DETERMINATION OF BIOFOULER SETTLEMENT ON DIFFERENT TYPES OF SUBSTRATES AND ENVIRONMENT CONDITIONS IN PULAU BETONG, PENANG

June 2009

JANTZEN CHAI

DETERMINATION OF BIOFOULER SETTLEMENT ON DIFFERENT TYPES OF SUBSTRATES AND ENVIRONMENT CONDITIONS IN PULAU BETONG, PENANG

by

JANTZEN CHAI

Thesis submitted in fulfillment of the requirements

for the degree of

Master of Science

June 2009

ACKNOWLEDGEMENTS

At here, I would like to express my deepest gratitude to my supervisor, Prof Zulfigar Yasin for his supervision, kind patience, guidance, advice and support throughout my Master program.

For those in Marine Lab, Sim, Kak Ain, Bee Wah, Chiew Peng, Hanis, Wala, and undergrads thank you for all your support and advice during my master program. I would like to thank School of Biological Sciences for their facilities and logistics.

To Mr. Wong and Pak Ali from the oyster farm in Pulau Betong, special thanks for helping and supporting me in this project. Aquatic Lab Mr. Hamzah, I would like to thank you for your support and kind assistance.

The last but not least is my beloved family; I really appreciated their patience in allowing pursuing my goal.

TABLE OF CONTENTS

TITLE	TITLE OF THESIS				
ACKN	KNOWLEDGEMENTS				
TABL	E OF C	CONTENTS	iii		
LIST OF TABLES					
LIST (OF FIG	URES	xi		
LIST (OF PLA	ATES	xii		
LIST (OF ABI	BREVIATIONS	xii		
ABST	RACT		xiv		
ABST	RAK		XV		
1.0	Chapter One				
	1.1	General introduction	1		
	1.2	What is biofouling?	2		
	1.3 How does biofouling form?				
	1.4 Types of biofoulers		6		
	1.4	4.1 Barnacle	6		
	1.4	4.2 Snail	8		
	1.4	1.3 Tubeworm	9		
	1.4	4.4 Bryozoan	10		
	1.4	4.5 Oyster Spat	11		
	1.4	4.6 Sea urchin	12		
	1.5	Objectives	13		
2.0	Chapter Two: Materials and Method				

2.1	Study site		14
-----	------------	--	----

	2.2	Envir	onment	al water parameters measurement	16
		2.2.1	Physi	cal Parameters	
		a.	Total	Suspended Solids (TSS)	16
		b.	Temp	perature (°C), Salinity (ppt), Dissolved Oxygen	17
			(mg/I	L) and Chlorophyll-a (μ g/L)	
		c.	Turbi	dity Index, FTU	18
		d.	Wate	r Velocity	18
		2.2.2	Chem	ical Parameters	20
	2.3	Biofo	uling ex	xperiment using different types of collectors	
		Exper	iment I	: Oyster shells as collectors	20
		Exper	iment I	I: Plastic trays as collectors	24
		Exper	iment I	II: Asbestos sheets as collectors	26
3.0	Chap	ter Thr	ee: Re	sults	
	3.1	Water	quality	y parameters	28
		3.1.1	Physi	cal Parameters	
			A.	Total Suspended Solids, Water Turbidity and	28
				Water Velocity	
			B.	Salinity and Temperature	29
			C.	Dissolved Oxygen	29
		3.1.2	Chem	ical Parameters	32
	3.2	Exper	iment I	: Oyster shells as collectors	
		3.2.1	Biofo	uling on oyster shells at different depths	35
			A.	0.5m depth	35
			B.	1.0m depth	35
			C.	1.5m depth	36

		D.	2.0m depth	36	
	3.2.2	The di	The differences in biomass of biofouler settlement on		
		oyster	shells at different depths		
		A.	Shaded area	39	
		B.	Unshaded area	39	
	3.2.3	The di	fferences in biomass of biofoulers settlement on	40	
		oyster	shells between shaded and unshaded area at		
		differe	ence depths		
	3.2.4	The co	prrelation between environmental factors and	41	
		bioma	ss of biofouler settlement		
3.3	Exper	iment II	ment II: Plastic trays as collectors		
	3.3.1	The bi	The biomass of the biofouling on plastic tray at different		
		depths	depths		
		A.	Unshaded area at 1m depth	42	
		B.	Unshaded area at 2m depth	44	
		C.	Shaded area at 1m depth	45	
		D.	Shaded area at 2m depth	47	
	3.3.2	The di	fferences in biomass of biofouler settlements on		
		plastic	trays at different depths		
		A.	Shaded area	52	
		В.	Unshaded area	53	
	3.3.3	Comp	arison of biofoulers settlement on plastic trays in	54	
		different conditions between shaded and unshaded area			
	3.3.4	The re	lationship between environmental factors and		
		biomass of biofoulers settlement			

