.9	71.7 ± 5.8
126	98.3 ± 2.9	76.7 ± 7.6	90.0 ± 5.0	80.0 ± 18.0	95.0 ± 5.0	78.3 ± 2.9	76.7 ± 2.9	71.7 ± 5.8
140	96.7 ± 5.8 ^a	76.7 ± 7.6 ^{b,c}	88.3 ± 7.6 ^a	80.0 ± 18.0 ^b	95.0 ± 5.0 ^a	78.3 ± 2.9 ^b	76.7 ± 2.9 ^{b,c}	71.7 ± 5.8 ^c

Means and standard deviations are presented. Analysis of variance and Duncan's multiple range test were performed on the means of survival rate, the same letter identifies the values that are not significantly different (P<0.05).

Table 27 Food conversion rate of *H. asinina* fed fresh *G. salicornia* and artificial diets formulae I-VII over a period of 140 days.

Diets	Number and total weight of abalone*				Total wet weight gain (g)	Total dry weight gain (g)	Dry weight intake of algae (g)	Food Conversion Rate ¹	Growth rate	
	Initial		Final						Length (±m/day)	Weight (mg/day)
	Number	Weight (g)	Number	Weight (g)						
<i>G. salicornia</i>	20 ± 0.0 ^a	8.4 ± 1.4 ^a	19 ± 1.2 ^a	34.8 ± 2.8 ^a	26.4 ± 2.2 ^a	9.6 ± 0.2 ^a	140.4 ± 6.4 ^b	14.6 ± 0.3 ^a	54.7 ± 3.7 ^a	9.4 ± 1.9 ^a
Formula I	20 ± 0.0 ^a	8.2 ± 1.4 ^a	15 ± 1.5 ^{b,c}	13.6 ± 1.2 ^c	5.4 ± 0.9 ^c	2.0 ± 0.3 ^c	78.8 ± 2.7 ^a	40.1 ± 5.1 ^c	8.3 ± 1.3 ^d	1.9 ± 0.6 ^c
Formula II	20 ± 0.0 ^a	8.4 ± 1.6 ^a	18 ± 1.5 ^a	13.2 ± 0.8 ^c	4.8 ± 0.1 ^c	1.7 ± 0.1 ^c	92.0 ± 5.7 ^b	53.2 ± 1.6 ^d	5.1 ± 2.0 ^{a,e}	1.7 ± 0.1 ^c
Formula III	20 ± 0.0 ^a	8.0 ± 1.6 ^a	16 ± 3.6 ^b	15.0 ± 0.7 ^c	7.0 ± 0.3 ^c	2.6 ± 0.1 ^c	73.2 ± 3.4 ^a	28.0 ± 1.1 ^b	2.4 ± 1.5 ^e	2.5 ± 0.2 ^c
Formula IV	20 ± 0.0 ^a	8.3 ± 1.8 ^a	19 ± 1.0 ^a	15.1 ± 0.9 ^c	6.8 ± 0.6 ^c	2.6 ± 0.2 ^c	95.5 ± 4.4 ^b	36.2 ± 2.6 ^c	7.6 ± 3.4 ^d	2.4 ± 0.4 ^c
Formula V	20 ± 0.0 ^a	8.4 ± 2.6 ^a	16 ± 0.6 ^b	25.2 ± 9.0 ^b	16.8 ± 6.4 ^b	6.0 ± 2.4 ^b	159.9 ± 8.0 ^c	29.8 ± 12.1 ^b	35.6 ± 6.5 ^b	6.0 ± 2.3 ^b
Formula VI	20 ± 0.0 ^a	9.0 ± 2.4 ^a	15 ± 0.6 ^{b,c}	25.2 ± 6.0 ^b	16.2 ± 3.6 ^b	6.2 ± 1.6 ^b	114.9 ± 9.2 ^b	19.2 ± 4.7 ^a	29.1 ± 1.1 ^c	5.8 ± 1.3 ^b
Formula VII	20 ± 0.0 ^a	8.2 ± 2.8 ^a	14 ± 1.2 ^c	26.2 ± 9.2 ^b	18.0 ± 6.4 ^b	6.6 ± 2.3 ^b	167.4 ± 8.4 ^c	27.5 ± 10.3 ^b	35.8 ± 0.3 ^b	6.4 ± 2.3 ^b

Means and standard deviations are presented. Analysis of variance and Duncan's multiple range test were performed on the means of growth rate, the same letter identifies the values that are not significantly different ($P < 0.05$); 1. FCR = g. algae to produce 1.0 g. abalone weight gain; * from three replication.

Table 28 Proximate composition of artificial diets formulae I-VII and *G. salicornia* over a period of 140 days.

Diets	Composition (% dry weight)				Moisture (%)
	Protein	Fat	Carbohydrate	Ash	
<i>G. salicornia</i>	16.0 ± 0.2 ^b	1.3 ± 0.0 ^c	31.0 ± 0.6 ^{d,e}	51.7 ± 0.7 ^a	90.7 ± 0.4 ^a
Formula I	4.5 ± 0.1 ^c	5.4 ± 0.1 ^b	61.6 ± 1.6 ^b	28.5 ± 1.7 ^b	82.9 ± 0.4 ^b
Formula II	5.5 ± 0.2 ^c	6.1 ± 0.1 ^a	56.0 ± 0.9 ^b	32.5 ± 1.0 ^b	81.3 ± 0.5 ^c
Formula III	4.8 ± 0.1 ^c	5.4 ± 0.1 ^b	66.5 ± 1.4 ^a	23.2 ± 1.5 ^c	80.2 ± 1.4 ^c
Formula IV	4.9 ± 0.2 ^c	5.4 ± 0.1 ^b	58.6 ± 1.6 ^b	31.1 ± 1.8 ^b	82.7 ± 0.7 ^b
Formula V	33.6 ± 1.4 ^a	5.4 ± 0.1 ^b	37.5 ± 0.9 ^e	23.6 ± 1.7 ^c	83.7 ± 0.7 ^b
Formula VI	33.8 ± 1.2 ^a	5.5 ± 0.1 ^b	29.6 ± 0.4 ^e	22.6 ± 1.1 ^b	83.6 ± 0.7 ^b
Formula VII	36.3 ± 1.1 ^a	5.3 ± 0.1 ^b	35.9 ± 0.4 ^{c,d}	51.7 ± 0.9 ^c	80.0 ± 0.5 ^c

Means and standard deviations are presented. Analysis of variance and Duncan’s multiple range test were performed on the means of each composition, the same letter identifies the values that are not significantly different (P<0.05)

Table 29 The size of egg, trochophore and completion of larval shell of some *Haliotis* spp.

	Egg (μm)	Trochophore (μm)	Completion of larval shell (μm)	References
<i>H. asinina</i>	131x149	102x131	133x165	The present
<i>H. coccinea canariensis</i>	103	201x160	264x206	Pena 1986 (198)
<i>H. cyclobates</i>	150-225	-	-	Shepherd, Laws 1974 (18)
<i>H. laevigata</i>	200-250	-	-	Shepherd, Laws 1974 (18)
<i>H. discus</i>	230	178x200	290	Ino 1952 (38)
<i>H. rufescens</i>	-	160x195	210x270	Ebert, Houk 1984 (45)
<i>H. sieboldii</i>	280	-	-	Ino 1952 (38)
<i>H. gigantea</i>	270	-	-	Ino 1952 (38)
<i>H. iris</i>	230	-	-	Harrison 1986 (199)
<i>H. rubra</i>	200	-	-	Harrison 1986 (199)
<i>H. sorenseni</i>	200	-	-	Leighton 1972 (31)
<i>H. tuberculata</i>	210	-	-	Koike 1978 (154)

Table 30 The biological zero point (°C) of some *Haliotis* spp.

Species	Biological zero point(°C)	References
<i>H. discus hannai</i>	7.6	Seki & Kan-no 1977 (46)
<i>H. discus</i>	8.5	Hahn 1989c (28)
<i>H. gigantea</i>	9.0	Seki & Kan-no 1977 (46)
<i>H. rufescens</i>	8.5	Seki & Kan-no 1977 (46)
<i>H. rufescens</i>	0.9	Hahn 1989c (28)
<i>H. fulgens</i>	9.9	Leighton 1974 (32)
<i>H. asinina</i>	15.0	The present

Table 31 The time of hatch out, 90° torsion, first epipodium tentacle formation and fourth tubule on cephalic tentacle formation of several abalone species.

Species	Temperature (°C)	Hatch out (hours)	Larval retractor muscle (hours)	Torsion (90° twisting)	First epipodial tentacle (hours)	Fourth tubules on cephalic tentacle (hours)	References
<i>H. discus hannai</i>	20	12.6	23.4	32.1	64.9	99	Seki and Kan-no 1977 (46)
<i>H. discus</i>	16-17	20	-	45-46	120	-	Hahn 1989c (28)
<i>H. sieboldii</i>	16-17	18	29	35	96	-	Ino 1952 (38)
<i>H. gigantea</i>	16-18	21-22	-	40-43	-	-	Ino 1952 (38)
<i>H. rufescens</i>	15	20	-	-	-	144	Ebert and Houk 1984 (45)
	15	20	-	-	-	175	Hahn 1989c (28)
	17	24	-	44	-	-	Leighton 1974 (32)
	17	25	36	48	-	-	Hahn 1989c (28)
	18	16-24	-	-	-	-	Owen et. al. 1984 (172)
<i>H. fulgens</i>	17	14	-	48	-	360	Lieghton 1974 (32)
<i>H. corrugata</i>	16	17	-	48	-	192	Lieghton 1974 (32)
<i>H. sorenseni</i>	15	24	-	-	-	240	Leighton 1972 (31)
<i>H. tuberculata</i>	20	13	20.5	-	84	120	Koike 1978 (170)
<i>H. coccinea canariensis</i>	15	21	-	52	-	-	Pena 1986 (198)
<i>H. diversicolor supertexta</i>	26.2	6	-	13	38	-	Oba 1964 (171)
<i>H. asinina</i>	25	9.5	13.5	18.0	45.5	65.0	The present study
	28	7.5	11.0	14.0	36.0	49.0	The present study
	31	6.0	9.0	11.5	28.0	41.0	The present study
	34	6.0	9.0	11.5	28.0	41.0	The present study

Table 32 Growth rates reported for various *Haliotis* species fed different species of algae.

