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ORIGINAL ARTICLE

Growth, morphology and reproductive phenology of *Gelidium adriaticum* (Gelidiales, Rhodophyta) from the Slovenian coast (Gulf of Trieste, northern Adriatic)

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Abstract: The occurrence, growth, external morphology and reproductive phenology of a *Gelidium adriaticum* settlement from the midlittoral zone in St. Simon Bay (Slovenian coast, Gulf of Trieste, northern Adriatic Sea) were investigated. The study was based on monthly sampling conducted for one year, from January to December 2023. The studied settlement was well developed in shaded rock crevices, forming dense turfs with a very patchy and irregular distribution. It was characterized by a slight predominance of tetrasporic thalli, with a ratio between the three reproductive stages (tetrasporic:sterile:cystocarpic) of 1.19:1.04:1.00. The mean values of the morphological characteristics studied (height and width of the thallus, width of the widest part of the thallus axis, width of the apical, middle and basal parts of the erect axes) varied between seasons and reproductive stages. In general, these values were lower in the cystocarpic thalli than those in the tetrasporic and sterile stages in all seasons.

Keywords: *Gelidium adriaticum;* midlittoral; growth; morphology; reproductive phenology; Slovenian coast; northern Adriatic

Sažetak: RAST, MORFOLOGIJA I REPRODUKTIVNA FENOLOGIJA VRSTE *GELIDIUM ADRIATICUM* (GELIDIALES, RHODOPHYTA) NA PODRUČJU SLOVENSKE OBALE (TRŠĆANSKI ZALJEV, SJEVERNI JADRAN). Ovaj rad istražuje pojavu, rast, vanjsku morfologiju i reproduktivnu fenologiju naselja vrste *Gelidium adriaticum* u mediolitoralu uvale sv. Šimuna (slovenska obala, Tršćanski zaljev, sjeverni Jadran). Istraživanje je temeljeno na mjesečnim uzorkovanjima provedenim tijekom jedne godine, od siječnja do prosinca 2023. Proučavana populacija bila je dobro razvijena u zasjenjenim pukotinama stijena, tvoreći guste nakupine s vrlo neujednačenom i nepravilnom raspodjelom. Karakterizirala ju je blaga dominacija tetrasporofitnih talusa, s omjerom triju reproduktivnih stadija (tetrasporofitni:sterilni:cistokarpni) od 1,19:1,04:1,00. Prosječne vrijednosti zabilježenih morfoloških obilježja (visina i širina talusa, širina najšireg dijela osi talusa, širina vršnog, srednjeg i bazalnog dijela uspravnih osi) varirale su između sezona i reproduktivnih stadija. Općenito, te su vrijednosti bile niže kod cistokarpnih talusa u odnosu na tetrasporofitne i sterilne taluse u svim godišnjim dobima.

Ključne riječi: *Gelidium adriaticum*; mediolitoral; rast; morfologija; reproduktivna fenologija; slovenska obala; sjeverni Jadran

INTRODUCTION

Gelidium J.V. Lamouroux is a polymorphic genus consisting mainly of highly branched algae with considerable morphological differences between species. Members of *Gelidium* populations are among the most common components of algal communities in the midlittoral zone worldwide, where they form large turfs that provide important habitats for benthic organisms (Schneider and Mann, 1991; Huang *et al*., 2007) and play an important role in sedimentation processes (Airoldi and Cinelli, 1997; Airoldi and Virgilio, 1998; Birrel *et al*., 2005). There are currently 145 taxonomically accepted species belonging to *Gelidium*, including one accepted subspecies, 16 accepted varieties and 10 accepted formae, distributed from the midlittoral to the deep infralittoral of cold to tropical waters worldwide (Guiry and Guiry, 2024). However, the identification of *Gelidium* species is notoriously difficult, mainly because of the high infra-specific morphological variation, especially in the small and morphologically similar species (Womersley and Guiry, 1994; Díaz-Tapia and Bárbara, 2014; Perrone *et al*., 2019). In the last inventory of the macroalgae of the eastern Adriatic coast, seven species and two varieties of the genus *Gelidium* were listed, of which *G. crinale* (Hare ex Turner) Gaillon, *G. minusculum* (Weber Bosse) R.E. Norris, *G. pusillum* (Stackhouse) Le Jolis, and *G. spathulatum* (Kützing) Bornet are characteristic of the midlittoral zone (Antolić *et al*., 2013). Regarding the Slovenian coast, three *Gelidium* species for the midlittoral zone have been reported, including *G. crinale* (Vukovič, 1980, 1982, 1984), *G. pusillum* (Matjašič and Štirn, 1975; Munda, 1991, 1993a, b; Battelli, 2002; Rindi and Battelli, 2005) and *G.*

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spathulatum (Matjašič and Štirn, 1975; Battelli, 2002; Lipej *et al*., 2004).

A new species of the genus, *Gelidium adriaticum* C. Perrone, A. Bottalico, G.H. Boo & S.M. Boo, was recently described based on samples from Torre a Mare (Bari, Italy) (Perrone *et al*., 2019). The epithet "*adriaticum*" refers to the Adriatic Sea, where the species was collected as a holotype. *Gelidium adriaticum*, previously misidentified as *G. pusillum*, is a species of the upper midlittoral zone of the coast of Apulia (Italy) and Koper Bay (Slovenia). Moreover, it was suggested that *G. pusillum* is not present in the Adriatic Sea (Perrone *et al*., 2019).

