

# Mapping Invasive Macroalgae in the Western Iberian Peninsula: A Methodological Guide

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and Celia Olabarria

  
Amalia

Algae-to-Market Lab IdeAs

Adding value to  
invasive seaweeds of the  
Iberian northwest

Co-funded by  
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(EASME BLUE LABS PROJECT)

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Disclaimer: The authors are responsible for the  
contents of this guide

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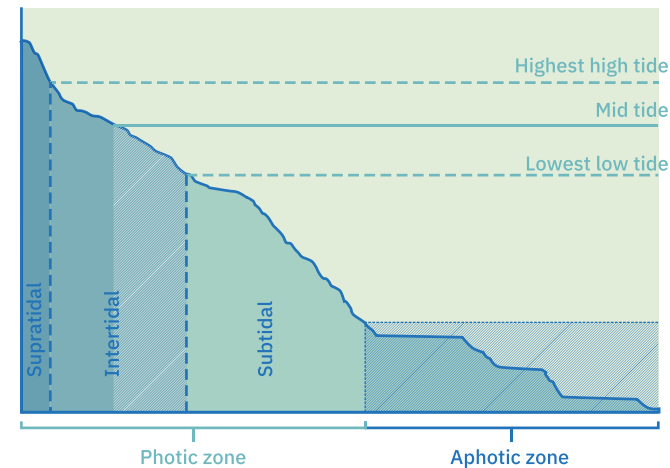


# 01 Preface

In the European Union member countries, more than 41% of marine invasive species of special concern are macroalgae. Invasive macroalgae, and invasive species in general, change the structure and functions of the invaded communities and ecosystems and thus have strong impacts on ecological processes and ecosystem services. Specifically, on the west coast of the Iberian Peninsula, the composition and structure of seaweed communities has changed dramatically in the last two decades, mainly due to climate change and biological invasions. In this context, the spread and establishment of invasive species is of growing concern to environmental managers as eradication of established invaders can be extremely difficult (if not impossible) and costly. Contingency plans should therefore focus on prevention rather than eradication. However, the implementation of effective management policies requires prior understanding of the factors involved in the distribution of non-indigenous marine species (NIMS). These factors include biotic, abiotic and anthropogenic variables. The aim of the present methodological guide is therefore to describe the steps involved in mapping the distributions of six well-established invasive marine macroalgae (*Grateloupia turuturu*, *Asparagopsis armata*, *Colpomenia peregrina*, *Sargassum muticum*, *Undaria pinnatifida* and *Codium fragile* subsp. *fragile*) as well as to gather information about the factors influencing the distributions. In addition, we briefly summarize the main characteristics and impacts of these invader species, including their potential applications.

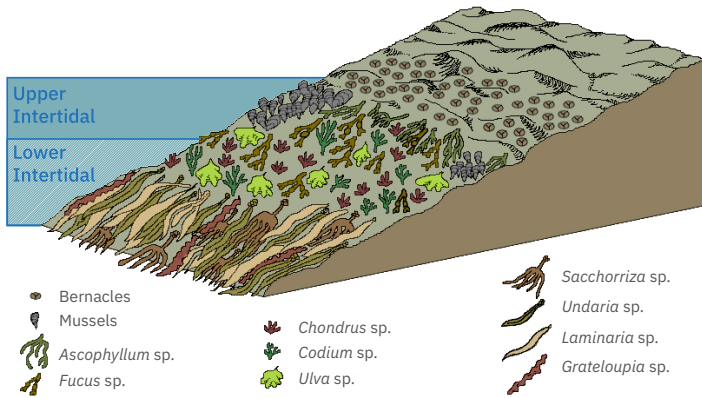
# 02 Macroalgae

Macroalgae or seaweeds are benthic marine algae that generally live attached to rocks or other hard substrata in marine or brackish water environments. Seaweeds are important primary producers of oxygen and organic matter in coastal environments as a result of their photosynthetic activity, and together with phytoplankton they form the bases of aquatic food webs. As photosynthetic organisms their survival depends on light availability. Thus, seaweeds are found in coastal regions between high tide and a depth where 0.01% of the photosynthetic light is available (Figure 1).



**Figure 1.** Coastal zonation by photosynthetic activity and tidal effects.

Both abiotic and biotic environmental factors play important roles in algal zonation, in terms of structure and function (Gómez and Huovinen 2011) pigmentation, photosynthetic parameters, photoinhibition, and thallus structure. Based on 32 seaweed species from the Pacific coast of southern Chile, intertidal assemblages exhibited higher light requirements for photosynthesis (Ek. Seaweeds have evolved according to these factors and have developed physiological mechanisms that enable them to survive and spread under particular conditions (e.g. high/low photosynthetic activity during immersion/desiccation events in algae from the upper littoral zones). This has led to the characteristic distribution of the macroalgal community, especially in the intertidal zone (Figure 2).

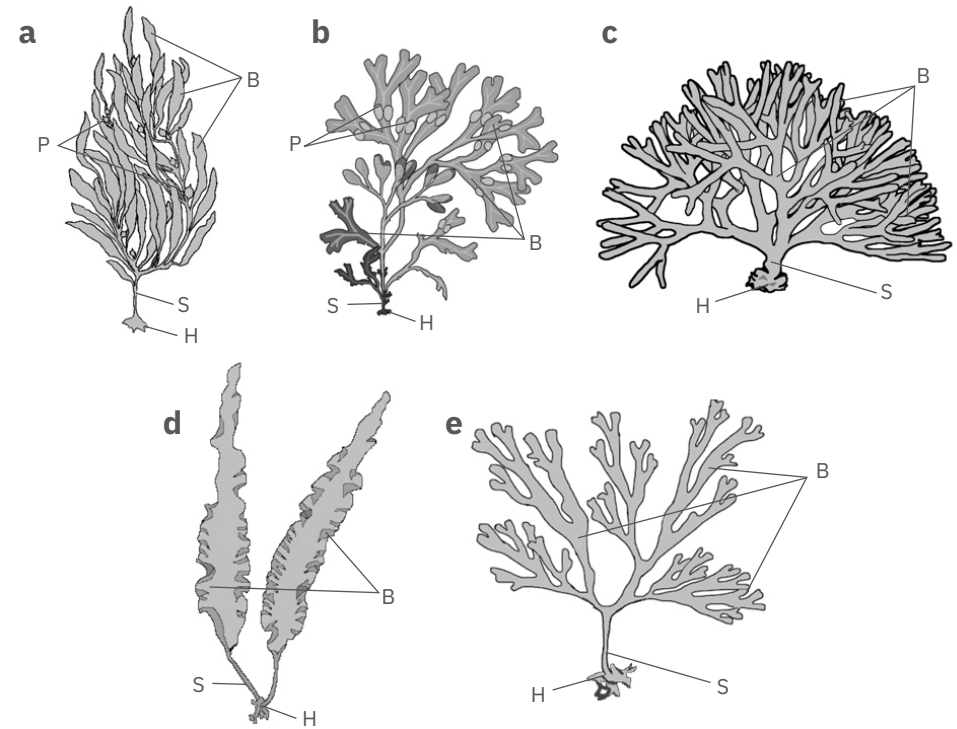


**Figure 2.** Example of cool-temperate type algae zonation in a slightly sloping coastline in Western Europe (based on Southward 1958).

The most important criteria used to distinguish the different groups of algae are photosynthetic pigments; thus, seaweeds are classified as Rhodophyta (red pigments), Ochrophyta (brown pigments) and Chlorophyta (green pigments). Morphologically, the whole body of a seaweed is called the thallus, which consists of the holdfast, stipe and blade (Figure 3).

The main structures of seaweed are as follows:

- Holdfast, the function of which is to attach macroalgae to the substratum; it can be discoidal, rhizoidal, bulbous or branched.
- Stipe, the main function of which is to support the blade, although it also has some photosynthetic and nutrient absorption capacity.
- Blade, the main functions of which involve photosynthesis and nutrient absorption. The blade is variable in shape (smooth, perforated, segmented, dented, etc.).
- Pneumatocysts, vesicles or air bladder, the main function of these gas-filled structures is to provide floatation or buoyancy for maximum display of photosynthetic tissue while avoiding abrasion by rock surfaces.
- Sporangia and gametangia, these reproductive structures are usually unicellular, but when they are multicellular, the spores and gametes are not enclosed within a wall formed by a layer of sterile or non-reproductive cells.



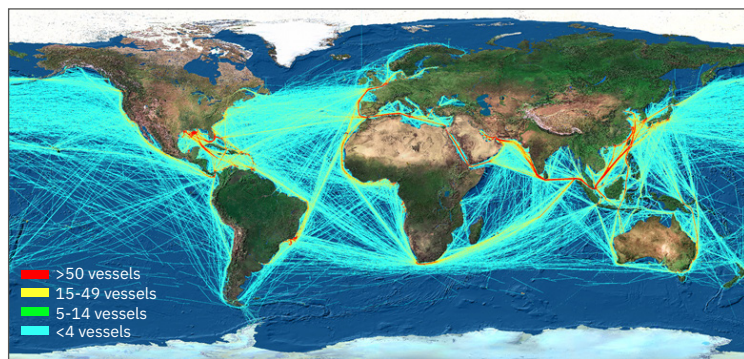
**Figure 3.** Morphological structure of representative macroalgae  
a) *Sargassum* sp.; b) *Fucus* sp.; c) *Codium* sp.; d) *Laminaria* sp.; and e) *Dictyota* sp..  
B= Blades; P= Pneumatocyst, air bladder or vesicle; S= Stipe; H= Holdfast.



## 02.01. Native, non-indigenous and invasive seaweeds

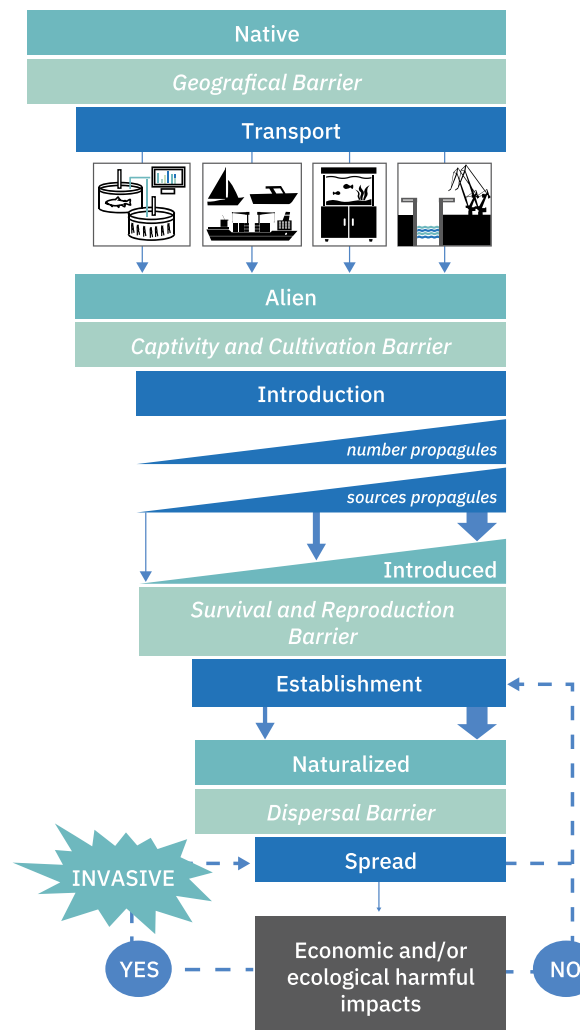
Seaweeds are often exposed to a wide variety of environmental stressors. Abiotic factors (e.g. temperature, desiccation, over-exposure to light and nutrient deprivation) and biotic factors (e.g. grazing, competition and disease) affect the production, release, dispersal, settlement, recruitment and growth of different taxa in different ways (Santelices 1990). These factors influence the capacity of a given macroalga to inhabit, colonize or invade a specific habitat. Despite most predictions, introductions of cosmopolitan species are less common than those of species with narrower distributional ranges (Williams and Smith 2007). However, it is difficult to establish whether a given species is native or has been introduced and became naturalized over time, because translocation of species through human actions has been occurring since boats have been used to cross seas and oceans. Furthermore, the increasing number of vessels in transit on an interoceanic scale in the last few decades has increased the uncertainty associated with natural distribution ranges (Carlton 1999).

