



In vitro negative effects of beach-cast invasive marine seaweed *Rugulopteryx okamurae* across life-stages of a native foundational species

Emma Gálvez-Pastor, Raquel Sánchez de Pedro ^{*} , María Jesús García-Sánchez, Antonio Flores-Moya, Elena Bañares-España 

Universidad de Málaga, Andalucía Tech, Departamento de Botánica y Fisiología Vegetal, Campus de Teatinos, 29010, Málaga, Spain

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ABSTRACT

The exotic invasive seaweed *Rugulopteryx okamurae* (Dictyotales, Ochrophyta) have posed a serious threat to coastal and marine habitats since its appearance in 2015 in the Strait of Gibraltar. This, together with multiple global change stressors, is leading to the disappearance of native foundational seaweeds. Here we explored the potential impacts that decomposing thalli of beach-cast *R. okamurae* (BCRo) might have on different life-stages of a native foundational seaweed (*Ericaria selaginoides*, Fucales, Ochrophyta). The *in vitro* effects of different biomass densities of BCRo and time exposure, in combination with three warming scenarios, were assessed across single- and few-celled stages and adult thalli of the native species. We hypothesized that *R. okamurae* might interfere in the survival and growth of *E. selaginoides* either by allelopathic interactions or water acidification, due to low intracellular pH of *R. okamurae* tissues. Sensitivity of *E. selaginoides* to the exposure of BCRo was stage- and thermal-dependent and decreased during early ontogenesis. Unfertilized oospheres (female gametes) exposed to BCRo experienced apoptotic-like cell death within less than 180 s, while embryo survival and germination dropped by 50 % and 36 %, respectively. Warming effects were not additive to that of BCRo, since higher growth reductions in 7-d old juveniles of *E. selaginoides* were detected at lower (20 °C) but not at higher temperatures (28 °C). Adults of *E. selaginoides* were the less sensitive stage but their growth was up to 7-times lower under BCRo addition. BCRo acidified natural sea water proportionally to the density and incubation time, reaching a pH of 7.2 after 24 h. However, that pH was not limiting for *E. selaginoides* juveniles and even enhanced their growth, suggesting an alternative chemical effect. This study highlighted the potential and overlooked deleterious effects that some species of beach-cast seaweeds can exert on the highly sensitive early developmental stages of native marine biota.

1. Introduction

Biological invasions are one of the main threats to marine ecosystems, due to their potential to shift community composition, alter function and cause ecosystem homogenization (Giakoumi et al., 2016; Mannino et al., 2017; Chan and Briski, 2017). Although several studies suggested that climate change can amplify the effects of biological invasions by facilitating the expansion of non-indigenous species (Castro et al., 2021; Sorte et al., 2010), the relationship between warming and invasions is complex, due to the differential responses of native and invasive species to environmental and anthropogenic stressors. For this reason, to gain a comprehensive understanding of the impacts of biological invasions requires a multi-faceted approach, including other

stressors involved in the global change (Hulme, 2017). In the oceans worldwide, seaweeds represent up to 40 % of invasive exotic species (Schaffelke et al., 2006; Crooks, 2002). Assessing the potential effects of invasive seaweeds on autochthonous species, with emphasis on single- and few-celled life-stages of macroalgae playing a structural role on seawater landscapes, urges to be understood in the context of global climatic changes and local environmental stressors.

Invasive seaweeds affect benthic communities in multiple ways, such as the displacement of native species, loss of genetic diversity, habitat alterations, shifts in community dynamics, and ecosystem functioning, potentially leading to human health impacts and significant economic consequences (Bachot and Riera, 2025). These effects can also be amplified at multiple impacted sites, as the experimental removal of invasive specimens may result in the establishment of algal turfs,

^{*} Corresponding author.

E-mail addresses: emmagalvezpastor@uma.es (E. Gálvez-Pastor), rsdpc@uma.es (R. Sánchez de Pedro), mjgs@uma.es (M.J. García-Sánchez), floresa@uma.es (A. Flores-Moya), elbaes@uma.es (E. Bañares-España).

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Abbreviations

AF	After fertilization
BCRo	Beach-cast <i>Rugulopteryx okamurae</i>
F _v /F _m	Optimum quantum yield of photosystem II
FW	Fresh weight
NSW	Natural seawater
PAM	Pulse amplitude modulated
PAR	Photosynthetically active radiation ($\lambda = 400\text{--}700\text{ nm}$)
RCP	Representative concentration pathway
RGR	Relative Growth Rate
UVR	Ultraviolet radiation ($\lambda = 280\text{--}400\text{ nm}$)

preventing the dynamic recovery of previous native communities (Gaertner et al., 2014).

The ability of invasive seaweeds to trigger regime shifts has been linked to changes in abiotic conditions, such as increased sediment trapping (Piazzi et al., 2007), reduced available irradiance, and nutrient depletion ((Schaffelke et al., 2006; Davidson et al., 2015; Pelechaty et al., 2022). Furthermore, direct effects on local species due to the release of toxins or allelopathic substances have also been reported in the early life stages of fishes and bivalves (Coelho et al., 2021; Young et al., 2022) and corals (Paul et al., 2011). Nevertheless, to the best of our knowledge, no studies have been conducted on the early life stages of habitat-forming macroalgae.

In particular, members of the order Dictyotales stand out for their deleterious effects on single- and few-celled stages of coral larvae, abalone, and young sea urchins (Casal-Porras et al., 2021; Paul et al., 2011; Suzuki et al., 2002). These effects have been attributed to their toxic and deterrent properties, which result from the presence of secondary metabolites (De Paula et al., 2011; Ninomiya et al., 1999) and a low intracellular pH due to sulfuric acid accumulation (Sasaki et al., 1999).

A particularly worrying case is represented by the exotic invasive seaweed *Rugulopteryx okamurae* (E.Y.Dawson) I.K.Hwang, W.J.Lee & H. S.Kim, with a native geographical distribution restricted to China, Korea, Japan, Taiwan and Philippines (Hwang et al., 2009). This species has caused an unprecedented invasion that originated at the Strait of Gibraltar (García-Gómez et al., 2020) and extended to the Alboran Sea (Mercado et al., 2022; El Aamri et al., 2018), western Mediterranean (Ruitton et al., 2021, Terradas-Fernández et al., 2023), Atlantic Moroccan coasts (El Madany et al., 2023) and Macaronesia (Bernal-Ibáñez et al., 2022a; Faria et al., 2022). Recently, this species has been found in the Eastern coast of Sicily and it is thought that it could invade into the Ionian Sea shortly (Marletta et al., 2024), as well as towards Northern Spain (Diaz-Tapia et al., 2025). Several traits and environmental conditions have contributed to its successful invasion (Souviron-Priego et al., 2024), namely its ability to colonize a huge variety of substrata across wide distributional ranges (García-Gómez et al., 2020, 2021a), its ability to overgrow and outcompete native species (Faria et al., 2022), its allelopathic mechanisms due to the presence of chemical deterrents (Casal-Porras et al., 2021), the occurrence of thermal and nutritional anomalies during its invasion (Mercado et al., 2022), as well as the capacity of detached thalli to survive in deep bottom habitats (Mateo-Ramírez et al., 2023). The impacts of *R. okamurae* are leading to ecosystem homogenization in heavily affected areas, with prominent biodiversity losses in benthic flora and fauna and changes in community assemblages (García-Gómez et al., 2021a, 2021b; Navarro-Barranco et al., 2019). Although its greatest cover and abundance occur in photophilic rocky habitats, their presence and impacts go beyond the photic zone (Mateo-Ramírez et al., 2023) and can be massively casted in coastal areas. As *R. okamurae* settles in multiple coastal habitats, an accumulation of detached material is

formed along the entire shoreline, including beaches (Roca et al., 2022). Consequently, large, decomposed biomass deposits were recorded on beaches, well-illuminated rocky bottoms, artificial substrates, and sheltered subtidal areas, including coralligenous (Sempere-Valverde et al., 2021) and precoralligenous assemblages (García-Gómez et al., 2020, 2021a). As a result, its expansion is generating multiple impacts on marine and coastal ecosystems, fisheries, and tourism (Bernal-Ibáñez et al., 2022a; García-Gómez et al., 2020; Ruitton et al., 2021). For instance, the invasion of *R. okamurae* caused a loss of more than €3.3 million per year in the village of Tarifa (Spain, Strait of Gibraltar), which represents 2 % of the total income in 2019 personal tax returns (Mogollón et al., 2024). *Rugulopteryx okamurae* was included in the Spanish Catalog of Exotic Invasive Species in 2020 (<https://www.boe.es/eli/es/o/2020/11/20/te1126>), and in the List of Exotic Species of the EU in 2022 (http://data.europa.eu/eli/reg_impl/2022/1203/oj).

One of the main impacts on marine communities in the Strait of Gibraltar and Alboran Sea is the overlap between current and predicted areas of *R. okamurae* expansion and the distribution of keystone *Cystoseira sensu lato* forests (i.e., *Gongolaria*, *Ericaria*, and *Cystoseira* species). This *Cystoseira s.l.* Complex represents the most relevant foundational species in the littoral and upper sublittoral rocky shores (Bermejo et al., 2016, 2018). This importance has led to most of these species being protected under several international instruments (see Falace et al., 2018) and their forests are considered indicators of good environmental coastal water quality in the Mediterranean (EC, 2000). In this context, these communities must cope with environmental and anthropogenic stressors, in addition to the invasion of *R. okamurae*.

In the Southern Iberian Peninsula, *R. okamurae* invades a region where the presence of *Ericaria selaginoides* (Linnaeus) Molinari & Guiry largely depends upon substrata availability, oceanographic conditions, and anthropic pressures (Bermejo et al., 2016). Additionally, thalli of beach-cast *R. okamurae* (BCRo) also accumulates in the eulittoral zone and shallow well-lit habitats, where *E. selaginoides* can develop. Even more concerning is that several populations of *E. selaginoides* in this region, especially those located within marine protected areas, occur in the upper sublittoral zone, where *R. okamurae* becomes the most successful competitor (García-Gómez et al., 2021) and may be subject to these previously overlooked impacts.

Sensitivity of marine benthic organisms to environmental and anthropogenic stressors changes across life stages (Alestra and Schiel, 2015; Coelho et al., 2000; Przeslawski et al., 2015). Single- and few-celled stages of the life-history of seaweeds are more susceptible to stress than multi-celled adult thalli, showing lower success of recruitment and higher mortality rates in their natural habitats (Brawley and Johnson, 1991; Vadas et al., 1992). This has been attributed to the action of several stressors, such as osmotic stress, solar ultraviolet radiation (UVR, $\lambda = 280\text{--}400\text{ nm}$), temperature, ocean acidification or pollution (Altamirano et al., 2003a, 2003b; Clausung et al., 2023; de Caralt et al., 2020; Guenther et al., 2018; Melero-Jiménez et al., 2017; Wiencke et al., 2000; Wright and Reed, 1990). Intra- and interspecific interactions can also limit recruitment, development, and survival of seaweeds, via physical (Brawley and Johnson, 1991), allelopathic mechanisms (Longo and Hay, 2017), or competitive mechanisms (Råberg et al., 2005; Worm et al., 2001).

In this study, we explore the effects of decomposing thalli of BCRo on different life-stages of the native foundational brown seaweed *E. selaginoides*, in combination with different warming scenarios for juveniles and adults. In microscopic stages of the life history of seaweeds, sensitivity to environmental stressors usually decreases during ontogenesis due to the development of adult-like metabolism (Altamirano et al., 2003b; Tarakhovskaya et al., 2017). Herein, we hypothesized that: 1) decomposing biomass of *R. okamurae* beach-cast affects uni- and few-celled stages of *E. selaginoides* life-history; 2) sensitivity of *E. selaginoides* life-stages to *R. okamurae* might decrease with the course of ontogenesis and 3) different warming scenarios can alter the outcome of that interaction. To address this, we performed five independent *in*

in vitro experiments to test the sensitivity of different life-stages of *E. selaginoides* to the presence of *R. okamurae*, in combination with actual and projected future thermal levels in the northern shores of Alboran Sea. If the presence of *R. okamurae* hampered survival of microscopic life-stages of *E. selaginoides*, it would be needed to ascertain whether the hypothetical deleterious effects of beach-cast *R. okamurae* on *E. selaginoides* early life-history stages would be attributable to alterations in physicochemical properties (pH) and/or chemical interactions (i.e., secondary metabolites).

