Kim Jang (Orcid ID: 0000-0001-7008-6768)

Exploratory evaluation of the effects of Kelpak® seaweed extract on cultivated kelp *Saccharina* spp. exposed to sublethal and lethal temperatures

Schery Umanzor

Department of Ecology & Evolutionary Biology, University of Connecticut, Stamford CT 06901-2315, USA

ORCID: 0000-0002-3812-4565

Sookkyung Shin

Department of Marine Science, Incheon National University, Incheon 22012, Korea

Research Institute of Basic Sciences, Incheon National University, Incheon 22012, Korea

*co-corresponding author: shin_78@naver.com

ORCID: 0000-0002-6087-1516

Charles Yarish

Department of Ecology & Evolutionary Biology, University of Connecticut, Stamford CT 06901-2315, USA

ORCID: 0000-0003-2683-5519

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/jwas.12687

Simona Augyte

Department of Ecology & Evolutionary Biology, University of Connecticut, Stamford CT 06901-2315, USA

ORCID: 0000-0001-7305-7532

Jang K. Kim

Department of Marine Science, Incheon National University, Incheon 22012, Korea

Research Institute of Basic Sciences, Incheon National University, Incheon 22012, Korea

*co-corresponding author: jang.kim@inu.ac.kr

Abstract

Kelpak® is a seaweed extract from the phaeophyte, *Ecklonia maxima*, used to enhance growth and production of terrestrial plants. It is also beneficial for some red and green seaweeds. As such, we assessed if Kelpak® could enhance growth and thermal tolerance of the brown seaweeds, *Saccharina latissima* and *S. angustissima*. Juvenile sporophytes were dipped (30 or 60 min) in Kelpak® solutions of different concentrations (0.001, 0.005, 0.05, 1 and 5ml L^{-1}). These sporophytes were then cultivated in half-strength PES at different temperatures (12, 16, 19, 23 and 25°C±1) for 20 days. The surviving sporophytes were then moved to 18 ±1°C and cultivated for 14 additional days. Results show that temperature was the main factor driving survival and growth. Both species exposed to the temperatures of 23 and 25°C died during the first seven days. Furthermore, no significant differences were observed in growth of sporophytes at the temperatures of 12 and 16°C. Results also show that treated sporophytes exposed to 18°C, the sublethal temperature, particularly of *S. angustissima* showed a higher survival and overall vigor than non-treated sporophytes. These results indicate that Kelpak® may enhance thermal tolerance and growth of *S. latissima* and *S. angustissima* exposed to sublethal temperatures.

Keywords: Biostimulant, Kelpak®, *Saccharina angustissima*, *Saccharina latissima*, seaweed aquaculture, thermal stress

Funding information: This work was supported by Macroalgae Research Inspiring Novel Energy Resources (MARINER, DE-FOA-0001726) to CY as a sub-award to Contracts DE-AR0000912, DE-AR0000911; and DE-AR000915. This work was also supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2017R1A6A1A06015181) and by the Ministry of Oceans and Fisheries of Korea (Project title: Improvement of management strategies on marine ecosystem disturbing and harmful organisms).

1. INTRODUCTION

Seaweed extracts have beneficial effects on terrestrial plants (Craigie, 2011; Panda et al., 2012). The extracts have been used extensively in agriculture and horticulture, mainly because the presence of growth regulators such as cytokinins and auxins enhances crop production (Khan et al., 2009; Craigie, 2011; Stirk et al., 2014). Some work has also examined the effects of seaweed extracts on seaweed crops (Robertson-Andersson et al., 2006; Hurtado et al., 2009a; Souza et al., 2018). The majority of these studies have examined the effects of seaweed extracts derived from the brown alga, *Ascophyllum nodosum* (Linnaeus) Le Jolis, on red seaweed crops such as *Kappaphycus*, *Gracilaria*, and *Laurencia* (Hurtado et al., 2009a, b; Loureiro et al., 2014; Tibubos et al., 2017; Souza et al. 2018); although extracts from different algal sources have also been applied to the green seaweed *Ulva* (Robertson-Anderson et al., 2006). In brief, these studies show that seaweed extracts significantly contribute to the overall vigor of red and green seaweed crops. Nonetheless, little is known about the effects and potential benefits of applying seaweed extracts such as Kelpak® on other economically important brown seaweed such as *Saccharina* spp.

Kelpak® (Kelp Products Pty Ltd, Simon's Town, South Africa) is a liquid concentrate derived from the stipes and laminae of the brown kelp, *Ecklonia maxima* (Osbeck) Papenfuss (van Staden et al., 1995). It is commercially used as a plant growth stimulator reducing nursery periods before out-planting and increases the yield and quality of a variety of terrestrial crops (Crouch et al., 1990; van Staden et al., 1995; Al-Hawezy, 2014; Kocira et al., 2018). It is also

known to aid producing stronger and healthier crops by enhancing root formation and reducing transplant shock resistance (Arthur et al., 2003; Bore & Ng'etich, 2007; Panda et al., 2012). Robertson-Andersson et al. (2006) carried out one of the few experiments assessing the effects of Kelpak® at different concentrations on the growth of *Gracilaria* and *Ulva.* They found that Kelpak® increased growth in both species and the optimal concentration of Kelpak® differed between species. Similar to other seaweed extracts, the beneficial effects of applying this product have been attributed to the presence of auxins and cytokinins and not necessarily to its nutritional content (Crouch & Van Staden, 1993; Robertson-Andersson et al., 2006). In fact, the effects of Kelpak® on *Gracilaria* and *Ulva* were tested using nutrient-enriched seawater as control media reducing the chances of a confounding effect. This approach of adding Kelpak® as complementary to enriched media highlights the feasibility of applying the product to other seaweed crops while growing in the nursery. The cultivation of *Saccharina* is typically undertaken at sea following an initial stage in the nursery where small sporophytes are produced in laboratory facilities (Flavin et al., 2013; Su et al., 2017). This phase is laborious and usually extends from four to six weeks in which resources are allocated to monitor and maintain adequate water quality, temperature, light, and nutrient levels (Flavin et al., 2013; Redmond et al., 2014). In North America and Europe, the kelp, *Saccharina latissima* (Linnaeus) C.E. Lane, C. Mayes, Druehl & G.W. Saunders, has been cultivated at commercial scales showing potential for larger off-shore seaweed aquaculture operations (Peteiro et al., 2014; Kim et al., 2015; Azevedo et al., 2016; Bak et al., 2018; Augyte et al., 2018). Therefore, reducing nursery periods

before deployment at sea could contribute to alleviating costs associated with the initial stages of *Saccharina* aquaculture, since labor is expensive in Western countries. Here we report on the effects of Kelpak® to enhance growth and thermal tolerance of *S. latissima* and *S. angustissima* (Collins), a kelp endemic to the Gulf of Maine (Augyte et al., 2017) ,while in the nursery.

2. MATERIALS AND METHODS

Two consecutive experiments were carried out to determine the effect of Kelpak® on growth and thermal tolerance of juvenile sporophytes (1 cm in length, 21 days old) of *S. latissima* and *S. angustissima*. Sporophytes were produced by crossing unisexual gametophytes isolated and cultivated in laboratory conditions from parental sporophytes collected in April and May, 2018 from Cape Cod Canal (41° 46' 25.7448" N; 70° 29' 58.0128" W) for *S. latissima* (SL18-UCONN-CC) and Casco Bay (43° 42' 42.48" N; 70° 11' 16.97" W) in the Gulf of Maine for *S. angustissima* (SA18-UCONN-CB).

2.1 Experimental design

A 10% stock solution of Kelpak® was prepared using filtered (1 µm) and sterilized seawater. The solution was then filtered through a 35-µm sieve to remove undisolved particles contained in the Kelpak® product. The manufacturer recommends applying Kelpak® maintaining the media solution at pH 7 to promote an active absorption. However, we used seawater with a pH of 8.1 to prepare the solutions that could affect the bioavailability of the product. We then prepared five

dilutions (0.001, 0.005, 0.05, 1 and 5 ml L^{-1}) and a control solution consisting of filtered and sterilized seawater (30 ppt) only, each one with a final volume of 100 ml. These Kelpak® concentrations were selected based on preliminary trials on *S. latissima* and *S. angustissima* and on previous work on *Ulva* and *Gracilaria* (see Robertson-Anderson et al 2006), which showed effects even when Kelpak® is used at concentrations lower and higher than that recommended by the manufacturer (see https://www.kelpakusa.com/support.html). Each Kelpak® dilution plus the control were divided equally into 25-ml beakers. Each one of the solutions was then used to dip either juvenile sporophytes of *S. latissima* or *S. angustissima*, where they remained for either 30 or 60 minutes (Fig. S1). After the completion of each dipping period, juvenile sporophytes were rinsed with seawater and two blades (pseudo-replicates) per treatment (Kelpak® concentration x dipping time) were transferred to individual wells within the first two rows of fifteen 24-well plates. All of the wells in these plates (four rows total) were filled with 10 ml of nutrient-enriched seawater (half-strength Provasoli Enriched Seawater (PES), Provasoli, 1968) plus germanium dioxide (GeO_2) to prevent the development of diatoms (Lewis, 1966).

Subsequently, each pair of blades (n = 3) was allowed to grow for 20 days at one of five temperature treatments (12, 16, 19, 23 and 25°C ±1; Fig. S1) using a temperature gradient table (Yarish et al., 1979). The fourth row in each plate was used to determine at which temperature 50% of additional non-treated blades died (LT_{50}). Light was maintained at 90 ± 10 µM photons m^{-2} s^{-1} PAR, with a photoperiod of 12:12 L:D.

Every five days, the juvenile sporophytes were photographed with a PixeLINK® camera (API Control, Barrington, NJ) mounted onto an Olympus (SZH, Tokyo, Japan) stereoscope. Each pair of blades was gently transferred from the wells to a microscope slide until an image was taken. Photographs were processed as black and white images to calculate blade area using Fiji by Image J (Schindelin et al., 2012). We used blade area as a surrogate to measure growth. This allowed us to evaluate possible differences in sporophyte growth as a function of Kelpak® concentration, dipping time, temperature or their interactions over time. We conducted seawater media changes every five days to avoid nutrient limitation.

After the 20 days described above, we transferred the surviving sporophytes to 75-ml Erlenmeyer flasks containing nutrient-enriched seawater (half-strength PES plus GeO_2) and gentle aeration. Blades were separated based on the concentration of Kelpak® and temperature that they were originally exposed to. Subsequently, the blades were allowed to grow for 14 days at 18˚C (LT_{50} temperature) in a Hotpak incubator (model 352632, US states). Immediately after the 14 days, the sporophytes were photographed, and the final area per blade was calculated.

For both experiments, the specific growth rate (SGR, expressed as percent increase day^{-1}) of the juvenile sporophytes was calculated as:

$$SGR = \frac{Ln(A2) - Ln(A1)}{T2 - T1}\ x\ 100$$

where A1 and A2 are the area (mm^2) at time T1 and T2, respectively (Kim et al., 2009). We used the Student T-test to analyze differences in survival as a function of species between *S. latissima* and *S. angustissima* growing at 18°C. We also used one-way ANOVAs to analyze differences in

the SGR as a function of either the concentration of Kelpak® or temperature. The interaction between both factors was not assessed due to limitations in the number of survivors.

