アジア太平洋域における大学院学生の国際連携教育プログラムーダブルディグリープログラムなどの推進ー最終報告書

理工学研究科 海洋自然科学 修士1年　Noelle Wenty Oldiais

推薦教員：土屋　誠

2.Research term:2012/10/6 to 2012/10/27

3.Research title, the detail and the results:

Research Title: Integration of biological and social evaluations of the Rock Island Southern Lagoon of Palau

Purpose: To collect socioeconomic data for evaluating rock island marine sites in Palau through key informant interviews of dive guides

Background:

Recreational activities on marine protected or conservation areas are increasingly becoming popular in the tourism industry. However they may lead to biological damage and decrease the values of popular recreational sites due to congestion (Davis and Tisdell 1995/1996). Palau is located in western Micronesia of the northwestern side of the Pacific Ocean with a land area of only about 456 km2 (Yukihira et al 2007) and a population of less than 21,000. It is one of the most famous diving destinations in the world specifically at the Rock Island Southern Lagoon, contributing to the nation's economy. In addition, the Lagoon provides opportunities for commercial and subsistence fishing that contributes to the economy and as an essential food source for the communities in Palau (Rock Island Southern Lagoon Management Plan 2012-2016).

Visitors to Palau have been increasing throughout the last decade from 57,732 in 2000 to almost double in 2011, 109,057 (Palau Visitors' Authority 2000/2011). The majority of the visitors involve themselves in marine activities especially in the Rock Island Southern Lagoon which 90% is owned by Koror State and the 10% by Peleliu State. In 1999, Koror State designated their entire area as a conservation area and was divided into different zones. The area covers approximately 859 km2, and includes all land areas within these waters except for the northern Koror Islands. The concern for managers is that with this increasing number of visitors, the degradation of biological and economic values of marine sites may soon be at risk. This includes the degradation of visitors experience due to congestion of sites.

Methods

1. Socioeconomic assessment

With a self administered questionnaire in English, Japanese, Chinese (Taiwanese characters), and Korean, the collection from snorkelers and divers of the Southern Lagoon began on August 2011 and ended on June 2012. The questionnaire explored expectations, experience, satisfaction, and perception of crowdedness of the visitors of marine sites in the Southern Lagoon. In addition, key informant interviews will explore problems, issues, and suggestions in regard to high quality tourism experience and low environmental tourism impacts on the sites among related tour operators.

2. Biological assessment

Data for fish size and abundance were collected quarterly from 2010-2011 along 5 50 meter transects (250m-2) at a depth of 50 meters. Coral cover was collected once in 2010 and again in 2011. They were collected by photographing a 0.50 x 0.50 m quadrat along the same transects starting at 0m in every meter interval. Coral recruitments (≤5 cm) were collected along the same transects in an area of .03m x 10m only in the first 10 meters of each of the transects. All these data were collected on the 3 different zones (dive sites, open/non-dive sites, Peleliu dive sites) with a total of 18 sites.

4.Achievements:

In this section of my overall research, my goal was to collect data by interviewing dive guides/masters in order to explore problems, issues, and suggestions that they may have in regards to tourism in Palau. These data will be added on to visitors' survey data and later on will be integrated to the biological data which have both been collected. I worked closely with the Koror State Conservation Enforcement office to schedule interviews in which they had sent out official letters prior to my arrival to all dive operators for their participation. I was able to work with 7 different tour operators for this research. Every other day, at least one or two interviews were collected which totaled to 15 interviews. After each interview, I had to enter all data (interview ran between 30 minutes to 1 hour) into a spreadsheet. During my last few days, I was able to begin my initial analysis for the data however; I would still need to transfer the data into QSR NVivo10 software to finalize the analyses which are going to be used for my master's thesis. In this report, I will share some preliminary results from both visitors' survey and key informant interviews from dive guides regarding crowding. Further analysis between biological and socioeconomic data will be done for my thesis research.

Results

According to about 50% of visitors' perceptions of crowdedness of boats at sites and of divers/snorkelers in water were on "moderate" as seen on both graphs below. There was a significant difference among the different ratings of crowdedness of boats and of divers with "moderate" having the highest residuals as seen on the table below.

When these data are compared to the key informant interviews that were collected, it is then understood further. Even though, a good number of key informant believe that crowding is generally a problem weather it is all the time or only during high peak season, the visitors' survey show that the perception of crowding was on "moderate". In addition, there was no significant difference in perception of crowding within different seasons. According to key informant interviews, it was explained that an internal communication between dive companies at sites before a dive, is the key and has helped control "crowding" or "congestion" in certain ways. For example, whoever is at the site first, will make the first dive entry while the next wait for a couple minutes before entering. Also, the installation of buoys has allowed more entry points instead of gathering at one area and going in at one time. It was further explained, that guests who do complain, do so on the boat because they see so many however once guest is in the water, it is no longer an issue. Other companies would explain to their guests about how certain sites can be a bit crowded due to popularity or it being a high peak season. Others would time their dive by either going very early or going at a different time when others are no longer at the sites to avoid congestion. This communication weather it is between tour companies or within guests, it seems to be the tool that is working and preferable by dive guides. When asked if they think that the Rock Islands Marine Park should establish limits of divers be at a site at one time, majority did not agree to this idea and that visitors would not respect this limitation.

