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Annual Cycle of the *Synechococcus* spp. and Picoeukaryotic Growth and Loss Rates in a Subtropical Coastal Ecosystem

Pei-Chi Ho [1,2], Gwo-Ching Gong [1,2], Vladimir Mukhanov [3], Zhi-Yu Zhu [1] and An-Yi Tsai [1,2,*]

[1] Institute of Marine Environment and Ecology, National Taiwan Ocean University, Keelung 202-24, Taiwan; bookwormpageho@gmail.com (P.-C.H.); gcgong@mail.ntou.edu.tw (G.-C.G.); ance4560@gmail.com (Z.-Y.Z.)
[2] Center of Excellence for the Oceans, National Taiwan Ocean University, Keelung 202-24, Taiwan
[3] A.O. Kovalevsky Institute of Biology of the Southern Seas, Russian Academy of Sciences, 299011 Sevastopol, Russia; v.s.mukhanov@gmail.com
* Correspondence: anyitsai@mail.ntou.edu.tw

Abstract: Seasonal variations in the picophytoplankton community structure (*Synechococcus* spp. and picoeukaryotes) were studied by flow cytometry in the coastal ecosystem of the subtropical western Pacific from October 2019 to September 2020. *Synechococcus* spp. was dominant in abundance during the study period, with its density ranging from 0.05 to 5.6 $\times$ 10^4 cells mL^{-1}; its maximum occurred in July 2020. Picoeukaryotes were less abundant, with their density ranging from 0.2 to 13.6 $\times$ 10^3 cells mL^{-1}. Their highest abundance was recorded in January 2020. The growth rates of *Synechococcus* spp. and picoeukaryotes ranged from -0.39 to 1.42 d^{-1} and 0.38 to 2.46 d^{-1}, respectively, throughout the study period. Overall, the growth rate of the picoeukaryotes was significantly higher than that of *Synechococcus* spp. It is interesting to note that the grazing mortality of *Synechococcus* spp. and picoeukaryotes during the warmer period (April to September) was relatively low. Based on this study, we suggest that mixotrophic nanoflagellates lowered their feeding activity that obtained nutrients from prey and instead used additional nutrients during the incubation experiments. Our study demonstrated that a shift in the picophytoplankton community composition and grazing activity of predacious nanoflagellates in cold and warm periods can impact on the seasonal dynamics of the microbial food web.

Keywords: picophytoplankton; *Synechococcus* spp.; picoeukaryotes; growth rate; annual cycle

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1. Introduction

Picophytoplankton mainly consist of picocyanobacteria (e.g., *Synechococcus* spp. and *Prochlorococcus*) and picoeukaryotes, and form an important component of the phytoplankton community in oligotrophic warm environments [1–4]. Picoeukaryotes are smaller in density than *Synechococcus* spp. [5], especially in the tropical and subtropical oceans [5,6]; however, they also contribute significantly to the primary production of biomass in the oceans [7]. Understanding the temporal changes of picophytoplankton abundance and its response to environmental drivers is essential for determining the lower trophic levels of the food web.

Given the importance of picophytoplankton in marine biogeochemical cycling and oceanic carbon flux, it is essential to gather information on their growth and mortality rates. The dynamics of picophytoplankton in aquatic ecosystems are not only strictly dependent upon temperature, nutrient, and light limitation through bottom-up controls [8–10] but are also regulated by mortality from grazing and viral lysis (top-down controls) [11,12]. Although the estimates of growth and mortality rates (from grazing or viral lysis) of *Synechococcus* and *Prochlorococcus* are from widely disparate oceanographic regimes [12–17], few growth and mortality rate measurements are available for photosynthetic picoeukaryotes, especially for seasonal observations [17]. In subtropical western Pacific coastal ecosystems, the seasonal trends of *Synechococcus* spp. abundance and its growth rate approximately

follow the annual cycle of temperatures [12,18]. Tsai et al. [15] found that pigmented nanoflagellates consume approximately 63% of the mean *Synechococcus* spp. production during the warmer seasons; thus, pigmented nanoflagellates are the key grazers of the *Synechococcus* spp. population in subtropical western Pacific coastal waters. Furthermore, Tsai et al. [16] confirmed that although nanoflagellate grazing was a significant cause of *Synechococcus* spp. mortality, viral lysis was also an important source of mortality, especially at night-time. Although there is much information regarding the influence of these processes on the seasonal and diel dynamics of *Synechococcus* spp., little is known about their influence on picoeukaryotes in this coastal environment.

In previous studies, picoeukaryotes were found to be better adapted to low temperatures and NO_3 availability whereas *Synechococcus* spp. generally favored high temperatures and NH_4 availability [19]. Moreover, the physiological characteristics of *Synechococcus* spp. and picoeukaryotes—such as their cell surface properties, cell size, and the nutritional content of the cells—could also influence the feeding behavior of protists [20,21]. Thus, we hypothesized that the abundance, growth, and mortality of *Synechococcus* spp. and picoeukaryotes exhibit distinct seasonal patterns in subtropical western Pacific coastal waters.

This study aims to explore the abundance, growth, and mortality rates (grazing and viral lysis) of two groups of picophytoplankton (*Synechococcus* spp. and picoeukaryotes) and their annual cycle at a sampling station located in subtropical western Pacific coastal waters. Here, we report for the first time the seasonal variability in growth and mortality of *Synechococcus* spp. and picoeukaryotes in order to better understand the complex trophic interactions in microbial food webs.

2. Materials and Methods

2.1. Water Sampling

Monthly field sampling was conducted from October 2019 to September 2020 (0900 am to 1000 am local time) from the surface waters at an established station located in coastal waters off the north-eastern Taiwan coast (25°09.4′ N, 121°46.3′ E) and all samples reached the laboratory within 30 min. The abundance of *Synechococcus* spp. and picoeukaryotes from natural seawater and incubation samples were fixed in paraformaldehyde (1% final concentration) and frozen prior to a flow cytometric analysis. The water samples were filtered (25 mm GF/F: Whatman glass microfiber filters) for a Chl *a* analysis and the Chl *a* concentration was measured after the extraction by an in vitro fluorometer (Turner Design 10-AU-005) (Turner Designs, Inc., San Jose, CA, USA) [22]. The concentrations of nutrients in the seawater were measured by the methods described by Gong et al. (2000).

