

# The phenology of epiphytic diatoms and epifauna observed on *Zostera marina* of Arikawa Bay, Nagasaki Prefecture, Japan

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**Abstract:** We present a descriptive account of the dynamics of epiphytic diatoms, epifauna, and the leaf surface area of *Zostera marina* in a shallow water ecosystem. We hypothesized that the growth stage of the host macrophyte (i.e., leaf surface area) influenced the presence of epiflora and epifauna, as well as that the leaf surface area and epifaunal population density affected the cell density and species composition of epiphytic diatoms. To evaluate this hypothesis, we quantified the leaf surface area of a host macrophyte (*Zostera marina*), the presence of epifauna, and the community of epiphytic diatoms that could be observed on the leaves of *Z. marina* during the period from May 2017 to December 2018. We conducted a descriptive analysis of the time-series observations of leaf surface area, epiphytic diatom density, and epifauna population density. Epiphytic diatom density was low and epifauna density was high during the growing season of *Z. marina*. Epiphytic diatom density was high and epifauna density was low during the maturation and senescence periods of *Z. marina*. Our analysis shows that epifauna densities lagged epiflora densities by at least four months, and that epiflora densities lagged leaf area by four months. Therefore, we hypothesized that herbivorous gastropods and amphipods could alter species composition via their preference of food items (active choice) or by ingesting more of the species that were structurally more available (passive preference).

**Key words:** seagrass, microalgae, leaf morphology, amphipod, gastropod

## Introduction

Seagrass meadows play an important role in the ecosystem, serving as spawning and nursery grounds for a wide variety of aquatic organisms (Heck & Orth 1980, Orth et al. 1984, Hashimoto et al. 2009). These meadows also provide additional ecosystem services, such as water purification, wave suppression, sediment stabilization, and nutrient absorption; they have the ability to significantly influence photosynthesis and primary production in coastal areas (Dan et al. 1998, Hemminga & Duarte 2000, Jackson et al. 2001, Arita et al. 2008, Nishihara & Terada 2010, Yoshida et al. 2011). The main primary producers in coastal eco-

systems are macroalgae and microalgae (e.g., phytoplankton, benthic microalgae, and epiphytic algae). In particular, benthic microalgae serve as a food source for epifauna, which in turn serves as prey for higher trophic levels (e.g., fish and crustaceans) (Fry 1984, Heck et al. 2003, Lewis & Hollingworth 2012, Östman et al. 2016, Sturaro et al. 2016). The extracellular organic matter produced by these microalgae also contributes to the flux of organic nutrients in the coastal ecosystem by contributing to the microbial loop, which serves as a substrate for bacteria (Goto 2002).

In shallow coastal waters, most microalgae observed on both sediments and macroalgae are diatoms that can significantly contribute to primary productivity (Fukuda et al. 2010). In seagrass ecosystems, associated microalgae are suggested to provide 22% to 61% of the total primary production (Hemminga & Duarte 2000). The dominant spe-

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cies of the attached diatom assemblages on macrophytes are believed to be dependent on the substrate (Nakaoka et al. 2001, Fredriksen et al. 2005, Totti et al. 2009). This is especially true for epiphytic diatoms (Rautiainen & Ravanko 1972, Nigorikawa 1997, Ohtsuka & Tsuji 1999, Worm & Sommer 2000). Indeed, the species composition of epiphytic diatoms observed on macrophytes may not relate only to host species specificity, but it could also be a result of phytochemical environmental conditions and the physiological state of the host (Ohtsuka & Tsuji 1998). It is difficult to determine the relationship between binding epiphytic diatoms, host macrophytes, and the aquatic environment because the environment of shallow coastal areas is highly dynamic and the precise taxonomic identification of the diatoms is challenging (Round et al. 1990, Gaiser et al. 2005). However, this dynamic environment allows benthic or epiphytic diatoms to be less susceptible to growth limitations due to water temperature, salinity, and nutrients; top-down growth constraints by herbivores are believed to dominate bottom-up effects (Kawamura 2004, Duffy 2006, Winder & Sommer 2012).

Herbivores (i.e., epifauna) that partly feed on epiphytic diatoms find refuge in macrophytes, and in terms of seagrass ecosystems, small crustaceans, gastropods, and polychaetes are the predominant groups (Lewis & Hollingworth 1982, Baden 1990, Nakaoka et al. 2001, Namba & Nakaoka 2018). The factors affecting grazer-epiphyte interactions in seagrass ecosystems are complex (Jernakoff et al. 1996), and the inclusion and exclusion of predators have immediate effects on grazers and epiphyte abundance (Reynolds et al. 2014, Östman et al. 2016). Reduced grazing by predators increased epiphyte abundance, and this effect was greater than that of fertilization (Duffy et al. 2015, Östman et al. 2016). Hence, grazing by epifauna is a dominant factor limiting the growth of epiphytic diatoms on host macrophytes.

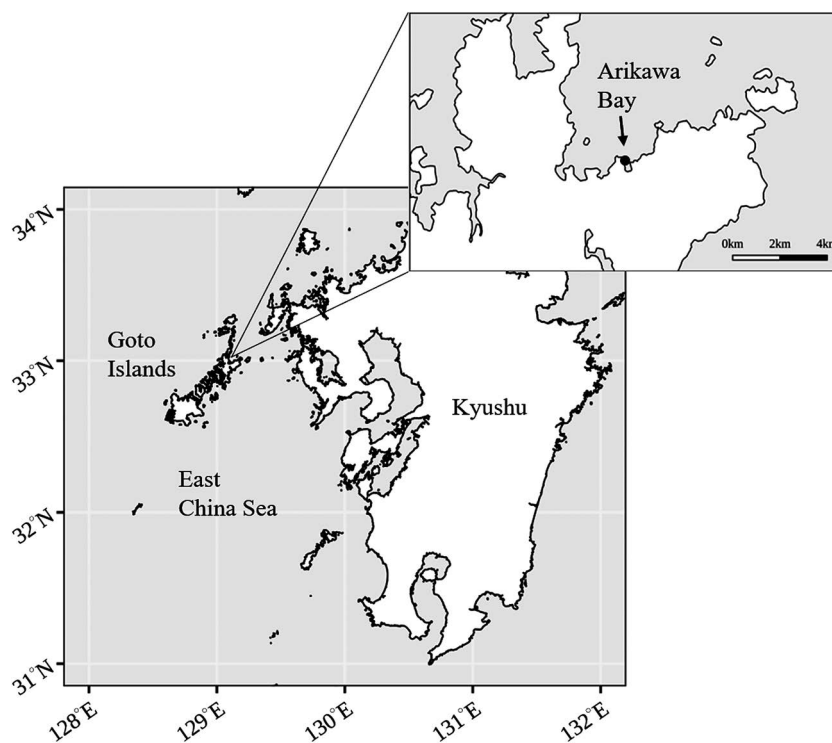
Kawamura (1994) classified benthic diatoms into eight colony types (i.e., Type A: motile prostrate, Type B: non-motile prostrate, Type C: solitary upright, Type D: colonial upright, Type E: solitary mucus thread, Type F: colonial mucus thread, Type G: mucus tube, and Type H: colonial thread) based on their ability to form colonies and four other characteristics (i.e., colony shape, mucus secretion form, motility, and adhesion). Using this classification, laboratory experiments using glass slides revealed that there was a progression of change in community structure under different conditions of light and herbivory (Kawamura 1995, 1998). However, the diatom communities attached to macrophytes are unlike those observed on glass slides. It is reasonable to expect that the surfaces of seagrass leaves and glass slides are dissimilar, and that the condition of the seagrass, along with the dominant epifauna, will influence the diatom community. The magnitude of these effects would be expected to vary with season.

