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Research Article

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Phenology of *Codium cylindricum* (Ulvophyceae, Bryopsidales) on the central Pacific coast of Japan

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Abstract: *Codium cylindricum* is a large green alga distributed along the temperate Northeast Pacific coasts. Although the species is edible and contains pharmaceutical substances, little is known about its ecology. In this study, we characterized the phenology of *C. cylindricum* by monthly observations in Tateyama on the central Pacific coast of Japan from March 2018 to February 2019. The average length of attached thalli in three quadrats (2 × 2 m) was 5 cm from April to July. After disappearing in August, recruits appeared in November and the maximum density (1.08 ± 3.5 inds·m^{-2}) was reached in February. On the sea bottom, detached thalli were present from April to October; the biomass monitored in a single quadrat (2 × 20 m) was high from June to August. Based on monthly observations of 50–100 utricles in each of 18–25 thalli, gametangium formation started in June and reached a peak (i.e., gametangia detected in 75% of thalli and 63.4% of utricles) in September, before the disappearance of the detached thalli. These results strongly suggest that *C. cylindricum* is annual and reproduces mainly while they are drifting. Therefore, growth during detached stage is critically important to increase biomass and dispersal.

Keywords: annual; Codiaceae; macroalgal bed; phenology.

1 Introduction

Green macroalgae in the genus *Codium* (Bryopsidales, Codiaceae) are distributed in much of the world from Arctic to tropical waters (Guiry and Guiry 2021). The genus contains approximately 150 species with substantial morphological variation, including mat, spherical, cylindrical, and flattened forms (Chapman and Chapman 1973; Guiry and Guiry 2021; Verbruggen et al. 2007). In general, *Codium* species lack a gametophyte stage in their lifecycle, and haploid gametes released from diploid sporophyte fuse immediately, as in animals (Lee 2008). Abnormal asexual reproduction in gametes (parthenogenesis: Borden and Stein 1969; Malinowski and Ramus 1973; Prince and Trowbridge 2004, propagation bud: Chang et al. 2003; Silva 1960) and sporophytes (vegetative bud: Scheibling and Melady 2008; Watanabe et al. 2009, fragmentation: Armitage and Sjøtun 2017; Silva 1957, vegetative growth from filamentous cells: Gonzalez et al. 2014; Hwang et al. 2008) are reported in several species.

The upright forms of *Codium* species are the main components of some macroalgal beds (e.g., Chavanich et al. 2006; Skriptsova and Levenets 2012; Steller et al. 2003). However, ecological studies of these species are rare, except for studies of the invasive species *Codium fragile* (Suringar) Hariot (including two subspecies, *C. fragile* ssp. *fragile* (Suringar) Hariot, and *C. fragile* ssp. *tomentosoides* (van Goor) P. C. Silva). In these subspecies, phenology (Bulleri and Airoldi 2005; Dromgoole 1975; Serisawa et al. 2020; Trowbridge 1996), competition with indigenous algae (Campbell 1999; Chapman 1999; Dromgoole 1975; Drouin et al. 2016; Lyons and Scheibling 2008; Neill et al. 2006), and relationships with herbivores (Lyons et al. 2007; Scheibling and Anthony 2001) and epifauna (Schmidt and Scheibling 2006) have been reported.

Codium cylindricum Holmes is a large branched cylindrical species (Figure 1a) with a thallus length of more than 10 m, comparable in size to a large Eastern Pacific congeneric species, *C. amplivesiculatum* Setchell *et* N. L. Gardner (Pedroche et al. 2002; Riosmena-Rodríguez and Holguín-Acosta 2008). It is a temperate species distributed in Asia-Pacific countries in the Northern Hemisphere (Korea: Lee and Kang 1986; Taiwan: Lewis and Norris 1987; Vietnam: Nguyen et al. 2013; Philippines: Silva et al. 1987; China: Tseng 1984; Japan: Yoshida 1998). In Japan, *C. cylindricum*