2.2. Incubation Experimental Design

To explore the seasonal changes in the growth and mortality rate of *Synechococcus* spp. and picoeukaryotics in our study site, we performed a two-point modified dilution experiment with a 24 h incubation to measure the *Synechococcus* spp. and picoeukaryotic growth and mortality rates by grazing and viral lysis [13,23]. The samples for the experiments were kept in 2 L polyethylene containers and transported to a local field laboratory. A total of 1 L of seawater was screened through a 200 μm Nitex mesh to remove the larger mesozooplankton grazers (200 μm filtered waters). First, a dilution was performed using 0.2 μm filtered water (47 mm diameter polycarbonate filters, AMD Manufacturing; operated at a low pressure <50 mm Hg), which represented the grazer-free diluents. Second, another dilution was produced by tangential flow filtration through a 30 kDa cartridge to generate virus-free water. Fresh 200 μm filtered water was then collected, as described above, and combined with grazer- and virus-free diluents in 25% filtered water of a 200 μm proportion. Nutrients were added to these bottles at a final concentration of 20 μM NO_3 and 2 μM PO_4^3 to eliminate the nutrient limitation of picophytoplankton growth during the incubation.

All treatments were incubated in triplicate for 24 h in 100 mL polycarbonate bottles under natural light in a water bath of an in situ temperature at the time of sampling. Subsamples were taken at the beginning (t_0) and the end of the 24 h incubation (t_{24}) to estimate the

abundance change of the picophytoplankton (*Synechococcus* spp. and picoeukaryotics). The net growth rate of the picophytoplankton (k, d^{-1}) was calculated as $k = (\ln N_t - \ln N_0)/t$ where t was the incubation time and N_t and N_0 were the picophytoplankton abundance at the end and the start of the experiments, respectively. The picophytoplankton was detected and measured by using flow cytometry (FCM) (BD FACSCalibur™; United States). The analysis of the abundance and structure of the picophytoplankton communities highlighted the presence of two distinct groups in the study area: *Synechococcus* spp. and picoeukaryotes (Figure 1).

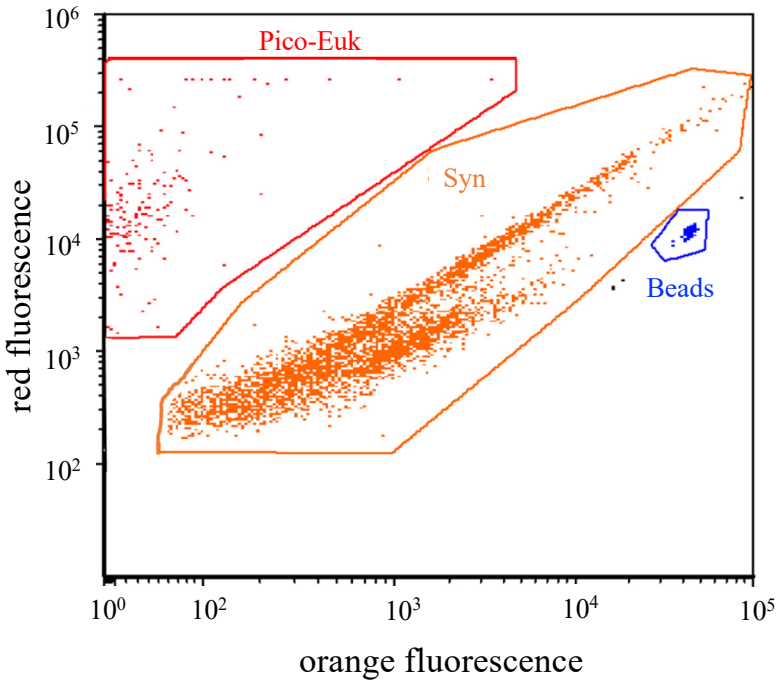

Figure 1. Resolution of picophytoplankton cell groups by flow cytometry. Typical red (chlorophyll *a*) versus orange fluorescence (phycoerythrin) cytograms exhibiting the resolution of *Synechococcus* spp. (Syn) and picoeukaryotes (Pico-Euk).

The net growth rate of picophytoplankton in the 200 µm filtered treatment signified the differences between the gross growth rate (µ) and the total mortality rate (grazing rates, M_g, and viral lysis, M_v). Thus, the net growth rate of picophytoplankton in the 200 µm filtered treatment (k (200 µm filtered)) was expressed as in Equation (1):

$$k\ (200\ \mu m\ filtered) = \mu - (M_g + M_v). \quad (1)$$

In the diluted water with 25% of 0.2 µm filtration treatments, the picophytoplankton mortality as a result of the grazing activity reduced to only 25%, as shown in the equation below:

$$k\ (0.2\ \mu m\ diluted) = (\mu - M_v) - 0.25 \times (M_g). \quad (2)$$

Finally, in the sample diluted with 25% of 30 kDa filtration treated water, the picophytoplankton mortality was reduced to 25% of the grazing and viral lysis (M_g + M_v) in this incubation experiment. Thus, the equation was expressed as:

$$k\ (30\ kDa\ diluted) = \mu - 0.25 \times (M_g + M_v). \quad (3)$$

The picoplankton grazing rate (M_g) was calculated from Equations (1)–(3):

$$M_g = \left(\frac{4}{3}\right) \times (k\ (0.2\ \mu m\ diluted) - k\ (200\ \mu m\ filtered)). \quad (4)$$

Furthermore, M_v was calculated from Equations (1)–(3):

$$M_v = \left(\frac{4}{3}\right) \times (k\ (30\ \text{kDa diluted}) - k\ (0.2\ \mu\text{m diluted})). \quad (5)$$

We thereby obtained the values of M_g and M_v; the value of the gross growth rate (μ) was calculated as μ = k (200 μm filtered) + (M_g + M_v).

