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Morphological and reproductive phenology of the non-native red alga *Caulacanthus okamurae* Yamada (Gigartinales, Caulacanthaceae) from the Slovenian coast (Gulf of Trieste, northern Adriatic)

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Abstract: This study reports the occurrence of the non-native red alga *Caulacanthus okamurae*, previously unknown on the Slovenian coast (Gulf of Trieste, northern Adriatic Sea). The distribution of the species, its abundance, morphological characteristics and reproductive phenology are described. The study is based on seasonal sampling conducted during one year, from winter to autumn 2021, on two different substrates (limestone and sandstone) of the midlittoral zone, and showed a moderate variation in the biomass of *C. okamurae* populations between the winter-spring and summer-autumn periods. The native algal assemblages of the two substrates impacted by *C. okamurae* were characterized by generally limited species diversity and consisted mainly of small-sized species with simple morphology. During the study, in addition to non-fertile thalli of *C. okamurae*, tetrasporophytic thalli with tetrasporocysts and female gametophytes with cystocarps were found.

Keywords: *Caulacanthus okamurae*; non-native species; midlittoral; biomass; reproductive phenology; Slovenian coast; northern Adriatic

Sažetak: MORFOLOŠKA I REPRODUKTIVNA FENOLOGIJA ALOHTONE CRVENE ALGE *CAULACANTHUS OKAMURAE* YAMADA (GIGARTINALES, CAULACANTHACEAE) NA PODRUČJU SLOVENSKE OBALE (TRŠĆANSKI ZALJEV, SJEVERNI JADRAN). Ovo istraživanje prikazuje nalaz alohtone crvene alge *Caulacanthus okamurae*, dosad nepoznate na području slovenske obale (Tršćanski zaljev, sjeverni Jadran). U radu se opisuje rasprostranjenost vrste, njezina brojnost, morfološke karakteristike te reproduktivna fenologija. Istraživanje se temelji na sezonskom uzrokovanju provedenom tijekom jedne godine, od zime do jeseni 2021., na dvije različite podloge (vapnenac i pješčenjak) mediolitoralne zone, a istim je utvrđena umjerena promjenjivost u biomasi naselja *C. okamurae* između zimsko-proljetnog i ljetno-jesenskog razdoblja. Zajednice zavičajnih algi dvaju supstrata pod utjecajem *C. okamurae* općenito su okarakterizirane ograničenom raznolikošću vrsta, uglavnom onih malih dimenzija s jednostavnom morfologijom. Tijekom istraživanja, osim sterilnih talusa *C. okamurae*, pronađeni su tetrasporofitni talusi s tetrasporocistama i ženski gametofiti s cistokarpima.

Ključne riječi: *Caulacanthus okamurae*; alohtone vrste; mediolitoral; biomasa; reproduktivna fenologija; slovenska obala; sjeverni Jadran

INTRODUCTION

Caulacanthus Kützing is a small genus (order Gigartinales, family Caulacanthaceae) generally found in warm temperate and tropical waters (Rueness and Rueness, 2000) and includes three taxonomically recognised species: *C. ustulatus* (Martens ex Turner) Kützing as the type species, *C. okamurae* Yamada and *C. salifugus* A.B. Cribb (Verlaque *et al*., 2015; Guiry, 2020). These are perennial, turf-forming red algae that grow in the upper to middle horizon of the midlittoral zone. The thallus of algae in this genus is characterized by short, tangled clumps up to 3 cm high, consisting of uniaxial, terete erect and prostrate branches. The laterally branched shoots arise from stoloniferous branches that give rise to multicellular rhizoidal discs from the cortical cells. The apical cell divides by oblique septa, each axial cell separating two periaxial cells that divide the compact cortex. The axes are attached to the substrate by numerous creeping stolons with attachment pads (Rueness, 1997). Algal turfs of *Caulacanthus* have been shown to increase habitat complexity, retain sediment and reduce desiccation at low tide; this may benefit some animals and algae by providing food, habitat and/or protection from desiccation (Smith *et al*., 2014).

Most records of *Caulacanthus* spp. populations, including *C. okamurae* (West and Calumpong, 1990; Zuccarello *et al*., 2002), traditionally refer to *C. ustulatus*, the type species of the genus, originally described from Spain. *C. okamurae*, first described from “Hokkaido down to Formosa [Taiwan]”, is native to the Pacific Northwest (Yamada, 1933; Choi and Nam, 2001; Choi *et al*., 2001), and is considered an invasive species in other parts of the world (Fofonoff *et al*., 2020), includ-

