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The Use of DNA Molecular Techniques for Mangroves Studies

Peter SAENGER¹⁾ and Maurizio ROSSETTO²⁾

Abstract : Biological and ecological studies of mangroves have provided limited insights in relation to the evolutionary processes in mangroves. However, with the development of molecular genetic techniques, a much better understanding of such evolutionary processes can be obtained. This review briefly introduces some of the molecular techniques available to researchers and then summarises their main applications. From an evolutionary perspective, genetics can be used in a hierarchical approach in order to appraise differences between species (phylogenetics), groups of populations (phylogeography) and individuals (population genetics). Some of the main theoretical and practical issues concerning each of these research areas will be briefly reviewed and illustrated with mangrove examples.

Key words : mangrove, phylogenetics, phylogeography, population genetics, polymorphism

1. Introduction

Biological and ecological studies of mangroves have provided limited insights in relation to the evolutionary processes in mangroves, i.e. the development, diversification or extinction of species. Understanding the evolutionary patterns and relationships between economically important species and their close relatives has long been recognised as a management and conservation priority. With the development of molecular genetic techniques, a much better understanding of such evolutionary processes can be obtained. These techniques are particularly useful to solve systematic and evolutionary questions as well as clarifying the extent and distribution of genetic variation within single taxa. As DNA technologies become more informative and less complex, an increasing number of laboratories are able to access them and benefit from their use.

Regardless of which specific molecular technique is used, the naturally occurring genetic variation at the population level is a useful tool for many applications, including conservation studies. Genetic investigations are useful in inferring the ancestral history of a group of organisms and, in some cases, merely the amount of genetic variation can be revealing. For instance, quan-

tifying and qualifying the overall genetic pool available to a species can be useful for assessing its evolutionary potential. Genetic polymorphisms can also be useful for investigating genetic relationships among subpopulations and identify possible links between geographic distribution and environmental adaptation. The principle is that alleles (different forms of the same gene) are shared among subpopulations because of migration, and as a result similarities in allele frequencies can be used to estimate migration rates. The absence of shared alleles between populations can either indicate the lack of movement between population or the presence of selective forces. From a more practical application, genetic polymorphisms can also be used to study mating systems in plants. Such studies rely on changes in genotypic frequencies to differentiate between random mating and self-fertilising individuals or populations.

The purpose of this review is to briefly introduce some of the molecular techniques available to researchers and then summarise their main applications. From an evolutionary perspective, genetics can be used in a hierarchical approach in order to appraise differences between species (phylogenetics), groups of populations (phylogeography) and individuals (popula-

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tion genetics). Some of the main theoretical and practical issues concerning each of these research areas will be briefly reviewed and illustrated with mangrove examples.

2. Molecular Techniques: the Options Available

Recent advances in molecular biology, especially the development of polymerase chain reaction (PCR) technology, have produced new and powerful techniques useful for the screening, characterisation and evaluation of genetic diversity. Some techniques can be more suited to specific purposes than others and when selecting the right tool for a genetic investigation it is important to consider various issues such as informativeness, simplicity, cost and ease of interpretation.

Molecular techniques can be broadly classified within three main groups: non-PCR based, targeted PCR based (including sequencing), and arbitrary PCR based. Some of these are briefly described below.

Non-PCR technology is principally limited to allozyme and restriction fragment length polymorphism (RFLP) analyses. The former methodology relies on protein rather than DNA polymorphisms. Small structural mutations within selected enzyme systems modify their electric charge and, as a consequence, their electrophoretic mobility across a gel. Allozyme analysis has been in use for many years, thus providing a large amount of comparative data to practitioners. Despite being simple and universally applicable, the use of this technique is not as widespread as in earlier years, mainly because of its low polymorphism and its susceptibility to environmental factors. RFLP was the first method capable of detecting polymorphisms at the sequence level. RFLP analysis involves digesting DNA with restriction enzymes and separating the resulting fragments by gel electrophoresis. A number of short universal sequences can then be used as probes (usually radioactively labeled) and, in the course of an hybridisation reaction, recognise and attach to complementary DNA fragments. Mutation at restriction sites will result in differential banding patterns which are highly reproducible, co-dominant (enabling the distinction between heterozygotes and homozygotes) and fairly polymorphic.

This technique is informative but fairly complex and lengthy.

Targeted PCR relies on the design of primers targeting specific regions of the genome. Amplified products from different individuals can be compared on a gel, where differences in size represent various mutational

events. An increasingly popular PCR-based technique is microsatellite analysis. Microsatellites, or simple sequence repeats (SSRs), are highly variable sites randomly dispersed across the DNA. Being co-dominantly inherited, highly reproducible and simple to use, SSRs are considered as one of the most useful population genetic tools. The main drawback of this technology is that it is not universal, and the identification of useful priming sites within the species of interest can be a complex matter. Nevertheless, the development of advanced enrichment techniques and transferability of primers across closely related species, make SSRs increasingly applicable to a variety of studies. Also fitting within the category of targeted PCR is DNA sequencing. When sequencing, PCR primers are selected to amplify a specific area of interest within the genome and each single nucleotide within the fragment obtained can be identified by way of a conventional sequencing reaction. Within plants there are three genomes and therefore three potential sources of sequence information: nuclear DNA (nDNA), chloroplast DNA (cpDNA) and mitochondrial DNA (mtDNA). These three genomes are inherited in different ways, have dissimilar mutation rates and thus can provide complementary evolutionary data. Finally, arbitrary priming PCR describes techniques such as random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP).

These procedures are based on the use of single, 'arbitrary' (i.e. not specific to a specific region of the genome) primers, they require no prior genomic knowledge and can therefore be applied to any organism. RAPD relies on the use of a single primers nine to 20 bp long. The multiple products obtained by a low stringency PCR reaction can be visualised on a conventional gel. RAPD is simple, cheap and produces highly polymorphic markers; however its robustness can be questionable if consistent protocols are not followed. AFLP is an intermediate technique between RAPD and RFLP and relies on restriction digestion followed by two selective PCR amplifications. Despite being more complex and expensive than RAPD, AFLP is highly polymorphic and more reproducible.

Unfortunately, both systems produce dominant markers and therefore are less informative for certain applications.

3. Investigating Phylogenies

3.1 Theory and Practice

The highest hierarchical level of molecular investigation is the unraveling of evolutionary relationships between species, genera and even families. Traditionally this type of phylogenetic surveys rely on conserved DNA regions that have diverged in a quantifiable manner across species, but for which no within-species diversity can be expected. As a result, sampling can be limited to a single accession per taxon being investigated. The optimal DNA region to be sequenced in phylogenetic studies must be conserved enough to enable the development of universal primers (i.e. primers that will work across all species), yet variable enough to discriminate between different taxa. When these conventional sequencing regions cannot distinguish among species, then molecular tools more appropriate for phylogeographic and population studies are likely to provide the necessary information. Conversely if these same regions differentiate between populations, then one might be dealing with cryptic species.

Ideally and when possible, the most informative phylogenetic approach is to combine sequence data from different genomes that evolve differently and follow distinct inheritance models. For instance, unlike nuclear DNA, chloroplast DNA (cpDNA) is uniparentally inherited (maternally inherited in most Angiosperms) and does not undergo recombination. Furthermore, evolutionary rates can vary within single genomes, so for instance genes (i.e. regions coding for a specific function) are more conserved than non-coding region. The former regions might be less informative as they have fewer variable sites, especially at the lower hierarchical levels, yet it might be impossible to design primers for the latter regions because of the lack of universally conserved sites. It is therefore essential to carefully select the most appropriate sequencing region through a preliminary investigation before launching into a phylogenetic study. Sampling strategies will depend on the questions being asked and outgroups need to be selected carefully in order to positively contribute to the final findings. Some of the most commonly used regions for DNA sequencing studies are cpDNA genes such as *rbcL*, *atpB* and *malk* and nuclear ribosomal DNA genes such as 18S to name a few. Frequently used non-coding regions include the *trnL* intron and the intergenic spacer between *trnL-F* from cpDNA and the internal trans-

cribed spacers (ITS1 and ITS 2) from nuclear ribosomal DNA.

3.2 Mangrove Studies

The fact that most mangrove taxa exist within larger, otherwise terrestrial clades supports the premise that taxa occupying the mangrove habitat arose independently from terrestrial clades in at least 15 plant families (Ricklefs and Latham, 1993). The fossil distributions of species occupying mangrove habitat support the generalisation that present distributions include their area of origin. When sister taxa (in this case species belonging to the same mangrove clade) are restricted to the same geographical region, one can surmise either that the ancestral taxon had similar distribution or that the ranges of the sister taxa have changed in parallel. The latter scenario is unlikely for mangroves whose sister taxa now occupy a substantially different terrestrial habitat. Parsimony would lead us to conclude that the common geographical distribution of sister mangrove taxa includes, in a very general sense, the place of the origin of the derived clade. Based on such cladistic analyses of mangroves and close, non-mangrove relatives, Ricklefs and Latham (1993) concluded that the distribution of Indo-West Pacific endemic mangroves originated in the Indo-West Pacific.

A similar conclusion was reached concerning the cladistic analysis of the Rhizophoraceae based on the morphological data of Juncosa and Tomlinson (1988).

Molecular data can provide a better understanding of the phylogeny of mangroves, as their characteristic features are phenotypically stable and not prone to environmental change. For example, on the basis of morphological and anatomical features, Rhizophoraceae has been included in the order Myrtales (Takhtajan 1980) or assigned to its own separate order Rhizophorales (Thorne 1992). However, a parsimony analysis of the DNA sequence data from the chloroplast gene *rbcL* (Conti *et al.* 1996), strongly suggests that Rhizophoraceae does not belong to the Myrtales. Rather, the analysis places Rhizophoraceae as part of a rosoid clade including Euphorbiaceae, Humiriaceae and Malpighiaceae. A more recent compilation of the phylogeny of the eudicots by Savolainen *et al.* (2000) reviewed the phylogeny of 589 *rbcL* sequences from 308 families including some of those containing mangrove species.

The study revealed that Rhizophoraceae and Erythroxylaceae form a distinct subclade within the Malpighiales (this order also includes Humiaceae, Euphor-

biaceae and Salicaceae to name a few). The Malpighiales are a sister group to the Oxalidales which includes families such as Elaeocarpaceae and Cunoniaceae (Savolainen *et al.* 2000). This *rbcL* based study also contained information on most of the other mangrove-containing families. For instance, Avicenniaceae was found to form a distinct subclade with Acanthaceae, Pedaliaceae and Gesneriaceae within the Lamiales and Myrsinaceae was included within the Ericales (Savolainen *et al.* 2000). This and other large studies, such as that of Soltis *et al.* (2000) based on three genes and 560 angiosperms, have proved extremely useful in depicting interfamilial relationships.

More detailed studies of Rhizophoraceae based on a combination of data from cpDNA, nuclear ribosome DNA and morphology, have shown Rhizophoraceae to consist of three well supported monophyletic tribes: Macarisieae, Gynotrocheae and Rhizophoreae (Schwarzbach and Ricklefs 2000). The latter two tribes form a strongly supported subclade and the overall molecular data is compatible with morphological observations (for example fruit characters circumscribe the three tribes). Interestingly, within the tribe Rhizophoreae the genus *Bruguiera* occupies a basal position in comparison to the other genera (*Kandelia*, *Ceriops* and *Rhizophora*) in this mangrove group. This suggests a possible ancestral origin for this genus, and supports morphological observations on dispersal mechanisms. In *Bruguiera* the seedling disperses initially with the fruit, whereas in the other more advanced genera only the seedling disperses.

Parani *et al.* (1998) used RAPD and RFLP to investigate the genomic relationships between various genera considered to be 'true' mangroves and 'associate' mangroves.

Not surprisingly, they found association between related mangrove species and distinction between unrelated groups. However this type of analysis is inadequate for such distantly related taxa and therefore the amount of information that can be obtained from it is limited.

4. Phylogeographic Studies

4.1 Theory and Practice

Just as there are substantial differences among species, there may be large differences between particular groups within species. Genetic structuring of plant populations is strongly influenced by common ancestry and current patterns of interpopulation genetic ex-

change. Phylogeography is concerned with the processes governing the geographic distribution of genealogical lineages among and within closely related taxa, thus fitting between other micro and macro-evolutionary disciplines. This is an area of growing interest especially in the animal kingdom, with the majority of studies conducted to date being based on mitochondrial DNA (mtDNA). MtDNA is the preferred genome for animal phylogeographic studies, because its genes evolve at a faster rate than nuclear genes. Such mutational rates are sufficient to discern between different intraspecific evolutionary groups but not between single individuals. This approach has been particularly useful in defining Evolutionary Significant Units (ESU) for conservation. Some controversy exists in relation to the exact criteria defining ESUs, but in general these units should represent populations that have been historically isolated thus having discrete adaptational potential.

Understanding such distinctions at the population level is particularly important, as optimal management strategies should insure that the full array of differentially adapted groups are maintained as distinct units.

Unfortunately the same high evolutionary rates are not shared by plant mtDNA thus limiting its application. In plants, phylogeographic studies are frequently based on selected regions of cpDNA which, despite being less variable than animal mtDNA, is also known to be clearly structured geographically and to possess most of its evolutionary properties. Because of the lower informativeness of cpDNA, plant phylogeographic studies have been uncommon despite the fact that circumstances such as reticulated speciation (i.e. speciation through hybridisation events) are more prevalent in plants than animals. In species where sufficient cpDNA variation has been detected to permit phylogeographic analysis, variation has been mainly revealed by restriction enzyme digests of whole cpDNA (RFLP), or of selected chloroplast loci (PCR-RFLP). Ultimately, direct sequencing of the variable cpDNA regions, as done for animal mtDNA, would be more desirable for studying relationships, however insufficient variation still restricts the more widespread use of this options. Other studies on plant population structure have relied on population genetic tools and nuclear DNA. In such studies, evolutionary divergence is measured by assessing differential allelic frequency or differential allelic fixation among populations. The majority of the examples below belong to this group.

4.2 Mangrove Studies

For speciation to occur reproductive isolation between two populations must be sufficient to cause genetic differentiation.

Such differentiation may eventually result in reproductive incompatibility.

Genetic differentiation can be brought about by a number of processes such as differing environmental stresses on the two populations, geographical separation, episodes of great extinction (bottleneck effects) or significant expansion from a small initial genetic pool (founder effects). Molecular techniques allow us to identify which of these processes are or have been active and to what extent they have resulted in genetic differentiation.

McMillan (1986) first used allozyme analysis to determine genetic differences amongst populations of *Avicennia germinans*. He found that allelic distribution from a number of loci differed between the plants from the western side and those on the Caribbean side of the Gulf of Mexico. Clearly, geographic separation and differing environmental conditions were limiting gene flow between the two populations thus resulting in genetic differentiation.

Genetic distinction based on allozyme analysis has also been described in other mangrove genera including *Bruguiera*, *Kandelia*, *Rhizophora* and *Sonneratia* (Baba *et al.* 1989, Goodall and Stoddart 1989, Huang 1993, Sun *et al.* 1998). Ballment *et al.* (1988) were able to differentiate three sibling taxa of *Ceriops* using allozymes, by detecting sufficiently high genetic divergence among taxa to justify the establishment of separate species. Their study showed that sympatric populations of *Ceriops tagal* and *C. australis* maintained fixed allelic differences at a number of loci, thus suggesting reproductive isolation and no interbreeding between two species indistinguishable on morphological grounds alone.

Also using allozyme analysis, Duke *et al.* (1998) confirmed the species status of *Avicennia alba*, *A. integra*, *A. marina* and *A. rumphiana* from the Indo-West Pacific and *A. germinans* from the Atlantic-East Pacific. As in the *Ceriops* study, the authors found fixed allelic differences at most loci even when sympatric populations were sampled. The authors were also able to confirm the distinction between three subspecific taxa: *A. marina* ssp. *marina*, *A. marina* ssp. *eucalyptifolia* and *A. marina* ssp. *australasica*. These subspecies differed in allelic frequencies which were revealed to be more reliable than the morphological characteristics

previously adopted, especially at the geographical conjunction of varieties, where partial evidence of introgression was detected (Duke *et al.* 1998).

Overall, the allozyme data did not support an ancient origin of the *A. marina* varieties, with divergence time being estimated at about 2 my BP. Rather, Duke *et al.* (1998) suggested that such varietal differentiation was the result of geographical structuring caused by limited dispersal of propagules.

In another study on *Avicennia* based on the analysis of DNA markers, 109 RAPDs and 84 RFLPs, it was shown that the widely distributed *A. marina* was more closely related to *A. alba* (genetic distance 0.22) than to *A. officinalis* (genetic distance 0.37 Parani *et al.*, 1997). Lakshmi *et al.* (1997) used the same molecular system to investigate intraspecific variability in *Acanthus ilicifolius* to find that intrapopulation differentiation was much greater than interpopulation diversity.

