

Research Article

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Effects of biostimulants, AMPEP and Kelpak on the growth and asexual reproduction of *Pyropia yezoensis* (Bangiales, Rhodophyta) at different temperatures

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Acadian marine plant extract powder (AMPEP) and Kelpak are commercial biostimulants derived from brown algae *Ascophyllum nodosum*. This study was to determine if AMPEP and Kelpak can induce thermal resistance in *Pyropia yezoensis*. *P. yezoensis* blades were exposed to different concentrations (control: 0, low: 0.001, high: 1 ppm) of AMPEP and Kelpak at 10°C for 6 and 7 days, respectively. Those blades were then cultivated in von Stosch enriched seawater medium at different temperatures (10, 15, 20, and 25°C) with 12 : 12 L : D photoperiod and 100 µmol m⁻² s⁻¹ of photosynthetically active radiation for additional 15 days. Results showed that *P. yezoensis* reproduced archeospores at 20 and 25°C at all biostimulant conditions within 15 days. At lower temperatures (10 and 15°C), only AMPEP-treated *P. yezoensis* reproduced archeospores. *P. yezoensis* exposed to 1 ppm Kelpak exhibited higher phycoerythrin and phycocyanin contents than control and 0.001 ppm conditions at 15°C. AMPEP-treated conditions showed higher phycoerythrin and phycocyanin contents than control at 10°C. These results suggest that AMPEP and Kelpak may not enhance the thermal resistance of *P. yezoensis*. However, AMPEP stimulated archeospores release at lower temperatures. The treatment of AMPEP and Kelpak also increased the pigment contents in *P. yezoensis*. These results suggest that the use of seaweed-derived biostimulants can provide some economic benefits in *P. yezoensis* aquaculture. The enhancement of archeospores formation by AMPEP at lower temperature may also increase the productivity since *Pyropia* farming relies on the accumulation of secondary seedlings via asexual reproduction.

Keywords: AMPEP; Archeospores; asexual reproduction; biostimulant; Kelpak; *Pyropia*; temperature

INTRODUCTION

Seaweed-derived biostimulants are mainly used in agriculture for an advance of the development of root capacities to improve nutrient use efficiency (Rayorath et al. 2008), to enhance resistance to abiotic stresses (e.g., frost and salinity) and to decrease the impacts of biotic

stresses (e.g., pests, fungal diseases, and insect infestation) (Jayaraj et al. 2008, Rayorath et al. 2008). One of the most widely known seaweed-based biostimulants is Kelpak (Kelp Products Pty Ltd., Simon's Town, South Africa), which is a liquid biostimulant derived from the



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brown alga, *Ecklonia maxima* (Osbeck) Papenfuss (Van Staden et al. 1995). Kelpak is known to improve the growth rate by stimulating phytohormones contents of plants (Featonby-Smith and Van Staden 1983, 1987). For example, Kelpak can improve yield of strawberry (Masny et al. 2004), fruit number, and size of pepper (Arthur et al. 2003). Higher plants, *Pelargonium peltatum* and *Brassica oleracea* treated with Kelpak showed significantly higher chlorophyll (Chl) *a* and Chl *b* contents than the treatment without this biostimulant (Krajnc et al. 2012, Rengasamy et al. 2016). Another higher plant species, *Festulolium braunii*, exposed to Kelpak also showed higher chlorophyll contents than control and therefore increased leaf greenness (Sosnowski et al. 2019). To seaweed, Kelpak increased the growth of *Gracilaria* and *Ulva* (Robertson-Andersson et al. 2006) and also enhanced thermal tolerance of *Saccharina* (Umanzor et al. 2020b). Another commercial seaweed-derived biostimulant, Acadian marine plant extract powder (AMPEP; Acadian Seaplants Ltd., Dartmouth, Nova Scotia, Canada) is made from the brown alga, *Ascophyllum nodosum* (Linnaeus). *Ascophyllum nodosum* extract is known to improve yield and nutritional quality of spinach (Fan et al. 2013) and yield and fruit quality of grapes (Norrie et al. 2002). Also, it improves phenolics, antioxidant, and chlorophyll contents (Norrie et al. 2002). In case of seaweed, AMPEP reduces epiphyte loads, and enhances growth and thermal tolerance on *Kappaphycus* (Hurtado and Critchley 2018). Also, AMPEP treatment increased the biosynthesis of carrageenan in *Kappaphycus* (i.e., yield, viscosity, and gel strength) (Ali et al. 2018). The growth of *Gracilaria corticata* and *G. salicornia* was also enhanced by AMPEP (Dawange and Jaiswar 2020, Jaiswar et al. 2021).

