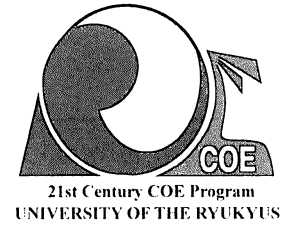


# 琉球大学学術リポジトリ

## ポスターセッション

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# **Poster Session**

Tropical Biodiversity Okinawa 2005

March 10, 2005

University of the Ryukyus Nishihara Campus  
Researcher Exchange Facility 50th Anniversary Memorial Hall

## Genetic Diversity

### PG-1 Preliminary studies on the distribution of morphotypes of the coral *Galaxea fascicularis* in reefs around Okinawa and Ishigaki Islands

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It has been suggested, based on nuclear and mitochondrial DNA analyses, that F1 hybrids of two *Acropora* species reproduce asexually and contribute to increasing the diversity of corals (Vollmer and Palumbi 2002). Colonies of *Galaxea fascicularis* were divided into three types, H, S, and mixed types, based on the shape of tentacular nematocysts (Wewengkang 2003). Watanabe et al (2005) found eight haplotypes of *G. fascicularis* based on an intergenic region of mitochondrial DNA. Eight haplotypes were grouped into Long or Short types based on the presence of 290 bp deletion. Nematocyst morphology is controlled by nuclear genomes. Hence studies on nematocyst morphology and mitochondrial haplotypes will help us to understand intraspecific variation in *G. fascicularis* and gene flow among populations. In the present study, we investigated the relative abundance of morphotypes based on tentacular nematocysts and two mitochondrial types (Long or Short) at five sites on Okinawa Island and two sites on Ishigaki Islands. A total of 201 small fragments were collected from reefs around Okinawa Island and Ishigaki Island. Relative abundance of the morphotypes differed greatly among populations and an interesting geographical pattern was observed among the Zampa, Sunabe and Odo populations. Morphotype H was abundant at Zampa, while the mixed type was the most abundant in Odo. In Sunabe, sited between Zampa and Odo, there was nearly equal numbers of H, S and mixed types. In contrast, the Shiraho population of Ishigaki island contained both H and S types but no mixed type. We identified the presence of mitochondrial haplotypes in 68 out of 201 samples. Generally type H colonies had Short haplotype (n=32) while S and mixed types had Long haplotype (n=17, 16). The present results suggest that the two morphotypes, H and S, are undergoing speciation though unidirectional hybridization that occurs between eggs of type S and sperm of type H resulting in the mixed type.

### PG-2 Parent-offspring relationship of the coral *Pocillopora damicornis*: histoincompatibility assay and microsatellite analysis

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There has been a debate about whether planula larvae of the coral *Pocillopora damicornis* are produced sexually or asexually. If planulae are produced sexually, sibling planulae are expected to be genetically different from each other and also from their mother colony. To detect possible genetic differences between sibling planulae and between planulae and their mother colony, we attempted to find histoincompatible reactions among siblings and between parent and offspring in *P. damicornis*. Sibling colonies derived from the same maternal colony always fused (74 pairs of 13 combinations). Parent-offspring grafting were also compatible in 16 pairs of 6 combinations, except for one pair, which showed

a non-fusion reaction. However, in two combinations, adult pairs showed fusion while young colonies derived from each of the paired colonies showed incompatible fusion or temporary fusion, which later transformed to non-fusion. This suggests genetic differences between planulae and their parent, though the number of observation was small. We also attempted to detect genetic difference between mother colonies and their offspring of *P. damicornis* using microsatellite markers developed for *P. verrucosa* by Magalon et al. (2004). Two (PV5 and PV7) out of 4 loci examined were polymorphic. There was a difference in PV7 between Hawaii and Akajima colonies. We found three alleles at PV5 locus from Bise samples. We are now trying to find evidence that supports sexual recombination during planula production in *P. damicornis* using this marker.

### PG-3 *Cassiopea* jellyfish infected with zooxanthellae of different genotypes shows different susceptibility to bleaching

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Corals and other cnidarians associated with zooxanthellae become bleached due to loss of zooxanthellae and/or chlorophyll pigments when exposed to environmental stress. Zooxanthellate cnidarians show different susceptibility to bleaching. However, it is not understood which partner of the cnidarian-zooxanthellae complex is responsible for the difference in the stress susceptibility. To answer this question, it is necessary to study the stress susceptibility of clonal hosts infected with zooxanthellae of different genotypes. Our objectives are to establish such an experimental system using *Cassiopea* jellyfish and to investigate how clonal jellyfish with different algae respond to stresses. We infected the clonal, aposymbiotic polyps (scyphistoma stage) of *Cassiopea* sp. with zooxanthellae isolated from various hosts; *Cassiopea* sp., *Aiptasia* sp., *Tridacna crocea*, and several hermatypic corals. We defined the genotype (clade) of isolates by 18SrDNA and/or 28SrDNA RFLP. Polyps infected with zooxanthellae generally produced medusae by strobilation. We exposed the medusae to high temperature in light or darkness for six hours, then allowed them to recover for 40 hours at normal condition. The photochemical efficiency ( $F_v/F_m$ ) and the release rate of zooxanthellae were measured during the stress treatment and recovery period. Medusae infected with zooxanthellae isolated from *Aiptasia* (mainly clade B) showed the largest decline in the  $F_v/F_m$  value. The  $F_v/F_m$  recovered little and many zooxanthellae were released during the recovery period. Those infected with isolates from *Pavona divaricata* (mainly clade D) showed a small but significant decline of the  $F_v/F_m$  value, which recovered almost completely. They did not release many zooxanthellae. The present results prove that the genetically identical jellyfish associated with different zooxanthellae show different susceptibility to stress. It is also suggested that the host expelled more zooxanthellae when the algae suffered severe PSII damage.

### PG-4 Ontogenetic changes in the branch forming capacity in a coral, *Pocillopora damicornis*

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Corals reefs are well known for their diversity in color and

structure. This diversity is due to the richness of coral species, showing various colors and growth forms. Furthermore, most colonial corals show intraspecific variation in their growth forms. Most striking is the diversity in branching morphology. Some corals even show both plate and branching morphology within the same colony.

While effects of environmental factors on growth forms were studied, there have been little studies on genetic control of the branching pattern of corals. If we find ontogenetic changes in the capacity to originate branching, we will be able to study genetic controls of branch formation.

During the course of experiments investigating contact reactions in the coral *Pocillopora damicornis*, we observed that colonies derived from primary polyps (settled planulae) and those regenerated from branch tips of adult colonies assumed different growth forms. Young colonies derived from primary polyps formed at least one branch from the central region of a colony, while colonies regenerated from adult branch tips (3-5 mm long) never formed branches during the nine month observation period. This pattern was always observed regardless of the types and outcomes of contact experiments or orientation of branch tips. However, some branch tips taken from one or two year-old colonies formed branches. The results suggest that the rate of branch origination of *P. damicornis* colonies decreases with age. The present findings open the way to analyze the mechanism of branch formation in corals at molecular level.

#### **PG-5 Plant hemoglobin: a key for seed germination at high temperature**

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Hemoglobins (Hbs) are well known as oxygen carriers in the blood of vertebrate animals. Recent progress in research has revised this simple notion to the concept that Hbs are not specific to animal blood but are more common than previously presumed. For example, Hbs and related proteins are present in many organisms including bacteria, protozoa, fungi, animals and plants. Thus, it is important to elucidate the physiological or biochemical functions of Hbs other than oxygen delivery. Here we report that *AtGLB3*, a non-symbiotic Hb of the plant *Arabidopsis thaliana*, is essential for seed germination at high temperature. We obtained the *AtGLB3*-deficient plant ( $\Delta$ GLB3) in which the *AtGLB3* gene was destroyed by a T-DNA insertion. Under favorable growth conditions,  $\Delta$ GLB3 did not display in phenotype any differences with the wild type. In fact, the mutant showed slightly faster germination and growth than the wild type under normal conditions. We found, however, that the mutant is extremely sensitive to high temperature during germinations. There was no substantial difference between the wild type and mutant at 22°C. In contrast, the mutant did not germinate at 32°C (0%) while the wild type still showed 100% germination. To investigate further the difference in heat sensitivity, we exposed the seeds to a wide range of temperature.  $\Delta$ GLB3 was roughly 5°C more sensitive to heat inactivation than the wild type. *AtGLB3* is a homologue of bacterial truncated hemoglobins (trHbs), but its function *in vivo* has been poorly understood. Some trHbs in bacteria are involved in the destruction of nitric oxide (NO). To see the failure of  $\Delta$ GLB3 to germinate at 32°C was associated with NO metabolism, the  $\Delta$ GLB3 seeds were treated with 2- (4-carboxyphenyl)-4,4,5,5-

tetramethylimidazole-1-oxyl-3-oxide (PTIO), a NO scavenger. PTIO partially restored the germination even at 32°C. This strongly suggests that the failure of  $\Delta$ GLB3 to germinate at high temperature is caused by a low NO scavenging activity. Therefore, *AtGLB3* may be necessary for germination by removing excess NO produce during heat stress.

#### **PG-6 Morphological variations of the zooxanthella *Symbiodinium microadriaticum* CCMP829 caused by differences in nitrogen source**

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Many marine invertebrates in tropics and subtropics have symbiotic dinoflagellates referred as to zooxanthellae. It is known that zooxanthellae show two distinct morphologies: one oval and the other spherical. The oval shape is observed when the zooxanthellae is isolated and cultured from its hosts. In contrast, spherical shapes are commonly observed *in hospite*. The molecule that induces morphological changes has not yet been identified. To identify the trigger molecule for zooxanthellae, here we used *Symbiodinium microadriaticum* (CCMP 829) as a model system. We cultured zooxanthellae in three different media: K-medium, NH<sub>4</sub><sup>+</sup>-medium, or NO<sub>3</sub><sup>-</sup>-medium. Zooxanthellae grown in the NH<sub>4</sub><sup>+</sup>-medium were morphologically distinct from those in the NO<sub>3</sub><sup>-</sup>-medium. In the NO<sub>3</sub><sup>-</sup>-medium, many cells showed oval shape whereas the cells grown in the NH<sub>4</sub><sup>+</sup>-medium were spherically shaped. Moreover, this morphological variation was reflected in cell size as determined by flow cytometry. These results suggest that the difference of nitrogen form in the culture media affects the cell morphology of zooxanthellae. We discuss the possibility that NO<sub>3</sub><sup>-</sup> in the medium induces the morphological change of zooxanthellae.

#### **PG-7 Quantification of FSH and LH receptors mRNA in gobiid fish (*Trimma okinawae*) during serial sex changes**

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Molecular cloning of goby FSH and LH receptors has been reported together with their expression throughout sex change. The cDNAs encoding the receptors were isolated from ovaries using RT-PCR and RACE procedures. The LH and FSH receptor cDNAs encoded 691 and 699 amino acids respectively. The identity between the two deduced amino acid sequences was only 39.4%; however, high similarity was found in the transmembrane region. Expression of the goby gonadotropin receptors was essentially restricted to the testis and ovary.

Minimal expression of the LH receptor was seen in the spleen. Transcript abundance of the two receptor genes in the ovary and testis of the same individual was measured by quantitative real-time RT-PCR. Expression appears to be related to sexual phase with quick location switching of the two genes after social manipulation to stimulate sex change. This differential expression of the two gonadotropin receptor genes may play a critical role in the sex change.

**PG-8 Mariner-like sequences classified into the Cecropia-ITR-MLE family and their various insertion sequences in the genomes of selected invertebrates and vertebrates inhabiting the Japanese archipelago and the wild silk moth *B. mandarina* in Japan and Korea**

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Mariner-like elements (MLEs) isolated from nearly all species are thought to transpose horizontally. However the mechanism by which they transpose into different species is not yet clearly defined. Moreover *mariner* elements, isolated by the degenerated primers corresponding to two conserved amino acid sequences, are nearly always incomplete, defective and partial segments. In a first approach to resolve these problems, we used an already developed novel PCR amplification method using the inverted terminal repeats (ITRs) of the *Hyalophora cecropia* MLE as PCR primers aiming at amplifying the full-length sequence of MLEs from some species.

Using this method, we isolated MLEs from several reptiles, frogs, *D. melanogaster* in the Japanese archipelago (primarily the Ryukyu Islands), and wild silk moth *Bombyx mandarina* distributed in Japan and Korea. Around the discrete 1.3-kbp bands expected to contain the full-length of MLEs or various different length of fragments other than 1.3-kbp bands seemed to have some insertion sequences into MLE were detected in all the specimens. The full-length MLEs isolated from species inhabiting relatively close regions of Japan were highly similar to each other, classified as the *Cecropia* MLEs subfamily as already reported. This seems to indicate that the horizontal transfer of MLEs takes place even among phylogenetically remote organisms and that these MLEs might have the ability to transfer horizontally even now. On the other hand, some of the clearly inactive MLEs forming a complex were situated in phylogenetic groups other than the horizontal *Cecropia* subfamily grouping. These complex structures were composed of other sequences such as the retrotransposable element or different types of MLE inserted into these types of MLE. We predicted the relationship, segregation pathway or time among the different species now living in geographically closely region by MLEs, inserting the sequences obtained by this method.

**PG-9 Monokaryotic chloroplast (moc) mutation has no effect on non-Mendelian transmission of chloroplast and mitochondrial DNA in *Chlamydomonas***

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We studied whether a monokaryotic chloroplast (moc) mutation affects the transmission of chloroplast and mitochondrial DNA in *Chlamydomonas*. We used a previously isolated mutant, mocG33, which has only one large chloroplast nucleus. To obtain zygotes we crossed the mutant cells with wild type cells, and mutant cells with receptive mates (females mt+ with males mt-). In these zygotes, we recorded preferential dissolution of mt- parental chloroplast nuclei and fusion of two cell nuclei. Antibiotic resistance markers of chloroplast DNA were maternally transmitted in all crosses. PCR analysis of cytochrome b (cob) gene sequence showed that mitochondrial DNA was paternally transmitted to offspring. These results suggest that moc mutation did not affect organelle DNA transmission.

**PG-10 Habitat function of the seagrass beds for fishes on coral reefs in the Ryukyu Islands, Japan**

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Seagrass beds often cover extensive areas surrounding coral reefs. Our past studies in the Yaeyama islands of the Ryukyu Arcipelago clarified that seagrass bed fish assemblage is largely comprised of seagrass bed residents and coral reef fishes which utilize the seagrass beds as a part of their foraging grounds and/or a nursery. However, it is not clear whether this habitat utilization pattern is commonly seen in the other regions of the Ryukyu Archipelago, which includes three distinct geographical locations covering the sub-tropical area. These are the southern region covering the Yaeyama islands, the middle region of Okinawa main island and its neighbors, and the northern region of the Amami's. To clarify whether seagrass habitat utilization patterns of coral reef fishes differs among the three regions, visual censuses were conducted in the seagrass beds and coral areas at each region over Nov-Dec., 2004. We observed that numbers of fish species and individuals per transect in the seagrass beds were lower in the Amami region compared with those in other two regions. Although the fauna of seagrass bed fish among the three regions were quite similar, region-specific occurrence of some fish species was observed, suggesting that habitat utilization patterns may be different among the regions. Such regional differences might have resulted from differences in seagrass species composition in the seagrass beds and/or development of the coral areas among the three regions. These observations are still in progress results, and further continuing research will be conducted to clarify habitat role of the seagrass beds for fishes in the Ryukyu Islands.

**PG-11 Preliminary studies on *mariner*-like elements of hermatypic corals**

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The *mariner* is a transposon, which is characterized by the possession of a transposase gene and inverted terminal repeats (IR) at the 3' and 5' ends. *Mariner*-like elements (MLEs), possessing IR but without complete transposase coding regions, were isolated from various organisms. Recently Nakajima et al (2002) found that MLEs isolated from a moth, *Attacus atlas*, and a coral, *Fungia* sp., both collected from Okinawa, had very similar sequences but also had complete transposase-coding regions. It is likely that the MLEs are of the active type. As a first step to using phylogenetic relationships among coral MLEs to better understand phylogenetic relationships and dispersal history among corals, we tried to determine whether the MLEs belonged to host corals or algal symbionts (zooxanthellae) and tried to study sequence diversity within species. We tried to amplify MLEs from DNA extracted from corals with symbiotic algae (*Fungia scutaria*: Hawaii and *Porites rus*: Sesoko), azooxanthellate corals (*Tubastraea coccinea* and *Tubastraea robusta*: Sesoko), coral sperm (*Fungia echinata*: Sesoko), and two cultured zooxanthellae. The primers used were designed from the IR region and conserved regions in the putative transposase-coding region. The expected lengths of amplified fragments were 1.3 kbps and 0.5 kbps, respectively. Over the course of several trials, we obtained amplicons of expected as well as unexpected lengths. To examine the possibility that we obtained the PCR products of unexpected length as a consequence of insertion, we sequenced cloned PCR products. We are now analyzing about 70 clones of the PCR products to find possible homology to reported MLEs sequences.

**PG-12 The genus *Pyramimonas* (Prasinophyceae, Chlorophyta) in the coastal waters around Okinawa Island**

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Okinawa Island was formerly surrounded by rich coral reefs. Now, however, the area is in a critical condition due to the direct or indirect impacts of human activity. The microbial component is a frequently neglected aspect of many marine floral studies, especially those done in this area. Consequently, unknown and unexplored microalgae in the vicinity of Okinawa Island may be extinct in the near future. This means there will be a decrease and loss of genes and genetic diversity. Hence, the need to study and collect microalgae in this area is urgent. This study presents some diversity of the genus *Pyramimonas* as found in the coastal waters of Okinawa Island. This is an example of microalgal diversity in this area.

The genus *Pyramimonas* comprises approximately 50 species, most of them reported from marine environments. It is a member of the Prasinophyceae, the earliest divergence within Viridiplantae. Therefore, genus *Pyramimonas* is considered to be phylogenetically important.

From October 2002 to December 2004, samples of coastal water and sediment were collected from a total of 20 sites

around Okinawa Island. From the samples, a total of 36 strains were isolated and cultivated. The light microscopic examinations suggested that half the strains might be yet undescribed and that at least six new species are included. Molecular phylogenetic and ultrastructural studies are in progress.