理工学研究科 海洋自然科学 修士2年　式場　はるか

推薦教員：James Davis REIMER

1. 研修先および研修受け入れ責任者：

National University of Singapore, The Marine Biology Laboratory

Peter A. Todd 助教授

2. 研修期間：平成24年10月10日-11月4日

3. 研究内容：

3-1. 研究内容

私は、イソバナ科*Melithaea*属の分類学的再検討を研究テーマとしている。

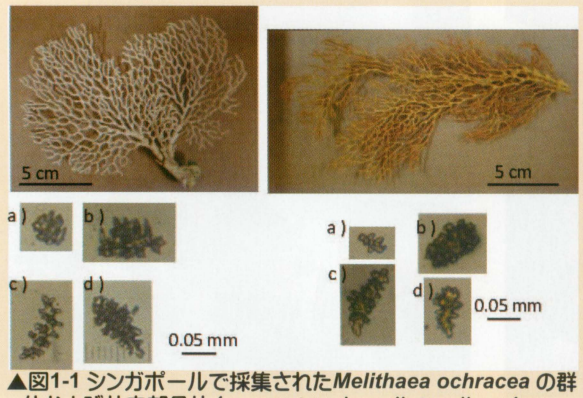
イソバナ科は、花虫綱八放サンゴ亜綱ウミトサカ目に属する固着性の群体性動物であり、インド太平洋域の水深1～600mに分布している。私の研究拠点である琉球列島沿岸の水深1～30mでは、普遍的に存在している。　本科は6属106種からなるが、それらの種を正確に同定することは非常に難しい。従来の分類体系では、群体の共肉部に含まれる骨片の形状が分類形質として重要視されてきた。しかし、共肉部の骨片は同一群体内でも形状や大きさに変異があることが普通で、種の判別基準は各研究者の主観による部分が大きい。そのため、分類学的問題が生じている。

私は本科のうち*Melithaea*属に着目し、従来の形態分類手法に、分子系統解析を組み合わせ、琉球列島産*Melithaea*属の分類学的再検討を行なっている。これまでの結果、琉球列島の*Melithaea*属は、骨片の形態から*Melithaea ochracea*に同定された。しかし、分子系統解析では、琉球列島産*M. ochracea*には複数のクレードが含まれ、その一部は近縁の別属と重複することが示された。このことは、少なくとも本属では骨片形態による分類法が系統を反映しないこと、*M.　ochracea*が2種以上からなる可能性を示している。この問題を解決するため、系統を反映する分類形質の精査および、新たに検討した形質に基づく分類体系の構築を試みている。また、琉球列島産*Melithaea*属内の分類を整理するためには、種同定の的確性について検討する必要がある。

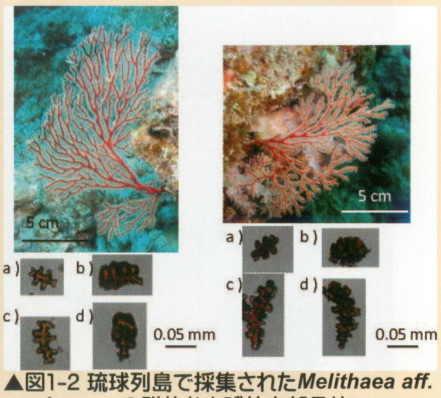
本調査の目的は、種同定の的確性の検討であった。*M.　ochracea*のタイプ標本は紛失しているため、*M. ochracea*として種同定された他の標本を参考にして比較を行った。シンガポールでは近年、イソバナ科について種レベルの同定が行われた。13群体の*M.　ochracea*がシンガポール大学内の博物館（Raffles Museum of Biodiversity Research）に乾燥標本として保存された（van Ofwegen et al., 2000）。そこで私は、実際に現地に赴き自ら標本を観察することで、琉球列島産*Melithaea*属との詳細な形態比較を試みた。また分子系統解析による比較も行うため、van Ofwegen et al.（2000）によってイソバナ科の調査が行われた地点を中心に、スキューバ潜水を用いて新たに標本を採集した。

3-2. 研究成果

博物館では、*Melithaea ochracea*の標本5群体を観察した。共肉部内には多様な形態の骨片が見られた。そこで、各標本において骨片の形態タイプの組成および大きさを調べた（図1）。骨片の形態タイプの組成については、シンガポール産標本において、「club」型骨片の割合が琉球列島産*Melithaea aff. ochracea*に比べて多かった。



▲図1-1 シンガポールで採集された*Melithaea ochracea* の群体および共肉部骨片（a: capstan, b: unilaterally spinose spindle, c: spindle, d: club）
（左：ZRC.1996.1677、右：ZRC.1995.7）



▲図1-2 琉球列島で採集された*Melithaea aff. ochracea* の群体および共肉部骨片
（a: capstan, b: unilaterally spinose spindle, c: spindle, d: club）

骨片の大きさについては、シンガポール産標本の間でも大きな差異が見られた。また、シンガポール産標本の共肉部は、琉球列島産標本に比べて薄かった。これらの差異が種内変異であるかについては、分子系統解析を用いて検討する予定である。

標本の採集は、シンガポール南部の9地点で行った（図2）。van Ofwegen et al.（2000）における採集地点から5地点、新規に4地点から採集を行い、88標本のイソバナ科を採集した。これらの骨片形態を博物館標本と比較した結果、*M. ochracea*と、その他数種のイソバナ科が混在していることが明らかになった。本調査で採集した*M. ochracea*とについては分子系統解析を行い、琉球列島産*Melithaea aff. ochracea*との比較を行う予定である。他種については、van Ofwegen et al.（2000）を参考にして属および種の同定を行う。

シンガポール滞在中は、ほぼ毎日Dr. Peter A. Toddの研究室へ通い、研究室のメンバーと昼食や夕食を共にすることで交流を深めた（図3）。また研究室メンバーを対象に、研究内容をゼミ形式で発表することで、本調査の必要性について理解してもらえるよう努めた（図4）。私の世話役となってくれたPhD学生の Neo Mei Lin（図5）とは、調査の計画、実行、本調査の結果を論文としてまとめるためのアイデア、また今後の研究の発展方法などについて議論した。

