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FIRST REPORT OF AN AEGAGROPILOUS FORM OF *RYTIPHILAEA TINCTORIA* FROM THE LAGOON OF STRUNJAN (GULF OF TRIESTE, NORTHERN ADRIATIC)

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ABSTRACT

The occurrence of an aegagropilous form of the red alga Rytiphlaea tinctoria in the Stjuža marine lagoon of Strunjan (Gulf of Trieste) has been reported for the first time. The distribution, mean diameter, mean density and morphological structure of this population of R. tinctoria are here described. During the study, it was observed that the ball-like form of this species differs from the attached form found on open shores, lacking a holdfast, having a radial arrangement of the branches, and a curled distal part of the branches. No reproductive structures were observed in any of the collected samples. The ball-like form of Rytiphlaea tinctoria can be considered as an ecotype.

Key words: *Rytiphlaea tinctoria*, ball-like form, Stjuža lagoon Strunjan, northern Adriatic

PRIMA SEGNALAZIONE DI *RYTIPHILAEA TINCTORIA* IN FORMA EGAGROPILA NELLA LAGUNA DI STRUGNANO (GOLFO DI TRIESTE, ALTO ADRIATICO)

SINTESI

La nota riporta alcune osservazioni su Rytiphlaea tinctoria in forma egagropila rinvenuta nella Laguna Schiusa di Strugnano (Golfo di Trieste). Vengono descritte la distribuzione, il diametro, la densità e le caratteristiche morfologiche delle forme a palla di questa specie. Lo studio comparativo tra la forma egagropila e quella fissata, non rinvenuta in Laguna, evidenzia delle differenze in quanto priva di strutture d'attacco, ramificazione radiale e ramuli distali più aggrovigliati. Non si sono osservate alcune strutture riproduttive nei campioni raccolti. La forma egagropila presente nella laguna potrebbe essere considerata un ecotipo di R. tinctoria.

Parole chiave: *Rytiphlaea tinctoria*, forma egagropila, laguna Schiusa Strugnano, Alto Adriatico

INTRODUCTION

The red alga *Rytiphlaea tinctoria* (order Ceramiales, family Rhodomelaceae) is a perennial species and can occur in attached or unattached forms, depending on environmental conditions. This alga usually grows attached on rocky substrate, often covered with a thin sandy layer in sheltered and shaded habitats of the upper infralittoral zone (Calvo & Ragonese 1982). It has been reported from Atlantic European coasts, from Great Britain, Spain and Portugal to northern Africa. It is also widespread in Mediterranean coastal areas. In Slovenian coastal waters, the attached form of *R. tinctoria* has been found in Koper Bay (Avčín *et al.* 1974; Vukovič 1982), Strunjan Bay (Avčín *et al.* 1974; Turk & Vukovič, 1994) and Piran Bay (Vukovič 1980; Munda 1993).

The species *R. tinctoria* was described by Clemente (1807) as *Fucus tinctorius*. It is cited in literature for the presence of a water-soluble red pigment in its plastids, called “ficoamatin” by Kützing (1843). Later, Feldman & Tixier (1947) named this pigment “floridorubin”. The type locality of this species is Castillo de Santa Catalina and Puerto de Santa Maria and Cádiz, Andalusia, Spain. The name derives from the Latin “*rytis*” = a wrinkle, “*phloios*” = cortex, referring to the transversely furrowed or striate appearance of the surface, and “*tinctorius*” refers to the fact that the alga was used as a source for red dye (Phillips & De Clerck 2005).

Floating balls or unattached mats of *R. tinctoria* have been found in several locations, although reports of the ball-like form of this species are very limited. According to the available literature, this form of *R. tinctoria* has never before been found in the Gulf of Trieste. One of the most studied unattached, ball-like forms of this species has been reported from the Stagnone Lagoon (Sicily, Italy) by Calvo *et al.* (1981), Calvo & Ragonese (1982), Orestano & Calvo (1985), Mercurio *et al.* (2006) and Bellissimo & Orestano (2014).