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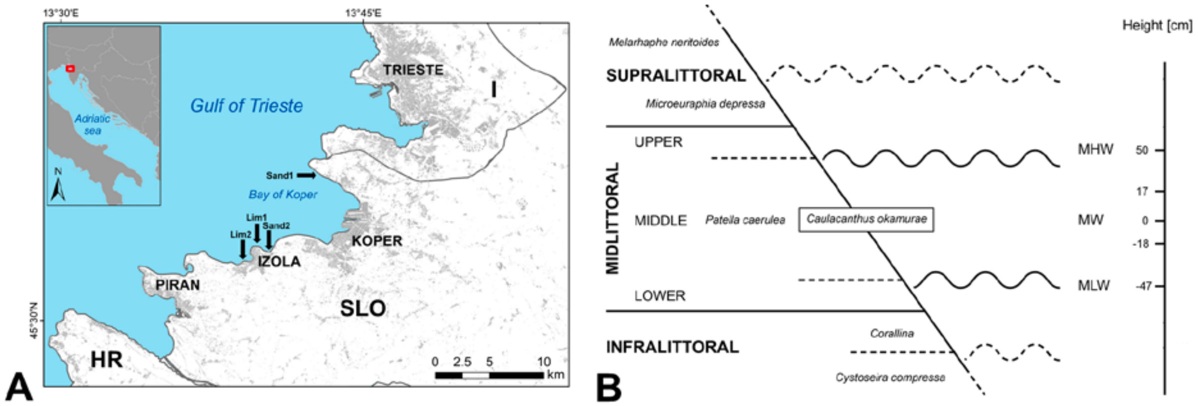

Fig. 1. Map of the study area. The visual assessment of *C. okamurae* is made along coastline between Izola and the border with Italy. Sampling site are located on limestone (Lim) and sandstone (Sand) (**A**); schematic presentation of zonation in study area with average sea levels during the period 1961–2020. Source of data: MOP, ARSO, 2022. MHW – Mean High Water; MW – Mean Water (0 cm); MLW – Mean Low Water (**B**).

ing the Mediterranean (Verlaque *et al*., 2015) where it was probably introduced by shipping, and was first found in Marseille, France, in 2004.

All records regarding *Caulacanthus* reported from the Adriatic Sea by different authors before 2020 were considered as *C. ustulatus*. For example, Ercegović (1980) reported the occurrence of *C. ustulatus* from the central Adriatic, Munda (1979) and Curiel *et al*. (2012) from the northern Adriatic and Pignatti and Giaccone (1967) from the Gulf of Trieste. Vukovič (1980, 1984) and Battelli (2013, 2016a) reported from several sites along the Slovenian coast the occurrence of *Caulacanthus* species such as *C. ustulatus* growing in the middle horizon of the midlittoral zone, mixed with red and green turf-forming algae, within chthamalids and as epibiont on mussel aggregates. Recently, Petrocelli *et al*. (2020) reported the first finding of *C. okamurae* in the Adriatic Sea in the Venice Lagoon (Northern Adriatic). The identity and Pacific origin of Mediterranean populations of *C. okamurae* were confirmed by molecular studies (Mineur *et al*., 2007). *C. okamurae* is morphologically similar to *C. ustulatus* in terms of plant height, branching pattern, shape of axial cells and number of pericentral cells, but observations of reproductive structures (Choi *et al*., 2001a; 2001b) and molecular analyses (Zuccarello *et al*., 2002; Petrocelli *et al*., 2020) support the validity of *C. okamurae* as a distinct species.

In this article we present the occurrence of *Caulacanthus okamurae* populations along the Slovenian coast (Gulf of Trieste, northern Adriatic Sea) in 2021. Following the first observation, we characterized the morphological traits and seasonal reproductive phenology of *C. okamurae* and analyzed the communities associated with *C. okamurae* from two different hard substrates (limestone and sandstone) to determine composition and abundance of native algal species in different seasons.

MATERIAL AND METHODS

Study area

The occurrence of *Caulacanthus* populations was visually assessed in the midlittoral zone along the Slovenian coast during low tide, while sampling was conducted in Bay of Koper (Fig. 1A). The study area is mainly influenced by winds blowing from the north-northeast, north-west and south-east. In 2021, the monthly average surface water temperature in the Gulf of Koper varied between a minimum of 8.0 °C in February and a maximum of 28.0 °C in August, while the average temperature over the year was 17.2 °C (ARSO, 2022). Salinity varied from 25.95 in December to 35.17 in April (ARSO, 2022). Tides are mixed. Half-day tides (two high tides and two low tides in a day) and day tides (one high tide and one low tide per day) alternate approximately every 15 days. The average tidal amplitude between mean low water (MLW) and mean high water (MHW) for the period 1961-2020 was 67 cm, with mean water (MW) considered to be 0 cm (ARSO, 2022) (Fig. 1B).

Fieldwork was conducted at four sites on two different hard substrates (limestone and sandstone), two sites on each substrate (Fig. 1A). Only limestone and sandstone were selected for this study because *Caulacanthus* populations did not occur on marl. Previous studies have shown that the type and structure of the hard substrate influences the composition of the macroalgal communities in the midlittoral of the Slovenian coast (Battelli, 2004, 2016b). All sites are designated Lim1 and Lim2 on limestone and Sand1 and Sand2 on sandstone. Site Sand1 (45.5874º N; 13.7070º W) consisted of sandstone rocky platforms exposed to waves generated by winds from northwest to northeast and south-east. Site Sand2 (45.5419º N; 13.6749º W) on sandstone was consisted of boulders of various sizes with a rough surface. The substrate of sites Lim1 (45.5417º N; 13.6571º W) and Lim2 (45.5339º N; 13.6468º W) (Fig. 1A) is generally more irregular and heterogeneous in structure, with many outcrops and fissures of different sizes. The wind exposure of the Sand2, Lim1 and Lim2, sites was northwest and northeast.

