

Seasonality of sexual reproduction in three photosymbiotic *Trididemnum* species (Didemnidae: Ascidiacea: Tunicata) in a subtropical sea grass bed

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The seasonality of sexual reproduction was studied in three photosymbiotic *Trididemnum* species, *T. miniatum*, *T. clinides* and *T. nubilum*, inhabiting the same site. In all species, the colonies lacked gonads and/or embryos during the cold season, while gonads were found in most colonies for several months in the warm season. There were some interspecific differences in the duration of the breeding season. Although water temperature is thought to be the principal factor confining sexual reproduction in these photosymbiotic ascidians, other factors, e.g. precipitation, potentially affect fecundity.

INTRODUCTION

In sessile chordates, i.e. ascidians, seasonality of sexual reproduction has been recognized in some species, while other species spawn gametes or larvae all year. For instance, some ascidians breed in spring and/or summer, whereas the season is restricted to winter in some Mediterranean species (cf. Turon, 1988; Turon & Becerro, 1992). While temperature is a crucial factor controlling sexual reproduction (cf. Millar, 1971), fluctuation of food availability also has a decisive effect on reproduction of benthic suspension feeders in temperate coastal community (Coma et al., 2000).

The occurrence of photosymbiosis is known in some colonial ascidians of the family Didemnidae. Since the photosymbionts are transferred from the parent colony to the embryo or larvae in most species, the didemnid-algal symbioses are supposed to be obligate. The photosymbiotic ascidians are exclusively distributed in tropical and subtropical waters probably due to the susceptibility of their photosymbionts to low temperature; photosynthesis is completely suppressed under 20°C (Dionisio-Sese et al., 2001). Therefore, the reproduction in photosymbiotic ascidians would be severely affected by water temperature. The photosymbiotic ascidian *Trididemnum solidum* (Van Name) releases larvae throughout the year on a tropical coral reef off Curaçao (van Duyl et al., 1981), and *Didemnum molle* (Herdman), another photosymbiotic species, released larvae on every monitoring day (every two weeks from August to December) at Lizard Island, Great Barrier Reef (Olson, 1983). In these cases, continuous gamete production in some tropical ascidians may be caused by the almost constant water temperature of tropical waters (~25–30°C).

Subtropical waters experience a hot summer and a cool winter. Water temperature varies from approximately 20–30°C in Okinawajima Island (Ryukyu Archipelago, Japan) where about 15 photosymbiotic species have been

recorded so far. Since only one species has been reported from the mainland of Japan, many of the photosymbiotic species in the Ryukyu Archipelago are nearby the northern extreme of their range in the north-west Pacific (cf. Oka & Hirose, in press). Recently, we found distinct seasonality in the sexual reproduction of some photosymbiotic species on subtropical coral reefs in Okinawajima Island, i.e. *Diplosoma virens* (Hartmeyer), *Trididemnum cyclops* Michaelsen and *Lissoclinum bistratum* (Sluiter). The colonies lacked gonads in the winter (Hirose et al., 2005, 2006a), and there were some interspecific differences in the duration and peak of the breeding season. However, these differences were possibly caused by differences in the micro-environments of the habitat of each species.

Here we studied the seasonality of sexual reproduction in three photosymbiotic *Trididemnum* species inhabiting the same site based on monthly sampling for more than 12 months. We studied *T. miniatum* Kott, *T. clinides* Kott and *T. nubilum* Kott. The photosymbiont of *T. miniatum* is *Prochloron* sp. (Hirose et al., 2006b), and the photosymbionts of *T. clinides* and *T. nubilum* are non-*Prochloron* cyanophytes, probably *Synechocystis* sp. (Münchhoff et al., 2007). We confirmed that seasonality of sexual reproduction exists in photosymbiotic ascidians inhabiting a subtropical coral reef lagoon and that the breeding season differs somewhat among species.

