



Comparative Ecological Characteristics of Two Potentially Toxic Epiphytic Dinoflagellate Species, *Ostreopsis* sp. and *Coolia canariensis*, Native to Jeju Island

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Abstract : Growth responses along the gradient of water temperature, salinity, and light intensity and cytotoxicity against *Artemia nauplii* were explored using *Ostreopsis* sp. and *Coolia canariensis* strains, representing the two potentially toxic epiphytic dinoflagellate (EPD) species from Jeju coastal waters of Korea. Variation in maximum growth rate (GR_{max}) and maximum biomass yield (Y_{max}) along the environmental gradients was quite contrasting between the two strains, which appears to be reflected in the *in situ* abundance distribution of the corresponding genera. The more eurythermal characteristics of *Ostreopsis* sp. strain were in good agreement with the relative distribution of *Ostreopsis* spp. and *Coolia* spp. in 520 macroalgal samples collected from 6 stations. The more stenohaline *C. canariensis* strain was well matched by a markedly narrower range of salinities in the *in situ* distribution of *Coolia* spp. than the salinity range for *Ostreopsis* species. The differences in light adaptation between the high light-preferring *Ostreopsis* sp. strain and the more euryphotoc *C. canariensis* strain were remarkably consistent with the distinct vertical profiles of *Ostreopsis* spp. and *Coolia* spp. abundance in the red alga *Amphiroa* sp. off Moom-seom. Cytotoxicity against *Artemia nauplii* in the *Ostreopsis* sp. preparation with 1000 cells ml⁻¹ was similar to that in *C. canariensis* preparation with 12000 cells ml⁻¹, which is noteworthy. Thus, the new potential cytotoxicity risks from *C. canariensis* along with the well-known toxic genus *Ostreopsis* may be introduced to Jeju coasts, which necessitates further exploration into the contrasting ecological niches occupied by EPD species in relation to their cytotoxicity.

Key words : *Ostreopsis*, *Coolia*, Growth characteristics, *In situ* distribution, Cytotoxicity

1. Introduction

In 2011, the occurrence and quantitative distribution of five epiphytic dinoflagellate (EPD) genera from Korean seas were first reported in the coastal waters of Jeju (Kim et al. 2011, 2021). EPD occurrence monitoring in Korean coasts (Baek 2012a; Jang 2013; Shah et al. 2013; Oh 2015; Park et al. 2020; Lee and Park 2020, 2022) was conducted in parallel with the strain-based studies on taxonomy and

ecophysiology of EPD species (Jeong et al. 2012a, 2012b; Lee et al. 2014; Yoo et al. 2015; Shah et al. 2016; Jang et al. 2018). In addition, several new organic molecules were reported from the *Ostreopsis* cf. *ovata* strains of Jeju Island origin (Yih et al. 2019) through the search for novel marine natural products of mass-cultured EPD strains (Hwang et al. 2013, 2018; Yang et al. 2017; Lee et al. 2019, 2020).

Among the five EPD genera from Jeju coastal waters (Kim et al. 2011), the two genera, *Ostreopsis* and *Coolia*, including toxin producing species (Rhodes and Thomas

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1997; Rhodes et al. 2000; Tibiriçá et al. 2020; Chomérat et al. 2022) commonly co-occur under bloom conditions (Gladan et al. 2019; Misurale et al. 2022). Therefore, a comparative study on the effect of the environmental variable on the growth rate and cytotoxicity of two strains representing the two genera, *Ostreopsis* and *Coolia*, in Jeju coastal waters would be worth conducting.

Epiphytic *Ostreopsis* species in the East Sea was first reported by Selina and Orlova (2010) from the Russian Ussuriiskii Bay. EPD species including two genera *Ostreopsis* and *Coolia* were later observed in the Jeju coastal waters (Kim et al. 2011; Jeong et al. 2012a, 2012b; Kang et al. 2013; Lee et al. 2013; Lim et al. 2013; Lee and Park 2020) and the East Sea (Baek 2012a, 2012b). Cytotoxicity of *Ostreopsis* species such as *Ostreopsis* cf. *ovata* and *O. ovata* isolated from Jeju coastal waters was identified and determined (Hwang et al. 2013, 2018; Shah et al. 2014) as was in the *Ostreopsis* spp. from Mediterranean (Gémin et al. 2020) and Adriatic seas (Gladan et al. 2019). Ciguatoxin-like toxin (*Ostreopsis* cf. *ovata*) and PSP toxin (*Coolia monotis*) were listed as the major toxins from 15 species of the alien/invasive Mediterranean microalgae (Marampouti et al. 2021). However, there are no literature reports on the toxicity of *Coolia* species inhabiting Korean seas (Yih et al. 2011; Lim and Jeong 2021). *Coolia malayensis* was the most prevalent and more toxic to larvae of *Artemia* and sea urchin than the other *C. canariensis*, *C. tropicalis*, and *C. palmyrensis* isolated from Hong Kong waters, where *C. canariensis* exhibited relatively higher toxicity to sea urchin larvae than that against *Artemia* larvae (Leung et al. 2017). Yessotoxin analogues, $C_{56}H_{78}O_{18}S_2$ and $C_{58}H_{86}O_{18}S_2$, were also determined in the Guam strain G6 of *C. canariensis* although no toxic effects of the water-soluble fraction of the strain on *Artemia* was observed (Phua et al. 2021).

Here, we compared the growth rate and biomass yield for the two toxic epiphytic dinoflagellate strains isolated from Jeju coastal waters, *Ostreopsis* sp. (strain KNUTP-OS 090203) and *Coolia canariensis* (strain CCJJ1; Jeong et al. 2012b) under the gradients of seawater temperature, salinity, and irradiance. Different cytotoxicity against *Artemia* larvae by *Ostreopsis* sp. and *C. canariensis* was also detected during the 72 h incubation experiments. At the same time, the contrasting *in situ* spatiotemporal distribution patterns of *Ostreopsis* spp. and *Coolia* spp. in Jeju coastal waters were considered to reflect the distinct and

contrasting growth characteristics of the two EPD strains along the gradients of T, S, and irradiance.