Quantifying the phenological response of epiphytic diatoms of macrophytes is challenging because of the diffi-

culty in identifying benthic and epiphytic diatom species. Hence, ecological studies are limited and tend to report readily extractable information, such as geographic distribution and diatom abundance (Worm & Sommer 2000, Suzuki 2015). Nevertheless, a few reports on the species composition and cell density of epiphytic diatoms for certain macrophyte species are available (Jacobs & Noten 1980, Tanaka 1986, Fredriksen et al. 2005, Totti 2009). In the Baltic Sea, the micro-epiphytic community was more affected by season than by host preference (Snoeijs 1994, 1995), whereas in Skagerrak, Norway, the host macrophyte generated a strong effect (Fredriksen et al. 2005). Therefore, we hypothesized that the host macrophyte growth stage (i.e., leaf surface area) influenced the presence of epiflora and epifauna, and along with epifauna population density affected the cell density and species composition of epiphytic diatoms. To evaluate this hypothesis, we quantified the leaf surface area of a host macrophyte (*Zostera marina* Linnaeus), the presence of epifauna, and the community of epiphytic diatoms observed on the leaves of *Z. marina*, during the period May 2017 to December 2018, and analyzed the data using a generalized additive model (GAM).

## Materials and Methods

*Zostera marina* was collected monthly from a seagrass meadow in Arikawa Bay, Nagasaki, Japan, from May 2017 to December 2018 (Fig. 1). One shoot of *Z. marina* (excluding the underground root) was carefully collected by snorkeling and placed inside a container with 1 L filtered seawater every month. Samples were fixed with a mixed solution (2 : 1) of formalin (37% aqueous solution) and glutaraldehyde (25% aqueous solution) to achieve a final concentration of 1%. Any attached organisms were carefully removed manually with rubber gloves from each leaf of the preserved seagrass specimens. Individual plants were further washed with filtered seawater, which along with the removed organisms, were passed through a 300  $\mu\text{m}$  net to separate the epifauna that remained on the net from the epiphytic diatoms. The seagrass surface area was measured from digital images using ImageJ version 1.51, after taking a photograph of one side of the seagrass (Schneider et al. 2012). The obtained value was doubled to obtain the total surface area. The diatom frustules were treated following the procedure outlined by Nagumo (1995). The pre-concentrated diatom sample and distilled water were placed in a centrifuge tube (Centrifuge Tubes, IWAKI, Tokyo, Japan) with an equal amount of cleaning agent. To enhance the effect of the cleaning agent, centrifuge tubes were placed in a 50°C water bath for approximately 30 min (Step 1). Centrifugation (Manual centrifuge, AS ONE, Osaka, Japan) was performed to precipitate the diatom sample, and the supernatant was discarded (Step 2). Distilled water (10 mL) was added and the tubes rested for 10 min (Step 3). Steps 2 and 3 were repeated five times to re-



**Fig. 1.** The study was conducted in Arikawa Bay, Nagasaki, Japan ( $32^{\circ}59.17.7' \text{ N}$ ,  $129^{\circ}07.03' \text{ E}$ , water depth ca. 3 m).

move the cleaning agent. Aliquots (1 mL) were placed under an inverted light microscope (IX-70, Olympus, Tokyo, Japan) for counting and identifying epiphytic diatoms and epifauna. A second inverted microscope (BX-51, Olympus, Tokyo, Japan) and a scanning electron microscope (JSM-6390LAKII, JEOL, Tokyo, Japan) were used to aid in the identification of diatoms, and a stereoscopic microscope (ST30RDL, AS ONE, Tokyo, Japan) was used to closely examine the epifauna where necessary. The following literature was utilized to identify diatoms and epifauna specimens: “Diatoms: Biology and morphology of the genera” (Round et al. 2007), “H. Kobayashi’s atlas of Japanese diatoms based on electron microscopy” (Kobayashi et al. 2006), “An illustrated guide to marine plankton in Japan” (Chihara & Murano 1996), and “Benthos of the tidal flat” (Suzuki et al. 2013).

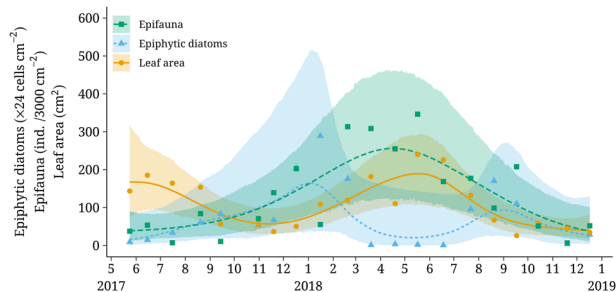
All statistical analyses were performed using R (R Core Team 2020). The epiphytic diatom total density, epifauna total density, and *Z. marina* leaf area time-series were examined independently using a GAM that applied a gamma distribution and log link function to the observations. A GAM was used, as the time series was too short to apply a population dynamics model. A generalized linear model (GLM) with the same distributional parameters of the GAM was fitted to each time series to serve as a null model. The smoothing functions used in the GAM was a p-spline with 15 basis functions (Eilers and Marx 1996). Each epiphytic diatom type and epifauna group were examined using a similar model; however, the p-spline was constructed with eight basis functions, the observations

were transformed using the natural logarithm, and the distribution was assumed to be Gaussian. A GLM was again used as the null model. The fitting was performed using Bayesian methods with the rstanarm (Goodrich et al. 2020) package, which uses Hamiltonian Markov Chains (HMC) to explore the posterior distribution. Weakly informative priors were applied to the intercept (i.e., a normal distribution with a location of 0 and a scale of 2), the coefficients of the smoothing functions (i.e., an exponential distribution with a location of 2), and the scale of the gamma distribution (i.e., an exponential distribution with a location of 0.5). Convergence, HMC mixing, and posterior validity were assessed visually. A total of four chains were run for 6000 iterations each. The two competing models were compared using the difference in the expected log pointwise predictive density of the leave-one-out cross-validation estimate,  $\text{elpd}_{100}$  (Vehtari et al. 2017).

