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Seirophora villosa (Ach.) Frödén: a multidisciplinary investigation on morphology, ecophysiology and ecology

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To my brothers

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Summary

Light regime, water availability and high salt concentrations are generally the main ecological factors modified by habitat fragmentation that could be detrimental to the survival and establishment of poikilohydric organisms such as lichens, causing rapid dehydration, ionic imbalances and the inhibition of photosynthesis. In the Mediterranean basin, coastal juniper habitats, priority habitat for nature conservation (Natura 2000 habitat code 2250), are known to host numerous epiphytic lichens, that living near the seashore need a morphological and chemical organization to adapt or acclimate to extremely variable disturbances. Among them, the macrolichen *Seirophora villosa*, which consists in a fruticose habitus characterized by the presence of compressed canaliculated laciniae covered by thin hairiness and the absence of secondary surface metabolites. Recently studies, has demonstrated the dependence of *S. villosa* on undisturbed *Juniperus* stands suggesting a significant effect of disturbance on the presence of this lichen species.

Moreover, it has been demonstrated that *S. villosa* is only occupying a small part of its colonizable niche because of a very limited propagation ability.

Since the presence and abundance of *S.villosa* are positive indicators of the conservation status of coastal nurseries, our study aimed to evaluate the effects of the main ecological variables on the eco-physiology of this species. Our first aim was to investigate whether the habitat structure affects the functional diversity and species richness of lichen communities along a coastal dune system and how affects the presence of *S. villosa*. Our second aim was to investigate how light regime and water availability affect individual specimens of *S. villosa*, by studying the relationship between photosynthetic activity and water content to thallus area in different sized. Furthermore, we investigated the role of the thin hairiness on thallus surface characterizing *S. villosa* to withstand the effects of seawater by continuous exposure to marine aerosol.

Our results showed that epiphytic lichens richness and habitat width used as proxy of habitat integrity. *Juniperus* stands with higher individuals and cover continuity, probably ensuring a major stability of microclimatic factors, supported richer and functionally more differentiated epiphytic lichen communities. In contrast, the presence of *S. villosa* resulted not directly related to the habitat structure, suggesting a major role of dispersal and establishment capability therefore suggesting a management tailored at the tree-level aimed to the conservation of mature (apotheciate) specimens. However, ongoing research indicate a possible role of microhabitat structure on the abundance of this species.

The experiments carried out showed that the size of the thalli influence the water retention capacity of *S.villosa* species, and consequently influence responses to strong exposure to light. Our outcomes showed that *S. villosa* are susceptible to sudden increases in light exposure, especially in the case of small specimens, which after photoinhibition exhibited a reduced ability to recover. Moreover, our work evidenced for the first time the relevance

of hair as a strategic morphological trait in lichens to face extreme environments. Our results suggest that hair could offer a passive, but selective, water control. Furthermore, hair could repel the salt dissolved in water, by activating a passive resistance mechanism, a real avoidance of stress, which by not allowing salt to enter, allows the thallus to tolerate the presence of salt.

In conclusion, from a conservation point it would be necessary to preserve all the growth stages of the population, providing a suitable habitat for the larger thalli that have the role of propagate the species and for the smaller ones giving them the opportunity to colonize and establish in fragment habitat without being photohinbited.

Chapter 1

General introduction

1.1. Lichen biology

Lichens represent a complex and unique symbiosis between a mycobiont, which is a heterotrophic fungus and one or more autotrophic photobionts, that are a green alga or cyanobacterium (Kranner et al. 2008). All the partners increase benefits from the association: the mycobiont offers a suitable habitat for the growth and the physiological activities to its photosynthetic partners. In return, the autotrophic organisms provide the lichen with fixed carbon derived from photosynthesis.

In a taxonomic perspective, the fungal partners are mostly *Ascomycota* (98%), the others belong to the *Basidiomycota* (0, 2%) and to the *Deuteromycotina* (8%) (Honegger 1991). The most frequent photobionts belongs to the genera *Trebouxia*, *Trentepohlia* and *Nostoc*. The genera *Trebouxia* and *Trentepohlia* have an eukariotic structure and belong to the green algae (sometimes referred to as "phycobionts", 90% of lichens), while the genus *Nostoc* belongs to the prokaryotic cyanobacteria (called "cyanobionts", 10% of lichens) (Nash III 2008). Depending on the partners involved in the lichen symbiosis, we can define three different lichen groups:

(i) chlorolichens, with a green alga as the only photobiont; (ii) cyanolichens, with cyanobacteria as main photobiont; and (iii) cephalolichens, with green algae as main photobiont and cyanobacteria as secondary photobiont, located in internal or external structures called cephalodia (Honegger 1993).

The appearance of a lichen thallus is primarily determined by the mycobiont the fungus hyphae containing the photobiont population, and usually consists of layers differing in thickness (heteromerous thallus) (Jahns 1988). The peripheral cortical layers (upper and lower cortex), which consist of compact hyphae, are involved in the defense, protection, shielding, mechanical stabilization processes of the thallus (Jahns 1988). On the other hand, the medulla is a system of hyphae involved in the transfer of water and solutes and in the maintenance of a system for the circulation of the air. The algae are often located at the top of the medulla, below the upper cortex. In this case, fungal hyphae and photobiont cells form a homogeneous and undifferentiated twine (homoiomerous thallus) (Nash III 2008).

Growth form can be a feature to classify lichens too. We can identify three main classes of lichens according to their morphology: (i) foliose lichens, with a thallus composed of leaf-like lobes and relatively loosely attached to its substrates, (ii) fruticose lichens, with a branched structure growing upward or hanging down from the substrate, and (iii) crustose lichens, tightly bound to the substrate and lacking a lower cortex.

The stable relationship between the photobiont and the mycobiont led to the development of organisms provided by high degree of organization and with singular characteristics not found in the two separate partners. In the symbiosis, the algal cells lose their sexual reproduction capacity and reproduce only asexually. The photobiont could be dispersal in the environment simultaneously with the mycobiont, by the production of vegetative propagules: isidia and soredia. Isidia are corticated thalline extroflextions. Contrary to isidia, soredia are never corticated, they consist of a mass of algae cells held by fungal hyphae. They are present widely in the thallus or only in localized areas and they have a powdery or grainy appearance. Otherwise, the sexual reproduction involves only the fungal partner and occurs through spores dispersion by apothecia or perithecia. These structures are characterized by a layer (hymenium) including asci (fertile hyphae containing the spores) and paraphyses (sterile hyphae supporting the spores). The apothecia, varying in color and size, are dispersed throughout the thallus surface. Generally, they are shaped like a flat, concave or convex disk, and generally have a central disc delimited by a more or less thickened margin.

The close relationship between the mycobiont and the photobiont/s results in their ability to survive in a wide range of habitats, where the symbiontic partners would not manage separately. Lichens occur in almost all terrestrial habitats of the world, including the most inclement environments (e.g., Larson 1987; Green et al. 2012; Zedda and Rambold 2015). They are physiologically adapted and chemically diverse to face abiotic and biotic environmental stresses and to survive in extreme environments (Upreti et al. 2015). They can tolerate hot dry places, arctic climate, salt spray and immersion in water and they colonize many types of substrata (Nash III 2008).

1.2. Ecosystem functions

Lichens contribute to determine the functionality of the ecosystem itself by providing a number of ecological functions. Several lichen morphological and physiological traits are linked to specific functions that mostly act at small-to-medium spatial scale. Actually, in contrast to higher plants, lichens are poikilohydric organisms. This means that they lack mechanisms that allow active regulation of water content, uptake and loss (Kranner et al. 2008). They absorb water, nutritive substances and gases from the atmosphere over their entire thallus surfaces and their water status changes passively with environmental conditions and thus, they can tolerate prolonged periods of dehydration and, as soon as the water becomes available again, they can recover the normal metabolic processes (Lakatos 2011). Lichen metabolism can thus only be activated when the thallus is hydrated by rain, dew or water vapour (Kershaw 1985). The water source is very important for type of lichen community, as shown in Gauslaa (2014). He related the spatial distribution of rain, dew and humid air to the occurrence of different water-related traits among epiphytic macrolichens, such as growth form and type of photobiont. Chlorolichens become metabolically active in the presence of not only rain events or dew but also when exposed to high levels of air humidity and they are in general a more widespread group compared to cyanolichens. In contrast, cyanolichens require direct exposure to liquid water for photosynthetic activation and they are restricted in their distribution to areas with rain and/or dew (Gauslaa 2014). Although lichens do not have any active mechanisms to control water content, the capacity of water retention could varies from species to species mainly in relation to their growth form, the type of photobiont and the internal structure of the thallus. The importance of lichens in forests has been shown in several studies. The ecosystem functions provided by lichens assume a greater relative importance on a small spatial scale. Lichens could contribute to the total biomass in a forest (Esseen et al. 1996); lichens are important food sources for small mammals and ungulates, as well as for many invertebrates. For example, lichen thalli are used as nesting or hunting areas for small invertebrates or birds (Seaward 2008). In other cases, the presence of secondary metabolites (lichen substances) can act as a deterrent for small herbivores and determines the composition of the intrathallin microbial communities (Benesperi and Tretiach 2004). As the spatial and temporal extension of ecosystem processes involving lichens is still not completely clear, the loss of species could lead to a little predictable loss of functionality. The development of appropriate conservation policies should therefore take into account the scale of effect of these organisms and the possible interactions they undertake with the ecosystem as a whole.

1.3. Conservation strategy

The diversity, spatial distribution and population dynamics of lichens are influenced by complex interactions between abiotic and biotic factors that, acting at different spatial and temporal scales, determine the possibility of colonization and survival of lichen species (Giordani 2006). Although the basic knowledge about the relationship between these organisms and the environmental and auto-ecological factors is relatively advanced (Ellis 2012; Giordani et al. 2012), its relevance in the planning of operative conservation activities on threatened lichen populations remains less investigated. This situation is probably related to the lack of attention received by these organisms in terms of international conservation policies. However, recent studies have shown that a more in depth knowledge of the effects of environmental factors, especially those acting at tree and stand scales, can significantly increase the likelihood of success of conservation and reintroduction of threatened epiphytic lichen species (Benesperi et al. 2018). Lichens are threatened by several factors acting at multiple spatial scales, from regional, as in the case of climate change, to landscape and local, as in the case of forest management

(Benesperi et al. 2018). Usually, different factors simultaneously interplay, determining interactive effects that exacerbate the impact on epiphytic lichens. For example, forest management and land-use changes might interplay with the negative effects of air pollution (Geiser and Neitlich 2007) and climate change (Ellis et al. 2007) resulting in a severe decline of several sensitive species (Nascimbene et al. 2016). Habitat loss has been identified as the most widespread threat to lichens (Scheidegger and Werth 2009). Deforestation and degradation of lichen habitats by the replacement of natural forests with plantation forests have both a drastic effect on species richness and composition of lichen communities.

Forest habitat fragmentation and tree exploitation cause a complete break in the availability of the primary habitat for epiphytic lichens (Whittet and Ellis 2013; Otálora et al. 2011), causing a dramatic decline in the ecological integrity, and affecting habitat structure and dynamics, as well as environmental parameters relevant to the lichen dispersal, establishment and maintenance (Nascimbene et al. 2013a).

Light received by a lichen photobiont, especially during thallus hydration, influences growth and metabolism of both the symbionts, although hydration during nights may also stimulate growth (Bidussi et al. 2013). At the same time, excessive light may be detrimental to lichens, strongly influencing water loss and air humidity, causing long term photoinhibition (Gauslaa and Solhaug 2000). Thallus dehydration is a common mechanism to avoid the harmful effects of excessive light. It activates energy-dissipating mechanisms (Heber et al. 2006), reduces the transmission of solar radiation through the upper cortex (Gauslaa et al. 2001) and interrupts dark reactions in photosynthesis (Kranner et al. 2008). The effects of excessive solar radiation on poikilohydric organisms, such as lichens, depend on the state of hydration of the thallus. It has been demonstrated that hydrated thalli are more photoinhibited than dehydrated thalli. However, in sensitive species these mechanisms do not prevent photoinhibition damage in the desiccated state (Gauslaa et al. 2012), since most of the damage occurs under high light intensities (Färber et al. 2014) and involves the formation of harmful reactive oxygen species (ROS). Gauslaa and Solhaug (2004) have shown that species adapted to shady habitats are more photoinhibited by the damage induced by high solar radiation compared to species adapted to sun-exposed environment. These differences are attributed to the reduced structure of the photosynthetic apparatus. When they receive an excess of light, with respect to their optimum, they are not able to obtain the maximum yield from it and disperse it in the form of heat or fluorescence. Thus, a deficit in their photosynthetic efficiency is observed. The species of sun-exposed habitats, on the other hand, have a well-developed photosynthetic apparatus capable of exploiting at best the light regime to which they are subjected and therefore are less susceptible to an increase in solar radiation. However, it has been demonstrated (Gauslaa and Solhaug 2004) that sun-adapted lichens are more sensitive to PAR if they are in a state of hydration, while species that live in the dark are more photoinhibited if dehydrated (Gauslaa and Coxson 2011). Furthermore, the size of the thalli affects the relationship between thallus hydration and physiological activity (Merinero et al. 2014). In general, adult thalli can store more water per thallus area (Gauslaa and Solhaug 1998) and their water holding capacity is higher than in juvenile thalli (Merinero et al. 2014), which tend to reach an equilibrium with the surrounding environment much more rapidly (Gauslaa and Coxson 2011). Habitat fragmentation causes an alteration of water and light regimes, so that both these factors can be detrimental, especially to the establishment and survival of recruits and juvenile thalli that are too small for holding sufficient amounts of water to sustain metabolic activity. In a lichen conservation perspective, even small cutting interventions can profoundly alter the availability of water and light, and can lead to negative consequences, especially if operated in the vicinity of fragile, physiologically weak populations with low capacity for resilience and/or near the population propagation core (Paoli et al. 2018). In the case of active transplantation interventions, it has been shown that exposure of propagules transplants or small thalli in conditions of high luminosity can easily determine a low probability of development (Paoli et al. 2018).

1.4. Outline of this thesis

In the Mediterranean basin, coastal dune systems are among the most threatened habitats by human activities, which cause their fragmentation, and loss of species and functional diversity (Picchi 2008; Prisco et al. 2012; Bertacchi and Lombardi 2014).

The coastal environment is an extremely dynamic ecosystem where natural processes interact and modify the environment characteristics. One of the environment most important features are strong environmental gradients, with rapid changes in the abiotic factors along gradient sea land.

If the effects on biodiversity loss are well known, the consequences of decreasing functional diversity are still poorly explored, but are expected to drastically affect ecosystem productivity and vulnerability. This latter is particularly evident for sensitive organisms, such as epiphytic lichens, that are symbiotic organisms representing ecologically significant component of *Juniperus* shrublands of coastal dune.

In natural conditions, coastal juniper habitats are known to host numerous epiphytic lichens and among them the red-listed macrolichen *Seirophora villosa* (Ach.) Frödén (Nascimbene et al. 2013), which is strictly associated with dune environments. The presence and abundance of this species are considered positive indicators of the conservation status of coastal nurseries (Benesperi and Ravera 2011).

Habitat degradation and loss may be the main causes of the decline and consequent clustered distribution of *S. villosa* populations, as suggested by (Benesperi et al. 2013). Moreover, even in optimal situations, *S. villosa* occupies only a small portion of the entire available area, suggesting the possible involvement of ecological and biological factors (Giordani et al. 2015) in limiting its dispersion.

In chapter 2, we aimed to investigate whether the habitat structure affects:

i) The functional diversity and species richness of lichen communities along a coastal dune system

ii) The presence of a model species of this habitat, the fruticose lichen *Seirophora villosa*.

Light regime, water availability and salt concentrations are generally the main ecological factors modified by habitat fragmentation that can be detrimental to the establishment, survival and maintenance of healthy conditions for individuals and populations of epiphytic lichens (Nascimbene et al. 2013a). These lichens live near the seashore, and need a morphological and chemical organization to adapt or acclimatize to these extremely variable disturbances.

In chapter 3 we investigated:

i) How water availability affects individual specimens of *S. villosa*, by studying the relationship between photosynthetic activity and water content per thallus area in different sized *S. villosa* thalli;

ii) How light regime affects different sized *S. villosa* thalli, comparing susceptibility of hydrated and desiccated thalli to light stress.

In chapter 4 we investigated the role of hairiness on the lichen thallus, as a characteristic morphological trait, using *S.villosa* as a model species:

i) comparing the effects of the presence/absence of hair on the physiological parameters of a set of samples during a dehydration cycle

ii) comparing the effects of the presence/absence of hair on the physiological parameters of a set of samples upon exposure to different salt (NaCl) concentrations.