3. RESULTS

Early juvenile sporophytes of *S. latissima* and *S. angustissima* showed the highest specific growth rates (SGR) at Day 5 post-treatment with Kelpak® (Fig 1a, d, g and Fig. 2a, d, g). Differences in SGR appear to be driven by temperature only, with no clear effect as a result of the concentration of Kelpak® nor dipping time ($p < 0.05$; Fig.1 and Fig. 2). On average, *S. angustissima* showed a higher SGR than *S. latissima* across temperature treatments and time (Fig. 1 and Fig. 2). Regardless of the concentration of Kelpak®, dipping time and species, there was an increase in blade bleaching and blade degradation in sporophytes exposed to 19°C, while a 100% mortality was recorded during the first seven days at temperatures of 23 and 25°C. High mortality, particularly after Day 10 at 19°C or higher, coupled with the limitation in the number of replicates, precluded further statistical analysis.

On the other hand, there was a differential response based on species, both on survival (Fig. 3) and growth (Fig. 4), after juvenile sporophytes were exposed to 18˚C. Survival of juvenile sporophytes of *S. angustissima* was significantly higher than that of *S. latissima* ($p < 0.005$; Fig. 3). *S. angustissima* exhibited survivals of up to 100% at high concentration treatments (i.e., 1 and 5 ml L^{-1}). Moreover, sporophytes of *S. latissima* previously exposed to 5 ml L^{-1} of Kelpak® showed significantly lower SGR than any other concentration treatment (Tukey $p < 0.05$; Fig.

4a), while those previously growing at 16°C also showed limited growth (Tukey $p < 0.05$; Fig. 4a).

On the contrary, we could not detect any effect of the concentration of Kelpak® on the SGR of *S. angustissima*, but we did detect a significant effect of temperature ($p < 0.001$). Sporophytes of *S. angustissima* previously exposed to 19°C and then moved to 18°C showed significantly higher SGR than any other temperature treatment regardless of the concentration of Kelpak® (Tukey $p < 0.001$; Fig. 4b), even for the only non-treated blade (control) transferred to 18°C (Fig. 4b).

By the end of the second experiment (i.e., 14 days of cultivation at 18˚C), we also observed that for both species, non-treated blades looked degraded, with some of them showing degraded borders. Despite their survival and growth, non-treated blades did not appear as vigorous as treated blades, particularly for *S. angustissima* at the higher concentration treatments (Fig. S2).

4. DISCUSSION

Seaweed commercial extracts are gaining momentum as enhancing agents to improve growth and overall vigor on a variety of seaweed mariculture crops (Hurtado et al., 2009a; Tibubos et al., 2017; Souza et al., 2018). Kelpak® has been used to improve the performance of *Gracilaria* and *Ulva* spp., showing a beneficial effect on their growth (Robertson-Anderson et al., 2006). To our knowledge, this is the first study applying a kelp-derived (i.e., *E. maxima*) biostimulant to

other non-related kelp mariculture crops (*S. latissima* and *S. angustissima*). Similar to our results, experiments assessing the effects of seaweed extracts, including Kelpak®, on terrestrial plants indicate that the extracts enhance growth of individuals even when subject to adverse environmental conditions, such as nutrient limitations, suboptimal temperatures, or pests (Verkleij, 1992; Zhang & Ervin, 2008; Hurtado et al., 2015; Ali et al., 2018).

Studies conducted on seaweed crops report that the optimum concentration of the extract may vary as a function of the seaweed species or even strain that is being treated (Hurtado et al., 2012; Robertson-Andersson et al., 2006). Experiments assessing the effects of Kelpak® on *Ulva* and *Gracilaria* growing in ideal temperature conditions described improvements on their specific growth rate two weeks post-treatment (Robertson-Andersson et al., 2006). On the contrary, our study did not show significant differences in blades growing at the ideal temperatures of 12-16 °C. Neither showed any enhancement that could allow blades to survive to lethal temperatures above 19 °C. The optimal growth temperature for *Saccharina* ranges from 10°C to 15°C with poor growth typically observed above 16°C, and total degradation above 21°C (Fortes and Lüning 1980, Bolton and Lüning 1982, Andersen et al., 2013, Borlongan et al., 2019, Augyte, 2019). However, results did show that blades treated with Kelpak® and transferred to the sub-lethal temperature of 18°C had a relatively higher survival and SGR than non-treated blades. This could be indicative that Kelpak® could provide enhancements in temperature tolerance up to a given threshold. Moreover, in our study, the Kelpak® concentration of 0.005 ml L^{-1} appeared to be optimal to enhance sublethal temperature tolerance and growth of *S. latissima,* while for *S.*

angustissima, both 0.005 and 5 ml L^{-1} showed similar results. These differences in the optimum concentration for both species further confirm the relevance of determining the ideal concentration of seaweed extracts before applying seaweed extracts as enhancing agents for seaweed crops.

Seaweed extracts provide a wide range of responses on treated individuals (Crouch & van Staden, 1993), suggesting the presence of more than one type of stimulant depending on the algal source (Craigie, 2011). Seaweed extracts contain high levels of phytohormones that influence the cellular metabolism of treated individuals, leading in part to an increased growth through cell division and elongation. (Verkleij, 1992; Khan et al., 2009; Hurd et al., 2014). Similar to terrestrial crops, Kelpak® may induce the production of endogenous cytokinins and bioactive compounds (Aremu et al., 2016) in *Saccharina*. Cytokinins influence several aspects of plant growth and development, including cell division, nutrient uptake, as well as the response to biotic and abiotic factors (Kieber & Schaller, 2014). Such roles could explain the differences in blade vigor observed between treated and non-treated blades when exposed to 18°C for 14 days.

Altogether, our outcomes highlight the importance of optimizing application methods based on the seaweed-extract and seaweed-crop system. In conclusion, our data provide evidence of the potential of Kelpak® as a useful biostimulant to enhance juvenile sporophytes of *Saccharina* while in the nursery. It also provides insights into practical applications to enhance the thermal tolerance of *Saccharina* before deployment at sea, which could result in a competitive advantage when compared to non-treated blades.

Acknowledgments

We thank D. Bailey and S. Lindell for their support in collecting the parental sporophytes.

References

Al-Hawezy, S. M. N. (2014). The Use of Kelpak to Seedlings Loquat (*Eriobotya Jappanica L*.). *International Journal of Scientific and Research Publications*, 4, 85–88.

Ali, M. K. M., Yasir, S. M., Critchley, A. T., & Hurtado, A. Q. (2018). Impacts of *Ascophyllum* marine plant extract powder (AMPEP) on the growth, incidence of the endophyte *Neosiphonia apiculata* and associated carrageenan quality of three commercial cultivars of *Kappaphycus*. *Journal of Applied Phycology*, 30, 1185–1195. https://doi.org/10.1007/s10811-017-1312-2

Aremu, A. O., Plačková, L., Gruz, J., Bíba, O., Novák, O., Stirk, W. A., Doležal, K., & Van Staden, J. (2016). Seaweed-Derived Biostimulant (Kelpak®) Influences Endogenous Cytokinins and Bioactive Compounds in Hydroponically Grown *Eucomis autumnalis*. *Journal of Plant Growth Regulation*, 35(1), 151–62. https://doi.org/10.1007/s00344-015-9515-8

Arthur, G. D., Stirk, W. A., van Staden, J., & Scott, P. (2003). Effect of a seaweed concentrate on the growth and yield of three varieties of *Capsicum annuum*. *South African Journal of*

Botany, 69(2), 207–211. https://doi.org/10.1016/s0254-6299(15)30348-3

Augyte, S., Yarish, C., Redmond, S., & Kim, J. K. (2017). Cultivation of a morphologically distinct strain of the sugar kelp, *Saccharina latissima* forma *angustissima*, from coastal Maine, USA, with implications for ecosystem services. *Journal of Applied Phycology*, 29(4), 1967–1976. https://doi.org/10.1007/s10811-017-1102-x

Augyte, S., Umanzor, U., Yarish, C., & Lindell, S. (2018). Enhancing marine ecosystem services via kelp aquaculture. Pages 10-14 in S. Nayar, editor. International Society for Applied Phycology Newslatters 2018 December Issue. https://www.appliedphycologysoc.org/

Azevedo, I. C., Marinho, G. S., Silva, D. M., & Sousa-Pinto, I. (2016). Pilot scale land-based cultivation of *Saccharina latissima* Linnaeus at southern European climate conditions: Growth and nutrient uptake at high temperatures. *Aquaculture*, 459, 166–172. https://doi.org/10.1016/j.aquaculture.2016.03.038

Bak, U. G., Mols-Mortensen, A., & Gregersen, O. (2018). Production method and cost of commercial-scale offshore cultivation of kelp in the Faroe Islands using multiple partial harvesting. *Algal Research*, 33, 36–47. https://doi.org/10.1016/j.algal.2018.05.001

Bore, J. K. & Ng'etich W. K. (2007). Influence of kelp based plant growth stimulants on nursery tea seedlings. *Tea*, 28, 3–7.

Craigie, J. S. (2010). Seaweed extract stimuli in plant science and agriculture. *Journal of Applied Phycology*, 23(3), 371–393. https://doi.org/10.1007/s10811-010-9560-4

Crouch, I. J., & Van Staden, J. (1993). Commercial Seaweed Products as Biostimulants in Horticulture. *Journal of Home & Consumer Horticulture*, 1(1), 19–76. https://doi.org/10.1300/j280v01n01_03

Crouch, I. J., Beckett, R. P., & van Staden, J. (1990). Effect of seaweed concentrate on the growth and mineral nutrition of nutrient-stressed lettuce. *Journal of Applied Phycology*, 2(3), 269–272. https://doi.org/10.1007/bf02179784

Flavin, K., Flavin, N., & Flahive, B. (2013). Kelp Farming Manual. A guide to the processes, tecniques, and equipment for farming kelp in New England waters. Portland, Maine

Hurd, C. L., Harrison, P. J., Bischof, K., & Lobban, C. S. (2014). Seaweed Ecology and Physiology, 2nd edn. Cambridge University Press, Cambridge, UK

Hurtado, A. Q., Critchley, A. T., Trespoey, A., & Bleicher-Lhonneur, G. (2009a). Growth and carrageenan quality of *Kappaphycus striatum* var. sacol grown at different stocking densities, duration of culture and depth. In M. A. Borowitzka, A. T. Critchley, S. Kraan (Eds.), Nineteenth International Seaweed Symposium (pp. 101–105). Springer, Netherlands, Dordrecht

Hurtado, A. Q., Neish, I. C., & Critchley, A. T. (2015). Developments in production technology of Kappaphycus in the Philippines: more than four decades of farming. *Journal of Applied Phycology*, 27(5), 1945–1961. https://doi.org/10.1007/s10811-014-0510-4

Hurtado AQ, Yunque DA, Tibubos K, Critchley AT (2009b) Use of Acadian marine plant

extract powder from Ascophyllum nodosum in tissue culture of *Kappaphycus* varieties. J Appl Phycol 21:633–639. doi: 10.1007/s10811-008-9395-4

Hurtado, A. Q., Joe, M., Sanares, R. C., Fan, D., Prithiviraj, B., & Critchley, A. T. (2012). Investigation of the application of Acadian Marine Plant Extract Powder (AMPEP) to enhance the growth, phenolic content, free radical scavenging, and iron chelating activities of *Kappaphycus* Doty (Solieriaceae, Gigartinales, Rhodophyta). *Journal of Applied Phycology*, 24(3), 601–611. https://doi.org/10.1007/s10811-011-9785-x

Khan, W., Rayirath, U. P., Subramanian, S., Jithesh, M. N., Rayorath, P., Hodges, D. M., Critchley, A. T., Craigie, J. S., Norrie, J., & Prithiviraj, B. (2009). Seaweed Extracts as Biostimulants of Plant Growth and Development. *Journal of Plant Growth Regulation*, 28(4), 386–399. https://doi.org/10.1007/s00344-009-9103-x

Kieber, J. J., & Schaller, G. E. (2014). Cytokinins. *The Arabidopsis Book*, 12, e0168. https://doi.org/10.1199/tab.0168

Kim, J. K., Kraemer, G. P., & Yarish, C. (2009). Research note: Comparison of growth and nitrate uptake by New England *Porphyra* species from different tidal elevations in relation to desiccation. *Phycological Research*, 57(2), 152–157. https://doi.org/10.1111/j.1440-1835.2009.00533.x

Kim, J. K., Kraemer, G. P., & Yarish, C. (2015). Use of sugar kelp aquaculture in Long Island Sound and the Bronx River Estuary for nutrient extraction. *Marine Ecology Progress*

Series, 531, 155-166

Kocira, A., Świeca, M., Kocira, S., Złotek, U., & Jakubczyk, A. (2018). Enhancement of yield, nutritional and nutraceutical properties of two common bean cultivars following the application of seaweed extract (*Ecklonia maxima*). *Saudi Journal of Biological Sciences*, 25(3), 563–571. https://doi.org/10.1016/j.sjbs.2016.01.039

Lewis, J. (1966). Silicon metabolism in diatoms. V. Germanium dioxide, a specific inhibitor of diatom growth. *Phycologia*, 6, 112.