Maguire and Saenger (2000) examined the tropical Indo-Pacific genus *Excoecaria* L. (Euphorbiaceae), which contains several poorly known components in Australia. In Australia, the most widespread species is the mangrove *E. agallocha* L. (type species) whose taxonomic and geographic limits are difficult to define from those of its closely related taxa. As a result, two additional taxa, *Excoecaria dallachyana* Baillon and *Excoecaria ovalis* Endl, have been described but could not be clearly differentiated from the type species. In order to determine the relationships between this closely related group of taxa, Maguire and Saenger (2000) investigated morphometric and DNA sequence data from the ITS1 region of nuclear ribosomal DNA. The authors showed that generally there were no within-species differences in either *E. agallocha* from eastern Australia or *E. ovalis* from Western Australia. However a sufficient number of between-species nucleotide substitutions were detected to justify their respective species status thus corroborating the morphometric data (Maguire and Saenger 2000). The only slight within-species difference was detected between the *E. ovalis* populations from the Gulf of Carpentaria and those from Western Australia, but such variation was not supported by morphometric differentiation. The ITS1 sequencing data also suggested that *E. dallachyana* is not closely related to either mangrove species (*E. agallocha* or *E. ovalis*) despite superficial morphological similarity.

5. Population Genetics and Breeding Biology

5.1 Theory and Practice

As it is important to recognise genetic distinction between groups of populations likely to represent local adaptation, it is important to understand the extent of overall genetic diversity representing evolutionary potential. Changes in gene diversity can be connected to environmental conditions as well as human impact, and generally are not distributed evenly throughout the range of habitats in which the species occurs.

The centres of diversity for single species, as well as the presence of rare genotypes within individual populations can be identified by using highly informative molecular tools.

Population geneticists study the changes in genetic structure, gene flow and overall diversity that occur within and among populations. Population genetic theory is used to quantify the amount, distribution and dynamics of genetic variation in populations and to elucidate their breeding and mating strategies. In the long term, the genetic pool available to a wild species must enable it to survive environmental pressures exceeding the limits of its developmental plasticity.

As a result, determining how much diversity exists within a species and understanding how it is maintained is essential for long term management and conservation. The mating system and breeding strategy of a species will influence the amount of gene flow within and across populations and as a result, the way genetic diversity is distributed. Being able to assess if a population is or is not mating randomly is particularly important.

For example selfing and inbreeding can cause loss of genetic diversity and evolutionary potential, especially in small populations. Similarly, isolation can reduce migration rates and gene flow, potentially causing genetic drift (i.e. directional fluctuations in allelic frequencies).

The extent of gene flow is particularly consequential to the understanding of population dynamics. Generally there are two main methods to acquire such information: the indirect way (historical levels of gene flow are measured based on the distribution of diversity among populations) and the direct way (contemporary gene flow is estimated by direct observation and the identification of immigrant genotypes). Molecular techniques that can provide highly polymorphic, co-dominant markers are especially useful in popula-

tion studies. A number of theoretical population genetics principles have been developed to facilitate the investigation of these and many other important mechanisms.

5.2 Mangrove Studies

Huang (1994) first studied the genetic variability between and within populations of the mangrove *Kandelia candel* in Taiwan using allozyme analysis. He concluded that a moderate level of genetic variation existed between the four mangrove populations studied as well as low within-population diversities. The author suggested that local environmental selection and restricted gene flow between the populations contributed to the limited genetic variability recorded for this species in Taiwan. Huang and Chen (1997) extended the study of *K. candel* to include the Ryukyu Archipelago. They found that the level of genetic variation in all six populations studied was lower than previously reported in other plant taxa. This low variation was largely due to high inbreeding rates, possibly a consequence of founder events in the recent past. As *K. candel* was previously shown to be predominantly outcrossing and as some genetic differentiation was recorded between populations, it is likely that the lack of diversity is also due to restricted gene flow between populations. The results suggest that on a macrogeographic scale, *Kandelia* populations consists of isolated and subdivided units with restricted gene flow. In a microgeographic study of 13 *K. candel* populations in Hong Kong, Sun *et al.* (1998) detected very low genetic diversity despite high outcrossing rates measured from the study of their mating system. As expected within such a small geographic scale, the authors found very low genetic differentiation between sites (with nearly four migrants per generation being estimated to move across populations). At that scale homogeneity is likely to be a result of recent coancestry (Sun *et al.* 1998).

Using allozymes, Duke *et al.* (1998) identified strong genetic structuring in *A. marina*, concluding that high levels of local diversity and outcrossing rates are not matched by high levels of gene flow between neighbouring populations. Gene flow was only recorded between geographically adjacent sites likely to be part of a single, large population. Overall there was strong evidence of isolation-by-distance, likely to be the consequence of reduced dispersal of propagules (Duke *et al.* 1998). Similar findings were reported in a study Maguire *et al.* (2000).

Table 1 : Intra- and interpopulation polymorphism in mangroves

Species	Method	Intra	Inter	Reference
<i>Acanthus ilicifolius</i>	RAPD	3.8-7.3	34.0	Lakshmi et al. 1997
<i>Acanthus ilicifolius</i>	RFLP	3.2-9.1	45.8	Lakshmi et al. 1997
<i>Avicennia alba</i>	Allozyme	0-9.1		Duke 1998
<i>Avicennia alba</i>	RAPD	37.8		Parani et al. 1997
<i>Avicennia integra</i>	Allozyme	0		Duke 1998
<i>Avicennia germinans</i>	Allozymes	0		Duke 1998
<i>Avicennia marina</i>	RAPD	17.8-38.9	76.7	Parani et al. 1997
<i>Avicennia marina</i>	RFLP		66.0	Parani et al. 1997
<i>Avicennia m. marina</i>	Allozyme	0-27.3		Duke 1998
<i>Avicennia m. eucalyptifolia</i>	Allozyme	18.2-45.5		Duke 1998
<i>Avicennia m. australasica</i>	Allozyme	9.1-54.5		Duke 1998
<i>Avicennia officinalis</i>	RAPD	32.3		Parani et al. 1997
<i>Avicennia rumphiana</i>	Allozyme	0		Duke 1998
<i>Bruguiera cylindrica</i>	RAPD	16.38-18.96	37.33	Lakshmi et al. in press
<i>Bruguiera gymnorhiza</i>	RAPD	12.68-16.98	31.08	Lakshmi et al. in press
<i>Bruguiera parviflora</i>	RAPD	14.16-19.36	32.67	Lakshmi et al. in press
<i>Ceriops decandra</i>	RAPD	16.23-19.62	27.77	Lakshmi et al. in press
<i>Ceriops tagal</i>	RAPD	14.68-16.28	39.33	Lakshmi et al. in press
<i>Excoecaria agallocha</i>	RAPD	21.4-31.0	65.0	Lakshmi et al. 1997
<i>Kandelia candel</i>	RAPD	14.45		Lakshmi et al. in press
<i>Kandelia candel</i>	Allozyme	11.1-16.7		Huang and Chen 1997
<i>Kandelia candel</i>	Allozyme	10.8		Sun et al. 1998
<i>Rhizophora apiculata</i>	RAPD	16.20-18.38	32.46	Lakshmi et al. in press
<i>Rhizophora lamarckii</i>	RAPD	17.10		Lakshmi et al. in press
<i>Rhizophora mucronata</i>	RAPD	18.02-19.68	33.33	Lakshmi et al. in press
<i>Rhizophora stylosa</i>	RAPD	16.43		Lakshmi et al. in press

In this study, microsatellite markers were used to assess genetic variation throughout the worldwide range of *A. marina*. Despite the greater informativeness of SSRs and evidence of the species being a preferential outcrosser, the authors generally detected low within-population genetic diversity and some evidence of inbreeding (especially in the most isolated populations). High levels of genetic structure were also measured ($F_{st} = 0.41$) with number of migrants being estimated at less than 1 in most pairwise population comparisons. Overall, the findings from this SSR-based study indicated that genetic structure in *A. marina* is not purely the result of geographical distance, but is the consequence of the formation of independent subunits caused by inadequate dispersal mechanisms (Maguire *et al.* 2000). Such findings support previous observations that propagule dispersal in *A. marina* is limited by longevity, ocean currents and the presence of suitable environmental conditions (Clarke and Myerscough 1991, Clarke 1992). A further study by the same researchers but based on a smaller number of *A. marina* individuals, compared the data obtained using two differ-

ent techniques : AFLPs and SSRs (Maguire *et al. in press*). The authors found that while the levels of polymorphism obtained were similar for the two techniques, SSRs were more adequate for population based investigations, whereas AFLPs were particularly useful for fingerprinting single individuals and monitoring propagation programs.

Finally, random amplified polymorphic RAPD and RFLP markers were used to estimate intra-and inter-specific variation in three species from the mangrove genus *Avicennia* (Parani *et al.* 1997).

Intrapopulation polymorphism among the 10 populations of *A. marina* varied between 17.8 and 38.9%. The authors found that when sympatric, populations of *A. marina* were less genetically variable than populations of *A. officinalis* (Pichavaram population, 32.3%) and *A. alba* (Coringa population, 37.8%). Interpopulation variation in *A. marina* (76.7% for RAPDs and 66% for RFLPs) was greater than the variation estimated within any individual population of this same species, confirming the high degree of divergence between the populations found in other studies (Table 1).

6. Conclusion

As a considerable amount of genetic research as been conducted on mangrove species, it is now possible to draw preliminary conclusions on the evolutionary processes that operate in mangrove species.

At the phylogenetic level, gene sequencing corroborated the observation that mangroves have evolved in parallel within very different families. Nevertheless, within their families, mangrove species form distinct clades often incorporating unique genera (eg. the Rhizophoraceae in the Rhizophoraceae). At a phylogeographic level, morphological distinction within taxa often corresponds to species or subspecies status (eg. *Avicennia*). In fact, all the molecular techniques used to date appear to agree in finding great genetic differentiation among populations. As geographic distance increases between populations, so does genetic identity. Most mangrove population genetic studies indicate that intrapopulation diversity is consistently smaller than interpopulation diversity. In other words, it appears that for most mangrove species, gene flow among population is limited even across relatively confined geographic distances. This suggests that the evolution of mangrove propagules is likely to have been directed more towards buoyancy than long-range dispersal.

Nevertheless, sporadic long-range dispersal events do occur. From a genetic point of view such rare episodes, known as founder events, establish populations with small genetic pools. Such processes explain the lack of intrapopulation diversity recorded in most studies despite the fact that most species are preferential outcrossers. Subsequent environmental stresses and lack of genetic flow ensure that geographically isolated populations remain genetically distinct.

From a conservation and management point of view, it is therefore important to reach a balance between the need to preserve local adaptation and the necessity to ensure optimal evolutionary potential. Overall the best strategy is to maintain local provenances distinct while ensuring that maximum genetic diversity is kept within them.

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Photosynthesis and Evapotranspiration of the Mangrove Forest in Eastern Thailand

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Abstract : The net photosynthetic rate of a mangrove forest was investigated under natural conditions in the rainy and dry seasons in Thailand. The evapotranspiration rate of the mangrove forest was also investigated in the rainy season. The net photosynthetic rate was determined as the total of the CO₂ flux at the plant canopy and the soil surface. The CO₂ flux was obtained from the gradient of CO₂ concentration above the plant canopy multiplied by the CO₂ transfer coefficient. The coefficient was obtained from the heat balance of the forest in the rainy season and estimated with the wind speed above the forest in the dry season. The CO₂ flux at the soil surface was measured with a closed chamber method. The evapotranspiration rate was obtained with the heat balance method. In the rainy season, the net photosynthetic and evapotranspiration rates increased with an increase in solar radiation. The effects of the wind speed on both the rates were significant as well as the solar radiation. The effect of the tidal level on the net photosynthetic and evapotranspiration rates was not significant during the measurement. In the dry season, the net photosynthetic rate decreased gradually in the afternoon. The wind speed negatively affected the net photosynthetic rate. The net photosynthetic rate was considerably lower in the dry season than in the rainy season.

Key words : evapotranspiration, mangrove forest, photosynthesis, soil respiration, Thailand

Introduction

Recently restoration of mangrove forests has become an urgent issue in tropical and sub-tropical coastal regions. In order to restore mangrove forests, it is important to understand the productivity of the mangroves. Mangroves generally form communities with several species of diverse growth forms and sizes. For determining the productivity of the mangrove forest, it is necessary to measure the net photosynthetic rate of the mangrove forest as a whole under natural conditions.

However it is difficult to determine the actual primary production of the mangrove forest under natural conditions by extrapolating the results from measurements for single leaves (e.g. Moore *et al.*, 1973 ; Attiwill and Glougii, 1980) or individual trees. Micro-

meteorological methods for measuring CO₂ flux above the canopy of the mangrove forest are useful for determining the net photosynthetic rate of the mangrove forest. The evapotranspiration of the mangrove forest can also be measured. However few measurements have been conducted in mangrove forests under natural conditions. We measured the net photosynthetic rate under natural conditions in the rainy and dry season. The evapotranspiration rate of the mangrove forest was also measured in the rainy season.

Materials and Methods

Measurements were carried out in Num Chew, Trat province, Thailand (Fig.1). The measurement site was located about two km far from the coastal line and about 90 m far from the forest edge facing a canal. The site was muddy and submerged with 2-3% saline water

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once a day. The highest tidal level was about 0.3 m and 1.0 m from the soil surface during the measurement periods in September and March, respectively. The dominant trees in the area of investigation were *Rhizophora apiculata*, *Rhizophora mucronata* and *Xylocarpus moluccensis*. The mean height of trees was about 12 m. The leaf area index was about three. The measurement in the rainy season was carried out for 10 days in September to October, 1985 and in the dry season for 7 days in March, 1986.



Fig. 1 The location of the research site.

The net photosynthetic rate, P ($\text{gCO}_2 \text{ m}^{-2} \text{ s}^{-1}$), of the mangrove forest in the rainy season was determined with the equation 1.

$$P = F + R \quad (1)$$

where F ($\text{gCO}_2 \text{ m}^{-2} \text{ s}^{-1}$) is the CO_2 flux from the atmosphere to the forest and R ($\text{gCO}_2 \text{ m}^{-2} \text{ s}^{-1}$) is the CO_2 flux from the soil surface to the atmosphere, generally called the soil respiration rate. The value F was determined with the equation 2.

$$F = D_z (dC / dz) \quad (2)$$

where D_z ($\text{m}^2 \text{ s}^{-1}$), is the gas transfer coefficient and dC / dz is the gradient of the CO_2 concentration, C (g m^{-3}), at a height of z (m). The gas transfer coefficient above the plant canopy is obtained from the heat balance of the forest. The heat balance of the forest is generally expressed as the equation 3.

$$S = H_s + H_L + G + B + W \quad (3)$$

where S (W m^{-2}) is the net radiation above the plant canopy, H_s (W m^{-2}) is the sensible heat flux, H_L (W m^{-2}) is the latent heat flux, G (W m^{-2}) is the sub-soil heat flux, B (W m^{-2}) is the flux of heat storage of plants, and W (W m^{-2}) is the flux of heat storage of water. The total of the sensible and latent heat flux is given by the equation 4.

$$H_s + H_L = D_z [H \rho (dT / dz) + 0.622 w \rho (dq / dz) / p] \quad (4)$$

where H ($\text{J g}^{-1} \text{ K}^{-1}$) is the specific heat of air under a constant pressure, ρ (g m^{-3}) is the density of air, dT / dz is the gradient of the air temperature, T (K), the constant of 0.622 is the ratio of molecular weights of water vapor and air, w (J g^{-1}) is the heat for vaporization of water, p is the atmospheric pressure (Pa) and dq / dz is the gradient of the water vapor pressure, q (Pa). The temperatures of the soil surface, the tree trunk surfaces and the water in the forest floor were almost the same as the surrounding air temperatures in the forest and fluctuated within 2°C in the daytime. The sum of G , B and W in the mangrove forest was estimated to be less than five percent of the net radiation (S), so it could be neglected. Therefore D_z was given by the equation 5.

$$D_z = S / [(H \rho (dT / dz) + 0.622 w \rho (dq / dz) / p)] \quad (5)$$

Then F is given by the equation 6.

$$F = S (dC / dz) / [(H \rho (dT / dz) + 0.622 w \rho (dq / dz) / p)] \quad (6)$$

Hence the CO_2 flux is given by measuring the net radiation, the gradients of the air temperature, the water vapor pressure and the CO_2 concentration above the plant canopy.

