POPULATION ECOLOGY OF A SEA URCHIN, *DIADEMA SETOSUM* FROM CORAL COMMUNITIES IN THE GULF OF THAILAND

JAMREARN BUARUANG

A THESIS PRESENTED TO RAMKHAMHAENG UNIVERSITY
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF MASTER OF SCIENCE
(BIOLOGY)
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นิเวศวิทยาประชากรของเม่นทะเล *Diadema setosum* บริเวณกลุ่มปะการังในอ่าวไทย

จำเริญ บัวเรือง

วิทยานิพนธ์เสนอต่อมหาวิทยาลัยรามคำแหง
เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญา
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Thesis Title	Population Ecology of a Sea Urchin, *Diadema setosum* From Coral Communities in the Gulf of Thailand	
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ABSTRACT

Thesis Title : Population Ecology of a Sea Urchin, *Diadema setosum* From Coral Communities in the Gulf of Thailand
Student's Name : Mr. Jamrearn Buaruang
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Studies on population ecology of a sea urchin, *Diadema setosum* from coral communities at Khang Khao Island, Chonburi Province (Inner Gulf of Thailand) and Samet Island, Rayong Province (Eastern Gulf of Thailand) were carried out during July, 2000 to September, 2002. Average of population densities of *D. setosum* at Khang Khao Island was 7.04 ± 0.56 individuals/m^2 whereas that at Samet Island was only 2.94 ± 0.24 individuals/m^2. Average of test diameters of *D. setosum* at Samet Island was 71.16 ± 0.79 mm which was statistically different from that of Khang Khao Island (only 43.63 ± 0.21 mm). Recruitment of *D. setosum* at Khang Khao Island was in a range of 0.69-3.13

individuals/m^2. High densities of recruits at Khang Khao Island were found in January, July, October, 2001 and September, 2002 while those at Samet Island were 0.27-0.49 individuals/m^2 with peaks of recruitment in July, 2000 and July, 2001. Sea urchin recruits found in the filed on dead coral were higher than on hard substrate (fragments of rock) and live coral, respectively. Gut contents of *D. setosum* were mainly sand, granule, algae and diatom. Mean of growth rates of newly settled of *D. setosum* from the experimental settlement devices was 2.66 ± 0.43 mm/month. On the other hands, the sea urchin growth rate estimated from the Brody-Bertalanffy model, by obtaining the data from tetracycline tagged individuals was 21.337 mm yr^{-1}. Mortality rates observed in the field were very low. However, the annual mortality rate from regression method was 51.6% yr^{-1}. The relationships between gonad weight and test diameter were also analyzed by using non-linear regression. The results revealed that there was a significant relationship at Khang Khao Island but there was not statistically significant at Samet Island The present study not only shows implications for coral reef management but also provides important basic data for commercial cultivation of *D. setosum* in the future.

บทคัดย่อ

ชื่อเรื่องวิทยานิพนธ์ : นิเวศวิทยาประชากรของเม่นทะเล *Diadema setosum* บริเวณกลุ่มปะการังในอ่าวไทย
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3. รองศาสตราจารย์สุภาวดี จุลละศร

การศึกษานิเวศวิทยาประชากรของเม่นทะเล *Diadema setosum* บริเวณกลุ่มปะการังเกาะค้างคาว จังหวัดชลบุรี (อ่าวไทยตอนใน) และเกาะเสม็ด จังหวัดระยอง (อ่าวไทยฝั่งตะวันออก) ตั้งแต่เดือนกรกฎาคม 2543 ถึงเดือนกันยายน 2545 พบว่าความหนาแน่นของประชากรเม่นทะเล *D. setosum* บริเวณเกาะค้างคาวมีค่าเฉลี่ย 7.04 ± 0.56 ตัวต่อตารางเมตร มากกว่าบริเวณเกาะเสม็ด ซึ่งมีความหนาแน่นประชากรเฉลี่ยเพียง 2.94 ± 0.24 ตัวต่อตารางเมตร อย่างไรก็ตามเม่นทะเล *D. setosum* บริเวณเกาะเสม็ดมีขนาดเส้นผ่าศูนย์กลางเฉลี่ยสูงถึง 71.16 ± 0.79 มิลลิเมตร ส่วนบริเวณเกาะค้างคาวมีขนาดเส้นผ่าศูนย์กลางเฉลี่ยเพียง 43.63 ± 0.21 มิลลิเมตร การทดแทนประชากรของเม่นทะเล *D. setosum* บริเวณเกาะค้างคาวมีความหนาแน่นเฉลี่ยอยู่ในช่วง 0.69–3.13 ตัวต่อตารางเมตร และมีความหนาแน่นมากในเดือนมกราคม กรกฎาคม ตุลาคม 2544 และเดือนกันยายน 2545 ส่วนเกาะเสม็ดมีความหนาแน่นเฉลี่ยอยู่ในช่วง 0.27–0.49 ตัวต่อตารางเมตร พบมากในเดือนกรกฎาคม 2543 และเดือนกรกฎาคม 2544 การทดแทนประชากร

เม่นทะเลพบมากบริเวณซากปะการัง พื้นแข็งที่มีก้อนหินขนาดเล็ก และบนปะการังมีชีวิต ตามลำดับ อาหารในกระเพาะของเม่นทะเลส่วนใหญ่ที่พบคือทราย เศษหินขนาดเล็ก ซากปะการัง สาหร่าย และไดอะตอม อัตราการเจริญเติบโตเฉลี่ยของเม่นทะเล *D. setosum* ที่วิเคราะห์จากอุปกรณ์ดักจับตัวอ่อนในทะเลมีค่า 2.66 ± 0.43 มิลลิเมตรต่อเดือน ในขณะที่ผลการวิเคราะห์จากแบบจำลองทางคณิตศาสตร์พบอัตราการเจริญเติบโต 21.337 มิลลิเมตรต่อปี อัตราการตายของเม่นทะเลในธรรมชาติมีค่าน้อยมาก อย่างไรก็ตามเมื่อวิเคราะห์ Aristotle's lantern ของเม่นทะเลจากการฉีดด้วย tetracycline พบว่าอัตราการตายต่อปีมีค่า 51.6 เปอร์เซ็นต์ จากการวิเคราะห์การถดถอยเชิงเส้นพบว่าน้ำหนัก gonad ของเม่นทะเลที่บริเวณกลุ่มปะการังเกาะค้างคาวมีความสัมพันธ์กับขนาดเส้นผ่าศูนย์กลางแต่บริเวณเกาะเสม็ดไม่มีความสัมพันธ์กัน การศึกษาครั้งนี้ไม่ได้มีความสำคัญเฉพาะด้านการจัดการแนวปะการังเท่านั้น แต่ยังใช้เป็นข้อมูลพื้นฐานที่สำคัญสำหรับการเพาะเลี้ยงเม่นทะเลเชิงพาณิชย์ ในอนาคตอีกด้วย

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Jamrearn Buaruang

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ABBREVIATIONS

µm = micrometer

ANOVA = analysis of variance

cm = centimeter

DNA = dexyribonucleic acid

e.g. = *exempli gratia* (for example)

etc = et cetera

et al. = *et alii*; and others

GI = gonad index

GIS = geographic information system

GPS = global positioning system

GW = gonad weight

i.e. = *id est* (that is)

ml= = milliliter

mm = millimeter

PIT = passive integrated transponder

RMA = reduced major-axis

SCUBA = self-contained underwater breathing apparatus

SE = standard error

SEM = scanning electron microscope

sp. = species

SST = surface seawater temperature

TD = test diameter

UV = ultraviolet

var. = varietas/variety

yr = year

CHAPTER 1

INTRODUCTION

Population ecology is based two attributes of living organisms: birth and survival (Ebert 1999, 1). Biotic and abiotic factors inherent to different environments can influence the characteristics of resident populations. Population characteristics including density, spatial distribution size frequency, age structure, and juvenile recruitment, as well as somatic and reproductive growth, reproductive periodicity and mortality may be influenced by habitat-specific factors. The structure of populations of sea urchins may differ even in similar areas of close proximity (Beddingfield and McClintock 2000, 18), and may be based on differential recruitment success and post-settlement survival (Ebert 1983, 169-302), differences in the quality or quantity of available food resources, or levels of predation. Population demographics may also be influenced by abiotic factors, particularly in the extreme limits of biogeographic distribution (Beddingfield and McClintock 2000, 235-240). Both asymptotic body size and variations in the initiation, timing, intensity and duration of reproductive events may be set by local environment conditions in sea urchin populations (Ebert 1968, 1075-1091; King et al. 1994, 95-106).

In marine systems, coral reefs are among the most diverse habitats on the globe (Bellwood and Hughes 2001, 1532), and one of the excellent resources for human especially the fisheries (Nisit Ruengsawang and Thamasak Yeemin 2000, 289). A recent trend in fisheries in many parts of the world is increasing on nearshore invertebrate species (Pfister and Bradbury 1996, 298). Populations of sea urchins are declining in regions with developed fisheries. It is evident that land-based aquaculture will be necessary for timely development and management of industry (Watts et al. 1998, 1591). Gonad production of wild-caught sea urchins has been achieved using prepared diets in a number of different sea urchin species including: *Evechinus chloroticus* (Barker et al. 1998, 1583), *Loxechinus albus* (Lawrence et al. 1997, 91), *Paracentrotus lividus* (Fernandez and Pergent 1998, 1574-1581; Spirlet et al. 2000, 85-99), *Strongylocentrotus droebachiensis* (Walker and Lesser 1998, 663-676; Pearce et al. 2002a, 301-323; 2002b, 307-332; Robinson et al. 2002, 289-303) and *S. franciscanus* (McBride et al. 1997, 357-365; 1999, 364-370). While the use of prepared diets to increase urchin gonad yield has been substantially documented, the quality of gonads from individuals fed these diets has received considerably less attention (Watts et al. 1998, 1591; Pearce et al. 2002b, 307-332; Robinson et al. 2002, 289). Gonad yield is an important factor in the commercial exploitation of sea urchins (Hågen 1998, 1533). Sea urchin gonads are considered a delicacy and fetch high prices in the Asian and Mediterranean markets (Jimmy et al. 2003, 261).

The most important market in the world is Japan. Sea urchin roe (both male and female gonads, *uni* in Japanese) are marketed under different forms: fresh (65%), but also dried, salted, frozen or cooked (35%). According to various authors, the main species exploited in Japan are *S. intermedius* (A.

Agassiz), *S. nudus* (A. Agassiz), *Heterocentrotus pulcherrimus* (A. Agassiz), *Pseudocentrotus depressus* (A. Agassiz), *Anthocidaris crassispina* (A. Agassiz) and *Tripneustes gratilla* (L.) (Hagen 1996a, 6-19). Both *S. droebachiensis* (Müller) and *S. franciscanus* (A. Agassiz) are imported from North America (Grosjean 2001), while *Loxechinus albus* Molina is imported from Chile (Lawrence et al. 1997, 91-96). The Japanese market is quite stable, around 60,000 tons of fresh echinoids per annum, since several years and accounts for more than 95% of the whole world sea urchin market. Average of current landings in Japan was 14,000 tons per year. Japanese imports a total of approximately 5,000 tons of roe under different forms, corresponding to 40,000 to 50,000 tons of live sea urchins (Hagen 1996a, 17).

The sea urchin, *Diadema setosum* (Echinoidea; Diadematoida; Diadematidae) is a monopyletic marine invertebrate which is widespread throughout of the tropical Indo-Pacific (Pearse 1998, 777), as well as in the Gulf of Thailand (Nisit Ruengsawang and Thamasak Yeemin 2000, 289), and often plays major roles in controlling macroalgal populations and organizing the structure of shallow subtidal communities (Harrold and Reed 1985, 1160). Nisit Ruengsawang and ThamasakYeemin (2000, 290) resurveyed the distribution and abundance of *D. setosum* in coral communities of Khang Khao Island and concluded that *D. setosum* is a dominant species in this area throughout thirteen years.

Studies on population ecology of a sea urchin, *D. setosum* from coral communities in the Inner and the Eastern Gulf of Thailand are very important because of its ecological roles in coral reefs, an application as a bioindicator of marine environment, its eggs for human consumption and high potential for commercial aquaculture. The study on populations of *D. setosum* at Khang Khao Island, Chonburi Province, and Samet Island, Rayong Province, focused on population structure, size-frequency distribution, recruitment, growth rate, mortality rate, gut content and gonad index.

Objectives

The major objectives of this study are as follows:

1. To study population ecology of a sea urchin, *D. setosum*, from coral communities in the Inner and the eastern Gulf of Thailand by focusing on population structure, size-frequency distribution, recruitment, growth rate, mortality rate and gonad index.
2. To provide important basic data for development of commercial cultivation of *D. setosum* in the future.
3. To apply the finding as fundamental data for management of living resources in coral communities.

Hypothesis

Population parameters of a sea urchin, *D. setosum*, from coral communities in the Inner Gulf of Thailand are different from the eastern Gulf of Thailand.

Scope of Research

This research concentrates on population ecology of a sea urchin, *D. setosum* from coral communities at Khang Khao Island, (Chonburi Province) in the Inner Gulf of Thailand and at Samet Island, (Rayong Province) in the Eastern Gulf of Thailand. Population structure, size-frequency distribution, recruitment, growth rate, mortality rate and gonad index of *D. setosum* are examined to obtain basic data for commercial cultivation in the future.

CHAPTER 2 .

LITERATURE REVIEW

Reproduction

Reproduction of the widespread Indo-Pacific sea urchin *Diadema setosum* (Leske) has been the subject of several investigations. Gametogenesis is synchronous among individual of *D. setosum*, and spawning occurs rhythmically, probably more or less monthly, during the spawning period (Pearse 1968, 247; Payad Mahamad 1997, 3-6; Chatchadaporn Sankanurak 1998). Seasonally changing seawater temperatures are often believed responsible for seasonal patterns of reproduction. Populations of *D. setosum* near the equator are reproductively active throughout the year and breeding in this species proceeds only when seawater temperature is above 25 °C (Pearse 1968, 247; 1970, 697-720). Sea urchin species living near the equator spawn for a longer period of time than the temperate species (King et al. 1994, 95-106; Guillou and Michel 1994, 97-111; Byrne et al. 1998, 305; Meidel and Scheibling 1998, 461; 2001, 97-110).

Lunar cycles also affect the gametogenesis in echinoid and apart from annual or semi-annual rhythms, monthly or semi-monthly rhythms of spawning are also known. Such rhythms are more pronounced in diadematoid populations. *D. setosum* and *D. mexicanum* gonads were full of gametes near the time of full moon (Lession 1984, 335; Pearse 1990, 311; Kobayashi

1994b, 95-98). While *D. antillarum* and *Centrostephanus coronatus* had mature gonads at the time of new moon (Lession 1984, 335-337), some populations of *D. setosum* and *D. antillarum* did not show any lunar rhythms. Some authors stated that lunar rhythm may also be depending on other factors like water temperature and moon light (Pearse 1968, 247-279). Lunar cycle was not a major factor for spawning of *Holopneustes purpurascens* (Williamson and Steinberg 2002, 527).

Hori (1991, 249-255) described that the sex ratio in *D. setosum* from Singapore was more skewed towards the male side in a sample of around 459 animals. Sex ratios deviating from 1:1 are also known for the same species originating from different localities. Alsaffar and Lone (2000, 852-853) reported that *D. setosum* sex ratio of male to female was 1:1, whereas this ratio for the *Echinometra mathaei* was one female to three male. There was a seasonal shift in the ratio of male and females in the population of *Lytechinus variegatus* at Key Biscayne (McCarthy and Young 2002, 165). *D. setosum* and *E. mathaei* had five stages of gametogenesis which could easily be defined in both male and female. These stages were (1) recovering, (2) growing, (3) premature, (4) mature and (5) spent, similar to reported for other urchin species (King et al. 1994, 95-106; Byrne et al. 1998, 305; Meidel and Scheibling 1998, 466-471; Walker and Lesser 1998, 669-672).

The synchronicity in the reproductive cycles noted for separate populations of the same species led to the observation that this entrainment was controlled by exogenous factors such as photoperiod or temperature (King et al. 1994, 95; Byrne et al. 1998, 305-310; Kelly 2000, 223-240; 2001, 67-80). The factors, which control the synchronization of spawning, have also been investigated in a number of species and are similarly often identified by studying coincident environmental parameters such as water temperature, food availability and day length (Byrne et al. 1998, 308; Guillou et al. 2000, 183-196). Pearse et al. (1986, 107) showed that photoperiod controlled both gametogenesis and growth in the echinoid *S. purpuratus* by influencing energy allocation between somatic and reproductive effort. On the Pacific coast of North America, this species spawns mainly in January and February, suggesting that either long days inhibit or short days enhance gametogenesis in this 'short day' species. A mass synchronous spawning of the sea urchin *Evechinus chloroticus* (Valenciennes) was occurred between 17.30 hrs and 18.30 hrs on 27 January 1994 (Lamare 1998a, 111), and coincided with a full moon, spring tide (Lamare and Stewart 1998b, 135; Wing et al. 2003, 109-123), and a period of decreasing sea temperature (Lamare and Stewart 1998b, 135). The spawning season of the green sea urchin, *S. droebachiensis* was occurred mainly in March and April, 2000 (Wahle and Gilbert 2002, 375). Further investigations into the role of factors such as population size and degree of aggregation, fecundity, and spawning rate, as well as flow regime on fertilization success in various echinoderms are required (Levitan 1991, 261-268).

Recruitment and Settlement

In marine benthic organisms with a pelagic larval phase, assessment of recruitment regulation necessitates estimates of larval supply and initial settlement densities (Pearce 1997, 283-341; Moksnes and Wennhage 2001, 257). A variety of factors influences recruitment patterns in benthic marine species. On a global scale current patterns and reproductive success of adult populations are the two important parameters. On a local scale substrate characteristics, water motion and established resident populations all play potential roles in determining recruitment success (Harris and Chester 1996, 212). A number of review articles have been published on echinoderm larval development and settlement (Ebert 1983, 169-203), but few of these have focused specifically on the cues that induce settlement and/or metamorphosis. Recruitment of marine invertebrates with a planktonic larval stage can be broadly divided into 3 components: (1) pre-settlement process comprising larval production and larval development, mortality and transport; (2) settlement and metamorphosis; and (3) post-settlement growth and mortality of juveniles (Lamare and Barker 2001, 153).

Nisit Ruengsawang and Thamasak Yeemin (2000, 291) reported that recruitment of *D. setosum* after the event of coral bleaching seems to increase in coral communities at Khang Khao Ialand. Settlement and recruitment of the sea urchin *Evechinus chloroticus* was determined by a series of field and laboratory experiments on the South Island of New Zealand. Settlement and recruitment were higher in Doubtful Sound (SE South Island) than in the Tory

Channel (NE South Island). A large settlement in Doubtful Sound was between August 1992 and February 1993 (1.14 settlers sample/day) (Lamare and Barker 2001, 153). Two populations of *Paracentrotus lividus* from the Northwestern Mediterranean were major recruitment in summer (Lozano et al. 1995, 179). Harris and Chester (1996, 207-215) reported that densities of *S. droebachiensis* recruiting in Casco Bay and the Isles of Shoals were two orders of magnitude higher than those from Eastport and Winter Harbor. Cameron and Schroeter (1980, 243-247) performed settlement assay, both in the laboratory and the field, with competent echinoplutei of *S. franciscanus* and *S. purpuratus*. They found that rock chips taken from urchin burrows and from nearby rocks without urchins induced similar percentage metamorphosis of larvae of *S. purpuratus*, whereas a significantly smaller percentage of larvae of *S. franciscanus* metamorphosed on the former than the latter. In California and Oregon, USA, recruitment of the sea urchins *S. franciscanus* and *S. purpuratus* varied spatially and between years (Ebert 1983, 169; Pearce and Hines 1987, 275; Ebert and Russell 1988c, 286-294; Ebert et al. 1994b, 41; Miller and Emlet 1997, 83). Newly settled *S. franciscanus* and *S. purpuratus* commenced feeding at 9 days (Miller and Emlet 1999, 67). Newly settled *E. chloroticus* normally feed on microbial surface films, filamentous algae and crustose *Coralline* algae for the first 150 to 200 days (Lamare and Mladenov 2000, 17-43). Red sea urchin (*S. franciscannus*), residing in shallow habitats in Bodega Bay, California are morphologically distinct and possibly enhance recruitment by spawning larvae and sheltering juvenile (Rogers-Bennett et al. 1995, 1171).

Certain aspects of settlement conditions have been observed such as growth, size-weight relationship, periodic increase, as well as data related to survival (Catoira Gomez et al. 1995, 255). Schroeter et al. (2002, 1-12) reported that the patterns of settlement of red (*S. franciscanus*) and purple (*S. purpuratus*) sea urchins may have important impacts on the commercial fishery. They have monitored weekly sea urchin settlement at multiple sites in southern and northern California since February 1990 and including an additional season in 2002. Significant findings to date include: (1) Settlement of both species of sea urchins is generally seasonal and discontinuous. The settlement "season" generally occurs between January and August, however, the timing of abrupt peaks in settlement vary from year to year. (2) Settlement peaks tend to occur within the same quarters of a given year over large geographical areas. Despite this, there are site-to-site differences in settlement and differences in the exact timing of settlement within a quarter. (3) Marked differences in settlement patterns occur between northern and southern California. Settlement is generally greater and more frequent in the Southern California Bight than in northern California, and the interannual variation in settlement is greater in northern California. (4) Settlement responds to the changes in large-scale oceanographic conditions associated with El Niños in both northern and southern California, but these responses differ markedly between regions. In northern California large settlement events occurred during the relaxation of upwelling associated with El Niño conditions in 1992, 1993, 1997 and 1998. By contrast, settlement in southern California was lower during the El Niños and increased following the end of the El Niño conditions.

Bak (1985, 267-272) used artificial substrate (white plastic sheet sandwiched between two such grates and white plastic grates with a size of 27 × 57 cm) to examine newly settlement of *D. antillarum*. Harrold et al. (1991, 81-94) used plastic matrix (i. e., polystyrene cube louver light diffuser) to examine settlement and metamorphosis of *S. purpuratus* both in the laboratory and the field. Ebert et al. (1994b, 41-52) used wood-handle scrub brushes to examine temporal and spatial settlement patterns of *S. franciscanus* and *S. purpuratus* in California, USA. Harris et al. (1994, 701-706) set up artificial turf collectors to study the temporal and spatial patterns of settlement of *S. droebachiensis* in the Gulf of Maine, USA and found that settlement densities varied with depth of the collector.

Density, Spatial Distribution and Size Structure

Population densities of *D. antillarum* are important in ecology of both intertidal and coral reef communities, and there is nothing in the literature on biological variations between geographically distant populations. Historical population densities of *D. antillarum* in the Florida Keys were similar to Caribbean reefs (Bauer 1980, 509-515), but declined after the 1983–1984 mortality event, even suffering a second mass mortality in 1991 (Forcucci 1994, 917-928; Chiappone et al. 2002a, 117). Adult *D. antillarum* populations were recently discovered up-current of the Florida Keys in the Dry Tortugas

(Chiappone et al. 2001, 137-138) and in the western Caribbean (Moses and Bonem 2001, 327-336). Despite urchin density increases in some up-current locations, densities in the Florida Keys remain dramatically lower than historical numbers (Chiappone et al. 2002b, 155-159). Size structure of the purple sea urchin *S. purpuratus* was sampled from central California to central Oregon. There were differences in the size frequency distribution of populations from the areas (Ebert and Russell 1988c, 286-294). Beddingfield and McClintock (2000, 17-40) studied the sea urchin, *Lytechinus variegatus* in three habitats of Saint Joseph Bay, Florida and found densities ranged as high as 35 individuals/m^2.

Diet and density of *Evechinus chloroticus* (Val.) were interacted in influencing both the size of gonads and their histological condition (Andrew 1986, 63). Pearse (1998, 777-782) reported that two species of long-spined sea urchins, *D. savignyi* (Michelin 1845) and *D. setosum* (Leske 1778), were sympatric throughout much of the Indo-Pacific and both species were found. They often form mixed species clumps, with higher proportions of *D. savignyi* in offshore locations and higher proportions of *D. setosum* in inshore locations. *Astropyga radiata,* a species of Diadematidae, has a wide-spread distribution in the Indo-West Pacific region (Nojima 1988, 93). Tsuchiya et al. (1986, 75-96) and Nisit Ruengsawang and Thamasak Yeemin. (1998, 215-220) studied on distribution of subtidal macrobenthic animals around Khang Khao and Thai Ta Mun Island. They found that *D. setosum* was extremely abundant and may play an important role in organization process of the subtidal community.

Certain experiments were performed in Discovery Bay (Jamaica, W. I.) to determine the relationship between behavior of threespot damselfish *Eupomacentrus planifrons* Cuvier, and distribution of the common shalow-water echinoid *D. antillarum* Philippi (Sammarco and Williams 1982, 58). Palacín et al. (1998, 281) studied interactions between urchins and algae in temperate areas and showned an important structuring effect of sea urchin populations. Some experimental studies on the effect of different densities of sea urchins on algal populations have been performed (Andrew and Underwood 1993, 282). A response to a complex set of physical and biological variables including size of populations, availability of food, presence of predators and water motion was examined (Carpenter 1984, 101-108). McClanahan and Shafir (1990, 233) found that at the lowest levels of predation a near-monoculture of the rock-boring sea urchin *Echinometra mathaei* can develop and reach a total wet weight >5000 kg/ha probably near the carrying capacity for this particular species. Nine species of herbivorous sea urchin commonly inhabit shallow-water coral reefs of the Indian Ocean. There was evidence for partitioning of the spatial refuge resource among species particularly for *Echinometra mathaei*, *Tripneustes gratilla* and *D. setosum* at the low to intermediate levels of predation. There was, however, a large overlap in the peak abundance of *D. savignyi*, *Echinothrix diadema*, *Toxopneustes pileolus* and *Stomopneustes variolaris* at the highest levels of predation (McClanahan 1998, 231). The availability and spatial arrangement of refuges can have important effects on the patterns of foraging at small spatial scales, both in subtidal habitats and in littoral rock pools (Benedetti-Cecchi and Cinelli 1995, 203-212; 1998, 203-211).

Growth and Mortality

Growth rate is a critical parameter of the population dynamics of a species. For many populations of marine invertebrate species, however, accurate estimates of growth rate are lacking. This is due, in part, to the difficulties associated with sampling, monitoring and tagging individuals in marine populations. A further problem is the application of a suitable growth model to marine invertebrate populations (Ebert and Russell 1993, 79-89). Pearse and Pearse (1975, 731-753) added each year for all sizes of sea urchins before these data can be used to measure growth reliably. Growth rates were determined from size frequency analyses of randomly sampled urchins at Boca Raton (Bauer 1976, 273). Rahman et al. (2000, 45) studied comparative viability and growth of hybrids between two sympatric species of sea urchins (Genus *Echinometra*) in Okinawa. The jaw length of demi-pyramids was considered a good indication of the relative size of an urchin's feeding apparatus (Ebert 1980, 467-474; Black et al. 1982, 101; 1984, 81-97; Morris and Campbell 1996, 777). Model II non-linear analog of reduced major-axis (RMA) regression that minimizes areas similar to triangles that are minimized in RMA regression. Data for two tropical sea urchins, *Salmacis belli* and *Heterocentrotus mammillatus*, illustrate the good method (Ebert and Russell 1994a, 367). Lamare and Mladenov (2000, 18) examined growth of the New

Zealand sea urchin, *E . chloroticus*, in two populations. Growth in very small sea urchins (<1 year old) was measured by in vitro rearing and the analysis of size frequency distributions of newly settled urchins in the field. Older sea urchins (>1 year old) were tagged in situ using a fluorescent dye, and growth after 1 year modelled using four growth models. Three of the models are asymptotic growth functions, the Brody-Bertalanffy growth model

$$S_t = S_\infty(1 - be^{-kt})$$

the Richards growth model,

$$S_t = S_\infty(1 - be^{-kt})^{-n}$$

and the Jolicoeur growth model

$$S_t = S_\infty(1 - be^{-kt})^{-1}$$

where S_t = size at time t, S_∞ = asymptotic size, b = scaling parameter to adjust for size $\neq 0$ at time 0, K = growth constant and n = shape parameter for the Richards model. The fourth model applied was the Tanaka growth model

$$S_t = \left(\frac{1}{\sqrt{f}}\right)\ln\left(\left|2f(t - c) + 2\sqrt{f^2(t - c)^2 + fa}\right|\right) + d$$

where S_t = size at time t, and f, c, a and d are growth parameters. In contrast to the three asymptotic growth models, the Tanaka model incorporates an initial period of slow growth, a period of exponential growth followed by an indefinite period of slow growth (Ebert 1998, 619-627; Russell 2001, 533-538).

The cohort separation method is commonly used with species displaying annual recruitment (Ebert and Russell 1993, 79; 1999). Cohorts are separated by time increments of one year. Usually, the youngest individuals recruited in the year form a well defined cohort whose peak displacement with time can be used to estimate mean growth rate (Lumingas and Guillou 1994, 671-686). The cohorts are implicitly assumed to be unimodal and normally, or at least, symmetrically distributed. Neither authors working on sea urchins tested the validity of this fundamental assumption, nor do they discuss implications of violations of this assumption. When individuals interact, cohorts can be asymmetrical, or even multimodal (Grosjean et al. 1996, 173-184). Ebert (1968, 1075-1091) observed wide variations in growth rate of *S. purpuratus* (Stimpson) in some habitats, and attributed it to food limitation. The results generated generally reveal a greater growth with formulated diets than with their natural counterparts (Fernandez and Caltagirone 1994, 655-660).

The method to estimate age of sea urchins by using the natural growth bands is common. The trabecules within the sea urchin skeleton are more or less densely packed depending on growth rate (Pearse and Pearse 1975, 731-753). A succession of fast and slow growth stages results in light and dark bands, respectively, in the stereom of the ossicles (Pearse and Pearse 1975, 731; Lumingas and Guillou 1994, 671). It is postulated that there is only one period of fast growth and another period of slow growth per year. If this is true, counting these growth bands allows determining the ages of the echinoids. If there is a single recruitment in a narrow time window during the year (Ebert 1983, 169-302).

Many authors considered that if they use both methods simultaneously cohort separation and growth rings analysis and get the same result (Lumingas and Guillou 1994, 671-686; Turon et al. 1995, 193-204). If the number of growth rings is correlated with the size, not the age, one would interpret a group of fast growing individuals as being older, and a group of slow growing ones as being younger and eventually mix animals of different age in a single cohort. This would result in an agreement between both methods although conclusions on size at age are incorrect (Grosjean 2001, 58).

Measuring relative growth (without knowing age) is an alternative to calculating growth rate of individuals in the field. Animals are tagged, field released and captured again one year later (Kenner 1992, 107-188; Ebert and Russell 1992, 31-41; 1993, 79-89; Lamare and Mladenov 2000, 17-43). Size increase of the ossicles can then be determined because a band of tetracycline-labeled skeleton, visible under ultraviolet light, indicates its size at tagging time. An allometric relationship between the size of the given ossicles and the body size allow estimating the latter (Ebert 1988a, 1407-1425). To fit such data, growth models need to be reworked, using a so-called Ford-Walford representation, or Walford plot (Ebert 1999) where size at time t + 1 year (at recapture) is expressed as a function of size at time t (at capture). Ebert (1999) reviewed such transformations for von Bertalanffy, Gompertz, logistic, Richards and Tanaka models.

Mass mortality of sea urchin *Echinometra mathaei* (Blainville) occurred on the reef flats of Okinawa, Japan in late June of 1986 (Tsuchiya et al. 1987, 375). Recurrent outbreaks of a disease results in mass mortalities of sea urchins, *S. droebachiensis* in the rocky subtidal zone of the Atlantic coast of Nova Scotia (Canada) (Scheibling and Hennigar 1997, 155). A massive mortality of *D. antillarum* occurred on the Caribbean coast of Panama in January, 1983. By January, 1984, it had spread the Caribbean; being the most widespread epidemic ever reported for a marine invertebrate (Lession et al. 1984, 335-337; Hunte et al. 1986, 135; Lession 1988, 371-393). Given its species specificity, the causative agent is thought to be a pathogen, perhaps similar to those which caused severe mortalities of the urchins *S. fransiscanus* in California (Pearse et al. 1977, 645-648). Mortality is described using a negative exponential curve including a seasonal variation to represent observed winter mass mortalities. A simplified demonstration version of this model implemented in Microsoft Excel (Grosjean and Jangoux 2000).

For populations that are seasonally stable and stationary, mortality can be estimated from the growth parameters and size distributions. Recruitment appears to be periodic in sea urchins, and such periodicity can be utilized to estimate the instantaneous mortality rate per individual. Estimates of mortality also can be obtained by estimating flow rates into and out of size classes (Ebert and Russell 1992, 32; Kenner 1992, 108). Mortality was estimated by 3 different methods that make use of the size distributions. Methods 1 uses the Richards function as a growth model and Method 2 uses size transitions without recourse to a specific model. Method 3 uses just size-frequency data (Ebert and Russell 1992, 33; 1993, 81; Ebert 1999).

Technique for Tagging Sea Urchins

Techniques for the identification of individual, or groups, of living aquatic animals have many uses in biology, ecology, fisheries and aquaculture; and a variety of marking and tagging techniques have been developed for specific application in studies of growth, behaviour, population dynamics and genetics (Hagen 1996b, 271). External sea urchin tags have been attached to the spines, wrapped around the test, or anchored in holes drilled through the test. In an excellent review of tagging methods, Hagen (1996, 271-272) concluded the techniques gave poor tag retention and increased mortality or reduced growth, except for species with large enough spines that a hole could be drilled through the spine.

Hagen (1996, 271-284) implanted PIT (passive integrated transponder) tags and found that survival and growth after 15 months were not different from the controls. Two internal and three external tags were tested on the green sea urchin (Duggan and Miller 2001, 155-122). The most common sea urchin tagging technique is group marking with tetracycline (Ebert 1980, 467-474; 1988b, 160-172; Ebert and Russell 1992, 32; 1993, 80; Kenner 1992, 108), florescent dye injections (Lamare and Maladenov 2000, 19-20) and attached by drilling holes in the skeleton (Ebert 1965, 193-194; Neill 1987, 92-94).

Grazing and Bioerosion

A common feature of the population biology of sea urchins is fluctuations in local abundance and, consequently, variability in the effects of grazing by sea urchins on the structure of communities (Benayahu and Loya 1977, 383; Chapman 1981, 307; Harrold and Reed 1985, 1160-1169; Bak 1990, 267-272; 1994, 99-103; Mokady et al. 1996, 367; Bulleri et al. 1999, 81; Hibino and van Woesik 2000, 1-14). The bioerosion impact of several species of fish, sponges and sea urchins have been estimated in the Caribbean (Griffin et al. 2003, 79). Reef morphology is a function of the balance between active growth of corals and algae and simultaneous erosion of the substrate (Scoffin et al. 1980, 475-508; Hutchings 1986, 239-252). Grazing studies on coral reefs have shown the importance of grazers and refuge from grazers on the distribution and abundance of marine plants and corals (Sammarco 1980, 245; McClanahan et al. 1994, 237-257). Sea urchins and scarid fishes were the most important grazers on the two reefs of La Saline on Réunion Island (Indian Ocean) and of Tiahura on Moorea Island (French Polynesia) (Peyrot-Clausade et al. 2000, 477). *Paracentrotus lividus* was considered to have a pivotal role in benthic ecology through control of algae by grazing (Barnes et al. 2002, 311). Conand et al. (1997, 953-958) indicated no significant difference in rate of daily erosion between the different types of *Echinometra*. The sea urchin *Centrostephanus rodgersii* (Agassiz) is the most important herbivore on subtidal reef in New South Wales, Australia. Grazing by *C. rodgersii* and associated molluscan herbivores removes foliose algae from large of reefs (Andrew and Underwood 1989, 89-90; 1993, 89-98). Nisit Ruengsawang and Thamasak Yeemin (2000, 289-294) reported that high bioerosion rates were found in the shallow zone at Khang Khao Island in the Gulf of Thailand.