Global aquaculture production of *Pyropia* was increased from about 1.8 million tons in 2015 to 2.8 million tons in 2021. Its commercial value was also increased from \$1.7 to \$2.5 billion at the same time (Food and Agriculture Organization of the United Nations 2023). *Pyropia* is one of the most cultivated species in the world, especially in Korea, Japan, and China (Kim et al. 2017, Food and Agriculture Organization of the United Nations 2023). *Pyropia yezoensis* grows well at 5–18°C (Kim et al. 2007), and therefore this alga is cultivated in the open water farms from December to March. Since climate change will continuously affect the *Pyropia* farming, technology development is critical to enhance thermal tolerance in *Pyropia* (Kim et al. 2022, Umanzor et al. 2022a).

Pyropia cultivation usually utilizes zygotospores from fertilized thalli to produce conchocelis, which grows in oyster shells (Kim et al. 2022). This process demands

space, time, and cost, and may also lead to diseases such as yellow spot and white spot (Ryu et al. 2001, Guan et al. 2013). Germination and growth of archeospores of *P. yezoensis* are faster than conchospores (Li 1989). Archeospores release amplifies crop biomass compared with conchospores seeding (Li and Cui 1980, Wang 1985). Archeospores require only 1 or 2 days to develop into the two-cell stage, whereas protoplasts from blade need 5 to 7 days. Also, growth of gametophytes via archeospores enables the rapid multiplication of individuals (Waaland et al. 1990, Chen et al. 1994). Archeospores rapidly attach to seeding ropes and develop into secondary stage (Duffield et al. 1972). This suggests that methods utilizing asexual reproduction may be more efficient in terms of time, labor, space, and cost. This study was to test the effect of Kelpak and AMPEP on *P. yezoensis* at different temperatures and to determine the optimal concentrations of Kelpak and AMPEP for thermal resistance.

MATERIALS AND METHODS

Algal materials

The strain of *P. yezoensis* (PY-JB-ST1) used in this study was originally collected from Mabawi, Jeju Island, Gyeonggi-do, Korea ($37^{\circ}15'69.07''$ N, $126^{\circ}61'53.80''$ E) in February 2018. After the collection, *P. yezoensis* blades were cut into 0.5 cm and cultivated under $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ at 20°C to stimulate archeospores release. The gametophytic blades from archeospores were then cultivated at 10°C, 12 : 12 L : D photoperiod and $80 \mu\text{mol m}^{-2} \text{s}^{-1}$ at the Marine Ecology and Green Aquaculture Laboratory, Incheon National University, Korea.

Experimental design

Kelpak and AMPEP experiments were conducted independently. The full profile of nutrients of these biostimulants can be found in Hurtado et al. (2009) and Lötze and Hoffman (2016). *P. yezoensis* blades (1 cm × 1 cm) were cultured at 0.1 g L^{-1} stocking density at 10°C in different concentrations (control: 0, low: 0.001, high: 1 ppm) of Kelpak and AMPEP for 7 and 6 days, respectively. Photosynthetically active radiation (PAR) and photoperiod were $90 \pm 10 \mu\text{mol m}^{-2} \text{s}^{-1}$ and 12 : 12 L : D, respectively. Nutrients were supplied by von Stosch enriched (VSE) medium, and 250 mg L^{-1} of germanium dioxide (GeO_2) was added to inhibit the development of diatoms (Lewin 1966). To prevent nutrient limitation, VSE medium was

added after 4 days. After exposure to Kelpak or AMPEP, all the blades were rinsed with filtered and sterilized seawater and transferred to 100 mL flask with VSE seawater at 0.5 g L⁻¹ stocking density, 30 psu salinity, 12 : 12 L : D photoperiod and 90 ± 10 µmol m⁻² s⁻¹ of PAR. *P. yezoensis* blades were then cultivated at four different temperatures, 10, 15, 20, and 25°C for additional 15 days. Continuous aeration was provided and the culture medium with VSE was changed every 5 days. To prevent nutrient limitation, VSE was added to the culture medium 3 days after the medium change.

Measurements of growth rate and pigments

Fresh weight (FW) of *P. yezoensis* in each cultivation condition was measured every 5 days. Water on the blades was removed with paper towels before weight measurement. Weight of each condition was restored to the initial stocking density after the measurement. The specific growth rate (SGR) was calculated by the following formula (Kim et al. 2007):

$$\text{SGR} (\% \text{ d}^{-1}) = \frac{(\ln S_2 - \ln S_1)}{(T_2 - T_1)} \times 100$$

, where S₁ and S₂ are the initial and final FW on days T₁ (initial day) and T₂ (final day), respectively.

