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Epizoic algae distribution on the carapace and plastron of the European pond turtle (*Emys orbicularis,* Linnaeus, 1758): A study from the Camargue, France

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Abstract – We investigated epizoic algal assemblages on the shell of European pond turtles (*Emys orbicularis*) during two years (2013-2014). A total of 60 *Emys orbicularis* were captured in the three shallow Mediterranean wetlands located in Camargue. Epizoic algae on the plastron (below the shell) and carapace (above the shell) were sampled, identified and counted. Seventy-seven epizoic algal species were identified on the carapace and plastron and comprised in 51 Bacillariophyta, 11 Chlorophyta, 7 Cyanophyta, 6 Euglenophyta, 1 Dinophyta and 1 Xanthophyta taxa. Our findings indicated a distinct distribution of epizoic algae according to taxonomical group density; Chlorophyta, and Cyanophyta were dominant on the carapace whereas the Xanthophyta (genus *Vaucheria* sp.) was dominant on the plastron. Turtle-associated algal assemblages did not differ among the wetlands

Epizoic algae / Epibionts / *Emys orbicularis* / plastron / carapace / temporary wetland / Camargue

Résumé – **Distribution des algues épizoiques sur la carapace et le plastron de la Cistude d'Europe *Emys orbicularis* (Linnaeus, 1758). Etude menée en Camargue, France** – Nous avons realisé une étude sur l'assemblage des algues épizoiques sur la carapace de Cistudes (Emys orbicularis) au cours de deux années (2013-2014). Soixante *Emys orbicularis* ont été collectés dans trois marais méditerranéens situés sn Camargue. Les algues épizoiques ont été identifiées et dénombrées sur le plastron (dessous la carapace) et sur la dossière (dessus la carapace). Au total, 77 espèces d'algues épizoiques ont été inventoriées sur la dossière et sur le plastron réparties en 51 Bacillariophyta, 11 Chlorophyta, 7 Cyanophyta, 6 Euglenophyta, 1 Dinophyta and 1 Xanthophyta. Nos recherches ont mis en évidence une distribution originale des algues épizoiques selon leur groupe taxonomique. Parmi les principaux groupes,

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les chlorophytes et les cyanophytes filamenteuses sont prédominantes sur la dossière tandis que le groupe des xanthophytes (genus *Vaucheria* sp.) se développe sur l'ensemble des plastrons étudiés. Aucune différence dans les assemblages algaux n'a été observée entre les 3 marais étudiés.

Algues épizoiques / epibiontes / *Emys orbicularis* / plastron / dossière / marais temporaires / Camargue

INTRODUCTION

Freshwater algae occur in aquatic and terrestrial habitats and on a wide variety of benthic substrates (Round, 1981; Burkholder, 1996; Graham and Wilcox, 2000). Algae colonize a variety of biological and non-biological substrates such as epilithic algae on stones (Entwisle, 1989; Uehlinger, 1991; Peterson, 1996; Biggs *et al*., 1999**;** Davie *et al*., 2012), epiphytic algae on aquatic plants (Comte *et al.,* 2001; Comte *et al.,* 2005) and epizoic algae on aquatic animals (Thiéry & Cazaubon, 1992). Epizoic algae have been described on the exoskeletons of many freshwater crustaceans living in temporary ponds (Bourrelly, 1959; Belk, 1973; Shelton, 1974; Thiéry, 1991; Thiéry & Cazaubon, 1992). In a study of some North American turtles, Edgreen *et al*. (1953) found that over half of the specimens examined were epizooitizied. Indeed, turtle shells may provide a favorable substrate for algae (Neill & Allen, 1954; Sheath &Wehr, 2003; Ziglar & Anderson, 2005; Tumlison & Trauth, 2006; Garbary *et al*., 2007, Akgül *et al*., 2014, Wehr *et al*. 2015). However, the carapace and a plastron of an amphibious turtle would be a challenging habitat for most aquatic organisms (Skinner *et al*., 2008). The role of turtles in seed dispersal via seeds adhering to their carapacial algal mat has been widely documented (Walker *et al.,* 1953; Moski, 1957; Proctor, 1958; Anjum *et al.,* 1980; Burgin & Renshaw, 2008). A diversity of algae has been described and observed to grow on the shell (carapace and plastron) and the head of many species of freshwater turtle (Edgren *et al*., 1953, Dixon, 1960; Belusz & Reed, 1969; Soylu *et al*., 2006; Garbary *et al*., 2007; Akgül *et al*., 2014). In particular, the green filamentous algae *Arnoldiella chelonum* and *Basicladia* species are widely represented and most common on turtles across most of North America (Bury *et al*., 2015). Edgren *et al*. (1953) detailed the range of turtles host then known in North America and the range of epizoic algae including *Rhizoclonium* and *Cladophora*. Two other genera in the Cladophoraceae comprise the other widely recorded macroalgae on turtle shell: the prostrate, spreading, endozoic (and possibly disease causing) *Dermatophyton radicans* and species of the heterotrichous genus *Basicladia* (Skinner *et al*., 2008). In the Camargue (south of France), populations of the European pond turtle (*Emys orbicularis*) are monitored by several researchers in the Camargue (south of France) (e.g., Olivier *et al*., 2010; Ficheux *et al*., 2014) within the context of biodiversity loss. Water management and anthropogenic impacts generate eutrophication and, consequently, algal development on the shells of European pond turtles because algae are generally nutrient-limited. In this context, the density of epizoic algae on turtle shells was investigated to determine whether the carapace (above) and the plastron (below) had the same or different algal assemblages. This is the first investigation of epizoic algae on European pond turtles in the Camargue and apparently also in France.

