Seasonal succession of living coccolithophores in the coastal waters of Tomari Port, Tottori, Japan

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Abstract Seasonal changes in coccolithophore abundances and assemblage compositions were investigated in the coastal waters of Tomari, Tottori, Japan from July 2007 to June 2014. The coccolithophores were less abundant in winter, slightly increased in abundance in early to mid spring, and became rare/absent in late spring. They increased again in early summer, but disappeared again from the studied area in mid to late summer, while sea-surface temperatures exceeded 28˚C. Coccolithophores flourished the most throughout autumn. This seasonal cycle in the abundance of total coccolithophores was controlled by *Emiliania huxleyi* and *Gephyrocapsa oceanica* – the dominant species in the nannofloras throughout the year. *Braarudosphaera bigelowii* and *Tergestiella adriatica* occurred in mid June, for only a short period (5–15 days), following the low abundance period in late spring. Species of *Umbilicosphaera* and members of the Rhabdosphaeraceae appeared in early autumn. Middle to lower photic-zone dwellers, such as *Algirosphaera robusta*, *Oolithotus antillarum* and *Florisphaera profunda*, appeared in the sea-surface samples of the studied area only in mid to late autumn. Their occurrences indicate a weakening of water stratification in the studied area during autumn.

Keywords Coastal coccolithophores, seasonality, Cretaceous/Palaeogene (K/Pg) boundary

1. Introduction

Coccolithophores are marine haptophytes that are characterised by calcareous scales called coccoliths. The Division Haptophyta that includes coccolithophores consists of two classes – the Pavlovophyceae and Prymnesiophyceae – that are differentiated from each other based on their cell, scale, flagella and haptonema morphologies (Green & Leadbeater, 1994). The Pavlovophyceae do not have body scales, and are considered to be earlier-diverged lineages of the haptophytes due to their relatively simple morphologies and molecular phylogenetic positions (e.g. de Vargas et al., 2007; Edvardsen et al., 2011). Members of the Pavlovophyceae are distributed in both fresh and brackish waters (Green, 1980). Most members of the Prymnesiophyceae that produce organic body-scales have an affinity with coastal/neritic environments (e.g. de Vargas et al., 2007; Edvardsen et al., 2011). Other Prymnesiophyceae members that produce calcareous body-scales – the so-called coccolithophores – predominantly occur in oceanic environments (e.g. Young et al., 2003). These biogeographic, morphological and molecular phylogenetic lines of evidence suggest that oceanic, calcifying coccolithophores evolved from coastal non-calcifying Prymnesiophyceae (de Vargas et al., 2007).

Coccolithophores first appeared in the fossil record in the Late Triassic, gradually diversifying through the Mesozoic (Bown, 1987; Bown et al., 2004). At the Cretaceous/Paleogene (K/Pg) boundary mass extinction event, the oceanic Mesozoic species were eliminated, while the coastal Mesozoic species survived selectively (Pospichal, 1996; Bown, 2005). The coastal survivors temporally flourished in oceanic waters 'immediately' following the K/Pg boundary event; however, they once again became constrained to coastal waters as newly-evolved Cenozoic oceanic species appeared (Bown, 2005). The new Cenozoic taxa were predominantly oceanic, but some Cenozoic species, such as placolith-bearing taxa, were distributed in both coastal and pelagic waters (e.g. Young, 1994). Hence, modern coastal coccolithophore assemblages are a mixture of species with Cenozoic and Mesozoic origins.

The floral compositions of pelagic coccolithophores have been extensively studied in the Atlantic, northern Indian and Central Pacific Oceans. They differ among water-masses (e.g. McIntyre & Bé, 1967; Okada & Hon-

jo, 1973; Kleijne, 1993), and change seasonally (e.g. Okada & McIntyre, 1979; Reid, 1980; Broerse, 2000; Cortés et al., 2001). Neritic coccolithophore assemblages are relatively rich in placolith-bearing species, and usually poor in umbelliform and floriform species (e.g. Young, 1994). Quantitative floristic studies on coastal coccolithophores are scarce, and seasonal changes in coastal coccolithophores have only been reported from the Portuguese coast, facing the Atlantic Ocean (Silva et al., 2008), from the NW Mediterranean (e.g. Cros, 2002) and from certain small bays of Andros Island, Aegean Sea, NE Mediterranean (Triantaphyllou et al., 2002; Dimiza et al., 2008).

Figure 1: (a) Location of Port Tomari, Tottori, Japan. (b) Sample localities along the coast and offshore of Port Tomari

Successions of coccolithophores were recorded from the coastal waters of Tomari, Tottori, Japan (Sea of Japan, adjacent to the NW Pacific), over a six-year period, as part of a morphological and molecular phylogenetic study of two coastal taxa with Mesozoic fossil records – *Braarudosphaera bigelowii* and *Tergestiella adriatica* – (Hagino et al., 2013, 2015, 2016). Occurrence data for all of the coccolithophore taxa in these samples were not discussed in these studies, however. Herein, we introduce the seasonality of the coccolithophores in the coastal waters of the Tomari area in order to better understand the characteristics of the coastal coccolithophore floras that have yielded *B. bigelowii* and *T. adriatica*. Differences in the occurrence patterns among the typical Cenozoic and Mesozoic species of coccolithophores in the studied area are also discussed.

2. Materials and methods
2.1 Floristic studies of coccolithophores
Surface seawater samples were originally collected for morphological and molecular phylogenetic studies of *B. bigelowii* and *T. adriatica* in Tomari Port and offshore of Tomari, Tottori Prefecture, Japan (Fig. 1; Hagino et al., 2013, 2015, 2016). Ten to 12L of seawater per sample were collected, using a bucket, from the quay and breakwaters of Tomari Port and from the Ishiwaki bathing beach, on 167 occasions, from July 2007 to June 2014,
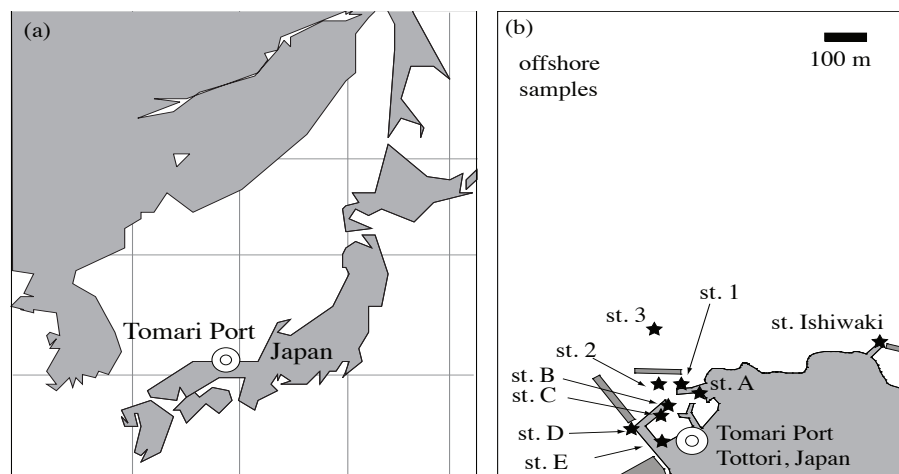
and offshore of Tomari, from the fishing boat *Ryoto-Maru*, on 65 occasions, from July 2007 to July 2013 (see Hagino et al., 2015, app. 1). In order to confirm the seasonality of the coccolithophores recorded during the opportunistic sampling in 2007–2012, sampling was conducted at Station 1 in Tomari Port at least once a month, from April 2012 to June 2014. Quantitative studies of the coccolithophore assemblages were conducted by identifying the specimens preserved on filters under a cross-polarised light microscope (LM). Selected filter samples were also examined using a scanning electron microscope (SEM) in order to evaluate the primary identifications made based on the optical microscopy (Plates 1–3). Preparation of the filter samples for floristic and morphological studies, procedures for the counting and identification of the coccolithophores, and the method of calculation of cell density of the major coccolithophore taxa are fully described in Hagino et al. (2015).

