Spatio-temporal occurrence patterns of epibiota along the leaves of the seagrass *Cymodocea nodosa* in the Northern Adriatic Sea

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ABSTRACT
We investigated the epibiotic community along *Cymodocea nodosa* leaves in a seagrass meadow in the Northern Adriatic Sea. In accordance with previous studies, we found that autotrophic organisms dominated throughout the study period (May–October 2014) and showed a clear temporal occurrence pattern with highest occurrence frequencies during summertime. Low relative occurrence of autotrophs was observed in spring, coinciding with higher values for sessile heterotrophs. In both autotrophs and heterotrophs, we observed a general trend towards increased epibiota cover on old leaves and towards the apical (i.e. older) parts of leaves, even though this pattern does not hold true for all species or sampling times. Most heterotrophs were rarely observed, but serpulid polychaetes, the foraminiferan *Tretomphaloides concinnus*, the hydrozoan *Clytia linearis* as well as gastropod clutches occurred frequently on all parts of the leaves and showed occurrence patterns putatively reflecting spatial competition with autotrophs and predator avoidance. Hitherto, few data have been available on the epifaunal diversity and community structure on *C. nodosa*. Thus, this study provides important baseline data for future studies investigating the impact of natural and anthropogenic stressors on epibiota communities associated with this ecologically important Mediterranean seagrass species.

Introduction

Seagrass meadows are among the most productive shallow coastal ecosystems (Duarte and Chiscano 1999; Hemminga and Duarte 2000) and as ecological engineers (Wright and Jones 2006) they provide numerous ecological services to the marine environment (Costanza et al. 1997; Duarte 2000; Barbera et al. 2005; Nakaoka 2005; Tuya et al. 2014). They increase seascape heterogeneity and consequently the net diversity of species, as the three-dimensional structures of seagrass meadows provide habitat for a great diversity of plants and vagile and sessile animals (Trautman and Borowitzka 1999; Hemminga and Duarte 2000; Piazzi et al. 2016; Hayes et al. 2020; Hoffmann et al. 2020). In addition, seagrass meadows offer an important source of nutrients, store large quantities of organic carbon (Orth et al. 2006) and play an important role as primary producers (Mazzella et al. 1993; Duffy 2006).

Epibiota initially colonize young leaves and increase their cover and biomass on old ones (Reyes et al. 1998;

Reyes and Sansón 2001). Growth dynamics, associated with certain life strategies, as well as competition among epibiota, determine their success along the leaf surface (Borowitzka et al. 1990). However, if the abundance of epibiota is too high, this has a strong negative effect on the photosynthetic capacity, life-span and growth rate of seagrass leaves (Sand-Jensen 1977; Bulthuis and Woelkerling 1983; Heijs 1985), which forces the release of older leaves (Ott 1980) and leads to a clear leaf-age gradient (Heijs 1985). Additionally, abiotic factors (e.g. water depth, light levels, seasonality) shape seagrass growth dynamics and meadow structure. With increasing water depth, light availability and, consequently, the photosynthetic capacity of the plants is reduced. On the other hand, growth and long-term survival of the leaves and epibiota is enhanced by reduced mechanical disturbances (Dring and Dring 1991; Reyes and Afonso-Carrillo 1995; Krause-Jensen et al. 2000; Duarte et al. 2007; Bračun et al. 2020). These complex interactions between biotic and abiotic factors shape

a whole community, characterized by quick turnovers, short lifespans and strong adaptability, that is very susceptible to changes in the environment. Therefore, seagrass meadows and their associated flora and fauna represent a promising monitoring tool for detecting coastal environmental changes (Orlando-Bonaca et al. 2015; Ben Brahim et al. 2020).

In temperate and subtropical climates, such as the Mediterranean Sea, seagrass meadows represent crucial coastal ecosystems and provide important habitats (Meinesz et al. 1991; Orth et al. 2006; Duarte et al. 2008; Tuya et al. 2014). Five seagrass species naturally occur in the Mediterranean Sea: *Posidonia oceanica* (Linnaeus) Delile, 1813, *Zostera marina* Linnaeus 1753, *Zostera noltei* Hornemann, 1832, *Cymodocea nodosa* (Ucria) Ascherson, 1870 and *Ruppia maritima* Linnaeus, 1753. While *P. oceanica* is certainly the best studied seagrass species considering its epibiota (e.g. Mazzella et al. 1989, 1992; Marbá et al. 2002; Balata et al. 2007; Lepoint et al. 2014), less attention has been devoted to the other species (Bračun et al. 2020). However, especially *C. nodosa* and its associated community play an important ecological role, for instance by preparing the soil for the re-establishment of larger seagrass species (Toccaceli 1990) and by contributing to the coastal ecosystem functioning (Orlando-Bonaca et al. 2015, 2016).

Overall, studies of epibiota on *C. nodosa* are scarce and biased towards autotrophic components (Reyes and Afonso-Carrillo 1995; Reyes et al. 1998; Reyes and Sansón 1996, 1997, 2001). Hence, little is known about sessile invertebrates inhabiting the leaves (Guidetti et al. 2001; Ben Brahim et al. 2020; Bračun et al. 2020). However, growth patterns of seagrass and epibiota are strongly connected and show interdependencies (Sand-Jensen 1977; Bulthuis and Woelkerling 1983; Heijs 1985; Borowitzka et al. 1990). Therefore, studies focusing on a temporal and spatial resolution of epibiota are crucial in order to understand the processes that underlie these dynamics, especially for assessing potential environmental impacts (e.g. Orlando-Bonaca et al. 2015).