The present study aimed to describe the epizoic algae distribution on the shells and answered the following questions: "Are the carapace and plastron of European pond turtles colonized by the same epizoic algal species? and are the algal assemblages distributed randomly?"

MATERIALS AND METHODS

The three temporary marshes studied are located in the Camargue (southern France) at the Tour du Valat natural reserve and research station (43°30' N, 4°40' E): The first site (site 1), the Faïsses (Moncanard in Olivier *et al*., 2010) covers an area of 100 ha. The second site (site 2) the Esquineau has a total area of 250 ha. and the third site (site 3), the Clos du Marteau has a total area of 150 ha. At all sites turtles inhabit two kinds of habitats: permanent and semi-permanent marshes dominated by reed beds of *Phragmites australis* and man-made irrigation and drainage canals (Olivier *et al*., 2010). At each site, twenty turtles (ten males; ten females) were captured between June-July 2013 and June-July 2014 with fish traps and by hand. Age, sex and morphological measurements were recorded for each captured turtle. Sex was identified by observing male secondary sexual characteristics: concave plastron, orange eyes (yellow in females), basic wide tail and cloacae away from the plastron (Zuffi & Gariboldi, 1995). Turtles were classified as adults if they had no visible growth rings (Castanet, 1988; Olivier, 2002).

For each turtle, epizoic algae were collected by scraping with a scalpel an area of 1 cm^2 on both the plastron and the carapace. The algae recovery rate was noted. Samples were stored in formaldehyde solution (35%).

Epizoic algae were identified and counted using "Olympus" inverted microscope (×400 magnification) and counted. Cleaned Bacillariophyta samples were mounted in the highly refractive medium Naphrax, accentuating the frustular details used in taxonomy. Most taxa were identified using the Süßwasserflora von Mitteleuropa volumes (Krammer & Lange-Bertalot, 1986, 1988, 1991a, 1991b, Komàrek & Anagnostidis 1999, 2005). Quantification of epizoic algae densities was performed using standard counting techniques (Uthermöhl, 1958; Lund *et al.*, 1958). Between-sites (S1, S2 and S3) and between-shells (n = 60) differences were determined for total cell density measured on the carapace and on the plastron during each of the two years, respectively. Data were log-transformed and analyzed using the Wilcoxon non-parametric test (R package pgirmess). To examine and compare the distribution of dominant epizoic algae (15 species with density > 5% of total density) on the plastron and on the carapace. A Principal Component Analysis (PCA) was performed using ade4 package for R software version 2.15.2.

