行政院國家科學委員會專題研究計畫　成果報告

南灣海域松藻及海葵族群大量擴張的生態機制及珊瑚群聚復育的研究﹝3/3﹞

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一、中文摘要

本計畫針對南灣海域大量生長的松藻及海葵探討其族群大量擴張的生態機制。經過詳細的形態分析和細胞核染色分類研究，我們鑑定了南灣海域的松藻共 9 種，包括一新種-南灣松藻(*Codium nanwanensis*)；其中最占優勢的食用松藻(*Codium edule*)經將藻體打碎、收集類似配子囊構造進行培養後，發現此一構造可在 3-4 星期後生成囊胞，而囊胞為松藻的主要結構，顯示該類似配子囊的構造，可視為具有繁殖能力的繁殖體（propagules），而此繁殖體極可能是促成食用松藻季節性大量繁生的主要機制。在海葵的族群生態研究方面，南灣海域大量繁生的海葵經形態比較研究，鑑定為新種-南灣結節海葵(*Condylactis nanwanensis*)的分類研究，其形態與加勒比海的巨大結節海葵(*C. gigantea*) 近似，推測可能為外來種入侵所造成。我們分析了南灣結節海葵的體型頻度分布，結果顯示其族群各月份都有小型個體 (＜0.5 g wet wt) 加入族群中，而各種體型的海葵全年都會進行分裂生殖，並以體型約 3-5.5 g 濕重的海葵個體行無性生殖的頻率較高 (> 5%)；在歷次所採集的二千餘隻標本中，僅發現雌性個體，未見雄性個體，顯示此種海葵可能行孤雌生殖；顯示無性的分裂生殖和單性的孤雌生殖是造成此海葵大量增值的主要機制。

關鍵詞：族群爆發、松藻、結節海葵、珊瑚礁變遷

Abstract

The population outbreaks macroalgae and sea anemones that causing the degradation of coral reefs in Nanwan Bay were studied. A detailed taxonomic study by morphological and karyological methods revealed 9 *Codium* species, including a new species *C. nanwanensis*. The results of culture of gametangium-like structures, named as the propagation buds, from *C. edule* showed that they have the potential to form new thalli and can be regarded as an effective reproductive structure. In the field, the abundance of both gametangia and propagation buds in the macrothalli of *C. edule* were the highest in the late period of its growing season in August. The propagation buds may serve as a persistent stage when the macrothalli disintegrate at the end of the growing season and act as a seed bank for vegetative reproduction of *C. edule* in the next season. Studies on the sea anemones from coral reefs of southern Taiwan revealed a new species, *Condylactis nanwanensis*. Most individuals of *C. nanwanensis* occur in dense aggregations on or below large living or dead branching corals, or in reef crevices. The species is similar to the Caribbean *C. gigantea* (Weinland, 1860) in number of tentacles and mesenteries, and features of the column, but differs in color, size, and cnidom. Based on the recency of its detection, its localized distribution, its high population density, its deleterious effect on the local fauna, and the fact that only females were found, the population is likely to be invasive, possibly introduced via the aquarium trade.

Keywords: population outbreak, *Codium*, *Condylactis*, reef degradation.

Introduction

Phase shifts of coral reefs from coral-dominated to algal-dominated or sea anemone dominated communities were reported from Nanwan Bay in southern Taiwan (Dai 1997, Dai et al. 2002, Chen and Dai 2004). Although the phase-shifts of coral reefs have been reported in many areas (Smith et al. 1981, Hughes 1994, Andres and Witman 1995, Shulman and Robertson 1996, Connell et al. 1997, Lapointe 1997), there was little direct experimental evidence demonstrating that the decrease of coral cover was due to the influence of macroalgae. Several reports have documented that filamentous algae may cause shading and abrasion effects on corals, inhibit the settlement of coral larvae, and kill juvenile corals by overgrowing them or entrapping sediment (Dart 1972, Bak and Engel 1979, Van Moorsel 1985, Hunte and Winttenberg 1992). However, in some macroalgal removal experiments, it has been shown that the cover of macroalgae is rarely detrimental to large coral colonies and only caused reduction of coral cover and growth rate (Tanner 1995, Miller and Hay 1996). On the other hand, some corals can inhibit the attachment and growth of macroalgae through preempting the space (De Ruyter van Steveninck et al. 1988; Hughes 1989). Furthermore, macroalgae may form canopies to prevent corals from bleaching (Jompa and McCook 1998).

