Fatemeh Zahmatkesh

Sampling, Mapping and Adding Value to Marine Invasive Seaweeds of the Iberian Peninsula

Tese de Mestrado em Ecologia,
orientada pelo Professor Doutor Leonel Pereira e pelo Doutor Rui Gaspar,
apresentada ao Departamento de Ciências da Vida da Universidade de Coimbra.

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Abstract

Non-indigenous species (NIS) can be defined as species that colonized new geographical areas where they were not present previously. Their increasing appearance has been causing considerable ecological and economic problems globally. Under this scope, biological invasion and invaders' success has been an important topic during the last decades. Hundreds of marine NIS have been introduced around the world and seaweeds (marine macroalgae) are a significant part of them. Studies conducted worldwide in the last decade have been reporting around 250 species of introduced seaweeds. The northeast part of the Atlantic is especially known to be home to many introduced species and more than 100 introduced species of seaweeds are reported in Europe. This thesis will go through the processes of seaweeds invasion, from their introduction stage and their introduction vectors, to their invasive success, focusing on the main NIS of seaweeds that can be currently found at the northwest of the Iberian Peninsula. Particularly, six NIS of seaweeds (*Sargassum muticum*, *Grateloupia turuturu*, *Asparagopsis armata* (including *Falkenbergia rufolanosa* stage), *Undaria pinnatifida*, *Codium fragile* subsp. *fragile* and *Colpomenia peregrina*) were previously selected and sampled – based on DAFOR (Dominant, Abundant, Frequent, Occasional or Rare) cover ranges scale – along intertidal sites located within the northwest of the Iberian Peninsula. As a result of the sampling, the distribution of those seaweeds has been updated for the area, producing a hotspot map of their presence and relative abundance. Furthermore, the environmental management of NIS was addressed, as well as their potential uses such as biofertilizers, bioactivities, feed and food, aiming to show how these species, which are jeopardizing the ecosystem, can be an excellent environmental resource of bioactive compounds with high industrial potential and high socio-economic revenue.

Keywords: Environmental Resources; Invasive Marine Macroalgae; Non-Indigenous-Species; Seaweeds

Resumo

As espécies não nativas (ENN) podem ser definidas como espécies que colonizaram novas áreas geográficas onde não estavam presentes anteriormente. O seu crescente aparecimento tem causado problemas ecológicos e económicos consideráveis a nível mundial. Neste âmbito, as invasões biológicas e o sucesso dessas invasões têm sido um tema importante nas últimas décadas. Centenas de ENN marinhas têm sido introduzidas em todo o mundo e macroalgas marinhas são uma parte significativa delas. Estudos realizados em todo o mundo na última década têm relatado cerca de 250 espécies de macroalgas introduzidas. O nordeste do Oceano Atlântico é especialmente conhecido por albergar muitas espécies introduzidas e mais de 100 espécies de macroalgas introduzidas estão relatadas na Europa. Esta tese considerará os processos de invasão de macroalgas, desde a fase de introdução e de seus vectores de introdução, ao seu sucesso invasivo, com foco nas principais ENN de macroalgas que podem ser encontradas no noroeste da Península Ibérica. Particularmente, seis ENN de macroalgas (*Sargassum muticum*, *Grateloupia turuturu*, *Asparagopsis armata* (incluindo a sua fase *Falkenbergia rufolanosa*), *Undaria pinnatifida*, *Codium fragile* subsp. *fragile* e *Colpomenia peregrina*) foram previamente seleccionadas e amostradas - com base na escala de cobertura DAFOR (Dominante, Abundante, Frequente, Ocasional ou Rara) – no espaço entre marés e ao longo de praias localizadas no noroeste da Península Ibérica. Como resultado da amostragem, a distribuição dessas macroalgas foi actualizada para a área de estudo, produzindo um mapa da sua presença e abundância relativa. Além disso, a gestão ambiental das ENN foi abordada, bem como seus potenciais usos, nomeadamente como biofertilizantes, alimentos, rações, compostos bioactivos, com o objectivo de mostrar como essas espécies, que estão comprometendo o ecossistema, podem ser um excelente recurso ambiental de compostos bioativos com alto potencial industrial e valor socioeconómico.

Palavras-chave: Recursos ambientais; Macroalgas Marinhas Invasoras; Espécies não nativas; Macroalgas

‘Ecology and spirituality are fundamentally connected. Because deep ecological awareness, ultimately, is spiritual awareness’

- Fritjoy Capra

1. Introduction

Non-indigenous species (NIS) can be defined as species that colonized new geographical areas where they were not present previously; that is, NIS are species that present new geographical ranges beyond their native areas of distribution (e.g. Boudouresque and Verlaque 2002, Vaz-Pinto et al. 2014). Several terms have been used by different authors to refer NIS, including exotic, alien, non-native, introduced or invasive (Trowbridge 2006). On one hand, not all introduced species may have an invasive impact but, on the other hand, invasive species may have multiple impacts on the diversity and ecosystem functioning of native communities. Furthermore, they also can cause negative economic impacts worldwide; actually, species invasions have been known as the second most serious threats to ecosystems and its biodiversity (Nyberg 2007).

Human interferences have been pointed out to be the cause of species colonization at new areas where they could never have been reached naturally. Moreover, the success of an introduced species in becoming established and then invasive happens mostly in communities that have previously been altered by human activities (Hobbs and Huenneke 1992, Bulleri et al. 2010). The introduction and subsequent spread of seaweeds (i.e. marine macroalgae) can be resultant from several vectors, including the transoceanic and interoceanic transfer of Pacific oysters with macroalgal epibionts, the local and global movements of ships with macroalgae fouling the hulls or in the ballast water, or due to constructions like canals or artificial waterways (Trowbridge 2006, Nyberg 2007, Vaz-Pinto et al. 2014).

Biological invasion and invaders success has been a hot topic during the last decades. It started with terrestrial and freshwater systems but the importance of marine systems lead to intensive studies for the last two decades (Nyberg 2007, Grosholz 2002).

Although some non-indigenous species cause no harm and they can have favorable impacts on the new ecosystem and its native species but the rest may become invasive. Further and deeper research is needed to fully understand invasion and its negative impacts in order to manage, prevent and hopefully reverse them (Schaffelke et al. 2006) which proposes the question that whether these impacts can ever be reversed or the population can reach a new equilibrium after the establishment (Zavaleta et al. 2001, Nyberg 2007).

1.1. The invasion process

Species invasion from its native range to the recipient area happens in four stages:

1. The organism moves from its native range by a vector and transported to the recipient area (Introduction*);

2. The organism is present in the recipient area and at least one individual reproduces there;

3. The population establishes temporary in the new area and grows to the minimum viable rate (Establishment);

4. The population colonizes permanently in the area and causes negative impacts (Invasion) (Nyberg 2007).

* The introduction phase can be both intentional and unintentional.

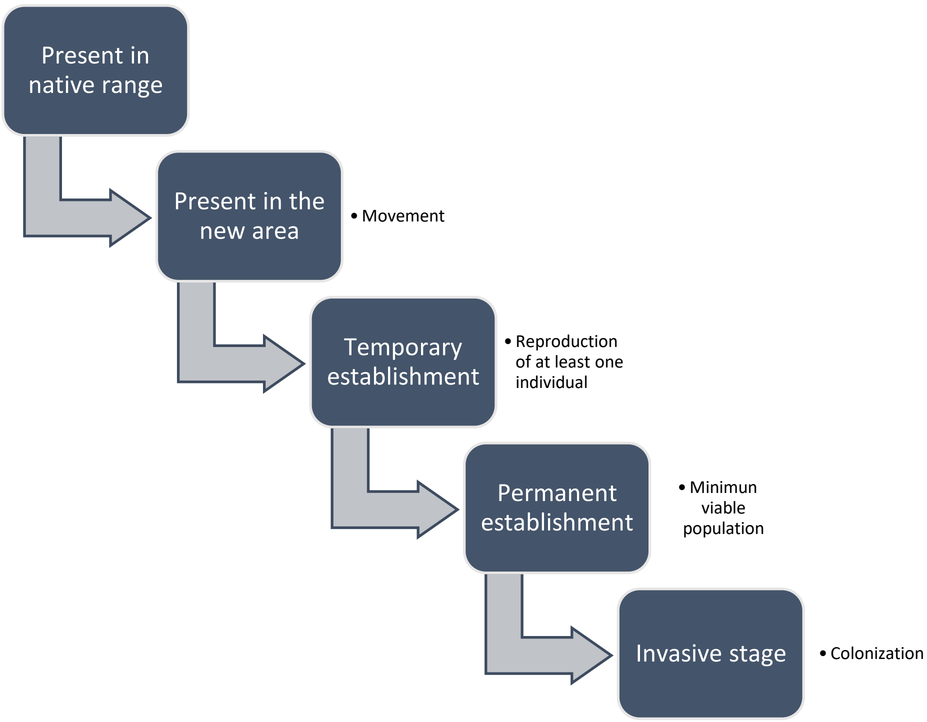


Figure 1. Steps and stages of an idealized species invasion. Modified from Heger and Trepl (2003).

At the introduction phase, the key point in the successful exchange between areas, i.e., between the native area and the new area, is for the species to finding suitable habitat with similar nutrient regimes, salinity, temperature and light. Then, to get to the establishment phase, it is vital that at least one of the introduced individuals reproduces and by the time that this new population gets to the point of self sustain it will be permanently established (Boudouresque and Verlaque 2002, Nyberg 2007) (Fig. 1).

There are a number of different factors affecting the success of invasion such as the species biology, the invasiveness of the community and propagule pressure (Lonsdale 1999). Some specific characteristics like size, propagules number and growth pattern which are directly affected by species life history traits can modify invasion and their importance differs in different invasion states (Nyberg 2007). In areas with different natural aspects comparing to the native ones, it is essential for species survival that they hold wide environmental tolerance in order to be able to tolerate environmental stress like fluctuations and extremes (Boudouresque and Verlaque 2002, Nyberg 2007).

1.2. Vectors of introduction

As species move from their native geographical range to a new colonized area, different vectors can be responsible for species introductions. In many cases, it is not likely to find the responsible vector for a particular introduction (Trowbridge 2006), although it can be done in some extent within some uncertainty levels (Tab. 1).

In Europe, a number of seaweed introductions have been related to the import business of juvenile Pacific oysters *Crassostrea gigas* (Verlaque 2001, Trowbridge 2006). Macroalgal propagules can be attached to oyster shells, being thus able to be carried from their origin to new places. In a study done by Ribera (1995) it was concluded that out of 44 species that have been introduced to the Mediterranean Sea, almost 10 were by oyster transportation.

Species can also be attached to the oceanic vessels and then be transported to new places. In areas with a high coastal traffic the hull of the floating structures can play an important role in transporting species (Vaz-Pinto et al. 2012). Although it is a very important source of species introduction, it is difficult and costly to control (Trowbridge 2006). It has been estimated by Ribera (1995) that approximately 39 species were transported this way. Studies on boat hulls have shown that mostly opportunistic and cosmopolitan species where subject to this transportation vector (Vaz-Pinto et al. 2012).

Table 1. Vectors of species introductions (adapted from the Invasive Pathways team; Final report; Campbell and Penny 2003).

Man Made Invasion Pathways	Transportation	Boat hull/trailer fouling
		Ship ballast water release
		Transportation of cargo
	Living Industries	Landscapeing/horticulture
		Agriculture
		Aquaculture
		Aquarium/pet trade
		Live seafood trade
	Miscellaneous Pathways	Plant and animal facilities
		Intentional release/stocking
		Biological control
		Government programs
		Release for religous/cultural reasons

Some species are able to survive and be transported in ballast water tanks or ballast rocks (Trowbridge 2006). From all the studies done only cosmopolitan species seem to be subject to this vector (Vaz-Pinto et al. 2012).

Also by shipping, fresh seaweeds are sometimes used to pack live seafood or bait, which is thrown afterwards into the water and then can spread in the new area (Trowbridge 2006). *Fucus spiralis* (Phaeophyceae) and *Polysiphonia fucoides* (Rhodophyta) are believed to have been introduced by this vector into the coasts of France (Verlaque and Rioulla 1989).

Species transportation can occur also on the fishing gear such as ropes, nets, buoys or boat anchors. These materials can shelter numerous propagules of different seaweeds and, when cleaned in a new area, the seaweeds attached to it can disperse there (Trowbridge 2006, Vaz-Pinto et al. 2014).

With the rapid growth of the human waste, different types of waste debris such as plastics can be easily moved by wind and currents, and this debris can represent another source of transport for species that are attached to it into new geographical areas (Trowbridge 2006).

As humans commonly remove geographical barriers by constructing canals and waterways, this can be an important source of species dispersal into new areas. A great example is the Suez Canal, where species had move mainly from the Red Sea to the Mediterranean Sea (Trowbridge 2006).

There are different species, including seaweeds, which can be sold in shops or websites to aquarium hobbyists. In this context, aquarium organisms can be released into the wild, unintentionally or not, but eventually spread into new areas (Vaz-Pinto et al. 2012). The growing need for food and resources can led to the aquaculture production of non-indigenous species, which ultimately may lead to species invasions (Trowbridge 2006).

Accidental escapes can happen in scientific research experiments, which can be another possible way in introduction (Trowbridge 2006). There are also some intentional introductions as Chemin (1930) was responsible for the introduction of *Asparagopsis armata* (Rhodophyta) to the Bay of Moraix, France (Vaz-Pinto et al. 2012).

1.3. Fate of an invasion

Species which are more efficient in using resources are more likely to be successful invaders as they grow faster than the native ones (Vaz-Pinto et al. 2012). Although species fitness is an important factor and can explain the success in some invasion cases, it is not enough to explain other outcomes. Many theories on species invasion agree that there is a fundamental difference between a successful invader and a native species (Daehler 2003). If an introduced species can invade new areas, this probably indicates that the introduced species is fulfilling empty niches in the new environment or is creating itself a new niche; ultimately the invasive species is a superior competitor, utilizing resources and responding to disturbance better than the existing native species (Myers and Bazely 2003).

An important factor to consider is the ecosystems` susceptibility to invasion. Gollasch and Leppakoski (1999) showed that disturbed environments are more likely to be affected by invasions. It also has been suggested that in communities with higher diversity, the efficiency in using resources is higher and as a result, the available niche is lower, which would decrease the probability of a successful invasion (Vaz-Pinto et al. 2012). The Diversity Resistance Hypothesis indicates that the chances for species invasions are higher in communities with less diversity (Nyberg 2007). Moreover, environmental resources are key factors on determining a successful invasion. Davis et al. (2000) introduced the Fluctuating Resources Theory, suggesting that, in a case of increasing availability of resources, the community becomes vulnerable to invasion. For example, in a study by Vaz-Pinto et al. (2012) done on the invasion of *Sargassum muticum* (Phaeophyceae) it was found that, during the process of invasion, the importance of propagule pressure was highest in the settlement period, whereas the success of colonization was mostly based on the availability of resources.

1.4. Introduced macroalgae

Hundreds of marine NIS have been introduced around the world and seaweeds are a significant part of them. They can cause substantial ecological and economic impacts (Schaffelke et al. 2006). The northeast part of the Atlantic is especially known to be home to many introduced species. As different consequences of their introduction are difficult to predict (Rueness 1989) further studies on their ecology, dispersal and use are essential. From many studies conducted worldwide in the last decade around 250 species of introduced macroalgae has been reported (Trowbridge 2006). There are 113 introduced species of marine macroalgae in Europe (Wallentinus 2002), 21 were found in French Atlantic coast (Goulletguer et al. 2002), 20 were reported on the coast of the North Sea (Nyberg 2007) and 83 species have been found in the Mediterranean Sea (Zenetos et al. 2005). Sorte et al. (2010)

explained the spread towards the pole as a result of the climate change but as a general fact there are more human related vectors which play role. In fact, in last two centuries, European region (Atlantic Ocean and Mediterranean Sea) has been hardly affected by the increasing number of introduced seaweeds at an increasing spreading rate (Mineur et al. 2010, Vaz-Pinto et al. 2012).

1.5. Known impacts of introduced macroalgae

Non-indigenous species are considered to have many major negative impacts both on biodiversity and also ecological processes (Pejchar and Mooney 2009). The importance of invasion by large seaweeds is the fact that they can damage the structure of an ecosystem and alter its function (Schaffelke et al. 2006). Some of the most important impacts caused by NIS species are enumerated below.

1.5.1. Effects on genetic level

Following an invasion it is vital to count for any possible genetic and evolutionary changes (Booth et al. 2007). With hybridization there is the possibility of losing the native genotypes and in some cases it can also lead to speciation (Vaz-Pinto et al. 2012).

1.5.2. Competition for limiting resources

Invaders compete for light, nutrients and ground with native species which leads to their growth reduction and reduce their reproduction. The availability of light which is a vital resource for the function of marine ecosystem can be altered as a result of seaweed invasion affecting other species and changing the nutrient cycle (Vaz-Pinto et al. 2012). The impacts caused by canopy forming species is more than just limiting light, they also affect sedimentation and the movement of water (Jenkins et al. 1999).