## Results

### Seasonal variation of seagrass surface area

The surface area of *Z. marina* leaves ranged from 26.00 to 239.84  $\text{cm}^2$  (Fig. 2) throughout the sampling period. In May 2017, the surface area of leaves was 143.60  $\text{cm}^2$  reaching a maximum in June (185.21  $\text{cm}^2$ ), which coincided with the development of fruiting. *Z. marina* and then entered senescence, gradually losing leaf area, which decreased to 153.6  $\text{cm}^2$  by August. Thereafter, a rapid decrease in surface area was observed with a minimum in September



**Fig. 2.** The leaf surface area of *Zostera marina* and the total density of epiphytic diatoms and epifauna observed on *Z. marina* leaves. The solid and dashed lines indicate a descriptive analysis of the time-series observations. The shaded region indicates the 95% credible interval.

(56.44 cm<sup>2</sup>). Following this, *Z. marina* entered a growth period, and the surface area began increasing again gradually from January to April 2018 (108.28 to 110.60 cm<sup>2</sup>, respectively). The maximum area in 2018 occurred in May (239.94 cm<sup>2</sup>), and a similar cycle of maturation and senescence was observed for the remainder of the survey period.

The difference in the  $\text{elpd}_{100}$  between the GAM and GLM was  $-8.7 \pm 2.5$  (expected value  $\pm$  standard error) suggesting that the GAM had a higher predictive ability and that the smooths were relevant. The expected value was bimodal, and the maxima were observed in May 2017 and in May 2018, when the surveys began, and the minima in November 2017.

### Seasonal variation of epiphytic diatoms

Forty-eight genera including seventy-six species of epiphytic diatoms were observed in *Z. marina* (including some unidentified species; Table 1). The dominant species were *Ardissonea formosa* (Hantzsch) Grunow, *Climacosphenia moniligera* Ehrenberg, *Cocconeis heteroidea* Hantzsch, *C. pseudomarginata* Gregory, and *Rhabdonema adriaticum* Kützing (Fig. 3). The total cell density of epiphytic diatoms ranged from 15 to 6940 cells cm<sup>-2</sup>, increasing gradually from May to November 2017 and then decreasing to marginally detectable levels from April to June 2018 (Fig. 2). Cell densities began to increase toward a second peak in August 2018. The maximum cell density was observed in January 2018.

After fitting the GAM and GLM to the time series, the difference in  $\text{elpd}_{100}$  between the two models was  $-3.9 \pm 1.6$ , suggesting that the GAM had a higher predictive ability than the GLM and that the smooths were relevant (Fig. 2). This time series was clearly bimodal, with peaks occurring in January 2018 and August 2018.

In our study, epiphytic diatoms were classified into eight types (Kawamura, 1994) (Type A: motile prostrate, Type B: non-motile prostrate, Type C: solitary upright, Type D: colonial upright, Type E: solitary mucus thread, Type F: colonial mucus thread, Type G: mucus tube, and Type H: colonial thread). The cell density (cells cm<sup>-2</sup>) for each type

ranged as follows: Type A, 1–614; Type B, 1–2229; Type C, 0–5384; Type D, 1–313; Type E, 0–580; Type F, 0–3724; Type H, 0–229 (Fig. 4). Type A (*Nitzschia* and *Navicula*) and Type B (*Cocconeis*) were often dominant throughout the year, with other forms of attachment dominating in the short term. Type G was not observed in this study. Type B dominated from May to August 2017, after which time they declined and Type C (*T. hennedyanum*) became dominant from August to September. Type F (*C. moniligera*) replaced Type C when they diminished from October to December. Type F then decreased, and Type C (*A. formosa*) became dominant in January 2018. For the remaining periods, Types A and B were the principal diatoms, except in November 2018.

A comparison of the GAM and GLM fitted to the log-transformed observations of individual types returned values of  $\text{elpd}_{100}$  that were smaller than the standard error, indicating that there was insufficient information in the data to make definitive conclusions regarding the relevance of a smooth (Fig. 4).

### Seasonal variation of epifauna

The main epifauna groups identified were Amphipoda (e.g., Aoridae and Ischyroceridae), Gastropoda (e.g., Barleeidae), and Polychaeta (e.g., Nereididae and Spirorbidae). Of these, the predominant grazers were Amphipoda and Gastropoda, and detritus feeders or filter feeders were Polychaeta and tubicolous Polychaeta. We only examined the effect of grazers on epiphytic diatoms and they were only identifiable to the genus level. Therefore, further references to epifauna only include grazers. A more detailed analysis of the taxonomic diversity and population size of each genus was difficult due to the poor condition of the epifauna samples. The density of individuals ranged from 0.02 to 1.17 ind. cm<sup>-2</sup> (Amphipoda) and 0 to 0.19 ind. cm<sup>-2</sup> (Gastropoda). Overall, the total density of epifauna steadily increased from May 2017 to a maximum in May 2018 (Fig. 2). The density of amphipod individuals was low from June to September 2017, and it increased from September to December 2017 (Fig. 5), with more rapid growth from January to February 2018, remaining high until May. Amphipod density gradually decreased in June and increased again slightly in December. The density of gastropods escalated rapidly from July to August 2017 and declined rapidly thereafter until September 2017, after which time, they increased until November. Gastropod abundance was regained rapidly from March to April 2018 and then declined until August. Subsequently, August and September showed a rapid increase followed by another decrease until December.