In a previous study we investigated the temporal growth patterns of *C. nodosa* in a seagrass meadow in the Northern Adriatic Sea and quantified the diversity of the epibiota on the leaves (Bračun et al. 2020). In this preceding study we revealed that the general structure and growth dynamics of the investigated patch are similar to other Mediterranean *C. nodosa* meadows. Additionally, we showed that overall autotrophs dominated over heterotrophs throughout the study period and at both investigated depths (1.5

and 5 m). Highest abundances of epibiota were observed during summer months and taxa differed among water depths. Additionally, the abundance of epibiota followed a clear leaf-age gradient for autotrophs but not for heterotrophs.

Building upon this, the present study aims to characterize the temporal dynamics of occurrence patterns of epibiota along single *C. nodosa* leaves. More specifically, we assessed differences in occurrence patterns of epibiota on basal, central and apical leaf segments throughout a period of six months, across two depths and two different leaf-ages, to provide a baseline characterization of this epibiota community on *C. nodosa* in the Northern Adriatic.

Materials and methods

The investigated *Cymodocea nodosa* meadow (Figure 1A) is located in the south-west of the Istrian peninsula (Valsaline/Pula/Croatia – 44°50′59.6″N, 13°50′10.0″E), in the Northern Adriatic Sea. Sampling (by means of snorkelling and scuba diving) was conducted monthly from May–October 2014 at a depth of 1.5 m (shallow) and 5 m (deep). Each monthly sample consisted of four replicates per depth of a standardized area of 0.25 m² where seagrass was collected using a custom-built 0.5 m × 0.5 m frame attached to a mosquito net (Bračun et al. 2020). In order to investigate the epibiota on the leaves, 13 shoots from each replicate were harvested in a star-like standardized way within the frame (i.e. $3 + 2 + 3 + 2 + 3$ samples per row = 13 shoots × sample-1 × 4 replicates × month-1 × depth-1 = 52 shoots × month-1 × depth-1) (Bračun et al. 2020). Leaves in a shoot were classified into three categories (old, young, others; Figure 1B), and for the present study we only focused on the oldest and youngest leaf of the shoot (Figure 1C). Determination of leaf-ages followed Reyes and Sansón (2001), with old leaves typically occupying the outer position of a shoot and young leaves originating in between them. Occurrence as well as the position of the epibiota were recorded for each replicate, sample and depth for young (Y) and old (O) leaves. For characterizing differences in epibiota occurrence along seagrass leaves, leaves were subdivided into three segments of the same length (Figure 1C; apical, central, basal). If possible, epibiota were determined to species level. Otherwise, higher taxonomic ranks were used to classify the specimens (Bračun et al. 2020).

The occurrence frequency was calculated as the count of occurrences per taxonomic unit for each depth, leaf-age and leaf-segment divided by the total
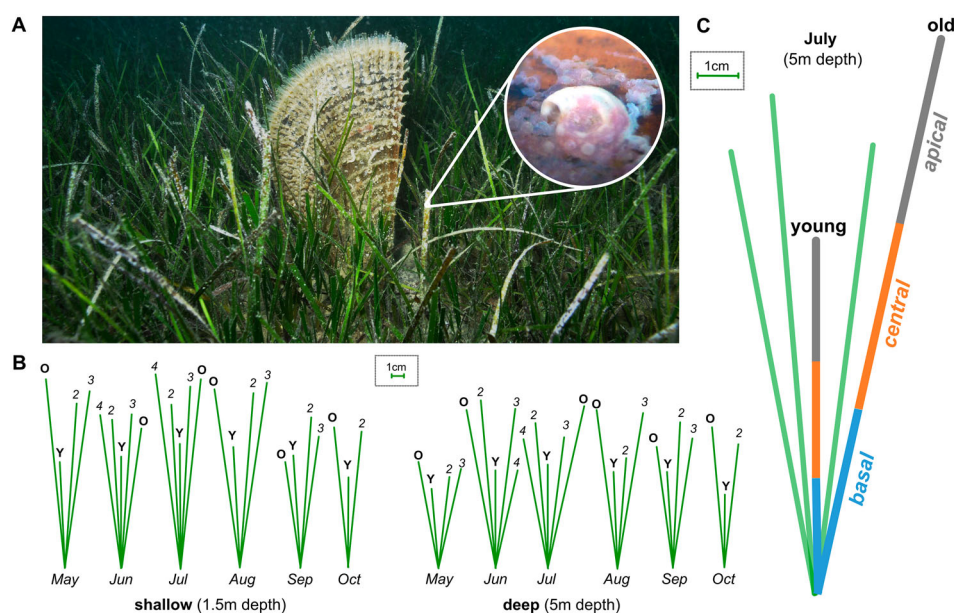
Figure 1. Study site, mean monthly (seasonal) variation of shoot structure (leaf length, number of leaves) and investigated leaf segments. (A) Picture of the investigated meadow (Valsaline; Pula; Croatia; 44°50′59.6″N, 13°50′10.0″E) and 'space competition' on leaves shown by a sessile Polychaeta, which is overgrown by Corallinaceae. (B) Monthly variation of shoots for the shallow and deep site. Numbers (1–4) indicate other leaves, beside the old (O) and young (Y). (C) One representative shoot (July at 5 m): basal (blue), central (orange), apical (grey).

number of investigated leaves per month (i.e. $N_{total} = 52$). To calculate pairwise comparisons of the occurrence frequency between months and the leaf position, a pairwise Wilcoxon rank sum test was applied and P-values were Bonferroni corrected. Kruskal–Wallis tests were used to compare the occurrence frequencies of autotrophs and heterotrophs for different leaf segments, as well as for epibiota inhabiting old and young leaves in different depths. Statistical analyses as well as graphical visualizations were done in R v3.3.2 (R. Core Team 2013).