2.2 Observation of living cells
The 10-L seawater samples from the studied area were prefiltered through a 50μm plankton net (Sefar Inc., DIN-110). The prefiltered samples were then concentrated using a piece of 1μm-mesh plankton net (Sefar Inc., NY1-HD). The motility of the major coccolithophore taxa in the concentrated seawater samples was examined under an inverted LM, and then selected living cells were isolated using a micropipette, placed on a 24-well culture plate with MNK medium (Noël et al., 2004), and stored in a 20°C incubator under an 14:10 light:dark regime, in order to grow them into clonal culture strains.
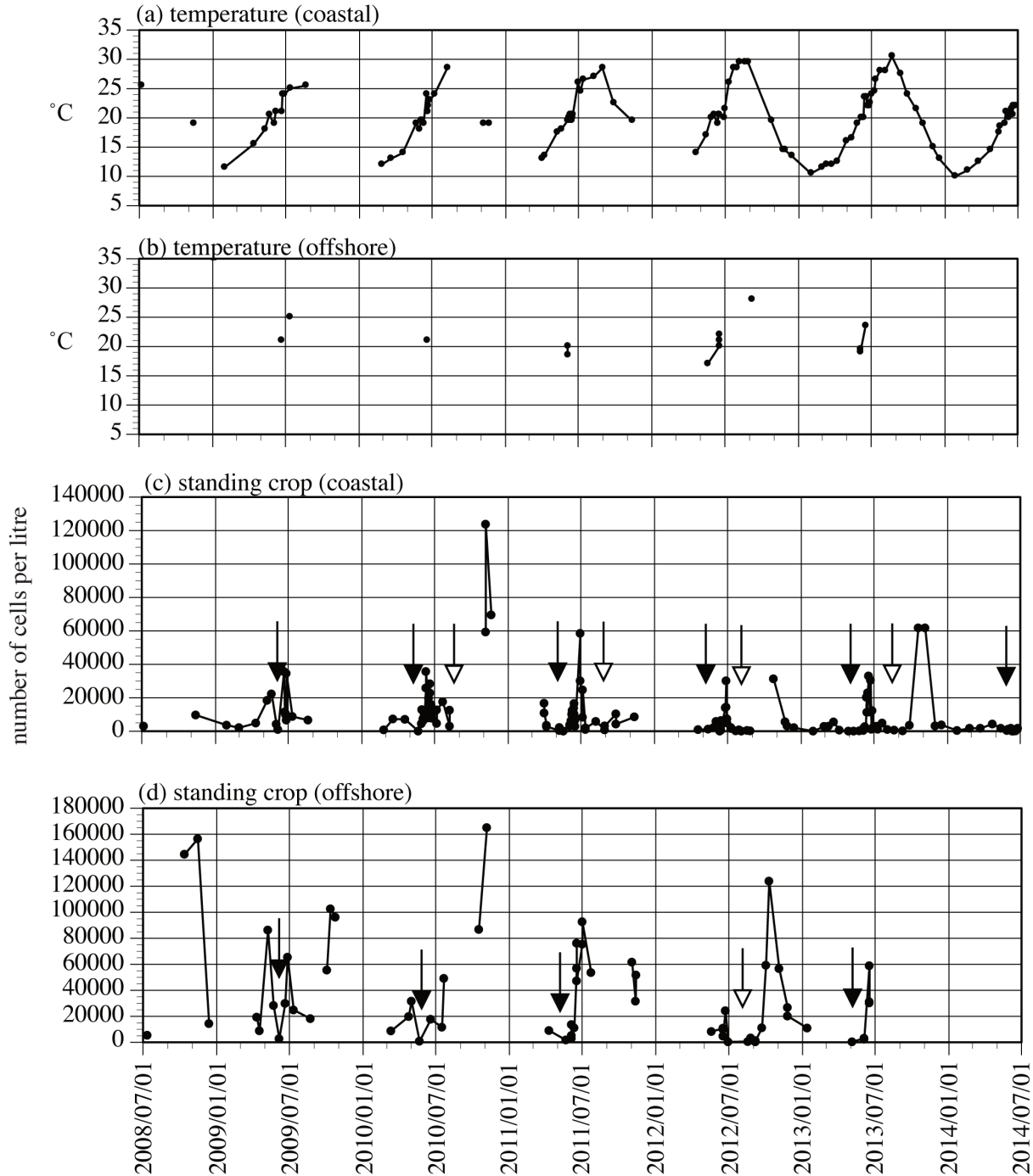
Figure 2: Seasonal changes in SSTs at (a) coastal and (b) offshore stations in the Tomari area, and seasonal changes in coccolithophore standing crops in (c) coastal and (d) offshore samples from Tomari. Arrows with solid and open heads in (c) and (d) indicate declines in cell density in May and August, respectively

3. Sea-surface temperatures (SSTs) in the Tomari area

The in situ SSTs of almost all of the coastal samples and some of the offshore samples were measured using a bar thermometer. The SSTs ranged from 27.0 to 30.5°C in summer (mid July–September), and from 10 to 12°C in winter (January–February; Fig. 2a, b; Hagino et al., 2015, app. 1).