Species of abalone	Optimal temperature (°C)	Initial		Species of algae	Growth rate		References
		Weight (g)	Length (mm)		Length (mg/day)	Weight (µm/day)	
<i>H. discus hannai</i>	18.9-20.6	1.9	24.0	<i>Laminaria japonica</i>	19.0	108.0	Uki 1981 (162)
		1.8	24.0	<i>L. religiosa</i>	18.0	101.0	
		1.9	24.0	<i>E. bicyclis</i>	16.3	48.0	
		1.9	24.0	<i>U. pinnatifida</i>	25.3	121.0	
<i>H. laevigata</i>	18	0.4	15.0	<i>Gracilaria confervoides</i>	6.7	-	Morrison & Whittington 1991 (163)
<i>H. rubra</i>	18	7.0	-	<i>Jeannerettia lobata</i>	19.0	-	Gorfine & King 1991 (164)
		7.0	-	<i>Phyllospora comosa</i>	51.0	-	Fleming 1995 (161)
<i>H. rufescens</i>	10-20	3.8	83.0	<i>Macrocystis</i> sp.	8.0	-	Gorfine & King 1991 (164)
<i>H. tuberculata</i>	10.6-14.2	0.2	10.0	<i>Palmaria palmate</i>	80.3	-	Ebert & Houk 1984 (45)
	20 ± 0.5	0.1	10.0	<i>Ulva lactuca</i>	2.9	-	Koike et al. 1979 (165)
		0.1	9.0	<i>Laminaria digitata</i>	2.1	-	
<i>H. iris</i>	15 ± 1	1.2	21.6	<i>Gracilaria chilensis</i>	1.7	-	
		1.1	21.0	<i>Macrocystis pyrifera</i>	9.7	45.5	Stuart & Brown 1994 (160)
<i>H. asinina</i>	25	-	0.2	<i>Gracilaria salicornia</i>	5.9	34.4	
	25-28	10.9	3.1	<i>Laurencia</i> sp.	51.1	51.2	Singhagraiwan 1991b (135)
		9.7	3.1	<i>Gracilaria fisheri</i>	24.0	98.1	Kunavongdate et al. 1995 (131)
		10.4	3.1	<i>Eucheuma</i> sp.	3.0	21.0	
					2.0	14.0	

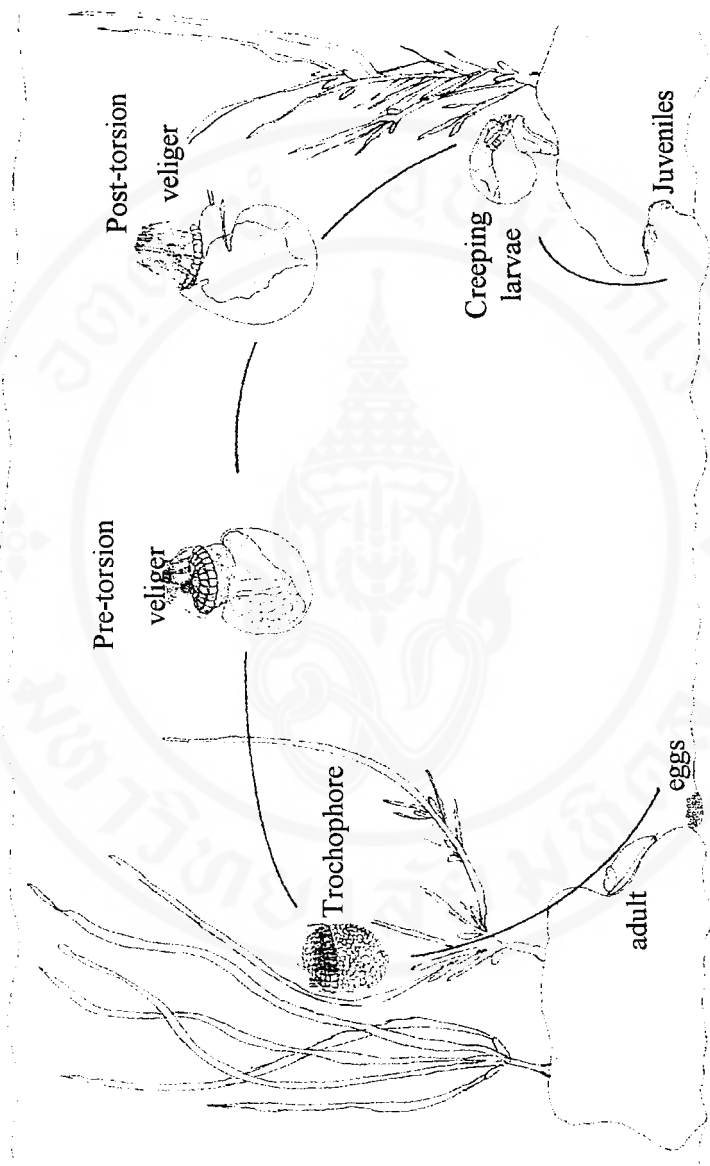


Figure 1 The life cycle of abalone larvae. Larvae are entrained near reef by complex reef topography and stands of kelp (17).

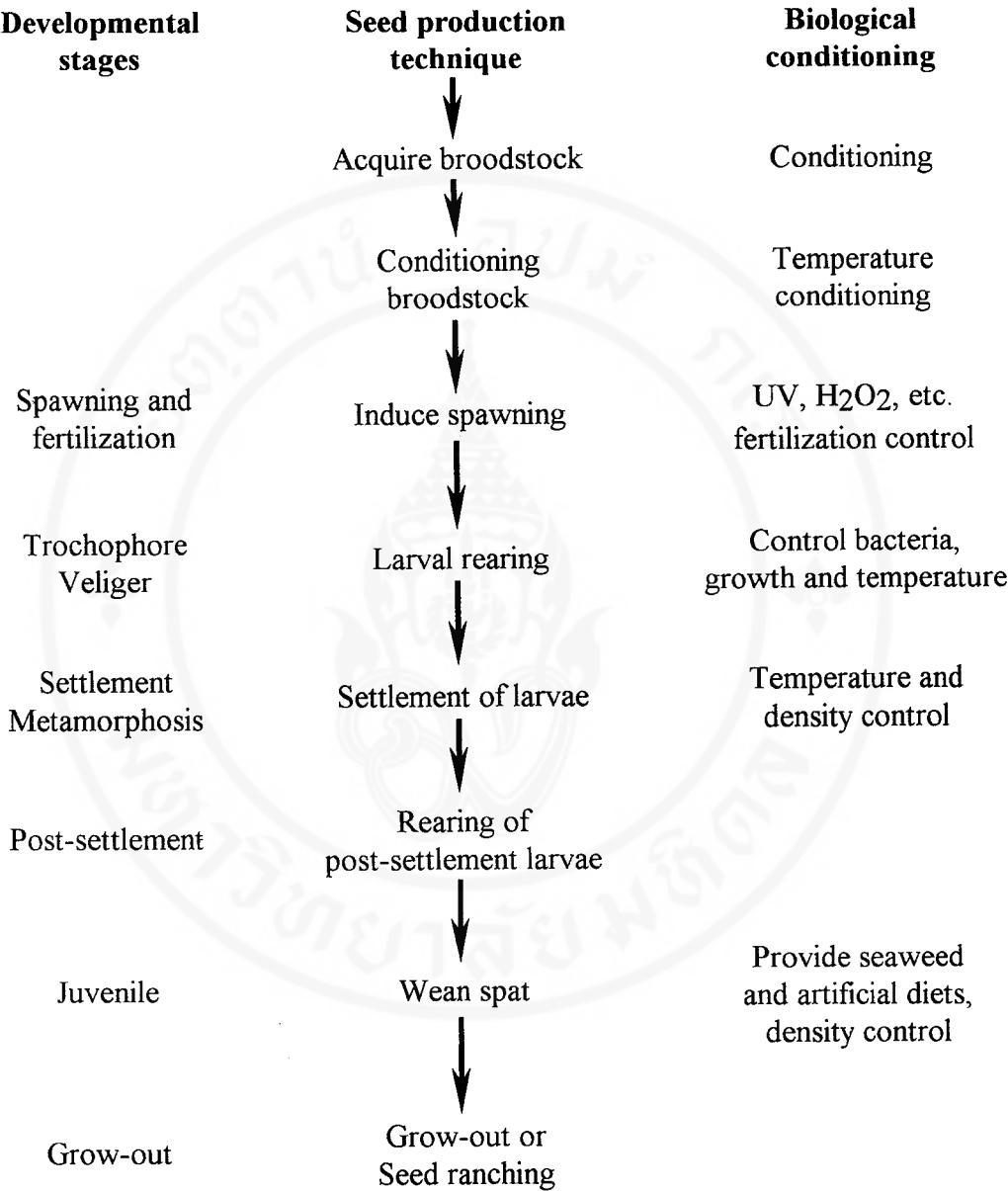


Figure 2 Abalone seed production (from: McCormick and Hahn 1983 (57) and Kufuku and Ikenoue 1983 (72)).

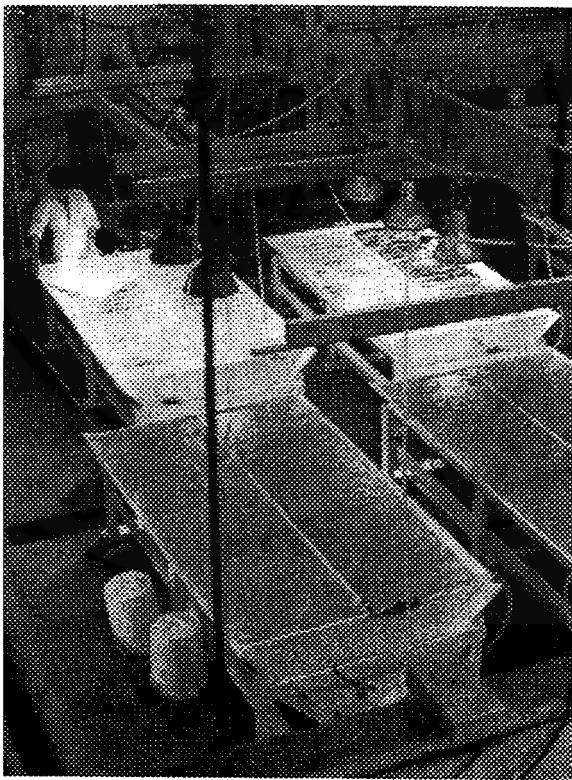
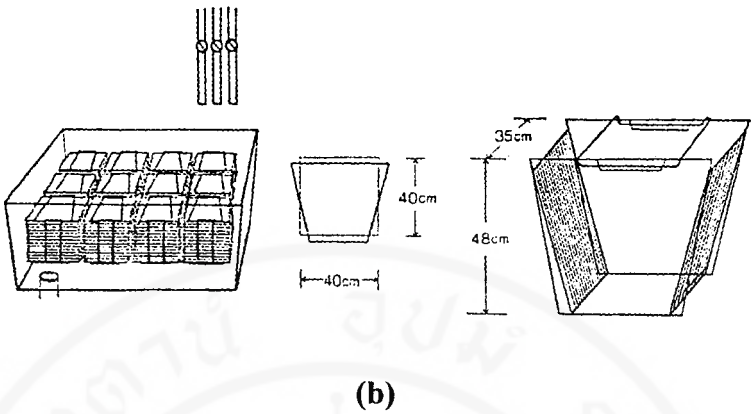


Figure 3 Settlement tanks and collectors

(a) From Kufuku and Ikenoue 1983 (72).

(b) From Tong and Moss 1992 (60).