RESULTS

Epizoic algal communities

Species Richness

77 epizoic algae taxa were identified from 60 sampled turtles (Table 1). Six divisions were identified and counted: Bacillariophyta (Diatoms), Chlorophyta, Cyanophyta, Euglenophyta, Xanthophyta and Dinophyta. Diatoms was the most

Table 1: Epizoic algae observed on *Emys orbicularis* in the Camargue, France

Taxon	*Site 1*	*Site 2*	*Site 3*
51 Bacillariophyta			
*Achnanthes lanceolata**	+	+	+
*Achnanthidium minutissimum**	+	+	+
*Amphora pediculus***	+	+	+
*Caloneis amphisbaena***	+	+	+
*Cocconeis pediculus**	+		
Cocconeis placentula	+	+	+
Cymatopleura solea	+	+	+
Cymatopleura elliptica	+	+	
Cymbella lanceolata	+	+	+
*Cymbella minuta**	+	+	
Cymbella affinis	+	+	+
*Denticula sp.***	+	+	
*Diatoma vulgaris**	+	+	+
*Diploneis didyma***	+		
Epithemia adnata	+	+	+
*Fragilaria capucina**	+	+	+
Fragilaria sp.	+	+	+
Fragilaria ulna		+	
Gomphonema acuminatum	+		+
*Gomphonema parvulum**	+	+	+
*Gomphonema truncatum***			+
Gomphonema olivaceum.	+	+	+
Gyrosigma attenuatum	+	+	+
*Gyrosigma balticum***		+	
Gyrosigma acuminatum.	+	+	+
*Melosira italica***	+		+
*Navicula capitatoradiata**		+	
Navicula cryptocephala	+	+	+
*Navicula cryptotenella**	+	+	
*Navicula cuspidata***		+	
*Navicula elegans***	+		
*Navicula gibbula***		+	
*Navicula lanceolata**	+		+
Navicula menisculus		+	
*Navicula minuscula***		+	
Navicula radiosa		+	
*Navicula rhyncocephala**		+	+
Navicula sp.	+	+	+
Navicula tripunctata	+	+	+
*Navicula tuscula***	+	+	+
*Nitzschia dissipata**	+	+	+
*Nitzschia flexa**	+		
Nitzschia linearis		+	
*Nitzschia obtusa***		+	
*Nitzschia sigmoidea**		+	+
Nitzschia sp.	+	+	+
Pinnularia divergens	+	+	+
*Pinnularia mesolepta***		+	
Pinnularia sp.	+	+	+
*Stauroneis sp.***		+	
*Surirella brebissonii**		+	

Taxon	*Site 1*	*Site 2*	*Site 3*
11 Chlorophyta			
Chaetophora sp.	+	+	+
*Chlorella sp.***	+	+	+
Cladophora glomerata	+	+	+
Closterium sp.		+	+
*Cosmarium sp.***	+	+	+
*Micrasterias sp.***	+	+	+
*Oedogonium sp.**	+	+	
Scenedesmus armatus	+	+	+
*Spirogyra varians**	+	+	+
Ulothrix zonata	+	+	+
*Uronema sp.***			+
7 Cyanobacteria			
*Arthrospira sp.**		+	
*Chlorogloea sp.***	+	+	+
*Komvophoron sp.***		+	+
Lyngbya sp.	+	+	+
Oscillatoria limosa	+	+	+
Oscillatoria sp.	+	+	+
*Phormidium sp.**	+	+	+
6 Euglenophyta**			
Euglena acus.	+	+	+
Euglena tripteris			+
Phacus orbicularis		+	+
Trachelomonas nigra	+	+	+
Kyste Trachelomonas	+		+
Kyste Euglena	+		+
1 Xanthophyta			
Vaucheria sp.	+	+	+
1 Dinophyta*			
Peridinium cinctum.	+		+

* species inventoried only in 2013
** species inventoried only in 2014

diverse group with 51 taxa identified, followed by Chlorophyta (green algae, 11 taxa), Cyanophyta (7 taxa), Euglenophyta (6 taxa), Xanthophyta (1 taxon), and Dinophyta (1 taxon). The growth of macroscopic filamentous epizoic algae was often extensive on the carapace and on the plastron (illustrated in Figs 1-2).