For phycoerythrin (PE) and phycocyanin (PC) analyses, approximately 0.05 g (FW) of fresh thalli was ground in 0.1 M phosphate buffer (pH 6.8). After centrifugation at 5,000 × g for 15 min at 4°C, the absorption of super-

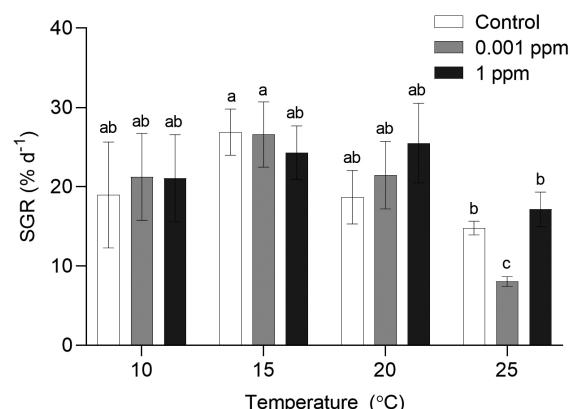


Fig. 1. Specific growth rates (SGRs) of *Pyropia yezoensis* in different Kelpak concentrations and temperatures at day 15. Each coordinate is the overall mean ± standard deviation (STD) of 9 replicates measured at 10 and 15°C. Since reproduction occurred at 20 and 25°C within 15 days, 6 replications were used for the analysis at these temperatures. Different letters on the bars indicate significant differences ($p < 0.05$). Error bars represent mean ± STD of 6 or 9 replicates.

nant was measured for PE and PC following Beer and Eshel (1985):

$$\text{Phycoerythrin (PE)} = [(A_{564} - A_{592}) - (A_{455} - A_{592}) \times 0.20] \times 0.12$$

$$\text{Phycocyanin (PC)} = [(A_{618} - A_{645}) - (A_{592} - A_{645}) \times 0.15] \times 0.15$$

Statistical analysis

All statistical analyses were conducted using IBM SPSS Statistics version 27 (IBM, Armonk, NY, USA). Two-way

Table 1. Results of analysis of variance examining the effects of different biostimulants concentrations (0, 0.001, and 1 ppm) and different temperatures (10, 15, 20, and 25°C)

Source		Concentration	Temperature	Concentration × temperature	Error
SGR (Kelpak)	df	2	3	6	60
	F	8.533	58.962	7.697	
SGR (AMPEP)	Sig.	0.001	<0.001	<0.001	
	df	2	3	6	60
Phycoerythrin (Kelpak)	F	12.500	75.636	6.950	
	Sig.	<0.001	<0.001	<0.001	
Phycoerythrin (AMPEP)	df	2	1	2	12
	F	1.384	33.937	1.364	
Phycocyanin (Kelpak)	Sig.	0.008	0.008	<0.001	
	df	2	1	2	12
Phycocyanin (AMPEP)	F	11.595	20.638	14.267	
	Sig.	0.002	0.001	0.001	
Phycoerythrin (Kelpak)	df	2	1	2	12
	F	7.027	29.959	1.166	
Phycocyanin (AMPEP)	Sig.	0.010	<0.001	0.345	
	df	2	1	2	12
	F	6.914	21.613	7.268	
	Sig.	0.010	0.001	0.009	

Significant differences are shown in bold with p-value.

SGR, specific growth rate; AMPEP, Acadian marine plant extract powder.