Unattached algae that grow in a more or less spherical form as free-floating balls are described by the term *aegagropilous*. This term was first used by Linnaeus (1763) as a specific name for a rolling-ball alga from the Baltic: *Conferva aegagropila* L., sin. of *Cladophora aegagropila* (L.) Rabenh., currently regarded as a synonym of *Aegagropila linnaei* Kützing, published by Kützing (1843) (Calvo & Ragonese 1982). Ball-like forms are also formed by several marine species of *Cladophora*, as well as at least 54 other algae, including 25 red, 18 green and 11 brown algae (Norton & Mathieson, 1983).

The present paper reports the occurrence of extensive ball-like aggregates of *Rytiphlaea tinctoria* (Clem.) C.Ag. in the Stjuža marine lagoon of Strunjan (Gulf of Trieste, northern Adriatic). It aims to provide general information on the extensive development of the mobile, free-living rolling balls of this alga observed in the Stjuža marine lagoon of Strunjan in the spring of 2019. Details on the distribution, morphology and morphological measure-

ments of the ball-like form of this alga are reported. Thus, our results contribute to expanding the current knowledge on the unattached, ball-like form of algal populations in this area. The possible factors that led to the formation of the ball-like form of *R. tinctoria* are discussed.

MATERIAL AND METHODS

Study area

The Strunjan Lagoon is a shallow, semi-enclosed oligotrophic brackish coastal lagoon situated in the eastern part of the Strunjan Bay (45° 31' 30" North, 13° 36' 20" East) (Fig. 1a and 1b), about approx. 10 hectares in surface area and divided into two sub-basins: a smaller discharge lagoon and the larger, main Stjuža Lagoon of the silted former fish-farming pond. Stjuža (from the Italian “*chiusa*”, meaning ‘closed’) is the only Slovene marine lagoon; it is not completely natural, rather the result of human activities. For about a half century, it has been an abandoned fish farm. After the construction of a dam over 400 years ago, the bay was artificially closed for the purpose of fishing; the newly created lagoon remained connected with the sea only by three tidal channels. The Stjuža Lagoon is characterized by a meadow consisting predominantly of the sea grasses *Cymodocea nodosa* (Ucria) Ascherson and *Zostera noltei* Hornemann on its margins (Vrišer 2002; Šajna & Kaligarič, 2005). Today, the lagoon area is an important part of the Strunjan Stjuža Nature Reserve, within the Natura 2000 network, the primary objective of which is to preserve biodiversity.

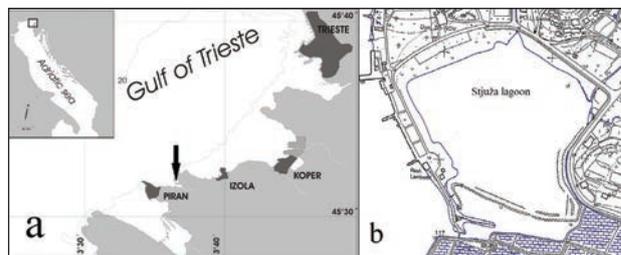


Fig. 1: Maps of the study area (a); Stjuža – Lagoon of Strunjan (b).

Sl. 1: Karta raziskovalnega območja (a); Stjuža – Laguna Strunjan (b).

Environmental parameters

Because of its shallow depth of about 0.5–1 m, the thermal conditions in the Stjuža Lagoon range seasonally from one extreme to the other: between 5 °C and 10 °C in wintertime and between 24 °C and 27 °C during the summer, while in the other seasons water temperatures are similar to the atmospheric temperatures. Salinity, oxygen content, and thermal conditions in the Stjuža Lagoon