The aim of this study is to improve our understanding of the biology of *Gelidium adriaticum*, with particular emphasis on habitat preferences, including the geological type of substrate and seasonal dynamics. To achieve this, we conducted a quantitative study of the growth, external morphology and reproductive phenology of the species on the Slovenian coast (Gulf of Trieste, northern Adriatic Sea). The specific goal of the research was to investigate whether seasonality influences the growth, morphology, and reproductive phenology of the different reproductive life stages of this species.

MATERIALS AND METHODS

Study area

The occurrence of *Gelidium adriaticum* along the Slovenian coast (Fig. 1A) was investigated after a preliminary sampling of *Gelidium* populations growing as small clumps or turfs, at randomly selected sites on various substrates (marl, sandstone and limestone) in the midlittoral zone. Preliminary analysis of the collected algal material revealed the presence of several *Gelidium* species, including *G. crinale* and *G. spathulatum,* growing on all these substrates, while the occurrence of *G. adriaticum* was restricted to the limestone substrate in the Izola area. For this study, we selected a sampling site in the bay of St. Simon (45º32'0.2" N, 13º38'51.0" E) (Fig. 1B). The substrate of this site is characterized by an irregular and heterogeneous structure, with many outcrops and fissures of different sizes. This area is mainly influenced by winds from the north-northeast and northwest. During the year 2023, the average monthly water surface temperature in the Gulf of Koper fluctuated between a minimum of 7.6 °C in February and a maximum of 30.1 °C in July, while the average temperature throughout the year was 18.3 °C (ARSO, 2024). Salinity fluctuated between 25.95 in December and 35.17 in April (ARSO, 2024). The tides are mixed. Half-day tides (two high tides and two low tides in one day) and day tides (one high tide and one low tide *per* day) alternate approximately every 15 days. In 2023, the tidal amplitude between the mean highest (MHWL) and the mean lowest water level (MLWL) was 153 cm (+77 and –76 cm), with the mean water level (MWL) set at 0 cm (Fig. 1C; ARSO, 2024).

Field sampling and laboratory procedure

Specimens of *G. adriaticum* were collected monthly at low tide in the upper midlittoral zone from January to December 2023. For the study of seasonal variations in growth, morphology and reproductive phenology, January, February and March were selected for winter, April,

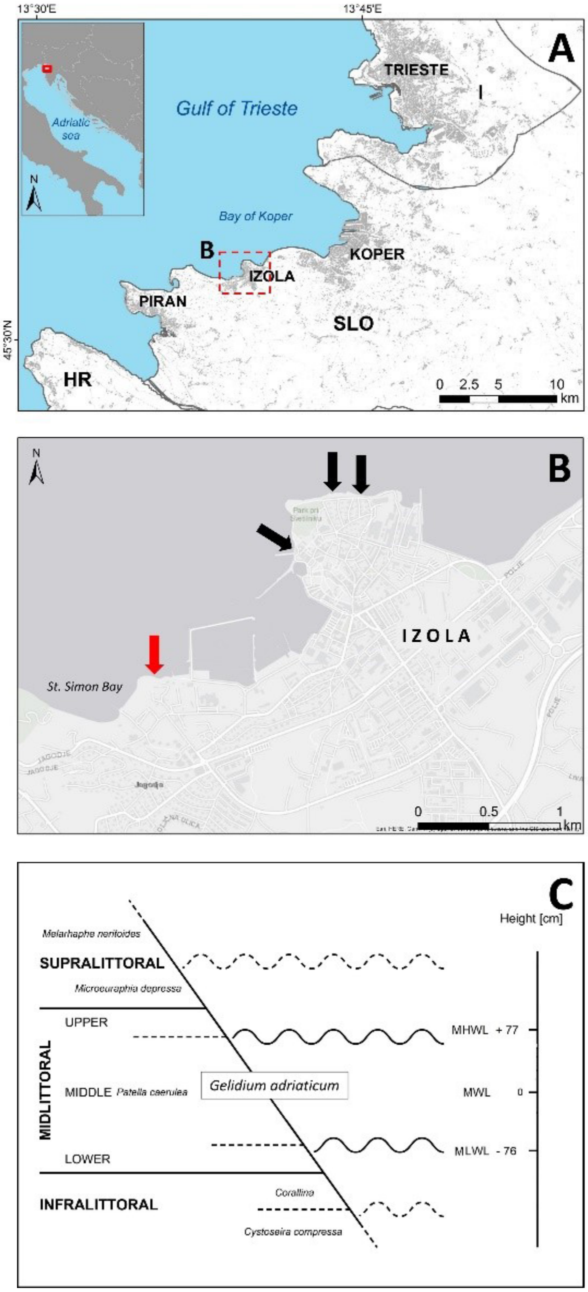

Fig. 1. Map of the study area on the Slovenian coast, with red frame to indicate the location in the wider Adriatic region **(A)**; wider area of occurrence with arrows to indicate sites where the occurrence of *Gelidium adriaticum* was confirmed, the sampling site is located on limestone in the bay of St. Simon (red arrow) near Izola **(B)**; schematic representation of the zonation in the study area with the average sea level in 2023, MHWL - Mean Highest Water Level; MWL - Mean Water Level (0 cm); MLWL - Mean Lowest Water Level, source of data: ARSO (2024) **(C)**.