**PG-13 Stress tolerance and zooxanthella genotypes of *Pavona divaricata* and *P. decussata***

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Coral bleaching, generally attributed to elevated sea surface temperatures, may lead to the death of the bleached corals. However, when corals recover from bleaching, they may acquire new symbionts that are more tolerant of stress. Thus, coral bleaching not only induces a breakdown of coral-algal symbiosis but also provides the host with an opportunity to adapt to the environment by changing the algal partner.

The objectives of this study are to investigate the possible seasonal changes in the algal genotypic composition in two *Pavona* corals and to study how changes in the algal composition affect the stress tolerance of the corals. We found that most colonies of *Pavona divaricata* at Bise, northern Okinawa, harbored clade D zooxanthellae while some others harbored clade C zooxanthellae. *Pavona decussata* at Bise was associated with clade C zooxanthellae. Fragments of both corals were exposed to high temperatures and high light stress for 3 hours, then allowed to recover at normal temperatures in darkness for 24 hours, then kept in a circulating seawater tank for an additional four 4 days. The damage to photosystem II (PSII) and recovery were estimated from the measurements of photochemical efficiency ( $F/F_m$ ) of PSII. We found no significant difference in the damage to PSII nor recovery between colonies associated with clade C zooxanthellae and those harboring clade D zooxanthellae. This was contrary to recent reports that clade D zooxanthellae are more tolerant than clade C zooxanthellae. On the other hand, *P. decussata* colonies harboring clade C zooxanthellae showed slower recovery from the PSII damage than *P. divaricata*. These results suggest that the physiological diversity of zooxanthellae belonging to the same clade, is large, or that the host plays a major role in determining stress tolerance. The corals studied were collected from the same shallow reef and might experience the same environmental history. We are planning to study why dominant genotypes were different among *P. divaricata* colonies and between the sympatric two species of *Pavona*.

**PG-14 Allozyme variation in *Dinodon semicarinatum* (Reptilia: Squamata), a colubrid snake endemic to the central Ryukyus**

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A group of islands sharing somewhat similar terrestrial fauna often provides a unique opportunity to study the effects of isolation on the diversification of conspecific populations. Based on the numerical dominance of highly relict species, the central Ryukyus is considered to have been isolated from the other land masses for a long period of time. The Ryukyu odd-tooth snake, *Dinodon semicarinatum*, is an endemic species of this region, and

occurs on almost all islands of the Amami and Okinawa Groups. Therefore, samples of this snake from these islands would be useful in investigating vicariance and dispersals of terrestrial vertebrates in the central Ryukyus. In this study, geographic genetic variations were examined for *D. semicarinatum* by use of allozyme electrophoresis. Allelic variations in 22 presumptive loci, controlling 18 enzymes, were investigated for a total of 144 specimens from 11 localities of ten islands. Seven of the 22 loci were polymorphic in at least one sample. At *Mpi*, there was a considerable allele frequency difference between the Amami and the Okinawa Group samples. Nei's unbiased genetic distance between the Amami and the Okinawa Group samples was greater than those between samples within each of the two groups. Such a geographic pattern of genetic divergence in *D. semicarinatum* is concordant with those reported for other reptiles and amphibians so far studied by the same method. This suggests an initial divergence in this snake between the two island groups. However, this contradicts the geographic pattern of morphological variation in this species, which does not suggest a primary divergence between the Okinawa and the Amami Group populations. Rapid morphological changes in some island populations under localized environments may be responsible for such an inconsistency between the morphological and genetic variations.

#### **PG-15 Differentiation of cellulolytic systems in termites**

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Cellulolytic systems in the guts of termites were examined by measuring hydrolysis of crystalline- and carboxymethyl-cellulose as well as performing reverse transcription-PCR (RT-PCR) and phylogenetic analyses of endogenous endo- $\beta$ -1,4-glucanase cDNAs from termites of diverse feeding habits. In cellulolytic flagellate-harboring termites, carboxymethyl-cellulose, a highly soluble cellulose ether, was primarily hydrolysed in the salivary glands, whereas microcrystalline cellulose was degraded primarily in the hindgut. In non-flagellate-harboring termites, a fungus-growing termite retains both the cellulolytic activities in the salivary glands, while other wood-feeding and soil-feeding termites possess both the cellulolytic activities primarily in the midgut. RT-PCR and phylogenetic analyses revealed that multiple endogenous endo- $\beta$ -1,4-glucanase genes are expressed in the salivary glands of flagellate-harboring termites as well as in the fungus-growing termite and are expressed in the midgut of the wood- and soil-feeding non flagellate-harboring termites, which are likely to be related to diversification of feeding habits in termites of the family Termitidae. Comparison of intestinal distributions of cellulolytic activities between the two substrates always showed significant differences in each species of the flagellate-harboring termites. These data strongly indicate that other cellulolytic enzymes such as cellobiohydrolases, which have different substrate specificities from endo- $\beta$ -1,4-glucanase (preferentially hydrolysing carboxymethyl-cellulose), also participate in cellulose digestion of flagellate-harboring termites. In the light of these results, as well as recently accrued phylogenetic data, we discuss scenarios for the evolution of cellulose digestion in termites.

#### **PG-16 Biochemical and genetic analyses of a hemolytic toxin isolated from a sea anemone *Actinaria villosa***

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*Actinaria villosa* is a rarely found sea anemone species whose existence has just recently been identified along the coastal areas of Okinawa islands. This marine animal, known to produce several biochemically still uncharacterized proteinous toxins, was implicated as a cause of accidental stings incurred while swimming. For the purpose of biochemical and genetic analysis of toxins produced by this animal, we purified several polypeptides from the tentacles and isolated a protein molecule with a potent hemolytic activity against mammalian red blood cells. This hemolytic toxin which we named Avt-I, had a molecular weight of approximately 19 kDa and *pI* of 9.3. A genetic analysis of a full-length cDNA obtained from the total RNA preparations from the tentacles indicated that it consists of a 1,046 bp gene with a single open reading frame having a predicted amino acid length of 226. Analysis of the predicted amino acid sequence indicated that it has very high homology with hemolytic toxins of other sea anemone species (i.e., 99% homology with Pstx20 of *Phyllo-discus semoni*, 61% with EqII of *Actinia Equina*, and 57% with both StII of *Stichodactyla helianthus* and HmT *Heteractis magnifica*). We also identified the existence of isoforms for Avt-I and Pstx20 genes, and we named these isoforms Avt-II and Pst-I, respectively. Both isoform genes having an identical length with 1,046 bp could conveniently be distinguished by restriction fragment length polymorphism analysis (using endonuclease HphI). Additionally, polymerase chain reaction amplifications of these toxin genes using genomic DNA as templates indicated that each of the four genes i.e., Avt-I, II, Pstx20 and Pst-I, contained two introns and three exons. This is the first report indicating the existence of introns within the actinoporin genes of sea anemones. We speculate that *A. villosa* and *P. semoni*, both belong to the Aliciidae family, may have evolved independently from other sea anemone species.

#### **PG-17 A novel cyanobacterium *Haromicronema* sp. colonized within massive coral skeleton**

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Hermatypic corals in the field harbor a considerable number of bacterial species. However, the biological significance of these bacteria for corals has remained largely unknown. Here we report a novel cyanobacterium *Haromicronema* sp. found within massive coral skeleton. Using a standard culture medium, we successfully isolated filamentous cyanobacteria that inhabited the *Goniastrea aspera* skeleton. Characteristics of the filamentous cyanobacterium were: (1) 1  $\mu$  m in diameter, (2) nonheterocyst-forming, (3) Chl *a* as the sole chlorophyll pigment, and (4) ability to grow in a nitrogen-free culture medium. 16S rDNA analysis affiliated the cyanobacterium with *Haromicronema* sp., a novel genera in marine cyanobacteria. The present study is the first report on the cyanobacterium *Haromicronema* sp. colonized within a coral skeleton. We will discuss the biological interaction between *Haromicronema* sp. and host coral in terms of a new form of symbiosis.

## Species Diversity

### PS-1 Biodiversity of the coral reef-associated Bryozoa: what to expect in Okinawa reefs?

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Bryozoans are known to be an important component of cryptic reef communities worldwide, with many species apparently circumtropical in distribution. For a long time, bryozoans were considered to be uncommon in reef environments. Their apparent rarity and low diversity in early studies were primarily artifacts of the collecting methods used and inadequate sampling. Winston (1986) compiled a global checklist of coral reef-associated bryozoans based on 32 early studies for 29 different reefs in the Caribbean, Gulf of Mexico, Tropical West Atlantic, East Africa, Red Sea, Indonesia, Great Barrier Reef, Eniwetak, Hawaii, Eastern Pacific, and Philippine Region, giving in total just 284 species. However, contrary to previous data, recent intensive studies have indicated a high biodiversity of bryozoans on coral reefs. Thus, 124 species were reported for the Heron Island reef-flat, Great Barrier Reef (Ryland and Hayward, 1992; Hayward and Ryland, 1995). Tilbrook (2001) recorded 92 cheilostome bryozoans for reef-flat in the Vanuatu Archipelago, Coral Sea. Though reef-associated bryozoans have been scantily studied in Okinawa, even a sporadic collection revealed over 40 species (Mawatari, 1987, 1991), with a high proportion of new species. Hence, Okinawa, lying in a region with a diverse bryozoan fauna, is likely to have hundreds of reef-associated species.

### PS-2 Molecular phylogenetic study on the genus *Cardiandra* (Hydrangeaceae) in the Ryukyu Archipelago and the vicinity: a preliminary report

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More than 100 endemic vascular plants are distributed throughout the Ryukyu Archipelago. Understanding the origins of these endemics will provide new insights concerning floral diversity in this area *Cardiandra amamioshimensis*, an endemic narrowly distributed in Amamioshima Island, is distinguished from other congeneric taxa by the absence of ornamental flowers. Although the genus *Cardiandra* is a small genus consisting of only two to four species distributed in eastern Asia, inter-specific relationships remained unclear until the present. The purpose of this study is to clarify the evolutionary process of *Cardiandra* in the Ryukyus with special reference to its relationship with continental and Taiwanese congeners. Here we report our preliminary findings on the molecular phylogenetic analysis of *Cardiandra* in the Ryukyus.

*Cardiandra alternifolia* (Hyogo Pref.), *C. amamioshimensis* (Amamioshima Is.) and *C. moellendorffii* (Iriomotejima Is.) were used for molecular analysis. *Hydrangea liukuensis* (Okinawa jima Is.) was used as the out-group. The internal transcribed spacer (ITS) of the nuclear ribosomal DNA was PCR amplified and sequenced. The aligned ITS was 672bp in length and pair sequence comparisons among the taxa of *Cardiandra* indicated sequence similarity ranging from 97.9% - 99.3%. Most parsimonious (MP) and neighbor-joining (NJ) trees showed the same topology. On the NJ tree, *C. alternifolia*, found between the cen-

tral part of Honshu and the Kyushu districts, was placed as a sister of *C. amamioshimensis*. On the other hand, *C. moellendorffii*, found in the southern part of China and on Iriomotejima Island, was at the most basal position. Although Ohba (1985) treated *C. alternifolia* and *C. moellendorffii* as a single species, our results indicate they are not monophyletic. It is possible that the ornamental flowers of *C. amamioshimensis* are secondarily absent when this species is geographically isolated from its congeners.

### PS-3 Cytogeographical and molecular phylogenetic study on the origins of tetraploid *Hydrangea liukuensis* (Hydrangeaceae), endemic to Okinawajima Island: a preliminary report

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In addition to genetic divergence influenced by paleogeographical dynamics, speciation processes such as interspecific hybridization and polyploidization appear to play an important role in creating plant diversity in the Ryukyus. *Hydrangea liukuensis*, endemic to Okinawajima Island, is an example in which intraspecific polyploidy has been reported. The aim of this study is to clarify the origin of polyploid *H. liukuensis*. Here, we report the preliminary results from the cytogeographical and molecular phylogenetic analyses of *H. liukuensis*.

Sixty *H. liukuensis* plants were collected from 14 localities for cytogeographical analysis. The tetraploid ( $2n = 72$ ) occurred at only two localities (Mts. Yae and Nekumachiji), and the diploid ( $2n = 36$ ) occurred at the remaining 12 localities. Intra-population variations in the ploidy level were not observed. Five diploid and four tetraploid plants were used for molecular analysis. Octoploid ( $2n = 144$ ) *H. yaeyamensis* (Iriomotejima Island) and diploid ( $2n = 30$ ) *Cardiandra alternifolia* (Hyogo Pref.) were used as out-groups. The internal transcribed spacer region (ITS) of nuclear ribosomal DNA was PCR amplified and sequenced. Most parsimonious (MP) and neighbor-joining (NJ) trees were then constructed based on the nucleotide sequences of the ITS. Both diploid and tetraploid *H. liukuensis* were monophyletic with high bootstrap values (90% and 98%, respectively) and they were not nested on both trees, suggesting that the tetraploid has a single origin. Since the fruit of *H. liukuensis* has no special organ suitable for long distance dispersal, limited and distant distribution of tetraploid seems to reflect its relict nature. Furthermore, *H. yaeyamensis* was a sister of tetraploid *H. liukuensis*, and diploid *H. liukuensis* was placed at the most basal position. It is possible that *H. liukuensis* is not monophyletic but a paraphyletic taxon. Further studies including other related taxa are needed to clarify the origin of tetraploid *H. liukuensis*.

### PS-4 Molecular phylogenetic study on the origin of *Eurya emarginata* var. *minutissima* (Theaceae), a shrub endemic to the central Ryukyus

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*Eurya emarginata* sensu lato (Theaceae) is an evergreen shrub widely distributed over an area covering mainland Japan and South Korea, through the Ryukyu Archipelago, to Taiwan and southern China. It shows great variety in leaf size and shape, and pubescence of the stems, petioles and fruits. *E. emarginata* var. *emarginata* usually prefers coastal habitats, while *E. emarginata*



var. *minutissima*, characterized by minute leaves and rather densely pubescent branchlets, only occurs in cloud forests in the mountainous areas of Amamioshima, Tokunoshima, and Okinawajima islands. To elucidate the relationship between *E. emarginata* var. *minutissima* and *E. emarginata* var. *emarginata*, we conducted molecular phylogenetic analysis based on the internal transcribed spacer (ITS) region of nuclear ribosomal DNA. On both the most parsimonious and neighbor-joining trees, 34 individuals of *E. emarginata* sensu lato including var. *minutissima* formed a monophyletic but somewhat polychotomous clade (clade A) together with *E. sakisimensis*. Considering that *E. sakisimensis* is endemic to the Yaeyama Islands, it is reasonable to assume that *E. emarginata* var. *minutissima* derives from *E. emarginata* var. *emarginata* in the central Ryukyus. In clade A, *E. emarginata* var. *minutissima* from five localities in Amamioshima (Mt. Yuwan), Tokunoshima (Mts. Amagi and Inokawa) and Okinawajima (Arakawa and Aha Rivers) islands, formed weakly supported subclades. However, because four individuals from two localities (Mts. Yonaha and Tamatsuji) on Okinawajima Island, formed polychotomies at the basal position of clade A, the monophyly of *E. emarginata* var. *minutissima* could not be elucidated. On Mt. Yonaha, *E. emarginata* var. *minutissima* and *E. emarginata* var. *emarginata* grow in close proximity and a morphological intermediacy exists between them. It is likely that hybridization with *E. emarginata* var. *emarginata* makes the monophyly of *E. emarginata* var. *minutissima* unclear on the molecular phylogenetic tree.

**PS-5 Genetic and environmental determinants of sex differentiation in the European sea bass (*Dicentrarchus labrax*)**

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The European sea bass is a gonochoristic teleost of the family Moronidae. Like most teleosts, the sea bass does not have morphologically recognizable sex chromosomes, but evidence based on sex-associated banding patterns suggest that pair 24 could be at an early stage of heterochromosome differentiation. Furthermore, there is a parental component in the sex ratios of different families, apparently being more influenced by dam than sire. The induction of triploidy or gynogenesis increases the number of females, suggesting that modifications in the balance of sex determining factors as a consequence of these manipulations, are responsible for the altered sex ratios. Sex differentiation takes place during the first year of life, although molecular, endocrine and histological data suggest that the crucial events occur between 90 and 120 days post-hatch (DPH). Sex-related growth in favor of females is evident in fish just initiating sex differentiation at about 150 DPH, with three possible phenotypes: 1) fast growing fish which are usually females, maturing at three years of age, 2) fast growing fish with a low gonadosomatic index, that will mature as precocious males just after completing sex differentiation towards the end of the first year, and 3) slow growing fish which will mature as regular males at the end of the second year. How many fish will develop each phenotype will depend on a genetic (parental) component that can be influenced by environmental conditions, particularly by rearing temperature, during approximately the first three months of life. This sexual plasticity makes the sea bass a useful model to study effects of temperature on fish sex ratios. The relationship between water temperature, growth and phenotypic sex, and the molecular and

endocrine changes characteristic of genotype-environment interactions in this species are currently being investigated.

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**PS-6 Species diversity and spatial distribution of trees in relation to stand stratification in a subtropical evergreen broadleaved forest on Okinawa Island**

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On the basis of forest stand stratification, woody species diversity, species composition and spatial distribution of trees in a subtropical evergreen broadleaved forest growing in a silicate area, were investigated in a 0.08 ha plot. A total of 26 families, 43 genera, 60 species and 4,684 individuals were encountered. The diversity index  $H'$  and equitability index  $J'$  were respectively 4.82 bit and 0.81 for total trees. The forest showed a high diversity, comparable to the diversity of subtropical forests grown in other limestone areas. The forest stand consisted of four layers. The  $H'$  and  $J'$  values increased with the decreasing height of the layers, with the exception of the bottom layer. It was shown that the diversity index  $H'$  for the total stand should be measured over a minimum sample area of 200 m<sup>2</sup>. *Castanopsis sieboldii* (Mak.) Hatusima was the most dominant species in terms of importance value, and it appeared over the four layers having the highest importance value. The expected maximum number of species was 62 per plot in the total stand, 36 in the top layer, 46 in the second layer, 59 in the third layer and 64 in the bottom layer. The spatial distribution patterns of trees for the total stand, and for each layer excepting the top layer, were found to be in single-individual, completely random distributions. In the top layer, there seemed to exist a triple-clump structure. Most of the species were common between the second and the third layers, and half the total number of species were common between the top layer and the other three layers. The degree of overlapping showed that the spatial distribution of trees almost overlapped between the second and the third layers, and it was nearly overlapped between the top and the other three layers.