4. Results
4.1 Total coccolithophore cell density

The coastal and offshore samples showed similar seasonal successions of coccolithophore standing crops, although the standing crops in the offshore samples were usually higher than those in the coastal samples (Fig. 2c, d). The standing crops at the coastal stations were usually <4000 cells/L in winter (late December–February). Unfortu-
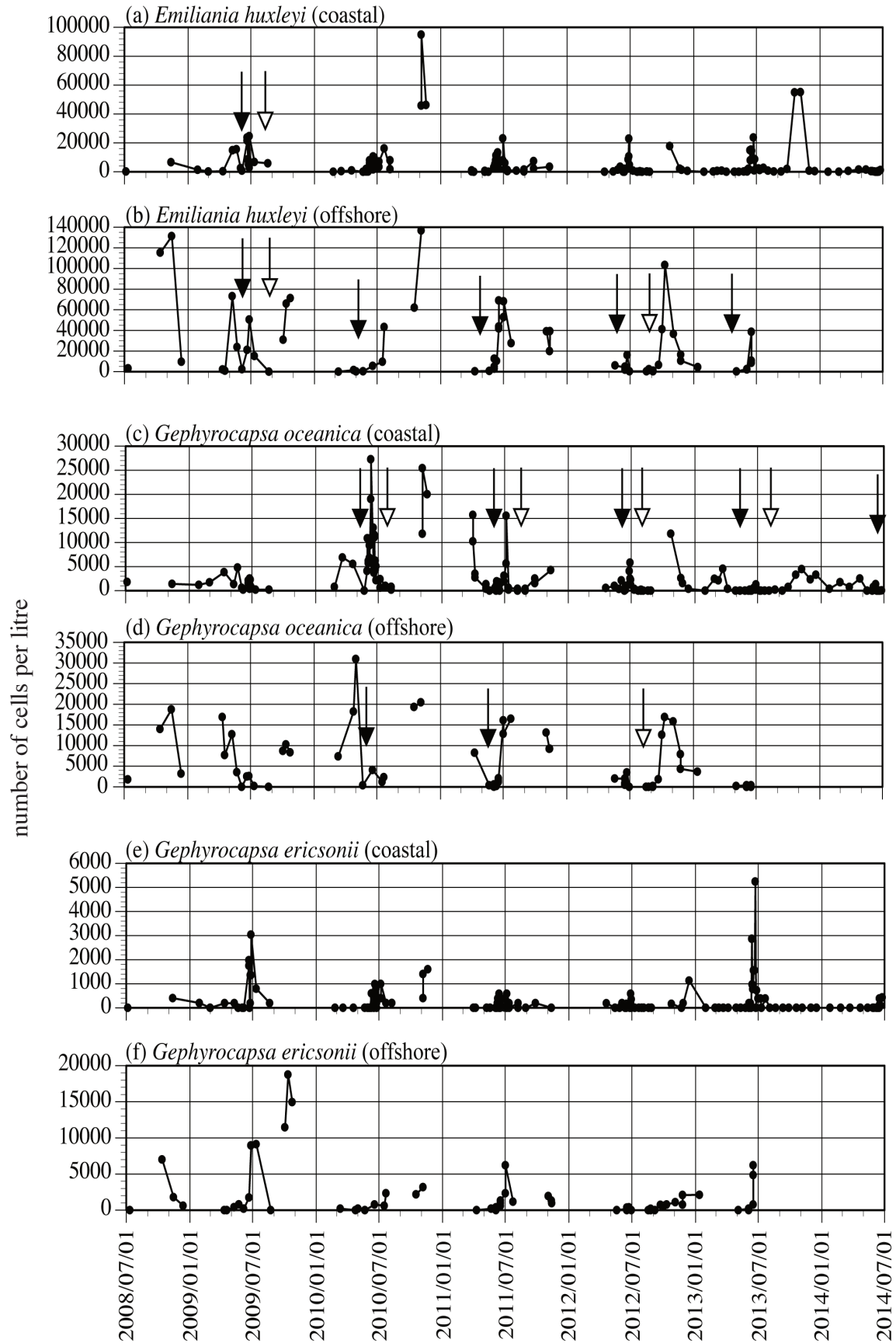
Figure 3: Seasonal changes in abundance of major coccolithophore taxa at coastal and offshore stations, Tomari area. Arrows with solid and open heads indicate a decline in cell density in May and August, respectively

nately, the coccolithophore abundances at the offshore stations during this period are not available because sampling was difficult due to high waves caused by strong seasonal winds. The standing crop increased to 2000–20,000 cells/L at the coastal stations, and 20,000–80,000 cells/L at the offshore stations, from March to early/mid May (Fig. 2c, d). The SSTs gradually increased from 13 to 16°C over this period (Fig. 2a, b).

The standing crop dropped to <2000 cells/L, or specimens were completely absent, at both coastal and offshore stations from mid May through late May/early June (solid arrows on Fig. 2c, d). In this period, the SSTs increased from c.16 to 19°C (Fig. 2a, b). The plankton net (50µm mesh size) used for the prefiltration of the seawater samples always caught the bioluminescent dinoflagellate *Noctiluca* during this period. Also at this time, the seawater was so transparent that the seafloor at Tomari Port was clearly visible from the pier of the port. This suggests that other phytoplankton (such as diatoms) were also rare.

Following the disappearance of *Noctiluca*, the coccolithophores recovered in abundance, occurring together with abundant diatoms (e.g. *Chaetoceros* spp.), from mid June to mid July. The plankton net used for seawater sample prefiltration was often clogged with *Chaetoceros* spp., and as a consequence of the relatively low water transparency, the seafloor in the port became invisible from the pier. During this period, the coccolithophore cell density was usually >10,000 cells/L at the coastal stations and >20,000 cells/L at the offshore stations (Fig. 2c, d), and the SSTs increased from 19 to 27°C (Fig. 2a, b).

The cell density again dropped to <1000 cells/L at both the coastal and offshore stations from mid July through mid September, when the SST exceeded 28°C (arrows with open heads on Fig. 2c, d). The standing crops were high from late September through early December, typically being >3000 cells/L at the coastal stations and >50,000 cells/L at the offshore stations (Fig. 2c, d), when the SSTs gradually decreased from 26 to 13°C (Fig. 2a, b).

4.2 Seasonal changes in major taxa at Tomari

Of the 22 taxa identified under the LM (Hagino et al., 2015, app. 1), the following eight taxa exceeded 2000 cells/L at least once during the six-year study period: *Emiliania huxleyi*, *Gephyrocapsa oceanica*, *G. ericsonii*, *B. bigelowii*, *T. adriatica*, *Syracosphaera* spp., *Algiros-phaera robusta* and *Calciopappus rigidus*.