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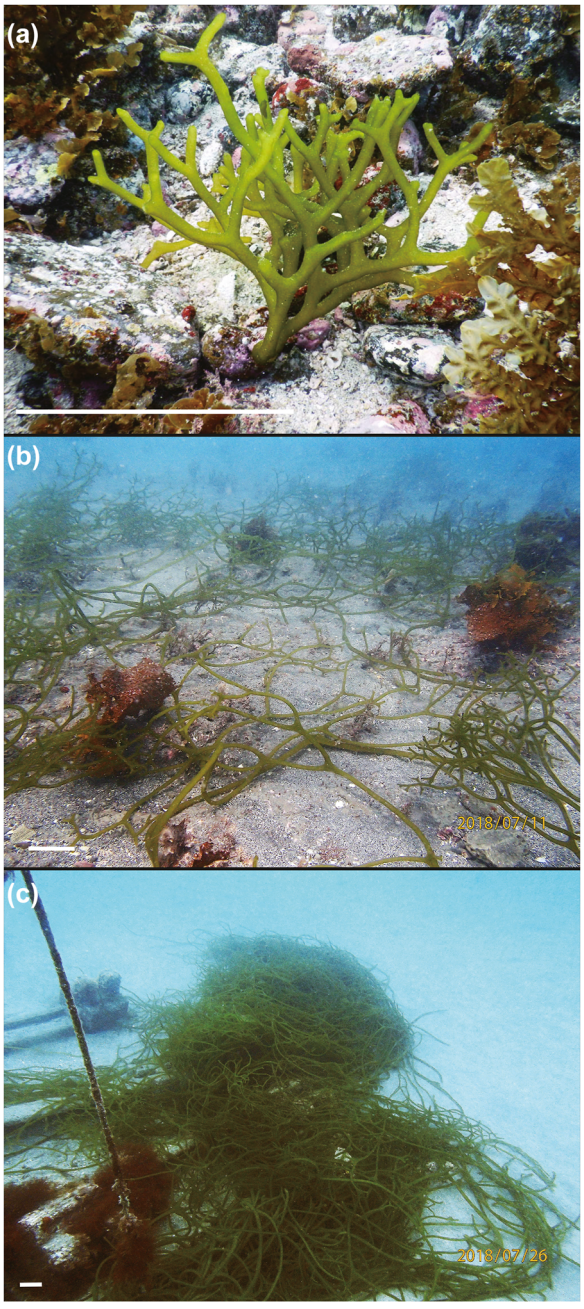


Figure 1: Photographs of *Codium cylindricum*. Thalli attached to bedrock (a), detached thalli (b), and an assemblage of detached thalli (c). Scale bars in each photograph: 10 cm.

is common on the Pacific coast from Kyushu to the Boso Peninsula (Yoshida 1998), with a disjunct population in Matsushima Bay, Miyagi Prefecture (Narita et al. 2008). Large populations have been reported in some inner bays, such as Ago Bay (Okamura 1915), Tateyama Bay (present study), and Nanao Bay (Harada et al. 2021). Further, marine organisms (Akagawa and Okiyama 1997; Chavanich et al. 2006), including a kleptoplastic sea slug (Hamatani 1967; Trowbridge et al. 2008), feed on this species. Given these characters, *C. cylindricum* is considered to be an important species for carbon sink and coastal ecosystems such as kelps and fucoids along temperate Pacific coasts (Fujita 2010). However, there is little fundamental ecological information available to guide conservation.

Chemicals extracted from *Codium* species have beneficial effects on human health (Alvarez-Hernández et al. 1999; Ohta et al. 2009). *Codium cylindricum* is a source of the keto-carotenoid siphonaxanthin (Ganesan et al. 2011; Li et al. 2018) and an antiangiogenic sulfated galactan (Matsubara et al. 2001, 2003). Siphonaxanthin induces apoptosis in human leukemia cells (Ganesan et al. 2011) and has anti-obesity effects in mice (Li et al. 2018). Sulfated galactan suppresses tumor growth and metastasis (Matsubara et al. 2003). However, further ecological information is needed for sustainable harvesting or stock improvement. In the present study, we investigated seasonal changes in the density, length of thalli, and biomass of *C. cylindricum* to reveal its detailed phenology.