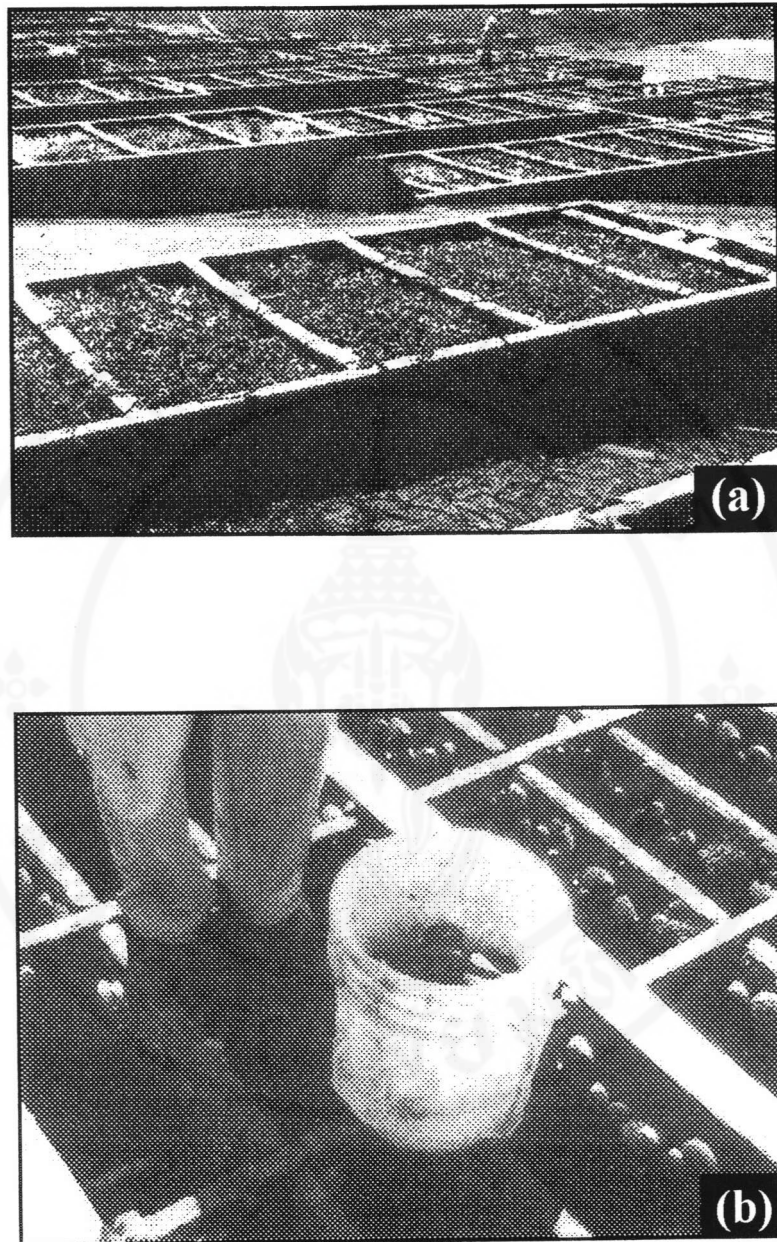


Figure 4 (a) The abalone farm, Cayucos, CA. Partitioned concrete tanks used for grow-out of red abalone, *H. rufescens* (60).

(b) Red abalone being harvested from the growing tanks (60).

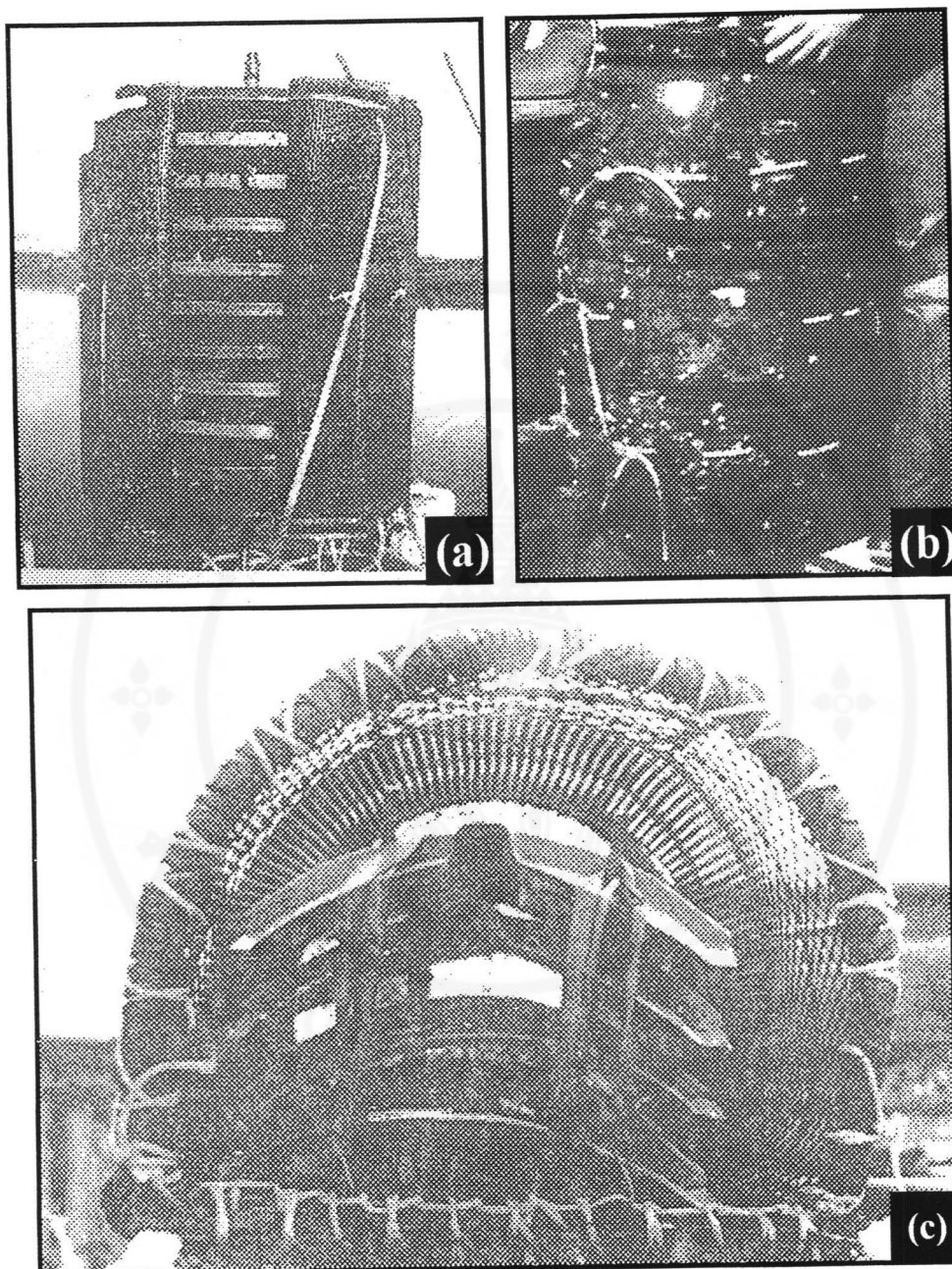


Figure 5 (a) Experimental cage used at S.R.L. (9).
 (b) The plastic culture barrel at Abalone Farm Inc., California (89).
 (c) Lobster pots modified for growing abalone (9).

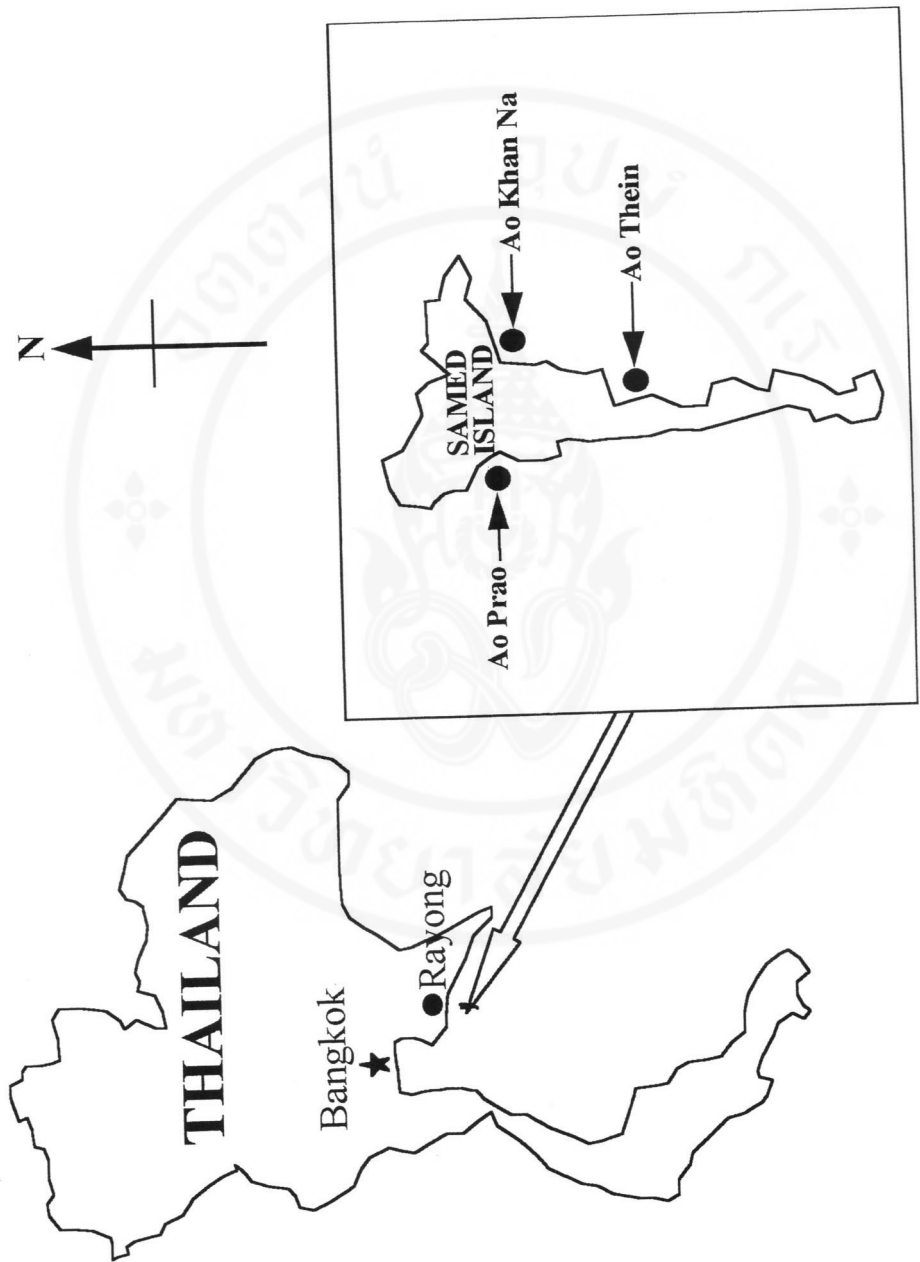


Figure 6 The sampling area of *H. asinina* at Ao thein, Samed Island, Rayong Province, Thailand.