2. Material and methods

2.1. Algal material collection

We used decomposing thalli of BCRO collected from washed-shore deposits in Calaburras (Mijas, Málaga, Spain; 004.328° W, 36.506° N). Samples were collected in September 2021, at low spring tide from the shoreline, and transported to the laboratory in natural seawater (NSW), under cold and dark conditions, in less than 1 h (see Sánchez de Pedro et al., 2023 for details). Once in the laboratory, thalli were cleaned with a soft-bristle toothbrush to remove epiphytic biota and debris. At that time of the year, *R. okamurae* exhibited an intermediate morphotype *sensu* Sun et al. (2006), characterized by wider basal thalli and thinner apical regions. The apical parts showed a high degree of degradation, which could lead to biomass loss and introduce errors in weight measurements. To standardize the material used, we selected only the basal to intermediate portions of the thalli, removing the thinner and degraded apical regions. The selected parts were then cut, weighed, and placed in multi-well plates. Selected parts were cut off, weighted, and put in several multi-well plates. To ensure that algal material was dead and non-photosynthetically active, we applied PAM fluorometry to ensure a zero value of optimum quantum yield of photosystem II (F_v/F_m ; Genty et al., 1989) (data not shown). Samples of *E. selaginoides* were collected from intertidal rocky platforms in La Araña (Málaga, Spain; 004.328° W, 36.700° N), at low spring tide in September 2021. Two types of branches were collected from 20 individuals: reproductive (bearing mature receptacles) and vegetative (apical branches) (*sensu* Roberts, 1970). To reduce the impact of the sampling on the standing population cover and biomass, we only took 2–3 cm sections from each branch type. Sampled branches were collected at least 50 cm apart to account for intraspecific natural variability and prevent pseudo-replication. This distance was chosen based on the low dispersal range observed in this genus and in other furoid seaweeds (Verdura et al., 2018). Vegetative fragments were kept in sealed zip bags filled with NSW. Fertile branches were cleaned *in situ* and wrapped in humid paper. All the algal material was transported under cold and dark conditions till laboratory arrival in less than 1 h. Vegetative thalli were cleaned as stated for *R. okamurae*, while fertile branches of *E. selaginoides* were preserved in the fridge at 4 °C for 24 h prior to gamete isolation. Experiments were conducted following algal collection, and no acclimation culture period was applied.

2.2. Isolation of early life stages of *E. selaginoides*

Ericaria selaginoides is a monoecious species with gametic life history, where mature receptacle branches bear conceptacles with basal oogonia and antheridia towards the ostiole (Roberts, 1970). Gamete release was induced in cold-preserved mature receptacle branches of *E. selaginoides* following protocols applied in other furoids (Falace et al., 2018; Verdura et al., 2018). After 24 h of storage at 4 °C, fertile branches were placed in trays with GF-C filtered and sterilized NSW (20 °C, pH 8.2 and salinity of 36). The contrast from cold, emerged and humid conditions to submersion induces the mechanical process of gamete release in furoids, based on osmotic and thermal shock (Brawley and Johnson, 1991). Based on our observations, after gamete release fertile branches can be again preserved at 4 °C and induce a new cycle of gamete release 24 h

later. We performed this process up to 5 times in consecutive days, but successful gamete release and fertilization was only possible within the first 4 d. Oospheres (female gametes) were extruded from conceptacles before antherozoids (45 and 60 min after re-submersion of mature receptacle branches, respectively). This fact allowed us to isolate a sub-sample of cell-wall free unfertilized oospheres with a Pasteur pipette, to test the effect of BCRO addition (see section 2.3, Experiment 1). Antherozoid release was detected by the presence of an orange mound around the ostiole, as defined by Roberts (1967). Antherozoid motility and fertilization was confirmed by direct observations under a Motic AE31 inverted microscope (Motic, Xiamen, China), and marked the initial time after fertilization (AF) for the different experiments (Nagasato et al., 2001). Fertilized oospheres (zygotes) were collected 30–45 min following re-submersion of fertile branches and pooled in a dense stock. Zygotes were immediately inoculated due to their rapid attachment to the substrata in this species (60–90 min). Multi-well plates or microscopy slides were used as substrate depending on the aim of each experiment.

2.3. Experimental design

We performed five independent experiments to investigate the effect of BCRO on different life-stages of *E. selaginoides*. It should be noted that the interactive effect of BCRO and temperature was only included in experiment 3. The following life-stages were selected for the different experiments: unfertilized oospheres (0 d, prior to antherozoid release), embryos (1 d AF), germlings (2 d AF), juveniles (7–14 d AF) and adults (vegetative branches). It should be pointed out that the main focus was to test the effect of BCRO within each stage, but as the experimental approach were different for juveniles/adults comparisons among stages would not be accurate.

2.3.1. Experiment 1: short-term effect of BCRO biomass density and exposure time on unfertilized oospheres

Oosphere stock 100 μ L aliquots with a density ca. 210 cells mL^{-1} (see section 2.2) were inoculated in six wells of a 24-multiwell plate ($n = 6$) for each treatment (BCRO biomass densities) and control. Different densities of BCRO were weighted and added to the plates in the wells (0.25, 0.75, 1.5, 3, 4.5 mg FW mL^{-1}), filled with 2 mL of GF-C filtered and sterilized NSW (pH 8.2 and salinity of 36). Survival of 21 ± 11 oospheres was monitored on each independent well at 0.5, 1, 1.5 and 24 h. A physical control using a mesh fragment with the same surface area than the highest BCRO biomass density was included to discard a negative interaction due to physical contact or shading (Paul et al., 2011).

2.3.2. Experiment 2: effect of BCRO exposure time on embryo survival and germination

The second experiment assessed the effect of BCRO on embryo survival and germination of 1-d old embryos of *E. selaginoides* at a fixed BCRO biomass density. Embryos were isolated as indicated in section 2.2., attached to microscopy slides ($n = 12$) and placed in independent Petri dishes filled with 20 mL of GF-C filtered and sterilized NSW (pH 8.2 and salinity of 36). Then, 5 mg FW of BCRO was added to half of the Petri dishes as BCRO treatment, with a final density 0.25 mg FW mL^{-1} . This density was selected as it was the minimum concentration tested in experiment 1, which exerted a significant effect on oospheres. Time course of individual embryo survival was monitored under the microscope and microphotographs were taken at 15, 30, 90 and 120 min (see section 2.4). Embryo survival was additionally checked 24 h after BCRO addition in the same slides, for which BCRO was removed after 24 h of exposure. To assess possible delays in germination due to previous BCRO exposure, germination rates were monitored at day 2 and 7 after its initial addition, corresponding to 3- and 8-d old embryos.

2.3.3. Experiment 3: interactive effects of BCRO and temperature on juveniles and adults

This experiment was designed to explore the possible interactive effects of BCRO addition under combinations of different temperatures (20, 24, 28 °C) on the growth of juveniles and adults of *E. selaginoides*. Despite similar designs, such effects were separately analysed for both life stages due to the different approaches in growth measurement in juvenile and adult thalli (see section 2.4). A fixed density of 4 mg mL⁻¹ BCRO was selected, lying in the range of maximal densities used in exp. 1 to make later comparisons feasible. Temperature levels were selected based on current (20 °C) and future (24 °C) warming scenarios under the Representative Concentration Pathway (RCP) 8.5, and the thermal extreme historically detected during marine heatwave events in the area of study (28 °C) (Campos-Cáliz et al., 2019).

For juveniles, fertilized zygotes of *E. selaginoides* were inoculated in 24-multiplate plates filled with 2 mL of GF-C filtered and sterilized NSW (pH 8.2 and salinity of 36). Each well was inoculated with an individual zygote (n = 20–22 zygotes per plate/temperature), and thus, they could be considered as an individual replicate. From day 0–7 all embryos grew without BCRO at the common culture conditions indicated in section 2.4. At day 7, 8 mg of BCRO (final density of 4 mg FW mL⁻¹) was added to 12 individuals, remaining 10 individuals as controls without BCRO addition. Microscopic stages length was monitored on an individual basis at 0, 7 and 14 d.

For adult individuals, 2–3 cm of vegetative branches (175 ± 14 mg FW) were placed in two multiwell plates filled with 10 mL of GF-C filtered and sterilized NSW at the previously indicated pH and salinity. At day 0, 40 mg FW of BCRO (final density of 4 mg FW mL⁻¹) was added to the treatment plate (n = 6) (Fig. S1). Biomass was weekly measured for 2 weeks to monitor algal growth under control and treatment conditions.

2.3.4. Experiment 4: effect of BCRO biomass density and exposure on seawater pH

To assess whether BCRO can modify the pH of seawater and identify critical pH values, we added two different densities of BCRO (0.75 and 4 mg FW mL⁻¹). A total of 7.5 or 40 mg FW of BCRO were added to two multiplate plates with each well filled with 10 mL of GF-C filtered and sterilized NSW (pH 8.2 and salinity of 36). Seawater pH was measured at 0, 0.5, 1, 2, 3, 24 h following BCRO addition with a Crison pH-Meter BASIC 20 electrode (Crison Instruments S.A., Barcelona, Spain).

2.3.5. Experiment 5: effect of pH on juvenile survival and growth

Since we found significant changes in NSW pH due to BCRO addition, we performed an additional experiment to test whether these pH levels affected growth and survival of microscopic stages of *E. selaginoides*. This experiment would allow us to understand if the negative effects of BCRO on microscopic stages might be solely attributed to changes in the pH or also to a possible chemical interference due to high concentrations of secondary metabolites (Casal-Porras et al., 2021). In this case, mature receptacular branches were directly placed on multiwell plates instead of pipetting zygotes from a stock, filled with 10 mL of GF-C filtered and sterilized NSW (salinity of 36) at three different pH: 7.2, 7.5 and 8.2 (control). Acidified treatments were selected following the final concentrations obtained in experiment 4, by adding drops of a sterilized 1 N HCl solution. Fertile fragments were left for 2 h for gamete release (Falace et al., 2018). Despite we used 6 initial replicates, 3 out of the 6 receptacular branches did not released any gametes only 3 replicates could be used. Microscopic stages size and survival were compared between days 0 and 14 on a culture well instead of on an individual basis, taking a total of three randomly chosen microphotographs per technical replicate (see details in section 2.4.).

2.4. Culturing conditions and growth and survival measurements

Multiwell plates containing different life-stages of *E. selaginoides*

under the different treatment combinations were kept in a growth chamber set at 20 ± 0.5 °C (EQUITEC EGCS 501 LED, Equipos Técnicos para Controles S.L., Spain). To set the experimental temperatures of 24 and 28 °C, two aquaria were placed inside the growth chamber, each of them thermostated with a submersible precision aquarium heater (75 W Eheim Jäger, Eheim, Deisizou, Germany, temperature control accuracy of ±0.5 °C). Plates were sealed with Parafilm® to minimize possible water evaporation or water leakage into the plates from the aquaria. All life stages were cultured in the same sterile GF-C filtered NSW reported in previous sections at a PAR irradiance of 150 μmol m⁻² s⁻¹, provided by LED lamps under a photoperiod of 18:6 light:darkness.

Growth and survival rates in early-post settlement stages (oospheres, embryos, germlings and juveniles) were estimated from photomicrographs on a Motic AE31 inverted microscope (Motic, Xiamen, PCR) coupled with a Moticam 10.0 Mp digital camera with Moticam Images Plus 3.0 ML software (Motic). Images were processed using the ImageJ distribution Fiji (Schindelin et al., 2012). Alive and dead oospheres, embryos or germlings were counted from the photomicrographs and survival rate was calculated as the proportion of alive cells. We based our selection criteria on the visual integrity of the cells. Growth of microscopic stages was calculated on a length basis, considering the length of the major axis and excluding the rhizoids (Altamirano et al., 2003a). Alternatively, spherical zygotes were counted and measured (zygote diameter) by using an automated procedure of selection of region of interest (ROI) based on image contrast in Image J distribution Fiji. Growth of adult individuals (vegetative apical branches) was followed by changes in biomass over the experiment. Relative growth rates (RGR) of microscopic stages and adults of *E. selaginoides* were calculated on a length or biomass basis, as:

$$RGR (\% d^{-1}) = 100 \cdot \frac{\ln(S_t) - \ln(S_0)}{\Delta t},$$

where S_t and S_0 are the final and initial size (length in μm or biomass in g FW), and Δt is the elapsed time.

2.5. Statistical analysis

Different statistical analyses were applied to test the effects of BCRO on microscopic stages of *E. selaginoides* throughout the five experiments, which are summarized in Table 1. When the sphericity assumption was not met in the repeated measures designs, we applied the Greenhouse-Geisser correction (1959), by using adjusted degrees of freedom and *P*-values. When normality and homoscedastic assumptions were not met in ANOVA designs, non-parametric tests were run (Kruskal-Wallis non-parametric ANOVAs). When ANOVA tests showed significant differences, *post-hoc* Tukey honest significant differences (HSD) analysis was run. All graphs were made using GraphPad Prism 8.01 (GraphPad Software Inc.) and statistical analyses were performed using STATISTICA 7.0 (StatSoft Inc., Tulsa, OK, USA).

3. Results

3.1. Short-term effect of BCRO biomass density and exposure time on the survival of oospheres

Exposure of oospheres of *E. selaginoides* to BCRO decreased their survival by 50–100 % depending upon exposure time and density of BCRO (Fig. 1, Table A.1). Oospheres from control plates without BCRO addition and control plates with a physical control (mesh addition) showed a survival rate of 96 ± 3 % (Fig. 1, at BCRO = 0), without any sign of cell damage. Survival of oospheres exposed to 0.25 mg FW mL⁻¹ of BCRO dropped by 50 % regardless of the exposure time (Fig. 1).

Survival of oospheres exposed for 30 min to increasing BCRO biomass densities (up to 4.5 mg FW mL⁻¹) decreased from 55 to ca. 25 % (Fig. 1). As exposure time increased (60, 90 min) all oospheres died at densities

Table 1
Statistical analyses applied to each of the experiments of the study.