Sampling procedure

Samples from *Caulacanthus* communities were collected in four seasons (February for winter, May for spring, August for summer, and November for autumn). Each sampling site was divided into four five-meters subsections so that each site had a horizontal extent of approximately 20 m (4x5 m). Five random quantitative samples were collected from one of the sub-areas at each site each season. Samples were collected by scraping algae in 100 cm^2 (10 cm x 10 cm) quadrants in the middle of the midlittoral zone in areas where *Caulacanthus* sp. was visible to the naked eye. Care was taken to ensure that samples were collected at least 1 m apart and that the same quadrats and subplots were not re-sampled during the sampling period. A total of 80 samples (10 samples per substrate in each season) were collected from winter to autumn 2021 and immediately processed in the laboratory. Some thalli were randomly collected monthly at each sampling site for reproductive status analysis. Algal material for morphological analysis was preserved in 25% alcohol in seawater and then transported to the laboratory at UP FAMNIT for laboratory analyses. Some of the collected specimens were stored in the personal herbarium of the first author (CB) and in the herbarium of UP FAMNIT.

Laboratory analyses

The biomass of *Caulacanthus* in the sample was measured using a precision balance KERN ABJ320-4NM and expressed as fresh weight (g fw dm^{-2}) after water removal. Seasonal dynamics of biomass were visualized in boxplots and tested using generalized linear models (GLM), with mass as the response variable and season and substrate 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” and “multcomp” (R Core Team, 2021; Fox and Weisberg, 2019; Wickham, 2016; Hothorn *et al*., 2008).

Morphological observations were made on fixed specimens using a Leica EZ4W stereo microscope, while sections for microscopic observations were made by hand using 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. Thallus height, main axis diameter, length, and diameter of the terminal branches were measured using the Digimizer program. Statistical differences between measurements were assessed by applying analysis of variance with the R package “stats” (R Core Team, 2021).

Composition and seasonal dynamics of the algal community with *C. okamurae*

Collected algal specimens in the samples were identified to species when possible and to genus when diacritical features were absent. To determine community structure, the collected algal specimens were divided into six morphological groups, roughly corresponding to the traditional classification of Steneck and Dethier (1994) and Balata *et al*. (2011): (i) the filamentous group (Fil.), (ii) the tubular group (Tub.), (iii) the corticated group (Cort.), (iv) the blade-like group (Blade), (v) the encrusting, non-calcified group (Enc.) and (vi) the encrusting, calcified group (Enc.-cal.).

To test similarities in sample composition by taxonomic and morphological identity over different time periods on different geological substrates, we applied hierarchical clustering using Ward’s method. Distance matrices were created for both datasets by calculating Euclidean distance. Analyses were performed and visualized using the R package ‘stats’ (R Core Team, 2021).

RESULTS

Based on morphological characteristics (details below), we identified the studied species as *Caulacanthus okamurae* Yamada.

Field observation

Throughout the sampling period, the non-native species *C. okamurae* populations formed dense, irregular mats on the hard substrate of limestone and sandstone in the middle of the midlittoral zone, where it was usually attached to rocks (Fig. 2A), often among chthamalids and as an epibiont mainly on bivalves (Fig. 2B), mixed

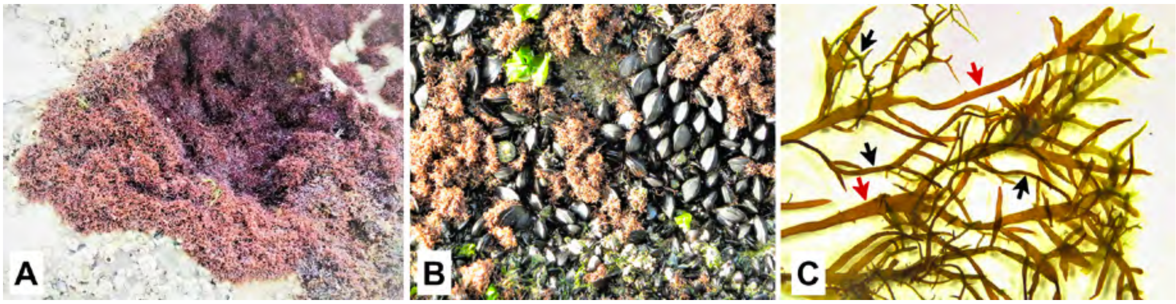

Fig. 2. *Caulacanthus okamurae* on different substrates: epilithic on hard limestone substrate (**A**); as epibiont on mussels (**B**); *C. okamurae* (black arrow) as an epiphyte on *Gelidium* sp. (red arrow) (**C**).

and intergrown with green and red algal turfs, and as epiphytes on *Gymnogongrus griffithsiae* (Turner) C. Martius, but mainly on *Gelidium* spp. thalli (Fig. 2C). The vertical slope of the belt of *C. okamurae* populations at the studied sites was about 35 cm: from about 18 cm below to 17 cm above mean sea level (MW).