Scleractinian corals are the major space occupants in the benthic community and the cover of living corals is about 46 % in Nanwan Bay, southern Taiwan (Dai 1993). *Codium edule* Silva, a prostrate, coenocytic and siphonous green alga, is the most abundant when macroalgae standing stocks increasing in spring. The increasing of macroalgae followed by sea anemones was presumed to be a primary factor in coral community degradation in the past ten years (Dai 1997, Dai et al. 2002). However, the causes and possible mechanisms are still unknown. In this study, we investigate the taxonomic status of species and possible mechanisms that are responsible for the degradation of coral reefs in Nanwan Bay, southern Taiwan.

Material and Methods

1. Codium edule

Macrothalli of *Codium edule* were collected from Nanwan Bay, southern Taiwan (21° 57’ N, 120° 46’ E) by scuba diving during the growing season in 2000 and 2001. They were transported in seawater to the laboratory within 6 h. Epiphytes on the thallus were removed, and the thalli were then kept in an aquarium with filtered seawater and proper aeration before observation and further culturing.

In order to determine the occurrence and abundance of reproductive structures, the apical section (3-5 cm) from the branches of each collected thallus of *C. edule* was cut and preserved in 4% formaldehyde. The reproductive structures in preserved samples were harvested by grinding the branches with 100 ml filtered seawater for 25-30 sec in a Waring blender (Churchill and Moeller 1972). The number of gametangia and gametangium-like structures from each thallus was counted respectively under a microscope.

For further cultivation of reproductive structures, gametangia and gametangium-like structures were obtained from flesh thalli following the method mentioned above (Churchill and Moeller 1972). The quick grinding effectively separated the reproductive structures from utricles without causing significant damage. The reproductive structures suspended in the solution were isolated using a capillary tube with the aid of a dissecting microscope and cleaned four to five times with sterile seawater. The gametangia were treated with low temperature (2-4 h in 10 ℃) following the procedures in Borden and Stein (1969) to stimulate the release of gametes.

The gametangia or gametangium-like structures were cleaned and cultured with Provasoli’s enriched seawater (PES) medium in petridishes respectively (Provasoli 1968). They were maintained in a growth chamber with the temperature set at 22℃ and the illumination at approximately 65 μmol/m^2/sec in a 12 : 12 h L : D photoperiod. The PES medium was changed every two days and the morphological changes of these reproductive structures were recorded by a digital camera (Coolpix 990, Nikon).

Microspectrophotometric studies were used to determine the ploidy of different stages in the life cycle of *C. edule*. The isolated utricles, gametangia, and gametangium-like structures were fixed immediately in 0.5% glutaraldehyde seawater solution for 24 h. Then the materials were transferred into 100% methanol for preservation at 4 ºC. Before staining, methanol was removed by evaporation and the gametangia or gametangia-like structures were crushed. Then they were stained with 0.5 μg·ml^{-1} DAPI (4', 6-diamidino-2-phenylindole) in McIlvaine buffer (pH 4.4) for 30 min, and examined with a Nikon fluorescence microscope (Chang and Carpenter, 1988). Photographs were taken using a digital camera with a fixed shutter speed and aperture to avoid possible influences of different exposure time on the relative fluorescence unit (RFU). The digitized photographs were then analyzed using an image analysis software (Image-Pro Plus, Media Cybernetics). The images of DAPI-stained nuclei were separated from the background with a threshold light intensity, and the integrated light intensity of the nuclear region was measured for each nucleus.