The procedure of the measurement was as follows. Equipments were attached on a 16 m high steel tower as shown in Fig.2. The net radiation was measured with a net pyrradiometer (model CN-1, Eiko Seiki Co. Ltd., Japan) set up at a height of 13.0 m, which is 1.0 m above the plant canopy. The air temperature and the water vapor pressure were measured with air-flow type wet- and dry-bulb thermometers set up at both heights of 13.0 m and 16.0 m. Sample air was inhaled continuously with polyethylene tubes at the two heights in order to measure the difference of CO_2 concentration between the two heights. The measurement

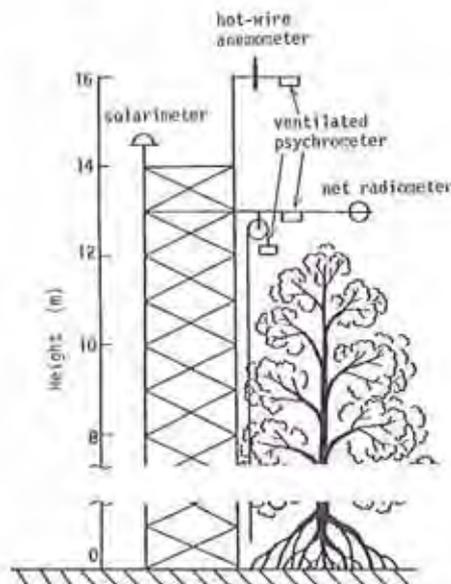


Fig. 2 Instrumentation for measuring the net photosynthetic and evapotranspiration rates of the mangrove forest and meteorological elements above the mangrove canopy.

of CO_2 concentration was conducted with a differential type infrared CO_2 analyzer (ZALDA 262-11, Fuji Electric Co., Ltd., Japan). A solarimeter (Noshi-Denshi type, Nakano Co., Ltd., Japan) and an anemometer (Anemomaster, Kanomax Co., Ltd., Japan) were set up at the height of 14.6 m and 16.0 m, respectively. Recorders and the CO_2 analyzer were set in a shelter under the tower. The air temperature and the water vapor pressure were measured every three minutes and others were measured continuously. Except for the solar radiation, the measurements were averaged every 15 minutes as a moving average.

The soil respiration rate was determined with the closed chamber method. The soil surface was covered with a plastic chamber (0.07 m^3 in volume) and the inside air was well-stirred with a fan. The chamber was impermeable to the light and the temperature inside the chamber was almost the same as the atmospheric temperature near the soil surface. The soil respiration rate was calculated from the rate of the CO_2 concentration increase with time inside the chamber. In the usual case, the CO_2 concentration inside the closed chamber increased from about $400 \mu\text{mol mol}^{-1}$ to about $500 \mu\text{mol mol}^{-1}$ for 8-15 minutes. The CO_2 concentration was measured with an absolute type infrared CO_2 analyzer (ZEP-5, Fuji Electric Co., Ltd., Japan). The measurement for the soil respiration was carried out at seven points on the forest floor nearby the tower.

The evapotranspiration rate, E ($\text{gH}_2\text{O m}^{-2} \text{ s}^{-1}$) was obtained from the latent heat flux ($-H_L$) above the plant canopy divided by the heat for vaporization of water (w) as the equation 7.

$$E = -L / w \quad (7)$$

In the dry season, the gas transfer coefficient (D_i) above the plant canopy was adequately determined as a function of the wind speed above the plant canopy, instead of using equation (5). The function was estimated with the relation of the gas transfer coefficient and the wind speed from the results in the rainy season. The wind speed and the gradient of CO_2 concentration above the plant canopy in the dry season were measured in the same manner as in the rainy season.

Results

It was cloudy or rainy every day during the measurement period in the rainy season. Diurnal changes in the net photosynthetic rate, the evapotranspiration rate and some meteorological elements on two representative days in the rainy season are shown in Fig. 3 and 4. Fig. 3 shows the result from the measurement on September 20. It was cloudy in the morning, and became clear temporarily at 12:30-14:00 but then cloudy again after 14:00. The air temperature at the height of 16.0 m, which was 4.0 m above the plant canopy, was 23°C at 6:00. It increased to 28°C at noon, which was the maximum in the day. The relative humidity in the early morning was 98%, mostly saturated, and decreased to the minimum value of 75% at midday.

The mean net photosynthetic rate was about $0.5 \text{ mg CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ from 8:00 to 10:00 when it was cloudy and the mean solar radiation was about 0.5 kW m^{-2} . The net photosynthetic rate increased to $1.5 \text{ mg CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ at noon when the solar radiation was 0.7 kW m^{-2} . It was about $1.0 \text{ mg CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ at around 13:00 when the sky was clear and the solar radiation was about 1.0 kW m^{-2} . The evapotranspiration rate also increased with an increase in solar radiation. It increased to 2-5 times when the solar radiation increased 2-3 times at around 8:00, 9:30 and 12:30. It ranged between 150 and 250 $\text{mg H}_2\text{O m}^{-2} \text{ s}^{-1}$ at 13:00-14:00 when the solar radiation was about 1.0 kW m^{-2} .

The tide was lowest at around 4:00 and highest at around 16:00. The water came to the tower at 11:20 and the soil surface was submerged until 20:00. The tidal level showed insignificant effects on the net photosynthetic and evapotranspiration rates during

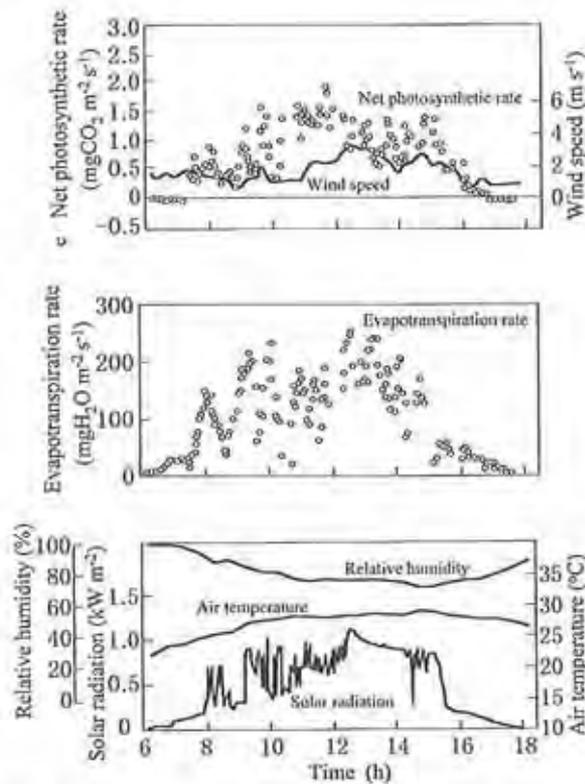


Fig. 3 Diurnal changes of net photosynthetic and evapotranspiration rates of the mangrove forest and meteorological elements measured at 4.0 m above the mangrove canopy on September 20, 1985.

the measurement.

Fig. 4 shows the result from the measurement on October 1. The sky was clear in the morning and the solar radiation reached up to 1.0 kW m^{-2} at noon, and then it began to rain heavily at 13:00. The air temperature at the height of 16.0 m was 23°C at 6:00. It increased with an increase in solar radiation and reached 32°C at noon, which was 4°C higher than the maximum temperature on September 20 shown in Fig. 3. The relative humidity in the early morning was 98% and decreased to 65% after 10:00, which was 10% lower than the minimum relative humidity on September 20.

The net photosynthetic rate increased rapidly with an increase in solar radiation, and was already $0.7 \text{ mg CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ at 7:30 when the solar radiation was 0.2 kW m^{-2} (Fig. 4). Then the net photosynthetic rate was $1.7 \text{ mg CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ at 9:30 when solar radiation was 0.7 kW m^{-2} , and increased up to about $2.7 \text{ mg CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ at noon when the solar radiation was 1.0 kW

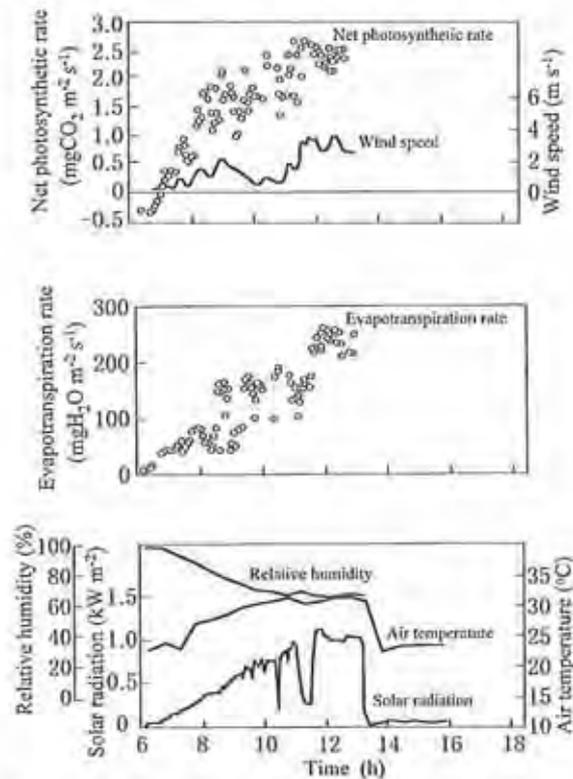


Fig. 4 Diurnal changes of net photosynthetic and evapotranspiration rates of the mangrove forest and meteorological elements measured at 4.0 m above the mangrove canopy on October 1, 1985.

m^{-2} . While the wind speed decreased from 3.0 m s^{-1} to 1.5 m s^{-1} at 9:00-11:00, the net photosynthetic rate showed no increase with an increase in solar radiation. There after it increased rapidly up to $2.7 \text{ mg CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ when the wind speed increased to 4.0 m s^{-1} . The net photosynthetic rate was accompanied by an increase in the wind speed.

The evapotranspiration rate also increased with an increase in solar radiation and air temperature. The evapotranspiration rate was $80 \text{ mg H}_2\text{O m}^{-2} \text{ s}^{-1}$ at 8:00 when the solar radiation was 0.3 kW m^{-2} , and increased up to $250 \text{ mg H}_2\text{O m}^{-2} \text{ s}^{-1}$ at noon when the solar radiation was 1.0 kW m^{-2} . It also showed the tendency to follow the change in the wind speed as well as the net photosynthetic rate.

Fig. 5 and 6 show effects of solar radiation and wind speed on the net photosynthetic rate and the evapotranspiration rate, respectively. Both the rates increased significantly with an increase in solar radiation and an increase in the wind speed. The net

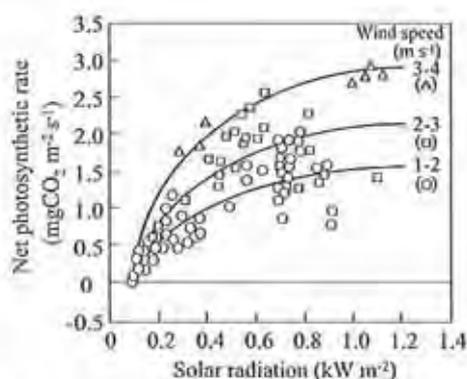


Fig. 5 Effects of solar radiation and wind speed on the net photosynthetic rate of the mangrove forest. Approximate curves at the wind speeds of 1-2, 2-3 and 3-4 m s^{-1} are also shown.

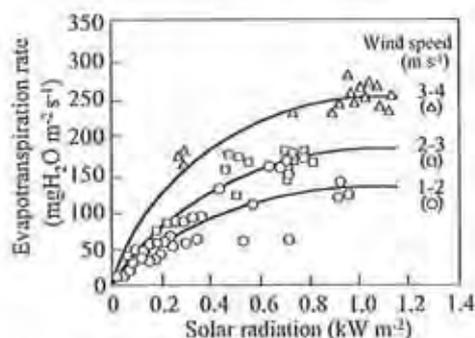


Fig. 6 Effects of solar radiation and wind speed on the evapotranspiration rate of the mangrove forest. Approximate curves at the wind speeds of 1-2, 2-3 and 3-4 m s^{-1} are also shown.

photosynthetic rates were 1.2 times and two times greater at wind speeds of 2.3 m s^{-1} and 3.4 m s^{-1} , respectively, than at a wind speed of 1.2 m s^{-1} regardless of the solar radiation (Fig.5). The evapotranspiration rate showed a similar tendency (Fig.6). These facts show that the wind enhances gas exchange between the mangroves and the atmosphere and thus the primary production of the mangrove forest in the rainy season.

We tried to estimate the gas transfer coefficient from the wind speed above the plant canopy. Fig.7 shows the gas transfer coefficient as affected by the wind speed above the mangrove canopy. These data was obtained from the measurements at 7:00-8:00, 12:30-14:00 on September 20 and at 7:00-13:00 on October 1 when the solar radiation was relatively stable and the error

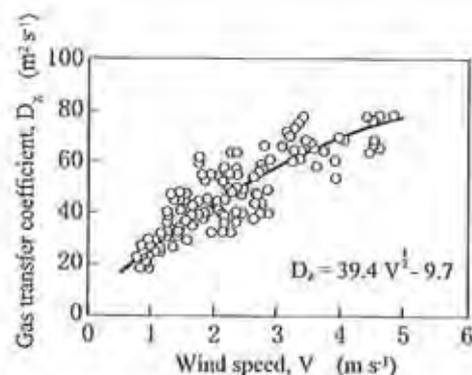


Fig. 7 Gas transfer coefficient as affected by the wind speed at 4.0 m above the mangrove canopy.

in the measurement of the transfer coefficient was considered to be relatively small. The relation was expressed as the function of the wind speed and the correlation coefficient was 0.85 in the range of wind speed from 1.0 to 5.0 m s^{-1} . Hence the gas transfer coefficient in the dry season was estimated from the wind speed with the equation in Fig.7 instead of the heat balance method employed in the measurement in the rainy season.

In the dry season, there was a clear sky all day long every day. Leaves of some species such as *Xylocarpus moluccensis* turned yellow and some were shed. Diurnal changes in the net photosynthetic rate and some meteorological elements on two representative days in the dry season are shown in Fig.8 and 9. Fig.8 shows the result from the measurement on March 11. The sky was clear all day long, and the air temperature was 24°C at 6:00 and reached 30°C at 10:00. The relative humidity was 98% at 6:00. It decreased rapidly with time down to 60% at noon. The maximum net photosynthetic rate was 0.7 $\text{mg CO}_2 \text{m}^{-2} \text{s}^{-1}$ at 9:00. Then it decreased to 0.5 $\text{mg CO}_2 \text{m}^{-2} \text{s}^{-1}$ at noon when solar radiation was 1.0 kW m^{-2} and furthermore decreased to 0.2 $\text{mg CO}_2 \text{m}^{-2} \text{s}^{-1}$ at 13:30. The net photosynthetic rate was considerably lower than that in the rainy season. The net photosynthetic rate positively followed an increase in the windspeed at around 9:00. However the net photosynthetic rate decreased with an increase in wind speed in the afternoon. At 14:00 when the wind speed decreased temporarily from 5.5 m s^{-1} to 3.5 m s^{-1} , it increased from 0.2 $\text{mg CO}_2 \text{m}^{-2} \text{s}^{-1}$ to 0.4 $\text{mg CO}_2 \text{m}^{-2} \text{s}^{-1}$.

Fig.9 shows the result from the measurement on March 10 in the dry season. The net photosynthetic

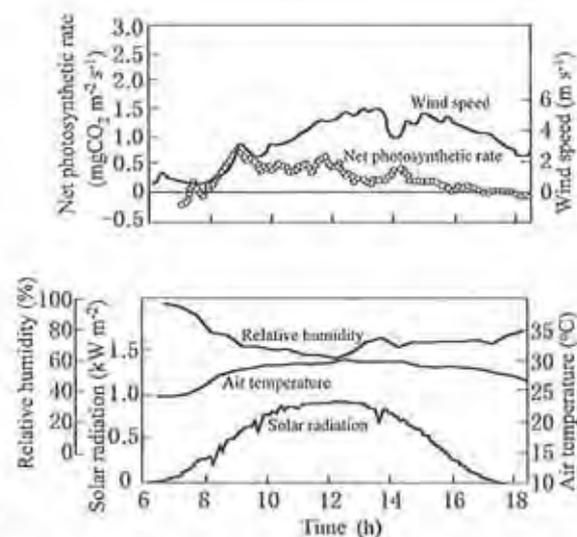


Fig. 8 Diurnal changes of net photosynthetic and evapotranspiration rates of the mangrove forest and meteorological elements measured at 4.0 m above the mangrove canopy on March 11, 1986.