Results

General and temporal growth patterns of epibiota along leaves

In both autotrophs (Kruskal–Wallis, $\chi^2 = 11.007$, df = 2, $p = 0.004$) and heterotrophs (Kruskal–Wallis, $\chi^2 = 7.130$, df = 2, $P = 0.028$), cover along leaves significantly increased from the basal to the apical segment. Pairwise comparisons between different segments revealed significant differences only in the apical vs. basal and the central vs. basal comparison for the autotrophs (Wilcoxon rank sum test with continuity correction; apical vs. central: $P = 1$; apical vs. basal: $P = 0.006$; central vs. basal: $P = 0.022$; Bonferroni corrected) and between the central and basal segment for the heterotrophs (Wilcoxon rank sum test with continuity

correction; apical vs. central: $P = 1$; apical vs. basal: $P = 0.111$; central vs. basal: $P = 0.035$; Bonferroni corrected). Additionally, with the exception of old leaves in the shallow transect (Kruskal–Wallis; $\chi^2 = 3.884$, df = 2, $P = 0.143$), a clear differentiation in epibiota occurrence patterns became evident across both depths and leaf-ages (Figure 2A, 3A).

Throughout the study period (May–October 2014), the occurrence of epibiota increased from the basal to the apical segment, but with no significant difference between the central and apical segment (pairwise comparisons Wilcoxon rank sum test with continuity correction; apical vs. central: $P = 1$; apical vs. basal: $P = 0.002$; central vs. basal: $P = 0.001$; Bonferroni corrected). Epibiota reached highest occurrences in July and August on the apical and central segment (Figure 4A, B). This pattern also holds true when looking at autotrophic and heterotrophic epibiota separately.

Growth patterns and diversity of epibiota in both investigated depths

In shallow water (Figure 2B, C), young as well as old leaves were dominated by autotrophic Corallinaceae, *Ceramium* spp., as well as diverse other Rhodophyta and heterotrophs, Polychaeta and the foraminifera *Tretomphaloides concinnus* (Brady, 1884). Occasionally occurring organisms included gastropod clutches, the hydrozoans *Clytia linearis* (Thorneley, 1900) and
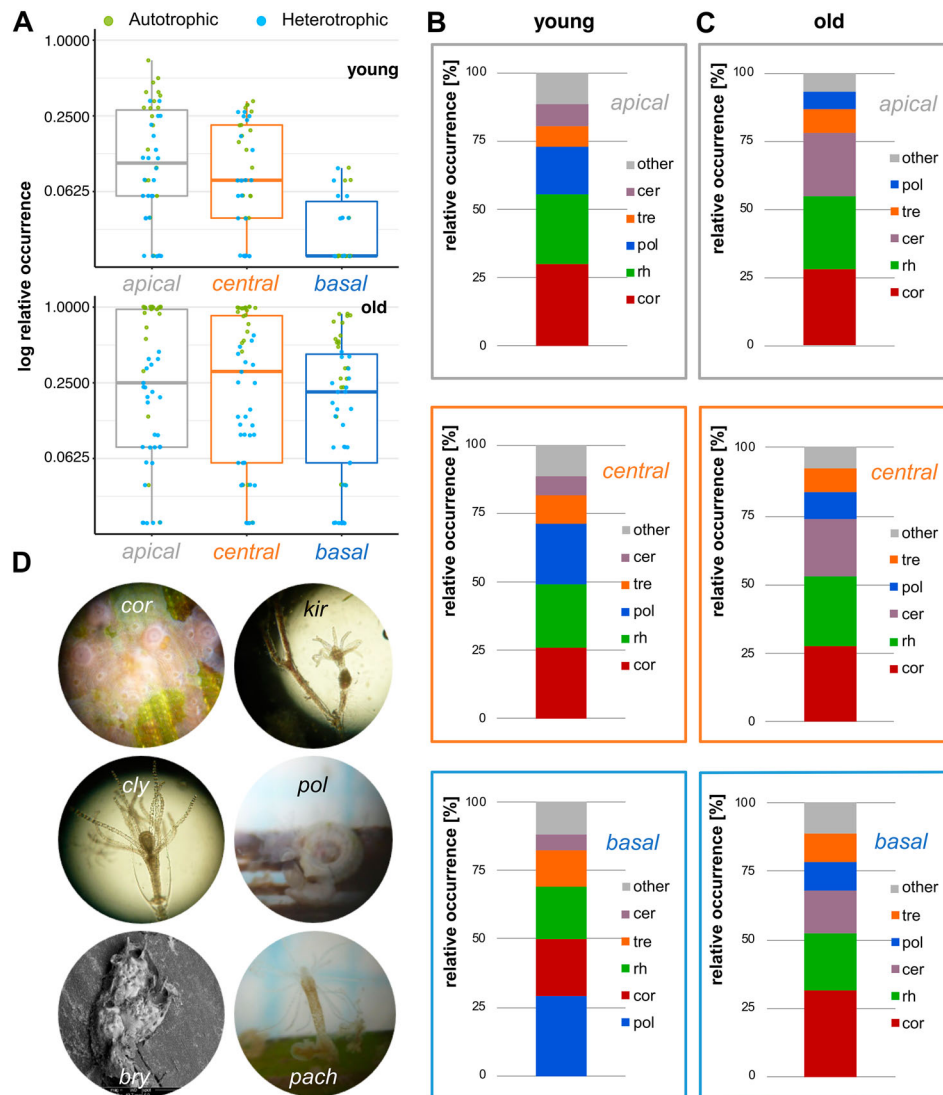
Figure 2. (A) Relative occurrence (log-scaled) of autotrophic and heterotrophic epibiota for each segment collected in the shallow transect (1.5 m depth). Relative occurrence in per cent for epiphytes on young (B) and old (C) leaves from apical (grey), to central (orange) and basal (blue) in shallow water. (D) Representative organisms found in the transect. Abbreviations of single taxa are ordered from bottom to top according to relative occurrence values in per cent. Abbreviations: bryozoan (bry), *Ceramium* (cer), *Clytia linearis* (cly), Corallinaceae (cor), *Kirchenpaueria pinnata* (kir), *Pachycordyle pusilla* (pach), Polychaeta diverse (pol), Rhodophyta diverse (rh), *Tretomphaloides concinnus* (tre). A detailed view on the relative occurrence of all other epibiota can be found in the Appendix (Figures S1, S3).