2 Materials and methods

2.1 Study site

This study was conducted at Banda (34°97′72.20″N, 139°76′83.34″E), central Pacific coast of Japan, near the tip of the Boso Peninsula facing the mouth of Tokyo Bay (Figure 2). The sea bottom was mostly rocky reef composed of bedrock and boulders at 1–3 m in depth, with a gradual shift to sand at 4–6 m in depth (Figure 2). We found upright thalli of *C. cylindricum* on the rocky reef covered with a low sediment load at a depth of 4–6 m (Figure 2). On this reef, several canopy-forming macroalgae were sparsely distributed, including *Ecklonia cava* subsp. *cava* Okamura, *Eisenia bicyclis* (Kjellman) Setchell, *Sargassum macrocarpum* C. Agardh, and *S. yamamotoi* Yoshida.

2.2 Abiotic factors

Water temperature, illuminance, and nutrient concentrations were monitored as environmental parameters. Water temperature and illuminance were recorded once an hour from April 2018 to March 2019 using the HOBO pendant logger (UA002-64; Onset Computer Co., Bourne, MA, USA) installed close to our survey area. Illuminance was converted to photon flux density by dividing by 54, a conversion factor for sunlight (Lee and Park 2013). To measure nutrient concentrations, 500 ml of seawater was collected monthly using a clean and sterilized plastic bottle at a consistent depth. The water samples were transported in a cool box to the laboratory, filtered using a glass microfiber filter (GF/C 90 mm: 1.2 µm, GE Healthcare Life Sciences, Buckinghamshire, UK), and stored in a deep freezer (−20 °C). Prior to the analysis, the seawater samples

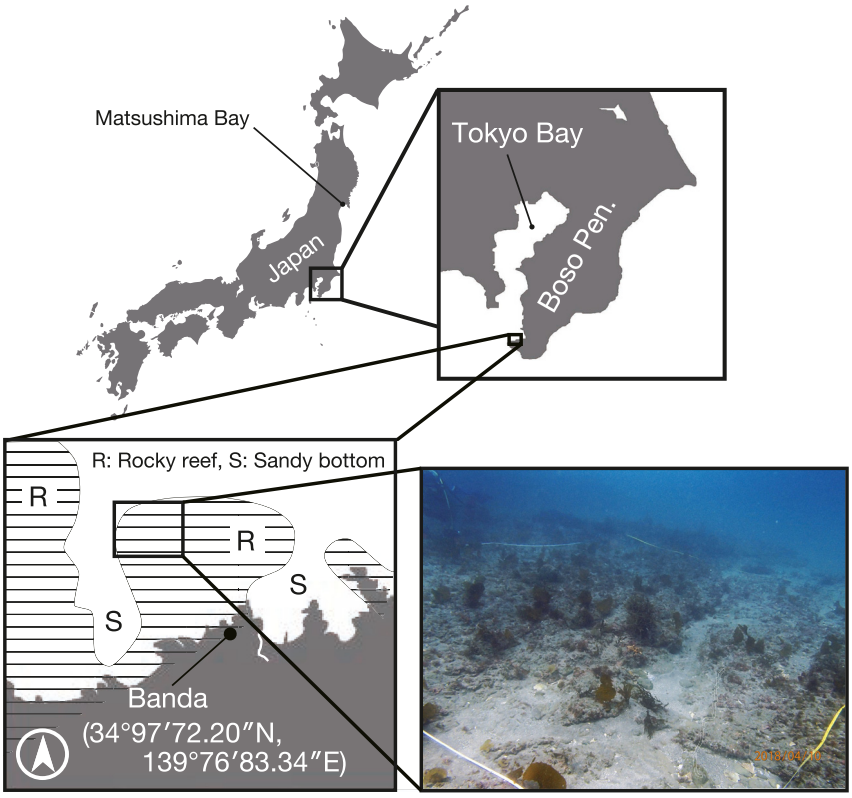


Figure 2: Map of study sites at Banda, Boso Peninsula, Japan and photograph of the *in situ* scenery.

were thawed at room temperature. Nutrients, including NO_3-N, NO_2–N, NH_4–N, and PO_4–P, were detected using an absorption spectrophotometer (DR2800, Hach Co., Loveland, CO, USA) in accordance with the method described in Akita et al. (2014). We summed concentrations of NO_3–N, NO_2–N, and NH_4–N as values of dissolved inorganic nitrogen (DIN).