**PS-7 Host specificity of crinoid-associated decapod crustaceans in the vicinity of Okinawa Island**

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Taxonomical and ecological studies were conducted to determine the host specificity of crinoid-associated decapod crustaceans in the vicinity of Okinawa Island.

The crinoids collected were included 42 species belonging to seven families and 21 genera. This collection may include at least four species not yet described: two *Comanthus* (Comasteridae), one *Comatella* (Comasteridae), and one *Dorometra* (Antedonidae). Field observations revealed 43% (18 of the 42) of the species to be nocturnal, and the number of species externally visible on the substratum increased at night over the numbers during the day. The crinoids can be categorized by the following four living positions: 1) those attached to the substratum by their arms or cirri, or both; 2) those inhabiting crevices or small holes on the substratum; 3) those attached to the underside of rocks by their arms and cirri; and

4) those attached by their cirri to other invertebrates such as gorgonian fans, soft or spiral corals.

Decapod crustacean associates included 13 pontoniines, three alpheid, five galatheids, and four eumedonines. Of these, one *Periclimenes* (Pontoniinae) and two *Galathea* (Galatheaidae) were recognized as undescribed species. The micro-habitats of decapods on their crinoid hosts differed as follows: pontoniine shrimp usually hide among the pinnules or arms of crinoids; alpheid snapping shrimp nearly always live on the oral disk; galatheids and eumedonine crabs usually hide between the cirri or at the base of the arms. From the 42 species of crinoids and 25 species of decapods collected, 148 symbiotic combinations were identified. Host specificity of the associated decapods varied from general to specialized in a single host species.

**PS-8 Histological observations of the larval tissue adapted for carrying photosymbionts in a colonial ascidian *Didemnum molle* (Didemnidae, Ascidiacea)**

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The larval tissue attaching the photosymbionts was histologically investigated in a *Prochloron*-bearing ascidian *Didemnum molle*. The *Didemnum molle* larva is about 3.5 mm long, and the surface of the posterior half of the trunk is smothered with *Prochloron* cells. In the posterior half of the larval trunk, the tunic, integumentary matrix of tunicates, has many folds that catch *Prochloron* cells, while a thin larval tunic layer covers only the anterior half of the larval trunk. The area where *Prochloron* cells are not distributed is almost identical to the area covered by the larval tunic. The tunic of posterior half of larval trunk may be adhesive to catch the *Prochloron* cells of the mother colony. The thin larval tunic layer is probably not adhesive and protects the anterior half of the trunk from the attachment of *Prochloron* that could interfere with sensory receptors and/or larval settlement of the substratum.

Although the forms of pre-hatching embryos/larvae much differ between *Diplosoma* spp. (Hirose, 2000; Hirose et al., 2005) and the present species, the histological features of the tunic tissues attaching *Prochloron* cells have very similar morphology. This suggests that the mechanism of the *Prochloron* attachment is essentially the same in these *Prochloron* host species. Since ascidian-*Prochloron* symbiosis is assumed to have had an independent origin at least once in each genus (Kott, 1980; 1982), the numerous tunic folding, an adaptation for vertical transmission of photosymbionts, might be independently acquired in *Didemnum* and *Diplosoma*.

**PS-9 Mirror image reactions in squid: behavioral divergence in cephalopod social systems**

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Direction to a glittering object or a polarized object is a shared characteristic among cephalopods. This could be related to their highly developed, vertebrate-like visual systems as well as their having the largest brains among invertebrates. But few examinations have studied cephalopods reactions to a 100% reflective object, i.e., a mirror, in which they encounter them-

selves. Considering the level of social recognition in this intelligent invertebrate, how they react to self-reflection is an important subject for examination.

We tested with a captive oval squid (*Sepioteuthis lessoniana*), a member of one of the highest socialized groups among cephalopods, on reactions to its own mirror image. The squid behaved very uniquely to its own mirror reflection; they directed their body toward the mirror, approached it, then followed by touching the mirror surface repeatedly. This behaviour was not induced by a control board, but in freely swimming squids. Episodes observed in captivity revealed that touching behaviour was neither seen in oval squid in any social situations involving conspecifics and/or other cephalopods. Furthermore, we isolated an oval squid in a tank for a month to see the effect of non-socialization on reactions to mirror images. The isolated squid behaved differently to the mirror compared to the grouped individuals. He stayed in front of the mirror but did not touch it at all. We discussed this mirror-mediated behaviour by the oval squid with respect to sociality and self-recognition that has been found only in great apes and dolphin.

**PS-10 Population dynamics of larvae and juveniles of fishes in a lagoon on Okinawa Island**

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Surf zones and seagrass beds in Okinawa Island are important nursery grounds for juvenile fish and larvae. However, the appearance of larvae and juveniles of coral reef fish species in these areas was few. They supposedly appear in coral reef lagoon; I therefore conducted larval sampling in the channel of the reef crest (at Ohdo Beach) and in lagoons (Ohdo Beach and Cape Zanpa) throughout a year. I analyzed the dynamics of larvae and fish juveniles in the lagoon.

The total number of sampled larvae and juveniles came to 17,933 (channel: 15,573 and lagoon: 2,360). At both reef environments, there were two peaks in appearances during the year: spring and autumn. The top five dominant families in the channel were Clupeidae (65.8%), Gobiidae and Microdesmidae (16.9%), Schindleriidae (5.0%), Syngnathidae (3.6%), and Pempheridae (3.3%). In the lagoon, these were Pempheridae (43.3%), Apogonidae (19.9%), Gobiidae and Microdesmidae (16.7%), Clupeidae (16.3%), and Atherinidae (4.5%).

The appearance patterns of larvae and juveniles were categorized as follows: Type 1; appeared all year round with random peaks; Type 2; appeared all year round with a peak between spring and summer; Type 3; appeared from winter to spring; Type 4; appeared from spring to autumn, and Type 5, appeared intermittently. Their patterns of lagoon utilization were divided into two types: Type A, appeared in the lagoon and other coastal environments, and Type B; appeared only in the lagoon. These types were applied to the dominant families: Clupeidae; 2-A, Atherinidae; 3-A, Sparinae; 4-A, Schindleriidae; 1-B, Gobiidae and Microdesmidae and Syngnathidae; 2-B, Pempheridae and Apogonidae; 4-B and Labridae and Scaridae; 5-B. Many coral reef fish families belonged to Type B, indicating that the lagoons are important places for larvae and juveniles of coral reef fish species as settlement ranges.

**PS-11 A natural hybrid mud crab (Decapoda: Portunidae) from Japan**○Hideyuki Imai<sup>1</sup>, Masatsune Takeda<sup>2</sup><sup>1</sup>Faculty of Science, University of the Ryukyus, Nishihara, Okinawa, 903-0213 Japan (imai@sci.u-ryukyu.ac.jp),<sup>2</sup>Department of Zoology, National Science Museum, Shinjuku, Tokyo, 169-0073 Japan

A presumed hybrid mud crab of the genus *Scylla* was examined. The presumed hybrid could not be easily separated morphologically from the three Japanese *Scylla* species, *S. serrata*, *S. paramamosain* and *S. olivacea*. An analysis of the first internal transcribed spacer (ITS-1) of the nuclear DNA revealed that the hybrid carried genomic DNA from both *S. serrata* and *S. olivacea*. Analysis of the maternally inherited 16S rDNA of the mitochondrial DNA demonstrated that the presumed hybrid carried *S. olivacea* mtDNA. Based on these results, we conclude that the examined animal is indeed the hybrid offspring of a female *S. olivacea* and a male *S. serrata*. The degree of genetic isolation among these species, and the possible causes of hybridization are discussed.

**PS-12 The Composition of endophytic fungi in shoots and propagules of mangroves in the Ryukyu Islands**

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The composition of endophytic mycobiota in leaves, twigs and propagules of five mangrove species was investigated in the Ryukyu Islands. In November and December 2004 samples of *Kandelia candel*, *Bruguiera gymnorrhiza*, *Rhizophora stylosa*, *Avicennia marina* and *Sonneratia alba* were gathered at sixteen mangrove stands in Tanega-shima, Amami-oshima, Okinawa-jima, Miyako-jima, and Iriomote-jima. At each stand, branch samples were collected from three to five trees per species. Young propagules of Rhizophoraceae species in different sizes were also collected randomly from fruiting trees.

Disks from the two parts of the leaf on the costa and near the margin, a petiole segment and an internodal segment of a current-year twig were taken after surface sterilization. Seven segments or disks from each part were taken randomly from five asymptomatic branches per tree. Propagules were sorted into four groups: unexposed hypocotyls, elongated hypocotyls with <2 cm-long exposed area, elongated hypocotyls with >2 cm-long exposed area, and hypocotyls with insect damage. Segments were taken from the asymptomatic portion of each hypocotyl and surface-sterilized. All segments were incubated at 15°C for two months in the dark on weakened PDA with a half concentration of nutrients. Isolates were grouped according to their cultural characteristics.

Isolation from the shoot revealed that two taxa were the major endophytic fungi common to five mangrove species, one of which was the dominant in three host species. No fungal taxon detected was clearly specific to the stands or to the island. All isolated fungi from *Avicennia marina* and *Sonneratia alba* were sorted into eight of ten taxa which we had found in the shoots of three Rhizophoraceae on Okinawa Island. These results may demonstrate not the difference but the similarity in composition of endophytic fungi among mangrove species throughout the Ryukyu Islands. Endophytic fungi in similar groups were also isolated from both exposed and unexposed hypocotyls.

**PS-13 Testing the reduced genetic diversity hypothesis for the evolution of unicoloniality in *Pheidole megacephala***

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Biological invasions by ants are a severe threat to biodiversity and human activities in areas where they occur, especially in the Pacific Islands. Unicoloniality is one of the main attributes thought to facilitate the success of invasive ants. Unicolonial species typically form spatially vast and competitively dominant supercolonies that lack territorial boundaries; they are polygynous (multiple queens), reproduce by colony budding, and exhibit high interspecific aggressiveness. Reduced genetic diversity in introduced population is considered as one of the evolutionary factors for unicoloniality.

The tramp species *Pheidole megacephala*, native from Africa, has been accidentally introduced throughout the Ryukyu Archipelago. While *P. megacephala* is a major pest in other Pacific Islands such as Hawaii and New Caledonia, little known about its biology. In this study, we investigated if *P. megacephala* exhibits unicoloniality in the Ryukyu islands. We performed behavioral experiments and genetic analyses of *P. megacephala* populations collected in four islands: Okinawa, Miyako, Minami Daito and Amami.

**PS-14 Variation in resistance to toad toxin by snakes of the genus *Dinodon* (Reptilia: Colubridae)**○Noriko Kidera<sup>1</sup>, Hidetoshi Ota<sup>2</sup><sup>1</sup>Department of Marine and Environmental Science, Graduate School of Engineering Science, University of the Ryukyus,<sup>2</sup>Tropical Biosphere Research Center, University of the Ryukyus

A toxic prey and its predator often provide an ideal model system to investigate organismal diversification in the processes of coevolution. In studies of coevolution using this advantageous system, the major focus has been placed upon the effect of changes in the quantity or quality of the toxin of the prey upon the anti-toxic resistance of the predator. In contrast, little attention has been paid to aspects of degeneration of such resistance in the predator following its isolation from the toxic prey. Toads of the genus *Bufo* have toxic secretion from the dermal glands that has a defensive function against predators. Predators of the *Bufo* species in Asia include the colubrid snakes of the genus *Dinodon*. In the Ryukyu Archipelago and adjacent islands, the isolation periods between the *Bufo* and *Dinodon* species show a few discrete grades, offering excellent opportunities to address the latter issues. To explore the relationship between the extent of the predator's ability to resist the toxin of the prey and the passage of time during isolation, we experimentally investigated the toad toxin resistance in three *Dinodon* species and subspecies (*Dinodon rufozonatum rufozonatum*, *D. r. walli* and *D. semicarinatum*) that form a monophyletic group and are sympatric with toads, or have been isolated from them since the Late or Early Pleistocene. By measuring negative increments of the swimming speeds of the snakes after administration of the toxin from *Bufo marinus*, we evaluated the degree of the snakes' resistance to the toad toxin, and examined its correlation with the estimated isolation period between this group of predators and toads.

**PS-15 Sex differentiation and the expression of cytochrome P450scc in the gonads during early gonadal differentiation in the golden rabbitfish, *Siganus guttatus* (Bloch)**

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In the coral reef in the tropics and subtropics, a variety of teleost fish species share limited habitats by diversifying their ecological strategies. Among those strategies, reproductive biology is thought to be one of the most critical factors in maintaining each species. While much research on reproductive ecology has been done, only limited information on reproductive physiology, which is fundamental and closely related with reproductive ecology, is available for coral reef fishes. The present study clarifies the process of gonadal sex differentiation in *Siganus guttatus*, Siganidae, in Okinawa. The expression of cytochrome P450 cholesterol side-chain cleavage enzyme (P450scc), the primary enzyme in a cascade converting cholesterol into steroid hormones including sex hormones, during early gonadal differentiation was also examined to elucidate the mechanism of sex differentiation in the species as a gateway into the highly diverse species of the area.

During gonadal sex differentiation, several conspicuous features first reported among teleost fishes, were observed. First, we found a sex cord-like structure giving a double-layered structure to the somatic element of gonads, suggesting the resemblance of early gonadal development in the species to that of higher vertebrates such as amphibians and amniotes. Second, there was delayed onset of oogenesis in the ovaries, totally different from other fishes for which the onset of oogenesis is one of the earliest criteria of ovarian differentiation. Third, there was the detachment of gonadal tissues, which progresses forming the ovigerous lamellae and regulating the number of oocytes to attain full maturation in the ovaries, while forming efferent duct network in the testes. The immunoreactivity of P450scc was undetectable in the ovaries from the onset of ovarian differentiation through to the complete formation of the ovarian cavity, inferring a delayed commencement of oogenesis. On the other hand, immunoreactivity was conspicuous in the testes from the onset of testicular differentiation. This suggests that steroid hormones including androgen have an important role in morphological differentiation of testis.

**PS-16 Taxonomy and our perception of biodiversity: North Pacific bryozoans as an example**

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The biological species is the basic unit that researchers want to recognize in the enumeration of biodiversity. A fundamental problem is how to recognize biological species in a timely manner. Taxonomists have historically employed a morphological species concept to detect what they hope are biological species. However, it is important to realize that taxonomy has never been an assumption-free enterprise. For example, bryozoan taxonomists working in the North Pacific from the mid 1800s through the mid-1900s made the tacit assumption that species tend to have broad distributions and show much intraspecific geographic variation. In contrast, taxonomists now tend to assume that if

two populations are widely geographically separate and show some consistent morphological differences, then they are as likely as not to be separate biological species. An *a priori* assumption that poorly dispersing benthic species tend to have broad distributions is as valid (or invalid) as the assumption that they have narrow distributions. Fortunately, taxonomists can now use DNA sequence data to evaluate these opposing assumptions. A drawback of this approach is that there is no sure correlation between genetic distance and degree of reproductive isolation. Examination of as many bryozoan taxa as possible in a phylogeographic context can begin to give a picture of where nominal species fall in a 3-dimensional space defined by axes of genetic distance, morphological distance, and geographical distance. The multiple pairs of nominal species occurring on both sides of the boreal North Pacific provide a fertile ground for testing this approach. Here we provide one example, *Rhynchozoon tumulosum* from Alaska, where we used DNA sequence data to address a problem in bryozoan alpha-level taxonomy, and another example, the *Porella acutirostris* complex, for which a phylogeographic approach would likely be productive.

**PS-17 Behavior, host-specificity and protection from sea anemone hosts in juvenile *Thalassoma amblycephalum* (Labridae, Teleostei)**

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A guild of at least 40 fish species are facultative symbionts of sea anemones. Most behavioral and physiological aspects of this type of association are unknown. We studied the behavior and host-specificity in the juvenile wrasse *Thalassoma amblycephalum* (Bleeker) which dwell within the tentacular sphere of sea anemones, at a local reef in the Ryukyus Archipelago southern Japan in daytime hours spanning 16 months from July 2003 to December 2004. There are two past records of juvenile *T. amblycephalum* dwelling in sea anemones, one from Kenya east Africa and one from the Komodo Islands, central Indonesia. This study is the first record from the eastern part of Indo-Pacific. Small juvenile *T. amblycephalum* frequently swam close to the host and occasionally touched the tentacles for a split-second when picking out (presumably) small food items. Large juveniles stayed close to the host, but never touched any tentacles. *T. amblycephalum* co-existed with the obligate anemonefish *Amphiprion frenatus* in the solitary type host anemone *Entacmaea quadricolor* and *A. ocellaris* in the host anemone *Heteractis magnifica*. By using forced host contact test *ex situ* followed by scanning electron microscopy examinations of the skin we show here that juvenile *Thalassoma amblycephalum* are protected from the host anemones *H. magnifica* and *E. quadricolor*. Based on past and our present results, we suggest a tentative evolutionary model of fish which are symbionts of sea anemones.

**PS-18 Two new cytotoxic diterpenes from a soft coral *Eleutherobia* sp.**Musri Musman<sup>1</sup>, Verawati Roring<sup>1</sup>, Junichi Tanaka<sup>1</sup>, Yehuda Benayahu<sup>2</sup>, Tatsuo Higa<sup>1</sup><sup>1</sup>University of the Ryukyus, Nishihara, Okinawa 903-0213, Japan, <sup>2</sup>Tel Aviv University, Ramat Aviv, Tel Aviv 69978, Israel

During our search for bioactive molecules from marine organisms of Okinawan waters, we found a soft coral *Eleutherobia* sp. whose lipophilic extract showed cytotoxic properties against NBT-T2 cells. Chromatographic separation of the extract resulted in the isolation of two new compounds 1 and 2. Their structures were elucidated by spectroscopic analysis and shown to be diterpenes of xenicane class and of eleutherobin class. We will present the isolation, structures, and biological activity of these compounds.

