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Cystoseira baccata meadows along the French Basque coast (Bay of Biscay) as a reference for the implementation of the Water Framework and Marine Strategy EU directives

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Abstract :

Cystoseira baccata, a biological quality element for the implementation of the Water Framework and Marine Strategy EU Directives, is a dominant species in the subtidal rocky bottoms of the Basque coast. As part of this issue and given the need to better understand the functional character of benthic rocky ecosystems and use them as a reference to anthropogenic changes, two samplings campaigns were conducted in 2014 and 2017. Several population parameters (i.e. frond density, frond length frond-length/total frond-length ratio and taxonomic richness of epibionts) of *C. baccata* were studied from three sites monitored within the Water Directive Framework in relation to bathymetry during the two campaigns. The results showed a significant influence of bathymetry on frond density, frond length and the epibiotic load, and also an effect of sampling time for the seaweed and epibionts (epiflora and epifauna). The characterization of these functional population parameters for *C. baccata* under natural conditions are extremely valuable for its application to monitoring programs evaluating the ecological status of coastal waters in this region.

Highlights

► Contribution of new knowledge on *C. baccata* habitat and population. ► Confirmation of interest of this engineering species in implementation MSFD. ► Swell effect influencing density and individual height. ► Ratio of length is pertinent metric to discriminate epibiosis considering levels.

Keywords : bathymetry, *Cystoseira baccata*, epiphytes, foundation species, population parameters, Water Framework Directive

1-Introduction

The marine environment provides valuable ecosystem services to humans but it is threatened by pressures from multiple anthropogenic activities and climate change (Marcos *et al.*, 2012; Duarte *et al.*, 2018). For the southeastern Bay of Biscay, the increase in sea surface temperature (SST) is estimated on average at around 0.01 °C per year (Le Treut, 2013). Anthropogenic pressure is particularly intense in coastal areas (Guillaud, 1992; Borja *et al.*, 2011) where around 60% of the human population is concentrated (Thomson *et al.*, 2002). Environmental changes occur not only as a consequence of physical alteration of the shoreline but also due to chemical impacts (e.g. industries, household products, farming and medicinal products) of marine waters (Borja *et al.*, 2013).

The Marine Strategy Framework Directive (MSFD) (2008/56/EC) and its interaction with other policies provide coverage of specific descriptors of the marine environment to achieve Good Environmental Status (GES) of European marine waters by 2020. Among these descriptors, "sea-floor integrity" is an important goal (Rice *et al.*, 2010). The capacity of resilience in canopy-forming macroalgae is a relevant aspect for the adoption of an ecosystem approach and evaluation of good habitat conservation. In addition, the Water Framework Directive (WFD - 2000/60/EC), following an adaptive management approach, establishes actions to reduce marine pollution from land-based sources and the protection of ecosystems as vital spawning grounds for many marine species in coastal and transitional waters, up to one nautical mile.

The rocky French Basque coast is considered as a marine protected area under the EU Birds and Habitat Directive (Natura 2000 zone FR7200813 Rocky Basque coast and offshore extension). The main factor resulting from human pressure is urban wastewater discharges which are increasing in number and flow, especially during touristic periods (Díez *et al.*, 2012; Borja *et al.*, 2016). In addition, the increase of the carbon concentration and the rise of the temperature of coastal waters, could also stimulate photosynthesis and the growth of algae (Celis-Plá *et al.*, 2017)

Concomitant with human pressure and disturbances driven by global warming, long life species of macroalgae (typically kelps and fucoids) are declining worldwide in favor of opportunistic species with short life cycles (Orfanidis *et al.*, 2003; Díez *et al*, 2009; Kregting *et al.*, 2011; Falkenberg *et al.*, 2015; Wernberg *et al.*, 2016). In temperate rocky bottoms, macroalgae, especially canopy-forming species, are the main benthic primary producers in near-shore marine rocky ecosystems (Littler and Littler, 1980; Wernberg *et al.*, 2011). The ecological role of these foundation species is of major importance as they are autogenic engineers (Schiel and Foster, 1986; Thomsen *et al.*, 2010; Wernberg *et al*., 2011) and provide much of the three-dimensional structure habitat and food resources for associated organisms (Mann, 1973; Dayton, 1985; Bedini *et al.,* 2014). The main large brown canopy forming macroalgae belong to the orders Laminariales *s.l.* and Fucales (Steneck *et al*, 2002). Despite kelps (Laminariales *s.l.*) dominating the north-eastern Atlantic Ocean, the large-brown algae dominating the Basque coast is *Cystoseira baccata* and the red one *Gelidium corneum*, both considered as resistant to wave exposure (Taylor et Shiel, 2003). In highly exposed coastal stretches (NW facing) *G. corneum* forms extensive stands from 2 to 9-12 m depth, being replaced deeper by *C. baccata* stands (Gorostiaga *et al.,* 2004). Also *C. baccata* also constitutes patches in the optimal *G. corneum* zone, on flattened rocky bottoms areas or depressions with a higher accumulation of sand (Gorostiaga 1995). Thus, bottom topography and sand deposits determine the distribution of this species. In exposed coastal stretches (N or NE facing) and generally in the shadow of coastal headlands and ledges, *C. baccata* is dominant both on sand-free substrates and sandy rocky substrates (Díez *et al.*, 2003). Although the stretch of coast studied in this research is NW facing, the shadow effect of Cape Higuer and the dominance of rocky bottoms would explain the predominance of extensive *C. baccata* stands.