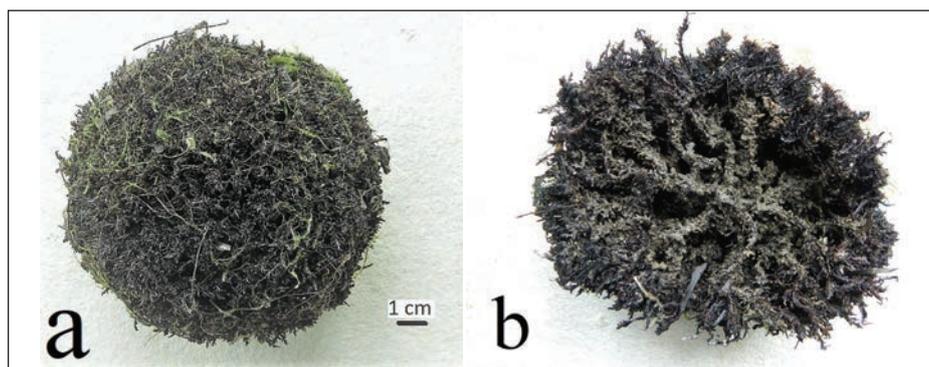


Fig. 2: *Rytiphlaea tinctoria* ball (a); section of *R. tinctoria* ball with branches radially arranged around the centre (b).

Sl. 2: Kroglica alge *Rytiphlaea tinctoria* (a); prerez kroglice alge *R. tinctoria* s prikazom radialno razporejenih poganjkov okoli središča (b).



Fig. 3: Branch of the alga *Rytiphlaea tinctoria* with curled apices (a); cross section of a branch showing the axial cell surrounded by 5 pericentral cells with medullary cells and darkly pigmented cortex (b).

Sl. 3: Stranski poganjek alge *Rytiphlaea tinctoria* s kaveljčastimi izrastki (a); prečni prerez poganjka prikaže osrednjo celico, 5 pericentralnih celic, medularne celice in temno pigmentirane kortikalne celice (b).

are related to the large water exchange and are usually similar to those of Strunjan Bay. The lagoon receives freshwater inputs from small canals from agricultural areas (Vrišer, 2002). The average tidal amplitude is 67 cm, with high water reaching 25–45 cm above the mean sea level, and low water 15–30 cm below the mean sea level (Malačič *et al.*, 2000).

Sampling procedure and data analysis

The fieldwork was carried out in the spring of 2019, when a dense aggregation of ball-like *R. tinctoria* was found in the Stjuža Lagoon of Strunjan. The study was conducted in separate parts of the lagoon margin on the northern, south-western and western shores. These sites were chosen because of the higher density of ball-like aggregations of this species than in the other parts of the

lagoon. The substrate of the entire research area is a soft sediment composed of compact-fine argillaceous silt with a slight admixture of sand, with a thin (0.5–1 cm) yellowish brown layer of flocculent organic detritus (Vrišer, 2002).

The algal material collected was carefully sorted and examined using a stereoscope, while a light microscope was used to check for the presence of reproductive structures. The anatomical observations were based on fresh material. Sections were cut by hand with a single-edged razor blade and photographed in the laboratory of the Natural History Museum of Trieste (Italy) using a microscope Leica MZ16 with camera Leica mc190 HD. All the specimens were identified at a species level, while taxonomically difficult taxa were summarized to genus level as 'spp.' due to the absence of reproductive structures and/or diacritical features that are traditionally used to identify a species.

The main resources used to identify the collected species were Maggs & Hommersand (1993), Bressan & Babbini (2003), Phillips & De Clerck (2005), Brodie *et al.* (2007), Sfriso (2010). The nomenclature follows Guiry & Guiry (2019).

Five randomly selected sampling frames (40 cm x 40 cm) were used to estimate the density of the *R. tinctoria* balls. The density was determined by counting the number of the ball-like forms directly from each frame during the field work in each of the three parts of the lagoon checked (northern, south-western and western).

Fifty balls of *R. tinctoria* from the studied area were randomly collected in each of the three parts of the lagoon checked in order to measure their diameter. The measures were taken directly to the nearest 0.1 mm using a caliper.

RESULTS AND DISCUSSION

Dense aggregates of ball-like forms of a red alga were recorded in the Stjuža Lagoon of Strunjan for the first time. Based on morphological features, we identified this red alga as *Rytiphlaea tinctoria*. Its morphology corresponded with the descriptions reported for this species in other parts of the Mediterranean (Phillips & De Clerck, 2005). The balls of *R. tinctoria* ranged in shape from roughly spherical to prolate spheroid (Fig. 2a). Branches were radially arranged around a very small branch segment (Fig. 2b) which formed the core of the thallus.