2. Material and Methods

Collection of macroalgal blades to set up clonal cultures of *Ostreopsis* sp. and *Coolia canariensis*

A single cell of *Ostreopsis* on a blade of macroalga *Gelidium amansii* was isolated and established as a unialgal clonal culture (*Ostreopsis* sp. strain KNUTP-OS 090203). The macroalgal substrate was collected at a depth of 3 m off Aeweol (33°28'04.78" N and 126°19'23.69" E; with water temperature and salinity of 14.8°C and 33.7, respectively), Jeju Island in February 2009. The morphological characteristics of the *Ostreopsis* sp. strain were rarely distinguished from *Ostreopsis* cf. *ovata* HJ-2013 (GenBank accession number HE793379; Kang et al. 2013), but the SSU rDNA sequence of the *Ostreopsis* sp. was, however, clustered with *Ostreopsis* sp. ORUS clone-A1 (GenBank accession numbers KC848711 and KC991331; Efimova et al. 2014) rather than HJ-2013 (Hae Jin Jeong, personal communication). Therefore, taxonomic position of the *Ostreopsis* strain is set to a temporarily unidentified *Ostreopsis* species.

From a different macroalgal blade of the same *Gelidium amansii* sample above a unialgal clonal culture of *Coolia canariensis* (strain CCJJ1) was also established by isolating single cells (Jeong et al. 2012b). In the field, each macroalgal substrates was collected *in situ* using a plastic bottle, transported to the laboratory, shaken vigorously by hand over 100 times to separate the dinoflagellates, and finally filtered through a 500 µm Nitex mesh to obtain live dinoflagellate samples for single cell isolation. All clonal cultures were serially transferred every 3 weeks to bottles with f/2 media at a temperature of 20°C and a salinity of 30 under continuous illumination of 60 µmol photons m⁻² s⁻¹.

Growth Response of the *Ostreopsis* and *Coolia* Cultures to Temperature, Salinity, and Illuminance Gradients

The unialgal cultures of *Ostreopsis* sp. and *C. canariensis* were experimentally grown in triplicate 500-ml PC bottles for 34 days under the test gradients of temperature (15, 20, 25, 30, and 35°C), salinity (15, 20, 25, 30, and 35), and light intensity (10, 20, 50, 100, 200 and 300 µmol photons m⁻² s⁻¹) (Table 1). Unialgal inoculation for the experimental batch cultivation of the two strains was

Table 1. The test gradients of temperature (T, °C), salinity (S), and irradiance (L, $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) for the unialgal cultures of *Ostreopsis* sp. and *Coolia canariensis* native to Jeju coastal waters

Gradient level	T (°C)	S	L ($\mu\text{mol photons m}^{-2} \text{ s}^{-1}$)
1	15	15	10
2	20	20	20
3	25	25	50
4	30	30	100
5	35	35	200
6			300

adjusted to obtain the initial target concentration of 50 dinoflagellate cells ml^{-1} in all the experimental bottles. A 5-ml subsample was collected daily from each bottle, and cell counting under a light microscope (Zeiss AxioCam HRc5, Carl Zeiss Ltd. Göttingen, Germany) was performed using a Sedgewick-Rafter counting chamber. Except for the respective environmental parameters (Table 1), the basic environmental conditions for all experimental cultures were 25°C and 30 salinity under continuous illumination with a cool-white fluorescent lamp at 50 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$.

The 6-day average daily specific growth rate (SDA-GR, μ) of each experimental culture was calculated as follows:

$$\mu = [\text{Ln}(C_{t2} / C_{t1})] / (t2-t1)$$

where C_{t1} and C_{t2} are the abundance of the unialgal dinoflagellates at incubation times $t1$ and $t2$, respectively.

Here, the time for $(t2-t1)$ is set to be 6d for the calculation of SDA-GR. During the 34d cultivation experiment, the maximum biomass yield (Y_{max}) of each bottle was determined as the unialgal concentration at the highest peak or maximum point on the population growth curve.

Cytotoxicity of the *Ostreopsis* and *Coolia* cultures against *Artemia* Nauplii

For the cytotoxicity experiments, 10 *Artemia* nauplii were added to each test glass tube containing a 5-ml volume of cell-free filtrate or live cells of an EPD species in $f/2$ medium. Then, the cytotoxicity of the two EPD species against *Artemia* nauplii was determined by counting the number of *Artemia* nauplii killed daily in each glass tube for a 72h period and calculating the survival rate of the *Artemia* nauplii. Initial concentration of EPDs in each set of triplicate glass tube was adjusted to 0, 1000, and 5000 and 0, 5000, and 12000 cells ml^{-1} for *Ostreopsis* sp.

and *C. canariensis*, respectively. The cell-free filtrate preparation in each glass tube also was also derived from the same initial EPD concentrations as above.

Spatiotemporal distribution of *Ostreopsis* and *Coolia* species in Jeju Coastal Waters

The vertical distribution of *Ostreopsis* and *Coolia* species was investigated at a sampling station off Moonseom (33°13'39.32" N and 126°33'50.97" E), an islet near Seogwipo-si, Jeju Island (Fig. 1). Macroalgal substrates of the thalloid and crustose red alga *Amphiroa* sp. were collected from five different depths (0.5, 5, 10, 15, and 20 m) in June and October 2011.

The quantitative distribution of *Ostreopsis* and *Coolia* species in relation to habitat temperature and salinity was explored at six stations around Jeju Island (Fig. 1 and Table 2) based on the macroalgae sampling for even-numbered months of 2011. From each substrate macroalga of the whole 520 macroalgal samples the abundance of *Ostreopsis* and *Coolia* species was determined by microscopic observation and cell counting.

For the *in situ* sampling of each macroalgal substrate divers placed each macroalga in a plastic bottle, then fixed it with buffered 3% formaldehyde, and later shook the fixed sample vigorously to detach the dinoflagellate cells before final filtration through a 500 μm Nitex mesh. The filtrate containing dinoflagellates was stained with Calcofluor-white (Sigma Aldrich), and cells were observed and counted using a light microscope (Zeiss AxioCam HRc5, Carl Zeiss Ltd. Göttingen, Germany) and a Sedgewick-Rafter counting chamber. EPDs belonging to *Coolia* and *Ostreopsis* genus were counted to obtain abundance data per unit mass of the host macroalgae (cells (gram wet weight) $^{-1}$, cells GWW $^{-1}$).