**PS-19 Seasonal variation in composition and quantity in the diet of the fish-egg-eating sea snake, *Emydocephalus ijimae* (Reptilia: Elapidae), in subtropical coral reefs**

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The sea snake *Emydocephalus ijimae*, feeding exclusively on eggs of reef fishes, should occupy a unique position in the food web of the coral reef ecosystem in Ryukyu waters. Their dietary habit is also of great interest in that it marks one extreme of dietary divergence in snakes. I thus investigated seasonal variations in dietary composition and quantity in *E. ijimae* by analyzing the stomach contents of preserved specimens from Sesokojima Island. Almost all individuals collected in spring and summer had fish eggs in their stomachs, while most snakes had empty stomachs in autumn. The median volume of eggs per stomach was largest in spring. Females showed a positive correlation between the volume of eggs from the stomach and body size in spring and summer, whereas in males the egg volume was consistently smaller and did not correlate to body size in these seasons. The fish eggs found in the snakes' stomachs obviously belonged to many species, with pomacentrids (damsel-fishes) occupying the greatest proportion. Eggs other than those belonging to the Pomacentridae could not be identified with certainty, but presumably belonged to the Gobiidae or Blenniidae. The percentages of damselfish eggs to all eggs from a stomach were 11%, 100%, 52-80%, and 100% in winter, spring, summer, and autumn, respectively. My field data suggest that the activity range of an individual sea snake, including that for foraging, is very small and stable. It is therefore likely that *E. ijimae* can stay in a fixed, small area for a long period by utilizing eggs of a number of fish species with variable seasonal spawning patterns.

**PS-20 A phylogeographical study of a coastal plant (*Limonium wrightii*: Plumbaginaceae) in the northwestern Pacific islands**

Shun-Ichi Matsumura

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The biota of the Ryukyu Archipelago is compounded with various organisms originating from tropics to temperate. I anticipate that the different species compositions between the islands would influence plant-animal interactions and in addition to geo-

graphical isolation, could generate plant speciation particularly through affecting pollination processes. In this study, I focused on the origin of flower color polymorphism within a coastal sea lavender, *Limonium wrightii*, distributed throughout islands in the northwestern Pacific. In addition, I wanted to clarify the genetic differences between populations on continental islands and those on oceanic islands. The flower color polymorphism showed a "leapfrog" distribution pattern, i.e., the distribution of pink flowers had two disjunctive areas intercalated by a distribution of yellow flowers. Molecular analysis showed that cpDNA haplotypes were not correlated to the difference in flower color. Thus, I inferred that this distribution pattern generated from genetic drift and/or natural selection. While the primary visitors to flowers of both pink and yellow colors were several species of small bees, only the pink flowers were also visited by various medium- to large- sized bees, wasps and butterflies. On the other hand, the ITS sequences were identical within *L. wrightii* except for those on the Daito Islands which were quite different from conspecific ones with nine apomorphic substitutions, indicating the distance-independent geographic structure of the genetic variations in the islands. I inferred that the geographic structure is mediated by the routes of seed dispersal and the differences in colonizing periods. The calibrated rate of nucleotide substitutions within *L. wrightii* ( $1.20 \times 10^{-8}$  substitutions per site per year) was higher than any other instance of substitution rates in the ITS region of plants. It might be accelerated by genetic drift in the small, isolated populations.

**PS-21 Diversity of Ca<sup>2+</sup>-dependent regulation of flagellar motility in the sperm of euryhalin tilapia *Oreochromis mossambicus***Morita, Masaya<sup>1</sup>, Takemura, Akihiro<sup>1</sup>, Gima, Shinichi<sup>2</sup>, Nakajima, Ayako<sup>3</sup>, Okuno, Makoto<sup>3</sup><sup>1</sup>Sesoko Station, Tropical Biosphere Research Center, University of the Ryukyus, 3422 Sesoko, Motobu, Okinawa 905-0227, Japan, <sup>2</sup>Radioisotope Laboratory, Joint-to use inter department institutes, University of the Ryukyus, 1 Senbaru, Nishihara, Okinawa 903-0213, Japan, <sup>3</sup>Department of Biology, Graduate School of Arts and Sciences, University of Tokyo, Komaba, Meguro-ku, Tokyo 153-0041, Japan.

Euryhalin tilapia *Oreochromis mossambicus* and most teleost sperm require an increase in intracellular Ca<sup>2+</sup> concentrations ([Ca<sup>2+</sup>]<sub>i</sub>) to activate/initiate flagellar motility. However, the downstream mechanism is unknown. Our goal was to identify the downstream mechanism that occurs following an increase in [Ca<sup>2+</sup>]<sub>i</sub> in tilapia sperm.

Demembrated tilapia sperm were reactivated in the presence of concentrations in excess of 10<sup>-6</sup> M Ca<sup>2+</sup>. Motility features changed when Ca<sup>2+</sup> concentrations were increased from 10<sup>-6</sup> to 10<sup>-4</sup> M. Although the beat frequency did not increase, the sear angle and wave amplitude of flagellar beating increased, suggesting that the sliding velocity of microtubules in the axoneme, which represents dynein activity, rises with an increase in Ca<sup>2+</sup>. Additionally, swimming speed increased with greater Ca<sup>2+</sup>. Thus, it is possible that Ca<sup>2+</sup> binds to flagellar proteins to activate flagellar motility as a result of the enhanced dynein activity. Ca<sup>2+</sup>-binding proteins were detected by <sup>45</sup>Ca<sup>2+</sup> overlay assays. One Ca<sup>2+</sup>-binding protein (18 kDa, pI 4.0) was detected. The isoelectric point and molecular weight of the 18 kDa Ca<sup>2+</sup>-binding protein was similar to those of calmodulin (CaM). A CaM antagonist, W<sup>7</sup>, suppressed the swimming speed and wave amplitude of flagellar beating, suggesting that the 18 kDa Ca<sup>2+</sup>-binding protein is CaM and that CaM regulates flagellar motility. It is

known that CaM mediates the activity of protein kinase(s)/phosphatase(s). Ca<sup>2+</sup>/CaM-dependent protein kinase(s) (CaMKs) were detected by Western blotting. CaM Kinase type IV (CaMKIV) was detected as a single 48 kDa band in both the fraction of low ion extract of the axoneme containing radial spoke heads/central pair and the remnant of the axoneme containing inner arm dynein, suggesting that CaMKIV binds to distinct positions in the axoneme. Immunofluorescence microscopic analysis showed that CaMKIV was localized along the flagella. It is possible that CaMKIV phosphorylates the axonemal proteins in a Ca<sup>2+</sup>/CaM-dependent manner for regulating the dynein activity. A <sup>32</sup>P-uptake in the axoneme showed that 75, 120, 150, 200, and 300 kDa proteins were phosphorylated in a Ca<sup>2+</sup>-dependent manner. It is possible that an increase in Ca<sup>2+</sup> induces Ca<sup>2+</sup>/CaM-dependent regulation, including protein phosphorylation for activation/regulation of flagellar motility.

**PS-22 Floral variation of the ground Jack bean, *Canavalia cathartica* (Fabaceae), and differences of pollinators in the Ryukyu Islands**

Shun-Ichi Matsumura

Graduate School of Engineering and Science, University of the Ryukyus

Numerous organisms originating in the tropics have extended their distribution range onto the Ryukyu Archipelago. These organisms may have generally small population sizes and change relationships with their mutualisms and competitors. Given the different plant-animal interactions, the question is whether or not those factors generate plant speciation especially through pollination processes. In this study, I turned attention to intraspecific variations of floral morphology within jack bean, *Canavalia cathartica*, and the differentiations of its pollinators. Whereas this species is widely distributed in the tropics and subtropics, part of the distribution boundary is situated just north of Okinawa Island.

Whereas the pollinators of *Canavalia* spp. in the tropics are reported as being anthophilid bees, e.g., carpenter bees, leaf cutter bees participated in the pollination in the Ryukyu Archipelago. Especially on Okinawa Island, the pollinators of *C. cathartica* consisted only of leaf cutter bees. In correlation to the difference of the pollinators on Okinawa Island, the standard petals of the flowers of *C. cathartica* bent downward, and this morphological change disabled carpenter bees from stepping on the platform-like petals. In contrast, female leaf cutter bees did not need the standard petals to pollinate the flowers of *C. cathartica*. They exhibited two kinds of behaviour related to modes of collecting resources at flower visitations, and pollinated flowers of *C. cathartica* only when collecting both pollen and nectar. In order to elucidate the evolutionary causes of this differentiation, I need to investigate the reproductive success of *C. cathartica* pollinated by each bee species and indirect effects by the other plants.

**PS-23 Preliminary list of the inland water crab fauna of the Ryukyu Islands, with special remarks on the distributional patterns**

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Brchyrura [IR1] is a very diversified group and has adapted from

deep sea to terrestrial environments. Crabs are one of the most suitable targets for comparisons of biodiversity with other areas, as their diversified habitats make it possible to evaluate whole ecosystems. In the present study covering nine families of inland water crabs, we preliminarily listed the constituent species of the Ryukyu Islands and compared crab fauna and distributional patterns with adjacent areas.

A total of 118 species representing 48 genera was recorded in the Ryukyu Islands, of which three undescribed species were discovered and three species were newly recorded. This revealed that the number of species is more abundant in the Ryukyu Islands than in Taiwan (44 genera 112 species) or Kyushu (28 genera 42 species). Among the inland water crabs of Taiwan and Kyushu, 52 species (46% of Taiwanese crabs) and 32 species (76%) are shared with Ryukyu Islands.

The Ryukyu Islands and Taiwan show similar composition in the distributional pattern of constituent species. On the other hand, Kyushu differs from Ryukyu and Taiwan in having significantly fewer endemic species. The compositions of the distributional pattern of unshared species showed that wide-ranging species (East Africa to French Polynesia or Hawaii) and Indo-East Asian species occur more abundantly in the in Ryukyus. On the other hand, East Asian species and endemic species are more abundant in Taiwan. This may imply that the fauna of Ryukyu Islands is much affected by species with high dispersal ability, while in contrast, Taiwan is under the strong influence of continental fauna.

[IR1]Check spelling. This doesn't seem right.

**PS-24 Phylogeography of *Ophiorrhiza japonica* (Rubiaceae), a perennial herb without long-distance dispersal capacity, in the Ryukyu Archipelago and vicinity**

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To examine the connection between the genetic differentiation of plant lineage in the Ryukyu Archipelago and the geographical history of this region, a molecular phylogenetic study of *Ophiorrhiza* (Rubiaceae) occurring in Japan and Taiwan was conducted on the basis of sequence variations in the internal transcribed spacer (ITS) region of nuclear ribosomal DNA (nrDNA) and *atpB-rbcL* and *matK* regions of chloroplast DNA (cpDNA). *Ophiorrhiza japonica* var. *japonica* (including var. *acutiloba* and var. *tashiroi*, diploid with 2n=22) and *O. japonica* var. *amamiana* (tetraploid with 2n=44, endemic to the central Ryukyus) grow on inland humid forest floors or along mountain streams. Both taxa disperse seeds by raindrops (Nakanishi 2002) and their capacity for migration is probably low. Therefore, it is supposed that genetic divergence of this species is influenced by segmentation of the distribution range. In the molecular phylogenetic trees, both *O. japonica* var. *japonica* and *O. japonica* var. *amamiana* were monophyletic respectively. Based on cpDNA haplotypes, *O. japonica* var. *japonica* was divided into three major groups: (1) Kanto-southern Kyushu group, (2) southern Kyushu-Okinawajima Island group, and (3) Ishigakijima-Iriomotejima Islands group. The border of the cpDNA haplotypes in north of the central Ryukyus was located in southern Kyushu, not at Tokara Strait nor Osumi Strait. On the other hand, many synapomorphic nucleotide substitutions were recognized for the samples from Ishigakijima and Iriomotejima Islands in the southern Ryukyus, which are isolated from the central Ryukyus by Kerama Strait. The genetic divergence of *O. japonica* var. *japonica* does not necessarily coincide

with the paleogeographical hypothesis that assumes long isolation of the central Ryukyus from the northern Ryukyus by Tokara Strait. *O. japonica* var. *amamiana* clade was sister to *O. japonica* var. *japonica* from the mainland of Japan to Taiwan. It is likely that *O. japonica* var. *amamiana* did not originate from *O. japonica* var. *japonica* of the central Ryukyus by polyploidization but migrated into the central Ryukyus separately.

#### **PS-25 Some additions to the inland water brachyuran fauna of the Ryukyu Islands**

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We surveyed the inland water crab fauna of the Ryukyu Islands. As a result, 118 species representing 48 genera of nine families were recorded, of which three species were not yet described and the other two species were new to Japan. We described morphological features of these five species, compared them with allied congeners and noted their ecological and distributional characteristics.

Two of the three species not yet described and one of the two newly recorded species were discovered from riverbeds of brackish waters. They are: *Moguai* sp. (Camptandriidae), *Pyxidognathus granulatus* (Varunidae), and *Ilyograpsus* sp. (Grapsidae). *Moguai* sp. which had been referred to *M. elongatum* in the previous literature, however our specimens differ remarkably from this species. *Ilyograpsus* sp. was discovered from Iriomote Island and Okinawa Island in sympathy with another congener, *I. nodulosus*. The three species, *M. sp.*, *P. granulatus*, and *I. sp.*, share the small body size and this may be responsible for the delay in their discoveries.

Both of the remaining two species, *Orcovita* sp. (Varunidae) and *Sesarmoides bohollano* (Sesarmidae), were found in the hypogean habitat. *Orcovita* sp., in an anchialine pool within a limestone cave, has gracile ambulatory legs, canescent body color, and somewhat smaller eyes. These specific characteristics state imply that this crab has adapted considerably to the hypogean life style.

#### **PS-26 The hermit crabs (Crustacea: Decapoda: Anomura) from shallow waters around the Ryukyu Islands, southwestern Japan**

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Owing to a number of taxonomic and faunal studies recently conducted, our knowledge on the hermit crabs from shallow waters around the Ryukyu Islands has been increasing in these years. However, examination of specimens recently collected from coral reefs by skin or SCUBA divers revealed that they still contain a number of species new to the Japanese fauna or new to science. This study supplements fauna of the shallow water hermit crabs in the Ryukyu Islands, exclusive of the species inhabiting estuaries. Specimens available included at least seven undescribed species of the Paguridae: one *Catapagurus*, four *Pagurixus*, one *Pagurus*, and one *Pylopaguropsis*.

Of these, *Pagurixus* is the most speciose genus among the Paguridae in shallow waters of the tropical or subtropical Indo-West Pacific region. However, re-examination of the type specimens of some known species disclosed that detailed

redescriptions are needed to either supplement or correct the original descriptions.

Specimens of uncertain specific identities belonging to the pagurid genera, *Decaphyllus* and *Enneopagurus*, were obtained. The known species of the two genera have been recorded in rather deep water of 75-289 meters (*Decaphyllus*) or 356-552 meters (*Enneopagurus*), in Indonesia, New Guinea, and Japan (Kagoshima Bay).

The small sizes and cryptic habitats of the species of the Paguridae have hindered their collection in the past. Continuous studies on the shallow water hermit crabs of the Ryukyu Islands clearly show that the pagurids in the coral reefs are more diverse than indicated by the previous literature.

#### **PS-27 Molecular cloning and expression of melatonin receptors (Mel<sub>1a</sub> and Mel<sub>1c</sub>) in the brain of golden rabbitfish**

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Melatonin, the principal hormone of the pineal gland, plays a central role in the adjustment of endogenous and exogenous rhythms in all vertebrates. Although it is known that its physiological roles are exerted after binding with melatonin receptors, few studies have been conducted on the involvement of melatonin receptors in exertion of rhythms in fishes. In order to clarify the physiological roles of melatonin receptors in circadian and circalunar cycles, we cloned two melatonin receptor genes (Mel<sub>1a</sub> and Mel<sub>1c</sub>) using rapid amplification cDNA ends (RACE) from the brain or the retina of the golden rabbitfish, *Siganus guttatus*, and examined daily changes in their expression in multiple organs. The full-length coding region of Mel<sub>1a</sub> (2281 bp) and Mel<sub>1c</sub> (1750 bp) cDNA contains open reading frames of 354 and 353 amino acids, respectively. Both receptors encode a protein with a 64- to 71% homology to melatonin receptors which have been previously isolated in non-mammals. A reverse transcription-polymerase chain reaction (RT-PCR) technique revealed that among the organs tested, transcripts for melatonin receptors were present in all organs. When the expression of the 2 genes were checked at 12:00 and 24:00, clear fluctuations of expression occurred in the retina, pineal gland and pituitary. These results demonstrate that the major sites of integration of melatonin signal in the golden rabbitfish are the retina, the pineal gland and the pituitary which act as biological clocks to influence behavioral and endocrine responses in the golden rabbitfish.

*Key words:* Melatonin, Mel<sub>1a</sub> receptor, Mel<sub>1c</sub> receptor, Golden rabbitfish, RT-PCR, circadian rhythm

#### **PS-28 Genetic variation in two camaenid tree snails, *Amphidromus atricallosus* (Gould, 1843) and *A. inversus* (Müller, 1774), from Thailand and Malaysia**

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This study deals with the genetic variation in two camaenid tree snails, *Amphidromus atricallosus* (Gould, 1843) and *A. inversus* (Müller, 1774). Although widely distributed in Southeast Asia, these species are believed to have limited dispersal abilities. Samplings were taken from a total of 25 localities in Thailand and Malaysia. The tissues from the resultant specimens were subjected to horizontal starch gel electrophoresis to identify any geographic variation in composition and frequency of allozyme alleles in each species. A total of 22 allozyme loci were screened, of which 11 exhibited polymorphism. Samples of *Amphidromus inversus* tended to show lower heterozygosity (0-0.023) than *A. atricallosus* (0-0.201). In contrast, genetic heterogeneity was much higher among populations of *A. inversus* (mean  $F_{st}$  = 0.965) than in *A. atricallosus*. In the latter the heterogeneity was significantly higher among samples from the south ( $F_{st}$  = 0.551) than among samples from the northeastern areas ( $F_{st}$  = 0.144). The high  $F_{st}$  and low heterozygosity values in *A. inversus* suggest that this species recently experienced strong bottlenecks accompanied with distinct geographic subdivisions. The low  $F_{st}$  and high heterozygosity in the northeastern Thailand samples of *A. atricallosus* suggest frequent gene flows among populations in this region. The southern Thailand populations of *A. atricallosus*, showing a relatively high  $F_{st}$  and high heterozygosity, may have been involved in an extensive local fragmentation under various evolutionary forces.