Gonads, Diets and Gut Contents

A recent review of the world's sea urchin fisheries has identified a peak or decline in most of the urchin producing nations (Keesing and Hall 1998, 1597-1604). Since consumer demand remains stable, many countries are now examining the feasibility of urchin aquaculture to supply future markets. While sea urchin cultivation has been practised for decades by the Japanese, echinoid aquaculture is still in its infancy in most other countries and is primarily at the experimental stage. Research has focussed on two distinct forms of culture: (1) full life-cycle grow out where larvae are produced in hatcheries and juveniles grown to commercial size either in land-based tanks or at sea in some sort of containment system (Grosjean et al. 1996, 173-184; 1998, 1523-1531) and (2) gonad enhancement where adult urchins are harvested from wild populations, maintained in captivity, and fed natural and/or prepared feeds in order to increase gonad yield and/or quality (Lawrence et al. 1997, 91-96; McBride et al. 1997, 357-365; 1998, 1563; 1999, 364-370; Barker et al. 1998, 1583-1590; Cook et al. 1998, 1549; Kelly et al. 1998, 157;Walker and Lesser 1998, 663-676; Vadas et al. 2000, 115-135; Atchaneey Chamnansinp 2002).

Much of the research on enhancement has focussed on the formulation of prepared feeds with the aim of optimizing gonad production. A number of studies utilizing prepared diets have documented how sea urchin gonad yield can be significantly affected by factors such as meal source, protein type, and pigment concentration (Barker et al. 1998, 1583-1590), but few have examined in detail the effect of prepared feed formulation on gonad qualities such as color or taste (Agatsuma 1998, 1541-1547; Vadas et al. 2000, 115-135; Robinson et al. 2002, 298-303). Liyana-Pathirana et al. (2002, 461) examined the effect of an artificial diet on the biochemical composition of the gonads of the sea urchin (*S. droebachiensis*). The frequency of feeding in the field is variable in sea urchins, ranging from nearly continuous to diel or intermittent. It is essential to know the effect of feeding interval on physiological and metabolic processes to understand the basis for production (Lawrence et al. 2003, 69). Kawamata (1997, 107) reported that modelling the feeding rates varied with a size dependent limit in the amount of annual feeding.

Sea urchins acquire the color for their gonads through their diet and this raises an important issue for the culture of this organism. If quality and price are tied to color, then what are the most practical options for producing a sea urchin with an appropriately colored gonad? Several studies on a number of species have noted the poor color of the gonads produced with some of the currently manufactured feeds for sea urchins (Barker et al. 1998, 1583; Grosjean et al. 1998, 1523; Walker and Lesser 1998, 663; Watts et al. 1998, 1591).

The gonads are the primary soft tissue and only edible part of the sea urchin. The harvested gonads of both female and male sea urchins are called "roe" regardless of sex (Hagen 1996a, 7). The color of the gonads ranged from light yellow to dark brown. There was no relationship between color and sex, the females generally had lighter gonadal coloration (different shades of yellow) than the males that exhibited dark colored gonad. However, there was some overlap present, as far as the gonad color was concerned (Hori 1991, 249-255; Alsaffar and Lone 2000, 852). Kobayashi (1994a, 91-94; 1994b, 95-98) reported that gonads from *D. setosum* in Thailand became increasingly lighter in color (form dark brownish yellow to light yellow or transparent) as the maturity stage increased. The color and texture of the gonads has an obvious commercial importance in the development of urchin fishery (King et al. 1994, 95-106; Walker and Lesser 1998, 663-676).

In the Japanese uni trade, (uni = urchin), gonad color, texture, firmness, and flavor are extremely important factors in establishing price and marketability of the product. Promotion of high gonad yields through the use of prepared feeds may be easily achieved, but this elevated yield will be of little use to the commercial aquaculture industry if the required color, texture, and flavor are not present. The Japanese uni consumer is familiar with the color and taste of roe from wild-caught sea urchins and any cultured product that does not meet with consumer standards will be rejected. The introduction of substandard roe from cultured sea urchins to the market place could be catastrophic for the aquaculture industry, as ''cultured product'' would then evoke negative connotations and commercial buyers would pay little, if anything, for cultivated sea urchins (Pearce et al. 2002a, 302-303; 2002b, 308-309).

Environmental Factors

Temperature is a basis factor that can interact with food and essential to document the effect of this interaction on gonadal production in sea urchins that are candidates for aquaculture (McBride et al. 1997, 357; Garrido and Barber 2001, 447-456). Guillou and Michel (1994, 98) reported that growth of echinoids depended on several factors including temperature and food quantity and quality. Temperature limits for fertilization and early development were examined in the tropical sea urchin *Echinometra lucunter* (Sewell and Young 1999, 291). Sea temperature is a known and important determinant of spawning (and recruitment) in a sea urchin, *Paracentrotus lividus* (Barnes and Crook 2001a, 1205-1212; Barnes et al. 2001b, 359). The effects of rapid salinity fluctuation was associated with freshwater canal discharge on survivorship and grazing rates of two common herbivores in South Florida seagrass beds, *Lytechinus variegatus* (Echinodermata) and *Lithopoma tectum* (Gastropoda) (Irlandi et al. 1997, 869). Reductions in salinity can have adverse on larval development and larval survival in a sea urchin, *Echinometra lucunter* (Metaxas 1998, 323). Spirlet et al. (2000, 86) studied the two objectives: (1) to determine the influence of the two major abiotic parameters (temperature and photoperiod) on gonadal growth and gametogenesis of cultured sea urchins in a closed-circuit facility; and (2) to control the reproductive cycle and determine the best combination of temperature and photoperiod to obtain individuals ready for marketing as soon as possible.

The bioaccumulation of heavy metals dissolved in water was assessed to measure the concentrations recorded within sea urchin larvae (*Paracentrotus lividus*, Lmk.) (Radenac et al. 2001, 151), in body compartments of the echinoid *D. setosum* (Flammang et al. 1997, 35-45), and in the gonads (Thamasak Yeemin et al. 1998, 215-220). In addition, embryos and larvae of *D. setosum* are sensitive to numerous pollutants, including heavy metals (Kobayashi 1994a, 91-94).

Genetics

The genetics of marine populations is a subject that has made little progress compared with the effort spent on the terrestrial environment. This is so despite ''applied'' aspects such as stock management, marine aquaculture, creation of reserves, conservation of the coastal zones, taxonomy, and protection of species. The crowded and dispersive marine environment, with its steep physical gradients, favors the existence of a planktonic larval stage for most species. The attendant high fecundity has important consequences for selection differentials and dispersal and therefore for the evolution of genetic structures (Féral 2002, 121). Lession and Pearse (1996a, 126) and Lession et al. (1996b, 133-142) reported the first case of hybridization between echinoid species evaluated through genetic marker and morphology. Debenham et al.

(2000, 49) evaluated genetic structure of populations in the red sea urchin, *Strongylocentrotus franciscanus* by using DNA sequence. Allozyme electrophoresis was used to characterize genetic variation within and among natural populations of the sea urchin, *S. franciscanus* (Moberg and Burton 2000, 773).

Sea Urchin Markets and Fisheries

In recent decades, fisheries for sea urchins have developed throughout the world in response to increasing demand for the roe of these animals (Hagen 1996a, 6-19; Keesing and Hall 1998, 1597; Perry et al. 2001; 2002, 253). More recently, several fisheries have seen declines in wild populations, suggesting sea urchins may be particularly vulnerable to exploitation (Pfister and Bradbury 1996, 298-310). This is surprising considering the ability of sea urchins to respond quickly to changes in food supply by increasing reproductive or somatic growth (Blount and Worthington 2002, 341-342). However, a variety of factors are now thought to combine to increase the vulnerability of sea urchin populations, including low rates of recruitment and natural mortality, that may be inversely density-dependent (Blount and Worthington 2002, 341). Further, these factors are exacerbated in some fisheries by the presence of older individuals, or individuals with restricted access to food, that produce roe that is not preferred by the markets (Blount and Worthington 2002, 341-348). Because the attributes of roe in a sea urchin are not externally apparent to divers, this can result in wasted harvest of roe.

The value of the roe from an individual sea urchin is not simply a function of the yield of roe from the individual. In fact, larger roe are less preferred at the premium end of the market, where there is more emphasis on the quality of the roe. A variety of attributes combine to determine the quality of sea urchin roe, including in particular color, size, texture and granularity (Blount and Worthington 2002, 342-348). Whilst attributes such as freshness and texture can be maintained or enhanced through processing of the roe after harvest, this is more difficult for color or size of the roe. As a result, an ability to identify sea urchins that are more likely to contain good quality roe may reduce both processing costs, and the harvest of sea urchins with less desirable roe that may otherwise have been sacrificed. Further, because poor quality roe may be caused by a limited supply of food, selective removal of individuals most likely to contain good quality roe may enhance growth rates and the quality of roe of those that remain. Such improvements in the efficiency of exploitation are important to the management of such a vulnerable resource (Blount and Worthington 2002, 341-348; Pearce et al. 2002a, 301; 2002b, 307; Robinson et al. 2002, 289-303). Sea urchins in Fringe generally have larger roe than sea urchins of a similar size in Barrens (Byrne et al. 1998, 305-318), presumably because of their increased access to food. Spawning appears synchronous in the two habitats (King et al. 1994, 95).

The average price of roe on the Japanese market ranges from 18.6 €/kg (750 BEF/kg) for the local production (fresh animals considered as top quality), to 7.9 €/kg (320 BEF/kg) of fresh imported echinoids. These figures equate to a total market of approximately 657 millions €/y (26.5 milliard BEF/y), 397 millions €/y of which is imported (Grosjean 2001, 32-33).

The second largest market is France. Its landings are much smaller: about 1,000 tons of live echinoids per year in the 1960s and 1970s. Since these peak levels of harvests have dropped to 250 to 350 tons per annum. Spain, Ireland and Greece exported to France and compensated for the reduced local production, so the market was kept at 500 to 600 tons from 1988 to 1990. According to the same author, 185 tons transited by Rungis (Paris) in 1991. The major species in the French market are *Paracentrotus lividus* (Lamarck), but *Psammechinus miliaris* (Gmelin) and *Sphaerechinus granularis* (Lamarck). In France, most sea urchins are consumed fresh during the period when gonads are in an adequate reproductive stage, i.e., between December and March. The season limits the importation market (Grosjean 2001, 33).

Wholesale prices in Rungis fluctuate according to the roe quality (freshness, size, color, maturity stage and taste). It ranges from 4.5 €/y (180 BEF/kg) to 17.8 €/y (720 BEF/kg). Briton, and to a lesser extent, Irish sea urchins are most valued. Mediterranean strains are of lower quality because they do not withstand travel as well as Briton or Irish strains (Grosjean et al. 1998, 1523-1531).

Fishing sea urchins is very profitable during the 5 to 10 years after starting harvesting new stocks. But after that short period of time, wild populations decline due to the high efficiency and selectivity of fishing techniques. Most exploitable natural stocks are easily picked by hand at low tide, or at least using simple equipment at shallow. Growth speed is also too low in some harvested species to allow replacement of large adults (Grosjean 2001, 34). Few rules exist to limit overexploitation. Indeed, the biggest problem is that animals must be collected before they fully mature, and they have no opportunity to spawn. A lack of recruitment results from intense fishing and, consequently, a rapid decline of the standing stock (Campbell et al. 2001).

An example of such a decline in landings is the Japanese fisheries which produced 23 to 28,000 tons of whole live sea urchins per year from 1967 to 1982 (Hagen 1996a, 6-19). Landings dropped to 14,000 since 1991, despite the establishment of hatcheries (to seed in the field) and of artificial feeding of sea urchins in harvested areas. Chile and the U.S.A. also produce less than before, and only Canada and Korea are still increasing harvests because the sea urchin fisheries are more recent there. Average worldwide landings are still stable but are obviously not sustainable in a near future. Aquaculture is a necessary alternative in all countries with sea urchin fisheries (Grosjean 2001).

Aquaculture Potentials for Sea Urchins

Japan was the first country to address the issue of overexploitation, and initiated stock enhancement programs very early. These techniques include habitat enhancement (artificial reefs), artificial feeding, translocation and building of hatcheries that produce several millions of seed a year that are transplanted to the field. For instance, a single hatchery in Hokkaido produces 11 million juveniles per year. Hatcheries may be a solution to ensure recruitment where harvesting eliminates adults before they spawn, but good natural habitats are required, like large tidal pools, to give enough protection to juveniles released in the field (Hagen 1996a, 6-19; Agatsuma 1998, 1541).

Another way to enhance production is through gonad enhancement. With an adequate artificial diet, it is possible to increase gonad size (Spirlet et al. 2000, 85-99). Gonad enhancement in culture is a necessity in Canada because sea urchins are at the right stage of maturity during the winter. At this time, the sea is frozen and the collection of sea urchins under the ice by SCUBA divers is a painful and dangerous activity. One solution is to collect animals during autumn, store them in tanks, and feed them with an adequate diet before marketing them (Grosjean 2001, 35).

The use of cages in sea ranching operations is also an alternative and may be used in mono or polycultures. As for any mariculture activity, degradation of cages by waves and storms is a major problem, and site location is critical. Suitable sites are limited, and there is often strong competition for space with other mariculture activities like salmoniculture or mytiliculture on long lines. Because of their grazing activity, sea urchins erode the cage nets and are also a direct cause of depredation which increases maintenance costs (Kelly et al. 1998, 1557-1562).

CHAPTER 3

MATERIALS AND METHODS

Historical Background of the Materials

Taxa and description of *Diadema setosum* (Leske)

Kingdom : Animal
  Phylum : Echinodermata
    Class : Echinoidea
      Order : Diadematoida
        Family : Diadematidae
          Genus : *Diadema*
            Species : *setosum*

Pearse (1998, 777-782) reported main morphological characters of *D. setosum* which are distinct in the field, the orange ring around the anus, 5 conspicuous white on the interambulacrals, and pattern of iridescent blue spots (rather than lines) around the periproct and down the interambulacrals (Figure 1).

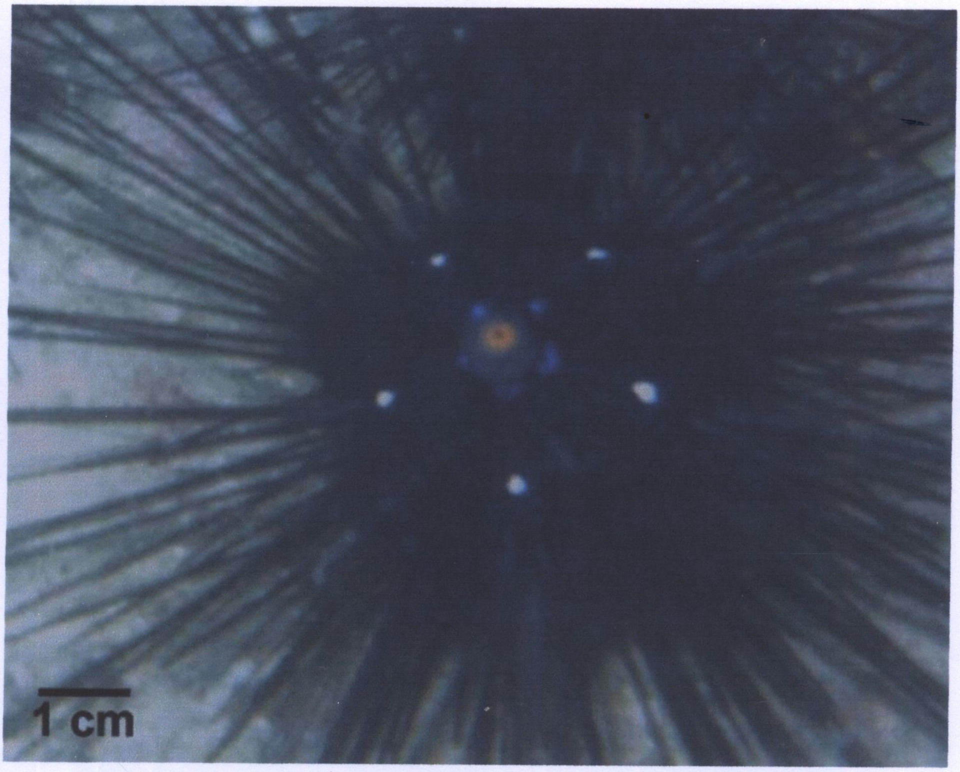


Figure 1 Living Specimen of *D. setosum*, Showing the Five Conspicuous White Spots on the Interambulacrals

Skeletal Morphology of Regular Echinoids

From an anatomical point of view, the body of the postmetamorphic echinoid has a quasi-spherical shape (for regular sea urchins such as *D. setosum*) with a pentaradial symmetry (Figure 2-3). Its shape is constrained by an endoskeleton, located just under the epidermis, composed of calcareous ossicles sutured together in a solid test. This test supports movable spines that cover the body of the animal and are the origin of the name Echinoidea, "like a hedgehog (porcupine)" (Grosjean 2001, 38).

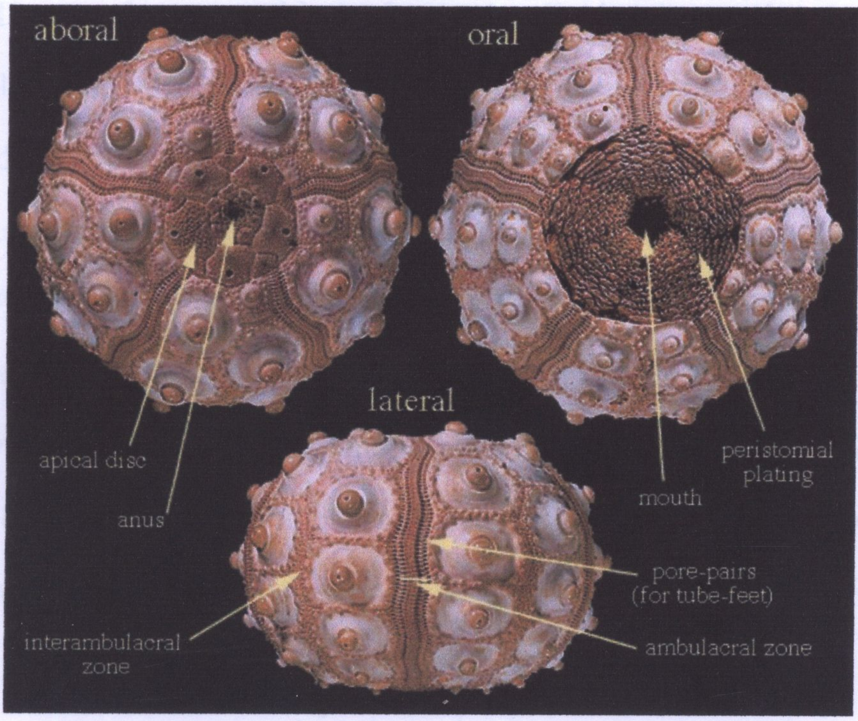


Figure 2 Skeletal Morphology of Regular Echinoids

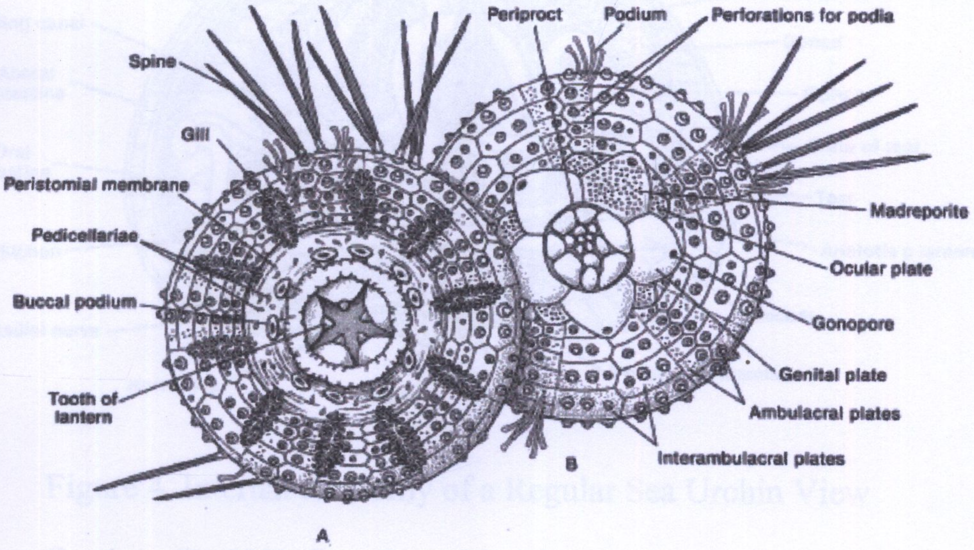


Figure 3 External Anatomy of a Regular Sea Urchin A: Oral View

B: Aboral View

Source: Grosjean, Ph. 2001. Growth model of the reared sea urchin *Paracentrotus lividus* (Lamarck, 1816). Ph. D. Thesis, Universite Libre de Bruxelles, Belgium.

The regular sea urchin body can be divided in two hemispheres: An oral pole where the mouth opens, directed towards the substratum, and an opposed aboral pole bearing the anus. The mouth opens in a short pharynx surrounded by a complex scraping apparatus recall that *D. setosum* is a grazer called Aristotle's lantern (Figure 4-6). It is composed of 5 pyramids radially arranged around the mouth and each holds one tooth (Figure 3A). The digestive tract forms two complete turns around the inner side of the test wall, one in one way and the other one in the opposite direction, leaving much space in the internal cavity for gonads (Figure 4) (Grosjean 2001, 38).

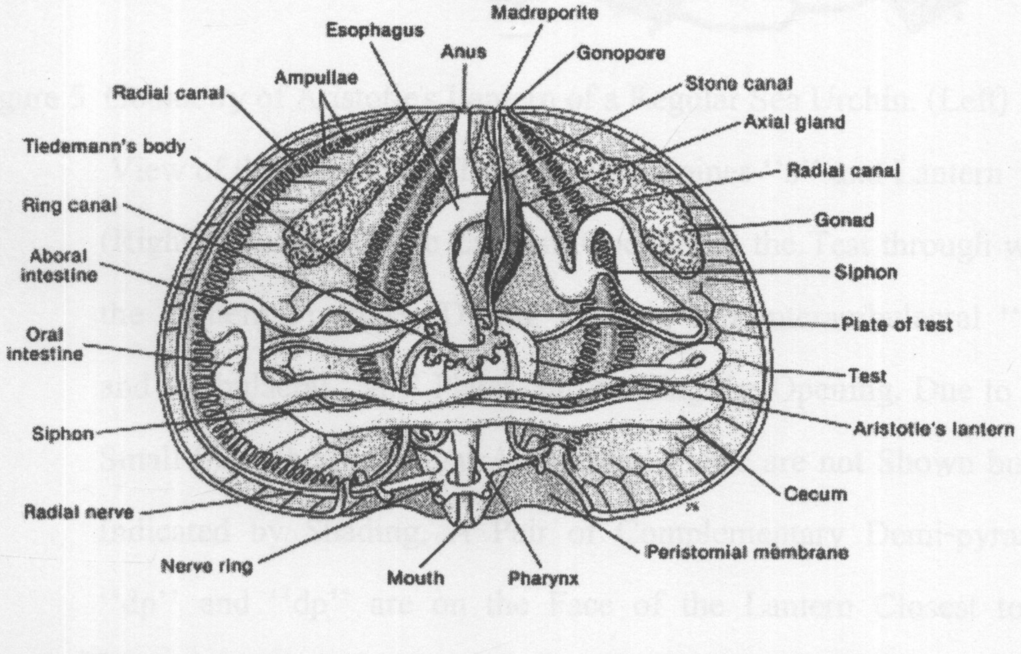


Figure 4 Internal Anatomy of a Regular Sea Urchin View

Source: Grosjean, Ph. 2001. Growth model of the reared sea urchin *Paracentrotus lividus* (Lamarck, 1816). Ph. D. Thesis, Universite Libre de Bruxelles, Belgium.

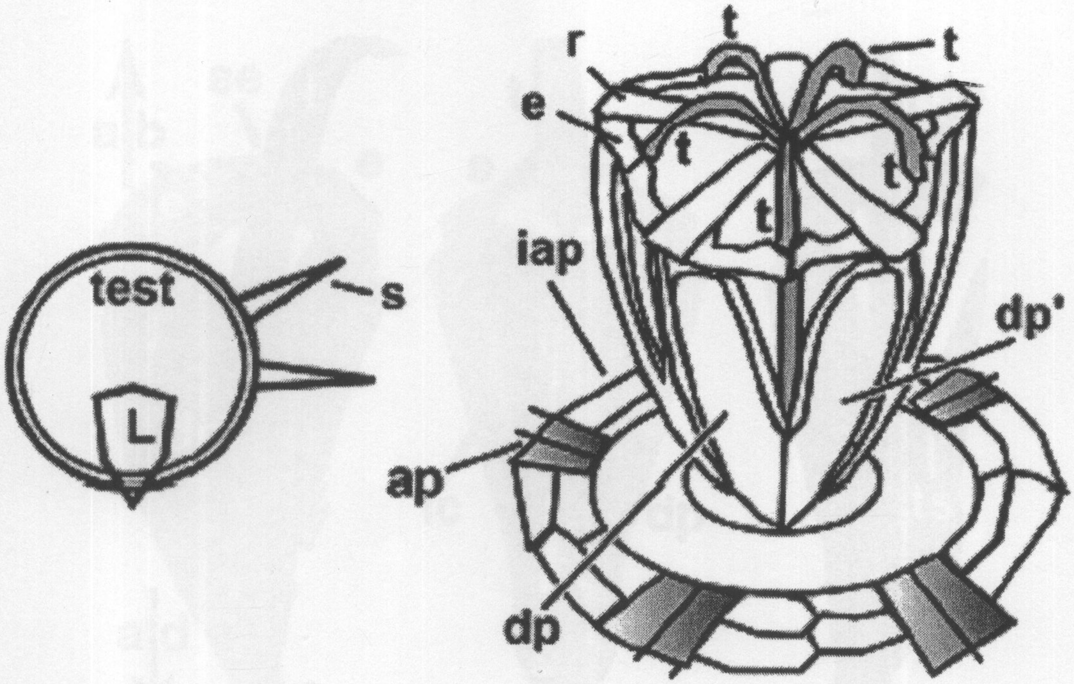


Figure 5 Geometry of Aristotle's Lantern of a Regular Sea Urchin. (Left) Side View of the Sea Urchin Test Showing Spines ‘‘s’’ and Lantern ‘‘L’’. (Right) Strucutre of the Lantern, Opening in the Test through which the Lantern Protrudes During Feeding and Interambulacral ‘‘ipa’’ and Ambulacral ‘‘ap’’ Plates Surrounding the Opening. Due to their Small Size, the Individual Ambulacral Plates are not Shown but are Indicated by Shading. A Pair of Complementary Demi-pyramids ‘‘dp’’ and ‘‘dp’’ are on the Face of the Lantern Closest to the Viewer, and all Five Teeth ‘‘t’’ of the Lantern are Labeled. An Epiphysis ‘‘e’’ and a Rotula ‘‘r’’ are also Labeled. The Aboral Ends of Lantern and Teeth Point Down in this Schematic

Source: Stock, S. R., S. Nagaraja, J. Barss, T. Dahl, and A. Veisc. 2003. X-ray microCT study of pyramids of the sea urchin *Lytechinus variegatus*. **Journal of Structural Biology** 141: 9-21.

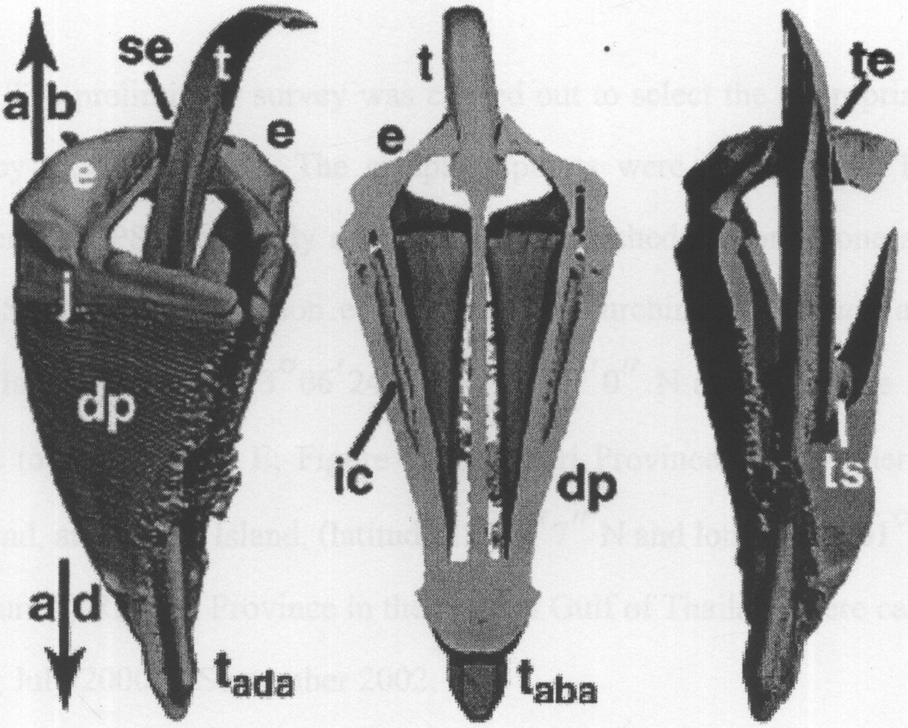


Figure 6 Three-Dimensional Renderings Based from the MicroCT Slices of the Intact Pyramid. In the Left-Hand Image, the Tooth ‘‘t’’ Spans 22.4 mm. The Middle and Right-Hand Images are Viewed after Numerical Sectioning to Expose Internal Structure (Stock et al. 2003, 10)

Source: Stock, S. R., S. Nagaraja, J. Barss, T. Dahl, and A. Veisc. 2003. X-ray microCT study of pyramids of the sea urchin *Lytechinus variegatus*. **Journal of Structural Biology** 141: 9-21.

Study Sites

The preliminary survey was carried out to select the appropriate study sites by SCUBA divers. The sampling points were then located by using differential GPS. The study sites were distinguished as coral zone and sand zone. Studies on population ecology of a sea urchin, *D. setosum* at Khang Khao Island, (latitude $13^{\circ}06'24''$ N to $13^{\circ}07'0''$ N and longitude $100^{\circ}48'45''$ E to $100^{\circ}49'0''$ E; Figure 7) Chonburi Province in the Inner Gulf of Thailand, and Samet Island, (latitude $12^{\circ}34'7''$ N and longitude $101^{\circ}28'22''$ E, Figure 8) Rayong Province in the Eastern Gulf of Thailand were carried out during July 2000 to September 2002.

Population Density

Population densities of *D. setosum* at Khang Khao Island, Chonburi Province, and Samet Island, Rayong Province were examined during June 2000 to September 2002. On each sampling date, twenty randomly placed 1-m^2 quadrats were sampled (Figure 9). The finding was used to assess density fluctuations of *D. setosum* in space and time during the present study.

Recruitment and Settlement

Recruitment was defined as the appearance of small individuals (<20 mm test diameter (TD)). All sea urchin recruits found in the filed were counted and measured their test diameters (Figure 10). The experimental settlement devices (basket plastic sandwiched and basket plastic a size of 16 × 22 cm^2) were also deployed to examine newly settlement of *D. setosum* in the field (Figure 11).

Size Frequency Distribution

Sea urchins were collected into a nylon net and the spines were trimmed with a pair of scissors (Figure 12). The sea urchin samples were preserved in 10% formalin with seawater and transferred to the laboratory. For size-frequency distribution studies, test diameter without spines of all samples were measured with vernier calipers to the nearest millimeters (Figure 13).

Relationship Between the Components of Diameter and Test Diameter

Body and gonad wet weights were also determined by analytical balance (g) (Figure 14). Then samples were dissected and removed the whole of gonad and gut content (Figure 15-16). The gonad index (GI) was computed according to the formula (Lamare and Stewart 1998b, 135-140; Meidel and

Scheibling 1998, 461-478; Walker and Lesser 1998, 664; Alsaffar and Lone 2000, 846-847) given below:

$$\text{Gonad index (GI)} = \frac{\text{Wet weight of the gonad (g)}}{\text{Drained wet weight of the urchin (g)}} \times 100$$

Sex Ratio

Sex ratio and the processes of spermatogenesis and oogenesis were examined from the histological sections (Figure 17). The samples were fixed immediately in 10% formalin – seawater, decalcified in a 1:1 solution of 10% acetic acid and 10% formalin (Parnhathai Nobchinwong 2001, 15-16; Yeemin Thamasak 1988, 5-6). The tissue were washed for 24 hours in running water to remove formalin, then dehydrated through a graded series of ethanol: 70%, 80%, 90%, 95%, absolute 1, absolute 2 for 30 minutes, respectively. The samples were transferred to ethanol–xylene (1:1), xylene1, xylene 2, for 30 minutes respectively, then passed to be impregnated in warm xylene–paraffin (1:1), pure paraffin 1, pure paraffin 2 and finally embedded in pure paraffin in plastic blocks (Figure 18). The samples were cut into serial sections (10 μm thick), and stained with hematoxylin and eosin. Both transverse and longitudinal sections were then examined with a light microscope for reproductive structures.

Gonad Color

Gonad Color: subjectively by eye with or without paint samples (rating 1-4).

1. Bright yellow or orange (equivalent to Grade A in commercial roe industry).

2. Paler yellow or orange, mustard (Grade A or Grade B).

3. Yellow-brown, orange-brown, red-brown, cream (Grade B or Grade C).

4. Any other color (e.g. dark brown, grey) (Grade C) (Pearce et al. 2002a, 301-323; 2002b, 307-332).

Growth Rate

Tetracycline Tagging

Growth rate of a sea urchin, *D. setosum* in the wild population was determined by marking individuals with tetracycline and by following the movement of size-frequency mode through time. Tetracycline produces a fluorescent mark on the actively growing portions of skeletal elements in sea urchin (Pearse and Pearse 1975, 731-753; Ebert 1980, 467-474; 1988b, 162; Kener 1992, 107-188) and these marks indicate the size of the skeletal part at the time of tagging.

The tetracycline solution consisted of 10 mg tetracycline per ml of sea water (Figure 19). Sea urchins had 1 ml of the tetracycline solution injected by syringe into the coelom through the peristomeal membrane (Figure 20) (Kener 1992, 107-188; Lamare and Mladenov 2000, 17-43). A total of 271 individuals of *D. setosum* was tagged on 11 April 2001 at Khang Khao Island. Tagged sea urchins were captured from the Khang Khao Island on 23 June 2002 (438 days post-tagging), with 194 urchins ranging in size from 31.5 to 68 mm. On these days, sea urchins were collected by SCUBA, bagged and returned to the field station for processing. For each sea urchin, the TD was measured to the nearest millimetre using knife-edged vernier calipers, and the Aristotle' s lantern was dissected out. Later, each Aristotle' s lantern was cleaned and disassembled by soaking in 5% sodium hypochlorite. The sample was then air dried for 3-4 day (Figure 21). The size (to the nearest 0.1 mm) of one of the demi-pyramids was measured the oral tip to the epiphysis junction. Each lantern was then inspected under a dissecting microscope that was illuminated by an external ultraviolet light source (Figure 22). If fluorescent tag was detected then the distance between the oral tip and the point where the tetracycline tag met the external edge of the demi-pyramids (*Jt*) was measured (Figure 23). Measurements were made to the nearest 0.01 mm using an ocular micrometer (Lamare and Mladenov 2000, 17-43).