Loureiro, R. R., Reis, R. P., Berrogain, F. D., & Critchley, A. T. (2013). Effects of a commercial extract of the brown alga *Ascophyllum nodosum* on the biomass production of *Kappaphycus alvarezii* (Doty) Doty ex P. C. Silva and its carrageenan yield and gel quality cultivated in Brazil. *Journal of Applied Phycology*, 26(2), 763–768. https://doi.org/10.1007/s10811-013-0210-5

Panda, D., Pramanik, K., & Nayak, B. R. (2012). Use of Sea Weed Extracts as Plant Growth Regulators for Sustainable Agriculture. *International Journal of Bio-Resource & Stress Management*, 3, 404–411.

Peteiro, C., Sánchez, N., Dueñas-Liaño, C., & Martínez, B. (2014). Open-sea cultivation by transplanting young fronds of the kelp *Saccharina latissima*. *Journal of Applied Phycology*, 26, 519–528. doi: 10.1007/s10811-013-0096-2

Redmond, S., Green, L., Yarish, C., Kim, J. K., & Neefus, C. (2014). New England Seaweed

Culture Handbook. Connecticut Sea Grant, Groton, CT

Robertson-Andersson, D. V., Leitao, D., Bolton, J. J., Anderson, R. J., Njobeni, A., & Ruck, K. (2006). Can Kelp Extract (KELPAK®) be Useful in Seaweed Mariculture? *Journal of Applied Phycology*, 18(3–5), 315–321. https://doi.org/10.1007/s10811-006-9030-1

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, T., Rueden, C. et al. (2012). Fiji: an open-source platform for biological-image analysis. *Nature Methods*, 9(7), 676–682. https://doi.org/10.1038/nmeth.2019

Souza, J. M. C., Castro, J. Z., Critchley, A. T., & Yokoya, N. S. (2018). Physiological responses of the red algae *Gracilaria caudata* (Gracilariales) and *Laurencia catarinensis* (Ceramiales) following treatment with a commercial extract of the brown alga *Ascophyllum nodosum* (AMPEP). *Journal of Applied Phycology*, 31(3), 1883–1888. https://doi.org/10.1007/s10811-018-1683-z

Stirk, W. A., Tarkowská, D., Turečová, V., Strnad, M., & van Staden, J. (2013). Abscisic acid, gibberellins and brassinosteroids in Kelpak®, a commercial seaweed extract made from *Ecklonia maxima*. *Journal of Applied Phycology*, 26(1), 561–567. https://doi.org/10.1007/s10811-013-0062-z

Su, L., Pang, S. J., Shan, T. F., & Li, X. (2017). Large-scale hatchery of the kelp *Saccharina japonica*: a case study experience at Lvshun in northern China. *Journal of Applied Phycology*, 29(6), 3003–3013. https://doi.org/10.1007/s10811-017-1154-y

Tibubos, K. R., Hurtado, A. Q., & Critchley, A. T. (2016). Direct formation of axes in new plantlets of *Kappaphycus alvarezii* (Doty) Doty, as influenced by the use of AMPEP K^+, spindle inhibitors, and plant growth hormones. *Journal of Applied Phycology*, 29(5), 2345–2349. https://doi.org/10.1007/s10811-016-0988-z

van Staden, J., Beckett, R. P., & Rijkenberg, M. J. (1995). Effect of seaweed concentrate on the growth of the seedlings of three species of *Eucalyptus*. *South African Journal of Botany*, 61(4), 169–172. https://doi.org/10.1016/s0254-6299(15)30513-5

Verkleij, F. N. (1992). Seaweed Extracts in Agriculture and Horticulture: a Review. *Biological Agriculture & Horticulture*, 8(4), 309–324. https://doi.org/10.1080/01448765.1992.9754608

Yarish, C., Lee, K. W., & Edwards, P. (1979). An improved apparatus for the culture of algae under varying regimes of temperature and light intensity. Botanica Marina, 22, 395–397.

Zhang, X., & Ervin, E. H. (2008). Impact of Seaweed Extract-Based Cytokinins and Zeatin Riboside on Creeping Bentgrass Heat Tolerance. *Crop Science*, 48(1), 364. https://doi.org/10.2135/cropsci2007.05.0262

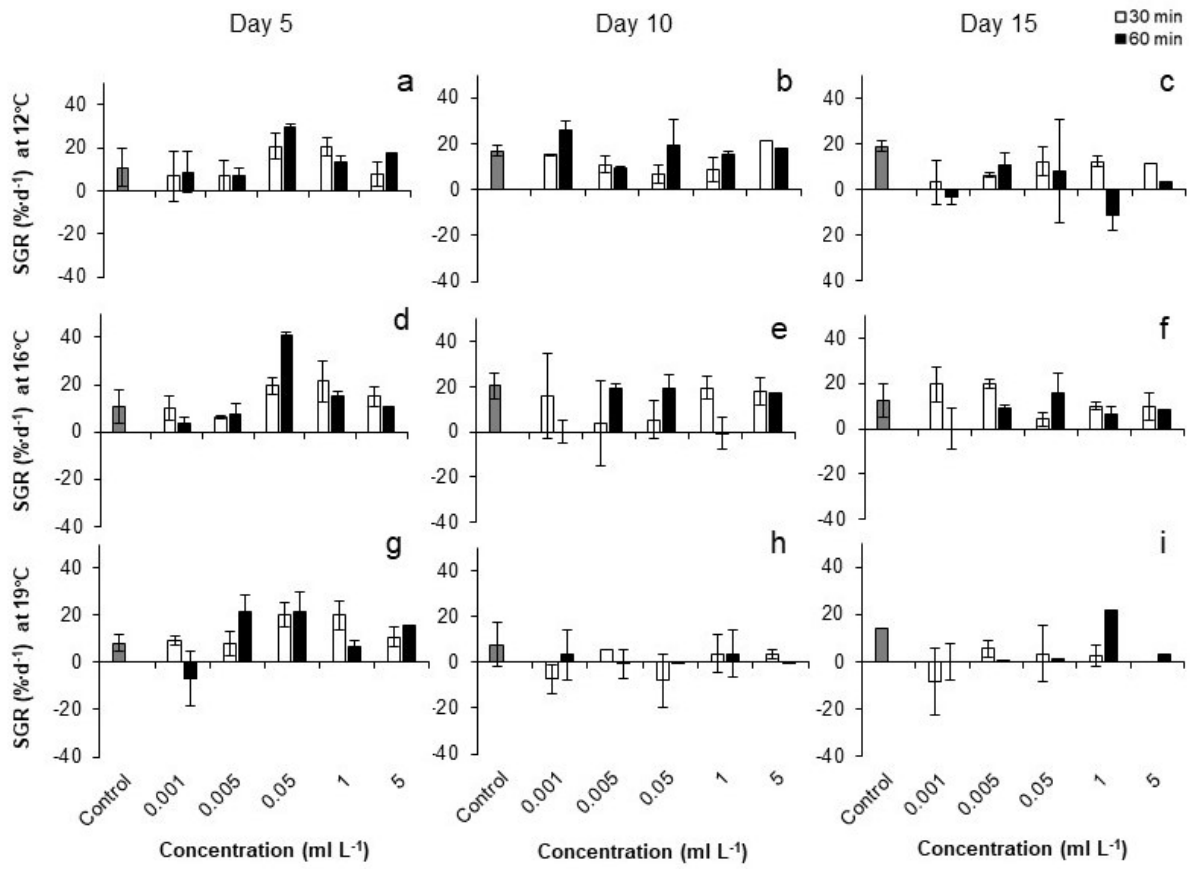

FIGURE 1 Specific growth rates (mean values ± SE, n = 3) of *Saccharina latissima* as a function of five post treatments (0.001, 0.005, 0.05, 1 and 5 ml L^{-1}) with Kelpak®, dipping time (30 min or 60 min), and temperature treatments (12, 16, and 19 °C) over 20 days. The absence of error bars indicates only one survivor per treatment.

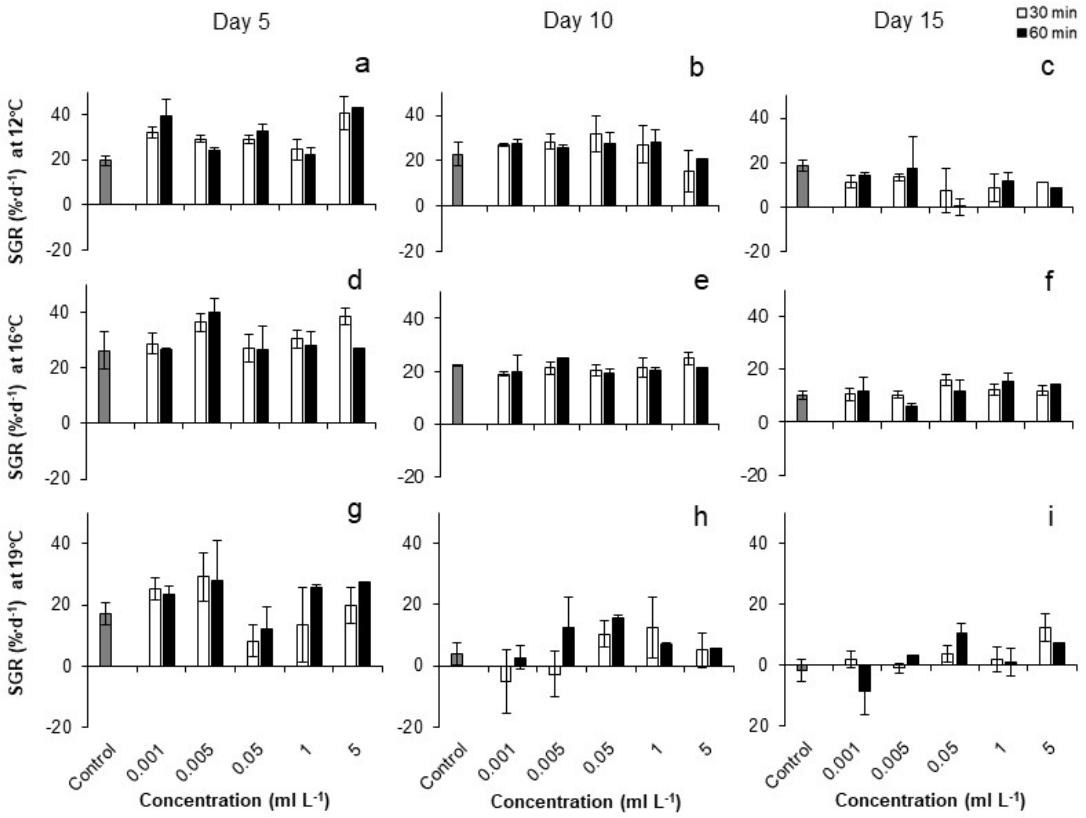

FIGURE 2 Specific growth rates (mean values ± SE, *n* = 3) of *Saccharina angustissima* as a function of five post treatments (0.001, 0.005, 0.05, 1 and 5 ml L^{-1}), with Kelpak® dipping time (30 min or 60 min), and temperature treatments (12, 16, and 19 °C) over 20 days. The absence of error bars indicates only one survivor per treatment.