The assumption was that grazing and viral mortality was linear with respect to the picophytoplankton concentration; when the picophytoplankton net growth rate (k) was lower in the diluted treatments than in the 200 μm filtered treatments, it pointed to a violation of a central assumption of the dilution method [24] and represented a negative value of the grazing or viral lysis rate.

A Kruskal–Wallis test was used to test the significant differences in the net growth rates (k) of the picoplankton among treatments. In addition, the differences in the means of any two treatments were tested by a Welch's *t*-test. Thus, in this case, the losses were considered to be undetectable. The significance level for all tests was set at < 0.05.

3. Results

3.1. Environmental Parameters

During the one year sampling period described in this study, the seawater temperature showed a regular seasonal trend and ranged from 17.5 °C (February) to 30.0 °C (August) (Figure 2A). The NO_3 concentrations showed the lowest values in the surface waters (0.7 μM for NO_3 in May 2020) (Figure 2B). The PO_4 concentrations did not vary seasonally, and the values were higher (>20 μM) during the rainy period, especially in April and July (Figure 2C). The total Chl *a* concentrations in the surface water were at a minimum during winter, increased in April, and maintained higher concentrations during summer (Figure 2D).

3.2. Seasonal Variations of Picophytoplankton Abundance

During the study period, *Synechococcus* spp. was numerically dominant during the study period, with its abundance ranging from 0.05 to 5.6 × 10^4 cells mL^{-1}. Its maximum was observed in July 2020 (Figure 3). Picoeukaryotes were less abundant; the values ranged from 0.2 to 13.6 × 10^3 cells mL^{-1} (Figure 3). The highest picoeukaryotic abundance was recorded in January 2020 (Figure 3). Figure 4 provides information on the initial Chl *a* concentration as well as the *Synechococcus* spp. and picoeukaryotic abundance in the experiments conducted. When considered separately, significant relationships were found between the *Synechococcus* spp. and picoeukaryotic abundance. The range of Chl *a* was <1 mg m^{-3} ($p < 0.05$) (Figure 4).

Furthermore, a significant correlation was found between *Synechococcus* spp. and the temperature ($r = 0.58$, $p < 0.05$, $n = 20$) (Supplemental Figure S1).

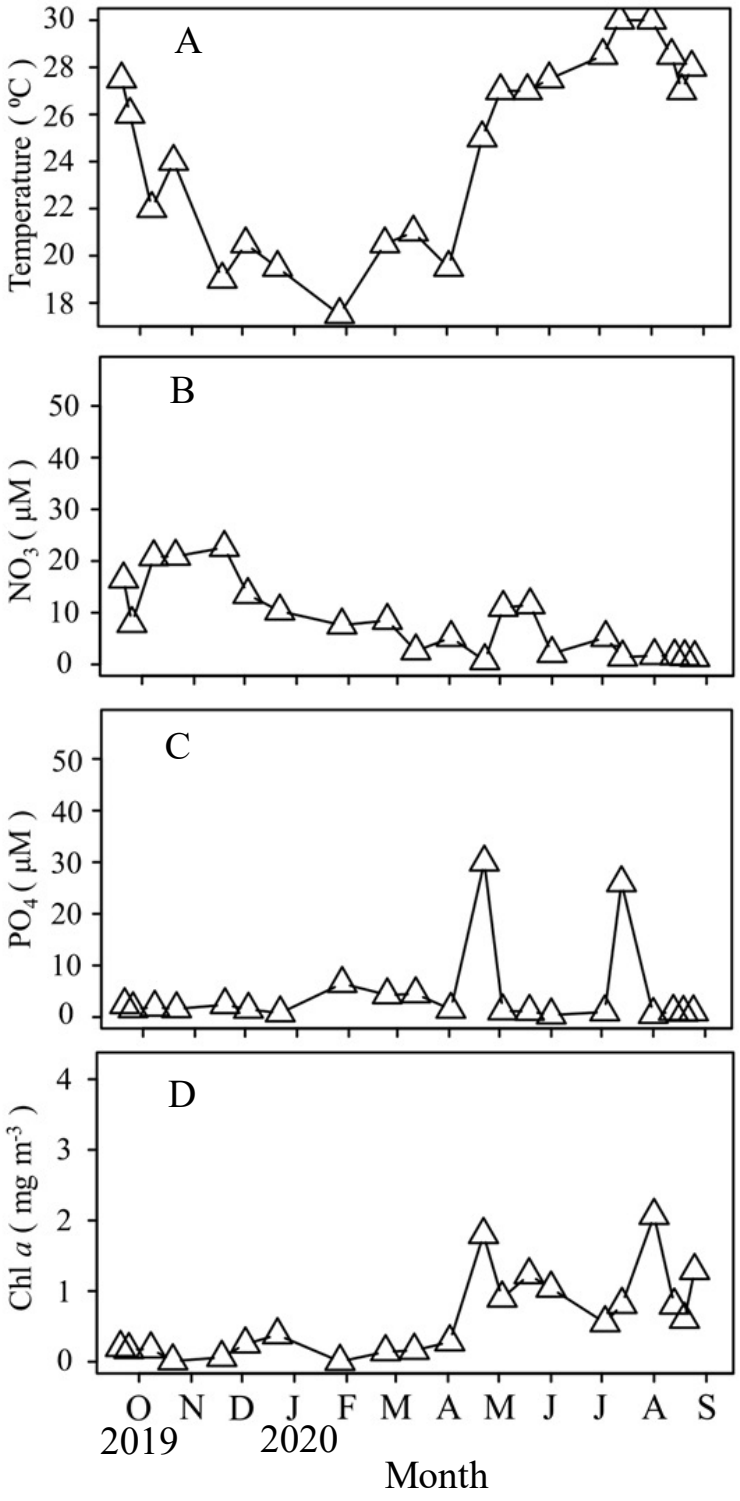

Figure 2. Seasonal variations of surface water temperature (**A**), NO_3 concentrations (**B**), PO_4 concentrations (**C**), and Chl *a* concentrations (**D**).