2. Condylactis nanwanensis

Nighty-eight specimens were collected from 3-10 m at Tiaoshi, Nanwan Bay, southern Taiwan in May and October 2002. The specimens were relaxed with menthol and fixed in 10% formalin in seawater for at least 24 h. Morphological features were observed under a dissecting microscope. For histological studies, paraffin sections 5-8 μm thick were stained with Heidenhain's Azan (Presnell and Schreibman 1997). Cnidae from tentacles, column, mesenterial filaments, and actinopharynx were measured in squash preparations at 1000× from preserved specimens; measurements are of undischarged capsules. Not all tissues were studied in all animals.

Comparisons were made with three specimens of *Condylactis gigantea* bought at a pet store in Lawrence, Kansas, USA. Specimens of the new species have been deposited in the National Museum of Marine Biology and Aquarium (NMMBA) in Taiwan, and the University of Kansas National History Museum (KUNHM), Lawrence, Kansas, USA.

Samples of the sea anemone were collected monthly from December 1999 to August 2001 in Nanwan Bay, southern Taiwan. Individuals in three quadrates (15 x 15 cm) haphazardly placed between 3 and 8 m in depth were sampled. The samples were narcotized with menthol and fixed with 10% formalin in sea water at least 24 h. The wet weight of each specimen was measured after the excessive water was removed by blotting. The wet weight was used to indicate the size of sea anemones.

The occurrence of asexual reproduction was recognized when the pedal disc of an individual showed a sign of fission. Samples were dissected to examine the development of gonads under a dissecting microscope. To determine the developmental stage of the gonads, ten samples in each month from January to June 2000 were prepared for histological studies. Tissue samples were dehydrated with increasing concentration of alcohol, cleared with xylene, embedded in paraffin, and sectioned into 7-9 μm serial sections. The sections were stained with Mayer's hematoxylin and eosin, and were examined under the compound microscope. The length and width of 20 oocytes in nucleolar section were measured for each sample by ocular micrometer. The average of two diameters in perpendicular direction was used to indicate the size of oocytes. The monthly variation of oocyte size was used to determine the seasonal pattern of gametogenesis and to estimate the possible spawning time.

Results

1. *Codium edule*

During the growing season from January to May 2000, the gametangia of *C. edule* were rarely found among approximately two hundred thalli. In the late portion of the growing season in August and September 2000, the gametangia and gametangium-like structures were found on the side of utricles from the remaining *C. edule* thalli. These two structures were ovoid to fusiform, and they could be distinguished on the basis of their size and color under a microscope. The gametangium-like structure was fresh-green, with an average length and width of 327.91 ± 23.25 µm and 184.17 ± 15.78 µm (n = 22), respectively. The gametangium was dark-green, and smaller in size with the average length and width of 297.98 ± 29.56 µm and 121.67 ± 15.61 µm, respectively (n = 14). There was a significant difference between the sizes of these two structures ($P < 0.01$, Mann-Whitney U test). In addition, the gametangium-like structures had protuberances and extending filaments, while the gametangia did not have these structure. After 1-3 days of cultivation in PES medium, the gametangium-like structures sprouted out filaments from the protuberances directly, mainly from those on the tip or bottom. The first utricle with a septum developed from the filament after culture for about 14 days. After 28 days, more utricles were found growing from the filaments, while observation was terminated at this point due to contamination by epiphytic red alga. From these observations, we considered that the gametangium-like structure (hereafter as propagation bud) could serve as a reproductive organ and can develop directly into a mature thallus. The developmental processes described above did not occur in those gametangia cultured in the same PES medium.

The occurrence of gametangia and propagation buds in *C. edule* collected from southern Taiwan was from June to October. The relative abundance of gametangia and propagation buds were the highest in August 2000 (240.0 ± 174.91 and 212.3 ± 157.1 individuals per gram, respectively).