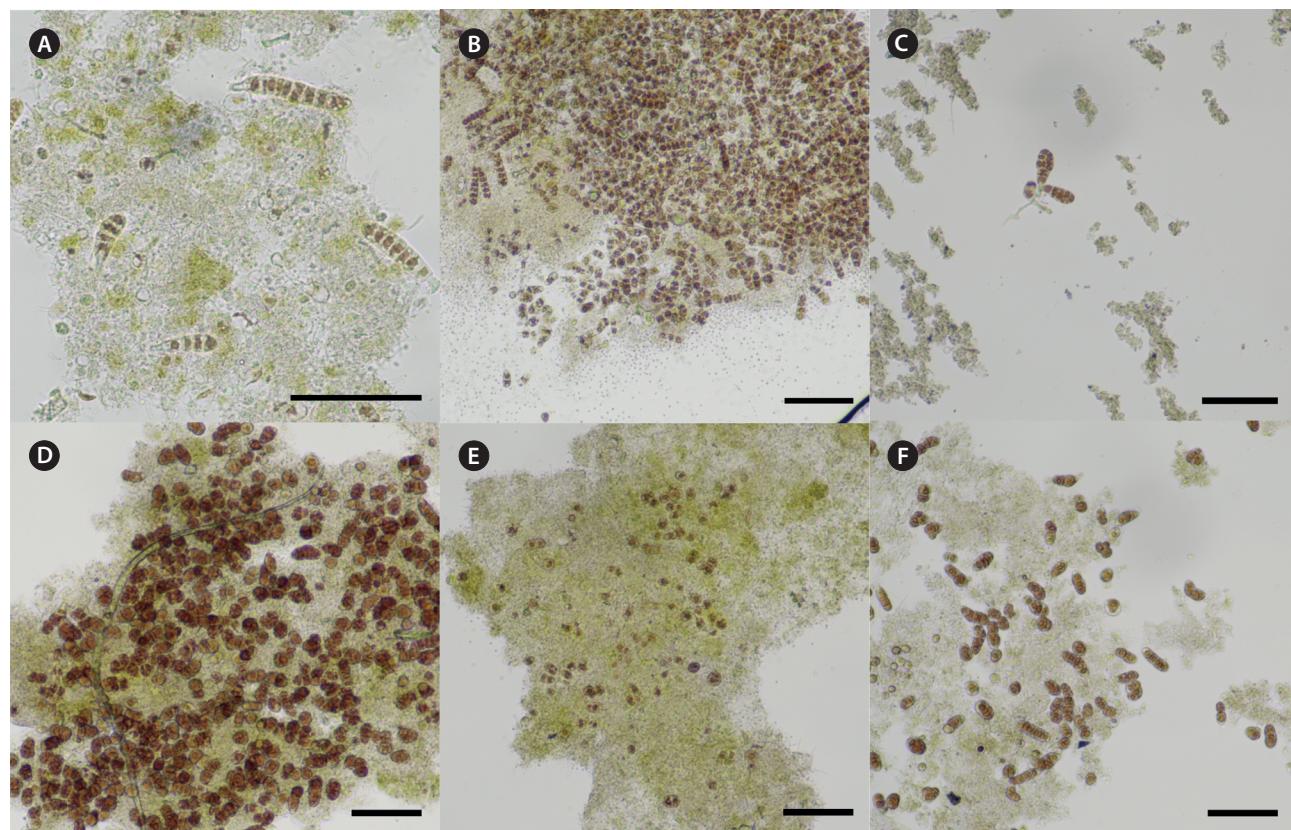


Fig. 2. Microscopic images of gametophytes of *Pyropia yezoensis* after 15 days of cultivation at 20 and 25°C at different Kelpak and temperatures. (A) Control at 20°C. (B) 0.001 ppm of Kelpak at 20°C. (C) 1 ppm of Kelpak at 20°C. (D) Control at 25°C. (E) 0.001 ppm of Kelpak at 25°C. (F) 1 ppm of Kelpak at 25°C. Scale bars represent: A–F, 100 µm.

ANOVA were performed to determine the effects of bio-stimulants concentrations and temperatures on SGR and PE and PC contents. Tukey's honest significant difference tests were conducted for *post-hoc* investigation. Differences between treatments were considered to be significant at $p < 0.05$.

RESULTS

Effect of Kelpak on *Pyropia yezoensis*

SGRs of *P. yezoensis* were significantly influenced by Kelpak ($p = 0.047$), temperature ($p < 0.001$) and the interaction of these two factors ($p = 0.002$) (Table 1). *P. yezoensis* grew well in a wide range of temperatures (10, 15, 20, and 25°C) for 10 days (Fig. 1). There was no significant difference in the SGR at 10–20°C. At control without Kelpak, the SGR at 15°C was significantly higher than that at 25°C. However, at 1 ppm, the SGR was similar at all temperature conditions. The lowest growth rate was

observed at 0.001 ppm and 25°C (Table 2). Archeospores release was observed in all conditions regardless of the Kelpak concentrations at 20 and 25°C in 15 days (Fig. 2). PE and PC contents of *P. yezoensis* were measured at 10 and 15°C only due to asexual reproduction at 20 and

Table 2. Specific growth rate of *Pyropia yezoensis*

Treatment	Specific growth rate (% d ⁻¹)		
	Control	0.001 ppm	1 ppm
Kelpak			
10°C	18.96 ± 6.67 ^{ab}	21.24 ± 5.48 ^{ab}	21.09 ± 5.51 ^{ab}
15°C	26.89 ± 2.90 ^a	26.60 ± 4.11 ^a	24.28 ± 3.39 ^{ab}
20°C	18.69 ± 3.38 ^{ab}	21.46 ± 4.26 ^{ab}	25.51 ± 5.02 ^{ab}
25°C	14.79 ± 0.85 ^b	8.06 ± 0.63 ^c	17.15 ± 2.17 ^b
AMPEP			
10°C	19.92 ± 4.54 ^{ab}	19.23 ± 4.38 ^{ab}	17.98 ± 6.70 ^{abc}
15°C	23.29 ± 3.63 ^a	25.48 ± 3.76 ^a	20.66 ± 5.02 ^{ab}
20°C	16.83 ± 3.63 ^{bc}	18.32 ± 2.22 ^{bc}	17.03 ± 2.60 ^{bc}
25°C	12.26 ± 1.95 ^c	11.95 ± 2.22 ^c	13.02 ± 2.38 ^c

AMPEP, Acadian marine plant extract powder.