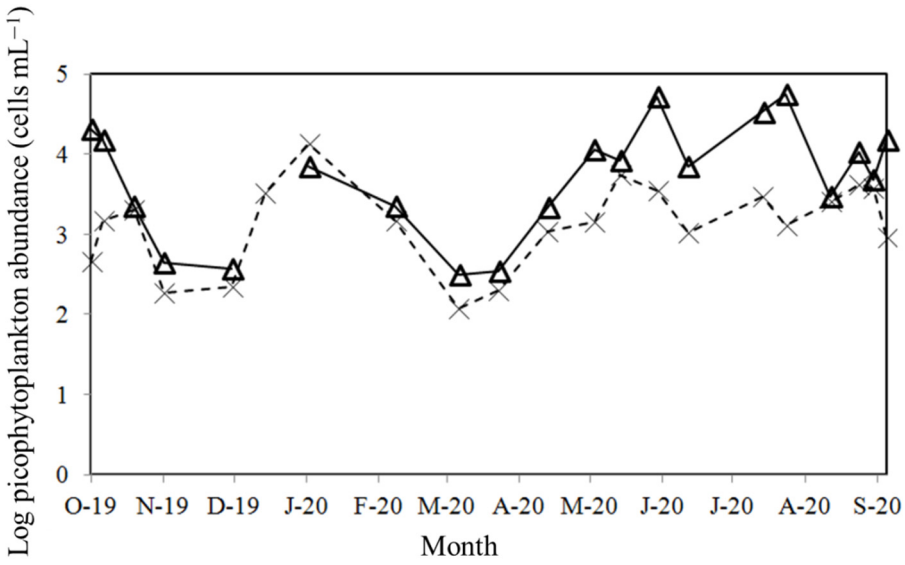

Figure 3. Seasonal variations of *Synechococcus* spp. (Δ) and picoeukaryotic (X) abundance.

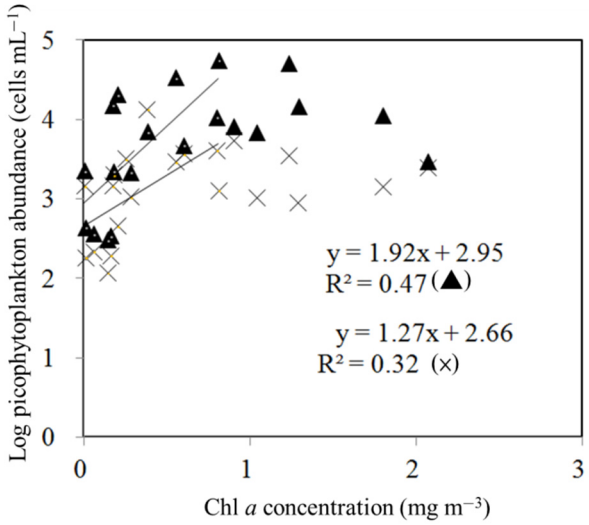

Figure 4. Relationship between Chl *a* concentrations and *Synechococcus* spp. (Δ) and picoeukaryotic (×) abundance. The data points of the linear regressions were below 1 mg m^{-3} of Chl *a* concentration.

3.3. Seasonal Variations of Picophytoplankton Growth Rates

The net growth rates of *Synechococcus* spp. and picoeukaryotes are summarized in Tables 1 and 2. Based on the calculations of Equations (1)–(5) from the data of Tables 1 and 2, the gross growth rates of *Synechococcus* spp. and picoeukaryotes ranged from −0.39 to 1.42 d^{-1} and 0.38 to 2.46 d^{-1}, respectively, throughout the study year (Figure 5A). The highest gross growth rate of *Synechococcus* spp. (1.42 d^{-1}) was found in September 2020 (Figure 5A). Furthermore, the higher gross growth rate of the picoeukaryotes (2.46 d^{-1}) was found in late March 2020, when the temperature was lower (20 °C) and dissolved inorganic nutrient concentrations were higher (NO_3: 8.5 μM; PO_4: 4.3 μM) during this study (Figures 2B,C and 5A). Overall, the gross growth rate estimates for the picoeukaryotes were significantly higher than for *Synechococcus* spp. (paired *t*-tests, $p < 0.05$, $n = 20$).

Table 1. Net growth rates of *Synechococcus* spp. in each treatment incubation: 200 µm filtered water (k (200 µm filtered)), 0.2 µm diluted (k (0.2 µm diluted)), and 30 kDa diluted (k (30 kDa diluted)). ND: no data.

***Synechococcus* spp.**			
Date	**k (200 µm Filtered) (d^{-1})**	**k (0.2 µm Diluted) (d^{-1})**	**k (30 kDa Diluted) (d^{-1})**
4 Oct	0.69 ± 0.07	−0.5 ± 0.12	−4.2 ± 0.31
9 Oct	0.73 ± 0.14	0.43 ± 0.17	−1.92 ± 0.48
22 Oct	−2.21 ± 0.24	−1.3 ± 0.14	−2.01 ± 0.51
4 Nov	−0.59 ± 0.27	0.34 ± 0.21	−0.13 ± 0.42
3 Dec	0.14 ± 0.31	−0.39 ± 0.33	−0.91 ± 0.17
17 Dec	ND	ND	ND
5 Jan	−0.77 ± 0.31	−0.12 ± 0.11	−0.52 ± 0.42
11 Feb	−0.39 ± 0.24	0.02 ± 0.04	0.12 ± 0.05
9 Mar	−0.01 ± 0.03	0.05 ± 0.02	0.1 ± 0.03
26 Mar	0.69 ± 0.14	0.71 ± 0.22	0.71 ± 0.31
16 Apr	0.37 ± 0.03	0.44 ± 0.02	0.45 ± 0.07
6 May	0.28 ± 0.02	0.32 ± 0.01	0.32 ± 0.08
17 May	0.66 ± 0.07	0.66 ± 0.01	0.68 ± 0.01
2 Jun	0.75 ± 0.04	0.86 ± 0.02	0.86 ± 0.07
15 Jun	0.17 ± 0.14	0.18 ± 0.07	0.19 ± 0.09
17 Jul	1.04 ± 0.04	1.13 ± 0.03	1.14 ± 0.08
27 Jul	0.7 ± 0.02	0.74 ± 0.01	0.78 ± 0.02
15 Aug	1.17 ± 0.08	1.19 ± 0.04	1.22 ± 0.01
27 Aug	0.46 ± 0.11	0.47 ± 0.09	0.47 ± 0.12
2 Sep	1.1 ± 0.24	1.11 ± 0.14	1.12 ± 0.48
8 Sep	1.42 ± 0.42	1.43 ± 0.17	1.43 ± 0.28

Table 2. Net growth rates of picoeukaryotes in each treatment incubation: 200 µm filtered water (k (200 µm filtered)), 0.2 µm diluted (k (0.2 µm diluted)), and 30 kDa diluted (k (30 kDa diluted)).