May and June for spring and July, August and September for summer. October, November and December were selected for autumn, which coincide with the seasonality of sea temperatures in the Gulf of Koper (ARSO, 2024). All specimens were collected from the same settlement. Specimens of *G. adriaticum* were collected with a knife, scraping the algal turfs from 5×4 cm (20 cm^2) patches randomly selected, avoiding the patches already sampled in previous sampling periods. The collected living algae material was immediately processed in the laboratory, where they were differentiated according to their reproductive stage based on the reproductive structures clearly visible and recognizable under a stereoscopic microscope: sterile, cystocarpic and tetrasporic thalli. The specimens of each detected stage, were separated and divided into two parts: one part was kept in 70% ethanol solution in seawater for species identification, morphological observations and measurements, while the second part was dried, pressed as herbarium specimens and kept in the personal herbarium of the first author (C.B.) and in the herbarium KP (University of Primorska). A thallus was characterized by a single main axis and branches extending straight from the basal creeping axes. It was considered reproductive when it was possible to identify reproductive structures, otherwise, it was considered a sterile thallus. Morphological observations were made using a Leica EZ4W stereomicroscope with a camera, while sections for microscopic observations were made by hand with a razor blade under a Leica EZ4W stereo zoom microscope. Photographs were taken with a digital camera connected to a Leica ICC50 W light microscope.

Seasonal growth dynamic of *G. adriaticum* thalli, was investigated by measuring: 1) the thallus height (determined by measuring the distance of the highest axis of the thallus, from the base attached to the stolon to the apex); 2) the thallus width (measuring the maximum width between the two extremes of the thallus); 3) the degree of branching; 4) the widest part of the thallus axis (determined by measuring the width of the widest part of the thallus axis) and the width of three different positions of the same thallus axis: the apical part, the middle part and the basal part (Fig. 2). Morphometric analyses were done on 10 randomly selected thalli for each reproductive stage (sterile, cystocarpic and tetrasporic) for each month and for each season. All measurements were carried out on the photos using the Digimizer Version 4.0.0.0 program.

Statistical analysis

The seasonal variation in the measured traits (height of the thallus, width of the thallus, width of the widest part of the thallus axis, width of three different positions of the same thallus axis) was analyzed for the three reproductive stages and for the total number of collected algae using the Kruskal-Wallis test, followed by Dunn's post hoc test. Prior to analysis, data distribution was assessed using the Shapiro-Wilk test, and homogeneity of variance was evaluated with Bartlett's test. The results were visualized using boxplots. Seasonal dynamics of morphometrical characters were tested using generalized linear models (GLM), with length or width as the response variable and season and generation as predictors. The response variable was fitted to a Gaussian distribution with an identity link function. The likelihood ratio test was performed to compare the full model with a restricted model by calculating P values using the $\chi 2$ distribution. Differences between levels of each effect were analyzed post hoc by multiple comparisons of means with Tukey contrasts. Statistical analyses were performed or visualized using the R packages "stats", "car", "ggplot2", "rstatix" and "multcomp" (Bretz *et al*., 2011; Wickham, 2016; Fox and Weisberg, 2019; Kassambara, 2023; R Core Team, 2024).

RESULTS

Based on morphological characteristics, we confirmed that the species under study was *Gelidium adriaticum*. The morphology was consistent with species descriptions for the specimens from Apulia (Italy) and Koper Bay (Slovenia; Perrone *et al*., 2019). The col-

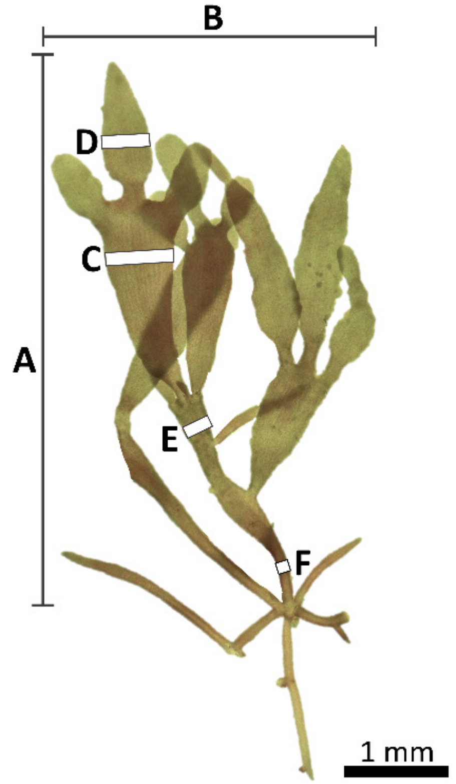

Fig. 2. *Gelidium adriaticum:* general aspect of the thallus with the parameters measured for morphological characterization: height of the thallus **(A)**; width of the thallus **(B)**; width of the widest part of the thallus axis **(C)**; width of the apical part of the thallus axis **(D)**; width of the middle part of the thallus axis **(E)**; and width of the basal part of the thallus axis **(F)**.

lected thalli were small (about 4.5 mm tall) and consisted of a branched system of prostrate axes (stolons), attached to the substratum by brush-like haptera, from which erect axes emerged. Branching was scarce and irregular, up to two orders. The erect axes showed parallel longitudinal striations when viewed on the surface under the microscope (Fig. 2). Thalli were well developed in shaded crevices, covering rock in the upper midlittoral zone of the exposed coast, and forming dense turfs with a very patchy and irregular distribution on limestone substrate. As for the vertical zonation, it was distributed at a height of about 10 cm above mean sea level (MW; Fig. 1C), where settlements of the red alga *Caulacanthus okamurae* Yamada are generally located directly below the *Catenella caespitosa* (Withering) L.M. Irvine. The thalli of *G. adriaticum* were also frequently found on the shells of the species *Mytilaster minimus* (Poli, 1795) and *Chthamalus* spp. During the study year, the collected thalli of *G. adriaticum* were frequently found densely overgrown by thalli of *C. okamurae*.