Experiment	Control variable	ANOVA analysis	Factors/levels	n
Exp. 1	Survival of <i>E. selaginoides</i> oospheres	1-way RM ANOVA	BCRo Biomass density (i = 6) Time (j = 3)	6
Exp. 2	<i>E. selaginoides</i> embryo germination rates	1-way RM ANOVA	Treatment (i = 2) Time (j = 3)	6
	Short term time course survival rates of embryos	1-way ANOVA	Time (i = 4)	6
	Embryo survival 24 h after BCRo addition	Mann-Whitney U	BCRo treatment (i = 2)	6
Exp. 3	Growth of adult tips of <i>E. selaginoides</i>	2-way RM ANOVA	Temperature (i = 3) BCRo treatment (j = 2) Time (k = 2)	6
	Growth rate of <i>E. selaginoides</i> juveniles	2-way crossed ANOVA	Temperature (i = 3) Treatment (j = 2)	10–12
Exp. 4	pH of natural seawater (NSW)	1-way RM ANOVA	BCRo Biomass density (i = 2) Time (j = 5)	6
Exp. 5	Survival and growth of juveniles of <i>E. selaginoides</i>		pH (i = 3)	3

Abbreviations: Repeated measures (RM) ANOVA. i, j and k indicate the number of levels within each factor.

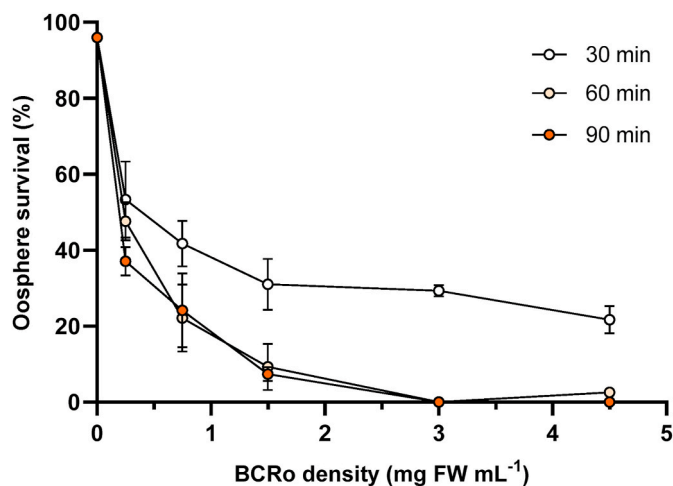


Fig. 1. Short-term survival of oospheres of *Ericaria selaginoides* exposed to five densities of beach-cast *Rugulopteryx okamurai* (BCRo, 0.25, 0.75, 1.5, 3, 4.5 mg FW mL⁻¹) and three exposure times (30, 60, 90 min).

above 3 mg FW mL⁻¹. There was no survival of oospheres under any treatment 24 h later, while those from the control remained without visible cell damage (data not shown). Fig. 2 shows oosphere death in the proximity of a piece of thallus of BCRo over a 15 min time span, at a BCRo concentration of 1.5 mg FW mL⁻¹.

To depict how fast this process occurred, the oosphere cell-death was recorded (Supplementary material, Video 1, Fig. 3). Oospheres experienced the extrusion of cytoplasmic vesicles through the plasmalemma within less than 3 min when exposed to BCRo (Fig. 3A), containing numerous vesicles of differing sizes (~0.3–15 μm). After cytoplasm extrusion, the oosphere nucleus remained inside the plasmalemma, and the membrane rapidly adjusted to the shrunk cytoplasm. However, in oospheres where cytoplasmic extrusion occurred in less than 30 s (Fig. 3B), the plasma membrane integrity was lost, and a surge release of

the cell content to the external medium was observed. Following this, broken sections of plasma membrane rapidly rearranged into single layered spheres (micelles) (Fig. 3B).

3.2. Effect of BCRo exposure time on the survival and germination of embryos

Survival of 1-d old embryos of *E. selaginoides* decreased from 87 % to 72 % during 2 h exposure to 5 mg FW mL⁻¹ of BCRo (Fig. B.1). A significant decline of survival of embryos was detected between 15 and 30 min, and then it progressively declined ($F_{3, 72} = 4.58, P = 0.018$).

In an independent assay, survival of 1-d old embryos exposed to BCRo for 24 h decreased by 50 % (Mann-Whitney U = 0, $P = 0.004$; Fig. 4A, Table A.2). In addition, germination rates of these same embryos significantly declined after 2- and 7- d of exposure to BCRo (3- and 8-d after fertilization, respectively), from ~91 to ~58 % (Table A.2). Germination rates did not significantly vary between experimental times (2 vs. 7 d) within the control or BCRo treatment (Fig. 4B, Table A.2). Furthermore, 7-d old germlings exposed to BCRo experienced delayed elongation, loss of regular shape of their apical hairs and rhizoidal weakness from which some of them got detached of the plates.

3.3. Interactive effects of BCRo and temperature on juveniles and adults

3.3.1. Juveniles (7 d-old embryos)

Growth of juveniles of *E. selaginoides* ranged from 10.5 to 12.4 % d⁻¹ between 0 and 7 d AF, when the apical hairs were developed (Fig. 5). Under control treatment, juvenile growth was unaffected by temperature (20–28 °C) between 0 and 7 d, but it decreased significantly from 2.7 to -1 % d⁻¹ at 28 °C between days 7 and 14 (Fig. 5). The addition of BCRo at day 7 A F had a negative effect on embryo growth at 20 °C (Fig. 5). Differences in embryo length between control (C) and treatment (R) were not significant at 24 or 28 °C (Table 2). Nonetheless, exposure to BCRo and/or warming led to distinct morphological features (Fig. 6). Exposure to BCRo decreased rhizoid number and thickness, causing weaker attachment and more frequent dislodgment. Moreover, both temperature and BCRo addition had a negative effect on the number and regular shape of apical hairs (Fig. 6). Lastly, embryos grown at optimal conditions (20 °C, without BCRo) had the greatest bilateral symmetry, while those exposed to warmer conditions and in the presence of BCRo presented more irregular shapes (Fig. 6).

3.3.2. Adults

Adults of *E. selaginoides* were significantly affected by the exposure to BCRo, showing similar or lower (up to 7-times) relative growth rate (RGR) than the control (Fig. 7, Table 3). In addition, an interactive effect was detected between temperature and time interval for both BCRo and control treatments. Relative growth rate of adult *E. selaginoides* were higher during the first week and were not affected by temperature. In contrast, RGR gradually decreased with temperature, reaching a minimal value at 28 °C during the second week of experiment. In addition, apical tips of *E. selaginoides* grown with BCRo showed damaged reddish tissues that easily broke and fell apart (Fig. B.2).

3.4. Effect of BCRo biomass density and exposure on seawater pH

Natural seawater pH decreased following the addition of BCRo but at a different pace depending on the biomass density ($F_{4,40} = 40.1$, Greiner-Greenhouse Adjusted $P < 0.001$) (Fig. 8). A significant reduction in pH was detected after 1 and 2 h at BCRo concentrations of 4 and 0.75 mg FW mL⁻¹ BCRo, respectively, and it declined by 1–2 % after 3 h (Fig. 8). NSW reached final pH values of 7.33 ± 0.03 and 7.64 ± 0.03 after 24 h at high and low BCRo concentrations, respectively, while pH of control treatment (without BCRo) remained at 8.31 ± 0.02 during the experiment (Fig. 8).

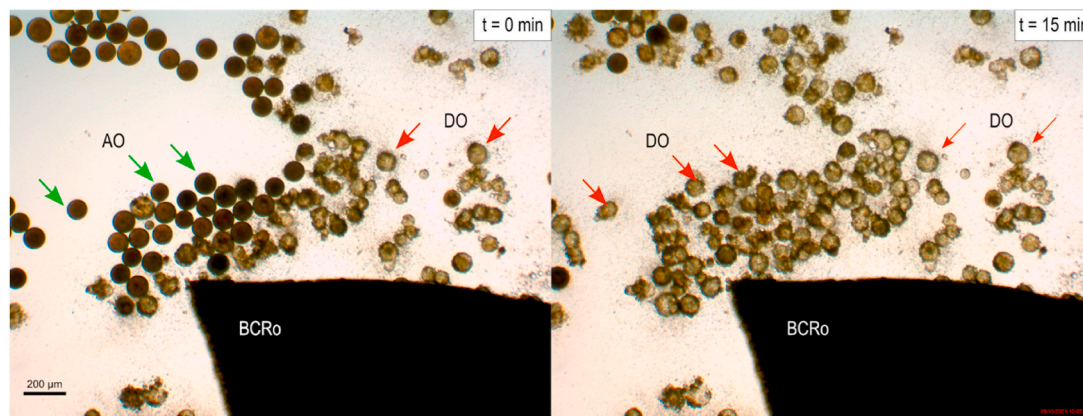


Fig. 2. Effect of 1.5 mg FW mL⁻¹ of beach-cast *Rugulopteryx okamurae* (BCRo) on oospheres of *Emericia selaginoides*. Elapsed time of 15 min between left and right micrographs (4× magnification). Beach-cast *R. okamurae* (BCRo), alive oospheres (AO, green arrows), dead oospheres (DO, red arrows). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.5. Effect of pH on growth and survival of juveniles of *E. selaginoides*

NSW pH did not affect the survival of juveniles of *E. selaginoides* ($F_{2,6} = 0.12$, $P = 0.89$), with an overall mean value of 62 ± 23 % (Fig. 9). Juveniles of *E. selaginoides* grown at pH 7.2 had 1.2-fold times higher RGR than at pH 7.5 or 8.2 (6.21 ± 2.2 % d⁻¹ vs. averaged pooled value of 5.4 ± 2.1 % d⁻¹) ($F_{2,319} = 5.40$, $P = 0.005$).

4. Discussion

Invasive species are considered the second biggest threat for biodiversity conservation, behind climate change. Among the most recent invasion events, the arrival of *R. okamurae* to the Strait of Gibraltar in 2015 and the expansion in the Western Mediterranean seems to be especially threatening to marine biodiversity, as well as a relevant problem for several economical activities (mainly, fisheries and tourism) (García-Gómez et al., 2021b; Mogollón et al., 2024). For this reason, we addressed the study of the impact of BCRo on structural, native seaweed species. Our study provided unquestionable evidence on the negative effects of decomposing BCRo across single- and few-celled stages of *E. selaginoides*. We demonstrated that exposure to BCRo was lethal for oospheres of *E. selaginoides*, and massive cell-death occurred within minutes. Nonetheless, during early development, *E. selaginoides* required longer exposure to BCRo to exhibit changes in survival, growth, and development, even under higher BCRo biomass conditions. Temperature, in the range which could be achieved according to future scenarios of climatic change, did not exert an additive effect to BCRo exposure in the physiological performance of juveniles of *E. selaginoides*. Despite adults of *E. selaginoides* were less sensitive to BCRo exposure than microscopic stages, their growth also decreased significantly. This effect aligns with previous studies demonstrating the inhibitory impact of various algal allelopathic compounds on photosynthesis by blocking the electron transport chain, ultimately affecting growth (Patterson et al., 1979; Patterson and Harris, 1983; Sudatti et al., 2020). Interestingly, the sesquiterpene elatol, extracted from the rhodophyte *Laurencia dendroidea* J. Agardh, exhibited stronger effects on the apical portions of its own thalli compared to the basal sections. This autotoxicity, which increases with higher population densities, regulates seaweed growth to prevent self-shading by structuring the population through density control (Sudatti et al., 2020). Although elatol is not present in *R. okamurae*, this species contains up to six different secondary compounds, most of them are terpenoids. In this context, future studies should investigate the effect of *R. okamurae* extracts on *E. selaginoides* photosynthesis along the adult thalli. Finally, we detected that pH of NSW proportionally decreased with the density and incubation time with BCRo, but contrary to our initial hypothesis, a pH level of 7.2

reached after 24 h did not limit the growth of *E. selaginoides* juveniles and rather enhanced it. In addition, this response might have been mediated by the carbonic-carbonate system, where growth would be enhanced via higher inorganic C uptake at lower pH. In fact, several studies point at the potential benefit experienced by brown algae under ocean acidification (Celis-Pla et al., 2015), including *E. selaginoides* if temperatures do not surpass 2–3 °C above optimum temperature and nutrients are not limiting (Celis-Plá, 2015). Nonetheless, we cannot rule out that pH changes by BCRo might be also the result of an allelopathic effect in this species that requires in-field demonstration.

The two species from this study were not collected at the same location since *E. selaginoides* population at the location of large strands of BCRo was highly reduced (it must be highlighted that the populations of this species were very abundant in the location where BCRo was collected, characterizing the beginning of the infralittoral zone; Conde and Seoane, 1983; Flores-Moya et al., 2021) and fertile branches were not detected. Nonetheless, through our experimental *in vitro* approach we evidenced a proof of concept with relevant ecological significance, as the extent of coastline where the two species overlap is rapidly increasing towards the Mediterranean and eastern Atlantic coast (Díaz-Tapia et al. 2024). This first approach highlighted the vulnerability of native foundational species, particularly in the few- and single-celled stages of the life history to the impacts of *R. okamurae*. Given the differing physiological status of beach-casted *R. okamurae* material depending on the distribution area and season (Roca et al., 2022), further studies should investigate how this variability may influence the activity of its bioactive compounds and their interactions with native communities.

The exposure time needed to detect a significant negative effect on the physiological traits (growth, survival, or germination) increased from minutes to days from oospheres to juveniles of *E. selaginoides*. Oospheres experienced rapid cell death within minutes, while young embryos had a similar fate but at higher BCRo concentrations and after longer exposure times. Stage-sensitivity have been reported for several stressors in other furoid algae, such as temperature, UVR or metals (Brawley and Johnson, 1991; Altamirano et al., 2003b; de Caralt et al., 2020). During early ontogenesis, furoid embryos experience multiple physiological, biochemical, and developmental changes, with apical hairs development as a milestone (Tarakhovskaya et al., 2017). At that point, juveniles acquire similar physiological performance than apical tissues of adult thalli (Falace et al., 2018; Sánchez de Pedro et al., 2022).