Picoeukaryotes			
Date	**k (200 µm Filtered) (d^{-1})**	**k (0.2 µm Diluted) (d^{-1})**	**k (30 kDa Diluted) (d^{-1})**
4 Oct	0.77 ± 0.52	0.62 ± 0.12	0.91 ± 0.14
9 Oct	1.55 ± 0.71	1.54 ± 0.42	−1.14 ± 0.78
22 Oct	−0.25 ± 0.04	−1.1 ± 0.15	−0.3 ± 0.11
4 Nov	−0.06 ± 0.07	1.19 ± 0.15	0.76 ± 0.32
3 Dec	0.38 ± 0.14	0 ± 0.09	0 ± 0.48
17 Dec	1.14 ± 0.42	1.39 ± 0.51	1.12 ± 0.34
5 Jan	−0.63 ± 0.62	0.43 ± 0.11	−0.12 ± 0.09
11 Feb	0.82 ± 0.47	0.82 ± 0.27	0.42 ± 0.14
9 Mar	1.58 ± 0.03	1.9 ± 0.02	2.02 ± 0.01
26 Mar	1.16 ± 0.04	1.26 ± 0.02	1.39 ± 0.03
16 Apr	0.43 ± 0.04	0.57 ± 0.02	0.6 ± 0.01
6 May	0.84 ± 0.03	0.96 ± 0.03	1.09 ± 0.04
17 May	0.93 ± 0.12	0.94 ± 0.01	0.96 ± 0.01
2 Jun	1.73 ± 0.01	1.76 ± 0.01	1.79 ± 0.02
15 Jun	1.17 ± 0.11	1.18 ± 0.02	1.23 ± 0.01
17 Jul	1.31 ± 0.01	1.33 ± 0.01	1.35 ± 0.01
27 Jul	0.8 ± 0.03	0.88 ± 0.02	0.98 ± 0.04
15 Aug	1.73 ± 0.02	1.78 ± 0.02	1.79 ± 0.07
27 Aug	0.87 ± 0.09	0.88 ± 0.04	0.9 ± 0.03
2 Sep	1.05 ± 0.08	1.07 ± 0.02	1.1 ± 0.01
8 Sep	1.6 ± 0.02	1.67 ± 0.03	1.69 ± 0.07

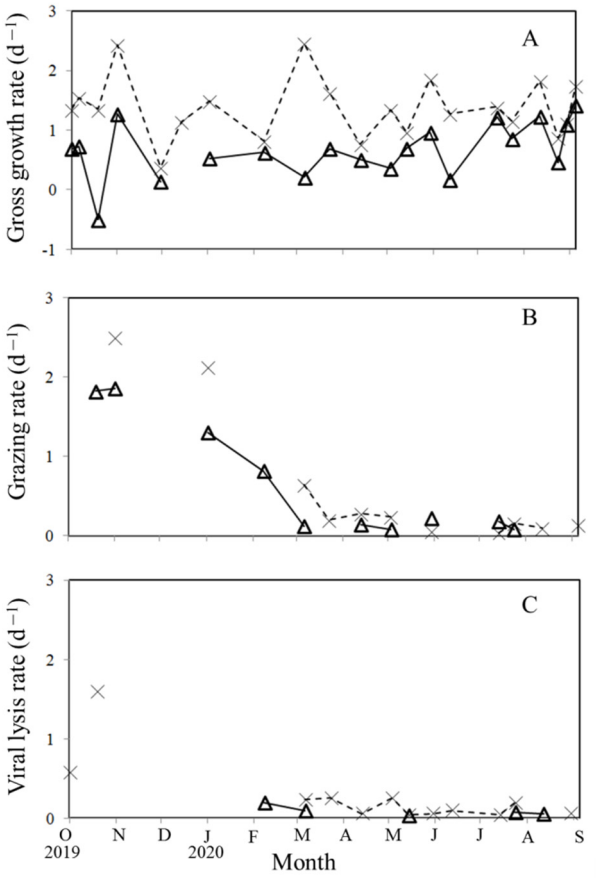

Figure 5. Seasonal variations of *Synechococcus* spp. (Δ) and picoeukaryotic (×) gross growth rates (**A**), grazing mortality (**B**), and viral lysis mortality rates (**C**).

The gross growth rates of *Synechococcus* spp. showed a statistically significant positive correlation with the water temperature ($p < 0.05$) (Figure 6A). Furthermore, a significant positive relationship with the temperature was also found for the gross growth rate of the picoeukaryotes when the temperature < 25 °C ($p < 0.05$); however, there was no significant relationship between the gross growth rate of the picoeukaryotes and the temperature when the temperature was >25 °C (Figure 6B).

3.4. Seasonal Variations of Picophytoplankton Mortality

When comparing the net growth rates in 200 μm filtered water (k (200 μm filtered)) and 0.2 μm diluted water (k (0.2 μm diluted)) (Tables 1 and 2), only 10 and 11 (48% and 52%) out of 20 and 21 dilution experiments conducted in this study generated a statistically significant grazing mortality of *Synechococcus* spp. and picoeukaryotes, respectively (Figure 5B). During the study period, the grazing mortality of *Synechococcus* spp. and picoeukaryotes ranged from 0.08 to 1.86 d^{-1} and 0.04 to 2.5 d^{-1}, respectively (Figure 5B). However, it was interesting to note that the grazing mortality of *Synechococcus* spp. and picoeukaryotes during the warmer period (April to September) was of a relatively low value. In our study, the grazing mortality of *Synechococcus* spp. and picoeukaryotes observed between April and September was lower than that between October and March (*t*-test, $p < 0.05$) (Figure 5B).