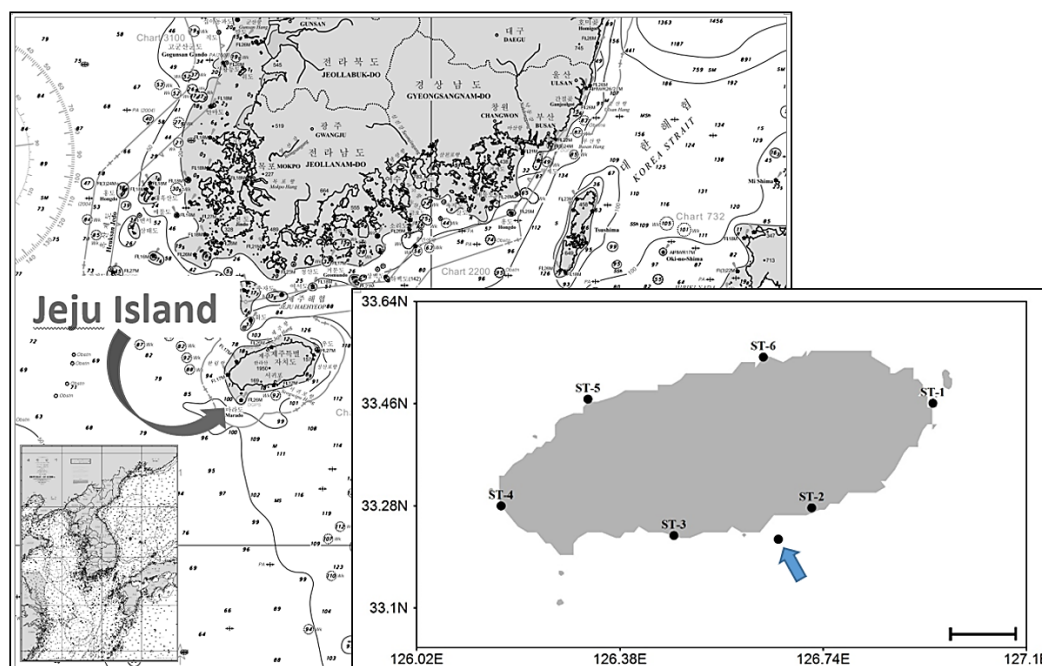


Fig. 1. An oceanographic map showing Korean seas surrounding Jeju Island (modified from the map published in 2011 by the Korea Hydrographic and Oceanographic Agency, Ministry of Oceans and Fisheries, Korea). The map in a separate box shows the sampling stations around Jeju Island and the Moom-seom, an islet in front of the arrowhead (scale bar = 10 miles)

Table 2. Location and name of the 6 sampling stations for bimonthly sampling around Jeju Island. Total number of macroalgal (MA) samples in each station during a whole year is shown in the last column

Sampling stations		Location	Number of MA samples
St.1	Seongsan	N33°27'38.28"/E126°56'03.64"	87
St.2	Namwon	N33°16'34.55"/E126°43'10.56"	89
St.3	Kangjung	N33°13'39.02"/E126°28'32.03"	85
St.4	Shindo	N33°16'46.89"/E126°10'09.98"	89
St.5	Aehwol	N33°28'04.78"/E126°19'23.69"	85
St.6	Joechun	N33°32'30.72"/E126°38'02.55"	85

3. Results and Discussion

Growth response to water temperature, salinity, and illuminance gradients by unialgal cultures of *Ostreopsis* sp. and *Coolia canariensis*

Ostreopsis sp. exhibited positive triplicate mean SDA-GR (6d averaged daily specific growth rate, μ) when the water temperature was lower than 35°C and peaked at 20°C. The growth rate of *C. canariensis* gradually increased as water temperature increased from 15°C to 25°C, but showed negative values at 30 and 35°C (Fig. 2). Trend of the triplicate mean Ymax (maximum biomass yield)

along the temperature axis was similar to that of triplicate mean SDA-GR for both strains (Fig. 3). However, the preferred temperature (20°C in Figs. 2a and 3a) of *Ostreopsis* sp. with wider temperature tolerance was lower than that of the relatively more stenothermal *C. canariensis* with best performance at 25°C (Figs. 2a and 3a).

Negative growth and negligible biomass yield were found for both strains at low salinities (15 and 20), with the highest mean SDA-GR and Ymax met at salinities 35 and 30 in *Ostreopsis* sp. and *C. canariensis*, respectively (Figs. 2 and 3). At 15, the lowest salinity tested, the *C. canariensis* population exhibited very sharp decline com-

pared to *Ostreopsis* sp. (Fig. 2b). Thus, *C. canariensis* seems to be more stenohaline than the *Ostreopsis* sp. strain (Figs. 2b and 3b).

C. canariensis showed positive growth at all 6 light intensities with the maximum of the triplicate mean SDA-GR at 50 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ whereas negative growth

of *Ostreopsis* sp. appeared only at 10 and 20 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (Fig. 2c). The Y_{max} of *Ostreopsis* sp. gradually increased at irradiances from 50 to 300 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (Fig. 3c). Although both the mean SDA-GR and Y_{max} of *C. canariensis* were positive at the 6 light intensities, Y_{max} at 200 and 300 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ were markedly