Macroalgae are considered as good indicators of the quality of the environment since they are sensitive and react quickly to perturbations, whether natural or anthropogenic (Juanes *et al.*, 2008; Díez *et al.*, 2012; Derrien-Courtel et al., 2013). Freshwater inputs such as domestic sewage, industrial effluents and diffuse pollution (e.g. agriculture drainage) are among the main factors affecting the presence and development of marine species. These local inputs promote increased turbidity and siltation rates and changes in nutrient availability and temperature (Díez *et al.*, 2009). Sensitiveness of anthropogenic impact is well known for *Cystoseira* spp. and they are considered as good indicators for good water quality under the European Directive (García-Fernández and Bárbara, 2016; Valdazo *et al*, 2017; Duarte *et al*., 2018).

The ecological traits of the genus *Cystoseira* have been clearly identified since the beginning of the 20th century (Sauvageau, 1912; Gómez Garreta, *et al.,* 2000.). Species diversity within the genus is higher in the Mediterranean Sea than in the Atlantic Ocean so as a consequence the majority of studies concern Mediterranean species (Ballesteros, 1988, 1990 a,b; Cormaci *et al*., 1990, 2012; Ballesteros *et al.,* 1998; Belegratis *et al.*, 1999; Gómez Garreta *et al.*, 2000; Faucci and Boero, 2000; Montesanto and Panayotidis, 2001; Sales and Ballesteros 2007, 2009, 2012; Draisma *et al.*, 2010; Sales *et al.*, 2011; Mačić and Svirčev, 2014; Devescovi, 2015; Lasinio *et al.*, 2017). By contrast, very few specific studies on *Cystoseira* have been conducted on the Atlantic coastline of the Bay of Biscay (van den Hoek and Donze, 1966; Hardy-Halos *et al.*, 1973; Gorostiaga, 1995; Jégou, 2011). In the southeastern corner of the Bay of Biscay (Basque coast), seven species of *Cystoseira* have been recorded from old data from the French side (Dizerbo and Herpe, 2007) and four from the Spanish side. (Gorostiaga *et al*., 2004). Among these species, *C. tamariscifolia* and *C. baccata* are the most abundant along the Basque coast. The first species is present from the infralittoral zone to 3 m depth and rarely deeper (Gorostiaga *et al*., 2004; de Casamajor *et al.*, 2018), whereas *C. baccata* shows a significant wider bathymetric range, from the infralittoral fringe to 25 m depth (Gorostiaga *et al*., 2004; Castro *et al.*, 2006). These large algae harbour a crustose and basal layer mainly composed of red algae (Díez *et al.*, 2003; Pinedo *et al*., 2007).

Since 2008, the methodology implemented for the macroalgal indicator in the subtidal zone in the context of WFD consider large brown algal belt (exept *Solieria chordalis* in turbid areas) as engineer species of coastal areas (Derrien-Courtel, 2008; Derrien-Courtel *et al,* 2013). This network was set up in Brittany and adapted locally with regards to specific environmental conditions and the particularly marked biogeographical factor for this water body. As a second phase of previous work, the present research aims to study the biological characteristics of the population of *C. baccata* on the Basque coast to assess their ecological status and the functional character of the habitat they constitute in the framework of the implementation of the European directives. For that purpose, several data sets not previously collected locally for this species, such as frond length, density and epibionts were considered for the present study. The acquisition of these data gathered from this habitat-forming species can be of great value for the assessment of possible alterations derived from human pressure. Those results should also make it possible to optimize sampling strategies in the coming years to improve the quality of the selected indicators.

2-Materials & Methods

2-1- Study area

The French Basque coast (Fig. 1) extends from the downstream zone between the mouths of the Adour and Bidassoa rivers (Borja and Collins, 2004). The rocky bottoms, composed of gravel and rocks, cover a 49% of the seabed. These areas are discontinuous and interspersed with sandy bays (Augris *et al.*, 2009). The mean tidal amplitude is between 1 and 5 m at mesotidal conditions (de Casamajor *et al.*, 2017) therefore the intertidal zone is of little relevance by in extension. Subtidal zones suitable for macroalgae colonisation are more extensive, with rocky bottoms formed by small boulders and large flat benches. The hydrographic network is dense and distributed along the coastline. Significant rainfall also contributes to regular freshwater supplies. The prevailing swell in this stretch of shoreline is from N and NW with an annual average height of 2m and 10s period (maximum height of 11 m for 20 s period) (Abadie *et al.*, 2005).

The study was carried out in a Marine Protected area (between Guéthary and Hendaye, Fig. 1) considered as representative of the FRFC11 "Basque coast" water body (according to the WFD classification). The three sites selected were surveyed in June 2014 and 2017, as part of the WFD monitoring using the subtidal macroalgae as biological quality element on the "Basque coast" water body. From south to north, the sampling sites were: 1) "Abbadia", just north of Hendaye Bay under the influence of Bidassoa river flows; 2) "Socoa", in the south of Saint-Jean-de-Luz Bay where the Nivelle is the main river flow; and 3) "Alcyons", located just north of Guéthary. The three sampled locations were considered as not being influenced by direct urban discharges and far from any direct pressure (Derrien-Courtel, S., Le Gal, A., 2014b). Nevertheless, they are influenced by mixed water from the coastal zone induced by river flow.

Figure 1. Sampling sites location of *Cystoseira baccata* along the French Basque coast.