Chapter 2

Effects of habitat structure on functional diversity of epiphytic lichen communities of coastal dune with *Juniperus* spp.

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Abstract

Dunal ecosystems are among the most threatened habitats by human activities, which cause their fragmentation, and loss of species and functional diversity. If the effects on biodiversity loss are well known, the consequences of decreasing functional diversity are still poorly explored, but are expected to drastically affect ecosystem productivity and vulnerability. This latter is particularly evident for sensitive organisms, such as epiphytic lichens, that are symbiotic organisms representing ecologically significant component of *Juniperus* shrublands of coastal dune. In this work, we selected a dunal system with *Juniperus* shrublands, located between Marina di Bibbona and Marina di Castagneto Carducci (Tuscany – Central Italy). We aimed i) to investigate whether the habitat structure affects the functional diversity and species richness of lichen communities and ii) the presence of a model species of this habitat, the fruticose lichen *Seirophora villosa*. By means of a Strip Adaptive Cluster Sampling (SACS), we sampled epiphytic lichens in randomly selected plots. We used UAV photogrammetric data to obtain information on the habitat structure and we performed Linear Mixed Models (LMM) to detect the relationship between *S. villosa* occurrence, functional richness and habitat structure. Both functional richness and species richness significantly depended on the habitat structure. On the contrary, the presence of *S.villosa* was not directly related to the habitat structure, suggesting a major role of dispersal and establishment capability on its current distribution. In a future perspective, the conservation of the structure and continuity of *Juniperus* stand will be crucial for preserving lichen and functional diversity.

2.1. Introduction

The loss of ecological functions is one of the main threats for ecosystem processes and services as much as the loss of species richness (Dìaz et al. 2001; Cadotte et al. 2011). Even though its formal definition and measurements are still under discussion, functional diversity is commonly assumed to be a better predictor of ecosystem productivity and vulnerability than species diversity (Tilman et al. 1997; Hulot et al. 2000; Dìaz et al. 2001; Heemsbergen et al. 2004). Functional diversity can be defined as "the value and range of those species and organismal traits that influence ecosystem functioning" (Tilman et al., 1997). In the last few decades, the number of publications based on functional diversity has been progressively increasing. It has been frequently used to assess how species respond to natural and anthropogenic disturbances, such as global change and forestry (Heemsbergen et al. 2004) or as predictor of the effects of human disturbance (e.g. Chapin et al. 2000 and Loreau et al. 2002).

Coastal dune systems are among the most threatened habitats by human activities. Italy hosts the largest surface area of this habitat in Europe, (39% of the total extent, Picchi 2008). Due to its very discontinuous distribution and hosting high plant and animal biodiversity, it was included into the priority habitat for conservation according to the Natura 2000 policies (habitat code 2250—Coastal dunes with *Juniperus* spp). The most impacting factors are urbanization, forestry, transport, tourism, alien species introduction and coastal erosion (Brown et al. 2008), causing habitat fragmentation, a dramatic decline of the ecological integrity of dunal ecosystems and loss of biodiversity (Arianoutsou et al. 2012). While the impact of human pressure on animals and vascular plants is already well documented, information is still scanty for other organisms such as epiphytic lichens. Epiphytic lichens are symbiotic organisms ecologically significant component of *Juniperus* habitats. Mediterranean shrublands host a peculiar lichen species assemblage of predominantly Mediterranean-distributed *taxa* restricted to coastal ranges (Nimis and Martellos 2017). *Seirophora villosa* (Ach.) Frödén, is one of the most representative species of this community, known as a red listed species (Nascimbene et al. 2013) sensitive to habitat disturbance (Benesperi et al. 2013). At large scale, the degradation and loss of the habitat represented the main causes of the clustered distribution of this community, of the loss of epiphytic lichen diversity and of the rarefaction of *S. villosa* (Benesperi et al. 2013). The role of specific environmental factors that lead to this loss of species at local scale are still unclear and not generalizable (Giordani et al. 2015). In particular, there is a lack of knowledge about the influence of these factors on the functional diversity of this epiphytic lichen community at habitat level, despite the fact that this information are crucial in a conservation-oriented management (Giordani et al. 2015).

In this work, we selected one of the core areas of *S. villosa* communities in Italy, located between Marina di Bibbona and Marina di Castagneto Carducci (Tuscany – Central Italy). Our aims were: i) to investigate whether and how the habitat structure (described by volume, mean height, maximum and minimum height of the vegetation), differently affects the distribution and the functional diversity and species richness of lichen communities in *Juniperus* shrublands *ii)* to evaluate the relationship between the habitat structure and the presence of *S. villosa*.

2.2. Materials and Methods

2.2.1. Study area

The study area, Marina di Castagneto Carducci (Livorno, Tuscany, Italy 43°9'42"12 N, 10°36'42"84 E) is included in the Mediterranean climatic region [12]. The locality is characterized by the presence of a continuous dune system with *Juniperus* spp. (Natura 2000 habitat code 2250*), a hotspot for several lichen species of conservation concern. Over the last years, human activities have been threatening this priority habitat, causing a degradation and a partial loss of the *Juniperus* spp. stands. *Seirophora villosa* is a fruticose epiphytic lichen with compressed canaliculated laciniae (0.5 mm at margin, up to 5-6 mm at base). Laciniae have a hispid villose and cinereous upper side, and a whitish, naked, lower side. Apothecia (2-5 mm diam.) are usually present and abundant, sub-apical, concave, and with a red disk. *S. villosa* is widespread but not common in Mediterranean basin (Benesperi and Ravera 2011). In Italy, this lichen showed a western distribution along the peninsula and it establishes in *Juniperus* stands both on and behind the dunes, growing on twigs of shrubs and trees exposed to frequent humid maritime winds and aerosol (Benesperi and Ravera 2011).

2.2.2. Sampling design

After the delimitation of the study area we proceeded to its digitalization (QGIS Development Team, 2017). Considering the clustered distribution of the *S. villosa* population, we applied Strip Adaptive Cluster Sampling (SACS), which in the case of non-homogeneous distribution of a target population gives better performance than the usual non-adaptive strategies (Pontius 1997). We randomly selected 5 primary units (strips), extending from the sea front to the back edge of the *Juniperus* shrubland across the dune. Each strip was divided into secondary units, which were represented by 47, 5×5 meters square plots. If *S. villosa* occurred in a secondary unit selected by the initial sample, we inspected every secondary unit in its neighborhood. Similarly, if *S. villosa* was found in a neighboring secondary unit, then every unit in that unit's neighborhood was inspected. This process continued according to the stop rule we set for sampling, after 2 laterally finding of the presence of the target species. In each plot, we annotated the presence of every overstore species and lichen species and the occurrence of mature thalli of *S. villosa* (i.e., those producing apothecia).

2.2.3. Unmanaged Aerial Vehicle (UAV) photogrammetric data

In the present study, a SenseFly eBee Ag fixed-wing UAVs were used for image acquisition in the study area. UAV images were collected in June 2017 under good conditions with sunny weather and wind speeds of <1 m/s. The flight altitude was set to 104 m. The images were acquired using a SONY camera DSCWX220RGB. The overlap was set to 85% along the tracks and 75% between the tracks. The quality of the images acquired was good without any problems related to light, atmospheric conditions, saturation, or blurriness. The UAV images were processed using Agisoft PhotoScan (Agisoft, 2017) to create a 3D point cloud. Elaborating cloud of points we obtained a Digital Surface Model (DSM). After that, we subtract the DSM taken by the drone with the Digital Terrain Model (DTM) downloaded from the site of Tuscany Region, from we extracted a Canopy Height Model (CHM) from which we extracted maximum (h_max) and minimum (h_min) heights, volume of the shrubland (Vol.m3), mean of heights (h_mean) for each plot.

2.2.4. Laboratory analysis

All the specimens we were not able to identify in the field were identified in laboratory under a dissecting microscope using chemical spot test. Anatomical observations of thallus and ascomata were carried out by light microscopy. For identification, the keys of (Claudaze et al. 1985; Nimis 1987, 1992; Purvis et al. 1992; Wirth 1995) were used. Nomenclature follows (Nimis and Martellos 20107).

2.2.5. Data analysis

We use functional richness and species diversity to assess the effects of anthropogenic disturbance on epiphytic lichen communities (and to test their sensitivity). We performed a Linear Mixed Model (LMM) to detect the relationship between species and functional richness at plot level and habitat structure (mean, maximum, minimum heights and volume of shrubland for each plot). We used the strip as random factor because of the SACS applied. We thus evaluated the significance of the fixed effects and of associated interaction factors using an ANOVA type III table, with Satterthwaite Degrees of Freedom Approximation (Goodnight 1976). The analyses were conducted in R environment (R version 3.4). LMM computations were performed using the lmer function of the lme4 package version 1.1-12 for fitting the models (Bates et al. 2017). To test the normality of data we used Shapiro-Wilk test (Shapiro and Wilk 1965). According with Villéger (2008) functional diversity (the number of functional group in a community) indices were worked out with the R package 'FD' version 1.0-12 (Laliberté et al. 2015). Thus, we performed an LMM to detect the relationship between functional richness and the Rao's quadratic entropy and the habitat structure, using the strip as random factor afresh. Finally, we performed a Generalized Linear Mixed Model to detect the relationship of structural variables and the presence/absence of *S. villosa*. In this case, we evaluated the significance using the Log Likelihood Ratio test compared with the distribution of $\chi2$.

2.3. Results

In this study a total of 91 *taxa* were detected. Among these, *Seirophora villosa*, *Bacidia parathalassica, Cerothallia luteoalba, Lecanora lividocinerea, Parmotrema stuppeum, Ramalina pusilla, Tornabea scutellifera* (Nascimbene et al. 2013) are species of conservation concern. According to LMM models, both Species richness and functional richness significantly depend on the habitat structure. (Table 2.1 and 2.2). Backwards, the Rao's quadratic entropy resulted not influenced by analyzed variables (Table 2.3). Similarly, the presence of *S. villosa* seems not to be directly influenced by the habitat structure (Table 2.4).

2.4. Discussion

Both functional richness and species richness significantly depend on the habitat structure. These results confirmed the correlation reported in (Benesperi et al. 2013) between epiphytic lichens richness and habitat width used as proxy of habitat integrity.

Juniperus stands with higher individuals and cover continuity, probably ensuring a major stability of microclimatic factors, supported richer and functionally more differentiated epiphytic lichen communities. Accordingly, these stands host some lichens of conservation concern (*Bacidia parathalassica, Cerothallia luteoalba, Lecanora lividocinerea, Parmotrema stuppeum, Ramalina pusilla, Tornabea scutellifera).* On the contrary, disturbed stands with scattered and smaller individuals, host a poorer and trivial lichen flora, less differentiated from a functional point of view. Therefore, in a future conservation strategies perspective the conservation of the structure and continuity of *Juniperus* stand will be crucial for preserving lichen and functional diversity. According to the theory that diversity is not just a byproduct of the abiotic environment, but that the numbers and types of organisms directly regulate ecosystem functioning (Cardinale et al. 2011), preserving lichen diversity we will contribute to preserve *Juniperus* habitat health. On the contrary, the presence of *S.villosa* resulted not directly related to the habitat structure. As reported also in (Giordani et al. 2015) *S. villosa* showed a clustered distribution occupying only a part of its colonizable habitat suggesting a major role of dispersal and establishment capability therefore suggesting a management tailored at the tree-level aimed to the conservation of mature (apotheciate) specimens as observed in (Benesperi et al. 2018) for *Lobaria pulmonaria*. However, ongoing research indicate a possible role of microhabitat structure on the abundance of this species.

Table 2.1 Results for the LMMs considering species richness and variables of habitat structure. NumDf: Numerator degrees of freedom; DenDf: Denominator degrees of freedom.

	Sum.Sq	NumDf	F value	DenDf	P value	
h_max	178.059	1.00	6.50	1.65	0.15152	
h_mean	195.639	1.00	6.72	45.00	0.01280	*
h_min	3.07682	1.00	0.11	44.98	0.74090	
Vol.m^3	0.30975	1.00	0.01	41.88	0.91614	

Table 2.2 Results for the LMMs on Functional richness and variables of habitat structure. NumDf: Numerator degrees of freedom;

	Sum.Sq	NumDf	F value	P value	
h_max	57.14652	1.00	11.99043	0.00118	**
h_mean	34.51064	1.00	6.54971	0.01392	*
h_min	21.56531	1.00	3.88095	0.05501	
Vol.m^3	10.28161	1.00	1.77042	0.19003	

Table 2.3 Results for the LMMs considering Rao's quadratic entropy and variables of habitat structure . NumDf: Numerator degrees of freedom

	Sum.Sq	NumDf	F value	P value
h_max	0.0088	1.00	0.9658	0.3310
h_mean	0.0049	1.00	0.5305	0.4702
h_min	0.0079	1.00	0.8716	0.3555
Vol.m^3	0.0032	1.00	0.3487	0.5578

Table 2.4 Results for the GLMMs on occurrence of *S. villosa* and variables of habitat structure. NumDf: Numerator degrees of freedom

	NumDf	Chisq	P value
h_max	1.00	1.16	0.28
h_mean	1.00	0.16	0.69
h_min	1.00	0.27	0.60
Vol.m^3	1.00	0.13	0.72

2.5. Supplementary materials

Figure 2.1 The regression line related to the species richness with shaded 95% confidence zone.

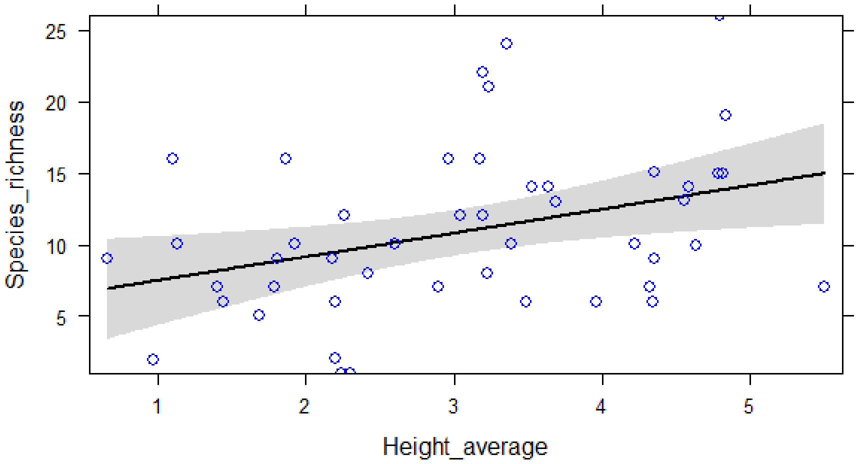

Figure 2.2 The regression line related to functional richness and maximum height.

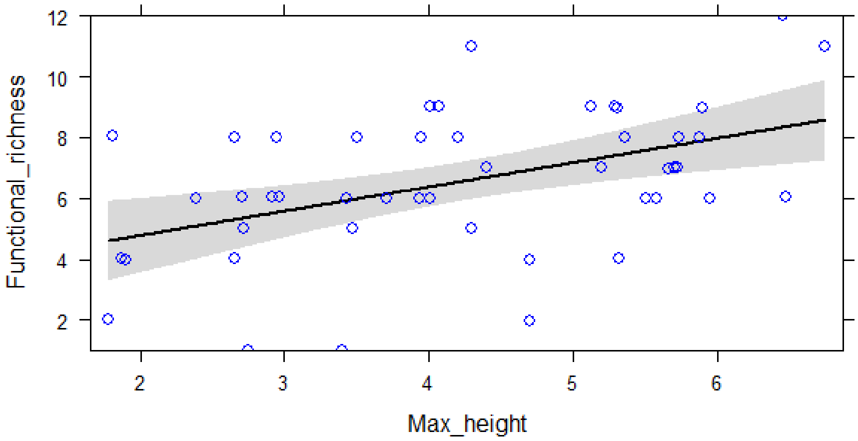

Figure 2.3 The regression line related to functional richness and maximum height.