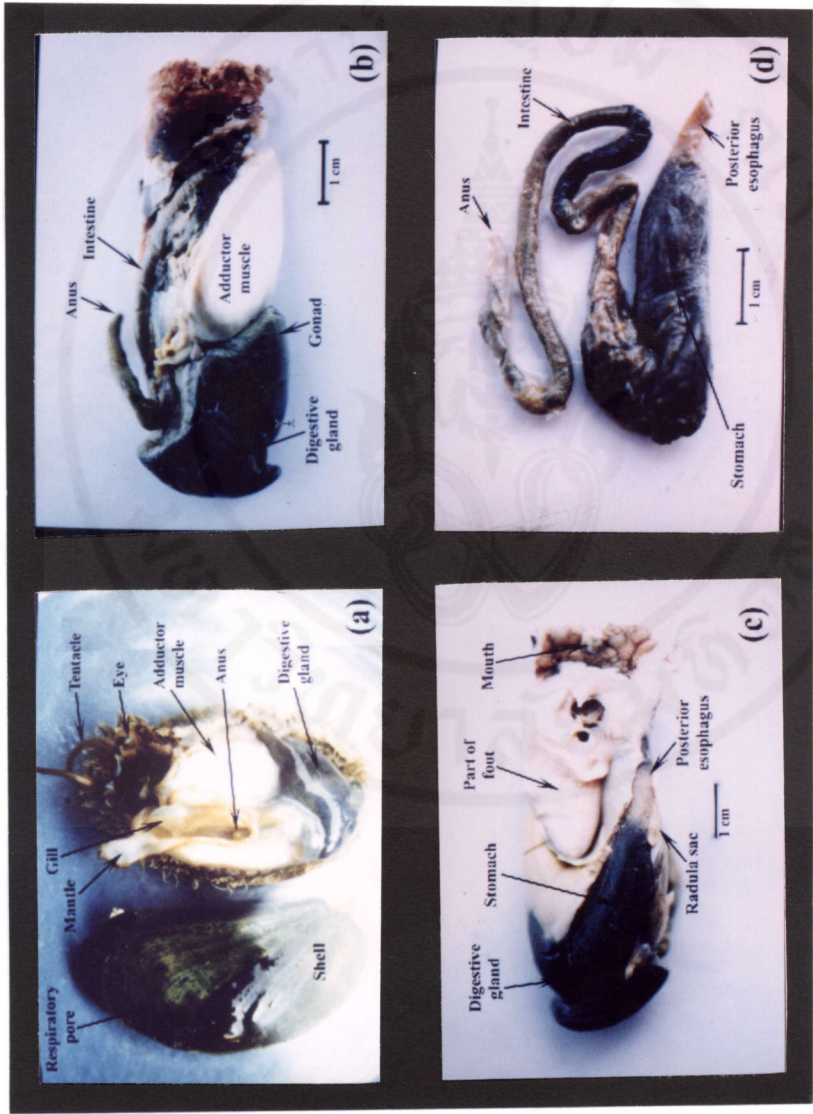


Figure 7 Gut dissection.

- (a) Dorsal view of *H. asinina* after remove the shell.
- (b) Dorsal view of *H. asinina* after remove the mantle, showing the parts of digestive organs.
- (c) Ventral view of *H. asinina*, showing the parts of digestive organs.
- (d) Showing the digestive organs from the posterior esophagus to the anus.



Figure 8 The experimental system of natural food utilization experiment.

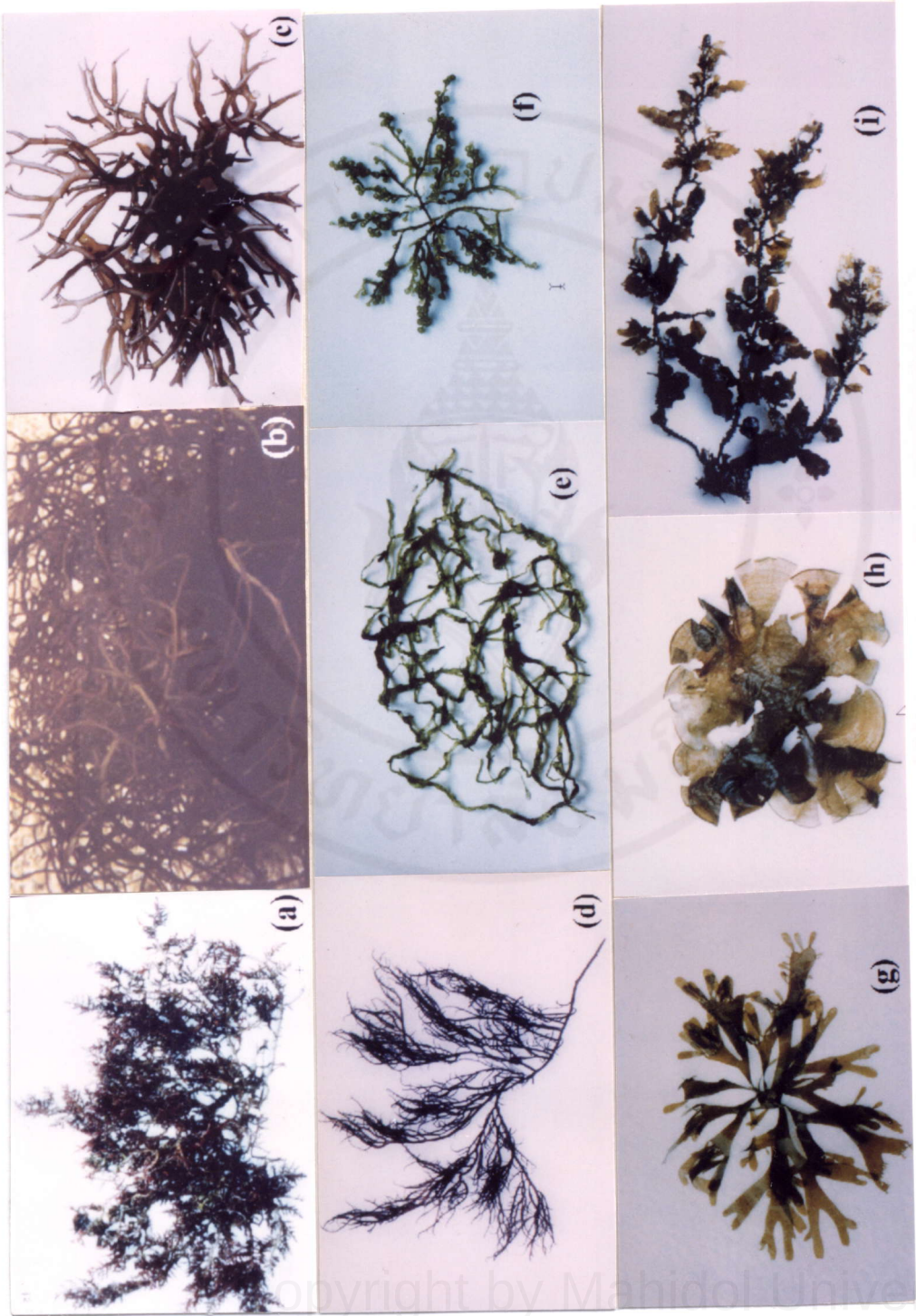


Figure 9 The macroalgae used to feed *H. asinina* in the experiment of natural food utilization.

(a) <i>A. spicifera</i>	(b) <i>G. fisheri</i>	(c) <i>G. salicornia</i>	(d) <i>G. tenuistipitata</i>	(e) <i>E. intestinalis</i>
(f) <i>C. racemosa</i>	(g) <i>D. dichotoma</i>	(h) <i>P. minor</i>	(i) <i>S. polycystum</i>	



Figure 10 The experimental system of artificial diet utilization experiment.

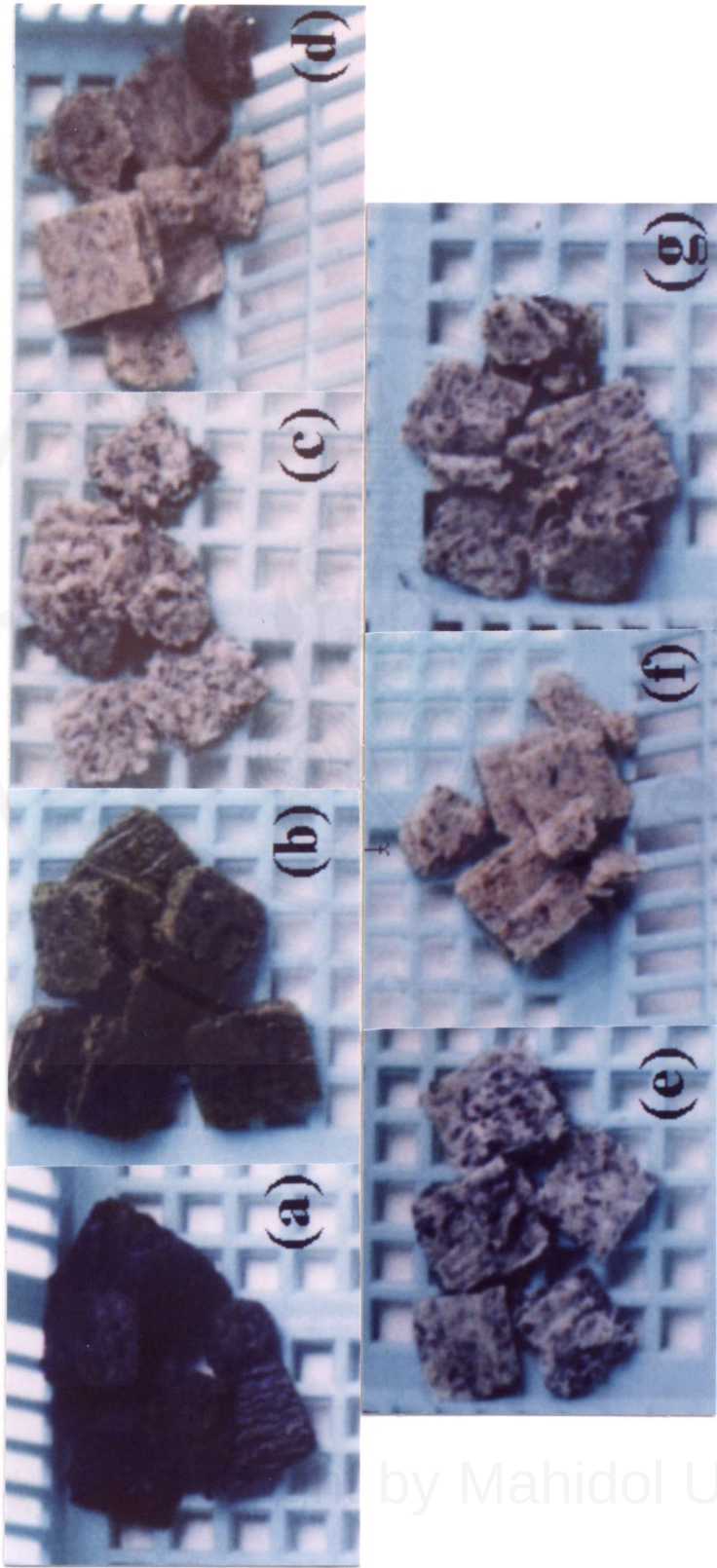


Figure 11 The artificial diets used in the experiment of artificial food utilization.