2-2-Response variables

Two sampling campaigns were carried out in 2014 and 2017. Three main parameters were considered to characterize *C. baccata* stands; belt depth limits, density and total length and epibionts. All data were recorded by scuba diving in a depth range from -3 to -23 m. Depths were measured with a digital depth gauge, and corrected according to the lowest astronomical tide considering the height of water at the time of the surveys (source: www.shom.fr). The reference port defined for the correction of water levels is Saint-Jean-de-Luz, the closest to the sampling sites.

2-2-1- Belt depth limits

For the determination of this parameter the density of *C. baccata* was estimated on randomly positioned quadrats (0.25 m^2) at different so-called "reference" depths (-3, -5.5, -8, -10.5, -13 -15.5 and -18 m). For Abbadia only, two depth were added (-20 et -23 m) due to large extension of C. baccata in this area. *Cystoseira baccata* is characteristic of the “slightly turbid” water body of the Basque coast (de Casamajor *et al.*, 2018). Establishing macrophyte belt depth limits is defined on the basis of the presence and density of the canopy-structuring species (Bertocci *et al.*, 2010). In the case of *C. baccata* two belts were distinguished: upper and lower infralittoral (N2 and N3, respectively (Table 1)).

Table.1 Algal belt definition for *Cystoseira baccata* along the French Basque coast.

Algal belts	Code	Algal belts definition
Upper infralittoral	**N2**	*Cystoseira* spp. $\geqslant$ 3 ind/m^2
Lower infralittoral	**N3**	*Cystoseira* spp. < 3 ind/m^2

The bathymetry of upper and lower infralittoral algal belt fluctuated from one site to another due to the different densities of *C. baccata*. The reproduction stage was not considered in this work.

2-2-2- Density and total length of *C. baccata*

For this parameter, five quadrats were randomly disposed at regular depth intervals (Table 2). The bathymetric interval selection criterion was a function of the maximum depth reached by the N2 level considering three sampling strategies (Derrien-Courtel and Le Gal, 2014a, b). Thus, three scenarios could be contemplated:

- 1. If the lower depth limit of N2 was less than -5.5 m, the five quadrats would be disposed at 1 m depth intervals (25 quadrats maximum);
- 2. If the lower depth limit of N2 was between -5.5 m to -18 m, the five quadrats would be disposed at 2.5 m depth intervals starting from -3 m (30 quadrats maximum);
- 3. If the lower depth limit of N2 extended beyond -18 m, the five quadrats were disposed at 5 m depth intervals starting from -3 m (30 quadrats maximum).

For the study area, the last two sampling approaches were applied (Table 2). For each quadrat density and frond length were obtained for *C. baccata*.

Table 2. Sampling and studied parameters of the structure of perennial algae *Cystoseira baccata* along the French Basque coast.

Algal belt	Code	Parameters	Sampling
Upper infralittoral	**N2**	density, frond length and length supporting epibionts	5 quadrats (0.25m²) every 1m, 2.5 or 5m (depending on lower limit of N2)

2-2-3- Epibionts on *C. baccata*

For this purpose, ten individuals of *C. baccata* individuals were selected randomly in each belt N2 and N3 for each site (Table 3). In Alcyons, N2 was the only sampled level (N3 being absent). Sampling was conservative with *in situ* measurements.

According to Derrien-Courtel and Le Gal (2014a) for each of the ten individuals, the following information was obtained by divers: (1) frond length of *C. baccata* thallus measured from holdfast to apex, (2) frond length of thallus supporting epibionts, (3) list of the five main species of epibionts (epiflora and epifauna) on frond surface, (4) epiphytic surface area (in cm^2) estimated visually (Table 3). The ratio (2)/(1) enables an estimate of the size effect of epibiosis colonization (RA_Length).

The five main species of epibionts, were collected *in situ* and transferred to the laboratory for further identification. They were classified according to http://www.marinespecies.org/ and http://www.algaebase.org/.

Table 3. Studied parameters of the epibioses of *C. baccata* along the French Basque coast.

Algal belt	Code	Sampling	Parameters
Upper infralittoral	**N2**	*C. baccata* Individuals (n = 10)	For each individual (1) Frond length of *C. baccata* thallus (2) Frond length of thallus supporting epibionts (3) Identification **of the 5 main** epibionts **species** (4) epiphytic surface area
Lower infralittoral	**N3**	*C. baccata* Individuals (n = 10)	

2-3- Data treatment

All data used in this study are available in the Quadrige[2] database (http://envlit.ifremer.fr). All uni- and multivariate statistical analyses of the data were achieved using R ® software.

Univariate one-way perm-ANOVA (permutational analysis of variance) analyses were conducted to study the variation of the density, frond length and epibiosis (epibiotic load; time for epibiosis) on *C. baccata* for each of the three factors considered: 1) site (fixed with 3 levels); 2) sampling time (fixed with 2 levels); and 3) bathymetry (fixed with 9 levels). Tests on interactions are presented in supplementary materials. These analyses were adapted to data coming from an unbalanced sampling effort between both years. One-way PERMANOVA (permutational multivariate analysis of variance) analyses were performed (Anderson, 2001) to assess the variation among epibiotic assemblages for the three factors mentioned above. For both univariate and multivariate analyses, the significance level selected was $\alpha < 0.05$. After a significant term in univariate and multivariate analyses, post-hoc analyses were conducted using pairwise permutation tests.

For epibiosis data (presence/absence), non-metric multidimensional scaling (nMDS) was used to produce two-dimensional ordinations to compare assemblages among sites, sampling time and bathymetry. Ordination analysis (based on Jaccard similarity) enables a visualization in multi-dimensional space of all replicated assemblages in terms of their relative similarity, based on taxon composition and assemblage structure estimated.