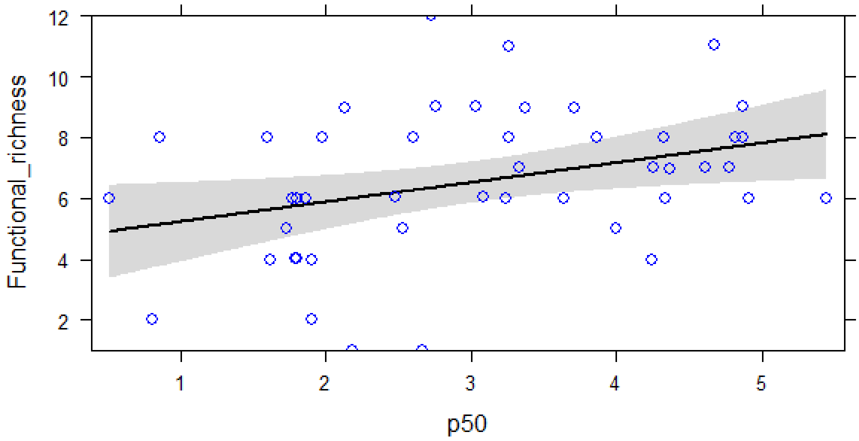

Figure 2.4 The regression line related to functional richness and average height.

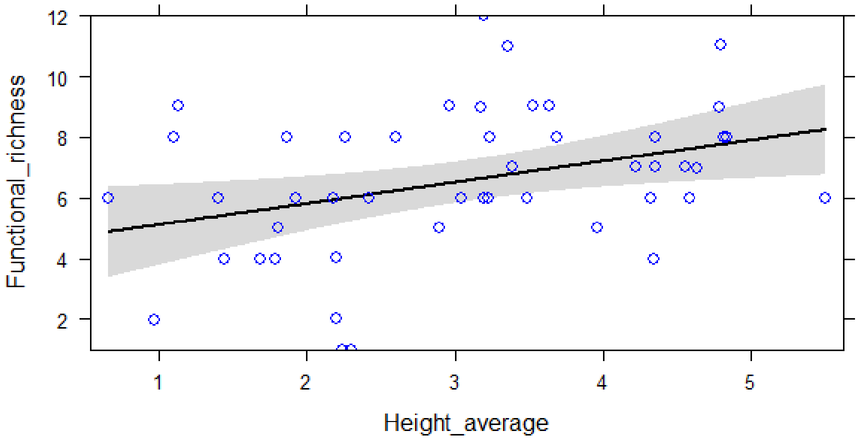

Table 2.1 *Table containing all the taxa detected and the related functional traits. All data refer to ITALIC 5.0 (Nimis and Martellos 2017)*

Specie	Forma di crescita	Fotobionte	Strategia riproduttiva
Alyxoria varia (Pers.) Ertz & Tehler	Cr	Tr	S
Amandinea punctata (Hoffm.) Coppins & Scheid.	Cr	Ch	S
Sp. 1. Apoteci neri			
Sp 2. Apoteci neri tallo bianco			
Sp 3. Apoteci tallo bianco			
Arhonia didyma cfr	Cr	Tr	S
Arhtonia sp.	Cr	Tr	S
Arthonia atra (Pers.) A. Schneid.			
Arthonia didyma Körb.	Cr	Tr	S
Arthonia punctiformis Ach.	Cr	Tr	S
Arthonia radiata (Pers.) Ach.			
Arthopyrenia sp.			
Athallia pyracea (Ach.) Arup, Frödén & Søchting	Cr	Ch	S
Bacidia laurocerasi (Duby) Zahlbr.	Cr	Ch	S
Bacidia rosella (Pers.) De Not.	Cr	Ch	S
Bacidia rubella (Hoffm.) A. Massal.	Cr	Ch	S
Blastenia ferruginea (Huds.) A. Massal.	Cr	Ch	S
Buellia griseovirens (Sm.) Almb.	Cr	Ch	S
Caloplaca aegatica Giralt, Nimis & Poelt	Cr	Ch	S
Caloplaca cerina (Hedw.) Th. Fr. s.lat.	Cr	Ch	S
Candelariella reflexa (Nyl.) Lettau	Cr	Ch	A.s
Candelariella sp			
Candelariella xanthostigma (Ach.) Lettau	Cr	Ch	S
Catillaria nigroclavata (Nyl.) J. Steiner	Cr	Ch	S
Cerothallia luteoalba (Turner) Arup, Frödén & Søchting	Cr	Ch	S
Chrysothrix candelaris (L.) J.R. Laundon	Lepr	Ch	A.s
Dendrographa decolorans (Sm.) Ertz & Tehler	Cr	Tr	A.s
Diploicia canescens (Dicks.) A. Massal.	Cr.pl	Ch	A.s
Diromma dirinellum (Nyl.) Ertz & Tehler	Cr	Tr	S
Evernia prunastri (L.) Ach.	Frut	Ch	A.s
Flavoparmelia caperata (L.) Hale	Fol.b	Ch	A.s
Flavoparmelia soredians (Nyl.) Hale	Fol.b	Ch	A.s
Gyalecta derivata (Nyl.) H. Olivier	Cr	Tr	S
Heterodermia obscurata (Nyl.) Trevis.	Fol.n	Ch	A.s
Heterodermia speciosa (Wulfen) Trevis.	Fol.n	Ch	S
Hyperphyscia adglutinata (Flörke) H. Mayrhofer & Poelt	Fol.n	Ch	A.s
Lecania cyrtella (Ach.) Th. Fr.	Cr	Ch	S
Lecania naegelii (Hepp) Diederich & van den Boom	Cr	Ch	S
Lecanora albella (Pers.) Ach.	Cr	Ch	S
Lecanora sp.	Cr	Ch	S
Lecanora carpinea (L.) Vain.	Cr	Ch	S
Lecanora chlarotera Nyl. subsp. Chlarotera	Cr	Ch	S

Lecanora chlarotera subsp. meridionalis (H. Magn.) Clauzade & Cl. Roux	Cr	Ch	S
Lecanora expallens Ach.	Cr	Ch	A.s
Lecanora horiza (Ach.) Linds.	Cr	Ch	S
Lecanora lividocinerea Bagl.	Cr	Ch	S
Lecanora strobilina (Spreng.) Kieff.	Cr	Ch	S
Lecanora symmicta (Ach.) Ach.	Cr	Ch	S
Lecidella elaeochroma var. juniperina (Poelt & Nimis)	Cr	Ch	S
Melanelixia glabratula (Lamy) Sandler & Arup	Fol.b	Ch	A.i
Melanelixia subaurifera (Nyl.) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch	Fol.b	Ch	A.s
Micarea prasina Fr.	Cr	Ch	S
Myriolecis hagenii (Ach.) Sliwa, Zhao Xin & Lumbsch	Cr	Ch	S
Naetrocymbe punctiformis (Pers.) R.C. Harris	Fu		S
Normandina pulchella (Borrer) Nyl.	Sq	Ch	A.s
Parmelia sulcata Taylor	Fol.b	Ch	A.s
Parmotrema hypoleucinum (J. Steiner) Hale	Fol.b	Ch	A.s
Parmotrema perlatum (Huds.) M. Choisy	Fol.b	Ch	A.s
Parmotrema reticulatum (Taylor) M. Choisy	Fol.b	Ch	A.s
Parmotrema sp.			
Parmotrema stuppeum (Taylor) Hale	Fol.b	Ch	A.s
Pertusaria hymenea (Ach.) Schaer.	Fol.b	Ch	A.s
Pertusaria pertusa cfr			
Phaeophyscia endophoenicea (Harm.) Moberg	Fol.n	Ch	A.s
Phaeophyscia hirsuta (Mereschk.) Essl.	Fol.n	Ch	A.s
Phaeophyscia nigricans (Flörke) Moberg	Fol.n	Ch	A.i
Phaeophyscia orbicularis (Neck.) Moberg	Fol.n	Ch	A.s
Phlyctis argena (Spreng.) Flot.	Cr	Ch	A.s
Physcia adscendens H. Olivier	Fol.n	Ch	A.s
Physcia biziana (A. Massal.) Zahlbr. var. biziana	Fol.n	Ch	S
Physcia clementei (Turner) Lynge	Fol.n	Ch	A.i
Physcia stellaris (L.) Nyl.	Fol.n	Ch	S
Physcia tribacia (Ach.) Nyl.			
Physciella chloantha (Ach.) Essl.	Fol.n	Ch	A.s
Physconia perisidiosa (Erichsen) Moberg	Fol.n	Ch	A.s
Physconia venusta (Ach.) Poelt	Fol.n	Ch	S
Porina aenea (Wallr.) Zahlbr.	Cr	Tr	S
Porina sp.			
Punctelia borreri (Sm.) Krog	Fol.b	Ch	A.s
Punctelia sp			
Punctelia subrudecta (Nyl.) Krog	Fol.b	Ch	A.s
Pyrrhospora quernea (Dicks.) Körb.	Cr	Ch	A.s
Ramalina canariensis J. Steiner	Frut	Ch	A.s
Ramalina farinacea (L.) Ach.	Frut	Ch	A.s
Ramalina fraxinea (L.) Ach.	Frut	Ch	S
Ramalina lacera (With.) J.R. Laundon	Frut	Ch	A.s
Seirophora villosa (Ach.) Frödén	Frut	Ch	S
Sp 4. Tallo bianco			
Tephromela atra var. torulosa (Flot.) Hafellner	Cr	Ch	S
Thelopsis isiaca Stizenb.	Cr	Tr	S
Xanthoria parietina (L.) Th. Fr.	Fol.b	Ch	S

Chapter 3

High-light stress in wet and dry thalli of the endangered Mediterranean lichen *Seirophora villosa* (Ach.) Frödén: Does size matter?

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Abstract

In the Mediterranean basin, coastal dune systems are a priority habitat for nature conservation. *Seirophora villosa* is strictly associated with undisturbed dune juniper formations and can be used as an indicator of the status of conservation. Light regime and water availability are generally the main ecological factors modified by habitat fragmentation that can be detrimental to the colonisation and survival of *S. villosa* populations in coastal dunes. This study aims to investigate how light regime and water availability affect individual specimens of *S. villosa*, by studying the relationship between photosynthetic activity and water content per thallus area in different sized *S. villosa* thalli and comparing susceptibility of hydrated and desiccated thalli to light stress. During dehydration, photosynthetic activity decreased, reaching low constant values in smaller thalli more quickly than in larger ones. During the exposure of dry thalli to high light, photoinhibition consistently occurred earlier in smaller specimens than in larger ones.

Moreover, larger thalli that were kept dried recovered to pre-treatment values within 3 days, while smaller ones took 5 days to recover initial values. On the other hand, both large and small wet thalli were photoinhibited within 1 day and recovered to pre-treatment values within 4 and 6 days respectively. Our results showed that *S. villosa* thalli are susceptible to sudden increases in light exposure, especially in the case of small specimens, which after photoinhibition exhibited a reduced ability to recover. Habitat fragmentation therefore represents a significant threat to the species, particularly in dispersal and establishment phases.

Keywords Chlorophyll fluorescence; Coastal dunes; Hydration; Photoinhibition; Mediterranean area

3.1. Introduction

Over the last hundred years, human activities have been threatening coastal dune systems characterised by juniper (*Juniperus* spp.) vegetation, a priority habitat for nature conservation (Natura 2000 habitat code 2250), located in Italy (Picchi 2008). The principal factors impacting here are urbanisation, agriculture, forestry, industrial activities, traffic and tourism (Brown et al. 2008), causing habitat fragmentation, a dramatic decline in the ecological integrity of dunal ecosystems and loss of biodiversity (Arianoutsou et al. 2012). In arid and semi-arid environments, epiphytic lichens are an ecologically significant component of coastal juniper habitats, dependent on landscape structure as well as the quality and size of habitat patches (Aragón et al. 2010a, b). In the Mediterranean basin, coastal juniper habitats are known to host numerous epiphytic lichens, among them the red-listed macrolichen *Seirophora villosa* (Ach.) Frödén, which is strictly associated with undisturbed dune environments (Nascimbene et al. 2013a). The presence and abundance of this species are considered positive indicators of the conservation status of coastal nurseries (Benesperi and Ravera 2011). Habitat degradation and loss are the main causes of the decline and consequent clustered distribution of *S. villosa* populations (Benesperi et al. 2013).

Moreover, even in optimal situations, *S. villosa* occupies only a small portion of the entire available area, suggesting the possible involvement of ecological and biological factors (Giordani et al. 2015) in limiting its dispersion, such as lichen hydration and the presence of excessive light.

Habitat fragmentation causes an alteration in water and light regimes, both of which can be detrimental to the establishment, survival and maintenance of healthy conditions for individuals and populations of epiphytic lichens (Nascimbene et al. 2013a). Light received by a lichen photobiont, especially during thallus hydration, influences growth and metabolism of both the symbionts, although hydration during nights may also stimulate lichen growth (Bidussi et al. 2013). At the same time, excessive light may be detrimental to lichens, strongly influencing water loss and air humidity, causing long-term photoinhibition (Gauslaa and Solhaug 2000). Thallus dehydration is a common mechanism to avoid the harmful effects of excessive light. It activates energy-dissipating mechanisms (Heber et al. 2006a), reduces the transmission of solar radiation through the upper cortex (Gauslaa et al. 2001) and interrupts dark reactions in photosynthesis (Kranner et al. 2008).

The effects of excessive solar radiation on poikilohydric organisms, such as lichens, vary, depending on the state of hydration of the thallus, and the relationship between thallus hydration and light damage needs to be critically assessed for vulnerable lichens. In sensitive species, these mechanisms do not prevent photoinhibition damage in the desiccated state (Gauslaa et al. 2012), since most of the damage occurs under high light intensities (Färber et al. 2014) and involves the formation of harmful reactive oxygen species (ROS). Gauslaa and Solhaug (2004) have shown that species adapted to shady habitats are more photoinhibited as a result of the damage induced by high solar radiation, compared to species adapted to sun-exposed environment. Species in sun-exposed habitats have a well-developed photosynthetic apparatus capable of exploiting the light regime to which they are subjected and are therefore less susceptible to an increase in solar radiation. However, it has been demonstrated (Gauslaa and Solhaug 2004) that sun-adapted lichens are more sensitive to PAR if they are in a state of hydration, while species that live in the dark are more photoinhibited if dehydrated. One way to assess the relationship between thallus hydration and light damage is to study the effect of thalli size, as demonstrated for example by Merinero et al. (2014). In general, large thalli can store more water per thallus area (Gauslaa and Solhaug 1996) and their water-holding capacity is greater than that of small thalli (Merinero et al. 2014), which tend to reach equilibrium with the surrounding environment much more rapidly (Gauslaa and Coxson 2011). To elucidate the above, we used *S. villosa* as a point of reference.

Our working hypothesis was that in fragmented environment, smaller thalli are more susceptible than larger ones to changes in water and light availability. The present study, comparing small and large samples of *S. villosa*, thus aims to (i) investigate the relationship between photosynthetic activity and water content per thallus area (mg H2O cm^{-2}), during a dehydration cycle and (ii) compare the susceptibility of hydrated and desiccated thalli to light stress.

3.2. Materials and methods

3.2.1. Lichen material

Seirophora villosa is a fruticose epiphytic macrolichen with compressed canaliculated laciniae (0.5mm at margin, up to 5– 6 mm at base). Laciniae have a hispid villose and cinereous upper side and a whitish, naked, lower side. Apothecia (2–5 mm diam.) are usually present and abundant, sub-apical, concave and with a red disk. In the Mediterranean basin, the species is widespread (but not common) along the coasts of Spain, Portugal, Italy, the Greek islands, Israel and North Africa (Frödén and Lassen 2004). In Italy, *S. villosa* has a distribution along the western peninsula (Nimis 2016), occurring only in Tuscany, Lazio, Campania, Sardinia and Sicily, where it grows on the stems of shrubs and trees (mainly *Juniperus* spp.) on sand dunes exposed to frequent, humid maritime winds (Benesperi and Ravera 2011). Thirty-seven healthy and intact thalli of *S. villosa* were collected in Marina di Castagneto Carducci, (Livorno, Tuscany, Italy 43°9′42″12 N, 10°36′42″84 E) in April 2017. The locality is characterised by the presence of a continuous dune system—a typical habitat for *Juniperus* spp. It represents one of the 'hotspots' of *S. villosa* in the Mediterranean basin. To preserve this rare species, the thalli were harvested only in non-critical areas (with a higher density population), omitting isolated reproductive individuals. The collection covered the entire size distribution, from the smallest to the largest available specimens. All specimens were brought to the laboratory and preconditioned up until treatment: slowly moistened by repeated spraying with distilled water while being maintained in a temperature-regulated chamber at 16 °C and dim light (70 µmol m^{-2} s^{-1}). Samples were then divided into two batches: large (136.9 ± 19.6 cm^2) and small thalli (27.4 ± 1.3 cm^2). (For more information, see Table 3.1).