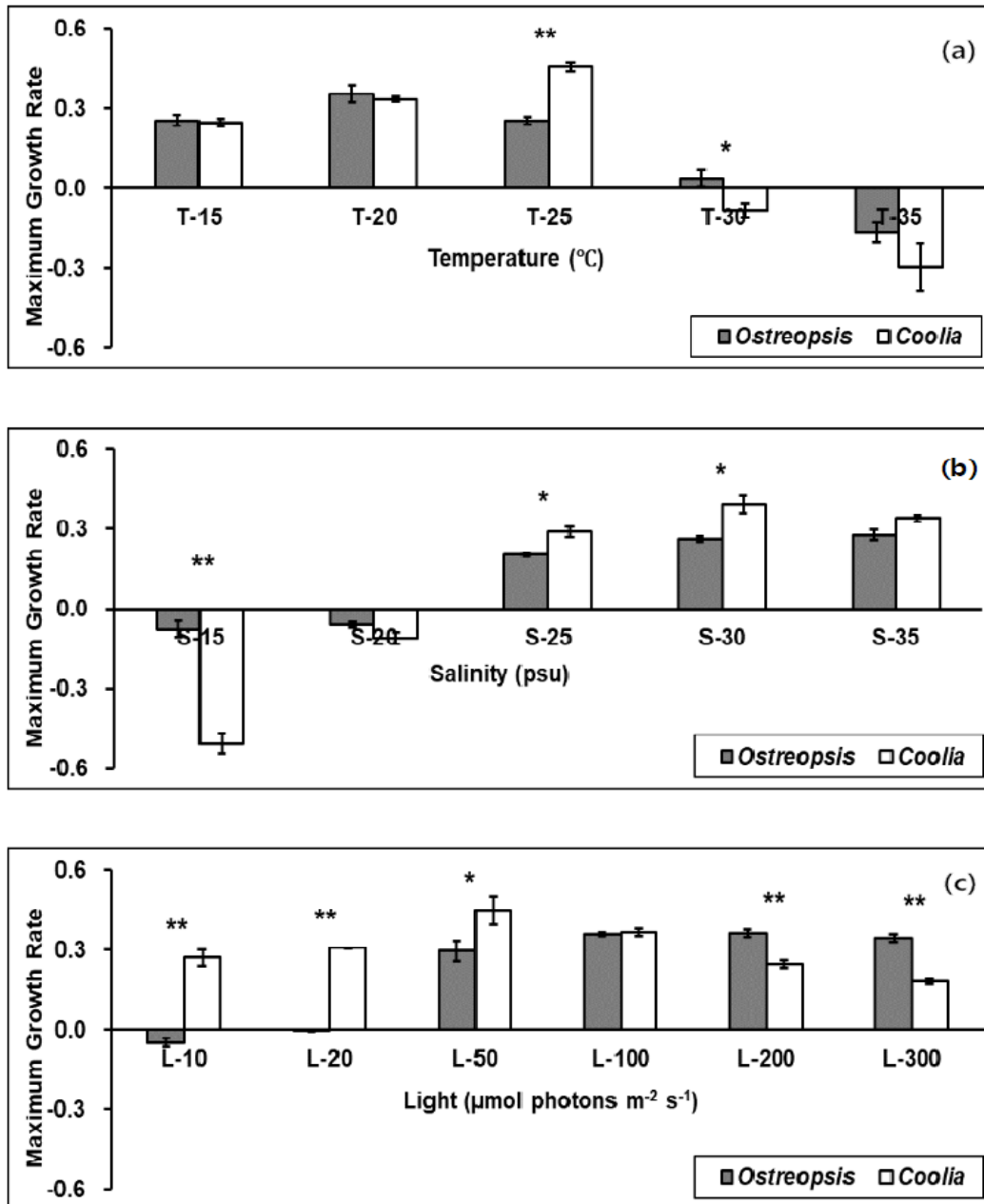


Fig. 2. Mean maximum growth rates (mean GRmax) of the epiphytic dinoflagellate strains *Ostreopsis* sp. and *C. canariensis* (a-c) along the gradients of water temperature (a), salinity (b) and light intensity (c). The mean GRmax is the mean of maximum SDA-GR (6d averaged daily specific growth rate) in each of the triplicate bottles during a 34d cultivation experiment. Error bar: standard error. Significant differences resulted from T-tests are indicated by asterisks (*: $p > 0.05$, **: $p > 0.01$) above the paired bars representing each environmental condition