Although the appearance of new species in new habitats has occurred repeatedly throughout history, the trend and speed of introductions have increased considerably during the last half of the 20<sup>th</sup> century as a result of the globalization of transportation (Figure 4) and the shift in environmental conditions caused by climate change (Occhipinti-Ambrogi 2007, Katsanevakis *et al.* 2014, Seebens *et al.* 2016). Fortunately, a downtrend in this process was observed in the 2000s, probably due to the implementation of EU regulations (Streftaris *et al.* 2005, Katsanevakis *et al.* 2013).



**Figure 4.** Worldwide marine traffic density map for June 2013 (data from the US Coastguard Automated Mutual-Assistance Vessel Rescue System website, [www.amver.com](http://www.amver.com)).

The IUCN defines a native species as one that “currently occurs within its natural range (past or present) and dispersal potential; i.e. within the range it occupies naturally, or could occupy without direct or indirect introduction or care by humans” (IUCN 2017). As such, the terminology regarding the presence of a new species in a new habitat is highly controversial (Blackburn *et al.* 2011, Pereyra 2016), being defined variously as introduced, non-indigenous, non-native, invasive, alien and exotic. In this respect Blackburn *et al.* (2011) aimed to establish a unified framework for discussing biological invasions (Figure 5).



**Figure 5.** The process of colonization of non-indigenous species in new habitats according to propagule pressure integrating the invasion stages (blue), barriers (light green) and terminology (turquoise). Adapted from Allendorf and Lundquist (2003) and Blackburn *et al.* (2011).

Thus, an introduced species is a species that 1) due to human activity colonizes a new area where it was not previously present, 2) is remotely dispersed with a wide geographic discontinuity, and 3) becomes naturalized by perpetuation of new generations without human intervention (Boudouresque and Verlaque 2002). Fortunately, most introduced species fail to become established, as result of mortality during translocation, suboptimal environmental conditions for the invader's physiological characteristics (tolerance and resistance, reproductive mode, etc.) and biotic resistance exerted by the host community in the new habitat (Lonsdale 1999). However, once established, a new species can become invasive when it has an adverse effect on biological diversity, ecosystem functioning, socio-economic values and/or human health in invaded regions at individual, population or community levels (Williamson and Fitter 1996, Boudouresque and Verlaque 2002, Bax *et al.* 2003, Lages *et al.* 2015).

The following different stages of the process of invasion by non-native species have been suggested (Allendorf and Lundquist 2003, Blackburn *et al.* 2011):

- 1) Introduction (arrival of the non-native species)
- 2) Colonization (survival of the new species in the new habitat)
- 3) Establishment (introduced populations that become self-sustaining)
- 4) Spread and replacement of native species by the introduced species.

Dispersion and establishment have been linked to propagule pressure, which includes both the number of individuals introduced and the number of release events (Allendorf and Lundquist 2003). Thus, a greater number of founding individuals and a larger number of release events (probably from different sources of populations) would generate greater genetic variation and thus facilitate the invasion process. Invasion success also depends on the ability of native communities to limit the success of established invaders (i.e. 'Biotic resistance', Elton 1958). The 'Biotic resistance' hypothesis suggests that highly diverse communities will be more resistant to invasion. As a mechanism underlying this relationship, it has been proposed that communities characterised by high levels of diversity would use almost all available resources and hence no ecological niche would be available for the invader to become established in the host community (Levine and D'Antonio 1999). However, an increase in diversity would also increase the occurrence of both suppressive and facilitative species of the invader (Wardle 2001) but some recent literature has claimed that these observational studies are confounded by extrinsic covarying factors while experimental studies are not. In this article I evaluate each of eight experiments from six recent publications in which the effect of varying plant diversity on the success of invasive species was investigated. In each case that invasibility was identified by the authors as being adversely affected by plant species richness, the result can be explained by factors that covaried with diversity in the experiment, most notably as a consequence of "sampling effect" (in which the most competitive species or species combination in the total species pool has a greater probability of occurring as species richness is increased. It has been suggested that the invasiveness of macroalgae is linked to

inherent characteristics of the family, as some of them include significantly more introduced species than would be expected by chance (Williams and Smith 2007).

Introductions of alien seaweeds have been associated with various pathways, such as shipping, navigational canals, aquaculture and the aquarium trade (Katsanevakis *et al.* 2014, de Castro *et al.* 2017). As a vector for the introduction of alien species, especially macroalgae, shipping includes both ballast water and biofouling on ships and has been cited as the most possible vector/pathway for introductions in the NE Atlantic, while natural dispersion has been cited as the least probable pathway (de Castro *et al.* 2017). In the marine environment, two important dispersal events are involved in the spread of non-indigenous marine species (Allendorf and Lundquist 2003). On the one hand, primary introductions occur beyond the natural distribution range of a species and are often related to transoceanic transportation via ballast water, hull fouling, aquaculture activities and the aquarium trade (Kelly *et al.* 2012) but we have no test of the hypothesis that recreational boats are also a vector of secondary spread of AIS among freshwater ecosystems via in-water transport i.e., while boating between interconnected waterways. In this study, we surveyed recreational boaters travelling into Lake Simcoe (44A degrees 25'N, 79A degrees 20'W). On the other hand, once the invasive species has become established, secondary spread is usually associated with domestic and foreign vessels, and both private and commercial fishing craft (Minchin *et al.* 2006).

## 02.02. Impacts

The impacts of invasive species are determined according to the environmental status of the recipient area (Occhipinti-Ambrogi and Savini 2003). Invasions usually have negative effects on genetic diversity, ecosystem functions/processes, and/or community structure and, consequently, they cause biotic homogenization and are a threat to global biodiversity (Allendorf and Lundquist 2003, Galil 2007).

An invasion can be understood as a source of "contamination". However, unlike other forms of marine contamination, invasions are usually irreversible (Streftaris *et al.* 2005, Thomsen *et al.* 2009). Management and control are therefore particularly important for marine conservation and together represent one of the biggest challenges faced by researchers worldwide. In this respect, distribution modelling is a useful tool for examining the potential spread of invasive species and optimizing methods of control. As native species have suffered in almost all cases where biological control has been used, the management of marine invasions should focus on prevention rather than eradication (Allendorf and Lundquist 2003).

Native species that share the same ecological niche as that of an introduced species will be more negatively affected than mere users of the niche (i.e. macrophytes will be more affected by macroalgal invasions than mobile animals that use native macroalgae as nurseries, food source, etc.).

The idea of “native good, alien bad” may be a mistaken concept as some alien species can enhance some ecosystem services or benefit some components of native biodiversity. In addition, the introduction of alien species can have economic benefits, e.g. *Undaria pinnatifida* (Harvey) Suringar 1873 mariculture (Peteiro *et al.* 2016). Moreover, native species that become pests can have even greater impacts than those of invasive species (Carey *et al.* 2012, Katsanevakis *et al.* 2014), e.g. the spread of *Caulerpa filiformis* J. Agardh, 1873 (Chlorophyta) into the natural habitat of *Sargassum* spp. (Phaeophyceae) (Zhang *et al.* 2014, Glasby *et al.* 2015) (Figure 6).



**Figure 6.**  
Natural spread of *Caulerpa filiformis* into *Sargassum* habitat.  
Photograph by Dilys Zhang.

Macroalgae make up a significant proportion of marine invasions, with up to 40 % of all introduced species that become invasive in marine ecosystems, which may reduce the resilience of native seaweed communities (Williams and Smith 2007, Vaz-Pinto *et al.* 2013) which subsequently may influence species invasions. We used a mesocosm experiment to test how increases in temperature and CO<sub>2</sub> partial pressure (pCO<sub>2</sub>). In addition to propagule pressure and the susceptibility of the native communities to invasion (i.e. invasibility), the invasive potential of alien macroalgae (i.e. invasiveness) is due to some common characteristics. Alien macroalgae are often more competitive than native species, and thus potentially effective invaders, due to characteristics such as vegetative reproduction through propagules, production of toxic metabolites, a high dispersal potential, phenotypic plasticity and the fact that most are perennial species.

## 02.02. 01 Impacts on ecosystem services

The economic value of the oceans, and especially coastal zones, is estimated to be more than 60% of the total economic value of the biosphere. Thus, the presence of new species that cause environmental shifts in ecosystem functions and/or structure will have significant economic impacts (Figure 7).



**Figure 7.**  
Human use of coastline affected by the presence of the invasive *Sargassum muticum*.  
Photograph by Eric Gay/AP.

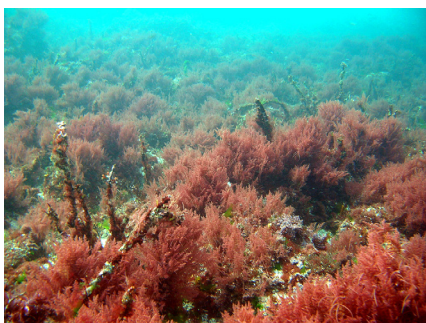
Some of the important positive impacts of introduced species include the cultivation of seaweed for the feed and food industries (Figure 8). Seaweed aquaculture has increased in the last few decades because of interest in the product for food provision and research on human health, as many macroalgae have potential benefits for pest and disease control. Thus, macroalgae have been used to study grazer-deterrent mechanisms, algal protection against abiotic stress, cytotoxic activity against human cancer cells and as anti-fouling materials. They have been also used as antioxidant, antifungal, antibacterial, antinociceptive and anti-inflammatory agents and can also be applied as insecticides or even to protect against disease in humans caused by protozoans of the genus *Leishmania*. In addition, some macroalgae have also been farmed to produce cosmetic compounds, food-derived substances (such as agar) and polyphenols, and others have been used to adsorb heavy metals or to purify water in aquaculture facilities (i.e. as biofilters).





**Figure 8.**  
Open sea farm of *Undaria pinnatifida* in New Zealand.  
©WakameFresh

However, macroalgal invasions can cause substantial economic losses through direct effects on habitats that are essential for fish stocks, although the substitution of former species and the creation of novel habitats may hamper assessment of the overall effect on food provision. Some macroalgae can have negative economic impacts on aquaculture and fisheries by fouling aquaculture facilities, fishing gear or interfering with harvesting, among other effects. Invasive species can also affect emblematic values by outcompeting native species and causing the depletion of symbolic habitats that are important for recreational activities such as snorkelling, scuba diving and recreational fishing, i.e. coralligenous habitats, kelp forests, seagrass meadows, sublittoral algal habitats and biogenic reefs (Figure 9).



**Figure 9.**  
*Asparagopsis armata* forest in a subtidal *Cystoseira baccata* habitat off the coast of A Coruña, Galicia, Spain, 2016.  
Photograph by Ignacio Bárbara.  
©Ignacio Bárbara.