The collected and analyzed algal material included 70 sterile, 80 tetrasporic and 67 cystocarpic thalli. Sterile thalli were observed from January to June and in August; while none was observed from September to December (Table 1; Fig. 3). Tetrasporic thalli were found in January, February, June, July, September, and from October to December. Cystocarpic thalli were found from October to March, while in April we observed only three thalli and in July four thalli. The cystocarpic specimens were not observed in some periods of the year; this may be explained by the fact that they were not present in the collected material or because they were difficult to detect before fertilization and are usually considered only after the development of the cystocarp. During the sampling period, it was observed that the proportion of *G. adriaticum* thalli identified as tetrasporic, sterile and cystocarpic was not uniform, but showed slight seasonal variations. Tetrasporic thalli were slightly dominant. The ratio between the three reproductive stages was 1.19:1.04:1.00 (tetrasporic:sterile:cystocarpic). Among the collected algae, male/spermatangial thalli of *G. adriaticum* were never considered and therefore analyzed because they were not detected under the stereomicroscope, due to the inconspicuousness of the reproductive structures during the sampling period.

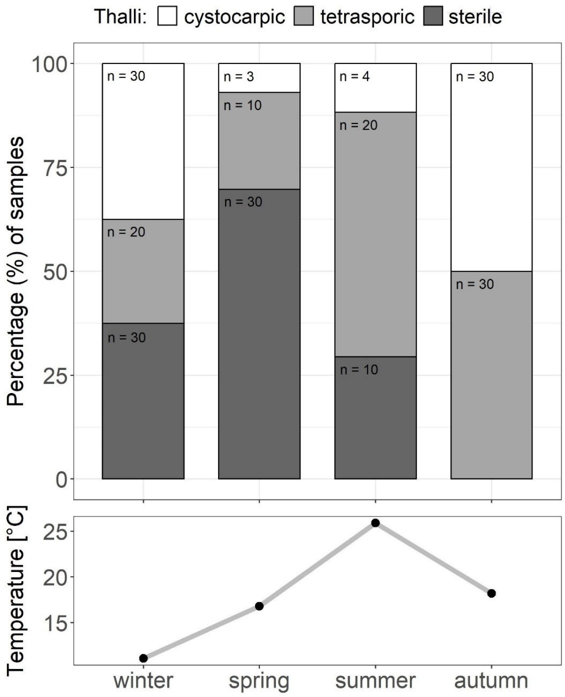

Fig. 3. Number and percentage values of thalli collected in each season for all three life stages (thalli) with seasonal cycle of sea temperature, source of data: ARSO (2024).

We found that thallus height, width, width of the widest part and width of the middle part of the axis were related to both season and different generations. We could not demonstrate a significant relationship between the width of the apical part of the axis and seasonal differentiation and the width of the basal part of the axis with both variables (Table 2). The cystocarpic thalli were smaller in winter and larger in autumn. However, a detailed analysis of size trends was not possible due to the limited number of thalli collected in spring and summer. The trend of the mean values of the thallus height of the tetrasporic individuals was lower in spring and summer than in winter; in autumn they were higher and reached the highest values (mean value 5.94 mm; SD 1.38; Fig. 4A). The mean values of the height of the observed thalli of the sterile reproductive stage of *G. adriaticum* were lower in winter and sum-

Table 1. Number and percentage of samples of *Gelidium adriaticum* for all three life stages (thalli) collected in each season at the site of the bay of St. Simon.

Seasons	**Winter**		**Spring**		**Summer**		**Autumn**		**Total**
Life stages	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**
Cystocarpic	30	44.8	3	4.5	4	5.4	30	44.8	**67**
Tetrasporic	20	25.0	10	12.5	20	25.0	30	37.5	**80**
Sterile	30	42.9	30	42.9	10	14.3	-	-	**70**
Total number	**80**	**36.9**	**43**	**19.8**	**34**	**15.7**	**60**	**27.7**	**217**

Table 2. Comparison of the full model with a restricted model based on χ2 (Chisq) distribution and P value. N.S. corresponds to not statistically significant.

	Thallus height		Thallus width		Widest part of axis		Width of the apical part of axis		Width of the middle part of axis		Width of the basal part of axis	
	Chisq	*P*	*Chisq*	*P*	*Chisq*	*P*	*Chisq*	*P*	*Chisq*	*P*	*Chisq*	*P*
Season	46.5	<0.05	8.7	<0.05	9.4	<0.05	4.5	N.S.	15.7	<0.05	1.4	N.S.
Life stages	32.2	<0.05	24.1	<0.05	27.5	<0.05	11.9	<0.05.	6.6	<0.05	3.0	N.S