Morphological observations

Thalli of *C. okamurae* from the study area are 15-20 mm high (Fig. 3A), cartilaginous, and dark red to brownish in color. The main axes are cylindrical to slightly compressed, branching irregularly to subdichotomously, with the smallest apical branches being curved and having a spine-like shape with a length of about 557 µm (SD 182 µm) and a width of 134 µm (SD 22 µm; Fig. 3B). The thalli are attached to the substrate by multicellular holding apparatuses with many attachment pads of 175-200 µm in diameter (Fig. 3C, 3D). The main axes are approximately 220 µm in diameter (SD 25 µm), and it has no internal rhizoids around the axial cell (Fig. 3G, H). The central axial cell, which is up to 45-50 µm in diameter, is characterized by a thick cell wall (Fig. 3H). The medulla consists of more or less round cells and becomes more compact toward the periphery (Fig. 3H). The cortex is compact and consists of small, pigmented, elongated cells that are twice as long as they are wide (Fig. 3E). Externally, the thalli are delimited by a thick and resistant cuticle. The apical part of the thalli is characterized by obliquely divided apical cells (Fig. 3F).

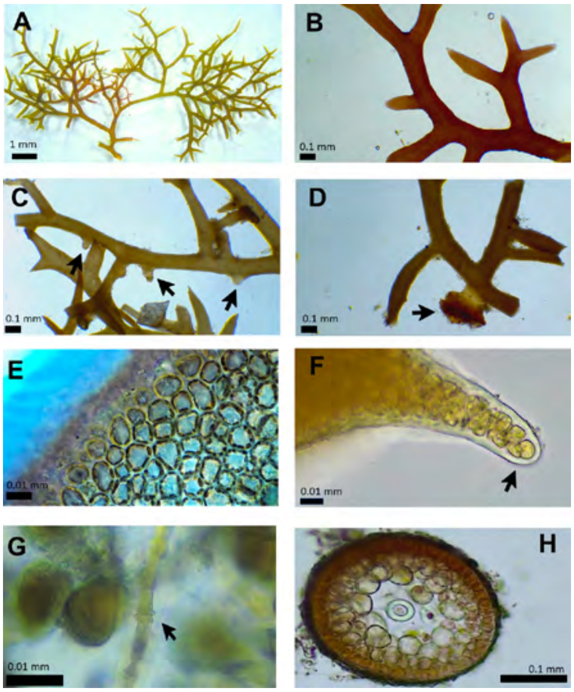

Fig. 3. Morphological characteristics of *C. okamurae*. General view of thalli (**A**); detail of a thallus showing the smallest branches, which are curved and have spine-like shape apexes (**B**); part of a branch forming attachment pad (**C**); detail of attachment pad (**D**); surface view of cortical cells (**E**); apical part showing obliquely divided apical cell (**F**); longitudinal section of branch with central cell (**G**); cross section of a branch with central cell, external cortical layer and internal medulla (**H**).

Two types of fertile thalli were observed in the study area: diploid tetrasporophytic thalli (Fig. 4B) and female gametophytic thalli with cystocarps (Fig. 4C). Both types were observed in summer, when the mean seawater temperature at the surface was 22.9 ºC, with a range between a minimum of 19.0 ºC and a maximum of 26.0 ºC (ARSO, 2022). No male gametophytic thalli were found. The different types of fertile thalli also differed in their morphological characteristics, especially in the size of the apical branches (Table 1).

The statistically significant differences (at $p < 0.05$) were found in the width of apical branches between sterile and sporophytic thalli, in the length of apical branches between sterile and sporophytic thalli, and in the length of apical branches between sterile and gametophytic thalli. However, the differences in the width of the main thalli were not statistically significant.

Tetrasporocysts (Fig. 5B) on tetrasporophytic thalli (Fig. 5A) appeared as swellings mainly in the central part of spiny branches (Fig. 5C). The tetrasporocysts were zonately divided, ovoid, 50-55 µm long and 30-32 µm in diameter (Fig. 5D).

Female gametophytic thalli (Fig. 5E) are characterized by the presence of cystocarps that occur at the tips of terminal branches and are 220-315 µm in diameter. The cystocarp is with an ostiole and without spines (Fig. 5F).

Seasonal dynamics

Mean seasonal biomass of *C. okamurae* populations, ranged from a minimum biomass of 0.76 g fw dm^{-2} (0.89 SD) in summer on limestone to maximum values of 3.62 g fw dm^{-2} (0.72 SD) in spring on sandstone ($n = 5$). Statistically significant differences were observed between seasons ($\chi^2 = 39.4$, $p < 0.001$). Post-hoc we observed statistically significant differences between spring – autumn ($p < 0.001$), winter – autumn ($p = 0.03$), summer – spring ($p < 0.001$) and winter – summer (p

Table 1. Comparison of some characteristics of sterile and fertile thalli of *C. okamurae*. Comparison of the dimensions (mean values in mm and standard deviation in parentheses) of the width of main axes and apical branches and length of apical branches of sterile, sporophytic and female gametophytic thalli of *C. okamurae*.