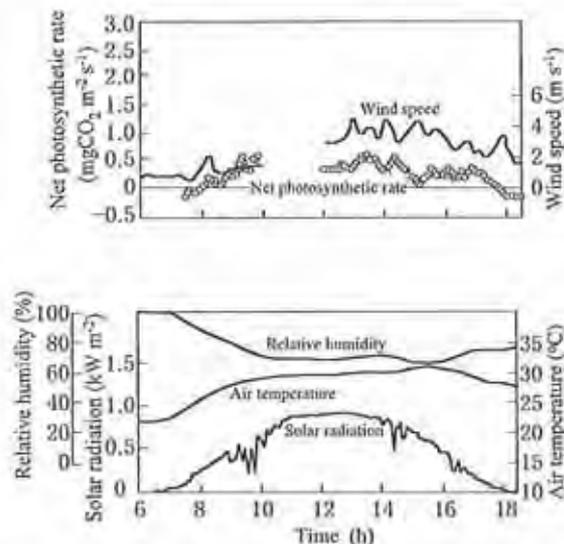


Fig. 9 Diurnal changes of net photosynthetic and evapotranspiration rates of the mangrove forest and meteorological elements measured at 4.0 m above the mangrove canopy on March 10, 1986.

rate increased with an increase in the wind speed at around 8:00. However negative effects of the wind speed on the net photosynthetic rate was observed from 13:00 to 16:00. The mean net photosynthetic rate at 12:00-17:00 was slightly greater on March 11 (Fig.9) than on March 10 (Fig.8) because of lower wind speed. The net photosynthetic rate in the dry season tended to decrease with an increase in the wind speed when the solar radiation was more than 0.7 kW m^{-2} . This phenomenon was in contrast with that in the rainy season, which showed the positive effect of the wind speed on the net photosynthetic rate.

The soil respiration rate varied from 0.08 to $0.14 \text{ mg CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ at the seven sampling plots and the averaged value was $0.11 \text{ mg CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ when the soil surface was exposed at a low tidal level. When the soil surface was submerged, CO_2 release from the soil was not detected.

Discussion

The maximum value of the net photosynthetic rate of the mangrove forest in the rainy season was about $2.5 \text{ mg CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ at the solar radiation of 1.0 kW m^{-2} (Fig.4). This value was approximately two times that of the tropical rain forest in Malaysia and the tropical dry evergreen forest in Thailand whose maximum values are 1.1 to $1.4 \text{ mg CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ (Aoki *et al.*, 1975; Yabuki *et al.*, 1983). Although the leaf area index of each forest was different, the photosynthetic ability of the mangrove forest may be high in comparison with other tropical forests. In the rainy season, the net photosynthetic rate was accompanied by a rise in the wind speed. Similar to the phenomena were reported for the other tropical forests (Yabuki *et al.*, 1983).

The net photosynthetic rate in the rainy season in this study was greater than that investigated by Monji *et al.* (1996). The reason would be partly due to the difference of tree size between the forests. The average height of trees was 12 m in the present study and about three times that in their study.

The depression of the net photosynthetic rate in the dry season was observed also in the tropical evergreen forest (Yabuki *et al.*, 1983). This phenomenon was due to the water stress on the plants because of decrease in water content of the soil and increase in water vapor pressure deficit of the atmospheric air. The relative humidity above the mangrove forest decreased to 60% in the dry season, which was 15% lower than in the rainy season. The high solar radiation might induce a raise of the leaf temperature. Since these fac-

tors would induce a large difference between the water vapor pressures of leaves and the atmosphere and a highly negative water potential in the plants, the mangrove trees may have water stress. The tidal level was higher and the period during which the forest floor was submerged was longer in the dry season than in the rainy season. In addition the salinity of the soil water was higher in the dry season than in the rainy season. In such conditions, the root respiration and thus water absorption by roots were considered to be suppressed. Therefore the water stress within the plants may be aggravated. The decrease in the net photosynthetic rate with an increase in wind speed in the dry season may be due to an enhancement of the plant water stress as mentioned by Yabuki (1985). The measurements indicate that the growth rate of the mangroves does not seem to be very high in the dry season, therefore the dry season is less suitable for planting mangroves than the rainy season.

The soil respiration rate in the mangrove forest observed in this study was similar to that in the crop vegetation in the summer (e.g. Yabuki, 1985). On the other hand, it was 1/2 to 1/3 times those in the temperate forest and the tropical dry evergreen forest, which were $0.25 \text{ mg CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ (Yoneda and Kirita, 1978) and $0.32 \text{ mg CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ (Yabuki *et al.*, 1983), respectively. This suggests that the organic matter of the soil in the mangrove forest would be decomposed more gradually than in other temperate and tropical forests, and therefore a large amount of humic substances would be accumulated in the soil.

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タイ国東部におけるマングローブ林の光合成および蒸発散

タイ国トラート県ナムチュウにあるマングローブ林の純光合成速度および蒸発散速度を、微気象学的方法を用いて測定した。雨季には、日射量および風速の増加にともない、マングローブ林の純光合成速度および蒸発散速度は増加した。それぞれの最大値は、日射量 1 kW m^{-2} において、純光合成速度 $2.5 \text{ mg CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ 、蒸発散速度 $250 \text{ mg H}_2\text{O m}^{-2} \text{ s}^{-1}$ であった。しかし乾季の純光合成速度は、雨季の1/3に低下した。

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The Distribution Pattern of the Macrobenthos in the Nagura Tidal Flat Enclosed by Mangrove Forest, Ishigaki Island, Southwest Japan

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Abstract : The distribution of macrobenthos was studied on a tidal flat of Ishigaki Island, Okinawa Prefecture. The tidal flat is enclosed by a mixed forest of *Bruguiera gymnorrhiza* and *Rhizophora stylosa*, and receives a large supply of decomposed leaf litter, from the inflow of turbid water. Such suspended organic matter arriving at the open space of the tidal flat, changes the conditions of the bottom sediment and the distribution pattern of the benthic animals. The macrobenthic communities investigated include 46 taxa and a larval form, in which 34% was allied to detritivorous species or groups of feeding types. The distribution pattern of the detritivores showed a reasonable trend to accommodate the habitat and corroborated the results of water quality and sediment analyses. Divisions of the benthic communities came from mathematical analysis of their similarity. These communities are expected to be relevant to the composition of the detritus-based ecosystem of the next step of the study.

Key words : macrobenthos, detritivore, Nagura tidal flat, Ishigaki Island

Introduction

The trend that particulate organic matter decomposed from mangrove litter, contributes to estuarine ecosystem as a part of the energy flow has been explained by Odum and Heald (1975) in a study of the Everglades. As stressed by Mcclusky(1981), it is the main source of the detritus-based food web in estuarine waters regardless of primary production by phytoplankton. The litter decomposition process has been demonstrated in only a few phanerogamous plants i.e. *Spartina* (Odum and de la Cruz, 1967), *Zostera* (Imai et al., 1951; Harrison and Mann, 1975) and mangroves (Odum and Heald, 1975), noting the consumption by estuarine animals such as macrobenthos at the secondary trophic level of the ecosystem. How much does the particulate organic matter contribute to the feeding of macrobenthos? Thus, the secondary production of mangrove waters should be evaluated. The objective of the present study is the distribution pattern of macrobenthos, with classification of the taxa by feeding types which include detritivore, the feeder of particulate organic matter precipitated as detritus.

The study area is the Nagura estuary located at

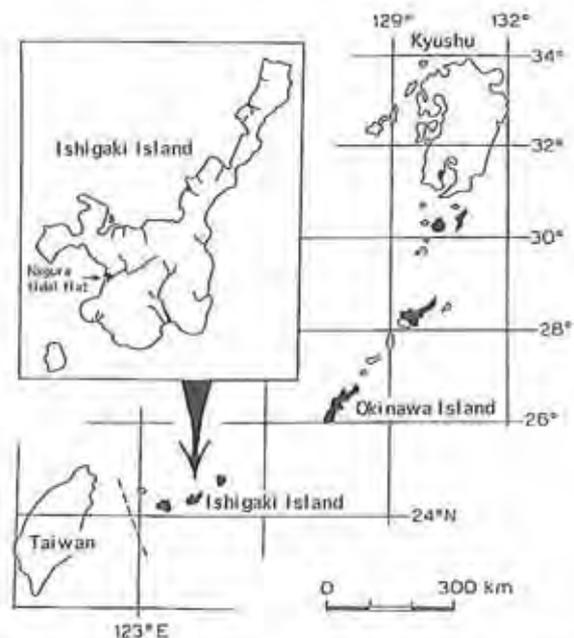


Fig. 1 Map showing the sampling site of Ishigaki Island and the islands of Nansei-Shoto, southwest Japan. The dark parts indicate mangal vegetation

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south coast of Ishigaki Island, Okinawa Prefecture. The estuary looks like a tidal flat at ebb tide and like an estuary when it floods, at which time streams flowing from the mangrove forest into the estuary have a high turbidity rich in particulate organic matter. Therefore, the macrobenthos distribution would be characterized by the source of food supply and would reflect the specialty feeding of macrobenthos such as detritivore.

Macrobenthos distribution in the mangrove swamps or in the watered areas have not often been reported in benthos studies, except in results by Walski (1967), Paphayasit and Setti (1985) and Nishihira et al. (1996) which are worthy of discussion in the results of the present study.

Materials and Methods

The study site is an elongated lagoon-like tidal flat with 43.3 ha surface area and a mixed forest of *Bruguiera gymnorrhiza* and *Rhizophora stylosa* consisting of 41.1 ha behind the inner coast. Its main inflow is the Nagura River and a few of smaller streams passing through the mangrove forest which connect to the waterways at the tidal flat and join as one opening into the outer sea. The shape and location (24° 4' N, 128° 8' 30" E) was shown in Fig.1 as the same as that of the previous report (Kuwabara and Shiroma, 1996).

Sampling of the macrobenthos was carried out on April 29-May 1, 1998 of 16 stations on the tidal flat (Fig.2), using frame-quadrates of 10cm × 10cm, 20cm × 20cm, or 50cm × 50cm, twice at each station at ebb tide. Samples were sieved immediately with a metal sieve with 0.5mm aperture. The animals collected were fixed with a 10% formalin solution for later sorting and counting of each species.

At the time of ebb and flood tides on July 27, 1999, water qualities for each station were determined with HORIBA Water Quality Checker U-10 (HORIBA Ltd., Japan). The stations were submerged at this time.

Sampling of the sediment was carried out on April 30, 1998, at the same time of sampling of the macrobenthos. Sediment analyses for grain size composition and for ignition loss as an indicator of organic matter were done. In grain size analysis, fractions of coarse to fine sand were measured by the sieve method. Silt-clay fraction was estimated from weight loss through 0.063mm sieve. Ignition loss was determined at 500°C for 2 hours.

The same species and the same genus or taxonomical similar group from the literature on the feeding

habits of the macrobenthos were identified, using Bienbaum (1979), Day (1967a, 1967b), Dorsey (1982), Enequist (1950), Fauchald and Jumars (1979), Gaston (1987), Graham (1955), Hawkins and Hartonall (1983), Kuwabara and Akimoto (1986), Kuwabara and Imai (1997), Kuwabara et al. (1997), Maurer and Leathem (1981), Whitlatch (1980).

Results and Discussion

1. Distribution of the macrobenthos

The result of the macrobenthos analysis is shown in Table 1. A list of species with a feeding type assigned to each taxon and a numerical density are outlined for each station. A total of 46 taxa and a larval form (zoaea larva) were identified. Of the major groups, Polychaeta had 10 taxa, Bivalvia had 4 taxa, Gastropoda had 18 taxa, and Crustacea had 10 taxa. Most gastropod species were epifaunal inhabitants. The designation of the feeding type was followed in the original descriptions. SD, DET and D/S included the same detritivore, at least in part of the feeding behavior. Sixteen taxa of the total 47 species and groups (34%) were detritivores.

Each station yielded 4-18 species, and 8.8 species in average, considerably fewer in comparison to estuary, or inner bay area. Numerical density showed a range of 625-77,400 ind/m², and the average showed 22,266 ind/m². Species showing higher than 10,000 ind/m² were two common gastropods, *Batillaria multiformis* and *B. zonalis*. They were concentrated at St. 4-7 at the northern site of the tidal flat close to an opening to the outer sea where two streams, namely, the Nagura River and the secondary big river flowed into the surrounding waterways. Another concentration of the species was seen at St. 14 close to the fringe of the central mangrove forest (Fig.2). Showing the highest numerical density at each station were the taxa *Langerhansia rosea*, *Arenimitra exasperata*, *Batillaria multiformis*, *B. zonalis*, *Corophium* sp. and a chironomid of Orthocladinae, in which the latter four species were detritivores. A species found at almost all the stations was the *Arenimitra exasperata*, a small epifaunal gastropod of which the feeding type is unknown but may be a detritivore because its habitat is similar to the *Batillaria*.

The distributions of a number of species and the numerical density at every station were illustrated in Fig.3. Although any tendency towards large numerical densities was hard to recognize, lower numerical densities were seen just at the inner site of the opening to the outer sea, and higher numerical densities at just behind the site. Thus the generalized pattern of nu-

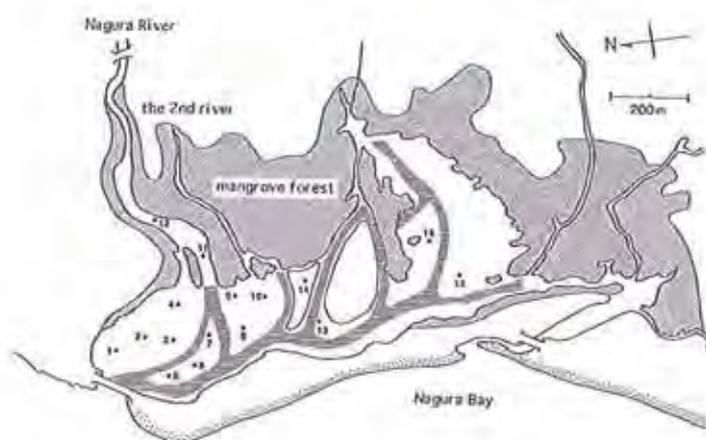


Fig. 2 The sampling stations of macrobenthos in the Nagura tidal flat, Ishigaki Island (stripes: waterway at ebb tide)

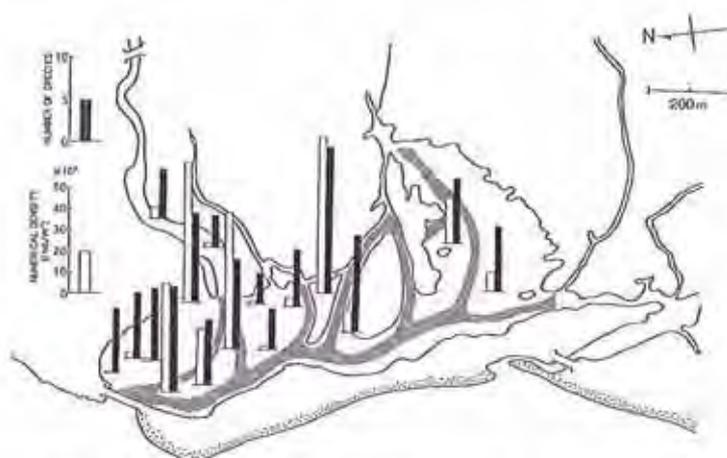


Fig. 3 Distributions of numerical density and number of species of the macrobenthos in the Nagura tidal flat on April 29-May 1, 1998 (stripes: waterway at ebb tide)

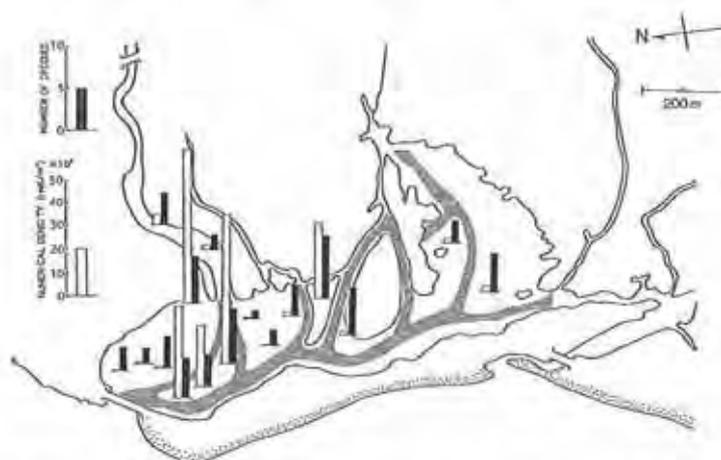


Fig. 4 Distributions of numerical density and number of species of detritivore macrobenthos in the Nagura tidal flat on April 29-May 1, 1998 (stripes: waterway at ebb tide)



Fig. 5 Distribution of salinity (%) in water of the Nagura tidal flat on July 27, 1999 (solid lines: ebb tide, dotted lines: flood tide, solid lines with convexes: tidal flat at higher level)