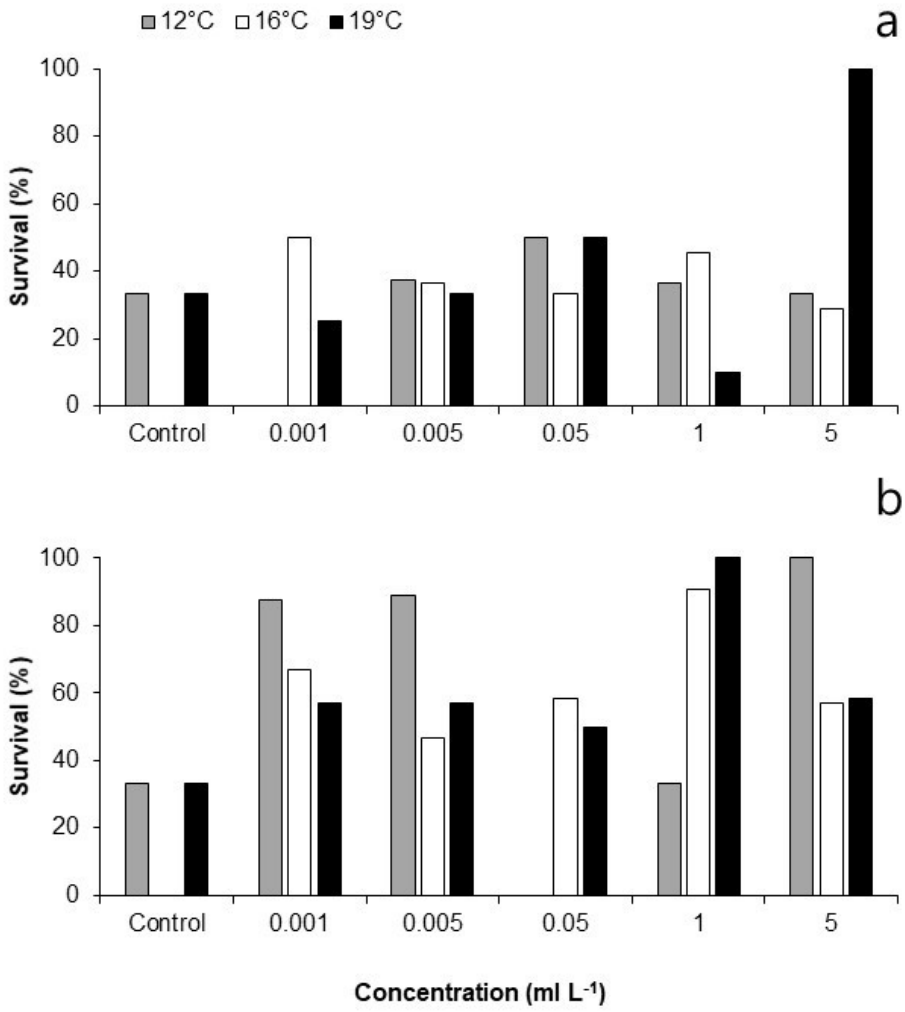

FIGURE 3 Percentage of survival of early juvenile sporophytes *Saccharina latissima* (a) and *S. angustissima* (b) growing at 18°C after exposure to different Kelpak® solutions and temperature treatments.

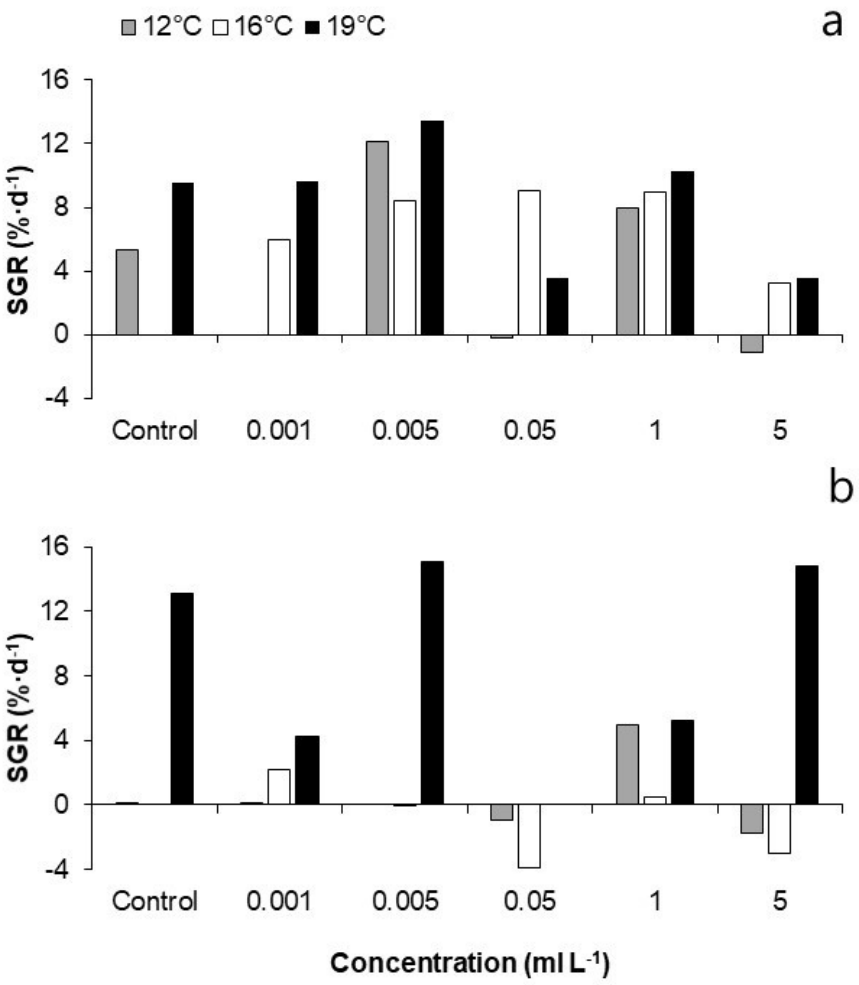

FIGURE 4 Average specific growth rate of *Saccharina latissima* (a) and *S. angustissima* (b) growing at 18°C after exposure to different Kelpak® solutions and temperature treatments.

TABLE 1 Number of surviving sporophytes transferred to 18°C for 14 days as a function of temperature and Kelpak® concentration (ml L^{-1}).

	Temperature (˚C)		
	12	**16**	**19**
Saccharina latissima			
Control	3	3	3
0.001 ml L^{-1}	8	4	4
0.005 ml L^{-1}	8	11	9
0.05 ml L^{-1}	10	6	4
1 ml L^{-1}	11	11	10
5 ml L^{-1}	6	7	2
Saccharina angustissima			
Control	3	2	3
0.001 ml L^{-1}	8	12	7

0.005 ml L^{-1}	9	15	7
0.05 ml L^{-1}	7	12	6
1 ml L^{-1}	12	11	4
5 ml L^{-1}	8	14	12

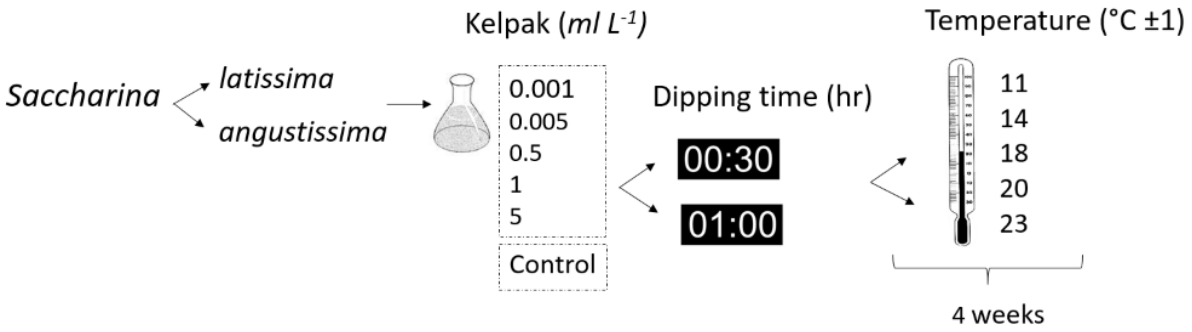

FIGURE S1 The experimental design consisted of testing the effect of Kelpak® in growth and thermal tolerance of *Saccharina latissima* and *S. angustissima* as a function of concentration, dipping time, and temperature.

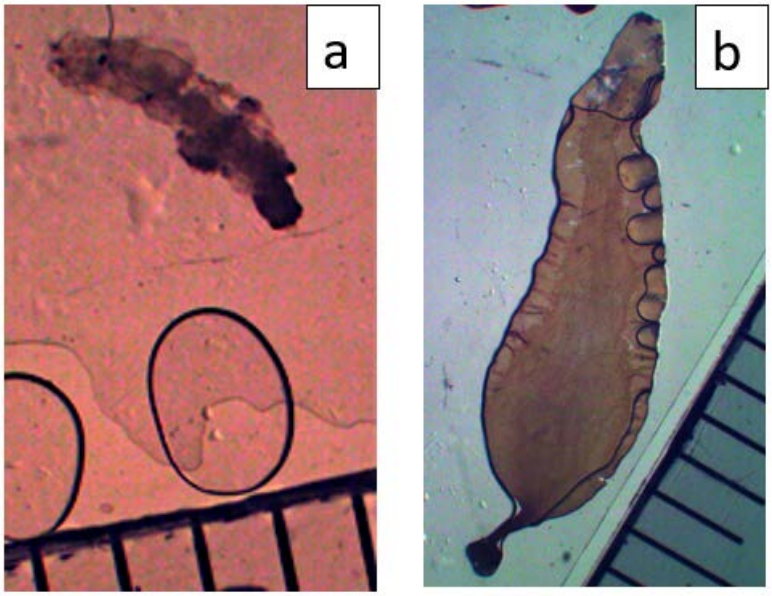

FIGURE S2 A) Non-treated and B) treated blade of *Saccharina angustissima* exposed to 18°C for a 14 day period.