In addition to the several impacts of *R. okamurae* on native marine species, our reported effects of BCRo across life-stages of *E. selaginoides* might extend across different life-stages of many others keystone foundation organisms (Cebrián et al., 2012; García-Gómez et al., 2020b; Sempere-Valverde et al., 2021). Considering that survival of propagules,

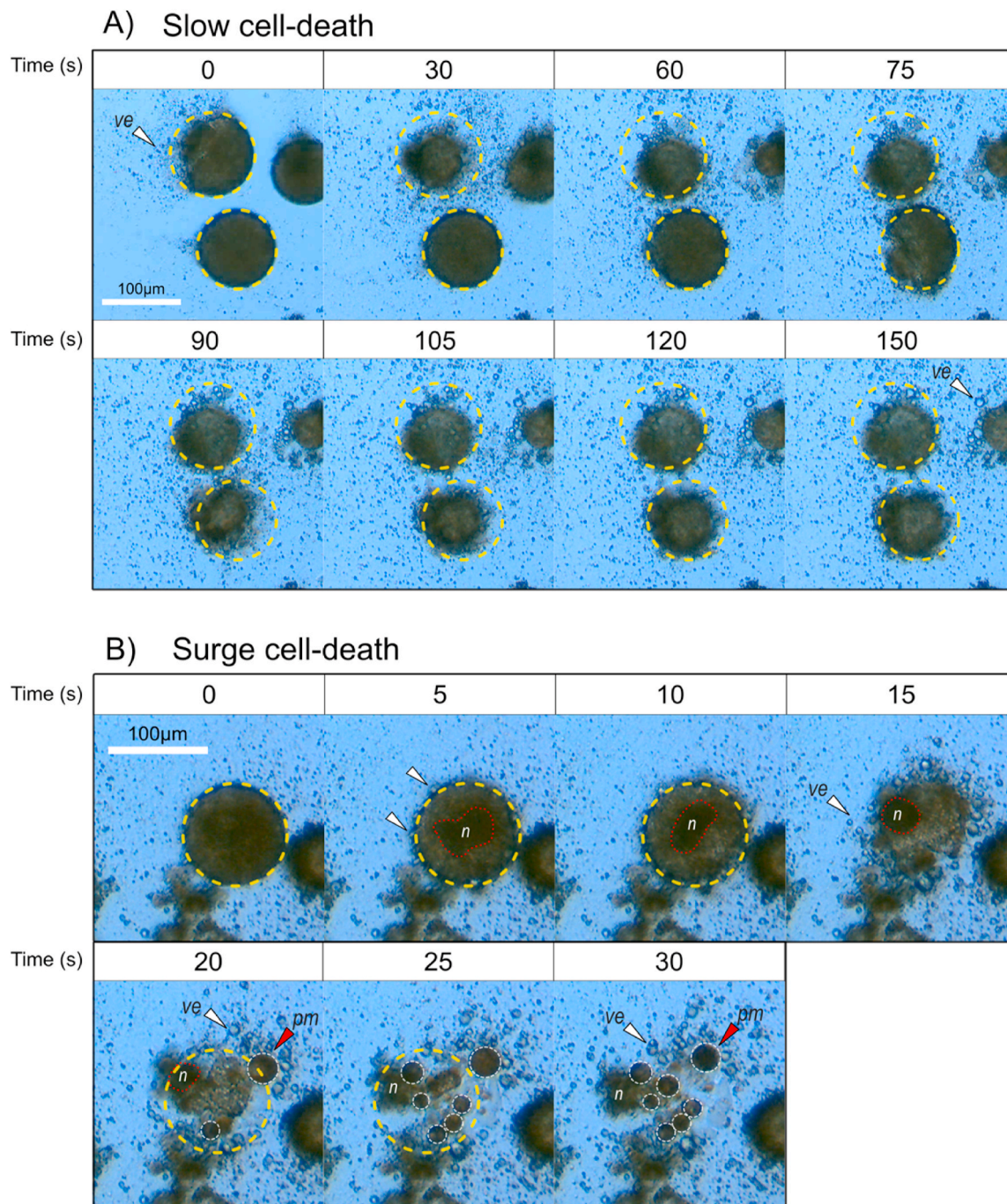


Fig. 3. Detailed micrographs of the effect of $1.5 \text{ mg FW mL}^{-1}$ of beach-cast *Rugulopteryx okamurae* (BCRo) on oospheres of *Ericaria selaginoides*. Time-lapse of “slow” (A, 150 s) and “surge” cell-death (B, 30 s) of oospheres of *E. selaginoides* exposed to 1.5 mg mL^{-1} BCRo. Dashed yellow circles depicted the initial shape of the cell. Dashed red shapes depicted areas with higher chromatin density in the oosphere. White arrowheads indicated extruded vesicles. Red arrowheads indicated formation of micelles (small, single layered spheres, dashed white shapes) following plasma membrane disruption in oospheres that experienced “surge” cell-death. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

spores and recruits in nature is very low (Vadas et al., 1992), an added stressor such as the interaction of an invasive species as *R. okamurae* would represent a critical bottleneck for population persistence and success of sexual reproduction, since recruitment ensures genetic variability within a population. *Cystoseira* s.l. Forests comprise species with varying connectivity among populations and unique pools of genetic diversity (Bermejo et al., 2018; Buonomo et al., 2017; Falace et al., 2018). Dispersal distances are low in this genus (Verdura et al., 2018), but recruits and rafted fertile branches may reach longer distances due to local currents (Reynes et al., 2021). In this regard, if a fast degradation

of oospheres as that found in our study would occur in nature, the chance of viable oospheres reaching longer distances would be heavily constrained under the presence of *R. okamurae*. Nevertheless, extrapolating the detected lethal dose from our study to the field is highly difficult, due to local currents, dilution effects and the interaction with other factors that might enhance decomposition of beach-cast materials. Areas with high densities of *R. okamurae*, as the coasts of Ceuta (Southern Strait of Gibraltar), up to 5000 tons of BCRo were detected only during fall 2015 (García-Gómez et al., 2020). In fact, in the northern shore of the Strait of Gibraltar, it has been suggested that such

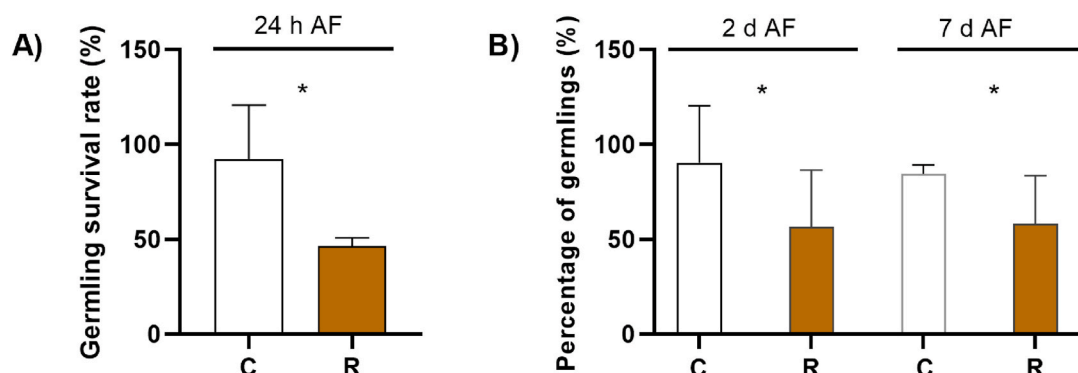


Fig. 4. A) Survival of 1-d old embryos of *E. selaginoides* 24 h after fertilization (AF) under control (C) and BCRo treatment (R). B) Germination rate of embryos of *E. selaginoides* 2- and 7-d AF of control samples (C) and exposed to 5 mg FW mL⁻¹ of beach-cast *R. okamuræ* (R). Asterisks indicate significant differences between treatments.

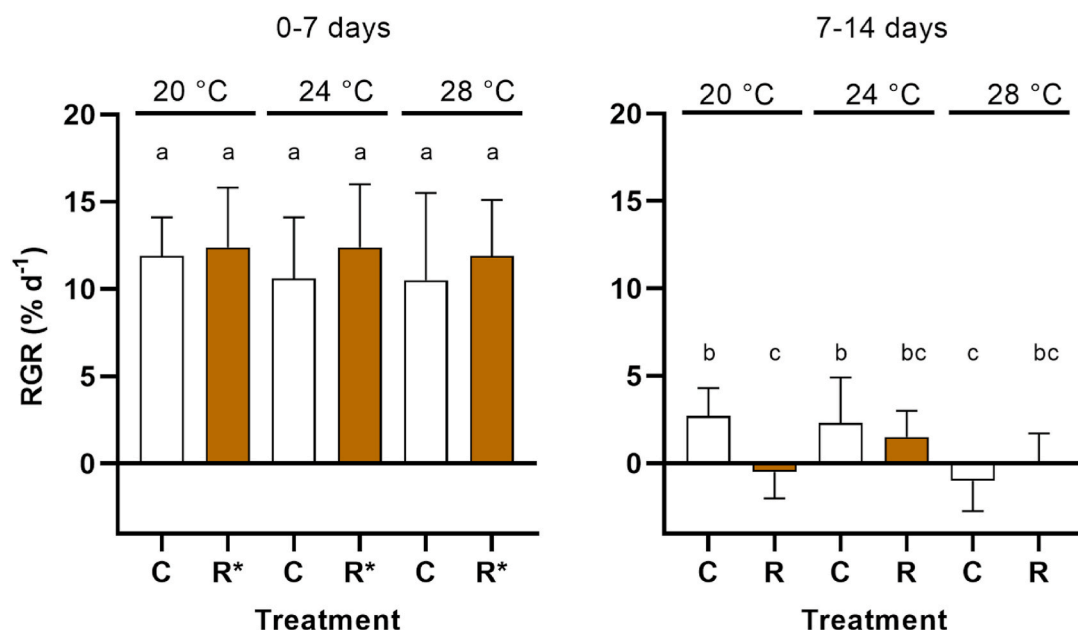


Fig. 5. Relative growth rates (RGR) of juveniles of *Ericaria selaginoides* (n = 10–12) between 0-7 and 7–14 d after fertilization, under two treatments: control (C) and exposed to 8 mg FW mL⁻¹ of beach-cast *Rugulopteryx okamuræ* (R) in combination with three temperature levels (20, 24 and 28 °C). Superscript letters indicate sub-homogeneous groups derived from Tukey’s HSD *post-hoc* comparisons within each time interval. Data are mean ± SD. Asterisks indicate that BCRo was not added for juveniles until day 7 A F.

Table 2

Two-way ANOVA results for the growth rates of juveniles of *Ericaria selaginoides* (n = 10–12) between 0-7 and 7–14 d after fertilization, under two treatments: control (C) and exposed to 8 mg FW mL⁻¹ of beach-cast *Rugulopteryx okamuræ* (R) in combination with three temperature levels (20, 24 and 28 °C).

Time interval	Source of variation	df	F	P	Rank order of main factors
0-7	Treatment	1	2.87	0.094	
	Temperature	2	0.51	0.602	
	Treatment × Temperature	2	0.31	0.734	
7-14	Treatment	1	6.51	0.012	C > R
	Temperature	2	12.6	< 0.001	20 = 24 > 28
	Treatment × Temperature	2	8.54	< 0.001	[C ₍₂₀₋₂₄₎] ≥ R ₍₂₄₎ ≥ [R ₍₂₀₋₂₈₎] ≥ C ₍₂₈₎
	Temperature				

Bold fonts indicate the statistical significance for each factor. Rank order of main factor is derived from sub-homogeneous groups of data obtained by Tukey’s HSD *post-hoc* comparisons.

accumulations of BCRo might be interfering with larval settlement of *Patella ferruginea* (Gmelin, 1791) (CAGPDS, 2019). Since we used small amounts of BCRo in our *in vitro* approach, the actual effects could be much bigger and act together with other competitive traits of this species, environmental stressors, and pollutants.