1.5.3. Altering the population dynamics

Being described as community dominance species non-indigenous macroalgae (Vaz-Pinto et al. 2012) change communities' abundance, structure, growth rate and distribution and might eventually make them extinct (Nyberg 2007).

1.5.4. Economic impacts

Generally, many cases of invasion are a result and consequence of an economic activity. It goes beyond just the costs and damages (Perrings et al. 2002) and they enforce real costs on society in two major areas: human disease and as pests and pathogens affecting agriculture and fisheries (Perrings et al. 2000). On the other side, there is all the costs which rise from the management of an invasion. Actually, using instruments and human labor to control or eradicate invasive species can be costly to governments worldwide.

1.5.5. Habitat modification

Non-indigenous species can have positive or negative impacts on habitat both directly or indirectly by acting as ecosystem engineers (Nyberg 2007). These impacts can happen in small or large scales.

- Changing the substrate. Animals like crabs or mussels dig holes in sediments which might lead to erosion of shore banks. Digging can improve denitrification by increasing bioturbation which in anoxic sediments will lead to oxygenation. Plants can also have a significant impact. Their roots can protect sediments from the disturbances caused by waves. They can also improve microbial mineralization and increase the oxygen content (Nyberg 2007).

- Changing habitat architecture. Colonization by large sessile organisms can affect the conditions of the substrate by changing the water movements in areas which were not vegetated before (Nyberg 2007).

- Changing foraging behaviors. Increase in the density of algae on the seabed can cause negative impacts for deposit feeders and other organisms by reducing the amount of suspended particles. The negative impacts caused by dense coverage leads to hard access to the sediments and in return would change the foraging (Nyberg 2007).

-Changing in the light climate. Another serious concern is the shading effect caused by the establishment of large amounts of algae. Even though in some cases the filtering capacity of some species have a positive effect and clears the turbid water (Nyberg 2007).

- Changing the availability of nutrients. The availability of nutrients can increase by some long lived species that store nutrients for long periods. On the other hand in some cases the increase in nutrient uptake by some introduced species have a negative impact on the availability of nutrients (Nyberg 2007).

- Changing toxic compounds. This can be caused by some species that have toxic secondary metabolites which can limit larvae from settling (Nyberg 2007).

Ecosystem processes are defined as the functions of interactions between different species and is not just due to the present or absence of them. This fact enables us to truly predict the potential impacts of the introduced non-indigenous species (Vaz-Pinto et al. 2012).

1.6. Objectives and structure of the thesis

The general objective of this thesis was to investigate the presence and relative abundance of the main NIS seaweeds that can be currently found at the northwest of the Iberian Peninsula.

In the introduction, the general information about invasive species, vectors of introduction and their fate plus the possible impacts were outlined. Next part is focused on the study area, sampling protocol and information on six NIS seaweeds that can be currently found at the northwest of the Iberian Peninsula (*Sargassum muticum*, *Grateloupia turuturu*, *Asparagopsis armata* (including *Falkenbergia rufolanosa* stage), *Undaria pinnatifida*, *Codium fragile* subsp. *fragile* and *Colpomenia peregrina*). The results of sampling have been organized for each species separately in order to find the existing hotspot areas. Finally, the environmental management of NIS was addressed, as well as their potential uses such as biofertilizers, bioactivities, feed and food, aiming to show how these species, which are jeopardizing the ecosystem, can be an excellent environmental resource of bioactive compounds with high industrial potential and high socio-economic income.

2. Materials and methods

2.1. Study area

This work was conducted at the coastal shoreline of the northwestern Iberian Peninsula, namely along intertidal rocky shore sites located in Spain (Galicia) and Portugal (Fig. 2).

Figure 2. Study area (red box) located at the northwest of the Iberian Peninsula, encompassing Spanish and Portuguese intertidal rocky shore sites.

2.2. Sampling protocol

Each intertidal rocky shore site was sampled across two representative subset areas; each area corresponded to 30 m width (parallel to waterline) and variable length (perpendicular to the waterline) according to the length of each site. Coordinates were taken from the middle point of each area, as well as from the land-based access point used for the site, using a GPS (Global Position System) device. Sampled areas were walked through in a zig zag way in order to record the presence/absence of non-indigenous species of marine macroalgae (Fig. 3).

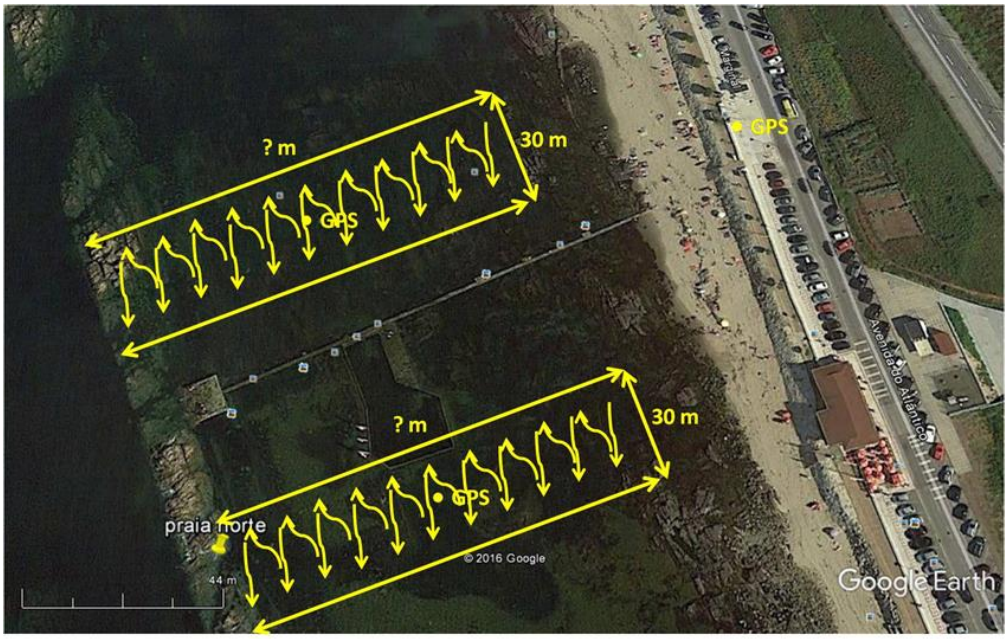


Figure 3. Sampling design used to record non-indigenous species of macroalgae at intertidal rocky shore sites. Each site is sampled from two subset areas, having each area 30 m width (parallel to waterline) and variable length (perpendicular to the waterline) according to the length of each site. Sites' access point and middle point of each sampled area is recorded by GPS. Sampled areas are walked though in a zig zag way to record the presence/absence of targeted species according to the DAFOR cover range scale (see Tab. 2).

Then, the presence of the non-indigenous species was classified according to the DAFOR (Dominant, Abundant, Frequent, Occasional, and Rare) cover range scale (Tab. 2).

Table 2. DAFOR (Dominant, Abundant, Frequent, Occasional, and Rare) cover range scale. N = Not seen.

Cover category	Abbreviation	Percentage cover
Dominant	D	50 – 100 %
Abundant	A	30 – 50 %
Frequent	F	15 – 30 %
Occasional	O	5 – 15 %
Rare	R	< 5 %
Not seen	N	0%

The type of habitat of each targeted species (rock pools and/or emergent substrate) was recorded. Simultaneously, information such as the type of substrate, weather conditions and time spending in sampling was also recorded in the field sampling sheet for each area (Appendix 1). Samplings occurred during spring low tides, from the end of March to the end of July 2017.

2.2.1 Laboratory identification of *C. fragile* subsp. *fragile*

Two native species of *Codium* (*C. tomentosum* and *C. vermilara)* can be found in the study area, plus the non-indigenous *C. fragile* subsp. *fragile.* The presence of pointed mucronate utricles is an indicator of *C. fragile* subsp. *fragile* (Silva 1955, Rojo et al. 2014) (Fig. 4).

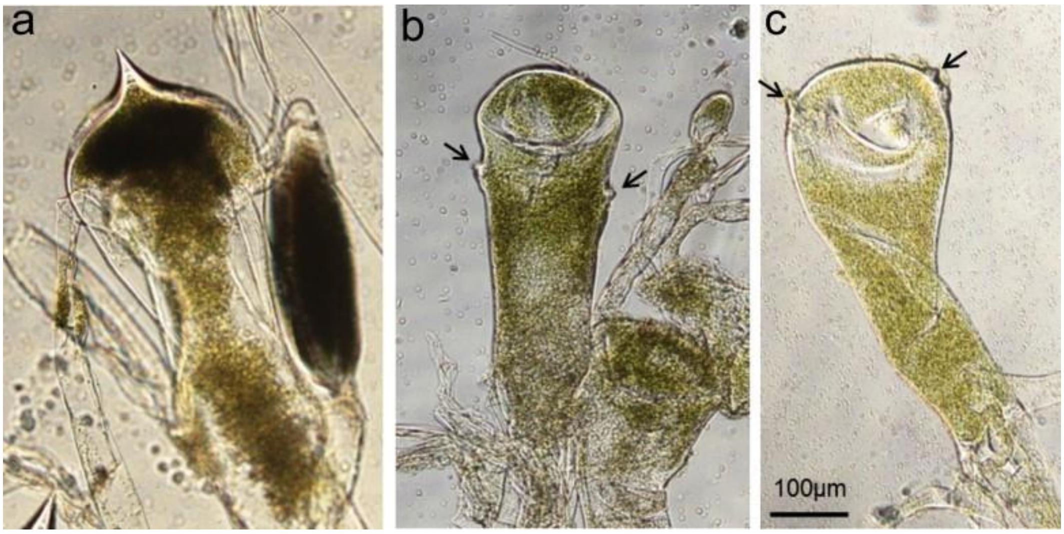


Figure 4. Illustration showing different utricle shapes and hair scars positions (indicated with arrows) of each *Codium* species taken with an Olympus Bx51 microscope: a) mucronate *Codium fragile* subsp. *fragile*; b) *Codium tomentosum*; c) *Codium vermilara* (according to Rojo et al, 2014).

Therefore, whenever *Codium* spp. was present in a sampled area, 50 (or, if abundant, 100) apices (small pieces of the thallus branch tip with about 3-4 cm) were cut randomly from different specimens, labelled in plastic bags and taken in an ice cooler to the laboratory for species identification. Samples were stored frozen at - 18 ^{0}C until examination (Rojo et al. 2014).

2.3. Sampled non-indigenous species of macroalgae

Six non-indigenous species of marine macroalgae were previously selected and sampled along the study area, namely one green species (Chlorophyta) – *Codium fragile* subsp. *fragile* (see 2.3.1), three brown species (Ochrophyta, Phaeophyceae) – *Sargassum muticum* (see 2.3.2), *Undaria pinnatifida* (see 2.3.3) and *Colpomenia peregrina* (see 2.3.4) and two Red species (Rhodophyta) – *Asparagopsis armata* (including *Falkenbergia rufolanosa* stage) (see 2.3.5) and *Grateloupia turuturu* (see 2.3.6).

2.3.1. *Codium fragile* (Suringar) Hariot (Chlorophyta) subsp. *fragile*

Codium fragile subsp. *fragile* (Fig. 5) is a dark-green algae which grows up to 15-100 cm. In wave-exposed areas it tends to be shorter due to fragmentation (D' Amours and Scheibling 2007). The segments look like dark green fingers. Its holdfast is a broad, sponge-like cushion of tissue. The tips of segments are blunt and the surface is soft, so it is sometimes mistaken as a sponge. Its body consists of interwoven, filamentous cells with incomplete cross-walls forming the inner part of the branches (Pereira 2017). This large branching species weigh can reach up to 3.5 kg (Trowbridge 1999). This species reproduces sexually and vegetatively by thalli fragmentation (Begin and Scheibling 2003).

Figure 5. *Codium fragile* (image source: MACOI website).

This species is native in Japan and Korea (Provan et al. 2005). It was first seen in the Netherlands in 1900 and by 1957 spread to the northeast part of the Atlantic and Mediterranean coast (Trowbridge 1999). In 1975, Dromgoole documented its presence in New Zealand. It has been noted that compared to populations recorded from New England, USA, Nova Scotia and Canada; European populations are much smaller (Chapman 1999).

Its tolerance to variety of habitats with different salinity and temperature ranges makes it successful in the introduced area. The species can travel long distances on waves reaching new locations. It is mostly present in harbors and bays which improve their chance of dispersal by human activities (Begin and Scheibling 2003).

C. fragile subsp. *fragile* has a negative economic impact in aquaculture (Provan et al. 2005). It’s overgrow can result in suppressing oyster beds and because of that it has been called as ‘oyster thief’ by Naylor et al. (2001). It is included in the list of five top risk species in Europe (Nyberg and Wallentinus 2005). Unfortunately, there are not many options to manage this species because the mechanical and hand removal is only temporary and expensive and the population grows back again. Chemical removal could be also an alternative although it does more harm than good. The best option is to prevent the spread of the species by the means of control (quarantine) and education (Trowbridge 1999).

2.3.2 *Sargassum muticum* (Yendo) Fensholt (Ochrophyta, Phaeophyceae)

Sargassum muticum (Fig. 6) is a large brown seaweed, varying in color from dark brown to pale, yellowish brown depending on the season and the growing conditions. *S. muticum* has regularly alternating lateral shoots or branches, on a central perennial stem. It attaches to the substrate with a disc-shaped holdfast. It has numerous small 2–3 mm round or pear-shaped air-bladders which sit on small stems and cause the alga to stand upright in the water or float if parts of the alga are detached from the basal stem (Pereira 2010).

S. muticum is a native species from Japan and China which was first found in English Channel in late 1960s (Farnham et al. 1980) and Farnham et al. (1973) noted that it was seen in Bembridge, Isle of Wight in 1971 and which was assumed to have dispersed from France

(Farnham et al. 1973). It was then spread to other areas of the Mediterranean Sea and Atlantic coast and now is present in Portugal, Spain, France, Denmark, Sweden, Belgium and Netherlands (Critchley 1983, Rueness 1989).

Figure 6. *Sargassum muticum* (image source: MACOI website).

The vector responsible for the introduction of the species into France is known to be an unintentional transport by Oysters. After that, it was assumed to be transported by natural means to other areas. Dispersal of spores also might occur due to transport of ballast water, rafting and floating (Critchley et al. 1990).

This species has a high reproduction rate (Norton and Deysher 1989) and rapid growth rate (Hales and Fletcher 1989) which makes it succeed after introduction. It produces fertile receptacles which can float and survive for 3 months after being cast off in summer (Farnham et al. 1980).

One of the main impacts resulting from the introduction of *S. muticum* is displacement of native species which is caused by its overgrowing which covers the surface and does not allow sunlight to get to the underlying species by shading (Critchley et al. 1986). Givernaud et al. (1991) reported a replacement of *Saccharina latissima* (formerly *Laminaria saccharina*) and *Zostera marina* in Atlantic coast of France. It can also cause some negative economic impacts specially when it is detached and is floating in waterways. It intervenes with recreational activities (Farnham 1980), causing problems for fishermen by attaching to their nets and blocks the propellers (Critchley et al. 1986).

Methods of removal to control *S. muticum* have been tried. Hand removal is very time consuming and probably needs to be repeated forever (Farnham 1980). Herbicides were used as a chemical method but failed. Until now there is no permanent method found for removing this species though cutting is still the preferred one (Critchley et al. 1986).

2.3.3 *Undaria pinnatifida* (Harvey) Suringar (Ochrophyta, Phaeophyceae)

Undaria pinnatifida (Fig. 7) is a brown seaweed, native in Japan, China and Korea. It grows up to 1.5m in nature and 3m in aquaculture (Silva et al. 2002). The blade is lanceolate and broad with a prominent midrib, and translucent with color ranging from green to yellowish-brown to dark brown (Pereira 2017c). It can tolerate many different range of environmental conditions, in temperatures from 0-27°C (Hay 1990) and salinities as low as 20 PSU. It is mostly present is sheltered areas such as harbors (Zabin et al. 2009). Its sporophytes can grow on any available substrate and on many different organisms (Silva et al. 2002). Many of the areas colonized by *U. pinnatifida* are close to urban sewage emissions, though it can also grow in places with organic pollution (Cecere et al. 2000).

Figure 7. *Undaria pinnatifida* (image source: MACOI website).

U. pinnatifida was first seen in Europe in 1971 in France (Boudouresque et al. 1985) and was assumed to be transported with Pacific oysters (Floc'h et al. 1991). It is now established in many places including Mediterranean Sea and the Atlantic coast, New Zealand, Argentina, United States and Mexico (Aguilar Rosas et al. 2004).

The vector responsible for the introduction of this species is mainly noted to be by boat fouling but accidental introduction by aquaculture is also documented (Silva et al. 2002, Thornber et al. 2004).

Their impact on native species and the ecosystem is more concerning when this species is present all year round (Zabin et al. 2009). Curiel et al. 1998 documented the negative impact of *U. pinnatifida* on native species richness and diversity by the means of competition.