Kirchenpaueria pinnata (Linnaeus, 1758), Bacillariophyceae, various bryozoans, athecate hydrozoans and bivalves (Figure 2D). Hardly any *Oscillatoria* spp. (Cyanobacteria) were recorded on young leaves, whereas they were rather common on old leaves. On the young leaves of the shallow transect (Figure 2B; Figure S1), the relative occurrence of polychaetes and *T. concinnus* decreased from the basal to the apical segments, whereas Rhodophyta, Corallinaceae and *Oscillatoria* spp. (on old leaves) increased. Old leaves were colonized by autotrophs such as Corallinaceae, Rhodophyta as well as *Ceramium* spp. and dominant heterotrophic taxa included the hydrozoans

Pachycordyle pusilla (Motz-Kossowska, 1905) as well as *C. linearis*, polychaetes and the foraminiferan *T. concinnus* (Figure 2D). Compared with young leaves, an increase of *Ceramium* spp. was found on old leaves (Figure 2C, Figure S2).

Similar results were obtained for deeper water (Figure 5B,C). On young and old leaves, various autotrophic Rhodophyta (incl. *Ceramium* spp., Corallinaceae) and heterotrophs such as Polychaeta, the foraminifera *T. concinnus* as well as gastropod clutches were most prevalent. Occasionally occurring organisms included other foraminifera, mucus cases of vagile polychaetes, several leptothecate and anthothecate
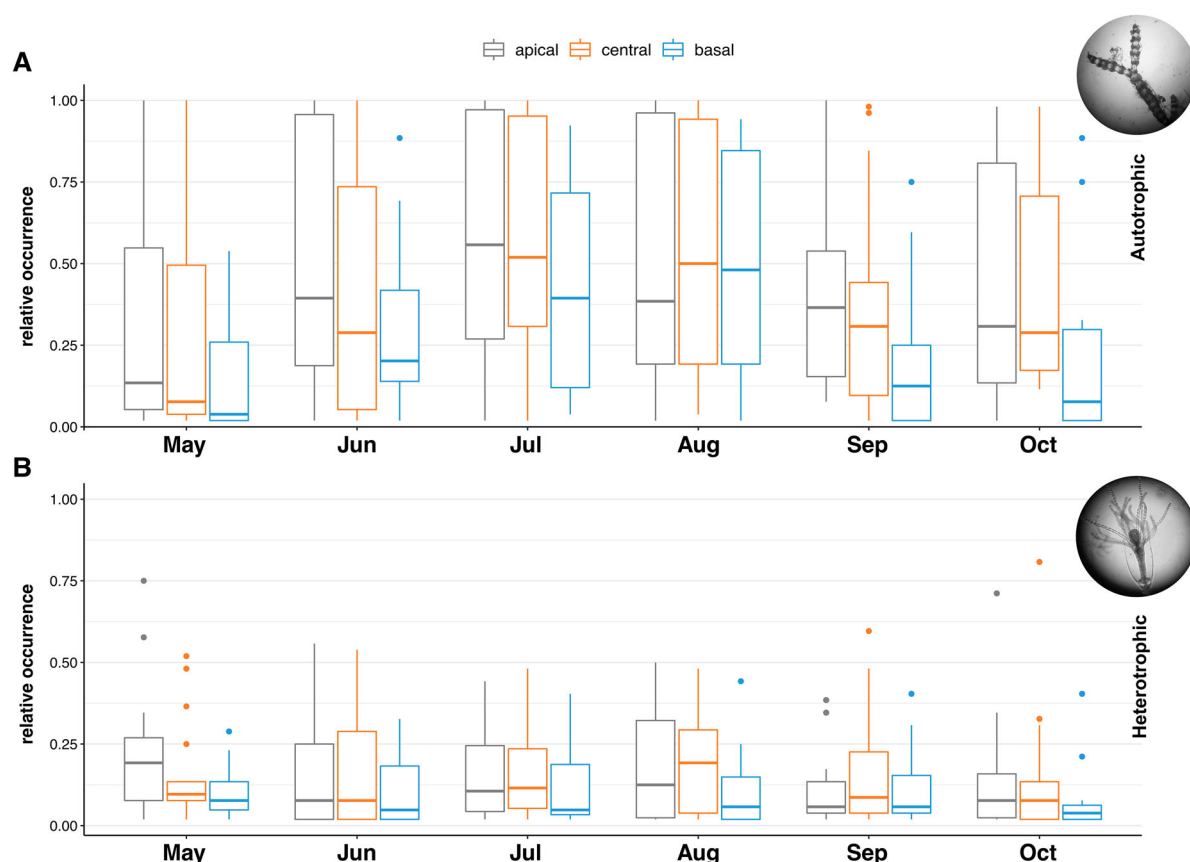
Figure 3. Seasonal occurrence frequency of (A) autotrophic and (B) heterotrophic epiphytes within the apical (grey), central (orange) and basal (blue) segment. Representative taxa for each group are shown in the figure: *Ceramium* spp. (A) and *Clytia linearis* (B).