Values are presented as mean ± standard deviation. Different letters indicate significant differences ($p < 0.05$).

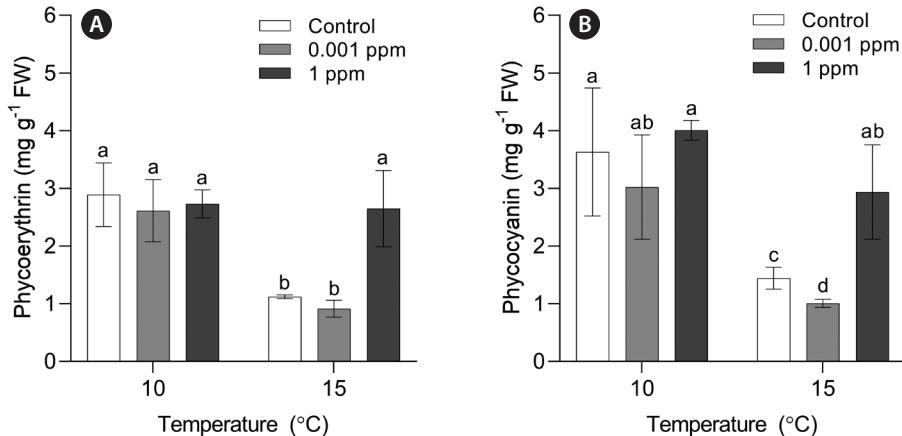


Fig. 3. Phycoerythrin (A) and phycocyanin (B) contents of *Pyropia yezoensis* at different temperatures (10 and 15°C) and different concentrations of Kelpak at day 15. FW, fresh water. Different letters on the bars indicate significant differences ($p < 0.05$). Error bars represent mean \pm standard deviation of 9 replicates.

25°C. PE content was significantly influenced by temperature ($p = 0.008$), Kelpak ($p = 0.008$), and the interaction of these two factors ($p < 0.001$) (Table 1). No significant effects of Kelpak were observed in PE content at 10°C. At 15°C, however, PE content at 1 ppm was significantly higher than control and 0.001 ppm of Kelpak, and similar to other conditions at 10°C (Fig. 3A). PC content was significantly affected by temperature ($p < 0.001$) and Kelpak ($p = 0.010$) (Table 1). PC content showed a similar pattern to PE content. No significant differences were observed in PC content at 10°C while the content in 1 ppm was the highest at 15°C (Table 3, Fig. 3B).

Effect of AMPEP on *Pyropia yezoensis*

SGR of *P. yezoensis* was significantly influenced by temperature ($p < 0.001$), whereas AMPEP and the interaction of temperature and AMPEP did not affect significantly ($p > 0.05$) (Table 1). Regardless of the AMPEP concentrations, the lowest growth rate was observed at 25°C (Fig. 4).

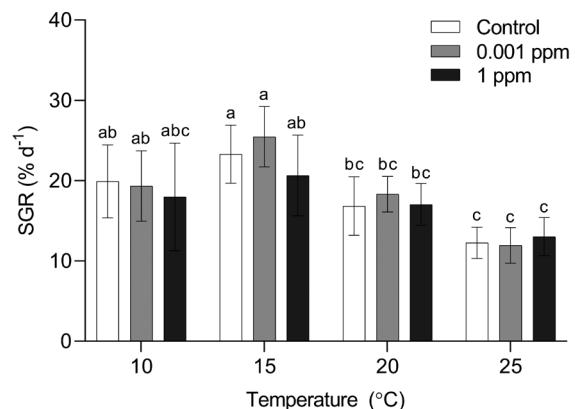


Fig. 4. Specific growth rates (SGRs) of *Pyropia yezoensis* in different Acadian marine plant extract powder (AMPEP) concentrations and temperatures at day 15. Each coordinate is the overall mean \pm standard deviation (STD) of 9 replicates measured at 10 and 15°C. Since reproduction occurred within 15 days, 6 replications were used for the conditions at 20 and 25°C. Different letters on the bars indicate significant differences ($p < 0.05$). Error bars represent mean \pm STD of 6 or 9 replicates.