### 02.02.02. Impacts on biodiversity

According to the Marine Strategy Framework Directive (MSFD) non-indigenous marine species in Europe are a major threat to marine biodiversity and marine ecosystem

health (Vandekerkhove and Cardoso 2010). The MSFD aims for Member States to reach Good Environmental Status for European underwater habitats.

Macroalgae, especially canopy-forming macroalgae, are considered ecosystem engineers and have strong impacts when colonizing new habitats, by either increasing habitat heterogeneity or by decreasing structural complexity and species richness in native assemblages. They can compete with native species (especially other seaweeds or sessile animals) for space, light or nutrients, and the competition usually creates monospecific stands and homogenized microhabitats (Figures 9 and 10). Invasive seaweeds can also reduce the coastal hydrodynamics or accumulate sediment particles, thus affecting the associated native infauna.

Invasive macroalgae can outcompete native macroalgae in different ways:

- 1) By modifying the community structure through creating novel three-dimensional habitats in which light penetration, water movement and oxygen levels are reduced and the photosynthetic capacity of other primary producer species is thus diminished (Figure 10).
- 2) By producing toxic secondary metabolites and chemical defences for protection against epiphytes and herbivores, resulting in shifts in the food-web structure. This may lead to massive shifts in ecosystem functioning by changing the direction of energy flow and nutrient cycling in food webs.

Invasive macroalgae can also have positive impacts as bioengineers, by providing new sites for shelter in previously unvegetated habitats or by providing spatial heterogeneity by increasing habitat diversity. However, invasive macroalgae usually substitute the biomass of existing macrophyte-dominated ecosystems and, thus, their impact on some ecological functions may remain relatively intact (Williams and Smith 2007, Thomsen *et al.* 2009).

Increasing extinction rates of small populations have been related to genetic drift and the inbreeding effect derived from the presence of invasive species, especially in already damaged ecosystems (Allendorf and Lundquist 2003). However, the resulting changes in the genetic integrity of native populations are limited to a few hybridization events (Vandekerkhove and Cardoso 2010).



**Figure 10.**  
Dense mats of the invasive species *Sargassum muticum* preventing penetration of light to underlying primary producers in Portonovo, Galicia, Spain, 2017.  
Photograph by Andreu Blanco.  
©Andreu Blanco.

# 03 Species of special concern

Several non-indigenous macroalgae are currently present along the northwest coast of the Iberian Peninsula. Some of the most abundant and widespread species include *Grateloupia turuturu*, *Asparagopsis armata* (Rhodophyta), *Colpomenia peregrina*, *Sargassum muticum*, *Undaria pinnatifida* (Phaeophyceae) and *Codium fragile* subsp. *fragile* (Chlorophyta), among others (Bárbara *et al.* 2005, Cremades *et al.* 2006, Araújo *et al.* 2009, Incera *et al.* 2011, Rojo *et al.* 2014, Veiga *et al.* 2014) and reproductive traits (presence of gametophytes and size at time of reproduction).

Despite the numerous negative impacts that they have on native macroalgal assemblages, these macroalgae are important sources of bioactive compounds with high industrial potential. Use of these macroalgae in the pharmaceutical and food industries may convert the problems associated with invasions into an opportunity for sourcing bioactive compounds and providing socio-economic benefits through the creation of jobs and increased profits of the associated industries. Furthermore, collection of these target species from the ocean may become a sustainable management practice contributing to the equilibrium and even restoration of the marine ecosystem as well as representing a viable solution that will foster relationships between industry, research, public demand and environmental conservation. It will also contribute to addressing Atlantic Action Plan Priority 2, i.e. to protect, secure and develop the potential of the Atlantic marine and coastal environment.

However, the use of invasive species as profitable products should be limited and carefully managed. According to the Regulation (EU) No 1143/2014, the management of invasive species aimed at eradicating, controlling or containing, can include temporary commercial use. In this context, it is essential to map the current distribution and hotspots of interest in order to focus management efforts.



## 03.01. Brief description of the species concerned

### 03.01.01. *Grateloupia turuturu*, Yamada 1941

Origin: Japan.

First recorded in Europe in Hérault (France), in 1985, as *G. doryphora* (Gavio and Fredericq 2002).

This species is a large, foliose irregular red alga with soft, gelatinous and undulate blades (Figure 11), and it grows in both sheltered and exposed areas. It belongs to the family Halymeniaceae and its worldwide distribution may be related to its adaptive capacity, including a high tolerance to variations in temperature and salinity and to eutrophic conditions (Verlaque *et al.* 2005, Araújo *et al.* 2011).



**Figure 11.**  
Blades of *Grateloupia turuturu*  
in a tidal pool on the Galician  
shore.  
Photograph by Andreu Blanco.  
©Andreu Blanco.

This alga uses multiple recruitment strategies and has a long fertile season, which has been reported to occur throughout the year in some locations (Simon *et al.* 2001). Its life cycle includes a monoecious gametophyte, which produces reproductive structures over the entire thallus, and a tetrasporophyte (diploid). It also has a third reproductive stage exclusive to female gametophytes, a carposporophyte (Verlaque *et al.* 2005, Araújo *et al.* 2011).

#### Negative impacts

*Grateloupia turuturu* can negatively affect aquaculture facilities and fishing nets by fouling and clogging shellfish and fishing nets (Streftaris and Zenetos 2006). Due to

its large blades, the presence of *G. turuturu* can strongly affect biodiversity by out-competing native macroalgal assemblages for light and space (Cecere *et al.* 2011) either accidental or voluntary, of alien species that may cause biological pollution. On the basis of morphological data and molecular analyses (rbcL and mitochondrial cox2-cox3 spacer sequences). The species has been reported to reduce the abundance and diversity of associated fauna and epiphytes (Jones and Thornber 2010).

#### Positive impacts or uses

*Grateloupia turuturu* is edible and is rich in fibre, proteins and polyunsaturated fatty acids. Extracts of this species contain anti-microfouling substances that protect against bacteria and fungi (Katsanevakis *et al.* 2014, Pereira 2016, Cardoso *et al.* 2019). Some extracts have been described to be useful for cell biological-immunological purposes and in the cosmetic industry (Denis, Ledorze, *et al.* 2009, Denis, Morançais, *et al.* 2009).

### 03.01.02. *Asparagopsis armata*, Harvey 1855

Origin: Western Australia.

First recorded in Europe in western France, in 1925 (Sauvageau 1925).

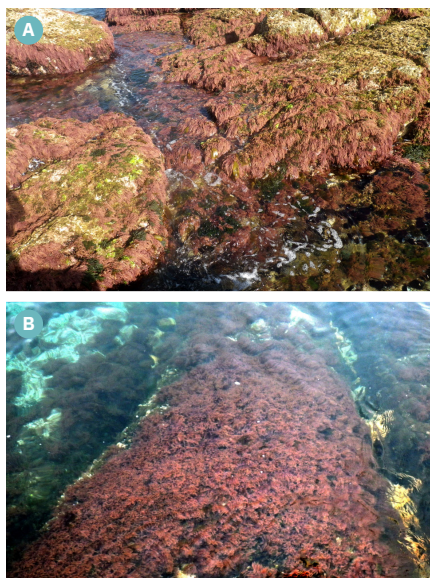
This red alga belongs to the family Bonnemaisoniaceae and has a heteromorphic life cycle with branched gametophytes up to 30 cm tall with some branches developing as conspicuous harpoon-like barbed structures up to 10 mm long and small, filamentous, tufted tetrasporophytes referred to as *Falkenbergia rufolanosa* (Harvey) F.Schmitz, 1897, which forms fine woolly balls 10 – 20 mm in diameter. It is pale purplish-red, although it quickly degenerates and becomes orange when is removed from the water. The gametophyte occurs between June and September, whereas the sporophyte occurs all year round, especially between October and March.

#### Negative impacts

The sporophyte *F. rufolanosa* usually occurs as an epiphyte covering the blades of other macroalgae. The presence of high densities of the sporophyte prevents light reaching the host blades and impedes growth of the host. In addition, the high surface area to volume ratio in *F. rufolanosa* provides the alga with a higher nutrient uptake capacity than the host, also affecting nutrient cycling (Katsanevakis *et al.* 2014).

The gametophyte is known to dominate many algal assemblages by producing monospecific coverage (Figure 12). This affects coastal fisheries by clogging fishing nets and gear and hindering filtration in filter feeders (Streftaris and Zenetos 2006, Mineur *et al.* 2007). High densities of dead algae accumulate in deeper tidal pools where they eventually rot and have negative impacts on human leisure activities (Katsanevakis *et al.* 2014). Relative to other native seaweeds, *A. armata* may have less associated fauna, in terms of number of species and abundance, due to the production

of toxic substances. Although some authors (Pacios *et al.* 2011) have demonstrated that *A. armata* generally maintains a diverse crustacean community throughout the year, other authors observed impoverishment of the peracarid crustacean community relative to the native algae *Ellisolandia elongata* (J.Ellis & Solander) K.R. Hind & G.W. Saunders, 2013 (formerly *Corallina elongata*), which is the main algal species affected by the introduction of *A. armata* (Guerra-García *et al.* 2012). Production of large amounts of a potentially toxic halogen may ultimately represent an additional danger to the ecological balance of the invaded community, leading to a reduction in the abundance of native species.



**Figure 12.**  
*Asparagopsis armata* dominating intertidal (A) and subtidal (B) habitats on the Galician coast (Spain).  
Photograph by Andreu Blanco.  
©Andreu Blanco.

### Positive impacts or uses

*Asparagopsis armata* is considered a potential natural biofilter in aquaculture facilities due to its rapid nutrient uptake and the production of ultraviolet (UV)-screening substances, i.e. mycosporine-like amino acids (Figueroa *et al.* 2008, Mata *et al.* 2010) mycosporine-like amino acids (MAAs).

Bioactive substances extracted from *A. armata* display antibacterial activity against marine *Vibrio* spp. and act as a deterrent to grazers. In addition, *A. armata* extracts can be used in both the medical and cosmetics industries. The extracts show effective antibacterial activity against human pathogens such as *Escherichia coli* Escherich 1885, *Pseudomonas aeruginosa* (Schroeter 1872) Migula 1900 and *Staphylococcus* spp. as well as potential antiprotozoal activity against Leishmaniasis in humans (Paul *et al.* 2006, Genovese *et al.* 2009). Importantly, *A. armata* also displays cytotoxic activity against human cancer cells, opening up an important avenue for oncological research (Zubia *et al.* 2009).

### 03.01.03. *Colpomenia peregrina*, Sauvageau 1927

Origin: NW Pacific.

First recorded in Europe in the Bay of Biscay (France), in 1908 (Blackler 1967).

This species is a brown alga belonging to the family Scytosiphonaceae. Compared to the native European species *Colpomenia sinuosa* (Mertens ex Roth) Derbès & Solier, 1851 (Figure 13), the invasive *C. peregrina* is thinner, forms smoother bladders and is greenish rather than yellowish brown in colour (Blackler 1967). This alga usually grows or occurs as an epiphyte on rocky surfaces where it settles and reproduces. *Colpomenia peregrina* is an annual alga with a heteromorphic life history, alternating between a globular gametophyte (5-10cm) and a crusting sporophyte (1-3 mm), and it has saccate thalli filled with water and air, which provide buoyancy (Cho *et al.* 2005).



**Figure 13.**  
*Colpomenia sinuosa* (A) and *C. peregrina* (B) on the Galician coast.  
Photograph by Andreu Blanco.  
© Andreu Blanco.