Measures	Sterile thalli	Sporophytic thalli	Gametophytic thalli
Width main axes	0.220 (0.025)	0.217 (0.038)	0.223 (0.028)
Width apical branches	0.134 (0.022)	0.109 (0.032)	0.117 (0.039)
Length apical branches	0.557 (0.182)	0.117 (0.069)	0.270 (0.157)

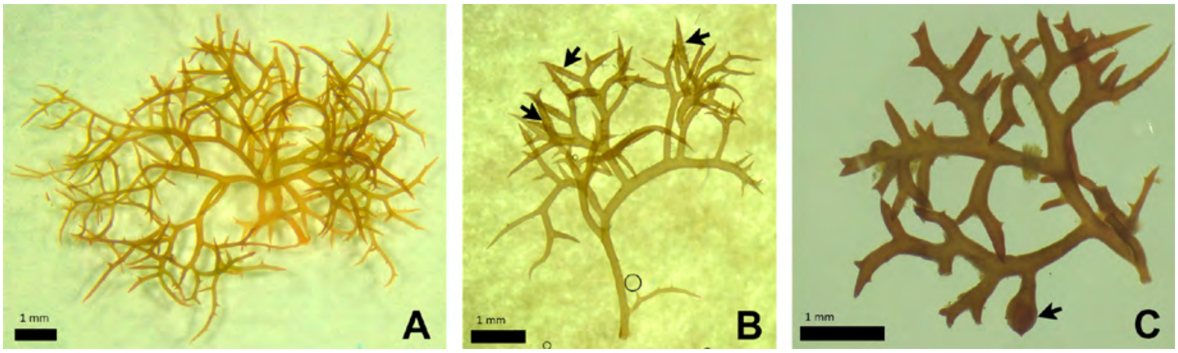

Fig. 4. Thalli of *C. okamurae* in non-reproductive and reproductive phases: general view of non-reproductive thalli (**A**); tetrasporophytic thalli with tetrasporocysts (black arrows) (**B**); female gametophytic thalli with cystocarps (black arrow) (**C**).

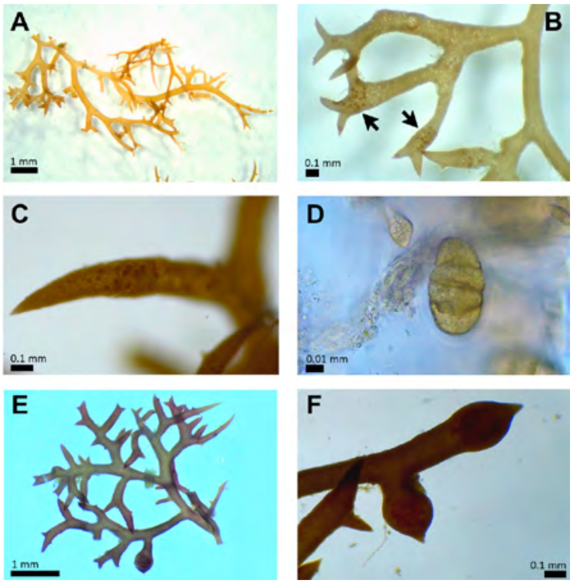

Fig. 5. Morphological characteristics of the reproductive thalli of *C. okamurae*. tetrasporophytic thalli and branchlets (**A**) with tetrasporocysts (black arrows) (**B**); terminal branchlets with tetrasporocysts (**C**); zonately divided tetrasporocyst (**D**); female gametophyte with cystocarps (**E**); terminal branchlets of female gametophyte with two cystocarps (**F**).

< 0.001). In general, the mean biomass of this species was moderately higher on sandstone than on limestone (Table 2). However, we did not detect statistically significant differences ($\chi^2 = 1.941$, $p = 0.164$).

The average biomass of *C. okamurae* populations increased from winter to spring, then declined sharply in summer and increased again slightly in autumn (Fig. 6A). Conversely, the average monthly seawater temperature increased steadily from winter trough summer and then decreased toward autumn (Fig. 6B).