Table 3.1. Mean values ± Standard error for thallus size (Area: cm^2; dry weight: g; laciniae thickness: mm)

	Area (cm^2)	**Dry weight (g)**	**Laciniae thickness (mm)**
Small	27.45 ± 1.28	3.31 ± 0.53	0.10 ± 0.02
Large	136.89 ± 19.6	0.36 ± 0.03	0.43 ± 0.38

3.2.2. Chlorophyll a fluorescence

In all experiments, chlorophyll a fluorescence induction curves were recorded with a portable fluorimeter (Plant Efficiency Analyser—Handy PEA, Hansatech Ltd., Norfolk, UK). After an acclimation period of 48 h at 70 photons µmol m^{-2} s^{-1}, initial fluorescence values of all thalli were determined. Thalli were dark-adapted for 15 min, and the maximum quantum yield of primary photochemistry $F_V/F_M = (F_M–F_0)/F_M$ was recorded.

Experimental design

3.2.3. Photosynthetic activity during a dehydration cycle experiment

After preconditioning, thalli were repeatedly sprayed on the upper surface with mineral water, until fully hydrated. Each thallus was shaken three times and weighed at the beginning of the dehydration cycle, and thereafter, the rate of water loss and the photosynthetic activity were assessed every 40 min on two different laciniae from each thallus, until the thalli were completely dry and, consequently, the fluorescence signal was no longer obtained. To obtain the final dry weight, thalli were left to dehydrate in a climatic chamber at 60% RH, 16 °C and 70 µmol m^{-2} s^{-1} light intensity, then dried for 48 h in a laboratory oven at 70 °C and measured. Twenty *S. villosa* specimens were used. To determine the water storage traits for this experiment, we determined wet mass (WM), spraying each thallus with deionised water until full hydration and then weighing them (WM_{max}). We then removed excess surface water by gently shaking them three times and weighed the wet mass ($WM_{shaking}$). Five random thalli were dried at 70 °C for 24 h to calculate a correction factor to estimate the oven-dry mass (DM). DM was calculated by multiplying air-dry masses of each thallus with the mean correction factor (DM/air-dry mass-ratio). The hydrated thallus area (A_{wet}) was estimated with ImageJ (version 46; Rasband 2014) as suggested by Esseen et al. (2015). This method was destructive for *S. villosa* thalli that, due to its fruticose growth form, were carefully broken to be scanned with a Canon (MF4320), excluding the possibility of re-using them for further experiments. Furthermore, recently, its conservation status was evaluated by Benesperi and Ravera (2011) and ranked as 'endangered' according to IUCN (2001) criteria in the red list of the epiphytic lichens of Italy (Nascimbene et al. 2013a, b). These aspects led us to consider sacrificing only a part of the samples used in this experiment to calculate A_{wet} and consequently the water holding capacity (WHC). The thallus size parameters, DM and A_{wet} were highly coupled in the subset of *S. villosa* (R^2 adj. = 0.90; p value ≤ 0.001, see Fig. 3.1) and based on this correlation, we estimated WHC at saturation after shaking, $WHC_{shaking} = (WM_{shaking} - DM) / A_{wet}$.

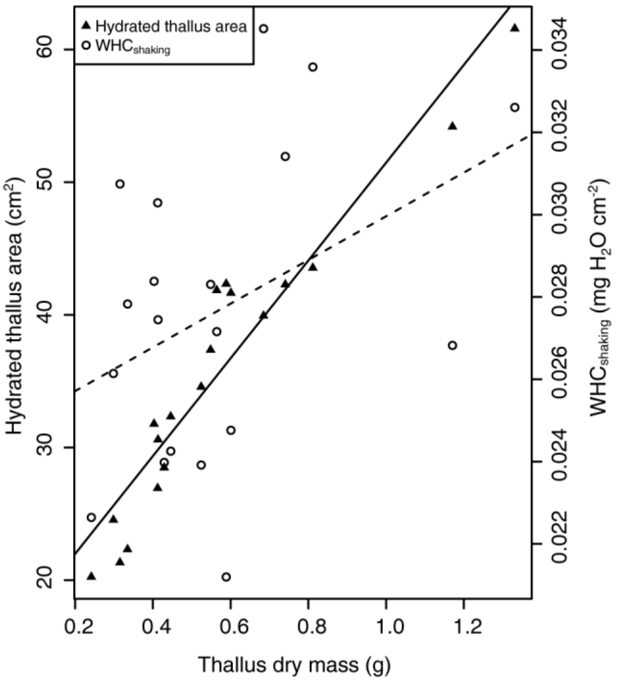


Figure 3.1 *Regression line.* Relationship between thallus dry mass (DM) and hydrated thallus area (A_{wet}) in nineteen thalli of *S.villosa*. Regression lines with corresponding 95% confidence intervals are given. (R^2_{adj} 0.90 and P-value ≤ 0.001).

3.2.4. Photoinhibition experiment in air-dry and wet thalli

Photoinhibition treatment comprised the above samples being exposed to light using a photon flux density of 2000 µmol m^{-2} s^{-1}. Light was provided by a Clorofilla Cree 3070 LED lamp with white light, consisting of two channels of the latest model COB Cree CXB 3070 chip, with real power factor of about 50W. Small and large thalli were treated in the hydrated and air-dry state in order to study the effect of intense light exposure, with a photoperiod of 8 h of high-level light (2000 µmol m^{-2} s^{-1}) and 16 h of low-level light (70 µmol m^{-2} s^{-1}). Light exposure was performed in a climatic chamber at 20 °C and 60% RH. The hydration status of each sample was maintained for the entire exposure time. Hydrated thalli were kept wet by means of regular spraying with deionised water during the treatment. Two chlorophyll a fluorescence measurements were recorded for each thallus until F_V/F_M values were lower than the threshold of 0.200, a reduced F_V/F_M value that can be considered consequent to the photoinhibition (Long et al. 1994). Once both dry and wet thalli were photoinhibited, they were hydrated in dim light and allowed recovery as reported in Solhaug (2018) in order to detect any long-term damage. Recovery following the high light treatments occurred at a low PAR (70 µmol m^{-2} s^{-1}) at 16 °C in the hydrated state, spraying them several times a day. Chlorophyll a fluorescence was measured on each thallus during the recovery period every 24 h. The overall experiment (photoinhibition + recovery) lasted 13 days. Twelve *S. villosa* specimens were used.

3.2.5. Data analysis

The results of both experiments were analysed fitting linear mixed models (LMMs) in a repeated measurement ANOVA design, using thallus identity as a random effect factor to account for the temporal correlation of observations. As the relationship between the variation in water holding capacity or quantum yield of primary photochemistry and time appeared quite far from a simple linear regression, we used time as an ordinal variable in all the analyses. To verify whether water content to thallus area during a dehydration cycle was affected by thallus size, and to verify whether the relation between the maximum of (F_V/F_M) was affected by exposure time and thallus size, an LMM was used in both cases. The effects of light were investigated using F_V/F_M as a response variable and size, hydration, time of exposure and time of recovery as explanatory variables in a full factorial design. We evaluated the significance of the fixed effects and of associated interaction factors using an ANOVA type III table, with Kenward-Roger Degrees of Freedom Approximation. The analyses were conducted in R environment (R version 3.3.1). LMM computations were performed using the lmer function of the lme4 package version 1.1–12 for fitting the models and car package to obtain probability (P) values. All plots were drawn using ggplot2 package version 2.1.0.

3.3. Results

3.3.1. Rate of water loss and photosynthetic activity during a dehydration cycle

Water loss kinetics changed significantly depending on the size of the thalli (size/time significant with P value < 0.001, Table 3.2). Once hydrated, smaller thalli reached their maximal water content per area (0.039 ± 0.003 mg H2O cm^{-2}), Fig. 3.2), with water rapidly evaporating and thalli reaching low water content per area values (10%) after 160 min. Larger thalli increased their water content per thallus area up to 0.060 ± 0.009 mg H2O cm^{-2}, remaining hydrated for a longer time and hence reaching low values (10%) after 240 min from the beginning of the experiment.

Throughout the experiment, the maximum of quantum yield of primary photochemistry (F_V/F_M) changed significantly in both larger and smaller thalli (size/time significant with P value < 0.001, Table 3.3). Initial F_V/F_M values were comparable for larger and smaller thalli but changed during the course of the experiment depending on size: F_V/F_M values in smaller thalli decreased rapidly, reaching a plateau of constant low values (0.110 ± 0.036 SE) 200 min from experiment outset, while larger thalli reached constant low F_V/F_M values (0.143 ± 0.036 SE) 280 min from the beginning of the experiment (Fig. 3.3).

Table 3.2 ANOVA Type III table with Kenward-Roger approximation for degrees of freedom (df) for repeated measures analysis of variance (rANOVA) for the relationship between loss in water content over time and thallus size. During the experiment thalli weights were measured every 40 minutes.

Factor	**F**	**df**	**Df.res**	**Pvalue**	
(Intercept)	581.581	1	27.245	< 0.001	***
Size	45.513	1	27.245	< 0.001	***
time	283.882	7	126.000	< 0.001	***
size:time	19.785	7	126.000	< 0.001	***

Figure 3.2 *Rate of water loss.* Decline in WHC in different sized thalli during a complete drying cycle. The interaction term time:size is statistically significant, according to a mixed model ANOVA with P value < 0.001, F = 25.311, df = 4. The dashed line = small thalli; the solid line = large thalli.

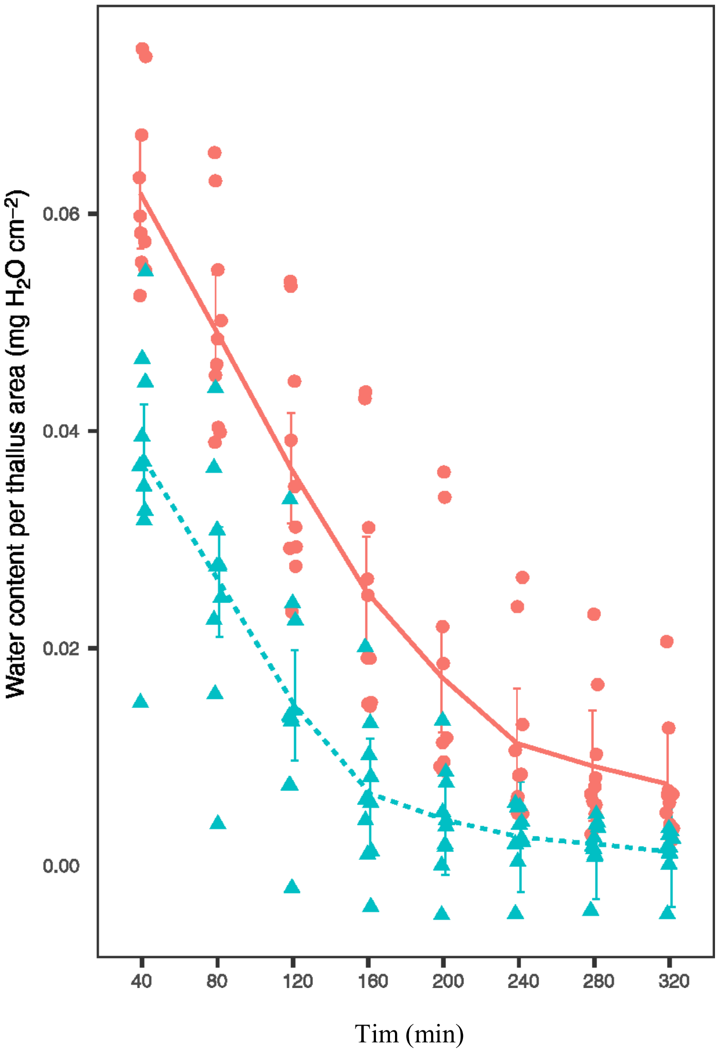
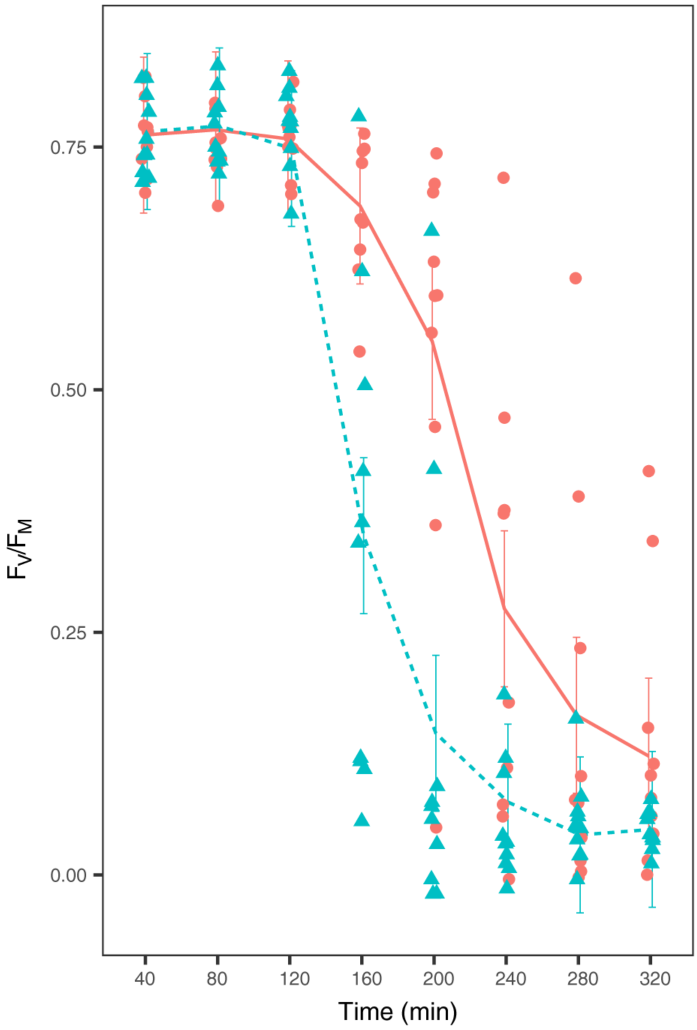


Table 3.3 ANOVA Type III table with Kenward-Roger approximation for degrees of freedom (df) for the repeated measures analysis of variance (rANOVA) for the relationship between efficiency of photosystem II (FV/FM) and time in different sized thalli. During the experiment FV/FM values were measured for each thallus every 40 minutes.

Factor	F	df	Df.res	Pvalue	
(Intercept)	351.8052	1	118.46	< 0.001	***
size	0.0041	1	118.46	0.9488	
time	57.7569	7	126.00	< 0.001	***
size:time	9.2121	7	126.00	< 0.001	***

Figure 3.3 *Reduction of F_V/F_M during a complete drying cycle*. Variation in different sized thalli's phosystem II (F_V/F_M) efficiency over time. The interaction term time:size is statistically significant, according to a mixed model ANOVA with P value < 0.001, F= 13.893, df=4. The dashed line = small thalli; the solid line = large thalli.