*Colpomenia peregrina* has two morphotypes, a relatively small globose form (usually occurring in tidal pools as an epiphyte) and a larger irregular form that mainly occurs on open rock surfaces in intertidal to upper subtidal zones (Clayton 1979). It has been suggested that the morphotypes may belong to two different species (Cho *et al.* 2005) and two morphotypes have been described. We used the protein-coding plastid *rbcL* and the nuclear ribosomal internal transcribed spacer (ITS). Despite the high dispersal rate, the abundance of this introduced species had apparently collapsed naturally to a very low and almost stable level (Boudouresque and Verlaque 2002). However, the abundance was reported to increase between 2007 and 2011 (Mathieson *et al.* 2016), suggesting a high degree of uncertainty regarding the future distribution, abundance and potential impacts of *C. peregrina*. This species presents broad tolerance to desiccation, temperature and salinity which likely explains its continued expansion worldwide (Green-Gavrielidis *et al.* 2019).

## Negative impacts

*Colpomenia peregrina* is considered a pest because it grows over and potentially smothers oyster beds (Katsanevakis *et al.* 2014). However, the impact has not been confirmed and no deleterious effects have been associated with the presence of this alga (Mathieson *et al.* 2016). Furthermore, as an epiphyte it can grow over native seaweeds, increasing drag and competing for space and limited resources (Green *et al.* 2012).

## Positive impacts or uses

Alginates (natural polymers) extracted from *C. peregrina* have been reported to be immunostimulants with antioxidant effects (Rostami *et al.* 2017). This seaweed contains chlorinated ethanes, which have defensive functions. This species also displays moderate antibacterial activity against *Staphylococcus aureus* Rosenbach 1884 (Kamenarska *et al.* 2009).

### 03.01.04. *Sargassum muticum*, (Yendo) Fensholt 1955

Origin: Japan.

First recorded in Europe on the south coast of England, in 1973 (Farnham *et al.* 1973).

This species is a brown alga belonging to the family Sargassaceae. It shows some morphological variations between its native habitats (where it is the smallest species of the genus) and the new habitats it colonizes (where it reaches up to 10 m in length). *Sargassum muticum* is a pseudo-perennial species that displays differences in colour between the primary basal axis (dark brown) and the secondary axis (light brown to yellowish) (Figure 14).



**Figure 14.**  
*Sargassum muticum*:  
A) dark-brown main axis (turquoise arrow) and B) yellowish secondary axis.  
Photograph by ©Teresa Mouga.

*Sargassum muticum* has a fertile peak between spring and summer, although this may extend until autumn. The number of blunt vesicles (air bladders) on new primary branches increases throughout the summer and then detach with fertile propagules at the end of the summer. The species is monoecious and has branches with reproductive receptacles bearing both male and female reproductive organs, which enable self-fertilization. Receptacles represent 50% of fertile individuals and each can release up to 300 embryos. Floating branches with fertile receptacles and the high reproductive potential provide *S. muticum* with a long-distance spreading capability and allow its rapid expansion worldwide (Arenas and Fernández 1998, Engelen *et al.* 2015).

## Negative impacts

Due to its fast growth rate, high fertility and great biomass settlement it produces highly density mats that hamper recruitment and growth of other seaweed species. It also strongly affects several abiotic factors such as sedimentation, light penetration, water movement, nutrient cycle and oxygen levels. It also alters community composition by decreasing faunal richness while increasing filamentous epiphytic algae (Thomsen *et al.* 2009) hereafter ES. Such impacts seem to be more intense in subtidal than in intertidal assemblages (Olabarria *et al.* 2009).

*Sargassum muticum* is one of the main invasive macroalgae affecting ecosystem services. It strongly affects coastal and recreational fisheries by clogging fishing lines, nets and gear. It also has negative impacts on boats by becoming entangled in propellers, pipes, hulls and even by blocking narrow sounds and harbours. Direct impacts on industry include clogging intake-cooling pipes, fouling aquaculture ropes and cages and devaluating the end-products of native algae used in horticulture, animal feed, alginates and biotechnology. In this respect, it also has a negative impact on the traditional lifestyle of seaweed harvesters in rural areas (Kraan 2008) Co. Waterford, for the first time in the Republic of Ireland in 2001. It was found at several other places in that year and in subsequent years. An intensive survey was carried out from April 2003 to November 2003 to map the geographical distribution and spread of this invasive brown macroalga, and data on distribution were collected in subsequent years through 2006. This study indicated that *S. muticum* most probably arrived in the early or mid 1990s and has spread all around the Irish coastline colonising Co. Donegal for the first time in 2006. The results indicate that spreading is facilitated by boating and perhaps via shellfish transport. A rough rate of spread of 2-3 km year<sup>-1</sup>. It also has a serious impact on recreational activities as long floating senescent canopies drift on to beaches where they rot and cause an unpleasant odour. Ecosystem services provided by the affected biotopes are also potentially affected (i.e. food, coastal protection, recreation, etc.).

## Positive impacts or uses

As a habitat engineer, *S. muticum* provides a new three-dimensional habitat for many epiphytic algae and/or smaller animals, potentially increasing the abundance and diversity. It thus provides protection for animals and facilitates settlement of invertebrates, which, in turn, attracts predators (Katsanevakis *et al.* 2014). In addition, it has also been shown to accumulate PAHs and other petroleum compounds from the water surface (Sfriso and Facca 2013).



*Sargassum muticum* is mainly used for the extraction of profitable compounds, particularly polyphenols, anti-microfouling substances against bacteria and fungi, and dichloromethane for biological regulation (Plouguerné *et al.* 2008, Jard *et al.* 2013). It has been also shown to efficiently remove heavy metals with positive results for the elimination of mercury (Carro *et al.* 2013).

### 03.01.05

#### *Undaria pinnatifida*, (Harvey) Suringar 1873

Origin: NW Pacific.

First recorded in Europe in Etang de Thau (France), in 1971 (Boudouresque *et al.* 1985)

This species is a brown alga belonging to the family Alariaceae. One of its distinctive characteristics is a simple stipe that continues upwards through the blade as a percurrent midrib, which is present from early developmental stages (Figure 15). Unlike other kelp species, *U. pinnatifida* has lanceolate and pinnately divided blades (Verlaque *et al.* 2015).



**Figure 15.**  
*Undaria pinnatifida*: adult (A-B) and young (C) individuals showing the presence of the midrib (turquoise arrows) throughout the different developmental stages.  
Photograph by Andreu Blanco.  
©AndreuBlanco.

The invasiveness of *U. pinnatifida* is due to its rapid growth and remarkable ability to colonize a wide range of habitats, ranging from artificial substrates (ropes, pontoons, buoys, etc.) and disturbed areas to shellfish beds or sandy bottoms. Its life cycle is annual and heteromorphic, alternating between the sporophyte, which is easily recognizable (Figure 15), and the gametophyte, which is microscopic and can survive out of the water for up to one month. Sporophytes have been found throughout the year, with large peaks occurring in spring and summer (Katsanevakis *et al.* 2014, Minchin and Nunn 2014). In addition to its life cycle, the rapid spread of this species is related to its tolerance to a wide range of exposure levels (from sheltered marinas to moderately exposed coastal areas).

In most of Europe, impacts on native macroalgal communities have been described as negligible (Katsanevakis *et al.* 2014), although *U. pinnatifida* has been considered one of the most invasive species worldwide (Lowe *et al.* 2000). Nevertheless, the impacts of the species are not well understood and are likely to vary considerably depending on the location. *Undaria pinnatifida* can alter the structure of ecosystems, especially in areas where native seaweeds are absent (Casas *et al.* 2004).

#### Negative impacts

Observations from sites where *U. pinnatifida* forms dense stands suggest that it can prevent light reaching smaller understory algal species (Curiel *et al.* 2002) the brown algae *Undaria pinnatifida* (kelp). Epibiotic assemblages found in association with *U. pinnatifida* are also different and less diverse than those found on native macroalgae. It also has strong negative impacts on shellfish aquaculture by fouling live molluscs or aquaculture structures (ICES 2007).

When present at high densities, detached individuals of *U. pinnatifida* washed up on beaches have deleterious effects on recreational activities when they rot, although dense, live meadows of *U. pinnatifida* may also have negative impacts on diving, angling, spear fishing, boating and other commercial activities (Irigoyen *et al.* 2011).

#### Positive impacts or uses

Sites with dense populations of *U. pinnatifida* experience substantial increases in production through both *in situ* and exported biomass (Sfriso and Facca 2013). For example, the presence of *U. pinnatifida* amongst native macroalgae in southern New Zealand more than doubled net primary production of recipient communities during its annual peak in abundance, between late winter and early summer (South *et al.* 2015).

As a foodstuff *U. pinnatifida* is economically valuable, and frequent intentional introductions have intensified the rapid colonization of European waters, especially in France and Spain (ICES 2007). Under specific conditions in unvegetated areas, *U. pinnatifida* may provide a nursery ground for small fishes and shelter for macrofauna, thus increasing species diversity and richness (Irigoyen *et al.* 2011).

Extracts from *U. pinnatifida* produce natural antioxidants, anti-fouling bioactive derivatives and antibacterial compounds (Katsanevakis *et al.* 2014). This alga is also considered a good biofilter for removing organic pollutants and inorganic metals (Sfriso and Facca 2013).

### 03.01.06.

#### *Codium fragile* subsp. *fragile*, (Suringar) Hariot 1889

Origin: Japan.

First record in Europe in County Donegal, Northern Ireland, in 1845 (Provan *et al.* 2008).

This species is a green alga belonging to the family Codiaceae. It exhibits a high degree of morphological/functional plasticity and has thus been misidentified on several occasions, leading to some debate about the number of subspecies existing and which of these are invasive. It is still reported as either *C. fragile* ssp. *fragile* or *C. fragile* ssp. *tomentosoides*, although the former is the name accepted by the International Code of Botanical Nomenclature (Provan *et al.* 2008). This alga is considered perennial or semi-perennial as the thallus or the holdfast can persist for years and regrow annually. *Codium fragile* has a simple life cycle and reproduces either sexually or parthenogenetically, providing the species with a high dispersal capacity as new individuals can reattach and grow from fragments of thallus or individual utricles (Chapman 1999). Under conditions of high photosynthetic activity, these fragments produce excess gas bubbles that accumulate in the thallus, thus producing positive buoyancy and enabling the fragments to disperse over long distances (several kilometres). Fortunately, many of those buoyant fragments will wash up on sandy shores thus reducing the spread of the species (Gagnon *et al.* 2011). In some areas, a decline in the invasive progress of *C. fragile* has been described in the last few decades, with a subsequent increase in native macroalgae congeners (Trowbridge and Farnham 2009, Watanabe *et al.* 2010).

The species has been found to coexist with native con-generics and conspecifics (Figure 16). This alga is characterized by a siphonaceous organization of the thallus with two differentiated regions, the medulla and the cortex. Microscopically the medulla is the inner part of the thallus and consists of imbricated multinucleated filaments. The cortex or outer part of the thallus consists of multiple bladder-like structures called utricles, by which species can be differentiated (Chapman 1999, Kusakina *et al.* 2006). Macroscopic distinction between *Codium* species may lead to misidentification, and microscopic examination of the utricle morphology is thus essential for species identification. In addition, *C. fragile* subspecies should be morphologically and genetically analyzed for correct identification (Rojo *et al.* 2014).



**Figure 16.**  
*Codium* spp. assemblages in exposed intertidal areas in NW Spain. Photograph by Andreu Blanco. ©Andreu Blanco.