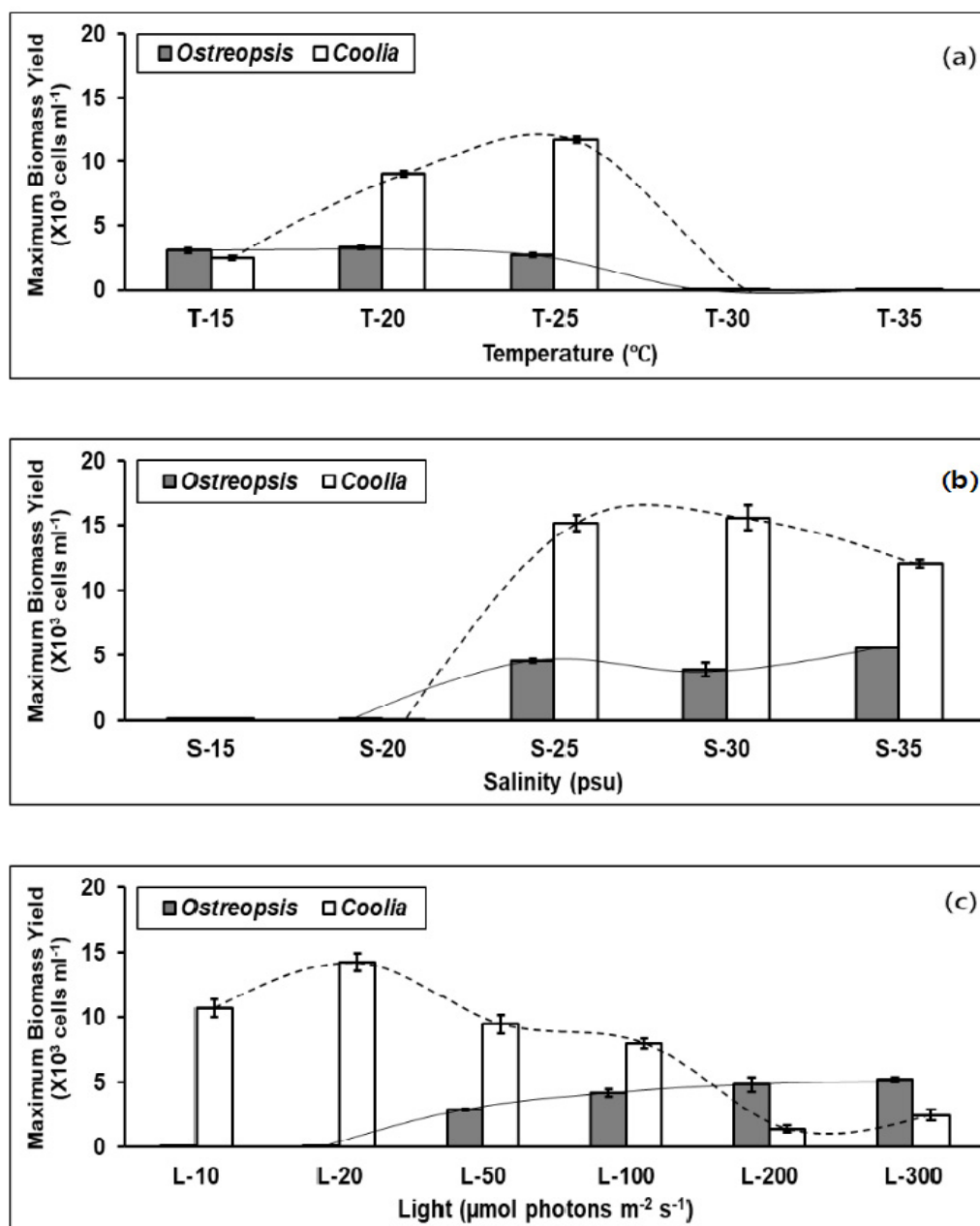


Fig. 3. Mean maximum biomass yields (mean Y_{max}) of the epiphytic dinoflagellate strains *Ostreopsis* sp. and *C. canariensis* (a–c) along the gradients of water temperature (a), salinity (b) and light intensity (c). The mean Y_{max} is the mean of the Y_{max} in each of the triplicate bottles during a 34d cultivation experiment. Error bar: standard error. The solid lines and dotted lines reflect the trend of Y_{max} variations along the gradients of water temperature (a), salinity (b) and light intensity (c) represented by *Ostreopsis* sp. and *C. canariensis*, respectively

lower than at the other irradiances (Fig. 3c). Thus, *Ostreopsis* sp. appears to be better adapted to high light intensities than *C. canariensis*, which has a wide light-adapting range.

In situ spatiotemporal distribution of *Ostreopsis* and *Coolia* species in Jeju coastal waters

In the plane of temperature and salinity axes, the two balloon diagrams of *Ostreopsis* and *Coolia* abundances (Fig. 4a and 4b) among the 520 macroalgae samples collected at 6 stations during 2011 were quite distinct from

each other (Fig. 4). In contrast to the large T-S (13.5–22.7°C, 29.2–34.1) space occupied by high *Ostreopsis* abundances, *Coolia* abundances were concentrated in the small T-S (16.2–22.6°C, 33.2–34.1) space (Fig. 4a and 4b). The T-S cores with the highest EPD abundance in *Ostreopsis* (20.3°C, 32.0) and *Coolia* (22.6°C, 34.1) differed significantly from each other. The above *in situ* distribution of *Ostreopsis* and *Coolia* matches strikingly well with the results from the experimental cultivation of the strains *Ostreopsis* sp. and *C. canariensis*, where maximum growth rate (GRmax) and Ymax were found at 20°C and 25°C in the strain *Ostreopsis* sp. and *C. canariensis*, respectively (Fig. 5a). Again, *Ostreopsis* species living in Jeju coastal waters was shown to be more eurythermal and with lower optimal temperatures than *Coolia* species (Fig. 4a and 4b). In addition, the growth limit temperature (see the positive growth of *Ostreopsis* sp. strain at 30°C in Fig. 5a) supports the eurythermal character of *Ostreopsis* species inhabiting Jeju coastal waters.

The highest mean SDA-GR of the experimental strains was found at salinity ranges of 30–35 in both *Ostreopsis* sp. and *C. canariensis* strains (Fig. 5b), which was also reflected by the *in situ* salinity for the distribution of high abundance core of *Ostreopsis* species (32.0) and *Coolia* species (34.1) (Fig. 4). Thus, *Ostreopsis* sp. strain with the highest GRmax at salinity 35 seems to be more euryhaline

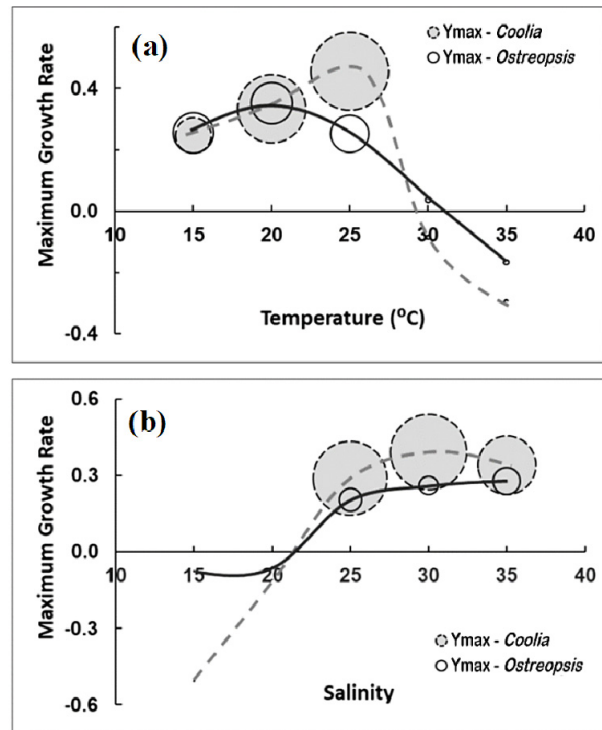


Fig. 5. Balloon diagrams of the mean maximum biomass yields (mean Ymax) in the plane of (a) (water temperature - GRmax) axes and (b) (salinity - GRmax) axes. Solid and dashed lines indicate the variations in GRmax of *Ostreopsis* sp. and *C. canariensis*, respectively