Microspectrophotometric measurements of RFU of nuclei in gametangia, propagation buds and utricles showed that their total DNA contents were variable. The peaks of RFU data showed that the nuclei of small cells from gametangia were haploid, and those from the nuclei of filaments in propagation buds and utricles were diploid. It suggests that meiosis is likely occurred in the gametangium and the propagation bud is possibly a transformed gametangium with incomplete gametogenesis or meiosis.

2. *Condylactis nanwanensis*

The new species was ascribed to the genus *Condylactis* (family Actiniidae) based on the following diagnostic features: elongate column with verrucae on its upper part; no sphincter muscle; no marginal spherules; long, simple tentacles that are hexamerously arranged; most mesenteries perfect and fertile; diffuse retractor muscles; no more mesenteries at the base than at the margin; a cnidom of spirocysts, basitrichs, and microbasic *p*-mastigophores (Carlgren 1949).

<u>Population structure</u>

The size-frequency distribution of *Condylactis* sp. showed that juvenile individuals (＜0.5 g wet weight) were found throughout the year and were more abundant in December 1999 and January, September, October, November 2000, and August 2001.

<u>Asexual reproduction</u>

The anemone propagates asexually by longitudinal fission. Fission was initiated as the elongation and extension of a pedal disc, then proceeded to the separation and formation of two pedal discs under one column of the same individual. Further steps of fission involved the division of column from aboral to oral direction, and the regeneration of missing part of the actinopharynx and the column. Longitudinal fission occurred throughout the year and in all size classes, although higher proportions (>5%) were found in the classes of 3-5.5 g wet weight.

Oogensis

Oocytes were found within the mesenteries and oogenesis occurred throughout the year. Monthly variations of oocyte size measured in the sections indicated that oogenesis started in May, and the maximum size was found in March and April. The disappearance of mature oocytes in April and May suggested that spawning possibly occurred in this period.

The individuals (collected in March 2001) larger than 0.5 g wet weight were found with oocytes and this size was considered as the minimum size of maturation.

Spermary was not found over two years of intensive examinations.

Discussion

1. *Codium edule*

The propagation bud is a reproductive structure newly described for *C. edule*. It differs from gametangium by its larger size, darker color and higher DNA contents. It could also be distinguished from the gametangium because they neither contain gametes nor bearing an opening at the tip. In addition, the propagation buds could germinate with extending filaments after culturing in PES medium, while the gametangia failed to do so. It is regarded as a reproductive organ because it can initiate filaments, and utricles can further develop from the distal ends of these filaments, which represent the initial stage of thallus formation. Structures similar to the propagation buds were reported in *C. vermilara* (Olivi) delle Chiaje (as *C. tomentosum* in Went 1889), and *C. ithmocladum* Vickers from the Caribbean (Schmidt 1923, Silva 1960). However, the present study is the first to show that the propagation buds can germinate and act as a reproductive structure.

Male and female gametangia of *Codium* species were known from the Pacific populations of *C. fragile* in British Columbia (Borden and Stein 1969) and Japan (Arasaki *et al*. 1955) based on size differences of their gametes. The propagation bud with different sized cells found in this study was similar to that described in Prince (1988), and it was regarded as an aborted gametangium (Throbridge 1998, pers. com. with P. C. Silva). However, the cells inside the propagation buds were not swimming cells and they contained the same amount of nucleic acids as utricles. It is speculated that incomplete meiosis may have occurred during the formation of these propagation buds. The propagation buds can then be regarded as transformed gametangia.
The propagation buds were mainly found in late period of the growing season of *C. edule*. The abundance of propagation buds in the field suggests that they may serve as a persistent stage or seed bank when the macrothalli disintegrated during the end of their growing season. Their potential to form new utricles and macrothalli further suggests that they may act as an effective mechanism of vegetative reproduction that lead to mass production of *C. edule* during the next growing season.