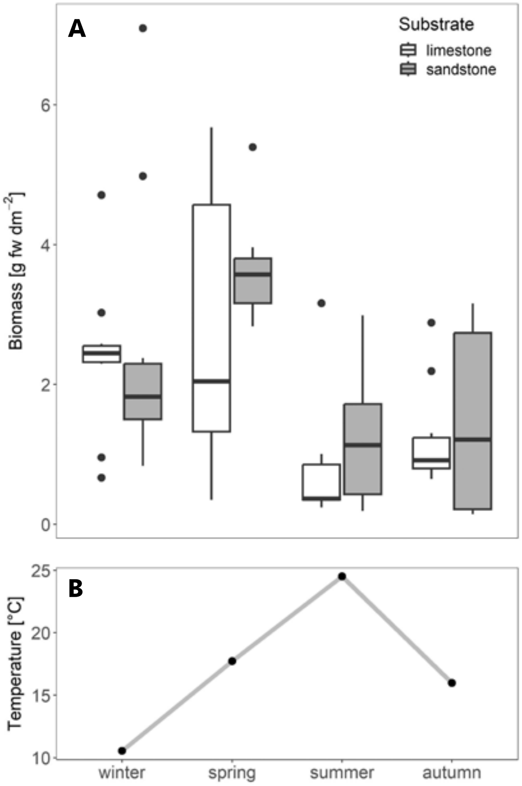

Fig. 6. Seasonal dynamics of *C. okamurae* biomass: boxplots showing the differences in biomass in different seasons on two different geological substrates (limestone and sandstone). The total number of samples collected for the analysis was 80, ten samples for each substrate in each season. Quartile groups 2 and 3 are represented by the boxes and delineated by the median line, quartiles 1 and 4 by the lower and upper whiskers, while the black dots represent the outliers (**A**); seasonal variations in seawater temperature at the surface (**B**).

Table 2. Seasonal variation in mean biomass of *C. okamurae* population samples on limestone and sandstone, expressed in grams fresh weight (g fw dm^{-2}), and in mean surface seawater temperature (°C; ARSO, 2022). The total number of samples is 80, ten samples for each substrate in each season. SD – standard deviation.

	Winter	Spring	Summer	Autumn	Average	SD
Sandstone	2.52	3.62	1.17	1.46	2.19	1.12
Limestone	2.40	2.82	0.76	1.22	1.80	0.97
Temperature	10.53	17.73	24.5	15.97	17.18	

Table 3. Seasonal variability of *C. okamurae* assemblages at the morphological group level. Number of presences of morphological groups on limestone and sandstone, in different seasons, in assemblages of *C. okamurae*. The encrusting calcified group was not considered because it occurs only as an epiphyte on *C. okamurae*. Wi = Winter; Sp = Spring; Su = Summer; Au = Autumn.

	Limestone (presence)				Sandstone (presence)			
Morpho-group	Wi	Sp	Su	Au	Wi	Sp	Su	Au
Filamentous	19	14	13	22	18	19	9	27
Corticated	10	19	3	7	13	23	9	1
Tubular	1	8	20	2	1	15	7	22
Encrusting	3	7	5	4	4	4	6	5
Blade-like	5	1	3	2	9	5	3	3

Structure and species composition

Midlittoral algal communities with *Caulacanthus okamurae* in the two substrates studied were characterized by generally limited species diversity. A total of 21 macroalgal taxa were identified, of which red algae dominated with 13 taxa; 6 belonged to green algae, while only 2 belonged to brown algae. The macroalgal assemblages consisted mainly of small-sized macroalgae with simple morphology. Thin turfs formed by filamentous and corticated algae were the most common algal growth form. The predominant macroalgal taxa consisted mainly of a cushion-like layer 2-3 cm high composed of various morphological algal groups, such as: corticated (including *Caulacanthus okamurae*, *Gymnogongrus griffithsiae* and *Gelidium* spp.); filamentous algae (mainly consisting of *Polysiphonia* sp., *Lophosiphonia* sp., *Ceramium* sp., *Cladophora* sp., *Chaetomorpha linum* (O.F.Müller) Kützing and *Sphacelaria* spp., and *Bangia fuscopurpurea* (Dillwyn) Lyngbye; tubular (with the most abundant *Ulva compressa* Linnaeus, *U. polyclada* Kraft and *Blidingia minima* (Nägeli ex Kützing) Kylin); blade-shaped (consisting of *Ulva australis* Areschoug, *U. rigida* C. Agardh and *Neopyropia leucosticta* (Thuret) L.-.E.Yang & J.Brodie (ex *Pyropia leucosticta*)). The encrusting calcified group was represented by only one taxa, the red alga *Titanoderma pustulatum* (J.V.Lamouroux) Nägeli (only as an epiphyte on *C. okamurae*), and the encrusting not calcified group by the brown alga *Pseudoralfsia verrucosa* (Areschoug) Parente, Fletcher & G.W.Saunders.

In general, the morphological groups composing the *C. okamurae* assemblages showed moderate seasonal variation in the number of occurrences, with significant differences noted between limestone and sandstone (Table 3).