3-Results

3-1-Depth extension of *Cystoseira baccata* belt

Although some variations were observed, the depth extension limits of *C. baccata* were broadly similar between the two years irrespective of the site and the bathymetric level. Moreover, there was a depth gradient from the northern site (Alcyons) to the southern one (Abbadia) for both years, with an increase in the depth extension of *C. baccata* regardless of the bathymetric level (Table 4).

Table 4. Depth limits of *Cystoseira baccata* along the French Basque coast (N2 upper; N3 lower infralittoral) for each site and year.

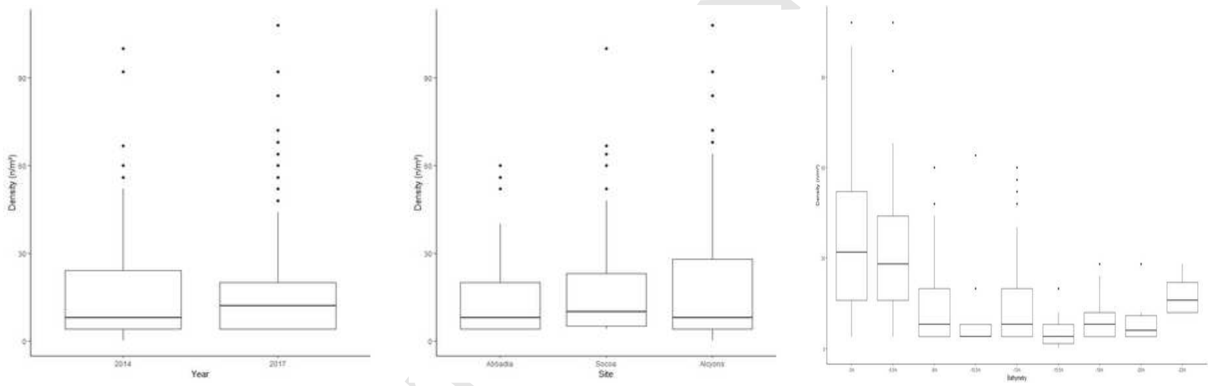
Sites	Limit < N2 (m)		Limit <N3 (m)	
	2014	2017	2014	2017
Alcyons	17.9	15.5	sand	sand
Socoa	19.6	19	21	20.5
Abbadia	21.5	22.2	24	26.5

For "Socoa" and "Abbadia" sites, the depth limits defined for N2 were respectively 19.6 and 21.5 m (Table 4). Therefore, the sampling effort for *C. baccata* parameters (frond density and frond length) was carried out at 5 m depth intervals (-3, -8, -13 and -18 m) as previously described (sampling strategy 3).

For the "Alcyons" site, the depth limit defined for N2 was less than -18 m in 2014 and 2017 (Table 4). Then, following to sampling strategy 2, quadrats were located at every 2.5 m depth intervals (-3, -5.5, -8, -10.5, -13 and -15.5 m) instead of every 5 m for the other sites. A total of 672 individuals of *C. baccata* individuals were measured in 2014 and 704 in 2017 (Table 5 in supplementary materials).

3-2- Density and frond length of *C. baccata*

Density of *C. baccata* was around 11.5 (+/- 10) ind.m^{-2} and it was 17.6 (+/- 12) ind.m^{-2}, in 2014 and 2017 respectively, (mean +/- SE). Differences in density between the two years and three sites were not significant ($\alpha > 0.05$) (Fig.2).



(a) *F value*=0.0042 Pr(>F)=0.955

(b) *F value*=2.4052 Pr(>F)=0.084

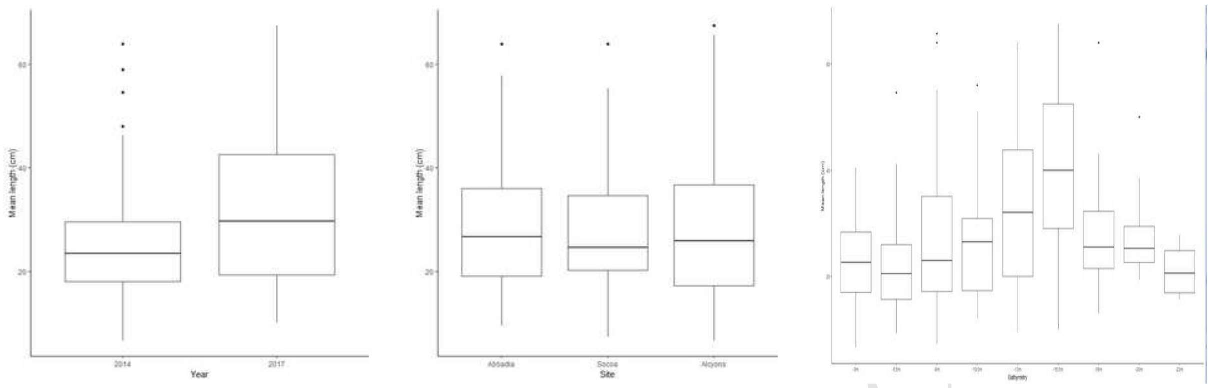
(c) *F value*=18.459 Pr(>F)=0.001 ***

Figure 2. *Cystoseira baccata* along the French Basque coast: box-plot diagrams showing the density (number of ind/m^2) of *C. baccata* individuals regarding time (a), site (b) and bathymetric level (c).