2. *Condylactis nanwanensis*

The sea anemone we found at Tiaoshi conforms to the genus *Condylactis* as defined by Carlgren (1949). *Condylactis aurantiaca* (Delle Chiaje, 1822), *C. gigantea* (Weinland, 1860), and *C. parvicornis* (Kwietniewski, 1898) are currently considered valid species of *Condylactis*, but other six species have been associated with that generic name in the past (Fautin 2003). Only two individuals had fewer than 50 mesenteries, a feature that distinguishes the species from *C. parvicornis*, which is characterized by 48 mesenteries (Kwietniewski 1898).

Mesentery number of *C. aurantiaca* is 96, which is similar to that of *C. nanwanensis*. However, its abundant microbasic *b*-mastigophores in the tentacles, column, actinopharynx, and filaments (Schmidt 1972) distinguish it from *C. nanwanensis.*

Condylactis gigantea is morphologically most similar to *C. nanwanensis*. They are alike in number of tentacles and mesenteries, column morphology, and the distinct white stripes on the tentacles. The two species also occur in similar habitats on reefs: *C. gigantea* generally occupies holes or crevices in or between rocks on reefs, where its body is entirely concealed (Corrêa 1964).

The two species can be distinguished by sexual and reproductive characteristics, which are commonly used to distinguish taxa of sea anemones (Fautin 2002). Jennison (1981) reported that most individuals of *C. gigantea* are gonochoric (one he studied was hermaphroditic) and the sex ratio is 1:1. Females contain oocytes with broad range of diameters throughout the gametogenic cycle (Jennison 1981). We found only female individuals of *C. nanwanensis*, with oocytes developing synchronously within a narrow range of diameters (Tsai et al. 2002). Further, *C. nanwanensis* undergoes frequent longitudinal fission (Tsai et al. 2002), but *C. gigantea* reportedly does not reproduce asexually (Jennison 1981).

The two species also differ in color and size, features that may vary intraspecifically in anemones (e.g. Stephenson 1928). Some specimens of *C. gigantea* have pink tips on their tentacles or an orange column whereas *C. nanwanensis* is always brown in all parts except for bleached individuals. In addition, *C. gigantea* is much larger than *C. nanwanensis* (Table 2).

They also can be separated by their nematocysts. The nematocyst composition of *C. nanwanensis* differs from that of *C. gigantea* as recorded in the literature as well as from three specimens identified as *C. gigantea* purchased from a pet store [NMMBA-ACT-079 (x2), KUNHM 001945 (x1)]. In particular, the purchased specimens had abundant microbasic *b*-mastigophores in their mesenterial filaments and actinopharynx.

The difference in cnidae between the specimens we examined and what is reported in the literature may imply there is more than one species of *Condylactis* in the Caribbean. Two have been described, *C. gigantea* (Weinland, 1860) and *C. passiflora* Duchassaing and Michelotti, 1864; they were synonymized by Carlgren (1949). Detailed study of specimens of this genus from throughout the Caribbean is clearly warranted. We include in Table 1 the few nematocyst data provided for *C. passiflora* by Watzl (1922). They do not resolve this taxonomic issue.

We infer from the sudden appearance and population explosion of *C. nanwanensis* that it is introduced. Chapman and Carlton (1991) listed attributes of an invasive marine species. Of the six for species known only locally or regionally, one definitely applies to *C. nanwanensis*: previously unknown in the local region (attribute 1). Another may apply as well. A nuclear power plant was built in 1982 at the head of Nanwan Bay, within 2 km of Tiaoshi. Virtual monocultures of sea anemones cover the walls of the impoundment for water used to cool the plant. A species of *Megalactis* being described from that habitat (Ardelean in press) formerly covered the walls; it is being displaced by another. The anemones on the impoundment walls may be introductions: association with new or artificial environments (attribute 5) is relevant to them. Thus, it may be that association with known introductions (attribute 4) applies to *C. nanwanensis*. As far as is known, *C. nanwanensis* has not spread (criterion 2), but that is not surprising because habitats like the one in which it is found are absent in the area.