The model fit to the epifauna time-series was examined with  $\text{elpd}_{100}$  that indicated a difference of  $-5.4 \pm 1.9$  between the GAM and the GLM, suggesting that the GAM had a higher predictive ability compared to the GLM and that the smooths were important. Unlike the previous two time-series, this time series was unimodal, with a peak

**Table 1.** Abundance of epiphytic diatoms species during the investigations. Shows taxa and each attached type.

Taxa	Type	Taxa	Type
<b>Class Bacillariophyceae</b>			
<b>Order Bacillariales</b>		<b>Order Mastgloiales</b>	
<b>Fam. Bacillariaceae</b>		<b>Fam. Mastroloiaceae</b>	
<i>Cylindrotheca closterium</i> (Ehrenberg) Reimann & J.C.Lewin	A	<i>Mastogloia</i> cf. <i>binotata</i> (Grunow) Cleve	B
<i>Nitzschia closterium</i> (Ehrenberg) W.Smith	A	<i>Mastogloia ovulum</i> Hustedt	B
<i>Nitzschia longissima</i> (Brébisson) Ralfs	A	<i>Mastogloia</i> sp.	B
<i>Nitzschia</i> sp.	A		
<i>Psammodictyon</i> sp.	A	<b>Order Naviculales</b>	
<i>Tryblionella</i> cf. <i>levidensis</i> W.Smith	A	<b>Fam. Amphipleuraceae</b>	
<i>Tryblionella</i> sp.	A	<i>Halamphora</i> sp.	A
<b>Order Cocconeidales</b>		<b>Fam. Diploneidaceae</b>	
<b>Fam. Cocconeidaceae</b>		<i>Diploneis</i> sp.	B
<i>Cocconeis</i> cf. <i>apiculata</i> (Greville) A.W.F.Schmidt	B		
<i>Cocconeis heteroidea</i> Hantzsch	B	<b>Fam. Naviculaceae</b>	
<i>Cocconeis pellucidan</i> Grunow	B	<i>Gyrosigma</i> sp.	A
<i>Cocconeis pseudomarginata</i> W.Gregory	B	<i>Navicula salincola</i> Hustedt	A
<i>Cocconeis scutellum</i> Ehrenberg	B	<i>Navicula</i> sp.	A
<i>Cocconeis</i> sp.	B	<i>Seminavis</i> cf. <i>strigosa</i> (Hustedt) Danieledis & Economou-Amilli	A
		<i>Trachyneis aspera</i> (Ehrenberg) Cleve	A
<b>Order Cycloporales</b>			
<b>Fam. Entopylaceae</b>		<b>Fam. Pinnulariaceae</b>	
<i>Gephyria media</i> Walker-Arnott	E	<i>Oestrupia powellii</i> var. <i>bartholomei</i> Cleve	A
<b>Order Cymbellales</b>		<b>Fam. Pleurosigmataceae</b>	
<b>Fam. Rhoicospheniaceae</b>		<i>Pleurosigma normanii</i> Ralfs	A
<i>Rhoicosphenia abbreviata</i> (C.Agardh) Lange-Bertalot	F		
		<b>Order Striatellales</b>	
<b>Order Eunotiales</b>		<b>Fam. Striatellaceae</b>	
<b>Fam. Peroniaceae</b>		<i>Striatella unipunctata</i> (Lyngbye) C.Agardh	D
<i>Peronia fibula</i> (Brébisson ex Kützing) R.Ross	C		
		<b>Order Rhaphoneidales</b>	
<b>Order Fragilariales</b>		<b>Fam. Rhaphoneidaceae</b>	
<b>Fam. Fragilariaceae</b>		<i>Rhaphoneis ampiceros</i> (Ehrenberg) Ehrenberg	B
<i>Fragilaria crotonensis</i> Kitton	B	<i>Rhaphoneis</i> sp.	B
<i>Fragilaria rumpens</i> (Kützing) G.W.F.Carlson	B		
<i>Podocystis adriatica</i> (Kützing) Ralfs	B	<b>Order Surirellales</b>	
		<b>Fam. Entomoneidaceae</b>	
<b>Fam. Staurosiraceae</b>		<i>Entomoneis</i> sp.	B
<i>Staurosira</i> sp.	D		
		<b>Fam. Surirellaceae</b>	
<b>Order Licmophorales</b>		<i>Iconella hibernica</i> (Ehrenberg) Ruck & Nakov	B
<b>Fam. Licmophoraceae</b>		<i>Stenopterobia</i> sp.	B
<i>Licmophora flabellata</i> (Greville) C.Agardh	F	<i>Surirella fastuosa</i> (Ehrenberg) Ehrenberg	B
		<i>Surirella</i> sp.	B
<b>Fam. Ulnariaceae</b>			
<i>Catacombas</i> sp.	C	<b>Order Talassiophysales</b>	
		<b>Fam. Catenulaceae</b>	
<b>Order Lyrellales</b>		<i>Amphora angusta</i> W.Gregory	B
<b>Fam. Lyrellaceae</b>		<i>Amphora ovalis</i> (Kützing) Kützing	B
<i>Lyrella lyra</i> (Ehrenberg) Karajeva	A	<i>Amphora</i> sp.	B
<i>Lyrella</i> sp.	A		



Table 1. Continued.