Table 3. Phycoerythrin and phycocyanin contents of *Pyropia yezoensis*

	Phycoerythrin (mg g ⁻¹ FW)			Phycocyanin (mg g ⁻¹ FW)		
	Control	0.001 ppm	1 ppm	Control	0.001 ppm	1 ppm
Kelpak						
10°C	2.89 \pm 0.55 ^a	2.61 \pm 0.54 ^a	2.73 \pm 0.24 ^a	3.63 \pm 1.11 ^a	3.02 \pm 0.91 ^{ab}	4.01 \pm 0.17 ^a
AMPEP						
10°C	0.46 \pm 0.18 ^c	1.47 \pm 0.23 ^a	1.13 \pm 0.21 ^{ab}	0.07 \pm 0.01 ^{bc}	0.18 \pm 0.06 ^a	0.12 \pm 0.03 ^{ab}
15°C	1.12 \pm 0.03 ^b	0.91 \pm 0.15 ^b	2.65 \pm 0.66 ^a	1.44 \pm 0.19 ^c	1.01 \pm 0.07 ^d	2.93 \pm 0.81 ^{ab}
15°C	0.71 \pm 0.16 ^{bc}	0.69 \pm 0.11 ^{bc}	0.51 \pm 0.15 ^c	0.07 \pm 0.02 ^{bc}	0.07 \pm 0.01 ^{bc}	0.03 \pm 0.01 ^c

FW, fresh weight; AMPEP, Acadian marine plant extract powder.

Values are presented as mean \pm standard deviation. Different letters indicate significant differences ($p < 0.05$).