hydrozoans (e.g. *C. linearis*, *P. pusilla*), various bryozoans (e.g. *Schizobrachiella sanguinea*) (Norman, 1868) and the anemone *Bunodeopsis strumosa* Andrès, 1881 (Figure 5D). On the young leaves (Figure 5B, Figure S1), the relative occurrence of gastropod clutches decreased towards the apical part whereas Polychaeta and Rhodophyta increased. *T. concinnus* was distributed evenly along all three segments. Compared with the young leaves, old leaves were dominated by autotrophic cover. On the old leaves (Figure 5C, Figure S2), *Ceramium* spp. was quite common in the central and apical segments, but rare in the basal segment. Other autotrophs were generally more common towards the apical end of the leaves. In contrast, gastropod clutches (despite being present in the other segments as well), occurred quite frequently in the basal segment. *T. concinnus* and Polychaeta were found in all three segments but were generally less common in the basal parts of the leaves. Compared with the young leaves, polychaetes were generally less common on older leaves. In general, for some taxa (e.g. *P. pusilla*, *K. pinnata*, Bacillariophyceae) only single or very few individuals were recorded (Figure S1, S2).

Discussion

Influence of depth and leaf age on epibiota occurrence pattern

Seagrass leaves offer a stable substrate for numerous organisms like algae and sessile invertebrates (Reyes and Sansón 2001). These epibiota represent an important component of the community structure of seagrass meadows and are major actors in food webs as well as the overall seagrass ecosystem functioning (Lepoint et al. 2014; Hoffmann et al. 2020). This is particularly true for *C. nodosa* that harbours a diverse flora and fauna, despite its relatively small leaf surface area (Mazzella et al. 1998; Guidetti et al. 2002).

In this study, we quantified the diversity and spatio-temporal occurrence patterns of epibiota along single *C. nodosa* leaves. Seagrass leaves grow from the base. Thus, the tips represent the oldest parts which are first exposed to and colonized by epibiota. Consequently, initial colonizers are found on young leaves and if they persist and survive, they are pushed 'conveyer-like' along the leaf to increasingly light-exposed, apical and thus older parts (Heijs 1985). Across the
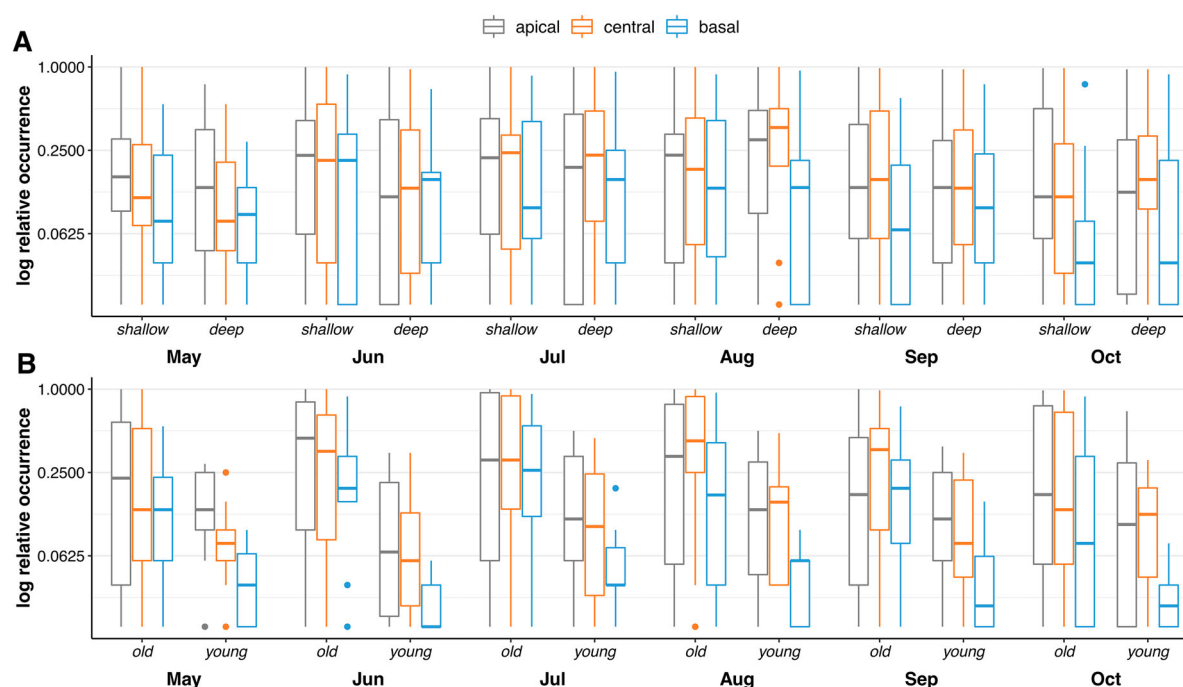
Figure 4. Seasonal occurrence frequency (log transformed) of epiphytes for different depths (A) and leafage (B) within the apical (grey), central (orange) and basal (blue) segment.