### Negative impacts

This species has been shown to alter benthic communities and habitats, either by affecting both macroalgae and invertebrate communities or by increasing sedimentation (DAISE 2017). When it occurs in high density, *C. fragile* has intense

negative impacts on ecosystem services such as aesthetic values in recreation and tourism as it usually detaches, drifts and decomposes on highly hydrodynamic beaches.

In aquaculture and shellfish fisheries, it may interfere by competing for space and hindering filtration (Neill *et al.* 2006).

### Positive impacts or uses

As an ecosystem engineer, *C. fragile* provides shelter, food and support to a large number of native herbivorous species and epiphytes (Jones and Thornber 2010, Katsanevakis *et al.* 2014).

It is very valuable in food production and cooking as it has high nutritional and functional value in both human and livestock diets (Hwang *et al.* 2008, Ortiz *et al.* 2009). In Integrated Multi-Trophic Aquaculture activities it is used as biofilter owing to its fast nutrient uptake rates (Kang *et al.* 2008). In addition, *C. fragile* can also have a positive impact on climate regulation due to its high contents of dimethylsulfoniopropionate.

## 03.02. Identification key for species of concern

### *Grateloupia turuturu*

*Grateloupia lanceola* (J.Agardh) J.Agardh 1851 was originally reported on the coasts of the southern Iberian Peninsula and north-western Africa. Its Iberian distribution is currently restricted to some locations in NW (A Coruña) and SE (Málaga) Spain. The closely-related species *G. turuturu* has been present in Galicia (NW Spain) since 1991 and in northern Portugal since 1997 (Bárbara and Cremades 2004). The following diagnostic characters are based on published data (Bárbara and Cremades 2004, Figueroa *et al.* 2007):

1. Small discoidal holdfast (1-3 mm diameter) with a short stipe (up to 5 mm), abruptly expanding into a short blade (5-20 x 2-7 cm); presence of few proliferations, usually in marginal eroded areas, never close to the base; purplish-red, purple or brownish, characteristic emerald green colour to the blade base, usually with scattered paler spots ..... *G. lanceola*
2. Large discoidal holdfast (5-15 mm diameter) with a long stipe (up to 15 mm) gradually expanding into a large blade (10-35 x 4-9 cm); abundant proliferations from apex to base, as well as in marginal eroded areas; red, pinkish, crimson or brownish-red, lacking paler spots and never emerald green colour at the blade base ..... *G. turuturu*



## *Asparagopsis armata*

Two species of the genus *Asparagopsis*, namely *A. armata* and *A. taxiformis* (Delile) Trevisan de Saint-Léon 1845, are distributed along the Atlantic and Mediterranean coasts of the Iberian Peninsula. Members of these taxa are highly invasive and are included in the list of the “Worst invasive alien species threatening biodiversity in Europe” (EEA 2007). The following diagnostic characters are based on published data (Chualáin *et al.* 2004):

### Gametophyte

1. Thalli possess modified stolons with apically arranged harpoon-like hooks and lack an obvious rhizomatous system ..... *A. armata*
2. Thalli lack hooked stolons, but possess a clear rhizomatous system, and side branches along the main axes of specimens are generally densely ramified ..... *A. taxiformis*

### Tetrasporophyte (*Falkenbergia* phase)

1. Cells short (40-43 µm) and narrow (19-20 µm) ..... *A. armata*
2. Cells large (41- 72µm) and wide (21-28 µm) ..... *A. taxiformis*

## *Colpomenia peregrina*

*Colpomenia* is a common genus comprising 12 species in habitats ranging from temperate to tropical waters. The genus includes two morphologically distinct groups: (i) thalli irregularly globular to convolute or elongate-ovoid, often bullate, usually not more than two (or three) times as high as broad, hollow, with a thin, crisp thallus wall (membrane), and (ii) elongate or tubular thalli. *C. sinuosa* is the most common species of the genus and commonly occurs in warm to tropical waters of the world. *C. sinuosa* was first described near Cádiz, Spain (see Silva *et al.* 1996). It is easily confused with the invasive species *C. peregrina*. These species, olive brown in colour, are also very similar to *Leathesia marina* (Lyngbye) Decaisne, 1842 but the latter is yellow brown, fleshy and mucilaginous, and it is globose and smooth when young becoming hollow and convoluted with age, growing up to 5 cm in diameter. It is easily distinguished as it is readily squashed when pressed between finger and thumb (Hiscock 1979). The following diagnostic characters of *Colpomenia* spp. are based on published data (Parsons 1982):

1. Sori punctate, usually surrounding a hair group, with an overall cuticular membrane ..... *C. sinuosa*
2. Sori extensive or irregular in shape, with scattered hair groups without an overall cuticular membrane ..... *C. peregrina*

## *Sargassum muticum*

The genus *Sargassum* contains around two hundred species, distributed in tropical and subtropical waters. In the Iberian Peninsula and the Balearic Islands, it is represented by six species: *S. flavifolium* Kützinger 1849, *S. trichocarpum* J. Agardh 1889, *S. vulgare* C. Agardh 1820, *S. acinarium* (Linnaeus) Setchell 1933, *S. hornschurchii* C. Agardh 1820 and *S. muticum*. The following species identification key has been published (Barceló Martí *et al.* 2001):

1. Reproductive structures simple ..... *S. muticum*
- Reproductive structures branched ..... 2
2. Reproductive structures fertile from the base to the apex; sometimes small sterile base not branched ..... 3
- Reproductive structures with a well-developed sterile branched base ..... 4
3. Reproductive structures thick and with wide base ..... *S. flavifolium*
- Reproductive structures thin and cylindrical ..... *S. trichocarpum*
4. Reproductive structures less than 1 cm long ..... *S. vulgare*
- Reproductive structures more than 1 cm long ..... 5
5. Reproductive structures cylindrical; foliaceous branches not very abundant, narrow especially in the apical part of the plant ..... *S. acinarium*
- Reproductive structures flattened or with triangular section; foliaceous branches abundant ..... *S. hornschurchii*

## *Undaria pinnatifida*

Kelp forests dominate subtidal shallow rocky coasts and are key components of coastal ecosystems in temperate to polar areas of the world. The following are the six most abundant kelp species along the Iberian Peninsula coast: *U. pinnatifida*, *Saccharina latissima* (Linnaeus) C.E. Lane, C. Mayes, Druehl & G.W. Saunders 2006, *Saccorhiza polyschides* (Lightfoot) Batters 1902, *Phyllariopsis purpurascens* (C. Agardh) E.C. Henry & G.R. South 1987, *Laminaria hyperborea* (Gunnerus) Foslie 1884, and *L. ochroleuca* Bachelot de la Pylaie 1824 (Araújo *et al.* 2016). The diagnostic characters of the most abundant kelp species are as follows:

1. Thalli foliated, straighter towards the tip, with a clearly defined, brown-green translucent medial nerve ..... *U. pinnatifida*
- Thalli lacking medial nerve ..... 2

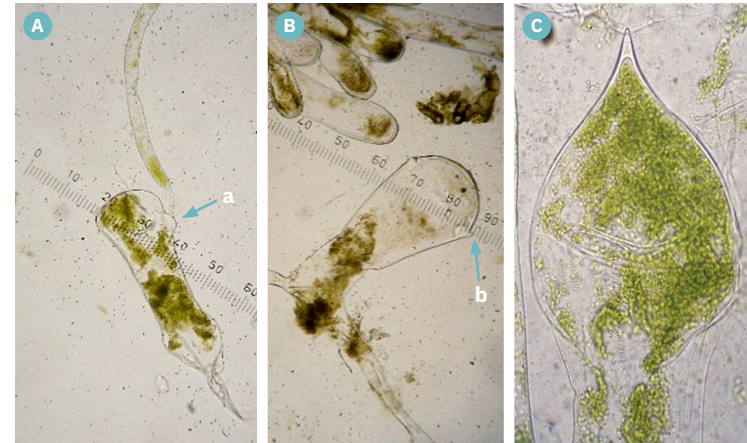
- 2. Initial part of thallus cylindrical; undivided frond with ruffled edges ..... *S. latissima*
- Initial part of thallus widens to form a large flat frond, which is divided into ribbon-like sections ..... 3
- 3. Stipe twisted at the base forming a bulb ..... *S. polyschides*
- Stipe lacking bulb at the base ..... 4
- 4. Stipe attached to substrate with a basal disc; a dark spot on the basis of the frond ..... *P. purpurascens*
- Stipe attached to substrate with rhizoids ..... 5
- 5. Stipe cylindrical, rough textured; often colonized by epiphytes ..... *L. hyperborea*
- Flat and smooth stipe; lacking epiphytes ..... *L. ochroleuca*

**Codium fragile ssp. fragile**

*Codium* spp. is a worldwide distributed complex of siphonous green algae, with different species and subspecies recognized in various parts of the world. In European waters the genus includes four native species (*C. adherens* C. Agardh 1822, *C. bursa* (Oliv) C. Agardh 1817, *C. tomentosum* Stackhouse 1797, *C. vermilara* (Oliv) Delle Chiaje 1829) and the invasive *C. fragile* including both *C. fragile* subsp. *fragile* and subsp. *atlanticum* (A.D. Cotton) P.C. Silva, 1955. The macroalga *C. fragile* subsp. *fragile* (previously known as subsp. *tomentosoides*) is a clear example of a cryptic invasion. The following diagnostic characters are based on published data (Bunker *et al.* 2017):

- 1. Thallus entirely crustose, forming extensive spongy patches ..... *C. adherens*
- Thallus erect, globose or branched, with or without crustose base ..... 2
- 2. Thallus globose, 2-20 cm in diameter; solid or hollow ..... *C. bursa*
- Thalli erect, with dichotomous branching ..... 3
- 3. Utricles with small or prolonged pointed tips in at least part of thallus... ..... 4
- Utricles without pointed tips ..... 5
- 4. Utricles with small umbos or short pointed tips < 15µm long; holdfast typically > 1 cm diameter ..... *C. fragile* subsp. *atlanticum*

- Utricles with prolonged pointed tips > 30µm in at least part of the thallus (Figure 17C); holdfast typically < 1 cm diameter ..... *C. fragile* subsp. *fragile*
- 5. Utricles with broad domed apex, hair scars borne below the widest part of utricle (Figure 17A); holdfast > 2 diameter ..... *C. tomentosum*
- Utricles with flat-topped apex, hair scars borne at the widest part of utricle (Figure 17B), holdfast < 1 cm diameter ..... *C. vermilara*



**Figure 17.** Microscopic differences in utricle structure of different species of *Codium*. A) *C. tomentosum*, B) *C. vermilara*, and C) *C. fragile*. Turquoise arrows indicate the relative positions of the scar of filaments or gametangia on (a) *C. tomentosum* and (b) *C. vermilara*. Photograph by Andreu Blanco. ©Andreu Blanco.

# 04

## Sampling methodology

An in-depth understanding of species occurrence is required in order to plan rapid actions in response to the arrival of new species, such as conservation measures that indirectly enforce the use of valuable resources that are currently only viewed as a threat and not as an opportunity. The creation of replicable monitoring programmes is encouraged. A monitoring programme that includes both qualitative and quantitative assessment of the invasive species of particular concern on the western coast of the Iberian Peninsula is therefore proposed. Quantitative, semi-quantitative and qualitative sampling can be carried out to characterize macroalgal assemblages within specific locations. Depending on the study objectives, one or more types of sample can be collected in the field.