The distal parts of the thalli consisted of flattened, regularly alternate branches with strongly incurved branch apices. The thallus was yellowish to dark red-brown in colour, darker towards the tips of the axes (Fig. 3a), and coriaceous to cartilaginous in texture, abundantly branched. Microscopic observations showed that the structure of the thallus was uniaxial. Cross section through young and mature branches showed the sub-terete to oval shape of the axes, composed of a central cell surrounded by five pericentral cells, further medullary cells and a darkly pigmented single-layered cortex (Fig. 3b). During our examination of the collected material, we did not detect any reproductive structures.

The values of the mean diameter and mean density of the ball-like *R. tinctoria* from the research area are illustrated in Table 1. The average density was 22.3 (N/1600 cm²) and it varied between 14 and 33 (N/1600 cm²). The balls ranged widely in size, their diameter on average 73.5 mm, from a minimum of 36.2 to a maximum of 145.3 mm. The described thalli are morphologically very similar to those described by Calvo & Ragonese (1982) from the Stagnone Lagoon (western coast of Sicily), as shown in Table 1.

The dissection of the balls revealed a solid, dense mass of intertwined branches, almost entirely of *R. tinctoria*, but also containing fragments of degraded material composed mainly of leaves of *C. nodosa* and *Z.*

Tab. 1: Average values of the density and comparative size (diameter) recorded for red alga *Rytiphlaea tinctoria* balls from the Stjuža Lagoon of Strunjan, and the Stagnone Lagoon (western Sicily).

Tab. 1: Povprečne vrednosti gostote in primerjalne velikosti (premer) kroglic rdeče alge *Rytiphlaea tinctoria* iz Lagune Stjuža Strunjan in Stagnone (zahodna obala Sicilije).

<i>Rytiphlaea tinctoria</i>			
Stjuža Lagoon (Strunjan)			Stagnone Lagoon (Calvo & Ragonese, 1982)
	Density (N/1600 cm ²)	Size (diameter/mm)	Size (diameter/mm)
Mean	22.3	73.5	100.0
SD	6.7	24.6	-
Min	14	36.2	40.0
Max	33	145.3	200.0

noltei. Some species of Rhodophyta (mainly filamentous Rhodomelaceae with *Polysiphonia*-like morphology), Chlorophyta (mainly Cladophoraceae and *Ulva*) were found as epiphytes on the branches of *R. tinctoria*. In some balls, the only non-organic matter was sand from the substrate. Some balls were kept in an aquarium and numerous small invertebrates were seen emerging from and retreating into the balls, as also reported by Sparla & Riggio (1983-84) and Ballantine *et al.* (1994).

List of the most abundant epiphytes of *R. tinctoria*:

Ceramium spp.

Chaetomorpha linum (O.F. Müller) Kützing

Cladophora spp.

Cystoseira foeniculacea f. *tenuiramosa* (Ercegovic)

A. Gómez Garreta, M.C. Barceló, M.A. Ribera & J.

Rull Lluch

Polysiphonia scopulorum Harvey

Polysiphonia spinosa (C. Agardh) J. Agardh

Ulva rigida-laetevirens complex

Titanoderma pustulatum (Lamouroux) Näegeli

Valonia utricularis (Roth) C. Agardh

In the Stjuža Lagoon, many algal species were present in both attached and unattached form. The soft bottom was clearly unsuitable for the development of a highly diverse attached macroalgal vegetation. The presence of the ball-like aggregations of *R. tinctoria* (Fig. 4b) was the consequence of an accumulation of detached material caused by winds and tidal currents flowing during the tidal switch, as illustrated in Fig. 4a, where the yellow arrows indicate the outflow and the red arrows the inflow of the seawater during the change of tides.