Emiliania huxleyi and *G. oceanica* are the dominant species in the studied area. Consequently, their seasonal abundance changes almost equate to those of the total coccolithophore assemblages. These species occurred at both the coastal and offshore stations almost throughout the year, but were rare (<2000 cells/L), or absent, during the period of *Noctiluca* occurrence (mid May to late May/early June; solid arrows in Fig. 3a–d) and in mid to late summer, when the SST exceeded 28°C (mid July–mid September; open arrows in Fig. 3a–d). The cell density of these species was higher at the offshore stations than at the coastal stations (Fig. 3a–d). Holococcolith-bearing haploid cells of several coccolithophore taxa coexisted with *E. huxleyi* and *G. oceanica* at both the coastal and offshore stations, with very low abundances almost throughout the year, except during the coccolithophore-barren period in late spring and mid to late summer (not illustrated).

The seasonal changes in the abundance of *G. ericsonii* resemble those of *E. huxleyi* and *G. oceanica*, being abundant in occurrence in late June and in the autumn, but differ from those of *E. huxleyi* and *G. oceanica* in their absence/very low cell density in early spring (March to early April; Fig. 3e, f). The cell density of *G. ericsonii* was higher at the offshore stations than the coastal stations.

Braarudosphaera bigelowii and *T. adriatica* regularly occurred, from mid June to early July, at both the coastal and offshore stations in 2008–2013 (Fig. 4a–d), following the coccolithophore-barren/low-density period from mid May through late May/early June (Fig. 2c, d). The cell densities of these species were almost equivalent between the coastal and offshore locations, or were occasionally higher at the coastal sites than the offshore sites. *B. bigelowii* and *T. adriatica* coexisted with *E. huxleyi*, *G. oceanica* and *G. ericsonii* following the disappearance of *Noctiluca*, but, unlike *E. huxleyi*, *G. oceanica* and *G. ericsonii*, were absent from mid July through early June of the following year (Figs 3–4). The only exception was one cell of *B. bigelowii*, found on 22 November, 2012 by Hagino et al. (2013). In June 2014, multiple loose coccoliths of *T. adriatica* and loose pentaliths of *B. bigelowii* were recorded from the coastal samples collected from 1–20 June and 15–18 June, respectively, but coccospheres of these taxa were not found in the final sample of this
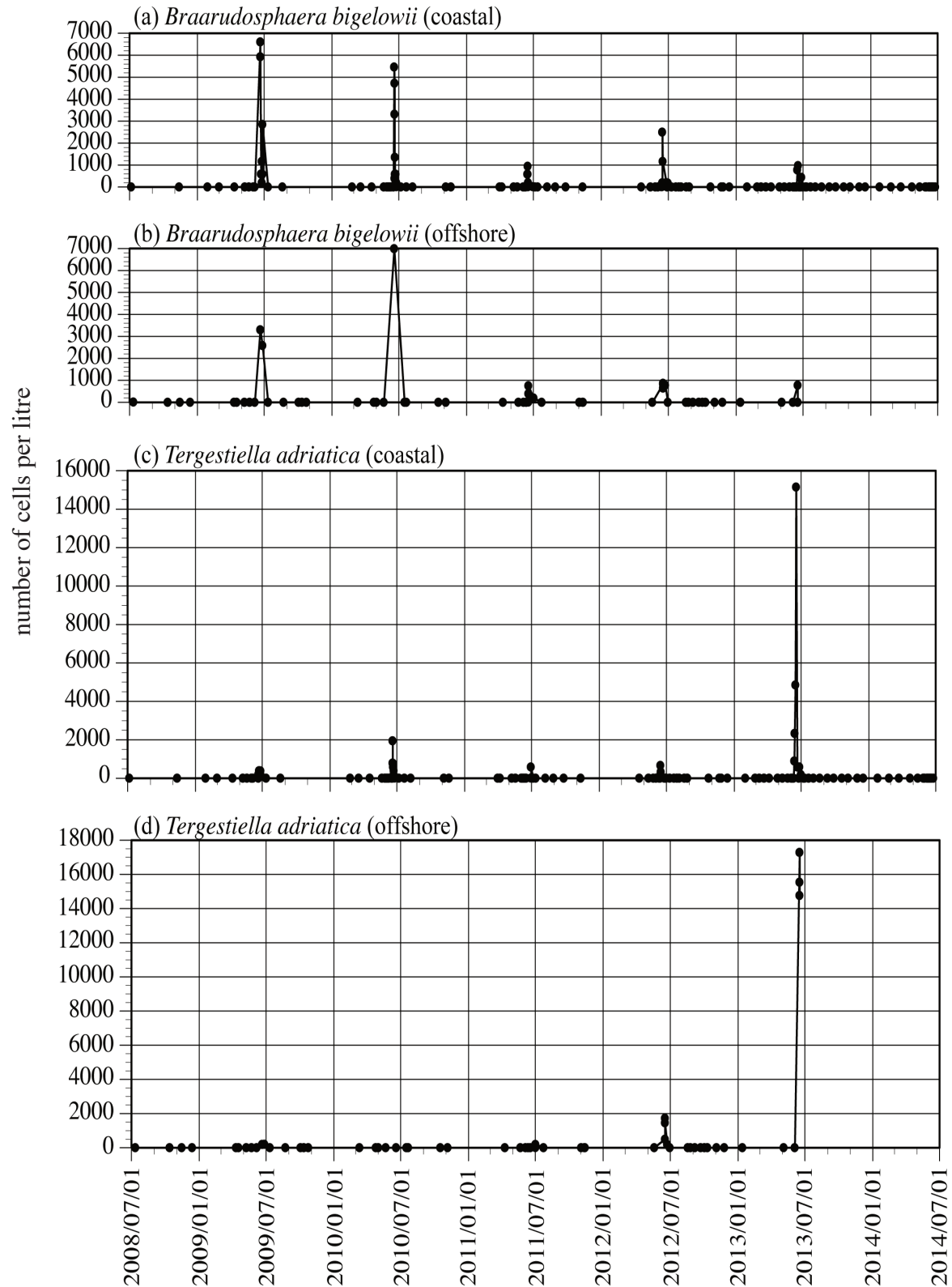
Figure 4: Seasonal changes in abundance of major coccolithophore taxa in coastal and offshore stations, Tomari area. *NB* This figure is a duplicate of Hagino et al. (2015, fig. 6)

study, collected on 25 June.

Syracosphaera spp. (mainly *S. pulchra*) occurred from late June or early July, and increased in abundance mainly in early summer (mid June–early July) and in autumn (late September–November), but did not occur in mid summer (mid July–mid September) or early spring

(March–May; Fig. 5a, b; Hagino et al., 2015, app. 1). The cell density of *Syracosphaera* spp. was usually higher at the offshore stations than the coastal stations. The seasonal succession of *Syracosphaera* spp. resembles that of *G. ericsonii*, but differs in the timing of its appearance in June. *G. ericsonii* appeared in mid June, together with *B. bigelowii*, *T. adriatica*, *G. oceanica* and *E. huxleyi*, while *Syracosphaera* spp. usually occurred from late June, as the numbers of *B. bigelowii* and *T. adriatica* declined (Hagino et al., 2015, app. 1).