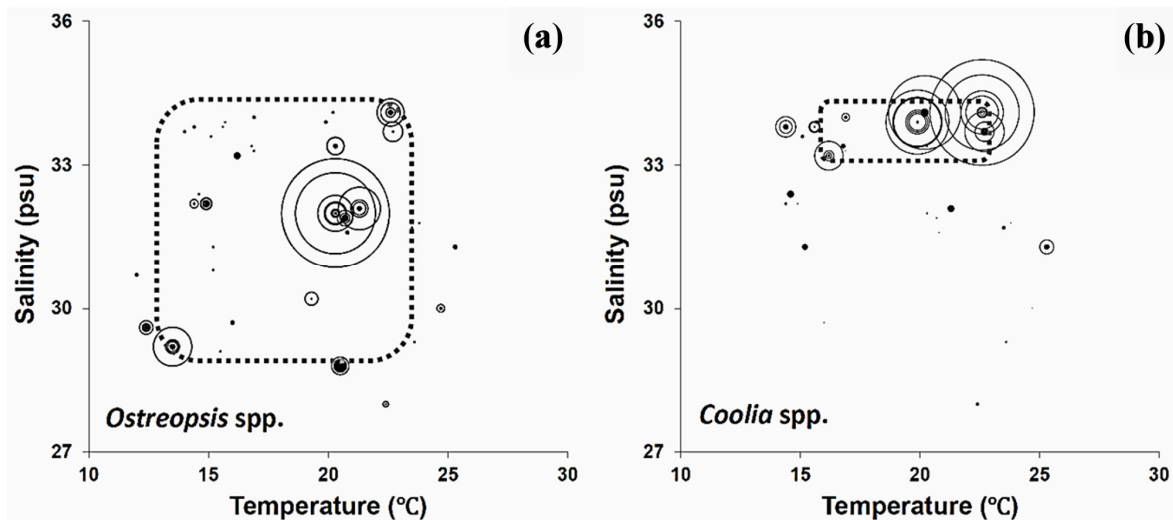


Fig. 4. Balloon diagrams of the epiphytic dinoflagellate (EPD) abundance from each of the 520 macroalgal substrates collected bi-monthly at 6 stations of Jeju coastal waters when plotted in the in the plane of water temperature axis and salinity axis. (a) *Ostreopsis* species. The dotted line indicates the T-S rectangular area with > 2500 *Ostreopsis* cells GWW^{-1} . (b) *Coolia* species. The dotted line indicates the T-S rectangular area with > 1000 *Coolia* cells GWW^{-1}

than *C. canariensis* strain (*Coolia* species with the highest GRmax at salinity 30 (Fig. 5b). The local adaptation of *Ostreopsis* species to lower salinities as well as the tolerance of *in situ* *Coolia* species to narrower salinity ranges (Fig. 4) again reflect the euryhaline character of *Ostreopsis* species inhabiting coastal waters of Jeju.

Vertical distribution of *Ostreopsis* and *Coolia* species in Moon-seom, an islet of Jeju Island

EPD abundances on the thalloid and crustose red alga *Amphiroa* sp. at 4–5 water depths of the sampling station off Moon-seom showed vertically significant profiles (Fig. 6). In June, the abundance of *Ostreopsis* spp. rapidly decreased from a surface maximum of 226 to 3.5 cells GWW⁻¹ at 15 m depth while *Coolia* spp. showed somewhat similar abundances from 5 m to 15 m depth (Fig. 6a). The

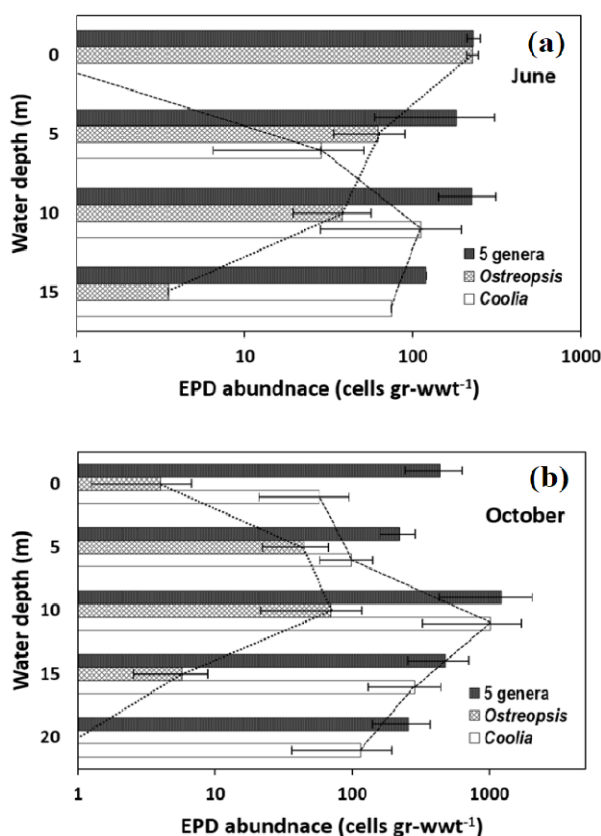


Fig. 6. Vertical distribution of *Ostreopsis* and *Coolia* abundances (cells GWW⁻¹) on the blades of a substrate red alga *Amphiroa* sp., respectively, in June (a) and October (b). The sum of the abundance of all the five genera ('5 genera' in a and b) exhibited vertically least variable profiles in both June and October. Error bar: standard error

maximum abundance of *Ostreopsis* and *Coolia* in October was at 10 m depth, with extreme vertical variation in *Ostreopsis* compared to less pronounced vertical variation in *Coolia* (Fig. 6b). Abundance sums of all five genera including three other additional genera, *Amphidinium*, *Gambierdiscus*, and *Prorocentrum*, exhibited relatively even vertical profiles in both June and October (Fig. 6a and 6a). In both June and October, minimal *Ostreopsis* and *Coolia* were found at the deepest sampling depths (lowest light intensities) and surface (highest light intensities), respectively (Fig. 6). These results for the *in situ* EPD distribution appear to clearly reflect the growth response of clonal cultures to different light intensities in the experimental cultures with *Ostreopsis* sp. and *C. canariensis* strains (Figs. 2 and 3). Better adaption to high light intensity by *Ostreopsis* sp. strain and the contrasting adaptation to wider range of light intensities by *C. canariensis* strains in the experimental cultivation (Fig. 7) allows for a better understanding of the differences in the vertical profiles of *in situ* abundance between *Ostreopsis* and *Coolia* species in the Moon-seom (Fig. 6).