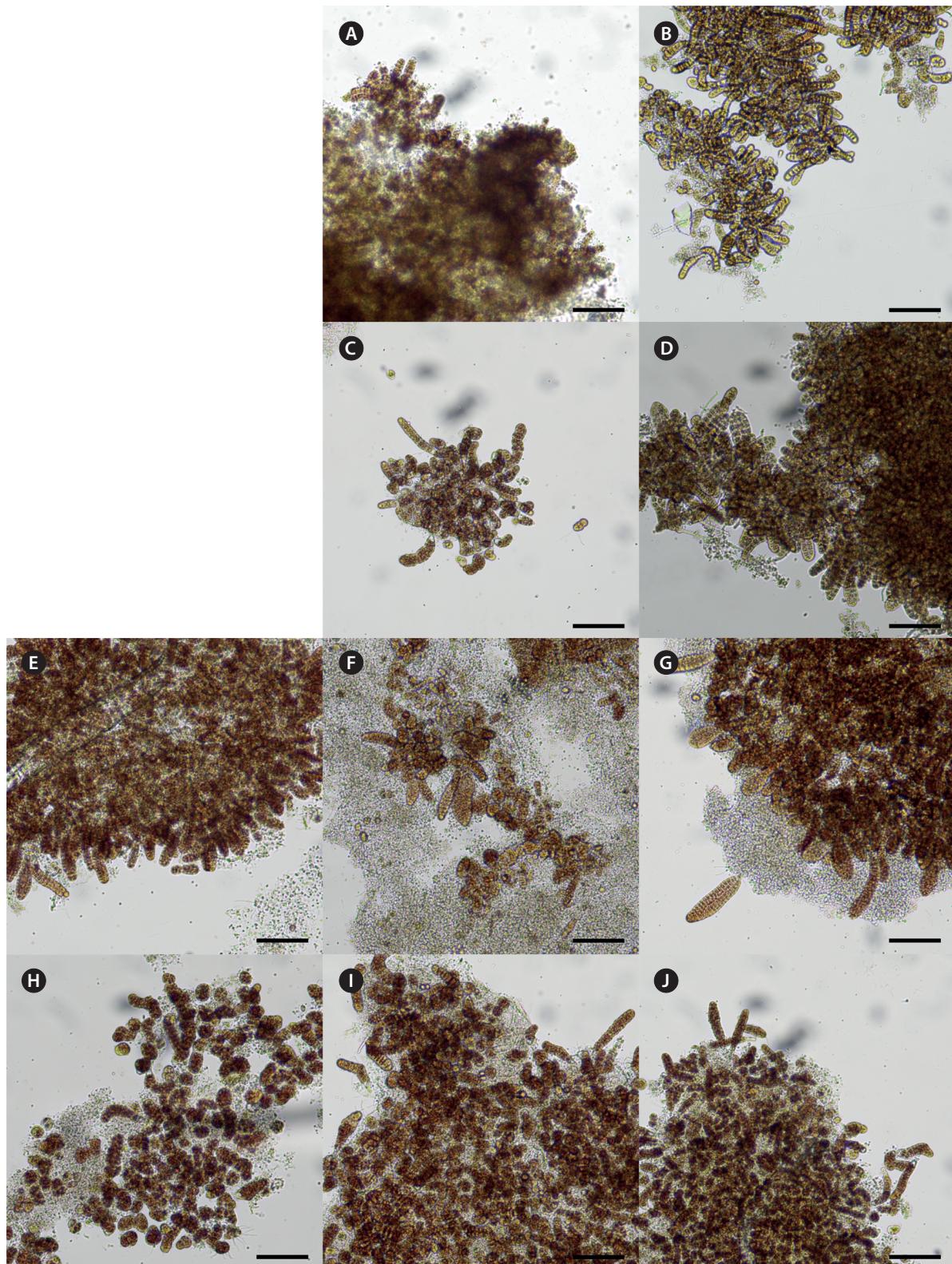


Fig. 5. Microscopic images of gametophytes of *Pyropia yezoensis* after 20 days (A–D) and 15 days (F, G, I & J) of cultivation at different Acadian marine plant extract powder (AMPEP) and temperatures. (A) 0.001 ppm of AMPEP at 10°C. (B) 1 ppm of AMPEP at 10°C. (C) 0.001 ppm of AMPEP at 15°C. (D) 1 ppm of AMPEP at 15°C. (E) Control at 20°C. (F) 0.001 ppm of AMPEP at 20°C. (G) 1 ppm of AMPEP at 20°C. (H) Control at 25°C. (I) 0.001 ppm of AMPEP at 25°C. (J) 1 ppm of AMPEP at 25°C. Scale bars represent: A–J, 100 µm.

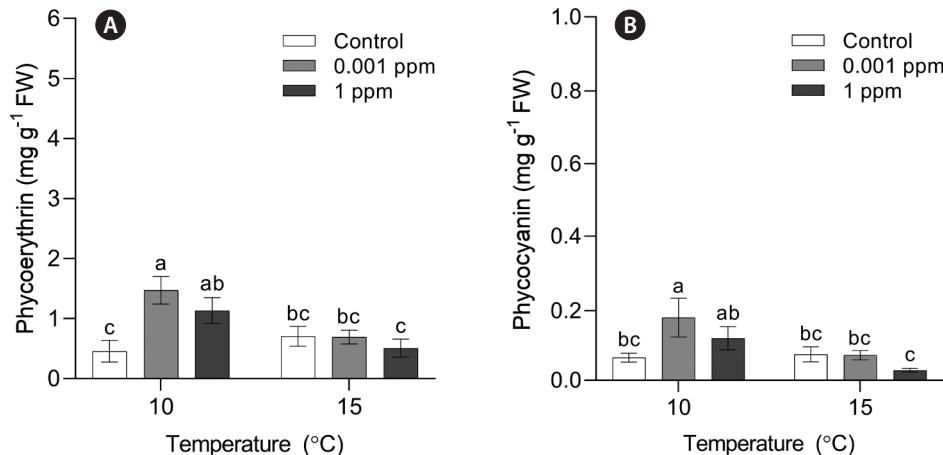


Fig. 6. Phycoerythrin (A) and phycocyanin (B) contents in *Pyropia yezoensis* at different Acadian marine plant extract powder concentrations and temperatures (10 and 15°C) at day 15. FW, fresh water. Different letters on the bars indicate significant differences ($p < 0.05$). Error bars represent mean \pm standard deviation of 9 replicates.

The SGR at control and 0.001 ppm was significantly lower at 20 than 15°C. Archeospores release was observed at 20 and 25°C regardless of the AMPEP concentrations in 15 days. At 10 and 15°C, archeospores release was also observed in AMPEP-treated conditions (Fig. 5).

Like the Kelpak experiment, PE and PC contents of *P. yezoensis* were measured at 10 and 15°C. PE content was significantly influenced by temperature ($p = 0.001$), AMPEP ($p = 0.002$), and the interaction of these two factors ($p = 0.001$) (Table 1). PE content in AMPEP-treated thalli was significantly higher than that in control at 10°C. There was no significant differences in PE content at 15°C (Fig. 6A). PC content was significantly affected by temperature ($p = 0.001$), concentration of AMPEP ($p = 0.010$) and the interaction of these two factors ($p = 0.009$) (Table 1). PC content showed a similar pattern to PE content. PE content in 0.001 ppm at 10°C was significantly higher than those in other conditions (Table 3, Fig. 6B).

DISCUSSION

Asexual reproduction (production of archeospores) occurred in *P. yezoensis* at high temperature conditions (20 and 25°C) in 15 days at all AMPEP and Kelpak concentrations. Archeospores were also observed in *P. yezoensis* treated with AMPEP in 15 days, even at 10 and 15°C, but was not observed in the samples pre-treated with Kelpak. These results suggest that AMPEP may enhance asexual reproduction in *P. yezoensis*. The formation of archeospores from *P. yezoensis* is influenced by various factors such as temperature, light intensity, wound, blade size,

and desiccation (Li 1989, Notoya et al. 1993, Suda and Mikami 2020). In the present study, higher temperature (20 and 25°C) clearly stimulated the release of archeospores. The promotion of the archeospores at higher temperature (20–24°C) was previously observed in the red alga *Bangia fuscopurpurea* (Wang et al. 2008). *Pyropia lacerata* and *Porphyra suborbiculata* also released archeospores at 20 and 25°C (Notoya et al. 1993). However, *P. yezoensis* cut to about 1 mm² formed callus cells, which are a three-dimensional cluster of cells randomly divided, without the formation of archeospores (Suda and Mikami 2020). The callus cells germinated into gametophytic thalli like those of archeospores (Suda and Mikami 2020). The present study cut the blade into 1 cm² in area, and all blades formed archeospores at higher temperatures. This result suggests that the blade size is an important factor to form archeospores.