Exploratory evaluation of the effects of Kelpak® seaweed extract on cultivated kelp *Saccharina* spp. exposed to sublethal and lethal temperatures

Schery Umanzor

Department of Ecology & Evolutionary Biology, University of Connecticut, Stamford CT 06901-2315, USA

ORCID: 0000-0002-3812-4565

Sookkyung Shin

Department of Marine Science, Incheon National University, Incheon 22012, Korea

Research Institute of Basic Sciences, Incheon National University, Incheon 22012, Korea

*co-corresponding author: shin_78@naver.com

ORCID: 0000-0002-6087-1516

Charles Yarish

Department of Ecology & Evolutionary Biology, University of Connecticut, Stamford CT 06901-2315, USA

ORCID: 0000-0003-2683-5519

Simona Augyte

Department of Ecology & Evolutionary Biology, University of Connecticut, Stamford CT 06901-2315, USA

ORCID: 0000-0001-7305-7532

Jang K. Kim

Department of Marine Science, Incheon National University, Incheon 22012, Korea

Research Institute of Basic Sciences, Incheon National University, Incheon 22012, Korea

*co-corresponding author: jang.kim@inu.ac.kr

Abstract

Kelpak® is a seaweed extract from the phaeophyte, *Ecklonia maxima*, used to enhance growth and production of terrestrial plants. It is also beneficial for some red and green seaweeds. As such, we assessed if Kelpak® could enhance growth and thermal tolerance of the brown seaweeds, *Saccharina latissima* and *S. angustissima*. Juvenile sporophytes were dipped (30 or 60 min) in Kelpak® solutions of different concentrations (0.001, 0.005, 0.05, 1 and 5ml L^{-1}). These sporophytes were then cultivated in half-strength PES at different temperatures (12, 16, 19, 23 and 25°C±1) for 20 days. The surviving sporophytes were then moved to 18 ±1°C and cultivated for 14 additional days. Results show that temperature was the main factor driving survival and growth. Both species exposed to the temperatures of 23 and 25°C died during the first seven days. Furthermore, no significant differences were observed in growth of sporophytes at the temperatures of 12 and 16°C. Results also show that treated sporophytes exposed to 18°C, the sublethal temperature, particularly of *S. angustissima* showed a higher survival and overall vigor than non-treated sporophytes. These results indicate that Kelpak® may enhance thermal tolerance and growth of *S. latissima* and *S. angustissima* exposed to sublethal temperatures.

Keywords: Biostimulant, Kelpak®, *Saccharina angustissima*, *Saccharina latissima*, seaweed aquaculture, thermal stress

Funding information: This work was supported by Macroalgae Research Inspiring Novel Energy Resources (MARINER, DE-FOA-0001726) to CY as a sub-award to Contracts DE-AR0000912, DE-AR0000911; and DE-AR000915. This work was also supported by the Basic

Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2017R1A6A1A06015181) and by the Ministry of Oceans and Fisheries of Korea (Project title: Improvement of management strategies on marine ecosystem disturbing and harmful organisms).

1. INTRODUCTION

Seaweed extracts have beneficial effects on terrestrial plants (Craigie, 2011; Panda et al., 2012). The extracts have been used extensively in agriculture and horticulture, mainly because the presence of growth regulators such as cytokinins and auxins enhances crop production (Khan et al., 2009; Craigie, 2011; Stirk et al., 2014). Some work has also examined the effects of seaweed extracts on seaweed crops (Robertson-Andersson et al., 2006; Hurtado et al., 2009a; Souza et al., 2018). The majority of these studies have examined the effects of seaweed extracts derived from the brown alga, *Ascophyllum nodosum* (Linnaeus) Le Jolis, on red seaweed crops such as *Kappaphycus*, *Gracilaria*, and *Laurencia* (Hurtado et al., 2009a, b; Loureiro et al., 2014; Tibubos et al., 2017; Souza et al. 2018); although extracts from different algal sources have also been applied to the green seaweed *Ulva* (Robertson-Anderson et al., 2006). In brief, these studies show that seaweed extracts significantly contribute to the overall vigor of red and green seaweed crops. Nonetheless, little is known about the effects and potential benefits of applying seaweed extracts such as Kelpak® on other economically important brown seaweed such as *Saccharina* spp.

Kelpak® (Kelp Products Pty Ltd, Simon's Town, South Africa) is a liquid concentrate derived from the stipes and laminae of the brown kelp, *Ecklonia maxima* (Osbeck) Papenfuss (van Staden et al., 1995). It is commercially used as a plant growth stimulator reducing nursery periods before outplanting and increases the yield and quality of a variety of terrestrial crops (Crouch et al., 1990; van Staden et al., 1995; Al-Hawezy, 2014; Kocira et al., 2018). It is also known to aid producing stronger and healthier crops by enhancing root formation and reducing transplant shock resistance (Arthur et al., 2003; Bore & Ng'etich, 2007; Panda et al., 2012). Robertson-Andersson et al. (2006) carried out one of the few experiments assessing the effects of

Kelpak® at different concentrations on the growth of *Gracilaria* and *Ulva.* They found that Kelpak® increased growth in both species and the optimal concentration of Kelpak® differed between species. Similar to other seaweed extracts, the beneficial effects of applying this product has been attributed to the presence of auxins and cytokinins and not necessarily to its nutritional content (Crouch & Van Staden, 1993; Robertson-Andersson et al., 2006). In fact, the effects of Kelpak® on *Gracilaria* and *Ulva* were tested using nutrient-enriched seawater as control media reducing the chances of a confounding effect. This approach of adding Kelpak® as complementary to enriched media highlights the feasibility of applying the product to other seaweed crops while growing in the nursery. The cultivation of *Saccharina* is typically undertaken at sea following an initial stage in the nursery where small sporophytes are produced in laboratory facilities (Flavin et al., 2013; Su et al., 2017). This phase is laborious and usually extends from four to six weeks in which resources are allocated to monitor and maintained adequate water quality, temperature, light, and nutrient levels (Flavin et al., 2013; Redmond et al., 2014). In North America and Europe, the kelp, *Saccharina latissima* (Linnaeus) C.E. Lane, C. Mayes, Druehl & G.W. Saunders, has been cultivated at commercial scales showing potential for larger off-shore seaweed aquaculture operations (Peteiro et al., 2014; Kim et al., 2015; Azevedo et al., 2016; Bak et al., 2018; Augyte et al., 2018). Therefore, reducing nursery periods before deployment at sea could contribute to alleviating costs associated with the initial stages of *Saccharina* aquaculture, since labor is expensive in Western countries. Here we report on the effects of Kelpak® to enhance growth and thermal tolerance of *S. latissima* and *S. angustissima* (Collins) Augyte, Yarish & Neefus – a kelp endemic to the Gulf of Maine (Augyte et al., 2017) – while in the nursery.

2. MATERIALS AND METHODS

Two consecutive experiments were carried out to determine the effect of Kelpak® on growth and thermal tolerance of juvenile sporophytes (1 cm in length, 21 days old) of *S. latissima* and *S. angustissima*. Sporophytes were produced by crossing unisexual gametophytes isolated and cultivated in laboratory conditions from parental sporophytes collected in April and May, 2018 from Cape Cod Canal (41° 46' 25.7448" N; 70° 29' 58.0128" W) for *S. latissima* (SL18-UCONN-CC) and Casco Bay (43° 42' 42.48" N; 70° 11' 16.97" W) in the Gulf of Maine for *S. angustissima* (SA18-UCONN-CB).

2.1 Experimental design

A 10% stock solution of Kelpak® was prepared using filtered (1 µm) and sterilized seawater. The solution was then filtered through a 35-µm sieve to remove undisolved particles contained in the Kelpak® product. The manufacturer recommends applying Kelpak® maintaining the media solution at pH 7 to promote an active absorption. However, we used seawater with a pH of 8.1 to prepare the solutions that could affect the bioavailability of the product. We then prepared five dilutions (0.001, 0.005, 0.05, 1 and 5 ml L^{-1}) and a control solution consisting of filtered and sterilized seawater (30 ppt) only, each one with a final volume of 100 ml. These Kelpak® concentrations were selected based on preliminary trials on *S. latissima* and *S. angustissima* and on previous work on *Ulva* and *Gracilaria* (see Robertson-Anderson et al 2006), which showed effects even when Kelpak® is used at concentrations lower and higher than that recommended by the manufacturer (see https://www.kelpakusa.com/support.html). Each Kelpak® dilution plus the control were divided equally into 25-ml beakers. Each one of the solutions was then used to dip either juvenile sporophytes of *S. latissima* or *S. angustissima*, where they remained for either

30 or 60 minutes (Fig. S1). After the completion of each dipping period, juvenile sporophytes were rinsed with seawater and two blades (pseudo-replicates) per treatment (Kelpak® concentration x dipping time) were transferred to individual wells within the first two rows of fifteen 24-well plates. All of the wells in these plates (four rows total) were filled with 10 ml of nutrient-enriched seawater (half-strength Provasoli Enriched Seawater (PES), Provasoli, 1968) plus germanium dioxide (GeO_2) to prevent the development of diatoms (Lewis, 1966).

Subsequently, each pair of blades (n = 3) was allowed to grow for 20 days at one of five temperature treatments (12, 16, 19, 23 and 25°C ±1; Fig. S1) using a temperature gradient table (Yarish et al., 1979). The fourth row in each plate was used to determine at which temperature 50% of additional non-treated blades died (LT_{50}). Light was maintained 90 ± 10 µM photons m^{-2} s^{-1} PAR, with a photoperiod of 12:12 L:D.

Every five days, the juvenile sporophytes were photographed with a PixeLINK® camera (API Control, Barrington, NJ) mounted onto an Olympus (SZH, Tokyo, Japan) stereoscope. Each pair of blades was gently transferred from the wells to a microscope slide until an image was taken. Photographs were processed as black and white images to calculate blade area using Fiji by Image J (Schindelin et al., 2012). We used blade area as a surrogate to measure growth. This allowed us to evaluate possible differences in sporophyte growth as a function of Kelpak® concentration, dipping time, temperature or their interactions over time. We conducted seawater media changes every five days to avoid nutrient limitation.

After the 20 days described above, we transferred the surviving sporophytes to 75-ml Erlenmeyer flasks containing nutrient-enriched seawater (half-strength PES plus GeO_2) and gentle aeration. Blades were separated based on the concentration of Kelpak® and temperature that they were originally exposed to. Subsequently, the blades were allowed to grow for 14 days

at 18˚C (LT_{50} temperature) in a Hotpak incubator (model 352632, US states). Immediately after the 14 days, the sporophytes were photographed, and the final area per blade was calculated.

For both experiments, the specific growth rate (SGR, expressed as percent increase day^{-1}) of the juvenile sporophytes was calculated as:

$$SGR = \frac{Ln(A2) - Ln(A1)}{T2 - T1}\ x\ 100$$

where A1 and A2 are the area (mm^2) at time T1 and T2, respectively (Kim et al., 2009). We used the Student T-test to analyze differences in survival as a function of species between *S. latissima* and *S. angustissima* growing at 18°C. We also used one-way ANOVAs to analyze differences in the SGR as a function of either the concentration of Kelpak® or temperature. The interaction between both factors was not assessed due to limitations in the number of survivors.