When considering picophytoplankton loss by viral lysis, and comparing the net growth rates in 0.2 μm diluted (k (0.2 μm diluted)) and 30 kDa diluted (k (30 kDa diluted)) water from Tables 1 and 2 in the present study, the estimated virus-mediated mortality rates for the natural communities of *Synechococcus* spp. and picoeukaryotes ranged from 0.04 to 0.20 d^{-1} and 0.04 to 0.58 d^{-1}, respectively (Figure 5C). The estimates indicated that, on

average, viruses were responsible for the mortality of approximately 5% of the production of *Synechococcus* and 10% of picoeukaryotes on a daily basis during the warmer seasons.

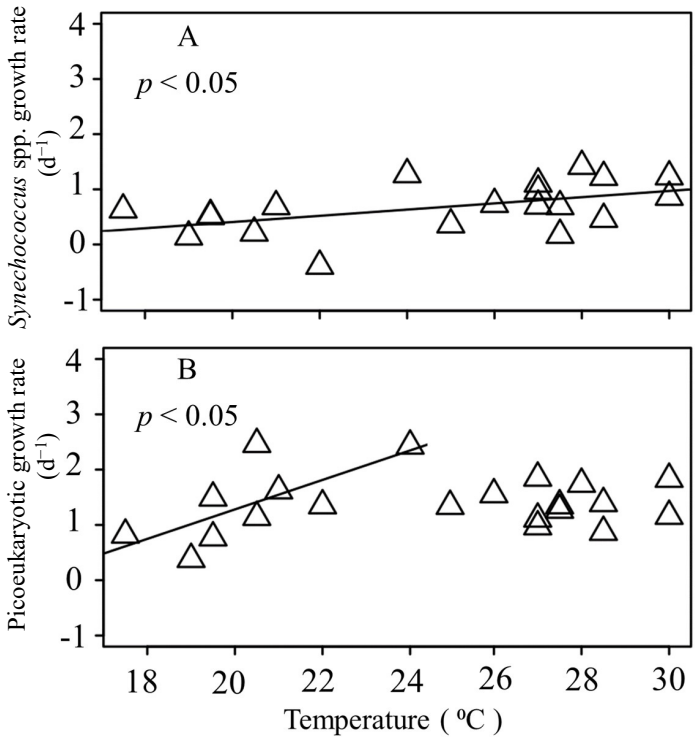

Figure 6. Relationship between *Synechococcus* spp. (**A**) and picoeukaryotic gross growth rates (**B**) and sea surface water temperature. Solid lines exhibit the fitted linear regressions.

4. Discussion

The coastal ecosystem of the subtropical western Pacific was particularly well-suited to this microbial ecological study because its seasonal water temperature variations are significant (17 to 30 °C), being cold and nutrient-enriched in winter and warm and nutrient-poor in summer. In this study, we showed the seasonal abundance changes of the picophytoplankton communities and their growth and mortality in the coastal ecosystem of the subtropical western Pacific. The major findings of this study were that a maximal *Synechococcus* spp. abundance of 5×10^4 cells mL^{-1} was observed during summer, which coincided with the high temperature and low nutrient conditions. In contrast, the picoeukaryotic abundance peaked in January 2020, at 20 °C. In addition, the growth rate for the picoeukaryotes was significantly higher than for *Synechococcus* spp. A strong seasonality in the growth and abundance of *Synechococcus* spp. related to the in situ temperature is reported here.

4.1. Seasonal Dynamics of Synechococcus spp. *and Picoeukaryotic Abundance*

Picophytoplankton have a competitive advantage for nutrient uptake in oligotrophic environments where they can be responsible for most of the primary production [8,25]. The seasonal variability of the *Synechococcus* spp. abundance from the same region was studied by Tsai et al. [12,18]. In this study, a maximum *Synechococcus* abundance (5×10^4 cells mL^{-1}) was observed in summer (Figure 3) and these abundances were similar to the previous field records by Tsai et al. [12,18], with its abundance showing a clear correlation with the surface water temperature. Our observations were also similar to the pattern in temperate waters where the seasonal variation of the *Synechococcus* spp. abundance usually reaches a maximum in summer and a minimum in winter [26]. However, information on the seasonal abundance of picoeukaryotes at our study site was limited.

A peak of picoeukaryote abundance of up to 13.6 × 10^3 cells mL^{-1} was observed during spring at a lower temperature (20 °C) and higher dissolved inorganic nutrients (NO_3: 8.5 μM; PO_4: 4.3 μM) (Figure 2B,C and Figure 3). In previous studies, the increase in picoeukaryote abundance was usually related to low temperatures and high nutrient concentrations [19,27]. Similar results were also shown from the Bay of Palma where the two picophytoplankton groups exhibited important seasonality and differed in the period of peak abundance: in summer, *Synechococcus* spp. reached a maximal density and in spring, the picoeukaryote abundance peaked [28]. Previous studies have suggested that picoeukaryotes appear to be better adapted to low temperatures and NO_3 availability whereas *Synechococcus* spp. generally favors high temperatures and NH_4 availability [19,29]. Moreover, the *Synechococcus* spp. preference for NH_4 over NO_3 has also been observed in laboratory experiments on isolates [30]. In this situation, the picoeukaryotic growth rates would have been overestimated in our experiments due to NO_3 being added to these bottles. Specific resources (inorganic nutrients) and temperatures have been reported to be the main limiting factors of growth in *Synechococcus* spp. [12,31] although the availability of nutrients appears to be the strongest factor in the warmer seasons [18]. Data from the study of Ayukai [32] suggested that *Synechococcus* spp. may depend on locally recycled nutrients and a similar study found that viral-induced ammonium regeneration resulted in an increased growth as well as the proportion of dividing *Synechococcus* spp. cells in the warmer seasons [33]. The above results could explain why *Synechococcus* spp. was present throughout the year, with a maximum abundance during summer. The increase of the picoeukaryotes at lower temperatures and *Synechococcus* spp. during summer could shift the picophytoplankton community composition and have a large impact on the microbial food web. If we assumed that all *Synechococcus* spp. and picoeukaryotes were retained on the GF/F filters, the importance of the picophytoplankton contribution to the total phytoplankton biomass was significant in the low Chl *a* periods in our study (Figure 4). Thus, the composition change of picophytoplankton should be systematically included in future phytoplankton biomass studies and carbon flux models.