Taxa	Type	Taxa	Type
<b>Class Coscinodiscophyceae</b>		<b>Class Mediophyceae</b>	
<b>Order Coscinodiscales</b>		<b>Order Biddulphiales</b>	
<b>Fam. Aulacodiscaceae</b>		<b>Fam. Biddulphiaceae</b>	
<i>Aulacodiscus kittonii</i> Arnott ex Ralfs	B	<i>Lampriscus kittonii</i> A.W.F. Schmidt	C
<i>Aulacoseira</i> sp.	H		
<b>Fam. Coscinodiscaceae</b>		<b>Fam. Bissulpiaceae</b>	
<i>Coscinodiscus radiatus</i> Ehrenberg	B	<i>Biddulphia pulchella</i> S.F.Gray, nom.illeg.	D
<i>Coscinodiscus</i> sp.	B	<i>Biddulphiopsis</i> cf. <i>titiana</i> (Grunow) von Stosch & Simonsen	D
<b>Fam. Hemidiscaceae</b>		<b>Order Eupodiscales</b>	
<i>Actinocyclus gallicus</i> F.Meister	B	<b>Fam. Odontellaceae</b>	
<i>Actinocyclus octonarius</i> Ehrenberg	B	<i>Amphitetras antediluviana</i> Ehrenberg	D
<i>Actinocyclus</i> sp.	B	<i>Odontella aurita</i> (Lyngbye) C.Agardh	D
		<i>Odontella granulata</i> (Roper) R.Ross	D
<b>Fam. Heliopeltaceae</b>		<b>Order Thalassiosirales</b>	
<i>Actinoptychus senarius</i> (Ehrenberg) Ehrenberg	B	<b>Fam. Thalassiosiraceae</b>	
		<i>Thalassiosira nodulolineata</i> (Hendey) Hasle & Fryxell	H
<b>Order Melosirales</b>		<i>Thalassiosira</i> sp.	H
<b>Fam. Melosiraceae</b>		<b>Order Toxariales</b>	
<i>Melosira nummuloides</i> C.Agardh	H	<b>Fam. Ardissonaceae</b>	
<i>Melosira</i> sp.	H	<i>Ardissona formosa</i> (Hantzsch) Grunow	C
<b>Order Paraliales</b>		<b>Fam. Climacospheniaceae</b>	
<b>Fam. Paraliaceae</b>		<i>Climacosphenia moniligera</i> Ehrenberg	F
<i>Paralia sulcata</i> (Ehrenberg) Cleve	H	<b>Fam. Toxariaceae</b>	
		<i>Toxarium undulatum</i> Bailey	C
<b>Order Triceratiales</b>		<b>Order Rhabdonematales</b>	
<b>Fam. Triceratiaceae</b>		<b>Fam. Grammatophoraceae</b>	
<i>Triceratium alternans</i> Bailey	B	<i>Grammatophora angulosa</i> Ehrenberg	D
<i>Triceratium dubium</i> Brightwell	B	<i>Grammatophora marina</i> (Lyngbye) Kützing	D
<i>Triceratium pentacrinus</i> (Ehrenberg) Wallich	B	<i>Grammatophora oceanica</i> Ehrenberg	D
		<b>Fam. Rhabdonemataceae</b>	
		<i>Rhabdonema adriaticum</i> Kützing	E

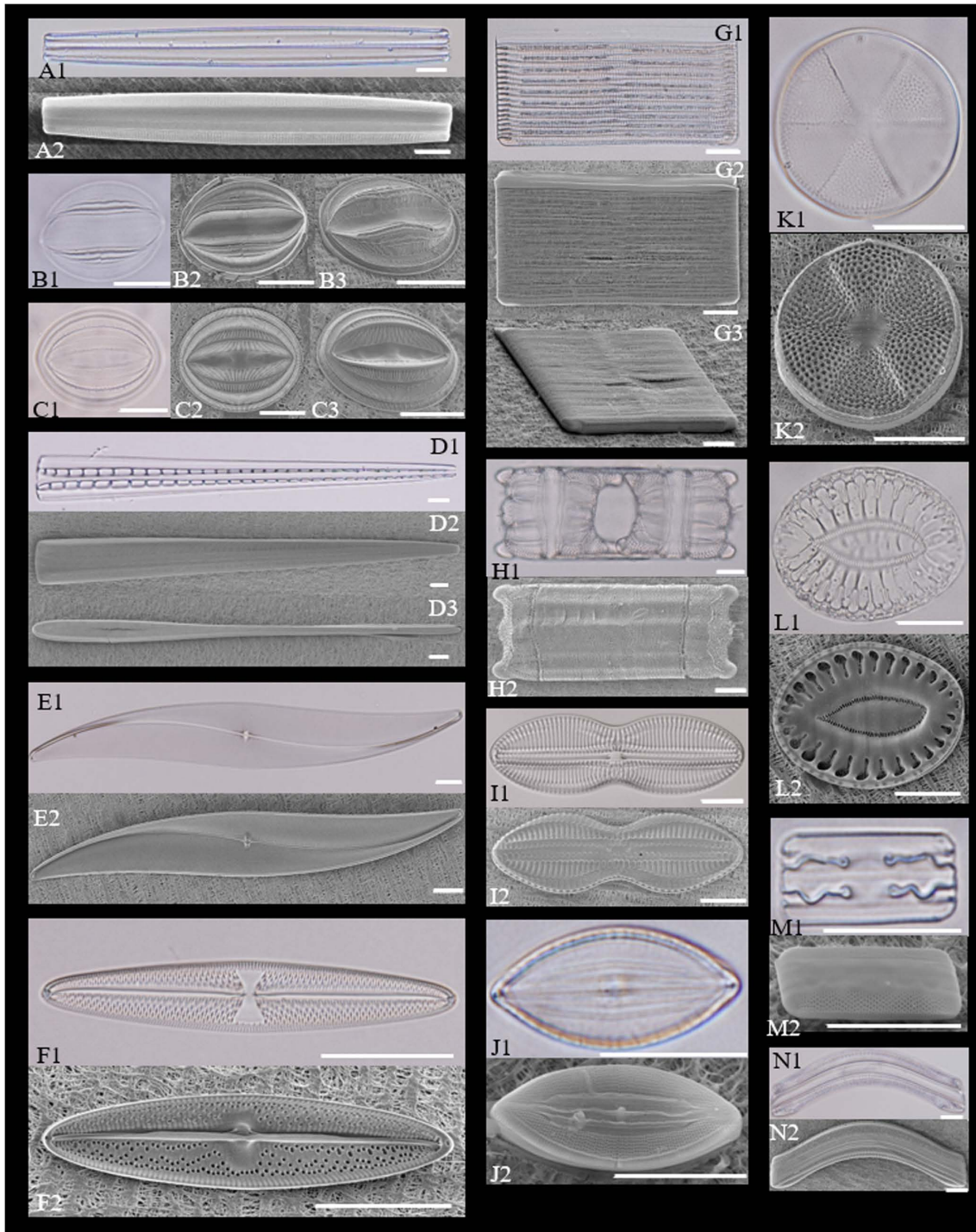
occurring in April 2018. However, when the two groups of epifauna were examined individually, there was insufficient evidence to support a smooth (Fig. 5).