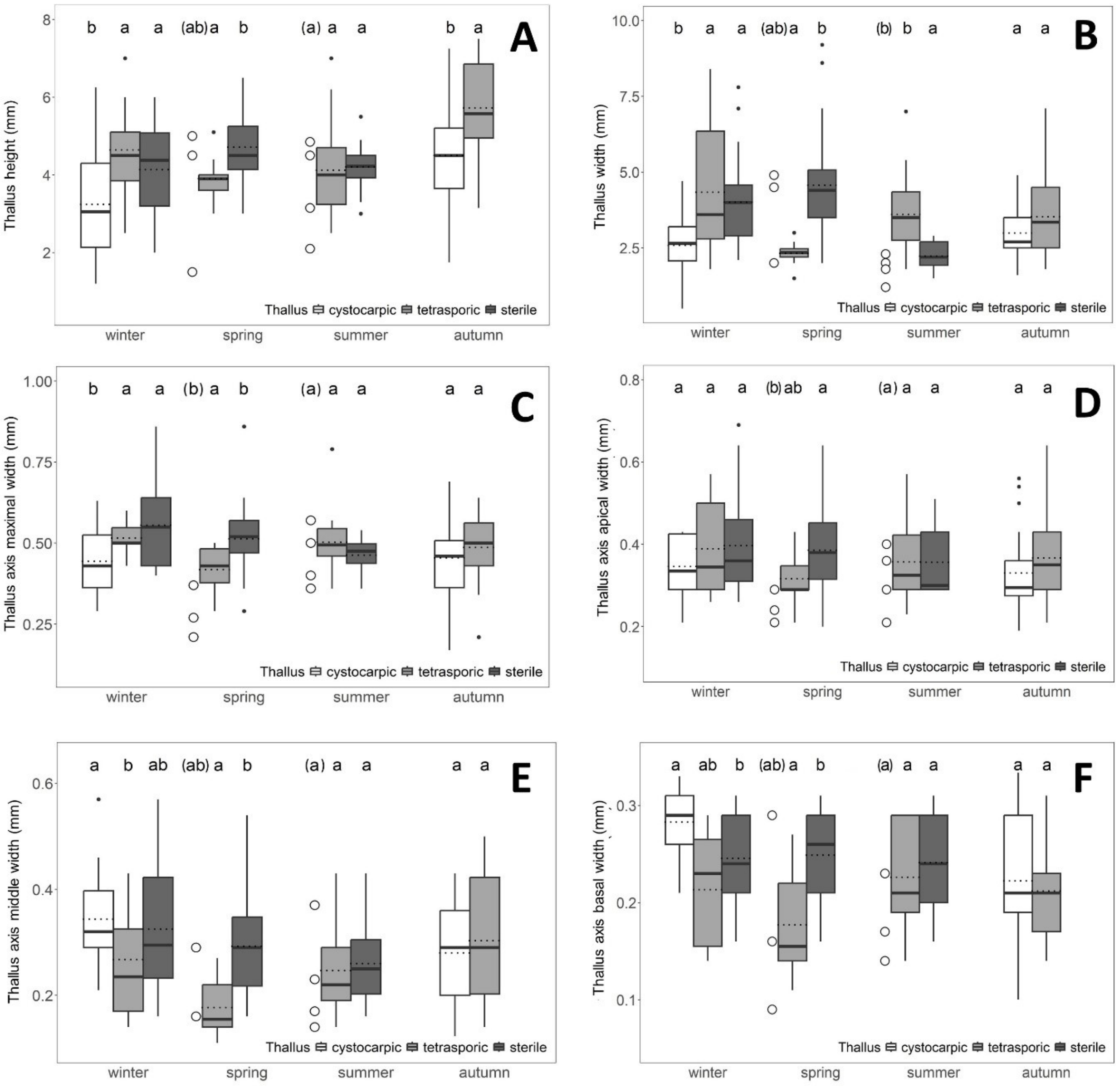

Fig. 4. Seasonal dynamics of morphological characters measured in specimens of *Gelidium adriaticum* during the year in individuals of all three life stages (thallus): thallus height **(A)**; width of the thallus **(B)**; widest part of the thallus axis **(C)**; width of the apical part of the thallus axis **(D)**; width of the middle part of the thallus axis **(E)**; and width of the basal part of the thallus axis **(F)**. Boxplots were only created if the number of samples was large enough (n≥ 10). The values of smaller samples are marked with white circles. The solid line within the boxplot indicates the median value, the dotted line the mean value. Letters above the boxplots indicate statistically significant differences between the observed variables (p<0.05). The differences were calculated between different life stages within seasons. Values in brackets were calculated for a smaller statistical sample.

mer, and slightly higher in spring (Fig. 4A). The width of the thalli did not vary over the seasons or within the different reproductive stages (Fig. 4B). The only statistically significant differences were observed between samples from winter and summer. The average values for the width of the widest part of the thallus axis of the tetrasporic generations were very similar between winter, summer and autumn, reaching the lowest values in spring (for cystocarpic thalli mean value 0.28 mm; SD 0.08; for tetrasporic mean value 0.42 mm; SD 0.08) (Fig. 4C). The trend of these values for the sterile thalli was different: the values decreased from winter to summer (mean value 0.46 mm; SD 0.10; Fig. 4C). For the width of the apical part of the thallus axis, we did not find statistically significant differences between reproductive stages and seasons, despite slight variations (Fig. 4D; Table 3). The observed mean values of the width of the middle part of the thallus axis of tetrasporic individuals decreased from winter to spring and then increased from summer to autumn (Fig. 4E). The values of cystocarpic thalli were higher in winter and autumn than in spring and summer. The seasonal trend of the values of sterile thalli was different: they were higher in winter and spring than in summer. Seasonal variation of the mean values for the width of the basal part of the thallus axis revealed no statistically significant differences between reproductive stages and seasons (Fig. 4; Table 3). Due to the low number of individuals for some of the reproductive stages during the different seasons or even due to the lack of individuals, we could not properly determine the growth pattern of the thallus of *G. adriaticum*. We observed larger thallus size in specimens collected in the autumn period than in other seasons (Fig. 4A; Table 3). However, no sterile thalli were collected during this period. Another pattern that emerges from our results is the width of the middle part of the thallus axis, which was wider in the winter months than in the spring and summer months (Fig. 4E; Table 3). There were no statistically significant differences in the widest part of the thallus axis between the sterile and the tetrasporic generation (Fig. 4A; Table 3). The same is true for the apical part of the thallus axis (Fig. 4D; Table 3). As for the values of the widest part of the thallus axis, the differences were statistically significant in all three generations. We found no differences in the width of the basal part of the thallus axis (Fig. 4C; Table 3), while the width of the middle part of the thallus axis was statistically significantly different between individuals of the sterile and tetrasporic generations.