- | | | | |
|---------------------|----------------------|------------------------|----------------------|
| (a) Diet formulae I | (b) Diet formulae II | (c) Diet formulae III | (d) Diet formulae IV |
| (e) Diet formulae V | (f) Diet formulae VI | (g) Diet formulae VII. | |

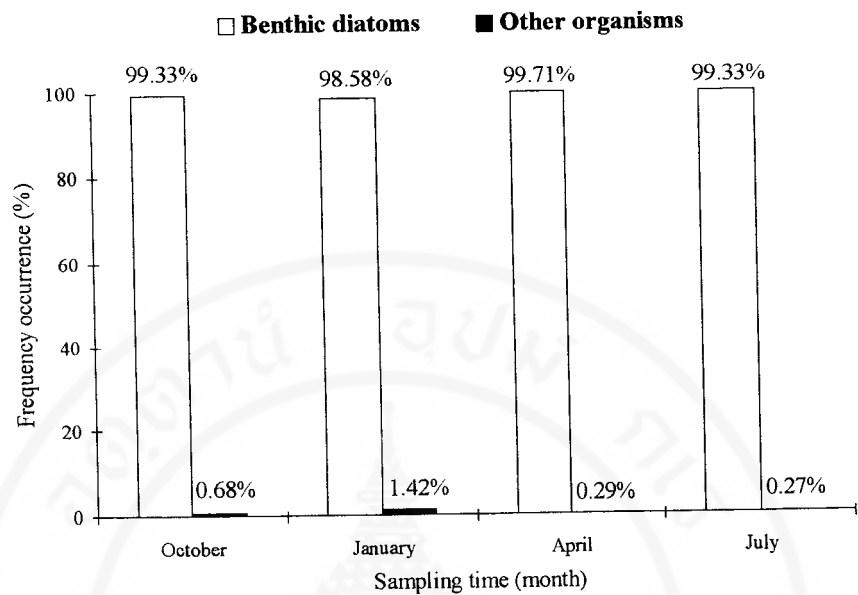


Figure 12 The micro-organisms (<120 μ m) in *H. asinina* gut during different sampling periods.

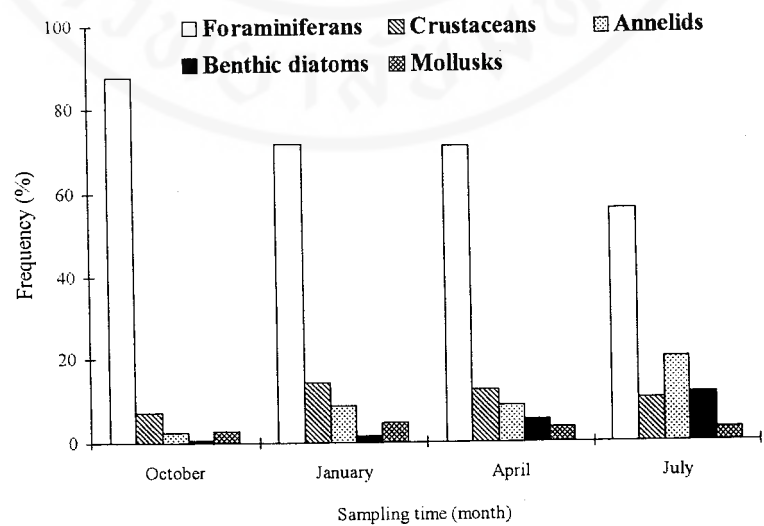


Figure 13 The macro-organisms (>120 μ m) in *H. asinina* gut during different sampling periods.

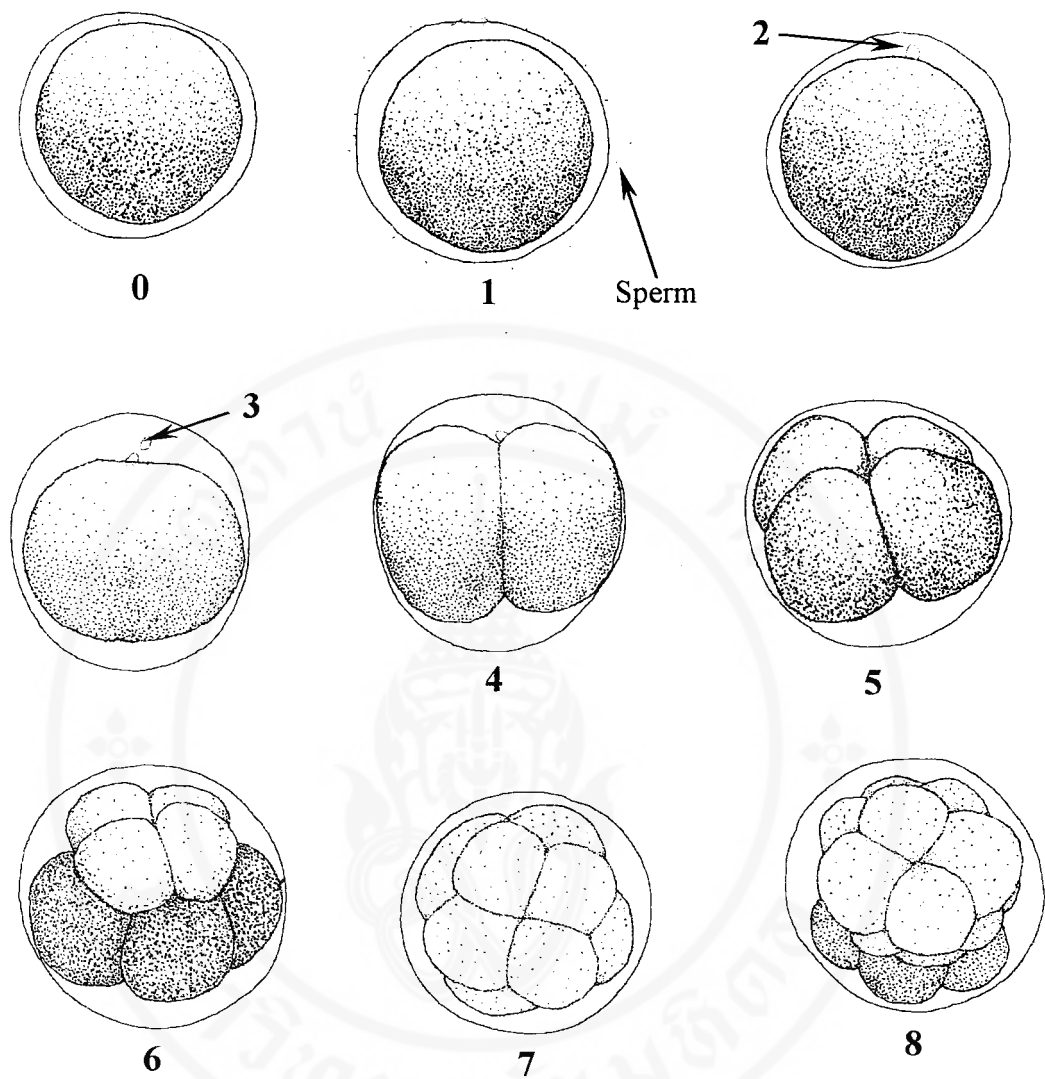


Figure 14 The development of *H. asinina* larvae from egg to the first respiratory pore formation stage

- | | |
|------------------------------------|--|
| 0 = Unfertilized egg | 4 = First cleavage (2 cells) |
| 1 = Fertilized egg | 5 = Second cleavage (4 cells) |
| 2 = Discharge of first polar body | 6 = Third cleavage (8 cells) |
| 3 = Discharge of second polar body | 7 = Fourth cleavage (12 cells)
(top view) |
| | 8 = Fifth cleavage (16 cells) |

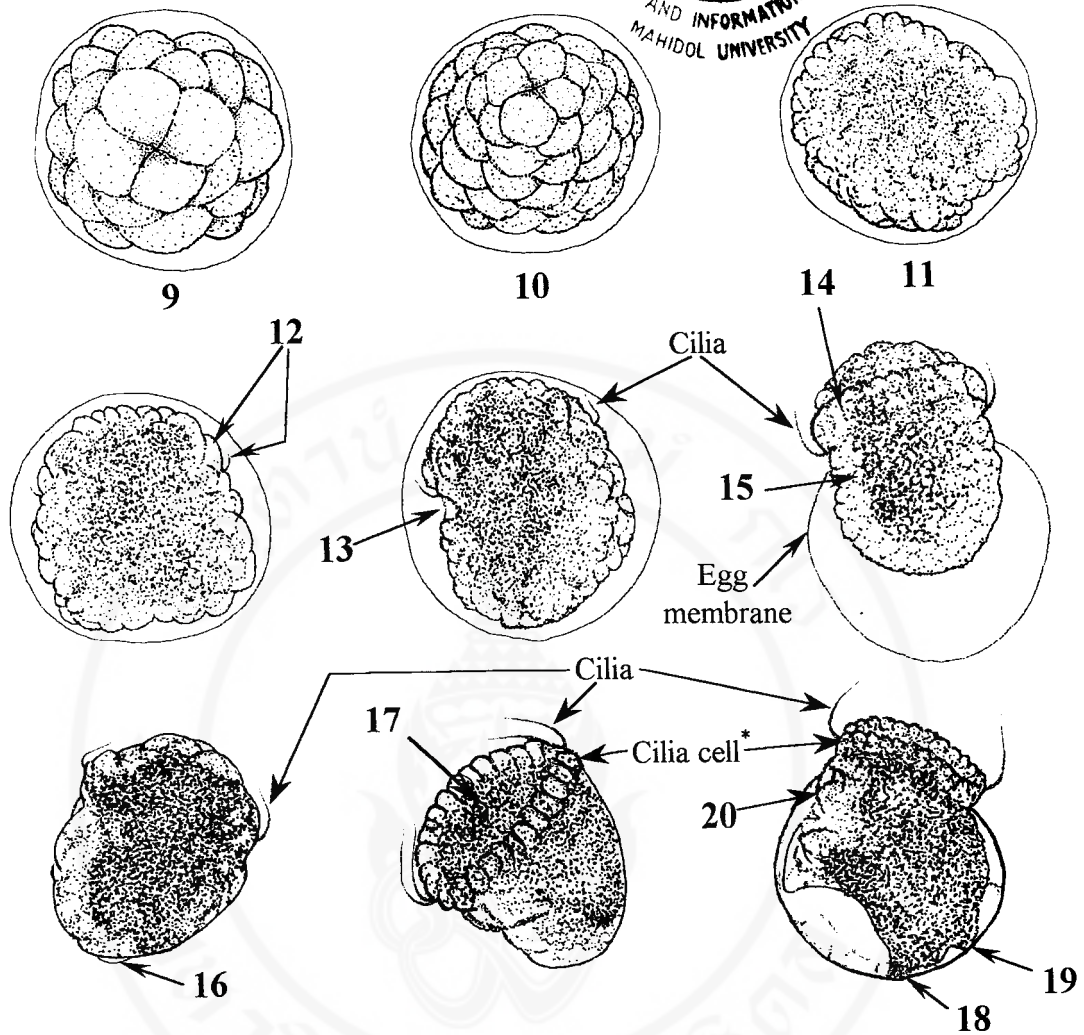


Figure 14 (Continued)

- | | |
|---|--|
| 9 = Morula | 15 = Hatch out |
| 10 = Blastula | 16 = Beginning of larval shell formation |
| 11 = Gastrula | 17 = The apical region becomes flats and the velum is completely developed with long cilia present |
| 12 = Appearance of prototrochal girdle and cilia along the prototrochal girdle* | 18 = Appearance of larval retractor muscle |
| 13 = Appearance of stomodeum (rotation) | 19 = Appearance of integumental attachment |
| 14 = Prototrochal girdle and cilia along the prototrochal are completely formed.* | 20 = Development of foot mass. |

* There are 24 cilia cell surrounds the velum along the prototrochal girdle.