## Discussion

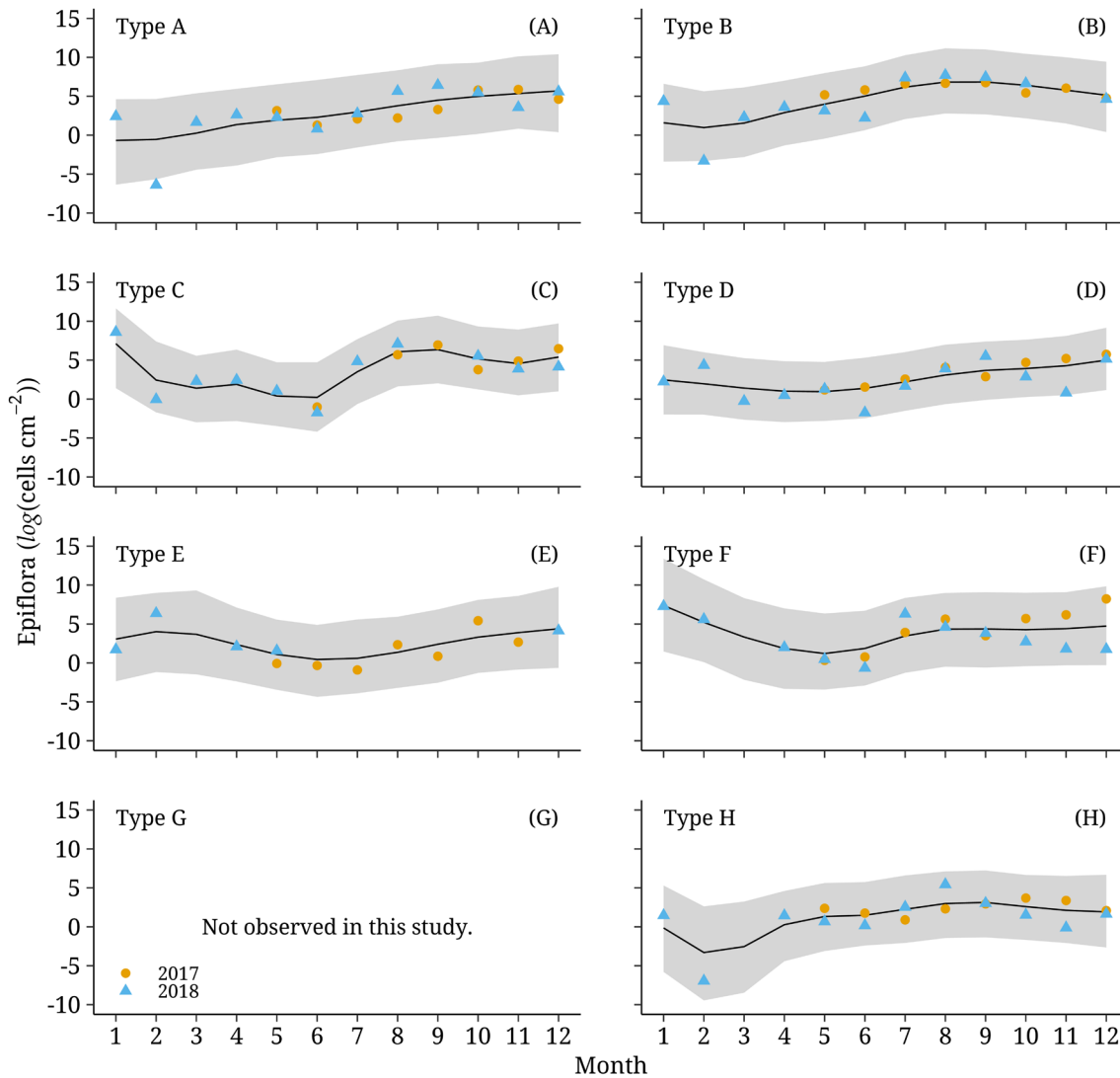
The phenological response of the state of the epiflora and epifauna of seagrass leaves is a well-researched topic (Jacobs et al. 1983, Nakaoka et al. 2001, Namba & Nakaoka 2018). Many studies have examined the interactions between epiflora and epifauna; however, evaluations on the interactions between seagrass leaves and epiflora are rare. For example, a study conducted in Otsuchi Bay, Japan, demonstrated that epiflora and epifauna population densities were not in phase and that the epifauna lagged the epiflora by a few months (Nakaoka et al. 2001). Nelson (1997)

modeled the dynamics of epiflora and grazers grown under controlled conditions by applying a Lotka-Volterra model with some success. An older study on the phenology of epiphytic diatoms on *Zostera marina* leaves suggested that leaf age strongly influences diatom abundance (Jacobs et al. 1983). We examined the seagrass-epiflora-epifauna relationship under natural conditions and proposed that the condition of the macrophyte (e.g., *Zostera marina*) drives epifloral population density, which in turn affects the epifauna population density (Fig. 2).

Given the abundance of data on epifauna, the lack of epifloral data, and the poor condition of our epifauna samples, we focused our examination on epiphytic diatoms, of which we recovered 48 genera, including 76 taxa. The number of genera observed was similar to a study from Roscoff, France that also reported 48 genera (Jacobs &



**Fig. 3.** The dominant epiphytic diatom species observed on *Zostera marina* collected from Arikawa Bay. **A1, 2:** *Ardissonaea formosa* (Girdle view, LM, SEM.), **B1–3:** *Cocconeis heteroidea* (B1: Valve view, LM. B2: External view of the valve showing partly broken valve mantle, SEM. B3: External oblique view of a whole valve showing the unique stria, SEM.), **C1–3:** *Cocconeis pseudomarginata* (C1: Valve view, LM. C2: External view of the valve, SEM. C3: External oblique view of a whole valve showing the unique stria, SEM.), **D1–3:** *Climacospheia monilifera* (D1, 2: Girdle view, LM, SEM. D3: Valve view, SEM.), **E1, 2:** *Pleurosigma normanii* (Valve view, LM, SEM.), **F1, 2:** *Trachyneis aspera* (Valve view, LM, SEM.), **G1–3:** *Rhabdonema adriaticum* (G1, 2: Girdle view, LM, SEM. G3: External oblique view of whole valve, SEM.), **H1, 2:** *Biddulphia pulchella* (Girdle view, LM, SEM.), **I1, 2:** *Diploneis* sp. (Valve view, LM, SEM.), **J1, 2:** *Lyrella lyra* (Valve view, LM, SEM.), **K1, 2:** *Actinoptychus senarius* (K1: Valve view, LM. K2: SEM.), **L1, 2:** *Surirella fastuosa* (Valve view, LM, SEM.), **M1, 2:** *Grammatophora oceanica* (Girdle view, LM, SEM.), **N1, 2:** *Gephyria media* (Girdle view, LM, SEM.), Scale bar=20  $\mu$ m.