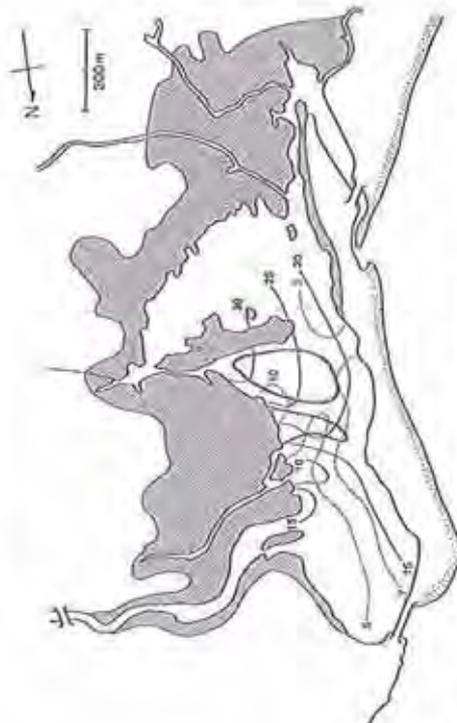


Fig. 6 Distribution of turbidity (NTU) in water of the Nagura tidal flat on July 27, 1999 (solid lines: ebb tide, dotted lines: flood tide, solid lines with convexes: tidal flat at higher level)



Fig. 7 Distribution of median diameter (mm) of the sediment in the Nagura tidal flat on April 30, 1998 (solid line with convexes: tidal flat at higher level, stripes: waterway at ebb tide)



Fig. 8 Distribution of ignition loss (%) of the sediment in the Nagura tidal flat on April 30, 1998 (solid line with convexes: tidal flat at higher level, stripes: waterway at ebb tide)

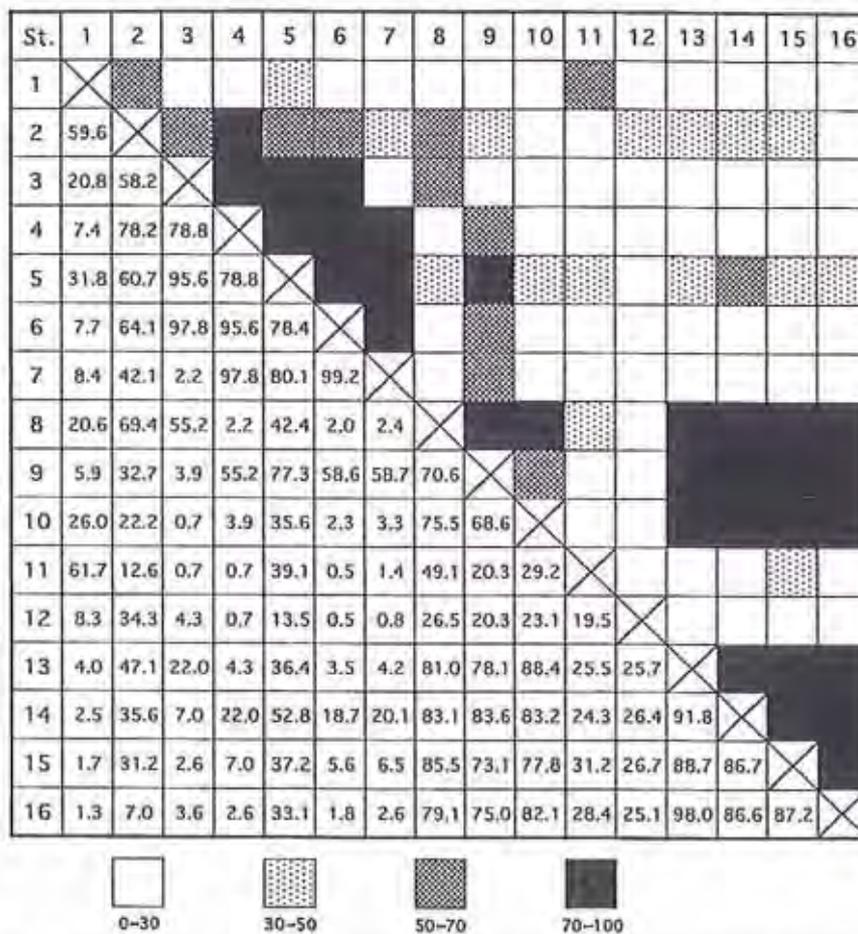


Fig. 9 Diagram showing similarity of macrobenthos community among total stations. (Each number indicates Kimoto's similarity index $C_{\pi} \times 100$)

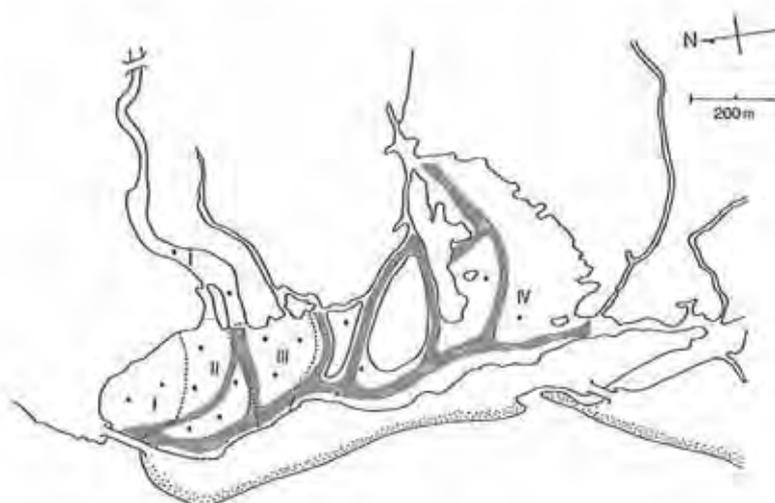


Fig. 10 Division of macrobenthic community pattern in the Nagura tidal flat, examined from diagram of similarity in Fig. 9 (dotted lines: approximate boundary of the divisions, stripes: waterway at ebb tide)

Table 1 Macrobenthic taxa with their feeding types and numerical densities obtained from the Nagura tidal flat, Ishigaki Island, April 29-May 1, 1998

SPECIES & FEEDING TYPE	STATION	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
OLIGOCHAETA																	
<i>Limnodrilus</i> sp.		100															
POLYCHAETA																	
<i>Aulolytus</i> sp.					100												
<i>Langerhansia rosea</i>	DEP	50	1,200	50	100	200											
<i>Ophiodromus</i> sp.	C	25															
<i>Neanthes japonica</i>	C,O											25					
<i>Carstonereis erythraensis</i>	O												125	500	1,000	1,300	300
<i>Hemipodus</i> sp.	C			100	400												
<i>Glycera lancedivae</i>	C					100											
<i>G. prashadi</i>	C										100						
<i>Heteromastus filiformis</i>	DEP	75	150					200						300	400	300	300
MALDANIDAE																	
	DEP		50														
NEMERTINEA																	
	DEP										100						
HEMATODA																	
	DEP														100		
BIVALVIA																	
<i>Musculista senhousia</i>	SF					200								200			
<i>Gafrarium tumidum</i>	D/S	125	50								1,300			600	500		
<i>Psammolaea minor</i>	D/S								200								
<i>Psammolaea elongata</i>	D/S				100							375	250			2,100	1,700
GASTROPODA																	
<i>Angaria distorta</i>							100										
<i>Nerita squamulata</i>	G				300	1,800	400	600									
<i>Ritena costata</i>	G					400											
<i>Cilithon retropictus</i>	G						200										
<i>Melanoides tuberculatus</i>	SD													100			
<i>Batillaria multiformis</i>	SD				21,200	4,900	3,800	12,200			100			100	6,600	200	
<i>Batillaria zonalis</i>	SD	25		450	44,100	22,300	21,300	47,400		600				100	6,600	200	
<i>Cerithidea rhizophorarum morchii</i>	SD			300	1,100		600	4,500									
<i>Cerithiopsis cingulata</i>	SD																100
<i>Cerithium kobelti</i>	SD																
<i>Proclava kochi</i>	SD				100			100									
<i>Clypeomonas pelmus</i>	SD				100	3,800	1,000	300	100		100			300	5,600	100	
<i>Niso nizenensis</i>	PA													100			100
<i>Japeuthia ferrea</i>	O							100									
<i>Picardularia bellula</i>																200	
<i>Arenimlira exasperata</i>			100	250	1,300	10,200	400	1,300	700	600	2,300	425	850	7,800	41,800	5,100	18,900
<i>Didontoglossa koyasensis</i>															100		
<i>Melampus nuxkastanae</i>	G													100			
CRUSTACEA																	
<i>Pholis</i> sp.	SF-SD (DET)			50										625	200	4,800	100
<i>Coryphum acherusicum</i>	SF-SD (DET)					300										7,200	
<i>C.</i> sp.	SF-SD (DET)												3,100				
<i>Idotea</i> sp.								100									
<i>Cirratina</i> sp.	C,O															100	
<i>Tanais cavolinii</i>	D/S															1,200	
<i>Myctylis brevidactylus</i>											100						
<i>Scoipimera globosa</i>		25	200	100		200			300	100							100
ANOMURA																	
<i>Zoea</i> larva	P															200	
																100	
INSECTA																	
<i>Enochrus</i> sp.				100													
ORTHOCLADIINAE																	
	DET	200	850	100		9,000		400	200		200	1,550	400		200		300
No. of species		8	8	9	11	13	8	11	5	4	7	4	6	12	18	8	8
Numerical density (Ind/m ²)		625	2,700	1,500	68,900	63,500	27,800	67,200	1,500	1,600	4,200	2,375	5,350	10,400	77,400	9,400	21,800

C: carnivore, O: omnivore, SF: suspension feeder, SD: surface detritivore, DEP: deposit feeder, DET: detritus feeder, D/S: deposit-suspension feeder, G: grazer, P: plankton feeder, PA: parasite

merical distribution was variegated in comparison to that of species number shown at the same time in Fig.3. However, the illustration for detritivores showed a fine locational difference as seen in Fig.4. Very high numerical density was recognized at the site showing high numerical density for the total number of species including St. 4-7 and St. 14, allied to the abundance of *Batillaria multiformis* and *B. zonalis* mentioned above. Looking at the geography, these sites are influenced significantly by the flow of the two rivers, the Nagura River and the second river.

Meanwhile, three reports have been published on the faunal and the distributional studies of the macrobenthos in mangrove waters under a different scale and a different condition for each other: 1) a riverine zone of 250m length in a Hawaiian mangrove swamp, Walsh (1967), 2) a big scale coastal and varied stony area in Pan-naga Bay, Thailand, Paphavasit and Setti(1985), 3) Klong Kone mangrove swamp in Samut Songkhram, Thailand, Nishihira et al.(1996).

In the study by Walsh (1967) under the conditions of black mud with gravel bottoms, and salinity 0.1-3‰ or

over, a total of 23 benthic taxa including 6 molluscan and 14 crustacean taxa was recorded from the riverine of two mangrove species, *Bruguiera sexangula* and *Rhizophora mangle*. After reviewing the data, 7 taxa (30%) can be attributed to detritivore type.

Paphavasit and Setti (1985) identified 80 taxa or more of macrobenthos including 30 polychaete, 6 bivalve, 15 gastropod and 27 crustacean taxa from three coastal stations where 12 species of mangrove trees were observed. Numerical density ranged from 115-546 ind/m² of the epifauna, and from 1,305-6,691 ind/m² of the infauna. The Shannon-index of species diversity for the infauna ranged from $H' = 0.7562-1.1892$ are considerably low against the richness of species. Comparing the result of the present study, polychaete and crustacean taxa were abundant. Otherwise, the numerical density and species diversity showed smaller values. The difference might be due to a sampling method such as line-transect sampling in their research of Thailand compared to the quadrat method of the present study.

Nishihira et al. (1996) also studied macrobenthic communities of an outer mud flat and an ex-mangrove fringe of mangrove forest composed of the species, *Avicennia alba* and *Sonneratia caseolaris* in Thailand. Although the report had no list of macrobenthos species, a total of 63 species have been identified with 9 polychaete, 6 bivalve, 9 gastropod and 18 crustacean species. Only 11-21 species occurred at each station. The average density was 578 ind/m², which is very small compared to the abundance of species. The Shannon-index ranged from $H' = 1.85-2.39$ except for a value of mangrove-less stations. A cockle, *Anadara* sp., and an ocyroid crab, *Macrophthalmus*, were common to the area studied.

Compared to the data of these publications, the result of the present study shows a moderate number of total taxa and of species in the main taxonomic groups. The high numerical density and low species diversity which is shown in the Section 3, are characteristic of the area studied. The community was occupied predominantly by epifaunal gastropods such as *Batillaria* spp. and *Arenimitra exasperata*.

2. Water and sediment environments

As hydrodynamics are the major agents forming the distribution pattern of the inhabitants of mangrove waters, physico-chemical data of water quality were requested at the study site. When the benthos sampling was carried out there in 1998, no water quality determination had been applied. Additional research

was done on the analyses of the water environment. Some of the data were discussed as follows.

Salinity ranged from 7.2-16.7‰ in ebb tide and 17.1-24.7‰ in flood tide on July 27, 1999. In the illustration of the results (Fig.5), the distribution of isometric lines in ebb tide showed that the freshwater flow from the Nagura River influenced from the inside of the barrier to the opening of the estuary to the outer sea. A similar reflex lines-pattern is seen in the flood time with a few modifications caused by the invasion of seawater. It is recognized that the repeated movement of water mass is greater in the outer part of the tidal flat where the macrobenthic inhabitants should be more or less tolerant of salinity changes. As suggested by Wolanski (1992), high salinities seen in 20, 22 and 24‰ of the isometric lines of the inner part of the tidal flat might be the effect of evapo-transpiration of the shallow and warmed water under the remained state.

Turbidity ranged from 8-33 NTU in ebb tide and from 4-10 NTU in flood tide in the same survey, which suggests that the high turbidities were derived from land-source origins. The distribution shown in Fig.6 recognizes that the high turbidity water advances from the fringe of the mangrove forest toward the central part of the tidal flat. The fact suggests that since there is almost no other source of producing turbidity except litter decomposition and accumulation around the mangrove forest, particulate organic matter would be dispersed from muddy sediment under the mangroves, which supply the litter leaves and the decomposed substance. Distribution of the isometric lines in the flood tide show a similarity to the distribution of salinity during the movement of the same water mass.

The sediment condition directly affects the inhabitants such as macrobenthos. The major particle sized physico-chemical elements and the nutritional substance are also analyzed and discussed in this study, using data taken at the same time as the macrobenthos sampling in 1998.

The median diameter of the sediment exhibited a range of 0.135-0.48mm of fine sand and medium sand at every station. As seen in Fig.7, while grain smaller than 0.2mm (within the range of fine sand) disperses in the inner part of the total area, grain larger than 0.35mm (within the range of medium sand) is isolated in the northern side toward the opening, the riverine of the Nagura River, and a central spot surrounded by two waterways flowing toward the opening to the outer sea.

Ignition loss as an index of organic matter content

exhibited a range of 0.73-3.16%. The distribution of the isometric lines is shown in Fig.8. The highest value and the next highest values were obtained from the inner wide part of the area where finer sands were distributed. In general, ignition loss shows a negative correlation to grain size, because a volume of smaller particles has a greater total surface area for adsorption of organic matter and colonization of bacteria. On the contrary, larger particle has a smaller surface area in the same unit of volume which accompanies lower organic content (Barnes, 1974). Accordingly, the inner area, rich in organic matter, has to yield a high density of macrobenthos, and its virtual abundance was observed mostly in the outer part of the area where ignition loss is lower. This area of well-arranged isometric lines shows a concentration of ignition loss which increases gradually the values. This means that particulate organic matter supplied from the Nagura River increases the successive precipitation of the opened space by decreasing the flow velocity. Thus, the suspending organic matter changes the condition to aggregative detritus-form in the bottom surface. Therefore, abundant macrobenthos of a particularly detritivore-type as comprehensible in Fig.4 would be raised. Actually, this is the domain of detritivorous *Batillaria* species.

3. The division of community pattern

From the data on macrobenthos species and the density, all possible pairs of stations can be compared by use of Kimoto's similarity Index C_c (Kimoto, 1967; 1976) in Fig.9. If two samples are the same composition and numerical density, $C_c = 1$ is derived. As the values were often very small, $C_c \times 100$ was used to show the results. Consequently, St. 3-7, St. 8-10, and St. 13-16 are seen as a group with high similarity values from 70-100. The remaining St. 1, 2, 11, and 12 are connected by location and values ranging from 50-70. The division of the community pattern and the location were summarized in Fig.10 showing partitions from I - IV. Waterways seen at the borders have a rapid current mostly in ebb tide, and the sediment conditions are different from the surface of the tidal flat. As simplified explanation of the partitions is described below.

As for the next step of study, the composition of a detritus-based ecosystem for I - IV needs to be researched. In that case, Partition II would be chosen because of the higher numerical density of a detritivorous gastropod and the moderate ranges of grain size and the organic matter content in the sediment.

