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**Site selection and dynamic modelling of
Manila clam, *Ruditapes philippinarum*,
in the Tagus Estuary**

Dissertação para obtenção do Grau de Mestre em
Engenharia do Ambiente

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Abstract

This thesis assesses the viability of shellfish aquaculture in the Tagus Estuary of the species Manila clam (*Ruditapes philippinarum*), by means of a GIS-based site selection and the application of a dynamic modelling software at farm-scale, in order to evaluate the potential production, environmental effects, and economic feasibility.

The site selection for shellfish aquaculture is based on the application of a Multi-Criteria Evaluation in a GIS software. Both are useful tools for site selection, as when combined they help decision-makers to identify the existing spatial constraints and take into consideration key variables, such as (i) the external environmental conditions that regulate shellfish growth, (ii) existing infrastructures and socioeconomic variables that are relevant for production, and (iii) contaminated areas that may reduce the shellfish quality as a food product.

The dynamic modelling software used, FARM (Farm Aquaculture Resource Management), was applied in accordance with the best areas identified as being suitable for shellfish aquaculture, where the production and profit of two different locations are estimated and compared at a farm-scale level, and further extrapolated to larger areas.

Keywords: Manila clam; Site selection; GIS; Multi-Criteria Evaluation; Dynamic modelling

Resumo

Esta tese de dissertação avalia a viabilidade da aquacultura da espécie Manila clam (*Ruditapes philippinarum*) no Estuário do Tejo, com base na seleção dos melhores locais para a sua prática, através do uso de um SIG e da aplicação de um software de modelação dinâmica, com o intuito de avaliar a produção, impactos ambientais e viabilidade económica.

A seleção dos melhores locais para a prática da aquaculture de bivalves baseou-se na integração de uma Análise Multi-Critério num SIG, sendo que são ferramentas úteis, dado que quando usadas em conjunto, servem de auxílio aos decisores na identificação de limites espaciais existentes, tendo por base variáveis-chave, tais como (i) condições ambientais que influenciam o crescimento dos bivalves, (ii) as infraestruturas e variáveis socioeconómicas relevantes para a produção e (iii) áreas contaminadas que possam reduzir a qualidade dos bivalves como produto alimentar.

A aplicação de um software de modelação dinâmica, FARM ((Farm Aquaculture Resource Management), baseou-se nos melhores locais identificados como adequados para a aquacultura, tendo sido estimadas e posteriormente comparadas as produções e lucros associados a duas localizações diferentes, numa escala local. Em seguida, os resultados obtidos foram extrapolados para áreas maiores.

Palavras-chave: Manila clam; Seleção de locais; SIG; Análise Multi-Critério; Modelação dinâmica

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Acronyms list

AA – Appropriate Assessment
AHP – Analytic Hierarchy Process
B2K – BarcaWin 2000
CI – Consistency Index
CLC – Corine Land Cover
CO07 – Portugal’s Land Use map 2007
CR – Consistency Ratio
DIN – Dissolved Inorganic Nitrogen
DO – Dissolved Oxygen
EC – European Commission
EWN – EcoWin
EPA – United States Environmental Protection Agency
ERL - Effects Range low
ERM – Effects Range Median
EU – European Union
FAO – Food and Agriculture Organization of the United Nations
FARM – Farm Aquaculture Resource Management model
GEV - Gross Economic Value
GIS – Geographic Information System
IAS – Invasive Alien Species
IDW – Inverse Distance Weighted
LWS4 – Live weight corresponding to Shell’s length of 4cm
MCE – Multi-Criteria Evaluation
MSFD – Marine Strategy Framework Directive
NPL – National Priority List
OSM – OpenStreetMaps
PEL – Probable Effect Level
POM – Particle Organic Matter
PORNET – Tagus Estuary Nature Reserve Land Use and Management Plan
psu – Practical Salinity Units
RI – Random Consistency Ratio
S – Suitable
TEL – Threshold Effect Level

TFW – Total fresh weight

TPA – Total Protection Areas

TPM – Total Particle Matter

t - tonnes

WFD – Water Framework Directive

WLC – Weighted Linear Combination

WWTP – Wastewater Treatment Plant

1. Introduction

1.1. Problem definition

In the last decade, the Manila clam (*Ruditapes philippinarum*) population in the Tagus Estuary has grown considerably, and it is starting to expand and further colonize the available habitats. Meanwhile, an illegal fishing problem has arisen due to the prosperity of the clams, disrupting the trade chain of live bivalve molluscs by illegally transporting the harvested clams to Spain where it is sold as local product or exported to Portugal. In turn, this may put public health at risk, since no treatments are applied to the harvested products in order to reduce possible contaminations.