Calciopappus rigidus occasionally occurred in the offshore samples, typically in low abundances (<600 cells/L) in autumn. It was usually absent from the coastal sites, although it was exceptionally abundant in the port on 30 June, 2011 (c. 48,000 cells/L; Fig. 5c, d; Hagino et al., 2015, app. 1).

Discosphaera tubifera, *Rhabdosphaera stylifera*, *Umbilicosphaera foliosa* and *U. sibogae* occurred as minor species (<2000 cells/L) from late September to November (not illustrated; Hagino et al., 2015, app. 1). Following the occurrence of these taxa, *A. robusta* regularly occurred from October to the end of November, when the SSTs decreased from 24 to 16°C. The cell density of *A. robusta* was higher in the offshore samples than the coastal samples (Figs 2a, b, 5e, f; Hagino et al., 2015, app. 1). *Florisphaera profunda*, *Helicosphaera walichii*, *Michaelsarsia* sp., *Oolithotus* spp., *Pontosphaera syracusana*, *Scyphosphaera apsteinii*, *Reticulofenestra sessilis* and/or *Ophiaster* spp. often co-occurred with *A. robusta* in low abundances (<2000 cells/L) in autumn (not illustrated; Hagino et al., 2015, app. 1).

4.3 Observation of living cells
We checked the motility/non-motility of the cells of the major coccolithophore taxa when isolating cells for the culture study, using an inverted LM. *E. huxleyi*, *Calcidiscus leptoporus*, *C. quadriperforatus*, *G. ericsonii*, *G. oceanica*, *R. sessilis*, *T. adriatica*, *U. foliosa* and *U. sibogae* were non-motile, and did not possess flagellae or haptonemas. *B. bigelowii* was non-motile, and did not have a flagella, but did have a haptonema, which it uses for adhesion to external substrates, as reported in Hagino et al. (2016). The following nine taxa were motile, and each had two flagellae and a haptonema: *A. robusta*, *D. tubifera*, *C. rigidus*, *H. wallichii*, *P. syracusana*, *S. apsteinii*, *R. stylifera*, *S. pulchra* and unidentified holococco-

lith-bearing taxa.

We established multiple clonal culture strains of *E. huxleyi*, *G. oceanica* and *S. pulchra* from the coastal and offshore waters of Tomari, but were not successful in culturing the other species.

4.4 SEM observation of selected species
We studied selected filters using an SEM in order to evaluate the identifications made in the LM, and to examine the detailed morphologies of selected species. Plates 1 and 2 show the major taxa and selected minor species observed in this study, respectively. Plate 3 shows examples of complete and collapsed specimens of *F. profunda* found in this study. The calcareous scales (nannoliths) of *F. profunda* were very well preserved, and the protococcolith-ring-like basal structure that was discovered by Young et al. (2010) was observed in several nannoliths of a collapsed coccosphere (Pl. 3, fig. c).

5. Discussion
Coccolithophores occurred from mid June to early July and from late September through the following April, and exhibited seasonal abundance changes. They were very rare or absent in mid to late summer (mid July through mid September) and in late spring (mid May through early June; Fig. 2c, d). The scarce occurrence of coccolithophores in mid to late summer was probably due to high SSTs (>28°C). This observation is consistent with the results of Hagino et al. (2000) who reported that coccolithophores, especially *E. huxleyi* and *G. oceanica*, were rare in the surface-waters of the Western Pacific Warm Pool, where SSTs exceeded 28°C. On the other hand, the scarcity of coccolithophores from mid May to early June was unexpected. The SSTs during this interval (16–19°C) seem to be suitable for coccolithophore growth because the temperatures are within the range where coccolithophores are common – before May (<16°C) and after mid June (>19°C). Therefore, SST was not a major controlling factor in the decrease in abundance of coccolithophores during late spring.

The decrease in abundance of coccolithophores in late spring coincided with the appearance of *Noctiluca* spp. This observation is consistent with those of previous studies that have shown a negative relationship in abundance between *Noctiluca scintillans* and other phytoplankton. The decrease in phytoplankton abundance during *N. scin-*
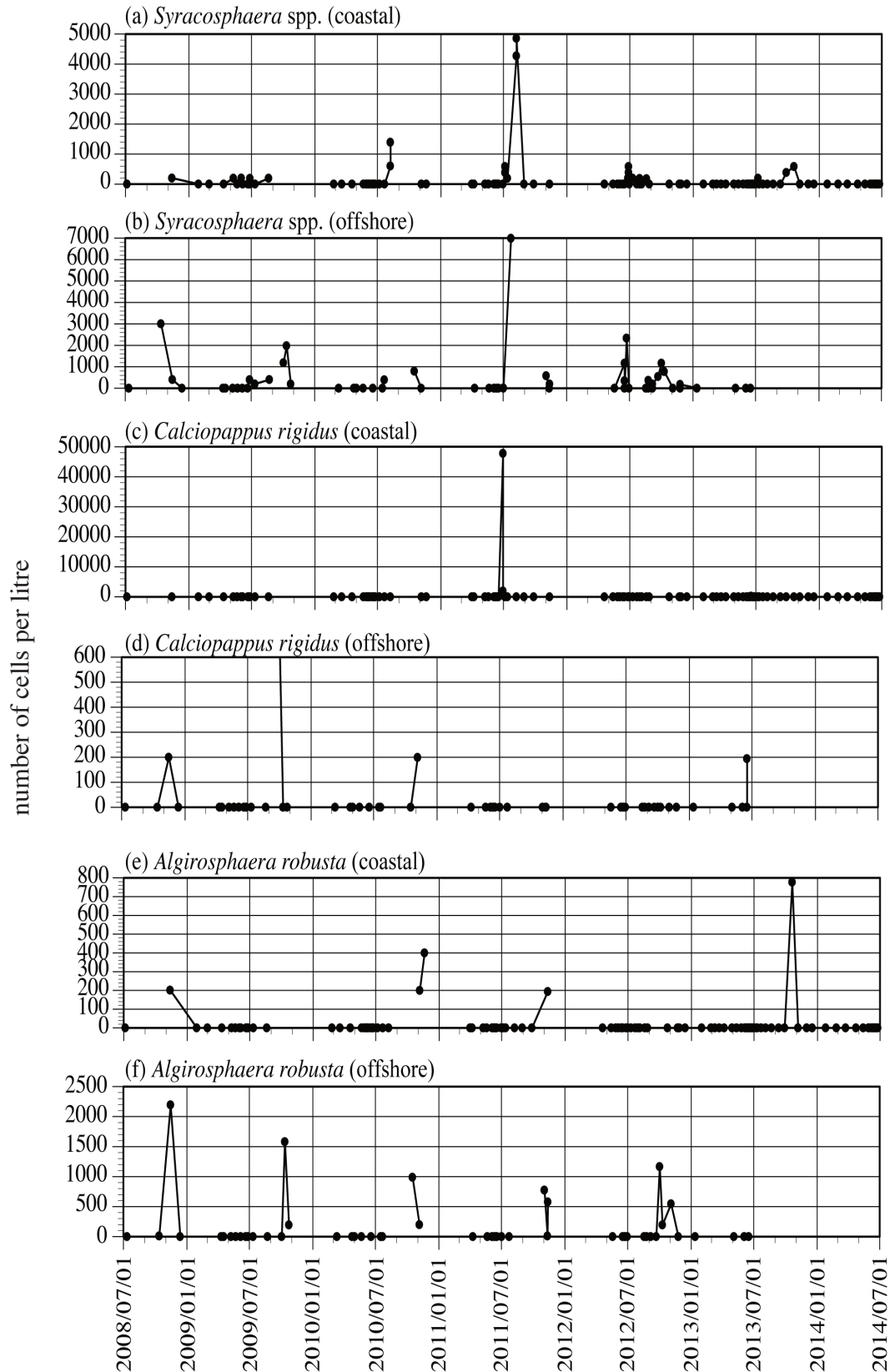
Figure 5: Seasonal changes in abundance of major coccolithophore taxa in coastal and offshore stations, Tomari area