### PS-29 Molecular cloning and expression of the period gene in the golden rabbitfish

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Circadian rhythms generated by an internal biological clock have been observed in a majority of living organisms. The main function of the circadian clock is to organize the time course of biochemical, physiological and behavioral processes by optimizing an organism's performance in anticipating changes in environmental conditions. In mammals, the molecular mechanism of circadian rhythm is an autoregulatory feedback loop in which a transcription factor (Clock and Bmal1) activates the transcription of Period and Cryptochrome genes. In teleost fish, melatonin is an important component of the circadian system. Melatonin controls the daily rhythm in circulating melatonin and function to photo adaptation. Melatonin rhythm generating systems consist of three elements: a photodetector, circadian clock, and melatonin synthesis machinery. As a first step to understanding the control of the melatonin rhythm by circadian clock genes in rabbitfish, *siganus guttatus*, we tried to clone Period genes (*per1* and *per2*) using rapid amplification cDNA ends (RACE) and analyzed their expression patterns in eye and brain tissues. The cloned full-length *per1* cDNA is approximately 4 kb in size and has 70% homology with zebrafish *per1*. The expression pattern of *per1* transcripts between day and night showed changes in the pineal and the pituitary glands; nighttime transcripts were detected at levels higher than in daytime. The result is consistent with the changes of melatonin concentration. The eye and other brain tissues showed no significant changes between daytime and nighttime. In contrast, the *per2* transcripts

showed higher levels of expression in daytime rather than nighttime; except for pineal and pituitary gland (not detected the transcripts in daytime and nighttime). These results suggest that period genes may play important roles in circadian rhythm, especially in melatonin changes in daily rhythm.

**Keywords:** Period gene, Circadian rhythm, Melatonin, Rabbitfish

### PS-30 Taxonomy of *Spratelloides gracilis* (Temminck & Schlegel, 1846) and allied species (Pisces: Clupeidae)

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The following four species of clupeid fish genus *Spratelloides* are currently recognized by most workers: *S. gracilis* (Temminck & Schlegel, 1846), *S. lewisi* Wongratana, 1983, *S. delicatulus* (Bennett, 1831), and *S. robustus* Ogilby, 1897. In the Ryukyu Islands, occurrence of an unidentified species of *Spratelloides* has been known in addition to the two well-known species (*S. gracilis* and *S. delicatulus*). This unidentified species is most similar to *S. gracilis* in having a bright silver band along the whole flank (not fading anteriorly) and discontinuous vertical striae on the scales, but is distinguishable from the latter in having fewer anal fin rays (10-13 vs. 12-14), pectoral fin rays (10-13 vs. 12-15), vertebrae (43-47 vs. 47-50) and lower gillrakers (20-24 vs. 30-39), and shorter head, snout and upper jaw. Two nominal species (*Clupea argyrotaeniata* Bleeker, 1849 from Indonesia and *Spratelloides atrofasciata* Schultz, 1943 from Samoa: specific epithet of the latter, *atrofasciatus* in the original description, is herein modified for correct ending in recognition of gender for the generic name as feminine) are currently treated as the junior synonyms of *S. gracilis*. Such taxonomic treatment is supported for *C. argyrotaeniata* by the fact that the count of lower gillrakers in its putative neotype (RMNH 7127) is 31 according to the literature description. In contrast, status of *S. atrofasciata* is uncertain because of the poor shape of its original description that lacks any direct comparison of this nominal species with *S. gracilis*, or detailed information regarding states of taxonomically important characters in its type specimen. Reexamination of the holotype of *S. atrofasciata* (USNM 115099) showed that the counts of vertebrae and lower gillrakers are 44 and 20, respectively. This indicates that *S. atrofasciata* is actually valid and corresponds to the unidentified *Spratelloides* found in the Ryukyu Islands.

### PS-31 Sex allocation in scleractinian corals

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The majority of scleractinian corals are hermaphroditic. The theory of sex allocation in hermaphroditic organisms predicts that if the fitness gain curve is saturated for a given sex, individuals with larger reproductive resources shall allocate more resources towards the opposite sex. When fertilization occurs in close proximity to the point of gamete release, the large number of sperm is generally in excess of the number needed to fertilize the eggs, and the fitness gain curve for sperm is expected to be saturated. Under this condition, the theory predicts that individuals that have larger reproductive resources would have relatively lower male/female allocation ratio. To examine this prediction, the sex allocation of *Goniastrea aspera* and *Favites chinensis* in Okinawa, and of



*Montipora capitata* in Hawaii was studied. Larger coral colonies were assumed to have more reproductive resource. A negative correlation between colony size and T/O ratio (testes to ovaries volume ratio) was detected in natural colonies of *F. chinensis*, but not in *G. aspera*. This appears to be explained by the different mode of reproduction: gamete spawning in *F. chinensis*, and planula brooding after gamete spawning in *G. aspera*. An experiment using small and large clonal fragments in *M. capitata* showed an opposite result to the prediction, i.e., higher T/O ratio in the large fragments than in the smaller ones. I would like to discuss the reason why the opposite result occurred in this species.

**PS-32 Intersexual dietary divergence in a Chinese soft-shelled turtle population, *Pelodiscus sinensis*, on Okinawajima Island, Ryukyu Archipelago, Japan**

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The Chinese soft-shelled turtle (*Pelodiscus sinensis*) exhibits the broadest geographic distribution among the East Asian turtles, ranging from boreal Russia to tropical Vietnam. Such a distribution makes this turtle an advantageous subject to examine the effects of varying climates on the ecological diversification in single evolutionary lineages. However, almost no studies have been carried out on ecological aspects of *P. sinensis*. To address this problem, we conducted a study on the feeding ecology of this turtle on Okinawajima Island. Sampling was made every month during the sampling period. As a result, the stomach contents of 76 specimens (38 males, 36 females and two immature turtles) were obtained. Of those specimens, 67 (86%) had at least one prey item in their stomachs. We obtained a total of 1,668 individual items that could be classified to 49 forms belonging to 33 families of 21 orders. The primary diets of males and females, while similar in autumn, dramatically differed in the other seasons of the year, and this is obviously due to the distinct seasonal dietary shift in males. From stomachs of males collected from spring to summer, we found various prey items, such as terrestrial insects, fishes, and frogs, whereas in autumn, aquatic shells were predominant among the contents of male stomachs. In contrast, the aquatic shells were remained the most dominant item in the female stomachs throughout the year. We suspect that the seasonally varying nutritional requirements are responsible for the seasonal dietary shift in males.

**PS-33 Oviposition and feeding stimulants for Okinawan Aristolochiaceae-feeding swallowtail butterflies: Pinitol and aristolochic acids from *Aristolochia Tagara* and *A. liukuensis***

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*Aristolochia liukuensis* (Japanese name: Ryukyu umanosuzukusa) and *A. Tagara* (Japanese name: koushun umanosuzukusa) belong to Aristolochiaceae plant and are widely distributed in Okinawa and the surrounding islands. Two Okinawan Papilionid butterflies, *Atrophaneura alcinous loochooana* (Japanese name: benimonageha) and *Pachilioptera aristolochiae* (Japanese name: jakou ageha) utilize *A. Tagara* and *A. liukuensis* as host plants. As a part of studies into the

chemical ecology between those butterflies and the host plants, I investigated the oviposition and feeding stimulants in the host plants for the butterflies. I now wish to describe the results. Fresh leaves of *A. Tagara* and *A. liukuensis* were collected at Okinawa, Ishigaki, Miyako, Amami Islands, and individually extracted with methanol to obtain oviposition and feeding stimulants. Each methanolic extract, except for the sample from Amami Island, was dissolved in water and then distributed with chloroform and 1-butanol, successively. The chloroform soluble fraction which showed strong activity for oviposition and feeding response with water soluble fraction, was subjected to several chromatographic separations yielding compounds 1-2 and the water soluble fraction gave 3-7. Adult butterflies were collected in the wild from Okinawa, Ishigaki, and Miyako Islands. All butterflies were reared in the laboratory under controlled temperatures and photoperiods. After a few days, bioassay was applied to only mature butterflies.

Compounds 1-7 were identified with aristolochic acids-I (1, AA-I), AA-II (2), *myo*-inositol (3), D-(+)-pinitol (4), glucose (5), fructose (6) and sucrose (7) respectively, by means of spectroscopic methods and by comparisons of these physical data with those of authentic samples. The combination of AAs and 6 lead to inducing strong stimulation on oviposition and feeding behavior of butterflies, but each compound alone was not effective enough to enhance behavior. The chemical characterization of *A. liukuensis* collected at Amami Islands is now under research.

**PS-34 The origin of the Crucian carp from the Ryukyu Islands and its ploidy**

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The hyper-variable segments of the mitochondrial D-loop of 432 Crucian carp from 32 open water areas throughout the Ryukyu Islands (Amami Oshima, Iheya, Izena, Okinawa, Tokashiki, Ishigaki, and Minami Daito islands) were amplified. Amplified products were sequenced directly and compared with sequences of Crucian carp collected in the waters of China, Russia, Kazakhstan, Taiwan, and mainland Japan. Crucian carps from each region were divided into different clusters and samples from the Ryukyu Islands except for Amami Oshima and Ishigaki islands, forming monophyletic groups showing endemic haplotypes. This finding means that the distribution of Crucian carp at in the middle Ryukyus comes as the result of natural events without artificial ingression. On the other hand, specimens of Amami Oshima island had haplotypes endemic in the Kyushu region of mainland Japan. At present, though it is difficult to determine the origin of the population in Amami Oshima (naturally or artificially), they appear to have a different origin from the populations found in the middle Ryukyus excepting Amami Oshima.

The appearance ratio of diploid and triploid varied in waters. Some waters had only diploid or triploid, and some had both. Generally, mature individuals belonging to groups consisting only of triploids reproduce gynogenetically, while those belonging to groups consist only of diploids reproduce sexually. It is thought that Crucian carp distributed in the Ryukyu Islands have both gynogenetic and sexual reproduction systems, but more research is needed to make clear the reproduction system.

**PS-35 The Late Pleistocene geoemydids (Reptilia: Testudines) from the Ryukyu Archipelago**

**Akio Takahashi**

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The Ryukyu Archipelago is a chain of continental islands located off the eastern coast of the Eurasian Continent. In terms of fauna, the archipelago is divided into three sections: (1) northern Ryukyus characterized by species common to the Japan Main Islands; (2) central Ryukyus characterized by a high ratio of endemic species, and (3) southern Ryukyus characterized by species common to Taiwan but with occasional slight divergences. Based on this geographic faunal pattern and stratigraphic data, it is assumed that isolation of the central Ryukyus from the surrounding land masses initiated as early as the Late Miocene or Plio-Pleistocene, whereas most of the northern and southern Ryukyus continued respectively to be connected to the Japan Main Islands and Taiwan until the Middle or Late Pleistocene. Extant three geoemydid turtle lineages, each having a representative in the Ryukyu Archipelago (*Geoemyda japonica*, *Cuora flavomarginata evelynae*, and *Mauremys mutica kami*), seemingly follow the above geohistorical scenario, because the central Ryukyu species, *G. japonica*, is obviously at a highly relict state, whereas the two southern Ryukyu subspecies, *C. f. evelynae* and *M. m. kami*, have conspecific populations in Taiwan. Recently, however, fossils of the genus *Cuora* were found from the latest Pleistocene deposits on Okinawajima and Kumejima Islands in the central Ryukyus. Fossils of what is generally referred to as the genus *Mauremys* were also found from deposits of comparable dates on Ishigakijima and Miyakojima Islands in the southern Ryukyus, as well as on Okinawajima Island. Phylogenetic relationships of these fossil species with their possible extant relatives (i.e., *C. f. evelynae* and *M. m. kami*, respectively) are of great biogeographical interest, because this may offer counterevidence towards the generalized phylogeographical pattern of the Ryukyu vertebrates noted above.

I examined some of those turtle fossils to infer their taxonomic status. Results strongly suggest that the fossil *Cuora* from Okinawajima Island differs from any extant species including *C. flavomarginata*. It also suggests that the fossils from Miyakojima Island in the southern Ryukyus, identified as the genus *Mauremys* by previous authors, actually represents a completely different lineage. The historical biogeographical significances of these findings are discussed.

**PS-36 Perception of light-dark conditions by the cultured pineal gland of a lunar-synchronized spawner, the golden rabbitfish**

○**Akihiro Takemura, Yoshiaki Nikaido, Satomi Ueda, Nanae Hiyakawa**

*Sesoko Station, Tropical Biosphere Research Center, University of the Ryukyus*

The golden rabbitfish, *Siganus guttatus*, is a definite lunar-synchronized spawner and repeats spawning around the first quarter moon during the spawning season. At present, it is not known how cues from the moon are recognized by this species and conveyed as endogenous rhythms. One possibility is that the fish can utilize changes in moonlight intensity for synchrony of reproductive activity. The aim of the present study was to evaluate moonlight perception in a cultured pineal gland, which is a photoreceptor organ and synthesizes the phototransducing hor-

none melatonin. We measured the melatonin secreted in the culture medium under various conditions using time-resolved fluoroimmunoassay. When the pineal gland was cultured under a cycle of 12 hours of light followed by 12 hours of darkness (LD 12:12), melatonin levels in the medium during conditions of darkness were higher than those found during the light conditions. Under continuous dark conditions (DD), melatonin seemed to increase during subjective night. Under continuous light conditions (LL), melatonin changed at low levels. The culture of the pineal gland under natural light conditions (from sunlight to moonlight) resulted in suppression of melatonin levels in the medium under moonlight conditions. After the pineal gland was exposed to moonlight conditions, melatonin levels decreased rapidly and significantly. These results suggest that the pineal gland of golden rabbitfish secretes melatonin according to light-dark cycles and can perceive 'brightness' of the night.

**PS-37 Actin-targeting drugs from Okinawan marine sponges and their action mechanisms**

**Junichi Tanaka**

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In this poster presentation, we will present the structures and biological activity of several actin-targeting drugs: kabiramide C (1), halichondramide (2), ulapualide A (3), swinholide A (4), and seragamide A (5).

With biochemical studies using fluorescent actin, three members of trisoxazole macrolides (1-3) were shown to bind G-actin irreversibly in two steps in addition to previously known monomer-sequestrations. Crystallographic investigation by Rayment group resulted in visualization of their binding sites. Thus, the side chain portion lies between a cleft between subdomain 1 and 3, while the macrolide portion recognizes the hydrophobic patch of the actin surface.

As reported by Spector, we also confirmed by fluorescent study that one molecule of swinholide A (4) sequestered two molecules of G-actin. Crystallographic study revealed this was true, but not in the manner proposed by Spector.

Our new entity of actin-targeting drug seragamide A (5) yielded actin-polymerization as for jaspamide and phalloidin.

We are also trying to utilize these drugs as biochemical reagents to specifically stain actin. By attaching fluorescent groups on halichondramide (2) and seragamide A (5) we were able to stain cultured cells as expected.

We will present the above findings.

**PS-38 A cytokinesis inhibitor from the sponge *Clathria mixta* and microbial fauna of Okinawan sponges containing bioactive molecules**

○**Chiaki Tanaka, Junichi Tanaka, Tatsuo Higa**

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A lipophilic extract of the Indonesian sponge *Clathria mixta* showed cytokinesis inhibition against NBT-T2 cells. Bioassay-guided separation provided an active principle which is now under further purification.

Coral reef sponges have been known to contain a diverse array of bioactive molecules with unique structures and modes of action. We collected a number of sponge specimens containing bioactive molecules and examined the microbial fauna on the surface and inside of the specimens using SEM.

In order to determine true producers of bioactive compounds,

we are examining their DNA contents in conjunction with a group under Prof. Sherman at the University of Michigan. We will present the current status of these collaborative projects.

### **PS-39 Studies on speciation of the Indo-Pacific *Echinometra***

**Tsuyoshi Uehara**

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De Blainville (1825) originally described two *Echinometra* species, *E. mathaei* and *E. oblonga* in the Indo-Pacific. Until 1984, although there was controversy as to the number of species those forms represent, all Indo-Pacific *Echinometra* are reported to as a single species *E. mathaei*, but differentiated between *E. mathaei mathaei* and *E. mathaei oblonga* (Mortensen, 1943; Clarke & Row, 1971). Gamete incompatibility among four Okinawan types of *E. mathaei* was the preface to studies on speciation of Indo-Pacific *Echinometra*. Since 1984, numerous studies concerning gamete morphologies, gamete incompatibility, energy metabolism and acrosome reaction in spermatozoa, embryology, karyotype, detailed adult morphologies, molecular structure and ecology, hybridization and F1 backcrosses of types from Okinawa, Kagoshima, Mauritius, Guam, Palau, Panama, Indonesia and Hawaii have shown that at least five species are represented in what used to be called *E. mathaei*. These are *E. sp. A* (Tsumajiro-nagauni), *E. mathaei* (Hon-nagauni), *E. sp. C* (Ryukyu-nagauni) and *E. oblonga* (Himekuro-nagauni) and *E. sp. E* (Kuro-nagauni). It was shown that there are disagreements among the phylogenetic trees of the five species based on the data of various characters. In other words, many visual characteristics have evolved at different rates from others. Five species are very closely related, since experimentally cross-fertilized eggs can be developed into adults having healthy gametes. Because of their close relationships, the *Echinometra* species in the Indo-Pacific provide an ideal system for studying "speciation" and "species concepts" for sea urchins.

### **PS-40 Two new cytotoxic diterpenes from a soft coral *Eleutherobia* sp.**

**Musri Musman<sup>1</sup>, Verawati Roring<sup>1</sup>, Junichi Tanaka<sup>1</sup>, Yehuda Benayahu<sup>2</sup>, Tatsuo Higa<sup>1</sup>**

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During our search for bioactive molecules from Okinawan waters, we found a soft coral *Eleutherobia* sp., whose lipophilic extracts demonstrated cytotoxic properties against NBT-T2 cells. Chromatographic separation of the extract resulted in the isolation of two new compounds 1 and 2. Using spectroscopic studies, their structures were elucidated to be diterpenes of the xenicane class (1) and of the eleutherobin class (2). We will present the structures of these compounds and their activities.