At lower temperatures, 10 and 15°C, asexual reproduction was observed when treated with AMPEP, suggesting that AMPEP influences the asexual reproduction of *P. yezoensis*. In plants, the extract of *A. nodosum* induces higher levels of abscisic acid (ABA) and Ca²⁺ contents (Wally et al. 2013, Shukla et al. 2018). Previous studies have shown that Ca²⁺ influx and exposure to H₂O₂ and allantoin can promote archeospores release in *P. yezoensis* (Takahashi et al. 2010). H₂O₂ has shown to facilitate the influx of Ca²⁺ (Gui et al. 2022), while allantoin can increase the levels of ABA and a phytohormone (Watanabe et al. 2014). Therefore, AMPEP may help induce the release of archeospores in *P. yezoensis* through a similar mechanism.

Asexual reproduction can help maintain desirable

traits in the population, as the offspring are genetically identical to parental plants. This is beneficial for commercial cultivation for crops, as it ensures that the quality and taste remain consistent (Li 1989, Waaland et al. 1990, Chen et al. 1994). Asexual reproduction is the principal seeding for commercial *Pyropia* cultivation (Wada 1941, Li 1984). Interestingly, new blades produced via asexual reproduction, when the parental plants were treated with AMPEP. These thalli showed higher growth rates than the control without AMPEP at 10°C, which is the optimal growth temperature for this species (Umanzor et al. 2022b). These results suggest that AMPEP not only promotes asexual reproduction but also enhances the growth of new blades, which is advantageous for commercial aquaculture of *Pyropia*.

This is the first study showing that the seaweed-derived biostimulants, Kelpak and AMPEP can enhance the PE and PC contents. PE and PC are pigment-protein complexes found in certain photosynthetic organisms, particularly in cyanobacteria (blue-green algae) and red algae. These complexes play a crucial role in light harvesting during photosynthesis (Bryant 1982). Recent studies reported that Kelpak increased photosynthesis and chlorophyll fluorescence of plants (Krajnc et al. 2012, Rengasamy et al. 2016). Umanzor et al. (2020b) observed a darker color on AMPEP-treated *Saccharina* sporophytes. Similar results were also found in *Kappaphycus alvarezii* thalli at 16°C (Loureiro et al. 2014). The higher contents of PE and PC in *P. yezoensis* are indicative of the efficiency of photosynthesis. This is significant because photosynthesis is the primary process that drives growth and productivity in these seaweed (Raven et al. 2014).

The PE and PC have wide applications in food, cosmetics and pharmaceutical industries due to their natural optical and biological properties (Kim et al. 2018). The pigment contents in *P. yezoensis*, specifically the amount per unit area or weight, is crucial in determining the quality due to a strong correlation between pigment content and the quality of dried *P. yezoensis* sheets (Aruga 1984). Higher PE contents in *Pyropia* lead to higher free amino acids contents, which are known to be the most significant factors influencing the taste of *Pyropia* (Yoshie et al. 1993). Therefore, exposure to AMPEP and Kelpak have the potential to enhance the market value of *P. yezoensis* by stimulating the synthesis of PE and PC.

Pyropia yezoensis exposed to Kelpak did not reproduce at 10 and 15°C. Whereas, AMPEP-treated samples released archeospores within 15 days following a 6-day pretreatment at 1 ppm. In contrast, Han et al. (2023) reported that *P. yezoensis* didn't reproduce when exposed

to the same condition for 10 days. In *Eucheumatopsis isiformis*, a longer exposure to AMPEP (28 days at 5 ppm) resulted in a positive effect compared to a shorter exposure (45 min) or no exposure. The longer exposure produced more lateral shoots in *E. isiformis* (Umanzor et al. 2020a), indicating a pronounced stimulatory effect on lateral shoot formation by AMPEP. This result suggests that exposure time is a crucial factor determining the effects of biostimulants.

In conclusion, this study showed the potential of seaweed-derived biostimulants as a novel technology to improve *P. yezoensis* cultivation. AMPEP induced the formation and release of archeospores in *P. yezoensis* more efficiently than Kelpak, increasing production. Additionally, the use of Kelpak and AMPEP can increase PE and PC contents in this alga, resulting in higher quality products of *P. yezoensis*.

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CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

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