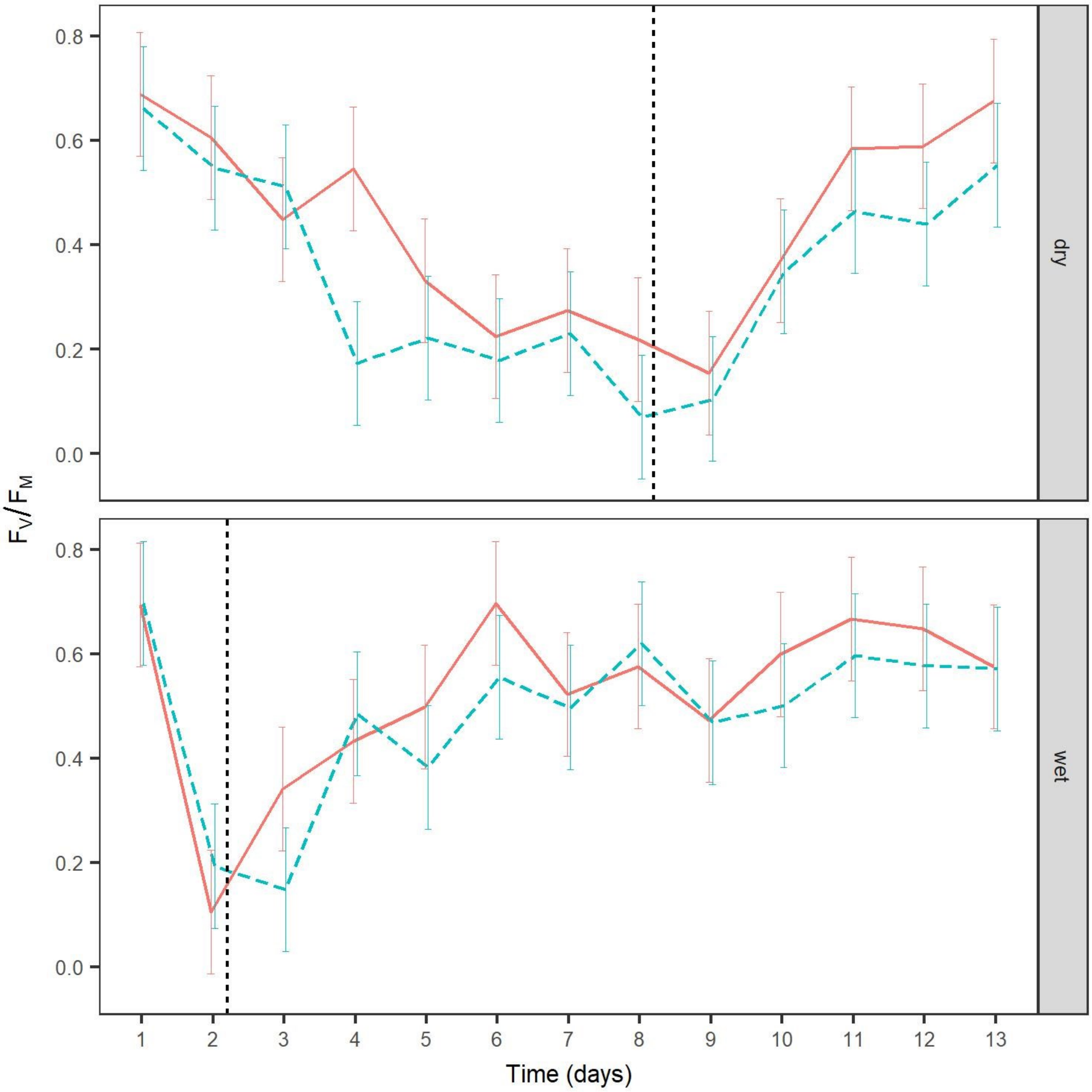
3.3.2. Time and irradiance dependencies of photoinhibition in dry and wet thalli

The exposure to high-light irradiance resulted in a significant decrease in the efficiency of the dry and wet thalli's PSII (F_V/F_M), the decrease depending on thallus size (Table 3.4). Figure 3.4 outlines the reduction and subsequent recovery of F_V/F_M during the treatment. In dry thalli , a photoperiod of 8 h high light (2000 µmol m^{-2} s^{-1}) and 16 h low light (70 µmol m^{-2} s^{-1}) caused a gradual decrease in F_V/F_M until photoinhibition took place (after 8 days of treatment). Smaller thalli reached the threshold of 0.200 after 4 days of treatment. After photoinhibition, larger thalli recovered normal (pretreatment) F_V/F_M values within 3 days in the hydrated state. Smaller thalli did not fully recover pre-treatment values. In wet specimens, F_V/F_M values of both larger and smaller thalli dropped by 70% of their initial values after 8 h of treatment and were photoinhibited within 2 days. Once the recovery period began, F_V/F_M values increased, returning to initial values after 4 days in larger thalli and 6 days in smaller ones.

Table 3.4 ANOVA Type III table with Kenward-Roger approximation for degrees of freedom (DF) for the repeated measures analysis of variance (rANOVA) for the effect of light on the efficiency of the photosystem II (F_V/F_M) of different sized thalli (size), different hydration during exposure (hydration), and different recovery times (time). F_V/F_M was measured daily during exposure time until photoinhibition took place (for F_V/F_M values 0.200), and during recovery time.

Factor	F	Df	df.res	P value	
(Intercept)	1317.404	1	8	< 0.001	***
size	7.361	1	8	0.026	*
hydration	20.461	1	8	0.002	**
time	17.170	12	96	< 0.001	***
size:hydration	1.083	1	8	0.328	
size:time	0.690	12	96	0.757	
hydration:time	19.165	12	96	< 0.001	***
size:hydration:time	2.019	12	96	0.030	*

Figure 3.4 *Reduction and subsequent recovery of F_V/F_M during thalli treatment*. Variation in different sized thalli's photosystem II (F_V/F_M) efficiency after combined dry and wet hydration states and light exposure (2000 μmol $m^{-2}s^{-1}$). Air-dry (a) and wet (b) thalli were exposed to light until fluorescence signals reached values of F_V/F_M equal to but not above 0.200; as such reduction in F_V/F_M was considered to be a measure of photoinhibition. F_V/F_M was assessed daily before high light treatment and during recovery time, with all thalli maintained in a hydrated state at low light (70 μmol $m^{-2}s^{-1}$). The vertical dashed line represents the end of exposure time and beginning of recovery time. The horizontal dashed line = small thalli; the solid line = large thalli. See Table 3 for ANOVA results.



3.4. Discussion

Our results suggest that larger *S. villosa* thalli store more water per thallus area than smaller ones, and hence remain hydrated for a longer time. The anatomical structure of a thallus plays a

major role in water movements between the lichen and its surrounding environment (Esseen et al. 2015), with smaller thalli reaching equilibrium much more rapidly than larger ones. The anatomical structure of *S. villosa* is typical of the desert species, being very similar to the species belonging to the genus *Teloschistes* (del Prado and Sancho 2007). Its fruticose morphology is characterised by the presence of highly branched laciniae covered by thin hairiness that could increase the absorption surface and break surface tension for dew drops, making their absorption easier (Ros and Werner 1997). This structural form increases the lichen's surface/ volume ratio and allows the thallus to maximise its use of dew, at the expense however of its ability to use water vapour (del Prado and Sancho 2007). During dry periods, such lichens hydrate at night through the uptake of fog, dew or water vapour. In fact, the first peak of positive net photosynthesis occurs immediately after sunrise (Lange et al. 2006), with higher air humidity causing a second peak in the late afternoon. This behaviour, typical of lichens from arid and semi-arid desert habitats (Kappen 1988), also occurs in Mediterranean (Lange and Kilian 1985) and temperate environments (e.g. Lange and Green 2003). Decline in the fluorescence parameters of *S. villosa* was first observed in the small specimens. Size dependency has been widely reported in the literature (Merinero et al. 2014), suggesting that the length of the photosynthetically active period during a dehydration cycle increases with the size of a thallus. Nevertheless, the transition between hydrated and dry states takes place gradually and is determined by light intensity (Paoli et al. 2010), particularly in lichens in sun-exposed environments, as coastal dune system. The amount of light received by the photobiont during periods of thallus hydration may determine lichen growth (Dahlman et al. 2003) and at the same time, depending on light intensity and the duration of sun irradiance, can also represent a significant stress factor causing long-lasting photoinhibition (Gauslaa and Solhaug 2000). In our study, *S. villosa* in both the hydrated and the dry state was susceptible to high light intensity to varying degrees, depending on the size of the thallus. A decrease in F_V/F_M was observed in all specimens following high light treatment. A high degree of resistance to excessive light has generally been assumed in relation to dry thalli, given that several mechanisms protect the lichen thallus during desiccation (Heber et al. 2006a, b).However, high light damage also occurs with dry thalli (Barták et al. 2006). Consistent with these findings, our results showed that both large and small hydrated thalli were photoinhibited within 1 day and recovered to pre-treatment values within 4 to 6 days respectively. On the other hand, dried large thalli remained active for up to 5 days of treatment and recovered after 3 days, while smaller ones inactivated after 3 days and recovered after 5 days and proved to be among the most susceptible to treatment. Our results showed that *S. villosa* thalli are susceptible to sudden increases in PAR, especially in the case of smaller specimens, regardless of the degree of hydration. Several studies have shown that after logging or habitat fragmentation, epiphytic lichens are exposed to both sudden increases in PAR and to rapid change in water availability, which when exceeding the normal ecological range can negatively impact on their photosynthetic performance (Larsson et al. 2012). Incorrect management of dune systems can cause vegetation loss and a resulting sudden increase in light exposure (Giordani et al. 2015). Under these conditions, young populations of *S. villosa* with their small-sized specimens are particularly vulnerable and less likely to return to a physiological healthy status, which in turn limits their ability to recolonise fragmented habitats (Giordani et al. 2015). Considering that *S. villosa* depends heavily on undisturbed juniper stands, that it has a patchy distribution, and that it colonises only a fraction of its niche (Giordani et al. 2015), it is evident that inappropriate environmental management risks triggering a decline of *Seirophora* populations.

Chapter 4

The multi-purpose role of hairiness in the lichen of coastal environments: Insights from *Seirophora villosa* (Ach.) Frödén

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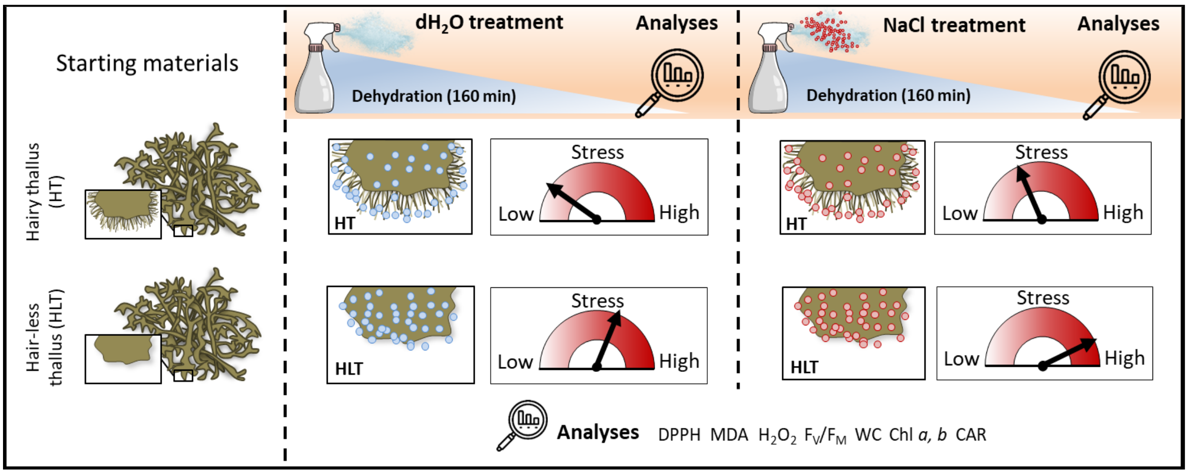
Abstract

Epiphytic lichens serve a number of ecological functions, and so contribute to determining the functionality of an ecosystem. Several morphological and physiological traits of lichen are linked to specific functions that mostly act on a small-to-medium spatial scale. The fruticose epiphytic lichen *Seirophora villosa*, an International Union for Conservation of Nature red-listed species strictly associated with *Juniperus* shrublands in the Mediterranean basin, was used as a model species to investigate the role of hairiness on a lichen thallus, as a characteristic morphological trait. We compared the effects of the presence or absence of hair on the physiological parameters of a set of samples, during a dehydration cycle and on exposure to different salt concentrations. Hairy thalli dehydrated faster than hair-less ones, and remained active (as suggested by F_V/F_M values) until their water content dropped below 20%; compared to 40% in the hair-less thalli. At the end of the dehydration cycle, the lack of hair appears to be associated with a whole series of stress signals, which consist of the degradation of chlorophyll a and b concentrations, along with an increase of malondialdehyde and hydrogen peroxide concentrations, a variation of carotenoids and free radical scavenging activity. Hairy thalli were less affected by salt, suggesting that during dehydration the presence of hair protects the thallus from oxidative stresses and the lipid peroxidation generated by free radicals, and could offer passive, but selective, water control. Hair could not only increase thallus surface and promote water absorption when availability is low, but could also repel the salt dissolved in water by activating a passive resistance mechanism which, by not allowing salt to enter, allows the thallus to tolerate its presence.

Keywords Antioxidant activity; Chlorophyll *a* fluorescence; *Juniperus* shrublands; Hair; MDA; Salt stress



4.1. Introduction

The close relationship between the mycobiont and the photobiont/s in lichens allows them to survive in a wide range of habitats, where symbiotic partners would not manage separately. Lichens occur in almost all terrestrial habitats of the world, including in the most inclement environments (Larson, 1987; Green et al., 2012; Zedda and Rambold, 2015). They are physiologically adapted and chemically diverse, and so are able to face abiotic and biotic environmental stresses and survive in extreme environments (Upreti et al., 2015). They can tolerate hot dry environments, arctic climate, salt spray, and immersion in water; and they colonize many types of substrata (Nash III, 2008).

Coastal environments are dynamic ecosystems influenced by prolonged sun exposure, rapid change in water availability, and wind-dependent fluctuations in salinity and temperature (Le Devehat et al., 2014). Stress factors such as those mentioned above induce an overproduction of reactive oxygen species (ROS) and can therefore unbalance the relationship between oxidants and antioxidants in favor of the former, thus increasing the level of oxidative stress (Green et al., 2011). Heat can denature proteins in lichens, so that they must activate detoxification mechanisms in order to re-establish homeostasis efficiently (Wang et al., 2003). Moreover, the accumulation of sea salt may induce strong dehydration, ion imbalances, and significant loss in net photosynthesis (Matos et al., 2011). A few studies have addressed the impact of salinity stress on lichens, mostly focusing on the cellular location of elements and its effects on net photosynthesis (Smith and Gremmen, 2001; Matos et al., 2011). Investigations on *Ramalina canariensis* Steiner, indicated that saline stress may irreversibly impair photosynthesis, thus compromising lichen vitality (Matos et al., 2011). In such saline stress coastal environments, sea spray is one of the main abiotic factors influencing lichen distribution (Fletcher, 1976), with lichens demonstrating considerable variability in salt (NaCl) sensitivity proportionate to their distance from the coast (Nash III and Lange, 1988). Winds can carry sea spray inland for considerable distances, thus not limiting marine influence to the immediate shore, but potentially extending it inland for many kilometers, creating small scale variations depending on local topography and shelter (Fletcher, 1976; Nash III and Lange, 1988).

Lichens living near the seashore need a morphological and chemical organization to adapt and/or acclimate to extremely variable disturbances (Delmail et al., 2013). For instance *Ramalina menziesii*, a typical Californian species of the aerohaline zone, is characterized by a morphological adaptation consisting of a reticulate structure that increases its surface, thereby enhancing the water-holding capacity of its thallus that protects it from fast dehydration when its host trees are ventilated by the ocean (Rundel, 1974). The coastal lichen *Xanthoria aureola*, a widespread species of sunny environments, has developed a protection system that includes the synthesis of photoprotective pigments, bringing a yellow pigmentation caused by parietin derivatives in the cortex (Smith et al., 2009). Some lichens have a super-hydrophobic upper surface that chemically repels water drops, as is the case with *Cladonia chlorophaea* (Hamlett et al., 2011). As a consequence of these various adaptation strategies, the ability to survive in stressful environments may vary among lichen species (Delmail et al., 2013).

In the Mediterranean basin, coastal juniper habitats are threatened by several anthropogenic and natural disturbances, such as urban coastal development, tourism, habitat fragmentation, the introduction of alien species, coastal erosion, forest fires, and polluted sea spray (McLachlan and Brown, 2006; Picchi, 2008; Prisco et al., 2012; Bertacchi and Lombardi, 2014). In natural conditions, coastal juniper habitats are known to host numerous epiphytic lichens, among them the Union for Conservation of Nature (IUCN) red-listed macrolichen *Seirophora villosa* (Ach.) Frödén (Nascimbene et al., 2013), which is strictly associated with dune environments, such as coastal *Juniperus* shrublands (a priority habitat for conservation according to Habitats Directive 92/43/EEC, habitat code 2250*) (Benesperi et al., 2013).

Seirophora villosa is a fruticose lichen characterized by the presence of compressed canaliculated laciniae covered by thin hairiness and the absence of secondary surface metabolites (Søchting and Frödén, 2002). The role of hair on the thallus has been only partially investigated. Since lichens do not have active mechanisms to regulate water content, hair is presumed to increase the absorption surface and break the superficial tension of drops, making their absorption easier (Ros and Werner, 1997; Esseen et al., 2015).

Our working hypothesis is that hair could regulate water penetration in the thallus, hence protecting the thallus from salt particles carried by sea spray. In this study, we investigated the role of the hair layer as a characteristic morphological trait of *S. villosa*, comparing the susceptibility of physiological parameters according to the presence or absence of hair i) during a dehydration cycle and ii) on exposure to different NaCl concentrations.