今回は多くの方に助けられて目的の調査を成し遂げることができた。そこで築いた人間関係は、これから研究活動を続けていく上でとても重要なものになるだろう。また、海外の研究室に一時的に所属するという経験から得られた知見は貴重であった。Dr. Peter A. Toddの研究室では、学生同士が日々活発に研究に対しての議論をし、互いの研究発展に努めていた。このような一面を見ることで、研究室の役割の重要性を再認識することができた。

今回研究室への受け入れを承諾して下さった、シンガポール国立大学助教授のDr. Peter A. Todd、調査の計画から実行にいたって親密になって考え、多大な手助けをしてくれたNeo Mei Lin、私を研究室の一員として受け入れてくれた研究室のメンバー、標本採集の機会を与えて下さったDr. Jeffrey Low、そして本調査をする上で全面的に支援をして下さった琉球大学の「アジア太平洋域の大学院学生の国際連携教育プログラム」に心から感謝いたします。

▲図2 イソバナ科の採集風景

▲図3 Dr. Peter Todd（左）と研究室メンバー

▲図4 研究発表の様子

▲図5 PhD学生のNeo Mei Lin（右）

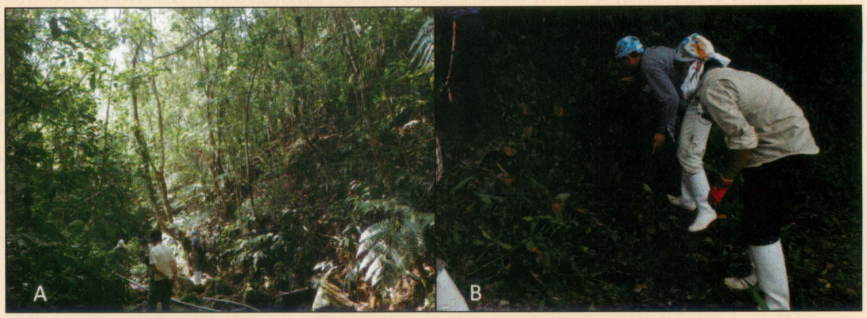
理工学研究科 海洋自然科学 修士2年　小林　峻

推薦教員：伊澤　雅子

1. 研修先および研修受け入れ責任者：
台湾，于宏燦（臺灣國立大學）

2. 研修期間：平成24年10月10日～10月20日

3. 研究内容：台湾各地（烏来，公館，石岡，日月潭，蓮華池，六亀，墾丁）のイルカンダMucuna macrocarpa（マメ科）の分布および種子の結実状況，葉の形態について調査を行い，各地の森林を管理する機関の職員から開花状況および周辺動物相についての聞き取りを行った（図1）．また，各地の大学（臺灣國立大學・東海大學・國立成功大學），林務局（烏来工作站），林業試驗所（六龜研究中心・恆春研究中心），特有生物研究保育中心の教授および職員の方に来年以降の台湾各地における本格調査の説明と調査拠点としての施設利用の依頼をし，各機関から快諾を得ることができた（図2）．



▲図1. 調査風景．A：蓮華池の調査地，B：六龜で果実を探索する様子．

花の裂開者および送粉者となり得る動物に関して，聞き取り調査を行った結果，地域によって生息している種構成が異なっており，台湾の中でも地域差があるのではないかと考えられる．調査した7地域中6箇所で種子の確認ができた．また，種子の大きさや葉の刺毛の密度は産地によって差異があったため，今後，気候や地質なども考慮してイルカンダの形態のバリエーションについても精査する必要があると思われた．また，種子散布者の候補となり得る動物についての聞き取り調査からは，種子散布者になり得る動物が生息している地域と，生息していない地域が考えられた．

▲図2. 研究の説明後の集合写真．A：烏来工作站，B：六龜研究中心．

台湾では保護対象種となっているツダナナフシ　*Megacrania tsudai*（ナナフシ科）に関する調査では，台湾南部（屏東）の2地点を林業試驗所恆春研究中心の職員の方に，緑島は東海大學リサーチアシスタントの方に案内していただき，分布地の確認を行った（図3）．聞き取り調査では，台湾本島には調査した2ヵ所以外に分布している地域が複数あるとのことであった．また，緑島では複数の分布地があったものの非常に局所的である印象であった．

▲図3. 台湾のツダナナフシ．A：緑島の成虫，B：緑島の警告看板．台湾では保護対象種になっており採集禁止である．

今回の調査では、台湾に調査協力者や友人ができた。長期の生態調査では人間関係は重要であり、今回多くの関係者に直接会い、話ができたことは今後研究を進めるうえで非常に有意義であった。

台湾／中央研究院生物多様性研究部門　Steve Doo

受入教員：藤田　和彦

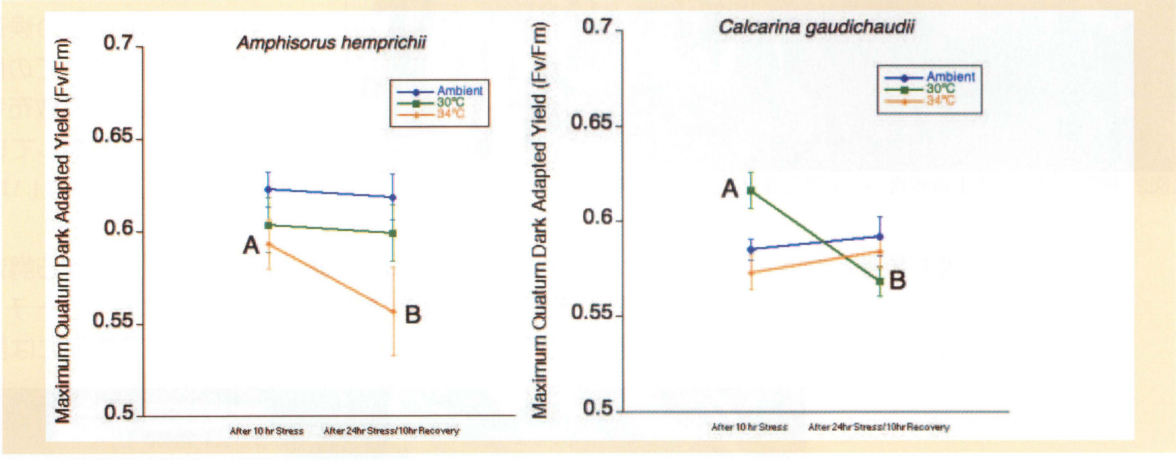
1.Research term: Summer 2012

2.Research title, the detail and the results:

Rationale: Recent evidence has overwhelmingly suggested that climate change stressors (eg. ocean warming, ocean acidification, eutrophication) will have deleterious effects on tropical benthic foraminiferal physiology, potentially affecting a significant portion of carbonate production on reef ecosystems (Fujita et al. 2009, Uthicke et al. 2012). Recently, few studies have reported varied responses to stressors of ocean acidification and eutrophication, and suggested that this is due to difference in symbiont type, and none to date have investigated the potential for recovery. In this study, we collected two common LBF species, *Amphisorus hemprichii* (dinoflagellate-bearing) and *Calcarina gaudichaudii* (diatom-bearing) from Sesoko and Ikei beaches and acclimated them in laboratory conditions for at least 2 days. Foraminifera were exposed to ambient, +4°C, and +8°C for 24 h. Half of the replicates were harvested for protein samples, and the other half were returned to ambient conditions for 24 h, at which point the remaining samples were preserved. In addition maximum quantum photosynthetic efficiency (Fv/Fm) measurements were taken 10 h after heat stress, and 10 h after return to ambient conditions.

Preliminary Findings:

1. The diatom-bearing species, *C. gaudichaudii* increased in photosynthetic efficiency at +4°C warming, suggesting mild warming may be beneficial to it's holobiont
2. The initial heat stress to *A. hemprichii* did not affect Fv/Fm, but even after a recovery period, significant deleterious effects were still seen.
3. It appears C. gaudichaudii is more robust to large fluctuations in temperature stress, as our +8°C temperature treatments had no affect on Fv/Fm values.



Continuing Research: This project is on-going at the National Museum of Marine Biology and Aquarium (NMMBA). Samples preserved for protein expression analyses will be probed for the photosynthetic enzyme RuBisCO using the semi-quantitative western blot technique. These results will provide a more complete picture of how molecular pathways affect overall photosynthetic efficiency in these two important symbiont-bearing species. After protein analyses, these data will be prepared for a short publication, with the goal of either a Coral Reefs note, or Nature Climate Change short letter.

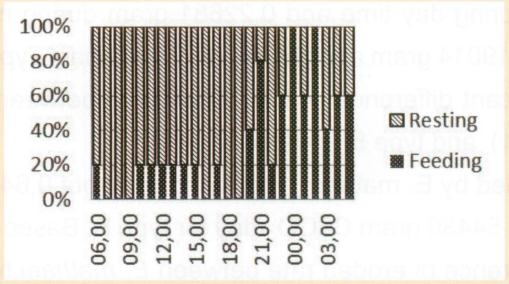
インドネシア／ボゴール農業大学　Noar Muda Satyawan

受入教員：土屋　誠

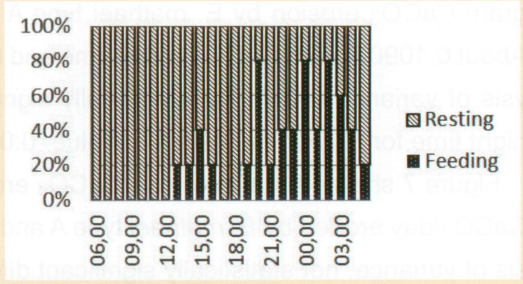
1.Research term: July 25, 2012 – August 27, 2012

2.Research title, the detail and the results:

Title of the research is Feeding Behahiour and Bioerrosion, Determining the Echological Role of Sea Urchin Echinometra mathaei Blainville on Okinawa Reef flat. The goal of this research is to broaden the knowledge base on the ecology role of *E. mathaei.* This will be achieved by adresing three objectives. The first, to obseving the feeding behaviour of *E. mathaei* on Okinawa reef. Second, to measuring the fecal pellet production and the last to measuring the bioerosion effect caused by feeding behaviour of *E. Mathaei*.

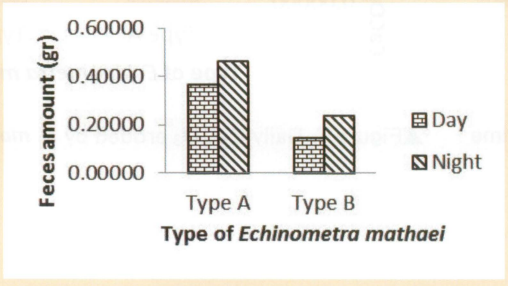


▲Figure 1. Feeding behaviour *E. mathaei* type A

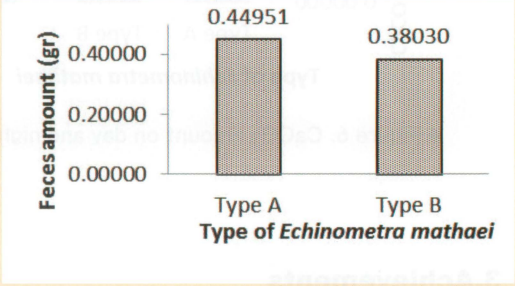


▲Figure 2. Feeding behaviour *E. mathaei* type B

In this study strongly suggest that *E. mathaei* is mainly a nocturnal grazer. Both type urchins mostly active during night time (19.00 – 05.00) which indicated by fecal pellet production and activity of ambulacral tube. Fecal pellet not only produced at night time only but also at day time with lower intensity than night time.