Overall, the results showed very little seasonal variation when all thalli were considered independently of their affiliation to individual reproductive stages (Fig. 5). The mean values of the height of thalli were slightly higher in autumn than in other seasons, among which the values were statistically not significant (Fig. 5A). The width of the middle part of the thallus axis showed higher values in autumn and winter (Fig. 5E), while the values of the thallus width were slightly higher in spring (Fig. 5B). Other values, such as the widest part of the thallus axis, the width of the basal part of the thallus axis and the width of the apical part of the thallus axis, showed no statistically significant differences between the seasons (Figs. 5C, D and F).

DISCUSSION

In agreement with previous study (Perrone *et al*., 2019), the settlements of *Gelidium adriaticum* observed in this study were more extensive in sites well exposed to wave action. They were particularly well developed in shaded crevices and moist fissures on limestone substrate, where they occur patchily and irregularly, making observation of this species very difficult. Based on our observations, settlements of other *Gelidium* species (*G. spathulatum* and *G. crinale*) were distributed along the

Table 3. Post hoc analysis of levels of singular predictor represented between seasons and life stages. N.S. corresponds to not statistically significant.

		Thallus height	Thallus width	Widest part of axis	Width of the apical part of axis	Width of the middle part of axis	Width of the basal part of axis
				P			
Seasons	spring - autumn	<0.05	0.054	N.S.	N.S.	0.07	N.S.
	summer - autumn	<0.05	N.S.	N.S.	N.S.	N.S.	N.S.
	winter - autumn	<0.05	N.S.	N.S.	N.S.	N.S.	N.S.
	summer - spring	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
	winter - spring	N.S.	N.S.	<0.05	N.S.	<0.05	N.S.
	winter - summer	N.S.	<0.05	N.S.	N.S.	<0.05	N.S.
Life stages	tetrasporic -cystocarpic	<0.05	<0.05	<0.05	N.S.	<0.05	N.S.
	sterile - cystocarpic	<0.05	<0.05	<0.05	N.S.	<0.05	N.S.
	sterile - tetrasporic	N.S.	N.S.	<0.05	<0.05	N.S.	N.S.