whole study period, epibiotic cover increased during the summertime and from the basal to the apical segment, independent of depth and leaf-age. If conditions are beneficial for autotrophs, they rapidly grow in size, while heterotrophs need time for establishment and are competing with autotrophs for leaf surface area (spatial competition) (Borowitzka et al. 1990).

The general trend of temporal changes in occurrence patterns of both autotrophs and heterotrophs in the three leaf segments followed the overall patterns reported by Bračun et al. (2020) for the entire leaves and shoots. Autotrophic organisms dominated throughout the study period and showed a clear temporal occurrence pattern with highest values during summertime, when the light conditions were best for growth and lowest values in spring and autumn (Bračun et al. 2020). Low occurrence of autotrophs was observed in May which coincides with higher values for heterotrophs. During springtime, a large amount of nutrients, caused by upwelling effects of nutrient-rich water to upper coastal habitats, is available (Gilmartin et al. 1990). Filter-feeding invertebrates grow in size, since a lot of food and leaf area unoccupied by autotrophic epibiota is accessible.

Considering the colonization dynamics along the leaf, autotrophic and heterotrophic organisms follow different trajectories. In general, autotrophs show a much clearer separation between apical (incl. central)

and basal leaf segments. In the apical parts of leaves more light for autotrophic organisms is available. This is also true for old leaves, that are, compared with young leaves, less affected by self-shading, due to their relative (i.e. outer) position in the shoot and overall longer leaf lengths and larger surfaces (Stoner 1980; Reyes et al. 1998). In a previous study on the same *C. nodosa* patch, growth parameters (e.g. leaf area index, shoot density and leaf length) revealed that old leaves offer one third more area for settlement than young leaves (Bračun et al. 2020). Additionally, the leaf area index (LAI) of the shallow transect is much higher (Bračun et al. 2020), which could explain the difference in autotroph occurrence patterns between depths. In deeper water light availability for photosynthesis is restricted (Dring and Dring 1991; Krause-Jensen et al. 2003), which leads to a reduced leaf area and lower growth rates (Bračun et al. 2020) of *C. nodosa* and its epibiota. Interestingly, the higher LAI values at shallow depths do not lead to an increased abundance of heterotrophs, which might be linked to stronger competition due to favourable conditions for autotrophic organisms.