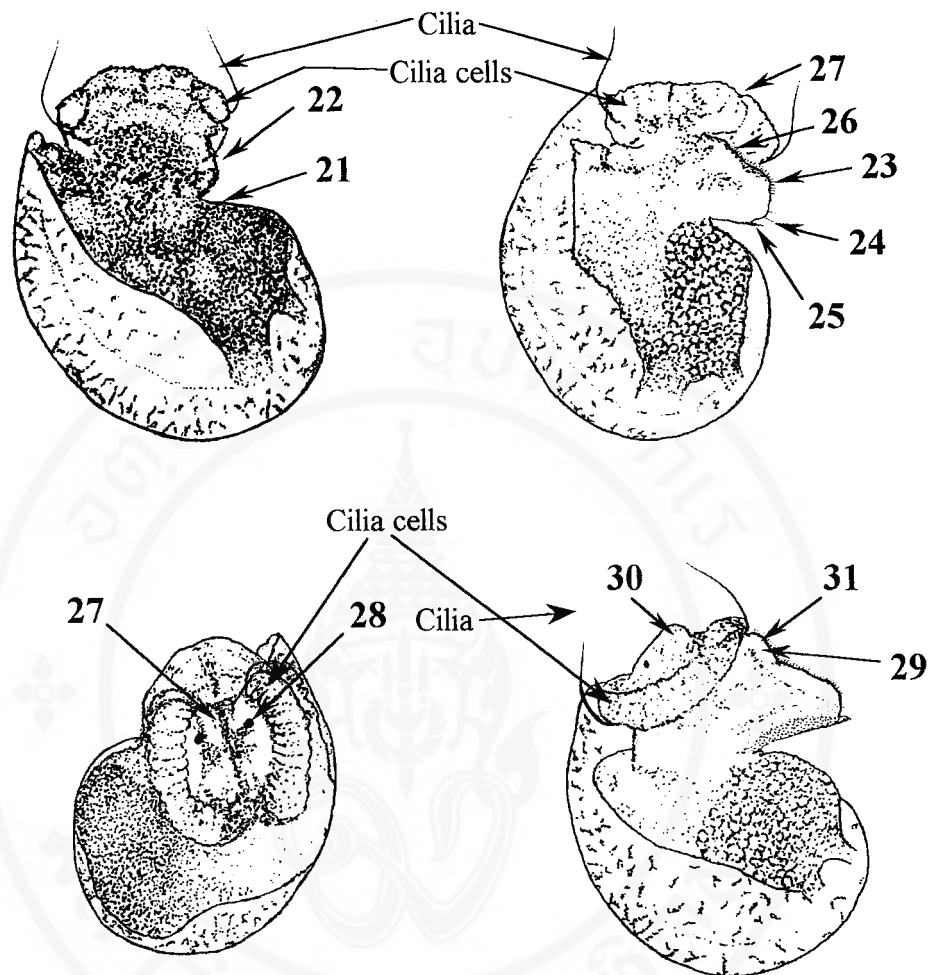


Figure 14 (Continued)

- 21 = Completion of larval shell
- 22 = Cephalo-pedal mass 90° torsion
- 23 = Foot muscle 180° torsion
- 24 = Appearance of spine on metapodium end
- 25 = Operculum
- 26 = Appearance of fine cilia on foot sole
- 27 = Vertical groove formation in velum
- 28 = Appearance of eye spot
- 29 = Appearance of propodium
- 30 = Appearance of cephalic tentacles
- 31 = Appearance of cilia on the propodium

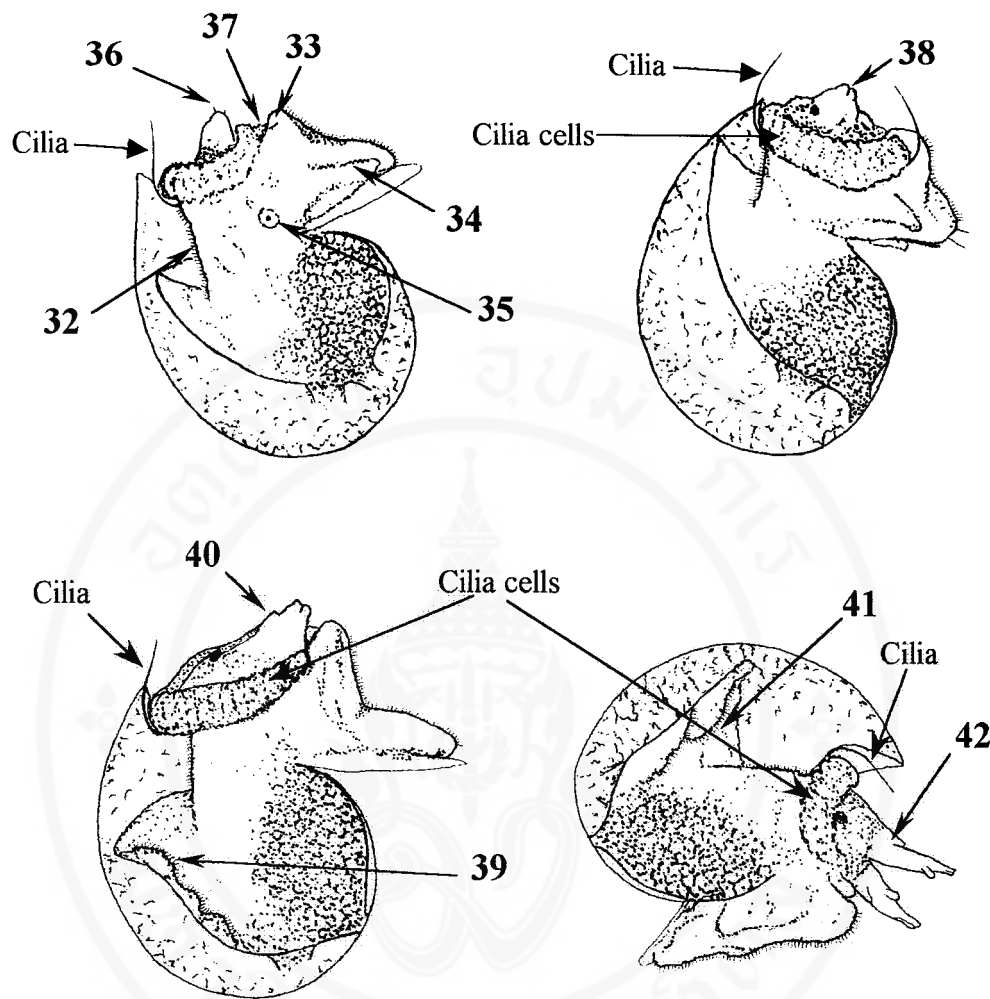


Figure 14 (Continue)

- 32 = Appearance of cilia in mantle cavity up to the anterior edge of the velum
- 33 = Formation of apophysis on propodium
- 34 = Formation of first epipodial tentacle
- 35 = Appearance of otolith
- 36 = Appearance of spine on cephalic tentacles
- 37 = Protrusion of snout underneath the velum
- 38 = Appearance of two tubules on cephalic tentacle
- 39 = Ciliary process forms on the roof of the mantle cavity
- 40 = Third tubule appearance on cephalic tentacle
- 41 = The retractor muscle attached to the larval shell and draws the enlarged mantle cavity toward the back of the she shell
- 42 = Fourth tubule appearance on cephalic tentacles

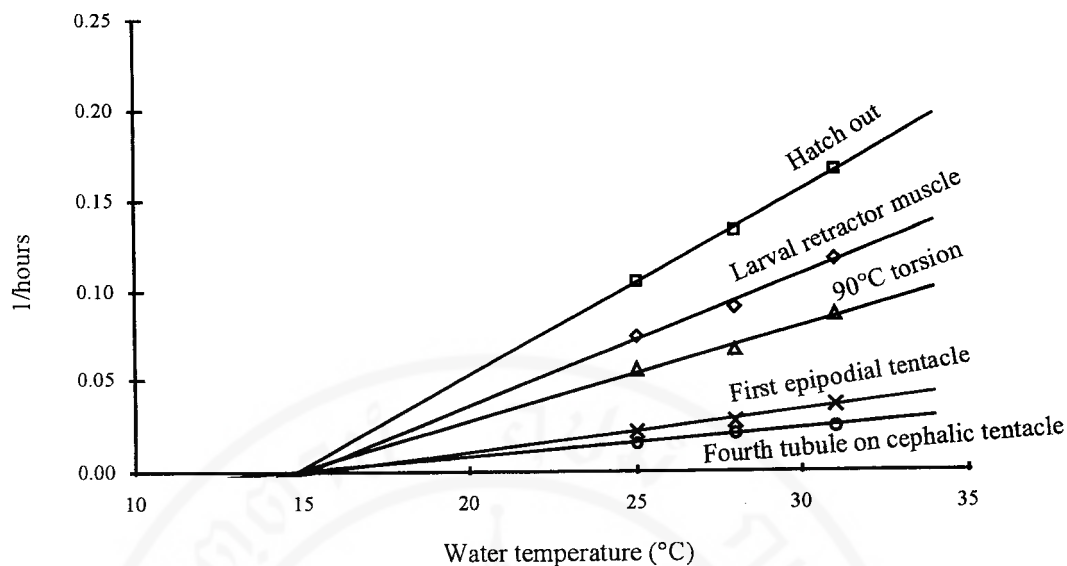


Figure 15 The relationship between water temperature and time inversion (1/hours) of beginning formation of the fifth larval development stage (hatch out, larval retractor muscle, 90°C torsion, first epipodial tentacle and fourth tubule on cephalic tentacle) of *Haliotis asinina*. The Biological point is the x-intercept of the five lines.