It is interesting to note that the number of occurrences for almost all morphological groups reached the higher values in the same seasons on both substrates, except for the tubular algae, which were more abundant in summer on limestone and in autumn on sandstone, and the encrusting taxa, which were more abundant in spring on limestone and in summer on sandstone. When analyzing the similarities between taxonomic assemblages using hierarchical clustering, we observed a grouping according to the same periods, regardless of

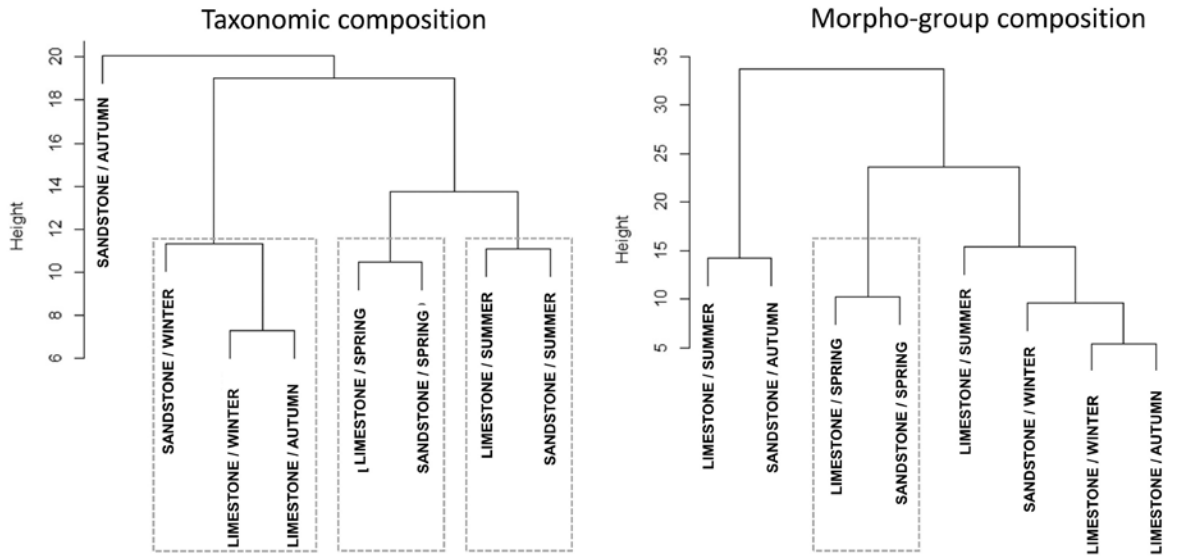

Fig. 7. Similarity of composition of taxonomic and morpho-groups of specimens collected seasonally on two different geological substrates. Dendrograms were generated by applying hierarchical clusters according to Ward's method. The dashed rectangles indicate the grouping of releves according to the same time period.

the geological substrate. This trend is less obvious when composition is considered on the basis of morphological groups (Fig. 7).

The most common epiphytes on *C. okamurae* were the red algae *Ceramium* spp., *Polysiphonia* spp., *T. pustulatum* and *N. leucosticta*, the brown alga *Sphacelaria* spp. and the green alga *Cladophora* spp. The number of macroinvertebrates found in the *C. okamurae* community is very low and mainly includes the bivalves *Mytilaster minimus* (Poli) and the chthmalids *Chthamalus montagui* Southward and *C. stellatus* (Poli), which mainly form the substrate to which the thalli of *C. okamurae* were attached as epibionts. Other animals were also found in these dense turfs of the *C. okamurae* assemblages: the small periwinkle, *Melarhaphe neritoides* (Linnaeus), the chiton *Acanthochitona fascicularis* (Linnaeus), the gastropod *Bittium reticulatum* (da Costa) and the juvenile specimens of pill isopod *Sphaeroma serratum* (J. C. Fabricius) that could find refuge, habitat or even food there.

DISCUSSION

The study, conducted in the period from winter to autumn 2021, reports the presence of the non-native red alga *Caulacanthus okamurae*, which has not been previously observed on the Slovenian coast (Gulf of Trieste, northern Adriatic Sea). Populations of *C. okamurae* grew in very compact pads or carpets, mainly near freshwater inflows as epiliths on various natural substrates (limestone and sandstone), mixed and matted with green and red algal turfs, on chthamalids and bivalves as epibionts and as epiphytes mainly on *Gelidium* spp. and *Gymnogongrus griffithsiae* thalli. It is interesting to note that *C. okamurae* was absent on marl (main component of flysch besides sandstone) as well as *Catenella caespitosa* (Withering) L.M. Irvine, the other representative of the same family, Caulacanthaceae, as reported by Battelli (2004), which is due to differences in geological structure of the two hard substrates on the Slovenian coast.

This study has reconfirmed that identification of native, but also non-native macroalgal species can be difficult, especially for taxa with cryptic and/or overlapping morphologies such as *Caulacanthus okamurae* and *C. ustulatus*. In fact, these two species have very similar thallus morphologies and differ mainly by: (a) the absence of internal rhizoids in *C. okamurae*, unlike *C. ustulatus*, (b) the fusion cell, which is clearly lateral to the central axial filament in *C. okamurae*, whereas in *C. ustulatus* the central fusion cell comprises several cells of the axial filament, and (c) the carposporangia are single and terminal; the cystocarps have a single ostiole and lack spines characteristic of *C. okamurae*. To distinguish these two species, we focused mainly on the absence of internal rhizoids, lateral cystocarps on terminal branches with single ostioles and without spines, which is consistent with Verlaque *et al*. (2015) and Petrocelli *et al*. (2020). The presence or absence of internal rhizoids allows us to make a relatively simple identification, since in cross section the space between axial and subcortical cells appears empty in *C. okamurae*. Another possibility concerns the cystocarps, which are always terminal. As Choi and Nam (2001) and Choi *et al*. (2001) have noted, sexual reproduction is very rare in these species, making it difficult to distinguish between the two taxa.