2.3 Seasonal changes in *Codium cylindricum*

In our preliminary observations, we observed attached and detached thalli of *C. cylindricum* on the rocky reef. Thalli were attached to hard substrata from the winter to early summer (Figure 1a) and detached thalli were observed on the sea bottom from the late summer to autumn (Figure 1b and c). The number and length of attached thalli were recorded once a month in three fixed quadrats (2 × 2 m) deployed at a depth of 5 m from March 2018 to February 2019. Density (individuals·m^{-2}) was calculated in each quadrat using the number of thalli and the quadrat area. The maximum length of thalli was measured from the base of holdfast to tip of the longest branch and was defined as the thallus length of *C. cylindricum*. For detached *C. cylindricum*, biomass was detected monthly from April 2018 to March 2019. A rectangular area (2 × 20 m) set close to the above three quadrats was used because a larger plot was preferable to several small fixed quadrats considering the nature of drifting (i.e., the distribution of the detached thalli varies due to wave and current). We collected all detached *C. cylindricum* found in the rectangular area using mesh bags, determined the wet weight onshore, and returned the thalli to the same area. The number and length of detached thalli were not measured owing to the difficulty in determining individuals because of their fragility (ease of fragmentation) and entanglement during drifting. All surveys were conducted by SCUBA diving.

2.4 Reproductive season

Seasonal changes in the formation of gametangia were monitored using thalli collected in the vicinity of quadrats once a month from March to September 2019. In 2019, thalli were absent in October. At each sampling point, 18–25 thalli were collected and brought to the laboratory. During collection, a thallus attached to substrata or a detached thallus with a holdfast was selected to avoid collection of thalli derived from same individual. In the laboratory, 50–100 utricles were removed from various parts of the thallus of each specimen and were examined under a microscope (BX50; Olympus, Tokyo, Japan) to determine the presence or absence of gametangia. A total of 900–1250 utricles were observed each month. The seasonal change in gametangium formation was evaluated based on the percentage of thalli that had utricles with gametangia and the percentage of utricles forming gametangia within each thallus. In addition, the sex was determined by the size of gametes (Lee 2008), which were released by crushing the gametangia and observed by microscopy. We treated gametangia including gel-like non-solid gametes as undeveloped, and utricles without gametangia as immature. According to this, all gametangia were assigned to four categories: immature, undeveloped, male, and female. Sex of thalli was also categorized based on the result of sex determination for the gametangia: immature, undeveloped, monoecious, male, and female. Relative frequencies of these categories were calculated for all gametangia and thalli examined.

3 Results

3.1 Abiotic factors

Water temperatures were between 14.4 and 28.4 °C. The daily average temperature exceeded 25.0 °C for 27 days from 20 August to 20 September and was colder than 16.0 °C from 22 February to 2 March. Fluctuations in water temperature within each day were higher in the summer (up to 7.7 °C on 9 July) than in the winter (Figure 3a).

Monthly averages were obtained for the daily fluctuation in photon flux density based on hourly data (Figure 3b). The maximum average photon flux density was higher from April to August than from September to February. Daylength was longest in June and July and shortest in November and December based on the average photon flux density.

DIN and dissolved PO_4–P fluctuated in the ranges of 4.11–0.28 μmol·l^{-1} and 0.46–0.01 μmol·l^{-1}, respectively (Figure 3c). Only DIN showed seasonal changes. The concentration increased rapidly from November to December and then dropped until March.

3.2 Seasonal changes in the length and density of attached thalli

The mean lengths of all thalli in the three quadrats and average values are shown in Figure 4a. One thallus was particularly long (i.e., 85.2 cm) in May. Thus, we excluded the individual from calculations of the average and standard error. The average length was approximately 5 cm until July. Thalli attached to the bedrock disappeared in August and tiny young thalli appeared in November, with an average length of less than 3 cm until March (Figure 4a).

The density of *C. cylindricum* attached to bedrock declined gradually from April to July (Figure 4b). Attached thalli were absent on the bedrock from August to October. After recruitment in November (mean of 3 quadrats, 0.08 inds·m^{-2}), the density increased until February. The density of thalli (1.08 inds·m^{-2}) was highest in February. During the study period, we did not find evidence for thallus regeneration from the basal parts.