4.2. Seasonal Dynamics of Synechococcus spp. *and Picoeukaryotic Growth Rates*

This study is the first annual high-resolution description of *Synechococcus* spp. and picoeukaryotic abundance and growth dynamics in the coastal ecosystem of the subtropical western Pacific. The growth rates over the study period for *Synechococcus* spp. and picoeukaryotes ranged from −0.39 to 1.42 d^{-1} and 0.38 to 2.46 d^{-1}, respectively (Figure 5A). Few rate measurements are available for photosynthetic picoeukaryotes. In this study, however, it was noteworthy that the growth rates of the picoeukaryotes tended to be higher than those of *Synechococcus* spp., which was in agreement with the study of the seasonal cycle of a Mediterranean coastal lagoon [34]. Bec et al. [34] found that picoeukaryotes always exhibited a higher growth rate than cyanobacteria. In a Pacific Ocean coastal site in the Southern California Bight, picoeukaryotes had a higher growth rate (0.71–1.29 d^{-1}) than *Synechococcus* spp. (0.52–0.86 d^{-1}) [14]. These results indicate that picophytoplankton do not have a universal photosynthetic response to environmental conditions [1] as the eukaryotic cell types perform better than the prokaryotic ones in coastal ecosystems.

Tsai et al. [12,18] found that both the abundance and growth rate of *Synechococcus* spp. increase as the water temperature increases in this study region. This phenomenon is similar to the early results of Agawin et al. [35], who indicated that a *Synechococcus* spp. abundance is closely related to the water temperature. Furthermore, a correlation between the picoeukaryotic growth rate and temperature was observed in the Pacific Ocean [14]. The analysis of our incubated data supported the existence of a significant correlation between the seawater temperature versus *Synechococcus* spp. and picoeukaryotic growth (Figure 6). However, it is worth noting that no significant relationship between the growth rate of the picoeukaryotes and temperature was found when the temperature was >25 °C in the present study (Figure 6B). The decoupling between the growth rate of the picoeukaryotes in response to the temperature during the warmer seasons highlighted the role of another

environmental factor that would influence its activity. A possible explanation could be the inhibition of the photosynthetic rates by UVR, which has been observed in many regions of the oceans such as tropical, temperate, and polar areas [36,37]. The underwater oceanic levels of UVR and visible light can induce significant cell death in the picophytoplankton communities. A previous study showed that the decay rate of living cells induced by solar radiation was the lowest for *Synechococcus* spp. [38] and that it had the highest resistance to solar radiation, which indicated that *Synechococcus* spp. had better photoprotection or repair systems than picoeukaryotes. It was likely that the higher levels of UVR and visible light were more advantageous for *Synechococcus* spp., which adapted to strong solar radiation in this coastal site during summer, than for the picoeukaryote competitors.

4.3. Seasonal Dynamics of Synechococcus spp. *and Picoeukaryotic Mortality Rate*

Microzooplankton consume a wide size range of phytoplankton cells, from picophytoplankton to microphytoplankton [39]. However, at our western subtropical Pacific coastal study site, microzooplankton (such as ciliates) have been reported to account for the removal of only 3% of the *Synechococcus* spp. production [15]. Furthermore, feeding experiments with fluorescent-labeled beads (with a size of 1 μm) have strongly suggested that pigmented nanoflagellates are the key grazers of *Synechococcus* spp. populations in subtropical western Pacific coastal waters [15]. Although the ingestion of picoeukaryotes by nanoflagellates was not measured in the study of Tsai et al. [15], we believed that the pigmented nanoflagellates frequently fed on picoeukaryotes (an average size of 1.02 ± 0.08 μm, unpublished data) as the potential ingestion impact on 1 μm fluorescent-labeled beads by pigmented nanoflagellates was shown in Tsai et al. [15].

Generally, phytoplankton growth rates and grazing mortality are closely coupled [18,33,39], which suggests a high transfer efficiency of picophytoplankton production to higher trophic levels. However, in the present study, it was interesting to note that the *Synechococcus* spp. and picoeukaryotic grazing mortality during the warmer period (April to September) was relatively low (Figure 5B) when a higher *Synechococcus* spp. growth was observed. The grazing activity may be driven by complex grazer–prey interactions, which can be modulated by the prey selectivity of the grazers (e.g., size or nutritional ratio) and this may result in mismatches between the grazers and their prey [40]. We plotted the relationship between the growth and grazing rates of *Synechococcus* spp. and picoeukaryotes; our grazing experiments showed that, in contrast, there was no response to the growth state of their prey, especially in the warmer seasons (>25 °C) (Figure 7). We were unable to assert exactly why low grazing mortality rates were often observed during this study but a variety of factors, including food selection, trophic cascades, prey switching, and dilution effects on grazers [41–43], may be responsible for such low mortality in the warm seasons. Another possible explanation for the low grazing mortality during the warm seasons could be the nutrients added to these bottles in this study: the mixotrophic pigmented nanoflagellates consumed additional nutrients and lowered their feeding ability to uptake nutrients from their prey during the incubation experiments. A similar result was discussed in another nutrient enrichment experiment with Sargasso Sea populations, which also produced a marked decline in phagotrophically-active pigmented nanoflagellates after the addition of phosphorus [44]. Furthermore, nutrient enrichment experiments in the Mediterranean Sea [45], which is oligotrophic, resulted in a significant decline in the phagotrophically-active pigmented nanoflagellates in the treatments. All of the evidence strongly supports our results that were obtained with natural pigmented nanoflagellate assemblages, suggesting that these mixotrophic nanoflagellates that inhabit these oligotrophic coastal waters could use their feeding capability to supplement nutrients but that they would take up nutrients primarily from a dissolved pool with high nutrient concentrations.