Cytotoxicity of the *Ostreopsis* sp. and *Coolia canariensis* strains against *Artemia* Nauplii

Cytotoxicity against *Artemia* nauplii was undetectable at 12h for all experimental preparations and undetectable at 24h for low density preparations (5000 cells ml⁻¹) of *C. canariensis* (Fig. 8). Cell filtrates were found to be less toxic than live cells for all the preparations except for one case (Fig. 8a), a low-density preparation of *C. canariensis*

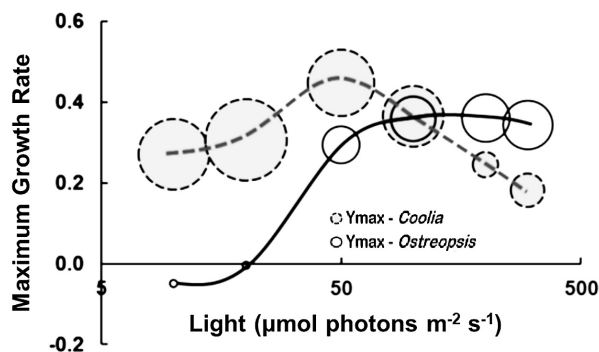


Fig. 7. Balloon diagram of mean *Y*_{max} in the plane of (light intensity - GRmax) axes. The solid and dashed lines indicate the trends of GRmax fluctuation along the light intensity gradients in *Ostreopsis* sp. and *C. canariensis*, respectively

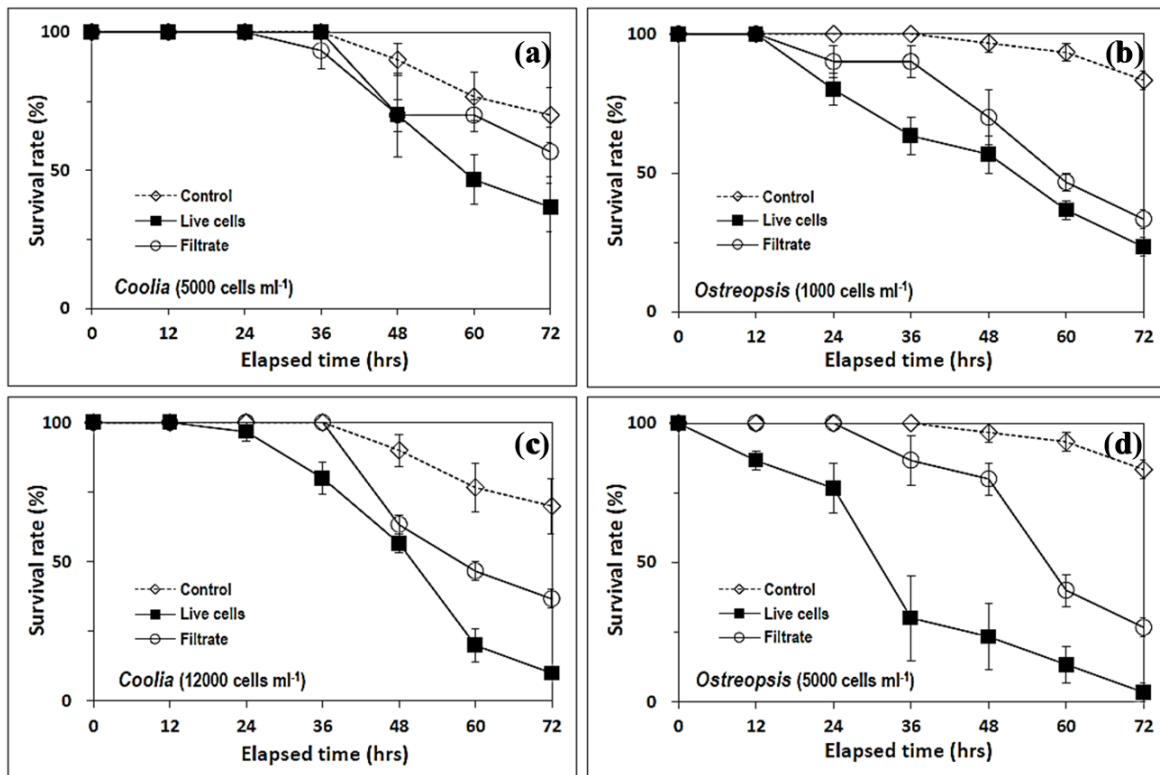


Fig. 8. Survival rate (%) of *Artemia* nauplii in the live cell and cell-free filtrate treatments of *Ostreopsis* sp. and *C. canariensis* cultures. Over 72 h exposure, live cell and cell-free filtrate treatments with 5000 (a) and 12000 (c) cells ml^{-1} culture of *C. canariensis* strain and 1000 (b) and 5000 (d) cells ml^{-1} culture of *Ostreopsis* sp. strain were tested for *Artemia* nauplii cytotoxicity. Error bar: standard error

(5000 cells ml^{-1} , at 36h). For the most toxic preparation (*Ostreopsis* sp., 5000 cells ml^{-1}) (Fig. 8d), the median lethal time, LT_{50} , was calculated to be 30.9 and > 57.0 h for live cell and cell filtrate treatments, respectively. On d2 and d3, the level of cytotoxicity against *Artemia* nauplii exerted by low-density (1000 cells ml^{-1}) preparation (Fig. 8b) of *Ostreopsis* sp. was similar to that by the high-density (12000 cells ml^{-1}) preparations (Fig. 8c) of *C. canariensis* (see treatments ‘Cc-12000’ and ‘Osp-1000’ in Fig. 9).