2.3.4. *Colpomenia peregrina* Sauvageau (Ochrophyta, Phaeophyceae)

Colpomenia peregrina (Fig. 8) is annual brown algae, has a globular shape and is found in temperate waters, yellowish-brown color, fixed to the substrate by filamentous rhizoids. Internally, the thallus is characterized by an outer cortex composed of small colored cells and inner pith composed of large pigmented cells (Lee et al. 2014, Pereira 2015). It has a vast tolerance to environmental conditions. It grows in the salinity range of 15 to 30 PSU (practical salinity unit) and temperatures between 13 to 20 °C, which makes it successful in establishing in new areas (Lee et al. 2014).

Figure 8. *Colpomenia peregrina* (image source: MACOI website).

It is usually established in intertidal zone mostly on rock or other surfaces like oyster shells (Pena and Barbara 2008). It is native to northwest Pacific and it was first seen in Europe in 1908 (Abbott et al. 1992). Then it spread throughout Europe very fast and is documented in Holland, Denmark, the Mediterranean and Norway. In the northwest Pacific, it can be seen all year round especially in the shallow subtidal zone (Green et al. 2012). Blackler 1967 noted that the introduction of *C. peregrina* to France caused a huge negative economic impact on the oyster industry in this country.

2.3.5 *Asparagopsis armata* Harvey (Rhodophyta)

Asparagopsis armata (Fig. 9) is a purplish-red seaweed with diplohaplontic and heteromorphic life cycle and it can be found mainly in summer and autumn (Kraan and Barrington 2005). Its native range includes Australia, Tasmania and New Zealand (Horridge 1951). In 1920s it was introduced in the Atlantic Ocean and Mediterranean Sea and after that was recorded from all over Europe like France, Spain, Portugal, down to Canary Islands and Senegal and south and west coast of England and Ireland (Kraan and Barrington 2005). The probable vector of introduction for *Asparagopsis armata* into Europe was noted to be in association with oysters' transportation and then rafting and floating into other areas like England and Ireland (Feldman and Feldman 1942).

The key characteristics of this species which makes it easier to recognize is the harpoon like hooks (it is commonly called as harpoon weed) and it is also known to be associated with macroalgal species from the genera *Ulva*. Gametophyte plants occur from June or July to August or September in North-eastern part of Europe (Pereira 2016).

A. armata is an opportunistic species with a fast growth rate. These characteristics combined with the lack of predators made the introduction of the species a success. *A. armata* also produces some toxic substances which affects other organisms like injuring fishes (Maggs and Stegenga 1998). The factors which most likely affect its distribution are temperature and light, which are the main requirements for its growth and reproduction (Santelices 1990). It has also been documented that floating objects can spread these species by providing a surface for the hooked branches to attach to (Farnham 1980).

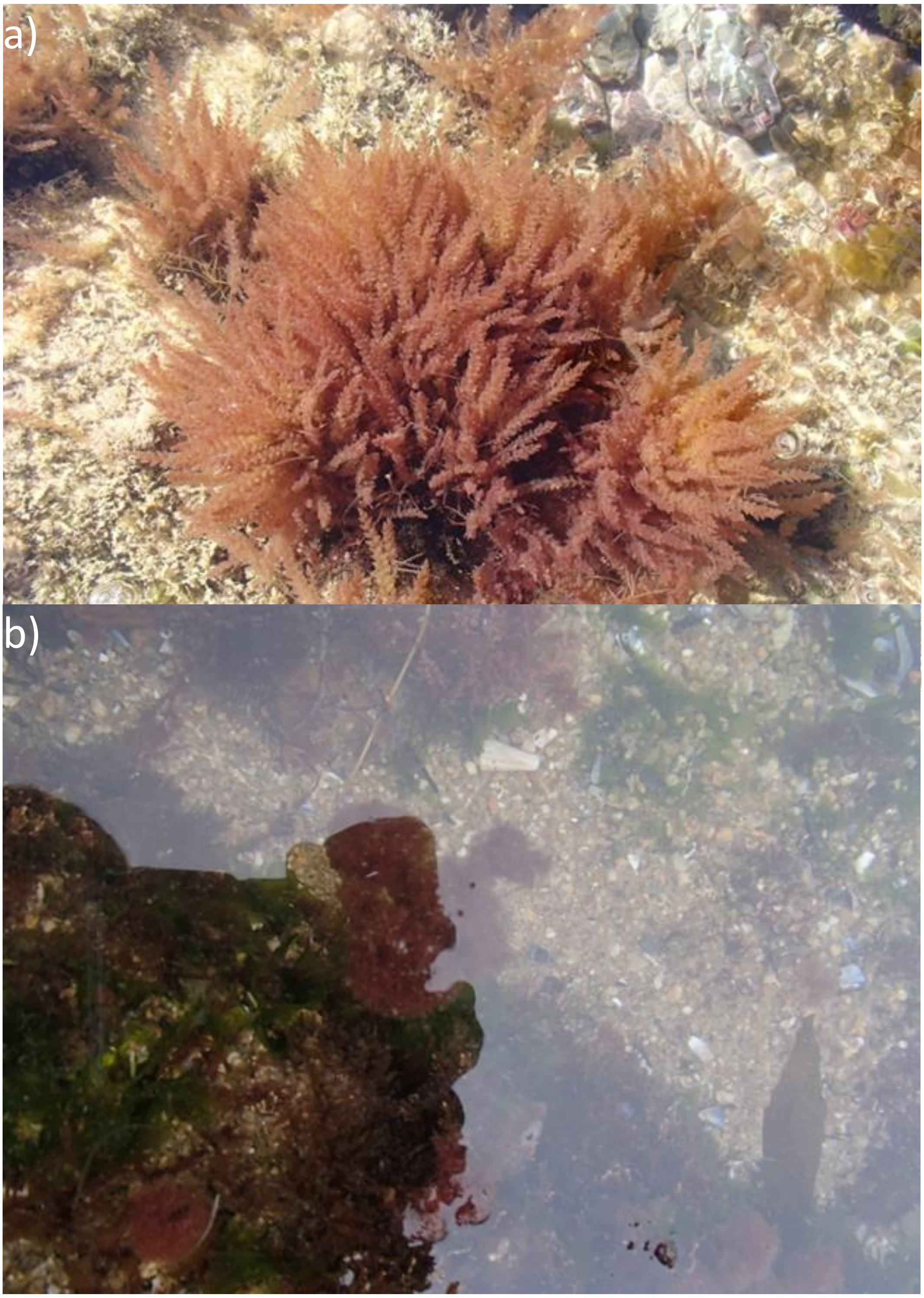


Figure 9. *Asparagopsis armata.* a) gamethophyte; b) tetrasporophyte (*Falkenbergia rufolanosa* stage) (image source: MACOI website).

This seaweed produces natural and valuable ingredients that can be used in medicine and for cosmetics (Schuenhoff et al. 2006). These ingredients are used by the species as a chemical defense and are natural antibiotics (Steinberg et al. 2001).

2.3.6 *Grateloupia turuturu* Yamada (Rhodophyta)

Grateloupia turuturu (Fig. 10) is red seaweed that grows fast and reproduces by sexual (spores) and vegetative reproduction. Lives in shallow subtidal zones and low intertidal areas where it competes with the native seaweeds and is mostly successful (Barille-Boyer et al. 2004). It mostly grows on rocky areas even on small loose stones. Its maximum growth in length and biomass occurs in late summer and early autumn (Harlin and Villalard-Bohnsack 2001). The perfect environment for *G. turuturu* to grow is nutrient enriched water with salinity of 22 to 37 PSU and in a temperature range of 4 to 29 °C (Simon et al. 2001).

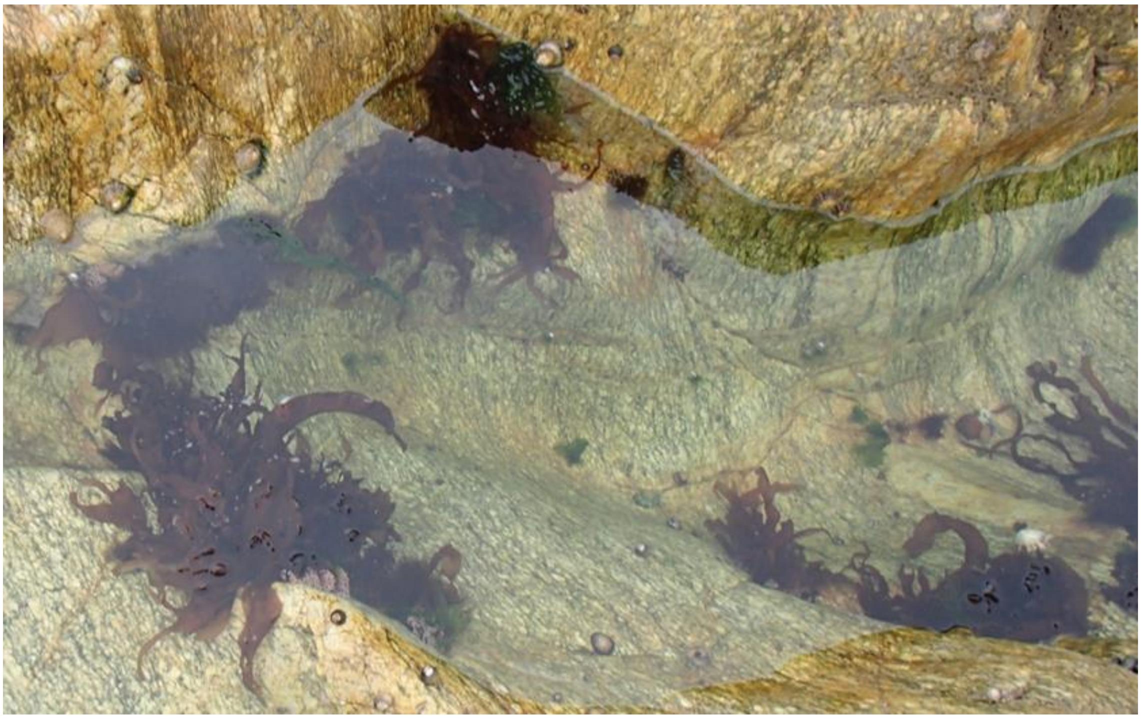


Figure 10. *Grateloupia turuturu* (image source: MACOI website).

G. turuturu is native from Pacific (China, Japan and Korea). In 1982 it was found in the Thau Lagoon in France and it is still present there, even though it was not considered invasive in the area (Verlaque et al. 2005). After two years, in 1984 it was observed close to an oyster farm in Milford Haven in Wales (Maggs and Stegenga 1998). By 1995 it was spread in many other areas like Portugal and Spain (Cremades et al. 2004).

G. turuturu is one of the five most threatening species with potential of becoming invasive because of its characteristics like its styles of reproduction, patterns of growth and physiological tolerance (Nyberg and Wallentinus 2005).

The vector of transport for this species is known to be the massive import of Japanese oysters from 1971 to 1976 (Verlaque 2001). The secondary transport was noted to be by boat to southern England (Farnham 1980). Since much of vectors responsible for the transportation of *G. turuturu* include aquaculture, deliberate transportation of shellfish and accidental transport by boats, reinforcement of legal responsibilities is crucial to scale down the possible introductions in the future. One of the main problematic vectors which has not been managed properly is hull fouling that can benefit from some monitoring agreements both in regional and international level. Having sanitary protocols in aquaculture appliances can prevent species transport *via* attaching to surfaces of shells. (Hewitt et al. 2007).

There are many possible negative impacts associated with the presence of this species. It prevents sunlight from reaching to understory vegetation due to its large stature (Simon et al. 2001). The only natural enemy mentioned in studies are *Lacuna vincta* which Villalard-Bohnsack and Harlin (1997) reported as an extensive grazer.

2.4. Data analysis

For each selected non-indigenous species, the following steps were followed.

Step 1. All data was organized according to the location of sites from north to south. Each sampled subset area of each site (a or b) was calculated based on the sampled measured length and its fixed width (=30m); i.e.:

$$\text{Sampled Subset Area}_{(\text{a or b})}\ (m^2) = \text{Width (fixed, 30m)} * \text{Length (variable, m)}$$

Step 2. An average of the percentage cover was attributed for each species, departing from the DAFOR cover range scale previously given for each species in the field (Tab. 3).

Table 3. DAFOR (Dominant, Abundant, Frequent, Occasional, and Rare) cover range scale and its respective average percentage cover. N = Not seen.

Cover category	Abbreviation	Percentage cover	Average of the Percentage cover
Dominant	D	50 – 100 %	75%
Abundant	A	30 – 50 %	40%
Frequent	F	15 – 30 %	22.5%
Occasional	O	5 – 15 %	10%
Rare	R	< 5 %	2.5%
Not seen	N	0%	0%

Step 3. Then, to calculate the actual coverage of each species the following formula was used:

Species cover area $_{(a\ or\ b)}$ (m^2) = Sampled Subset Area $_{(a\ or\ b)}$ (m^2) * average of the DAFOR percentage cover) / 100

After this phase, two cover area values for each site (one for each subset) were obtained. To reach a final value of cover area per species to represent each site, the average of the two subset cover areas was calculated by the following formula:

Species average cover area (m^2) = (Species cover area $_{(a)}$ (m^2) + Species cover area $_{(b)}$ (m^2)) / 2

All data from *Codium* is based on *Codium* spp., which was found on the field to be examined further in the laboratory. The laboratory work is still ongoing since each individual that was collected should be examined under microscope to determine whether it is the non-indigenous *Codium fragile* or not. Since it is not possible to have a complete data on *Codium fragile,* the analysis conducted regards solely *Codium* spp.

3. Results

A total of 110 sites were sampled at the coastal shoreline of the north-western Iberian Peninsula (with 2 subset areas sampled per site). 85 sites were located in Spain and 25 were in Portugal. The results obtained per each non-indigenous species are presented below.

3.1. *Codium fragile* subsp. *fragile*

In almost 64% of the sites, *Codium* was found both in Spain and Portugal. The final cover area varied but was mostly between 1-100 m^2 (Tab. 5).

Table 5. Summary of data on the number of sites for different average cover area ranges for *Codium* spp.

Cover area range (m^2)	Number of sites in Spain	Number of sites in Portugal	In Total
0	28	12	40
1-100	38	9	48
101-200	12	3	15
201-300	4	0	4
301-400	1	0	1
401-500	0	1	1
601-700	1	0	1

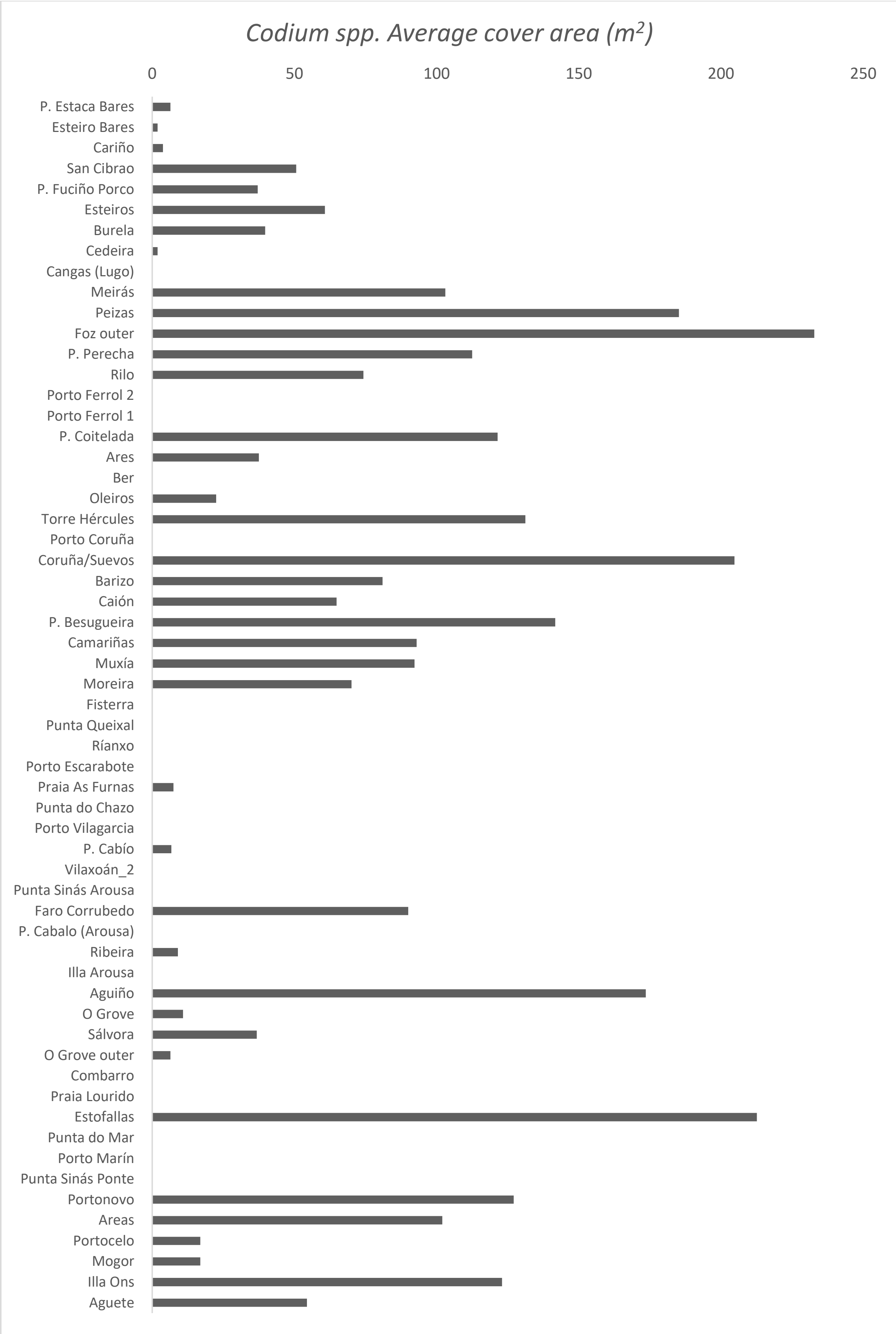

Codium spp. Average cover area (m2)
0
50
100
150
200
250
P. Estaca Bares
Esteiro Bares
Cariño
San Cibrao
P. Fuciño Porco
Esteiros
Burela
Cedeira
Cangas (Lugo)
Meirás
Peizas
Foz outer
P. Perecha
Rilo
Porto Ferrol 2
Porto Ferrol 1
P. Coitelada
Ares
Ber
Oleiros
Torre Hércules
Porto Coruña
Coruña/Suevos
Barizo
Caión
P. Besugueira
Camariñas
Muxía
Moreira
Fisterra
Punta Queixal
Ríanxo
Porto Escarabote
Praia As Furnas
Punta do Chazo
Porto Vilagarcia
P. Cabío
Vilaxoán_2
Punta Sinás Arousa
Faro Corrubedo
P. Cabalo (Arousa)
Ribeira
Illa Arousa
Aguiño
O Grove
Sálvora
O Grove outer
Combarro
Praia Lourido
Estofallas
Punta do Mar
Porto Marín
Punta Sinás Ponte
Portonovo
Areas
Portocelo
Mogor
Illa Ons
Aguete