## 04.01. Sampling plan and permission

Research teams must prepare a sampling plan prior to conducting any fieldwork. A sampling plan defines the sampling objectives, describes the roles and responsibilities of team members, lists sampling methods and procedures, and defines activities appropriate to the study. The sampling plan should describe when fieldwork will be performed and should include a process for making alternative plans in the event of adverse weather or hydrological conditions. All members of the sampling team should be familiar with the standard protocols for collecting and processing macroalgal samples. In addition, all members should sample macroalgae together at a given location in order to standardize the sampling protocol and resolve any doubts about the sampling plan. For security and safety reasons, intertidal and subtidal surveys should be conducted, when possible, by teams of at least two people.

The sampling team leader is responsible for obtaining the necessary permits prior to sampling. Permission from landowners may also be required when sampling on or crossing private property. In some cases, multiple permits may be required to satisfy private, National and Autonomous government (e.g. National Park Service or Environmental Service) regulations.

## 04.02. Type and size of sampling units

The choice of the sampling unit depends on the goals of the sampling programme, especially the species to be sampled. Quadrats and line transects are widely used sampling units on rocky shores. The advantages of quadrats are that they cover an area of substratum. They are widely used to estimate cover, density or biomass of seaweeds and provide a good combination of convenience and the appropriate scale for the organisms under study (Murray *et al.* 2006).

The following two main issues must be considered in determining sampling unit size: 1) maximization of the number of species included in the sample, and (2) minimization of the variance of the mean for abundance data. Although the sample unit sizes have not been standardized for rocky shores, quadrats of 0.25 - 1 m<sup>2</sup> are commonly used.

## 04.03. Location and number of sampling units

Reaching a decision about how the sampling units should be placed in the study area is complex and to certain extent determines the nature of the information collected and thus the accuracy and the inferences that can be drawn from the data. Random sampling is one of the best ways of ensuring independence of errors, which is one of the most critical assumptions in the statistical analysis of data. Nevertheless, a good alternative to random location is to place the sampling units systematically throughout the study area.

It is also important to determine the number of sampling units, i.e. replicates. The number of replicates required depends on several factors such as abundance and distribution of species, the level of precision desired and the aim of the study (Murray *et al.* 2006). Ideally, the number of replicates is sufficiently large when the cumulative mean becomes insensitive to the variations in the data. An index of precision D (in % of the mean) can be defined, from which the number of samples can be calculated as follows:

$$D = 1/\bar{x}\sqrt{S^2/n}$$

where  $\bar{x}$  is the mean, S is the standard deviation, and n is the number of sampling units.

A systematic sampling design in which the sampling units, i.e. 50 x 50 cm quadrats, are placed uniformly across the study area is proposed. The main advantages of this method are that (1) the estimate of the mean is more accurate than with random sampling, and (2) it is easier than random sampling (Murray *et al.* 2006).

## 04.04. Qualitative sampling

The presence and relative abundance of the target seaweeds are recorded and estimated following the DAFOR-N scale:

- **D** refers to the **dominant** presence of the species at the sampling site, representing more than 50% of the total macroalgal abundance in the area. In practice, this class is rarely used.

- **A** should only be used if the species is **abundant** and very common in a site, i.e. representing between 30 to 50% of the total macroalgal abundance. **LA** can be used if the species is **locally abundant** in a particular part of the site.

- **F** represents **frequent** occurrence of the species in a given area. It should be used when the abundance of the macroalga ranges between 15 and 30% of the total macroalgal abundance in the area.

- **O** represents **occasional** and is used for species that occur in a few places in a site, and the populations are usually not very large, i.e. representing 5-15% of the total abundance. **O** is likely to be used for many species in many sampling areas.

- **R** represents **rare** and is used for any species that occur as small numbers of individuals, usually less than 5% of the total abundance. This small number of individuals may be located in one place, or scattered over the sampling site.

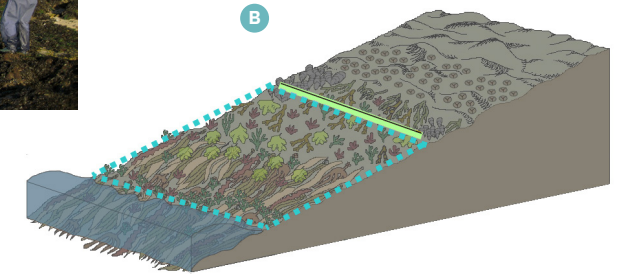
- **N** represents **not present** and indicates that no individuals of the species have been observed in the sampling site.

In this type of sampling, a checklist of the target species should first be drawn up. After the area is surveyed, the researcher should assign a DAFOR letter to each species by examining the list quickly and assigning the first score that comes to mind. The best option is to coordinate DAFOR scores between members of the team, and if a species seems intermediate between two categories, the lower category should be chosen (e.g. when it is not clear whether a species is occasional or frequent, it should be recorded as occasional).

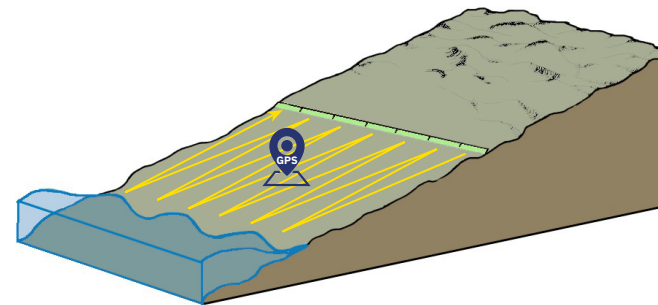
Cryptogenic species (a species that is not demonstrably native or introduced) such as *Codium* spp. must be correctly identified to prevent misinterpretation and underestimation of the impacts of invasion events on natural and/or previously invaded communities (Carlton 1996). Hence, *Codium* spp. must be morphologically identified in the laboratory by microscopic examination the utricles of the tips of the apices (Figure 17) (Rojo *et al.* 2014, Bunker *et al.* 2017). For the correct identification of *C. fragile* subsp. *fragile* population, sufficient numbers of *Codium* ssp. individuals should be identified for correct estimates of invasiveness. Up to 100 apices should be sampled when the relative abundance of *Codium* ssp. in the sampled area is dominant or abundant, and up to 50 apices when the relative abundance is frequent, occasional or rare.

#### 04.04.01. Intertidal sampling

In each location, two areas separated by tens to hundreds of metres should be randomly chosen and sampled. Each area will consist of a square/rectangle from the mid intertidal shore to the water line in lowest spring-summer tides. The area will be determined by a 30 m transect located on the mid intertidal shore; the whole surface should be sampled from this point to the water line in a zigzag pattern (Figure 18 and Figure 19).



**Figure 18.** Intertidal sampling methodology: A) Field sampling in the mid intertidal zone by two people; B) Sampling representation of the 30 m transect (green line) and placement of the sampling area (blue dotted line).



**Figure 19.** Zigzag sampling (yellow lines) on an intertidal slope along the 30 m transect (green line). The GPS position is estimated in the middle of the sampling area (dark blue icon).

The following information should be recorded at each site for homogenization of the type of data collected (Annex I, Table 1):

- Location: the sampling location, as outlined above.
- Area: two areas (1 or 2) separated tens to hundreds of metres (the number of metres should be noted).
- Date: according to standard calendar.

Recorder: a team of recorders is essential for resolving possible misunderstandings.

- Coordinates of the access point to the site. The GPS unit datum should always match the datum of the map being used (i.e. ETRS89, WGS84) as it is an important component of a coordinate. The Global Positioning System uses an earth centred datum called the World Geodetic System 1984 or WGS 84, which is adopted as a world standard from a datum called the North American Datum of 1983 or NAD 83. There are typically only one or two metres of difference between WGS 84 and NAD 83 in the Continental United States.



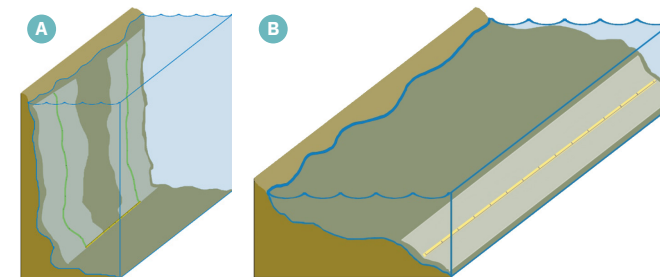
- Coordinates of the centre of the sampling area (Figure 19).
- Width of the area: measured between the midpoint of the transect (15 m) and the water line; however, the average shore shape must be taken into account, as it may vary significantly from one point to another.
- Visibility: usually good, although particular weather and time conditions may affect the visibility (darkness, sunrise, heavily raining, thick fog, etc).
- Weather: recorded for a better understanding of the sampling conditions.
- Shore exposure: Considering the shore-related variability, the presence and abundance of macroalgae may be affected by the degree of exposure (exposed, semi-exposed or sheltered).
- Substrate composition: estimated percentages of the origin (metamorphic, granite, sand, mud, etc) and type (platform, boulder, blocks, gravel, etc) of substratum present in the area.
- Slope: an estimated value of shore slope (very steep, steep, slightly sloping or flat) can facilitate interpretation of the results.
- Sampling time: the time since the start (placing the transect) and the end of sampling (prior to leaving the area).
- Presence: yes (Y) or no (N). Appearance of the target species and also whether present in intertidal pools (P) or in emergent (E) habitats.
- DAFOR abundance scale: as outlined above (D = Dominant, A = Abundant, F = Frequent, O = Occasional, R = Rare).

Sampling record	Sampling material
Coordinates (access and site)	<i>GPS</i>
Area measurements (length and width)	<i>2 Measuring tapes (at least 50m)</i>
Sampling conditions (weather, visibility, slope, substrate composition, shore exposure, sampling time, presence and abundance)	<i>PVC board, record sheet, pencil, pencil sharpener, rubber, DAFOR scale sheet, camera, chronometer or watch</i>
Sample collection	<i>Resealable zipped plastic bags and permanent marker</i>

Table 1. List of the materials needed for the sampling.

#### 04.04.02. Subtidal sampling

Subtidal sampling requires specific simple methodologies for underwater censuses. As scuba diving requires trained personnel and is costly, only a small number of subtidal sampling sites are usually surveyed. In addition, as scientific diving is considered under professional diving law, specific regulations and permission are required accordingly (both/either at a regional, national and/or international level). In order to optimize results of underwater sampling, underwater locations should be selected according to the intertidal results. Hence, sites where the abundance of species in the intertidal area falls into D, A or F DAFOR-N scale categories should be sampled.



**Figure 20.** Placement of the sampling area of A) Cliff seafloor (two transects of 15 m) and B) Flat seafloor (one transect of 30 m). The lightly shaded area represents a sampling area of 4 m width (2 m at each side of the sampling transect).

According to the sea-floor topography, underwater censuses require at least two different methodologies: very steep/cliff vs slightly sloping/flat (Figure 20).

A total area of 120 m<sup>2</sup> is sampled at each site following the DAFOR scale. Transects of 30 m (one transect of 30 m in flat sea-floors and two transects of 15 m separated by 30 m in cliffs) are randomly chosen and recorded by at least two scuba divers. In order to minimize the diving time, each diver covers and visually inspects the area along two meters on each side of the transect (Figure 21). The areas are sampled at a depth (standardized to the 0 m tide level) of 10 m in flat seafloors, and between 0 and 15 m in cliff areas.