Our results also pointed out the negative effect of warming especially on juveniles of *E. selaginoides* and showed that BCRo effects were not additive to warming. For instance, within the BCRo treatment, juveniles grew similar but slower at all temperatures; in contrast, in its absence, juveniles of *E. selaginoides* grew optimally at 20 °C and showed an upper thermal limit at 28 °C. These responses to warming were like those reported for other *Cystoseira* s.l. species, being unable to survive at 28 °C (Bernal-Ibáñez et al., 2022b; Falace et al., 2021; Verdura et al., 2021). Within the Northern Alboran Sea, this temperature corresponded to the RCP 8.5 warming scenario and the extreme historical temperature (Campos-Cáliz et al., 2019). However, these authors also revealed that thermal sensitivity of *E. selaginoides* varied along an Atlantic-Mediterranean thermal gradient. Although we obtained growth values in juveniles like those reported by Campos-Cáliz et al. (2019)

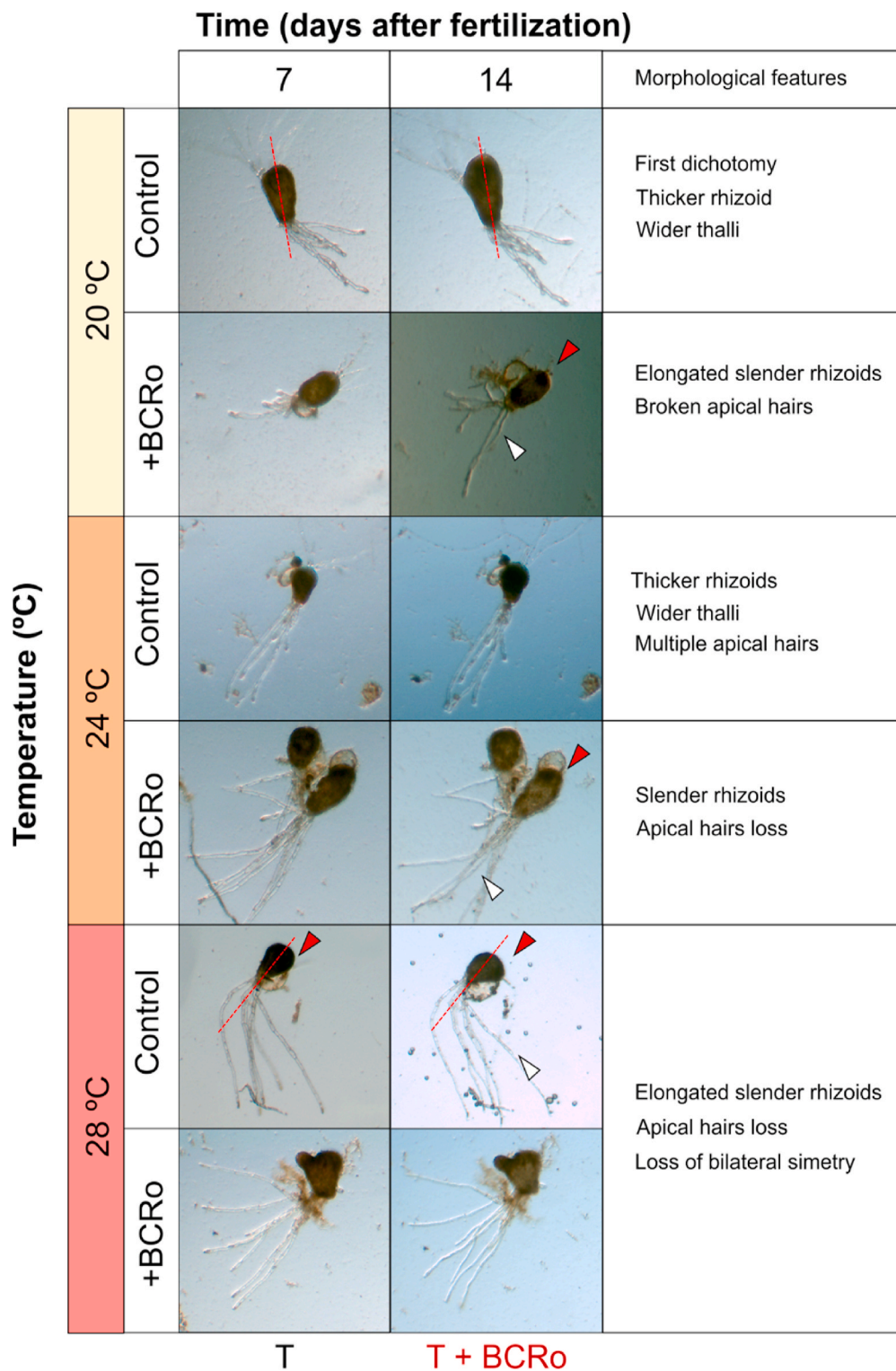


Fig. 6. Morphological features of juveniles of *E. selaginoides* grown for 14 d under the combination of three temperatures (T, 20, 24, 28 °C) and with and without exposure to 4 mg FW mL⁻¹ of the invasive beach-cast *R. okamurai* (BCRo). The treatment T + BCRo indicates the combination of both variables. Note that BCRo was present in the culture plates between 7 and 14 d after fertilization. Red arrows indicate loss or absence of apical hairs. White arrows indicate slender and debilitated rhizoids. Dashed red lines depict the axis of bilateral symmetry for the optimum (20 °C, control) and less favourable conditions (28 °C). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

between 20 and 24 °C (RGR 0–14–7.3 % d⁻¹), that study indicated positive but reduced growth for juveniles at 28 °C. These differences might be attributed to the different season at which the specimens were collected (winter 2019 vs. late-summer 2021). Seasonality plays a major role in modulating thermal sensitivities of early life stages which might

be indicative of a greater warming tolerance in winter recruits, as occur with another intertidal fucoid of this region, *Fucus limitaneus* (Montagne) Montagne (Melero-Jiménez et al., 2017; Sánchez de Pedro et al., 2022, 2023).

Ocean acidification can negatively affect recruitment of some

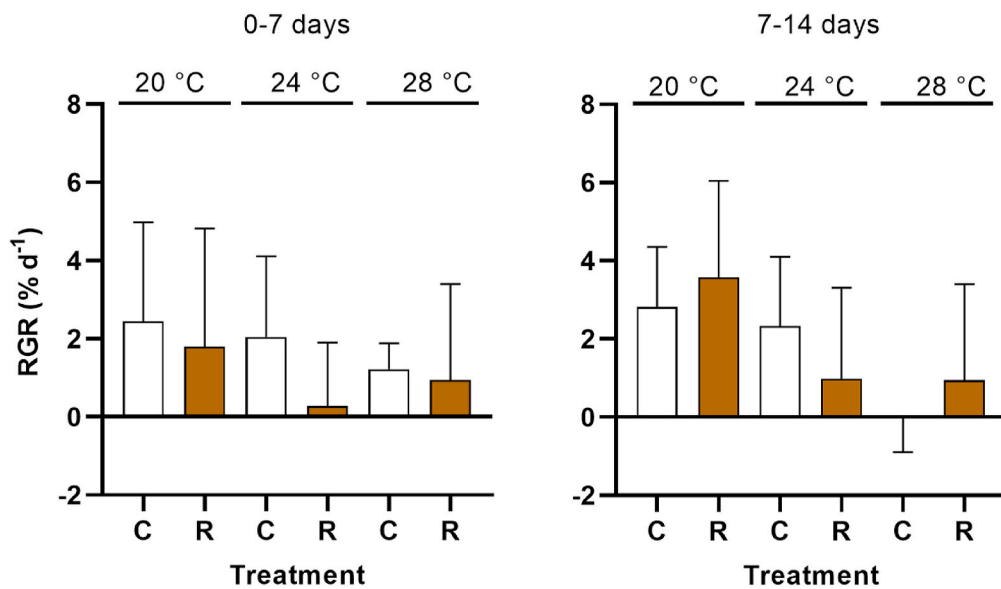


Fig. 7. Relative growth rates of adult apical tips of *Ericaria selaginoides* ($n = 6$) calculated between two-time intervals (0–7, 7–14 d), under control (C) and exposed to 4 mg FW mL⁻¹ of beach-cast *Rugulopteryx okamuræ* (R) in combination with three warming conditions (20, 24, 28 °C).

Table 3

Two-way repeated measures ANOVA results for the growth rates of adult apical tips of *Ericaria selaginoides* ($n = 6$) between two-time intervals (0–7, 7–14 d) under control (C) and exposed to 4 mg FW mL⁻¹ of beach-cast *Rugulopteryx okamuræ* (BCRo) in combination with three warming conditions (20, 24, 28 °C).

Source of variation	df	F	P	Rank order of main factors
Treatment	1	4.62	0.040	C > BCRo
Temperature	2	5.70	0.008	20 > 24 = 28
Treatment × temperature	2	2.48	0.101	–
Time interval	1	28.7	< 0.001	0–7 > 7–14
Time × Treatment	1	3.64	0.066	–
Time × Temperature	2	4.50	0.020	[0–7 (20=24=28)] ≥ 7–14 (20) ≥ 7–14 (24) ≥ 7–14 (28)
Time × Treatment × Temperature	2	2.66	0.086	–

Bold fonts indicate the statistical significance for each factor. Rank order of the main factor is derived from sub-homogeneous groups of data obtained by Tukey's HSD *post-hoc* comparisons.

seaweeds by weakening and delaying spore adhesion (Guenther et al., 2018), but sensitivities depend upon species, with higher impacts on calcifying species (Kroeker et al., 2010), and its early life stages (Ordoñez et al., 2019). Our study demonstrated that small amounts of BCRo in a closed system could acidify water from 8.2 to 7.2 within 24 h. This change has been previously mainly attributed to the release of exudates and intracellular compounds from decomposing and broken tissues of other brown algae (Sieburth and Jensen, 1969). Such exudates can induce significant changes in seawater chemistry for some species, altering the structure and function of associated microbial communities (Lozada et al., 2021; Paine et al., 2021). The acidification of NSW was initially explored based on the low vacuolar pH of many Dictyotales (up to 0.5) due to the presence of H₂S (Sasaki et al., 1999). This implies that BCRo might acidify surrounding waters, particularly in enclosed areas such as bays, which would agree with the reported impacts of *R. okamuræ* on coralligenous communities (Sempere-Valverde et al., 2021). Using decomposing and non-photosynthetically active thalli of BCRo also allowed us to discard the interference of the algal metabolism itself and to eliminate a possible confounding effect by resource competition (i.e., nutrients, light) (Carpenter, 1990). Contrary to our expectations, we found that a pH of 7.2 (the lowest value reached after 24 h incubation of BCRo) promoted juvenile growth and no visible

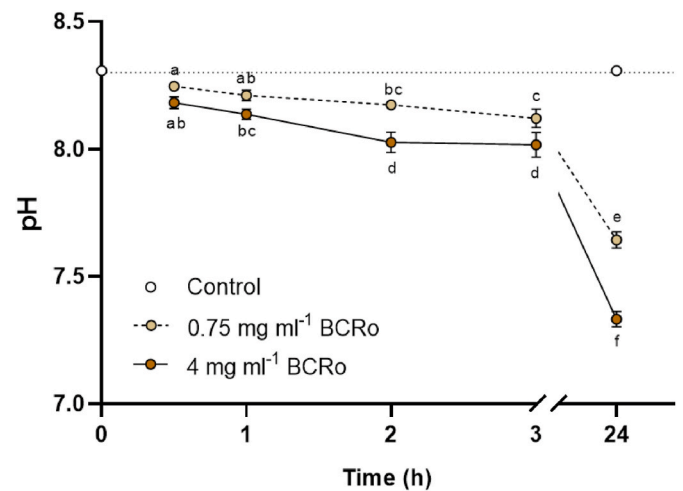


Fig. 8. Time course of pH of natural seawater between 0 and 3 h and 24 h after addition of beach-cast *Rugulopteryx okamuræ* (BCRo) at two concentrations (0.75 vs. 4 mg FW mL⁻¹), in a total volume of 10 mL. Superscript letters indicate sub-homogeneous groups derived from Tukey's HSD *post-hoc* comparisons.

differences in adhesion were observed between pH levels. This suggested that the lethal and physiologically limiting effects of BCRo on *E. selaginoides* should be driven by the action of its secondary metabolites rather than through changes in pH. Nevertheless, it cannot be ruled out that other indirect variables that have not been yet addressed might mediate these responses, as for example indirect microbiome modifications by algal exudates (Haas et al., 2013) or decomposing algal biomass (Quilliam et al., 2014).

Chemically mediated interactions have been reported between macroalgae and different life stages of several marine benthic organisms (Longo and Hay, 2017; Jormalainen and Honkanen, 2008; Paul et al., 2011; Paul and Puglisi, 2004). Here we reported new evidence to this field, evidencing a prominent negative *in vitro* effect of BCRo on microscopic life stages of *E. selaginoides*. *Rugulopteryx okamuræ* present more than 20 secondary metabolites, such as dictyterpenoids, sesquiterpenes and unique newly discovered compounds, all of them with the

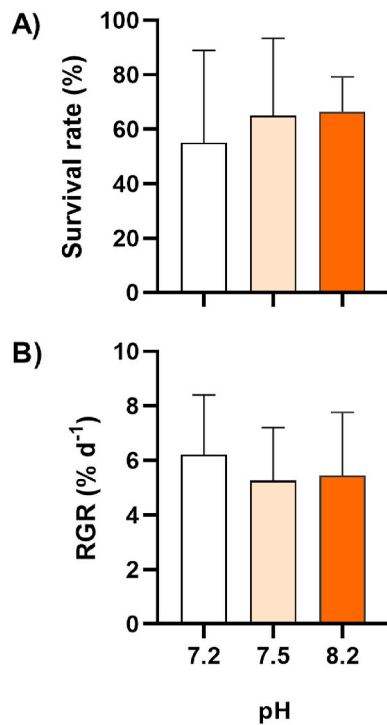


Fig. 9. Survival and relative growth rates (RGR) of embryos of *Ericaria selaginoides* at natural pH of seawater (8.2) slightly acidified NSW (pH 7.5) and acidified medium (pH 7.2, mean final pH detected in seawater) 14 days after fertilization. Bars are mean \pm SD (n = 3).

potential to chemically interact with other marine organisms (Casal-Porras et al., 2021; Cuevas et al., 2021; Paul et al., 2011; Suzuki et al., 2002). Terpenoids from Dictyotales possess feeding-deterrent activity for generalist herbivores (Casal-Porras et al., 2021; Suzuki et al., 2002), although recent studies show the capacity of sea urchins to feed on *R. okamurai* (Hachero-Cruzado et al., 2024). Those terpenoids are used in medicine as cytotoxic for anticancer therapy as cell-cycle stopper and cellular apoptosis inducer (Chen et al., 2010; Prakash, 2018). In this regard, we hypothesize that cytotoxic chemicals derived from *R. okamurai* induced cell-death in the most sensitive oospheres of *E. selaginoides*, and to a lesser extent on successive developmental stages. A lethal dose 50 of 0.25 mg FW mL⁻¹ of BCRO after 30 min of exposure, and a DL of approximately 3 mg FW mL⁻¹ when the exposure time was extended up to 60 min, represents a significant threat to *E. selaginoides* recruitment. Recent studies demonstrated the presence of six overlapping cohorts of *R. okamurai* throughout the year, with individual densities ranging from 3285 to 888 individuals m⁻². Biomass increased from November (approximately 15 g DW m⁻²) to July, reaching a peak of 170 g DW m⁻² (Rosas-Guerrero et al., 2025). This suggests that *E. selaginoides* populations could be permanently exposed to higher lethal doses for their oospheres, considering that the doses tested in the laboratory were based on fresh weight. According to our measurements (*pers. obs.*), DW was 42.33 % of DW for BCRO ($R^2 = 0.56$, n = 30), which means these figures would equal ca. 35–401 g FW m⁻².