"Bathymetry level" (including wave exposure, light penetration etc.) was the only significant factor to explain fluctuations in terms of densities. At shallow waters (-3 to -5.5 m), the algal belt was denser (Fig. 2). Between -8 to -20 m depth, the density was quite similar, increasing slightly at -23 m; -8 m depth appeared as a transition zone (Fig. 2).

Frond length of *C. baccata* was around 23.8 cm (+/- 12.17) and 27.8 cm (+/- 17.2) in 2014 and 2017 respectively, (mean +/- SE). This parameter showing a higher variability in 2014. Time and bathymetry highly influenced significantly on frond length (Fig. 3).

The average size of *C. baccata* was between 17-25 cm, with larger individuals at -13 and -15 m depths (Fig. 3). Lower frond lengths were found at shallow (-3 and -8 m depth) and deepest water (-23 m).



(a) *F value*= 23.529 Pr(>F)= 0.001 ***

(b) *F value*= 0.2041 Pr(>F)=0.819

(c) *F value*= 6.5769 Pr(>F)= 0.001 ***

Figure 3. Frond length of *C. baccata* along the French Basque coast: box-plot diagrams showing the mean length (cm) regarding time (a), site (b), and bathymetric level (c).

3-3- Epibiosis

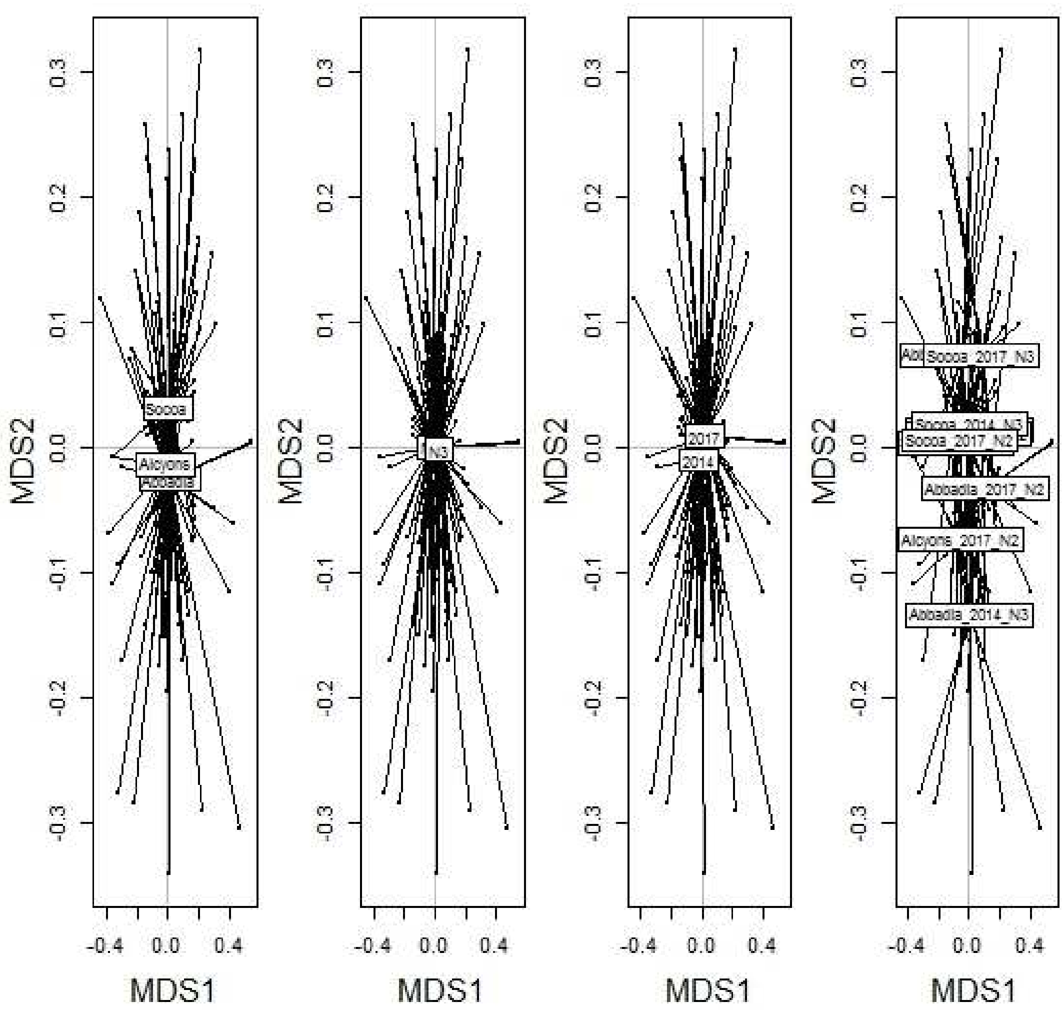
Flora and fauna associated to *C. baccata* fronds was diverse, housing 23 red algae, 4 brown algae and 13 invertebrates (Table 6).

Table 6. Epiphytic taxonomic diversity on *C. baccata* along the French Basque coast covering two campaigns (2014 and 2017), three sites and depth range of -3 to -23 m (*crustose algae not identified).