Another morphologically similar species is *Feldmannophycus rayssiae* (Feldmann & G. Feldman) H. Augier & Boudouresque, but it has terminal carposporangia in short chains and a broader bathymetric distribution (Choi and Nam, 2001; Verlaque *et al*., 2015).

Despite its importance in understanding the life history of species, information on the reproductive structure of macroalgae is often lacking because of limited field and laboratory research. Such information is especially important when the species is non-native and potentially harmful. Here we provide the first information on the presence of female gametophytes of *C. okamurae* in the Mediterranean. Choi and Nam (2001) and Choi *et al.* (2001) found that reproduction of *C. okamurae* was directly related to seawater temperature. In their study, the optimal temperature for sexual reproduction of *C. okamurae* in laboratory cultures was found to be between 19 °C and 27 °C. Tetrasporangia, gametangia, and cystocarps were formed at 19 °C and above. In our study, the most favorable seawater temperature for sexual reproduction occurred during summer when we observed female gametophytic thalli with cystocarps and tetrasporophytic thalli when the mean seawater temperature was 22 ºC, reaching a minimum of 19.0 ºC and a maximum of 26.0 ºC. Male gametophytic thalli were never observed during our study. The presence of fertile thalli during this period is also consistent with the study of Petrocelli *et al.* (2020) conducted in the Mar piccolo of Taranto and in the Venice Lagoon (Italy), with the difference that tetrasporophytic thalli were common in these areas, while gametophytic thalli were never observed. From this we can conclude that reproduction of *C. okamurae*, at least in the northern Adriatic, is essentially by spores.

The aim of our study was also to investigate the abundance of *C. okamurae* populations in different seasons of the sampling period. The results showed that the average biomass of this species increased from autumn to spring, then decreased toward autumn and reached the highest average value in spring, when the average temperature of seawater at the surface was 17.73 °C (from a minimum of 11.0 °C to a maximum of 27.0 °C). These results partially contradict the studies conducted in Korea by Choi *et al.* (2001), who reported that the average biomass of *C. okamurae* increased with increasing seawater temperature from winter to summer and then decreased sharply, with minimal biomass recorded in winter. Conversely, our results were consistent with the results of the study from the Venice Lagoon (Adriatic Sea), where *C. okamurae* reached the highest biomass in late spring (Petrocelli *et al*., 2020).

Caulacanthus okamurae is an invasive red alga that forms extensive mats in protected marine habitats worldwide. The species is native to China, Japan, South

Korea and Taiwan, but is invasive in other parts of the world (Fofonoff *et al*., 2020). Turfs of this species have been shown to increase habitat complexity, retain sediment, and reduce desiccation at low tide. This could benefit some animals and algae by providing food, habitat, and/or protection from desiccation. However, these dense swards may restrict the movement of larger animals and the settlement and feeding of barnacles. Introduced populations have been shown to reduce the numbers of larger midlittoral fauna (periwinkles, limpets and barnacles) but benefit meiofauna (copepods and ostracods) and larger fleshy algae, including genera *Ulva*, *Gelidium* and *Chondracanthus*, by retaining water and reducing desiccation (Smith *et al*., 2014). In the Venice Lagoon, *C. okamurae* can be considered invasive because it has colonized every suitable substrate in the area and almost completely displaced *Gelidium spathulatum* (Kützing) Bornet, *Gelidium pusillum* (Stackhouse) Le Jolis and *Gymnogongrus griffithsiae* (Petrocelli *et al*., 2020).

The non-native alga *C. okamurae* poses a potential threat to native midlittoral communities, as previously observed and reported by other authors (Petrocelli *et al*., 2020). Our results are state of the art because there are no past data that would allow us to determine the impact of the species on this habitat. However, past experience has shown us that global change is already impacting midlittoral habitats (Battelli, 2016b) and the emergence of alien species will only exacerbate this process. Our results and those of other authors on the structure of the studied algal community of *C. okamurae*, allow us to hypothesise that changes in community composition may be due to the competition between native and non-native macroalgae.

CONCLUSIONS

The obtained results provide detailed quantitative data on the abundance of macroalgal species on different substrates of the midlittoral zone in the northern Adriatic invaded by *C. okamurae*. Morphological analyses allowed us to identify the collected specimens as a non-native species *C. okamurae*, previously unknown on the Slovenian coast. The characteristics of the midlittoral of the northern Adriatic Sea are unique in the Mediterranean due to its northern location and associated special tidal regimes. Therefore, these findings represent an important contribution to the understanding of patterns and processes of biological invasions, especially in such sensitive ecosystems as the intertidal zone. It is therefore clear that further studies, repeated over time, are needed to understand the impact of the occurrence of non-native species on the composition and structure of the algal and animal communities, especially in the face of climate change and the strong pressure on the coastal environment from human activities.

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