tillans bloom/abundant periods has been interpreted as being the result of high predation pressure by *N. scintillans* (e.g. Dharani et al., 2004; Mohamed & Mesaad, 2007). These studies have also reported that blooms of *N. scintillans* resulted in a decrease in seawater pH due to the emission of CO_2 by the respiration of *N. scintillans*. The sensi-

tivity of coccolithophores to the lowering of seawater pH has been widely studied in the last decade, since the effects of ongoing ocean acidification on marine calcifying organisms has been of great concern. Culture experiments have shown that the calcification of coccoliths is affected by acidification, although the response varies among species, and even among strains in identical species (e.g. Langer et al., 2006, 2009; Iglesias-Rodríguez et al., 2008). Based on the summation of previous experiments, Meyer & Riebesell (2015) concluded that acidification has a negative effect on the calcification and cellular particulate inorganic to organic carbon ratios in *E. huxleyi* and *G. oceanica*. Because these two species are dominant in the studied area, the change in pH, in tandem with the high occurrence of *Noctiluca*, is possibly a controlling factor in the absence/ scarcity of coccolithophores in late spring.

The compositions of the coccolithophore assemblages changed seasonally (Fig. 6). *A. robusta* co-occurred with *Michaelsarsia* spp., *Oolithotus antillarum*, *Ophiaster* spp. and *F. profunda* from early October through December (Hagino et al., 2015, app. 1). All these species are known to be middle to lower photic-zone dwellers in stratified open-ocean locations (e.g. Okada & Honjo, 1973; Hagino et al., 2000; Cortés et al., 2001; Cros et al., 2001; Dimiza et al., 2015), and so it was unexpected to find these taxa regularly in the coastal surface-waters in this study, since they were thought to be rare in the neritic waters of the continental shelves (e.g. Young, 1994). In this study, most specimens of *A. robusta* observed in the concentrated seawater samples destined for the culture study were motile (alive). We could not find any cells of *F. profunda* in the concentrated seawater samples using an inverted LM; therefore, it is unclear whether the *F. profunda* cells observed in the filter samples with both a cross-polarised LM and SEM were actually alive in the coastal surface waters. The presence of protococcolith-ring-like basal structures on the nannoliths of *F. profunda* (Pl. 3, fig. c), which are easily dissolved/lost during preservation (Young et al., 2010), however, suggests that the observed specimens of *F. profunda* were likely living locally, and were not dead cells transported from a long distance away.

The co-occurrence of these middle to lower photic-zone dwellers in the surface-waters suggests that mixing of the water-column likely occurred in the studied area in autumn. *U. sibogae* regularly appeared in low abundances (<2000 cells/L) from late September, a few weeks prior to the appearance of *A. robusta* (Hagino et al., 2015, app. 1). *U. sibogae* is known to prefer moderately eutrophic conditions, between an upwelling centre and stratified oligotrophic waters (Hagino & Okada, 2004, 2006). Therefore, the appearance of *U. sibogae* prior to the occurrence of middle to lower photic-zone taxa is likely to indicate a weakening of stratification, starting in late September.

Among the 22 taxa identified in the cross-polarised LM, *B. bigelowii* and *T. adriatica* have Mesozoic fossil records, whereas the remaining 20 taxa evolved in the Cenozoic (Bown, 1987, 2005; Hagino et al., 2015). A comparison of the cell densities of the major taxa among the coastal and offshore stations showed that *B. bigelowii* and *T. adriatica* were adapted to both coastal and neritic waters, while the other 20 taxa preferred offshore waters to coastal ones, although they do live in coastal waters, but in significantly lower abundances.

These results confirm the findings of previous studies – that K/Pg survivors had an affinity for coastal environments, while typical Cenozoic coccolithophores preferred oceanic waters (e.g. Young et al., 2003; Bown, 2005). Here, *B. bigelowii* and *T. adriatica* occurred regularly in mid June to late June/early July during the SST increase from 19 to 26°C, but they did not occur in autumn, when the SSTs decreased from 26 to 19°C. The factors inducing their co-occurrence in early summer are still uncertain, as discussed in Hagino et al. (2015); however, their specific occurrence patterns, their short-lived flourishes after the intervals of absent/rare oceanic taxa, are reminiscent of the episodic high abundances of coastal K/Pg survivors, including *B. bigelowii* and the ancestor of *T. adriatica*, after the extinction of the majority of Mesozoic oceanic taxa following the K/Pg event.