Total cell density

Mean total cell densities (calculated from 2013-2014 data) on the carapace were similar at the three sites: 279239 cell.cm^{-2} at site 1, 297794 cell.cm^{-2} at site 2 and 299941 cell.cm^{-2} at site 3 (Fig. 3). These among-site densities were not significantly different (Wilcoxon test, p-value = NS). Mean density was slightly lower on the plastron than on the carapace at each site, with plastron densities of 248962 cell.cm^{-2} at site 1, 285621 cell.cm^{-2} at site 2 and 272025 cell.cm^{-2} at site 3.

Fig. 1. Macroscopic view of green filamentous epizoic algae on an adult male carapace of *Emys orbicularis* (Photo A. Olivier).

Fig. 2. Macroscopic view of algae (genus *Vaucheria sp.*) on the plastron of *Emys orbicularis* (Photo A. Olivier)

Total densities of epizoic algae were not significantly different between the carapace and plastron over the sites (Wilcoxon test, p-value = NS).

Density of algal groups

Algae were distributed among six taxonomic groups: Bacillariophyta (diatoms), Chlorophyta, Cyanophyta, Euglenophyta, Dinophyta and Xanthophyta (Fig. 4). Cell density of only the Dinophyta group did not differ significantly between plastron (mean = 144 cell.cm^{-2}) and carapace (mean = 245 cell.cm^{-2}, Wilcoxon test, p-value = NS). All other algal groups had significant differences in cell density (Figure 4): diatoms ($p < 0.05$), Chlorophyta ($p < 0,01$) Cyanophyta ($p < 0,001$) Euglenophyta ($p < 0,05$) and Xanthophyta ($p < 0,001$). Cell density of the first four above-listed groups had significantly fewer cells on the plastron relative to the carapace (Fig. 4). Indeed, diatom density mean values were 7324 cell.cm^{-2} on the plastron and 19897 cell.cm^{-2} on the carapace. For Chlorophyta, Cyanophyta, and Euglenophyta, mean values were respectively 27705 cell.cm^{-2}, 32947 cell.cm^{-2} and 549 cell.cm^{-2} on the plastron, and 117139 cell.cm^{-2}, 151932 cell.cm^{-2} and 3210 cell.cm^{-2} on the carapace. In contrast, to these algal groups Xanthophyta (*Vaucheria* sp.) was strongly dominant on the plastron (200118 cell.cm^{-2}) relative to the carapace (< 5 cell.cm^{-2}). Although epizoic algae colonization was significantly different between the plastron and the carapace, there was no significant difference among the three sites (NS, N = 60).

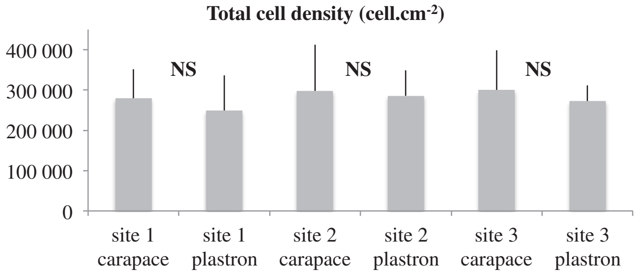

Fig. 3. Total cell density of epizoic algae (cell.cm^{-2}) monitored on the carapace and on the plastron at site 1, site 2 and site 3. Different letters indicate significant differences (Wilcoxon test; $p < 0.05$, NS = non significant).