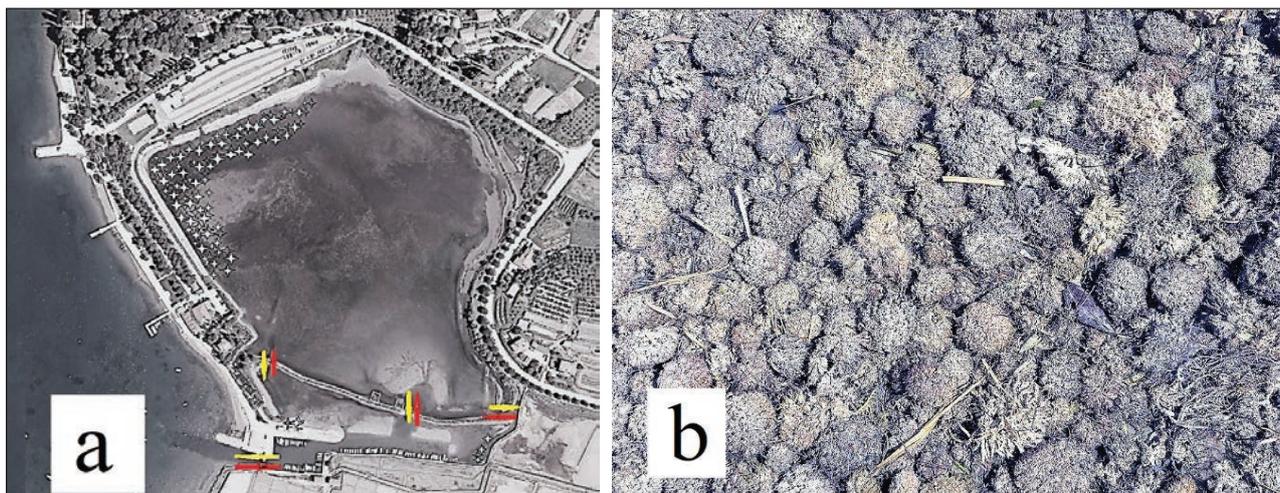


Fig. 4: Distribution of *Rytiphlaea tinctoria* ball-form (white stars) occurring in the Stjuža Lagoon of Strunjan and the direction of the currents of the seawater during tidal movement. The yellow arrows indicate the output flow and the red arrows the entry flow (a). Extensive aggregates of *Rytiphlaea tinctoria* balls (b).

Sl. 4: Razporeditev kroglic vrste *Rytiphlaea tinctoria* (bele zvezdice) v laguni Stjuža v Strunjanu in smer toka morske vode med plimovanjem. Rumene puščice predstavljajo smer izhoda, rdeče pa smer vhoda morske vode med bibavico (a); goste gruče kroglic vrste *Rytiphlaea tinctoria* (b).

Due to the lack of hard substrata in the lagoon, we found the attached algae mainly on small pebbles, shells, man-made objects and seagrass rhizomes. Some species were present only in the unattached form, floating above the bottom as benthopleustophytes. We observed only two species of algae with typical ball-like forms: *R. tinctoria* and the green alga *Lychaete echinus* (Biaioletto) M.J. Wynne. Their morphologies were different from those of the attached forms. All unattached macroalgae share some typical morphological features: they lack a basal holdfast and are smaller and more branched than the conspecific attached thalli. They usually exhibit curled or screw-like distal parts of branches.

The pleustophyte populations, rich in ball-like forms (with a free spherical structure) typical of lagoon environments, are frequent in the Mediterranean. Among the ball-like forms typical of lagoon environments in the Mediterranean, *Valonia aegagropila* C. Agardh, *R. tinctoria*, *L. echinus* and *Chaetomorpha linum* (O.F. Müller) Kützing were the most diffused (Calvo *et al.*, 1980; Orestano & Calvo, 1985; Cecere *et al.*, 1992).

Among the most abundant unattached forms of algae found in the Stjuža Lagoon were green algae of the genus *Ulva*, with the species *U. rigida* and *U. laetevirens* forming mostly unattached accumulations. Sfriso (2006) reported that it would be more correct to refer to the *Ulva rigida-laetevirens* complex, because the two species are indistinguishable when they are in the unattached form. Among these accumulations, *Enteromorpha*-type forms of *Ulva* (with the species *U. compressa* and *U. intestinalis*), *Chaetomorpha* (with the species *C. linum*), *Lychaete* (with the species *L. echinus*) and *Cladophora* (with the species *C. lehmanniana* and *C. liniformis*) were also present.