As tetracycline is incorporated into the sites of active calcium deposition (Figure 23), the position of the tag represents the size of the lantern at the time of tagging, *Jt*. The size of lantern at the time of measurement represents size at $Jt + \Delta t$, in this case Δt beging 1.2 years for the Khang

Khao Island. In order to convert these data on changes in lantern size to changes in sea urchin size, the relationship between the dimi-pyramid length and test diameter was established over the range of *D. setosum* samples. For each population, demi-pyramid length (*J*) was converted to test diameter (TD) using the non-linear, logarithmic equation

$$\mathrm{TD} = c + m\ln J$$

Thus *Jt* was converted to test diameter at the time of tagging (TD*t*) and harvest (TD$t + \Delta t$) (Figure 23).

Estimating Growth Model Parameters and Brody-Bertalanffy Growth Function

The paired measurements of initial (TD*t*) and final (TD$t + \Delta t$) test diameters were analysed using Walford plots (Lamare and Mladenov 2000, 22). The relationship between the initial and final sizes can be established for growth models using the appropriate linear or non-linear difference (or regression) equation.

Parameters for the Brody-Bertalanffy function;

$$S_t = S_\infty(1 - be^{-kt})$$

were estimated using the linear regression model, $y = c + mx$ of initial and final sizes, where y = final size (TD$t + \Delta t$) and x = initial size (TD*t*), and the slope of the regression, $m = e^{-kt}$ Therefore

Maximum size $S_\infty = c/(1-m)$

Growth constant $K = -\ln m/t$

were t= time between initial and final measurements and

$$b = 1 - S_o / S_\infty$$

where S_o = size at settlement. Therefore, S_o was replaced by 3 mm when estimating growth of the test diameter and the difference model only estimated S_∞ and K. Instantaneous growth rates (I) were calculated using the equation

$I = S_\infty K b e^{-Kt}$ (Lamare and Mladenov 2000, 22-23).

Mortality Rate

Techniques for estimating the instantaneous mortality, Z, from growth parameters and a size distribution were using regression models that made of the right descending slope of a size distribution to estimate Z (Ebert 1999, 252).

Regression Methods

Ebert (1999, 252-254) used the right descending slope of a size distribution with the Brody-Bertalanffy growth equation to estimate survival. The regression equation is

$$ln(N_s) = m\,ln(X) + C$$

When K as the Brody-Bertalanffy function growth-rate constant and m the slope of the regression, the mortality coefficient, Z is

$$Z = K\,(m+1)$$

The Brody-Bertalanffy growth model was assumed. Annual mortality rate and Z are related:

Annual mortality rate = $1-e^{-z}$ (Ebert and Russell 1992, 35)

Gut Contents

Gut contents of each urchin were washed from the stomach tissue into a small beaker of freshwater. Each sample was placed in a Petri dish and suspended in water, 1 cm deep. The Petri dish was placed over a 1 cm^2 grid on the stage of a dissecting microscope. The amount of each food type was then counted and calculated as the percent of the number of food pieces from three grid squares (Harrold and Reed 1985, 1162; Kenner 1992, 109).

Environmental Factors

Temperature

Surface Seawater Temperature (SST) of Khang Khao Island and Samet Island were detected during July 2000 to September 2002 by using a digital underwater thermometer.

Salinity

Salinity of seawater of Khang Khao Island and Samet Island were examined during July 2000 to September 2002. by using a digital underwater refractometer.

Statistical Analysis

Population densities of *D. setosum* from coral communities of Khang Khao Island and Samet Island were investigated. The number of individuals of *D. setosum* was counted from twenty 1-m^2 quadrats randomly placed at each station, coral zone and sand zone, of Khang Khao Island and at Aow Look Yoan, Samet Island. Two-way ANOVA was used for statistical analysis of mean population densities and mean recruitment of *D. setosum* between Khang Khao Island and Samet Island. The size-frequency distribution data were evaluated by using a single-factor (one-way ANOVA) at a significance level of 5%. The relationship between test diameter (TD) with the drained body weight, gonad weight (GW) and gonad index (GI) were also analyzed by using non-linear regression (Alsaffar and Lone 2000, 847; Carr and Biedenbach 1999, 413-418; deBruyn and Meeuwig 2001, 307-310; Ebert and Russell 1994a, 367-372; Laakso et al. 2003, 247-258; Zar 1999, 231). Differences in mean of recruit density on three types of substrate, newly settled sea urchins, position of experimental settlement devices, population density and gonad index between zones were statically tested by using Two-way ANOVA. For each population, demi-pyramid length was converted to TD by using the non-linear, logarithmic equation (Lamare and Mladenov 2000, 19-25).

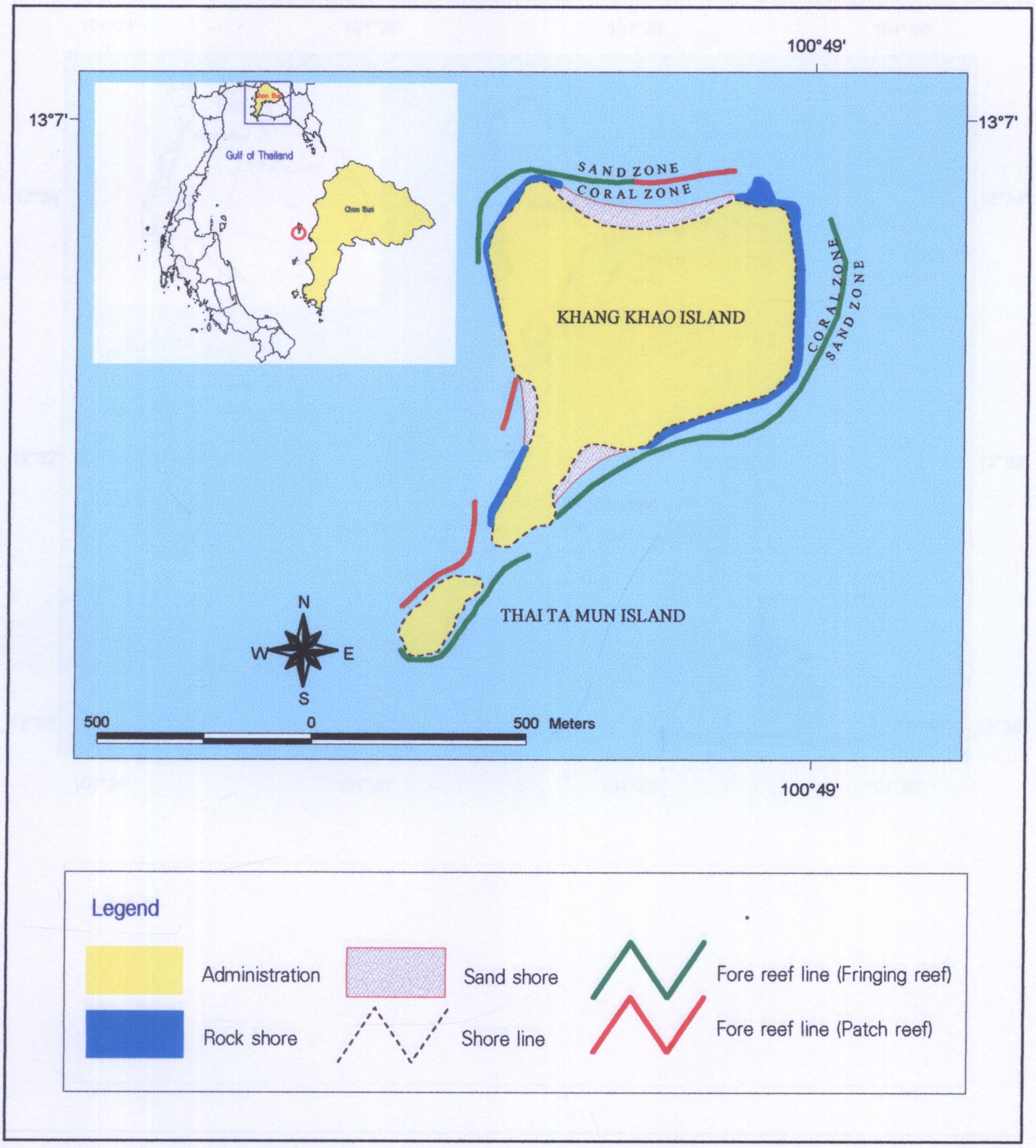


Figure 7 Map of Khang Khao Island

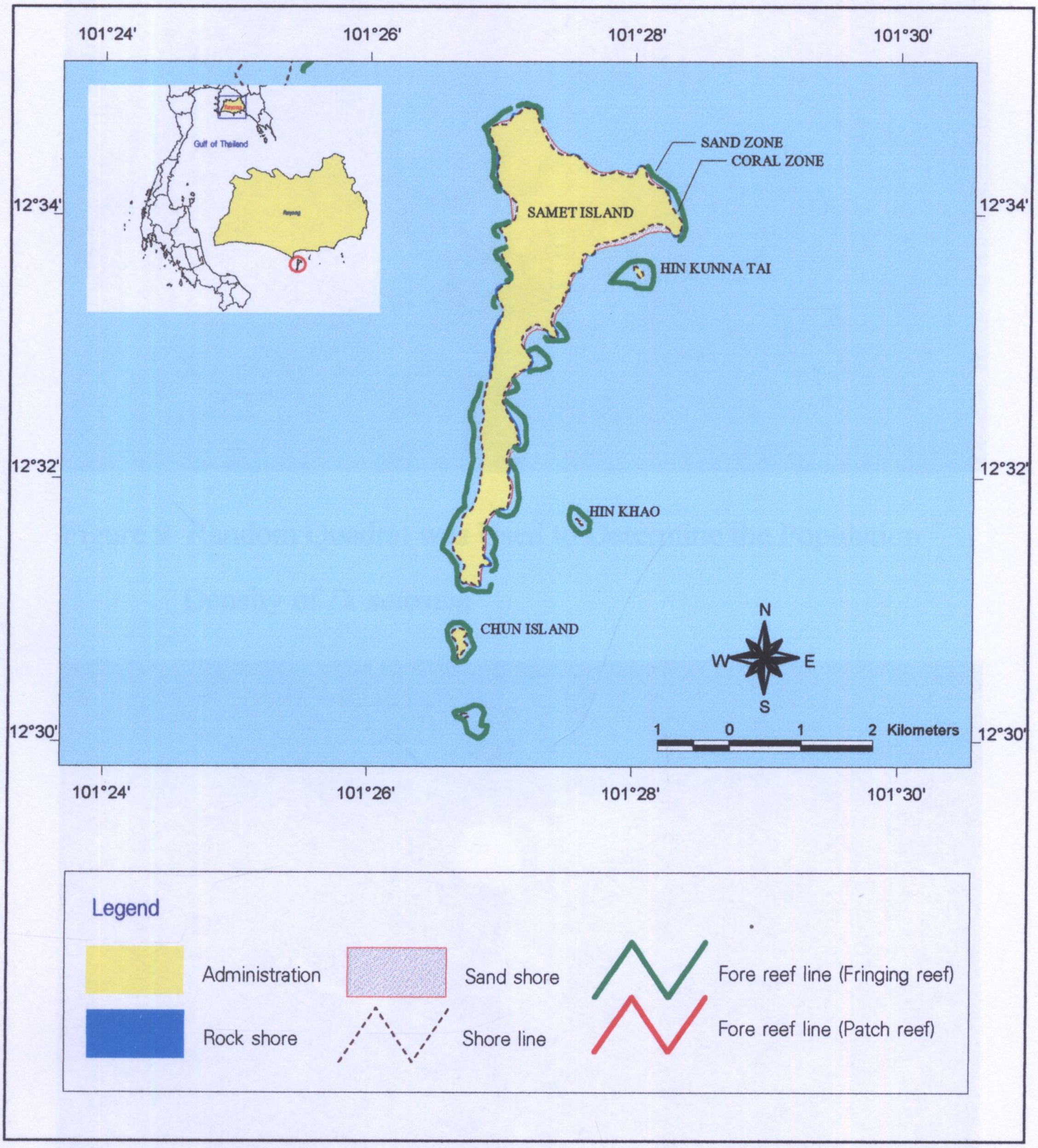


Figure 8 Map of Samet Island

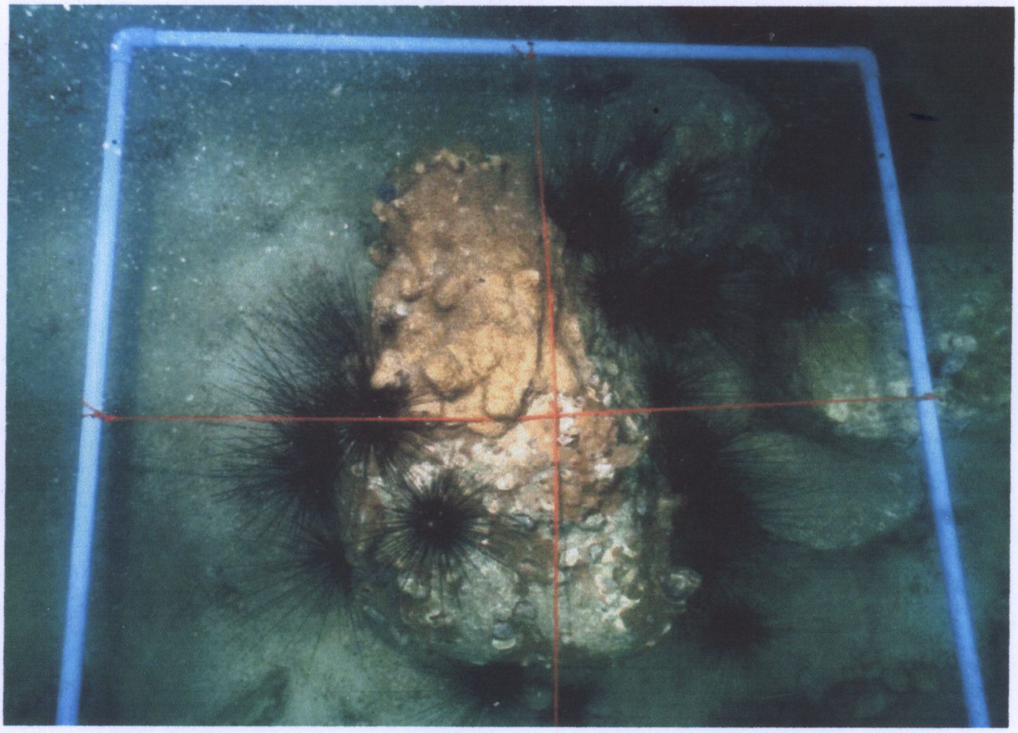


Figure 9 Random Quadrat was Used to Determine the Population Density of *D. setosum*

Figure 10 Test Diameters (TD) of *D. setosum* were Counted in the Field

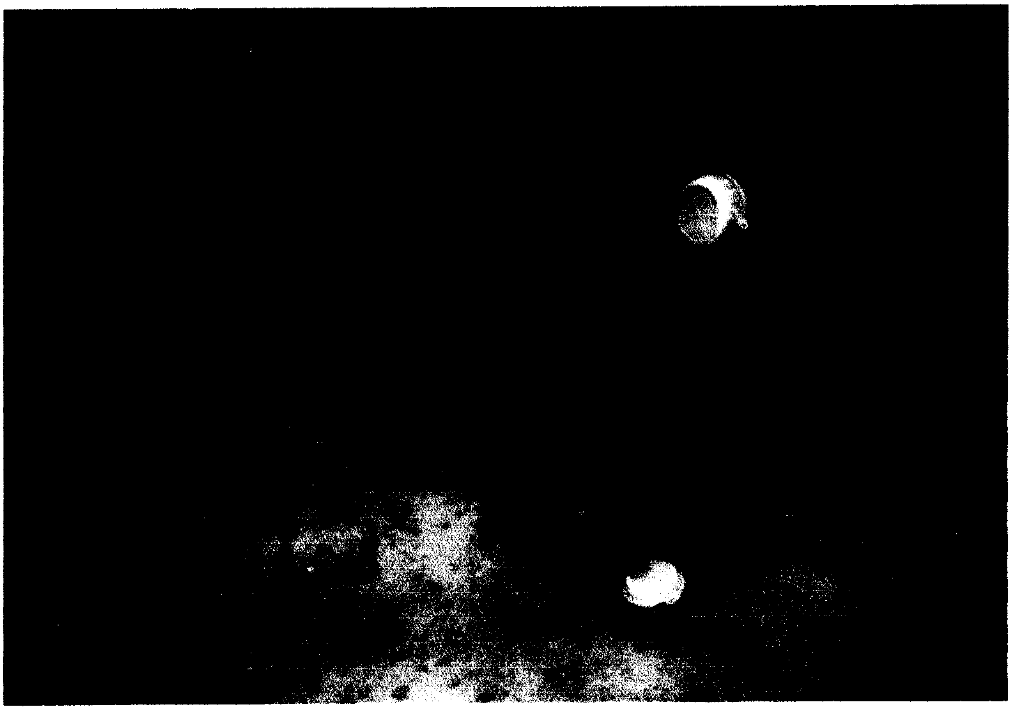


Figure 11 The Experimental Settlement Devices (Basket Plastic Sandwiched) for Study on *D. setosum* Settlement and Recruitment

Figure 12 The Spines were Trimmed with a Pair of Scissors

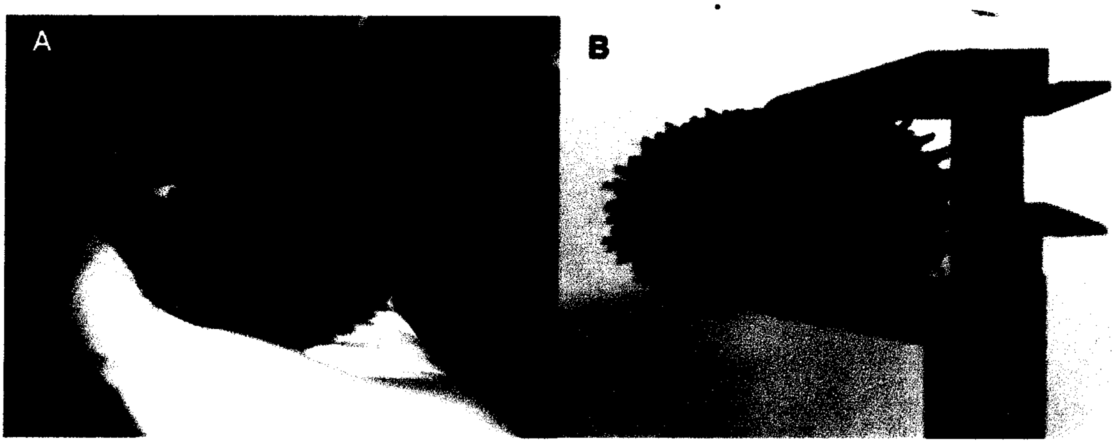


Figure 13 All Samples of *D. setosum* without Spines were Measured of with Vernier Calipers to the Nearest Millimeters (mm)

A: Test Diameter

B: Test Height

Figure 14 *D. setosum* was Weighted an Analytical Balance (g)

A: Body Wet Weight

B: Gonad Wet Weight

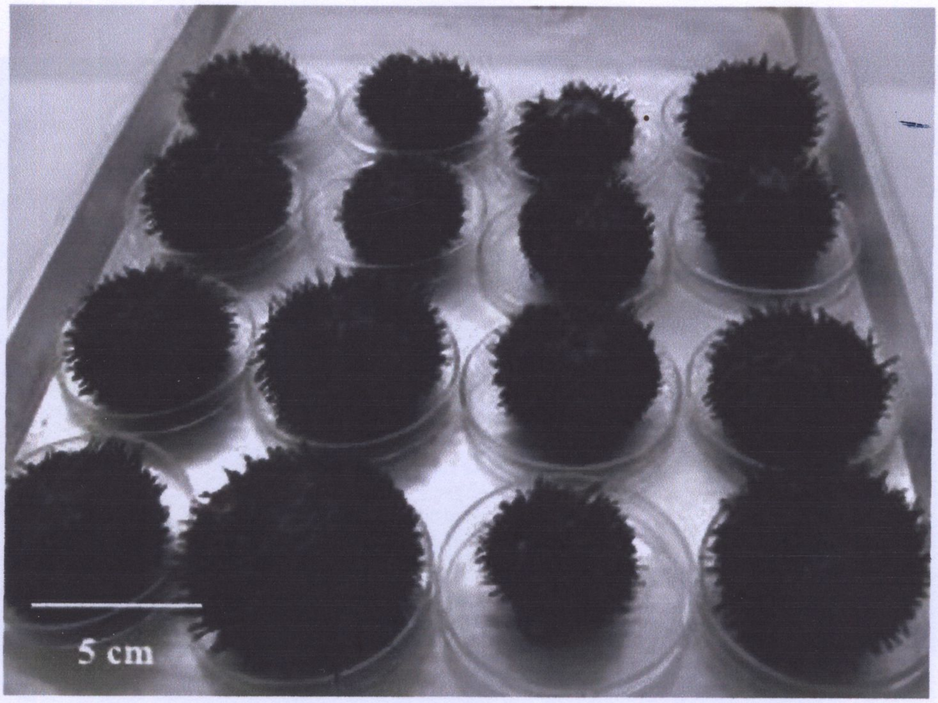


Figure 15 Before Dissection, Sea Urchins was Placed in the Petri Dish with Label

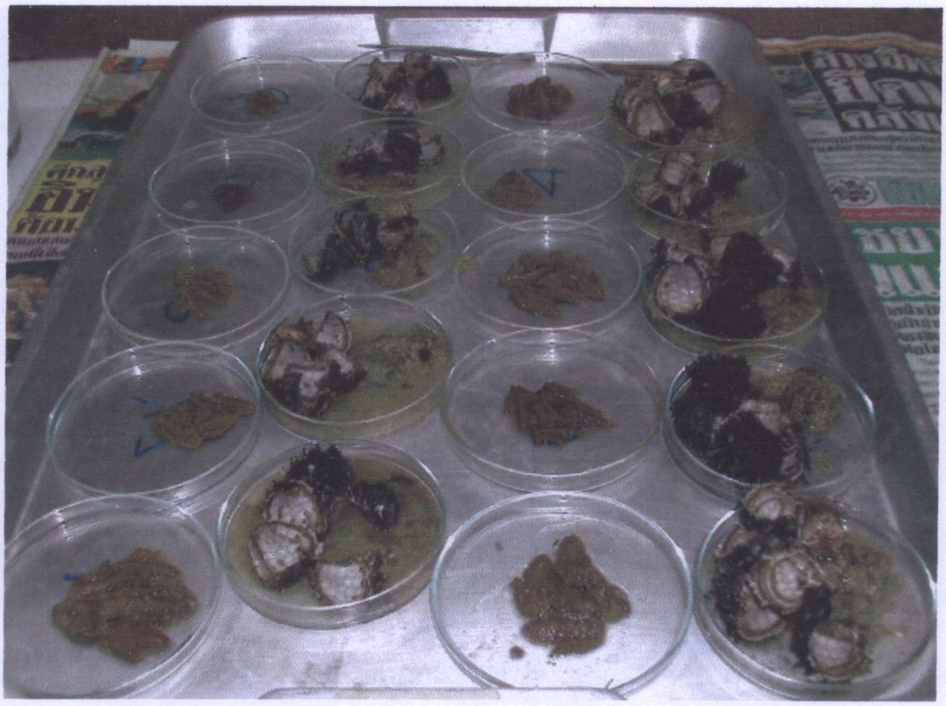


Figure 16 After Dissection, Gonad and Gut Content were Placed in the Petri Dish with Label

Figure 17 Gametogenesis Examination of *D. setosum* by Standard Microtechnique in the Laboratory

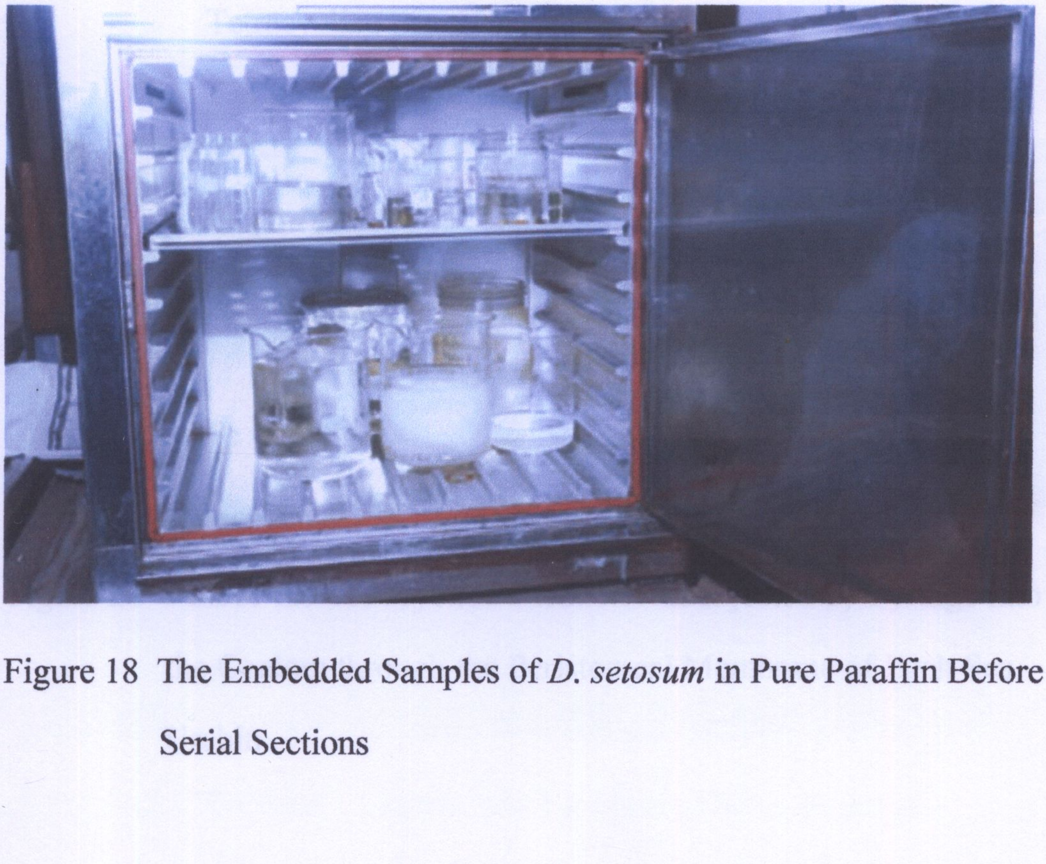


Figure 18 The Embedded Samples of *D. setosum* in Pure Paraffin Before Serial Sections

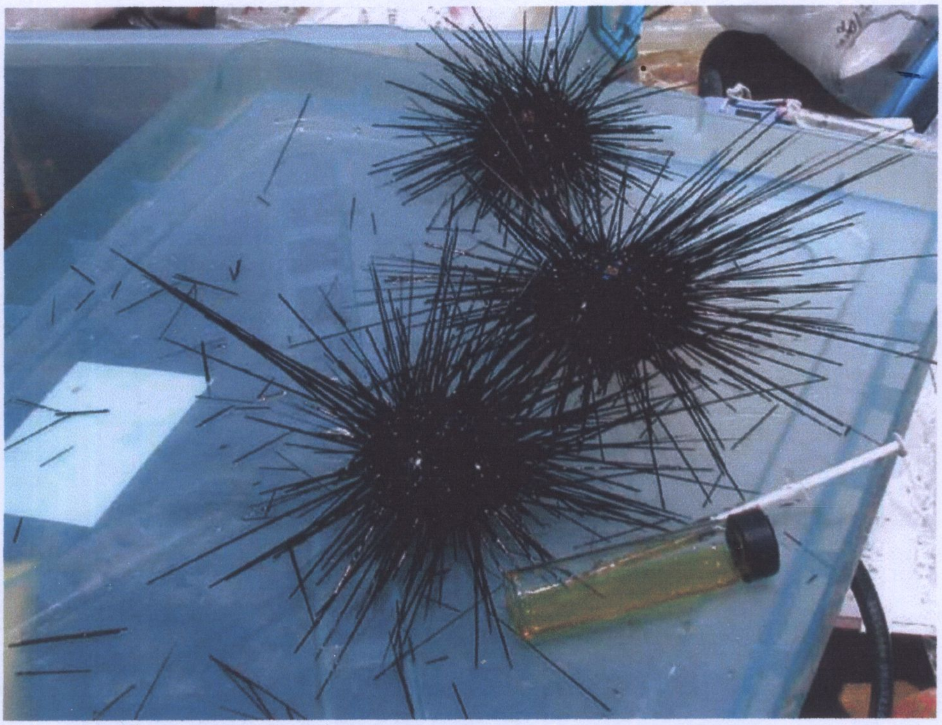


Figure 19 Syringe and the Tetracycline Solution, 10 mg Tetracycline per ml Sea Water

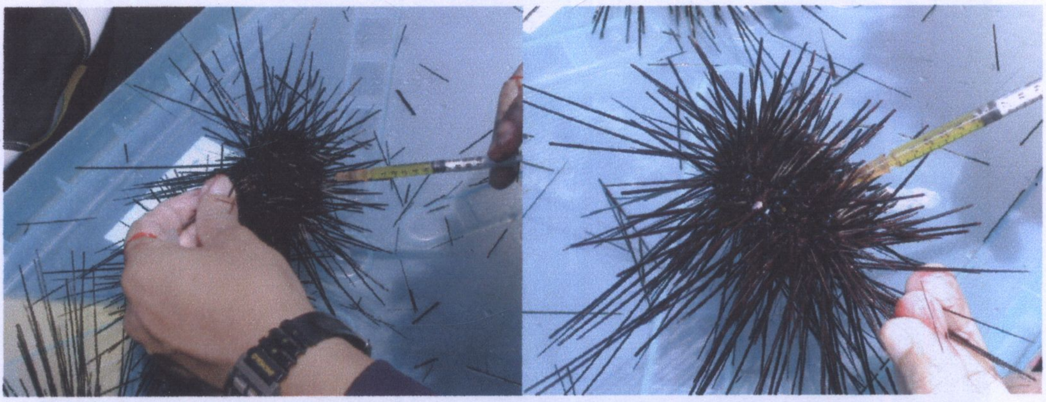


Figure 20 1 ml of the Tetracycline Solution was Injected by Syringe into the Coelom through the Peristomeal Membrane of Each Sea Urchin

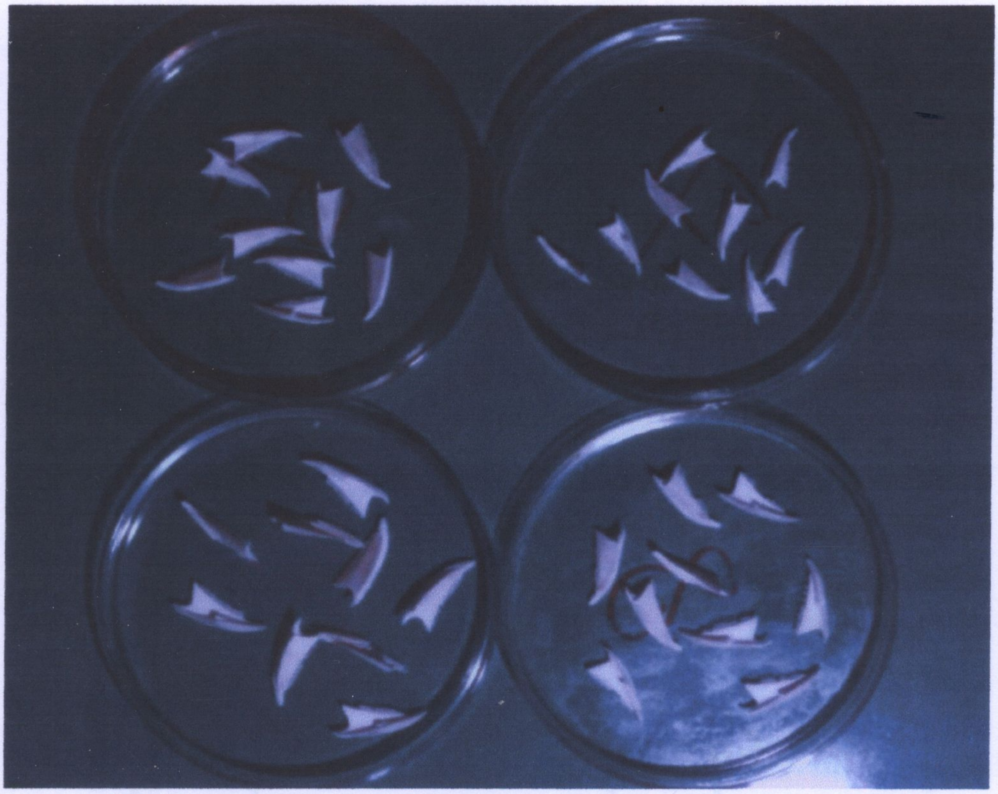


Figure 21 Aristotle' s Lantern was Cleaned and Air Dried for 3-4 Days

Figure 22 Aristotle' s Lantern was Illuminated by an External Ultraviolet Light Source (CAMAG; 254 nm, 366 nm)

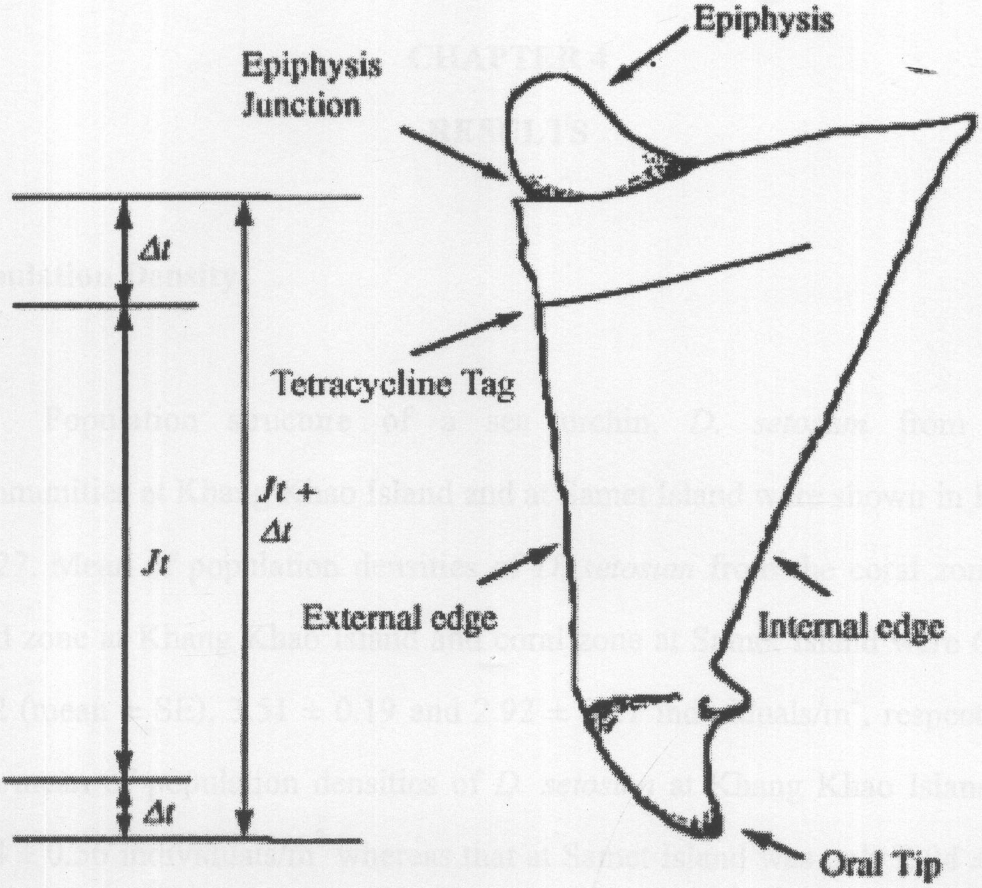


Figure 23 Measurements Made on the Aristotle's Lantern Demi-Pyramid to Estimate Change in Lantern Size in *D. setosum* Following Tetracycline Tagging. The Previous Position of the Epiphysis Junction (*Jt*) is Represented by the Location of the Tetracycline Tag, and is Used to Calculate the Change in Demi-Pyramid Size Over Time ($Jt + \Delta t$). (Modified After (Lamare and Mladenov 2000, 21)

Source: Lamare, M. D., and P. V. Mladenov. 2000. Modelling somatic growth in the sea urchin *Evechinus chloroticus* (Echinoidea: Echinometridae). **J. Exp. Mar. Biol. Ecol.** 243: 17-43.