Epiphytic taxonomic diversity on *Cystoseira baccata* (five dominant species)	
FLORA	**Ochrophyta**
RHODOPHYTA	Crustose brown*
Acrosorium sp. Zanardini ex Kützing	*Dictyota dichotoma (Hudson) J.V.Lamouroux*
Asparagopsis armata Harvey stadium Falkenbergia rufolanosa (Harvey) F.Schmitz	*Halopteris filicina (Grateloup) Kützing*
Bonnemaisonia asparagoides (Woodward) C.Agardh	*Halopteris scoparia (Linnaeus) Sauvageau*
Calliblepharis ciliata (Hudson) Kützing	
Callophyllis laciniata (Hudson) Kützing	
Ceramium sp. Roth, 1797	**FAUNA**
Drachiella spectabilis J.Ernst & Feldmann	*Actinothoe sphyrodeta* (Gosse, 1858)
Gracilaria multipartita (Clemente) Harvey	*Aglaophenia pluma* (Linnaeus, 1758)
Halymenia latifolia P.Crouan & H.Crouan ex Kützing	Annelida (tube worms)
Heterosiphonia plumosa (J.Ellis) Batters	Bryozoa
Jania rubens (Linnaeus) J.V.Lamouroux	*Campanularia* Lamarck, 1816
Lithophyllum incrustans Philippi	*Didemnum* sp. Savigny, 1816

Lomentaria articulata (Hudson) Lyngbye	*Electra pilosa* (Linnaeus, 1767)
Peyssonnelia sp. Decaisne, 1841	Hydrozoa
Phyllophora crispa (Hudson) P.S.Dixon	*Sabella discifera* Grube, 1874
Plocamium cartilagineum (Linnaeus) P.S.Dixon	*Sepia officinalis* (eggs) Linnaeus, 1758
Polysiphonia sp. Greville, 1823	*Serpula* sp. Linnaeus, 1758
Pterothamnion plumula (J.Ellis) Nägeli	*Sertularia distans* (Lamouroux, 1816)
Rhodymenia pseudopalmata (J.V.Lamouroux) P.C.Silva	*Sycon* sp. Risso, 1827
Crustose red*	
Sphaerococcus coronopifolius Stackhouse	
Sphondylothamnion multifidum (Hudson) Nägeli	

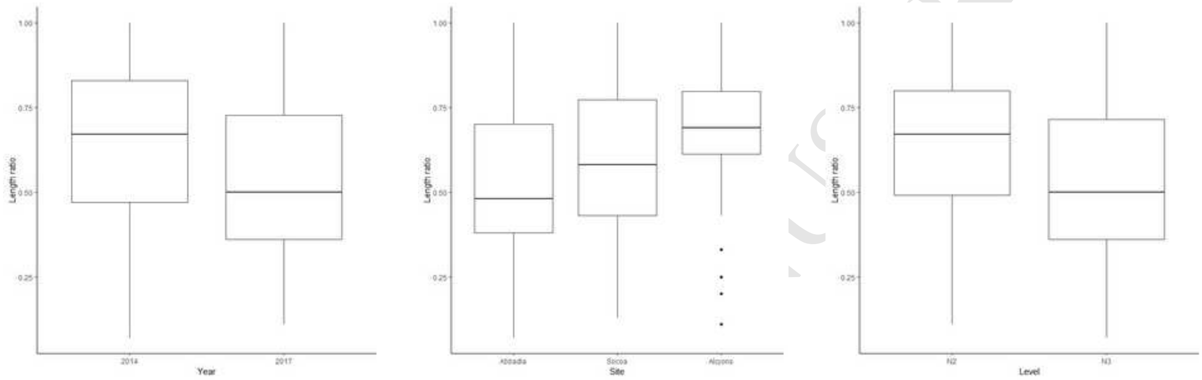
Results of non-metric multidimensional scaling (nMDS) did not allow for discrimination of taxa assemblages regarding sampling time, sites and bathymetric levels. There was an overlap of associations for each of the factors and their combination (Fig. 4).



(a) (b) (c) (d)

Figure 4. nMDS on epibiontic assemblages for a) sites, b) bathymetric levels, c) sampling time, and d) interactions (sites x bathymetric levels x sampling time).

Epibiotic frond-length/total frond-length ratio (RA_length, Fig.5) showed significant differences (one way perm-ANOVA, (p-value < 0.05)) for the three studied factors. According to pairwise tests, the ratio was higher in the northern sampling site (Alcyons), in 2014 and in shallow waters (N2). Nevertheless, the variability of this ratio was high for the three studied factors (Fig. 5).



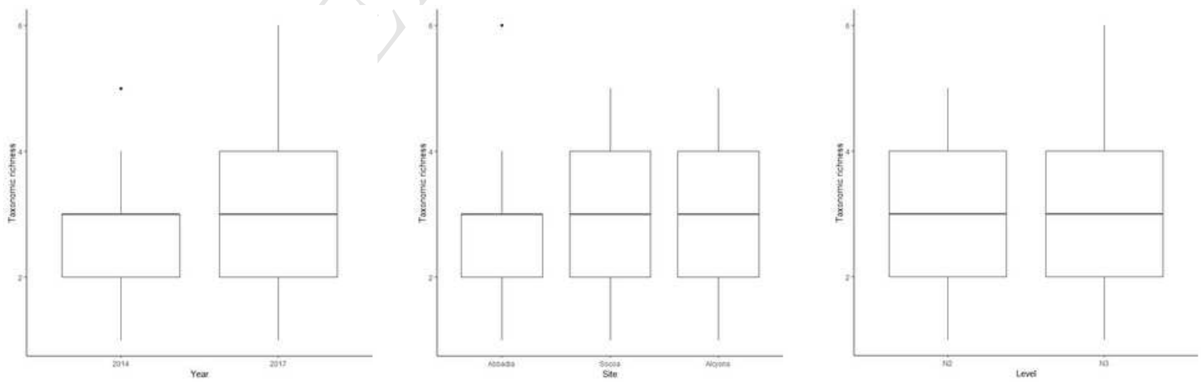
(a) *F value*=9.3647 Pr(>F)=0.001 ***

(b) *F value*=3.6031 Pr(>F)=*0.032**

(c) *F value*=10.359 Pr(>F)=0.004 **

Figure 5. Box-plot diagrams showing the epibiotic frond-length/total frond-length ratio (RA_LENGTH) according to sampling time (a), site (b), and bathymetric level (c).