We provided a microscopic and morphological approach to the cell-death process of unfertilized oospheres of *E. selaginoides* (Suppl. Video, see also Fig. 2). Mechanisms regulating programmed cell-death are highly evolutionarily conserved (Kasuba et al., 2015). Underlying molecular and biochemical pathways and types of cell-death (i.e., apoptotic, necrotic) are well-described in animal cells, and to a lesser extent in plant cells (Reape and McCabe, 2010). Despite they undergo distinct morphological and biochemical changes or “hallmarks”, they share many others. For algae, cell-death has been mainly studied in cyanobacteria and microalgae (Kasuba et al., 2015), but not for single-

and few-celled stages of macroalgae. Nagasato et al. (2001) detected the mechanism of nuclei degeneration in two brown algae species, where hydrolysis of cell contents is a natural mechanism. Here, we identified several key morphological changes during the cell-death of oospheres of *E. selaginoides* exposed to BCRO. Exocytosis is a process already documented for fucoid gametes (Allen and Brawley, 1984), and in eukaryotic cells this process is triggered by Ca²⁺, which occurs after rupture of the plasma membrane (Cooper and McNeil, 2015). In this line of reasoning, cytotoxic effects of terpenoids have been directly associated with plasma membrane disruption and oxidative stress responses (i.e., lipid peroxidation, ROS, mitochondrial impairment) (Agus, 2021). In parallel, overload of Ca²⁺ inside the cell leads to mitochondrial dysfunction and further ROS production (Shubin et al., 2016). Similar effects have been reported for the group of “pore-forming toxins”, a group of bacterial-derived toxins that act as ions channels, thus triggering vacuolization and osmotic stress (Shubin et al., 2016). The mode of action of terpenoids for plasma membrane disruption has been investigated in other unicellular eukaryotic cells (i.e., yeasts). Due to their lipophilic nature, terpenoids can perforate the plasma membrane lipid bilayer, disconnecting the cell wall and the cytoplasm and causing cell wall damage (Martínez et al., 2014). Among seaweeds, exposure to other toxic lipid substances (i.e., oil) increased cell membrane permeability and damaged tissues (Reddin and Prendeville, 1981).

We found two distinct morphological cell-death patterns depending upon the speed of exocytosis, both with the same fate of non-surviving oospheres. Under slow exocytosis, the cytoplasm shrunk, and plasma membrane rearranged to the new cell size, while under surge exocytosis (30 s), we reported plasma membrane disruption and cytolysis. The reported morphological changes were in good agreement with the hallmarks of apoptotic-like cell-death in plants. Chiboub et al. (2021) also detected apoptotic-like cell death in two unicellular parasites when exposed to diterpenoids of *Dictyota spiralis* Montagne, where the cell-death was defined by these authors as “instantaneous and catastrophic”, just as it occurred in *E. selaginoides* oospheres. In the “surge” cell-death, the plasma membrane disruption and cell lysis could lie into the category of necrotic-like cell death. Several morphological forms were reported during massive cell-death in the model algae *Chlamydomonas reinhardtii* P. A. Dangeard (Yordanova et al., 2010), where the cell form depended on the external inducer. In our study, the two distinct responses might be due to the simultaneous action and probable synergistic effects of the different and abundant terpenoids of *R. okamurai*.

A developmental factor explaining the extremely lethal effect of BCRO on oospheres might be their lack of cell wall (Levring, 1952). Cell wall and cellulose is formed in fucoids ~20 min after fertilization (Stevens, 1974) and increases in thickness over ~16 h prior to polarization and settlement. This implies that oospheres were less protected than zygotes or embryos from any external stressor, including chemical interactions, explaining why we needed longer exposures and BCRO concentrations to detect significant impacts. Nevertheless, lethal dosage for each terpene depends upon time and dose (Agus, 2021), a fact we also detected in our experiments. Similar deleterious effects have been reported for larval stages of marine invertebrates by other Dictyotales (Olsen et al., 2015; Paul et al., 2011). Moreover, direct exposure to living thalli of another Dictyotales had the same effect than extracts on coral recruit survival (Kuffner et al., 2006). That would suggest that terpenoids can keep their action outside of living cells. Since we used decomposing biomass from BCRO, release of such cytotoxic terpenoids might have been facilitated, a fact already suggested by Mateo-Ramírez et al. (2023). While these authors reported photosynthetically active thalli of *R. okamurai* at bathyal habitats, *R. okamurai* used in our study was decomposed, probably because of the environmental stress of beach-cast biomass at the eulittoral zone. A combination of the experimental approaches of Paul et al. (2011), with the isolation of key cytotoxic compounds from *R. okamurai* (Casal-Porras et al., 2021; Cuevas et al., 2021) together with ultramicroscopic and biochemical evaluations will contribute to elucidate the relative effect of these

chemical weapons on microscopic stages of marine organisms.

5. Conclusion

The beach-cast thalli of the invasive seaweed *Rugulopteryx okamurae* (BCRo) had severe adverse effects on the early life stages of the native, ecosystem-structuring seaweed *E. selaginoides*.

- **Rapid Cell Death:** Oospheres exposed to BCRo underwent rapid cell death within less than 3 min.
- **Inhibited Germination and Survival:** Zygote germination and embryo survival decreased significantly by 36 % and 50 %, respectively.
- **Temperature Independence:** Increasing the temperature from 20 °C to 28 °C did not exacerbate the negative effects of BCRo on the growth rate of 7-day-old juvenile thalli.
- **Stage-Specific Vulnerability:** While adult thalli of *E. selaginoides* exhibited greater resilience, early developmental stages proved highly susceptible to BCRo exposure.

These findings underscore the severe impact of *R. okamurae* on the early growth stages of *E. selaginoides*, providing a potential explanation for the rapid expansion of *R. okamurae* and its displacement of native structural species in the Strait of Gibraltar and adjacent areas of the Western Mediterranean.

CRedit authorship contribution statement

Emma Gálvez-Pastor: Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis. **Raquel Sánchez de Pedro:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **María Jesús García-Sánchez:** Writing – review & editing, Resources. **Antonio Flores-Moya:** Writing – review & editing, Resources, Funding acquisition. **Elena Bañares-España:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

☒ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Antonio Flores-Moya reports financial support was provided by Ministerio de Ciencia e Innovación, Spain. Emma Gálvez-Pastor reports financial support was provided by University of Málaga. Elena Bañares-España reports financial support was provided by Instituto de Estudios Ceutíes. Raquel Sánchez de Pedro reports financial support was provided by Ministerio de Ciencia e Innovación, Spain. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.marenvres.2025.107105>.

Data availability

Data will be made available on request.

References

- Agus, H.H., 2021. Chapter 4 - terpene toxicity and oxidative stress. In: Patel, V.B., Preedy, V.R.B.T.-T. (Eds.), *Toxicology. Oxidative Stress and Dietary Antioxidants*. Academic Press, pp. 33–42. <https://doi.org/10.1016/B978-0-12-819092-0.00004-2>.
- Alestra, T., Schiel, D.R., 2015. Impacts of local and global stressors in intertidal habitats: influence of altered nutrient, sediment and temperature levels on the early life history of three habitat-forming macroalgae. *J. Exp. Mar. Biol. Ecol.* 468, 29–36. <https://doi.org/10.1016/j.jembe.2015.03.017>.
- Allen, N.S., Brawley, S.H., 1984. Observations of exocytosis in *Fucus vesiculosus* gametes using video-enhanced light microscopy: a video report. *Cell. Motil.* 4, 25–27. <https://doi.org/10.1002/cm.970040104>.
- Altamirano, M., Flores-Moya, A., Figueroa, F.L., 2003a. Effects of UV radiation and temperature on growth of germlings of three species of *Fucus* (Phaeophyceae). *Aquat. Bot.* 75, 9–20. [https://doi.org/10.1016/S0304-3770\(02\)00149-3](https://doi.org/10.1016/S0304-3770(02)00149-3).
- Altamirano, M., Flores-Moya, A., Kuhlenskamp, R., Figueroa, F.L., 2003b. Stage-dependent sensitivity to ultraviolet radiation in zygotes of the brown alga *Fucus serratus*. *Zygote* 11, 101–106. <https://doi.org/10.1017/S0967199403002132>.
- Bachot, X., Riera, R., 2025. How the invasive algae *Rugulopteryx okamurae* affect coastal biodiversity? Insights from coastal fish communities of gran Canaria (NE Atlantic Ocean). *J. Sea. Res.*, 102568.
- Bermejo, R., Chefaoui, R.M., Engelen, A.H., Buonomo, R., Neiva, J., Ferreira-Costa, J., Pearson, G.A., Marbà, N., Duarte, C.M., Airoldi, L., Hernández, I., Guiry, M.D., Serrão, E.A., 2018. Marine forests of the Mediterranean-Atlantic *Cystoseira tamariscifolia* complex show a southern Iberian genetic hotspot and no reproductive isolation in parapatry. *Sci. Rep.* 8, 1–13. <https://doi.org/10.1038/s41598-018-28811-1>.
- Bermejo, R., de la Fuente, G., Ramírez-Romero, E., Vergara, J.J., Hernández, I., 2016. Spatial variability and response to anthropogenic pressures of assemblages dominated by a habitat forming seaweed sensitive to pollution (northern coast of Alboran Sea). *Mar. Pollut. Bull.* 105, 255–264. <https://doi.org/10.1016/j.marpolbul.2016.02.017>.
- Bernal-Ibáñez, A., Chebaane, S., Sempere-Valverde, J., Faria, J., Ramalhosa, P., Kaufmann, M., Florido, M., Albert-Fonseca, A., Canning-Clode, J., Gestoso, I., Cacabelos, E., 2022a. A worrying arrival: the first record of brown macroalga *Rugulopteryx okamurae* in Madeira Island and its invasive risk. *BioInvasions Rec* 11, 912–924. <https://doi.org/10.3391/bir.2022.11.4.10>.
- Bernal-Ibáñez, A., Gestoso, I., Ramalhosa, P., Campanati, C., Cacabelos, E., 2022b. Interaction of marine heatwaves and grazing on two canopy-forming algae. *J. Exp. Mar. Biol. Ecol.* 556, 151795. <https://doi.org/10.1016/j.jembe.2022.151795>.
- Brawley, S.H., Johnson, L.E., 1991. Survival of fucoid embryos in the intertidal zone depends upon developmental stage and microhabitat. *J. Phycol.* 27, 179–186.
- Buonomo, R., Assis, J., Fernandes, F., Engelen, A.H., Airoldi, L., Serrão, E.A., 2017. Habitat continuity and stepping-stone oceanographic distances explain population genetic connectivity of the brown alga *Cystoseira amantacea*. *Mol. Ecol.* 26, 766–780.
- CAGPDS, 2019. Programa de gestión sostenible del medio marino. Consejería de Agricultura, Ganadería, Pesca y Desarrollo Sostenible, Informe Regional. Sevilla, Spain.
- Campos-Cáiz, A., Fernández, A.N., Sánchez de Pedro, R., Bañares-España, E., 2019. Physiological responses of adults and juveniles of *Cystoseira tamariscifolia* to projected warming scenarios along Alboran Sea populations. In: II International Congress of Young Marine Researchers, pp. 426–430.
- Carpenter, R.C., 1990. Competition among marine macroalgae: a physiological perspective review. *J. Phycol.* <https://doi.org/10.1111/j.0022-3646.1990.00006.x>.
- Casal-Porras, I., Zubía, E., Brun, F.G., 2021. Dilkamural: a novel chemical weapon involved in the invasive capacity of the alga *Rugulopteryx okamurae* in the Strait of Gibraltar. *Estuar. Coast. Shelf. Sci.* 257, 107398. <https://doi.org/10.1016/j.ecss.2021.107398>.
- Castro, N., Ramalhosa, P., Cacabelos, E., Costa, J.L., Canning-Clode, J., Gestoso, I., 2021. Winners and losers: prevalence of non-indigenous species under simulated marine heatwaves and high propagule pressure. *Mar. Ecol. Prog. Ser.* 668, 21–38. <https://doi.org/10.3354/meps13710>.
- Cebrián, E., Linares, C., Marschal, C., Garrabou, J., 2012. Exploring the effects of invasive algae on the persistence of gorgonian populations. *Biol. Invasions* 14, 2647–2656.
- Celis-Plá, P.S.M., 2015. Vulnerability and acclimation of Mediterranean Sea macroalgae, to environmental stress related to climate change: use of indicators physiological state. Universitat. de Barcelona, p. 304. Doctoral thesis. <http://hdl.handle.net/2445/67327>. B 26074-2015.
- Celis-Plá, P.S., Hall-Spencer, J.M., Horta, P.A., Milazzo, M., Korbee, N., Cornwall, C.E., Figueroa, F.L., 2015. Macroalgal responses to ocean acidification depend on nutrient and light levels. *Front. Mar. Sci.* 2, 26.
- Chan, F.T., Briski, E., 2017. An overview of recent research in marine biological invasions. *Mar. Biol.* 164, 121.