3.3 Seasonal changes in the biomass of detached thalli

We observed the detached thalli from March until October (Figure 4c). The wet weight increased from 38.8 wwg·m^{-2} in May to 247.5 wwg·m^{-2} in June and decreased from 283.8 wwg·m^{-2} in August to 37.5 wwg·m^{-2} in September. Although we could not measure individual detached thalli

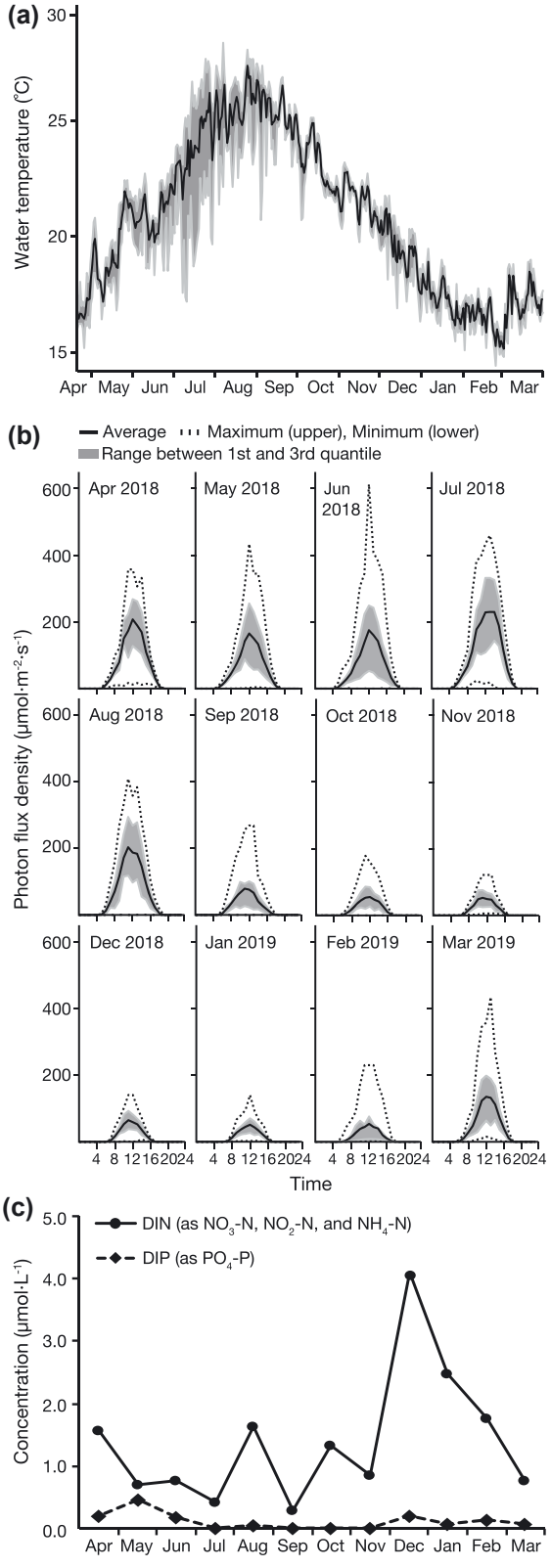


Figure 3: Seasonal changes in water temperature (a), photon flux density (b), and concentrations of nutrients (DIN and PO_4–P) (c) at the study site. Black line in (a) shows mean daily water temperature; gray area indicates daily fluctuations. For photon flux density, black line, dotted line, and gray areas show mean, maxima and minima, and 1st to 3rd quartile for each month, respectively.