		A. At 1m depth		56	
		B. At 2m depth		59	
3.4	Exper	ment III: Asbestos sheets as	collectors		
	3.4.1	Oyster spat coverage on the	top surface of the	62	
		asbestos sheets at the shade	d area		
	3.4.2	Oyster spat coverage on the	bottom surface of the	64	
		asbestos sheets at the shade	d area		
	3.4.3	Oyster spat coverage on the	top surface of the	66	
		asbestos sheets at the unsha	ded area		
	3.4.4	Oyster spat coverage on the	bottom surface of the	68	
		asbestos sheets at unshaded	area		
	3.4.5	Comparison of oyster spat settlement on asbestos			
		sheets in different condition	s at the unshaded and		
		shaded area			
	3.4.6	Oyster spat settlement on th	e top and bottom		
		surface of asbestos sheets a	t different depths		
		A. Unshaded and shade	ed area.	73	
		B. Top and bottom sur	face of the asbestos	73	
		sheets.			
	3.4.7	The relationship between er	vironmental factors		
		and oyster spat settlement a	t top and bottom		
		surfaces of the asbestos she	ets at the shaded area		
		A. At 0.5m depth		74	
		B. At 1.0m depth		76	
		C. At 1.5m depth		78	

			D.	At 2.0m depth	79
		3.4.8	The re	lationship between environmental factors and	
			oyster	spat settlement coverage at the top and bottom	
			surface	es of the asbestos sheets at the unshaded area	
			a.	At 0.5m depth	80
			В	At 1.0m depth	82
			C.	At 1.5m depth	83
			D.	At 2.0m depth	84
4.0	Chapt	ter Fou	r: Discu	ission	
	4.1	Water	Quality	Parameters	85
		4.1.1	Physic	al Parameters	85
		4.1.2	Chemi	cal Parameters	87
	4.2	Experi	iment I:	Oyster shells as collectors	88
	4.3	Experi	iment II	Plastic trays as collectors	92
	4.4	Experi	iment II	I: Asbestos sheets as collectors	102
5.0	Concl	usion			106
	5.1	Recon	nmenda	tions for further studies	108
6.0	Refer	ences			109

	LIST OF TABLES	Page
Table 2.1	Reagent and wavelength of nutrient analysis by using	20
	HACH Kit Spectrophotometer Model DREL 2000	
Table 3.1	Correlation of biofoulers with water parameters at 1 m	58
	depth at the unshaded area	
Table 3.2	Correlation of biofoulers with water parameters at 1 m	58
	depth at the shaded area	
Table 3.3	Correlation of biofoulers with water parameters at 1 m	61
	depth at the unshaded area	
Table 3.4	Correlation of biofoulers with water parameters at 1 m	61
	depth at the shaded area	
Table 3.5	Correlation between water parameters and oyster spat	75
	settlement at 0.5 m depth on the top surface of the asbestos	
	sheets at the shaded area	
Table 3.6	Correlation between water parameters and oyster spat	75
	settlement at 0.5 m depth on the bottom surface of the	
	asbestos sheets at the shaded area	
Table 3.7	Correlation between water parameters and oyster spat	77
	settlement at 1.0 m depth on the top surface of the asbestos	
	sheets at the shaded area	
Table 3.8	Correlation between water parameters and oyster spat	77
	settlement at 1.0 m depth on the bottom surface of the	
	asbestos sheets at the shaded area	
Table 3.9	Correlation between water parameters and oyster spat	78

settlement at 1.5 m depth on the top surface of the asbestos sheets at the shaded area