Figure 11. (Continued)

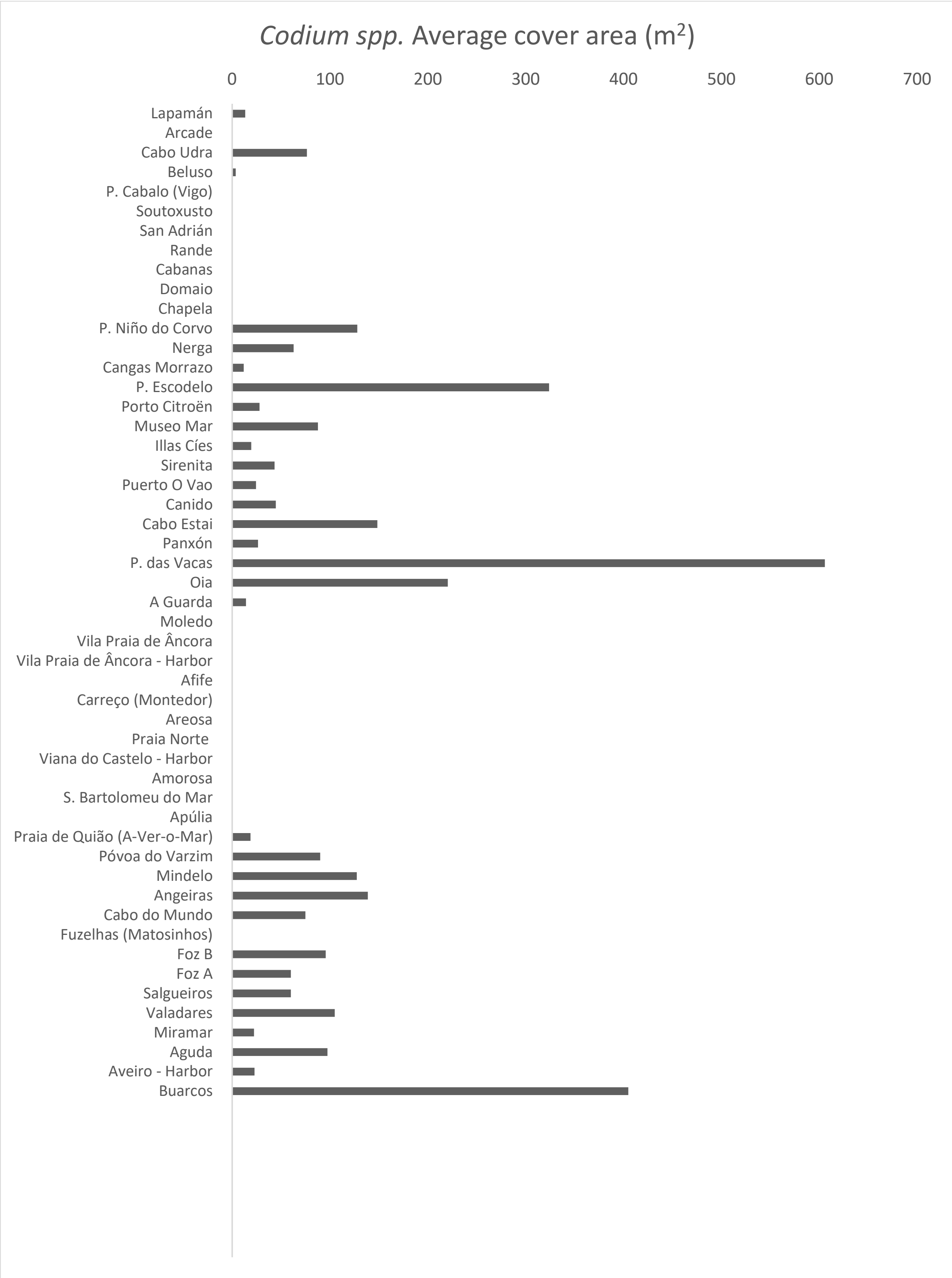


Figure 11. Average cover area of *Codium* spp. for 110 sites located in Portugal and Spain. Sites are shown from north to south.

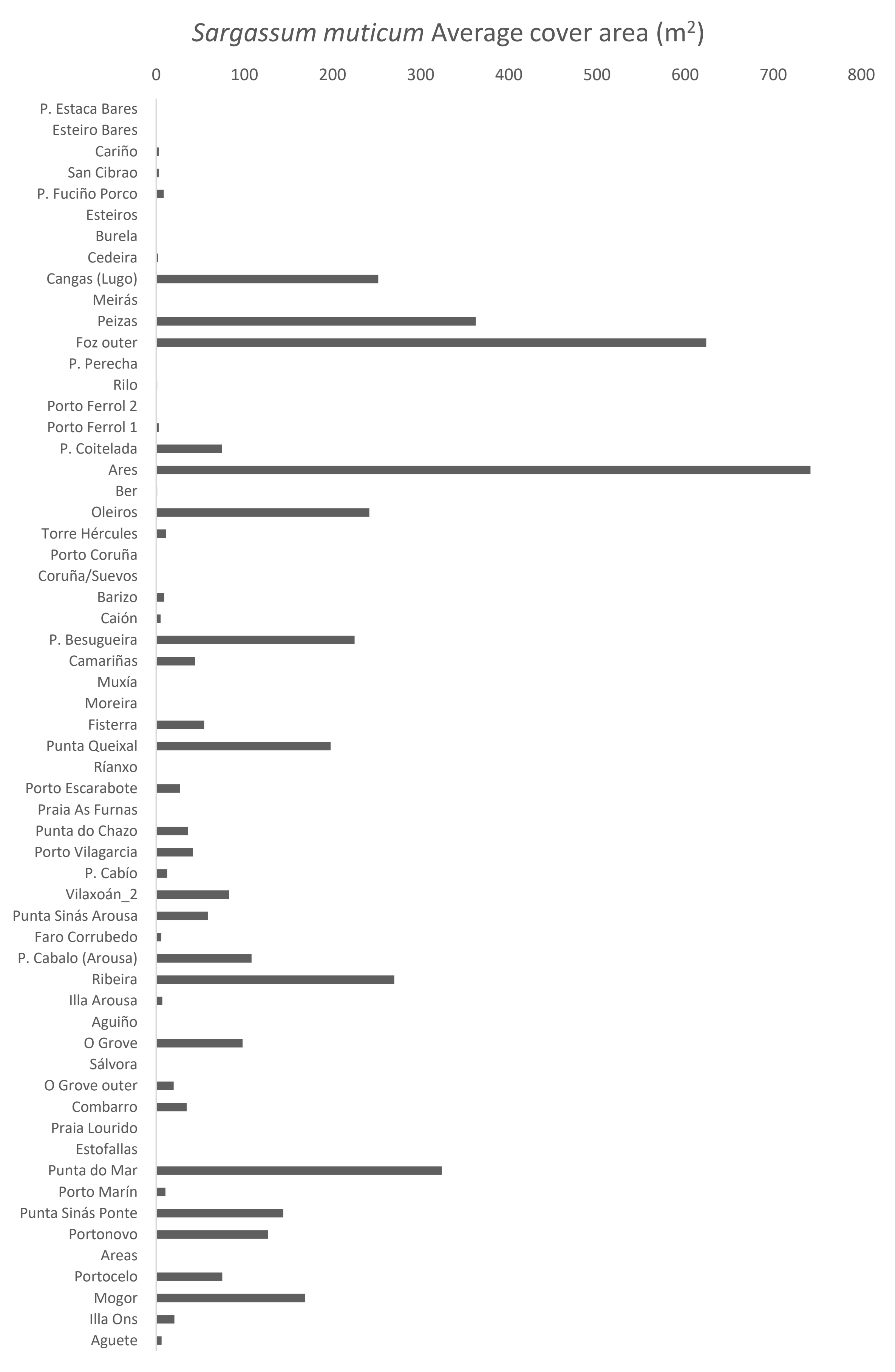
3.2. *Sargassum muticum*

Sargassum muticum was present in more than 70% of the sites both in Spain and Portugal. The average cover area for *S. muticum* varied between different sites but was mostly in 1-100 m^2 range (Tab. 6).

Table 6. Summary of data on the number of sites for different average cover area ranges for *Sargassum muticum*.

Cover area range (m^2)	Number of sites in Spain	Number of sites in Portugal	In Total
0	24	8	32
1-100	41	10	51
101-200	8	2	10
201-300	6	2	8
301-400	2	1	3
401-500	0	1	1
601-700	2	0	2
701-800	1	0	1
>1000	1	1	2

Data also shows some hot spot areas with 2 peaks in P. Niño do Corvo, Spain and Praia Norte, Portugal (Fig. 12). In Spanish sites Foz outer, Ares and Cangas Morrazo have also high coverage of *S. muticum.*



Sargassum muticum Average cover area (m2)
0
100
200
300
400
500
600
700
800
P. Estaca Bares
Esteiro Bares
Cariño
San Cibrao
P. Fuciño Porco
Esteiros
Burela
Cedeira
Cangas (Lugo)
Meirás
Peizas
Foz outer
P. Perecha
Rilo
Porto Ferrol 2
Porto Ferrol 1
P. Coitelada
Ares
Ber
Oleiros
Torre Hércules
Porto Coruña
Coruña/Suevos
Barizo
Caión
P. Besugueira
Camariñas
Muxía
Moreira
Fisterra
Punta Queixal
Ríanxo
Porto Escarabote
Praia As Furnas
Punta do Chazo
Porto Vilagarcia
P. Cabío
Vilaxoán_2
Punta Sinás Arousa
Faro Corrubedo
P. Cabalo (Arousa)
Ribeira
Illa Arousa
Aguiño
O Grove
Sálvora
O Grove outer
Combarro
Praia Lourido
Estofallas
Punta do Mar
Porto Marín
Punta Sinás Ponte
Portonovo
Areas
Portocelo
Mogor
Illa Ons
Aguete

Figure 12. (Continued)

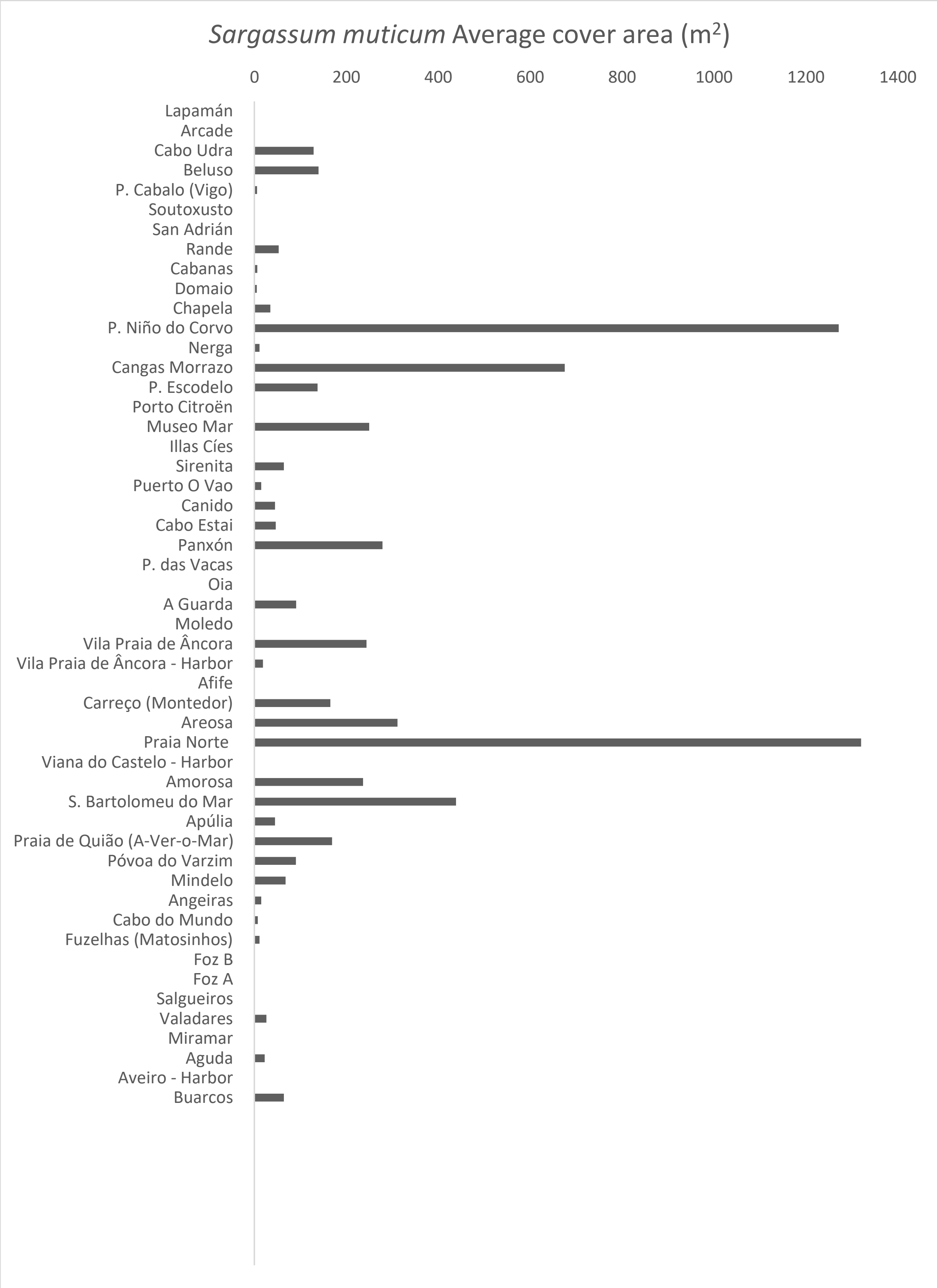


Figure 12. Average cover area of *Sargassum muticum* for 110 sites located in Portugal and Spain. Sites are shown from north to south.

3.3. *Undaria pinnatifida*

Undaria pinnatifida was only found in about 42% of the sites mostly in Spain. It was found in just 2 sites in Portugal with a coverage between 0-35 m^2. In Spain also the coverage was mostly between 1-100 m^2 (Tab. 7).

Table 7. Summary of data on the number of sites for different average cover area ranges for *Undaria pinnatifida*.

Cover area range (m^2)	Number of sites in Spain	Number of sites in Portugal	In Total
0	41	23	64
1-100	36	2	38
101-200	5	0	5
201-300	2	0	2
601-700	1	0	1

There was one hot spot point found in P. Niño do Corvo, Spain which showed considerably more coverage than all other sites with an average cover of 678 m^2 (Fig. 13).