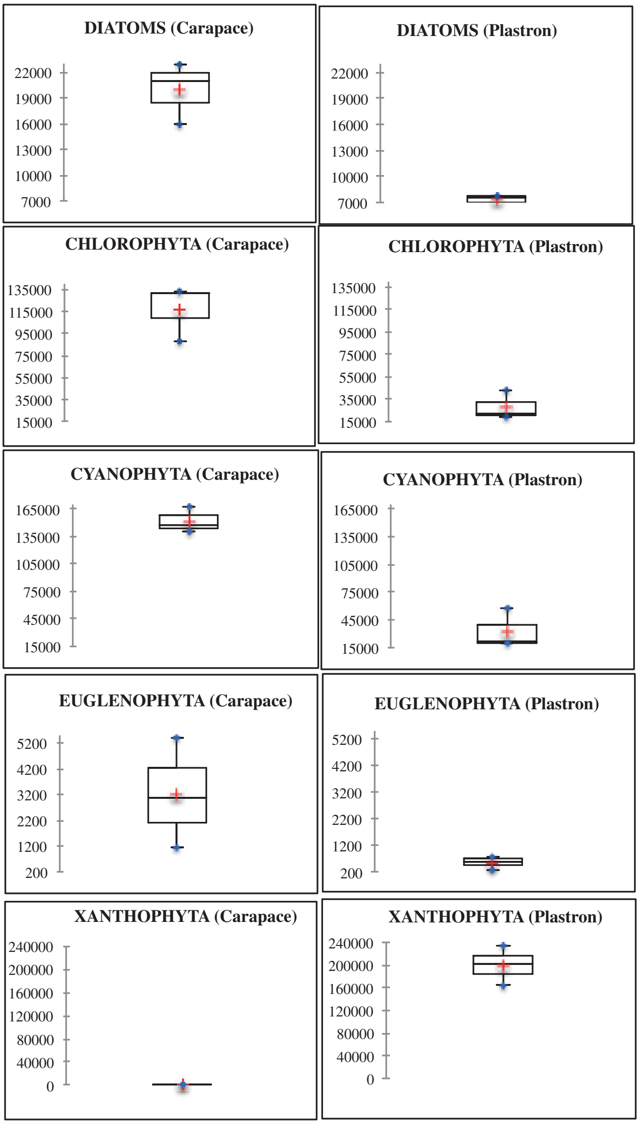

Fig. 4. Box and whiskers plots of cell density (cell.cm^{-2}) of each algal group observed on the carapace and plastron of European pond turtles and calculated from cell density obtained from 60 individuals at the three study sites. The vertical lines represent the range of observations (minimum and maximum). The box represents the interquartile range. The horizontal line within the box is the median and the cross is the mean.

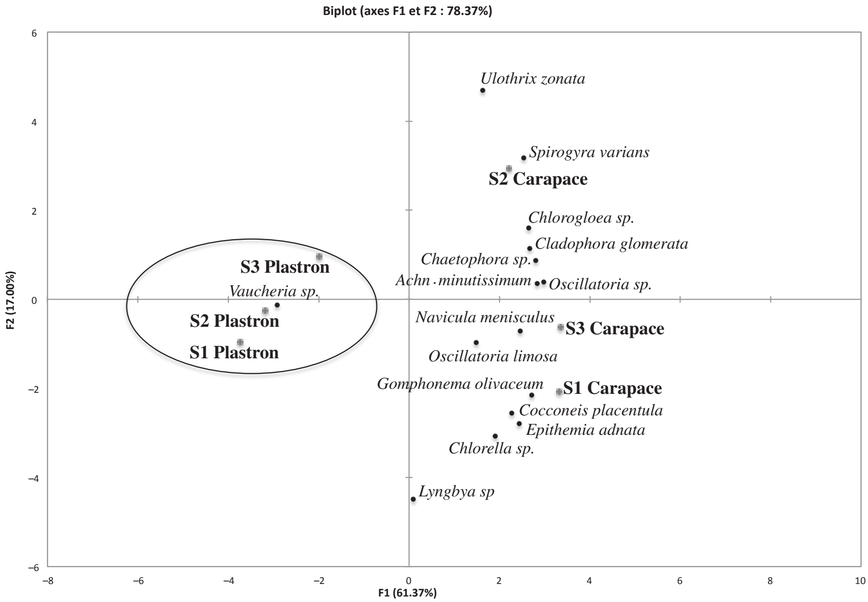

Fig. 5. Principal Component Analysis (biplot) – Representation of the epizoic algae species and sites from sampling the carapace and plastron of turtles.