MATERIALS AND METHODS

Colonies of *Trididemnum miniatum*, *T. clinides* and *T. nubilum* were attached to sea grass (*Thalassia hemprichii*), sometimes on the same leaves, in a shallow coral reef lagoon (<0.5 m deep at low tide) at Bise (26°42'30"N 127°52'45"E: Motobu, Okinawajima Island, Japan). Colonies of the three species were usually attached to the basal part of the leaves, and *T. miniatum* colonies were often found at relatively higher leaf sites than the other two species (Figure 1). Colonies

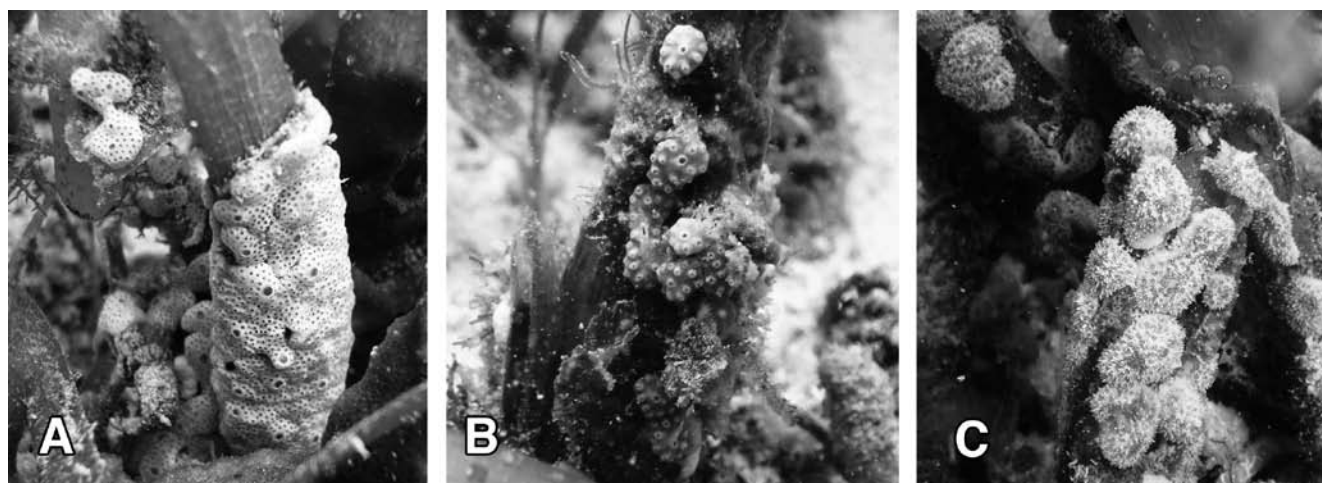


Figure 1. Colonies of (A) *Trididemnum miniatum*; (B) *T. clinides*; and (C) *T. nubilum* at the collection site.

were collected monthly by hand from September 2005 to September 2006 (13 months) for *T. miniatum* and *T. clinides*, and from September 2005 to October 2006 (14 months) for *T. nubilum*. Colonies were immediately brought to the laboratory and were fixed with 10% formalin–seawater after anaesthesia with menthol and $MgCl_2$. Specimens were identified according to Kott (2001).

Fixed colonies were dissected under a stereomicroscope to determine the presence or absence of testes, eggs and embryos. Because it was difficult to distinguish between eggs and early embryos, they were combined into a single category. During the embryogenesis, the embryos were moved from the zooids into the basal tunic of the colony for brooding. These embryos with tails, if present, were also recorded. Ten colonies and more than ten zooids per colony were examined each month. If the colony consisted of less than ten zooids, all zooids were examined. When a single zooid possessed a testis (and/or egg), the whole colony was recorded as sexually mature.

Water temperature data from coastal observations at Sesoko Station ($26^{\circ}38'8''N$ $127^{\circ}51'55''E$) were provided by the Tropical Biosphere Research Center, University of the Ryukyus, Japan. The data of precipitation in Motobu and hours of sunshine in Nago were obtained from the website of the Okinawa Meteorological Observatory (<http://okinawa-jma.go.jp/>).