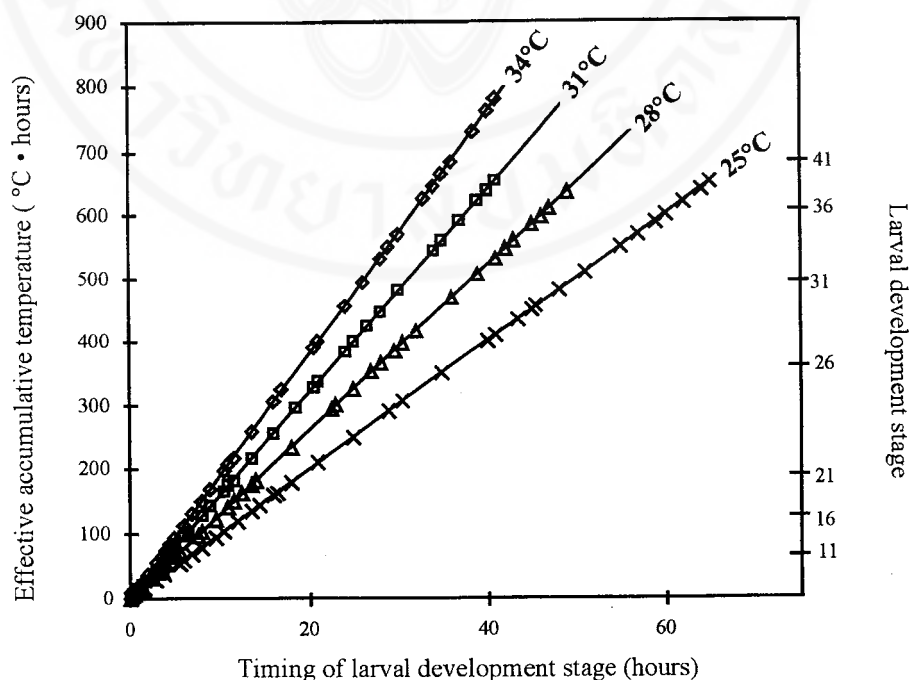


Figure 16 The relationship between timing of larval stage development (hours) and effective accumulative temperature (EAT, °C · hours) in 25, 28, 31 and 34 °C. The right axis correspond to larval development stage.

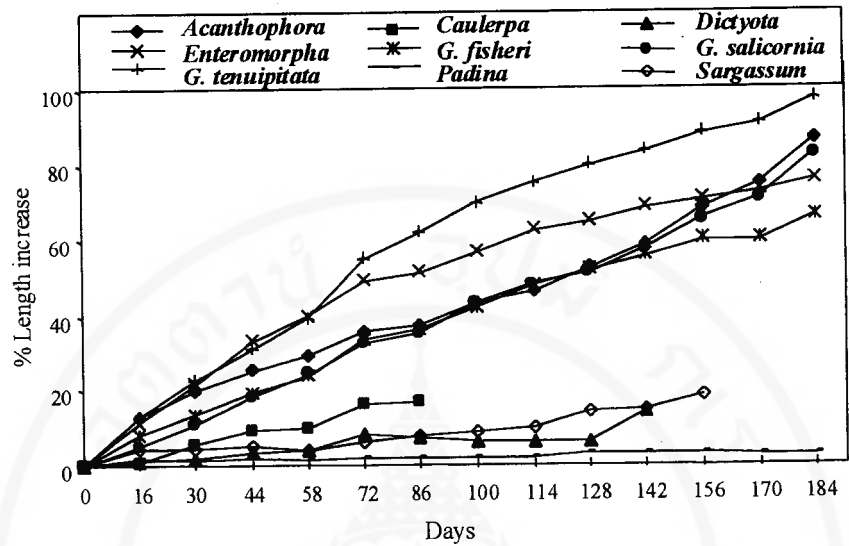


Figure 17 Shell lenght (mm) of *H. asinina* fed nine species of macroalgae.

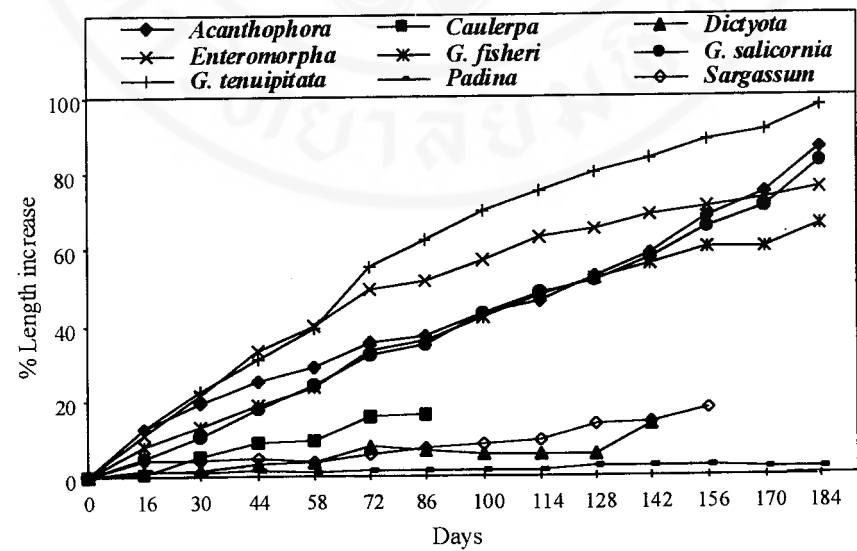


Figure 18 Percent shell length increase of *H. asinina* fed nine species of macroalgae.

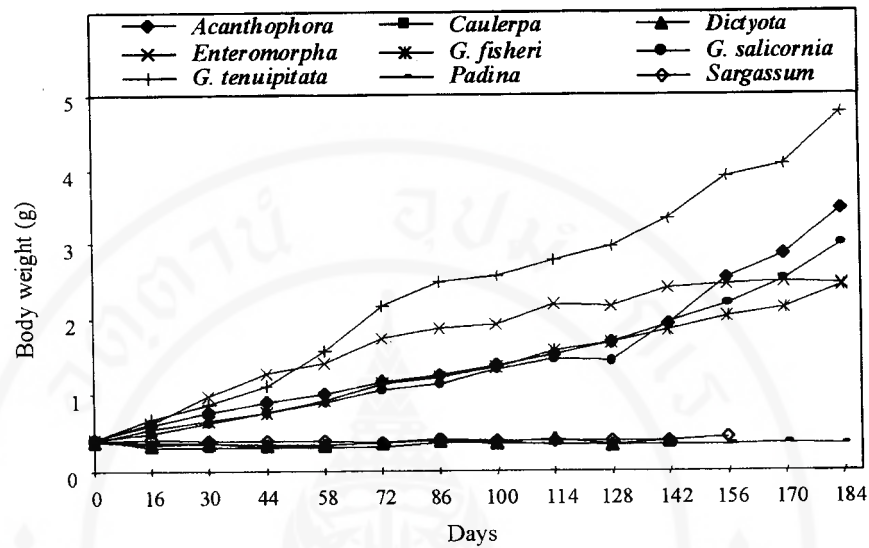


Figure 19 Body weight (g) of *H. asinina* fed nine species of macroalgae.

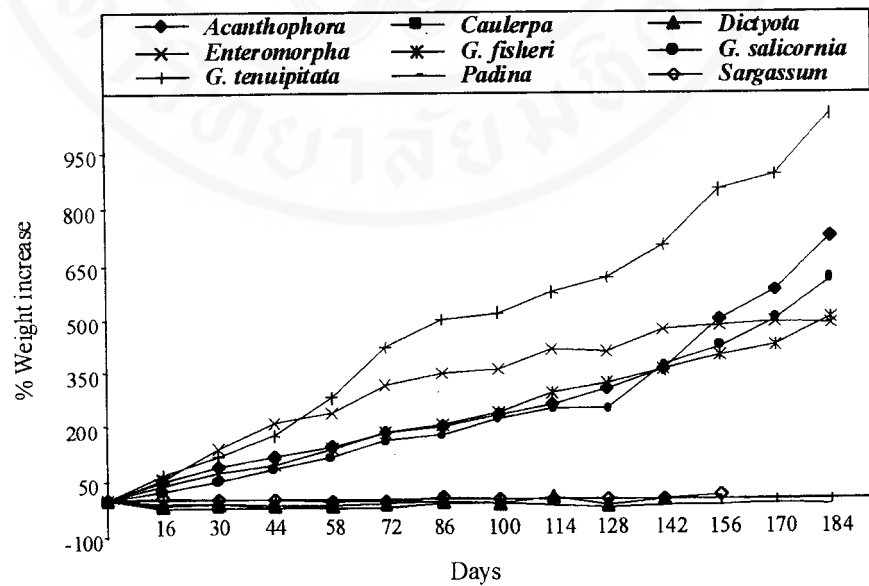


Figure 20 Percent weight increase of *H. asinina* fed nine species of macroalgae.

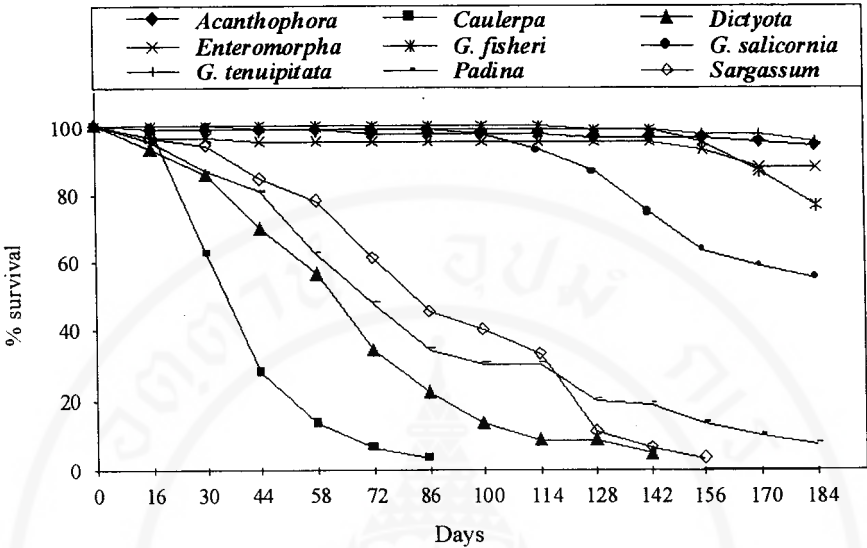


Figure 21 Percent survival of *H. asinina* fed nine species of macroalgae.

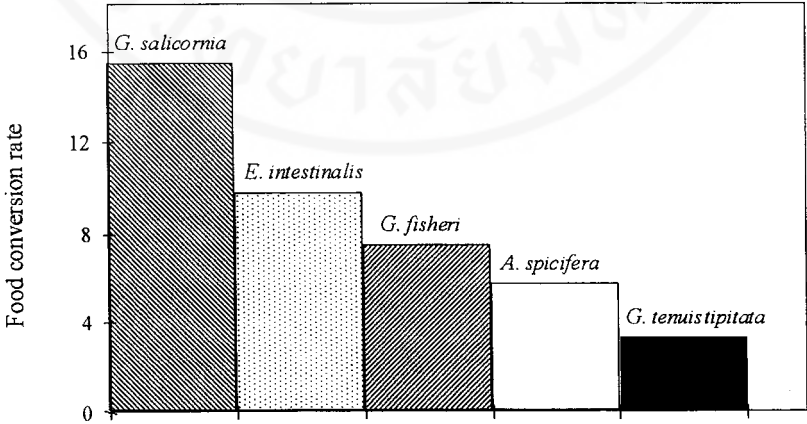


Figure 22 Food conversion rates of *H. asinina* fed nine species of macroalgae.