## Ecosystem Diversity

### PE-1 A long-term observation of metabolic changes of coral incubated in a continuous-flow complete-mixing experimental system

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We conducted a long-term coral incubation experiment using a continuous-flow complete-mixing (CFCM) experimental system to study the impacts of high seawater temperatures on coral metabolism. The CFCM system is a newly designed experimental system, which combines a chamber incubation system and a flow-through system. The CFCM experimental system is designed to minimize the stress a closed experimental system may impose on the coral, but at the same time enables us to obtain quantitative data on the coral's daily metabolic changes under well controlled experimental conditions (e.g., light conditions, seawater temperatures, nutrient levels, etc.). Our incubation experiment lasted 25 days and showed that when seawater temperature was maintained at 27°C, the metabolisms of the coral were relatively stable, indicating that the coral did not suffer significant stress by being in a glass incubation tank. We have observed clear increase of pH and decrease of total alkalinity during the light periods (when a light of a fixed intensity was shone), and clear decrease of pH and increase of total alkalinity during dark conditions. When we gradually raised seawater temperatures from 27°C to 31°C, we observed sudden large releases of ammonium ion, increase of respiration rates, and decrease of calcification rates. We propose to use the CFCM experimental system as a tool for quantitative investigation of coral metabolic changes. With the CFCM experimental system, we can further investigate impacts of various environmental factors such as nutrient levels, UV-light intensity levels and seawater temperatures on different coral species under the same controlled experimental conditions.

### PE-2 Sensory biodiversity of coral reef fish larvae for senses used in searching for their species' settlement habitats

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The senses (touching, seeing, feeling, tasting, hearing) keep an organism informed of the conditions of its surrounding environment. Small movements of a few centimetres or over thousands of kilometres enables the best choices to be made in search of food, sexual partner or settlement habitat. Some coral reef fish larvae have swimming abilities sufficient to control their patterns of ocean dispersion and return to adult habitats. However, these swimming abilities will be useful only if larvae can detect habitats suitable for settlement, because it is unlikely that successful settlement is solely a matter of chance. Perhaps one of the greatest challenges facing the majority of marine reef organisms with larval stages that potentially disperse and develop in offshore waters, is how to locate the rela-

tively rare patches of coral reef habitat on which they can settle and ultimately reside as adults. The answer must lie partly in the sensory modalities of fishes.

Larval recognition of potential settlement habitats can be based on detection of conspecifics already settled and/or of characteristics of coral habitats using visual (e.g. shape of a coral colony), chemical (e.g. an odour of anemone) and mechanical (e.g. vibratory or sound waves of fish) cues. The present study worked to estimate, by experiments in aquaria, the sensory modalities of coral reef fish larvae for the senses used to search for species-appropriate settlement habitats.

For this study, larvae captured with crest nets at Moorea Island (French Polynesia), were introduced into experimental tanks to allow separate testing of each type of cue (visual, chemical or mechanical). Among the 18 species studied, 13 chose their settlement habitat due to the presence of conspecifics and not based on characteristics of coral habitat. Five species did not move toward their settlement habitat (e.g. *Scorpaenodes parvipinnis*, *Apogon novemfasciatus*). Among the different sensory cues tested, two species (*Parupeneus barberinus* and *Ctenochaetus striatus*) used three types of cues, six (e.g. *Myripristis pralinia*, *Naso unicornis*) used two types, and five species (e.g. *Chrysiptera leucopoma*, *Pomacentrus pavo*) used only a single type. These results demonstrate that many coral reef fish larvae might use sensory cues for effective habitat selection at settlement, and have the ability to discriminate species-specific sensory cues. This study highlights the biodiversity in the sensory world of coral reef fish in the search of settlement habitats.

### PE-3 Coral recruitment and growth in Fiji after the coral bleaching in 2000 and 2002

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Mass Coral bleaching in Fiji in 2000 and 2002 began in mid-February but became progressively widespread into April. Bleaching varied geographically and was most severe in the south in 2000 but affected the northern areas above the two main islands in 2002 coinciding with SST HotSpot durations and reaching temperatures greater than 30°C. Bleaching was more severe in offshore areas characterized by clear water and was less severe inshore where the best recruitment and growth has now been observed. Some offshore areas have been very slow to re-establish living coral cover.

HotSpots in 2000 began to appear around Fiji February 5 peaking in the period of February 1-April 1. In 2002, elevated temperatures began in late January and were 2.5+deg above average to the south of the group by February 16. Elevated warm water conditions continued until mid-April.

In situ temperature loggers at 10m depth since 1996 are providing valuable in situ comparisons with satellite derived SSTs. Three peaks over 30°C in early 2000 and one in 2002 correlate with the bleaching events. Bleaching occurs in Fiji during the onset of the La Nina phase of the El Nino/Southern Oscillation (ENSO).

Though prone to bleaching, recent images of a new colonies (*Acropora*, *Millepora*, *Pocillopora*, *Sarcophyton sp.* and *Lobophytum sp.*) now proliferate on the reef flat. Surviving colonies of the soft coral *Lobophytum sp.* grow over the sclerite base ("spiculite") laid down by the colony before the bleaching.

#### PE-4 Effects of light environment and tree density on crown forms and stem volume in a subtropical evergreen broad-leaf forest in the northern part of Okinawa island

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To quantify the direct and indirect effects of light environment and tree density on the morphology of individual trees, we evaluated the relationship between canopy openness and tree density vs. crown traits and stem volume in reference to tree size in a subtropical evergreen broad-leaved forest in the northern part of Okinawa Island, southwest Japan.

The dependence on light environment and tree density on the crown form differed with species and tree height. The crown form of smaller sized trees (<4m height) did not change with canopy openness or tree density, except for *Syzygium buxifolium* which showed an increase in crown area with decrease in canopy openness. For larger trees (>4m height), the two canopy species (*Daphniphyllum glaucescens* ssp. *teijsmannii* and *Eraecarpus japonicus*) increased stem volume along with increase in canopy openness. Two subcanopy species of *S. buxifolium* and *Rapania neriifolia* decreased crown area with increase in tree density. Differences in the morphological response to the environment can contribute to structural complexity of the forest stand.

#### PE-5 Organic and inorganic carbon productions of a larger foraminifera-microalgal endosymbiont association estimated by the alkalinity anomaly technique

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Larger foraminifera are one of the most prolific carbonate (inorganic carbon) producers in coral reef environments in the present day as well as in the geological past. They are also host to endosymbiotic microalgae. Thus, a larger foraminifera-microalgal endosymbiont association may act as a primary producer in the coral reef ecosystem, releasing organic matters (organic carbon) to the environment. However, its role in the carbon cycle of the coral reef ecosystem is unquantified. In this study, we first applied the alkalinity anomaly technique to estimate organic and inorganic carbon production rates (OP and IP, respectively) of two ecologically important species of larger foraminifera, *Baculogypsina sphaerulata* (Parker and Jones) and *Calcarina gaudichaudii* d'Orbigny, which harbor diatom endosymbionts, and live abundantly on reef flats in the western Pacific. Approximately 1,000 live individuals of >1.0 mm in diameter for each species were isolated and placed onto a 100 μm mesh in a clear airtight container (ca. 250 ml), which contains natural filtered seawater. The cultures were kept in an incubator maintained at 27°C, subjected to different levels of light intensities (0, 80, 165, 500, and 1000 μmol photons/m<sup>2</sup>/s). pH, DO and total alkalinity in the continuous mixing of seawater were measured before and after incubation for about three hours. OP and IP rates were calculated by the alkalinity anomaly technique. In light conditions, OP rates of both species increased exponentially with increasing light intensity due to the photosynthesis of algal endosymbionts. In the dark, the OP rates

were lower than zero due to the respiration of host and symbionts. IP rates of *B. sphaerulata* and *C. gaudichaudii* were higher at light intensities of 80 and 165 μmol photons/m<sup>2</sup>/s respectively than at other light intensities. In the dark, IP rates were at nearly zero for both species, indicating that both calcification and dissolution did not occur in darkness. These results clearly show that the larger foraminifera-microalgal endosymbiont association acts as a primary producer in the coral reef ecosystem, and that calcification is enhanced in a relatively low light intensity, suggesting that the mechanism for calcification in larger foraminifera is different from that in reef corals, which show enhanced calcification with increasing light intensity.

#### PE-6 Willingness to pay for coral reefs in the Kerama Islands

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The purpose of this study is to estimate the value of the coral reefs in the Kerama Islands in Okinawa. This research was conducted at Zamami village in March 2003 and the respondents to the questionnaires were passengers on ships between Naha and Zamami. The questionnaire is based on the Contingent Valuation Method (CVM), which is used to evaluate the non-market value of environmental attributes. In this questionnaire, we used the double-bounded two-choice approach in order to determine the passengers' willingness to pay (WTP) for the conservation of Kerama's coral reefs.

Coral reefs are very important resources for ecology and the economy, especially in island areas like Okinawa. They have several public functions that provide material and spiritual benefits to human beings including fisheries, ocean biodiversity value, purification of the ocean, underwater views, coastal protection, resources for tourism and recreation and so on. Recently, however, drastic changes in certain environmental conditions have caused considerable damage to coral reefs. We should recognize the necessity of protecting coral reefs in order to preserve their public functions; hence, we attempted to define the value of conservation of the Kerama coral reefs.

We estimated the passengers' WTP for the conservation of the Kerama coral reefs using the Turnbull method, a type of survival analysis. The results revealed that the average WTP per person was 3,994 yen and the total annual value for passengers traveling to Zamami village was 367,473,347 yen.

#### PE-7 A 5-year systematic study of community metabolism transitions using water flow box model in the coral reef ecosystem of Sesoko Island

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As a consequence of global warming and environmental changes, coral reefs throughout the world have faced serious threats of degradation. To understand the present conditions of coral reefs and predict future changes, it is important to systematically study the relationship between global environmental changes and the community metabolism in coral reefs. Coral reef ecosystems are characterized by the high carbon productivity released in photosynthesis-respiration and calcification. These parameters represent the conditions of reef environment and must be continued to be monitored in the future.

In this study, we introduced a model to estimate the daily organic and inorganic carbon production rates of coral reefs in a natural water flow system.

The study area for the model calculations was the fringing reef in front of Sesoko Station, Tropical Biosphere Research Center, Okinawa, Japan. About 20 boxes were affixed in this area to monitor the variations in oxygen and alkalinity concentrations at the end point of the water flow, where we continuously monitor water quality using a multi-parameter sensor (YSI-6600 or YSI-6920). Dissolved oxygen (DO), pH, temperature, salinity and water current were measured every 15 minutes. Total alkalinity was measured by Gran titration. These concentrations were affected by benthic community metabolism. The degree of the impact depended on the irradiance and depth, and residence time of reef water. Photosynthesis-irradiance and calcification-irradiance curves for the community metabolism were determined to fit the reconstructed variations from the model with monitored real variations of oxygen and alkalinity.

Community metabolism calculated by the water flow model was within the ranges of other coral reefs. Net primary production was lower than zero during summer due to high respiration rates of benthic metabolism. This suggests that the Sesoko coral reef ecosystem has significant seasonal variations that are autotrophic during winter and heterotrophic in summer. The results indicated the water flow box model could be successfully used in estimating community metabolisms in natural reef environments and applied to long-term monitoring of reef ecosystems.

#### **PE-8 Effect of development activities on the aquatic ecosystem in Iriomote Island**

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##### **Introduction**

Iriomote (about 284km<sup>2</sup>) is the second largest island in the Okinawa Islands. The population is about 1,800 and the density is only 6.3 persons/km<sup>2</sup> (vis a vis a density of 880 persons/km<sup>2</sup> on Okinawa Island). On the Island, there is a river with the largest basin in the Okinawa Islands. Near the river mouth, a hotel accommodating 1,000 guests was constructed. Therefore, the effect by the people to environments is recently concerned. The first objective of the present study is to determine the chemical compositions of water samples from the river (the Urauchi River) and sandy beach (Tsukigahama). Analyzed species were nutrients (NH<sub>4</sub>N, NO<sub>2</sub>N, PO<sub>4</sub>P), major cations (Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>) and major anions (Cl<sup>-</sup>, NO<sub>3</sub><sup>-</sup>, SO<sub>4</sub><sup>2-</sup>). The basic chemical data are useful to understand aquatic ecosystem in Iriomote Island for the future. The second objective is to discuss the relationship between change in aquatic ecosystem and biodiversity at islands.

##### **Results and Discussion**

The results of the analysis of river waters showed that average concentrations of NH<sub>4</sub>N and PO<sub>4</sub>P in the Urauchi River are 0.78μg/L and 1.39μg/L, respectively. These values are lower

than those in the river waters of urban area of Okinawa Island. It means that the Urauchi River is not polluted by human activities at present. The concentration of dissolved oxygen (DO) in the interstitial water decreased with increasing depth of the holes at Tsukigahama sandy beach. Furthermore, DO decreased with increasing A250 substances indicating the concentration of organic matter. It suggests that DO was consumed to oxidize the organic matter by the decomposer etc.

#### **PE-9 The chemical composition of Okinawan red soil and its effects on pH, aluminum and dissolved silica when encountering seawater**

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The contamination of river and coastal water by red soil is a major environmental concern in the Okinawan islands. We conducted laboratory and field studies to examine the effects of red soil when encountering seawater. We determined chemical composition of bulk soils (<2mm) and seawater. Different weights (3, 10 and 20 g) of soil were mixed with 100 mL of seawaters, 10, 100, and 1000 times diluted seawater by milli-Q water. The mixture was then shaken for 4 hours with a shaking machine to obtain extracts. Water samples were collected from four red soil-impacted estuaries along salinity gradient from the river to the sea. They were analyzed for pH, EC, salinity, dissolved SiO<sub>2</sub> and Al<sup>3+</sup> concentrations.

The pH values of soils were 4.65 and 5.06, SiO<sub>2</sub> composed of 75.9 and 64.6% and Al<sub>2</sub>O<sub>3</sub> contained 10.7 and 17.8% for two examined red soils. The pH value of the seawater sample from the Cape Zampa was 8.23 with salinity of 34.4 g/kg. Aluminum concentration in the seawater was 0.262 mg/L while dissolved silica concentrations were 0.388 mg/L. After interaction of the red soil and the seawater, pH decreased to the lowest value of 4.02 in seawater extracts. Aluminum concentrations rose with the soil/solution ratio at 3-20 g/100 mL, showing the ranges of 5.66-36.6 mg/L in seawater extracts, 3.08-12.4 mg/L in one-tenth seawater extracts, 0.860-1.39 mg/L in one-hundredth seawater extracts and 0.45-0.760 mg/L in one-thousandth seawater extracts. Dissolved SiO<sub>2</sub> increased with the soil solution ratio at 3-20g/100 mL to the highest concentration of 10.3 mg/L in seawater extracts followed by one-tenth seawater extracts of 9.81 mg/L, one-hundredth seawater extracts of 6.06 mg/L and 3.88 mg/L in one-thousandth seawater extracts. The salinity strongly influences Al in comparison to dissolved SiO<sub>2</sub> but pH of the extract solutions contributed its influence on both parameters. The field observations at affected red soil estuaries revealed that Al concentration was <0.180 mg/L<sup>-1</sup> in river water and the highest value was 0.474 mg/L in seawater. The highest value of dissolved SiO<sub>2</sub> concentration was 17.8 mg/L in the river water and the lowest of 0.128 mg/L in the seawater. The Al concentration could be largely attributed to metal exchange while the release of dissolved SiO<sub>2</sub> could be due to chemical weathering of the silicates.

#### **PE-10 Ecology of *Vibrio parahaemolyticus* at estuaries and fishing ports with special reference to the effects of tide and rainfall**

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*Vibrio parahaemolyticus* is an estuarine bacterium which can cause gastroenteritis in human after consumption of contaminated seafood. After grown at estuaries, the bacterium is suspected to flow out to sea and contaminate fishes and shellfishes at fishing ports. Although the conditions enabling the bacterium to flow into the ports have not yet been fully explained, the level of the bacterium in port water seems to be related to the tidal cycle and rainfall. We have surveyed the bacterium around two estuaries; Sada Estuary in which Etomo Fishing Port is located, and Murotsu Estuary where Murotsu Fishing Port is adjacent. The level of the bacterium in the water of Etomo Port fluctuated along with the tidal variation, though it was consistently lower than that found in water at the lower part of Sada Estuary in any tidal phase. In water from the mouth of Murotsu Port close to the estuary, the level of the bacterium was highest at the mid-point of the rising tide, while it was high not on the rising tide but at full tide at the innermost station of Murotsu Port. The level of the bacterium in waters at the two fishing ports was found to be correlated to the amount of rainfall in the preceding 10 days at the upper streams of the estuaries ( $R_s=0.640$ ,  $p<0.01$ ). From this evidence, it was concluded that in these regions entry of the bacterium to fishing ports is enhanced by an increased water level due to tidal variation and rainfall.

#### **PE-11 Benthic microbial diversity and sedimentary fatty acids in subtropical mangrove forest - Manko tidal flat, Okinawa**

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The sediment burrows of various macro invertebrates have been recognized as sites of high microbial activity in aquatic ecosystems. A field study was conducted to determine how burrowing activities of grapsid crab (*Helice formosensis*) affect microbial community structure of the burrow walls and the fate of sedimentary fatty acids in mangrove estuary.

The results showed that sediments surrounding the grapsid crab burrow walls had a relative higher concentration of bacteria marker branched odd-carbon number ( $\Sigma C_{15}$  and  $C_{17}$ ), common in gram positive bacteria,  $C_{18:1\omega7}$  (vaccenic acids) and  $C_{16:1\omega7}$  that indicate sulfate reducing bacteria. Fungi biomass ( $\Sigma C_{18:2\omega6}$ ) decreased with increase in the burrow depth. The concentration of fungi to bacteria ratio changed between categories indicating a change in the microbial community structure. Using ANOVA test, the significant effects of *Helice formosensis* burrows on the fatty acids composition (PUFAs, MUFAs, and EFAs) were revealed between the burrow wall sediments and surface sediments ( $p<0.01$ ). The patterns of essential fatty acid ( $\omega3$  and  $\omega6$ ) varied between burrow wall sediments of the opening shaft, at the bottom and the surface sediments (ANOVA test,  $p < 0.05$  and  $< 0.01$  respectively). Where detected the percentage concentration of  $\omega3$  were greater in the surface sediments ( $8.8\% \pm 1.9$ , mean value  $\pm$  SE) and  $\omega6$  were higher in the burrow wall sediments ( $4.2\% \pm 0.3$ , mean value  $\pm$  SE). We conclude that burrowing activities by grapsid crab has significant impacts on microbiology and fatty acids distribution of intertidal sediments.