As of 2017, there is no management plan concerning Manila clam fishing, although studies are starting to address the issue and seeing it, not only as an invasive species, but as a possible source of increasing the economic relevance of poorer fishing communities located in Tagus margins. Manila clam fishers have been asking for local and regional authorities to develop ways to facilitate and further legalize Manila clam harvesting (e.g. depuration units), without putting consumers at risk.

This way, in order to support a possible framework for the implementation of shellfish aquaculture in the Tagus Estuary, it is here proposed the development of a site selection methodology to identify suitable areas for aquaculture practices, followed by FARM's model application, which allows the assessment of the economic and environmental impacts that shellfish aquaculture is likely to have.

1.2. Objectives and expected outcomes

The development of an aquaculture site selection methodology and application of dynamic farm-scale modelling relies on the following established objectives:

- Identify key factors for further develop shellfish aquaculture in the Tagus Estuary, as well as existing spatial and legal constraints;
- Evaluate shellfish aquaculture suitability, according to the GIS-based site selection;
- Quantify the potential environmental and economic impacts that shellfish production may have in a small area and extrapolate it to the entire estuary;

In the end of this thesis, the following questions should be answered, in order to assess the viability of the shellfish aquaculture implementation:

- What are the major spatial constraints for shellfish aquaculture?
- Which areas gather the best conditions for shellfish growth?
- Which areas have better accessibility? On which areas shellfish production is most facilitated?
- Which areas are most contaminated and may reduce product's quality?

By answering these previous questions, it is possible to reach the conclusion of whether shellfish aquaculture is viable.

1.3. Thesis layout

This thesis is divided into 5 key Chapters. The first chapter consists in the problem definition and tries to answer the question "Why should this thesis matter and why was it done?" and also includes the objectives and potential outcomes.

Chapter 2 includes the state-of-the-art, which encompasses the theoretical rationale for aquaculture's importance worldwide and in Europe, focusing on shellfish aquaculture and an invasive species mostly introduced for aquaculture, Manila clam. This topic also addresses the

importance of an appropriate site selection regarding aquaculture feasibility, licensing and deployment, using GIS-based Multi-Criteria Evaluation.

The methodology is described in Chapter 3 and is divided into four different steps, where the first three are related to a Multi-Criteria Evaluation by means of a GIS tool and the last, the application of the FARM model to the more suitable areas.

Chapter 4 contains results and discussion, where the thematic maps based on the Multi-Criteria Evaluation are presented and discussed. FARM's outputs are also analyzed in order to exemplify shellfish aquaculture environmental and economic relevance. Possible limitations are also identified and emphasized.

Finally, conclusions are presented in Chapter 5, where the question "Is shellfish aquaculture viable in the Tagus Estuary" will be answered, according to the final outcomes.

2. State of the art

2.1. World

Human population is growing, coastal urban areas are expanding and it is still being experienced new emerging economies in the developing world (e.g. China), where the majority of fisheries and aquaculture production take place (deFur and Rader, 1995; Grealis et al., 2017). Due to the rising incomes, emerging countries are starting to become not only sellers, but also consumers (FAO, 2014, 2016; NIR, 2014), with a new record high world per capita fish supply of 20.1 kg (FAO, 2016). With the inherent increase of seafood consumption as well (FAO, 2003; Kearney, 2010), many authors (e.g. Costa-Pierce, 2002; Dumbauld et al., 2009) are highlighting aquaculture's growing importance as a way to meet growing demand for food from the sea. In turn, this is further increasing stress conditions to what were already deemed as declining fishery stocks throughout the world and respective ecosystems (Simard et al., 2008), even considering that part is also a result of efforts to keep fisheries to sustainable levels (PRB, 2016).