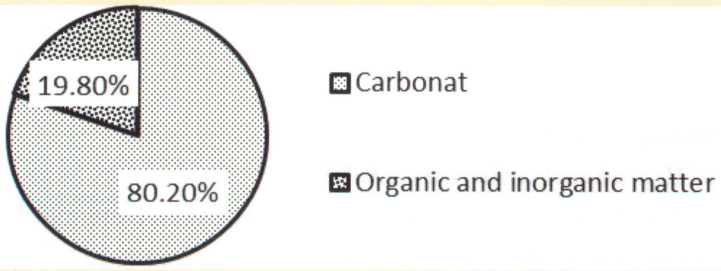
▲Figure 3. feces amount on day and night time



▲Figure 4. Daily feces amount of *E. mathaei*

Fecal pellet production determining the feeding activity by urchins. Figure 3 shows the difference amount of urchins feces on day and night time. At day time E. mathaei type A estimated produced0.36392 gram and type B produced 0.14365 gram. At the night time both of urchins type produced bigger feces amount than day time. During night time, type A produced 0.46194 gram feces and 0.23665 gram for type B.

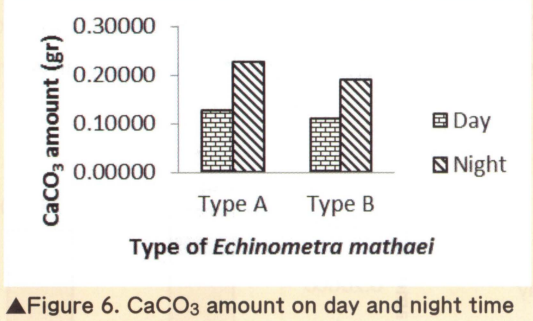
Total average of fecal pellet production by both type of Echinometra shown on figure 4.*E. mathaei* type A producing 0.44951 gram feces/day and type B producing 0.38030 gram feces/day. Analisi of Variance shows the statistically significant difference between day and night time fecal pellet production amount for type A (p-value= 0.008) and type B (p-value= 0.007) but not ststistically significant differece for total daily amount between *E. mathaei* type A and E. mathaei type B.



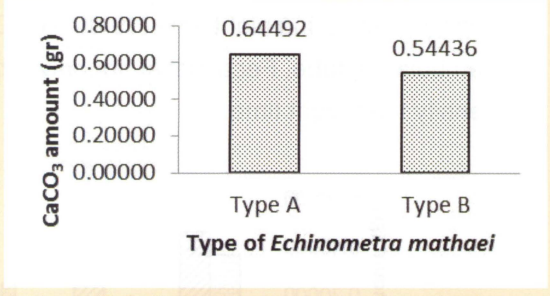
▲Figure 5. Proportion of Carbonat in feces and gut content

Figure 5 shows the proportion of calcium carbonat ($CaCO_3$) in the feces and gut content of E. mathaei. Feces and gut content of E. mathaei contained 80,20 % carbonat and 19,80 % organic and inorganic matter. $CaCO_3$ erosion at day and night time shown on figure 6. Approximately 0.12786 gram $CaCO_3$ erosion by E. mathaei type A during day time and 0,22681 gram during night time. About 0,10907 gram $CaCO_3$ at day time and 0,19014 gram at night time for E. mathaei Type B. Analysis of variance shown the statistically significant difference of $CaCO_3$ erosion between day and night time for *E. mathaei* type A (p-value=0.001) and type B (p-value=0.010).

Figure 7 shows the average of $CaCO_3$ eroded by E. mathaei type A and B. About 0,64492 gram $CaCO_3$/day eroded by *E. mathaei* type A and 0.54436 gram $CaCO_3$/day for type B. Based on analysis of variance, not statistically significant difference of eroded rate between *E. mathaei* type A and type B.



▲Figure 6. $CaCO_3$ amount on day and night time



▲Figure 7. Daily $CaCO_3$ eroded by *E. mathaei*

3.Achievements

In this experiment i had successfully collected the Echinometra mathaei from Minatogawa beach, Okinawa Island. During my experiment i had study more about this species from Tsuchiya sensei. I had realy wonderfull research experience both in laboratory and field. From this research i known the research method to measuring impact of seaurchins behaviour to aquatic environtment as the echological role of sea urchins.

フィリピン／ビコール大学大学院　Christian Cabiles

受入教員：竹村　明洋

Research title, the detail and the results:

Introduction

Histology (compound of the Greek words: ior6g "tissue", and -ALyyia :logia) is the study of the microscopic..anatomy ofcells and tissues ofplants and animals. It is commonly perfbrmed by examining cells and tissues by sectioning and staining, fbllowed by examination under a light microscope or eleotron-microscope. Histology is perfbrmed with defue purpose, 1ike in this activity histology is used to determine the gonadal stage of the Rabbidish Siganus gutatus from Philippine Waters. To better understand the condition of the gomad of a species, histological observation is very significant. It can be used to justify the brcedng season ofevery organisms. Histology gives the best infbrmation and viewed out the characteristics of egg through diffl:rent stages of development.Ten (10) samples of gomad from the species of Rabbitfish (Siganus gutatusp were examined to determine its gonadal development.

Materials and Method

The ten pieces of gomad sample were preserved using the 10% fbrmalin solution. For dehydration purposes, it undergoes series of Ethanol (Etoh) 70 -- 100% respectively, and embedded in histoparaffin as a clearing agent. The embedded samples was serially sectioned at 7 microns and stain with Mayers hematoxt1yn - eosin. Sectioned gonads were viewed using a high power microscope (Olympus BX50) for determination ofegg development.

The step of tissue processing involves:

Fixation

Fixation of the sample could be done tlMrough the use of different preservative chemicals 1ike formalin and Bouins solution. Fixation of samples to these preservatives helps to maintain the tissue in a good condition and also prevents from deterioration.