Taxonomic richness showed significant differences (perm-ANOVA analyses (p-value < 0.05)) for years (2017 > 2014) and sites (Abbadia < Socoa = Alcyons according pairwise tests) (Fig.6).



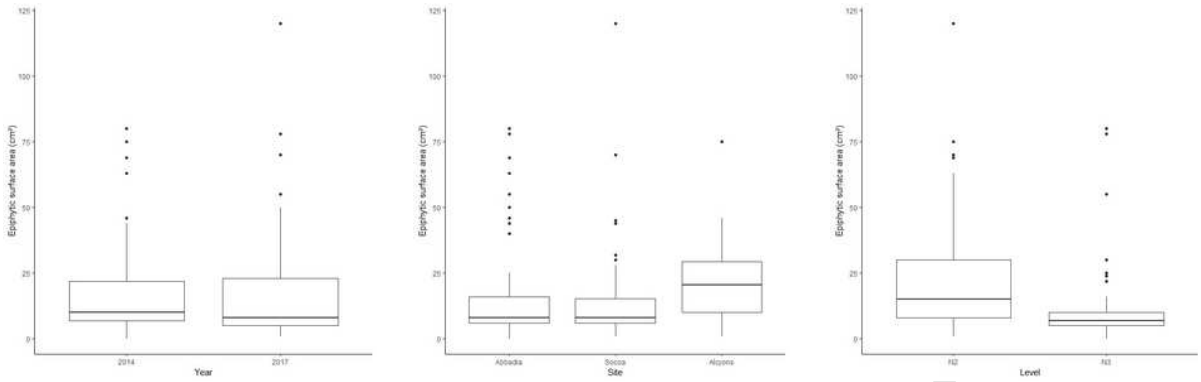
(a) *F value*=8.102 Pr(>F)=0.004**

(b) *F value*=4.3705 Pr(>F)=0.017 *

(c) *F value*=0.4831 Pr(>F)= 0.502

Figure 6. Box-plot diagrams showing the taxonomic richness of epibionts on *C. baccata* individuals regarding time (a), site (b), and bathymetric level (c).

Epiphytic surface area was only significantly [one way perm-ANOVA analyses (p-value < 0.05) affected by depth (N2-shallow > N3-deeper waters according pairwise tests)] (Fig. 7).



(a) `F value=0.155 Pr(>F)=0.701`

(b) `F value=1.2969 Pr(>F)=0.283`

(c) `F value=11.29 Pr(>F)=0.001 ***`

Figure 7. Box-plot diagrams showing the epiphytic surface area (AREA_EPI) on *C. baccata* individuals regarding time (a), site (b), and bathymetric level (c).

4-Discussion

These results provide the first biological information available for this engineering species in the southeastern corner of the Bay of Biscay. They contribute to including valuable metrics using macroalgae as a biological quality element (WFD) as it is already done in Brittany (Derrien-Courtel and Le Gal, 2014a; Le Gal and Derrien-Courtel, 2015). In addition, collection of data series is of great interest for the conservation and assessment of a coastal area in the MSFD context. *Cystoseira* spp - as slow growing perennial algae - are considered to be declining and pollution has been incriminated as a major contributing factor (Sales *et al.* 2011). This is why metrics selected in this study can provide valuable information on the characteristics of the current populations and establishing the basis for its long-term evolution. Moreover, it is important to consider this information to evaluate the functionality of these macroalgal population as an important structuring component of the benthic ecosystem (Rodriguez-Prieto and Polo, 1996).

Response variables

Among the analysed factors (time, site and bathymetric level) the bathymetry is the most significant. Results show that the height of individuals is inversely proportional to its density. Thus, shallow zones are characterized by lower individual heights and higher densities while the opposite trend occurred at deeper zones. The genus *Cystoseira* has a high sensitivity to anthropic environmental disturbances (Sales *et al.*, 2011) and a high plasticity to cope with changing environmental parameters such as depth, topography, swell and light (Devescovi, 2015). Bathymetry includes steep gradient of several environmental parameters; such as light penetration, hydrodynamics and temperature at the main ones. Swell (height and period) is an important factor for its colonization, particularly for zygote fixation during reproduction (Vadas *et al.*, 1990). On the Basque coast, swells are very frequent throughout the year. Swell conditions can show interannual fluctuations, but the occurrence of favorable periods of low swell usually occur in spring and summer (Abadie *et al.*, 2005) corresponding to the fertility period of *Cystoseira* and thus achieving a good recruitment. Success depends therefore on quiet episodes at the time of fertilization (Taylor and Schiel, 2003). The higher frond density of *C. baccata* found in this study at shallow waters is indicative that this macrophyte may adapt to strong surf environment reducing its size. The strong mechanical action generated by swells influences distribution and composition of algal communities (Kingsbury, 1962). *Gelidium corneum* and *Cystoseira baccata* are the dominant subtidal macrophytes on the Basque coast, an area subject to strong swell (Gorostiaga *et al.*, 1998). The lack of significant difference in the study area between sites might result from their geomorphologically similarity. Devescovi (2015) found that in the Adriatic Sea that growth and level of recruitment of *Cystoseira* spp. may be dependent on sampled site topography.