RESULTS

Water temperature and precipitation

Our study site was considered to have a ‘subtropical’ summer and winter: the water temperature was less than $22^{\circ}C$ from December to March and more than $29^{\circ}C$ from July to September (Figure 2A). Typically, abundant precipitation falls from April to June because of the spring rainy season and from August to September because of typhoons. However, the actual precipitation recorded varied greatly. For instance, precipitation in September 2005 was extraordinarily low (53 mm) and that in September 2006 was high (303 mm); the average precipitation in September from 1981 to 2000 was about 200 mm (Figure 2B). The hours of sunshine were significantly correlated with water temperature (Spearman’s correlation coefficient by rank test).

Trididemnum miniatum

Gonads and embryos were not found in February or March (Figure 3A). In the other months, some of the colonies possessed testes. Eggs/embryos were found from June to December. Sexual maturation peaked in December; eight of the ten colonies possessed both testes and eggs/embryos in December. Some colonies contained embryos with tails from August to December, except for September

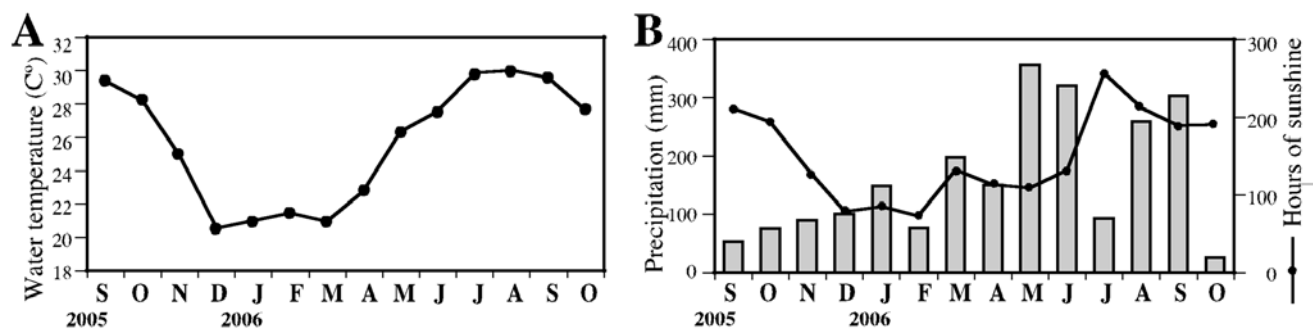


Figure 2. (A) Surface water temperature at Sesoko Station provided by the Tropical Biosphere Research Center (University of the Ryukyus); (B) precipitation in Motobu and hours of sunshine in Nago provided by the Okinawa Meteorological Observatory.