3. RESULTS

Early juvenile sporophytes of *S. latissima* and *S. angustissima* showed the highest specific growth rates (SGR) at day five post-treatment with Kelpak® (Fig 1a, d, g and Fig. 2a, d, g). Differences in SGR appear to be driven by temperature only, with no clear effect as a result of the concentration of Kelpak® nor dipping time ($p < 0.05$; Fig.1 and Fig. 2). On average, *S. angustissima* showed a higher SGR than *S. latissima* across temperature treatments and time (Fig. 1 and Fig. 2). Regardless of the concentration of Kelpak®, dipping time and species, there was an increase in blade bleaching and blade degradation in sporophytes exposed to 19°C, while a 100% mortality was recorded during the first seven days at temperatures of 23 and 25°C. High mortality, particularly after day ten at 19°C or higher, coupled with the limitation in the number of replicates, precluded further statistical analysis.

On the other hand, there was a differential response based on species, both on survival (Fig. 3) and growth (Fig. 4), after juvenile sporophytes were exposed to 18˚C. Survival of juvenile sporophytes of *S. angustissima* was significantly higher than that of *S. latissima* ($p < 0.005$; Fig. 3). *S. angustissima* exhibited survivals of up to 100% at high concentration treatments (i.e., 1 and 5 ml L^{-1}). Moreover, sporophytes of *S. latissima* previously exposed to 5 ml L^{-1} of Kelpak® showed significantly lower SGR than any other concentration treatment (Tukey $p < 0.05$; Fig. 4a), while those previously growing at 16°C also showed limited growth (Tukey $p < 0.05$; Fig. 4a).

On the contrary, we could not detect any effect of the concentration of Kelpak® on the SGR of *S. angustissima*, but we did detect a significant effect of temperature ($p < 0.001$). Sporophytes of *S. angustissima* previously exposed to 19°C and then moved to 18°C showed significantly higher SGR than any other temperature treatment regardless of the concentration of Kelpak® (Tukey $p < 0.001$; Fig. 4b). Even for the only non-treated blade (control) transferred to 18°C (Fig. 4b).

By the end of the second experiment (i.e., 14 days of cultivation at 18˚C), we also observed that for both species, non-treated blades looked degraded, with some of them showing degraded borders. Despite their survival and growth, non-treated blades did not appear as vigorous as treated blades, particularly for *S. angustissima* at the higher concentration treatments (Fig. S2).

4. DISCUSSION

Seaweed commercial extracts are gaining momentum as enhancing agents to improve growth and overall vigor on a variety of seaweed mariculture crops (Hurtado et al., 2009a; Tibubos et

al., 2017; Souza et al., 2018). Kelpak® has been used to improve the performance of *Gracilaria* and *Ulva* spp., showing a beneficial effect on their growth (Robertson-Anderson et al., 2006). To our knowledge, this is the first study applying a kelp-derived (i.e., *E. maxima*) biostimulant to other non-related kelp mariculture crops (*S. latissima* and *S. angustissima*). Similar to our results, experiments assessing the effects of seaweed extracts, including Kelpak®, on terrestrial plants indicate that the extracts enhance growth of individuals even when subject to adverse environmental conditions, such as nutrient limitations, suboptimal temperatures or pests (Verkleij, 1992; Zhang & Ervin, 2008; Hurtado et al., 2015; Ali et al., 2018).

Studies conducted on seaweed crops report that the optimum concentration of the extract may vary as a function of the seaweed species or even strain that is being treated (Hurtado et al., 2012; Robertson-Andersson et al., 2006). Experiments assessing the effects of Kelpak® on *Ulva* and *Gracilaria* growing in ideal temperature conditions described improvements on their specific growth rate two weeks post-treatment (Robertson-Andersson et al., 2006). On the contrary, our study did not show significant differences in blades growing at the ideal temperatures of 12-16 °C. Neither showed any enhancement that could allow blades to survive to lethal temperatures above 19 °C. The optimal growth temperature for *Saccharina* ranges from 10°C to 15°C with poor growth typically observed above 16°C, and total degradation above 21°C (Fortes and Lüning 1980, Bolton and Lüning 1982, Andersen et al., 2013, Borlongan et al., 2019, Augyte, 2019). However, results did show that blades treated with Kelpak® and transferred to the sub-lethal temperature of 18°C had a relatively higher survival and SGR than non-treated blades. This could be indicative that Kelpak® could provide enhancements in temperature tolerance up to a given threshold. Moreover, in our study, the Kelpak® concentration of 0.005 ml L^{-1} appeared to be optimal to enhance sublethal temperature tolerance and growth of *S. latissima*, while for *S.*

angustissima, both 0.005 and 5 ml L^{-1} showed similar results. These differences in the optimum concentration for both species further confirm the relevance of determining the ideal concentration of seaweed extracts before applying seaweed extracts as enhancing agents for seaweed crops.

Seaweed extracts provide a wide range of responses on treated individuals (Crouch & van Staden, 1993), suggesting the presence of more than one type of stimulant depending on the algal source (Craigie, 2011). Seaweed extracts contain high levels of phytohormones that influence the cellular metabolism of treated individuals, leading in part to an increased growth through cell division and elongation. (Verkleij, 1992; Khan et al., 2009; Hurd et al., 2014). Similar to terrestrial crops, Kelpak® may induce the production of endogenous cytokinins and bioactive compounds (Aremu et al., 2016) in *Saccharina*. Cytokinins influence several aspects of plant growth and development, including cell division, nutrient uptake, as well as the response to biotic and abiotic factors (Kieber & Schaller, 2014). Suchs role could explain the differences in blade vigor observed between treated and non-treated blades when exposed to 18°C for 14 days.

Altogether, our outcomes highlight the importance of optimizing application methods based on the seaweed-extract and seaweed-crop system. In conclusion, our data provide evidence of the potential of Kelpak® as a useful biostimulant to enhance juvenile sporophytes of *Saccharina* while in the nursery. It also provides insights into practical applications to enhance the thermal tolerance of *Saccharina* before deployment at sea, which could result in a competitive advantage when compared to non-treated blades.

Acknowledgments

We thank D. Bailey and S. Lindell for their support in collecting the parental sporophytes.

References

Al-Hawezy, S. M. N. (2014). The Use of Kelpak to Seedlings Loquat (*Eriobotya Jappanica L*.). *International Journal of Scientific and Research Publications*, 4, 85–88.

Ali, M. K. M., Yasir, S. M., Critchley, A. T., & Hurtado, A. Q. (2018). Impacts of *Ascophyllum* marine plant extract powder (AMPEP) on the growth, incidence of the endophyte *Neosiphonia apiculata* and associated carrageenan quality of three commercial cultivars of *Kappaphycus*. *Journal of Applied Phycology*, 30, 1185–1195. https://doi.org/10.1007/s10811-017-1312-2

Aremu, A. O., Plačková, L., Gruz, J., Bíba, O., Novák, O., Stirk, W. A., Doležal, K., & Van Staden, J. (2016). Seaweed-Derived Biostimulant (Kelpak®) Influences Endogenous Cytokinins and Bioactive Compounds in Hydroponically Grown *Eucomis autumnalis*. *Journal of Plant Growth Regulation*, 35(1), 151–62. https://doi.org/10.1007/s00344-015-9515-8

Arthur, G. D., Stirk, W. A., van Staden, J., & Scott, P. (2003). Effect of a seaweed concentrate on the growth and yield of three varieties of *Capsicum annuum*. *South African Journal of Botany*, 69(2), 207–211. https://doi.org/10.1016/s0254-6299(15)30348-3

Augyte, S., Yarish, C., Redmond, S., & Kim, J. K. (2017). Cultivation of a morphologically distinct strain of the sugar kelp, *Saccharina latissima* forma *angustissima*, from coastal Maine, USA, with implications for ecosystem services. *Journal of Applied Phycology*, 29(4), 1967–1976. https://doi.org/10.1007/s10811-017-1102-x

Augyte, S., Umanzor, U., Yarish, C., & Lindell, S. (2018). Enhancing marine ecosystem services via kelp aquaculture. Pages 10-14 in S. Nayar, editor. International Society for Applied Phycology Newslatters 2018 December Issue. https://www.appliedphycologysoc.org/

Azevedo, I. C., Marinho, G. S., Silva, D. M., & Sousa-Pinto, I. (2016). Pilot scale land-based cultivation of *Saccharina latissima* Linnaeus at southern European climate conditions: Growth and nutrient uptake at high temperatures. *Aquaculture*, 459, 166–172. https://doi.org/10.1016/j.aquaculture.2016.03.038

Bak, U. G., Mols-Mortensen, A., & Gregersen, O. (2018). Production method and cost of commercial-scale offshore cultivation of kelp in the Faroe Islands using multiple partial harvesting. *Algal Research*, 33, 36–47. https://doi.org/10.1016/j.algal.2018.05.001

Bore, J. K. & Ng'etich W. K. (2007). Influence of kelp based plant growth stimulants on nursery tea seedlings. *Tea*, 28, 3–7.

Craigie, J. S. (2010). Seaweed extract stimuli in plant science and agriculture. *Journal of Applied Phycology*, 23(3), 371–393. https://doi.org/10.1007/s10811-010-9560-4

Crouch, I. J., & Van Staden, J. (1993). Commercial Seaweed Products as Biostimulants in Horticulture. *Journal of Home & Consumer Horticulture*, 1(1), 19–76. https://doi.org/10.1300/j280v01n01_03

Crouch, I. J., Beckett, R. P., & van Staden, J. (1990). Effect of seaweed concentrate on the growth and mineral nutrition of nutrient-stressed lettuce. *Journal of Applied Phycology*, 2(3), 269–272. https://doi.org/10.1007/bf02179784

Flavin, K., Flavin, N., & Flahive, B. (2013). Kelp Farming Manual. A guide to the processes,

tecniques, and equipment for farming kelp in New England waters. Portland, Maine

Hurd, C. L., Harrison, P. J., Bischof, K., & Lobban, C. S. (2014). Seaweed Ecology and Physiology, 2nd edn. Cambridge University Press, Cambridge, UK

Hurtado, A. Q., Critchley, A. T., Trespoey, A., & Bleicher-Lhonneur, G. (2009a). Growth and carrageenan quality of *Kappaphycus striatum* var. sacol grown at different stocking densities, duration of culture and depth. In M. A. Borowitzka, A. T. Critchley, S. Kraan (Eds.), Nineteenth International Seaweed Symposium (pp. 101–105). Springer, Netherlands, Dordrecht

Hurtado, A. Q., Neish, I. C., & Critchley, A. T. (2015). Developments in production technology of Kappaphycus in the Philippines: more than four decades of farming. *Journal of Applied Phycology*, 27(5), 1945–1961. https://doi.org/10.1007/s10811-014-0510-4

Hurtado AQ, Yunque DA, Tibubos K, Critchley AT (2009b) Use of Acadian marine plant extract powder from Ascophyllum nodosum in tissue culture of *Kappaphycus* varieties. J Appl Phycol 21:633–639. doi: 10.1007/s10811-008-9395-4

Hurtado, A. Q., Joe, M., Sanares, R. C., Fan, D., Prithiviraj, B., & Critchley, A. T. (2012). Investigation of the application of Acadian Marine Plant Extract Powder (AMPEP) to enhance the growth, phenolic content, free radical scavenging, and iron chelating activities of *Kappaphycus* Doty (Solieriaceae, Gigartinales, Rhodophyta). *Journal of Applied Phycology*, 24(3), 601–611. https://doi.org/10.1007/s10811-011-9785-x

Khan, W., Rayirath, U. P., Subramanian, S., Jithesh, M. N., Rayorath, P., Hodges, D. M., Critchley, A. T., Craigie, J. S., Norrie, J., & Prithiviraj, B. (2009). Seaweed Extracts as Biostimulants of Plant Growth and Development. *Journal of Plant Growth Regulation,*

28(4), 386–399. https://doi.org/10.1007/s00344-009-9103-x

Kieber, J. J., & Schaller, G. E. (2014). Cytokinins. *The Arabidopsis Book*, 12, e0168. https://doi.org/10.1199/tab.0168

Kim, J. K., Kraemer, G. P., & Yarish, C. (2009). Research note: Comparison of growth and nitrate uptake by New England *Porphyra* species from different tidal elevations in relation to desiccation. *Phycological Research*, 57(2), 152–157. https://doi.org/10.1111/j.1440-1835.2009.00533.x

Kim, J. K., Kraemer, G. P., & Yarish, C. (2015). Use of sugar kelp aquaculture in Long Island Sound and the Bronx River Estuary for nutrient extraction. *Marine Ecology Progress Series*, 531, 155-166

Kocira, A., Świeca, M., Kocira, S., Złotek, U., & Jakubczyk, A. (2018). Enhancement of yield, nutritional and nutraceutical properties of two common bean cultivars following the application of seaweed extract (*Ecklonia maxima*). *Saudi Journal of Biological Sciences*, 25(3), 563–571. https://doi.org/10.1016/j.sjbs.2016.01.039

Lewis, J. (1966). Silicon metabolism in diatoms. V. Germanium dioxide, a specific inhibitor of diatom growth. *Phycologia*, 6, 112.