The brown alga *Cystoseira foeniculacea* f. *tenuiramosa* was detected in two attached forms: as epiphyte on *R. tinctoria* balls (Fig. 5a) and on small pebbles (Fig. 5b). The occurrence of this canopy-forming alga in the Stjuža Lagoon was observed for the first time during this study. Moreover, the species *C. foeniculacea* (Linnaeus) Greville was reported just once in Slovenian coastal waters by Avčín *et al.* (1974) as *Cystoseira discors* (Linnaeus) C. Agardh. Due to the high ecological value of *Cystoseira* spp., this finding is quite important, and the presence and abundance of *C. foeniculacea* f. *tenuiramosa* in the Stjuža Lagoon should be regularly monitored in the future.

Two theories about the formation of the ball-like form of *R. tinctoria* have been proposed. According to the first, the phenomenon can be considered the result of a dynamic action of the waves' motion (Fritsch, 1965; Smith, 1950). The second supports the active role of the alga (Van den Hoek, 1963; Austin, 1960).

Based on the information available for other parts of the Mediterranean, we suggest that some environmental conditions characteristic of the Stjuža Lagoon favour the formation of the ball-like form of *R. tinctoria*, such as: shallowness (with an average of about 0.5–1 m of depth), which permits continuous exposure to sunlight and consequently the growth of algal thalli in all directions; superficial and bottom water currents produced by winds blowing from the North-North-East (burja) and from the South-East (jugo); a wide tidal range, of about 67 cm; and a soft sedimentary bottom unfavourable for the development of attached macroalgae. This is in agreement

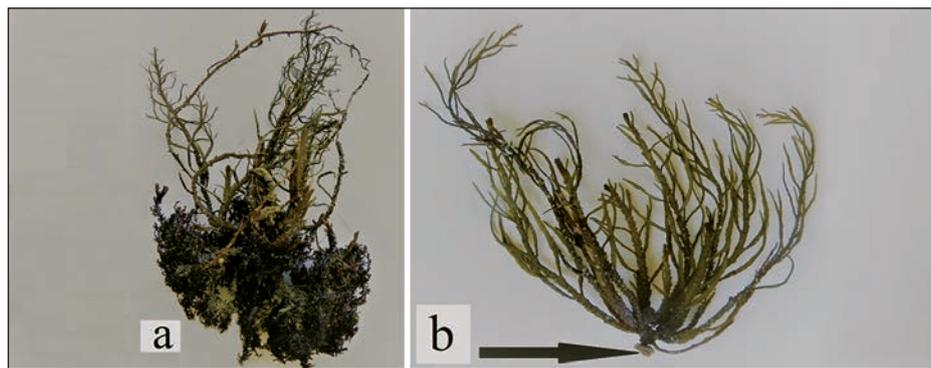


Fig. 5: *Cystoseira foeniculacea* as epiphyte on *Rytiphlaea tinctoria* ball (a); the attached form of *C. foeniculacea*, the arrow indicates the basal disc (b).

Sl. 5: *Cystoseira foeniculacea* kot epifit na kroglici alge *Rytiphlaea tinctoria* (a); alga v pritrjeni obliki, puščica prikazuje pritrtilno ploščico.

with Calvo & Ragonese (1982) and Orestano & Calvo (1985) who, during their studies on the ball-like form of *R. tinctoria* from the Stagnone Lagoon (Sicily, Italy), argued that the formation of the ball-like form is a consequence of two factors: the presence of bottom water currents, which allows rolling, and an intense proliferation of laterals and lateral hooks which, partially imbricate, increase the compactness of the alga. It is generally assumed that the unattached form can develop as spherical, entangled, free-rolling balls under certain hydrographic and topographic conditions. In their study of the formation of ball-like aggregations of the green alga *Aegagropila linnaei* Kützing, Togashi *et al.* (2014) suggested that these aggregations are an adaptative strategy to increase biomass in the extremely limited environments suitable for the growth of this alga.