Diversity and distribution of epibiota along leaves of *Cymodocea nodosa*

In the present study, a total of 18 taxa were identified, six of which were determined to species level (Table
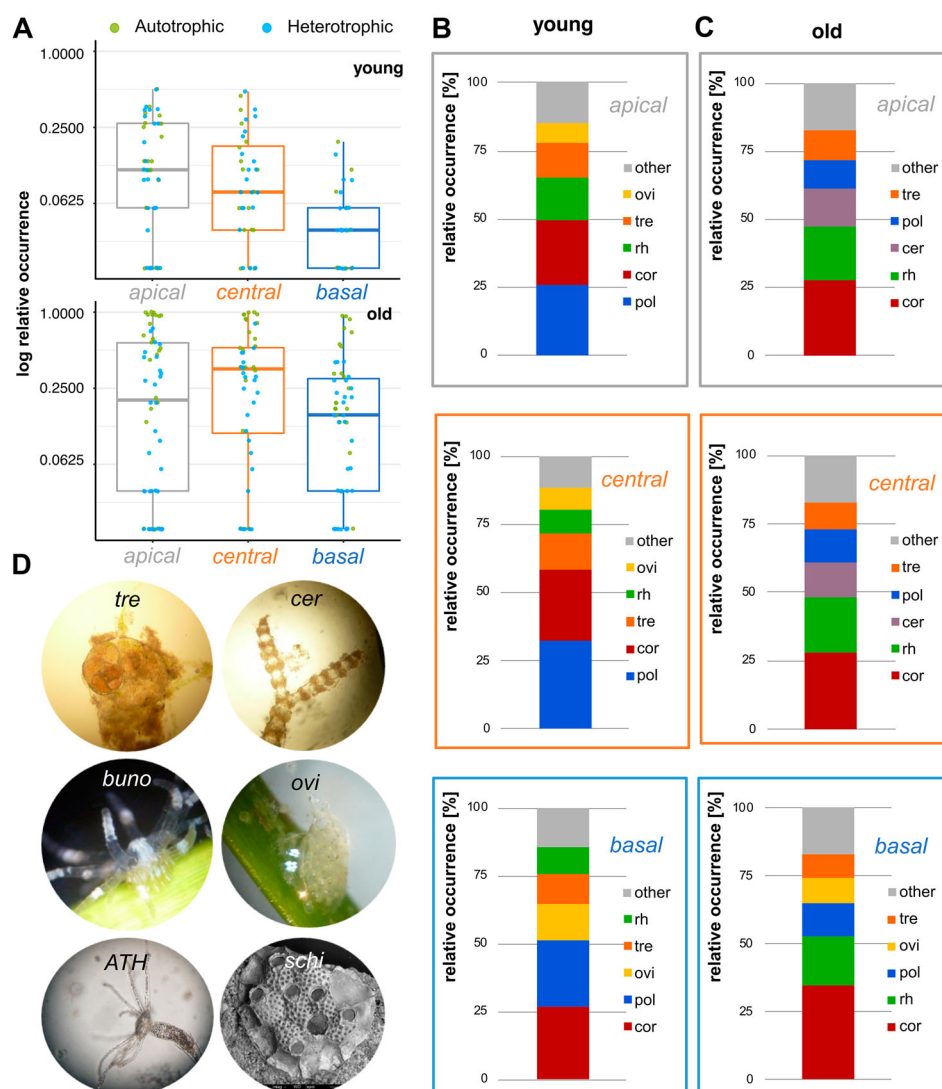
Figure 5. (A) Relative occurrence (log-scaled) of autotrophic and heterotrophic epibiota for each segment collected in the deep transect (5 m depth). Relative occurrence in per cent for epiphytes on young (B) and old (C) leaves from apical (grey), to central (orange) and basal (blue) in shallow water. (D) Representative organisms found in the transect. Abbreviations: Athecate Hydrozoan (ATH), *Bunodeopsis strumosa* (buno), *Ceramium* (cer), Corallinaceae (cor), Foraminifera (fora), gastropod clutches (ovi), Polychaeta diverse (pol), *Schizobrachiella sanguinea* (schiz), *Tretomphaloides concinnus* (tre). A detailed view on the relative occurrence of all other epibiota can be found in the Appendix (Figures S2, S4).

SV; Bračun et al. 2020). We classified algal epibiota into three main groups: Corallinaceae, represented by crustose algae, *Ceramium* spp., as erect algae and Rhodophyta (e.g. Reyes and Sansón 2001; Ben Brahim et al. 2020).

Corallinaceae were the dominant taxon throughout the study period, both depths and leaf-ages, as well as in all three leaf segments. This is in line with previous studies that recorded Rhodophyta as the by far most common phylum (up to 99.2% cover) on seagrass leaves (Reyes and Sansón 2001). Among the Rhodophyta, Corallinaceae are known as primary colonizers of seagrass leaves (Borowitzka et al. 1990) and considered as very resistant against mechanical

disturbances and unfavourable light conditions. Calcareous deposits in the cell walls of Corallinaceae could also prevent or reduce predation pressure by small invertebrates such as isopods compared with other, softer bodied red algae such as *Ceramium* spp. (Anderson et al. 1998).

However, *Ceramium* spp. occurred in all leaf segments across the whole study period and both depths but reached highest relative occurrences in the shallow transect on old leaves and lowest occurrence frequencies in the deep transect on young leaves, particularly in the basal segments. Hence, if established, a slight increase of the relative occurrence of *Ceramium* spp. from the basal to the apical segment

was detected, suggesting that it is well able to compete with calcareous rhodophytes for space on the leaves. Indeed, young *Ceramium* spp. develop erect thalli that tower over more planar growing forms such as Corallinaceae, which could lead to a photosynthetic advantage.

Additional autotrophic taxa included Bacillariophyceae (diatoms) and *Oscillatoria* spp. (Cyanobacteria). Bacillariophyceae live epiphytically, fixed with mucus, on seagrass leaves and occurred only on old leaves. Their absence on young leaves might be explained by self-shading effects due to the central position of the young leaf. *Oscillatoria* spp. appeared throughout the season, but preferentially in deeper water and on old leaves, and like the autotrophs they were most common towards the apical part of the leaves.

Thus far, most studies were confined to the investigation of algal cover on *C. nodosa* leaves (e.g. Reyes et al. 1998; Reyes and Sansón 2001; Mabrouk et al. 2014). Very little is known about the epifauna on *C. nodosa* leaves, but it is very likely a subset of that found on larger and longer-living seagrass species, such as *P. oceanica* (e.g. Reyes and Sansón 2001). A recent study on *C. nodosa* from Tunisian waters found 12 invertebrate species, including seven bryozoans, two hydrozoans and three annelids (Ben Brahim et al. 2020). In our recently published study (Bračun et al. 2020), which is also the foundation for this study, we detected three bryozoans (*S. sanguinea*, *Collarina* cf. *balzaci*, *Puellina* cf. *gattyae*), three hydrozoans (*C. linearis*, *K. pinnata*, *P. pusilla*), several annelids of the order Sabellida (*Janua* cf. *pagenstecheri*, *Spirorbis* cf. *borealis*, *S.* cf. *corallinae*) and mucus cases of vagile polychaetes. Apart from these, we found molluscs (bivalves and gastropod clutches), Anthozoa (*B. strumosa*) and foraminifera (*T. concinnus*, *Massilina* cf. *secans*, *Peneroplis* cf. *plan*).