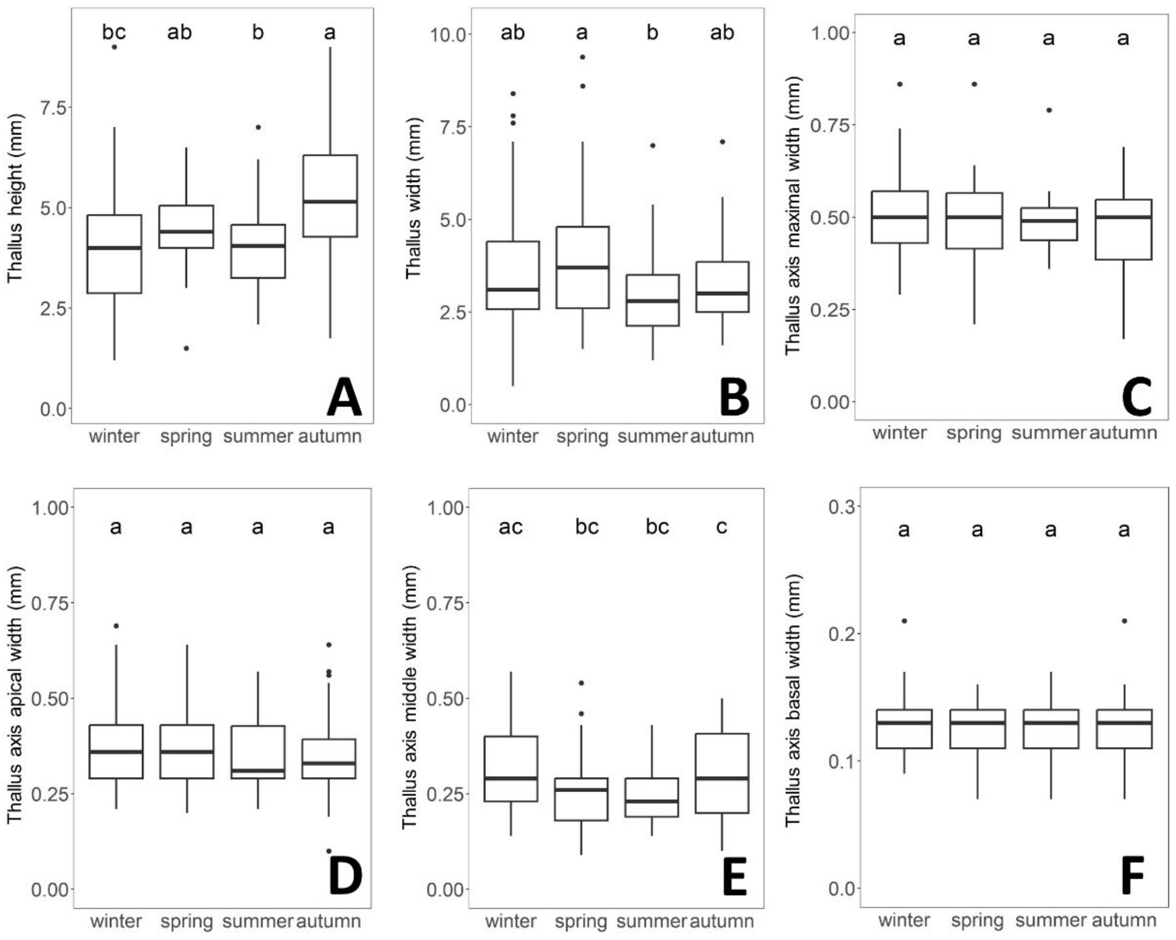

Fig. 5. General seasonal variations in morphological characters measured over the year in individuals of *Gelidium adriaticum*: thallus height **(A)**; width of the thallus **(B)**; widest part of the thallus axis **(C)**; width of the apical part of the thallus axis **(D)**; width of the middle part of the thallus axis **(E)**; and width of the basal part of the thallus axis **(F)**. The letters above the boxplots indicate statistically significant differences between the observed variables ($p < 0.05$). The differences were calculated between the seasons.

Slovenian coast on all substrates (marl, sandstone and limestone). Therefore, in our opinion, the type of rocky substrate is a potentially important factor determining the occurrence of *G. adriaticum* settlements along the Slovenian coast. Indeed, some previous studies have shown that the type and structure of the hard substrate influences the composition of macroalgal communities in the midlittoral zone of the Slovenian coast (Battelli, 2004; Rindi and Battelli, 2005; Battelli, 2016). *Gelidium* species seem to prefer certain types of substrates, and most species occur on limestone substrate where spores or fragments can germinate or adhere more easily (Santelices, 1988, 1991).

With regard to the influence of light intensity, it should be noted that the settlements observed in this study were not exposed to direct light, but were always located in the shaded crevices. It is in accordance with Dixon (1958), who suggested that the creeping axes of British intertidal populations of *Gelidium* can only survive summer light in rock crevices.

Seasonal variations of different reproductive stages

The algal material collected during the study year included individuals of *G. adriaticum* in the form of sterile, cystocarpic and tetrasporic thalli. Male and female thalli were not considered, as their separation in the collected algal material was not possible due to the inconspicuousness of the reproductive structures. For Polifrone *et al*. (2012), this could be explained by the fact that most thalli considered sterile could be immature or senescent gametophytic or tetrasporic individuals. Although these collected and studied thalli (tetrasporic, sterile and cystocarpic) showed a similar organization and were exposed to the same environmental conditions, no common pattern could be observed in their seasonal distribution. Sterile thalli were found in winter/spring, when the mean seawater temperature was at its lowest, while in summer, they were only found in August, when the mean seawater temperature reached its highest values. No sterile thalli were found in autumn. Thus, the decrease in the number of sterile thalli observed from summer to autumn, was certainly in favor of mature tetrasporic or gametophytic thalli (the sterile thalli become tetrasporic). Tetrasporic thalli were observed in all seasons, especially in summer-autumn, which is consistent with several authors, such as Akatsuka (1986), Santelices (1990), Sosa *et al*. (1993), and Croce and Parodi (2013), who reported that a predominance of tetrasporic thalli is a common feature of *Gelidium* species. For example, in their study on some *Gelidium* species in Tenerife, Polifrone *et al*. (2012) reported that the reproductive pattern of *G. canariense* is characterized by a high percentage of tetrasporic thalli, which increases from winter to autumn, which is very close to our results regarding *G. adriaticum*. Polifrone *et al*. (2012) also reported that *G. arbuscula* had a higher percentage of tetrasporic thalli in summer-autumn, which decreased in winter-spring, which is also consistent with our results. Regarding the collection period, Perrone *et al*. (2019) reported that tetrasporic thalli of *G. adriaticum* were collected on the Ionian coast of Apulia (south-eastern Italy) from September to January, while in our study, tetrasporic thalli of this species were found throughout the year. Perrone *et al*. (2019) reported in their work that cystocarpic thalli of *G. adriaticum* were collected only in January, while in our study period, cystocarpic thalli were observed in the autumn-winter period (from October to April), in spring (only in April), and in summer (only in July). From the study of Díaz-Tapia and Bárbara (2014), cystocarpic thalli of *G. spathulatum* from the Atlantic Iberian Peninsula were observed in March, June and October, while in our study, cystocarpic thalli of *G. adriaticum* were observed from October to April and in July. Díaz-Tapia and Bárbara (2014) also reported that tetrasporic thalli of *G. spathulatum* were observed in February, March, June, October and November, which is consistent with our observations on *G. adriaticum*. Numerous studies reported that the dominance of tetrasporophytes is a common feature of *Gelidium* (Montalva and Santelices, 1981; Carter, 1985; Akatsuka, 1986; Macler and West, 1987; Santelices, 1990; Melo and Neushul, 1993; Sosa *et al*., 1993). Our results also suggest that tetrasporic thalli slightly dominated in the studied settlement of *G. adriaticum*, with a reproductive ratio between the three reproductive stages of 1.19: 1.04: 1.00 (tetrasporic: sterile: cystocarpic), which is different from the ratio described by the authors mentioned above. This could be due to the fact that most thalli considered sterile could be immature or senescent gametophytes or tetrasporophytes and should contribute to the reproductive ratio, as pointed out by Ardito and Gómez (2005). Abbott (1980), for example, suggested that this could be due to the lack of variability in tetraspores, which are the sources of gametophytes and/or the increased ability of carpospores to produce tetrasporic thalli. It is interesting to note that the frequency and abundance of male and female gametophytes are very low in Gelidiales (Montalva and Santelices, 1981; Akatsuka, 1986; Ardito and Gómez, 2005), mainly because male gametophytes are often difficult to distinguish from sterile thalli, and female gametophytes are difficult to recognize in the field before fertilization and are usually only considered after cystocarp development (Guzmán del Próo *et al*., 1972; Melo and Neushul, 1993; Ardito and Gómez, 2005; Carmona and Santos, 2006).