4.2. Materials and methods

4.2.1. Lichen material and experimental conditions

Thalli of the epiphytic macrolichen *S. villosa* were collected in Marina di Castagneto Carducci, (Livorno, Tuscany, Italy 43°9’42"12 N, 10°36’42"84 E) in April 2017, and then transferred to the laboratory. Residual tree bark pieces were carefully removed and the thalli slowly moistened by repeated spraying with distilled water. Samples were kept at

16°C in dim light (200 photons µmol $m^{-2}s^{-1}$) until treatment. For each treatment, five replicates were used.

4.2.2. Anatomical investigation

Thalli of *S. villosa* were used to obtain fresh sections: samples were divided into rough sections of about 4 mm using a very sharp razor, and were then frozen at -20°C. Once the required temperature was reached, sections were reduced using a freezing microtome (CRYO-CUT), up to a size of 30-40 µm. Observations were done with an optical microscope (Leitz DMRB, Leica).

4.2.3. Salt treatments

Different types of thallus were used in the salt treatment. Samples were randomly divided into three batches: i) thalli with hair (hereafter referred as HT); ii) thalli from which hair had been removed prior to NaCl treatments (HLT); and iii) thalli where hair had been removed after NaCl treatments (HLT_{after}).

Hair was removed from the thallus surface under a stereomicroscope, using a razor and taking care not to damage the surface. Five different thalli were used for each of the three following experimental conditions using different prepared solutions containing NaCl in deionized water at nominal concentrations: 0 (control), 0.5 and 1 M. The upper surface of the thalli was repeatedly sprayed with each solution, until fully hydrated. The thalli were then left to dehydrate in a climatic chamber at 60% RH, 16°C and 200 photons µmol $m^{-2}s^{-1}$ light intensity. In parallel with dehydration, chlorophyll *a* fluorescence emission was recorded as described in par. 2.4. At the end of the dehydration cycle, samples were stored at about -18°C, the recommended way to preserve dry lichens (Honegger, 2003; Paoli et al., 2013) for the later chemical and physiological measurements carried out in this study (par. 2.5; 2.6; 2.7; 2.8).

4.2.4. Chlorophyll *a* fluorescence

During the dehydration cycle, measurements of the rate of water loss and fluorescence were carried out every 40 minutes from the beginning of salt treatment. Chlorophyll *a* fluorescence emission was recorded with a portable fluorimeter (Plant Efficiency Analyzer – Handy PEA, Hansatech Ltd, Norfolk, UK) on two different laciniae per thallus, until the thalli were completely dry and, consequently, a fluorescence signal was no longer obtained. Fluorescence was measured on well-saturated and dark-adapted samples, which were exposed to light for 1 sec with a saturating excitation pulse (3000 μmol s^{-1} m^{-2}) of red light (650 nm). Thalli were then dark adapted for 15 minutes before taking measurements.

4.2.5. Chemical analysis

At the end of the dehydration cycle after salt treatment, sodium concentrations were determined in all three types of *S. villosa* thalli. To obtain the internal fraction, half of the samples were shaken in 5 mL of nickel chloride ($NiCl_2$ 20 mM) for 20 min to remove the adsorbed sodium from the cell wall (Matos et al., 2011). The other half were shaken in deionized water for 20 min and used to determine the total sodium fraction. Aliquots of oven-dried material were weighed (about 100 mg) and mineralized with concentrated nitric acid (HNO_3) (Applichem, 65%) at 200°C for 20 min in a microwave digestion system (Mars 6, CEM, Matthews, North Carolina, USA). After the process of digestion, solutions were adjusted to a volume of 25 mL with deionized water, and Na concentrations were determined by atomic absorption spectrophotometry (AAnalyst 200, Perkin Elmer). Certified reference materials (LGC No 7162) were used to verify the accuracy and precision of the methods, whose values were <10% and <5% RDS, respectively.

4.2.6. MDA and H_2O_2 concentration assay

Malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) concentrations were determined as indicators of membrane lipid peroxidation. MDA was estimated using thiobarbituric acid reactive substances (TBARS) assay (Taiti et al., 2016). Fresh samples (about 0.5 g) were homogenized with 2.5 mL of 0.1% (w/v) trichloroacetic acid (TCA). 1.5 mL of the homogenate was put in Eppendorf tubes and centrifuged at 12,000 g for 20 min. 0.5 mL of the supernatant was collected and added to 1.5 mL of 0.6% thiobarbituric acid in 10% TCA and put in glass tubes. Tubes were put in the oven at 95°C for 30 min, cooled in an ice bath, and then solutions were centrifuged again at 12,000 g for 10 min. The absorbance of the supernatant was measured at 532 nm and corrected for non-specific absorption at 600 nm. Concentration of MDA was calculated using the extinction coefficient for the TBA–MDA complex (155 $mM^{-1}cm^{-1}$) and the results expressed as μmol g^{-1} (dw). H_2O_2 is one of the reactive oxygen species that accumulates as a consequence of oxidative stress. For H_2O_2 assay, 1 mL of the supernatant was added to 1 mL of 10 mM potassium phosphate buffer (pH 6.8) and 2 mL of 1 M KI. The reaction mixture was incubated for 1 h in darkness. H_2O_2 concentrations were calculated based on the supernatant absorbance at 390 nm and on a standard curve with known concentrations (Alexieva et al., 2001).

4.2.7. Antioxidant activity

The free radical scavenging activity of samples was measured with the 1,1-diphenyl-2-picrylhydrazyl free radical (DPPH), according to Hatano et al. (1988): 0.5 mL of the extract at five different concentrations was added to 0.5 mL of a DPPH ethanolic solution. The mixture was shaken vigorously and after 30 min of incubation at room temperature, the absorbance was read at 517 nm in a UV/Vis spectrophotometer (Lambda 35, Perkin-Elmer), against a blank. Percentage of inhibition free radical DPPH was calculated in the following way: DPPH scavenging effect (%) = $[(A_{blank} - A_{sample})/A_{blank}] \times 100$, where A_{blank} is the absorbance at the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound. The inhibition concentration at 50% inhibition (EC_{50}) was the parameter used to compare the radical scavenging activity. A lower EC_{50} corresponds to a higher antioxidant activity of the lichen extract.

4.2.8. Measurement of chlorophylls and carotenoids

Total chlorophyll and carotenoid concentrations were determined in *S. villosa* samples. Cold 100% methanol was added to frozen ground lichen material. Samples were left being shaken in darkness at 4°C for 30 min to extract pigments. After that, they were centrifuged at 1000 g for 10 min. The supernatant was collected and used to read the absorbance at 665, 652 and 470 nm using an UV/Vis spectrophotometer (Lambda 35, Perkin-Elmer). Chlorophyll *a* (Chl*a*), chlorophyll *b* (Chl*b*) and carotenoid concentrations were determined according to Wellburn (1994).

4.2.9. Data analysis

To verify whether loss of water content during a dehydration cycle was affected by the presence or absence of hair and salt treatments, a Linear Mixed Model (LMM) was fitted in a Repeated Measurement ANOVA design, using thallus identity as a random effect factor to account for the temporal correlation of observations. The water content and F_V/F_M

were used as response variables and hairiness, NaCl concentrations and time as explanatory variables in a full factorial design. LMMs served to also verify whether the chlorophyll *a* fluorescence (F_V/F_M) was affected by the presence or absence of hair and salt treatments. As the relationship between the variation in water content or chlorophyll *a* fluorescence and time appeared quite far from a simple linear regression, in all the analyses we used time as an ordinal variable. We evaluated the significance of the fixed effects and of associated interaction factors using an ANOVA type III table, with Kenward-Roger degrees of freedom (df) approximation. LMM computations were performed using the lmer function of the *lme4* package version 1.1-12 for fitting the models and *car* package to obtain probability (p) values. The means and standard errors (SE) of all studied parameters were checked by one-way and two-way ANOVA and Tukey post hoc test was used for a posteriori comparison of individual means (with at least $p < 0.05$ as the significance level). The analysis was conducted using the statistical program R environment (R version 3.3.1). All plots were drawn using *SigmaPlot* 8.0.

4.3. Results

4.3.1. Anatomical investigation

The microscopical observation of the fresh sections highlighted the heteromeric structure of the thallus, characterized by a loosely intertwined upper cortex, a scleroplectenchymatic medulla, and a reticulate lower cortex that leaves portions of the medulla exposed (Fig. 4.1). The algal partner lies below the upper cortex, well distinct from the hyphal region. The upper hyphal layer is considerably thickened by the formation of plectenchyma, making a compact layer free of gaps. Of particular note, the agglutinated conformation of the hyphae and the arrangement of the photosynthetic partner is a typical scleroplectenchymatic structure of the lichen thallus.

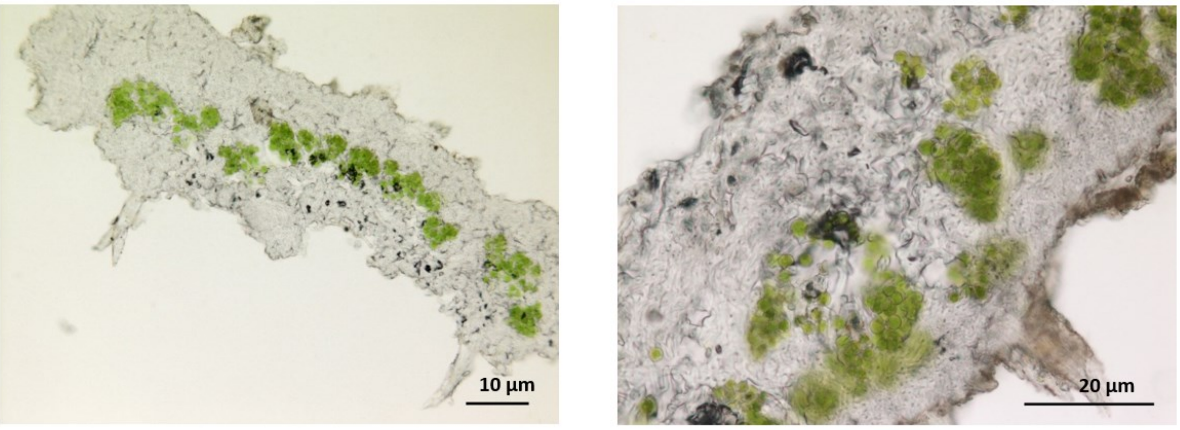


Figure 4.1 Cross section (10 µm and 20 µm, thick) of *Seirophora villosa* thalli.

3.2. Rate of water loss and photosynthetic activity during a dehydration cycle

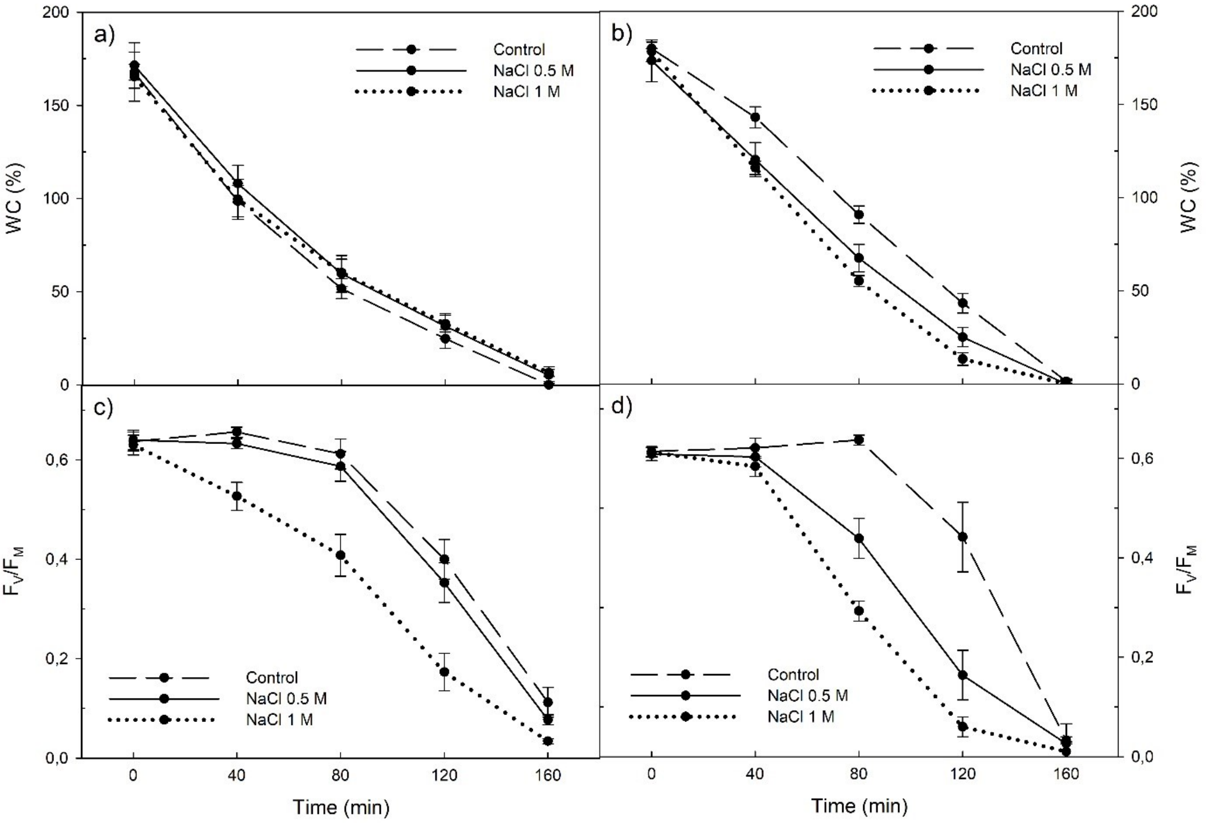
After spraying with water, HT and HLT reached water contents of about 168 % and 180 % dry weight respectively (Fig. 4.2 a, b). Water loss kinetics changed significantly over time for HT and HLT ($p < 0.001$) (Table 4.1): Complete dehydration was achieved in both cases after 160 minutes, decreasing faster in HT than in HLT ($p < 0.001$). Following NaCl treatments, HT did not show significant differences with respect to the control, while HLT reached a lower water content more rapidly than the control samples ($p < 0.001$).

Along with dehydration, the parameter F_V/F_M (used as a proxy for the photosynthetic activity) decreased significantly both in HT and HLT ($p < 0.001$) (Table 4.1), with F_V/F_M values in HT generally higher in comparison with HLT, both at 0.5 M and 1 M NaCl ($p < 0.001$) (Fig. 4.2 c, d). Initial F_V/F_M values were comparable for HT and HLT, but changed in different ways depending on salt treatment and the presence of hair: at 1 M NaCl, F_V/F_M values in HT decreased more rapidly compared to the control, reaching low values (0.173 ± 0.038 HT; 0.400 ± 0.049 control) in 120 minutes from experiment outset (Fig. 4.2 c). No difference in the controls was observed in HT treated with 0.5 M solution. In regard to HLT, significant differences with controls emerged both at 0.5 M and 1 M NaCl, with samples reaching low values (0.164 ± 0.054, 0.060 ± 0.024 respectively) in 120 minutes from the beginning of the experiment (Fig. 4.2 d).

Table 4.1 ANOVA Type III table with Kenward-Roger approximation for degrees of freedom (df) for repeated measures analysis of variance (rANOVA) for the relationship between loss in water content and efficiency of photosystem II (F_V/F_M) over time and the presence or absence of hair. During the experiment thalli weights were measured every 40 minutes. Significance codes: p value < 0.001 '***'; p value < 0.01 '**'; p value < 0.05 '*'.