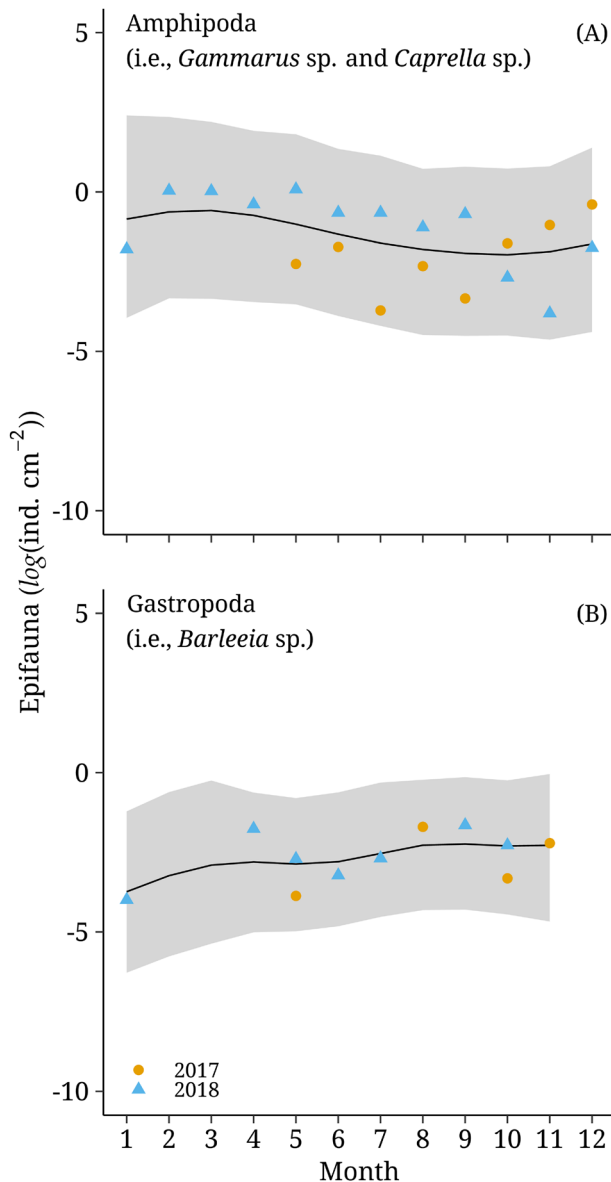
**Fig. 4.** The epiphytic diatoms observed on *Zostera marina* were classified into eight types (Kawamura 1994). The eight types were as follows. (A) Type A: motile prostrate, (B) Type B: non-motile prostrate, (C) Type C: solitary upright, (D) Type D: colonial upright, (E) Type E: solitary mucus thread, (F) Type F: colonial mucus thread, (G) Type G: mucus tube and (H) Type H: colonial thread. The cell density is given in the natural logarithm scale, where the line indicates the expectation of a generalized additive model and the shaded region indicates the 95% credible interval.

Nelson 1980). However, the number of taxa we observed was intermediate between the 199 from Roscoff (Jacobs & Nelson 1980) and the 35 from Skagerrak, Norway (Fredriksen et al. 2005). Cell densities were highly variable (15 to 6940 cells cm<sup>-2</sup>), and the expected values increased and decreased with time (Fig. 2). The cell densities observed in our study were substantially lower than those reported by Tanaka (2009), who showed diatom cell densities on *Z. marina* ranging from 12700 to 169000 cells cm<sup>-2</sup>; however our values were higher than those from Otsuchi Bay, which did not exceed 40 cells cm<sup>-2</sup> (Nakaoka et al. 2001). Tanaka (2009) cut *Z. marina* leaf sections from the seagrass and quantified diatom cell densities from the collected sections. Such a sampling method is biased towards higher cell densities. Variations in spatial distribution along the entire *Z. marina*

individual should be expected, as senescence begins at the leaf tip and diatom cell densities decrease towards the base of the leaves (Nagle 1968). Results can also be influenced by the sampling period because we show that variations in diatom cell density occur depending on the season, as has been validated by Snoeijs (1995).

Space and time are not the only factors that influence the cell density of epiphytic diatoms. *Z. marina* serves as a substrate for diatoms and has its own phenological response. At our study site, new shoots germinated in October and rapidly grew until March, when maturation occurred. Flowers can be observed beginning in March and by June seeds are readily apparent, which is a typical pattern observed for *Z. marina* meadows found along the coasts of Kyushu Island, Japan (Kawano et al. 2012). With the changes in the growth stage of *Z. marina*, altera-





**Fig. 5.** The density of epifauna observed on *Zostera marina*. (A) Density of amphipods increased from December 2017 to March 2018. (B) Density of gastropods increased in August 2017 and March and September 2018. The population density is given in the natural logarithm scale, where the line indicates the expectation of a generalized additive model and the shaded region indicates the 95% credible interval.

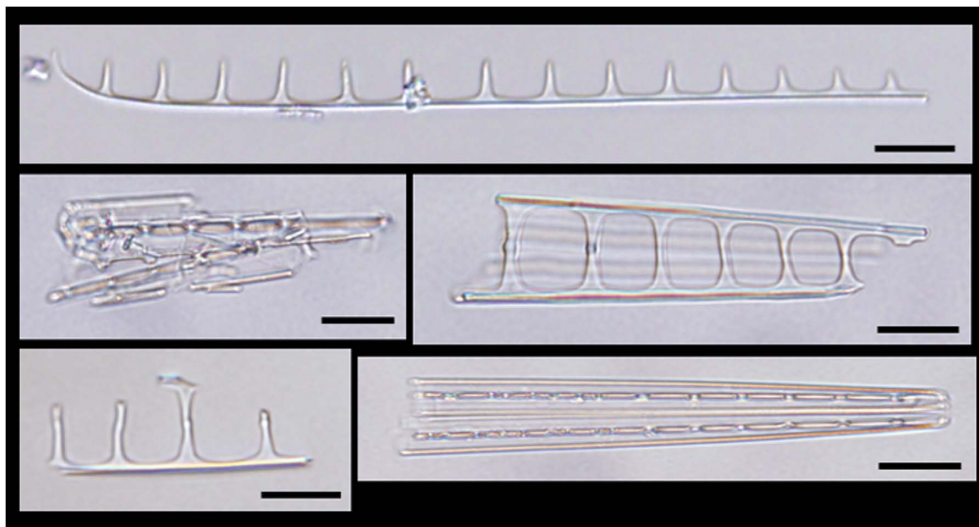
tions of the surface area of leaves can be associated with new growth beginning from late winter to spring (January to May) until early summer (June). The decrease in leaf surface area of *Z. marina* occurred due to leaf senescence during summer to early autumn (July to September). Such seasonal variation in leaf surface area in our study is not uncommon (Izumi et al. 2002, Abe et al. 2004, Kawano et al. 2012).