In our study, a significant decrease in the frond length of *C. baccata* was found between both years. This is probably an example of interannual variation of natural environmental conditions. However, under the influence of a permanent stress (i.e. pollution, climate change), populations may experience a regression reflected by a decrease in density or frond length. A reduction of the frond size in *Cystoseira abies-marina* from Canary Islands has been recorded in the past decades analysing herbarium specimens from a long temporal series (1960-2010) (Sansón *et al.*, 2013). A decrease of *C. baccata* forest could impact the nursery value for littoral fishes, particularly during the recruitment period (Cheminée *et al*., 2013) and mobile fauna (Bedini *et al.*, 2014).

Epibiosis

Epiphytism is a strategy for benthic species in marine environments to cope with competition for colonization of hard substrata. In this context, *C. baccata* constitute a suitable habitat considering the seasonal turnover of the frond.

In the study area, the epibiosis taxonomic composition was mainly composed of algae species, nevertheless no particular assemblages could be highlighted. The same results have been found in the Mediterranean Sea on other *Cystoseira* species (Belegratis *et al.*, 1999). The scale currently used for *Laminaria hyperborea* (Derrien-Courtel and Le Gal, 2010) cannot be applied to *C. baccata* because the biological characteristics of both species are not comparable: blades are located on the top of the stipe for *L. hyperborea* and all along the main axis for *C. baccata*. No reference works exist on the *C. baccata* epibiosis for the Basque coast and therefore data from this study constitute new knowledge. Some studies exist on the epiflora of *Gelidium corneum* (Quintano *et al.*, 2015) and show similarities with the taxonomic composition observed in *C. baccata*. Common and abundant species were *Plocamium cartilagineum*, *Dictyota dichotoma* and *Acrosorium ciliolatum*. Furthermore, studies have been carried out on epibiosis of the genus *Cystoseira* in Galicia, and *C. baccata* is one of the studied species (Arrontes, 1990; Otero-Schmitt, 1993; Bárbara, 1994; Bárbara *et al.* 1995; Otero-Schmitt and Pérez-Cirera., 1996; García-Fernández and Bárbara, 2016; García-Fernández and Bárbara, 2017). However, it is difficult to compare taxonomic composition from colder biogeographic areas with different hydrographic conditions. The swell impact is highly relevant with regards to the colonization rate; it has been particularly well-studied for hydrozoa communities (Faucci and Boero, 2000; Fraschetti *et al*., 2002). Regarding the lack of taxonomic assemblages in our results and the variability observed in this study, new and more data (more C. *baccata* individuals over a longer period for example) should be collected to understand the colonization mechanism for epibiosis and their integration in monitoring programs and evaluation of environmental changes. In this study, interannual significant differences were found in the epiphytic load of the fronds (measured as epibiotic frond-length ratio) and taxonomic richness. In addition, it was remarkable the differences found for epiphytic load in relation to the bathymetry (higher in shallow waters). Interannual epibiosis differences found in this study could be linked to the spatio-temporal variability of environmental conditions. In this regard, physical parameters (wave exposure, turbidity and nutrient availability) are considered in the Aegean Sea and Montenegro to be important in modeling the composition and abundance of epibiosis among others (Belegratis *et al.*, 1999; Mačić and Svirčev, 2014). The epiphytic load decrease from shallow to deeper waters observed in *C. baccata* could be related to the interaction between the effect of the swell and light attenuation such as has been reported for *G. corneum* (Quintano *et al.*, 2015). However, the results of the present study have not found significant variations of epiphytic assemblages composition and taxonomic richness with depth. This could perhaps be related to the fact that epibiosis sampling was focused on the five most abundant species. If the overall species had been recorded extending the sampling time, the results might have been different.

The epibiosis effects on their hosts are poorly known and could be the subject of further research to determine their impact on populations and growth. The study of the *C. baccata* epibiota could be of major relevance in environmental monitoring programs since fast changes in its composition could be correlated with a worsening of the physiological state of the fronds in response to some perturbation (Karez *et al.*, 2004). Slower changes in the epibiota, recorded in long time series, could be a consequence of the alteration of climatic variables.

5- Conclusion

Data collected and results obtained during the two sampling campaigns on *Cystoseira baccata* stands constitute a contribution of new knowledge about this high ecological value habitat. These results will help to develop improved protocols that will be more relevant for the evaluation of the conservation status of subtidal habitat as for the purposes of the MSFD. Data on density and individual height show a bathymetric structure of the populations.

In the near future, the metrics achieved in this study could be compared with those from other areas, exposed to disturbances (for example discharges from wastewater treatment plants) in order to evaluate the effectiveness of corrective measures. The sampling effort should be improved through a higher number of sites and by considering the overall epiphytic list. For complementary studies, a better understanding of the influence of swell exposure levels is important. The present study aimed to obtain diverse biological metrics on *C. baccata* stands from the "Basque water body" in the WFD and MSFD context; it would seem suitable to apply it to other areas with analogous "engineer" species in European Atlantic waters.

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Highlights

- Contribution of new knowledge on *C. baccata* habitat and population
- Confirmation of interest of this engineering species in implementation MSFD
- Swell effect influencing density and individual height
- Ratio of length is pertinent metric to discriminate epibiosis considering levels