CHAPTER 4

RESULTS

Population Density

Population structure of a sea urchin, *D. setosum* from coral communities at Khang Khao Island and at Samet Island were shown in Figure 24-27. Mean of population densities of *D. setosum* from the coral zone and sand zone at Khang Khao Island and coral zone at Samet Island were 6.76 ± 0.22 (mean ± SE), 3.51 ± 0.19 and 2.92 ± 0.21 individuals/m^2, respectively. The mean of population densities of *D. setosum* at Khang Khao Island was 7.04 ± 0.56 individuals/m^2 whereas that at Samet Island was only 2.94 ± 0.24 individuals/m^2. The variation among sites and during sampling months was significantly different (Two-way ANOVA, $P < 0.05$) (Figure 28). Mean of population densities in coral zone at Khang Khao Island was higher than that of sand zone. According to the statistical analysis, means of population densities of *D. setosum* from coral zone and sand zone were highly statistical different (Two-way ANOVA, $P < 0.05$) (Figure 29). However, the difference of monthly population densities of *D. setosum* between coral zone and sand zone at Khang Khao Island were not statistically different (Two-way ANOVA, $P > 0.05$).

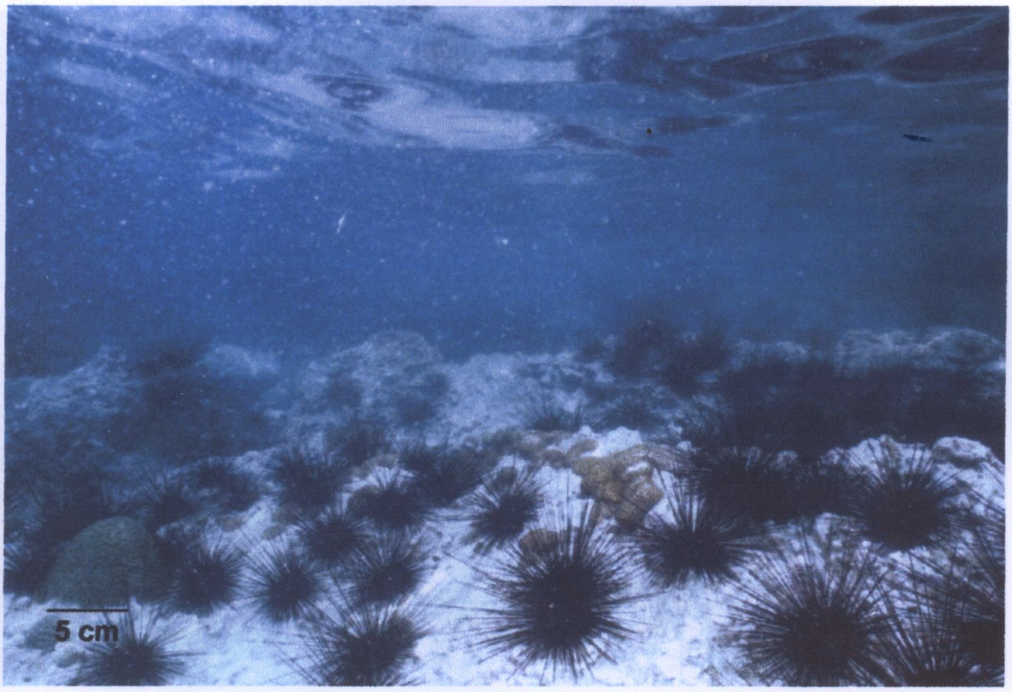


Figure 24 Population Density of *D. setosum* in the Coral Zone at Khang Khao Island

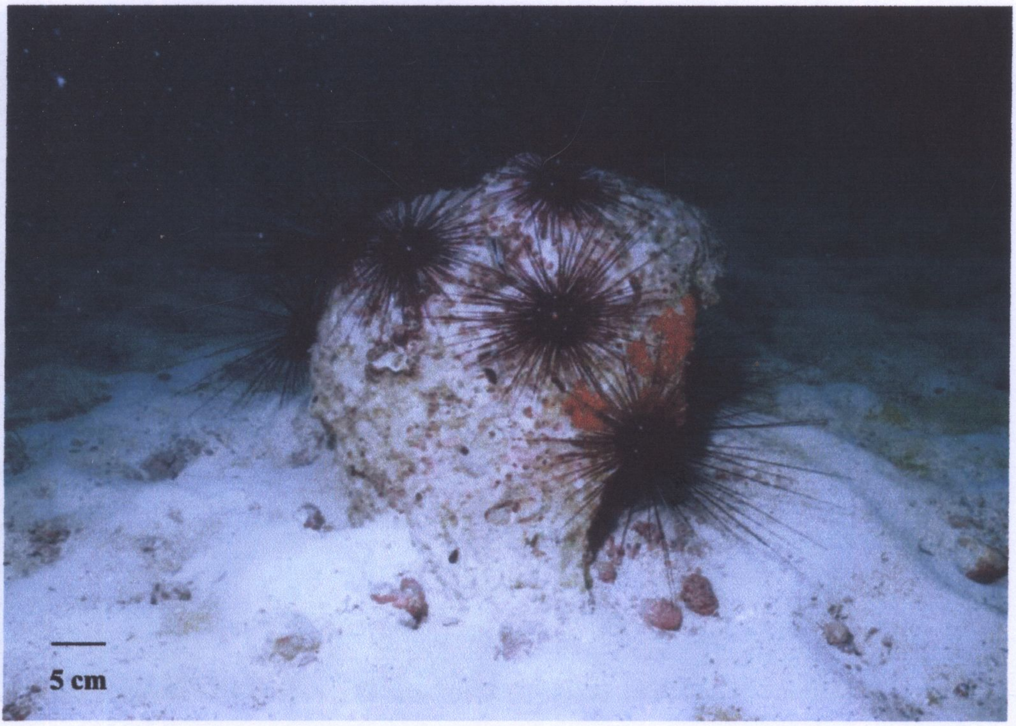


Figure 25 Population Density of *D. setosum* in the Sand Zone at Khang Khao Island

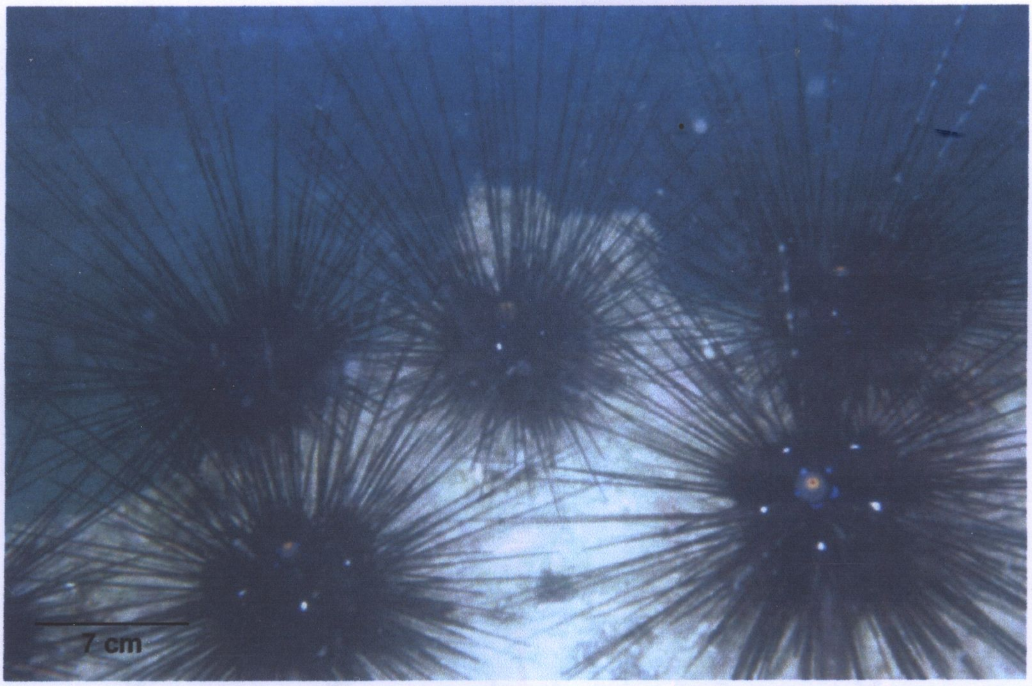


Figure 26 Population Density of *D. setosum* in the Coral Zone at Samet Island

Figure 27 Very Low Density of *D. setosum* in the Sand Zone at Samet Island

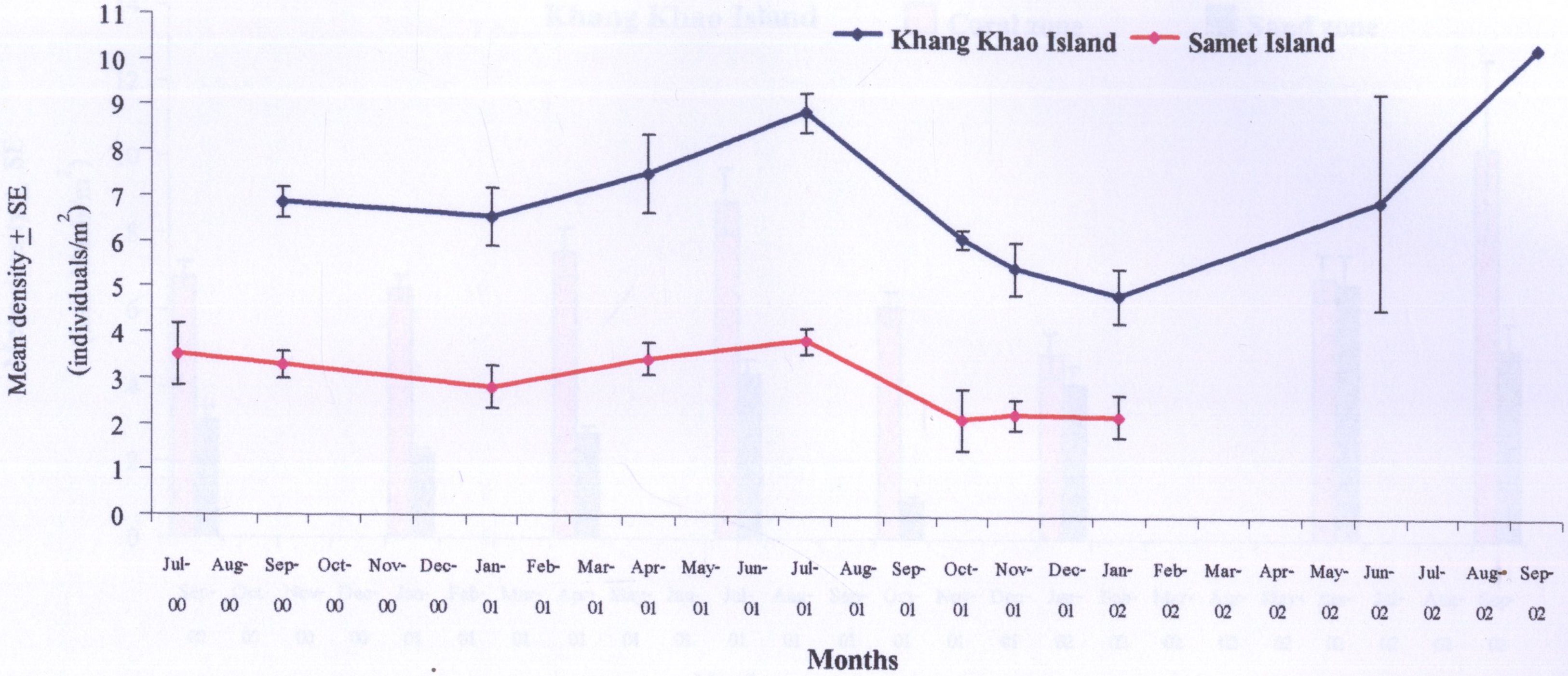


Figure 28 Population Densities (mean ± se) of *D. setosum* at Khang Khao Island and Samet Island

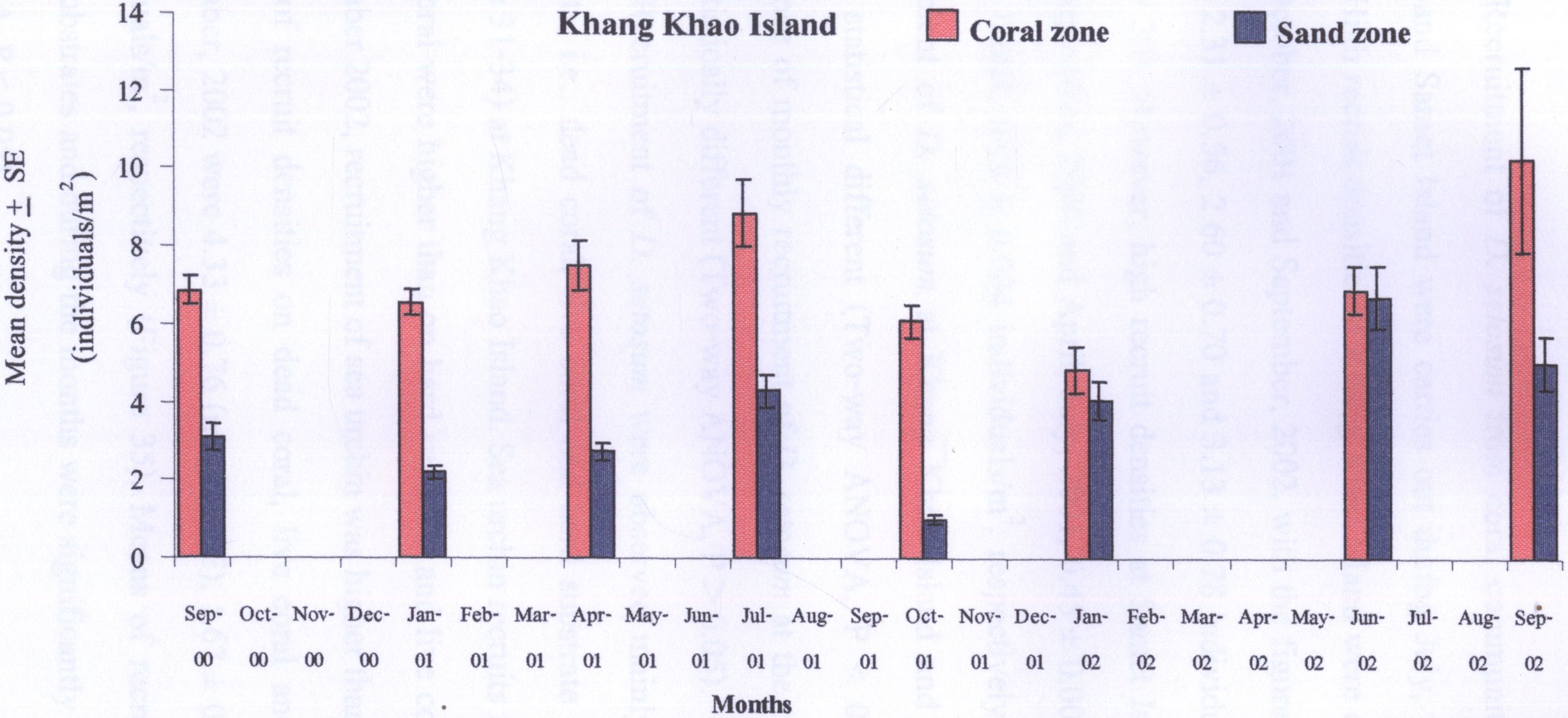


Figure 29 Population Densities (mean ± se) of *D. setosum* in the Coral Zone and Sand Zone at Khang Khao Island

Recruitment and Settlement

Recruitment of *D. setosum* from coral communities at Khang Khao Island and Samet Island were carries out during July, 2000 to September, 2002. High recruit densities at Khang Khao Island were observed in January, July, October, 2001 and September, 2002, with the figures 2.33 ± 0.41 (mean ± SE), 2.31 ± 0.56, 2.60 ± 0.70 and 3.13 ± 0.78 individuals/m^2, respectively (Figure 30). However, high recruit densities at Samet Island were found in July, September, 2000 and April, July, 2001, 0.45 ± 0.001, 0.42 ± 0.005 and 0.44 ± 0.003, 0.49 ± 0.004 individuals/m^2, respectively (Figure 30). Mean recruitment of *D. setosum* at Khang Khao Island and Samet Island were highly statistical different (Two-way ANOVA, $P < 0.05$). However, the difference of monthly recruitment of *D. setosum* at the two study sites were not statistically different (Two-way ANOVA, $P > 0.05$).

Recruitment of *D. setosum* were observed mainly on three types of substrate, i.e., dead coral, live coral and hard substrate (fragments of rock) (Figure 31-34) at Khang Khao Island. Sea urchin recruits found in the filed on dead coral were higher than on hard substrate and live coral, respectively. In September 2002, recruitment of sea urchin was higher than the previous years. Mean of recruit densities on dead coral, live coral and hard substrate in September, 2002 were 4.33 ± 0.76 (mean ± SE), 1.67 ± 0.42 and 3.40 ± 1.16 individuals/m^2, respectively (Figure 35). Means of recruit densities on the three substrates and during the months were significantly different (Two-way ANOVA, $P < 0.05$).

Settlement of *D. setosum* were also examined from the experimental settlement devices in the coral zone (Floating, Bottom) and sand zone (Floating, Bottom) at Khang Khao Island. Newly settled sea urchins were found in the bottom settlement devices of both coral and sand zones (Figure 36-37). No settled *D. setosum* was observed in the floating devices. The period of high newly settled *D. setosum* were July to October, 2001 and June to September, 2002 (Figure 38). Means of densities of newly settled sea urchin during the study periods were not statistically different (Two-way ANOVA, $P > 0.05$). However, means of newly settled *D. setosum* densities between the positions of experimental settlement devices were significantly different (Two-way ANOVA, $P < 0.05$).

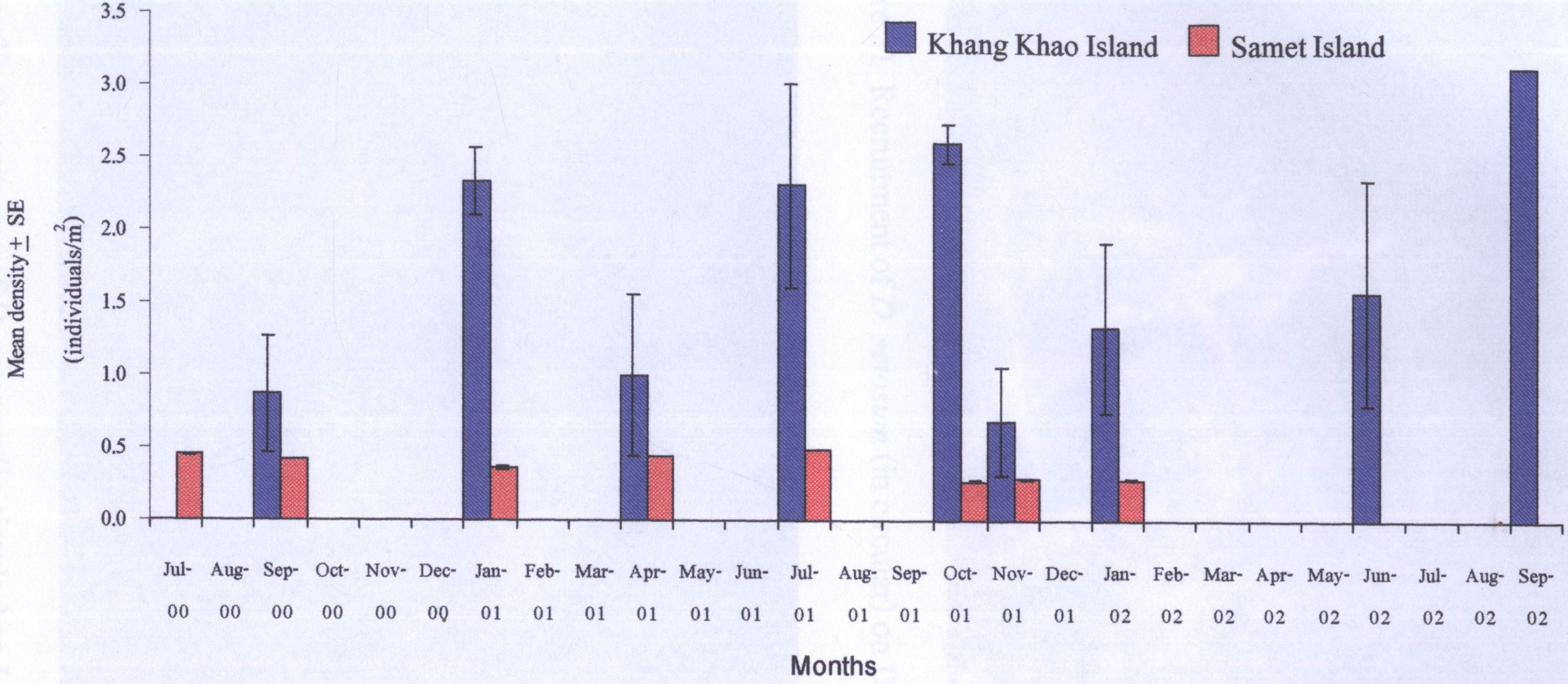


Figure 30 Densities of *D. setosum* Recruits at Khang Khao Island and Samet Island

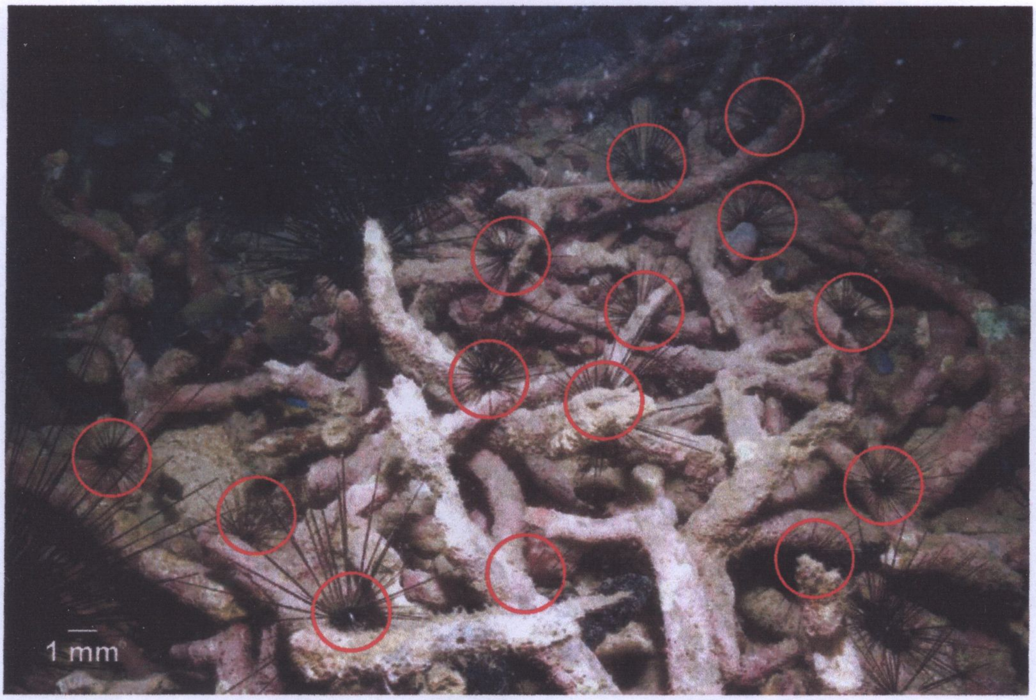


Figure 31 Recruitment of *D. setosum* (in circular) on Dead Coral

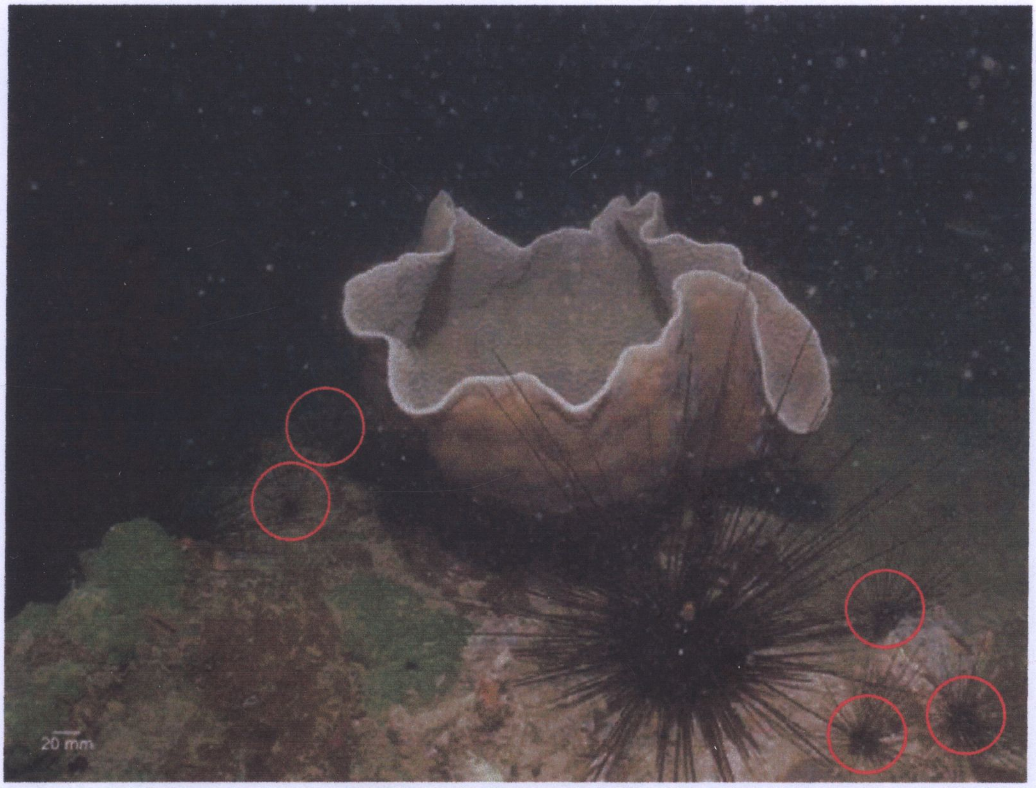


Figure 32 Recruitment of *D. setosum* (in circular) on Live Coral

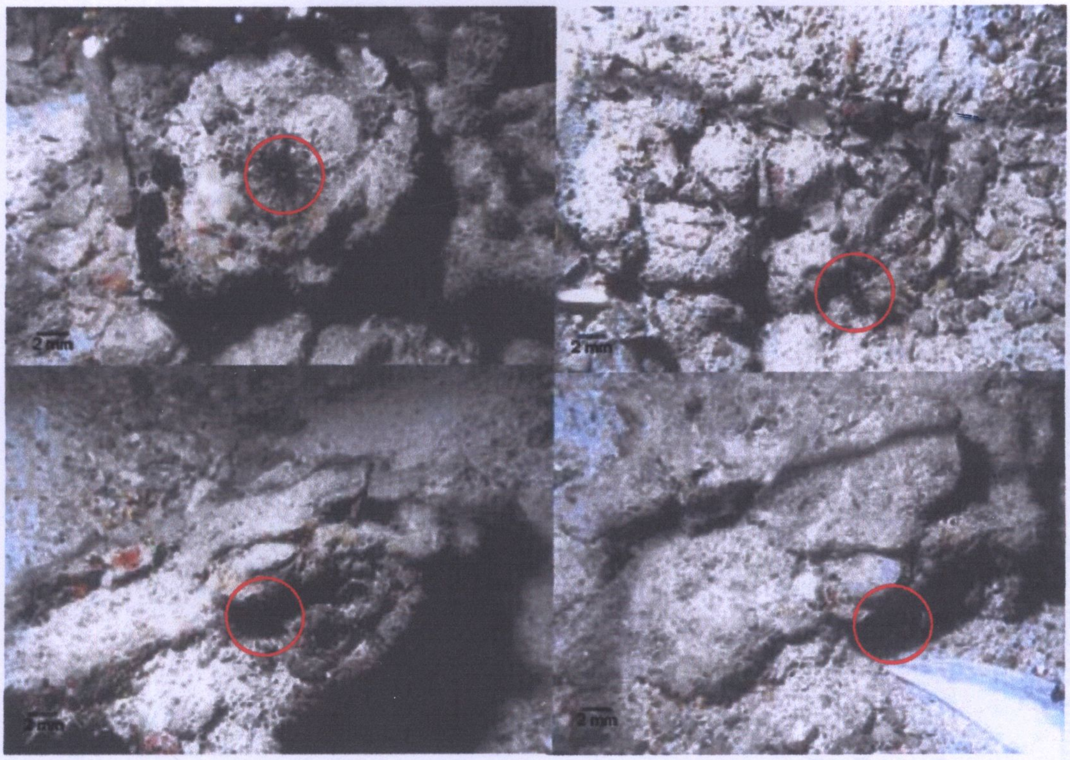


Figure 33 Recruitment of *D. setosum* (in circular) on Hard Substrate

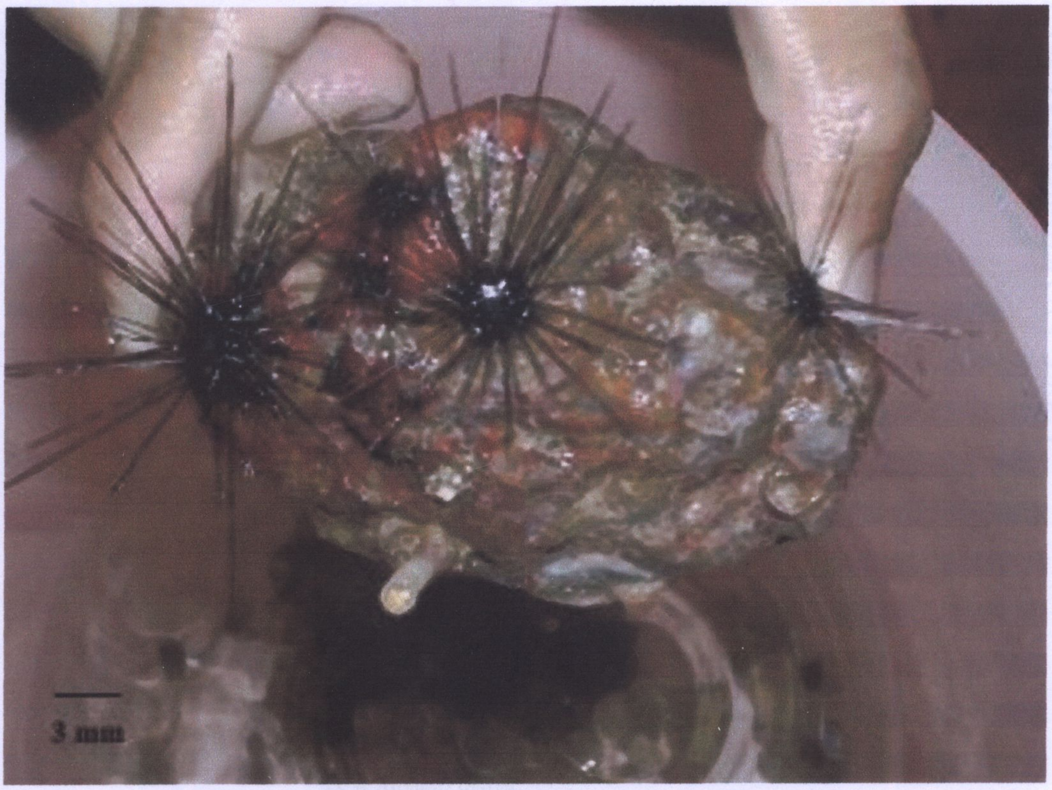


Figure 34 Juvenile of *D. setosum* was Collected from Coral Communities

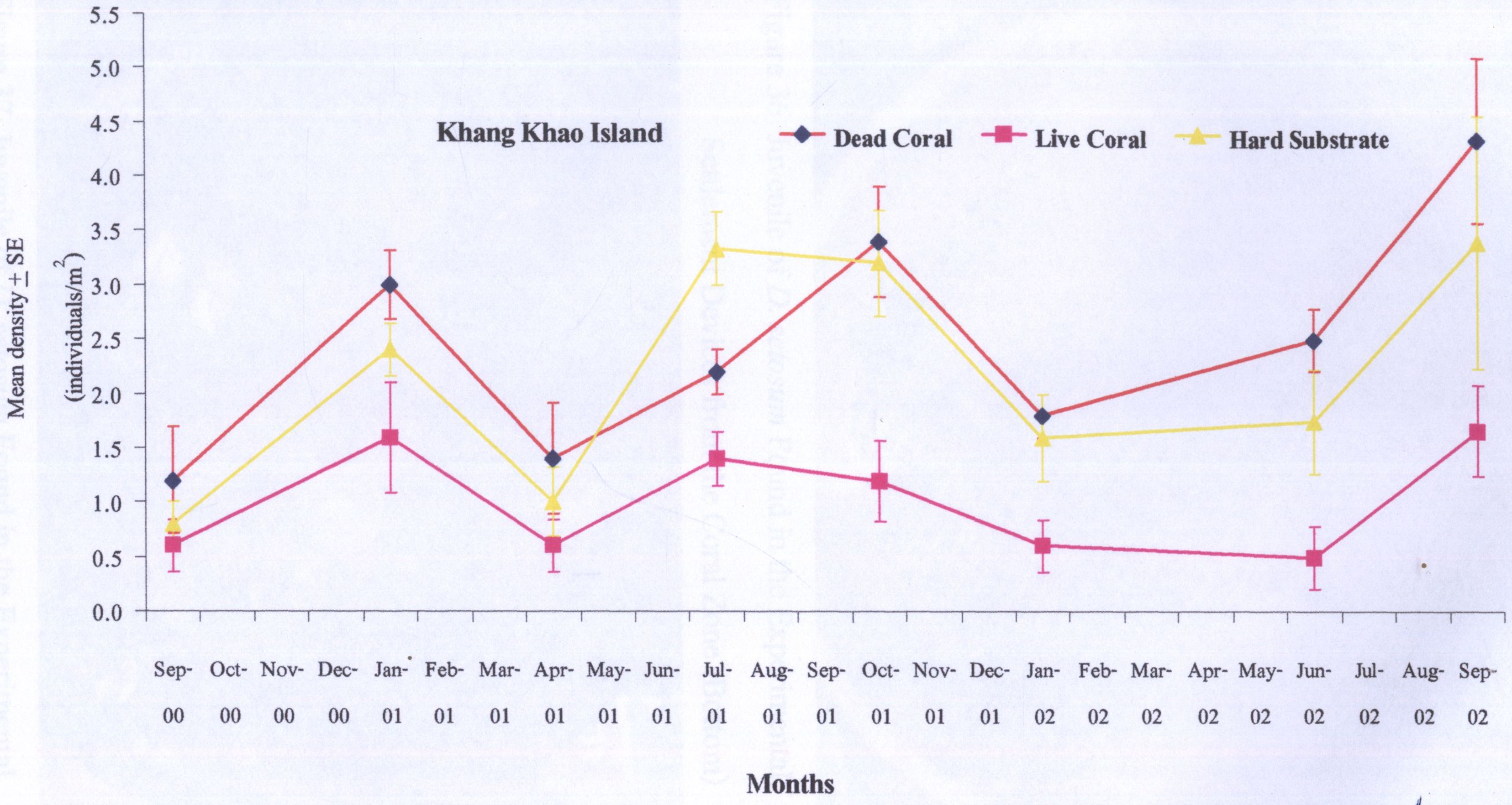


Figure 35 Densities of *D. setosum* Recruits (mean ± se) on Three Substrates

Figure 36 Juvenile of *D. setosum* Found in the Experimental Settlement Devices from the Coral Zone (Bottom)

Figure 37 Juvenile of *D. setosum* Found in the Experimental Settlement Devices from the Sand Zone (Bottom)

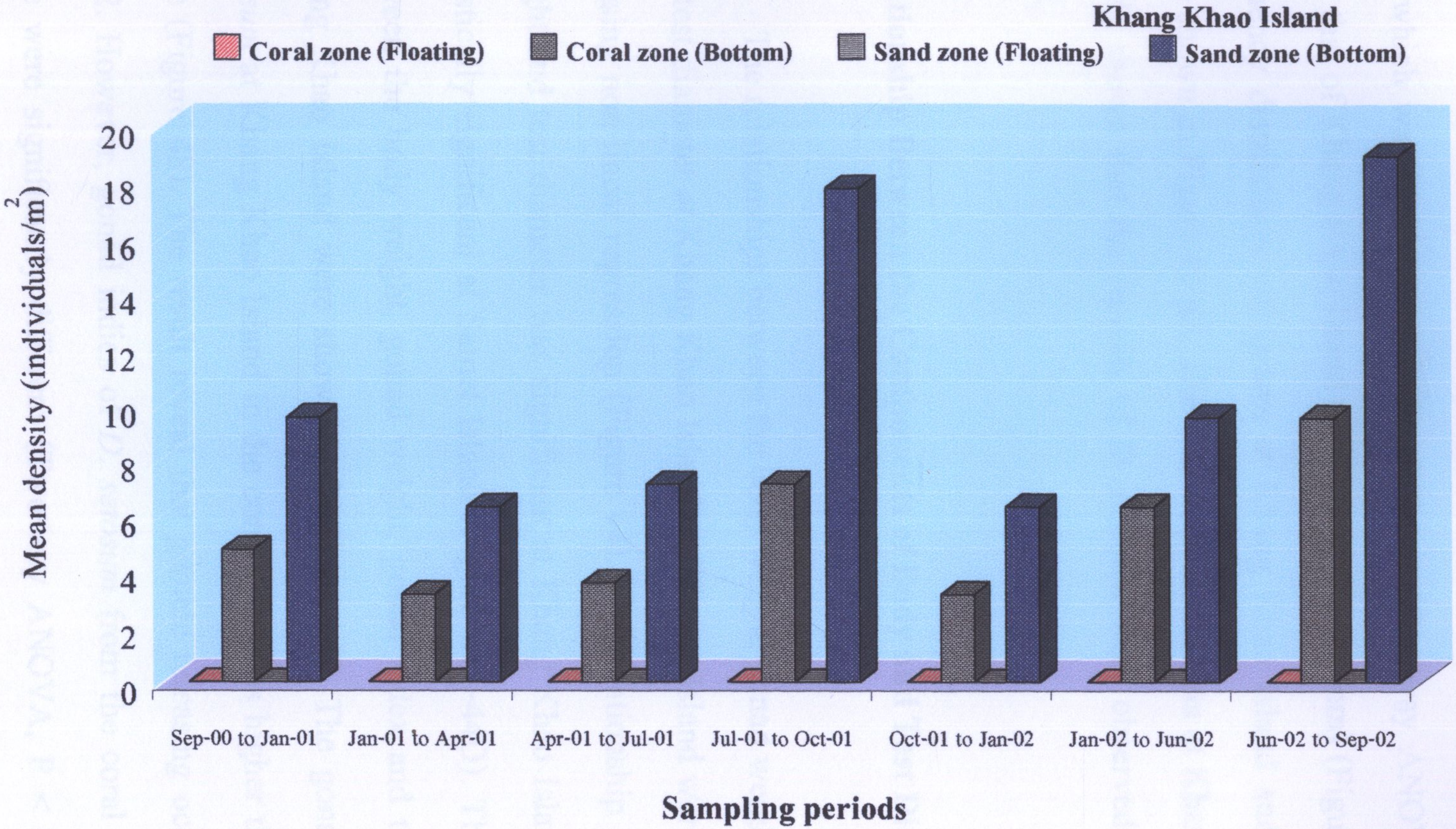


Figure 38 Densities of *D. setosum* Recruits (mean ± se) in the Experimental Settlement Devices from the Coral Zone and Sand Zone

Size Frequency and Distribution

Average test diameter of *D. setosum* at Samet Island was 71.16 ± 0.79 mm which was highly statistically different (One-way ANOVA, $P < 0.05$) from that of Khang Khao Island (only 43.63 ± 0.21 mm) (Figure 39-40). Size frequency distribution histograms at Khang Khao Island and Samet Island were shown in Figure 41-43. The size distribution data at Khang Khao Island clearly shown that the recruits of *D. setosum* were observed in September, 2002.