- Chen, H.-L., Lin, K.-W., Huang, A.-M., Tu, H.-Y., Wei, B.-L., Hour, T.-C., Yen, M.-H., Pu, Y.-S., Lin, C.-N., 2010. Terpenoids induce cell cycle arrest and apoptosis from the stems of *Celastrus kusanai* associated with reactive oxygen species. *J. Agric. Food Chem.* 58, 3808–3812.
- Chiboub, O., Sifaoui, I., Abderrabba, M., Mejri, M., Fernández, J.J., Díaz-Marrero, A.R., Lorenzo-Morales, J., Piñero, J.E., 2021. Apoptosis-like cell death upon kinetoplastid induction by compounds isolated from the brown algae *Dictyota spiralis*. *Parasit. Vectors* 14, 1–13.
- Clousing, R.J., De La Fuente, G., Falace, A., Chiantore, M., 2023. Accounting for environmental stress in restoration of intertidal foundation species. *J. Appl. Ecol.* 60, 305–318. <https://doi.org/10.1111/1365-2664.14334>.
- Coelho, S.M., Rijstenbil, J.W., Brown, M.T., 2000. Impacts of anthropogenic stresses on the early development stages of seaweeds. *J. Aquat. Ecosyst. Stress. Recover.* 7, 317–333.
- Conde, F., Seoane, J.A., 1983. Aspectos de la vegetación y zonación macrofitobentónica en las costas malagueñas. *Anales. Jard. Bot. Madrid* 39, 465–487.
- Cooper, S.T., McNeil, P.L., 2015. Membrane repair: mechanisms and pathophysiology. *Physiol. Rev.* 95, 1205–1240.
- Crooks, J.A., 2002. Characterizing ecosystem-level consequences of biological invasions: the role of ecosystem engineers. *Oikos* 97, 153–166.
- Cuevas, B., Arroba, A.I., de los Reyes, C., Gómez-Jaramillo, L., González-Montelongo, M. C., Zubía, E., 2021. Diterpenoids from the brown alga *Rugulopteryx okamurae* and their anti-inflammatory activity. *Mar. Drugs* 19. <https://doi.org/10.3390/md19120677>.
- Davidson, A.D., Hewitt, C.L., Kashian, D.R., 2015. Understanding acceptable level of risk: incorporating the economic cost of under-managing invasive species. *PLoS. One* 10, e0141958.
- de Caralt, S., Verdura, J., Vergés, A., Ballesteros, E., Cebrián, E., 2020. Differential effects of pollution on adult and recruits of a canopy-forming alga: implications for population viability under low pollutant levels. *Sci. Rep.* 10. <https://doi.org/10.1038/s41598-020-73990-5>.
- De Paula, J.C., Vallim, M.A., Teixeira, V.L., 2011. What are and where are the bioactive terpenoid metabolites from Dictyotaceae (Phaeophyceae). *Rev. Bras. Farmacogn.* 21, 216–228.
- Díaz-Tapia, P., Alvirte, N., Bañón, R., Barreiro, R., Barrientos, S., Bustamante, M., Carrasco, S., Cremades, J., Iglesias, S., López-Rodríguez, M., Mugerza, N., Piñero-Corbeira, C., Quintano, E., Tajadura, F.J., Díez, I., 2025. Multiple introduction events expand the range of the invasive brown alga *Rugulopteryx okamurae* to northern Spain. *Aquatic Botany* 196, 103830. <https://doi.org/10.1016/j.aquabot.2024.103830>.
- EC, 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. *Official. Journal. L* 327, 22/12/2000.
- El Aamri, F., Idhalla, M., Tamsouri, M.N., 2018. Occurrence of the invasive brown seaweed *Rugulopteryx okamurae* (E.Y.Dawson) I.K.Hwang, W.J.Lee & H.S.Kim (Dictyotales, phaeophyta) in Morocco (Mediterranean sea). *Mediterr. Fish. Aquac. Res.* 1, 92–96.
- El Madany, M., Hassoun, M., El Amri, F., El Mtili, N., 2023. Recent occurrence and expansion of the non-indigenous alga *Rugulopteryx okamurae* in Morocco (Mediterranean and Atlantic shores). *Aquat. Bot.* 130784 <https://doi.org/10.1016/j.aquabot.2023.103722>.
- Falace, A., Kaleb, S., De La Fuente, G., Asnaghi, V., Chiantore, M., 2018. *Ex situ* cultivation protocol for *Cystoseira amenacea* var. *stricta* (Fucales, Phaeophyceae) from a restoration perspective. *PLoS. One* 13, 1–16. <https://doi.org/10.1371/journal.pone.0193011>.
- Falace, A., Marletta, G., Savonitto, G., Candotto Carniel, F., Srijemsi, M., Bevilacqua, S., Tretiac, M., Alongi, G., 2021. Is the south-Mediterranean canopy-forming *Ericaria giaeconae* (= *Cystoseira hyblaee*) a loser from ocean warming? *Front. Mar. Sci.* 8, 1–12. <https://doi.org/10.3389/fmars.2021.760637>.
- Faria, J., Prestes, A.C.L., Moreu, I., Cacabelos, E., Martins, G.M., 2022. Dramatic changes in the structure of shallow-water marine benthic communities following the invasion by *Rugulopteryx okamurae* (Dictyotales, Ochrophyta) in Azores (NE Atlantic). *Mar. Pollut. Bull.* 175. <https://doi.org/10.1016/j.marpolbul.2022.113358>.
- Flores-Moya, A., Moreno, D., de la Rosa, J., Altamirano, M., Bañares-España, E., 2021. Seaweeds and seagrasses: the marine forests from the Alboran Sea. In: Báez, J.C., Vázquez, J.T., Camiñas, J.A., Malouli Idrissi, M. (Eds.), *Alboran Sea - Ecosystems and Marine Resources*. Springer, Cham, pp. 247–284. https://doi.org/10.1007/978-3-030-65516-7_8.
- Gaertner, M., Biggs, R., Te Beest, M., Hui, C., Molofsky, J., Richardson, D.M., 2014. Invasive plants as drivers of regime shifts: identifying high-priority invaders that alter feedback relationships. *Divers. Distrib.* 20, 733–744.
- García-Gómez, J.C., Florido, M., Olaya-Ponzzone, L., Sempere-Valverde, J., Megina, C., 2021a. The invasive macroalga *Rugulopteryx okamurae*: substrata plasticity and spatial colonization pressure on resident macroalgae. *Front. Ecol. Evol.* 9. <https://doi.org/10.3389/fevo.2021.631754>.
- García-Gómez, J.C., Florido, M., Olaya-Ponzzone, L., Rey Díaz de Rada, J., Donazar-Aramendía, I., Chacón, M., Quintero, J.J., Magariño, S., Megina, C., 2021b. Monitoring extreme impacts of *Rugulopteryx okamurae* (Dictyotales, Ochrophyta) in El estrecho natural park (biosphere reserve). Showing radical changes in the underwater seascape. *Front. Ecol. Evol.* 9. <https://doi.org/10.3389/fevo.2021.639161>.
- García-Gómez, J.C., Sempere-Valverde, J., González, A.R., Martínez-Chacón, M., Olaya-Ponzzone, L., Sánchez-Moyano, E., Ostalé-Valriberas, E., Megina, C., 2020. From exotic to invasive in record time: the extreme impact of *Rugulopteryx okamurae* (Dictyotales, Ochrophyta) in the strait of Gibraltar. *Sci. Total. Environ.* 704, 135408. <https://doi.org/10.1016/j.scitotenv.2019.135408>.
- Genty, B., Briantais, J.-M., Baker, N.R., 1989. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochim. Biophys. Acta Gen. Subj.* 990, 87–92. [https://doi.org/10.1016/S0304-4165\(89\)80016-9](https://doi.org/10.1016/S0304-4165(89)80016-9).
- Giakoumi, S., Guilhaumon, F., Kark, S., Terlizzi, A., Claudet, J., Felling, S., Cerrano, C., Coll, M., Danovaro, R., Fraschetti, S., 2016. Space invaders: biological invasions in marine conservation planning. *Divers. Distrib.* 22, 1220–1231.
- Guenther, R., Miklasz, K., Carrington, E., Martone, P.T., 2018. Macroalgal spore dysfunction: ocean acidification delays and weakens adhesion. *J. Phycol.* 54, 153–158.
- Haas, A.F., Nelson, C.E., Rohwer, F., Wegley-Kelly, L., Quistad, S.D., Carlson, C.A., Leichter, J.J., Hatay, M., Smith, J.E., 2013. Influence of coral and algal exudates on microbially mediated reef metabolism. *PeerJ* 1, e108.
- Hachero-Cruzado, I., Castilla-Gavilán, M., Muñoz-Martínez, M., Zuasti, E., Jimenez, T., 2024. Assessing grazing of *Rugulopteryx okamurae* (Ochrophyta) by the sea urchin *Paracentrotus lividus* (Echinodermata): a prospect for the biocontrol of the alien species? *E. J. Phycol.* 59, 332–349.
- Hulme, P.E., 2017. Climate change and biological invasions: evidence, expectations, and response options. *Biol. Rev.* 92, 1297–1313.
- Hwang, I.K., Lee, W.J., Kim, H.S., De Clerck, O., 2009. Taxonomic reappraisal of *Dilophus okamurae* (Dictyotales, phaeophyta) from the western Pacific ocean. *Phycologia* 48, 1–12.
- Jormalainen, V., Honkanen, T., 2008. Macroalgal chemical defenses and their roles in structuring temperate marine communities. In: Amsler, C.D. (Ed.), *Algal Chemical Ecology*. Springer, Verlag/Heidelberg, Germany, pp. 57–89.
- Kasuba, K.C., Vavilala, S.L., D'Souza, J.S., 2015. Apoptosis-like cell death in unicellular photosynthetic organisms—a review. *Algal Res.* 12, 126–133.
- Kroeker, K.J., Kordas, R.L., Crim, R.N., Singh, G.G., 2010. Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms. *Ecol. Lett.* 13, 1419–1434. <https://doi.org/10.1111/j.1461-0248.2010.01518.x>.
- Kuffner, I.B., Walters, L.J., Becerro, M.A., Paul, V.J., Ritson-Williams, R., Beach, K.S., 2006. Inhibition of coral recruitment by macroalgae and cyanobacteria. *Mar. Ecol. Prog. Ser.* 323, 107–117.
- Løvring, T., 1952. Remarks on the submicroscopical structure of eggs and spermatozooids of *Fucus* and related genera. *Physiol. Plant.* 5, 528–539.
- Longo, G.O., Hay, M.E., 2017. Seaweed allelopathy to corals: are active compounds on, or in, seaweeds? *Coral Reefs* 36, 247–253. <https://doi.org/10.1007/s00338-016-1526-9>.
- Lozada, M., Diéguez, M.C., García, P.E., Bigatti, G., Livore, J.P., Giarratano, E., Gil, M.N., Dionisi, H.M., 2021. *Undaria pinnatifida* exudates trigger shifts in seawater chemistry and microbial communities from Atlantic Patagonian coasts. *Biol. Invasions* 23, 1781–1801.
- Mannino, A.M., Balistreri, P., Deidun, A., 2017. The marine biodiversity of the Mediterranean Sea in a changing climate: the impact of biological invasions. *Mediterr. identities-environment. Soc. Cult.* 101–127.
- Marletta, G., Lombardo, A., Serio, D., 2024. First record of the invasive alien species *Rugulopteryx okamurae* (phaeophyceae, Dictyotales) along the eastern coast of Sicily (Italy, Mediterranean sea): is it ready to expand into the Ionian Sea? *Diversity* 16 (7), 424. <https://doi.org/10.3390/d16070424>.
- Martínez, A., Rojas, N., García, L., González, F., Domínguez, M., Catalán, A., 2014. *In vitro* activity of terpenes against *Candida albicans* and ultrastructural alterations. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol.* 118, 553–559. <https://doi.org/10.1016/j.oooo.2014.07.009>.
- Mateo-Ramírez, Á., Iñiguez, C., Fernández-Salas, L.M., Sánchez-Leal, R.F., Farias, C., Bellanco, M.J., Gil, J., Rueda, J.L., 2023. Healthy thalli of the invasive seaweed *Rugulopteryx okamurae* (Phaeophyceae) being massively dragged into deep-sea bottoms by the Mediterranean Outflow Water. *Phycologia* 62, 1–10. <https://doi.org/10.1080/00318884.2023.2177057>.
- Melero-Jiménez, I.J., Salvo, A.E., Báez, J.C., Bañares-España, E., Reul, A., Flores-Moya, A., 2017. North Atlantic Oscillation drives the annual occurrence of an isolated, peripheral population of the brown seaweed *Fucus guiryi* in the Western Mediterranean Sea. *PeerJ* 5, e4048.
- Mercado, J.M., Gómez-Jakobsen, F., Korbee, N., Aviles, A., Bonomi-Barufi, J., Muñoz, M., Reul, A., Figueroa, F.L., 2022. Analyzing environmental factors that favor the growth of the invasive brown macroalga *Rugulopteryx okamurae* (Ochrophyta): the probable role of the nutrient excess. *Mar. Pollut. Bull.* 174. <https://doi.org/10.1016/j.marpolbul.2021.113315>.
- Mogollón, S.L., Zilio, M.I., Buitrago, E.M., et al., 2024. Economic impact of *Rugulopteryx okamurae* (Dictyotales, Ochrophyta) along the andalusian coastline: the case of Tarifa, Spain. *Wetlands. Ecol. Manage.* 32, 19–32. <https://doi.org/10.1007/s11273-023-09951-2>.
- Nagasato, C., Motomura, T., Ichimura, T., 2001. Degeneration and extrusion of nuclei during oogenesis in *Silvetia babingtonii*, *Cystoseira hakodatensis* and *Sargassum confusum* (Fucales, Phaeophyceae). *Phycologia* 40 (1), 411–420. <https://doi.org/10.2216/i0031-8884-40-5-411>.
- Navarro-Barranco, C., Muñoz-Gómez, B., Saiz, D., Ros, M., Guerra-García, J.M., Altamirano, M., Ostalé-Valriberas, E., Moreira, J., 2019. Can invasive habitat-forming species play the same role as native ones? The case of the exotic marine macroalga *Rugulopteryx okamurae* in the Strait of Gibraltar. *Biol. Invasions* 21, 3319–3334. <https://doi.org/10.1007/s10530-019-02049-y>.
- Ninomiya, M., Hirohara, H., Onishi, J., Kusumi, T., 1999. Chemical study and absolute configuration of a new marine secospatane from the Brown alga *Dilophus okamurae*. *J. Org. Chem.* 64, 5436–5440. <https://doi.org/10.1021/jo9902190>.
- Olsen, K., Paul, V.J., Ross, C., 2015. Direct effects of elevated temperature, reduced pH, and the presence of macroalgae (*Dictyota* spp.) on larvae of the Caribbean coral