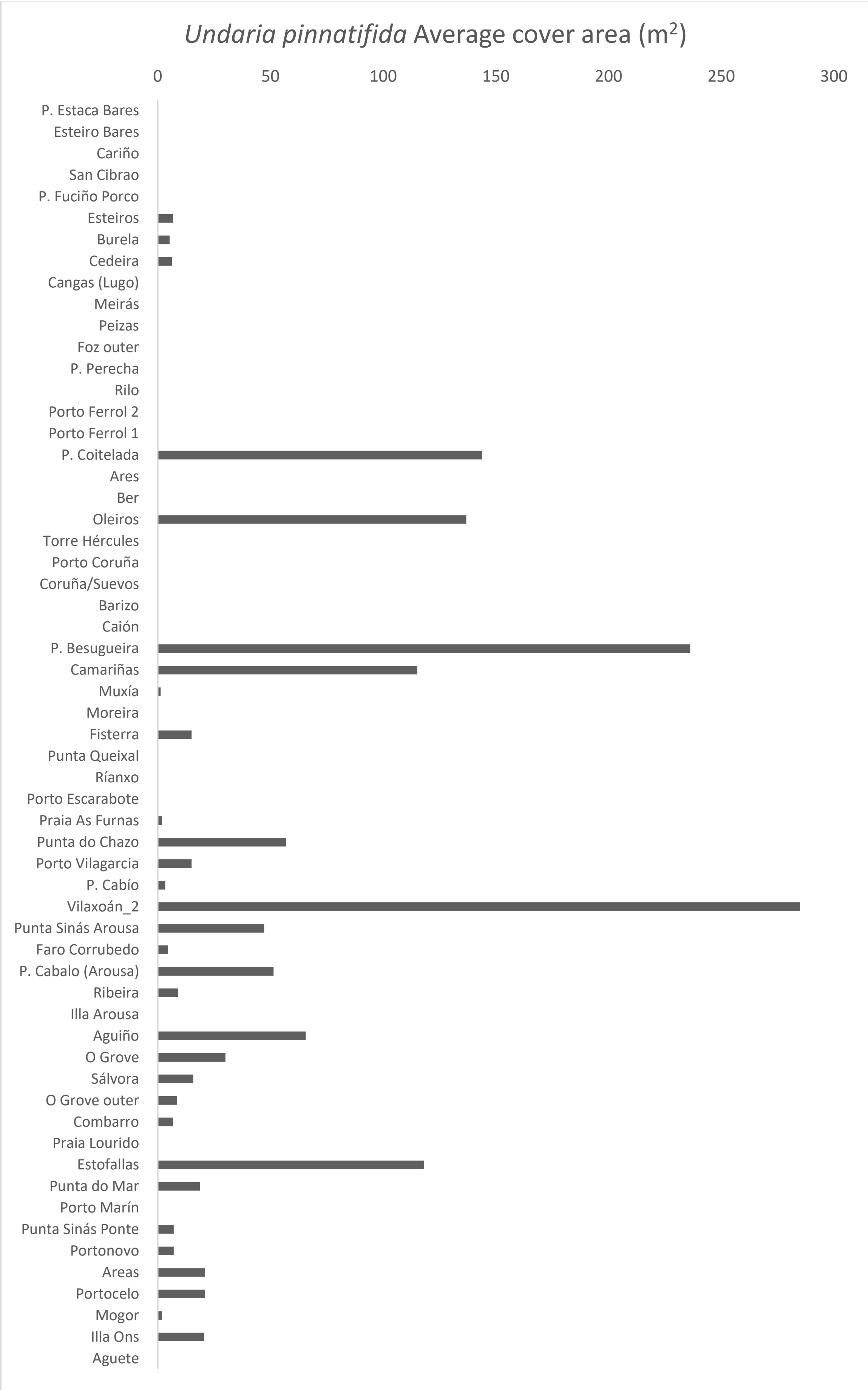

Undaria pinnatifida Average cover area (m²)
0
50
100
150
200
250
300
P. Estaca Bares
Esteiro Bares
Cariño
San Cibrao
P. Fuciño Porco
Esteiros
Burela
Cedeira
Cangas (Lugo)
Meirás
Peizas
Foz outer
P. Perecha
Rilo
Porto Ferrol 2
Porto Ferrol 1
P. Coitelada
Ares
Ber
Oleiros
Torre Hércules
Porto Coruña
Coruña/Suevos
Barizo
Caión
P. Besugueira
Camariñas
Muxía
Moreira
Fisterra
Punta Queixal
Ríanxo
Porto Escarabote
Praia As Furnas
Punta do Chazo
Porto Vilagarcia
P. Cabío
Vilaxoán_2
Punta Sinás Arousa
Faro Corrubedo
P. Cabalo (Arousa)
Ribeira
Illa Arousa
Aguiño
O Grove
Sálvora
O Grove outer
Combarro
Praia Lourido
Estofallas
Punta do Mar
Porto Marín
Punta Sinás Ponte
Portonovo
Areas
Portocelo
Mogor
Illa Ons
Aguete

Figure 13. (Continued)

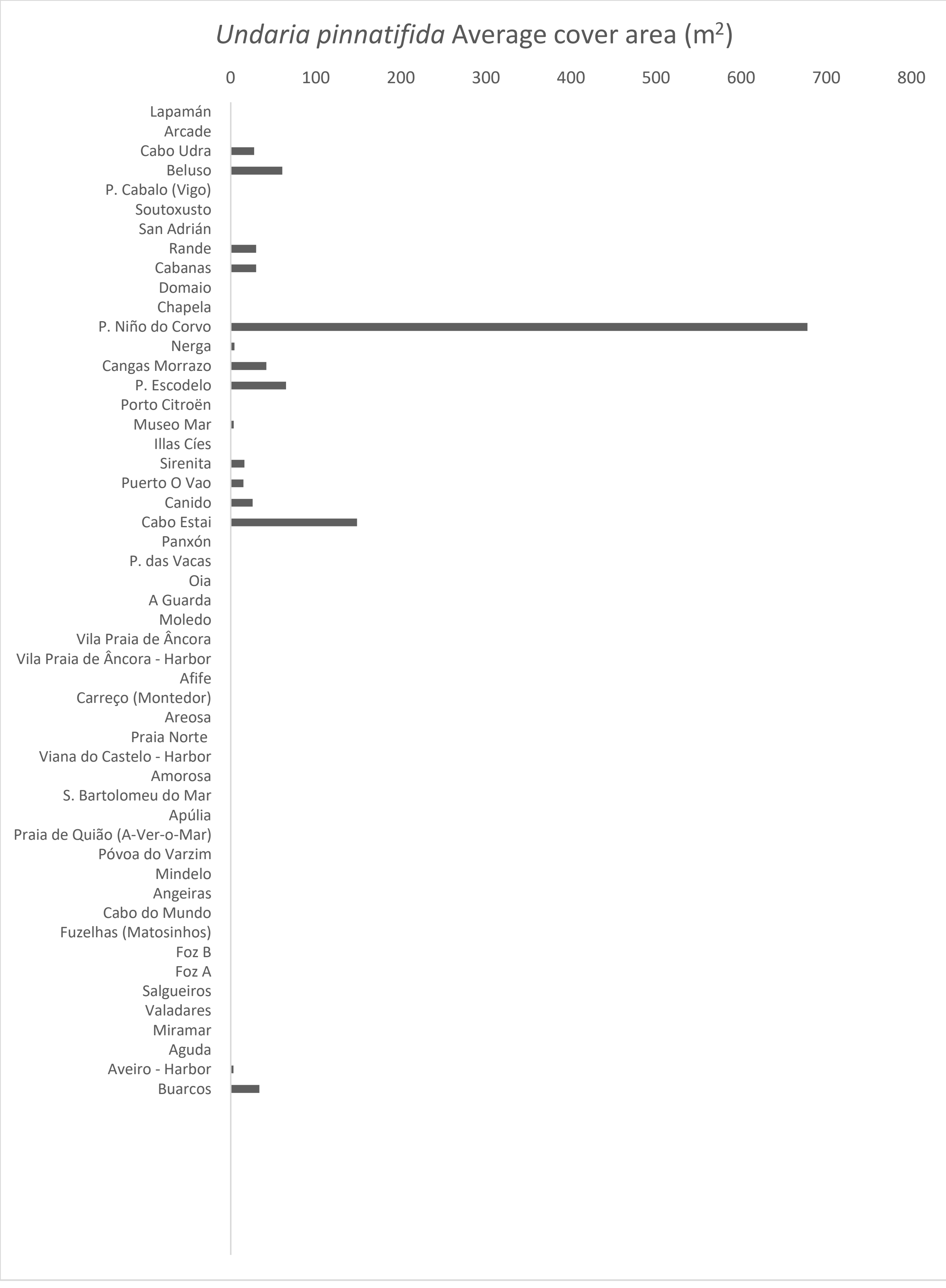


Figure 13. Average cover area of *Undaria pinnatifida* for 110 sites located in Portugal and Spain. Sites are shown from north to south.

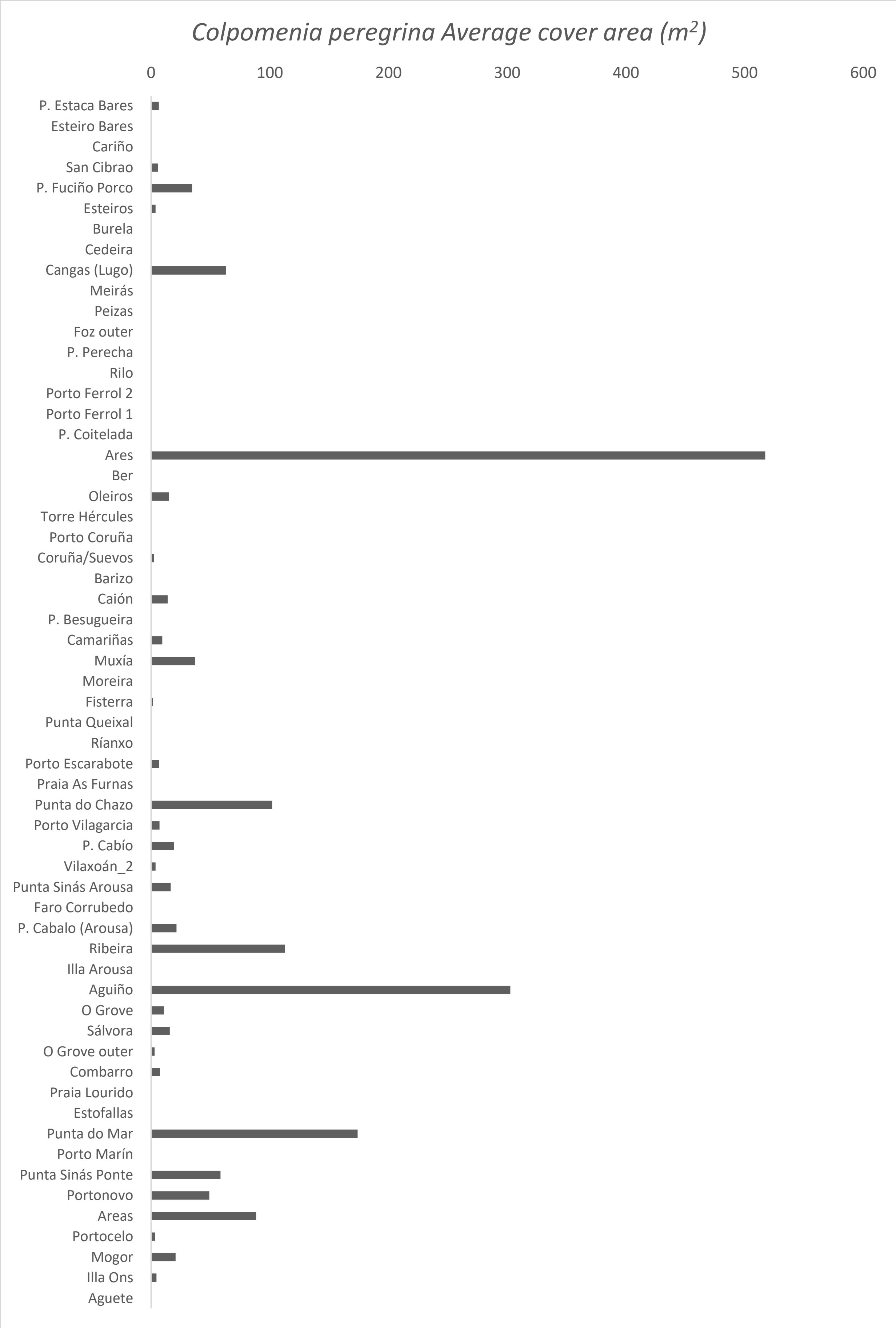
3.4. Colpomenia *peregrina*

Colpomenia peregrina was present in around 45% of the visited sites mostly in Spain. Praia Norte was the only site in Portugal which *C. peregrina* was observed with an average cover of 37 m^2. In Spain, also most of the sites with *C. peregrina* present in them had an average cover between 1-100 m^2 (Tab. 8).

Table 8. Summary of data on the number of sites for different average cover area ranges for *Colpomenia peregrina*.

Cover area range (m^2)	Number of sites in Spain	Number of sites in Portugal	In Total
0	36	24	60
1-100	41	1	42
101-200	3	0	3
301-400	3	0	3
401-500	1	0	1
501-600	1	0	1

Ares and P. das Vacas in Spain were the peaks with highest coverage (Fig. 14).



Colpomenia peregrina Average cover area (m2)
0
100
200
300
400
500
600
P. Estaca Bares
Esteiro Bares
Cariño
San Cibrao
P. Fuciño Porco
Esteiros
Burela
Cedeira
Cangas (Lugo)
Meirás
Peizas
Foz outer
P. Perecha
Rilo
Porto Ferrol 2
Porto Ferrol 1
P. Coitelada
Ares
Ber
Oleiros
Torre Hércules
Porto Coruña
Coruña/Suevos
Barizo
Caión
P. Besugueira
Camariñas
Muxía
Moreira
Fisterra
Punta Queixal
Ríanxo
Porto Escarabote
Praia As Furnas
Punta do Chazo
Porto Vilagarcia
P. Cabío
Vilaxoán_2
Punta Sinás Arousa
Faro Corrubedo
P. Cabalo (Arousa)
Ribeira
Illa Arousa
Aguiño
O Grove
Sálvora
O Grove outer
Combarro
Praia Lourido
Estofallas
Punta do Mar
Porto Marín
Punta Sinás Ponte
Portonovo
Areas
Portocelo
Mogor
Illa Ons
Aguete

Figure 14. (Continued)

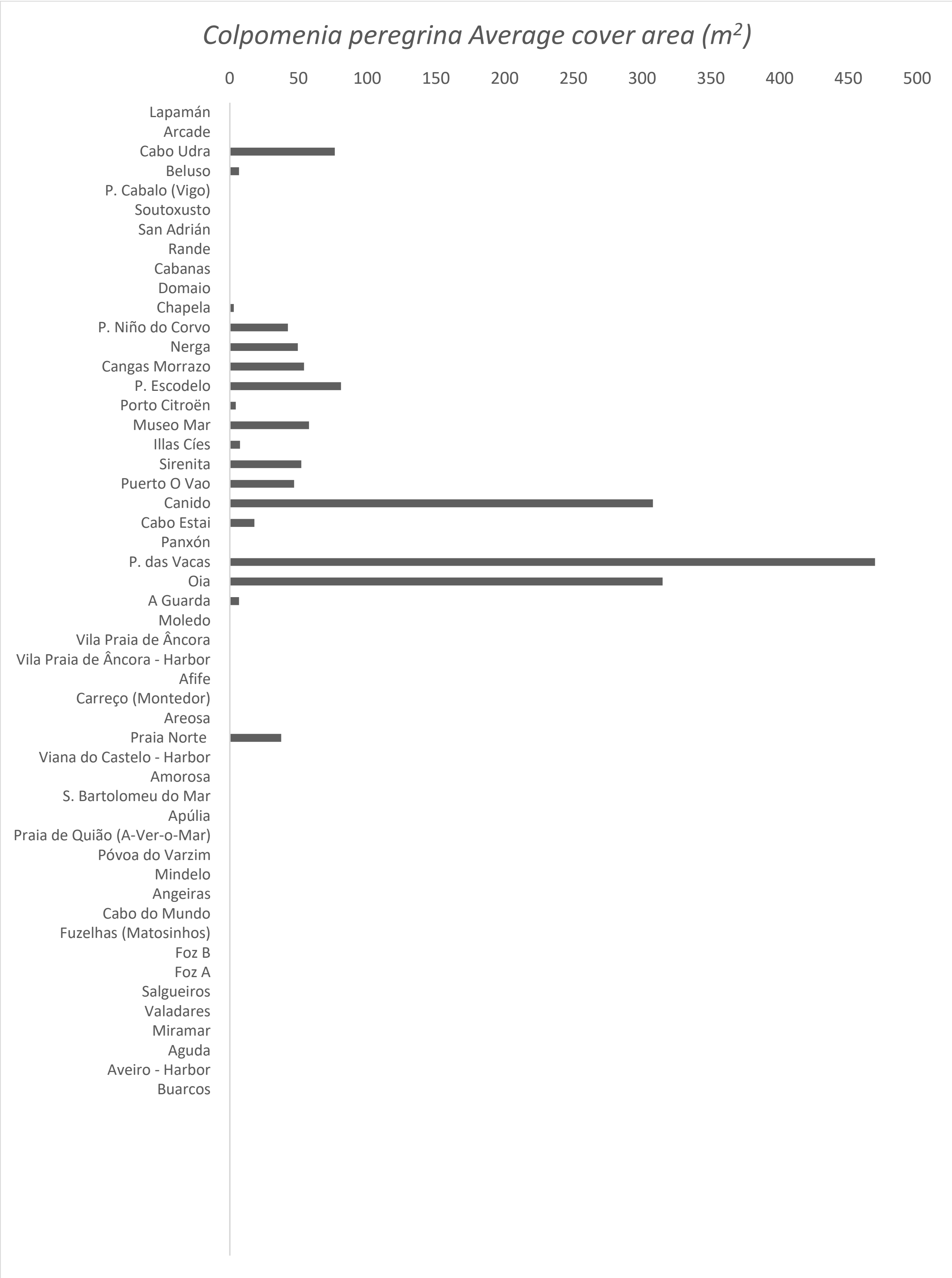


Figure 14. Average cover area of *Colpomenia peregrina* for 110 sites located in Portugal and Spain. Sites are shown from north to south.