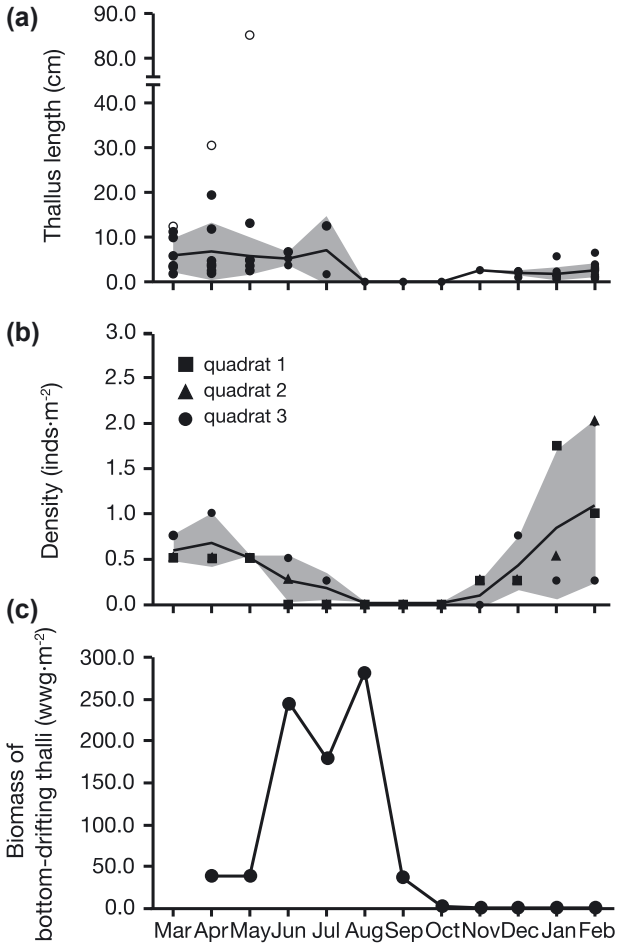


Figure 4: Seasonal changes in thallus length (a) and density (b) of *Codium cylindricum* attached to bedrock and in biomass (c) of detached *C. cylindricum*. Gray areas show standard errors for thallus length (n = 6–8, Mar.–May.; n = 2–3, Jun.–Jul.; n = 5–13, Dec.–Feb.) and density (n = 3).

owing to the difficulty of separating fragile individuals in the entangled mass, the thallus length reached a few meters in June to August (Figure 1b and c). The detached thalli fragmented in September (Figure 5).

3.4 Reproductive season

We first observed the formation of gametangia in June (Figure 6). The percentage of thalli with utricles bearing gametangia increased rapidly from 12.5% in June to 75.0% in July and reached 100% in September. The percentage of utricles producing gametangia within each thallus was 0.8% in June and increased from approximately 20% in July and August to 63.4% in September (Figure 6a).



Figure 5: Fragmented detached thalli of *Codium cylindricum*.

Thalli bearing undeveloped gametes were dominant from June to July but decreased thereafter, accounting for only 4% in September. The sex ratio of thalli was close to 1:1. The frequencies of male thalli were 4.1% in June, 16.8% in July, 32.0% in August, and 48.0% in September. The frequencies of female thalli were 12.5% in July, 24.0% in August, and 44.0% in September. Approximately 4% of thalli were monoecious every month from June to September (Figure 6b). Immature and undeveloped gametangia were dominant throughout the reproductive period. Similar to the thalli, the sex ratio of gametangia was close to 1:1. Male gametangia accounted for 0.6% in June, 3.0% in July, 6.5% in August, and 16.9% in September, and female gametangia accounted for 0.5% in June, 1.6% in July, 2.7% in August, and 13.0% in September (Figure 6c).

4 Discussion

We characterized the phenology of *Codium cylindricum* in detail for the first time. Unusual fluctuations in water temperature, photon flux density, and nutrient concentrations were not observed during the study period. Our measurements of attached and detached thalli indicated that the accumulation of *C. cylindricum* begins in early winter, biomass increases until summer, and reproductive organs are produced from early summer to fall, followed by its disappearance. These findings suggest that *C. cylindricum* is an annual species, despite the formation of a large thallus. However, whether the lifecycle is annual (Bulleri and Airoldi 2005; Serisawa et al. 2020) or perennial (Dromgoole 1975; Fralick and Mathieson 1972; Trowbridge 1996) varies among sites in the well-studied species *C. fragile*. Since our study was conducted on a temperate coast, further studies on tropical coasts are needed to establish whether southern populations of this species may have a perennial lifecycle.