Partition I. No. of species : 4-8, dominant species : *Langerhansia rosea*, *Arenimitra exasperata*, *Corophium* sp. and Orthocladinae, numerical density : 625-5,350 ind/m², median diameter of sediment : 0.255-0.40mm, ignition loss : 0.73-1.79%

Partition II. No. of species : 8-13, dominant species : *Batillaria zonalis*, numerical density : 1,500-68,900 ind/m², median diameter of sediment : 0.275-0.48mm, ignition loss : 1.49-1.73%

Partition III. No. of species : 4-7, dominant species : *Batillaria zonalis*, numerical density : 1,500-4,200 ind/m², median diameter of sediment : 0.21-0.23mm, ignition loss : 1.37-1.91%

Partition IV. No. of species : 8-18, dominant species : *Arenimitra exasperata*, numerical density : 9,400-77,400 ind/m², median diameter of sediment : 0.17-0.21mm, ignition loss : 2.35-3.16%

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桑原 連*：マングローブ林に囲まれた石垣島名蔵干潟のマクロベントス（底生動物）分布

マングローブ林の落葉由来有機物は河川水中の懸濁態粒子として干潟水域に運ばれデトリタスの形で沈積し、そこに分布するマクロベントスの餌料として生態系の二次生産段階に供されるものと予測される。名蔵干潟における1998年4月29日～5月1日のマクロベントス定量採集で得られた46種群、1幼生型について、ベントス食性に関する文献でその食性を照合すると、34%の種類がデトリタス食であった。デトリタス食種のみで再編成した種類数と個体数密度の分布は、干潟に流入する名蔵川河川水の直接影響域と重なり、落葉由来有機物の供給プロセスを示唆する結果となっている。水質・底質各項目の分布パターンもこれに沿った傾向を示した。

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The Role of the centroid in viviparous seeds of mangroves for transportation and dispersal

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Abstract : The position of the centroid in viviparous seeds of three mangrove species, having elongated hypocotyl, *Bruguiera gymnorhiza*, *Rhizophora stylosa* and *R. mucronata* were measured for the inquiry of the seed dispersal under natural conditions. The position of the centroid shown by the percentage of length from the proximal point of the hypocotyl to the total length was 48.9% in *B. gymnorhiza*, 63.1% in *R. stylosa* and 73.3% in *R. mucronata*, on average. When the detached seeds from the parent trees take the dispersal process in the field, the positioning of the centroid in the hypocotyl would be functional either by sticking to ground or floating in water for the settlement and germination on the ground. The operation was discussed with circumstantial evidence, and from the results of a diving experiment and the observation of the parent trees bearing the viviparous seeds.

Key words : viviparous seeds, centroid, mangrove

Introduction

The factors that effect the condition of the transportation for the detached viviparous seeds of mangroves are form, specific gravity and the position of the centroid. The former two elements have been noted by Macnae (1968), Rabinowitz (1978a), Nishihira and Urasaki (1983), Urasaki et al. (1986), Tomlinson (1986), and others. On the latter element i.e. the centroid, however, no description has been given up to the present. But perhaps, we may need the real evidence of where the centroid of viviparous seed is located, in relation to the basic studies of the other two factors.

On the dispersal of the viviparous seeds of mangroves for the development of the forest, the position of the centroid in the hypocotyl discharges its operations when detaching and after detaching. There are two styles on the first step of the dispersion, such as 1) sticking to the ground under the parent tree as in the "self-planting theory" by Dawes (1981) and Tomlinson (1986), and drifting in water mostly downward as the 'stranding theory' by Rabinowitz (1978a,b), Dawes (1981) and Tomlinson (1986). The criticism is seen in Van Speybroeck (1992). For both of the processes to settlement, the centroid must conduct some reasonable work of dynamics, particularly in the drifting in water. At this stage, the specific gravity is changeable and

the centroid may be engaged to the mechanism, which could not be concentrated in this study due to insufficient data. However, the considerations on the efficiency of sticking and floating for the dispersion of viviparous seeds required, in part, the location of the centroid. Because, at the basis, the centroid would be controlled by the form of hypocotyl as suggested by Rabinowitz (1978a), and after that, the changing of gravity in the tissue would modify the style of hanging in water. In this paper, the analysis of the centroid and the observation of the positioning in the tree in viviparous seeds were discussed by examining of three mangrove species.

Materials and methods

Viviparous seeds of three species having long hypocotyl, of *Bruguiera gymnorhiza*, *Rhizophora stylosa* and *R. mucronata* were obtained from the ground or at the bottom of the water around the parent trees, and by direct picking off the trees (only *B. gymnorhiza*) during the fruiting seasons. Namely, the hypocotyls of *Bruguiera gymnorhiza* were collected on March 26, 1997, *Rhizophora stylosa* on August 27, 1995 from Nagura lagoon, Ishigaki Island, and *Rhizophora mucronata* on March 3, 1997 in Pan-nga Bay, Thailand.

For the determination of the centroid, an entire

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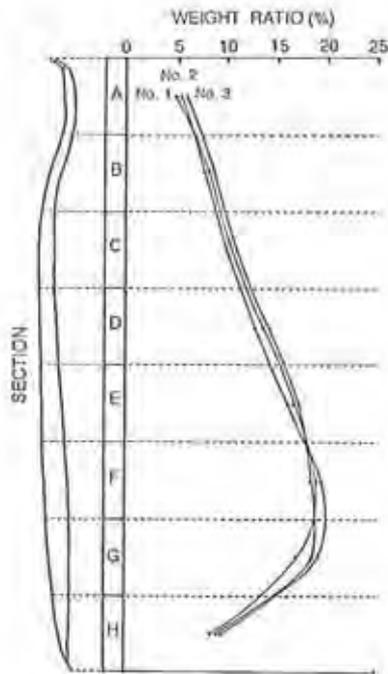


Fig. 1 Weight ratio of sections in the viviparous seed of *Burghiera gymnorhiza*. (Material No. 1 : 6.7cm length, 6.2 g; No. 2 : 9.9cm length, 8.3 g; No. 3 : 12.9cm length, 13.7 g)

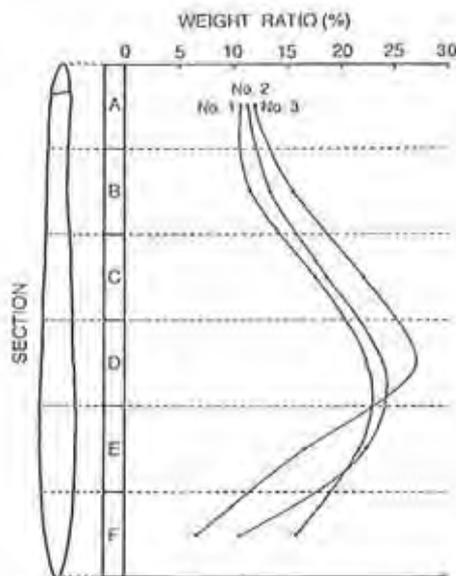


Fig. 2 Weight ratio of the sections in the viviparous seeds of *Rhizophora stylosa*. (Material No. 1 : 20.3cm length, 12.6 g; No.2 : 24.1cm length, 17.4 g; No. 3 : 29.2cm length, 27.6 g)

hypocotyl was cut off into six or eight cross-sectional parts of equal length, and each was weighed. The value as a percentage of the total hypocotyl weight was plotted on a section paper. And the position of the centroid was estimated from a curve tracing the plots (Fig.1-3). According to Maxwell (in press) who examined the buoyancy of the hypocotyl of *Rhizophora stylosa* collected from Nagura lagoon, it has held its horizontal floating position for several months in salt water without an additional increase of weight to its body. Therefore, any changes in the centroids of the studied materials would be rather few, even if they show the individual differences.

As related to the positioning of the hypocotyl in the tree, the frequency of seeds that made a successful sticking to wet medium sand (median diameter = 0.43mm) by throwing down from various heights, and range of height found seeds in pioneer trees were examined to *R. stylosa* on August 27, 1995 and May 1, 1998 in Nagura lagoon. Additional observation on the height of seeds borne in examined trees was done on March 26, 1997 in the Experimental Field of Iriomote Station of Tropical Biosphere Research Center, University of the Ryukyus, Iriomote Island.

The grain size analysis of the sediment was followed using a regular method in geography (Briggs, 1977).

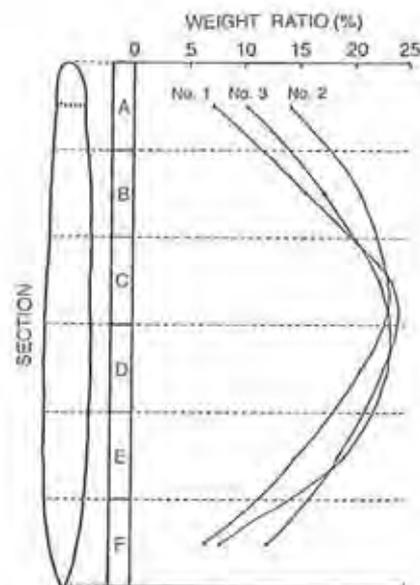


Fig. 3 Weight ratio of the sections in the viviparous seeds of *Rhizophora mucronata* (Material No. 1 : 61.6cm length, 107.0 g; No. 2 : 62.1cm length, 119.6 g; No. 3 : 64.6cm length, 113.4 g)

Results

An illustration of weight changes in the separated parts of the hypocotyl was shown to obtain the centroid in each examined species as in Fig.1-3. Using these, the position of the centroid was exhibited by the percentage of the length from the proximal point of the hypocotyl to the total length. It showed 48.9% in average in *B. gymnorrhiza* ($N = 9$, av. 11.6cm, 12.6g except part of cotyledon), 63.1% in *R. stylosa* ($N = 18$, av. 22.3cm, 17.4g), and 73.3% in *R. mucronata* ($N = 3$, av. 64.9cm, 113.3g). The results indicate that a form of hypocotyl showing antero-posterior symmetry (ex. *B. gymnorrhiza*) has the centroid at almost the middle position, and another asymmetric form swelling at the distal direction (ex. *R. stylosa* and *R. mucronata*) situates its centroid toward the distal side. By comparing both *Rhizophora* species, as seen in the values of centroid positioning, the larger seed is likely to have the centroid at a more distal portion than the smaller seed. The difference should be attributed to some advantages of dispersal by floating as discussed later. Seeing the relationship between the size of the seed and the position of the centroid among species and individuals in a species (Fig.4), a fact that there is no clear tendency within each species, but a resemblance between species of similar form as in *Rhizophora* species is recognizable.

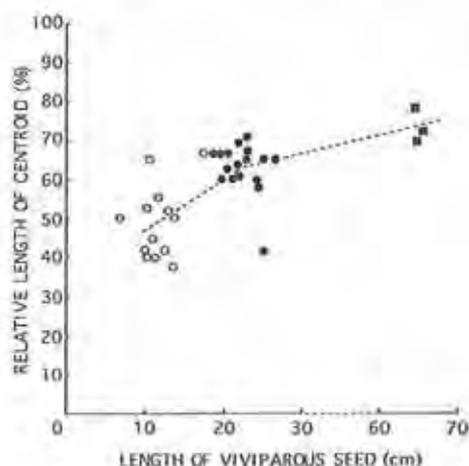


Fig. 4 Relation between the seed length and the relative length of the centroid from the proximal point of hypocotyle in three species of mangroves (○ : *Bruguiera gymnorrhiza*, ● : *Rhizophora stylosa*, ■ : *Rhizophora mucronata*, the broken line connects the each mean value for three species)

The positioning of the centroid is an important factor in rewarding the drop from the tree for the dispersal, with the pointing of the distal end, as already mentioned by local scientists. Then, a trial to drop collected seeds of *Rhizophora stylosa* from various heights of the air was examined to find the probability of its successful standing on the surface of medium sand, in Nagura lagoon, Ishigaki Island. The result showed a high frequency of success was almost within the range of 1.0 to 1.5m heights (Fig.5). The slightly curved form of the seed caused irregular dropping and unsuccessful standing on the sand when it dived through the air. Nevertheless, the result was partially significant, supported by an observation of seed number rate in tree-height ranges of *B. gymnorrhiza* and *R. stylosa* in Iriomote Island during the fruiting period (Fig.6). It showed that the high frequency of observed seed numbers has a range of 1.0-2.0 m in tree height for the both species and almost coincided with the result of the diving test of Fig.5, accommodated to a successful height. The effect on the difficulty of a successful drop is understood so that the seedling at a higher part is unable to drop directly because of crowding branches and that at a lower part has a lesser gravitational force which allows it to stick to the surface of the land.

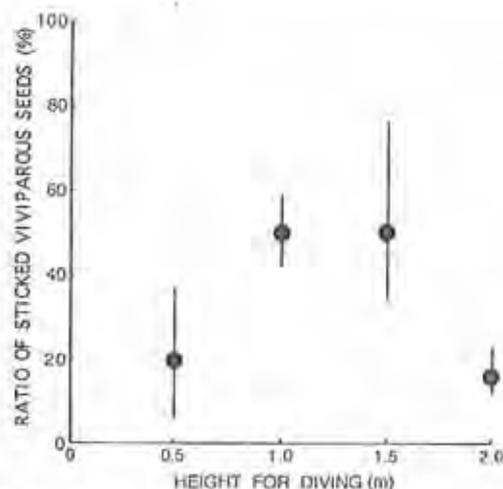


Fig. 5 Frequency of the artificially stuck viviparous seeds from various heights in *Rhizophora stylosa* on August 27, 1995 in Nagura lagoon, Ishigaki Island (vertical bar : range, black circle : mean value, the substratum : wet medium sand)

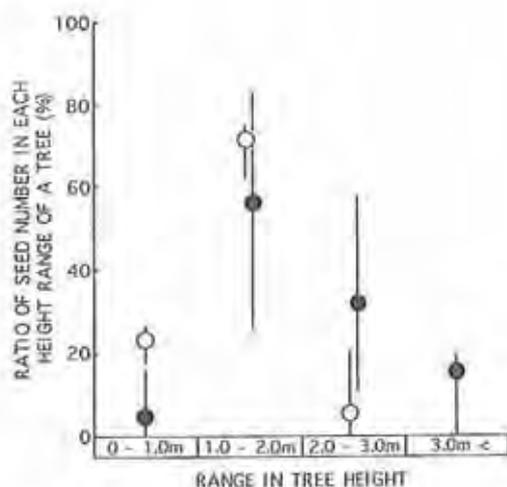


Fig. 6 Positional composition of the numeric rate of seeds borne in *Bruguiera gymnorrhiza* (bar : range, ○ : mean), and *Rhizophora stylosa* (bar : range, ● : mean), observed on March 26, 1997, in Funaura, Iriomote Island

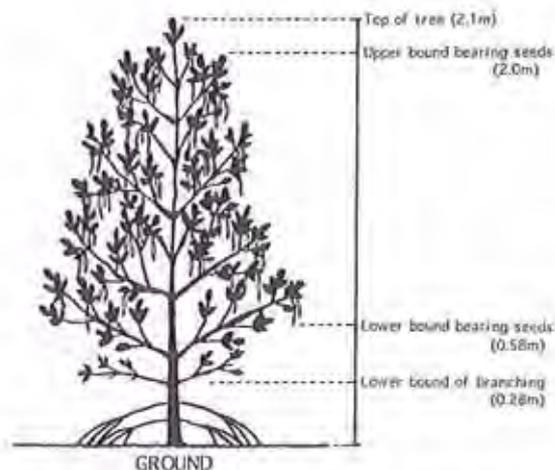


Fig. 8 A pioneer tree of *Rhizophora stylosa* showing a range of the height bearing the viviparous seeds at the fruiting time, May 1, 1998, in Nagura lagoon, Ishigaki Island

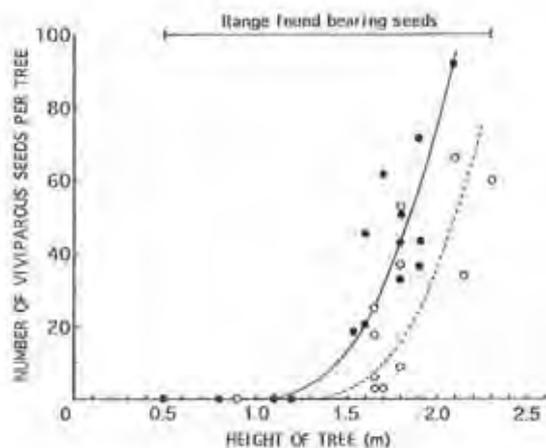


Fig. 7 Relation between the height of tree and the number of viviparous seed in the pioneer trees of *Rhizophora stylosa* on May 1, 1998, in Nagura lagoon, Ishigaki Island

Another observation of seed position was made for fruiting pioneer trees of *R. stylosa* in two tidal zones of Nagura lagoon. The result was shown as the relationship between the height of the tree and the number of seeds borne in a tree (Fig.7). There, the smaller trees of less than 1 meter in height had no seeds, but the larger trees of more than 1.6 meters in height substantially increased seeds with a growth in tree height, though there was a difference of the regression between two observed zones, as land ward and seaward. In the pioneer trees, the range of height bearing the seeds was very wide (Fig.8). But it was mostly installed within a range mentioned on Fig.6.