Relationship Between the Components of Body and Test Diameter

The relationships between the body weight, gonad weight, gonad index and test diameter at Khang Khao Island and Samet Island were also analyzed by using non-linear regression (Figure 44). The relationship between gonad weight and test diameter was significant at Khang Khao Island but was not statistically significant at Samet Island (Figure 44C-44D). The relationships between the body weight, gonad weight, gonad index and test diameter at Khang Khao Island were shown in Figure 45-47. The gonad index of *D. setosum* at Khang Khao Island in the coral zone was higher than in the sand zone (Figure 48). The result reveal that gamete releasing occurred in June, 2002. However, gonad indies of *D. setosum* from the coral zone and sand zone were significantly different (Two-way ANOVA, $P < 0.05$). Average values of the gonad index of *D. setosum* at Khang Khao Island and Samet Island were 12.70 and 15.15 respectively.

Figure 39 Size Class of *D. setosum* at Samet Island

Figure 40 Size Class of *D. setosum* at Khang Khao Island

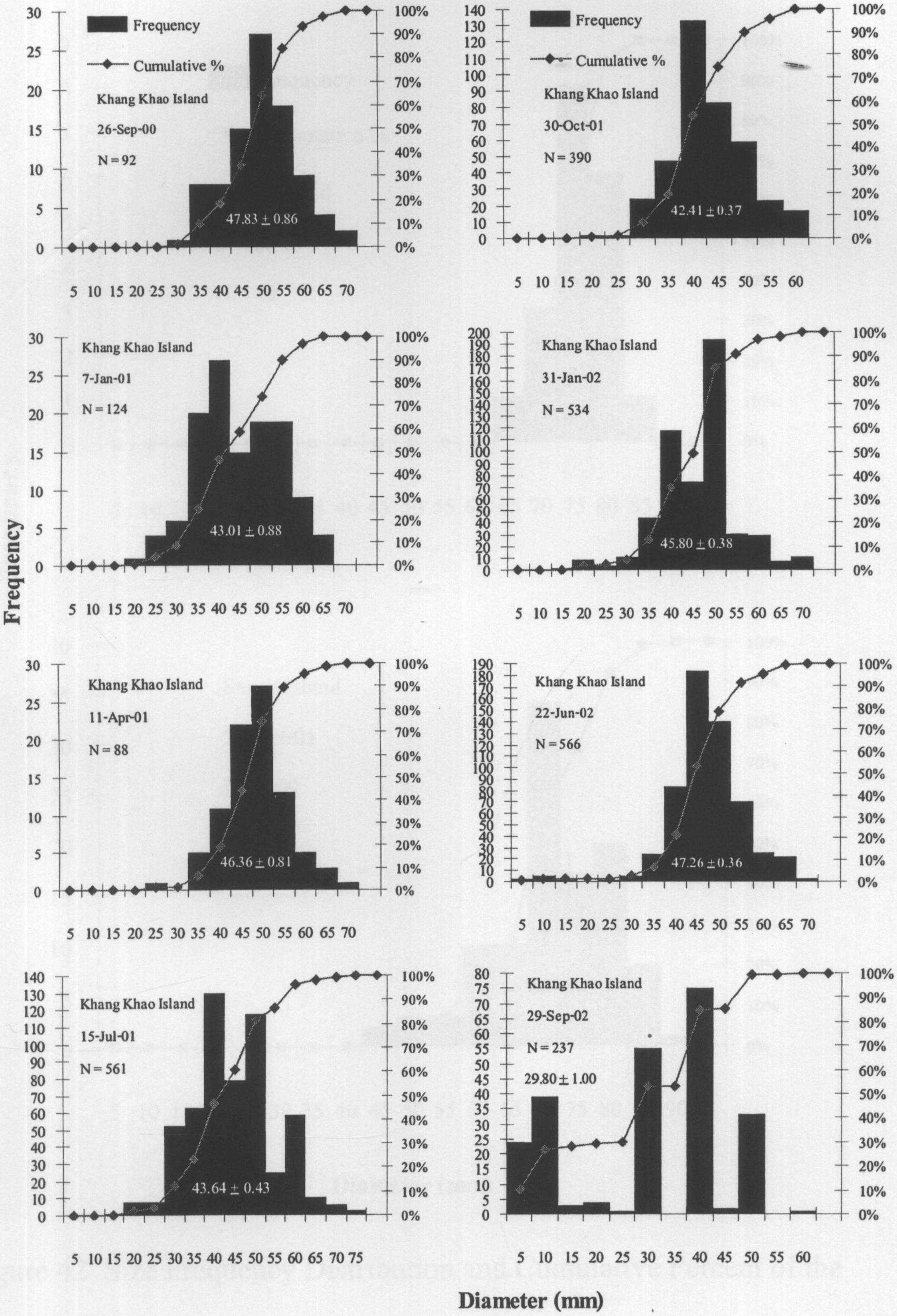


Figure 41 Size Frequency Distribution and Cumulative Percent of the *D. setosum* at Khang Khao Island

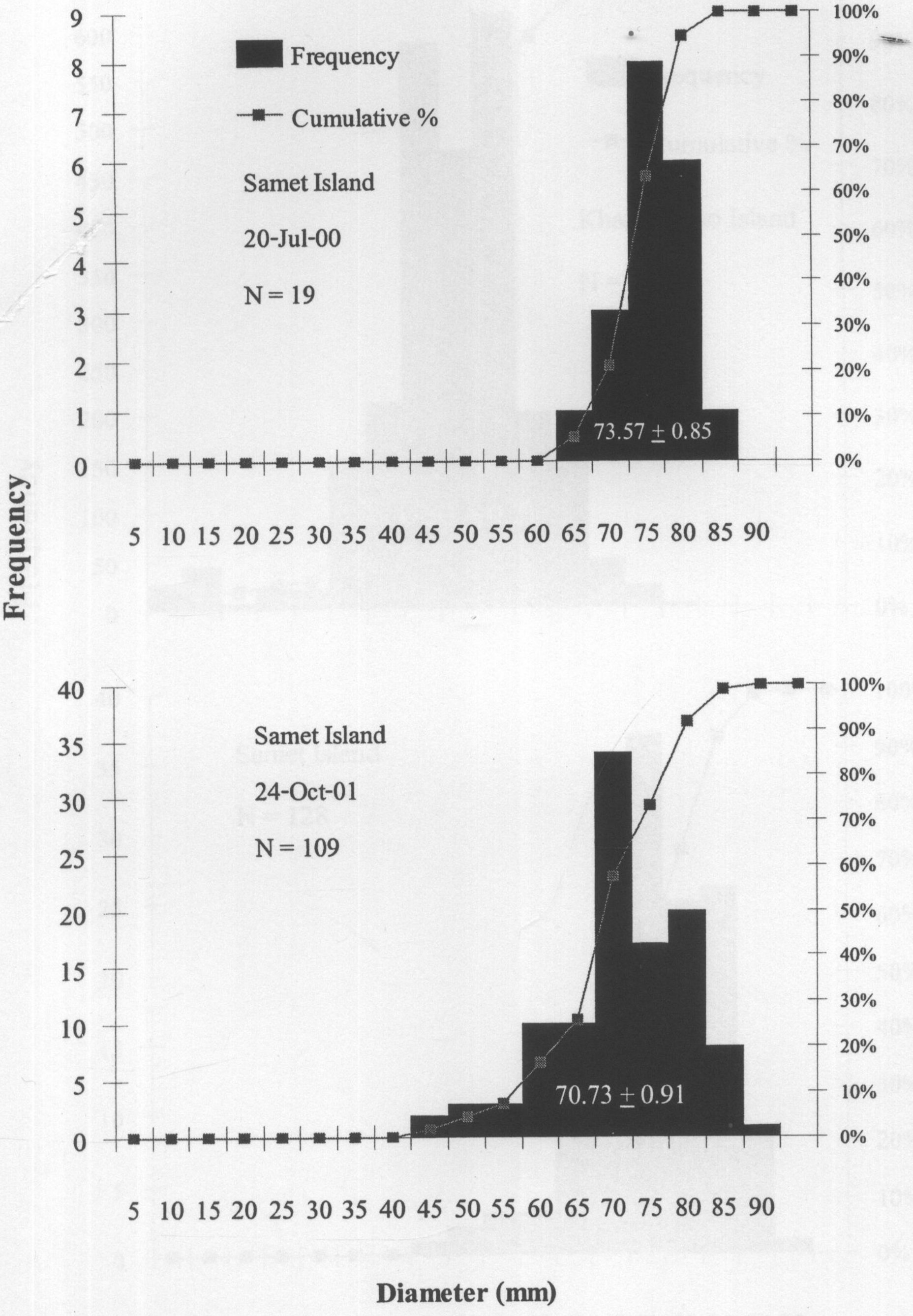


Figure 42 Size Frequency Distribution and Cumulative Percent of the *D. setosum* at Samet Island

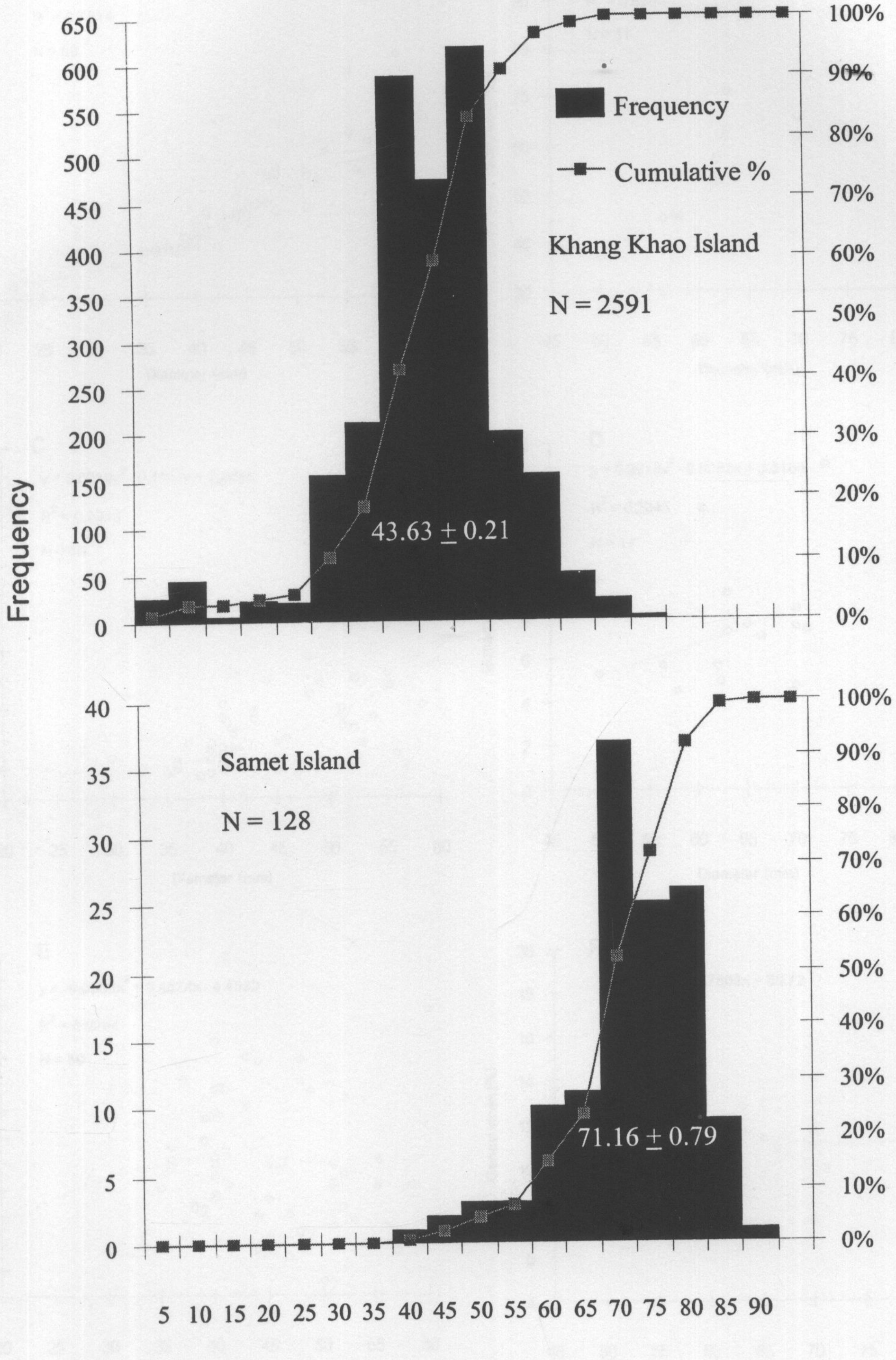


Figure 43 Size Frequency Distribution and Cumulative Percent of the *D. setosum* at Khang Khao Island and Samet Island

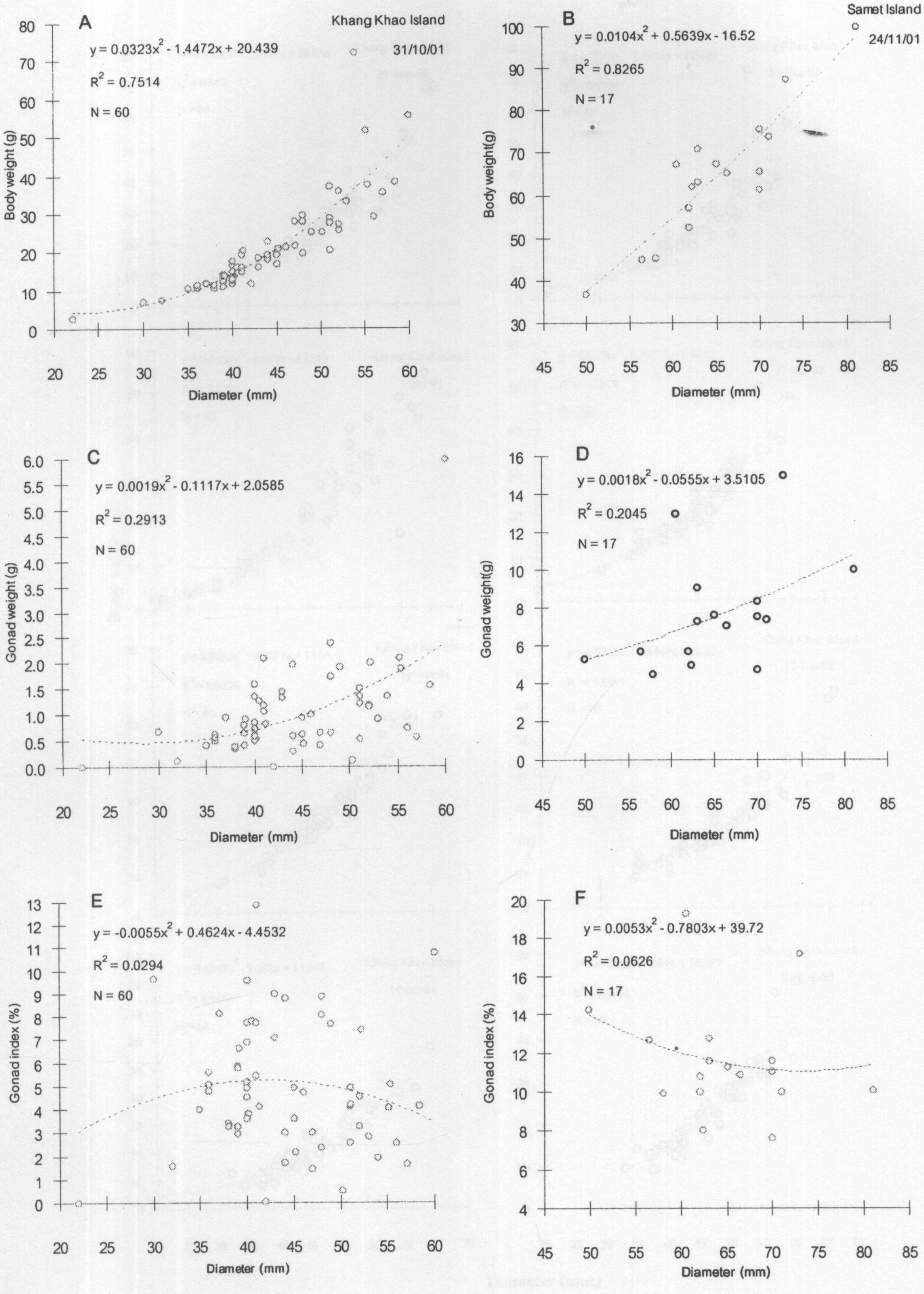


Figure 44 Regression Line Describing the Relationships Between Body Weight, Gonad Weight, Gonad Index and Test Diameter of *D. setosum* at Khang Khao Island and Samet Island

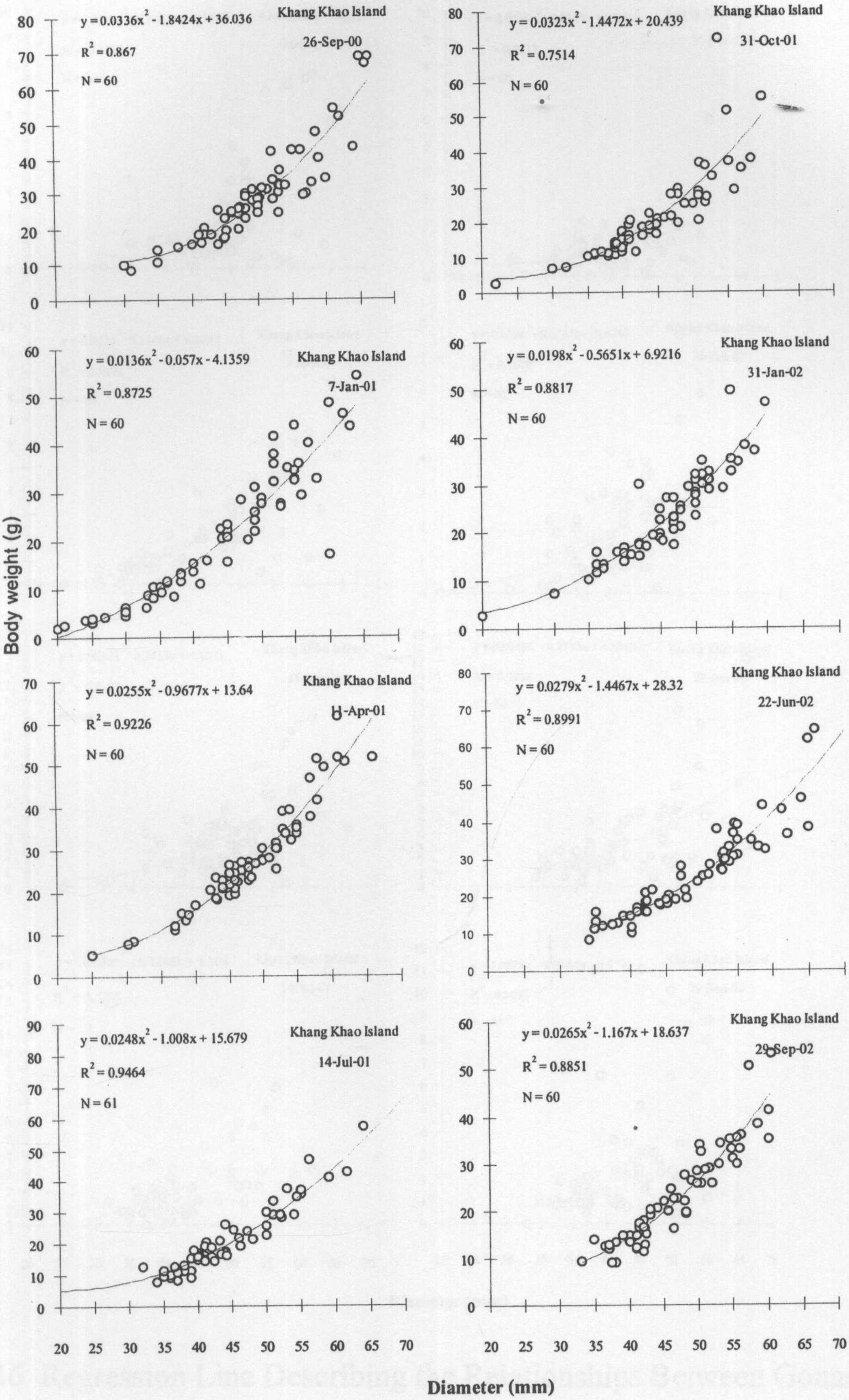


Figure 45 Regression Line Describing the Relationships Between Body Weight and Test Diameter of *D. setosum* at Khang Khao Island, During September, 2000 to September, 2002

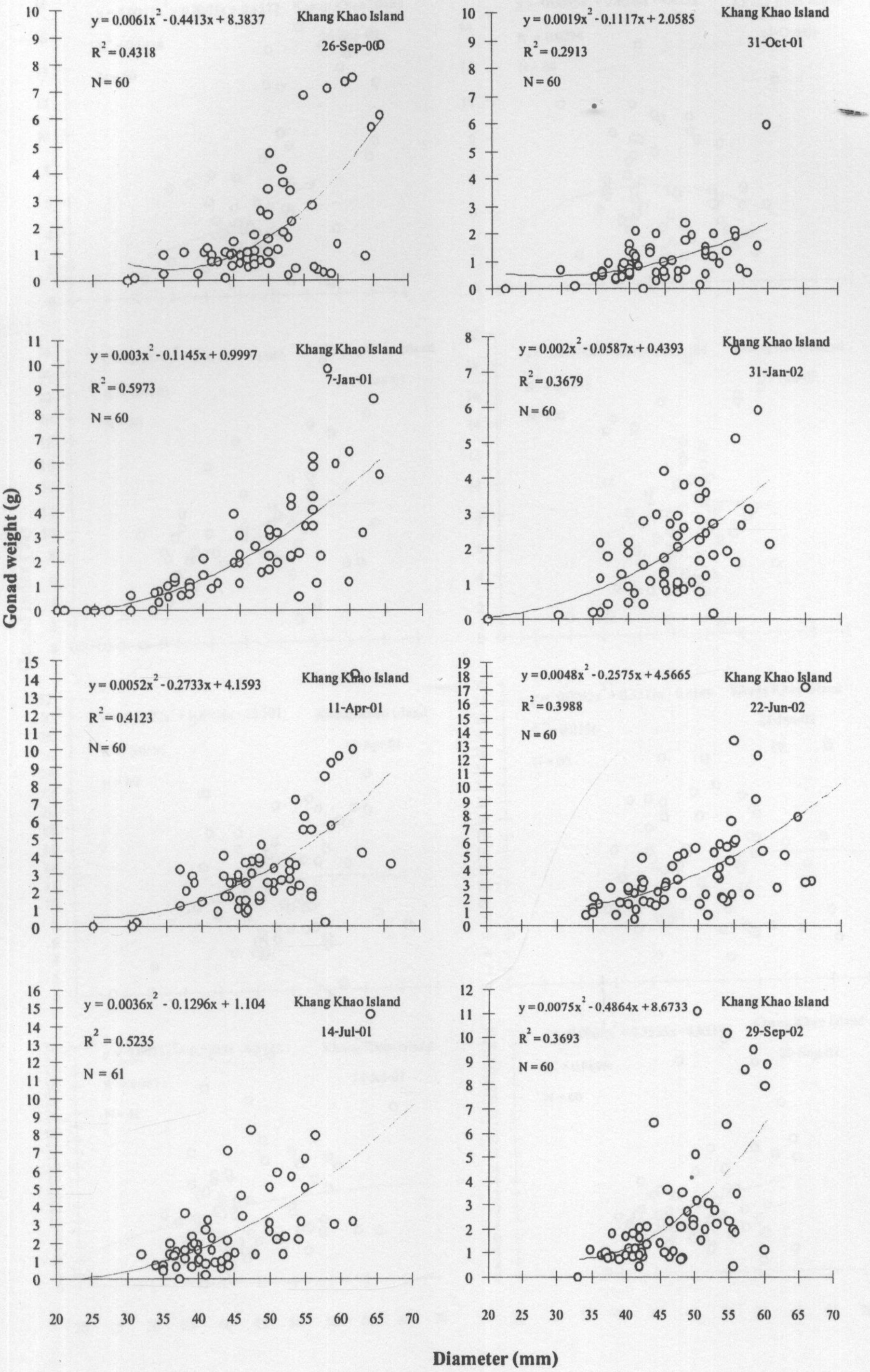


Figure 46 Regression Line Describing the Relationships Between Gonad Weight and Test Diameter of *D. setosum* at Khang Khao Island, During September, 2000 to September, 2002

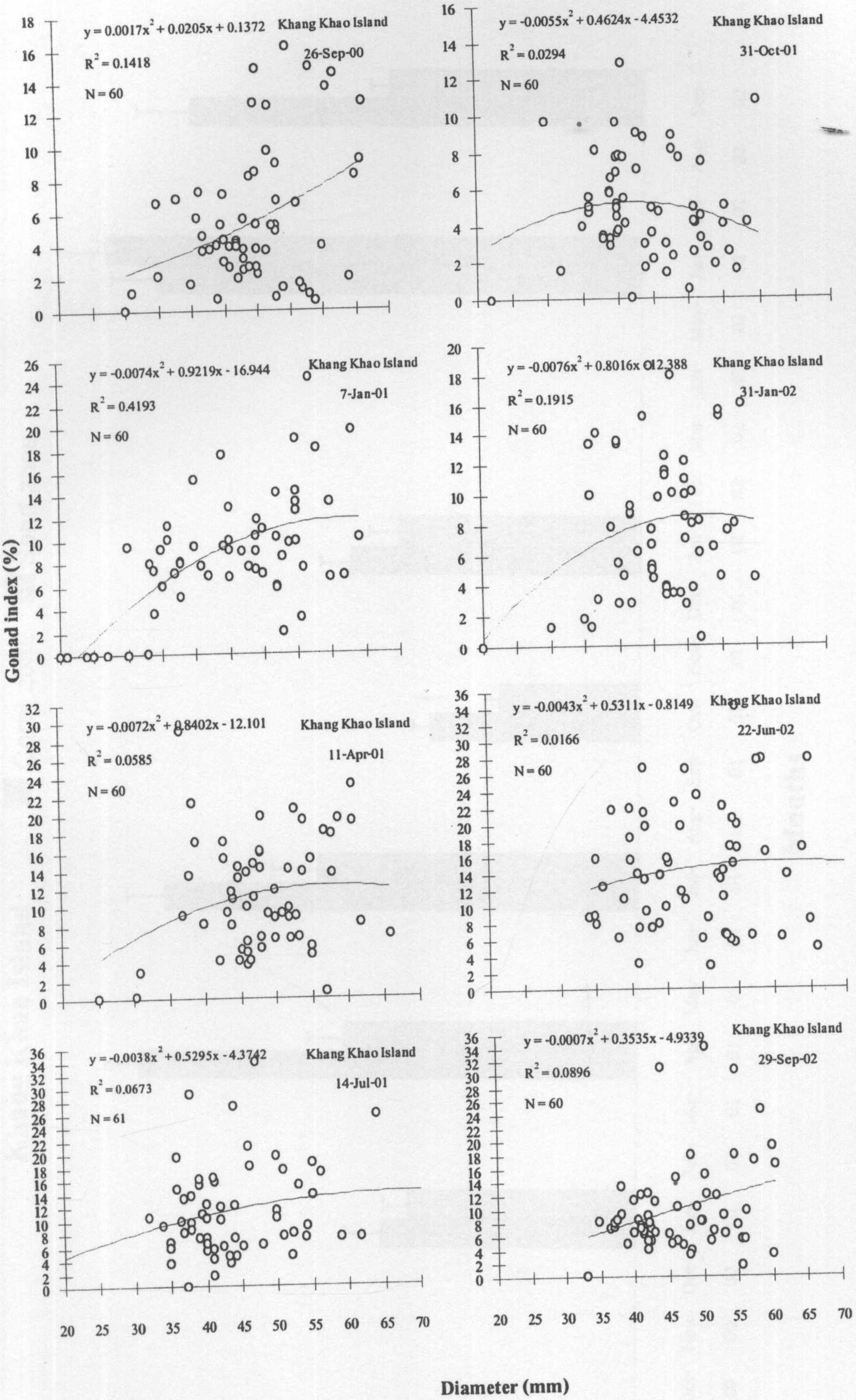


Figure 47 Regression Line Describing the Relationships Between Gonad Index and Test Diameter of *D. setosum* at Khang Khao Island, During September, 2000 to September, 2002

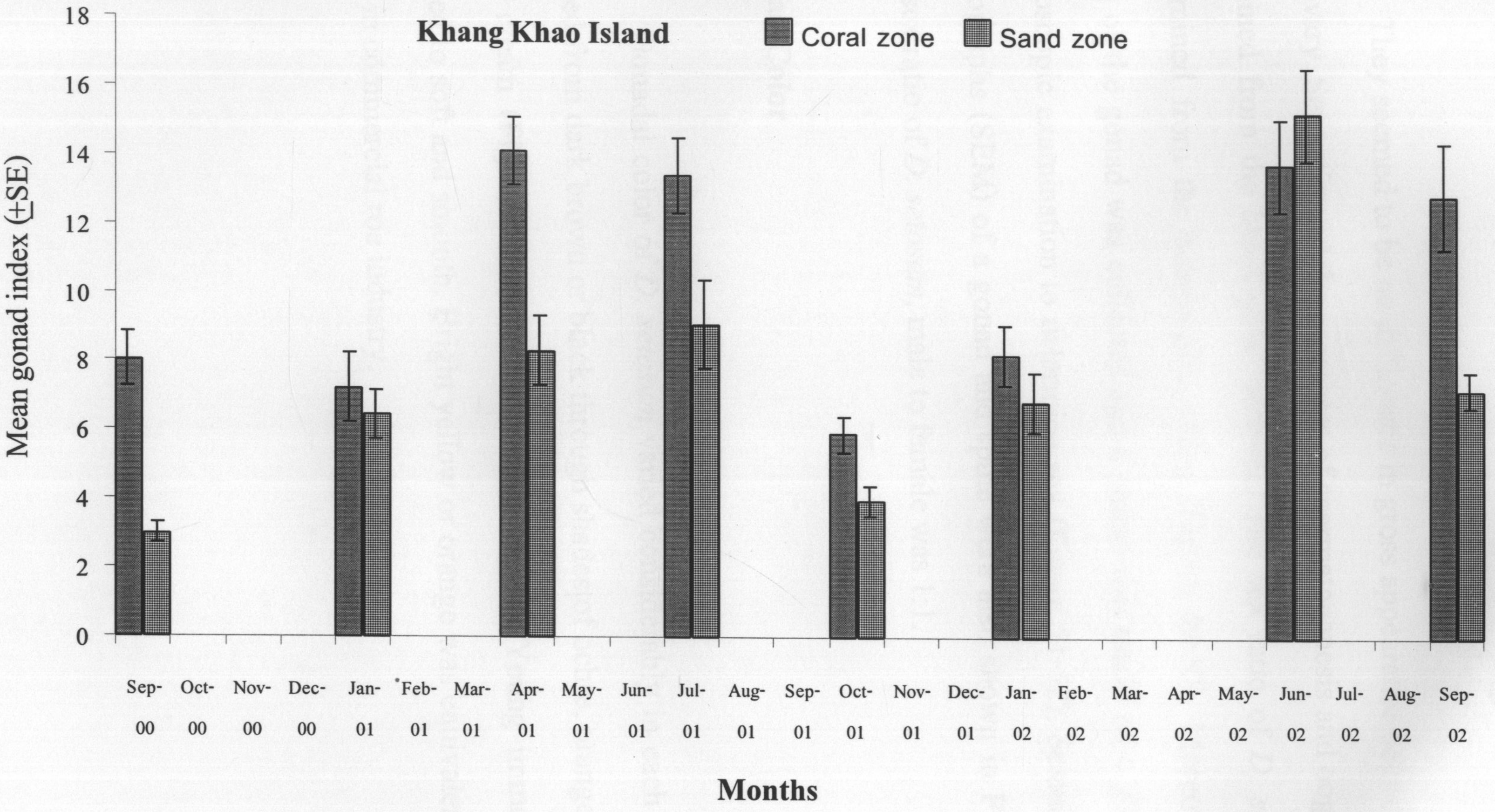


Figure 48 Mean Gonad Indies of *D. setosum* in the Coral Zone and Sand Zone at Khang Khao Island

Sex Ratio

They seemed to be no difference in gross appearance between the testis and ovary. Sex ratio and the processes of spermatogenesis and oogenesis were examined from the histological sections. The sex ratio of *D. setosum* was determined from the histological slides (Figure 49-50). Moreover a small piece of the gonad was collected and a smear was made on a glass slide for microscopic examination to determine sex (Figure 51-52). Scanning electron micrographs (SEM) of a gonad and spine were also shown in Figure 53-54. The sex ratio of *D. setosum*, male to female was 1:1.