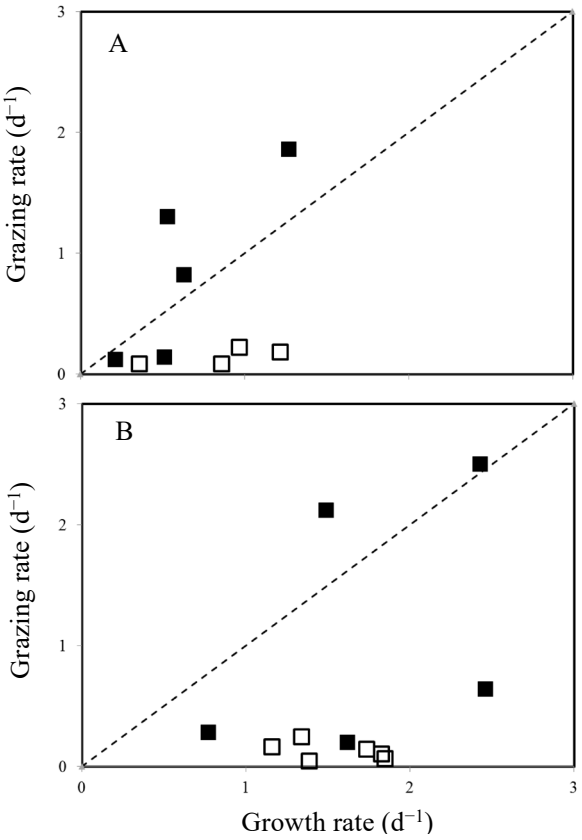

Figure 7. Comparison between the *Synechococcus* spp. (**A**) and picoeukaryotic growth rates (**B**) and nanoflagellate grazing rate during the study period. Dotted lines represent a 1:1 ratio. (■): cold seasons <25 °C, (□): warm seasons >25 °C.

This study is the first description of the influence of seasonality on the viral-mediated mortality of picophytoplankton in subtropical western Pacific coastal waters. Viral lysis alone was detected in 5 and 12 of the analyses of *Synechococcus* spp. and the picoeukaryotes in this study (Figure 5C). However, we did not find that the seasonal or environmental factors affected viral lysis in these experiments. Although viral infections of *Synechococcus* spp. are commonly found in marine waters [46–49], the lysis of *Synechococcus* spp. was rarely detected in our experiments, which implied that most of the *Synechococcus* spp. population was resistant to infection or that the efficiency of the infection was low.

Other factors were likely to influence the weak viral-mediated processes of the picophytoplankton in our study. Viral infections are host specific, and if there were no specific virus strains infecting the picophytoplankton in our dilution experiments or their density was not sufficient to affect the picophytoplankton [50], mortality by viral lysis would be low. One previous study conducted at our study site observed virus subpopulation groups by analyzing the flow cytometry (FCM) data and reported that two virus subpopulations, VLP1 and VLP2, were detected [51]. VLP1, which emits the lowest green fluorescence, is considered to be mostly bacteriophages [52]; VLP2, which emits a higher green fluorescence, is assumed to be cyanophages [53]. In the study of Tsai et al. [51], it was suggested that a VLP1 abundance, ranging from 84–89% of the viral density, was significantly associated with bacterial abundance. VLP1 dominated the viral community of the marine environments in our study region and implied that the viruses mostly comprised bacteriophages. This might explain why viral lysis was rarely strong over the annual cycle of the picophytoplankton groups in this study. Considering the dominance of bacteriophages and their hosts (bacteria), the low viral mortality of the picophytoplankton may not be surprising. An alternative explanation might be that the incubation period used in our dilutions might have been too short to capture the viral infection as the viral lytic cycle can often exceed

24 h [11,54]. Finally, nanoflagellate grazers would have a positive influence on the viruses. In a study in a reservoir, it was found that the presence of nanoflagellates could stimulate the viral production and viral infection of bacterioplankton [55,56]. A possible explanation was that there could have been a general stimulation of the picoplankton growth by increasing the supply of substrates from nanoflagellate excretion, which then increased the viral infection. Thus, in this study, we found that a lower nanoflagellate grazing rate may induce a lower viral lysis of picophytoplankton during the warmer seasons.

In summary, a picophytoplankton community dominated by *Synechococcus* spp. was present throughout the year in subtropical western Pacific coastal waters. Our study demonstrated that the picophytoplankton community composition shifted in cold and warm seasons. Picoeukaryotes increased when the temperature was low and *Synechococcus* spp. reached a maximal density during summer. The two picophytoplankton groups showed different seasonal growth rate patterns in these coastal waters and our results indicated that the abundance of *Synechococcus* spp. was closely related to the water temperature; however, there was no significant relationship between the growth rate of the picoeukaryotes and temperatures >25 °C during the warmer seasons. The grazing activity might be driven by complex grazer–prey interactions and the low grazing mortality of *Synechococcus* spp. and the picoeukaryotes during the warmer seasons could be due to the nutrients added to the incubations. This suggested that the mixotrophic nanoflagellates inhabiting these oligotrophic coastal waters could feed on picophytoplankton to supplement their nutrients in a low nutrient environment but they would take up nutrients primarily from a dissolved nutrient pool with a sufficient nutrient supply. The further study of the viral and prokaryotic community compositions is needed to explain why the virus-mediated mortality of the picophytoplankton is low in this oligotrophic subtropical coastal area.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d14010049/s1, Figure S1: Relationship between *Synechococcus* spp. (Δ) and picoeukaryotic abundance (×) and sea surface water temperature. Solid lines exhibit the fitted linear regressions.

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