	Hairy thalli					**Hair-less thalli**				
Parameter	**Loss in water content**					**Efficiency of photosystem II (F_V/F_M)**				
Factor	**F**	**df**	**Df.res**	**Pvalue**		**F**	**df**	**Df.res**	**Pvalue**	
(Intercept)	800.432	1	188.82	< 0.001	***	2143.626	1	104.56	< 0.001	***
NaCl	43.302	2	269.00	< 0.001	***	322.3045	5	245.00	0.991	
Time	283.882	1	269.00	< 0.001	***	0.8411	1	245.00	< 0.001	***
Hairness	432.750	1	269.00	< 0.001	***	0.0090	2	245.00	0.359	
NaCl:Time	13.539	2	269.00	< 0.001	***	1.4101	5	245.00	< 0.001	***
NaCl:Hairness	17.882	2	269.00	< 0.001	***	8.6286	10	245.00	0.981	
Time:Hairness	6.918	1	269.00	0.009	**	0.0187	2	245.00	0.221	
NaCl:Time:Hairness	3.899	2	269.00	0.021	*	1.9268	10	245.00	0.042	*

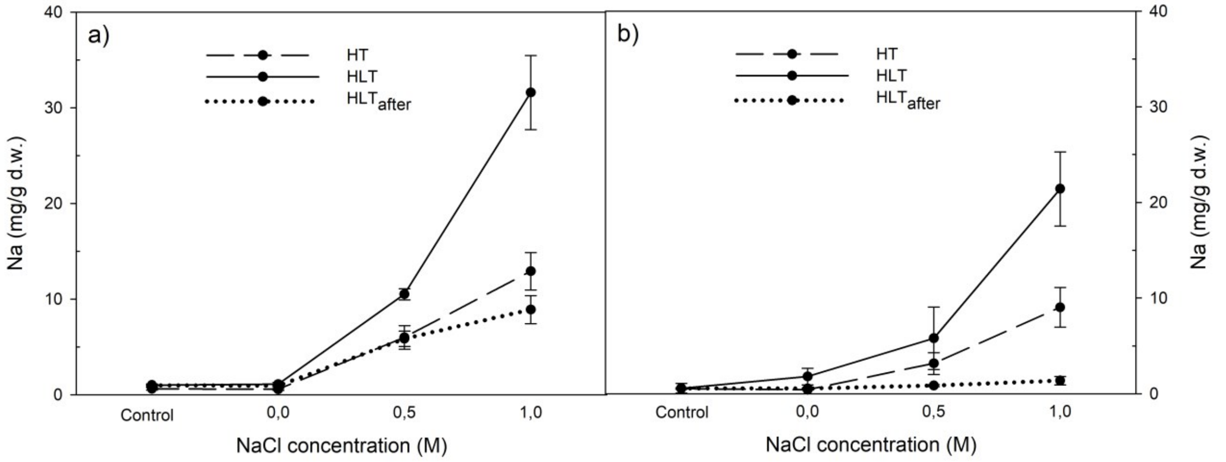
Figure 4.2 Rate of water loss and reduction of F_V/F_M during a complete drying cycle in *S. villosa* thalli after having been sprayed with different NaCl concentrations. The plots depict a decline in water content in hairy thalli (a) and in hair-less thalli (b) during a complete drying cycle, variation of photosystem II (F_V/F_M) efficiency in hairy thalli (c) and in hair-less thalli (d) over time. The interaction terms are statistically significant, according to a mixed model ANOVA with p value < 0.001. The dashed line = control; the solid line = NaCl 0.5 M; the dotted line= NaCl 1M.



4.3.3. Sodium accumulation

Total Na concentration increased progressively in HT, HLT and HLT_{after} as the concentration in the treatment solution increased, with the same trend observed for intracellular concentrations ($p < 0.001$) (Fig. 4.3). At treatment end, total Na concentration was significantly higher than the intracellular Na concentration ($p < 0.001$). There was also no significant difference in total Na concentrations between HT and HLT_{after}, which accumulated significantly lower amounts of Na than HLT both at 0.5 M and 1 M NaCl ($p < 0.05$). A similar pattern was also observed for intracellular Na accumulation, which was significantly higher in HT and HLT than in HLT_{after} both at 0.5 M and 1 M NaCl ($p < 0.05$).

Figure 4.3 Sodium concentrations of *S. villosa* thalli sprayed with different NaCl concentrations. The plots depict a) the total Na concentration b) the intracellular Na
error (at least, $p < 0.05$). The dashed line = hairy thalli (HT); the solid line = hair-less thalli (HLT); the dotted line = hair-less thalli after salt treatment ($\text{HLT}_{\text{after}}$).



4.3.4. Chlorophyll and carotenoid concentrations

In control samples, Chl*a* and Chl*b* concentrations were significantly higher in HT than HLT after 160 minutes of dehydration, as reported in Table 5.2; while higher carotenoid concentrations were measured in HLT than in HT ($p < 0.05$) (Table 4.2). In HT, in the presence of increasing salt treatments, the concentration of Chl*a*, Chl*b* and total carotenoids did not change compared to control samples. In HLT, both Chl*a* and Chl*b* significantly decreased at the highest salt concentration (1 M NaCl), while carotenoids were significantly affected both at 0.5 M and 1 M NaCl, compared to control values (Table 4.2).

Table 4.2 Mean values (± ED, N = 5) of chlorophyll and carotenoid concentration in hairy thalli and hair-less thalli treated with different NaCl concentrations. Lower case letters denote significant differences between intra-population means; upper case denotes inter-population means ($p < 0.05$).

Treatment	**Hairy thalli**			**Hair-less thalli**		
	Control	**0.5 M NaCl**	**1 M NaCl**	**Control**	**0.5 M NaCl**	**1 M NaCl**
Chlorophyll *a* (µg mg^{-1} d.w.)	946 ± 130 a A	1106 ± 83 a A	1090 ± 36 a A	426 ± 44 a B	439 ± 40 a B	191 ± 22 b B
Chlorophyll *b* (µg mg^{-1} d.w.)	444 ± 22 a A	471 ± 20 a A	379 ± 66 a A	222 ± 63 a B	221 ± 63 a A	105 ± 5 b B
Carotenoid (µg mg^{-1} d.w.)	60 ± 11 a A	61± 12 a A	57 ± 10 a A	140 ± 25 a B	35 ± 6 b A	55 ± 6 b A

4.3.5. MDA and H_2O_2 concentrations and antioxidant activity

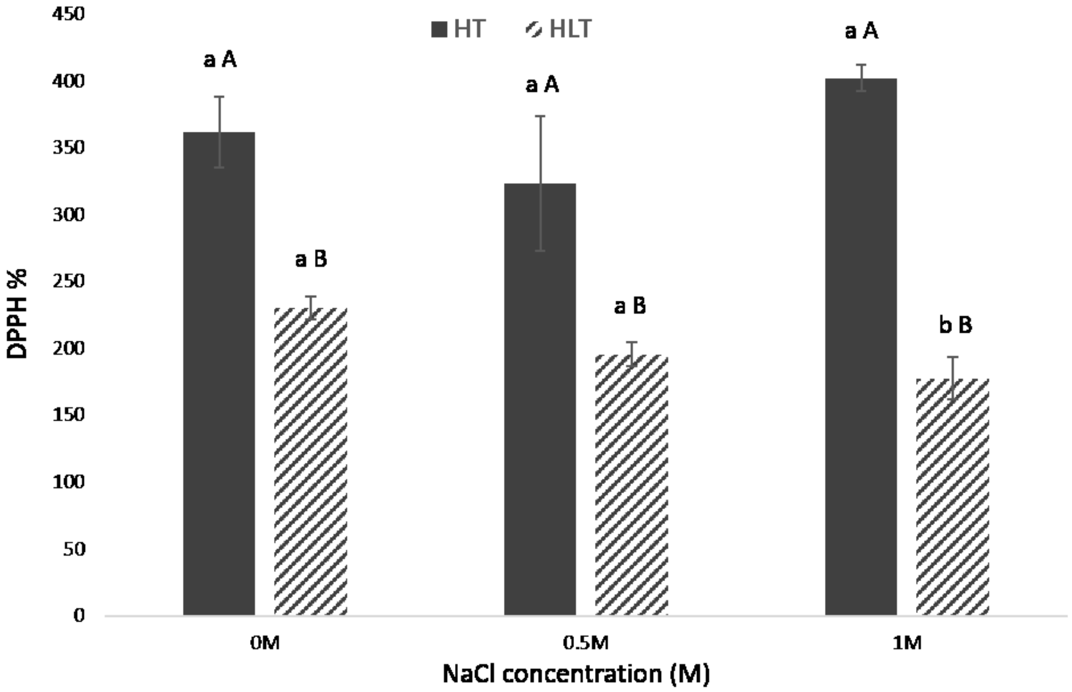
In control samples, MDA production and H_2O_2 concentration were higher in HLT compared to HT ($p < 0.05$) (Table 4.3). MDA production and H_2O_2 concentration were not affected by NaCl in HT and seemed reduced in HLT, both at 0.5 M and 1M NaCl, compared to the controls.

The free radical scavenging activity of the samples, as reflected by DPPH, significantly decreased in HLT compared to in HT ($p < 0.05$) (Fig. 4.4). DPPH values were not affected by salt treatments in HT and showed a significant decrease in HLT treated with the highest salt concentration.

Table 4.3 Mean values (± ED, N = 5) of MDA and H_2O_2 content of lichen samples treated with different NaCl concentrations. Lower case letters denote significant differences between intra-population means; upper case denotes inter-population means ($p < 0.05$).

Treatment	**Hairy thalli**			**Hair-less thalli**		
	Control	**0.5 M NaCl**	**1 M NaCl**	**Control**	**0.5 M NaCl**	**1 M NaCl**
MDA (µmol/g)	0.85 ± 0.09 a A	0.852 ± 0.03 ab A	0.72 ± 0.06 b A	1.23 ± 0.12 a B	0.94 ± 0.12 ab A	0.82 ± 0.07 b B
H_2O_2 (ug g^{-1})	0.41 ± 0.03 a A	0.41 ± 0.10 a A	0.25 ± 0.08 a A	0.57 ± 0.04 a B	0.24 ± 0.01 b A	0.27 ± 0.08 b A

Figure 4.4 Antioxidant activity of hairy thalli and hair-less ones after having been sprayed with different NaCl concentrations. The lower value indicates higher antioxidant activity. Values are means ± standard error (n = 3) and are expressed in mg of the dry weight needed to reduce by at least 50% 1 mg of the stable free radical DPPH. Lower case letters denote significant differences between intra-population means; upper case denotes inter-population means ($p < 0.05$). The solid black bar = hairy talli; the striped bar = hair-less thalli.



4.4. Discussion

The anatomical investigation highlighted a heteromeric structure characterized by a loosely intertwined upper cortex, a scleroplectenchymatic medulla, and a reticulate lower cortex that leaves portions of the medulla exposed. This structure and the morphological characteristics (such as the presence of hair) are considered typical for desert fog-zone lichens, as the other species belonging to the genus *Seirophora* (del Prado and Sancho, 2007). This fruticose growth allows the thallus to use the dew (del Prado and Sancho, 2007) as well as water vapor. The presence of hair further increases the absorption surface due to the greater surface to volume ratio (Ros and Werner, 1997; Esseen et al., 2015). Of note, our results show that hairy thalli of *S. villosa* store less water than hair-less ones. We therefore hypothesize that in our model species, hair plays a principal role in regulating the absorption of water in cases of both scarcity and excess. Hair could not only increase the thallus surface and promote water absorption in case of low availability (Kärnefelt, 1989), but also repels excess water, to prevent oversaturation. Considering that *S. villosa* does not produce hydrophobic metabolites on the hyphal reticulum (Søchting and Frödén, 2002), the presence of hair can play an important role in limiting the uptake of excessive water that could be detrimental, since it increases the resistance of gas diffusion in lichen thalli by constricting the pore space for gas exchange (Cowan et al., 1992).

Moreover, our results showed that hairy thalli dehydrated faster than hair-less ones, suggesting that hair could favor a rapid evaporation due to a greater thallus surface in contact with the air. These results support those reported in the literature for species living in arid environments, which tend to go into cryptobiosis more quickly to escape photoinhibition (Lange et al., 1990). In addition, *S. villosa* does not synthesize photoprotective pigments to defend the cortex against UV radiations, suggesting that the involvement of the mycobiont in protecting the photobiont might not be determined by the presence of secondary chemical compounds in the thallus.

In Mediterranean ecosystems, such as *Juniperus* shrublands in the Mediterranean basin, periods of excess thalli hydration and rapid dehydration are linked to their distance from the sea, habitat fragmentation, daily and seasonal fluctuations of microclimatic parameters, and to wet and rainy winters and hot and dry summers (Castro et al., 2004). During dry periods, lichens of dry environments hydrate at night through the uptake of fog, dew or water vapor. The first peak of positive net photosynthesis is seen to occur immediately after sunrise (Lange et al., 2006), with higher air humidity causing a second peak in the late afternoon. This behavior could allow thalli to be fully photosynthetically active even with low water content (Lange, 2006). As expected, our results showed that the length of the photosynthetically active period decreased during the dehydration cycle. Hair-less thalli maintained high photosynthetic performances (as suggested by F_V/F_M values) at 90 % of their water content, remaining active at up to 40%; hairy thalli maintained high photosynthetic performances even at 50% of their water content and remained started to deactivate when water content reduced below 20%. Hence, hair could be a strategic morphological trait for this species, not only enhancing a faster dehydration but also a differential evaporation between the hyphal network and the photosynthetic layer. The presence of hair could regulate the evaporation of water initially from the hyphal network, thus enhancing water persistence in the photosynthetic layer.

During a dehydration cycle, the absence of hair affected the concentrations of photosynthetic pigments as well as of oxidative stress markers. None of the parameters investigated changed in hairy thalli. By contrast, chlorophyll *a* and *b* concentrations significantly decreased in hair-less thalli at the end of the dehydration cycle, along with increases in MDA and H_2O_2 concentrations. These results suggest that the presence of hair protects the thalli during dehydration from oxidative stress and the lipid peroxidation generated by free radicals. ROS may accumulate in lichen tissues and represents a major cause of damage during desiccation, especially in photosynthetic organisms (Le Devehat et al., 2014). When desiccated in the light, chlorophyll molecules continue to be excited, but the energy not used in carbon fixation will cause singlet oxygen to form (Kranner et al., 2008). Moreover, in hair-less thalli the amount of oxidative stress markers increased, which triggered an antioxidative response in our samples. In effect, an increase of carotenoid concentrations was present, given that these molecules are key lipid-soluble antioxidants in membranes (Munne-Bosch and Alegre, 2002) and perform an essential photoprotective role by quenching triplet state chlorophyll molecules and scavenging singlet oxygen and other toxic oxygen species formed within the chloroplast (Kranner et al., 2008). In addition, an increase in the antioxidant response was suggested also by the low DPPH levels measured in hairy thalli. However this response was not sufficient to counteract oxidative stress, as shown by the increase of MDA concentrations found in hair-less thalli with respect to hairy ones, suggesting that the presence of hair protected the thallus. In fact, the levels of H_2O_2 in hair-less thalli were higher and contributed to the oxidative stress involved in these processes. Therefore, the presence of hair is seen to regulate water absorption and dehydration in order to keep the thallus photosynthetically active, even when water content is low, and at the same time to slow the onset of oxidative stress.

Spraying *S. villosa* thalli with two different NaCl concentrations had a significant effect on the accumulation of Na in the thallus and it is likely that this effect depends on the presence of hair. The amount of Na in hairy thalli and in hair-less thalli after salt treatment was significantly lower than hair-less ones, confirming that the presence of hair served to repulse excess water droplets, as well as the salt particles therein. These results were also confirmed by lower intracellular Na concentrations in hair-less thalli after salt treatment, suggesting that the cell wall also contributed to salt exclusion from the cytosol. Indeed, the cell wall was seen to act as an immobilizing filter in hairy thalli and in hair-less thalli after salt treatment, prevent a high amount of Na from entering the cell. In samples exposed to the highest NaCl concentration, observed changes in vitality depended mainly on the intracellular Na uptake. At the highest level of salt treatment, the performance of hairy thalli was also affected (reduction of F_V/F_M values), suggesting that even if hair could act as a barrier to salt entering, some Na still penetrates, compromising the activity of the samples. By contrast, in hair-less thalli all physiological parameters investigated were affected at both salt concentration levels. As demonstrated by Delmail et al. (2013), the first consequence of salt exposure is faster dehydration in hair-less samples. Our study found that a reduction of F_V/F_M values at 0.5 M and 1 M NaCl impacts on photosynthetic performances. The salt treatment also caused significant decrease in photosynthetic pigments in hair-less thalli. Nevertheless, hair-less thalli showed a high antioxidant activity after salt treatments, presumably scavenging the ROS accumulated in lichen tissues. Indeed, salt treatments when combined with the dehydration cycle activated physiological processes involved in desiccation tolerance that facilitate survival in a desiccated state, as well as the rapid re-establishment of normal physiological activity upon rehydration (Kranner et al., 2008).

4.5. Conclusions

Our work evidenced for the first time the relevance of hair as a strategical morphological trait in lichens facing extreme environments. The presence of hair in *S. villosa* was found to improve the performance of this species. Our results suggest that hair could offer a passive, but selective, water control. Hair was found to not only increase the thallus surface and promote water absorption when water availability was low, but could also repel excessive water to avoid oversaturation. Furthermore, hair was seen to favor a slower dehydration of the algal layer; keeping the photosynthetic process active, even at low water content. We can also conclude that in the presence of salt, hair activates a significant stress avoidance mechanism which, by preventing salt from entering, allows the thallus to tolerate that presence.