Loureiro, R. R., Reis, R. P., Berrogain, F. D., & Critchley, A. T. (2013). Effects of a commercial extract of the brown alga *Ascophyllum nodosum* on the biomass production of *Kappaphycus alvarezii* (Doty) Doty ex P. C. Silva and its carrageenan yield and gel quality cultivated in Brazil. *Journal of Applied Phycology*, 26(2), 763–768. https://doi.org/10.1007/s10811-013-0210-5

Panda, D., Pramanik, K., & Nayak, B. R. (2012). Use of Sea Weed Extracts as Plant Growth Regulators for Sustainable Agriculture. *International Journal of Bio-Resource & Stress Management*, 3, 404–411.

Peteiro, C., Sánchez, N., Dueñas-Liaño, C., & Martínez, B. (2014). Open-sea cultivation by transplanting young fronds of the kelp *Saccharina latissima*. *Journal of Applied Phycology*, 26, 519–528. doi: 10.1007/s10811-013-0096-2

Redmond, S., Green, L., Yarish, C., Kim, J. K., & Neefus, C. (2014). New England Seaweed Culture Handbook. Connecticut Sea Grant, Groton, CT

Robertson-Andersson, D. V., Leitao, D., Bolton, J. J., Anderson, R. J., Njobeni, A., & Ruck, K. (2006). Can Kelp Extract (KELPAK®) be Useful in Seaweed Mariculture? *Journal of Applied Phycology*, 18(3–5), 315–321. https://doi.org/10.1007/s10811-006-9030-1

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, T., Rueden, C. et al. (2012). Fiji: an open-source platform for biological-image analysis. *Nature Methods*, 9(7), 676–682. https://doi.org/10.1038/nmeth.2019

Souza, J. M. C., Castro, J. Z., Critchley, A. T., & Yokoya, N. S. (2018). Physiological responses of the red algae *Gracilaria caudata* (Gracilariales) and *Laurencia catarinensis* (Ceramiales) following treatment with a commercial extract of the brown alga *Ascophyllum nodosum* (AMPEP). *Journal of Applied Phycology*, 31(3), 1883–1888. https://doi.org/10.1007/s10811-018-1683-z

Stirk, W. A., Tarkowská, D., Turečová, V., Strnad, M., & van Staden, J. (2013). Abscisic acid, gibberellins and brassinosteroids in Kelpak®, a commercial seaweed extract made from *Ecklonia maxima*. *Journal of Applied Phycology*, 26(1), 561–567.

https://doi.org/10.1007/s10811-013-0062-z

Su, L., Pang, S. J., Shan, T. F., & Li, X. (2017). Large-scale hatchery of the kelp *Saccharina japonica*: a case study experience at Lvshun in northern China. *Journal of Applied Phycology*, 29(6), 3003–3013. https://doi.org/10.1007/s10811-017-1154-y

Tibubos, K. R., Hurtado, A. Q., & Critchley, A. T. (2016). Direct formation of axes in new plantlets of *Kappaphycus alvarezii* (Doty) Doty, as influenced by the use of AMPEP K^+, spindle inhibitors, and plant growth hormones. *Journal of Applied Phycology*, 29(5), 2345–2349. https://doi.org/10.1007/s10811-016-0988-z

van Staden, J., Beckett, R. P., & Rijkenberg, M. J. (1995). Effect of seaweed concentrate on the growth of the seedlings of three species of *Eucalyptus*. *South African Journal of Botany*, 61(4), 169–172. https://doi.org/10.1016/s0254-6299(15)30513-5

Verkleij, F. N. (1992). Seaweed Extracts in Agriculture and Horticulture: a Review. *Biological Agriculture & Horticulture*, 8(4), 309–324. https://doi.org/10.1080/01448765.1992.9754608

Yarish, C., Lee, K. W., & Edwards, P. (1979). An improved apparatus for the culture of algae under varying regimes of temperature and light intensity. Botanica Marina, 22, 395–397.

Zhang, X., & Ervin, E. H. (2008). Impact of Seaweed Extract-Based Cytokinins and Zeatin Riboside on Creeping Bentgrass Heat Tolerance. *Crop Science*, 48(1), 364. https://doi.org/10.2135/cropsci2007.05.0262

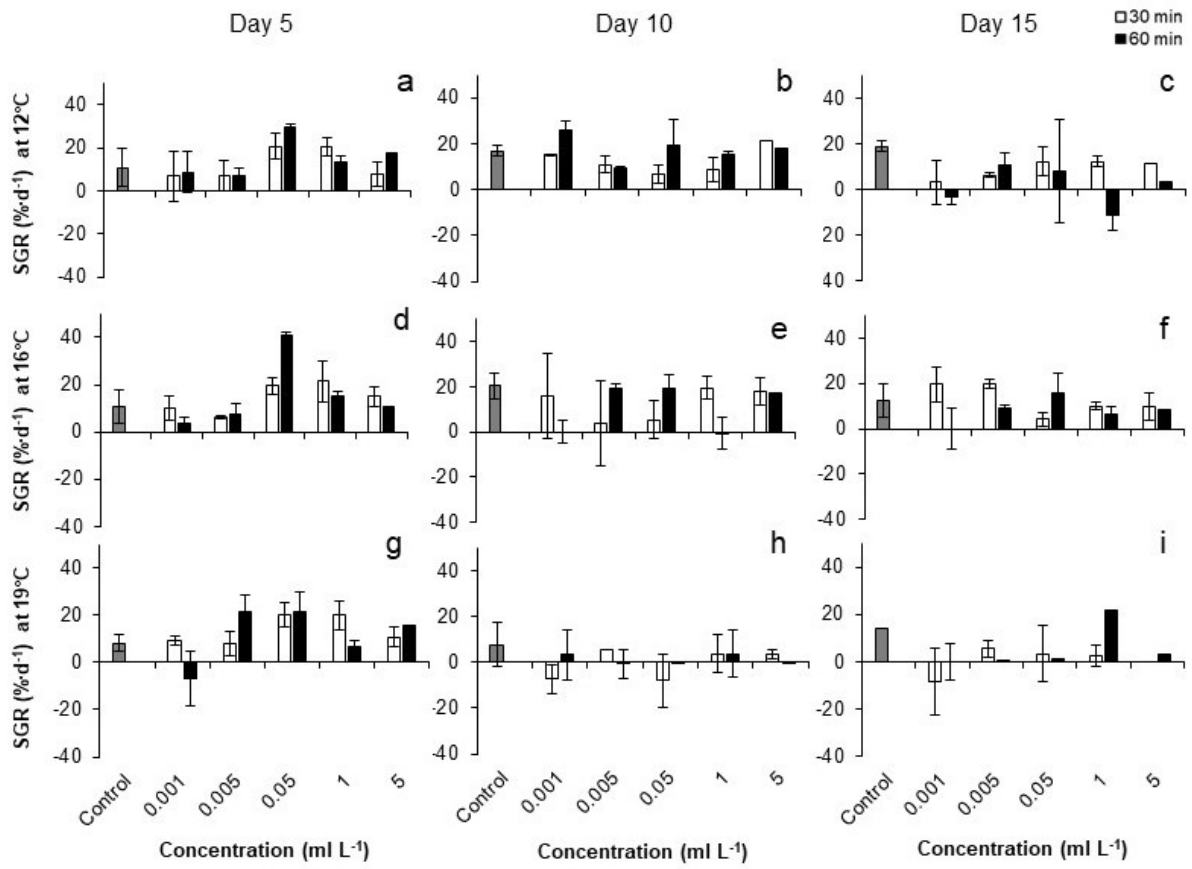

FIGURE 1 Specific growth rates (mean values ± SE, n = 3) of *Saccharina latissima* as a function of five post treatments (0.001, 0.005, 0.05, 1 and 5 ml L^{-1}) with Kelpak®, dipping time (30 min or 60 min), and temperature treatments (12, 16, and 19 °C) over 20 days. The absence of error bars indicates only one survivor per treatment.

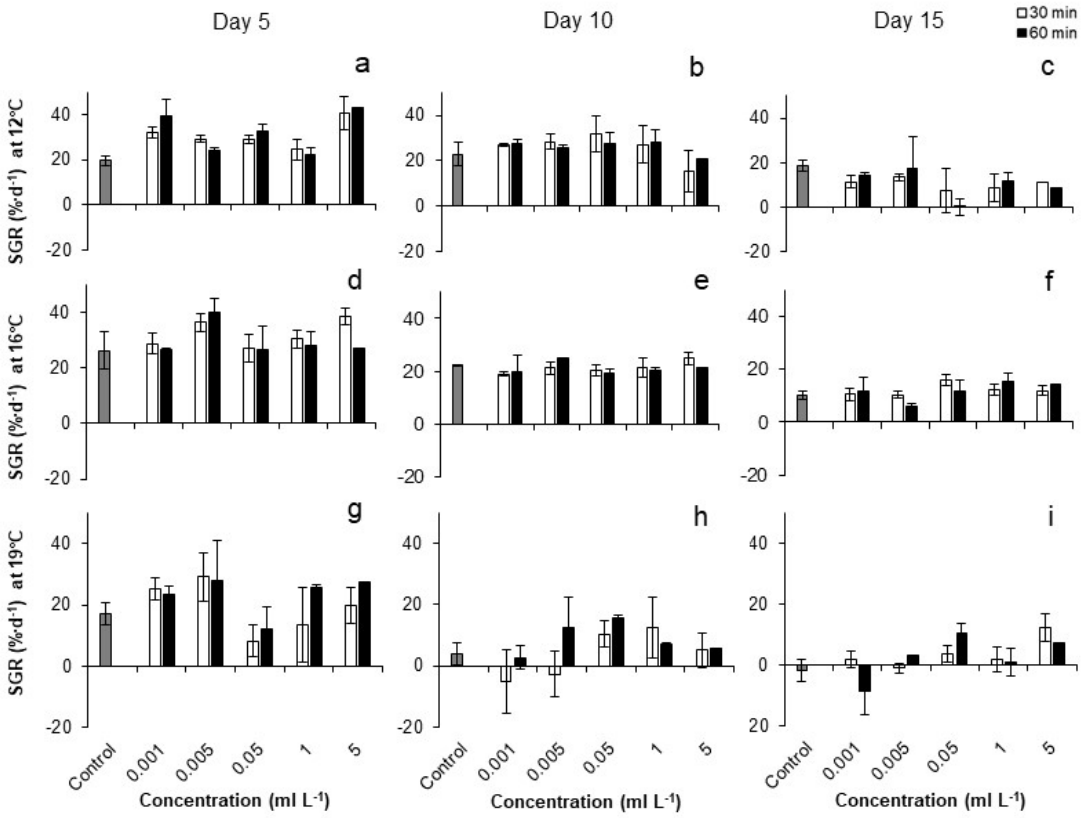

FIGURE 2 Specific growth rates (mean values ± SE, *n* = 3) of *Saccharina angustissima* as a function of five post treatments (0.001, 0.005, 0.05, 1 and 5 ml L^{-1}), with Kelpak® dipping time (30 min or 60 min), and temperature treatments (12, 16, and 19 °C) over 20 days. The absence of error bars indicates only one survivor per treatment.