- Porites astreoides. *Bull. Mar. Sci.* 91, 255–270. <https://doi.org/10.5343/bms.2014.1050>.
- Ordoñez, A., Wangpraseurt, D., Lyndby, N.H., Kühl, M., Diaz-Pulido, G., 2019. Elevated CO₂ leads to enhanced photosynthesis but decreased growth in early life stages of reef building coralline algae. *Front. Mar. Sci.* 5, 1–11. <https://doi.org/10.3389/fmars.2018.00495>.
- Paine, E.R., Schmid, M., Boyd, P.W., Diaz-Pulido, G., Hurd, C.L., 2021. Rate and fate of dissolved organic carbon release by seaweeds: a missing link in the coastal ocean carbon cycle. *J. Phycol.* 57, 1375–1391.
- Paul, V.J., Kuffner, I.B., Walters, L.J., Ritson-Williams, R., Beach, K.S., Becerro, M.A., 2011. Chemically mediated interactions between macroalgae *Dictyota* spp. and multiple life-history stages of the coral *Porites astreoides*. *Mar. Ecol. Prog. Ser.* 426, 161–170. <https://doi.org/10.3354/meps09032>.
- Paul, V.J., Puglisi, M.P., 2004. Chemical mediation of interactions among marine organisms. *Nat. Prod. Rep.* 21, 189–209.
- Patterson, G.M.L., Harris, D.O., Cohen, W.S., 1979. Inhibition of photosynthetic and mitochondrial electron transport by a toxic substance isolated from the alga *Pandorina morum*. *Plant. Sci. Lett.* 15, 293–300.
- Patterson, G., Harris, D.O., 1983. The Effect of *Pandorina morum* (Chlorophyta) toxin on the growth of selected algae, bacteria, and higher plants. *Br. Phycol. J.* 18, 259–266.
- Petechaty, M., Zhapparova, B., Brzozowski, M., Pucak, A., 2022. Impact of *Nitellopsis obtusa* (Desv.) J. Groves, a regionally alien and invasive charophyte, on macrophyte diversity in the species native range. *Hydrobiologia* 849, 63–76.
- Piazzì, L., Balata, D., Foresi, L., Cristaudo, C., Cinelli, F., 2007. Sediment as a constituent of Mediterranean benthic communities dominated by *Caulerpa racemosa* var. *cylindracea*. *Sci. Mar.* 71, 129–135.
- Prakash, V., 2018. Terpenoids as cytotoxic compounds: a perspective. *Pharmacogn. Rev.* 12, 166–176.
- Przeslawski, R., Byrne, M., Mellin, C., 2015. A review and meta-analysis of the effects of multiple abiotic stressors on marine embryos and larvae. *Glob. Chang. Biol.* 21 (6), 2122–2140. <https://doi.org/10.1111/gcb.12833>.
- Quilliam, R.S., Jamieson, J., Oliver, D.M., 2014. Seaweeds and plastic debris can influence the survival of faecal indicator organisms in beach environments. *Mar. Pollut. Bull.* 84, 201–207. <https://doi.org/10.1016/j.marpolbul.2014.05.011>.
- Råberg, S., Berger-Jönsson, R., Björn, A., Granéli, E., Kautsky, L., 2005. Effects of *Pilayella littoralis* on *Fucus vesiculosus* recruitment: implications for community composition. *Mar. Ecol. Prog. Ser.* 289, 131–139. <https://doi.org/10.3354/meps289131>.
- Reape, T.J., McCabe, P.F., 2010. Apoptotic-like regulation of programmed cell death in plants. *Apoptosis* 15, 249–256.
- Reddin, A., Prendeville, G.N., 1981. Effect of oils on cell membrane permeability in *Fucus serratus* and *Laminaria digitata*. *Mar. Pollut. Bull.* 12, 339–342.
- Reynes, L., Aurelle, D., Chevalier, C., Pinazo, C., Valero, M., Mauger, S., Sartoretto, S., Blanfuné, A., Ruitton, S., Boudouresque, C.-F., 2021. Population genomics and Lagrangian modeling shed light on dispersal events in the mediterranean endemic *Ericaria zosteroides* (= *Cystoseira zosteroides*) (Fucales). *Front. Mar. Sci.* 8, 683528.
- Roberts, M., 1970. Studies on marine algae of the British Isles. 8. *Cystoseira tamariscifolia* (Hudson) papenfuss. *Br. Phycol. J.* 5, 201–210. <https://doi.org/10.1080/00071617000650261>.
- Roberts, M., 1967. Studies on marine algae of the British Isles. 4. *Cystoseira baccata* (Gmelin) silva. *Br. Phycol. Bull.* 3, 367–378. <https://doi.org/10.1080/000716167000650251>.
- Roca, M., Dunbar, M.B., Román, A., Caballero, I., Zoffoli, M.L., Gernez, P., Navarro, G., 2022. Monitoring the marine invasive alien species *Rugulopteryx okamurae* using unmanned aerial vehicles and satellites. *Front. Mar. Sci.* 9, 1004012. <https://doi.org/10.3389/fmars.2022.1004012>.
- Rosas-Guerrero, J., Carmona, R., De la Rosa, J., Zanolli, M., Altamirano, M., 2025. A population dynamics approach to understand the invasiveness of the seaweed *Rugulopteryx okamurae* (Ochrophyta, Dictyotales). *NeoBiota* 97, 1–17. <https://doi.org/10.3897/neobiota.97.137873>.
- Ruitton, S., Blanfuné, A., Boudouresque, C.-F., Guillemain, D., Michotey, V., Roblet, S., Thibaut, D., Thibaut, T., Verlaque, M., 2021. Rapid spread of the invasive Brown alga *Rugulopteryx* in a national park in provence (France, mediterranean sea). *Water* 13.
- Sánchez de Pedro, R., Fernández, A.N., García-Sánchez, M.J., Flores-Moya, A., Bañares-España, E., 2023. Parental environment modulates offspring thermal tolerance in a foundational intertidal seaweed. *Eur. J. Phycol.* 58, 121–144. <https://doi.org/10.1080/09670262.2022.2081731>.
- Sánchez de Pedro, R., Fernández, A.N., García-Sánchez, M.J., Flores-Moya, A., Bañares-España, E., 2022. Seasonal and ontogenetic variability in the photosynthetic thermal tolerance of early life-stages of *Fucus guiryi* (Phaeophyceae, Fucales). *Phycologia* 61, 354–362. <https://doi.org/10.1080/00318884.2022.2053640>.
- Sasaki, H., Kataoka, H., Kamiya, M., Kawai, H., 1999. Accumulation of sulfuric acid in Dictyotales (Phaeophyceae): taxonomic distribution and ion chromatography of cell extracts. *J. Phycol.* 35, 732–739.
- Schaffelke, B., Smith, J.E., Hewitt, C.L., 2006. Introduced macroalgae—a growing concern. *J. Appl. Phycol.* 18, 529–541.
- Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.-Y., White, D.J., Hartenstein, V., Eliceiri, K., Tomancak, P., Cardona, A., 2012. Fiji: an open-source platform for biological-image analysis. *Nat. Methods* 9, 676.
- Sempere-Valverde, J., Ostalé-Valriberas, E., Maestre, M., Aranda, R.G., Bazairi, H., Espinosa, F., 2021. Impacts of the non-indigenous seaweed *Rugulopteryx okamurae* on a Mediterranean coralligenous community (Strait of Gibraltar): the role of long-term monitoring. *Ecol. Indic.* 121, 107135.
- Shubin, A.V., Demidyuk, I.V., Komissarov, A.A., Rafieva, L.M., Kostrov, S.V., 2016. Cytoplasmic vacuolization in cell death and survival. *Oncotarget* 7, 55863.
- Sieburth, J.M., Jensen, A., 1969. Studies on algal substances in the sea. II. The formation of Gelbstoff (humic material) by exudates of Phaeophyta. *J. Exp. Mar. Biol. Ecol.* 3, 275–289.
- Sorte, C.J.B., Williams, S.L., Zerebecki, R.A., 2010. Ocean warming increases threat of invasive species in a marine fouling community. *Ecology* 91, 2198–2204.
- Souviron-Priego, L., Márquez, A.L., Korbee, N., Figueroa, F.L., Real, R., 2024. Understanding the invasion of the macroalga *Rugulopteryx okamurae* (Ochrophyta) in the northern Alboran Sea through the use of biogeographic models. *Sci. Total Environ.*, 176851 <https://doi.org/10.1016/j.scitotenv.2024.176851>.
- Stevens, P., 1974. Cell Wall Formation in *Fucus* Zygotes: Cellulose Synthesis and Deposition after Fertilization. Oregon State University. <http://hdl.handle.net/1957/44432>.
- Sudatti, D.B., Duarte, H.M., Soares, A.R., Salgado, L.T., Pereira, R.C., 2020. New ecological role of seaweed secondary metabolites as autotoxic and allelopathic. *Front. Plant Sci.* 11, 347. <https://doi.org/10.3389/fpls.2020.00347>.
- Sun, Z., Hasegawa, K., Tanaka, J., 2006. A morphological study of *Dilophus okamurae* (Dictyotales, Phaeophyceae) in Japan. *Journ. Bot.* 81, 235–244.
- Suzuki, M., Yamada, H., Kurata, K., 2002. Dictyterpenoids A and B, two novel diterpenoids with feeding-deterrent activity from the brown alga *Dilophus okamurae*. *J. Nat. Prod.* 65, 121–125. <https://doi.org/10.1021/np010234b>.
- Tarakhovskaya, E., Lemesheva, V., Bilova, T., Birkemeyer, C., 2017. Early embryogenesis of brown alga *Fucus vesiculosus* L. is characterized by significant changes in carbon and energy metabolism. *Molecules* 22, 1509. <https://doi.org/10.3390/molecules22091509>.
- Vadas, R.L., Johnson, J.S., Norton, T.A., 1992. Recruitment and mortality of early post-settlement stages of benthic algae. *Br. Phycol. J.* 27, 331–351. <https://doi.org/10.1080/00071619200650291>.
- Verdura, J., Sales, M., Ballesteros, E., Cefali, M.E., Cebrián, E., 2018. Restoration of a canopy-forming alga based on recruitment enhancement: methods and long-term success assessment. *Front. Plant Sci.* 1832.
- Verdura, J., Santamaría, J., Ballesteros, E., Smale, D.A., Cefali, M.E., Golo, R., de Caralt, S., Vergés, A., Cebrián, E., 2021. Local-scale climatic refugia offer sanctuary for a habitat-forming species during a marine heatwave. *J. Ecol.* n/a. <https://doi.org/10.1111/1365-2745.13599>.
- Wiencke, C., Gómez, I., Pakker, H., Flores-Moya, A., Altamirano, M., Hanelt, D., Bischof, K., Figueroa, F.L., 2000. Impact of UV-radiation on viability, photosynthetic characteristics and DNA of brown algal zoospores: implications for depth zonation. *Mar. Ecol. Prog. Ser.* 197, 217–229. <https://doi.org/10.3354/meps197217>.
- Worm, B., Lotze, H.K., Sommer, U., 2001. Algal propagule banks modify competition, consumer and resource control on Baltic rocky shores. *Oecologia* 128, 281–293. <https://doi.org/10.1007/s004420100648>.
- Wright, P.J., Reed, R.H., 1990. Effects of osmotic stress on gamete size, rhizoid initiation and germling growth in fucoid algae. *Br. Phycol. J.* 25, 149–155. <https://doi.org/10.1080/00071619000650141>.
- Yordanova, Z.P., Iakimova, E.T., Cristescu, S.M., Harren, F.J., Kapchina-Toteva, V.M., Woltering, E.J., 2010. Involvement of ethylene and nitric oxide in cell death in mastoparan-treated unicellular alga *Chlamydomonas reinhardtii*. *Cell. Biol. Int.* 34, 301–308.