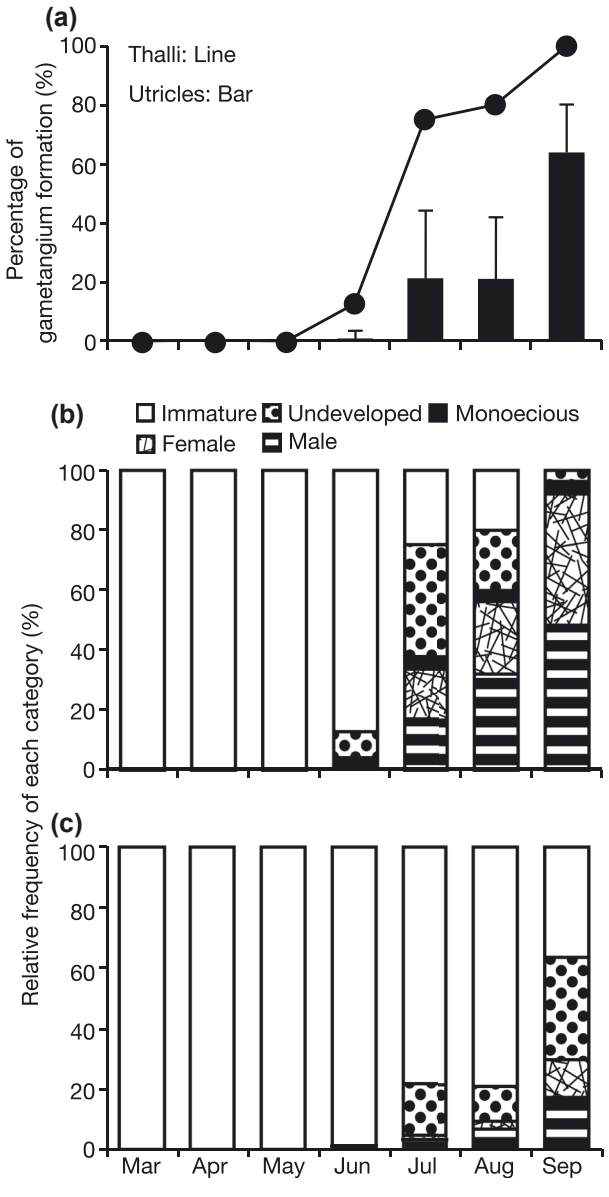


Figure 6: Seasonal changes in percentage of thalli (line graph) or utricles within each thallus (bar graph) showing gametangium formation in *Codium cylindricum* (a) and relative frequencies of sex types for thalli (b) and gametangia (c) of *C. cylindricum*. Error bars in (a) indicate standard errors (n = 24–25).

At the study site, we observed thalli attached to bedrock and drifting on the sea bottom. The density of attached thalli decreased from April to August, and a high biomass of detached thalli continued from June to August. As a reference, when we converted the length and density of attached thalli to wet weight biomass (wwg·m^{-2}) using eight detached thalli collected from the same area in May 2021 (length: 51.8 cm, wet weight: 113.05 g), the maximum wet weight biomass was 9.9 wwg·m^{-2} in April 2018, accounting for approximately 4% of detached thalli during the summer. In addition, the formation of gametangia was first detected in early summer and was highest in September, just one month before the disappearance of fragmented detached thalli. Therefore, the detached stage must be critically important for biomass growth and reproduction in *C. cylindricum*. If detached thalli disappear due to extreme events, such as large waves, they will fail to produce progeny at the study site. Given that most thalli attached to bedrock were less than 10 cm in length, the holdfast may be too weak to anchor a large thallus. An Eastern Pacific species, *C. amplivesiculatum*, also reaches a few meters in length without attaching to substrata (Pedroche et al. 2002; Riosmena-Rodríguez and Holguín-Acosta 2008). As the fragmentation in *C. fragile* contributes to the dispersal of gametes or utricles (Drouin et al. 2016), the detached stage of *C. cylindricum* might be important for the dispersal of gametes.