Gonad Color

Gonadal color of *D. setosum* varied considerably in each sample, and ranged from dark brown or black through shades of ochre, orange, yellow and pale cream (Figure 55), with no relation to sex. Young immature gonads tended to soft and smooth. Bright yellow or orange was equivalented to grade "A" in commercial roe industry.

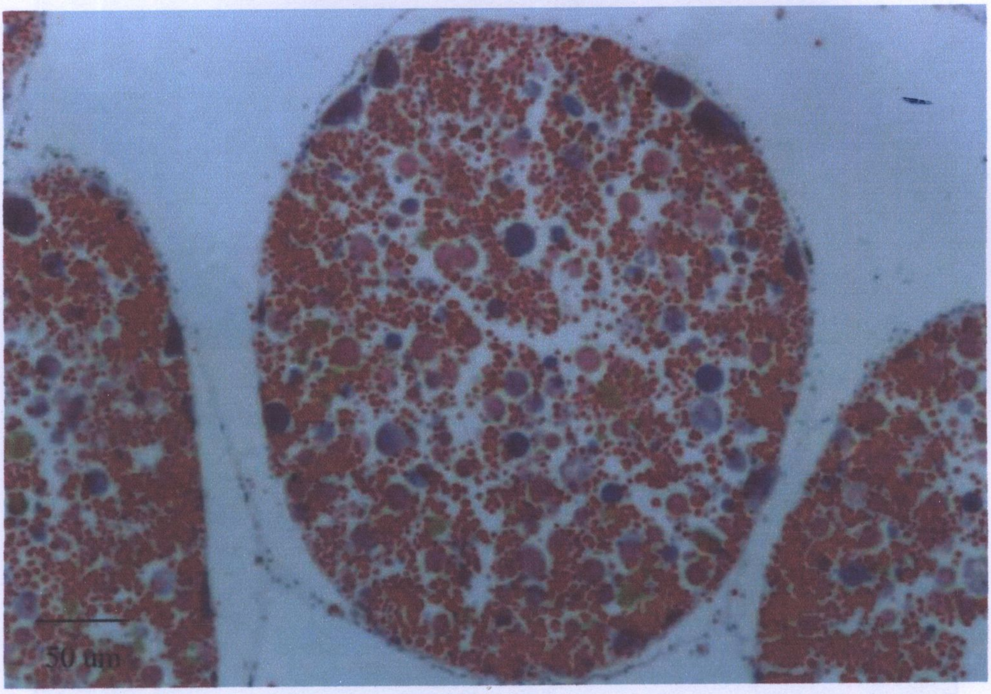


Figure 49 Histological Sections of the Gonads of *D. setosum* (Ovaries)

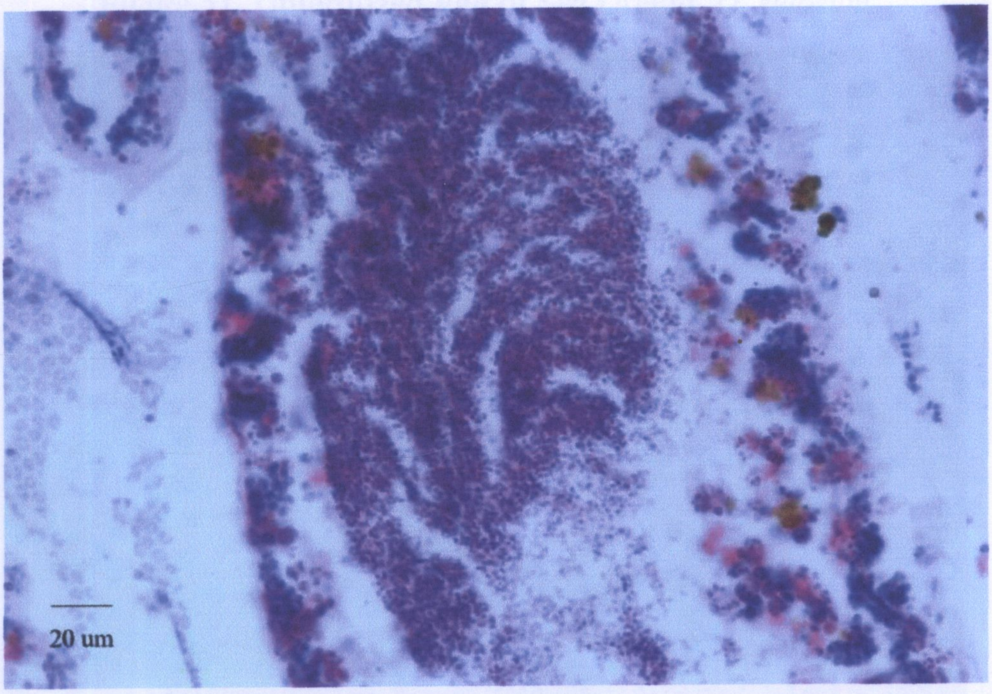


Figure 50 Histological Sections of the Gonads of *D. setosum* (Testes)

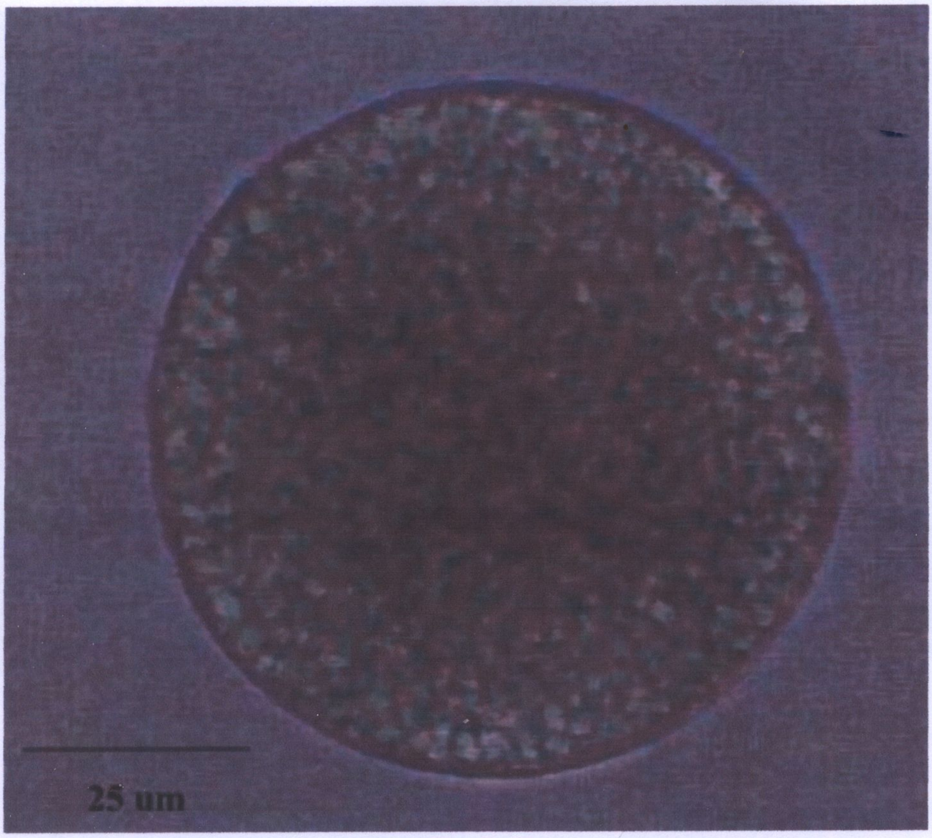


Figure 51 Unfertilized Egg of Sea Urchin, *D. setosum*

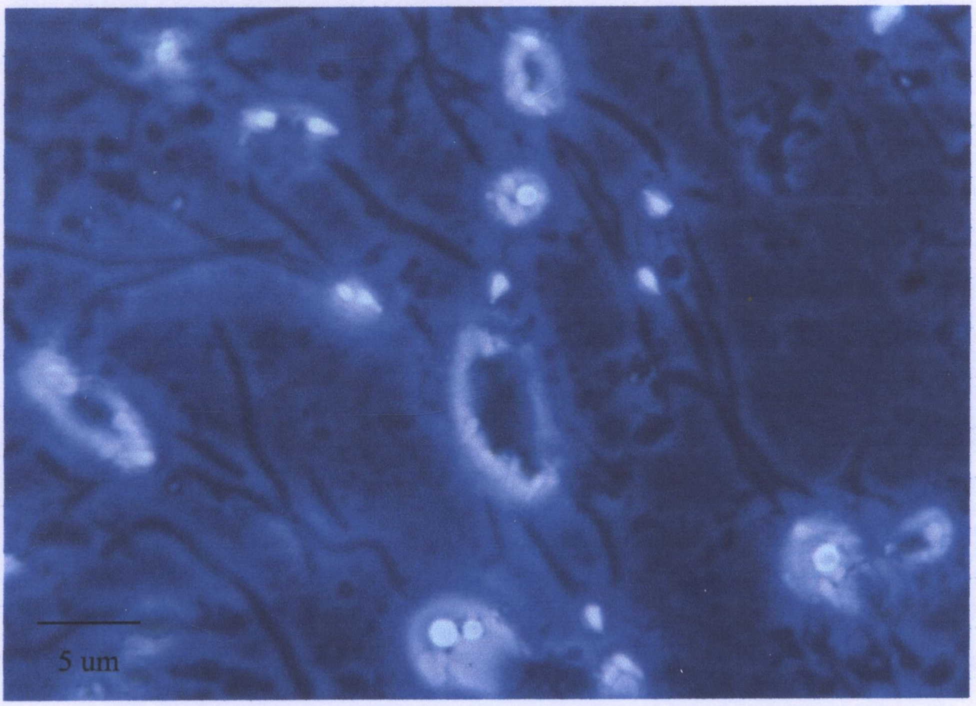


Figure 52 Sperm of Sea Urchin, *D. setosum*

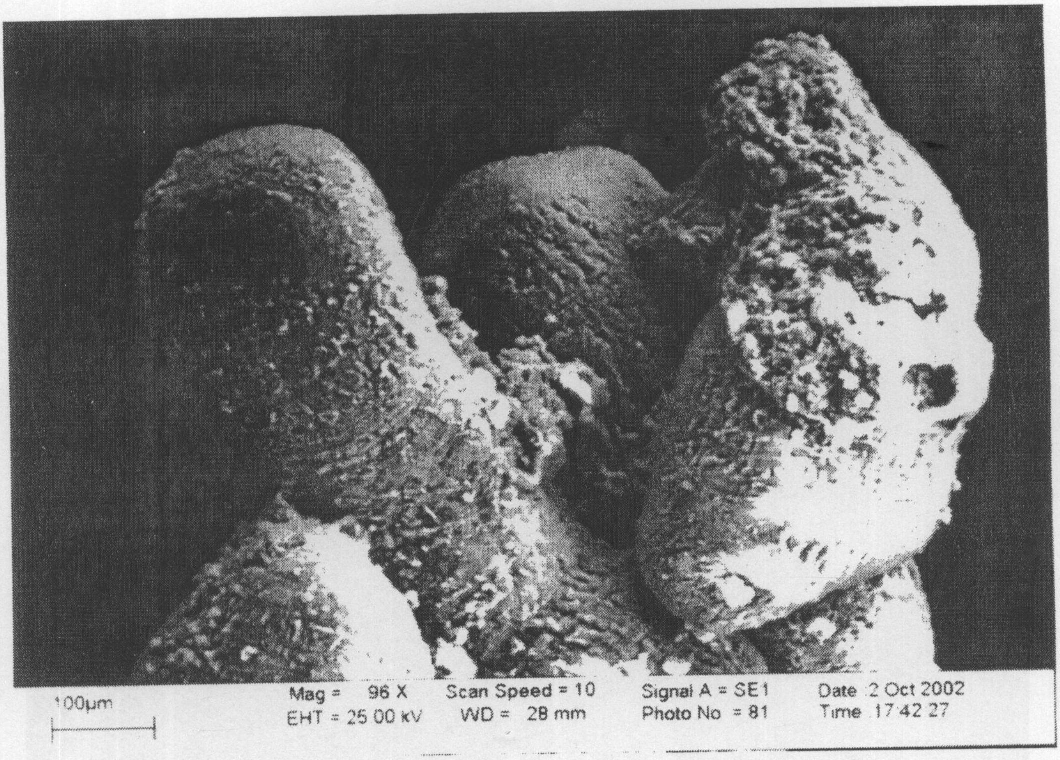


Figure 53 Scanning Electron Micrograph of Gonads in *D. setosum*

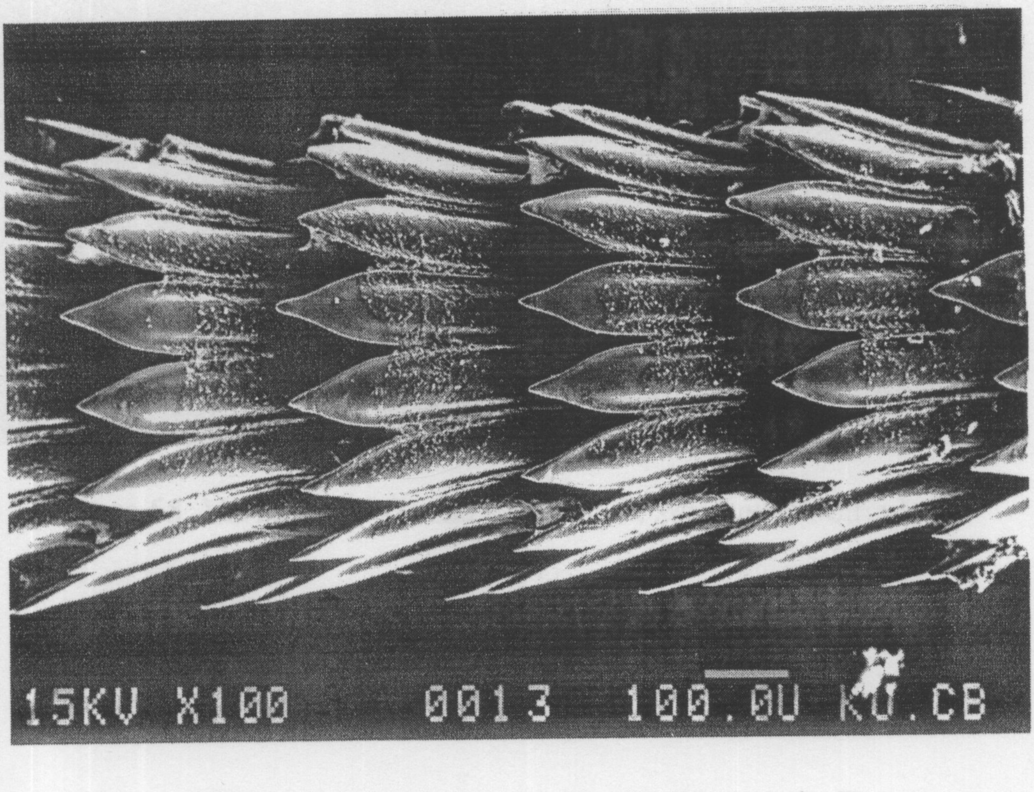


Figure 54 Scanning Electron Micrograph of Spines in *D. setosum*

Figure 55 Variation of Gonad Color of *D. setosum* (in circular)

Growth Rate

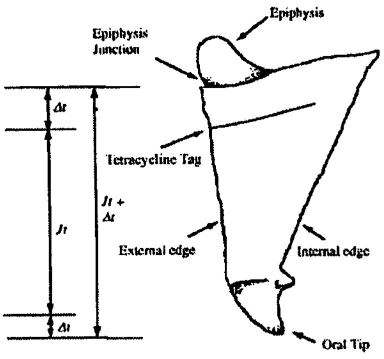
Mean growth rate (TD) of newly settled *D. setosum* from the experimental settlement devices in the field at Khang Khao Island was 2.66 ± 0.43 (mean ± SE) mm/month (Figure 56). Tetracycline tagged individuals were recaptured 1.2 years after tagging (Table 1, 2 and Figure 57). The growth parameters for the jaw were converted to growth parameters for test diameter first by determining the allometric relationship between test diameter and jaw length (Table 3).

Table 1 Summary of the Tagging and Recovery of *D. setosum* at Khang Khao Island Using the Fluorescent Dye Tetracycline

Summary of Tagging	Results
No. of sea urchin tagged (date)	271 (11th April, 2001)
Size range tagged (TD) (mm)	25-66
No. of sea urchins collected (date)	194 (23rd June, 2002)
Timing of tagged urchins	438 days post-tagging (≈1.2 years)
No. with Tetracycline tags	51
% tagged	26.29
Size range collected (TD) (mm)	31.5 - 68
Size range of tagged urchins (TD) (mm)	39-68

Table 2 Demi-Pyramids (Jaw) of *D. setosum* Tagged with Tetracycline in April, 2001 at Khang Khao Island and Collected in June, 2002

Jt	$Jt + \Delta t$	Jt	$Jt + \Delta t$	Jt	$Jt + \Delta t$
6.15	11.15	11.00	15.63	13.00	18.00
8.00	13.23	11.00	16.52	13.00	19.50
8.63	14.63	11.08	17.58	13.10	19.18
9.00	16.00	11.33	18.33	13.47	18.57
9.05	13.78	11.50	16.55	13.50	19.30
9.08	16.42	11.50	17.00	14.00	19.77
9.50	15.10	11.50	17.60	14.00	20.00
9.50	15.50	11.50	18.50	14.10	19.43
9.58	14.58	12.00	15.65	14.50	20.05
9.63	16.68	12.00	17.28	14.50	20.05
10.10	15.10	12.00	17.47	14.67	21.88
10.50	15.13	12.00	17.50	15.00	20.65
10.50	16.00	12.03	18.20	18.00	22.10
10.50	16.00	12.03	19.15		
10.50	16.13	12.07	17.97		
10.50	16.65	12.50	18.00		
10.50	16.70	12.50	18.00		
10.58	19.08	12.50	18.05		
10.60	17.15	12.55	19.05		



(Jt) is Represented by the Location of the Tetracycline Tag; ($Jt + \Delta t$) is Used to Calculate the Change in Demi-Pyramid Size Over Time; Measurements in Millimeters

Figure 56 Newly Settled *D. setosum* from the Experimental Settlement Devices

Figure 57 Ultraviolet Light Reveals the Size of the Skeletal Element (Jaw) at the time of tagging

Table 3 Demi-Pyramids (Jaw) of *D. setosum* was Converted to Test Diameter (mm) at the Time of Tagging (TDt) and Harvest (TD$t + \Delta t$)

TD$t + \Delta t$	**TDt**	**(TD$t + \Delta t$) - (TDt)**		**TD$t + \Delta t$**	**TDt**	**(TD$t + \Delta t$) - (TDt)**	
39.08	25.86	**13.21**		46.60	40.71	**5.90**	
42.87	31.70	**11.16**		48.80	40.71	**8.09**	
45.10	33.37	**11.73**		49.04	40.71	**8.34**	
47.10	34.32	**12.78**		49.09	40.71	**8.38**	
43.77	34.44	**9.33**	**11.40**	49.96	40.77	**9.19**	
47.67	34.52	**13.14**		51.09	40.77	**10.32**	
45.81	35.52	**10.29**		49.67	40.83	**8.84**	
46.39	35.52	**10.87**		49.71	41.61	**8.10**	
45.02	35.69	**9.33**		49.71	41.61	**8.10**	
48.01	35.81	**12.20**		49.77	41.61	**8.16**	
45.81	36.88	**8.93**		50.97	41.70	**9.27**	
45.85	37.74	**8.10**		49.71	42.48	**7.23**	**7.92**
47.10	37.74	**9.35**		51.49	42.48	**9.00**	
47.10	37.74	**9.35**		51.11	42.65	**8.46**	
47.27	37.74	**9.53**		50.40	43.27	**7.13**	
47.98	37.74	**10.24**		51.26	43.32	**7.94**	
48.05	37.74	**10.30**		51.79	44.13	**7.66**	
51.00	37.90	**13.10**		52.05	44.13	**7.92**	
48.64	37.95	**10.68**	**9.65**	51.41	44.29	**7.12**	
46.57	38.77	**7.79**		52.11	44.91	**7.20**	
47.80	38.77	**9.03**		52.11	44.91	**7.20**	
49.18	38.93	**10.25**		54.05	45.16	**8.89**	
50.12	39.44	**10.68**		52.76	45.66	**7.10**	
47.85	39.76	**8.08**		54.27	49.71	**4.56**	
48.44	39.76	**8.68**					
49.21	39.76	**9.45**		**(mean ± SE)**		**9.18 ± 0.26**	
50.32	39.76	**10.56**					

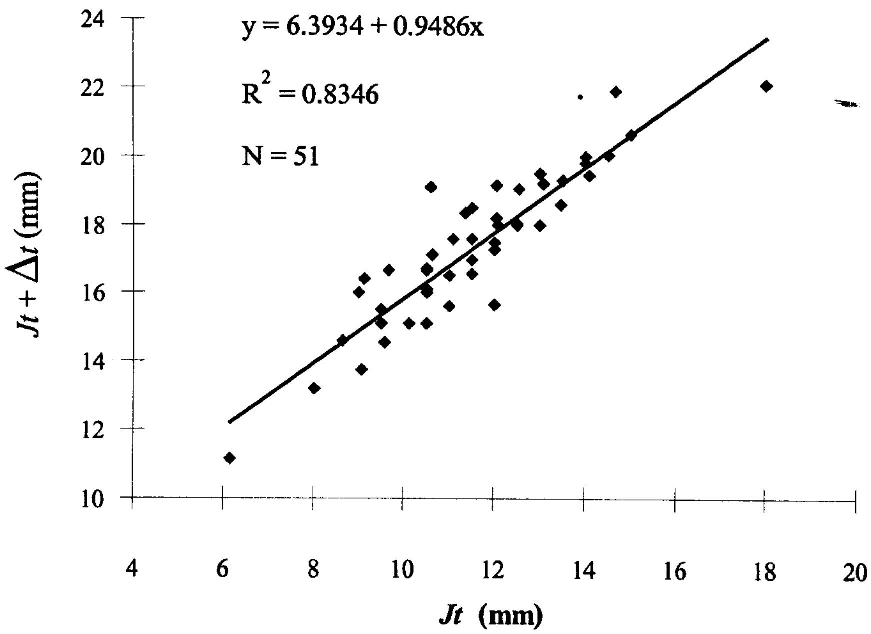


Figure 58 Initial (*Jt*) and Final Jaw Size (*Jt* + Δt) of *D.setosum* Tagged at Khang Khao Island

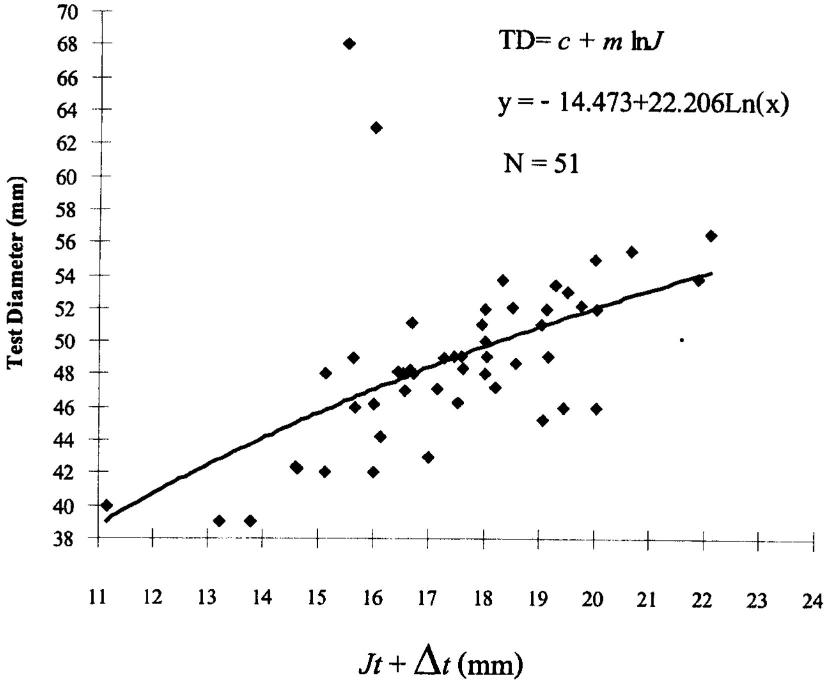


Figure 59 Relationship Between Test Diameter and Demi-Pyramid Length for *D. setosum* from Khang Khao Island

Estimating Growth Model Parameters from Brody-Bertalanffy Growth Function

The relationship between the initial (Jt) and final jaw Size ($Jt + \Delta t$) of *D. setosum* can be established for growth models by using the appropriate linear regression equation (Figure 58).

Parameters for the Brody-Bertalanffy function;

$$Jt + \Delta t = J_\infty(1 - be^{-kt})$$

were estimated using the linear regression model, $y = c + mx$ of initial and final sizes, where y = final size ($Jt + \Delta t$) and x = initial size (Jt), and the slope of the regression, $m = e^{-kt}$ Therefore

Maximum size $J_\infty = c/(1\text{-m})$

Growth constant $K = -\ln m/t$

were t= time between initial and final measurements and

$$b = 1 - (J_R / J_\infty)$$

where J_R = the size of the jaw at recruitment. Therefore, J_R was replaced by 1.5 mm when estimating growth of the jaw length and the difference model only estimated J_∞ and K. Therefore

The linear jaw size 1 year after tagged $= Jt + \Delta t = J_\infty Kbe^{-Kt}$

The parameters can be estimated by linear regression (Figure 59):

Maximum size $J_\infty = 6.3934/(1-0.9486)$

$J_\infty = 124.385$ mm

Growth constant $K = -\ln(0.9486)/1$

$K = 0.053\ \text{yr}^{-1}$

$$b = 1 - (1.5/124.385)$$

$$b = 0.988$$

$$Jt + \Delta t = J_\infty K b \mathrm{e}^{-\mathrm{Kt}}$$

$$Jt + \Delta t = (124.385)(0.043)(0.988)\mathrm{e}^{(-0.053)(1)}$$

$$Jt + \Delta t = (124.385)(0.043)(0.988)(0.948)$$

$$Jt + \Delta t = 5.016 \text{ mm}$$

The demi-pyramid length (*J*) was converted to test diameter (TD) using the non-linear, logarithmic equation (Figure 59).

$$\mathrm{TD} = c + m\ln J$$

$$\mathrm{TD} = -14.473{+}22.206\ln(Jt + \Delta t)$$

$$\mathrm{TD} = -14.473{+}22.206\ln(5.016)$$

$$\mathrm{TD} = 21.337 \text{ mm yr}^{-1}$$

Thus demi-pyramid length converted to test diameter (TD) for Brody-Bertalanffy model was 21.337 mm yr^{-1}.

Mortality Rate

When K as the Brody-Bertalanffy function growth-rate constant and m the slope of the regression (Figure 60), the mortality coefficient, Z is

$K = -\mathit{ln}\,m/t$

$K = -\mathit{ln}(0.6434)/1$

$K = 0.441$

$Z = K\ (m+1)$

the mortality coefficient Z for *D. setosum* is

$Z = 0.441\ (0.6434+1)$

$Z = 0.725\ \mathrm{yr}^{-1}$

The annual mortality rate are

$\text{Annual mortality rate} = 1\text{-}e^{-z}$

$1\text{-}e^{-z} = 1\text{-}e^{-0.725} = (1\text{-}0.484)$

$= 0.516 \text{ or } 51.6\%\ \mathrm{yr}^{-1}$

Therefore, mortality rates observed in the field were very low (Figure 61).

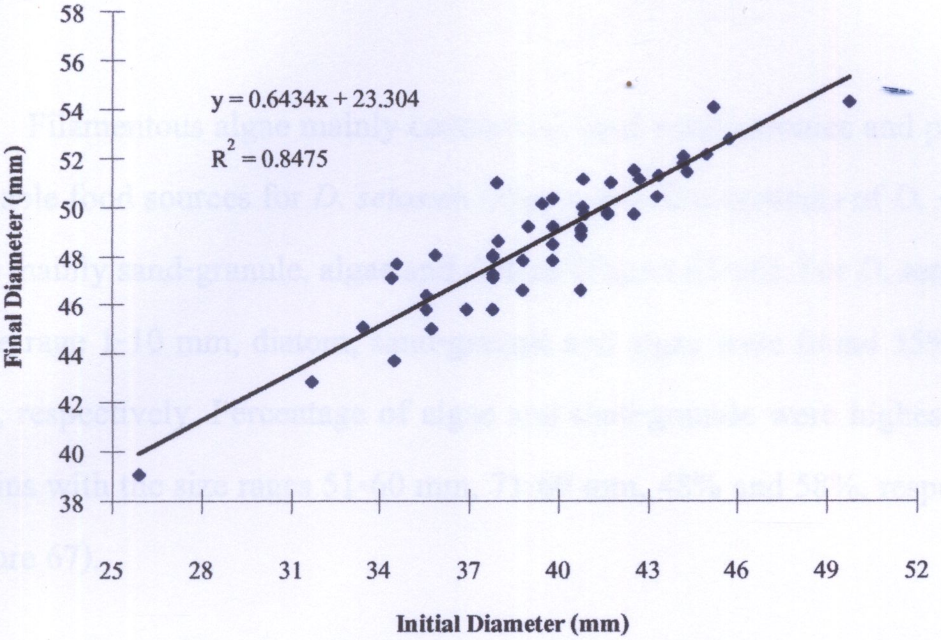


Figure 60 Relationship Between Initial Diameter and Final Diameter of *D. setosum* After Converted Jaw length to Test Diameter

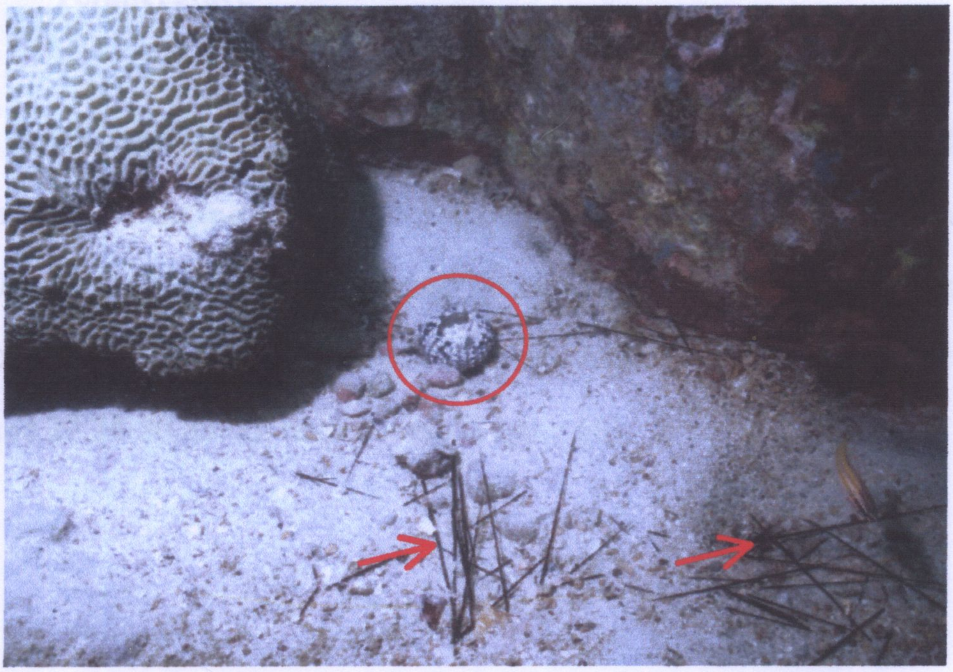


Figure 61 Mortality of *D. setosum* in the Field: Body Wall (in circular); Spine (arrow)

Foods and Gut Contents

Filamentous algae mainly covered on dead coral colonies and provided available food sources for *D. setosum* (Figure 62). Gut contents of *D. setosum* were mainly sand-granule, algae and diatom (Figure 63-66). For *D. setosum* in a size rage 1-10 mm, diatom, sand-granule and algae were found 35%, 32%, 28%, respectively. Percentage of algae and sand-granule were highest in sea urchins with the size rages 51-60 mm, 71-80 mm, 48% and 58%, respectively (Figure 67).

Environmental Factors

Temperature

The surface seawater temperatures (SST) of Khang Khao Island and Samet Island during July, 2000-September, 2002 were high in June and July, 2001 and June, 2002 for the former and in June and July, 2001 for latter. The maximum SST at Khang Khao Island and Samet Island ranged 29.30-30.71 °C and 29.24-30.83 °C, respectively (Figure 68).

Salinity

Maximum salinity of Khang Khao Island and Samet Island are showed in Figure 69. Khang Khao Island (salinity range 30.56-32.76 ppt) had less salinity than Samet Island (salinity range 30.79-33.05 ppt) because the former is located near the Chao Phraya River mouth.

Aquaculture

In recent decades, fisheries for sea urchins have developed throughout the world in response to increasing demand for the roe of these animals. *D. setosum*'s eggs for human consumption (Figure 70-72), are high potential for commercial aquaculture. In addition, two species of sea urchins, *Temnopleurus toreumaticus* and *Toxopneutes pileolus,* at Khang Khao Island were also found especially in the sand zone (Figure 73). Therefore they, as *D. setosum* are species of economic interest.