Table 3.10	Correlation between water parameters and oyster spat	78
	settlement at 1.5 m depth on the bottom surface of the	
	asbestos sheets at the shaded area	
Table 3.11	Correlation between water parameters and oyster spat	79
	settlement at 2.0 m depth on the top surface of the	
	asbestos sheets at the shaded area	
Table 3.12	Correlation between water parameters and oyster spat	79
	settlement at 2.0 m depth on the bottom surface of the	
	asbestos sheets at the shaded area	
Table 3.13	Correlation between water parameters and oyster spat	81
	settlement at 0.5 m depth on the top surface of the asbestos	
	sheets at the unshaded area	
Table 3.14	Correlation between water parameters and oyster spat	81
	settlement at 0.5 m depth on the bottom surface of the	
	asbestos sheets at the unshaded area	
Table 3.15	Correlation between water parameters and oyster spat	82
	settlement at 1.0 m depth on the top surface of the asbestos	
	sheets at the unshaded area	
Table 3.16	Correlation between water parameters and oyster spat	82
	settlement at 1.0 m depth on the bottom surface of the	
	asbestos sheets at the unshaded area	
Table 3.17	Correlation between water parameters and oyster spat	83
	settlement at 1.5 m depth on the top surface of the asbestos	

sheets at the unshaded area

Table 3.18	Correlation between water parameters and oyster spat	83
	settlement at 1.5 m depth on the bottom surface of the	
	asbestos sheets at the unshaded area	
Table 3.19	Correlation between water parameters and oyster spat	84
	settlement at 2.0 m depth on the top surface of the asbestos	
	sheets at the unshaded area	
Table 3.20	Correlation between water parameters and oyster spat	84
	settlement at 2.0 m depth on the bottom surface of the	
	asbestos sheets at the unshaded area	

	LIST OF FIGURES	Page
Figure 2.1	Map of Penang showing the sampling site, Pulau Betong	15
Figure 3.1	Water physical parameters in Pulau Betong from April	31
	2005 until September 2006 for 18 months	
Figure 3.2	Water chemical parameters in Pulau Betong from April	34
	2005 to September 2006 for 18 months	
Figure 3.3	Monthly weight of fouling organisms in Pulau Betong	38
	from April 2005 until September 2006 (except January	
	2006) at different depths	
Figure 3.4	Biofoulers settled on the plastic tray 2006 for 18 months	50
	from April 2005 until September	
Figure 3.5	Biofoulers settled on the plastic trays for 18 months from	51
	April 2005 until September 2006	
Figure 3.6	Oyster spats settled at the shaded area from April 2005	70
	until September 2006 for 18 months	
Figure 3.7	Oyster spat settled at the unshaded area from April 2005	71
	until September 2006 for 18 months	

LIST OF PLATES Page

Plate 1.1	Barnacle (Balanus sp.)	7
Plate 1.2	Snail (Thais sp.)	8
Plate 1.3	Tubeworm (Hydroides sp.)	9
Plate 1.4	Lacy crust (Membranipora sp.)	10
Plate 1.5	Close up image of oyster spat on substrate (Crassostrea sp.)	11
Plate 1.6	Sea urchin (Asteroidea sp.)	12
Plate 2.1	Oyster shells were cleaned and dried	22
Plate 2.2	Drilling process of the oyster shell	22
Plate 2.3	Oyster shells were set at shaded area	23
Plate 2.4	Oyster shells were set at unshaded area	23
Plate 2.5	The experiment setup using plastic trays as collectors	25
Plate 2.6	Biofoulers on an asbestos sheet	27

LIST OF ABBREVATIONS

- cm Centimeter
- mm Millimeter
- m Meter
- L Liter
- cm² Centimeter
- m² Meter square
- mL Milliliter
- mg Milligram
- ms Millisecond
- m/s Meter per second
- μg/L Microgram per liter
- mg/L Milligram per liter
- FTU Formazin turbidity unit
- °C Degree Celsius
- ppt Part per thousands
- ° Degree

4

Minute

DETERMINATION OF BIOFOULERS SETTLEMENT ON DIFFERENT TYPES OF SUBSTRATES AND ENVIRONMENT CONDITIONS IN PULAU BETONG, PENANG