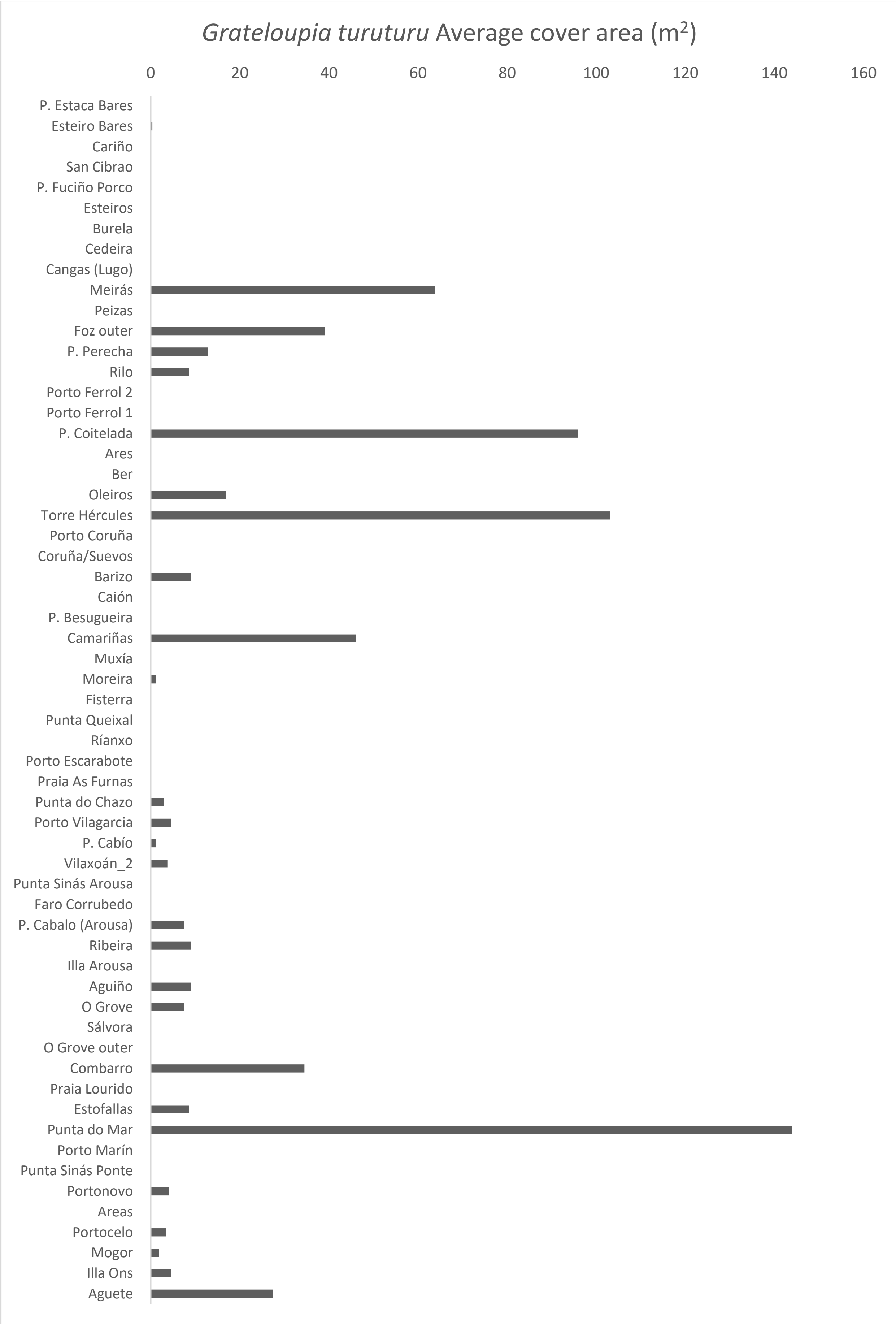
3.5. *Grateloupia turuturu*

Grateloupia turuturu was found in 46% of visited sites with the average cover between 1-100 m^2 in most areas (Tab. 9).

Table 9. Summary of data on the number of sites for different average cover area ranges for *Grateloupia turuturu*.

Cover area range (m^2)	Number of sites in Spain	Number of sites in Portugal	In Total
0	49	10	59
1-100	34	12	46
101-200	2	1	3
201-300	0	1	1
301-400	0	1	1

G. turuturu appears to be more concentrated in the southern areas with two peaks in Aguda and Valadares, Portugal (Fig. 15).



Grateloupia turuturu Average cover area (m2)
0
20
40
60
80
100
120
140
160
P. Estaca Bares
Esteiro Bares
Cariño
San Cibrao
P. Fuciño Porco
Esteiros
Burela
Cedeira
Cangas (Lugo)
Meirás
Peizas
Foz outer
P. Perecha
Rilo
Porto Ferrol 2
Porto Ferrol 1
P. Coitelada
Ares
Ber
Oleiros
Torre Hércules
Porto Coruña
Coruña/Suevos
Barizo
Caión
P. Besugueira
Camariñas
Muxía
Moreira
Fisterra
Punta Queixal
Ríanxo
Porto Escarabote
Praia As Furnas
Punta do Chazo
Porto Vilagarcia
P. Cabío
Vilaxoán_2
Punta Sinás Arousa
Faro Corrubedo
P. Cabalo (Arousa)
Ribeira
Illa Arousa
Aguiño
O Grove
Sálvora
O Grove outer
Combarro
Praia Lourido
Estofallas
Punta do Mar
Porto Marín
Punta Sinás Ponte
Portonovo
Areas
Portocelo
Mogor
Illa Ons
Aguete

Figure 15. (Continued)

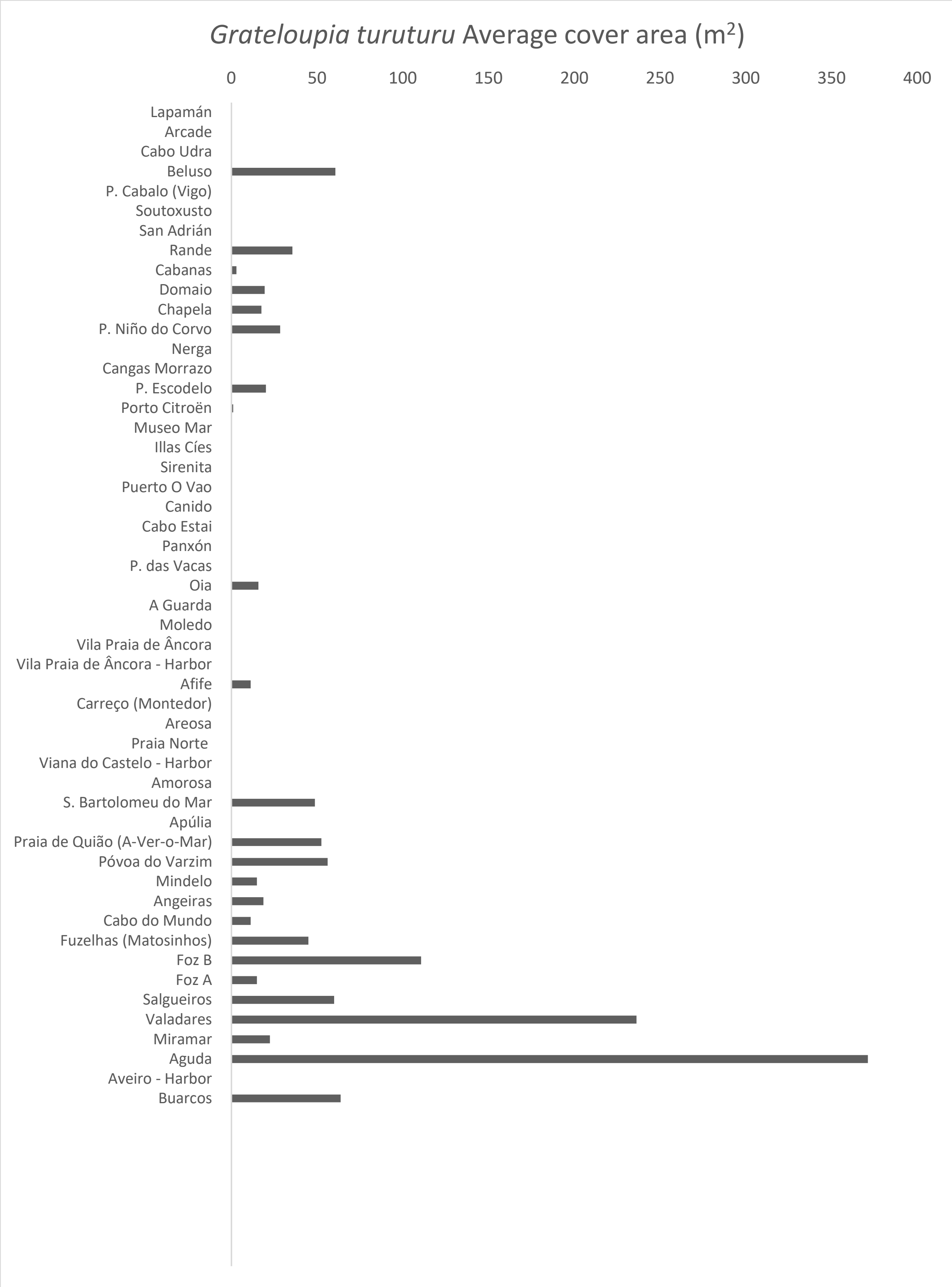


Figure 15. Average cover area of *Grateloupia turuturu* for 110 sites located in Portugal and Spain. Sites are shown from north to south.

3.6. *Asparagopsis armata*

Asparagopsis armata was present in about 35% of the visited sites mostly in Spain and was found only in 2 sites in Portugal with a low coverage (Tab. 10).

Table 10. Summary of data on the number of sites for different average cover area ranges for *Asparagopsis armata*.

Cover area range (m^2)	Number of sites in Spain	Number of sites in Portugal	In Total
0	47	23	70
1-100	25	2	27
101-200	6	0	6
201-300	3	0	3
301-400	2	0	2
601-700	1	0	1
>1000	1	0	1

A. armata appears to be more concentrated in southern part of Spain with high peaks in P. das Vacas and Canido (Fig. 16).

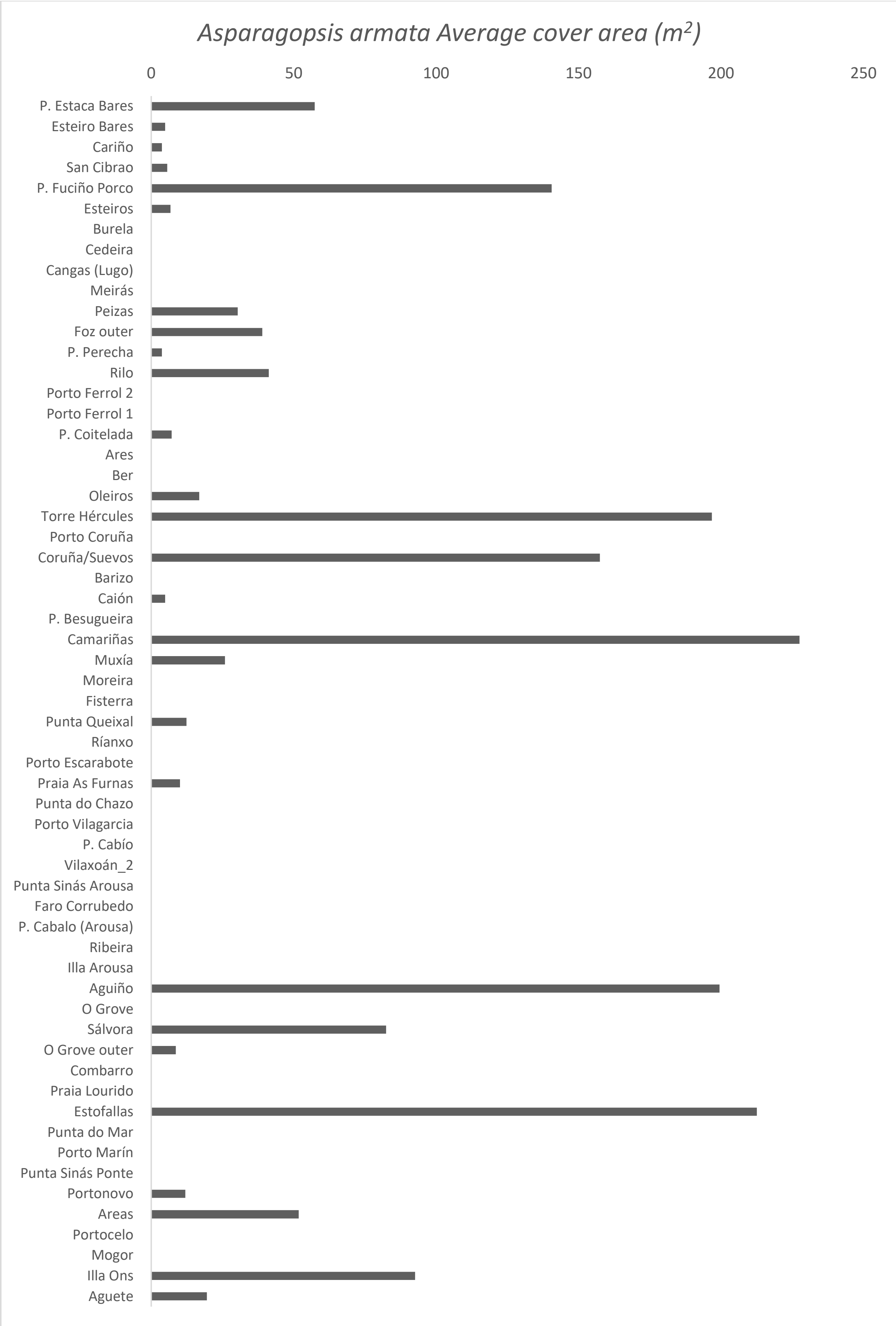

Asparagopsis armata Average cover area (m2)
0
50
100
150
200
250
P. Estaca Bares
Esteiro Bares
Cariño
San Cibrao
P. Fuciño Porco
Esteiros
Burela
Cedeira
Cangas (Lugo)
Meirás
Peizas
Foz outer
P. Perecha
Rilo
Porto Ferrol 2
Porto Ferrol 1
P. Coitelada
Ares
Ber
Oleiros
Torre Hércules
Porto Coruña
Coruña/Suevos
Barizo
Caión
P. Besugueira
Camariñas
Muxía
Moreira
Fisterra
Punta Queixal
Ríanxo
Porto Escarabote
Praia As Furnas
Punta do Chazo
Porto Vilagarcia
P. Cabío
Vilaxoán_2
Punta Sinás Arousa
Faro Corrubedo
P. Cabalo (Arousa)
Ribeira
Illa Arousa
Aguiño
O Grove
Sálvora
O Grove outer
Combarro
Praia Lourido
Estofallas
Punta do Mar
Porto Marín
Punta Sinás Ponte
Portonovo
Areas
Portocelo
Mogor
Illa Ons
Aguete

Figure 16. (Continued)