Discussion

The dispersal of viviparous seeds of mangroves is dependent on physical factors for its transportation, such as the flow of river water, tidal current, the force of stormy weather including mixed factors, and others. On the other hand, the form of the hypocotyl showing some features such as being elongated, curved, or pointed at the distal tip is also affected by the environment. Rabinowitz is one of a few scientists who mentioned the form of the seeds in relation to the distribution of mangrove forests. She found some genera having small seeds such as *Laguncularia* and *Avicennia* distributed on higher ground of a landward zone and genera having large and heavy and elongated

hypocotyle such as *Rhizophora* and *Pelliciera* in a swampy seaward zone, influenced the floating behavior for the transportation (Rabinowitz, 1978a). Urasaki et al. (1986) pursued the settlement pattern of the floating seeds in *Kandelia candel* (Rhizophoraceae), which included change of the specific gravity in the process of the germination. In the present study, remarkable points on the form of viviparous seeds should be such as:

- 1) size; large or small.
- 2) longitudinally symmetric or asymmetric,
- 3) curved or not, and
- 4) pointed distally or not.

These are concerned with the positioning of the centroid. Firstly, the small and shorter hypocotyl of *B. gymnorrhiza* has its centroid at the center of the long axis. It is not required for the straight drop and sticking, with the rounded end of the seed. And, as mentioned by Tomlinson (1986), cotyledons of *Bruguiera* do not separate from the hypocotyl even after detachment from the parent tree. I have often observed the same condition on *B. gymnorrhiza* on the ground. But whether it is kept through the drifting time in water or not was unconfirmed. When the seed of *B. gymnorrhiza* is accompanying cotyledons in floating time, its longitudinal symmetry and the centroid in the central position are unlikely to be functional. Because, a slight addition of weight to one side of the long axis can easily cause it to stand vertically in water.

Regarding comparison of the size of seeds for the studied two species of *Rhizophora*, the centroid of the larger *R. mucronata* was at a more distal part than smaller *R. stylosa* as seen in Fig.4. In the floating condition, seeds of this genus drift taking inclined style with the proximal end being toward the surface of the water. The difference of the centroid between the species may be counteractive to buoyancy.

In every species having elongated hypocotyl, it curves more or less along the long axis. I think that the reason should not be attributed to adaptation for the transportation by floating. As mentioned by Tomlinson (1986), the characteristic curvature in the hypocotyl of *B. parviflora* is helpful to erect the axis on the ground, which will be a part of the establishment strategy. Otherwise, as it is well known among fruit culturists, the flow of nutrient supply from the roots of a tree to its branches and flowers leans toward the underside when those are inclining, probably by the force of gravity. They know that trees standing perpendicularly have difficulty to ripen fruit. Thus, by the

effect of an unbalanced nutrient supply, many kinds of fruit show more or less a curved form. A slight curvature of the hypocotyl in *Bruguiera* and *Rhizophora* was presumed as the result of an unequal expansion of the cortical cells (Tomlinson, 1986). Therefore, on the contrary, we have to consider why viviparous seeds of mangroves should have approximately a straight form. A probable concept would be attributed to a summarization on the transportation of the seeds of various sizes, in relation to the environmental considerations mentioned by Rabinowitz (1978) as above. The state that the elongated hypocotyl of *Rhizophora* group provides the straight form might help in hanging vertically in water for the preparation of the coming settlement. As discussed by Urasaki et al. (1986) and Nishihara and Urasaki (1983), the floating seed of *Kandelia candel* varies the angle to the water surface from horizontal to vertical, taking several stages with a change of the specific gravity. Its straight form will be adaptive to vertical standing and traversing in deeper water with use of the pointed tip which makes hold when the depth of water decreased. Also positioning of the centroid controls the behavior. In the shorter seeds having a round tip as in *B. gymnorrhiza*, a change of the specific gravity, if it is possible, in a side of the long axis, can easily make a vertically hanging style in water, because its centroid is located in the middle of the axis as seen in Fig.1. However, a buoyancy test of *Rhizophora stylosa* hypocotyl by Maxwell (in press) showed its horizontal floating in 2.3% salt water has continued for six months which means no more gravity change than the moving of the centroid (if it has) to resist the buoyant force of the water.

It is a general opinion as explained by Baba (1990) that the germinated seedling standing under the parent tree does not result from sticking but mostly from rising up by help of the radicles after placement. Also he did not deny its probability of the chance to stick, as the same as my attention to the pointed hypocotyl of *Rhizophora*. However, the main conduct of transportation on the seed dispersal would be floating in water as mentioned by many reporters (Baba, 1990 ; Rabinowitz, 1978a, 1978b ; Tomlinson, 1986; Tagawa, 1982a, 1982b ; Urasaki et al., 1986), and by an indirect expression of Odaki (1997) who tried an artificial diving of detached seeds of *Kandelia candel*, but it was seldom successful. As already shown in Fig.5, the trial to dive on seeds of *R. stylosa* was partially effective to stick on the ground, though it showed a lower rate of

success. It might be dependent on the larger size with pointed distal end of the hypocotyl in this species.

A remarkable finding was that there was no settlement of seeds stuck under every pioneer tree of *Rhizophora stylosa* bearing a lot of viviparous seeds in the Nagura lagoon. Looking at the condition as shown in Fig.8, it is suggested that the success of the seed sticking would be at a very low probability because the illustrated 2.1 m tree has been bearing 66 seeds without any germinated young trees of previous years at the surface under the crown. As there is a sandy tidal flat, the flushing out of the dropped seeds might be a special case in the normal forest vegetation of *R. stylosa* located in a muddy zone. Meanwhile, Yamashiro (1961) observed that 11% of *Kandelia candel* seeds fall straight into mud, while 89% finish up flat and later become standing which showed that a high probability of the dispersal depends on once floating. Thus, I cannot but understand that most of the detached seeds of *Rhizophora stylosa* found in Nagura lagoon once have been transported by estuarine flush or tidal current. Thus, the floating would be recognized as the main style to disperse viviparous seeds in mangrove species having elongated hypocotyl. Nevertheless, remarking the riverine distribution of the mangrove forest, its considerable depth apart from the riverine area suggests that the extension of the forest has not only resulted from the floating of seeds.

There have been few researchers who have judged which are more important, either sticking or floating, of the viviparous seeds for the efficiency of new germination and growth. Tomlinson (1986) did not assign an adaptation for viviparous seedlings to a single factor, because the mangal environment itself is accepted as a combination of factors. If the style of the dispersal is limited to the seed's sticking on the ground, new germination is limited to a small area under the crown of the parent tree. And if it is so, the development of the forest goes very slowly by repeating new germination making small extensions of the forest fringe. Therefore, sticking only to the ground is surely inefficient for development of the forest. But it may be useful to the gradual replacement of the forest generation, mostly inside the forest.

The role of the centroid in viviparous seeds is the potential to both sticking and floating, but is a considerable element for theoretical development of the viviparity.

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桑原 連* マングローブ胎性種子の重心がその輸送に果たす役割

マングローブの胎性種子が落下して河川水に運ばれ或いは直接に地面に突き刺さり、新たな出芽・成長によって次世代の樹林形成に係わる要因として、その重心の位置が関係するであろう。そのために、オヒルギ、ヤエヤマヒルギ、オオバヒルギの胎性種子を材料として、胚軸の等分割区分の重量比を結ぶ曲線から重心を求めた。また、“突き刺さり”の効果を各高度からの落下実験および樹上の実生種子の位置などから検討し、それも有効であるが、むしろ水に浮いて輸送されることが伝搬手段として大きな意味を持つことを論じた。

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Morphometric studies on the leaves of *Bruguiera gymnorrhiza* and *Rhizophora stylosa* in relation to the numerical litter decomposition process

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Abstract : Morphometric and volumetric measurements of eleven items in the leaves of *Bruguiera gymnorrhiza* and *Rhizophora stylosa* were done by materials collected from the Nagura lagoon, Ishigaki Island, southwest Japan. Using the results, the number of decomposed silt-size particles for a leaf was calculated as 3,716,581 in *Bruguiera gymnorrhiza* and 3,185,641 in *Rhizophora stylosa* respectively. The object to obtain these values was to estimate the quantitative output of organic particles decomposed from leaves of mangrove to estuarine waters as a food resources of the benthic animal community, particularly of filter-feeders. The particle number of a leaf obtained is, therefore, a basement of the further step of the study.

Key words : morphometry, mangrove leaves, decomposed organic particles, Ishigaki Island

Introduction

In a previous paper, the author estimated the litter productivity of mangroves in a mixed forest of *Bruguiera gymnorrhiza* and *Rhizophora stylosa* by counting the leaves of living trees (Kuwabara and Shiroma, 1996). The objective was to obtain the quantitative output of organic particles decomposed from leaves of mangrove trees to estuarine waters as a food source of the benthic community, particularly of filter-feeders. This process has been suggested by Odum and Heald (1975) as a part of the process of primary production replacing phytoplankton in an estuarine ecosystem. If the number of decomposed leaf particles is known, the supply to a productive zone of benthic organisms can be estimated from the number of trees in a measured area of mangrove forest at any given time by assuming their transport by estuarine water flow and the turbidity. For the purpose of completing the process, the author needs to know how many particles can be produced from a mangrove tree leaf. As the number of leaves per tree can be estimated from the tree height (Kuwabara and Shiroma, 1996), the next step involved a study of the material flow.

The volume of a dried mangrove leaf can be used as a base to calculate the amount of particles decomposed from it. However, the volume of dried leaves is very

difficult to investigate due to irregular shrinkage during oven-drying. Hence, the volume was measured indirectly from the difference between green leaf volume and the water content available from the drying. The study does not actually trace the transfer of produced organic particles in the energy flow. It presents the estimated level at an optimum supply through calculations. As a supplementary work to the previous report (Kuwabara and Shiroma, 1996), the study involved morphological analyses on leaves of two mangrove species, *Bruguiera gymnorrhiza* and *Rhizophora stylosa*.

Materials and Methods

The materials were randomly collected from several trees of *Bruguiera gymnorrhiza* and *Rhizophora stylosa* in a mixed mangrove forest in Nagura estuary (24° 24' N, 124° 8' 30" E), Ishigaki Island, southwest Japan on August 27, 1996. Eighty leaves of each species were examined for morphometric and volumetric characteristics. Morphometric characteristics included leaf length, leaf breadth, petiole length, the ratio of leaf breadth to leaf length, and the ratio of leaf length to the petiole length (Fig.1, I). Volumetric measurements included green leaf weight (wet w.), dry weight, wet volume, dry volume, surface area, and water content for calculation of dry volume.

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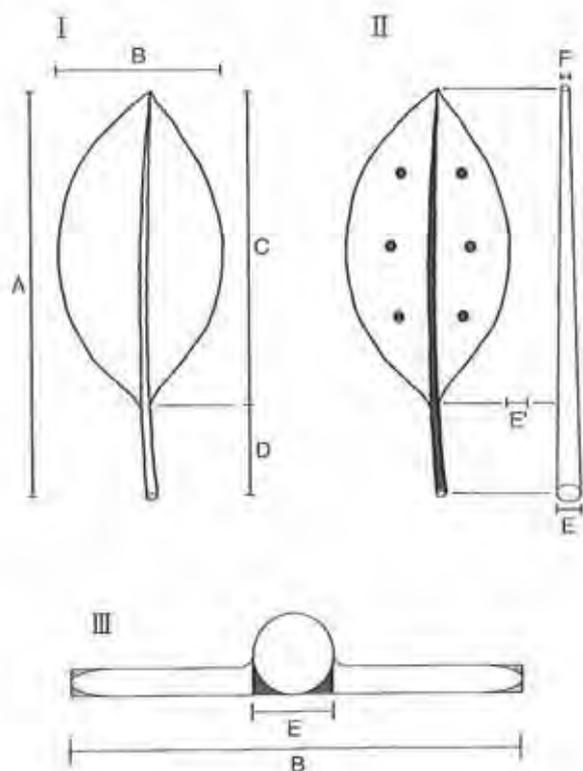


Fig. 1 Recording length measurements of a mangrove leaf.

I : plane figure, II : positions for the thickness and the midrib diameter measurements, III: longitudinal section,
 A: midrib length with petiole, B: leaf breadth, C: leaf length, D: length of petiole,
 E: diameter of petiole at the base,
 F: diameter of midrib at the tip.

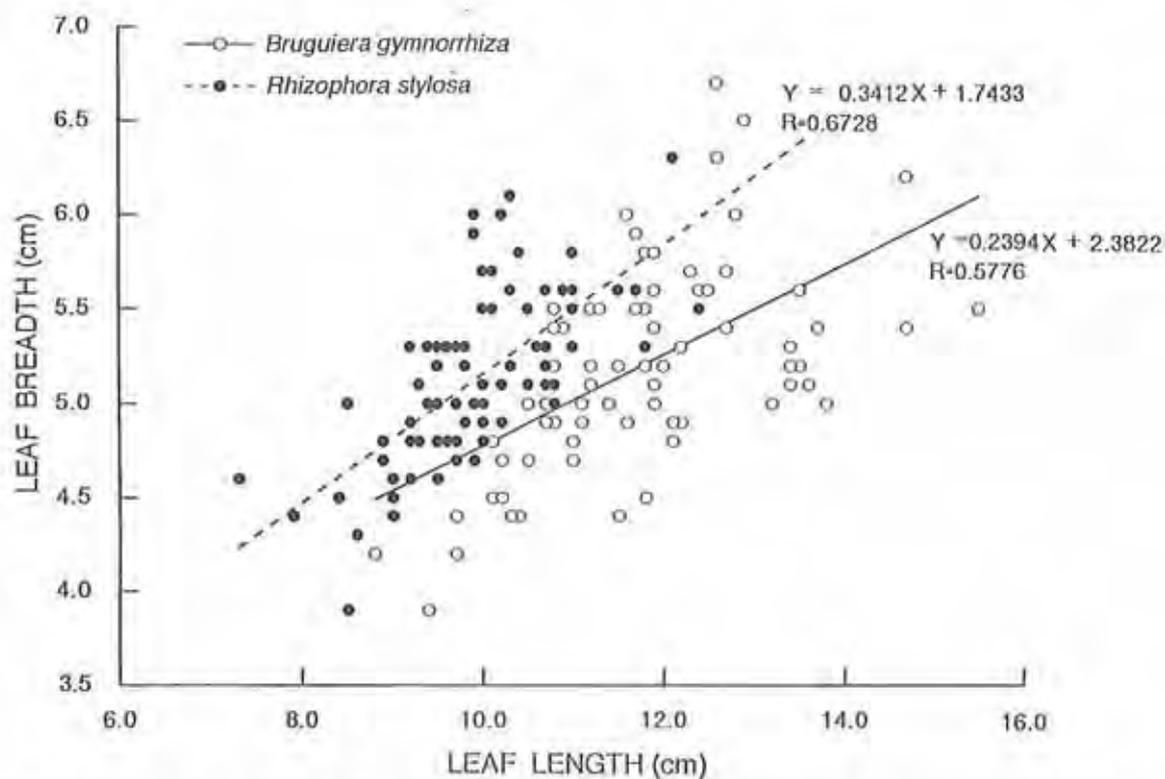


Fig. 2 Relationship between leaf length and leaf breadth in *Bruguiera gymnorrhiza* and *Rhizophora stylosa*

The dry weight of a green leaf was determined by drying it at 105°C for 4 hours in an oven. The wet volume of a green leaf was calculated from the measurements of its surface area, thickness, and midrib volume. The surface area was estimated by a paper weight method which calculates the ratio between the weight of paper cut off along the outline of the leaf and the weight of the same type of paper of which the surface area was known. The weight of the paper traced for each leaf was shown as the average of the measured values of six papers. The leaf thickness was measured at six points evenly distributed on the leaf (Fig.1, II). The total average from 20 leaves of each species was 0.44mm in *B. gymnorrhiza* and 0.53mm in *R. stylosa* and along the estimated surface area were used to calculate the volume of a cube. The volume of the midrib with petiole was calculated separately as a frustum with the length of the midrib as the height (Fig.1, I - A), the bottom area obtained from the diameter of the petiole at the base (Fig.1, II - E), and the top area from the diameter of the midrib at the top (Fig.1, II - F). The diameter at the top of the petiole (Fig.1, II - E) was just on the side-line of the midrib's cast shadow and was deleted from the calculation. A hidden area was previously excluded from the surface area of the leaf during the calculation of the frustum volume (Fig.1, III, black zones). The calculation was offset by inclusion of over-calculated zones at the fringe of the leaf (Fig.1, III, oblique lines) against the surface area of the leaf cubically calculated. The calculations of each partial volume were finally combined with the volume of a wet leaf.

As the dry volume of the leaf could not be determined due to irregular shrinking during drying, it was obtained from the difference between the wet volume and the volume of water content as (wet weight-dry weight) \times 1.002 being density of pure water at room temperature, for each leaf. The water content of the green leaf was obtained from the weight difference between wet and dried leaves.