Abstract

A study on the determination of biofoulers settlement on different types of substrates and environment conditions have been conducted at Pulau Betong, Penang. A total of six biofoulers have been recorded. The biofoulers consist of barnacles, tubeworms, oyster spat, bryozoans, snails and sea urchins. The highest biomass of biofoulers settlement on oyster shells were at 0.5 m and 1.0 m depths at both shaded and unshaded area. Water turbidity and temperature were found to be affecting the settlement of the biofoulers on the shells. The biomass of barnacles showed the highest among the rest of the biofoulers when using plastic trays as collectors. At the unshaded area, most of the biofoulers tend to settle at 2 m depth on the plastic trays while at the shaded area, the biofoulers tend to settle at 1 m depth on the plastic trays. Water temperature was found to be the main factor affecting the settlement of biofoulers on plastic trays. The top surface of asbestos sheets has the highest settlement of ovster spat compared to the bottom surface of the asbestos sheet at both shaded and unshaded areas using asbestos sheets. The settlement of oyster spat was higher at the shaded area compared to the settlement of oyster spat at the unshaded area. The settlement of oyster spat on the asbestos sheets was affected slightly by salinity and chlorophyll-a. Meanwhile ammonia concentration showed strong influence on the settlement of the oyster spats on the asbestos sheets.

PENENTUAN PEMENDAPAN "BIOFOULERS" KE ATAS JENIS DAN KEADAAN PERSEKITRAN YANG BERLAINAN DI PULAU BETONG, PULAU PINANG

Abstrak

Kajian penentuan pemendapan "biofoulers" ke atas jenis dan keadaan persekitaran yang berlainan telah dijalankan di Pulau Betong, Pulau Pinang. Sebanyak enam organisma "biofoulers" telah direkodkan iaitu teritip, cacing tiub, benih tiram, bryozoa, siput dan landak laut. Pemendapan biojisim "biofoulers" yang tertinggi direkodkan di atas cengkerang tiram pada kedalaman 0.5 m dan 1.0 m di kedua-dua kawasan terteduh dan tidak terteduh. Kekeruhan air dan suhu didapati telah memberi kesan kepada pemendapan "biofoulers" di atas cengkerang tiram. Manakala, biojisim teritip menunjukkan pemendapan tertinggi di antara semua "biofoulers" apabila menggunakan bakul plastik sebagai pengumpul. Di kawasan yang terteduh, kebanyakan "biofoulers" termendap pada bakul plastik pada kedalaman 2.0 m. Manakala di kawasan yang tidak terteduh, "biofoulers" termendap pada kedalaman 1.0 m. Suhu air merupakan faktor utama yang memberikan kesan kepada pemendapan "biofoulers" pada bakul plastik. Permukaan atas plat asbestos mencatatkan pemendapan benih tiram yang tertinggi berbanding dengan permukaan bawah plat asbestos di kawasan terteduh dan tidak terteduh. Pemendapan benih tiram adalah lebih tinggi di kawasan terteduh berbanding dengan kawasan tidak terteduh dengan menggunakan plat asbestos. Saliniti dan klorofil-a mempunyai perkaitan yang rendah dengan pemendapan benih tiram pada plat asbestos berbanding dengan kepekatan ammonia di dalam air.

CHAPTER 1

1.0 General introduction

1.1 Introduction

Fouling refers to the accumulation and deposition of living organisms (biofouling) and certain non-living materials on hard surfaces, most often in an aquatic environment. This can be the fouling of ships, pilings, and natural surfaces in the marine environment (marine fouling). Fouling communities are community organisms found on the sides of docks, harbors, marinas, ships and boats throughout the world (Cheah and Chua 1979). These communities are characterized by the presence of a variety of sessile organisms including ascidians, bryozoans, mussels, tube building polychaetes, sea anemones, and more. Common predators on and around fouling communities include small crabs, starfish, fish, limpets, chitons, and other gastropods.

Fouling is any material, biological or non-biological, which smothers the cultivated stock. Excessive fouling may completely envelop bivalve and prevent access to water, food and oxygen. In addition, biofouling species may compete with bivalve for resources such as food and space.

1.2 What is biofouling?

Surfaces immersed in the marine environment became colonised by marine organisms, a process which is called biofouling (Railkin, 2004). There are several organisms that cause biofouling on many different types of surfaces affected and due to the work of scientists, engineers and others, scores of solutions to the problem.