A descriptive analysis of the time-series observations of leaf surface area, epiphytic diatom density, and epifauna population density provide more insight into the phenol-

ogy of diatom density. The GAM analysis was superior to GLM, indicating that the observations of the total cell density of epiphytic diatoms and leaf surface area of the host macrophyte were bimodal during the survey period, and that the total density of epifloral individuals was unimodal (Fig. 2). The peak of the epiphytic diatoms lagged that of the leaf area by approximately three to four months and increased cell density only when the leaf area was declining, which was hypothesized by Jacobs et al. (1983). The epifaunal peak lagged that of the leaf area by approximately eleven months and the epifloral peak by four months. In Otsuchi Bay, the epifauna peak also lagged the epifloral peak by four months (Nakaoka et al. 2001). The duration of the survey period prevented any detection of further peaks in the epifauna time series that could be expected to occur in 2019. Although we could not sufficiently examine the hypothesis that the presence of epifauna and the physiological state of the macrophyte affected the epiphytic diatom species composition, we were able to demonstrate that the increase and decrease in leaf area over time was inversely related to the increase and decrease in the total cell density of epiphytic diatoms. This is consistent with the increase in epifloral cell density observed on older leaves of *Zostera marina* (Jacobs et al. 1983).

The analysis of the individual time series for epiphytic diatom type and epifauna groups was inconclusive. Nelson (1997) stated that the precision of predictions decreases when the epifloral biomass (i.e., cell density) increases. Unlike the results of the aggregate analysis described in the previous paragraph, our analysis did not provide clear evidence that a smooth had any statistical importance in describing the trend in the observations. Although log-transformation can stabilize variances, the low quantity of data and the wide range of values greatly affect the quality of the model fit. A much larger quantity of observations, taken at a high frequency and for a longer duration, is needed.

Ecological, physical, and chemical characteristics can affect the abundance of epiflora. *Z. marina* contains allelopathic substances that can inhibit the attachment of fauna and microflora due to phenolic compounds such as zosteric acid (Jendresen & Nielsen 2019) and rosmarinic acid (Guan et al. 2017). Zosteric acid content can increase as seagrasses mature (Ina 1991, Kuroda 2008) and inhibit diatom growth. Rosmarinic acid inhibits bacterial growth and is believed to inhibit the settlement of microfoulers on *Z. marina* leaves (Guan et al. 2017). Under the natural conditions of our study, it is unlikely that chemical or physical mechanisms caused the decrease in epifloral density; moreover, we hypothesize that active grazing by epifauna was likely the main reason for the of epifloral population suppression (Fig. 2). Studies concerning allelopathic substances with extracts under artificial conditions (i.e., laboratory) showed subtle effects (Guan et al. 2017); however, shear stress related to water motion may have caused physical detachment of the epiflora, and this remains to be



**Fig. 6.** Diatom frustule found in the stomach contents of amphipods observed with a light microscope. Only fragments of *Climacosphenia moniligera* were observed.

examined in detail (Jacobs et al. 1983). Nevertheless, top-down control of epiphytes by grazers has previously been demonstrated (Jernakoff et al. 1996, Reynolds et al. 2014, Östman et al. 2016), and additional data should provide a much more detailed insight into the population dynamics and interactions among these three states (i.e., substrate, producer, and grazer) in *Z. marina* ecosystems in Japan.

A more detailed examination of the effects of epifauna on the fluctuations of diatom cell density was explored (Fig. 4); however, it was difficult to provide compelling statistical arguments. In August 2017, the individual density of gastropods increased, whereas the cell density of type B diatoms decreased. In contrast, the greater gastropod density in March 2018 was coupled with an increase in type B diatoms. After May, the gastropods tended to decline, while the percentage of type B diatoms escalated to over 50%. In September and October, gastropod density increased again followed by a decrease in type B diatom density in November. Non-motile prostrate type diatoms such as *Cocconeis* are readily consumed by gastropods and are known to be used as food in the production of abalone seedlings (Kawamura 1998), suggesting that the feeding pressure of gastropods affected the populations of type B diatoms.

Amphipods increased in abundance between December 2017 and March 2018. Type F diatoms (*C. moniligera*) were the most dominant type in December 2017 and the second most dominant from January to July 2018. In general, amphipods feed on diatoms attached to surfaces and are known to be fragment feeders (Hosomi et al. 2006). It is thought that upright diatoms are consumed first in comparison with prostrate types; however, as little is known about the feeding habits of these amphipods (Farlin et al. 2010), we examined the stomach contents of amphipods under a microscope and observed only *C. moniligera* fragments (Fig. 6). This suggests that amphipods might have

a feeding preference, which influences the population of Type F diatoms (*C. moniligera*). Hence, we hypothesize that the state of the seagrass (i.e., leaf area) greatly affects the change in cell density of epiphytic diatoms and that the timing and appearance of groups of epifauna also modify the community composition. According to Hillebrand et al. (2000), erect and chain-building microalgal species (e.g., *Melosira moniliformis* and *M. nummuloides*) are preferred by herbivores.

Our study suggests that herbivores such as gastropods and amphipods could alter species composition by preferring food items (active choice) or by ingesting more of the species that were structurally more available (passive preference). In turn, food availability may influence herbivore diversity, as mouthpart morphology appears to be correlated with trophic ecology (Michel et al. 2020). More conservatively, herbivory and substrate availability both serve to control the cell density of epiphytic diatoms in a *Z. marina* meadow (Jacobs and Noten 1980, Nelson 1997, Nakaoka et al. 2001). Given that epiphytic diatoms are ephemeral, it may be of no consequence that over-growth of epiphytes may be detrimental to the host macrophyte, causing early senescence of seagrasses and macroalgae. However, the relationships and dynamics between organisms in the seagrass ecosystem remain to be determined. Given the acceleration at which environmental changes occur in our coastal environments, both physical and chemical environmental factors (e.g., water temperature and nutrients) should be considered in future models to predict the phenology of epiphytic microalgae and trophic interactions within macrophyte ecosystems.

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