Results and Discussion

I. Morphological description and morphometry

The results of morphometric and volumetric measurements of eleven items in the leaves of *Bruguiera gymnorrhiza* and *Rhizophora stylosa* were summarized in Table 1. As the samples were randomly collected from well-grown trees, the difference of values between two species would be characteristic of a leaf

Table 1 Morphometric measurements of leaves in two mangrove species:

Items	<i>B. gymnorrhiza</i>	<i>R. stylosa</i>
Leaf length (cm)	11.68 \pm 1.26	9.92 \pm 0.91
Leaf breadth (cm)	5.17 \pm 0.52	5.12 \pm 0.45
Length of petiole (cm)	3.46 \pm 0.63	2.04 \pm 0.38
Leaf length : Leaf breadth	1 : 0.45 \pm 0.04	1 : 0.52 \pm 0.04
Length of petiole : Leaf length	1 : 3.45 \pm 0.54	1 : 5.05 \pm 1.32
Wet weight (g)	2.29 \pm 0.64	2.76 \pm 0.47
Dry weight (g)	0.67 \pm 0.21	0.91 \pm 0.18
Wet volume (cm ³)	2.30 \pm 0.45	2.46 \pm 0.41
Dry volume (cm ³)	0.70 \pm 0.19	0.60 \pm 0.18
Surface area (one side, with petiole, cm ²)	43.22 \pm 7.86	39.12 \pm 6.04
Water content (%)**	70.66 \pm 2.98	67.06 \pm 4.56

*N=20 for each species. **Weight percentage

specific to species of the both.

The shape of a mangrove leaf can be oblong or narrowly oblong as in *B. gymnorrhiza* and elliptic as in *R. stylosa* (Nakamura and Fukuoka, 1990). Hence, there is no clear difference in shape for distinguishing between the species. But, the difference is easily recognizable in macroscopic observation, since the leaf of *R. stylosa* is broader than that of *B. gymnorrhiza*. As seen in Table 1, the leaf of *B. gymnorrhiza* is longer than *R. stylosa*, though the breadth is almost the same for both. This can be seen from the calculated ratio of the leaf length to the leaf breadth, showing 1 : 0.45 in *B. gymnorrhiza* and 1 : 0.52 in *R. stylosa*. Correlation analysis in present materials also indicated such differences (Fig.2). Bigger leaves tend to be broader as shown by differences in the slope of the regression lines. The petiole is relatively longer in *R. stylosa* than in *B. gymnorrhiza* as shown in its ratio to leaf length being 1 : 0.52 in the former and 1 : 0.45 in the latter. Another difference between the species is the existence of brown dots on the ventral side of the leaf of *R. stylosa* while that of *B. gymnorrhiza* is plain.

As seen in the comparison of volumetric items of the leaves between the two species (Table 1), wet and dry weights and wet volume showed higher values in *R. stylosa* than in *B. gymnorrhiza*. On the other hand, dry volume, surface area and water content were higher in *B. gymnorrhiza* than *R. stylosa*.

2. Amount of particles decomposed from a leaf

In estimating the amount of decomposed particles from a leaf, the value of the dry volume (Table 1) was used as a cube, namely $0.70 = 0.8872^3 \text{cm}^3$ in *B. gymnorrhiza*, and $0.60 = 0.8146^3 \text{cm}^3$ in *R. stylosa*, showing the number of a cubic root. The number of particles present in a cube can be estimated as:

$$N = (\sqrt[3]{V} \times 10 / r)^3 = V \times 10^3 / r^3 \quad (1)$$

where N : amount of particles

V : volume of cube (cm^3)

r : diameter of particle (mm)

and a side of a cube is $\sqrt[3]{V} \times 10$ (mm). When the size of the particles is assumed to be uniform, the vacant space index of a cube is derived from the packing index $\sqrt{2} \pi / 6$ (Kittel, 1976), such that $1 - \sqrt{2} \pi / 6 = 0.26$. Then, an additional number of particles rearranged from the volume of the vacant space is

$$N' = 0.26V \times 10^3 / r^3 \quad (2)$$

Because there are considerably less particles in vacant space than in packed space, twice the additions using rearranged cubes from the vacant space would be sufficient. Therefore, the final calculation is

$$N = (V + 0.26V + 0.26 \times 0.26V) \times 10^3 / r^3 \quad (3)$$

The diameter of particle (r) must be specified from an actual condition. Photo 1 shows microscopic particles of suspended matter filtered from water of the Nagura river surrounded by a large mangrove forest (Photo 1, A) and detritus washed out from the bottom sand in a part of the mangrove covered (Photo 1, B). This indicates that decomposed particles attain a smaller size below 0.1mm diameter. Based on the classification of standard size grades of sedimentary particles (Briggs, 1977), it is assumed that particles having a diameter of 0.063mm are at the upper limit of coarse silt. This is generally considered a normal sized particle providing a moderate sinking speed does not easily allow particles to be supplied to estuarine bottoms for benthic production. Nor does it allow particles to further decompose to the finer grains needed for food for the benthos.

Using $r = 0.063$ and $V = 0.70$ in *B. gymnorrhiza* and 0.60 in *R. stylosa* to equation (3),

$N = 3,716,581$ and $3,185,641$ respectively, an average of $N = 3,451,111$ is obtained. A mixed forest of the both

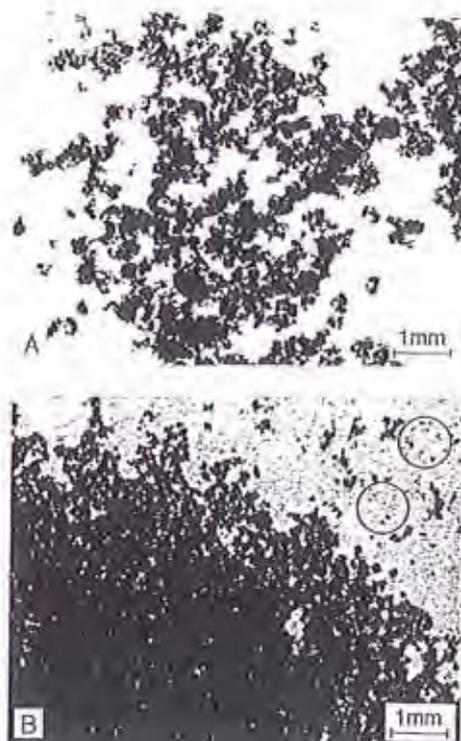


Photo. 1 Suspended matter in the river water (A) and detritus washed out from the bottom sand (B) of the Nagura lagoon, Ishigaki Island (Circle indicates particles of silt-clay size)

species contains approximately $3,451 \times 10^3$ silt-size particles per leaf.

3. Amount of particles contained in turbid water

It is undeniable that the turbid river water flowing among mangrove forests supplies the decomposed particles of mangrove leaves, to the downstream and the estuarine area. It would be particular in the 'riverline forest' classified by Lugo and Snedaker (1974), which is suffered washing by tides and land surface drainage. On the assumption that the turbid substance is only the decomposed particles of mangrove leaves, an estimate of how much particles are contained in a known turbidity is necessary to understand the process of mangrove litter supply to estuarine ecosystems.

In general, turbidity is shown by NTU, equal to mg/l which means some amount of dried substance per liter of water. Accordingly, using values of the dry weight in Table 1, one liter of water showing 10 NTU is equivalent to 1.5% and 100 NTU to 14.9% of a dried leaf of *B. gymnorrhiza*. Likewise, one liter of 10 NTU

is equivalent to 1.1% and 100 NTU to 11.0% of a dried leaf of *R. stylosa*. If a flux of river water and the turbidity are known under the assumption that the turbid substance is derived from decomposed particles of mangrove litter, the supply of the particles to a domain of benthic community can be estimated as an energy flux for the ecosystem. These trial is only a step of the process of the decomposed litter supply to the estuarine ecosystem, for the future study.

Conclusion

The litter production of trees is generally expressed in an amount per hectare. Its flux into the ecosystem is often estimated on a macro-scale area. Nevertheless, the receiver is usually not uniform but variable within the area including semi-divisions geomorphologically and hydrologically. Therefore, for understanding the consideration of optional mangrove forest, the smallest available unit as the base of litter supply would be a leaf. As the author has already estimated the litter productivity of mangroves in a mixed forest of *Bruguiera gymnorhiza* and *Rhizophora stylosa* at a riverine area of the Nagura lagoon (Kuwabara and Shiroma, 1996), how many leaves are possessed in a tree of mangroves is approximately known.

This study investigated a part of the process of numerical litter decomposition focusing the estimation of the leaf amount. Although, even on a macro-scale analysis, each calculation flow contributed insights into subsystems for benthos as the consumer of litter-derived particles in the estuarine water still could not be confirmed. As the next step to complete the litter flow process, it is necessary to subdivide the lagoon area according to the distribution pattern of benthic communities.

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桑原 連* : オヒルギおよびヤエヤマヒルギ葉体形質の計測と落葉分解粒子量の推定

沖縄県石垣島名蔵ラグーン域で採集したオヒルギ及びヤエヤマヒルギの成長した葉体の各形質すなわち葉長・葉幅・葉柄長・それらの比率、並びに生重量・乾重量・生時の体積・乾燥体積・表面積・水分含有率を測定した。

一方、マングローブ落葉分解粒子が河川水で移送され濾過摂餌型ベントスなどの二次生産者に供給されるエネルギー収支を考えると、一枚の葉から生じる粒子数を基本とする必要がある。葉体各形質の測定値を用いて計算した粒子数は、オヒルギでは3,716,581粒、ヤエヤマヒルギでは3,185,641粒であった。

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短報 Short Communication

Chill shock tolerance differentiates Hong Kong and Thai ecotypes of *Avicennia marina*

Gordon S. MAXWELL¹⁾

On 22 December 1999, HKSAR experienced its coldest winter solstice on record, when temperatures fell to 6.7°C in urban areas and < 4.0°C in some elevated and exposed low lying areas in the countryside. This was followed by the coldest Christmas Eve ever recorded at the Hong Kong Observatory, which is located in urban Kowloon. On the night of 24 December 1999, the temperature reached 7.0°C and shattered the previous lows recorded on December 24 in 1904 and 1929, when the temperature was 7.1°C (South China Morning Post, 24. December 1999 and Hong Kong Observatory). Cold snaps have occurred before e.g. in 1893 (Skertchly, 1893) and 1948 (Bannister, 1948) with reports of frost-covered hills in January and, more recently, on December 28, 1991 when a low of 4.6°C was recorded at the urban site of the Hong Kong Observatory. On this occasion, in 1991, these unusually cold temperatures were quite widespread in the South China region, when the associated cold front extended from just southeast of Japan and the Ryukyu Islands to the southern tip of Taiwan and to the Vietnam coast, where Hanoi recorded 9°C (Peart and Dongshen, 1992).

In Hong Kong, such cold spells or snaps can have harmful effects on poultry and pond fish, resulting in mortalities (Cheng, 1970). In December 1999, the HKO issued a frost warning to alert farmers, especially vegetable crop growers to step up crop protection measures. In anticipation of a future cold shock, an experimental transplant of both local (Hong Kong) and tropical (Thai) seedlings of the mangrove *Avicennia marina* had been set up on 21 October 1999, at Kei Ling Ha Hoi (Three Fathoms Cove), a mangrove fringed Bay located along the Eastern Seaboard of HKSAR (22° 25' 08" N and 114° 17' 02E). A thermohydrograph (Sato, Sigma-II Model NS-11 No.7210)

was protected by a semi-open box cage made from drift wood and placed under coastal vegetation ~ 1.5 m above Mean High Water level within 20 m of the transplant site. Mature *Aegiceras corniculatum* and *Lycoecaria agallocha* trees of 2.4 and 3.3 m tall respectively, were adjacent to the transplant site. The *Avicennia marina* seedlings had been grown to the 6 to 8 leaf stage in a potting mix of sand, mud and small pebbles previously taken from the target transplant habitat (Kei Ling Ha Hoi, KLHH) in preparation for the transplant experiment.

The principal attributes of these seedlings are shown in Table 1 together with post December cold shock survival rates. As Table 1 shows, the results are dramatic. The seedlings displayed overt differences in symptoms of chill shock. The local seedlings exhibited some foliage dieback (around 3 out of 8 leaves showed marginal curling and discolouration especially in the youngest leaves) but retained a vertical stem and the ability to regrow replacement foliage. Those in the Thai group however, quickly succumbed to a post chill shock : they wilted with stem collapsing to a horizontal position and leaves blackening within 3 to 10 days following the chill shock.

Corlett (1992) and Maxwell (1993) had recorded similar foliage dieback patterns with *A. marina* at Mai Po and Tsim Bei Tsui (22° 29' 14" N and 114° 00' 51" E) in the north west New Territories of HKSAR. Using both Bruneian (north Borneo, 05° 02' N and 115° 01' E) transplants of *A. marina* and *Kandelia candel*, Maxwell (1993 and 1995) also demonstrated marked differences in chill tolerance at Mai Po (Hong Kong) between true tropical (Brunei) and subtropical (Hong Kong) ecotypes within these species. The differences being most pronounced with *Avicennia marina* : the

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Table 1 Differential survival rates of local and Thai ecotypes of *Avicennia marina* seedlings exposed to an overnight temperature of 3.8°C at Kei Ling Ha Hoi (KLHH) Sai Kung Hong Kong on 22 and 25 December 1999.

Ecotype	# in field (KLHH)	\bar{X} leaf no. per seedling (\pm SD)	\bar{X} stem length cm (\pm SD)	age of seedling (months)	no. surviving † (%)
Hong Kong*	10	6.7 \pm 0.4	19.13 \pm 1.19	5	10 (100%)
Thai	10	8.0 \pm 0.4	30.67 \pm 0.88	7	0 (0%)

* Hong Kong populations of *A. marina* propagules were obtained in August 1999 from both KLHH and Chok Keng in Sai Kung East Country Park. The Thai propagules were collected from Samat Prakan (13° N) near Bangkok, Thailand.

† no. surviving refers to number alive (erect stems, normal non-blackened/discoloured foliage on 25 Feb. 2000, when the experiment was deemed completed).

Bruneian transplants, which were three years old in 1991, defoliated and died following the December, 1991 cold snap. This response contrasted with the local, Hong Kong, ecotype of *A. marina* which exhibited some foliage discoloration and dieback especially in the young foliage, but was able to recover. Interestingly, the *Kandelia* transplants, which came to Hong Kong from Thailand and Brunei (Maxwell, 1993 ; 1995) survived, despite exhibiting some foliage dieback. As with the local *Avicennia marina* ecotypes, those of *Kandelia candel* were able to cope with these limited cold snaps. The full ecogeographic and eco-physiological dimensions of these observations remain to be elucidated. In review literature (e.g. Hutchings and Saenger, 1987 ; Sakai and Larcher, 1987 and Lüttge, 1997) and even in the limited research literature (e.g. Sakai and Wardle, 1978 ; Markley et al, 1982) the chill tolerant species of *Avicennia*, i.e. *A. marina* and *A. germinans* tend to be treated and discussed as if they were single uniform species, especially with respect to ecotypicity. Today, the notion of ecotypicity including differential cold tolerance remains with few exceptions (e.g. Maxwell, 1993 ; 1995) a relatively neglected area.

Some recent papers hint at the possible biogeographic limits and associated cool temperature tolerance of mangroves at their extreme latitudinal ranges (e.g. Suzuki and Saenger, 1996). Recent work reported by the writer (Maxwell, 1999) before the Academic An-

nual General Meetings of JAM together with this present paper, may help to stimulate more interest in chill shock on mangroves. At present, aspects of this Hong Kong based investigation of differential chill shock tolerance in geographically isolated populations of *Avicennia marina* are ongoing. The report here is essentially a topical progress report on work that is continuing. These findings do however, underscore the unique nature of Hong Kong as a biogeographically special place. For example, where else in the tropics are such cold snaps (of \sim 4-5°C) experienced at sea level as they did on 22/23 Dec 1999 ? The answer is probably no where else ! At present (winter of 2000 / 2001) more chill shock experiments are underway. These involve the New Zealand as well as Hong Kong and Thai populations of *Avicennia marina*. A full report of this work will be the subject of an additional paper.

It is sincerely hoped that this short communication will perhaps encourage scientists in the Japanese biogeographic region to, for example, conduct transplant experiments with mangroves from the global northern limit at Kiire (31° 22N), Kyushu Japan (Suzuki and Saenger, 1996) to both subtropical (e.g. Hong Kong) and tropical locations (e.g. Thailand) and investigate both cold and hot thermal tolerance regimes. As suggested by Maxwell (1993, 1995 and 1999) field based studies of mangroves at their biogeo-

graphic extreme is a fertile area of research. With projected extreme changes in climatic patterns in the future, such work may have considerable importance to our knowledge of the sustainable conservation and management of coastal vegetation resources worldwide.

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