Biofouling occurs worldwide in various industries, from offshore gas and oil industries, to fishing equipment and to cooling systems (Rolland and DeSimone, 2003). The most common biofouling site is on the hulls of ships, where biofoulers are often found. The problem of biofoulers growth on a ship is the eventual corrosion of the hull, leading to the ship's deterioration (Rolland and DeSimone, 2003). Before the corrosion occurs, biofoulers growth can increase the roughness of the hull, thereby decreasing its maneuverability and increasing drag (Rolland and DeSimone, 2003). This effect could cause the ship's fuel consumption increase (Younglood et al., 2003). This has caused consequences to the economic and environmental, as the increased of fuel consumption leads to increased output of greenhouse gases (Anderson, 2002). Parts of a ship other than the hull are affected as well: propellers, water-cooling pipes, heat exchangers and even the ballast water (Brizzolara, 2002). Biofouling can cause problems to the heating and cooling systems in power stations or factories. Just like a clogged drain in your bathroom or kitchen where buildup of matter inside cooling system pipes will decrease the performance. This may cause the equipment to be cleaned frequently, at times with harsh chemicals, and the obstruction in piping can lead to a shutdown of plants causing economic losses (De Rincon et al., 2001).

Fish farming and fishing are also affected, with trawl and mesh cages harboring fouling organisms. In Australia, settlement of biofoulers would cost for about 80% of the pearling industry's costs. The machines in the papermaking and underwater instrumentation and pulping industries contain silicon-based and goldbased components of micro electrochemical drug delivery devices are susceptible to biofouling (Anderson *et al.*, 2003). The piping and sprinkler system nozzles of fire protection systems are common places for settlement of biofoulers (Lewis *et al.*, 1997).

Biofouling includes sedentary sessile motile and sedentary species. For the purpose of fouling control, biofouling species may be classified as hard or soft bodied. Soft-bodied species include ascidians, algae, sea anemones, sponges and annelids whereas hard bodied species include the encrusting barnacles, serpulid worms, bryozoans and other species of bivalve molluscs (Littlewood, 1990). These groups are considered as the most common biofoulers. The presence of most of these is assumed to have a detrimental effect on cultured oysters (Angell, 1973). Although some biofoulers such as the filter-feeding ascidians may act as sediment traps, thereby protecting oysters from heavy silt loads (Maurer and Watling, 1973). Yet some biofoulers species can quickly overgrow and kill cultured oysters, e.g. the colonial ascidian, *Didemnum psammathodes* (Wade *et al.*, 1981; Littlewood, 1988).

In pearl oyster and oyster cultivation, biofoulers have caused a lot of problems to the farmers. There were few studies have been reported on the fouling and predation on growth and survival of pearl oysters cultivation (Taylor *et al.*, 1997; Friedman and Southgate 1999a, b; Southgate and Beer 2000). There were a few drawbacks which include: reduced water flow rate to the culture unit which cause food availability decreased (Taylor *et al.*, 1997); available food competition among filter feeding biofoulers and culture animals (Claereboudt *et al.*, 1994); oxygen availability decreased which may affect the cultivation animals growth rate (Wallace

and Reisness, 1985); the excess weight from biofoulers settlement will caused increased in strain on culture equipment. Fouling has shown affect on the growth and survival of pearl oysters (Alagarswami and Chellam 1976; Mohammad 1976; Taylor *et al.*, 1997). Meanwhile, biofoulers could cause the growth rate of shell length decrease and increase the mortality if fouling developed on the ventral edge of the upper shell of *Crassostrea rhizophorae* (Lodeiros *et al.*, 2007).

The ecology of fouling communities associated with mangrove roots and cultivated mangrove oysters is very poorly understood. As Ellison and Farnsworth (1990) noted, "despite centuries of research on mangrove ecosystems, studies of mangrove root epibenthic fouling communities have rarely proceeded beyond taxonomic enumeration and description". Consequently, until more is known about these communities those involved in bivalves culture should assess the disadvantages of fouling cover for them, bearing in mind that fouling communities may add significant weight to collectors, compete with bivalves for food and space, and harbour predators and possibly parasites of bivalves.

Biofouling may act as a source of physiological stress on oysters although this has never been investigated, e.g. heavily fouled collectors presumably add to the oxygen demand in oyster culture sites. The carrying capacity for aquaculture sites is generally based on the demands of the cultivated organism. However, the carrying capacity of bays, longlines, raft and racks must surely be compromised by a greater mass of biofouling.

1.3 How does biofouling form?

Biofouling is not as simple a process as it sounds. Organisms do not easily stick onto a substrate like a suction cup. The complex process often begins with the production of a biofilm.