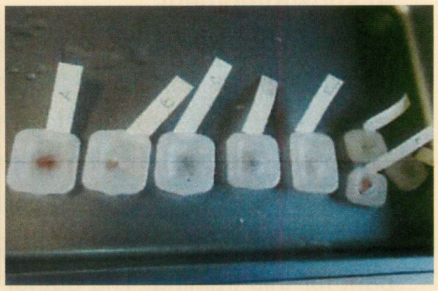
Dehydration

Dehydration is done through different series of ethanol (Etoh) say 70-100% respectively. Dehydration 1ikewise is very important in histology as the medium to remove the moisture content of the samples so that it can be inrutrated with paraflrm, (wet fixed tissue cannot be directly infiltrated with paraffu).

Clearing

After the samples are been dehydrated the next step is clearing. It is merely done with the used of xylene to removed the dehydrant (Etoh) from the samples. Xylene is used as the clearing agent because it can be easily miscible with paraffin.

▲Figure 2. Clearing ofthe samples with Xylene I and II



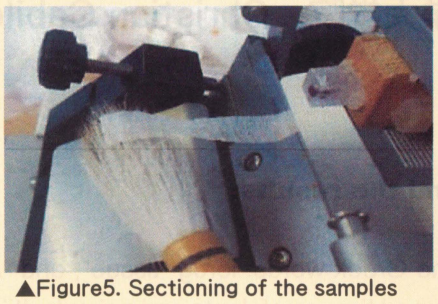
▲Figure 4. Samples inside the paraflin Bloch

Embedding

After the removal of the dehydrant (Etoh) from the sample with the aid of xylene, the next step is the embedding tlrrough paraflin. The technique of getting fixed dssue into para£fin is cailed tissue processing. This process is done inside the paraffin oven to rnaintain the liquidity of the paraffin, (60℃ is the meldng point of the paraffin)

Sectioning

After in the embeddmg procedure with paraflin, and paraffin bloch is akeady done, the next step is the sectioning with the aid of the microtome. Tissue embedded in parallfin which is similar in density to tissue can be sectioned at anywhere from 3 to 10 mierons, usually 6-8 routinely.

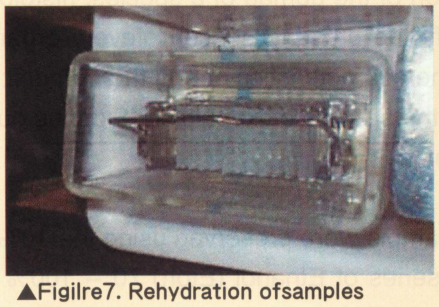


▲Figure5. Sectioning of the samples

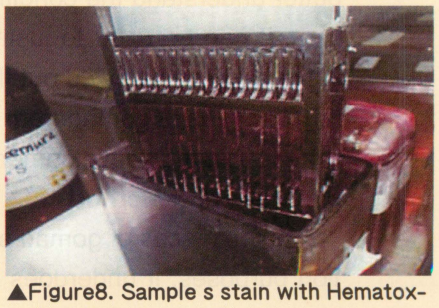
▲Figure7. Sectioned samples at the paraffin stretcher

Staining

Staining is done through series of step; lirstly the sample will undergo rehydration process with the down series from xylene to 70% Etoh and disulled water. This is prior for the proper staining of Hematoxylin and Eosin to the samples. Then after the samples are been rehydrated and akeady embed with Hematoxylin and Eosin the last step is dehydration. Dehydration is done to keep the samples for the long period of time.



▲Figilre7. Rehydration ofsamples (Down series)



▲Figure8. Sample s stain with Hematoxylin

Mounting

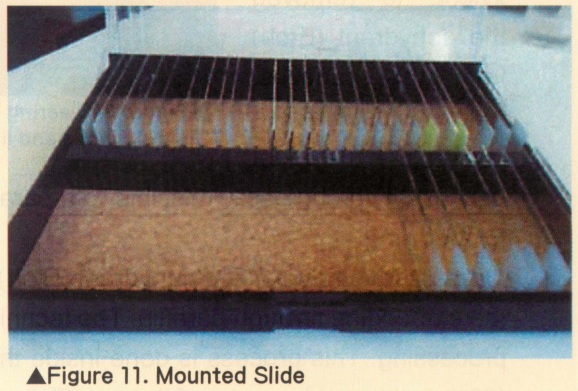
Mounting is done to attach the cover slip to the slide for protection of the samples. This is done with the used ofdifferent mounting glue.

Results

The ptocess of oocyte fomiation for rabbitfishes was divided into eight stages namely; the chromatin-nucleolus stage, peri-nueleolus stage, oil droplet stage, primary yolk stage, secondary yolk stage, teniary yolk stage, migratory nucleus stage and maturation stage (Takemura et aL, 1997). Four out of the ten samples are male; two of them are observed to be in the post spawning stage, one is in the spawning and also one was observed to be in irnmature stage. Characterization ofthe male gomad is based on Hoque et aL, 1998. in the six remaining female samples, three ofthem were observed to be in the perinucleolus stage, two are in the secondary yolk stage and one is in the prirnary yolk stage respectively.

AckuowRedgment

The author would like to express his fu11est gratitude to the entire person who is in one way to accomplish this activity. To the entire person who help him and be a part of his life in a one month stay in Okmawa. First and foremost to the University of the Ryukyus through the effort of Akihiro Takemura Ph. D. for having 1 here in the one of the best training ground for research and to Victor Soliman Ph. D fbr his strong collaboration in this miversity and for recommendmg me as participant for this training. The author would 1ike also to extend his grateful thanks to Bapary san for showing a helping hand always to help and teach the best he can. To all those names not mention but in one way to accomplish this works, He bwes this humble work to all of you.