The presence of cystocarps in the algal material collected during this study indicates that fertilization has occurred, although male gametophytic thalli were never observed in the specimens collected in the year of this study. Perrone *et al*. (2019) found that the cystocarpic thalli of *G. adriaticum* from Apulia had spherical, bilocular cystocarps, which is consistent with our observations, but while theirs had one central circular ostiole *per* chamber, we found some specimens with two ostioles *per* chamber, but these were very rare.

Seasonal variations in growth and morphology

In the collected specimens of *G. adriaticum*, which are assigned to the reproductive stages based on the development of the reproductive structures (sterile, cystocarpic and tetrasporic), there are considerable seasonal variations in growth and morphology. This is also consistent with the several studies, including Croce and Parodi (2013) on the species *Gelidium crinale* from Atlantic Patagonia. Variations in morphology depending on seasons and environmental conditions have also been observed in other *Gelidium* species (Santelices, 1988; Rueness and Fredriksen, 1989; Santelices, 1991; Prathep *et al*., 2009). Santelices (1991), for example, has shown that the size of thalli is related to light intensity and water movement. This could also be the case here, as the settlement studied was located in an area exposed to wave action, but in a site protected from both waves and light intensity. Echegaray-Taborga and Seoane-Camba (1982) correlated the development of *Gelidium crinale* and *G. spathulatum* algae with the seasons and sea temperature. Their work shows that the development of both species begins in late winter-spring and reaches its maximum in summer, when the temperature reaches its highest values. This differs from our results, where the thalli of *G. adriaticum* start their development in late winter-spring, but reach their maximum development in winter, when the sea temperature drops. In general, the mean values of the examined morphological characteristics of the cystocarpic thalli were lower than those of the tetrasporic and sterile phases in all seasons. This could be due to very early fertilization of the carpogonia.

The most significant differences concerned the width of the largest part of the thalli axis, the width of the apical part of the thalli axis, and the width of the middle part of the thalli axis, with the mean values of these morphological characteristics being significantly lower in the cystocarpic specimens in all seasons. This is consistent with the results of the study conducted by Perrone *et al*. (2019) on *G. adriaticum* from Apulia, in which it was reported that the cystocarpic thalli were the smallest in the population of this species. The differences in morphological characteristics during the seasons were less marked between the tetrasporic and sterile thalli. As for the differences in morphological characteristics between seasons for each reproductive stage, the thallus height values of the tetrasporic thalli were higher in the specimens collected in the autumn-winter, when the sea temperature was lower than in other seasons, than in the thalli collected in the other seasons, especially in summer, when the sea temperature values were higher. The thallus height values of the sterile thalli were higher in spring than in winter, when the temperature was the lowest, and in summer, when the temperature was the highest. This can be explained by the fact that the gametophytes mature earlier and grow less. It is interesting to note that the thallus height values were very similar in all seasons. Based on the data from our study, it appears that sea temperature has no effect on thallus height, as the differences between this parameter were minimal despite the wide range of temperatures. However, variations in morphology related to seasons and environmental conditions have also been observed in other species of Gelidiales (Macler and West 1987; Santelices, 1988; Rueness and Fredriksen, 1989; Prathep *et al*., 2009). Santelices (1978) reported that the growth of *Gelidium* species is regulated by complex interactions between irradiance, temperature, nutrients and water movement. For example, the external morphology of *Gelidium crinale* was seasonally variable. Both thalli length and branching were higher in specimens collected in summer (Santelices, 1991), while in our study, it was observed that the values of length of collected thalli were higher in autumn. During this study, the presence of sterile reproduction and regeneration was also observed in the collected specimens. Regeneration and growth of erect fronds from creeping axes are common in *Gelidium* species, and sterile propagation is a common method of colonization (Dixon, 1958; Santelices, 1991). The prostrate axes grow and colonize new surfaces, from which erect axes develop to form new mats. During this study, the presence of cut branches with regenerated parts was also observed in the collected algal material, confirming that regeneration occurs, as has been reported for other Gelidiales (Dixon, 1958; Felicini and Arrigoni, 1967; Felicini, 1970; Felicini *et al*., 2002).

CONCLUSIONS

The present study has shown that the observed settlements of *Gelidium adriaticum* from the Slovenian coast developed exclusively on limestone hard substrate. All collected and analyzed specimens of *G. adriaticum* proved to be fertile during the entire study year, with a slight dominance of tetrasporic thalli within the studied settlement. The distribution of tetrasporic, sterile, and cystocarpic thalli varied throughout the year and followed a non-uniform pattern. Furthermore, not all reproductive stages were present or recognizable in every month of the study year, while male gametophytic thalli were never observed.

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