According to FAO's latest report (FAO, 2016), employment in the fisheries and aquaculture sector have shown a small reduction, which in the same report is pinpointed as being a consequence of a stabilization of the sector, although it can also be attributed to the mentioned fisheries effort. Despite this, it is a major source of income for many million people around the world, with focus on more rural areas (Haylor and Bland, 2001; FAO, 2009; Halwart et al., n/d), with FAO's most recent estimates indicating that 56.6 million people found employment in it (FAO, 2016): the numbers concerning fish farmers accounted for 18 million, where the majority is located in China (94%), followed by Latin American and the Caribbean (1.9%) and Africa (1.4%) (Seafish, 2016; FAO, 2016), reinforcing the dependence on aquaculture in more rural areas.

Production of aquatic animals from aquaculture in 2014 amounted to 73.8 million t, with an estimated first-sale value of approximately 151.8 billion €, encompassing approximately 49.8 million t of finfish, 16.1 million t of molluscs, 6.9 million t of crustaceans and 7.3 million t of other aquatic animals, such as amphibians (FAO, 2016; Seafish, 2016).

As seen in Table 1, China totaled an aquaculture production of about 45.5 million t, which is more than 60% of global aquaculture production. All continents have shown a general trend of an increasing share of aquaculture production in total fish production (Table 1), except Oceania between 2010-2012.

Table 1: Aquaculture production by regional and major producers (as of 2014) (FAO, 2016) *mainland

| | Aquaculture production (thousand t) | | | | | | |
|----------|-------------------------------------|----------|----------|----------|----------|----------|--------------|
| World | 1995 | 2000 | 2005 | 2010 | 2012 | 2014 | % (2014) |
| Africa | 110.2 | 399.6 | 646.2 | 1,285.6 | 1,484.3 | 1,710.9 | 2.32 |
| Egypt | 71.8 | 340.1 | 539.7 | 919.6 | 1,017.7 | 1,137.7 | 1.54 |
| Americas | 919.6 | 1,423.4 | 2,176.9 | 2,514.2 | 2,988.4 | 3,351.6 | 4.54 |
| Chile | 157.1 | 391.6 | 723.9 | 701.1 | 1,071.4 | 1,214.5 | 1.65 |
| Asia | 21,677.5 | 28,422.5 | 39,188.2 | 52,439.2 | 58,954.5 | 65,601.9 | 88.91 |
| China* | 15,855.7 | 21,522.1 | 28,120.7 | 36,734.2 | 41,108.3 | 45,469.0 | 61.62 |
| Europe | 1,580.9 | 2,050.7 | 2,134.9 | 2,544.9 | 2,852.3 | 2,930.1 | 3.97 |
| Norway | 277.6 | 491.3 | 661.9 | 1,019.8 | 1,321.1 | 1,332.3 | 1.81 |
| Oceania | 94.2 | 121.5 | 151.5 | 189.6 | 186.0 | 189.2 | 0.26 |

2.2. Europe

In the EU, aquaculture production is an important economic activity in many coastal and continental regions but has been stagnating in recent years (e.g. EUMOFA; 2016), despite that

European aquaculture is known to “offer excellent quality products, respect environmental sustainability, animal health and consumer protection standards” (EC, 2013).

Hofherr et al. (2012) have reached the conclusion that EU aquaculture is stagnating, because of governance problems within the Member States, where few licenses were given in the last few years; Lado (2016) have also concluded that European coasts do not have enough physical space for aquaculture settings, even though Hofherr et al. (2012) study have disproven this, by giving emphasis to a proper identification of suitable sites (site selection) for aquaculture practices.

Overall labour productivity has increased in European aquaculture (Hofherr et al., 2012), given the development of new technologies and the inherent production intensification, which has led to a declining of employment, as shown by FAO (2016): values from 2014 accounted for 413,000 fishers and fish farmers, of which 66,000 were fish farmers, representing a declining of 47% when compared to 2000 (FAO, 2016).

Notwithstanding, Europe is the top trader of aquaculture products in the world (EUMOFA, 2016), alongside the United States of America and Japan (FAO, 2016), with a total extra-EU trade (export + import) of 26.8 billion € in 2015, albeit the trade deficit (export – import) totaled minus 17.8 billion €, with an increase of 7% when compared to 2014 (EUMOFA, 2016). In addition, Europe’s self-sufficiency rate has been increasing (47.5% in 2014), meaning that EU production can satisfy almost 50% of the total apparent consumption (EUMOFA, 2016).