A closer look at the distribution of epifauna along leaves revealed Polychaeta, the foraminiferan *T. concinnus* and gastropod clutches as the most common heterotrophic organisms. In both depths, especially the young leaves were inhabited by sessile tube-building polychaetes of the family Serpulidae. They are easily overgrown by calcareous algae (own observation) and if their aperture is affected, the feeding activity will be reduced or impeded. Therefore, it is advantageous for polychaetes to settle on little-covered fresh leaf parts (young, basal). However, during the lifetime of the leaf they are pushed towards the apical parts and run into risk of being overgrown by dominant algal components that find beneficial conditions in the upper segments (e.g.

light availability). Furthermore, quivers or mucus cases of vagile polychaetes, built up from sand, shell detritus, sludge or cement (Brito et al. 2005; Westheide and Rieger 2013), were rarely found and likely do not influence the overall spatio-temporal growth dynamics of other epibiota.

The foraminiferan *T. concinnus* was common on all segments of the investigated seagrass leaves. Whereas their smooth umbilical (bottom) side is perfectly adapted for settling on seagrass surface, they are also easily overgrown by the dominant algal community.

Gastropod clutches were found in all segments, in both depths and throughout the season. Snails are common components in seagrass meadows and are known for their grazing activities on seagrass leaves, which also influences the growth of epibiota (Rueda and Salas 2007; Bračun et al. 2020). No significant temporal difference across the whole study period was observed, but overall, young parts seem to be preferred by the snails. Two reasons could explain this pattern. Young leaves are generally less overgrown than old leaves, which means that more space is available on which to lay eggs. Secondly, young parts (i.e. basal, central in a shoot) are less exposed to putative predators that could feed on the adult snails or the clutches.

Several hydrozoans, including *C. linearis*, *K. pinnata* and *P. pusilla*, were found. Highest occurrences were recorded for *C. linearis*. This species showed no preference for a particular leaf segment. Usually, reproduction rates of *C. linearis* are highest during summer and autumn. Their planktonic medusae can drift for several weeks (Lindner and Migotto 2002) or, considering the large distribution range, even longer (Lindner et al. 2011). In our study, highest relative occurrences were obtained in the months May and October. In previous studies, *C. linearis* and *K. pinnata* were documented from different substrates and found to be particularly common on the brown algae *Cystoseira* spp., but not on *C. nodosa* (Morri and Bianchi 1999). The athecate hydroid, *P. pusilla*, occurred on old leaves, in all segments, later in the season and especially in deeper water. Other anthoathecate hydroids colonized old leaves, but predominantly the basal part. A theca, which is missing in athecate hydroids, enables the polyp retraction and protects the vulnerable parts of the animals. Hence, anthoathecate hydroids are highly sensitive against mechanical disturbances and predation in more exposed parts of the leaves, which could explain their absence at the apical and central segment.

Scarcely found epibiota included bryozoans (e.g. *S. sanguinea*), bivalves or anthozoans (e.g.

B. strumosa). *S. sanguinea* is a warm temperature species, often found in the Mediterranean Sea (Hayward and McKinney 2002; Mariani et al. 2005) and also inhabits *P. oceanica* (L.) Delile (Balata et al. 2007).. Even though larvae of this bryozoan species are efficient swimmers and show a photophilic behaviour (Mariani 2002), the single record of this species might have been rather accidental and could be explained by the presence of *P. oceanica* patches in deeper water close to the investigated *C. nodosa* site. Bivalves (i.e. putatively juvenile *Mytilus edulis* Linnaeus, 1758) were found only in June in both depths, on the central (shallow) and apical (deep) segment. Larvae of *M. edulis* attach preferentially on filamentous algae and until they reach a size of 5 cm a change of substratum is possible (Bayne 1964). *B. strumosa* was recorded only in October, on the basal segment of old leaves in 5 m depth. This is somewhat surprising, as this hemisessile anthozoan is typically found on leaves of *Z. noltii*, *C. nodosa* and *Ruppia* sp. in the summer months (Riemann-Zürneck 1998).

Conclusions

This study provides important insights into the spatio-temporal distribution of epibiota organisms along the leaves of *C. nodosa*. We conclude that the temporal patterns of changes in epibiotic community composition on differently aged leaves are mirrored in the patterns observed along single leaves. Thus, we found an increasing number of taxa and elevated relative occurrences on the older parts of a leaf (old leaves > young leaves, apical > basal segments), a pattern concordant with findings from previous studies that focused on the epiphytic algal communities on *C. nodosa* and other seagrass species (e.g. Humm 1964; Jacobs et al. 1983; Heijs 1985; Reyes et al. 1998). Notably, this general pattern not only holds for the autotrophic, but also for heterotrophic organisms (except for polychaetes or the hydrozoan *C. linearis*). Only few studies are available that focused not only on the algal epibiota but also on the epifauna associated with seagrass leaves. Thus, our study provides some important baseline data for increasing our understanding of the spatio-temporal structure of epibiota communities along seagrass, and in particular *C. nodosa*, leaves. Investigating potential changes in community composition and structure in the face of climate change or multiple other anthropogenic stressors with complex impacts on the structure and functioning of seagrass communities could be the key to preserve these important ecosystems and the whole associated flora and fauna (e.g. Grech et al. 2011; Siciliano et al. 2019; Vieira et al. 2020).

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