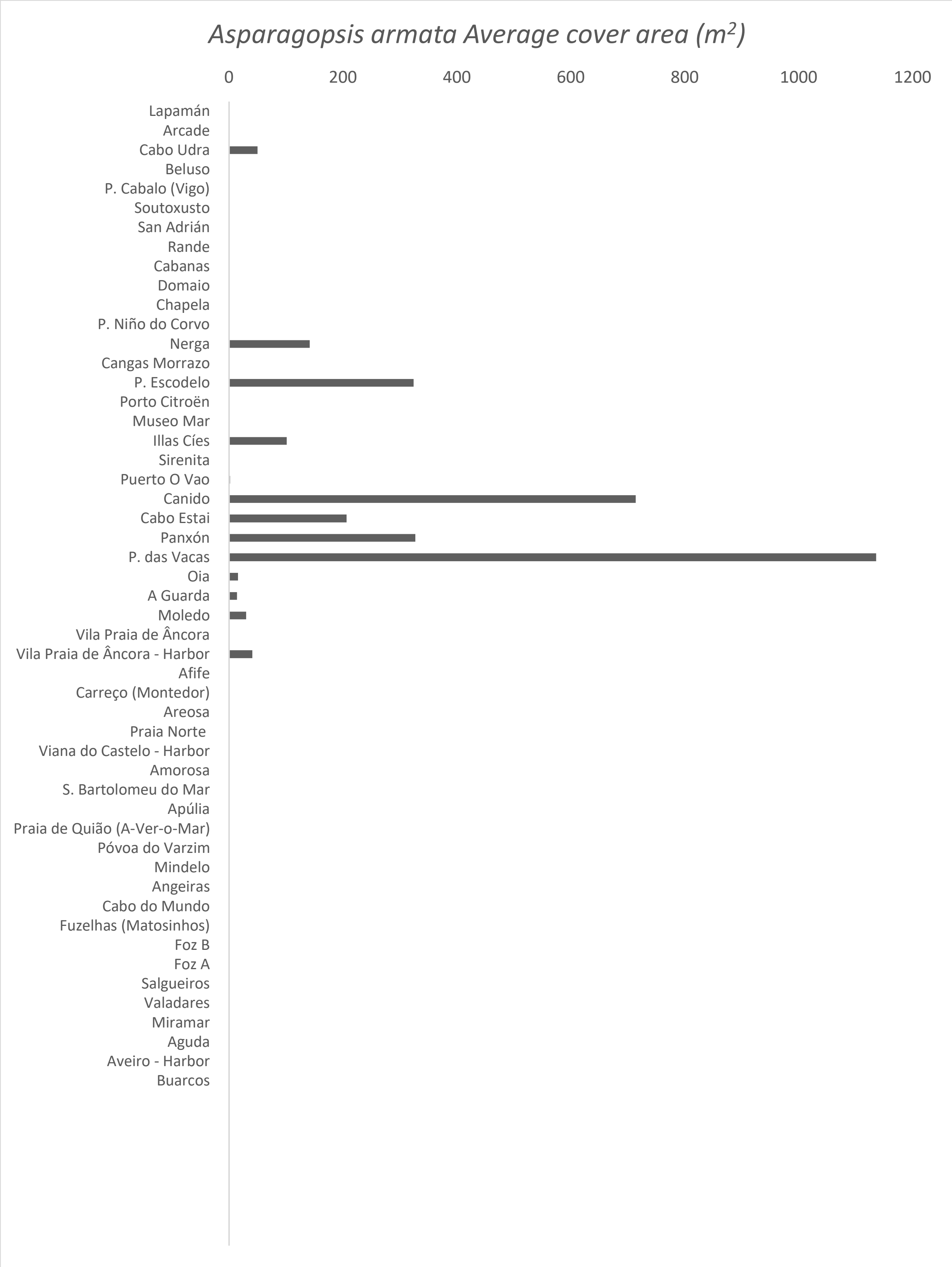


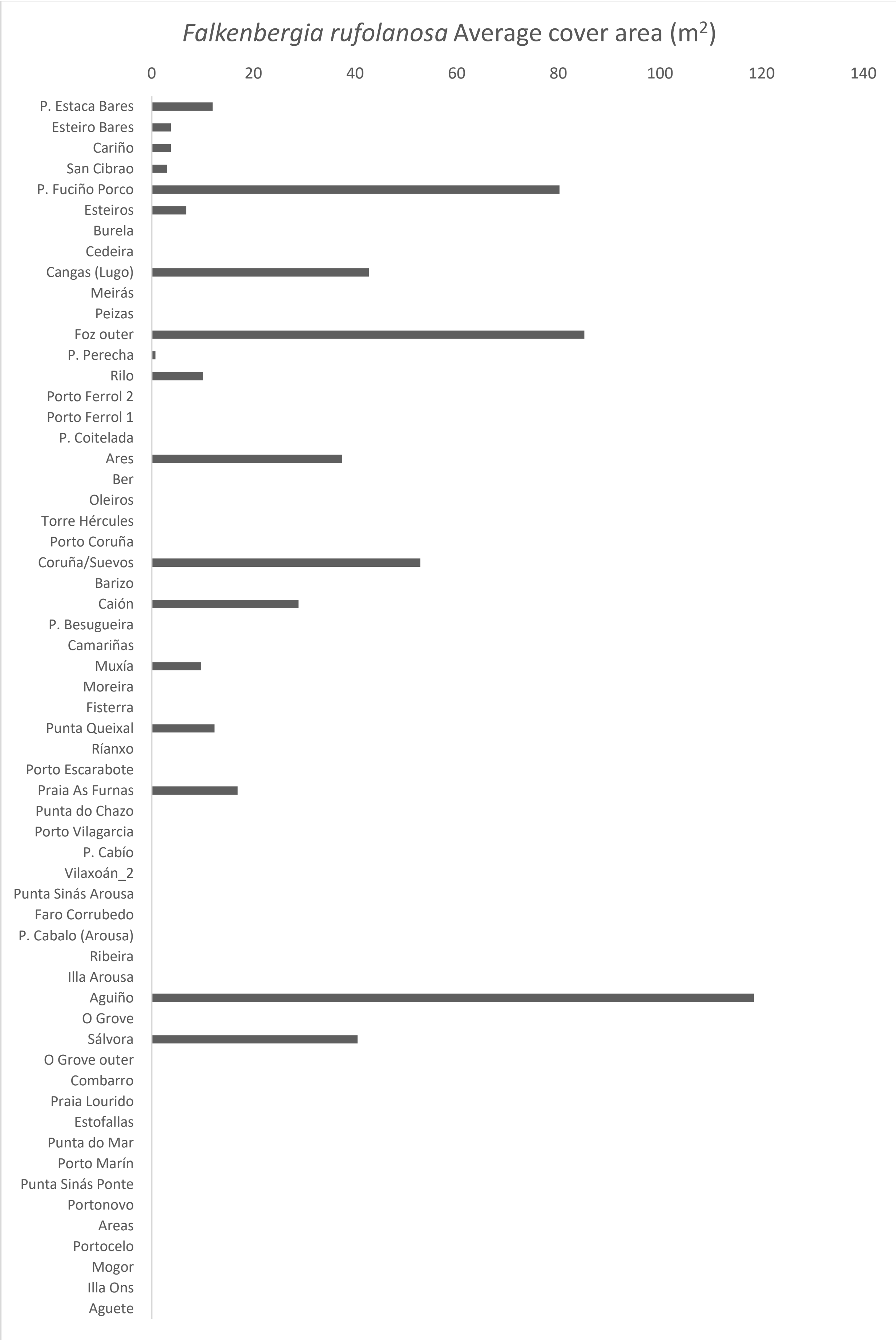
Figure 16. Average cover area of *Asparagopsis armata* for 110 sites located in Portugal and Spain. Sites are shown from north to south.

3.7. ***Falkenbergia rufolanosa* stage**

This phase of *Asparagopsis armata was* sampled separately. It was seen in about 26% of the sites mostly in Spain. The only site in Portugal where it was found was Moledo with 30m^2 coverage. In Spain also the coverage was mostly less than 100 m^2 (Tab. 11).

Table 11. Summary of data on the number of sites for different average cover area ranges for *Falkenbergia rufolansoda stage*.

Cover area range (m^2)	Number of sites in Spain	Number of sites in Portugal	In Total
0	57	24	81
1-100	26	1	27
101-200	2	0	2



Falkenbergia rufolanosa Average cover area (m²)
0
20
40
60
80
100
120
140
P. Estaca Bares
Esteiro Bares
Cariño
San Cibrao
P. Fuciño Porco
Esteiros
Burela
Cedeira
Cangas (Lugo)
Meirás
Peizas
Foz outer
P. Perecha
Rilo
Porto Ferrol 2
Porto Ferrol 1
P. Coitelada
Ares
Ber
Oleiros
Torre Hércules
Porto Coruña
Coruña/Suevos
Barizo
Caión
P. Besugueira
Camariñas
Muxía
Moreira
Fisterra
Punta Queixal
Ríanxo
Porto Escarabote
Praia As Furnas
Punta do Chazo
Porto Vilagarcia
P. Cabío
Vilaxoán_2
Punta Sinás Arousa
Faro Corrubedo
P. Cabalo (Arousa)
Ribeira
Illa Arousa
Aguiño
O Grove
Sálvora
O Grove outer
Combarro
Praia Lourido
Estofallas
Punta do Mar
Porto Marín
Punta Sinás Ponte
Portonovo
Areas
Portocelo
Mogor
Illa Ons
Aguete

Figure 17. (Continued)

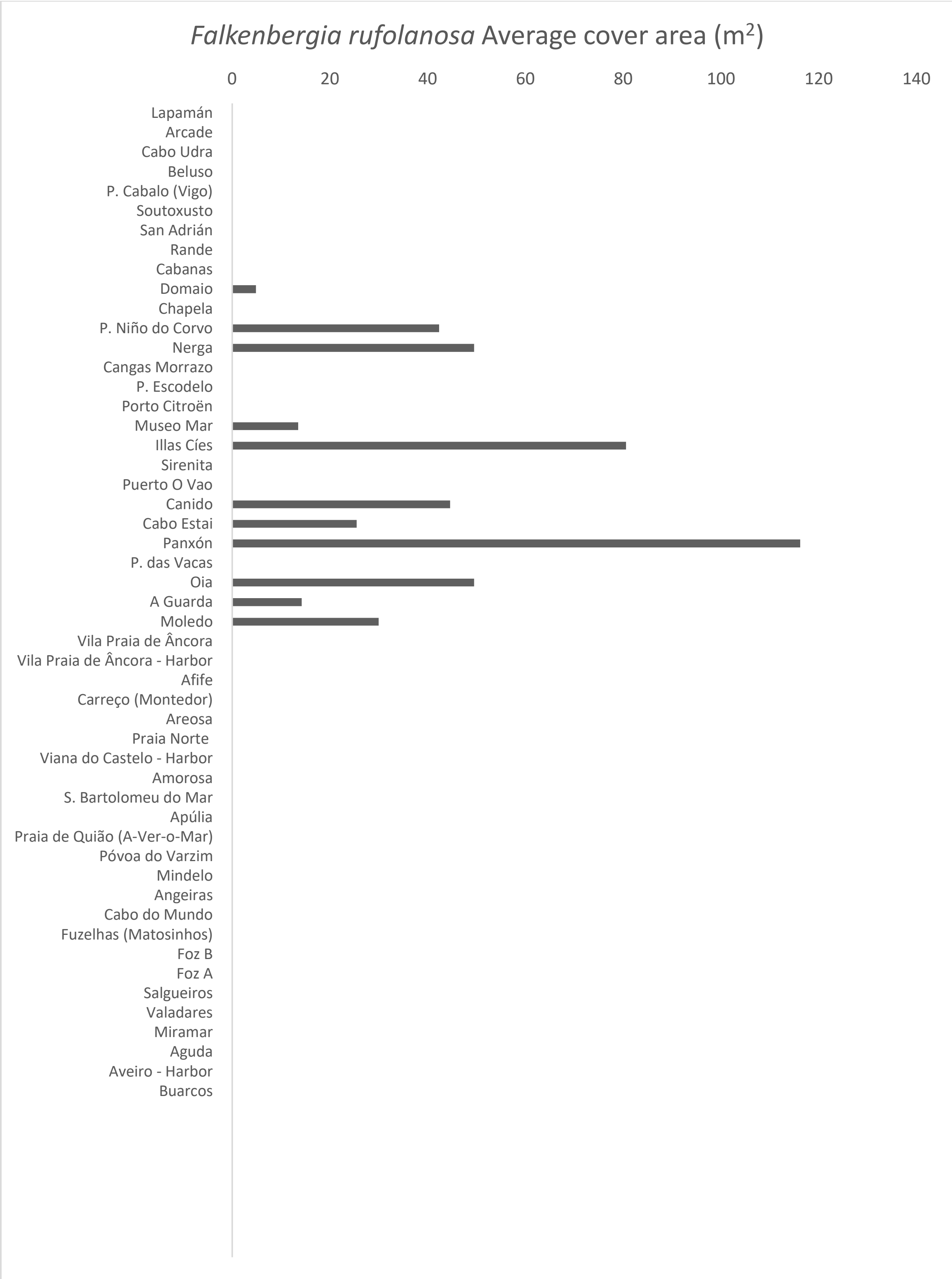


Figure 17. Average cover area of *Falkenbergia rufolanosa* stage for 110 sites located in Portugal and Spain. Sites are shown from north to south.

4. Discussion

Although most of the time introduced species do not become invasive but when they do, they can have enormous ecological and economic impacts. If identified early in their introduction and spread process, then the costs would be minimized and the early detection and management is the most powerful tool to control the damage.

Since eradication becomes really difficult once they are established, the importance of monitoring and updating the data on invasive species in different areas seems to be vital in all cases.

Here in this thesis, the results shows us all areas which are not yet invaded; made them areas to be watched and managed to prevent any future invasion. Places which have already been invaded by these non-indigenous species are prone to other types of managements which are addressed in the following pages (see section 4.1. Prediction and management).

In areas with low coverage, we are in the early detection stage which is also easier to manage and control.

In cases where the non-indigenous species are already having high coverage, besides the typical management practices which are explained below we have the option of using these species. This uses can bring economic income and also give us so many other benefits. Some possible uses are also addressed (see section 4.2. Potential uses of the invasive seaweeds of the Iberian Peninsula).

4.1. Prediction and management

Eliminating non-indigenous species can be really hard and expensive so knowing which species might become invasive can help us to aim all resources towards the target species (Hewitt et al. 2005). In doing so there are different approaches, the very first step would be knowing the history of invasion in different parts of the world and creating an information bank with the species names and places that they invaded. This information would be a great base on predicting which of them might potentially invade which new place (Hayes and Sliwa 2003). Some macroalgae invaders are considered as cryptic which means that they need to be examined by specialists to be detected otherwise their appearance including their small size or similar taxonomy and morphology keeps them undetected for years or decades (Trowbridge 2006) which would then lead to underestimation of the impacts and their actual number (Vaz-Pinto et al. 2012).

Pheloung et al. (1999) developed a system to screen the invasive species by creating a questionnaire which includes 49 questions. These questions target the essential attributes and impacts of the species. Their system could successfully predict serious weeds in Australia. Climate matching model can also be used for estimating the invasion by predicting the possible new range of the introduced species. This model is limited due to the fact that species can evolve and adapt to the new environments (Nyberg 2007).

Another important factor to consider is the ecosystems characteristics which prone them to invasion. Gollasch and Leppakoski (1999) showed that disturb environments under stress are more likely to be affected by invasion. The Diversity Resistance Hypothesis also indicates that the chances of an invasion is higher in communities with less diversity (Nyberg 2007).

There are many seaweed species which are considered to be high impact pests. They can establish in different marine environments and eradicating them is costly and even in some cases impossible (Thomsen et al. 2009). To have a successful eradication, firstly it needs to be followed by a fast response and it also works best when the population is small and restricted to a defined area , all this plus the presence of financial resources and human labor are the necessities (Wotton et al.2004). Eliminating invasive species as an intensive management act can only work if the entire range is being treated because otherwise the risk of reappearance is very high. And also in this method it is very important to look for the responsible vector and deal with that too (Schlaepfer et al. 2005). It is important to note that this method is very expensive and needs very careful consideration and analysis.

There is an action plan by the Global Invasive Species Program (GISP) which includes four steps, namely

1- Prevention; 2- Early detection; 3- Eradication; 4- Control.

The ideal management practice would be to control the species and preventing them from entering new areas (Nyberg 2007). There is no doubt that it is the best policy which also has the benefit of being cost effective (Olenin et al. 2011). In prevention phase all the attention should be towards the vectors to eliminate the factor of transportation (Williams and Grosholz 2008).

The best next option would be the early detection. When prevention fails, to deal with the establishment phase it is important to detect the invader as soon as possible, assess the situation fast and respond to it rapidly (Williams and Grosholz 2008). In order to be successful in early detection phase an ongoing monitoring program is essential. Public awareness and educating people would also help in this matter (Olenin et al. 2011). In this stage a rapid detection can be very essential in evaluating the use of eradication (Wittenberg and Cock 2001). Because at an early stage the likelihood of it being successful is much higher since there is a time lag between the first stage which is introduction and the settlement and population growth. Since seaweeds disperse really fast, control and eradicating them is very difficult.

Different removal methods include:

- Mechanical removal. With machine or hand which is very labor intense but also the most target specific one;

- Chemical removal. With toxins like pesticides, herbicides. It can be expensive and is rarely target specific. There is also the risk of the species becoming resistance to the specific chemical or toxin;

- Biocontrol. Using parasites, grazing; it is categorized as one of the best approaches since it is cost efficient and in many cases very successful and obviously sustainable but also it can be a little unpredicted since we are dealing with living organisms and sometimes they might not act as we expected.