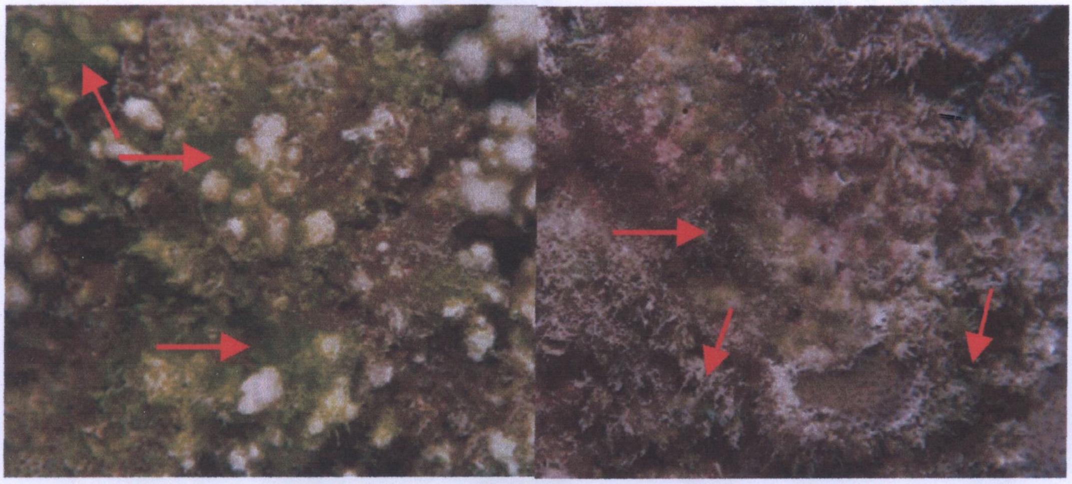


Figure 62 Filamentous Algae (arrows) were Main Food Sources for *D. setosum*

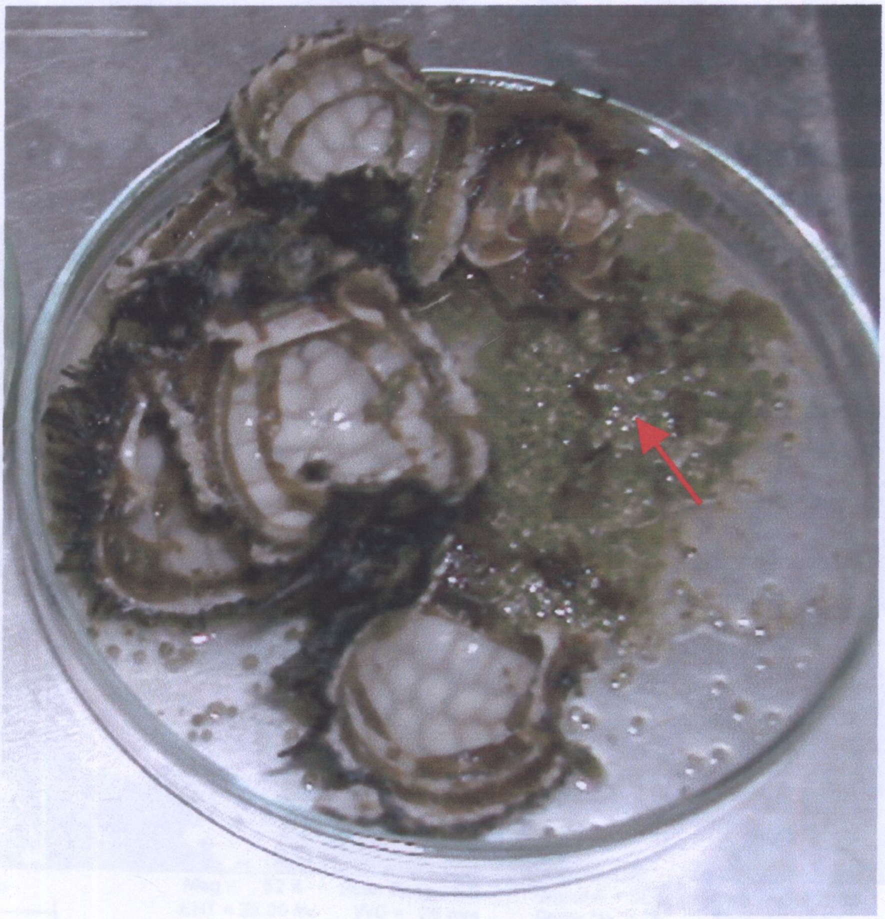


Figure 63 Gut Contents of *D. setosum* (arrow) after dissection

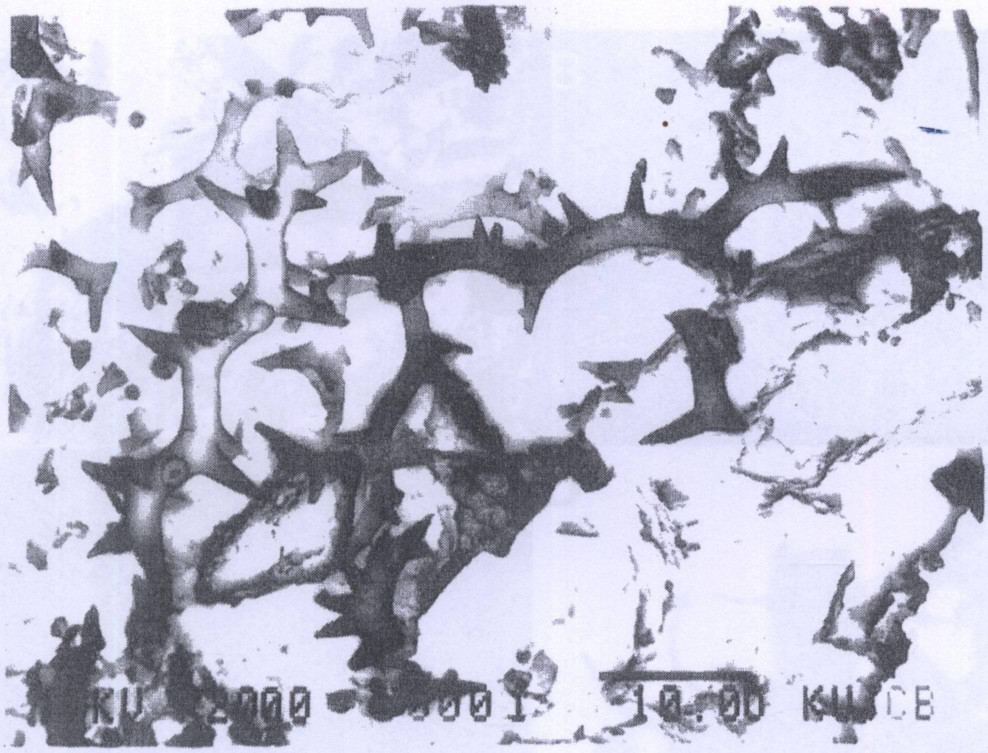


Figure 64 SEM of Algae in Gut Contents of *D. setosum*

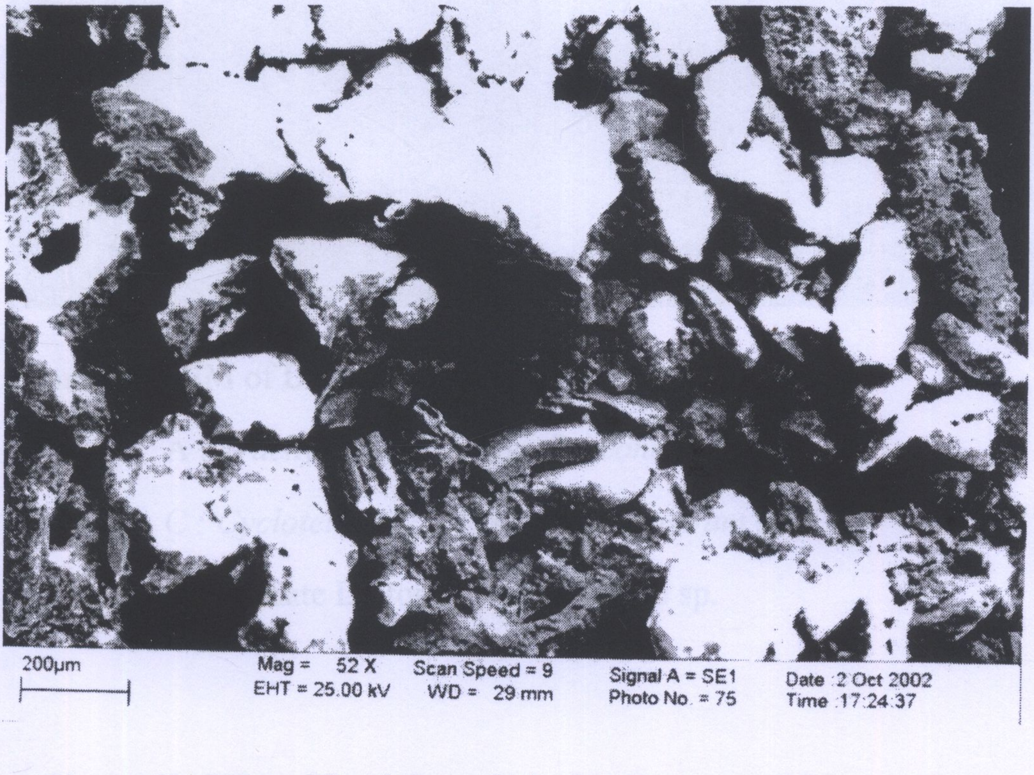


Figure 65 SEM of Sand-Granule in Gut Contents of *D. setosum*

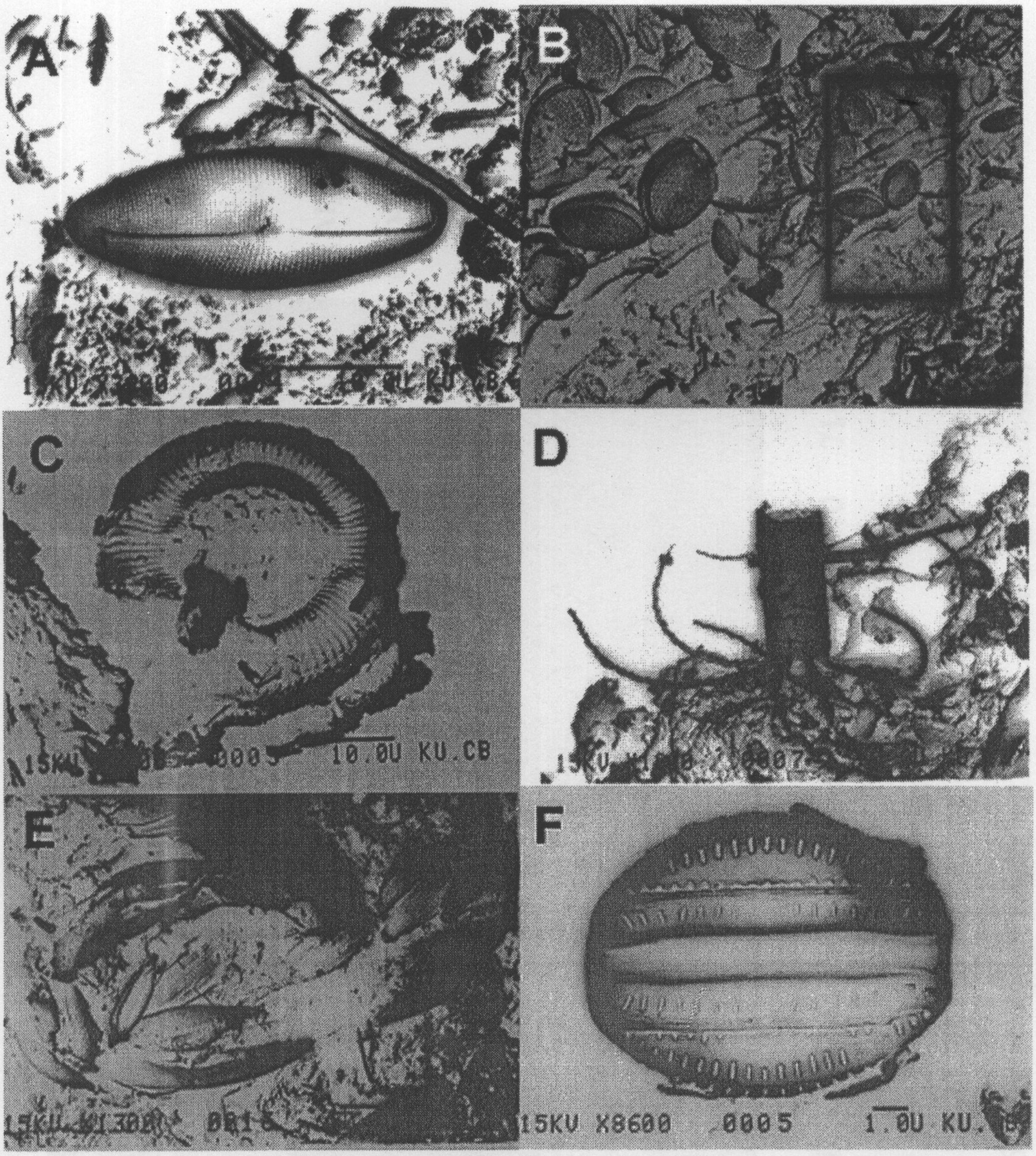


Figure 66 SEM of Diatoms in Gut Contents of *D. setosum*

A : *Trachyneis* sp. B : *Cocconeis* sp.

C : *Cyclotella* sp. D : *Bacteriastrum* sp.

E : Pennate Diatom F : *Amphora* sp.

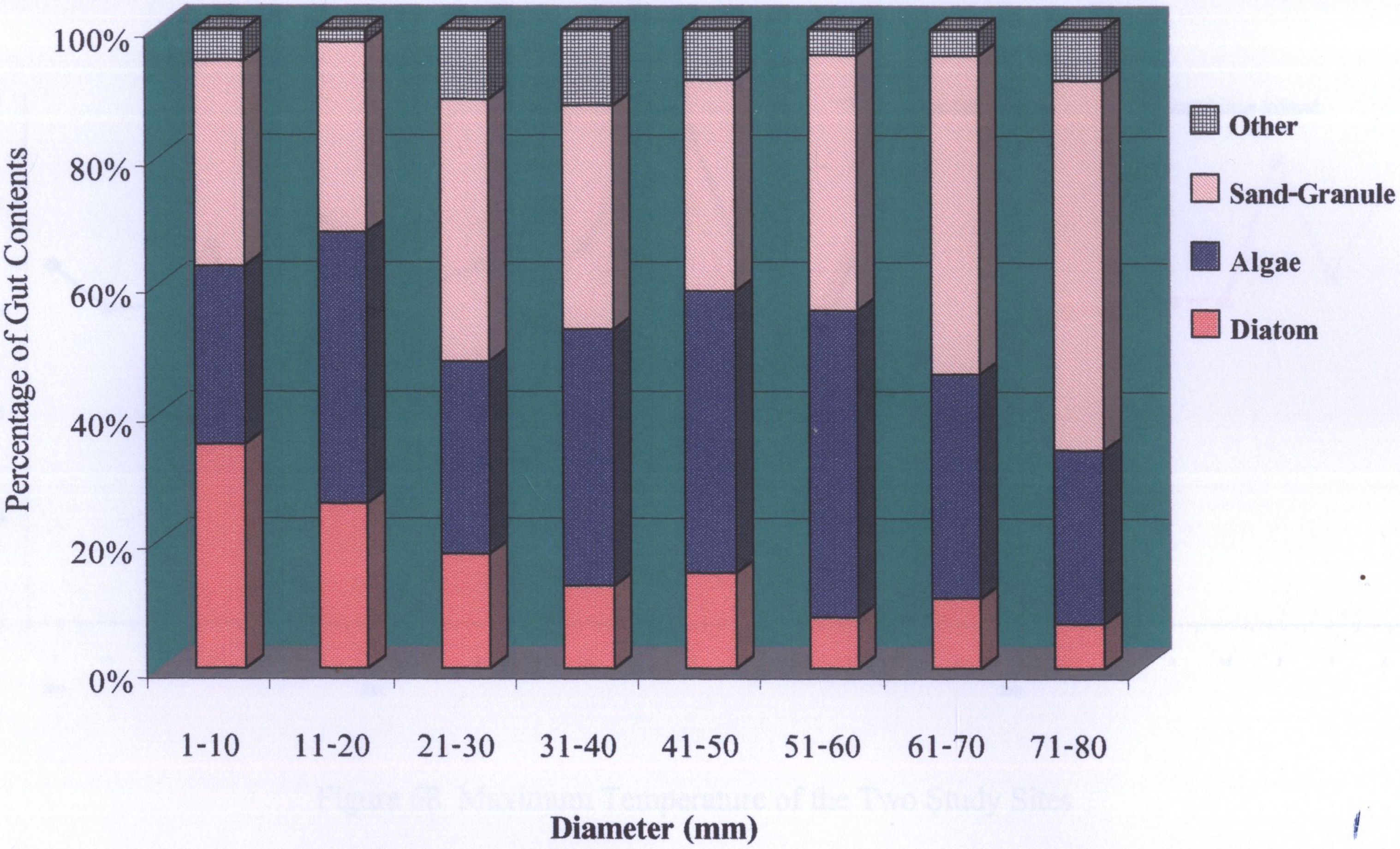


Figure 67 Percentage of Foods in the Gut Contents of *D. setosum*

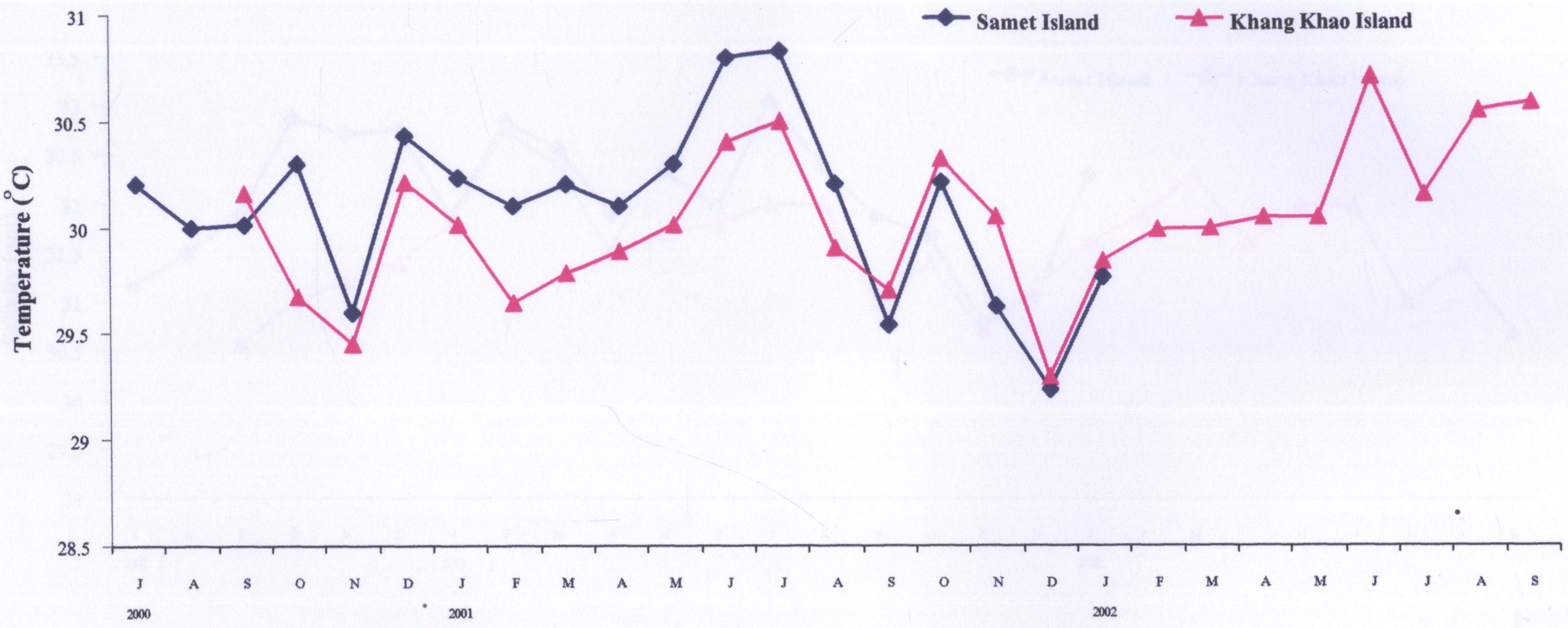


Figure 68 Maximum Temperature of the Two Study Sites

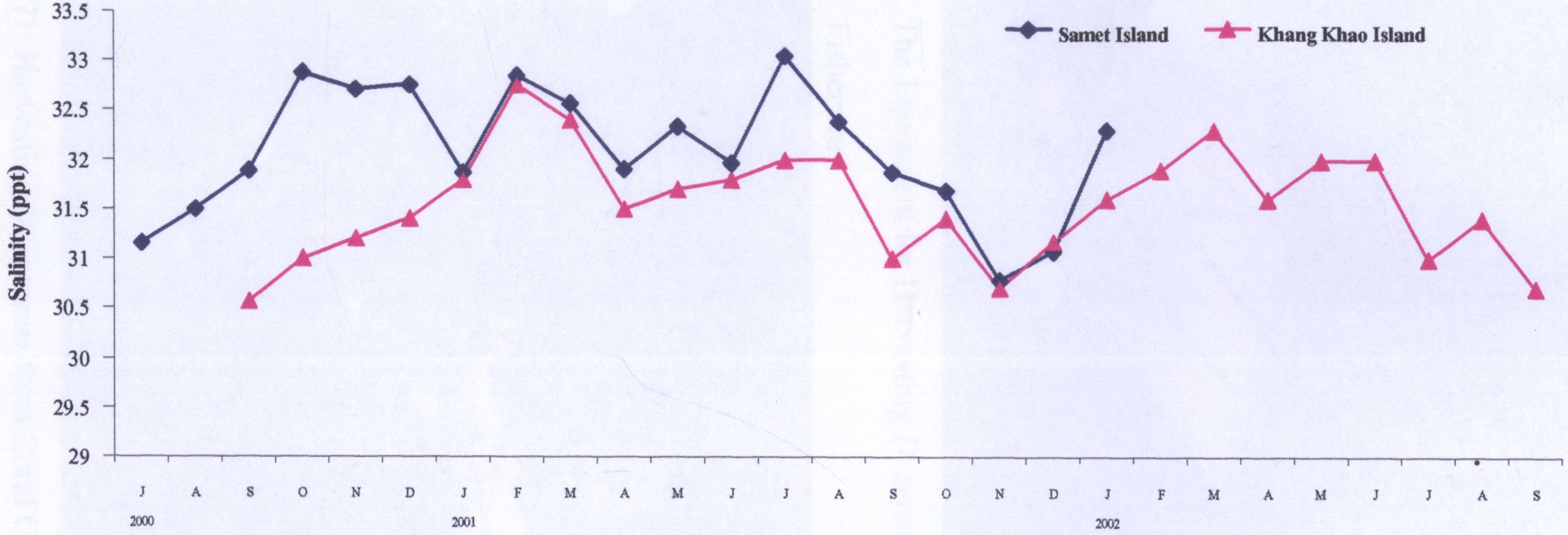


Figure 69 Maximum Salinity of the Two Study Sites

Figure 70 The Equipment for Harvesting *D. setosum* by Local Fisherman

Figure 71 Harvesting *D. setosum* from Coral Comminities of Chumporn Province by a Fisherman

Figure 72 Cleaning Gonads of *D. setosum* by a Fisherman

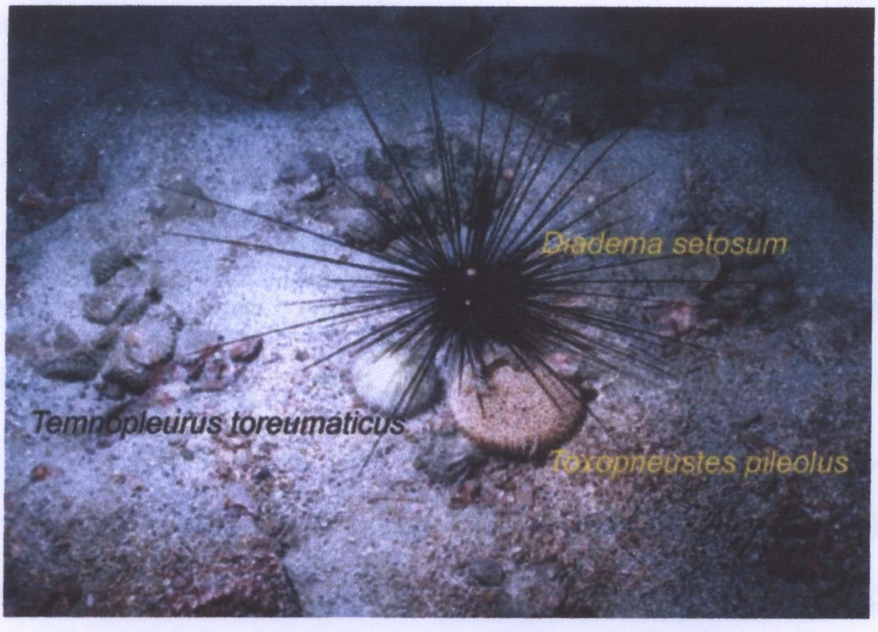


Figure 73 Three Species of Sea Urchins found at Khang Khao Island

(Sammarco 1982, 53-59). Miller et al. (2003, 181) reported *Diadema* abundance on back reefs and patch reefs in the Caribbean that have been well studied for >25 years. From June 2000 to June 2001, populations on back reefs have increased >100% (June 2001 mean densities 0.004–0.368 individuals/m^2), while patch reef populations increased >350% (June 2001 densities 0.236–0.516 individuals/m^2). However, the recovery in Jamaica is much more advanced than that in St. Croix (densities of ~5 individuals/m^2) in urchin zones (Edmunds and Carpenter 2001, 5068). Mean test diameter is not correlated with population density across sites; this suggests that population densities are well below trophic carrying capacity, with no intraspecific competition (Levitan 1988, 167-178). The population densities were not atypical, though; green sea urchins are often found aggregated at comparable densities at feeding fronts, living on vertical and horizontal surfaces ranging from rock walls to pier pilings and even stacked vertically along kelp stipes (Wahle and Peckham 1999, 127-137).

Test diameter of *D. setosum* at Samet Island was 71.16 ± 0.79 mm which was highly different from that of Khang Khao Island (only 43.63 ± 0.21 mm). The size distribution data at Khang Khao Island clearly shown that the juvenile recruits of *D. setosum* were in September, 2002. Size distribution data before the 1991 event indicated a bimodal distribution, with a cohort in the 31 to 40 mm class and large individuals in the 61 to 70 mm TD class, with the larger size classes more frequent (Forcucci 1994, 921).

Recruitment and Settlement

Peaks of newly settled *D. setosum* were found in January, July, October, 2001 and September, 2002 at Khang Khao Island. While those at Samet Island were in July, September, 2000 and April, July, 2001. Sea urchin recruits were found more on dead coral than on hard substrate (fragments of rock) and live coral, respectively. A comparison with other sea urchins are given in Table 4. Newly settled *D. setosum* from the experimental settlement devices were observed in the bottom of the coral and sand zones. No *D. setosum* settlement was found in the floating devices of both coral and sand zones. Recruitment studies combined with the GI data clearly showed that spawning period of *D. setosum* in the Gulf of Thailand were year round. Spawning time of *Diadema* spp. in various parts of the would are shown in Table 5.

The populations were dominated by small urchins, suggesting high recent recruitment. Increased *Diadema* densities appear to be affecting macroalgae abundance. The general spatio-temporal pattern of recovery around St. Croix seems to be following that of the die-off, suggesting that the same oceanographic features that spread *Diadema*'s pathogen are now carrying urchin larvae (Miller et al. 2003, 181). Intertidal field observation have shown that juvenile purple sea urchins *S. purpuratus* are found in higher densities near adults. The same is true for subtidal of juvenile red sea urchins *S. franciscanus* (Cameron and Schroeter 1980, 243). Settlement and metamorphosis behavior of competent larvae of sea urchin *Evechinus* *chloroticus* were examined in the laboratory and showed a preference for natural substrates (i. e. *Coralline* algae>oyster>shell>aged>rock) and for surfaces with older biofilms (Lamare and Barker 2001, 153). Densities of *S. franciscanus* remained very low (1 urchin/m^2), whereas the population of *S. purpuratus* had one substantive recruitment event in 1975-1976 when densities increased 25-fold from about 2 to about 50 urchins/10m^2 (Pearse and Hines 1987, 275). Takahashi et al. (2002, 763) used to promote larval settlement and metamorphosis of the sea urchin *S. intermedius* and *S. nudus*. They investigated chemical inducer(s) for larval settlement and metamorphosis of these sea urchins with extracts of the green alga *Ulvella lens*. Ebert (1983, 169) was unable to detect significant correlations between recruitment patterns and those of water temperatures, water run-off from the land, and upwelling indexes.

Table 4 Studies That Have Shown Sea Urchins Settlement and Metamorphorsis in Response to Natural Substrata

Species	**Natural Substrate**	**Reference**
Anthocidaris crassispina	Benthic diatoms such as *Achnanthes, Amphora, Navicula,Nitzschia*	Kitamura et al. (1993)
Diadema setosum	Dead coral, coral skeleton, sand and hard substrate (fragments of rock), filamentous algae, adult habitats	**Present study**
Evechinus chloroticus	Microbial film, algal-encrusted rock	Walker (1984)
Loxechinus albus	Microbial film	Gonzalez et al. (1989)
Lytechinus pictus	Microbial film	Hinegardner (1969, 1972, 1975) Cameron and Hinegardner (1974, 1978) Hinegardner and Tuzzi (1981) Satterlie and Cameron (1985)
	Ammonia-producing bacteria	Gilmour (1991)
	Rocks and shells from adult habitats	Burke (1980)
Lytechinus variegatus	Microbial film	Michel (1984)
	Microbial film	Cameron et al. (1989)
	Ammonia-producing bacteria	Gilmour (1991)
	Macroalgae and encrusted algal mats	Roller and Stickle (1993)
	Rocks and shells from adult habitats	Burke (1980)
	Variety of mollusc shells	Pawson and Miller (1982)
Paracentrotus lividus	Microbial film	Catoira Gomez et al. (1994)
Parechinus angulosus	*Patella* shell previously attached to conspecific adult	Cram (1971)
Pseudocentrotus depressus	Benthic diatoms	Tani and Ito (1979)
	Benthic diatoms *Navicula ramosissima*	Ito et al. (1987)
	Benthic diatoms such as *Achnanthes, Amphora, Navicula, Nitzschia*	Kitamura et al. (1993)
	Coralline alga *Corallina pilulifera*	Kitamura et al. (1992, 1993)
Sterechinus neumayeri	Sediments from adult habitats	Bosch et al. (1987)

Table 4 (continued)

Species	**Natural Substrate**	**Reference**
Strongylocentrotus droebachiensis	Ammonia-producing bacteria	Gilmour (1991)
	Microbial film, possibly microalgae, a variety of coralline and noncoralline macroalgal species, microbially-filmed cobble and sediments	Pearce and Scheibling (1991)
	Coralline alga *Lithothamnion glaciale*	Raymond (1985)
	Coralline alga *Lithothamnion glaciale*	Hart and Scheibling (1988)
	Macroalgae and encrusted algal mats	Roller and Stickle (1994)
	Rocks and shells from adult habitats	Burke (1980)
Strongylocentrotus franciscanus	Microbial film	Cameron and Schroeter (1980)
	Pseudomonad	Miller (1989)
Strongylocentrotus intermedius	Diatoms	Dept. of Mariculture, Hokkaido Central Fisheries Experimental Station 1985
	Sea grass *Zostera marina* colonized by calcareous alga *Melobesia* sp.	Naidenko and Dzyuba (1982) Naidenko (1983, 1991) Naidenko et al. (1991)
Strongylocentrotus pallidus	Macroalgae and encrusted algal mats	Roller and Stickle (1994)
Strongylocentrotus purpuratus	Microbial film, coralline alga, probably *Lithothamnium* sp.	Cameron and Schroeter (1980)
	Microbial film	Leahy (1986)
	Microbial film, coralline algae (*Lithothamnium, Lithophyllum, Pseudolithophyllum*), red algal turf (*Rhodymenia californica, Gigartina* spp., *Gymnogongrus* sp.)	Rowley (1989)
	Microbial film, coralline algae (*Lithophyllum, Lithothamnium, Calliarthron, Bossiella*)	Harrold et al. (1991)
Tripneustes esculentus	Ground algae, sand and fragments of rock	Lewis (1958)

Source: Pearce, C. M. 1997. Induction of settlement and metamorphosis in echinoderms. **Recent Advances in Marine Biotechnology** 1: 283-341.

Table 5 Spawning Times of *Diadema* spp. in Various Parts of the World

Species	**Location**	**Spawning**	**Referennce**
Diadema antillarum	Bermuda	April-November	Iliffe and Pearse 1982
	Barbados	January-April	Lewis 1966
	Curacao	March-December	Randall et al. 1964
	Panama (Caribbean)	Year round	Lession 1981
	Virgin Island	Year round	Randall et al. 1964
Diadema mexicanum	Panama (Pacific)	September-November	Lession 1981
Diadema savignyi	South Africa (east coast)	December-January	Drummond 1995
Diadema setosum	Australia	January-March and November-December	Stephenson 1934
	Egypt	June-August	Pearse 1969
	Honshu, Japan	June-August	Yoshida 1952
	Philippines	Year round	Tuason and Gomez 1979
	Singapore	Year round	Hori et al. 1987
	Kuwait	April-May	Alsaffar and Lone 2000
	Thailand (Gulf of Thailand)	Year round	**Present Study**

Source: Alsaffar, A. H., and K. P. Lone. 2000. Reproductive cycles of *Diadema setosum* and *Echinometra mathaei* (Echinoidea: Echinodermata) from Kuwait (Northern Arabian Gulf). **Bull. Mar. Sci.** 67: 845-856.

Growth and Mortality

Tagging Sea Urchins

Marking individuals is imperative for monitoring animals' movement patterns and behavior and for certain methods of estimating population size (Neill 1987, 92). Tetracycline tagged individuals of *D. setosum* recaptured 1.2 years after tagging were found 26.29%. A comparison with other tagging techniques for sea urchins is shown in Table 6. Annual mortality rate calculated from the regression method at Khang Khao Island was 51.6% yr^{-1}. Duggan and Miller (2001, 115-122) found anchor tags anchored in a hole drilled in the test caused over 50% mortality in only 1 month. Ebert (1965, 193-194) found that a nylon line threaded through two holes in the test was held firm by recalcification. The PIT tag (Hagen 1996b, 271) had potential but would be more useful if the detector could be submerged. Ebert (1968, 1075-1091) measured growth of urchins tagged by threading monofilament line through two holes drilled in the test. However, both reported slower growth than measured by size frequency analyses. Tetracycline injections, used extensively by Ebert (1980, 467-474), and florescent dye injections (Lamare and Maladenov 2000) leave a permanent mark on the test plates and jaws and appear not to injure the urchins or retard growth.