The high cytotoxicity of *C. canariensis* against *Artemia* nauplii was previously unknown. The cytotoxicity of *C. canariensis* against *Artemia* nauplii in this study was somewhat higher than that of *C. canariensis* isolated from Hong Kong waters (see Fig. 5 in Leung et al. 2017). Lysate solutions of *C. canariensis* at 0.5 mg ml^{-1} equivalent to 0.47 and 0.32 million cells ml^{-1} of the two Hong Kong strains, W039 and Ve011, respectively, was shown to be non-lethal against *Artemia* nauplii (Leung et al. 2017). However, for the prism larvae of sea urchin (*Heliocidaris crassispina*) the two Hong Kong strains of *C. canariensis*,

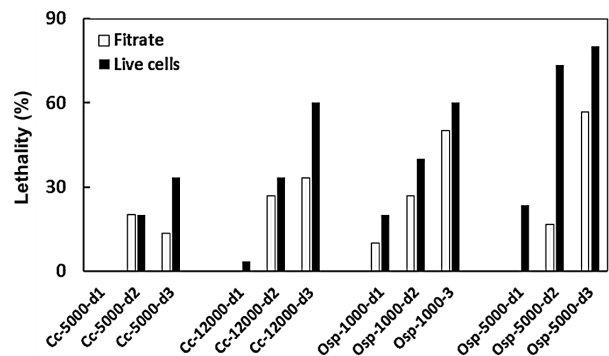


Fig. 9. Triplicate mean lethality (%) at d1 (24 h), d2 (48 h), and d3 (72 h) of live cell and cell-free filtrate treatments of *Ostreopsis* sp. (Osp) and *C. canariensis* (Cc) cultures to *Artemia* larvae. Live cell (black bar) and cell-free filtrate treatments (empty bar) with 5000 (Cc-5000) and 12000 (Cc-12000) cells ml^{-1} culture of *C. canariensis* strain and 1000 (Osp-1000) and 5000 (Osp-5000) cells ml^{-1} culture of *Ostreopsis* sp. strain were tested for *Artemia* nauplii cytotoxicity. The lethality (%) in the bottles was adjusted to calibrate the lethality in control bottles after 36 h (Fig. 8) to be “0”

W039 and Ve011, exhibited LC_{50} at lysate concentrations 0.082 and 0.064 mg ml⁻¹ (equivalent to 78 and 40 thousand cells ml⁻¹), respectively (Leung et al. 2017). Although the yessotoxin analogues, C₅₆H₇₈O₁₈S₂ and C₅₈H₈₆O₁₈S₂, were determined in the Guam strain G6 of *C. canariensis*, no toxic effects of the water-soluble fraction of strain G6 on *Artemia* was observed (Phua et al. 2021).

4. Conclusion

The cytotoxicity against *Artemia* nauplii (Fig. 8) as well as the growth responses to different water temperature, salinity, and light intensity (Figs. 2 and 3) were explored and compared through culture experiments of *Ostreopsis* sp. and *C. canariensis* strains from Jeju coastal waters. The difference in ecological characteristics of the two strains derived from the cultivation experiments was found to be in good agreement with the *in situ* distribution pattern of the corresponding EPD genera in Jeju coastal waters (Figs. 4 and 6).

Ostreopsis sp. strain with a lower preferred temperature (20°C) was also more temperature tolerant than the relatively stenothermal *C. canariensis* strain that performed best at 25°C (Figs. 2 and 5). The distribution pattern of *Ostreopsis* spp. and *Coolia* spp. in the 520 macroalgal samples collected bimonthly from 6 stations in Jeju coastal waters (Fig. 4) matched well with the eurythermal characteristics of *Ostreopsis* sp. strain in compared to *C. canariensis* strain (Figs. 2 and 5). The more stenohaline *C. canariensis* strain compared to the *Ostreopsis* sp. strain (Figs. 2 and 5) also matched well with a much narrower salinity range in the *in situ* distribution of *Coolia* spp. than that of *Ostreopsis* spp. in the 520 macroalgal samples (Fig. 4).

The preference of *Ostreopsis* sp. strain for high light intensities contrasted markedly with the better growth of *C. canariensis* strain at lower light intensities, including 10 and 20 μmol photons m⁻² s⁻¹, even though the Y_{max} of *C. canariensis* was significantly lower in high light intensities above 200 μmol photons m⁻² s⁻¹ (Figs. 2 and 3). Differences in light adaptation between the two strains (Figs. 2 and 3) are likely reflected in the *in situ* vertical profiles of *Ostreopsis* spp. and *Coolia* spp. from the thaloid and crustose red alga *Amphiroa* sp. off Moom-seom in June and October (Fig. 6). In October *Coolia* spp. exhibited a somewhat more even vertical distribution compared to the absence ('0') of *Ostreopsis* spp. at a depth of 20 m

(Fig. 6b). Again, abundance of *Ostreopsis* spp. gradually decreased with increasing depth while the abundances of *Coolia* spp. peaked at 10 m in June (Fig. 6a).

Cytotoxicity against *Artemia* nauplii was detected as percent lethality for all preparations containing live cell as well as cell-free extracts of *Ostreopsis* sp. and *C. canariensis* strains during a 72 h incubation experiments. Lethality (%) to *Artemia* nauplii in the *Ostreopsis* sp. preparations at 1000 cells ml⁻¹ (Osp-1000 in Fig. 9) was similar to *C. canariensis* preparations at 12000 cells ml⁻¹ (Cc-12000 in Fig. 9) on d2 and d3. Thus, the cytotoxicity of *C. canariensis* against *Artemia* nauplii in this study was somewhat higher than that of *C. canariensis* isolated from Hong Kong waters (Leung et al. 2017). Such a high cytotoxicity of *C. canariensis* against *Artemia* larvae was previously not known. Therefore, both strains from Jeju coastal waters were found to be toxic to *Artemia* nauplii in this study, which is noteworthy in terms of food safety and leisure activities in Jeju coastal areas.

In conclusion, the growth characteristics of the two EPD strains under different temperature, salinity, and light intensity derived from the laboratory experiments were found to be very important in understanding the spatiotemporal distribution of EPD species and abundance in the Jeju subtidal habitats. In other words, the difference in growth characteristic of the two EPD strains was useful in explaining the contrasting spatial and temporal distribution patterns of *Ostreopsis* spp. and *Coolia* spp. in Jeju coastal waters. The strain *C. canariensis* isolated from Jeju coastal waters showed notably higher cytotoxicity against *Artemia* nauplii than previous reported. Therefore, in the near future, a new potential cytotoxicity risk may be introduced by *C. canariensis* inhabiting Jeju coastal waters, in addition to the present risks of the genus *Ostreopsis*, a currently well-known potentially toxic EPD species. In order to prepare for the hazards of EPD cytotoxicity in the coastal waters of Jeju Island in the future, it is necessary to further explore the contrasting ecological niches occupied by EPD species in relation to the cytotoxicity risks of the dominant EPD species.

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