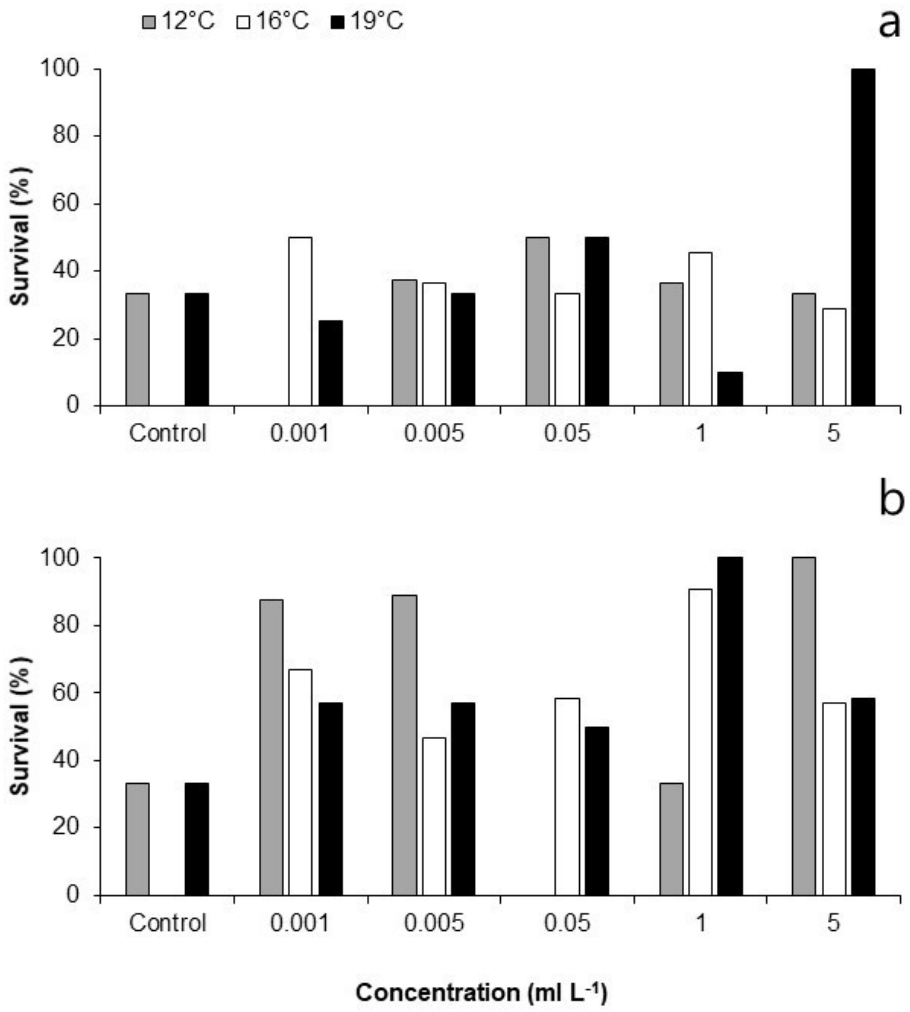

FIGURE 3 Percentage of survival of early juvenile sporophytes *Saccharina latissima* (a) and *S. angustissima* (b) growing at 18°C after exposure to different Kelpak® solutions and temperature treatments.

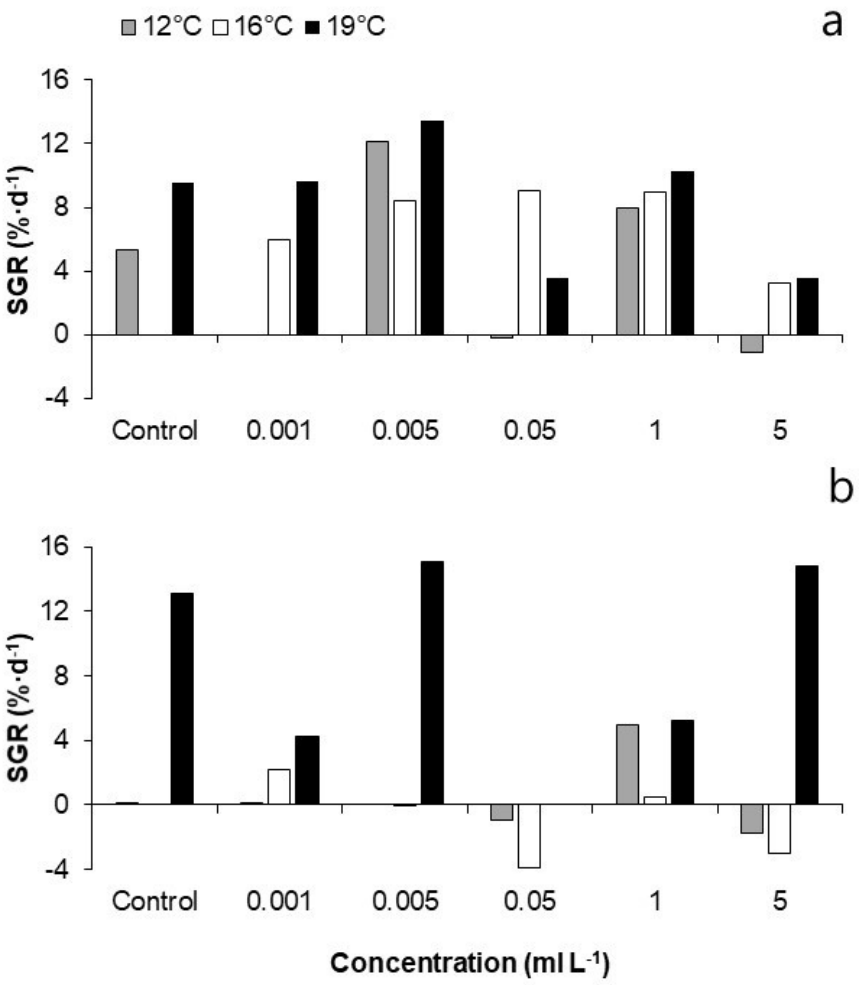

FIGURE 4 Average specific growth rate of *Saccharina latissima* (a) and *S. angustissima* (b) growing at 18°C after exposure to different Kelpak® solutions and temperature treatments.

TABLE 1 Number of surviving sporophytes transferred to 18°C for 14 days as a function of temperature and Kelpak® concentration (ml L^{-1}).

	Temperature (˚C)		
	12	**16**	**19**
Saccharina latissima			
Control	3	3	3
0.001 ml L^{-1}	8	4	4
0.005 ml L^{-1}	8	11	9
0.05 ml L^{-1}	10	6	4
1 ml L^{-1}	11	11	10
5 ml L^{-1}	6	7	2
Saccharina angustissima			
Control	3	2	3
0.001 ml L^{-1}	8	12	7
0.005 ml L^{-1}	9	15	7
0.05 ml L^{-1}	7	12	6
1 ml L^{-1}	12	11	4
5 ml L^{-1}	8	14	12

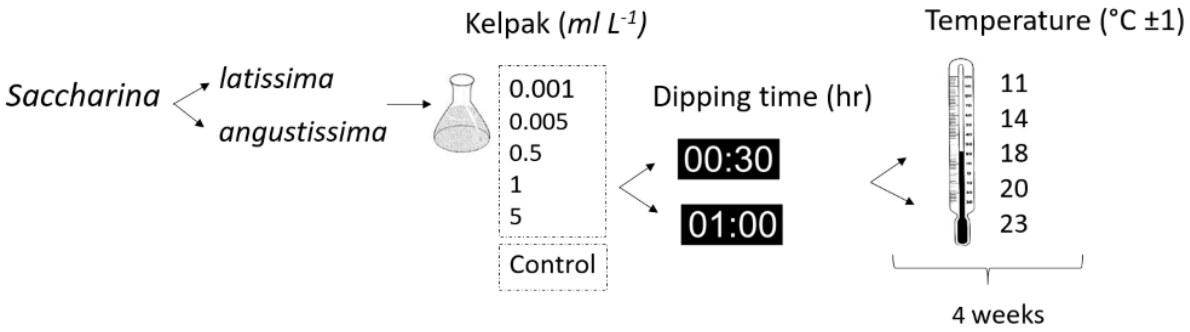

FIGURE S1 The experimental design consisted of testing the effect of Kelpak® in growth and thermal tolerance of *Saccharina latissima* and *S. angustissima* as a function of concentration, dipping time, and temperature.

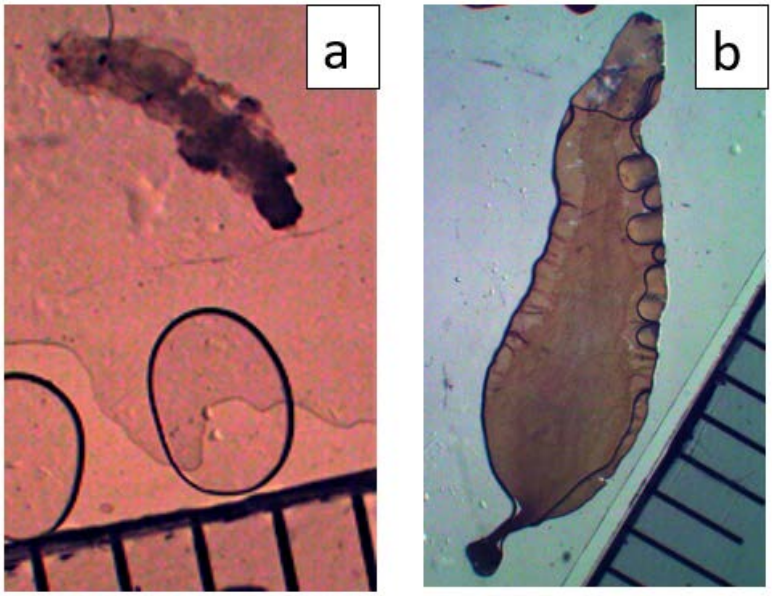

FIGURE S2 A) Non-treated and B) treated blade of *Saccharina angustissima* exposed to 18℃ for a 14 day period.