Carlton (1996a: 1653) advocated the adjective “cryptogenic” for “a species that is not demonstrably native or introduced.” Although this might seem prudent for *C. nanwanensis* using the criteria of Chapman and Carlton (1991), other of its features convince us that *C. nanwanensis* is invasive in southern Taiwan. Shick (1976) noted that invasive sea anemones commonly tolerate adverse environmental conditions, and, more generally, Carlton (1996b) recognized that environmental change in the recipient region could allow invasion by species tolerant to the new conditions. Increased sedimentation in Nanwan Bay was proposed by Dai et al. (1998) as a cause of the change in its coral community between 1987 and 1997. That these anemones tolerate turbidity is manifest by their burgeoning population in Nanwan Bay; moreover, they have been difficult to maintain in clean water of the nearby National Museum of Marine Biology and Aquarium where corals thrive.

Among attributes of invaders not enumerated by Carlton and colleagues are high population density and disruption to the local ecology, which characterize marine invaders (e.g. Purcell et al. 2001) as well as terrestrial ones, and apply to *C. nanwanensis* (the former certainly, the latter possibly). For species capable of clonal reproduction (plants and many marine invertebrates), high population density can be achieved rapidly; the ability of *C. nanwanensis* to reproduce asexually was probably an important factor in its domination of space in Nanwan Bay (Tsai et al. 2002).

An additional characteristic of introduced species – low genetic diversity – is implicit in the idea of introduction. This criterion can be used diagnostically, and potentially has predictive power in establishing the source population. Proxies for low genetic diversity may be manifest in morphology, and we regard the unisexual nature of the population of *C. nanwanensis* in Nanwan Bay as convincing evidence of its being invasive. Low genetic variability is consistent with the lack of variability we found in color and size as well.

Attribute 3 of an invader according to Chapman and Carlton (1991) is a human mechanism of introduction. We consider it unlikely that *C. nanwanensis* arrived in ballast water or attached to a ship, because it lacks attributes for such transportation and because Nanwan Bay does not have a cargo port – it is a port only for local fishing boats. However, *C. nanwanensis* may have come from accidental or purposive release of one or more aquarium pets. *Condylactis* anemones are widely sold in pet stores, including in Taiwan. Thus it is likely that the natural home range of *C. nanwanensis* is far from its type locality and the place for which it is named.

<u>計畫成果自評</u>

本計畫目前已發表４篇 SCI 論文，一篇研討會論文，另有二篇已投稿，在審稿中。本計畫結果在增進我們對珊瑚礁變遷的瞭解、我國海域的生物多樣性，以及外來種的生態效應上，都有重要成果，對於促進我國海域的珊瑚礁保育也有貢獻。發表成果如下列：

1. Chang JS, Chang J, Dai CF (2002) A taxonomic and karyological study of the three *Codium* species (Chlorophyta) in southern Taiwan, including the description of *Codium nanwanense* sp. nov. Bot Bull Acad Sin 43:161-170. (SCI)
2. Chang JS, Dai CF, Chang J (2003) A novel reproductive organ as the propagule of *Codium edule* (Bryopsidales, Chlorophyta). Bot Mar 46: 431-437. (SCI)
3. Tsai WS, Dai CF, Fan TY, Fautin D (2004) *Condylactis nanwanensis* n. sp., a new species of sea anemone. Bull Mar Sci (in press). (SCI)
4. Chen CA, Dai CF (2004) Local phase shift from *Acropora*-dominant to *Condylastis*-dominant community in the Tiao-shi reef, Kenting National Park, southern Taiwan. Coral Reefs 23 (in press) (SCI)
5. Tsai WS, DaiCF, Fan TY (2003) Asexual reproduction and population dynamics of the sea anemone *Condylactis nanwanensis* in Nanwan Bay, southern Taiwan.

(submitted to Marine Biology).

6. Tsai, W.H., C.F. Dai, T.Y. Fan (2002) Population structure and reproduction of the sea anemone *Condylactis* sp. In Nanwan Bay, southern Taiwan. Proceedings of IUCN/WCPA-EA4 Taipei Conference, p. 743-751, Taipei.

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