Chapter 5

General discussion

Coastal dune systems are among the most threatened habitats by human activities, which cause their fragmentation, and loss of species and functional diversity (Picchi 2008; Prisco et al. 2012; Bertacchi and Lombardi 2014). In natural conditions, they are known to host numerous epiphytic lichens and among them the red-listed macrolichen *Seirophora villosa* (Ach.) Frödén (Nascimbene et al. 2013), which is strictly associated with dune environments. The presence and abundance of this species are considered positive indicators of the conservation status of coastal nurseries (Benesperi and Ravera 2011).

The coastal environment we studied is an extremely dynamic ecosystem where natural processes interact and modify its characteristics. Light regime, water availability and high salt concentrations are generally the main ecological factors modified by habitat fragmentation that could be detrimental to the survival and establishment of poikilohydric organisms such as lichens, causing rapid dehydration, ionic imbalances and the inhibition of photosynthesis.

In the Mediterranean basin, coastal juniper habitats are known to host numerous epiphytic lichens, that living near the seashore need a morphological and chemical organization to adapt or acclimate to extremely variable disturbances. Recently studies, has demonstrated the dependence of *S. villosa* on undisturbed *Juniperus* stands suggesting a significant effect of disturbance on the presence and abundance of this lichen species. Moreover, it has been demonstrated that *S. villosa* is only occupying a small part of its colonizable niche because of a very limited propagation ability. The presence and abundance of this species are considered positive indicators of the conservation status of coastal nurseries.

This study aims to evaluate the effects of the main ecological variables on the eco-physiology of *S.villosa* to characterize its ecological niche.

In chapter 2, we showed that both functional richness and species richness significantly depend on the habitat structure. Confirming the correlation reported in (Benesperi et al. 2013) between epiphytic lichens richness and habitat width used as proxy of habitat integrity. *Juniperus* stands with higher individuals and cover continuity, probably ensuring a major stability of microclimatic factors, supported richer and functionally more differentiated epiphytic lichen communities. Accordingly, these stands host some lichens of conservation concern (*Bacidia parathalassica, Cerothallia luteoalba, Lecanora lividocinerea, Parmotrema stuppeum, Ramalina pusilla, Tornabea scutellifera).* On the contrary, disturbed stands with scattered and smaller individuals, host a poorer and trivial lichen flora, less differentiated from a functional point of view.

In contrast, the presence of *S.villosa* resulted not directly related to the habitat structure. As reported also in Giordani et al. (2015) *S. villosa* showed a clustered distribution occupying only a part of its colonizable habitat suggesting a major role of dispersal and establishment capability therefore suggesting a management tailored at the tree-level aimed to the conservation of reproductive specimens as observed in (Benesperi et al. 2018) for *Lobaria pulmonaria*. However, ongoing research indicate a possible role of microhabitat structure on the abundance of this species.

The experiments carried out in chapter 3 and 4 showed that the size of the thalli influence the water retention capacity of this species, and consequently influence responses to strong exposure to light. Our outcomes showed that *S. villosa* thalli are susceptible to sudden increases in PAR, especially if protracted over time and in the case of smaller specimens, exhibited reduced ability to recover after photoinhibition. Moreover, our work evidenced for the first time the relevance of hair as a strategic morphological trait in lichens to face extreme environments. The presence of hair in *S.villosa* was found to improve the performance of this species. Our results suggest that hair could offer a passive, but selective, water control. Hair could not only increase the thallus surface and promote water absorption when its availability is low but could also repel excessive water to avoid oversaturation. Furthermore, hair could favor a slower dehydration of the algal layer, to keep the photosynthetic process active, even at low water content. We can also conclude that as hair repels, water drops also repels the salt dissolved in water, by activating a passive resistance mechanism, a real avoidance of stress, which by not allowing salt to enter, allows the thallus to tolerate the presence of salt.

Several studies have shown that after logging or habitat fragmentation, epiphytic lichens are exposed to both sudden increases in PAR and to dry conditions, which, when exceeding the normal ecological range, can negatively influence their photosynthetic performance at different stages of growth. Our results demonstrate and confirm this hypothesis. Therefore, from a conservation point it would be necessary to preserve all the growth stages of the population, providing a suitable habitat for the larger thalli that have the role of propagating the species and for the smaller ones giving them the opportunity to colonize and establish in fragment habitat without being photohinbited. Thus, in a future conservation strategies perspective the conservation of the structure and continuity of *Juniperus* stand will be crucial for preserving lichen and functional diversity. According to the theory that diversity is not just a byproduct of the abiotic environment, but that the numbers and types of organisms directly regulate ecosystem functioning (Cardinale et al. 2011), by preserving lichen diversity we will contribute to preserve *Juniperus* habitat health. In the absence of these conditions, young populations of *S. villosa* with their small-sized specimens are particularly vulnerable and less likely to return to a physiological healthy status, which in turn limits their ability to recolonize fragmented habitats (Giordani et al. 2015). Considering that *S. villosa* depends heavily on undisturbed juniper stands, that it has a patchy distribution, and that it colonises only a fraction of its niche (Giordani et al. 2015), it is evident that inappropriate environmental management risks triggering a decline of *Seirophora* populations.

Chapter 6

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Final Report

Phd Course:
“Evolutionary biology and ecology”

Cicle XXXI

Candidate: Elisabetta Bianchi

Supervisor: Prof. Alessio Papini

Publications on IF Journals or on Scopus

- Favero-Longo, S. E., Benesperi, R., Bertuzzi, S., **Bianchi**, E., Buffa, G., Giordani, P., ... & Ravera, S. (2017). Species-and site-specific efficacy of commercial biocides and application solvents against lichens. *International Biodeterioration & Biodegradation*, *123*, 127-137.
- Ravera S., Vizzini A., Cogoni A., Aleffi M., Benesperi R., **Bianchi E**., Von Brackel W., Cataldo D., D'antonio C., Di Nuzzo I., Favero-longo S.E., Gheza G., Isocrono D., Matteucci E., Martellos S., Morosini L., Nimis P.L, Ongaro S., Poponessi S., Puntillo D., Sguazzin F., Tretiach M. (2018) Notulae to the italian flora of algae, bryophytes, fungi and lichens: 5. Italian botanist 5: 31-43. Https://doi.org/10.3897/italianbotanist.5.24852
- Benesperi, R., Nascimbene, J., Lazzaro, L., **Bianchi**, E., Tepsich, A., Longinotti, S., & Giordani, P. (2018). Successful conservation of the endangered forest lichen Lobaria pulmonaria requires knowledge of fine-scale population structure. *Fungal Ecology*, *33*, 65-71.
- Lazzaro L., Ferretti G., **Bianchi** E., Benesperi R. (2018) Treatment by glyphosate-based herbicide allowed recovering native species after *Oxalis pes- caprae* L. invasion: Indications from a Mediterranean island. *Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology*, 1-9.
- **Bianchi** E., Paoli L., Colzi I., Coppi A., Gonnelli C., Lazzaro L., Loppi S., Papini A., Vannini A., Benesperi R., (in press) High-light stress in wet and dry thalli of the endangered Mediterranean lichen *Seirophora villosa* (Ach.) Frödén: Does size matter? Mycologial Progress [Accepted] DOI: https://doi.org/10.1007/s11557-018-1451-0.

Publications on Journals without IF

- Caporale S., Benesperi R., **Bianchi E**., Catalano I. Et al., 2016. Contributo alle conoscenze lichenologiche della rnr cascate del verde e del bosco di Montalto (Borrello, Chieti). Notiziario della società lichenologica italiana 29:87-94.
- **Bianchi E**. 2017, "il sapore e il colore dei licheni. Una guida agli usi" di paolo modenesi: quando la scienza si trasforma in passione. Notiziario della società lichenologica italiana 30: 69-71.
- Paoli L., Vannini A., **Bianchi E**. 2017, I licheni della Riserva Naturale del Monte Labbro (Grosseto). Notiziario della Società Lichenologica Italiana 30: 67-68.
- Paoli L., Benesperi R., **Bianchi E**., Bruinalti G., Di Nuzzo L., Fackovcova, Frati I., Giordani P., Maccelli C., Ravera S., Zedda L., 2018. Impact of forest managment on threatened macrolichens. Incontro del gruppo di Lavoro per l'Ecologia della SLI. Notiziario della società lichenologica italiana 31: 61-62.

- Benesperi R., **Bianchi E**., Chirici G., Di Nuzzo L., Giannetti F., Giordani P., 2018 (In press). Effects of habitat structure on functional diversity of epiphytic lichen communities of coastal dune with Juniperus spp. Seventh International Symposium Monitoring of Mediterranean Coastal Areas: problems and measurement techniques.

Courses

- 2016 Corso di Scienza della vegetazione, Prof. Daniele Viciani, Corso di laurea magistrale in Scienze della Natura e dell'Uomo, Università degli Studi di Firenze
- 2016 Conservazione e gestione delle risorse vegetali, Corso di laurea magistrale in Scienze della Natura e dell'Uomo, Prof. Bruno Foggi, Università degli Studi di Firenze
- 2016 Introduzione all'uso di Gis, Offerta didattica per dottorandi, Prof. Bruno Foggi, Dipartimento di Biologia, Università degli Studi di Firenze
- 2016 Corso di statistica ed uso di R, Offerta didattica per dottorandi, Prof. Giacomo Santini Dipartimento di Biologia, Università degli Studi di Firenze

Congress attendance

- 28-30/09/2016 29° Convegno della Società Lichenologica Italiana (Trieste), -Poster- Caratterizzazione morfologica di due specie simpatriche: *Seirophora villosa* (ach.) Froden e *Ramalina canariensis* Steiner **Bianchi E**., Papini A., Tani C., Benesperi R.
- 28-30/09/2016 29° Convegno della Società Lichenologica Italiana (Trieste), -Poster- Trapianto di *Seirophora villosa* (ach.) Froden: un intervento di habitat restoration in ambiente dunale **Bianchi E**., Lazzaro L., Benesperi R.
- 28-30/09/2016 29° Convegno della Società Lichenologica Italiana (Trieste), -Poster- Revisione del genere *Parmelia* ach. S.str. In italia attraverso dati molecolari, chimici e morfologici, Benesperi R., **Bianchi E**., Castellani M.B., Coppi A.
- 28-30/09/2016 29° Convegno della Società Lichenologica Italiana (Trieste), -Poster- Contributo alla flora lichenica della riserva naturale dell'isola di Montecristo (Toscana-livorno), **Bianchi E**., Esposito C., Benesperi R.
- 13-15/09/2017 30° Convegno della Società Lichenologica Italiana (Torino), -Poster- Influenza dello stress idrico sull'attività fotosintetica di *Seirophora villosa* (ach.): quanto contano le dimensioni? **Bianchi E**., Colzi I, Gonnelli C., Loppi S., Paoli L., Vannini A., Benesperi R.
- 13-15/09/2017 30° Convegno della Società Lichenologica Italiana (Torino), -Poster- l'effetto dell'habitat sull'allocazione riproduttiva delle specie di *Lobaria* s. Lat. In Italia, Benesperi R., **Bianchi E**., Nascimbene J., Giordani P.
- 13-15/09/2017 30° Convegno della Società Lichenologica Italiana (Torino), -Poster- Il Progetto Co.L.D (COppice and Lichen Diversity). Effetti della ceduazione sulle

comunità licheniche epifite nelle foreste italiane. Stato di avanzamento. Benesperi R., **Bianchi E.**, Castellani B., Di Nuzzo L., Giordani P., Longinotti S., Paoli L., Puntillo D., Puntillo M., Nascimbene J., Zedda L.

- 13-15/09/2018 30° Convegno della Società Lichenologica Italiana (Torino), -Poster- Diversità funzionale delle comunità licheniche epifite di ginepreti dunali: quanto conta la struttura dell'habitat? **Bianchi E.**, Di Nuzzo L., Giannetti F., Giordani P., Chirici G., Benesperi R.
- 26-28/09/2018 31° Convegno della Società Lichenologica Italiana (Pistoia) –Oral presentation- The thin hairiness surface plays an important role in *Seirophora villosa* performances **Bianchi E.**, Colzi I., Gonnelli C., Morandi F., Benesperi R.
- 26-28/09/2018 31° Convegno della Società Lichenologica Italiana (Pistoia) –Oral presentation- *Seirophora villosa* (Ach.) Frödén: a multidisciplinary investigations on morphological, ecophysiological and ecological aspects, **Bianchi E.**, Coppi A., Colzi I., Gonnelli C., Giordani P, Lazzaro L., Loppi S., Paoli L., Papini A., Vannini A., Benesperi R.
- 26-28/09/2018 31° Convegno della Società Lichenologica Italiana (Pistoia) –Poster- Devitalizzazione di licheni sui monumenti in pietra: ab-, ad- e de-sorbimento di biocidi fra talli e substrato lapideo. Favero.longo S.E., Benesperi R., **Bianchi E.**, Giordani P., Loppi S., Matteucci E., Paoli L., Ravera S., Roccardi A., Tonon C., Vannini A.,
- 19-21/09/2018 Settimo simposio "il monitoraggio costiero mediterraneo: problematiche e tecniche di misura" (Livorno) -Oral presentation- Effects of habitat structure on functional diversity of epiphytic lichen communities of coastal dune with *Juniperus* spp. Benesperi R., **Bianchi E.**, Chirici G., Di Nuzzo L., Giannetti F., Giordani P.
- 12-15/09/2018 SBI Conference (Fisciano) -Oral presentation- Adaptations to environmental stresses: A case study *Seirophora villosa* (Ach.) Fröden **Bianchi E.**, Coppi A., Colzi I., Gonnelli C., Lazzaro L., Loppi S., Paoli L., Papini A., Vannini A., Benesperi R.
- Neobiota 2016, -Poster- A field experiment for the evaluation of an effective treatment to control/eradicate the invasive weed oxalis pes-caprae: indications from a Mediterranean island. Lazzaro L., Ferretti G., Foggi B., **Bianchi E.**, Benesperi R.

Master students co-supervision

- A.A. 2018. Federica Morandi, Laurea Magistrale in Biologia, "The thin hairiness surface plays an important role in *Seirophora villosa* performances", in corso.

Seminars

- Seminario sulla biologia dei licheni ed uso dei licheni al 1°anno di Scienze Biologiche, Corso di Botanica, Prof. Alessio Papini, anni 2016-2017
- Seminario sulla biologia dei licheni e uso dei licheni al 1°anno di Scienze Naturali Corso Lichenologia e Micologia, Prof. Renato Benesperi, anni 2016-2018

Other research activities

- 2016 Partecipazione ad un progetto internazionale con l'Università Rey Juan Carlos di Madrid: "Impact of global change on epiphytic organisms: from populations to communities at multiple spatial scales".
- 2015-2018 Partecipazione al progetto RESTO CON LIFE – Island conservation in Tuscany restoring habitat not only for birds. (LIFE13 NAT/IT/O00471- REST0 CON LIFE).
- 2016-2018 Partecipazione al Gruppo di Lavoro della Biologia Società Lichenologica Italiana (SLI). Attività sperimentali per la realizzazione di saggi comparativi sull'efficacia di diversi trattamenti biocidi per la devitalizzazione dei talli sui monumenti.
- 2016-2018 Partecipazione al Gruppo di lavoro per il Biomonitoraggio (SLI) Stesura di norma europea per la regolamentazione dei protocolli di bio-accumulo di elementi in traccia mediante licheni in situ e mediante trapianti.
- 2016-2018 Partecipazione al Gruppo di lavoro per l'Ecologia (SLI). Progetto Co.L.D. che si propone di analizzare gli effetti delle diverse tipologie di ceduazione sulla diversità lichenica epifita in foreste di *Quercus* spp. Decidue in Italia. Partecipazione allo studio relativo agli effetti della gestione forestale sulle comunità licheniche e alle problematiche di conservazione di specie indicatrici, quali *Lobaria pulmonaria*.
- 2018 Partecipazione al Gruppo di lavoro della Floristica (SLI) Si propone di condurre ricerca scientifica e divulgazione sulla presenza e distribuzione dei licheni in Italia.

Awards

- Settembre 2018 risultato vincitore della Settima edizione del premio "Carlo Gaggi", dedicato alla memoria del Prof. Carlo Gaggi, rivolto a studenti iscritti a un corso di dottorato di ricerca che svolgano ricerche con attinenza lichenologica – presso Società Lichenologica Italiana