Another interesting observation made in this study is the absence of reproductive structures in the ball-like form of *R. tinctoria*. It is our general opinion that the benthopleustophyte forms, which derive from the attached form, lost contact with the hard substrate and consequently the capability of development of reproductive cells (Burrows, 1958).

The present observations were limited to a single sampling date. Unfortunately, we did not have more data on the environmental conditions that may have favoured the unusual formation of the ball-like form of this red alga and others, such as *Lychaete echinus* in the Stjuža Lagoon of Strunjan. Our assumptions are based only on the observations made during the short research period and the study of the available literature. It is therefore evident that further investigations, repeated in time, will be necessary for a deeper understanding of this phenomenon.

CONCLUSIONS

On the basis of the cited literature and our observations during the study, we suppose that the formation of the ball-like form of the unattached red alga *R. tinctoria* in the Stjuža Lagoon of Strunjan may be interpreted as a consequence of (a) mechanic processes through a consistent water movement influenced by the winds and tidal current between the high and low tide and (b) features intrinsic to the species which allow the radial growth of the thallus by rolling it on the bottom and thus continuously vary its exposure to light.

On the bases of our field observations on the occurrence of the ball-like form of *R. tinctoria* in the Stjuža Lagoon of Strunjan, which form ball-like aggregations that remain lying or slowly rolling on the bottom, we propose the ball-like form of *R. tinctoria* be considered as an ecotype of this species.

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PRVI ZAPIS O POJAVLJANJU VRSTE *RYTIPHLAEA TINCTORIA* V KROGLIČNI OBLIKI V STRUNJANSKI LAGUNI (TRŽAŠKI ZALIV, SEVERNI JADRAN)

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POVZETEK

Avtorja opisujeta primer prvega pojavljanja kroglične oblike rdeče alge *Rytiphlaea tinctoria* iz morske lagune Stjuža v Strunjanu (Tržaški zaliv). Terensko delo sta izvedla spomladi 2019 v strunjanski laguni Stjuža, kjer sta opazila visoko gostoto kroglične oblike alge *Rytiphlaea tinctoria*. Ta se je z večjo gostoto kroglic pojavljala predvsem vzdolž robov lagune na severnem, jugozahodnem in zahodnem delu. Opisujeta porazdelitev, povprečni premer, povprečno gostoto in morfološko zgradbo kroglic *R. tinctoria*. Za oceno gostote kroglic *R. tinctoria* sta uporabila pet naključno izbranih kvadrantov (40 cm x 40 cm). Izmerila sta premer petdesetih naključno izbranih kroglic. Ugotovila sta, da je mehko dno lagune za razvoj pritrjene makroalgalne vegetacije očitno neugodno, zato so bile v času študije pritrjene oblike alg prisotne predvsem na majhnih kamenčkih, školjkah, umetnih predmetih in na koreninah morske trave. Avtorja razlagata, da je pojav velike gostote kroglične oblike *R. tinctoria* v laguni, posledica nanosov, ki so ga povzročili tokovi v laguni zaradi delovanja vetrov in plimovanja. V študiji opisujeta tudi pojav nastanka kroglične oblike *R. tinctoria*, ki ga skušata razložiti z dvema hipotezama in sicer, pojav lahko obravnavamo kot rezultat dinamičnega delovanja tokov in valov v laguni ali kot rezultat aktivne vloge alge z namenom povečanja biomase v prostorsko omejenem prostoru, kot je laguna. Med študijo sta opazila, da je kroglična oblika te vrste drugačna od značilne pritrjene oblike, ki ni prisotna v laguni, saj nima pritrjenih struktur in ima radialno razporejene poganjke z ukrivljenimi končnimi deli. V nobenem zbranem vzorcu nista opazila reproduktivnih struktur, kar razlagata kot posledico izgube stika s trdnim substratom in možnostjo za razvoj reproduktivnih struktur. Kroglična oblika *Rytiphlaea tinctoria* bi lahko veljala za ekotip.

Ključne besede: *Rytiphlaea tinctoria*, kroglična oblika, laguna Stjuža Strunjan, severni Jadran

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