Table 6 Techniques for Tagging and Marking Sea Urchins

Technique	Species	Source
Wrapping with		
Elastic band	*Echinus esculentus*	Moore, 1935
	Paracentrotus lividus	Dance, 1987
Nylon line or brass wire	*Centrostephanus rodgersii*	Sinclair, 1959
	Psammechinus miliaris	Cuenca,1988
Steel wire	*Paracentrotus lividus*	
	Tripneustes ventricosus	McPherson, 1968
	Eucidaris tribuloides	
	Lytechinus variegatus	Moore et at. 1963; Gamble, 1966
		Neill and Larkum, 1966
Writing on test surface		
With lead pencil	*Meoma ventricosa*	Cheser, 1969
Injection of		
Various days	*Strongylocentrotus droebachiensis*	Swan,1961
	Strongylocentrotus echinoides	
	Strongylocentrotus franciscanus	
	Strongylocentrotus purpuratus	
Tetracycline	*Diadema setosum*	Ebert, 1980a; Ebert, 1980b Ebert, 1982
		Present study
	Diadema paucispinum	Ebert, 1982
	Centrostephanus rodgersii	
	Echinotrix calamaris	
	Echinotrix diadema	
	Stomopneustes variolaris	
	Salmacis belli	
	Toxopneustes pileolus	
	Tripneustes gratilla	
	Tripneustes sp.	
	Colobocentrotus atratus	
	Heliocidaris erythrogramma	
	Heliocidaris trigonarius	
	Echinometra oblongata	
	Heliocidaris mathaei	Ebert, 1982; Ebert, 1988a
	Heterocentrotus mammillatus	Ebert, 1982; Ebert, 1988b

Table 6 (continued)

Technique	**Species**	**Source**
	Echinus esculentus	Gage, 1992a; Gage, 1992b
	Paracentrotus lividus	Märkel,1975
	Psannecgubys miliaris	Gage, 1991
	Strongylocentrotus intermedius	Kobayashi and Taki, 1969;Taki, 1971; Taki, 1972a; Taki, 1972b; Taki, 1978
	Strongylocentrotus franciscanus	Ebert, 1977; Ebert and Russell, 1992; Ebert and Russell, 1992
	Strongylocentrotus purpuratus	Pearse and Pearse, 1975; Ebert, 1977 Russell, 1987; Ebert, 1988a; Kenner, 1992
Calcein	*Evechinus chloroticus*	Lamare and Madenov, 2000
Spine attachments		
Rubber balloon pieces	*Phyllachantus parvispinus*	Sinclair, 1959
Surgical tubing	*Diadema antillarium*	Carpenter, 1984
Plastic insulation	*Paracentrotus lividus*	Dance, 1987
Hole in spine threaded with plastic-covered wire or brass wire	*Phyllachantus parvispinus*	Sinclair,1959
Two holes in skeleton threaded with		
Nylon line	*Strongylocentrotus purpuratus*	Ebert, 1965; Ebert, 1967; Ebert, 1968
	Strongylocentrotus intermedius	Kawamura and Hayashi, 1964
	Echinometra mathaei	McClanaham and Muthiga, 1989; Ebert, 1968
	Evechinus chloroticus	Dix, 1970; Walker, 1981
	Hemicentrotus pulcherrimus	Fuji, 1962; Fuji, 1963; Hur et at. 1985
	Paracentrotus lividus	Fenaux et al. 1987
Steel wire	*Centrostephanus coronatus*	Nelson and Vance, 1979
	Strongylocentrotus franciscanus	Lees, 1968
One hole in skeleton with		
T-bar anchor tag	*Echinus esculentus*	Dix, 1970
	Strongylocentrotus franciscanus	Olson and Newton, 1979
	Three species of Indo-Pacific echinoids	Neill, 1987
Barbed steel wire tag	*Centrostephanus coronatus*	Nelson and Vance, 1979

Table 6 (continued)

Technique	**Species**	**Source**
Insertion of		
Aluminium tag	*Paracentrotus lividus*	Fenaux and Nouguier, 1976
PIT tag	*Strongylocentrotus droebachiensis*	Hagen, 1991
External dyeing with		
Fluorescent pigments	*Strongylocentrotus droebachiensis*	Swan, 1961b
Nile blue	*Strongylocentrotus* sp.	
	Strongylocentrotus intermedius	Kawamura and Hayashi, 1964
Isolation chamber	*Lytechinus pictus*	Hinegardner, 1975
	Lytechinus variegatus	Klinger et al. 1994
	Paracentrotus lividus	Frantzis et al. 1992
	Eucidaris tribuloides	Lares and McClintock, 1991a; Lares and McClintock, 1991b

Source: Hagen, N. T. 1996b. Tagging sea urchins: a new technique for individual identification. **Aquaculture** 139: 271-284.

Modelling Sea Urchins Growth

Table 7 summarizes sea urchin studies using growth models. Numerous works using growth rate only (final–initial size) are not included. Most species of economic interest (*Tripneustes gratilla, Sphaerechinus granularis, Psammechinus miliaris, Paracentrotus lividus, Strongylocentrotus droebachiensis, S. intermedius, S. nudus, S. franciscanus, Hemicentrotus pulcherrimus*) were considered by one or more authors. They focused either on population dynamics aiming to provide management criteria for fisheries, or on growth in cultivation. Other species studied were either key-species in some biotopes (*Diadema antillarum* Philippi, *Echinus esculentus* L.), or animals occupying some particular biotopes [for instance, deep-sea urchins like *Echinosigra phiale* (Thompson) or *Hemiaster expergitus* Loven] (Grosjean 2001, 52).

The Richards model was first proposed by Ebert (1973) as a better alternative to the von Bertalanffy 1 curve to fit echinoid growth data. It was intensively used by the same author (Ebert 1973, 281-298; 1980, 464-474; 1999, 181-206; Ebert and Russell 1992, 31-41; 1993, 79-89) as well as by some others (Kenner 1992; Turon et al. 1995; Lamare and Mladenov 2000). The Gompertz model is also a favorite when there seems to be a lag phase in growth and it has been used in various studies (Turon et al. 1995; Ebert 1999).

Seasonal differences in growth of sea urchins has been previously suggested for other sea urchin species (Barker et al. 1998, 1583; McBride et al. 1998, 1563-1570). Mean growth rate (TD) of newly settled of *D. setosum* from the experimental settlement devices in the field at Khang Khao Island was 2.66 ± 0.43 (mean ± SE) mm/month. The Demi-pyramid length converted to test diameter (TD) for Brody-Bertalanffy model was 21.337 mm yr^{-1}. A comparison with growth rate for other sea urchins is given in Table 8. The growth of juvenile *E .chloroticus* in the first year was found to be an average of 8.05 mm yr^{-1} in the laboratory, and an estimated 10.5 mm yr^{-1} in the field. Growth in the field appears to be biphasic, with an initial period of slow growth (0–200 days post-settlement), followed by a period of accelerating growth. In contrast, growth rate in the laboratory increased in the first 150 days, but remained constant after this time (Lamare and Mladenov 2000, 40). This individual variability is not genetic but is attributable to a reversible size based intraspecific competition (Grosjean et al. 1996, 173).

Table 7 Model Used to Fit Sea Urchins Growth Data

Species	**Growth model**	**Reference**
Family Cidaridae		
Eucidaris tribuloides (Lamarck)	von Bertalanffy1	McPherson, 1968
Family Diadematidae	Richards	Ebert, 1980a
Diadema setosum (Leske)	von Bertalanffy1, Richards, Gompertz, logistic	Ebert, 1999
	Brody-Bertalanffy	**Present study**
Diadema antillarum Philippi	von Bertalanffy1	Ebert, 1975
Echinotrix diadema (L.)	von Bertalanffy1	Ebert, 1975
	Richards	Ebert, 1982
Centrostephanus rodgersii (A. Agassiz)	Richards	Ebert, 1982
Family Stomopneustidae		
Stomopneustes variolaris (Lamarck)	Richards	Ebert, 1982
Family Temnopleuridae		
Salmacis belli Döderlein	Richards	Ebert, 1982
Family Toxopneustidae		
Lytechinus variegatus (Lamarck)	von Bertalanffy1	Ebert, 1975
Tripneustes gratilla (L.)	von Bertalanffy1, Gompertz, logistic, Johnson	Dafni, 1992
Tripneustes ventricosus (Lamarck)	von Bertalanffy1	McPherson, 1965
Sphaerechinus granularis (Lamarck)	von Bertalanffy1	Lumingas & Guillou, 1994
	von Bertalanffy1	Jordana et al, 1997
Family Echinidae		
Echinus esculentus L	Richards	Ebert, 1973
	logistic	Nichols et al, 1985
	logistic	Sime & Cranmer, 1985
	Gompertz	Gage et al, 1986
	von Bertalanffy1	Gage, 1992
Echinus acutus Lamarck	logistic	Sime & Cranmer, 1985
	von Bertalanffy1, Gompertz, logistic	Gage et al, 1986
Echinus elegans Düben & Koren	von Bertalanffy1, Gompertz, logistic	Gage et al, 1986
Echinus affinis Mortensen	von Bertalanffy1, Richards, Gompertz, logistic	Gage & Tyler, 1985
	Gompertz	Gage et al, 1986
	linear	Middleton et al, 1998
Psammechinus miliaris (Gmelin)	von Bertalanffy1	Jensen, 1969a
	von Bertalanffy1	Allain, 1978
	von Bertalanffy1	Gage, 1991

Table 7 (continued)

Species	Growth model	Reference
Paracentrotus lividus (Lamarck)	von Bertalanffy1	Allain, 1978
	(von Bertalanffy1), Gompertz	Cellario & Fenaux, 1990
	Gompertz, logistic, Richards	Turon et al, 1995
	von Bertalanffy1	Sellem et al, 2000
	von Bertalanffy1, von Bertalanffy2, Gompertz, logistic, 4p-logistic, Weibull, original model	Grosjean, 2001
Loxechinus albus Molina	von Bertalanffy1	Gebauer & Moreno, 1995
Family Strongylocentrotidae		
Strongylocentrotus droebachiensis (Müller)	von Bertalanffy1	Munk, 1992
	von Bertalanffy1	Hagen, 1996
	logistic	Meidel & Scheibling, 1998
	Tanaka	Russell et al, 1998
Strongylocentrotus intermedius (A. Agassiz)	von Bertalanffy1, Gompertz	Fuji, 1967
Strongylocentrotus nudus (A. Agassiz)	von Bertalanffy1	Ebert, 1975
Strongylocentrotus purpuratus (Stimpson)	von Bertalanffy1	Ebert, 1977
	Richards	Russell, 1987
	Richards	Kenner, 1992
Strongylocentrotus franciscanus (A. Agassiz)	von Bertalanffy1	Ebert, 1977
	Richards	Ebert & Russell, 1992
	Richards, Tanaka, Jolicoeur	Ebert & Russell, 1993
Hemicentrotus pulcherrimus (A. Agassiz)	Tanaka	Ebert, 1998
Allocentrotus fragilis (Jackson)	Richards, Tanaka	Ebert, 1999
	von Bertalanffy1	Fuji, 1963
	von Bertalanffy1	Sumich & McCauley, 1973
Family Echinometridae		
Evechinus chloroticus (Valenciennes)	von Bertalanffy1, Richards, Tanaka, Jolicoeur	Lamare & Mladenov, 2000
Anthocidaris crassispina (A. Agassiz)	von Bertalanffy1	Chiu, 1990
Heliocidaris erythrogamma (Valenciennes)	Richards	Ebert, 1982
Echinometra mathaei (de Blainville)	von Bertalanffy1	Ebert, 1975
	Richards	Ebert, 1982
Echinometra oblonga (de Blainville)	von Bertalanffy1	Ebert, 1975
	Richards	Ebert, 1982

Table 7 (continued)

Species	**Growth model**	**Reference**
Heterocentrotus mammillatus (Klein)	Richards	Ebert, 1982
Heterocentrotus trigonarius (Lamarck)	Richards	Ebert, 1982
Colobocentrotus atratus (L.)	von Bertalanffy1	Ebert, 1975
	Richards	Ebert, 1982
Family Mellitidae		
Mellita quinquiesperforata (Leske)	von Bertalanffy1	Lane & Lawrence, 1980
Mellita grantii Mortensen	von Bertalanffy1	Ebert & Dexter, 1975
Encope grantis L. Agassiz	von Bertalanffy1	Ebert & Dexter, 1975
Family Pourtalesiidae		
Echinosigra phiale (Thompson)	von Bertalanffy1, Gompertz, logistic	Gage, 1987
Family Hemiasteridae		
Hemiaster expergitus Loven	von Bertalanffy1, Gompertz, logistic	Gage, 1987
Family Spatangidae		
Spatangus purpureus Müller	von Bertalanffy1, Gompertz, logistic	Gage, 1987
Family Loveniidae		
Echinocardium cordatum (Pennant)	von Bertalanffy1	Duineveld & Jenness, 1984
Echinocardium pennatifidum Norman	von Bertalanffy1, Gompertz, logistic	Gage, 1987

Sourec: Grosjean, Ph. 2001. Growth model of the reared sea urchin *Paracentrotus lividus* (Lamarck, 1816). Ph. D. Thesis, Universite Libre de Bruxelles, Belgium.

Table 8 Size (Horizontal Diameter in mm) of Sea Urchin Species at an Age of 1-Year

Species	**Size**	**Reference**
Order Diadematoida		
Family Diadematidae		
Diadema antillarum	40	Lewis 1966
Diadema setosum[a]	45	Drummond 1993
	32 (experimental settlement devices)	**Present Study**
	22 (growth model)	
Order Phymosomatidae		
Family Stomechinidae		
Stomopneustes variolaris	15	Drummond 1993
Order Temnopleuroida		
Family Toxopneustidae		
Lytechinus variegatus[a]	50	Moore et al. 1963
	50	Allain 1975
	50-70	Oliver 1987
Tripneustes gratilla[a]	50	Shokita et al. 1991
	70	Dafni 1992
	40	Maharavo 1993
Tripneustes ventricosus[a]	80-90	Lewis 1958
Order Echinoida		
Family Echinidae		
Echinus esculentus[a]	35	Comely and Ansell 1988
	15	Gage 1992
Loxechinus albus[a]	35	Bustos et al. 1991
	20	Gebauer and Moreno 1995
Paracentrotus lividus[a]	7-15	Cellario and Fenaux 1990
	12	Brias and LeGall, in Leighton 1995
	8-11	Willis, in Leighton 1995
	5-16	Leighton 1995

Table 8 (continued)

Species	**Size**	**Reference**
	8	Jangoux (pers. comm.)
	20	Shpigel (pers. comm.)
Family Echinometridae		
Echiometra mathaei	25	Drummond 1993
Family Strongylocentrotidae		
Strongylocentrotus droebachiensis[a]	10-15	Miller and Mann 1973
	15	Fletcher et al. 1974
	15-20	Sivertsen and Hopkins 1995
	5	Meidel and Scheibling 1998
Strongylocentrotus franciscanus[a]	20	Bernard and Miller 1973
	13	Ebert and Russell 1993
Strongylocentrotus intermedius[a]	15	Fuji 1960
	<10	Kawamura 1964
	18	Taki 1986
Strongylocentrotus nudus[a]	30	Fuji 1960
	16	Kawamura 1966
Strongylocentrotus purpuratus[a]	18	Kenner 1992

[a] Species of economic interest

Source: Lawrence, J. M., and A. Bazhin. 1998. Life-history strategies and the potential of sea urchins for aquacultrure. **Journal of Shellfish Research** 17: 1515-1522.

Relationship Between the Components of Body and Test Diameter

The relationship between gonad weight and test diameter found was significant at Khang Khao Island. The gonad indies of *D. setosum* at Khang Khao Island in the coral zone were higher than in the sand zone. Gamete releasing occurred in June, 2002. Average values of the gonad index of *D. setosum* at Khang Khao Island and Samet Island were 12.70 and 15.15, respectively.

The previous studies on *Sphaerechinus granularis* in Brittany carried out by Guillou and Michel (1994, 97-111) showed the seasonal changes observed in their somatic and gonadal growth which could be to split into two stages: the pre and post spawning. Three or four months before the spawning (maturity period), there is no variation in weight and test diameter while GI is increasing; this implies an allocation of energy to only gonadal growth. Post spawning, there is a short rest period (August), then the recovery period starts over the autumn, and energy is allocated to both somatic (body wall increase) and gonadal growth. (Ebert 1968, 1075-1091) showed that during the maturity period neither gonadal growth, nor test growth as for the fed treatment, nor significant diameter decrease as pointed out by several authors after prolonged starvation.

Gonads, Foods and Gut Contents

Gut contents of *D. setosum* at Khang Khao Island and Samet Island were composed mainly sand-granule, algae and diatom. Filamentous algae mainly covered on dead coral colonies and provided available food sources for *D. setosum.* A small size (1-10 mm) of *D. setosum* had diatom in gut contents higher than the bigger sizes (51-60 mm and 71-80 mm). The diets of urchins from the southwest end of San Nicolas Island and Venture County, California were composed primarily of brown algae although fleshy red algae initially constituted a higher proportion of the diet in sea urchins from the barren site (Harrold et al. 1985, 1165). The rate of food consumption per day of *Lytechinus variegatus* and *Stronogylocentrotus franciscanus* was inversely related to the concentration of protein in the food (McBride et al. 1999, 364). McBride et al. (1999, 364-370) found a similar gonad production efficiency for *Stronogylocentrotus franciscanus* fed a similar prepared feed. However, Lawrence et al. (2003, 74) found little change in gonad production efficiency with the quantity of food available when food was always available. Again, the difference between in response to frequency of feeding and quantity of food available indicates a basic difference in physiological response to these two modes of feeding. For example, Russell (1998, 1-14) fed frozen algae to green urchins from food-limited populations during June and observed significant increases in gonad wet weight after 3 and 6 weeks. Lawrence et al. (1997, 91) fed post-spawned *Loxechinus albus* natural and prepared diets over a 3-month period and observed significant increases in gonad indices with both treatments relative to starved controls. They indeed indicated that diet containing animal-derived proteins and lipids did not significantly increase somatic growth during the pre-spawning period of *Strongylocentrotus droebachiensis* (Walker and Lesser 1998, 663) and *Evechinus chloroticus* (Barker et al. 1998, 1583). Cook et al. (1998, 1557) suggested, however, that a high energetic animal food can, all over the year, support and enhance both somatic and gonadal growth in *Psammechinus miliaris*. *P. miliaris* larvae fed a high ration (Kelly et al. 2000, 223) were also disadvantaged, but this was attributed to a dramatic shortening of the post-oral arms associated with the microalgae *Pleurocrysis elongata*. Natural biofilms (bacteria and algae) and coralline algae are known to be inducers of settlement and metamorphosis in echinoderm larvae (Cameron and Schroeter 1980, 243-247).

Cook et al. (1998, 1549-1555) reported that the gonadal growth was very high when urchins were fed on protein and lipid-rich artificial feeds compared to a macroalgal diet resembling urchin's preferred natural diet. Another study, on the sea urchin *Paracentrotus lividus*, suggested that diets rich in proteins induce high gonad production (Fernandez et al. 1995, 269-275). Agatsuma (1998, 1541-1547) showed that sea urchins fed on an artificial diet containing fish meal had increased moisture levels in gonads. Urchins on the *Codium* diet showed no gonadal production over the annual reproductive cycle, compared to a marked rise in gonad index on the *Laminaria* and mixed diets (Meidel and Scheibling 1998, 461-478; 2001, 97-110; Scheibling and Anthony 2001, 139). Guillou et al. (2000, 192) showed that high food availability changed the pattern of gonadal and somatic growth of *Sphaerechinus granularis*. These changes were much more noticeable over the recovery period than over the maturity stage. During the maturity period, while GI increased in a pattern similar to that of the natural environment, neither the test diameter nor the weight increased. However the changes in organic matter levels indicated that excess of food was converted into nutrient reserves in the test and gut. During the recovery period, test growth rate and gonad indices were higher than those observed in the natural environment, but without reserve storage in the test and gut. Percent gonad yield of urchins fed prepared diets increased significantly more than that of individuals fed kelp or those sampled from the wild. Percent yield increases per week reached a maximum of 1.3% for prepared diets (i.e. 15: 29 treatment), but was only 0.5% for the kelp control (Pearce et al. 2002b, 327). Feeding, burrowing and ventilation of *Brissopsis lyrifera* are suggested to contribute substantially to the turnover of organic matter and element cycling within the sediment (Hollertz 2002, 968). Absorption of food occurred throughout the day with urchins digesting food outside of the feeding period. A total of 73% of the fecal pellets consisted of $CaCO_3$ eroded from the reef, 20% consisted of organic matter and 7% the refractory organic matter (Mills et al. 2000, 71). Food consumption and fecal production declined significantly in individuals exposed to medium and high concentrations of inorganic phosphates and all levels of organic phosphates (Bottger et al. 2001, 741).

Gonad Color

Production of appropriate gonad colors in sea urchins fed prepared diets had been problematic if appropriate levels of certain dietary pigment sources were not included in the diet (Barker et al. 1998, 1583-1590; Grosjean et al. 1998, 1523-1531; Watts et al. 1998, 1591-1595; Pearce et al. 2002a, 301-323). Recent research by Robinson et al. (2002, 289-303) with *S. droebachiensis* has shown that ß-carotene is a very effective pigment source for producing the bright yellow/orange coloration sought after by the commercial market, but that the most effective concentration is ~200–250 mg kg^{-1}. Robinson et al. (2002, 289) have also suggested that pigment origin can affect color production. They tested two sources of ß -carotene: (1) natural, spray-dried preparation of the phytoplankton *Duneliella salina* and (2) synthetically produced by chemical means and sold as an animal feed supplement. Pearce et al. (2002a, 301), working with *S. droebachiensis*, found that prepared diets bound with corn starch produced significantly better gonad color than prepared diets formulated with other binders such as gelatin, guar gum, or sodium alginate and hypothesized that starch may have led to greater production of storage glycogen and, thus, a lighter or whiter gonad background and a brighter gonad color. In the present study, gonadal color of *D. setosum* varied considerably in each sample, and ranged from dark brown or black through shades of ochre, orange, yellow and pale cream with no relation to sex. Young immature gonads tended to soft and smooth.

Environmental Factors

The surface seawater temperatures (SST) of Khang Khao Island and Samet Island during July, 2000-September, 2002 were high in June and July, 2001 and June, 2002 for the former and in June and July, 2001 for latter. The maximum SST at Khang Khao Island and Samet Island ranged 29.30-30.71 ° C and 29.24-30.83 °C, respectively. The distribution of *Echinometra lucunter* may instead be limited by factors such as adult temperature tolerances, temperatures needed for growth or spawning, or hydrographic features that limit larval settlement and juvenile survival (Sewell and Young 1999, 291). McBride et al. (1997, 357-365) found gonadal production of *S. franciscanus* to be independent of temperature and explained that increased catabolism at higher temperature balanced the increase of food intake. The enhancement of green urchin roe in the absence of short-day cues suggests that photoperiodic induction is superfluous for roe enhancement during summer, but not necessarily for gametogenesis. It is possible that nutritive, rather than gametogenic cells.

The maximum salinity of Khang Khao Island (salinity range 30.56-32.76 ppt) was less than Samet Island (salinity range 30.79-33.05 ppt) because the former is located near the Chao Phraya River mouth. Metaxas (1998, 323) examined the effect of six levels of salinity (15, 18, 21, 24, 27 and 33 PSU) on survival and rate of development of larvae in the subtropical sea urchin *Echinometra lucunter*. Urchins suffered 100% mortality when subjected to a short-term change in salinity from 36 to 2 to 36%, a salinity change characteristic of a major canal discharge event (Irlandi et al. 1997, 869).

Aquaculture

D. setosum was species of economic interest. Its eggs are good for human consumption and high potential for commercial aquaculture. The ultimate step in the aquaculture production of sea urchin is independence from natural resources, that is, to control the whole life cycle in culture, from spawning to gonad enhancement (Hagen 1996a, 6-19). Somatic growth of juveniles until reaching market size is a process that requires major improvements in current technology and is the key of successful development of closed-cycle echinoculture (Grosjean et al. 1998, 1523).

The quality of sea urchin roe determines its value at market, but it is not visible to commercial divers during harvest. To aid efficiency of harvesting, the relationship between three important attributes of the quality of roe (color, texture and granularity) and several covariates was investigated for *Centrostephanus rodgersii* in New South Wales, Australia. The covariates included three physical attributes of the sea urchin (roe weight, test diameter and total weight), as well as the habitat, location and reproductive period. There were significant relationships among the measures of roe quality and among the covariates. The preferred color of roe was associated with poor granularity and texture, and roe weight was strongly associated with test diameter, reproductive period and habitat. Generalised linear models were used to estimate the probability of selecting each category of roe quality for a sea urchin with a given set of covariates. In combination, roe weight and test diameter accounted for most variation in roe quality explained by the covariates. Sea urchins with smaller test diameters and heavier roe were more likely to contain roe of a preferred color, but less likely to contain roe of a preferred granularity and texture. Despite this contrast, physical characteristics of sea urchins and their local habitat can be used by commercial divers to enable the selection of individuals with a greater probability of containing high-quality roe (Blount and Worthington 2002, 341).

APPENDIX I

Summary Statistics and Results of ANOVA Analyzed

Table 9 Summary Statistics for *D. setosum* at Khang Khao Island and Samet Island

Khang Khao Island		**Samet Island**	
Mean	43.63	Mean	71.16
Standard Error	0.21	Standard Error	0.79
Median	45.00	Median	70.00
Mode	50.00	Mode	70.00
Standard Deviation	10.71	Standard Deviation	8.90
Sample Variance	114.81	Sample Variance	79.23
Kurtosis	2.29	Kurtosis	1.40
Skewness	-0.86	Skewness	-0.91
Range	72.00	Range	50.00
Minimum	3.00	Minimum	40.00
Maximum	75.00	Maximum	90.00
Sum	113101.75	Sum	9107.85
Count	2592.00	Count	128.00
Largest(1)	75.00	Largest(1)	90.00
Smallest(1)	3.00	Smallest(1)	40.00
Confidence Level(95.0%)	0.41	Confidence Level(95.0%)	1.56

Table 10 Results of Two Factor ANOVA on Population Densities of *D. setosum* Between at Khang Khao Island and at Samet Island

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows (Months)	21.57	8	2.70	4.46	0.025	3.44
Columns (Sites)	75.54	1	75.54	124.90	0.000	5.32
Error	4.84	8	0.60			
Total	101.95	17				

Table 11 Results of Two Factor ANOVA on Population Densities of *D. setosum* Between Coral Zone and Sand Zone at Khang Khao Island

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows (Months)	28.43	7	4.06	2.07	0.179	3.79
Columns (Zones)	51.67	1	51.67	26.36	0.001	5.59
Error	13.72	7	1.96			
Total	93.82	15				

Table 12 Results of Two Factor ANOVA on Mean Recruitment of *D. setosum* Between Khang Khao Island and Samet Island

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows (Months)	3.81	9	0.42	0.73	0.675	3.18
Columns (Sites)	8.27	1	8.27	14.28	0.004	5.12
Error	5.21	9	0.58			
Total	17.29	19				

Table 13 Results of Two Factor ANOVA on Mean Recruitment of *D. setosum* on Three Substrate Types and During Months at Khang Khao Island

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows (Months)	14.00	7.00	2.00	8.35	0.000	2.76
Columns (Substrates)	9.52	2.00	4.76	19.87	0.000	3.74
Error	3.35	14.00	0.24			
Total	26.87	23.00				

Table 14 Results of Two Factor ANOVA on Newly Settled *D. setosum* during the Study Periods and Between the Positions of Experimental Settlement Devices at Khang Khao Island

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows (Period Months)	87.38	6.00	14.56	2.20	0.09	2.66
Columns (Position of Experimental Settlement Devices)	557.32	3.00	185.77	28.11	0.00	3.16
Error	118.94	18.00	6.61			
Total	763.64	27.00				

Table 15 Results of Single Factor ANOVA on Size-Frequency Distribution of *D. setosum* at Khang Khao Island and Samet Island

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	92379.9	1.00	92379.89	816.47	0.00	3.84
Within Groups	307530	2718.00	113.15			
Total	399909	2719.00				

Table 16 Results of Two Factor ANOVA on Gonad Index of *D. setosum* Between the Coral Zone and Sand Zone at Khang Khao Island

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows (Months)	152.93	7.00	21.85	6.22	0.01	3.79
Columns (Zones)	34.01	1.00	34.01	9.68	0.02	5.59
Error	24.58	7.00	3.51			
Total	211.52	15.00				

APPENDIX II

Stepwise Multiple-Regression Results

Table 17 *Diadema setosum.* Stepwise Multiple-Regression Results for Relative Amounts of Body Weight (g) and Test Diameter (mm) at Khang Khao Island

ANOVA 31-Oct-01 $R^2 = 0.707$

Source	*df*	*SS*	*MS*	*F*	*Significance F*
Regression	1	6320.054	6320.054	140.075	0.000
Residual	58	2616.913	45.119		
Total	59	8936.967			

Table 18 *Diadema setosum.* Stepwise Multiple-Regression Results for Relative Amounts of Gonad Weight (g) and Test Diameter (mm) at Khang Khao Island

ANOVA 31-Oct-01 $R^2 = 0.259$

Source	*df*	*SS*	*MS*	*F*	*Significance F*
Regression	1	11.500	11.500	20.273	0.000
Residual	58	32.901	0.567		
Total	59	44.401			

Table 19 *Diadema setosum.* Stepwise Multiple-Regression Results for Relative Amounts of Gonad Index and Test Diameter (mm) at Khang Khao Island

ANOVA 31-Oct-01 $R^2 = 0.003$

Source	*df*	*SS*	*MS*	*F*	*Significance F*
Regression	1.000	1.501	1.501	0.198	0.658
Residual	58.000	439.717	7.581		
Total	59.000	441.217			

Table 20 *Diadema setosum.* Stepwise Multiple-Regression Results for Relative Amounts of Body Weight (g) and Test Diameter (mm) at Samet Island

ANOVA 24-Nov-01 $R^2 = 0.824$

Source	*df*	*SS*	*MS*	*F*	*Significance F*
Regression	1	3092.267	3092.267	70.169	0.000
Residual	15	661.029	44.069		
Total	16	3753.296			

Table 21 *Diadema setosum.* Stepwise Multiple-Regression Results for Relative Amounts of Gonad Weight (g) and Test Diameter (mm) at Samet Island

ANOVA 24-Nov-01 $R^2 = 0.202$

Source	*df*	*SS*	*MS*	*F*	*Significance F*
Regression	1	26.880	26.880	3.803	0.070
Residual	15	106.012	7.067		
Total	16	132.892			

Table 22 *Diadema setosum.* Stepwise Multiple-Regression Results for Relative Amounts of Gonad Index and Test Diameter (mm) at Samet Island

ANOVA 24-Nov-01 $R^2 = 0.044$

Source	*df*	*SS*	*MS*	*F*	*Significance F*
Regression	1	6.150	6.150	0.691	0.419
Residual	15	133.436	8.896		
Total	16	139.587			

Table 23 *Diadema setosum.* Stepwise Multiple-Regression Results for Relative Amounts of Body Weight (g) and Test Diameter (mm) at Khang Khao Island was During September, 2000 to September, 2002

ANOVA	26-Sep-00	R^2= 0.812			
Source	*df*	*SS*	*MS*	*F*	*Significance F*
Regression	1	8281.661	8281.661	250.441	0.000
Residual	58	1917.959	33.068		
Total	59	10199.620			
	7-Jan- 01	R^2= 0.855			
Source	*df*	*SS*	*MS*	*F*	*Significance F*
Regression	1	9274.070	9274.070	341.201	0.000
Residual	58	1576.480	27.181		
Total	59	10850.549			
	11-Apr-01	R^2= 0.880			
Source	*df*	*SS*	*MS*	*F*	*Significance F*
Regression	1	7287.537	7287.537	426.839	0.000
Residual	58	990.251	17.073		
Total	59	8277.788			
	14-Jun-01	R^2= 0.838			
Source	*df*	*SS*	*MS*	*F*	*Significance F*
Regression	1	8166.035	8166.035	305.457	0.000
Residual	59	1577.296	26.734		
Total	60	9743.331			

Table 23 (continued)

ANOVA 31-Oct-01 $R^2 = 0.707$

Source	*df*	*SS*	*MS*	*F*	*Significance F*
Regression	1	6320.054	6320.054	140.075	0.000
Residual	58	2616.913	45.119		
Total	59	8936.967			

31-Jan-02 $R^2 = 0.822$

Source	*df*	*SS*	*MS*	*F*	*Significance F*
Regression	1	4922.961	4922.961	268.433	0.000
Residual	58	1063.696	18.340		
Total	59	5986.657			

22-Jun-02 $R^2 = 0.863$

Source	*df*	*SS*	*MS*	*F*	*Significance F*
Regression	1	8702.086	8702.086	365.065	0.000
Residual	58	1382.551	23.837		
Total	59	10084.638			

29-Sep-02 $R^2 = 0.868$

Source	*df*	*SS*	*MS*	*F*	*Significance F*
Regression	1	5284.759	5284.759	381.787	0.000
Residual	58	802.845	13.842		
Total	59	6087.604			

Table 24 *Diadema setosum.* Stepwise Multiple-Regression Results for Relative Amounts of Gonad Weight (g) and Test Diameter (mm) at Khang Khao Island During September, 2000 to September, 2002

ANOVA 26-Sep-00 $R^2 = 0.364$

Source	*df*	*SS*	*MS*	*F*	*Significance F*
Regression	1	98.924	98.924	33.165	0.000
Residual	58	173.001	2.983		
Total	59	271.924			

7-Jan- 01 $R^2 = 0.563$

Source	*df*	*SS*	*MS*	*F*	*Significance F*
Regression	1	157.465	157.465	74.738	0.000
Residual	58	122.200	2.107		
Total	59	279.665			

11-Apr-01 $R^2 = 0.378$

Source	*df*	*SS*	*MS*	*F*	*Significance F*
Regression	1	158.695	158.695	35.276	0.000
Residual	58	260.924	4.499		
Total	59	419.619			

14-Jun-01 $R^2 = 0.472$

Source	*df*	*SS*	*MS*	*F*	*Significance F*
Regression	1	201.086	201.086	52.716	0.000
Residual	59	225.056	3.815		
Total	60	426.142			

Table 24 (continued)

ANOVA 31-Oct-01 R^2= 0.259

Source	*df*	*SS*	*MS*	*F*	*Significance F*
Regression	1	11.500	11.500	20.273	0.000
Residual	58	32.901	0.567		
Total	59	44.401			

31-Jan-02 R^2= 0.342

Source	*df*	*SS*	*MS*	*F*	*Significance F*
Regression	1	45.020	45.020	30.099	0.000
Residual	58	86.753	1.496		
Total	59	131.773			

22-Jun-02 R^2= 0.382

Source	*df*	*SS*	*MS*	*F*	*Significance F*
Regression	1	248.119	248.119	35.837	0.000
Residual	58	401.562	6.923		
Total	59	649.681			

29-Sep-02 R^2= 0.349

Source	*df*	*SS*	*MS*	*F*	*Significance F*
Regression	1	142.707	142.707	31.135	0.000
Residual	58	265.841	4.583		
Total	59	408.548			

Table 25 *Diadema setosum.* Stepwise Multiple-Regression Results for Relative Amounts of Gonad Index and Test Diameter (mm) at Khang Khao Island During September, 2000 to September, 2002

ANOVA 26-Sep-00 R^2=0.140

Source	*df*	*SS*	*MS*	*F*	*Significance F*
Regression	1	138.504	138.504	9.469	0.003
Residual	58	848.365	14.627		
Total	59	986.869			

7-Jan-01 R^2=0.325

Source	*df*	*SS*	*MS*	*F*	*Significance F*
Regression	1	487.123	487.123	27.900	0.000
Residual	58	1012.649	17.459		
Total	59	1499.773			

11-Apr-01 R^2=0.046

Source	*df*	*SS*	*MS*	*F*	*Significance F*
Regression	1	102.245	102.245	2.796	0.0999
Residual	58	2120.984	36.569		
Total	59	2223.229			

14-Jul-01 R^2=0.058

Source	*df*	*SS*	*MS*	*F*	*Significance F*
Regression	1	163.494	163.494	3.662	0.061
Residual	59	2634.033	44.645		
Total	60	2797.527			

Table 25 (continued)

ANOVA 31-Oct-01 R^2=0.003

Source	*df*	*SS*	*MS*	*F*	*Significance F*
Regression	1	1.501	1.501	0.198	0.658
Residual	58	439.717	7.581		
Total	59	441.217			

31-Jan-02 R^2=0.153

Source	*df*	*SS*	*MS*	*F*	*Significance F*
Regression	1	209.586	209.586	10.477	0.002
Residual	58	1160.272	20.005		
Total	59	1369.858			

22-Jun-02 R^2=0.014

Source	*df*	*SS*	*MS*	*F*	*Significance F*
Regression	1	41.909	41.909	0.805	0.373
Residual	58	3018.189	52.038		
Total	59	3060.098			

29-Sep-02 R^2=0.090

Source	*df*	*SS*	*MS*	*F*	*Significance F*
Regression	1	244.740	244.740	5.709	0.020
Residual	58	2486.359	42.868		
Total	59	2731.099			

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