#### **PE-12 Distribution of benthic animals and feeding activity of migratory birds on two tidal flats, Okinawa, Japan.**

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Many migratory birds utilize Okinawan tidal flats as resting and/or feeding sites during their migration periods and to pass the winter season. To clarify the characteristics of these tidal flats and their roles in providing for migratory birds, surveys on the distribution of benthic animals and feeding behavior of migratory birds were started with reference to the biodiversity in ecosystem level. Two tidal flats, Yone, in the south part of Okinawa island, and Nagura on Ishigaki Island, were selected. General surveys were conducted at Yone in November 2004 and at Nagura January 2005. A bird census was also conducted in January 2005 at the Yone tidal flat. Four gastropod, three bivalve, ten crustacean and other polychaete species were collected in Yone, while nine gastropod, five bivalve, seven crustacean and other polychaete species were collected in Nagura. Gastropods were abundant at both study sites, but polychaetes and crustaceans, the primary food items of migratory birds, were more abundant in Yone than in Nagura. Abundant but small sized gastropods such as *Batillaria* sp. *Clypeomorus* sp. *clypeomorus irrorata*, were found in Nagura vis a vis the populations in Yone. Although the species composition of crustaceans was almost same at both sites, fiddler crabs showed wider distribution in Yone, and were restricted to the surroundings of the mangrove estuary in Nagura. The kentish plover *Charadrius alexandrinus* (シロチドリ) feeding on the soldier crabs *Mictyris brevidactylus* (ミナミコメツキガニ) and the ocy-podid *Scopimera globosa* (コメツキガニ) appeared abundantly in both areas. The American golden plover *Pluvialis dominica* (ムナグロ) feeding on polychaetes appeared in Yone in January, but did not appear at Nagura. Differences in species composition of benthic animals and feeding habits of migratory birds between these tidal flats were recognized. Research on the feeding ecology of bird species focusing on the biogeochemical cycle might contribute to analyzing the biodiversity of tidal flat ecosystems.

#### **PE-13 Effects of water quality on biodiversity in Suva lagoon, Fiji**

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Suva Lagoon encompasses Laucala Bay and Suva Harbour and is enclosed by a barrier reef. At least five rivers discharge into the Lagoon transporting sediments, nutrients and trace metals. The Lagoon also receives effluent from a sewage plant that services the city of Suva (pop. approx. 150,000) and which constitutes another source of nutrients. Industries located in the catchment area of Laucala Bay are an additional albeit minor source of trace metals in the Lagoon. The ecological impacts of these pollutants are minimized by tidal dilution coupled with relatively small nutrient loads. The biodiversity of the reef/lagoon system is thus maintained and is evident from the flora and fauna on the reef and adjacent ecosystems such as the lagoon and mangroves. Such biodiversity supports important subsistence and artisanal fisheries. Recent observations of ecosystem changes associated with resource depletion have highlighted the need for management of the Lagoon by the local communities, the private as well as Government sectors. An integrated, interdisciplinary approach is required to understand and manage the lagoon system. A symposium is planned in March 2005 to bring together the following stakeholders: Traditional fishing rights owners; Local users; Oceanographers;

Geologists; Marine biologists; Botanists; Town planners; Fisheries officers; Researchers and Non-governmental organizations. The intent of the meeting is to determine how research activities can be incorporated into the management of the Lagoon so that decisions regarding current and future developments in the immediate catchment of the Lagoon are not based solely on economic, social and political issues. A strategic plan for further research will also be developed.

**PE-14 Comparison of the home ranges of the Iriomote cat *Prionailurus bengalensis iriomotensis* between different habitats**

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The Iriomote cat, *Prionailurus bengalensis iriomotensis*, is a small felid inhabiting only Iriomote-jima Island in the Ryukyu Archipelago, Japan. The coastal lowlands (< 50 m a.s.l.) are known to be suitable habitat for this species because of the presence of a higher diversity and abundance of prey animals in the various lowland forests, such as mangrove forests and other swampy forests. Previous studies on behavioral patterns and social relationships showed the male home ranges to be larger than the females and encompassed several females in the habitat. At the same time, the cats are found also in less suitable habitats on the island. However, it is still not clear what the behavioral patterns and social relationships are for the male and female cats in less suitable habitats. The home range size and its seasonal variations were studied by radio-trackings and camera-traps on Iriomote-jima Island. The study was conducted in the Shirahama area in the western part of the island. This area has smaller suitable habitats for the Iriomote cats as compared with other parts of the island. Two cats, a male and a female, were confirmed the residents in this area. We compared the results of these observations with some in the suitable habitat (Funaura) and discussed what factors affected the male home range size. We found that the home range size of a male cat in Shirahama area was half as large as that in Funaura area, though it showed a similar seasonal fluctuation to range size in Funaura. On the other hand, the home range size for a female was similar between two areas. These results provided empirical confirmation of the influence of the number and distribution of females on the male home range size in a solitary felid.

**PE-15 Functional morphology of planula larvae involved in coral dispersal: A scanning electron microscope study**

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We observed the ultrastructure of the cilia of planula from *Acropora digitifera*, *Oulastrea crispate*, *Favites chinensis*, *Goniastrea aspera*, *Pseudosiderastrea tayamai*, *Stylophora pistillata* and *Pocillopora damicornis* using scanning electron microscopy. The planula was cultured in aquariums after spawning/release and prepared each day for microscopy. We identified standard, straight shape cilia (St-cilia), and a uniquely shaped cilia with a spatula like tip (Sp-cilia) on the planula. St-cilia were observed on the surface of the planula of *A. digitifera*, *O. crispate*, *F. chinensis*, and *G. aspera*. Sp-cilia

were observed on the surface of the planula of *S. pistillata*, *P. damicornis*, *P. tayamai* and the front face (settlement side) of mature planula of *A. digitifera*. St-cilia were decreased on the surface of the metamorphosed planula of *A. digitifera*. *S. pistillata* and *P. damicornis* are brooders, and *P. tayamai* is a surface brooder (Nakano unpublished data). On the other hand, *A. digitifera*, *O. crispate*, *F. chinensis* and *G. aspera* are spawners. We suggest that St-cilia is used for swimming/buoyant and Sp-cilia is used for settlement.

**PE-16 The role of the Ryukyu flying-fox as a seed dispersal agent on Okinawa-jima Island, Japan**

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Fruit bats are known as an important seed dispersal agent in tropical ecosystems. Particularly, flying-foxes are likely to play a keystone role in maintenance and regeneration of a forest community of an island, where large frugivores animals are few (Rainey et al. 1995). However, the interaction between the Ryukyu flying-fox and plants in Okinawa-jima Island is not fully known. Here, we examined the role of the Ryukyu flying-fox as a seed dispersal agent by radio-tracking and seed germination experiments. The Ryukyu flying-fox fed on sixteen native plant species, and the seeds of the fruits they consumed were dropped beneath fruiting tree in the form of pellets and feces. However, fruits of three plant species, *Ficus harlandii* var. *kotoensis*, *Actinidia rufa* and *Terminalia catappa*, were sometimes carried to feeding roosts near the feeding tree in the mouths of the bats. Ten plant species with small-seeded fruits (mostly *Ficus*) were transported to other sites through the digestive tract. We estimated that the potential dispersal distance of seeds would be about three kilometers from the source tree, based on night home range size. The maximum dispersal distance of mouth-carried fruits were 126m for *Terminalia catappa* and the mean dispersal distance for ingested seeds was estimated as 397 m based on flight speed, digestion time and foraging patterns. We estimated the number of dispersed seeds for some species to evaluate the extent of the Ryukyu flying-fox as a seed dispersal agent. Additionally, germination rates of some plants were higher when the flying-fox ate the fruits. These results suggested that the Ryukyu flying-fox has a positive effect on at least 16 native plants in Okinawa-jima Island, and that they play a unique role as a long-distance disperser of *Ficus*, as limited agents of plants with large fruits.

**PE-17 Geochemical study on the Taketomi submarine hot spring - influences on the coral reef ecosystem**

○Takuroh Noguchi<sup>1</sup>, Nobuya Sano<sup>1</sup>, Naoto Taira<sup>1</sup>, Mayo Agarie<sup>2</sup>, Tamotsu Oomori<sup>2</sup>, Hiroyuki Yamamoto<sup>3</sup> and research group members  
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The Taketomi submarine hot spring is located at the bottom of the Sekisei Lagoon (approximately 20m below the ocean surface), 500m east of Taketomi Island (Fig.1). Hydrothermal fluid and gas vent from this hot spring field widely distributed with fringing coral reefs. Chemosynthetic organisms in the



shallow hot spring field can find habitats similar to those of deep sea hydrothermal vent fields. Our interest was in the relationship between the chemosynthetic and photosynthetic ecosystems. First, we observed the physical and chemical conditions at the Taketomi hot spring, and discussed the detection of chemosynthetic activities.

Divers with SCUBA gear collected hot spring vent fluid samples in two-liter polyethylene bags using a hand-pump. Sediment samples were collected using an M-BARI core sampler (L 30cm × Dia 10cm) for pore water analyses. Gaseous samples were collected in 100mL glass bottles. The pH was determined by ion-selective electrode and the alkalinity was calculated by Gran titration with a Radiometer auto-titrator. Sulfate concentration and major cations were respectively identified using ion chromatography and atomic absorption spectrometry. Dissolved silicates and ammonium concentrations were determined using spectrophotometric methods. The chloride analysis was done with silver nitrate titration. Gaseous compositions were determined by GC-TCD methods.

Fig.2 shows the SO<sub>4</sub> Mg relationship in the hot spring water. It is evident that the SO<sub>4</sub> concentrations in sea water lie below the mixing line of sea water and hydrothermal waters. This suggests SO<sub>4</sub> reduction due to microorganisms activity in the Taketomi submarine hot spring field. Habitats of both photosynthetic and chemosynthetic organisms were found in close proximity in the Sekisei Lagoon.

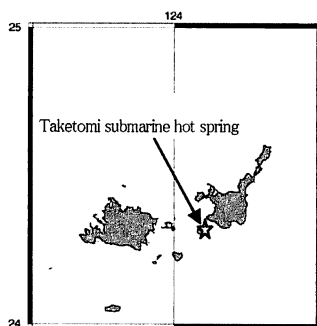


Fig.1 Map of Taketomi-jima hot spring

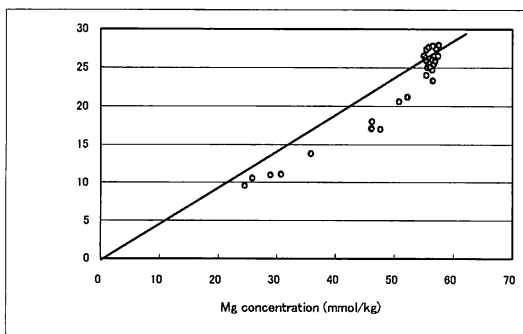


Fig.2 Relationship between Mg and SO<sub>4</sub> concentration

### PE-18 Industrial structure and wastes in Okinawa

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Our vision is to establish a low-environmental-burden-based society or a sustainable-recycling-type society.

The amount of industrial wastes in Okinawa was 3,729,000 T/year in 1999, representing a 13.4 % decrease relative to 1994 (4,309,000 T/year). The reason for the reduction of industrial

wastes in 1999 was due to the decrease in construction scrap and debris, livestock feces from agriculture and in animal-plant scraps from manufacturing. Farming, public utility, construction and manufacturing generated 97 % of the amount of wastes in 1999. 89% of the wastes consisted of livestock feces, debris, sludge and animal-plant wastes.

Local demand on Okinawan industry generated 2,704,000 T/year of industrial wastes that marked 72.5% of the wastes in 1999. Overseas demand from exportation generated 1,025,000 T/year that marked 27.5% of the total amount of industrial wastes in 1999.

The ratio of sludge to animal-plant wastes marked 55.0% and 60.3% respectively in the private consumption expenditure. The ratio of livestock feces marked 47.1% in export.

Three major sources of industrial wastes in Okinawa were livestock feces, sludge and animal-plant wastes. In addition, the intra-firm demand induced more than 70% of the amount of industrial wastes. Therefore, it is necessary for Okinawa to make efforts at reducing sludge and animal-plant wastes with private consumption expenditure, and at reducing the generation and the discharge of livestock feces with export.

### PE-19 Long-term monitoring of carbonate system at a coral reef

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Assisted by : Nobuya Sano, Keiko Tsuha, Tomihiko Higuchi, Taeko Kuwano, Miyo Ikeda, Mayo Agarie

The carbonate system in coral reefs is a typical biogeochemical process which is controlled by photosynthesis/respiration, calcification and hence relates to global atmospheric CO<sub>2</sub> change. Global environmental change, including global warming and the degradation of biodiversity, has been recognized worldwide in recent decades. In order to understand the present state of the environment and coral reef ecosystems, long term monitoring of the carbonate system has been carried out for ca. 5 years at a fringing reef at Sesoko Island, and additional comparative studies have been carried at Reunion Island and Sekisei Lagoon.

Proximity studies of historical change in marine environments show that the effects of anthropogenic activity such as fossil fuel combustion on marine carbonate chemistry have been remarkable in the last 40 years. This phenomenon is consistent with other phenomena such as increases in seawater temperature, shrinking of ice sheets at polar regions and extinction of biological species and ecosystems in various parts of the world. It is possible that further drastic changes in the global environment may occur in the future. Toxic substances derived from anthropogenic activity also affect marine ecosystems and biodiversity. The scope of the above topics will be presented.

### PE-20 Biogeographical variation in distribution of mangrove gastropods (Gastropoda: Potamididae)

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Potamidid mollusks, in particular those of genus Terebralia,

form a major group of mangrove gastropods that feed on mangrove leaves. In certain mangals, the potamidid mollusks outnumber the decapod crustaceans. This study was carried out in order to address regional trends in distribution and diversity of the potamidid gastropods along Okinawa main island and in mangroves of the Yaeyama Islands. We measured shell size and densities of the mangrove gastropods along transects located perpendicular to the shorelines for the mangroves of Oura Bay and Okukubi (Okinawa Island), Nagura (Ishigaki Island) and Urauchi (Iriomote Island). Only two species of the potamidid genus *Terebralia* and about five species of the genus *Cerithidea* were found during this investigation. The species of potamidid genus *Terebralia* were: *Terebralia sulcata*, found only in the Okinawa main island mangroves, and *Terebralia palustris*, found only in the Yaeyama Island mangroves. The absence of *T. sulcata* in Ishigaki and Iriomote Islands, despite its wide geographic distribution in the low latitudes, suggests that there is a regional discontinuity in the distribution of this species. While the absence of *Terebralia palustris* in Okinawa main island mangroves and their presence in the Yaeyama Islands is consistent with previous findings, the northernmost limit of this species distribution occurs in the Yaeyama Island mangroves. Results further indicate that there is a difference in composition of fatty acids in the tissues of the two species, as was indicated by an unusual high percentages of arachidonic acid (20:4 $\omega$ 6), 18:0 anteiso and 28:0 in the tissues of *T. palustris*. These acids were not found in *T. sulcata* tissues suggesting differences in lipid utilization and storage strategies between the two gastropod species, which can potentially influence competitive interactions among species. However, in terms of diet, both species were found to utilize similar food resources, i.e., macroalgae detritus, diatoms and mangrove detritus as indicated by the respective fatty acid biomarkers in their tissues.

**PE-21 The dietary sources of the Japanese oyster, *Crassostrea gigas* in a subtropical mangrove-estuary**

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Fatty acid markers have been successfully used to discriminate the food sources of marine organisms and also as trophic markers to trace the trophic transfers within the food webs in marine ecosystems. Japanese oysters, *Crassostrea gigas*, were examined using fatty acids in order to identify the dietary components of the oyster from different tidal zones and sites in a mangrove-estuary and also to assess in situ environmental conditions. Samples were collected at the beginning of winter, near the end of November 2004, in the Manko estuary, Okinawa. The result showed that dietary components of the Japanese oyster were dominated by diatoms (20:5 $\omega$ 3 fatty acid) and dinoflagellates (18:4 $\omega$ 3 and 22:6 $\omega$ 3 fatty acids). The other dietary components were bacteria (iso and anteiso 15:0 and 17:0, and 18:1 $\omega$ 7 fatty acids), green micro algae (18:3 $\omega$ 3 fatty acid), zooplankton (20:1 $\omega$ 9 fatty acid), vascular plants (26:0, 28:0 and 30:0 fatty acids) and possibility domestic waste matter (18:1 $\omega$ 9 and 18:2 $\omega$ 6 fatty acids).

The contributions of diatoms were slightly higher in the oyster from the upper tidal zone. This trend also held for dinoflagellates from two (2) stations, except from one station relatively close to the sea. This result indicates that tidal cycles have an important role for food supply in estuarine ecosystem. On the

other hand, bacteria and domestic waste markers were detected in higher concentrations at the stations where closer to the point sources of domestic waste effluents. Furthermore, vascular plant markers were also detected in considerable amounts in oysters collected either from lower or upper zones at all sites, suggesting that mangrove materials were transported from the original sites more than one kilometer to seaward and had an important role in the diets of oyster as well.

**PE-22 The coral gall crab *Pseudohapalocarcinus ransoni* (Decapoda: Cryptochiridae): population structure and effects of the ectoparasite *Carcinione platypleura* (Isopoda:Bopyridae) infestation.**

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Coral reefs are considered to be one of the most diverse and productive ecosystems on the planet. Symbiotic interactions between organisms play a very important role in coral reef ecology.

The gall crab, *Pseudohapalocarcinus ransoni*, is an obligate symbiont of living hermatypic coral *Pavona frondifera*. Each gall is occupied by a single crab, which eats coral mucus and tissue. Galls are formed from deformation of coral as the result of mechanical and chemical abrasion by crabs. Females never leave their galls and it is assumed that males visit female gall for mating. A total of 357 crabs (281 females, 68 males and 8 unidentified) were collected between August 2003 and October 2004. Positive and significant relationships between crab carapace width (males: 1.15-2.41 mm; females: 1.25- 4.18 mm) and length ( $R=0.925$ ,  $P<0.0001$ ) were observed. Male crabs and immature females tend to show light body colors (light yellow to light brown), small body size, and occupied early gall stages, while mature females tend to present dark body color (brown to black), large body size and late gall stages. The bopyrid parasite, *Carcinione platypleura*, was observed in the gill chamber of crabs with infestation rate of 10.64% for the whole population. Nearly all the infested female crabs (96.55%) did not carry eggs, strongly suggesting that infestation negatively affected the reproduction capabilities of the host crab (parasite castration). Bopyrid parasites were found as heterosexual pairs occupying the whole gill chamber and deforming the carapace of their host crabs. Parasite dwarf males were usually attached on the post-ventral part of ovigerous female body by process of uropod. Infested crabs showed dark body color, which could suggest that color changing is also an effect caused by parasitism.