According to FAO (2016), Europe produced a total of 2.9 million t regarding both inland and marine and coastal aquaculture in 2014, with 630 thousand t resulting from the production of molluscs. Europe’s major bivalve producers were Spain (0.22 million t), France (0.16 million t) and Italy (0.11 million t). In table 2 it is displayed the total production for each European region, from 1995 until 2014.

Table 2: Total aquaculture production between 1995 and 2014 for each European region (FAO, 2016)

| European region | Aquaculture production (thousand t) | | | | | | |
|------------------------------------|-------------------------------------|---------|---------|---------|---------|---------|-------------|
| | 1995 | 2000 | 2005 | 2010 | 2012 | 2014 | % (2014) |
| Europe | 1,580.9 | 2,050.7 | 2,134.9 | 2,544.9 | 2,852.3 | 2,930.1 | 100 |
| Eastern Europe | 183.5 | 195.9 | 239.0 | 251.3 | 278.6 | 304.3 | 10.4 |
| Norway | 277.6 | 491.3 | 661.9 | 1,019.8 | 1,321.1 | 1,332.3 | 45.5 |
| Northern Europe (excluding Norway) | 205.6 | 309.0 | 327.6 | 363.5 | 391.3 | 402.8 | 13.7 |
| Southern Europe | 480.6 | 640.8 | 541.5 | 573.5 | 579.3 | 595.2 | 20.3 |
| Western Europe | 433.6 | 413.7 | 365.0 | 336.0 | 282.0 | 295.3 | 10.1 |

In recent years, the development of EU aquaculture is being increasingly identified as a possible way to enhance economic growth, overcoming dependency on imports, which e.g., the EU’s Blue Growth Strategy (Remotti and Damvakeraki, 2015) and the current reform of the Common Fisheries Policy are aiming to achieve (EC, 2012a), as well as EATIP’s initiative (www.eatip.eu), whose goal is to “define a long-term vision for European aquaculture in 2030”. EU legal requirements are starting to address aquaculture, as well as the associated environmental impacts, by account for water quality, biodiversity protection and sustainable development (EC, 2013). In the table below are represented relevant EU legal requirements for aquaculture (Table 3).

Table 3: Relevant legal requirements for aquaculture in Europe

| Legal Requirements | Description |
|---|--|
| <p>Marine Strategy Framework Directive (MSFD)</p> | <p>The MSFD aims to protect more effectively the marine environment across Europe (EC, 2016a). MSFD requires EU Member States to achieve 'Good Environmental Status' for their marine waters by 2020, as judged against a range of 11 descriptors (Directive 2008/56/EC) and to protect resources based upon on which marine-related economic and social activities depend (EC, 2016a).</p> <p>Therefore, marine strategies must be developed and implemented in order to i) protect and preserve the marine environment, prevent its deterioration or, where practicable, restore marine ecosystems in areas where they have been adversely affected and ii) prevent and reduce inputs in the marine environment and possible pollution sources, to ensure that there are no significant impacts or risks to marine ecosystems and inherent biodiversity, as well as human health (Directive 2008/56/EC).</p> |
| <p>Water Framework Directive (WFD)</p> | <p>The WFD aims to improve and protect the chemical and ecological status of surface waters and groundwater bodies throughout a river basin catchment (rivers, lakes, groundwater, coastal waters and estuaries) (EC, 2016b) – regarding ecological status, coastal waters extent to one nautical mile out to sea, while chemical status applies also to territorial waters extending out to 12 nautical miles (EC, 2016b).</p> <p>Member States must prevent deterioration of the ecological and chemical status of surface waters, and to restore polluted surface waters and the ecological conditions necessary to achieve good status in all surface waters by 2021 and then 2027 (Directive 2000/60/EC).</p> |
| <p>Natura Network (Habitats and Birds Directives)</p> | <p>The Natura 2000 is a network of core breeding and resting sites for rare and threatened species (Birds Directive 2009/147/EC), and some rare natural habitats types which are protected in their own right (Habitats Directive 92/43/EEC), stretching across all 28 EU countries, both on land and at sea (EC, 2012a, 2016c). Natura 2000 sets the standard for nature conservation within Europe, enabling State Members to work together toward the same goals and within the same strong legal framework to protect valuable habitats and species within Europe, irrespective of political or administrative boundaries (Nature Link EEIG, 2008).</p> <p>The Habitats Directive (EC, 2012a) established protective measures that must be applied to plans and projects that are likely to have a significant effect on a Natura 2000 site. In order to determine whether a plan or project may have a significant effect, an Appropriate Assessment (AA) must be made: if plans or projects have negative effects, they can be authorized if there is no alternative solution on the basis of overriding public interest, although Member States must take the necessary compensation measures to ensure that the overall coherence of Natura 2000. In Articles 6(3) and 6(4) it is defined the step-by-step procedure for plans and projects within a Natura 2000 site, where each step further determines whether a next one is needed (EC, 2012a).</p> <p>In the light of the conclusions of the assessment of the implications for the site, the competent national authorities shall agree to the plan or project after assurance that it will not adversely affect the integrity of the site concerned and, if appropriate, after having obtained the opinion of the general public (EC, 2012a).</p> |