▲Figure 11. Mounted Slide

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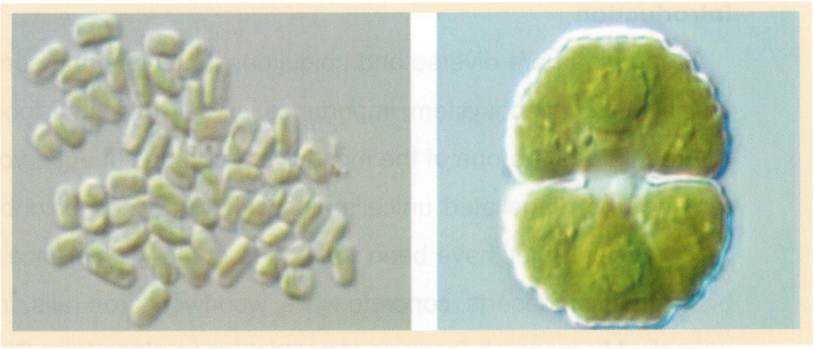
受入教員：須田　彰一郎

1.Research term: September 18,2012 - November 20, 2012.

2.Research title, the detail and the results:



Title of the research is Terrestrial green algae from Sueyoshi Park, Okinawa, Japan. It is reported the diversity of microalgae at Sueyoshi park. After that green algae were isolated by Pasteur pipette washing method. Culture conditions were 24± 2℃, 14:10 light dark cycle with 60μmol photon/m²/s. First, observed to collect 24 strains in 8 groups by microscopy and check to book. Second, culture strains in 2 different media BBM, AF6 and observed to grow.

Table1. Division of terrestrial green algae groups that have similar morphological characters observed microscopy

No.	Characters			Sp.
Group	Morphological cell	Size (x100)	Strains	Preliminary
1	Unicellular, the cells are deeply divided in the middle by a short isthmus. The front view of the semicells is reniform. The cell wall may be smooth. Each semicell has a single chlorpolast and the pyrenoids in the axial portion	41-42 μm long 19-27 μm wide	T16, T21, T22	*Cosmarium* spp.
2	Cells are solitary, ellipsoidal or broadly oval, lack mucilage. Chloroplast is single and has a pyrenoid. In young cells, plastid is entire.	6-11 μm long 12-18 μm wide	T17, T20	*Scotiellopsis* spp.
3	Cells are solitary, subpherical or subcylindrical. Chloroplast is sample, parietal at one or both ends, and has one pyrenoid.	5-7 μm long 2-5 μm wide	T11, T24	*Nannochloris* spp.
4	Cells are solitary, cylindrical in shape. Chloroplasts 1 to several in number, cup-shaped or plate-like, without pyrenoids	3-6 μm long 2-4 μm wide	T4, T5, T7, T10, T12, T23	*Stichococcus* spp.
5	Unicellular, the cells are deeply divided in the middle by a short isthmus. The front view of the semicells is semicircular.The cell wall may be not smooth Each semicell has a single chlorpolast and the pyrenoids in the axial portion	23-26 μm long 20-22 μm wide	T1, T3	*Cosmarium* spp.
6	Unicellular cells, spherical, and have parietal chloroplast, one to several pyrenoid.	16-22μm diameter	T2, T6, T19	*Neochloris* spp.
7	Cells are single, spherical, cell has a single cup-shaped chloroplast bright green parietal chloroplast.	4-8μm diameter	T8, T9, T14, T15, T13	*Heveochlorella* spp.
8	Cells are spherical. The chloroplast is cup-shaped, with a pyrenoid.	7-10μm diameter	T18	*Parachlorella* spp.

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1.Research term: September 18, 2012 . November 20, 2012

2.Research title, the detail and the results:

Introduction

Green algae are diverse and ubiquitous in aquatic and some terrestrial habitats. They play role crucial in global ecosystem, importance in applications for food and nutritional purposes of human.

Green algae are one of the most diverse groups of eukaryotes and include morphological forms ranging from flagellated unicells, coccoids, branched or unbranched filaments to multinucleated macrophytes. They have been isolated from many difference environment, includes natural rocks, biotic crusts in deserts, concrete walls, woodwork, iron rails, tree bark, leaves and fruits and hair of animals. My research focus on terrestrial green algae from Sueyoshi Park, Okinawa, Japan.

Materials and methods

* Sampling place: Sueyoshi Park-a forest park in Okinawa, Japan.

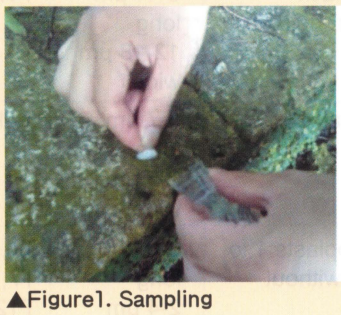
* Culture media: AF6 and BBM.

Greenish stains on the land, rock event wall were taken and scarped with small pieces of sponge. Sponge were placed in a petri dish and enriched by BBM medium for 2-3 days. 4 sample were selected to enrich.

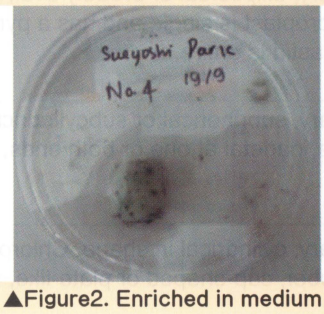
After that green algae was isolated by pipette Pasteur washing method. Culture conditions were 24± 2℃, 14:8 light dark cycle with 60μmol photon/m2/s. Then, different species continued to culture at 2 media-BBM, AF6, and observe the development.