One of the possible mechanisms that could have caused the selective extinction of oceanic calcareous nannoplankton is a short-lived ocean acidification interval after the K/Pg event (e.g. D'Hondt et al., 1994; Alegret et al., 2012; Ohno et al., 2014). Blooms of *Noctiluca* can cause episodic decreases in the pH of surface seawater (Dharani et al., 2004; Mohamed & Mesaad, 2007). This could explain the lowered abundances of *E. huxleyi* and *G. oceanica*, which are sensitive to water acidification (e.g. Meyer & Riebesell, 2015), in the Tomari area during late spring. Here, we propose a working hypothesis for
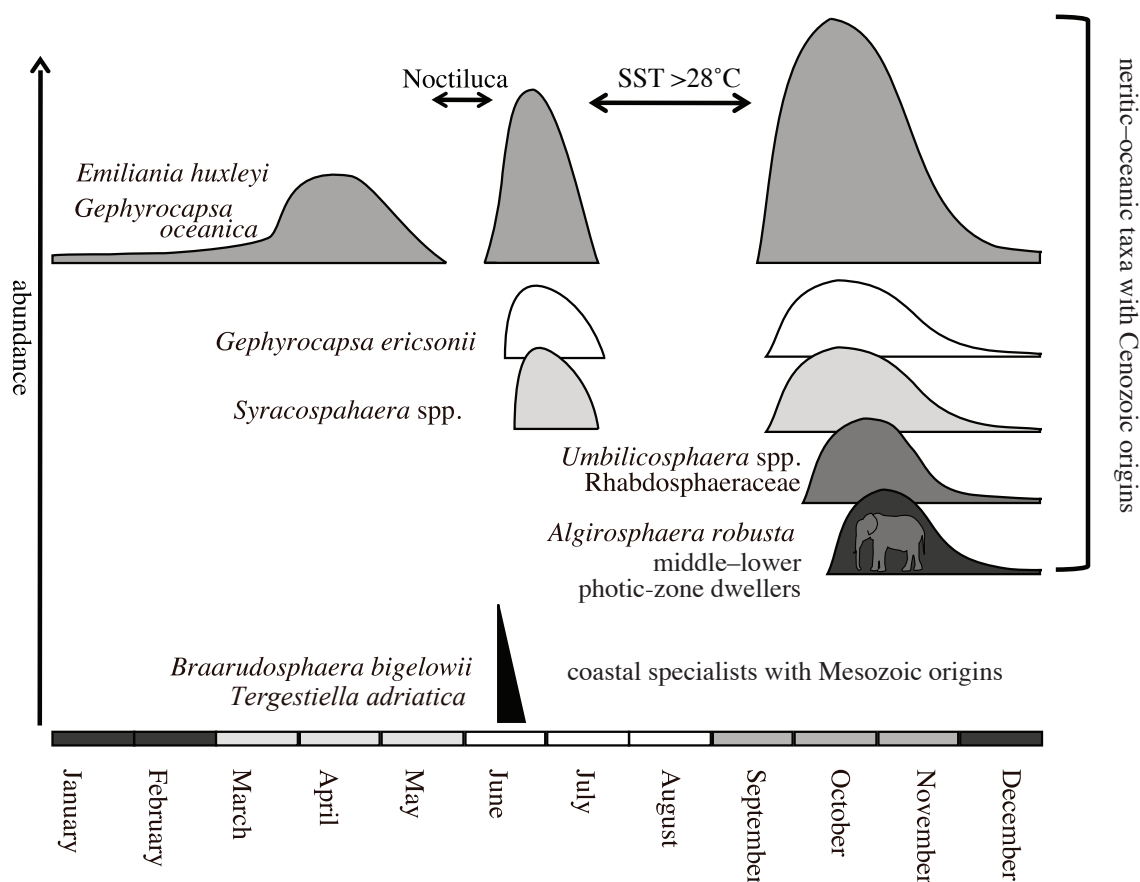
Figure 6: Schematic diagram showing the seasonal succession of coccolithophores in the Tomari area

the occurrence of the Mesozoic coastal taxa *B. bigelowii* and *T. adriatica* in mid June that involves them being triggered by a decrease in their competitors (*E. huxleyi* and *G. oceanica*), likely related to the abundance of *Noctiluca* spp. In order to confirm this hypothesis, further studies on seasonal changes in hydrographic conditions, as well as in the total plankton community, in the Tomari area will be necessary.

Acknowledgements

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Plate 1

SEM images of major coccolithophore species observed in this study

a. *Emiliania huxleyi* st. 3, 27 June, 2009

b. *Gephyrocapsa oceanica* st. 1, 27 May, 2012

c. *Gephyrocapsa ericsonii*, st. 3, 27 June, 2009

d. *Braarudosphaera bigelowii* st. D 15 June, 2012

e. *Tergestiella adriatica* offshore of Tomari 17 June, 2012

f. *Calciopappus rigidus* offshore of Tomari 25 November, 2012

g. *Syracosphaera pulchra* culture strain TMR-18 isolated from seawater 8 August, 2011