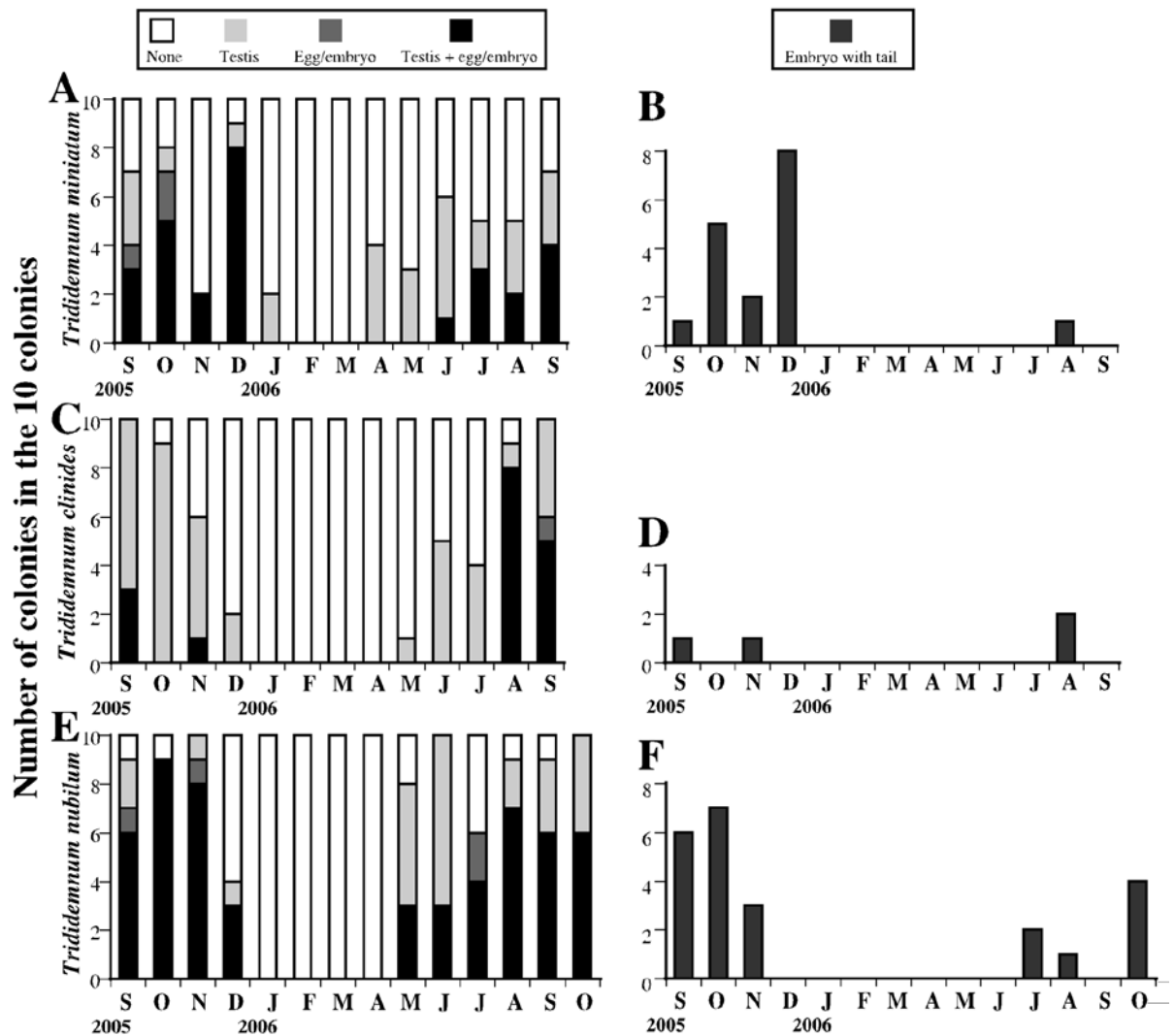


Figure 3. Number of colonies possessing gonads and/or early embryos (A, C, E) and embryos with tails (B, D, F) in the ten colonies examined for (A, B) *Trididemnum miniatum*; (C, D) *T. clinides*; and (E, F) *T. nubilum*.

2006 (Figure 3B). In December 2005, embryos with tails were found in eight of the ten colonies.

Trididemnum clinides

Colonies possessed neither gonads nor embryos from January to April (Figure 3C). Testes were found from May to December and eggs/embryos were found in August, September and November. Sexual maturation peaked in September; eight of the ten colonies possessed both testes and eggs/embryos. The number of colonies with gonads/embryos gradually increased from May (one colony) to September (ten colonies), and gradually decreased from September to December (two colonies). Embryos with tails were recorded in August, September and November (Figure 3D).

Trididemnum nubilum

There were no gonads or embryos in any colonies examined from January to April (Figure 3E). Some colonies possessed testes and/or eggs/embryos from May to December. Sexual maturation peaked in October 2005; nine of the ten colonies possessed both testes and eggs/embryos. Although nine or ten of the ten colonies possessed testes in

June and from August to November, only four colonies had testes in July 2006. Embryos with tails were recorded from July to November, except for September 2006 (Figure 3F). In October 2005, embryos with tails were found in seven of the ten colonies.