Results

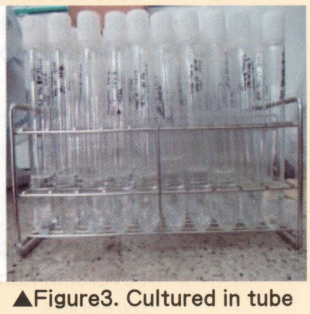
From 4 enrich samples, 20-25 days after isolated and cultured, 26 strains were grown well. Including 5 strains grew well in BBM medium, 5 trains grew well in AF6 medium, and 16 strains grew well in both BBM and AF6 medium.



▲Figure1. Sampling



▲Figure2. Enriched in medium



▲Figure3. Cultured in tube

26 strains that grew well, were observed under microscopy with 100 magnification, were taken photograph, and measured sizes. Morphological features is based on Wehr John D., Sheath Robert G., 2003; Robert Edward Lee, 2008 and Fabio Rindi et al, 2009.

8 groups within different morphological features were observed.

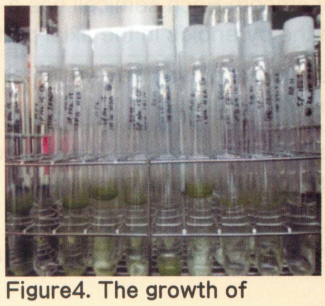


Figure4. The growth of strains

Figure5. The growth in BBM and AF6 medium

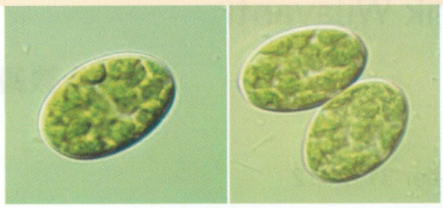


Figure 6. Goup 1

Strains	Size	Characters	Spp.
H2 H6 H20	22-34 µm long, 15-27 µm wide	nearly ellipsoidal, several chloroplasts parietal plate-like, with out pyrenoid	*Oocystis* spp.

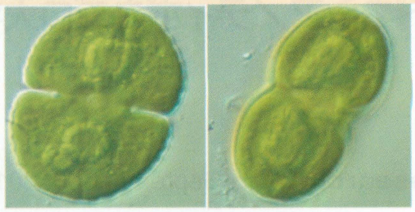


Figure 7. Goup 2

Strains	Size	Character s	Spp.
H8, H16	41-42 µm long, 19-27 µm wide	Unicellular, the cells are deeply divided in the middle, *semicells is reniform.*	*Cosmarium* spp.

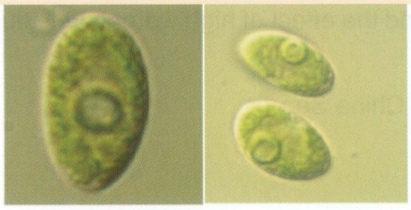


Figure 8. Group 3

Strains	Size	Character s	Spp.
H17, H15	6-11 µm long, 12-18 µm wide	Cells are solitary, ellipsoidal or broadly oval, lack mucilage. Chloroplast is single and has a pyrenoid	*Scotiellopsis* spp.

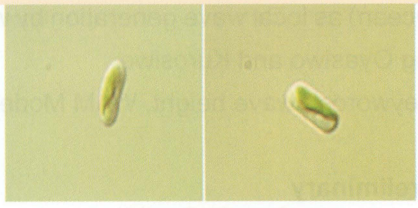


Figure 9. Group 4

Strains	Size	Character s	Spp.
H26	5-7 µm long 2-5 µm wide	Cells are solitary, subpherical or subcylindrical. Chloroplast is sample, and has one pyrenoid	*Nannochloris* spp.

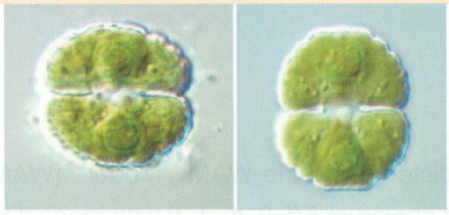


Figure 10. Group 5

Strains	Size	Characters	Spp.
H21, H24, H25	23-26 µm long, 20-22 µm wide	Unicellular, the cells are deeply divided in the middle by a short isthmus, *semicells is semicircular*	*Cosmarium* spp.

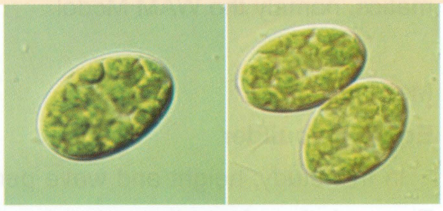


Figure 6. Goup 1

Strains	Size	Characters	Spp.
H2 H6 H20	22-34 µm long, 15-27 µm wide	nearly ellipsoidal, several chloroplasts parietal plate-like, with out pyrenoid	*Oocystis* spp.

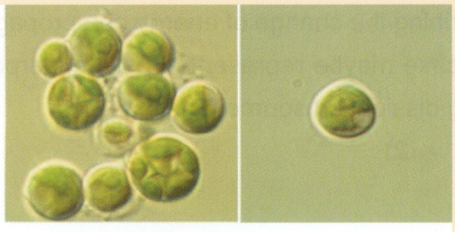


Figure 12. Group 7

Strain	Size	Character	Spp.
H23	4-8 µm dia-meter	Cells are single, spherical, cell has a single cup-shaped	*Heveochlorella* spp.

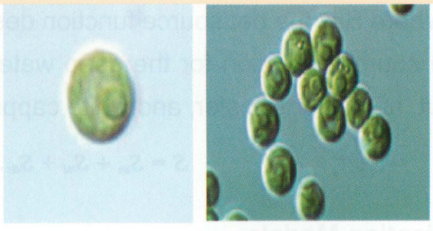


Figure 13. Group 8

Strain	Size	Character	Spp.
H1, H4, H7, H9, H10, H12, H13, H14, H18	7-10µ mµm dia-meter	Cells are spherical. The chloroplast is cup-shaped, with a pyrenoid	*Parachlorella* spp.