The following information should be recorded at each site (Annex II, Table 2):

- Location: the sampling location according to the selection criteria.
- Date: according to standard calendar.
- Recorder: a team of recorders is essential for solving possible misunderstandings.
- Coordinates of the site access point.
- Coordinates of the sampling site (immersion and emersion points). All coordinates are recorded to an UTM standardized datum (e.g. WGS84). One of two methodologies

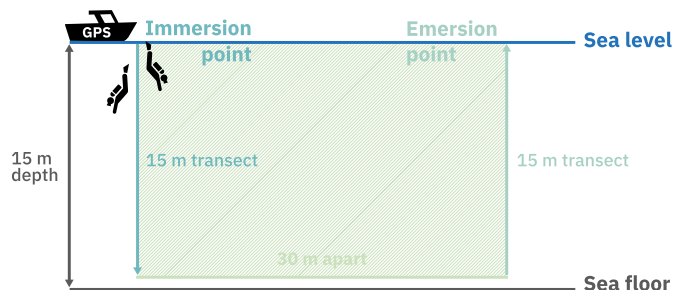
is used depending on the slope of the shore. In very steep or cliff areas, the immersion point is recorded by the scuba divers prior to immersion and the emersion point is recorded by the scuba divers once they emerge. In slightly sloping or flat areas, the immersion and the emersion points are marked with a rope and buoy from the initial and final sampling point of the seafloor while the support staff on the boat registers the GPS position of the buoys (Figure 21).

- Visibility: water turbulence or up-welling phenomena might hamper the visual censuses.
- Weather: recorded for a better understanding of the sampling conditions.
- Shore exposure: considering the variability of the shoreline, the presence and abundance of macroalgae may be affected by the degree of exposure (exposed, semi-exposed or sheltered).
- Substrate composition: origin (metamorphic, granite, sand, mud, etc) and type (platform, boulder, blocks, gravel, etc) of substratum are recorded by noting the length of the patches in substratum composition.
- Sampling time: the time between placing the transect (start) and leaving the area (end).
- Presence: yes (Y) or no (N).
- DAFOR abundance scale: as outlined above (D = Dominant, A = Abundant, F = Frequent, O = Occasional, R = Rare).

**A** Flat bottom (i.e. Galicia)



**B** Cliffs (i.e. Portugal)



**Figure 21.** GPS record of immersion and emersion points in (A) slightly sloping sea floor and (B) very steep sea floor.

Sampling record and needs	Sampling material
Coordinates (access, immersion and emersion points)	<i>GPS, rope, buoys and boat</i>
Area measures (transect length and transect separation)	<i>2 Measuring tapes (at least 50 m)</i>
Sampling conditions (weather, slope, substrate, shore exposure, sampling time, presence and abundance)	<i>PVC board, record sheet, pencil, pencil sharpener; rubber; DAFOR scale sheet, camera, chronometer or watch</i>
Sampling conditions (visibility)	<i>Secchi disk</i>
Scuba diving gear	<i>Suit, regulator, octopus, mask, tank, weight belt, swim fins, jacket, diving computer, depth and pressure gauge, lantern, knife, underwater camera</i>
Sample collection	<i>Resealable zipped plastic bags and permanent marker</i>

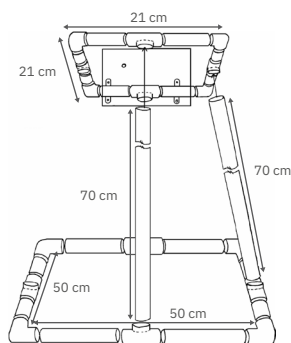
Table 2. List of material needed for sampling.

## 04.05. Quantitative sampling

Quantitative samples of macroalgae are collected from a known area of substrate to provide estimates of abundance of different macroalgae present in a targeted location, based on the previous qualitative sampling. We propose the use of both destructive and non-destructive sampling strategies.

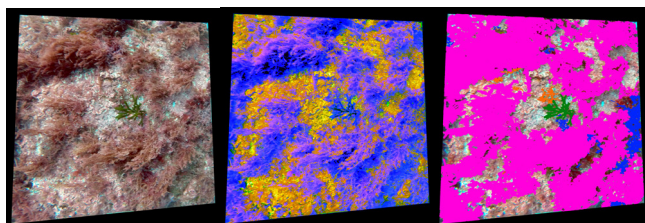
### 04.05.01. Non-destructive sampling

Species cover, both native and invasive, is also estimated along a 30 m transect in fifteen (n=15) quadrats of 50 x 50 cm, once every two metres. Every quadrat along each transect (see next section for detailed description of sampling) is photographed. A modification of a photo-quadrat (Preskitt *et al.* 2004) can be used for taking images of each 50 x 50 quadrat (Figure 22).



**Figure 22.** Photoquadrat for 50 x 50 photographs using a Pentax Optio WG-1 camera (adapted from )

The photographs are then analyzed in the laboratory using image software (e.g. Image J) to estimate cover and presence of macroalgae in assemblages (Figure 23).



**Figure 23.** Image processing using Image J software. Color-modified photographs are used to calculate the cover by each color. Each color corresponds to different macroalgae and/or substrate.

#### 04.05.02. Destructive sampling

The total biomass of the target macroalgae is estimated at each location by extrapolation of the density of target species and their relative fresh and dry weight. Biomass per quadrat should be estimated differently for macroalgae with short and dense fronds (*A. armata*) and macroalgae with long and sparse fronds (the other target species):

A) For *G. tututuru*, *C. peregrina*, *S. muticum*, *U. pinnatifida* and *Codium* sp. the total number of individuals per quadrat is recorded in the 50 x 50 cm quadrats in which the target species are present (minimum  $n = 5$ )<sup>1</sup>.

- Randomly selected individuals are removed with a scraper ( $n = 30$ ). The biomass of each quadrat is then estimated from the biomass of the total number of individuals sampled and the estimated density per quadrat.
- For *Codium*, additional tips are collected along the transect for identification of the species; 50 individuals if F, O or R (< 30%; DAFOR scale), and 100 if it is D or A (> 30%; DAFOR scale).

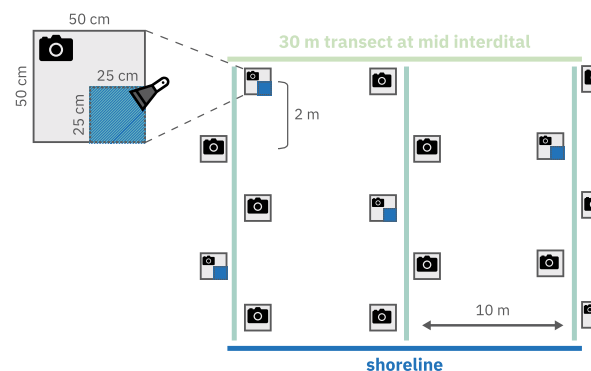
<sup>1</sup> Note that all species might not be present in the same quadrat and therefore the number of individuals of each species will probably have to be counted in different quadrats.

B) For *A. armata*/*F. rufulanosa*, the total biomass of a sub-quadrat (25 x 25 cm) is removed from each quadrat with a scraper (Figures 26 and 27).

The individual specimens of each target species are then washed, dried slightly by blotting with paper hand towels and weighed to the nearest 0.01 g (fresh weight). The samples are then oven-dried (at 65 °C until constant weight) and weighed to the nearest 0.01 g (dry weight).

#### Intertidal sampling

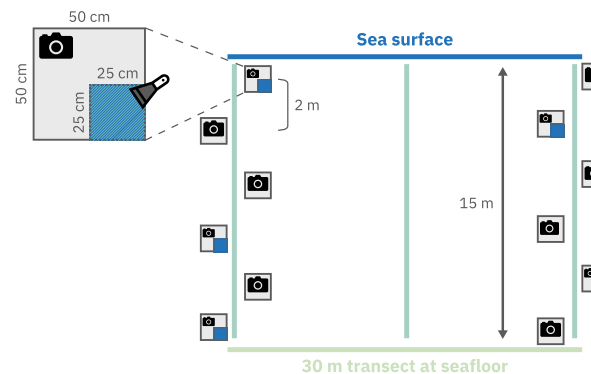
The macroalgal biomass (see qualitative sampling section) is estimated in 10 quadrats of 50 x 50 cm placed alternatively on both sides of 3 transects (separated by 10 m) perpendicular to the shoreline (Figure 24).



**Figure 24.** Sampling scheme for quantitative assessment of intertidal macroalgae community. Destructive (black and blue squares) and non-destructive (black squares) methods are shown.

#### Subtidal sampling

Depending on the sea-floor topography (see subtidal qualitative sampling section), two different sampling methods should be used (Figures 21 and 25), both of which involve use of a total of fifteen ( $n = 15$ ) sampling quadrats of 50 x 50 cm, as described above. In slightly sloping or flat sea-floor areas, one transect of 30 m is sampled, whereas in very steep or cliff areas, two transects (15 m) separated by 30 m are sampled from the 0 tidal level to a depth of 10-15 m.



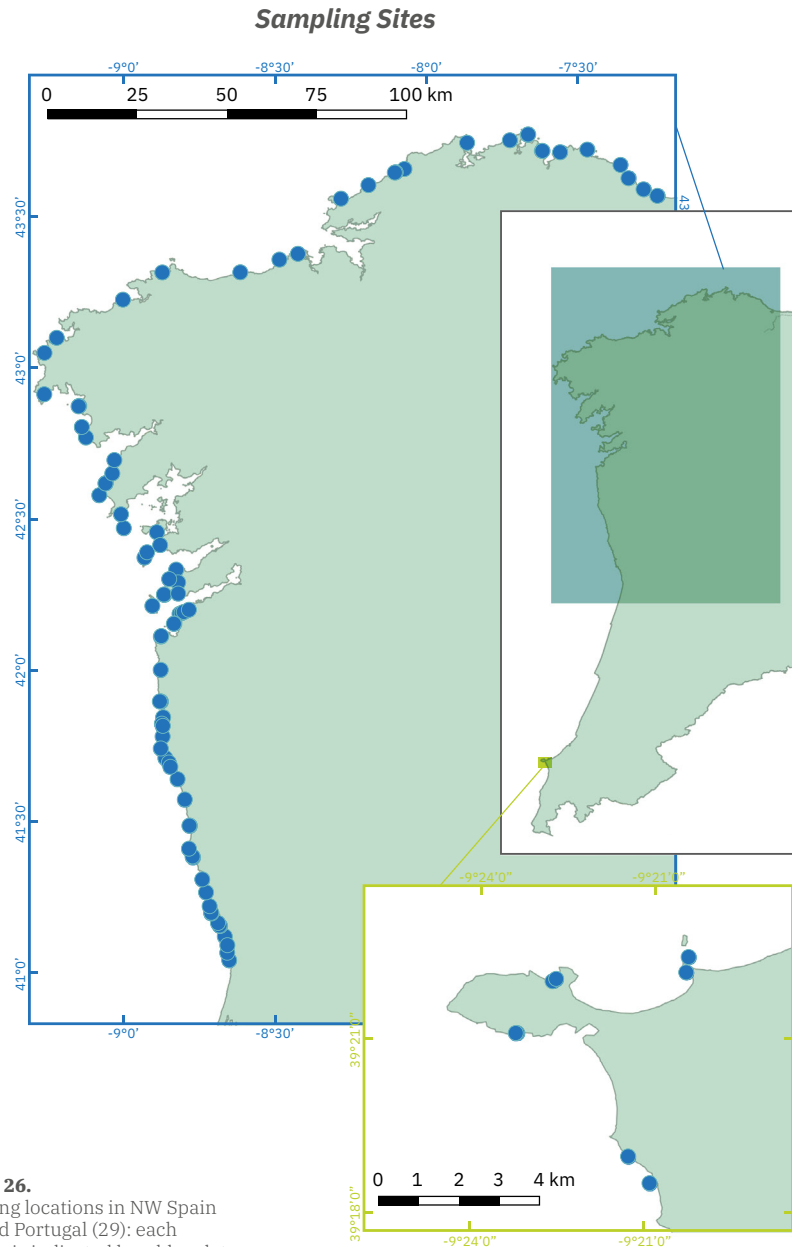
**Figure 25.** Sampling scheme for quantitative assessment of subtidal macroalgae community. Destructive (black and blue squares) and non-destructive (black squares) methods.