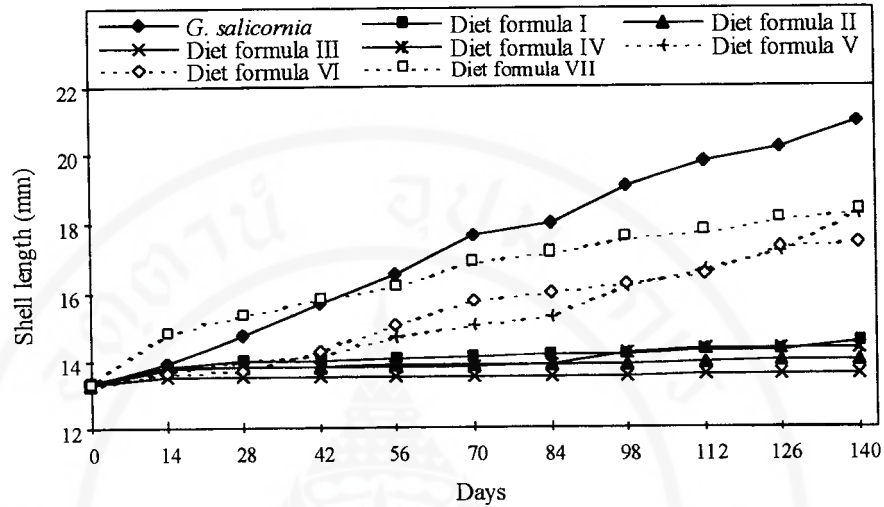


Figure 23 Shell length (mm) of *H. asinina* fed fresh *G. salicornia* and artificial diet formula I-VII over a period of 140 days.

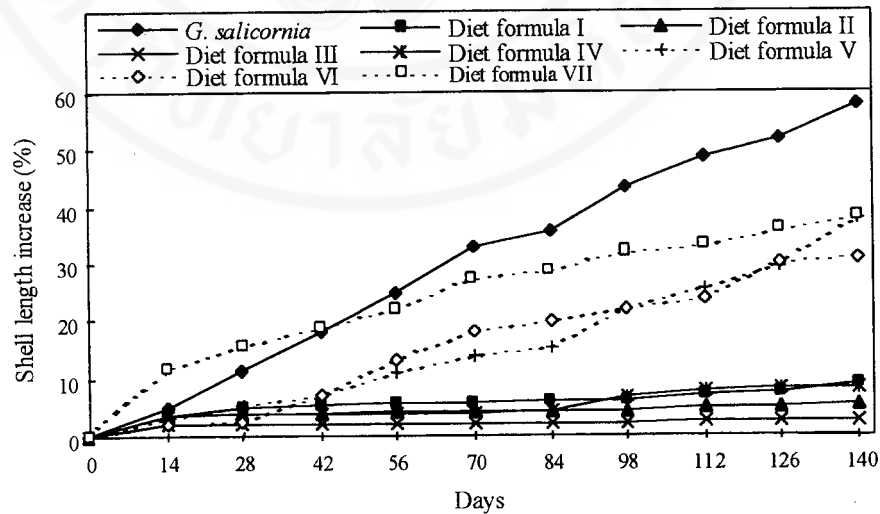


Figure 24 Percent shell length increase of *H. asinina* fed fresh *G. salicornia* and artificial diet formula I-VII over a period of 140 days.

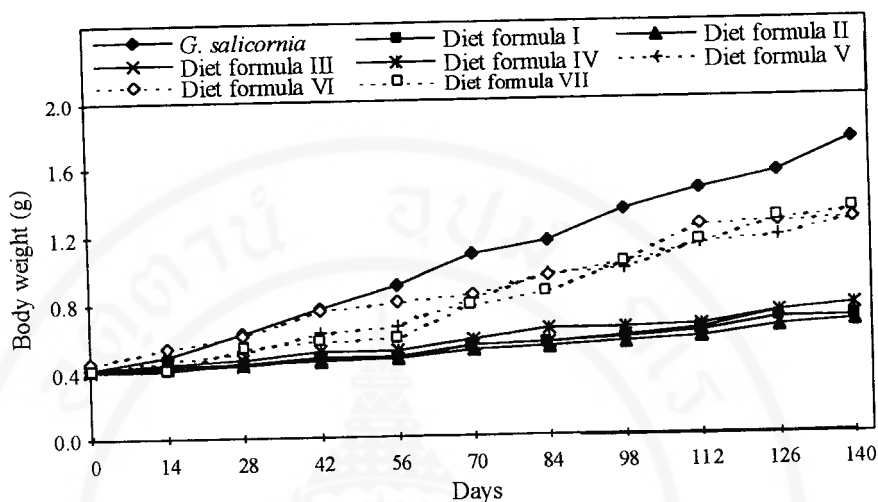


Figure 25 Body weight (g) of *H. asinina* fed fresh *G. salicornia* and artificial diet formula I-VII over a period of 140 days.

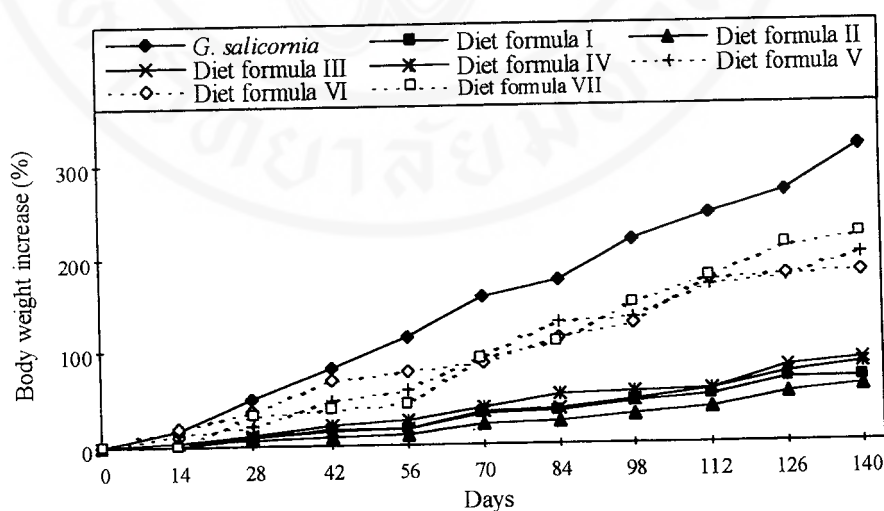


Figure 26 Percent weight increase of *H. asinina* fed fresh *G. salicornia* and artificial diet formula I-VII over a period of 140 days.

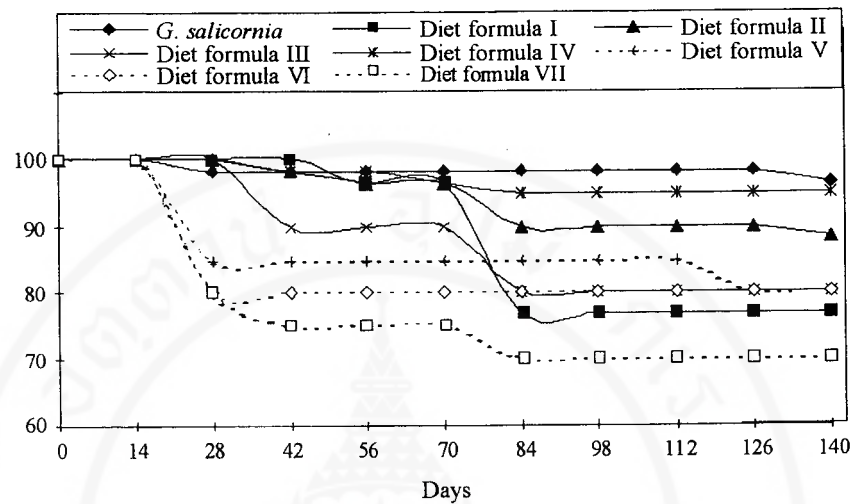


Figure 27 Percent survival of *H. asinina* fed fresh *G. salicornia* and artificial diet formula I-VII over a period of 140 days.

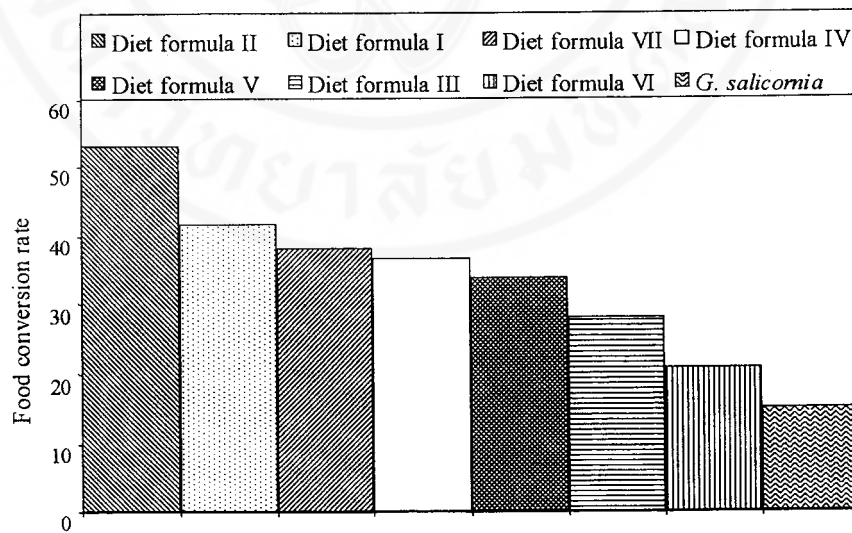


Figure 28 Food conversion rate of *H. asinina* fed fresh *G. salicornia* and artificial diet formula I-VII over a period of 140 days.



BIOGRAPHY

NAME Miss Saowapa Sawatpeera

DATE OF BIRTH 6 April 1954

PLACE OF BIRTH Nakhornrajasima, Thailand

INSTITUTIONS ATTENDED Silpikorn University, (1977-1980):
 Bachelor of Science (Biology)
 Chulalongkorn University, (1982-1986):
 Master of Science (Marine Biology)
 Mahidol University, (1995-1999):
 Doctor of Philosophy (Biology)

FELLOWSHIP / RESEARCH GRANT Institute Strengthening Program of National Science
 and Technology Development Agency,
 Thailand, 1995-1999
 Thailand Research Fund (PG2/015/2539),
 Thailand, 1996-1998

POSITION & OFFICE 1983-Present, Institute of Marine Science,
 Burapha University, Chonburi, Thailand.
 Position : Scientist, Research Section

PUBLICATION Determination of Gut Contents of Thai abalone
Haliotis asinina Linneaus. Sawatpeera S,
 Upatham ES, Kruatrachue M, Chitramvong
 YP, Singhagraiwan T, Parkpoomkamol K.
 J. Shellfish Res. 1998. (Inpress).
 Food utilization by *Haliotis asinina* Linneaus.
 Upatham ES, Sawatpeera S, Kruatrachue M,
 Chitramvong YP, Singhagraiwan T.
 Pumthong T, Jarayabhand P.
 J. Shellfish Res. 1998. (Inpress).