2.3. Shellfish aquaculture

Although extensively studied (e.g Rice, 2008; Rose et al., 2014; Deal, 2005; Byron et al., 2011; Carswell et al., 2006; Silver, 2014), it seems that shellfish aquaculture concept is not defined beyond the “shellfish production through aquaculture practices”. Shellfish aquaculture typically occurs in the shallower coastal waters near the coastline, whose culture practices can be based off-bottom, also called suspended cultures, such as longlines, rafts or floating bags; or on-bottom, in either the intertidal or subtidal areas (e.g. Shumway, 2011). Shellfish harvesting can be done by hand or mechanical means.

According to O'Beirn et al. (2012), shellfish production includes both crustaceans (e.g. shrimp and crab) and molluscs (e.g. clam and oyster), which fundamentally differ on the food system required for production: crustaceans are omnivorous, needing a higher quality diet requiring additional food such as fishmeal and fish oil (Bondad-Reantaso et al., 2012), whereas bivalve molluscs, as filter feeders, can sustain their growth with only the existing phytoplankton and other detritus in the water column. In aquaculture settings, this is called an extensive culture system (FAO, 1989).

Since the present work will only focus on intertidal cultures in an extensive culture system for a bivalve mollusc production, only those will be highlighted and mentioned from now on, starting on what is a bivalve.

Bivalve molluscs are suspension feeders that are mostly located in coastal marine ecosystems, namely estuaries or lagoons, feeding on organic particles, such as phytoplankton and other detritus, suspended in the water column (Gosling, 2004). Afterwards, the organic and nutritious food particles are digested, while the remaining particles are released as byproducts, in the form of either urine, faeces or pseudofaeces that are then deposited to bottom sediments (e.g. Newell and Jordan, 1983; Konrad, 2013): the transfer of biodeposits containing nutrients is a process known as pelagic-benthic coupling (Newell and Mann, 2012; Rice, 2008).

By filtering and consequently controlling phytoplankton populations, bivalves exert a process known as “top-down” control; in addition, bivalves also control the available urinary ammonia and inorganic nitrogen, which is a key factor for the growth of phytoplankton populations (i.e. “bottom-up” process), as inorganic nitrogen controls organic matter production in coastal marine environments (Rice, 2008; Gallardi, 2014).

Therefore, positive environmental effects of bivalves can be summarized as:

- Avoiding the excessive growth of phytoplankton populations, bivalves help to minimize the associated eutrophication problems, such as the formation of hypoxic environments and water quality deterioration, due to algae bloom, that when dead, are consumed by benthic bacteria (Xia et al., 2015);
- Shells that accumulate in bottom sediments (from both living and dead bivalves) can be used in aquaculture settings, as it provides a suitable substrate (Washington Sea Grant, 2005; DFO, 2011), as well as in the contribute to geochemical processes by capturing carbon in the form of calcium carbonate (NRC, 2010);
- Decreasing water clarity by filtering suspended particles, which in turn increases light penetration to benthic plants, such as seagrasses (Newell and Koch, 2004), that as mentioned in Gallardi (2014), can be important nurseries for crustaceans and molluscs.

Intertidal production of shellfish benefits from its proximity to land access (Huntington et al., 2006), even though boat navigation can sometimes be hard (e.g. mudflats), with the same author describing intertidal areas as a “dynamic physical environment”. However, despite the fact that shellfish aquaculture may bring positive effects, apart from the economic benefits, poor siting or bad practices can lead to serious disturbances to the shellfish and may jeopardize the harvested product's quality.