Epizoic algae species

The principal component analysis of the 15 dominant epizoic species on the shell (PCA biplot, Fig. 5) indicated that the carapace and plastron of European pond turtles are distinguished by differences in epizoic algal taxa. Axes 1 and 2 of the biplot accounted for 78.37% of the total variability and hence could be unambiguously considered as the two main structuring components. Axis 1, which accounted for 61.37% of the total variability, was correlated with the presence of *Vaucheria* sp. species on the plastron of turtles at all three sites, in contrast to the many algal species associated with the turtle carapaces, such as as *Spirogyra varians* at site 2. Axis 1 could be considered as indicative of epizoic algal distribution on regions of the European pond turtle shell. Axis 2 accounted for 17.00% of the total variation and revealed that the three sites were distinguished by differences in epizoic species. The results obtained during two years provided a synthetic classification in response to the respective importance of algal density on the plastron or the carapace turtles. Several epizoic species contributed to the observed algal growth on the turtle carapaces at the three sites: *Oscillatoria* sp. (Cyanophyta), *Cladophora glomerata, Chaetophora* sp., and *Spirogyra varians* (Chlorophyta). Filamentous algae was also visible on the plastron, where the genus *Vaucheria* sp. (Xanthophyta) dominated.

DISCUSSION

Similar to the findings of Soylu *et al*. (2006), we found that all turtles supported epizoic algal growth. Shell selectivity was evident in algae distributions on European pond turtles. The plastron was colonized largely by *Vaucheria sp* for the greater part whereas the carapace was colonized by diatoms, chlorophyta and cyanophyta. Cyanophyta predominance on carapace suggests eutrophication pressures. High cyanophyta numbers can also indicate high invertebrate grazing because cyanophyta are less consumed, but this isn't likely because of the high abundance of filamentous green algae, which would be consumed or even tolerance to frequent aerial exposure. Several authors described the presence of cyanophyta from the carapace of the snapping turtle *Chelydra serpentina,* among these cyanophytes were *Plectonema tenue*, reported by Belusz & Reed (1969) and *Oscillatoria* sp., *Lyngbya* sp. and *Trichodesmium sp* which were reported by Ernst & Barbour, (1972). According to Burgin & Renshaw (2008), the distribution and the abundance of resident algae are determined by turtle behavioural patterns as burrowing and hibernation. Moulting, light intensity and desiccation also influence epizoic algae distribution (Protor, 1958). Predation, physical stress, disturbance, recruitment dynamics and competition also contribute to the distribution of epibionts on turtles (Soylu *et al.*, 2006). According to Edgren *et al*. (1953) and Ersanli & Gonulol (2014) the turtles (Genus *Macrochelys*, *Chelydra*, *Sternotherus*, *Kinosternon*, *Emys*) colonized frequently by epizoic algal are these who capture active preys such as frogs, tadpoles, fishes, crawfishes and insects contrary to the turtles more herbivores (*Pseudemys*).

Freshwaters turtles are an important component of aquatic ecosystems and play a primary role in the dispersal of algae and seeds (epizoochory) among aquatic habitats (Burgin & Kenshaw, 2008). Epizoic algae on turtle shell could have several benefits including cryptic advantage. The camouflage afforded by macroscopic epizoic algal growth may offer a selective advantage in avoiding predators (Harper, 1950) and by mimicking aquatic plants and non-turtle-associated algal growths (Neil & Allen, 1954) and for improving the predation of preys. It is hypothesized that a detrimental relationship exists between epizoic algae and their turtle hosts (Allee *et al*. 1949; Edgren et *al*. 1953; Ersanli et Gonulol 2014), but symbiosis may also be possible. We are unable to confirm either type of association from our data. More investigation is needed to resolve the many uncertainties involved. The importance of epizoic *Vaucheria* sp. development on the plastron is not known. This algal species is slippery and sticky and seems not to be detrimental to the turtle host. Xanthophyta are generally found in freshwater, wet soil and tree trunks, but there are several marine species. Most of the species occur singly and are found around other algae, making it difficult to find the same species twice. They typically do very well at low pH in habitats that are rich in iron and phosphorus. Many of them are found in late winter among floating mats in still water. In our study, water management could support developments of these algae in spring and summer. These algae might remain on the wetland soil (as reproductive structures) during dry periods and colonize the plastron of European pond turtle following the turtle's hibernation phase.

Our study confirms the hypothesis of distinct differences in algal distribution between carapace and plastron. We can thus conclude that the turtle "*Emys orbicularis*" shell constitutes a selective support for epizoic algae and consequently the algal assemblages were not distributed randomly.

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