h. *Algirosphaera robusta* offshore of Tomari 12 October, 2012

$1\,\mu\mathrm{m}$

Plate 2

SEM images of selected minor coccolithophore species observed in this study

b. *Syracosphaera tumularis*, offshore of Tomari, 25 November, 2012

a. *Calciosolenia murrayi*
offshore of Tomari
25 November, 2012

c. *Syracosphaera bannockii*
offshore of Tomari
17 June, 2012

f. *Syracosphaera anthos*
offshore of Tomari
25 November, 2012

d. *Syracosphaera molischii*
offshore of Tomari, 23 June, 2012

e. *Syracosphaera ossa*
offshore of Tomari
12 October, 2012

g. *Syracosphaera rotula*
offshore of Tomari
4 October, 2012

h. *Syracosphaera corolla*
offshore of Tomari, 12 October, 2012

i. *Cyrtosphaera aculeata*
offshore of Tomari
23 June, 2012

j. *Acanthoica quattrospina*
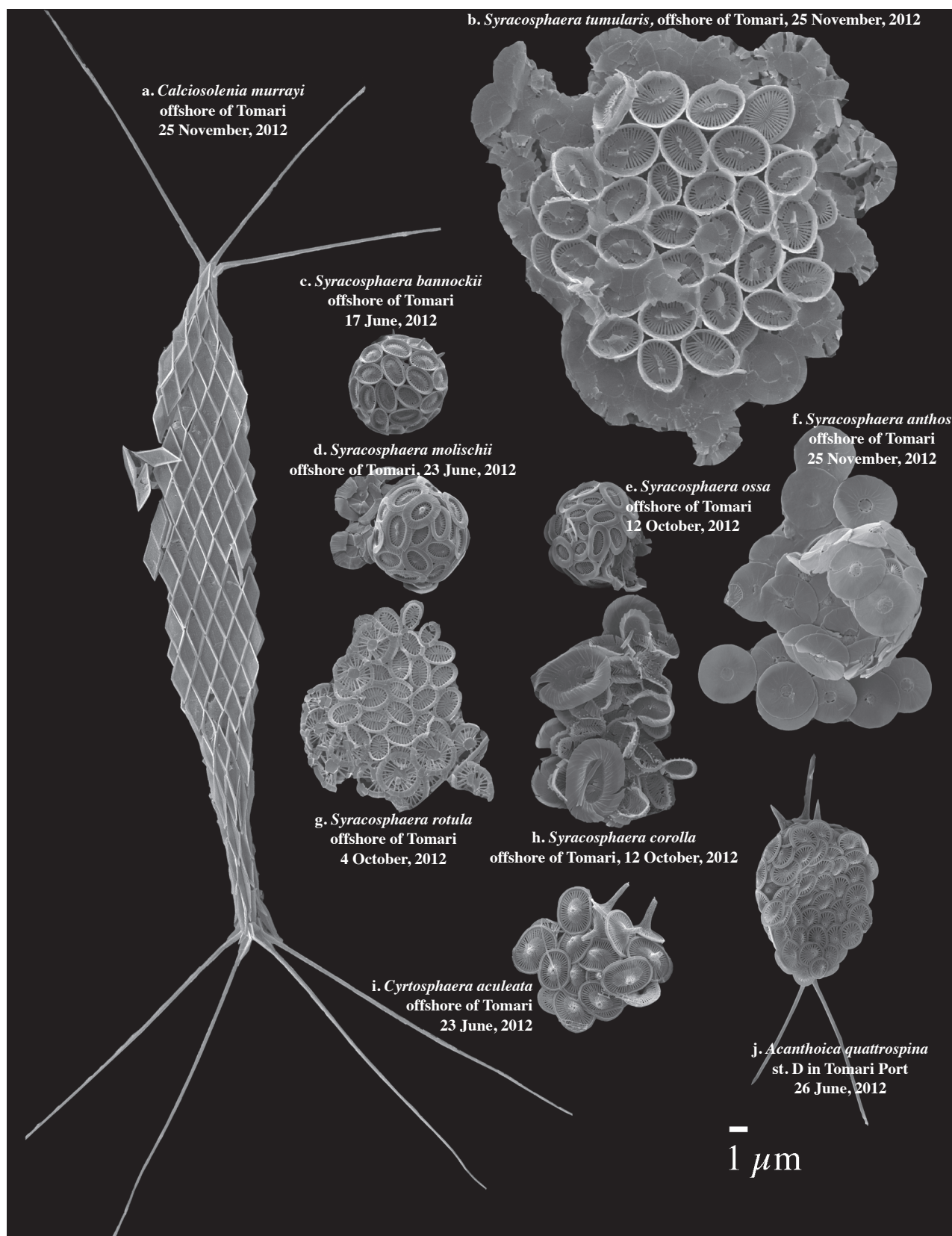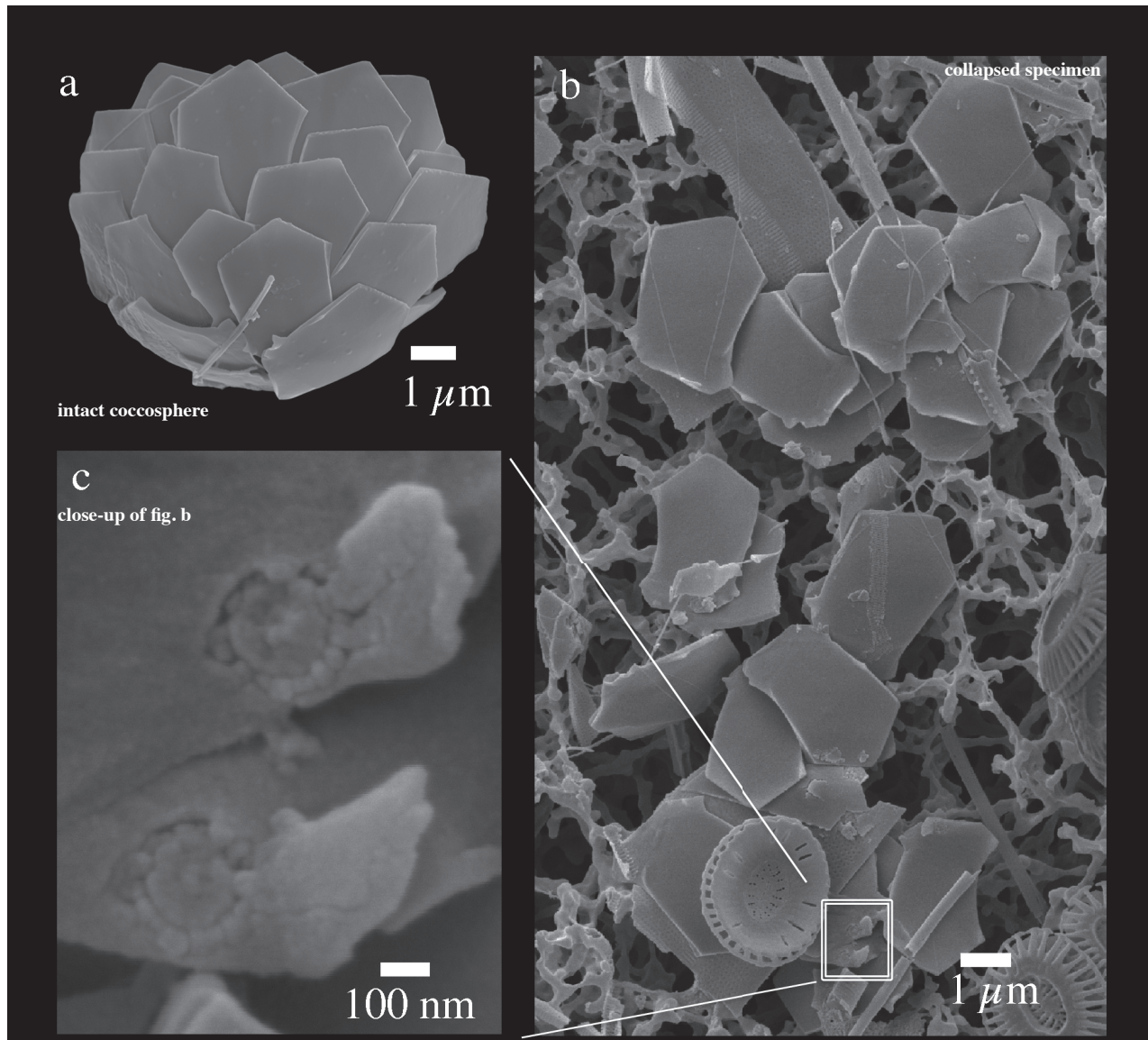st. D in Tomari Port
26 June, 2012

1 μm

Plate 3

SEM images of *Florisphaera profunda*, offshore of Tomari, 10 October, 2012

1957–1981.

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