**PE-23 Soil runoff analyses using wepp model in Okinawa, Japan**

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In Okinawa, red soil runoff from developed lands and consequent ocean pollution soil has been a major issue since the 1970's. To better counter the problem, the Okinawa Prefectural Government established an ordinance in 1994 that regulates the upper limit of suspended sediment concentrations (SSC) from development projects. Since passage of the Ordinance, there has been some reduction in the soil runoff from areas of development projects, but there has been no significant reduction in the

runoff from farmlands. In the near future, farmlands may also fall under the Ordinance. The Okinawa Prefecture Department of Agriculture must move to introduce some soil runoff prevention measures. For such countermeasures to be properly formulated, we must be able to estimate their efficiency in the early planning stages. To this end, the USLE (Universal Soil Loss Equation) has been in use but this model has shortcomings in its inability to calculate short-term runoff or runoff on the scale of entire watersheds. The USDA (United States Department of Agriculture) which originally developed the USLE also developed the WEPP (Water Erosion Prediction Project) model in 1985. In the WEPP model, watersheds are composed of slopes and channels. Changes of vegetation and soil condition on the slopes are calculated to yield seasonal changes in erosion or soil loss. Calculations for the channel include considerations of detachment and deposition in consonance with flow rates. Therefore, the WEPP model can be used to calculate spatial and temporal soil erosion processes. However, there are few examples of applications of the WEPP model in monsoon Asia. Then, WEPP model have been applied to an agricultural watershed in Okinawa and affectivity of the model is checked. In the application, parameters on soil and farming schedules were drawn from observations made in the watershed and the other parameters were defaults shown in the WEPP model. As the results demonstrate, the WEPP model can be used to calculate changes of crop and soil conditions clearly. Calculated soil erosion was almost the same as actual observed parameters in the event scale.

#### **PE-24 Flux of atmospheric deposition into forest ecosystems in Okinawa's subtropical islands**

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It has been noted that atmospheric depositions such as anthropogenic and acid depositions may accelerate forest degradation or acidification of various ecosystems. The investigations into atmospheric depositions such as aerosol are extremely important to clarify the influence of depositions on the forest. The purpose of this study is to estimate the amount of substances deposited from the atmosphere into the ecosystems, and to clarify the substance capture potential of the forest ecosystem. Rainwater and aerosol samples were simultaneously collected every seven (7) days±1day from November 2004 to January 2005 from the rooftop of the Faculty of Science building (outside the forest area) and from the experimental forest station (inside the forest) of the University of the Ryukyus. Samples were analyzed for major cations (Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, NH<sub>4</sub><sup>+</sup>), major anions (Cl<sup>-</sup>, NO<sub>3</sub><sup>-</sup>, SO<sub>4</sub><sup>2-</sup>) and heavy metals. Total Mass (TM) concentration of aerosol in the forest was lower than that outside the forest. Especially, concentrations of NO<sub>3</sub><sup>-</sup> and nss-SO<sub>4</sub><sup>2-</sup> (regarded as the cause of acid rain) of aerosol inside the forest were respectively about 48% and 73% of concentrations outside the forest. However, the concentration of nss-SO<sub>4</sub><sup>2-</sup> in rainwater inside the forest was three times as high as that outside the forest. There was no trend in NO<sub>3</sub><sup>-</sup> concentration in rainwater between outside and inside the forest. It is supposed that NO<sub>3</sub><sup>-</sup> is more easily captured on the forest canopy and more difficult to release to the surroundings. These results suggest that the forest ecosystem captures substances as dry deposition such as aerosol, and releases those to the surroundings in rainfall.

#### **PE-25 Forest structure and photosynthetic characteristics of mangrove species along the Okukubi River, Okinawa Island**

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For our study of matter production in the mangrove forest along the Okukubi River, we conducted a tree census, soil assay and diurnal photosynthesis measurement. Five belt-transect plots (5 m wide) perpendicular to the stream were established. The diurnal changes in net photosynthesis of sun leaves were measured for *Bruguiera gymnorrhiza* (L.) Lamk. and *Kandelia candel* (L.) Druce during the period from June to August 2004 in four plots. The number of individuals in *B. gymnorrhiza*, *K. candel* and *Rhizophora stylosa* Griff. were respectively 750, 289 and 7 within the total study area of 900 m<sup>2</sup>. The maximum tree height decreased from the landward to the seaward zone in each species. The relative basal area (RBA) of *K. candel* increased from the landward to the seaward zone. Soil salinity and pH increased from the landward to the seaward zone. Although the maximum net photosynthesis decreased from the landward to the seaward zone in each species, the maximum net photosynthesis was higher in *K. candel* than in *B. gymnorrhiza* in each plot. On the other hand, the light compensation point was higher in *B. gymnorrhiza* than in *K. candel*. The leaf and soil nitrogen concentration decreased from the landward to the seaward zone. The development process of vegetation near the seaward zone might be depressed, because the utilization of nitrogen in photosynthetic process was limited there. Therefore, the pioneer species, *K. candel*, showed a higher value in RBA near the seaward zone than the landward zone.

#### **PE-26 Sediment transportation by the deposit feeding sea cucumbers *Holothuria leucospilota* and *H. atra* in coral reefs: bioturbation or biopurification?**

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In coral reefs, sea cucumbers play an important role as deposit feeders in maintaining a beautiful coral reef environment. A huge amount of sand or fine particles is transported onto the coral reefs, and the amount of organic material present in the sand particles is reduced by sea cucumbers feeding activity. In the reefs of Okinawa, Japan, *Holothuria leucospilota* is the most common sea cucumber species followed by *H. atra*. We studied their sediment reworking process from the viewpoint of their feeding activity. Comparison of the amount of organic material in the sediments, consumed as food, and in the feces, showed a decrease of carbon and nitrogen contents for both species. *H. atra* consumed significantly smaller particles than *H. leucospilota*. For example, in *H. leucospilota*, granule gravels of 2-4 mm in diameter were frequently found in the fecal pellets together with sandy particles. In a coral reef of ca. 15 ha, 11,500 individuals (0.08 inds./m<sup>2</sup>) of *H. leucospilota* were counted and the amount of sediment consumed by this population was estimated to be 92 kg/day or 33.6 tons/yr. When sediment containing 10.6 mg C/g or 1.5 mg N/g was fed to the sea cucumber population, these organic materials were assimilated and feces containing 2.5 mg C/g or 0.6 mg N/g was produced.

In this coral reef, 272.4 kg of carbon and 31.3 kg of nitrogen were removed from the sediment surface over the course of a year, thus helping to maintain a beautiful and a healthy coral reef environment. Since sea cucumbers remove significant amounts of organic matter in the coral sediments through their feeding activity, we regard this as biopurification rather than bioturbation. The fatty acid composition of the tissue was also analyzed to identify their food sources.

### PE-27 Behavioral analysis of small felids using a miniaturized acceleration data logger

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Many forest-dwelling mammalian species, e.g. the Iriomote cat, *Prionailurus bengalensis iriomotensis*, are difficult to observe because of their nocturnal habits and thick-covered habitat. In order to remedy this, we adopted a new technique using a motion detector (acceleration data-logger) for monitoring the activity and behaviour of free-ranging domestic cat, *Felis catus*, and the Iriomote cat in captivity to test its efficiency.

From the experimental trial for a domestic cat, a total of 3,615 min of acceleration was measured along the longitudinal body axis. The cat's behaviour was also filmed for 113 min, used to correlate the logger's signals with the cat's behaviour. An acceleration data-logger attached to the cat with a collar recorded the acceleration signals which were influenced by both the gravitational acceleration resulting from the body posture and the dynamic acceleration resulting from the dynamic activity of the cat. By applying spectral analysis based on a fast Fourier Transform to acceleration signals, body postures and some of the dynamic activity of the cat such as drinking, eating, and several paces of travelling, were efficiently determined.

From the experimental trial on an Iriomote cat in captivity, 48 hours of the cat's behaviour was recorded by the data-logger and was monitored by remote control. From the results, the cat's behaviours were determined by the acceleration data; the time allocation for particular movements was assessed by the second. The cat was inactive in 81.8% of the 48-h observation as sitting or lying. While the cat was active, the cat spent 49.9% of the time in walking, 25.5% for grooming, 15.4% for eating food, 1.18% for drinking water, and 8.0% engaged in other miscellaneous activities.

The present study shows that acceleration data-loggers represent a useful and effective system for accurately assessing various activities and behaviours of free-ranging small felids. We expect application to felids in wild in the next step in our research.

### PE-28 Population analysis of marine viruses in coastal surface waters of the Okinawa Islands

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Marine viruses are the most abundant genetic particles in the ocean, the majority of which are assumed to be phages. Although the viruses certainly play important roles in microbial gene transfer and global carbon cycle, their biodiversity and evolutionary relation with the other viruses in non-marine biomes have been virtually unknown. Because tropical and subtropical coastal environments consist of distinct sub-environments such

as coral reefs and seagrass beds and are susceptible to the impacts of human activities, it is expected that the diversity of marine viruses there is high and the population is dynamic. In the present study, we analyzed the populations of marine viruses in the surface water of coastal marine environments of the Okinawa islands (reefs, seagrass beds and mangrove-contiguous areas) as well as polluted ones (a port area and a bay area into which red soil erodes). Marine viruses were extracted from ~1mL seawater using a membrane filter with a pore size of 200 nm, immobilized onto a glass substrate or an anodic alumina membrane after being stained with fluorescent dyes, and then directly enumerated using a fluorescence microscope and a cooled CCD camera. Similarly, the size distributions of marine microbes were analyzed. The population of marine viruses in the reef area is low,  $4.3 \times 10^5$  /mL. In the seagrass bed and near-mangrove areas, the populations are high and almost the same,  $\sim 5 \times 10^6$  /mL. The population in the highly polluted port area is  $1 \times 10^7$  /mL at most, which is comparable with a typical abundance in surface seawater. The population ratio of RNA virus to DNA virus was estimated to be 0.4 by using the difference in nucleic acid recognition between fluorescent dyes. Marine microbes in the same type of marine environment show the same size distribution. However, the size distribution in the bay area contaminated by red soil is unique and the population of marine viruses rather high, suggesting that nutrients and/or microbes involved in the soil changed the balance between host and virus.

We will also present microfluidic flow cytometry to achieve rapid population analysis of marine viruses under development.

### PE-29 Phytomass and growth process of mangrove *Kandelia candel* (L.) Druce trees in the Manko Wetland, Okinawa Island - Characteristics of dwarf-shaped trees

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The mangrove *Kandelia candel* (L.) Druce is the most dominant species in the Manko Wetland. At the study site, two types of tree shapes were observed, i.e. upright-shaped trees are distributed near the land, areas, whereas dwarf-shaped trees are distributed near the sea. The  $D_{0.1}/H$  ( $D_{0.1}$ , stem diameter at a height of  $H/10$ ;  $H$ , tree height) ratio of the dwarf-shaped trees was five times as large as that of the upright-shaped trees. The leaf nitrogen content on a leaf area basis significantly increased from the land to the sea. However, the net photosynthesis on a leaf area basis decreased from the land to the sea. It was concluded that the dwarf-shaped trees have lower nitrogen use efficiency in photosynthetic process.

The top/root ratio of the dwarf-shaped trees was the same as that of other mangrove forests. The leaf weight, branch weight and leaf area of the dwarf-shaped trees showed strong simple allometric relationships when plotted against  $D_B^2 H$  and  $D_B^3$  ( $D_B$ , stem diameter at a height of clear bole length). The stem weight, root weight and aboveground weight showed strong simple allometric relationships when plotted against  $D_{0.1}^2 H$  and  $D_{0.1}^3$ . These simple allometric relationships may be valid only for the dwarf-shaped trees. In the stem analysis, stem volume has not yet been saturated. This means that the *K. candel* dwarf-shaped trees are still in the developmental stage. The annual rings of the *K. candel* dwarf-shaped trees hypertrophied against the tidal current.

### PE-30 Ecological linkages between coral reef and deep sea: site survey data of Sekisei coral reef to Kuroshima knoll

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The seas surrounding the Ryukyu archipelago harbour typical photosynthetic ecosystems based on coral reef and planktonic production. The geological setting of the Ryukyu archipelago has led to the development of prominent chemosynthetic ecosystems. The Okinawa Trough (depth: 1200-2000 m), back-archipelago basin spreading between the Ryukyu archipelago and continental shelf of the East China Sea, contains active hydrothermal vent zones. The thermal fluid contains methane, hydrogen sulfide and carbon dioxide. The Kuroshima Knoll (depth 630 m) located on the south side of the Sekisei coral reef of Ishigaki Island in the southern Ryukyu archipelago, is a methane seep zone with associated bubbling. The chemicals emitted from these vents and seeps sustain copious deep-sea biota based on chemosynthesis. Such geological activity also appears within the Sekisei coral reef. A submarine hot spring and methane bubbling site at a 13-20 meter depth within a depression in the reef creates a peculiar ecotone between phototrophic and chemotrophic biota. The reef and deep-sea have quite different bathymetric topography and community structure. The deep-sea biota around the vents and seeps depend on chemosynthesis and the coral reef biota depend on photosynthesis. These two types of material and energy supply may combine to play an important role in the whole marine ecosystem. The linkages between reef and deep remain obscure due to a dearth of data from the deep. To begin this research project, the first step was construction of an oceanogeographic map that combined a fine scale topographic map and oceanographic profile information. Combining such high-resolution bathymetric profiles with photographs and high resolution imagery from a newly-developed Deep-sea HDTV video camera provides much information on the biota and their habitats. This information will greatly improve the quality of the site survey data in order to define targets and plan in-depth scientific studies of the Ryukyu archipelago marine ecosystem.

### PE-31 Recesson of Ryukyu limestone coastal cliffs

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Recesson of Ryukyu limestone coastal cliffs was studied from a dynamic viewpoint. Several rocky coasts of Okinawa-jima, Miyako-jima and Kikai-jima were selected as investigation areas. Field measurements were carried out to determine cliff height, the depth of notches, the length of fallen blocks and longitudinal wave velocity in rock mass. Cliff height, notch depth and block length ranged from 2 to 23m, 2 to 9m and 4 to 13m, respectively. Physical and mechanical properties of the cliff material were measured in laboratory tests. Rock strength drastically decreases as size of specimen increases i.e., the rock strength of Ryukyu limestone is subject to scale effect. Combining the laboratory results of strength reduction due to scale effect and the results of the longitudinal wave velocity obtained from both laboratory and field tests, an equation was derived for estimating the rock mass strength.

Cliff instability was analyzed using a beam analysis model. Stability analysis using the strength of rock mass ignoring scale effect could not fully explain cliff failures. A new equation for stability analysis was derived that takes into consideration tension cracks behind cliffs. Substituting the estimated rock mass strength and unit weight of cliff material into the equation, we obtain the critical stability lines in cliff height and critical notch depth relationships. Results show that the data from the present cliff profile were plotted in the stable area, while the length and breadth of fallen blocks were plotted on the critical lines. This leads to the conclusion that beam failure analysis, which takes account of rock mass strength and tension cracks, would provide a useful framework for predicting the critical notch depth of Ryukyu limestone cliffs.

### PE-32 Aboveground net primary production in a pioneer mangrove *Kandelia candel* (L.) Druce at Manko Wetland, Okinawa Island

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Aboveground net primary production in a mangrove stand of *Kandelia candel* (L.) Druce was quantified using the summation method. Aboveground biomass and its increment were estimated using allometric relationships and stem analysis. Direct grazing of mangrove leaves by insects and crabs usually accounts for a small proportion of leaf production. Therefore, the grazing effect in this study was not quantified. Litterfall in a 12 months period was taken into account. Stem volume without bark of harvested trees showed a strong allometric relationship with  $D_{0.1}^2H$  ( $D_{0.1}$ , stem diameter at a height of  $H/10$ ;  $H$ , tree height) ( $R^2 = 0.924$ ). Annual stem volume increment without bark also showed a strong allometric relationship with  $D_{0.1}^2H$  ( $R^{20} = 0.860$ ). Branch biomass increment per tree was indirectly estimated using the allometric coefficients from the relationships of branch weight, stem volume, stem volume increment and stem weight to  $D_{0.1}^2H$ . Litterfall rate was lower in winter (December and January) and showed a peak in the end of summer (August and September). Total annual litterfall was estimated as 10.68 Mg ha<sup>-1</sup> yr<sup>-1</sup> in which 68.26% was contributed by the leaves. Using stem analysis, stem and branch biomass increments were estimated as 11.13 Mg ha<sup>-1</sup> yr<sup>-1</sup> and 8.41 Mg ha<sup>-1</sup> yr<sup>-1</sup>, respectively. Using field measurements of  $D_{0.1}$  and  $H$  increments over a one-year interval the stem and branch biomass increments were estimated as 12.08 Mg ha<sup>-1</sup> yr<sup>-1</sup> and 9.46 Mg ha<sup>-1</sup> yr<sup>-1</sup>, respectively. Aboveground net primary production in the *K. candel* stand was 30.22 Mg ha<sup>-1</sup> yr<sup>-1</sup> and 32.22 Mg ha<sup>-1</sup> yr<sup>-1</sup> using the stem analysis and field measurements, respectively. These results suggest that the mangrove *K. candel* is a fast growing and highly productive species.

### PE-33 Requirement of water flow for sustainable growth of the pocilloporid corals during high temperature period

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Reef-building corals are threatened worldwide by mass-scale coral bleaching episodes that are pronounced in high sea surface temperature (SST) conditions. Although water-flow has been suggested to be a mitigating factor for bleaching, long-term effects of flow-mediated bleaching suppression are yet not

fully understood. In order to investigate flow effects, we monitored the corals, *Pocillopora damicornis* and *Stylophora pistillata* (Pocilloporidae), grown for 20 months in experimental outdoor flumes with the flow rates of 20 cm s<sup>-1</sup> (flow) and <3 cm s<sup>-1</sup> (still). Although bleaching was observed under high SST conditions, both species showed a shorter period or entirely no visible bleaching under the flow conditions. A better colony growth was confirmed in the flow conditions, whereas significant growth suppression and higher mortality were observed in still conditions. We conclude that water-flow is an essential environmental factor for the corals *P. damicornis* and *S. pistillata* especially under high SST conditions.