The final stage in the GISP recommended plan is control. This means controlling species abundance and their spreading rate and try to keep them in an acceptable and manageable boundary.

Another management tool is using risk assessment which measures and assesses risks in order to establish a plan for future events. Using risk assessment for non-indigenous species is different from other risk categories. For example when trying to assess pollution we know they would be diluted by time and also distance whereas in communities invaders can actively reproduce and disperse (Nyberg 2007).

This is vital to remember that the success for any management strategy against invasive species depends on the fact that it should be handled both globally and regionally.

4.2. Potential uses of the invasive seaweeds of the Iberian Peninsula

Invasions by exotic species can cause numerous economic and ecological problems. The effects of these invasions by alien species are influential key issues in ecosystems due to the accelerating rates of invasions caused by human activities, and the resultant loss of native biodiversity occurring worldwide. Invasive species disrupt communities through many mechanisms (competition, predation, parasitism, nutrient cycling, habitat alteration, etc.), and through these processes can extirpate native species, alter their spatial distributions, or change their roles in food webs.

Nevertheless, it is widely known that some seaweeds are important sources of bioactive compounds with high industrial potential, however, the majority of seaweeds which are screened for bioactive compounds do not occur in large amounts and its collection from the ocean may cause highly negative impacts in the marine ecosystem. Thus, using invasive species with a potential to harm the ecosystems, this problem may turn out to be an excellent opportunity of source of bioactive compounds, providing high socio-economic revenue, while its collection from the ocean may be included in effective and sustainable management practices, contributing to the marine ecosystem equilibrium or even site restoration.

The Northwest coast of the Iberian Peninsula is also a region under the threat of invasive seaweeds such as *Asparagopsis armata*, *Sargassum muticum*, *Codium fragile* subsp. *fragile*, *Colpomenia peregrina*, *Grateloupia turuturu*, *and Undaria pinatifida*, among others- a concern that has been highly stressed in EU directives. Nowadays pharma, food and aquaculture feed are important markets for a myriad of unexploited marine bio compounds mainly coming from macroalgae. The project AMALIA (2017) will contribute towards reversing a seaweed commercial trade deficit in this Atlantic area and contribute to European competitiveness by supporting the development of a sustainable blue economy by boosting the commercial exploitation of the new products, and its consequent job creation and other business opportunities, whilst promoting the preservation of marine ecosystems.

4.2.1. Biofuels

There is a growing concern about the use of fossil fuel and its relation to climate change. This made the production of biofuel from algae a hot topic and attracted more attention to it than other possible products (Van Iersel and Flammini 2010).

Algae-based biofuel has many advantages to the biofuels that are produced from agricultural feedstocks. Firstly as it has been noted by Tredici (2010), biochemical pathways and cellular composition in microalgae can be modified. Other benefits include their low requirement to freshwater and agricultural land, high productivity and year round production, their potential in treatment of wastewater (Van Iersel and Flammini 2010). They also do not need herbicides and pesticides which is also makes it a great option (Brennan and Owende 2010).

The use of algae (micro and macroalgae) for biofuels production is expected to play an important role in securing an alternative energy supply in the next decades. The potential of algae as fuel of the future is a very important topic given the shortage of fossil fuel reserves and its environmental impact. Algae are presented as a viable alternative because the production of algae for fuel should not compete with the food, preventing the increase in food prices, and is the third generation of biofuels (Pereira 2017).

Cho et al. (2013) concluded that due to *Undaria pinnatifida'*s rapid growth and high productivity, it is a great substitute to produce bioethanol. In another study done by Kim et al. (2013), ethanol was produced from *U. pinnatifida* using yeast acclimated to specific sugars. Macroalgae saccharified broths contain a much-diversified range of sugars, whose fermentability by *Saccharomyces cerevisiae* or others microorganisms were not yet studied in most cases. Use of *S. cerevisae* is desirable, since it is viable up to concentrations of ethanol of 12-14% (v/v), contents of which are required for successful separation of ethanol with high purity. Uronic acids are referred to be not fermentable by yeast, but not many studies have been published in the literature on the subject (Kraan 2012). In natural systems, endo- and exo- alginate lyases saccharify the alginate, which is then converted to 4-deoxy-L-erythro-5hexoseulose uronic acid (DEH). This is the key intermediate considered not to be fermented by general yeasts (Wang et al. 2014). Genetic engineering techniques allowed this intermediate to suffer glycolysis with incorporation in *S. cerevisiae* of enzymes from *Asteromyces cruciatus* (Wei et al. 2013). It is also known that bacteria can convert uronic acids to pyruvate and glyceraldeheyde-3-Phosphate, which can be fermented to ethanol by the glycolysis pathway (Wei et al. 2013, van Maris et al. 2006), suggesting that fermentation of hydrolysates from macroalgae can efficiently be converted to ethanol in aseptic conditions yeast fermentation. Results reinforcing this synergetic activity are obtained by Nazaré (Nazaré 2015), in which fermentation of sulfuric acid hydrolysates from *Sargassum muticum* with *S. cerevisae*, under non-septic conditions resulted in yields on ethanol conversion more than 95%.

4.2.2. Biofertilizers

In coastal areas around the world seaweeds have been used as fertilizers. They have mineral content and they can help to improve the water-binding capacity of soil. For cultivating rice, the species which fix nitrogen have high value (Pulz and Gross 2004, Pereira 2010).

Producing chemical fertilizers is expensive and energy demanding. Using organic fertilizers is more efficient, as the nutrients would be released slowly which benefits plant growth and reduces the Greenhouse gas (GHG) emissions resulting from microbial activities (Mulbry et al. 2008). A considerable number of nutrients would be still present in seaweeds leftover biomass after extracting oil or carbohydrates. It would be economical to use them as biofertilizers (Van Iersel and Flammini 2010).

Sargassum muticum is often used as compost and a nutrient rich fertilizer (Pereira 2016). Shekhar et al. (2012) reported that in both Mung Bean and Pak Choi plants, *S. muticum* extracts stimulate higher dry matter yield. They also mentioned that it contains rooting factors like indole acetic acid.

In the works carried out by Silva (2015), was assessed the potential that the extracts obtained from *S. muticum* have an agricultural fertilizer, in an attempt to encourage the reduction of the local economy of coastal areas and also the creation of industry production of liquid fertilizers. The evaluation of the potential of algae extracts as agricultural fertilizer, was made with rice plants (*Orysa sativa* L.) and lettuce (*Lactuca sativa* L.), in germination bioassays, culture of rice and lettuce plants in pots, and culture of lettuce plants in hydroponics. On these bioassays were used algae liquid extracts with different concentrations in different bioassays, where it was concluded that extracts with minor concentration (25%) have a positive effect on germination and plant development.

Undaria pinnatifida is also included in some fertilizers and agricultural treatments (Pereira 2017c).

4.2.3. Food

In many East Asian countries like Japan, Korea, China and some Pacific Islands and some parts of Europe; seaweeds, or sea vegetables, are used as food in daily life (Rao et al. 2007). Just in Japan the consumption of seaweed is 1.4 kg per person in a year (Pereira 2016). East Asian migrants from Japan, China and Korea where algae are part of their everyday meal brought it with them and expand its use all over the world (Barsanti and Paolo 2014). One of the first countries in Europe that instituted regulation for using seaweeds as a non-traditional food substance is France. 21 macroalgae and 3 microalgae has been authorized to be used as a food source there (Burtin 2003).

These days, people are surrounded by not healthy options such as fast foods which are high in calories and low in nutrient value. Algae can be the answer to solving this issue since it contains broad range of minerals like iron and calcium, vitamins, fiber and protein (Pereira 2016). Yamori et al. (2001) noted the role of dietary algae as a factor in Okinawan Japanese populations' longevity and health.

Grateloupia turuturu is an edible seaweed with high protein content which is also a great source of fiber and low lipid content (Denis et al. 2010, Pereira 2016). It is used as food in China (Pereira 2016) and known as a sea vegetable in Japan (Munier et al. 2013). People in Korea use it in salads (Sohn 1998). It is also considered edible in Portugal (Rodrigues et al. 2015a, Pereira 2016).

Undaria pinnatifida is considered as a luxury food in Japan and Korea. They sell it boiled or dried and it is also being used as an ingredient for salad and soup (Ohno and Largo 1998). People of China and Vietnam also use it both baked and raw (Chapman and Chapman 1980, Pereira 2016).

One of the most recent and innovative uses of some of the invasive species of the Iberian Peninsula, was developed in the academic field, along with the collaboration of masters (Biodiversity and Plant Biotechnology, FCTUC). “Medronhos” (*Arbutus unedo* plant fruits), wild berries and algae (*G. turuturu* and *U. pinnatifida*) are the ingredients that make up the “Gratô”, an eco-innovative food product like a gelatin with pulp, produced by a team of students of the Faculty of Sciences and Technology of the University of Coimbra (FCTUC), with the collaboration of the Superior Agrarian School of Coimbra (ESAC), two Portuguese higher education institutions. The idea of creating a new food product that could be ingested by everyone - children and adults, lactose and gluten intolerants, vegetarians, diabetics, etc. - was created in a Roadshow held in the framework of a partnership between the University of Coimbra (UC) and Portugal Foods promoted by the office of the Vice-Rector for Research and Innovation of University of Coimbra, Prof. Amílcar Falcão. “Gratô” consists of a combination of fruit and seaweed that is 100% free of additives and of vegetable origin. The “medronho” is the main ingredient to promote the consumption of this fruit, with much potential but little explored besides liquors. It also contains wild amber and two invasive seaweeds. The students of the Masters in Biodiversity and Plant Biotechnology at FCTUC point out that "the introduction of algae in the combination is a nutritional value added because they are very rich in iodine (essential for cognitive development in children) and in dietary fibers”. The “Gratô” authors also emphasize that they intend to promote a healthy diet and, therefore, the combination "does not have in its composition refined sugars, has only natural fruit sugar and a small portion of stevia (natural sweetener)" (UC News 2017).

Though not a commercial use, many coastal populations make use of *S. muticum* as edible seaweed. It is reported to be bitterer than other seaweed species, but is high in minerals and nutrients and can be made palatable through various preparation techniques. All kelps are also sources of alginate, a compound used in a variety of applications such as thickening and stabilizing agents in the food industry (Pereira 2011, Rodrigues et al. 2015a, 2015b, Pereira 2016).

Codium fragile is used as food in China (Bangmei and Abbott 1987, Harrison 2013). In Korean cuisine is used in various seasoning by soy sauce and vinegar, mix to Kimchi (Sohn 1998). Used as food in the form of vinegared and winter vegetable (Lembi and Waaland 1988), dried or salt cured (Levring et al. 1969). Used in sweets, salads and soups in Japan, Korea and Philippines (Chapman and Chapman 1980, Arasaki and Arasaki 1983, Zemke-White and Ohno 1999, Rao et al. 2007, Harrison 2013, Pereira 2016).

Asparagopsis armata and *Colpomenia peregrina* have been documented as an edible species (Green 2012, Pereira 2016).

4.2.4. Feed

Using algae to feed animals is recent and many experiments have been conducted resulting in the fact that it is a great feed supplement and also a good substitute for conventional protein sources. It is mostly used for poultry because it improves the skin color and yolks in eggs (Becker 2007).

Using algae as a food source for livestock also have many benefits like improving their immune system, help to control their weight and improve their fertility which as a result would lead to improvement in meat and milk for human consumption (Van Iersel and Flammini 2010).

Spolaore et al. (2006) reported using algae as a source of food for aquarium fish, ornamental birds, cows and horses, cats and dogs.

Sargassum muticum is used in China for sea cucumber and abalone as an aquaculture feed (Liu et al. 2013). It is also used as fish bait and animal feed (Pereira 2016).

Hwang et al. (2014) reported the nutritional value of *Undaria pinnatifida* for domestic animals as a feed supplement and they found a positive impact of using it as a dietary supplement on growth, immune statues and reduction in cholesterol concentration in Hanwoo steers.

4.2.5. Other uses

Algae is a source in many industries to produce medicine, vaccines, and vitamins and as an alternative cheaper plant or animal based product (Van Iersel and Flammini 2010). Pigments from algae are currently used as natural food colorants added to orange juice and candies, chewing gum and dairy products (Spolaore et al. 2006). They are the source for all essential vitamins like A, B_1, B_2, B_6, B_{12}, C and E. it also contains nicotinate, biotin and folic acid (Richmond 2007, Pereira 2011).

The invasive species was also being examined for their potential as a source of pharmaceutical and bioactive agents. For example, *A. armata* extracts contain anti-*Leishmania* (Genovese et al. 2009), antioxidant (El Abd Mageid et al. 2009), antiviral (Haslin et al. 2001, Bouhlal et al. 2011), antifungal (Ballesteros et al. 1992), and antimicrobial (Ballesteros et al. 1992, Paul et al. 2006, El Abd Mageid et al. 2009, Kolanjinathan et al. 2009) compounds. *A. armata* is also harvested or grown to produce phycocolloid (Haslin et al. 2000). In both cold and warm blooded animals, sulfated polysaccharides are used as an anti-adhesive against bacterial infection (Banerjee et al. 2002). *A. armata* extract is a powerful antioxidant with antibacterial qualities and is a valued ingredient in many cosmetic products. This invasive species present also strong cytotoxicity against human cancer cell lines (Ballesteros et al. 1992, Zubia et al. 2009, Alves et al. 2011).

The extracts of *Colpomenia peregrina* have cytotoxic and antibacterial activity (Kamenarska et al. 2009); the extracts obtained from *Grateloupia turuturu* have antiviral

(Hudson et al. 1998), antifouling (Plouguerné et al. 2008), and antioxidant (Kang et al. 2005) activity.

Sargassum muticum is used to treat fever, skin ailments and high cholesterol in Asia (Pereira 2016). This species is the source of Laminaran (Pereira 2017b), and extracts have antioxidant (Le Lann et al. 2008), antifungal (Peres et al. 2012), antitumor, antibacterial (Villarreal-Gomez et al. 2010), antialgal (Hellio et al. 2002), antibiotic (Glombitza et al. 1982), and antifouling (Plouguerné et al. 2008, Bazes et al. 2009) activity.

Undaria pinnatifida is an anti-hypertensive which is used to cure nicotine poisoning (Takagi 1975). It has many other medicinal uses like in treating hemorrhoids, urinary diseases, anal fistulas and stomach ailments (Chengkui et al. 1984). This species is economically important as a food crop, next to Nori, on the Japanese menu, and is eaten both dried and fresh. In East Asian countries, the seaweed is known as Wakame and is treated as a delicacy, often added to miso soup (Pereira 2016). Extracts of this species have antihypertensive, immunomodulating, antidiabetic, antiviral, cytotoxic, antioxidant, antitumor, antiedema, antiplasmodial, anti-osteoporotic, anti-inflammatory, anti-obesity, antihypertensive, and antithrombotic activity (Pereira 2015).

5. Future outlook

Species diversity, nutrient regimes, and anthropogenic disturbance are suspected to influence communities' vulnerability to invasion but these possibilities cannot be fully evaluated due to the general lack of quantitative surveys and experimental research. The unpredictable responses of NIS in their new regions and the irreversibility of algal incursions are of particular concern. The taxonomy, genetics, population biology and invasion ecology of non-indigenous macroalgae are priority areas of future phycological research (Trowbridge 2006).

It is important to have regular programs to collect and update data on invasive species worldwide. There are many invasive species and a way forward could be to find some beneficial use for them. As far as macroalgae is concerned, many possibilities can be underlined. They can be used as inputs in many industries to make products such as fertilizers and biofuels. In many developing countries, seaweeds can turn into a great source of income and can be established in both small and large scale business practices. They can be used in wastewater treatments as biofilter, cultivated for human food or be a source of animal feed, being thus a too much valuable resource to ignore.

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8. Appendix

8.1. Appendix 1 – Field Sampling recording sheet

Date: ______________ Geographical area: _____________________ **Site name:**______________________

Time of low-tide: ________ Low-tide size (m): ________

GPS coordinates of access point: ______________________________

Sampling area (1 or 2) at each site: _____

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

Recorder name: _______________________

Sampling area recorded (m^2):_____ m^2: **Size: 30 m parallel to de sea X _____ m^2 perpendicular to the sea**

Time spent in recording area: ________ **Start time:** ________ **Final time:** ________

Weather conditions: ________________ Visibility: _______________ Wave Exposure:________________

Surface Inclination (horizontal platform, very steep slope, steep slope, slight steep slope): _______________

Type of substrate	Granite boulder	Granite blocks	Granite platform	Sand	Mud	Gravel	Shale	
%								

Species		**100-50% D**	**50-30% A**	**30-15% F**	**15-5% O**	**<5% R**	**Not seen**	**Comments**
Codium fragile ssp *fragile*	rock pools emergent substrate							
Undaria pinnatifida	rock pools emergent substrate							
Sargassum muticum	rock pools emergent substrate							
Colpomenia peregrina	rock pools emergent substrate							
Asparagopsis armata / Falkenbergia rufulanosa (sporophyte)	rock pools emergent substrate							
Grateloupia turuturu	rock pools emergent substrate							