DISCUSSION

In the *Trididemnum* species studied here, colonies lacked gonads and/or embryos during the cold season, whereas gonads were found in most colonies for several months in the warm season. We observed some interspecific differences in the duration of the breeding season, and the cold season appears to be shorter for *T. miniatum* than for the other two species. The peak in breeding differed among the species; it was December in *T. miniatum*, August to September in *T. clinides*, and around October in *T. nubilum*. Since the habitats of the three *Trididemnum* were almost the same, these differences are probably specific to each species. All three species possessed embryos with tails for several months, but the month with the most embryos differed among species. Among these three species, the colonies of *T. clinides* are very small and often consist of less than ten zooids because

colonies frequently divide into smaller ones following fission of the colonies. Since the number of zooids per colony in *T. clinides* was much smaller than those in the other two *Trididemnum*, the probability of the presence of embryos in a colony should be lower in *T. clinides* than in the others. This is probably why *T. clinides* colonies often did not possess embryos with tails, even at the breeding peak.

Seasonality of sexual reproduction has been reported in some photosymbiotic didemnids inhabiting subtropical coral reefs, i.e. *Diplosoma virens*, *T. cyclops* and *Lissoclinium bistratum* (Hirose et al., 2005, 2006a). Our results are consistent with these previous findings, supporting the hypothesis that the sexual reproduction of photosymbiotic ascidians in the subtropics is suppressed in winter, with some interspecific differences in the duration and peak of the breeding season. Since the photosymbiotic ascidians *T. solidum* and *Didemnum molle* release larvae all year in tropical waters, where water temperature is almost invariable (van Duyl et al., 1981; Olson, 1983), water temperature is thought to be the principal factor confining sexual reproduction in photosymbiotic ascidians. Because the hours of sunshine were significantly correlated with the water temperature in the present study, they may also affect on the seasonality of the sexual reproduction. On the other hand, chlorophyll-*a* and nutrient concentrations in coral reef waters were low almost year-round off Sesoko Island located near the present sampling sites (Tada et al., 2003), similar to those in some other coral reefs. Therefore, food availability would not be crucial for controlling sexual reproduction in subtropical waters.

Other factors may also affect sexual reproduction. In the photosymbiotic ascidian *T. solidum*, colonies release larvae throughout the year in Curaçao, where water temperature is almost constant (26–28°C; van Duyl et al., 1981). In contrast, colonies of *T. solidum* possess fully-developed larvae exclusively from June to November in Galeta, Panama, where water temperature is also almost constant (27–29°C; Millar, 1974), suggesting that water temperature is not the only factor confining the breeding season. For instance, at that study site in Panama, seawater was turbid with suspended matter from the adjacent seabed during the windy season, from mid-December to mid-April (Millar, 1974). In our study, no colonies possessed embryos with tails in September 2006, whereas some colonies possessed them in September 2005. As shown in Figure 2B, precipitation was extraordinarily low in September 2005 and unusually high in September 2006; thus, high precipitation probably decreased the formation of embryos. Since the collection site was a shallow lagoon, heavy rain may have lowered the salinity of the seawater.

In November 2005, the number of *T. miniatum* colonies with gonads and/or embryos was much smaller than those in the adjacent months, i.e. October and December. It is unclear what happened to the *T. miniatum* population in November 2005 and why only *T. miniatum* was susceptible to the unknown factor(s). The effects of the unknown factor(s) may be greater at shallower sites, because *T. miniatum* colonies were usually attached to relatively higher parts of the sea grass leaves or in shallower sites than the other two species. The fecundity of these ascidians appears to be susceptible to water temperature, precipitation and other factors that

vary yearly. Fecundity may also vary due to environmental differences among microhabitats.

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