A biofilm is a film made of bacteria or other microorganisms which forms on a substrate when conditions are suitable (Gehrke and Sand, 2003). Nutrient availability is an important factor where bacteria require uronic acid, humic substances and dissolved organic carbon for optimum biofilm growth (Griebe and Flemming, 2000). Biofilms do not contain living material; they may instead contain such once living material as dead bacteria or secretions. Bacteria are not the only organisms that can create this initial site of attachment; diatoms, seaweed, and their secretions are also culprits.

The biofilm growth can provide as a foundation for the growth of barnacles, seaweed and other organisms. In other words, microorganisms such as diatoms, algae and bacteria form the primary slime film to which the macroorganisms such as bryozoans, seasquirts, sponges, polychaetes, mollusks, tube worms, sea anemones and barnacles attach.

This process does not occur in a random fashion. Conditions must be favorable, including proper humidity, nutrient and pH availability (Gomez de Saravia *et al.*, 2001). Organisms appear to be particular; for example, bacteria creating biofilms on carbon steels and stainless and recirculating cooling systems are similar physiologically (Dobrevsky *et al.*, 2000). In the case of *Vibiro alginolyticus*, a bacterium which produces organic compounds sensitive to changes in pH and temperatures, the biochemistry of the bacterium may be determined if and where the

21

biofilm attach (Muralidharan and Jayachandran, 2003). Biology and chemistry also determine which organisms attach to the biofilm.

1.4 Types of biofoulers

There were many types of biofoulers in the marine water. According to Chai *et al.* (2008), macrofoulers consists of tubeworms, barnacles, bryozoans, algae, hydroids, mussels and oysters. In this research, four biofoulers, namely barnacles, tubeworms, oyster spat and bryozoans were chosen. Meanwhile, snails and sea urchins were chosen as anti-foulers in this research at Pulau Betong.

1.4.1 Barnacle

Barnacles are hard-shelled crustaceans found on most substrates immersed in seawater and brackish water (Plate 1.1). Barnacles are distributed worldwide and are found in all the marine environments from high intertidal zone to the depth of the ocean. Barnacles have gained economic importance owing to their presence in the hard fouling community and have been widely used in studies (Barnes and Barnes, 1975; Hurley, 1973; Crisp, 1974; Wethey, 1986).

Most of the barnacles are hermaphrodites (Charnov, 1987), they are usually capable of producing eggs and sperms and fertilization is internal at the same time. Sperms exchange between adjacent individuals which leads to cross fertilization appears among acorn barnacles. However, incidences of self-fertilization have also been reported (Barnes and Crisp, 1956; Furman and Yule, 1990; El-Komi and Kajihara, 1991).

In a polluted area, barnacles are resistant to chemical pollution where it can serve as a bioindicator organism (Niyogi *et al.*, 2001). Thus, barnacles are used as

biomonitors whereby the level of metal pollution can be determined by the accumulation of concentrations of poisonous metals such as cadmium, lead, chromium, copper and zinc, which in turn, helps in management plans. The sources of high metal availability can also be traced which has been applied in the significantly contaminated Hong Kong Harbour (Masala *et al.*, 2001; Rainbow *et al.*, 2003).



Plate 1.1: Barnacles (Balanus sp.).

1.4.2 Snail

Snail is undoubtedly the most harmful enemy among biofoulers. *Urosalpinx* sp. is a snail which was first discovered in 1920, after it had been accidentally introduced into England among the American oyster imported for relaying (Hancock, 1969). In Pulau Betong Malaysia, *Thais* sp. was found in this area (Tan and Sigurdsson, 1996) with enormous number and does tremendous damage to the aquaculture industries.

Generally *Thais* sp. (Plate 1.2) preys on barnacles, bivalves and other gastropods (Taylor, 1980). This snail feeds on oysters by drilling a hole through the shell and sucking out the flesh, and it prefers oyster spat of thumbnail size to any other food. In River Crouch (England), during only a few months over 500,000 oyster spat were drilled (Hancock, 1969). The snails themselves fall through the rigging of the dredges, and newly-born snails of little more than pinhead size are usually not detected. Drill holes may be difficult to see and the drilled valves of spat quickly disintegrate.



Plate1.2: Snail (Thais sp.).