Codium cylindricum biomass was highest during the detached stage in June to August. These results suggest that the species is favored by summer conditions (e.g., strong light, high water temperature, and long daylength). Water temperature or photon flux density could be limiting factors for the growth of this species because nutrient concentrations are relatively high in the winter. Indeed, *C. cylindricum* showed the highest relative growth rate under 14L:10D, 50 µmol m^{-2} s^{-1}, and 25 °C in an analysis of various conditions for photon flux density (10, 30, 50 µmol m^{-2}·s^{-1}), water temperature (15, 20, 25 °C), and daylength (14L:10D, 10L:14D) (Miyadai unpublished data). The influence of recent temperature rises on the physiology of this species would be small.

With respect to asexual reproduction in *C. fragile* and its subspecies, fragmented thalli can re-attach to the substrata under certain conditions (e.g., when caught between stones or in a seagrass meadow), although reattachment takes several weeks (Gagnon et al. 2015; Scheibling and Melady 2008). Regeneration from the basal thallus and holdfasts left on bedrock has been recorded after the tip of the thallus had detached (Dromgoole 1975; Fralick and Mathieson 1972; Trowbridge 1996). Isolated utricles of *C. fragile* can form new upright thalli based on culture studies (Yotsui and Migita 1989). We did not find the evidence of thallus regeneration from the remains, or the reattachment, of fragmented drifting thalli in *C. cylindricum*. At least in this study site, the recruits appearing in the winter are apparently not derived from utricles but from zygotes because gametangia formation was highest in September. Further studies are desirable to establish whether this species lacks the ability to re-attach.

Codium cylindricum maturation started in June, and gametangia within thalli and among utricles reached peak

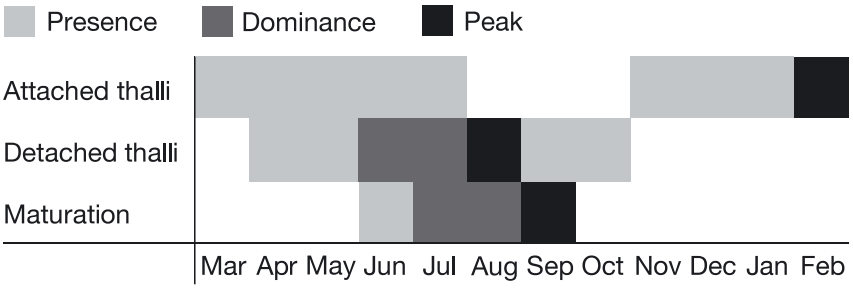


Figure 7: Summary of the phenology of *Codium cylindricum*. Gray, dark and black indicate presence, dominance, and peak, respectively.

levels in September. During this period, detached fragmented thalli were dominant, while thalli attached to the sea bottom were absent. This is consistent with findings with other species: maturation starts in early summer and peaks in autumn in *C. amplivesiculatum* (Riosmena-Rodríguez and Holguín-Acosta 2008), *C. fragile* (Arasaki et al. 1956; Serisawa et al. 2020), and *C. fragile* ssp. *tomentosoides* (Malinowski and Ramus 1973). The timing of this peak corresponds to the period of fragmentation in *C. amplivesiculatum* (Riosmena-Rodríguez and Holguín-Acosta 2008) and *C. cylindricum* (this study). Therefore, there is some overlap between fragmentation and the formation of gametangia in these giant *Codium* species. Unusual female-dominant populations resulting from parthenogenesis (Borden and Stein 1969; Churchill and Moelloer 1972) have been reported in *C. fragile* and *C. fragile* ssp. *tomentosoides*. We did not observe such cases in *C. cylindricum*. We detected monoecious thalli every month, although these accounted for only 4% of thalli. Further studies are needed to examine whether self-fertilization can occur between male and female gametes produced by monoecious thalli.

Our results reveal that the phenology of *C. cylindricum* can be summarized as in Figure 7. The lifecycle strongly relies on the detached drifting stage in early summer to autumn, during which period biomass increases and reproductive organs are produced. There is a risk of the loss of detached thalli; however, maturation in detached thalli can result in long-distance dispersal. Although the utilization of this species as food is locally limited and pharmaceutical applications have not yet been commercialized, the rapid growth in early summer of this species of *Codium* has a potential for commercial cultivation.

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