# 05 Sampling locations: NW Iberian Peninsula

A detailed understanding of species occurrence is essential for mapping the species distribution and abundance along the NW Iberian Peninsula coast. The current distribution maps range from north-west Spain (Ría de Foz) to south-west Portugal (Lisbon), encompassing almost 2000 km of temperate coast, between  $43^{\circ} 47' 20.35''$  N and  $38^{\circ} 58' 43.6''$  N (Figure 26). The sampling strategy includes sampling rocky shores in more than 120 locations (97 in Spain and 29 in Portugal), at low spring tide during the macroalgae growing seasons, i.e. spring and summer. The linear distance between two consecutive locations varies between 1 and 23 Km depending on the type of shore, i.e. the presence and accessibility of rocky shores. The species distribution is modelled at a resolution of 0.002083 degrees (around 230 m), which correspond to the finest resolution of the original environmental variables.

All locations were selected following an environmental stratification design based on variables that determine the distribution of the species of interest (wave exposure, open coast, estuarine areas, distance to river mouths, salinity, temperature, etc.). Sampling programmes should ideally start with a pilot study to assess accessibility and representation of different coastal habitats.





**Figure 26.** Sampling locations in NW Spain (97) and Portugal (29); each location is indicated by a blue dot.

## 05. 01. Habitat models

Species Distribution Models (SDMs) and ecological niche models (ENM) are commonly used to calculate habitat suitability from species occurrence and macroecological data. These predictive models can be applied to anticipate whether invasive species are likely to become established in an area, to identify critical routes and arrival points, to construct risk maps and to predict the extent of potential spread following an introduction. SDMs and ENMs have been used in terrestrial systems, but their application to predict suitable habitat for invasive marine macroalgae, or invasive marine species in general, is still rare. Fitting presence-absence models through a 3-step modelling process is advantageous because of a full sampling methodology:

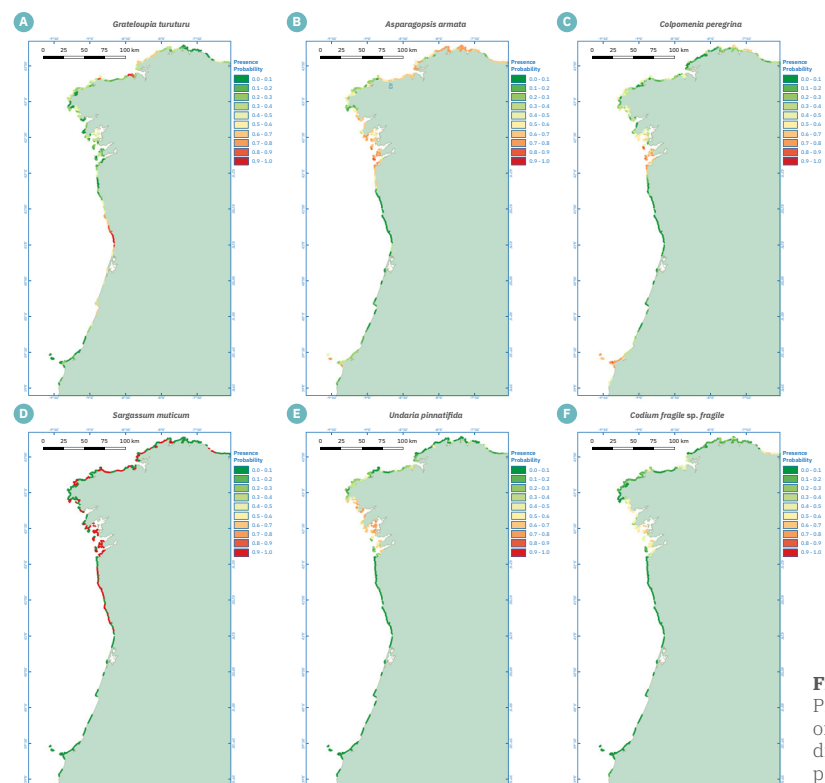
- Pre-processing of input variables, both presence-absence records (from field sampling) and the explanatory environmental variables (calculating derived variables, and adjusting formats, scale and resolution of the raw variables)
- Model building, by comparing different modelling techniques and model validation (by means of jackknife techniques). The most common modelling techniques are Generalized Linear Models (GLM), Generalized Additive Models (GAM), Random Forest (RF), Annealing Models (ANN) and Classification Tree Analysis (CTA). A combination of different SDMs (with different modeling algorithms) within an ensemble modelling framework with a consensus approach (Araújo and New 2006) provides the best fit from among several methods tested (Aguirre-Gutiérrez *et al.* 2013).
- Constructing final maps of species distributions (either by selecting the best fit model or by combining the results of different models, and hence considering uncertainty in model selection).

**GIS data sets.** These are valuable for storing, mapping and managing spatial data. SDMs and ENMs rely on GIS data sets that describe the environment. The data sets are typically raster layers, i.e. they are composed of a geographical grid of square pixels in which each pixel represents the value of the variable at that location.

**Presence-absence data.** Consist of georeferenced locations (latitude/longitude) where the species has been detected. Locations where the species does not occur, called absence records, will be also incorporated into models to improve predictions of species distributions (Elith *et al.* 2011, Marcelino and Verbruggen 2015). Both SDMs and ENMs are often modelled from a presence-only approach. However, disregarding the absence data from a planned sampling protocol such as ours results in loss of information and model bias (Elith *et al.* 2011). Hence, we propose fitting different models to the whole presence-absence information obtained from our sampling.

**Modelling technique and accuracy.** The algorithms available mainly differ in their mathematical principles, underlying assumptions and type of input data (e.g. presence-only, presence-absence, presence-background data). Algorithms that are suitable when presence and absence data are available (e.g. GLMMs, GAMs, SVMs, RFs) are used to build ensemble models. Model accuracy is then tested by checking the correct and incorrect classification of predicted values (for detailed procedure, see Marcelino and Verbruggen, 2015).

**Predictive maps.** The predictive equation is applied to the cells for which biological data are not available by using environmental values as inputs. The performance of favorability values (independent of the prevalence in the sample; Real *et al.* 2006) with raw suitability values is compared. Threshold selection by means of ROC plots and independent metrics threshold (Lawson *et al.* 2014) is used. Predictive maps of species distribution provide policy-makers with relevant information (Figure 27) to identify the main factors involved in invasive success and to estimate potential changes in species distribution (i.e. local extinctions or spread of invasions) and thus assess early detection of non-native species in potential susceptible areas (Thomsen *et al.* 2009, Gallardo *et al.* 2015).



**Figure 27.** Probability maps of presence of the different species of particular concern.

## 05.02. Modelling variables

For macroalgae, maps of sea surface temperature (SST), photosynthetically active radiation (PAR), chlorophyll a, air temperature, type of substratum, wave exposure, salinity, bathymetry and productivity serve as predictor variables in the models. In the case of the invasive macroalgae, some particular aspects must be considered. Their occurrence is not only determined by the environment, but also by other factors such as dispersal ability, colonization time lags or disturbance (Marcelino and Verbruggen 2015). For example, invasive macroalgae tend to be abundant in harbours and degraded habitats (Kaplanis *et al.* 2016). Information about proximity to ports, traffic density in ports, maritime routes, density of human population in coastal areas and different water quality parameters must then be incorporated into models. Moreover, invasive species tend to shift niches (Klein and Verlaque 2009), although modelling tools seem to be useful for predicting areas at risk, even for niche-shifting species (Medley 2010).

A wide set of pre-candidate variables with potentially important effects on the presence of the targeted species are selected on the basis of current knowledge on seaweed ecology and demography, community structure and invasion ecology. In this context, five types of variables are considered:

1. Dispersal from extant populations: The dispersal probability is defined as the probability of colonization of one cell from any of the previously known cell populations. We use the same presence record data set used by Kelly *et al.* (2014) to derive the dispersal functions, which were originally provided by Mineur *et al.* (2010).
2. Primary productivity: ocean productivity as chlorophyll a concentration and photosynthetically active radiation (PAR).
3. Physical conditions: Sea bottom habitat, water velocity, slope, sea surface temperature (including maxima, minima, mean, etc.) and air temperature (as when modelling intertidal marine species is essential as these species have to deal with overheating and desiccation during low tide, especially during summer time).
4. Human stressors: Human population density, fishing sales, distance to ports (fishing, commercial, recreational), and further ship/vessel factors (number, weight, number of lines).
5. Chemical conditions: oxygen, nitrates, silicates, phosphates, particulate inorganic carbon and ammonium concentration.

Because autocorrelation and multicollinearity among several environmental predictors is expected, the variable clustering or proxy set identifying approach is used, and one variable per cluster is retained (Dormann *et al.* 2013). Proxy sets are identified from a matrix of Spearman rank correlation coefficients among all variables. A subset of variables that yields strong predictions, i.e. with higher AUC values, is finally selected (Guisan and Thuiller 2005, Martínez *et al.* 2012).



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# 07 Annexs

## AMALIA Recording Sheets

Site name:

Date:

GPS coordinates of access point

Type of  
substratum

Wave Exposure:

0 m

Recorders:

GPS immersion point

Time spent:

Weather conditions:

Visibility (Secchi Disk):

GPS emersion point

Species	DAFOR	Quadrant (Q)				Comments
		Number of individuals				
<i>Codium fragile</i>	Q = N =	Q = N =	Q = N =	Q = N =		
<i>Undaria</i>	Q = N =	Q = N =	Q = N =	Q = N =		
<i>pinnatifida</i>	Q = N =	Q = N =	Q = N =	Q = N =		
<i>Sargassum muticum</i>	Q = N =	Q = N =	Q = N =	Q = N =		
<i>Colpomenia peregrina</i>	Q = N =	Q = N =	Q = N =	Q = N =		
<i>Asparagopsis armata</i>	Q = N =	Q = N =	Q = N =	Q = N =		
<i>Falkenbergia rutilanosa</i>	Q = N =	Q = N =	Q = N =	Q = N =		
<i>Grateloupia tururu</i>	Q = N =	Q = N =	Q = N =	Q = N =		

30 m

## 07.02. Annex II

### AMALIA Recording Sheets

Site name

GPS coordinates of access point

Area

GPS coordinates of the survey site (taken at the centre of the area)

Date

Area recorded (~m<sup>2</sup>):

Size: 30 m parallel to sea x

m perpendicular to sea

Time spent in recording area:

Start time:

Final time:

Weather conditions:

Wave Exposure:

Visibility

Type of substrate (granite rocks/boulders, shale platform):

Surface inclination (horizontal platform, very steep slope, steep slope, slight steep slope):

Species	D	A	F	O	R	Not seen	Comments
<i>Codium fragile</i> ssp <i>fragile</i>							
<i>Undaria pinnatifida</i>							
<i>Sargassum muticum</i>							
<i>Colpomenia peregrina</i>							
<i>Asparagopsis armata</i>							
<i>Falkenbergia rutilanosa</i> (sporophyte)							
<i>Grateloupia tururu</i>							

## 07.01. Annex I







*Amalia*

Algae-to-Market Lab IdeAs

Adding value to  
invasive seaweeds of the  
Iberian northwest

Co-funded by  
the European Union