Even though intertidal practices require low maintenance, harvesting techniques by means of mechanical extraction have been shown to disrupt infaunal communities (Spencer et al., 1998), modifying or even destroying the respective habitats (Spencer et al., 1996), whereas hand harvesting, albeit being less physical disruptive to the benthic habitats, is more labour-intensive and perturbs the sediment (DFO, 2017). Moreover, harvesting techniques may also negatively impact bird foraging and nesting areas, as reported in Huntington et al. (2006), due to disturbance, habitat modification, adding to the disturbances caused by boat traffic and noise (Forrest et al., 2009; Zydulis et al., 2009). Notwithstanding, Dumbauld et al. (2009) have

highlighted that the response of birds to aquaculture are relative to the bird species, as well as to the dynamic processes inherent to the analyzed areas, such as tides.

Introduction of alien species is also associated with shellfish aquaculture, and in Europe, it is regulated by Council Regulation (EC) No. 708/2007. Huntington et al. (2006) classified introduction of alien species as possibly having a “medium” impact concerning the Habitat Risk Index assessment carried by the authors. If the appropriate management measures are not taken, alien species might negatively influence benthic habitats, native species and ultimately ecosystem services.

Chapter 2.4 will address more thoroughly the impact of alien species on aquaculture.

2.4. Invasive alien species on aquaculture

An invasive alien species (IAS) is considered to have been introduced either intentionally or unintentionally to a specific area or region where it does not occur in normal conditions (i.e. alien species) and established itself in a way that threatens the environment (e.g. ecosystems and the inherent native species) and may cause negative impact to economic activities or even to human health, i.e. invasive species (Sicuro et al., 2016; GISP, 2005).

Bartley and Casal (1998) have divided possible impacts caused by alien species into two main categories: i) ecological, which may include biological and genetic effects, deriving from either competition or predation (Gallardo et al., 2016), habitat modification by ecosystem engineer species (Ruesink et al., 2005) or by hybridization of the native species with alien species closely related (Pérez and Rylander, 1998), which weakens the genetic pool and subsequently the fitness of wild populations (Goldburg et al., 2001); and ii) socioeconomic, which can be attributed to changes in prices of products sold in markets (e.g. native species may experience a drop in price after the introduction of an alien species) or changes in ecosystem services, such as water purification (e.g. increased biofiltration) (EEA, 2012; Katsanevakis et al., 2014). However, these two categories can be seen as interdependent, as socioeconomic impacts can further aggravate ecological impacts, as well as the opposite, as highlighted in Bartley and Casal (1998).

Aquaculture is known to be a major pathway for the introduction of alien species (e.g. Kochmann, 2012), as alien species are reported to have higher adaptability to new ecosystems (Vidthayanon, 2005) and faster growth than the native species (Keller et al., 2011). This is considered the main reason for the use of alien species instead of the native ones in aquaculture settings throughout the world (Cagauan, 2007), disregarding biological conservation for a more intensive production, in order to achieve higher profit yields, of which Atlantic salmon, Pacific oyster and Manila clam are good examples (Table 4).

Table 4: Gross estimation for worldwide total production for the three invasive species

| Invasive species | Production (million t) | Economic value (million €) | Source |
|------------------|------------------------|----------------------------|--------------------------|
| Atlantic salmon | 2.32 | 12,500 | FAO, 2017a |
| Pacific oyster | 0.63 | 2,520 * | FAO ,2017b; Richez, 2012 |
| Manila clam | 4.01 | 16,042* | FAO, 2017c |

* assuming a market price of 4 € kg⁻¹

Atlantic salmon (*Salmo solar*) is considered to be among the most pervasive alien species in the world, being farmed both within and outside its native areas (Thorstad et al., 2008). Ecological and socioeconomic impacts regarding the introduction of Atlantic salmons, escaping and further interactions with native species are well documented (Taranger et al., 2015; Waknitz et al., 2002; Sepúlveda et al., 2013; Thorstad et al., 2008; Naylor et al., 2005; Green et al., 2012) and can

range from direct interactions through competition or interference at its spawning grounds (e.g. Thorstad et al., 2008), hybridizing with the native species and weakening the gene pool (Bailey, 2014), and the introduction of new diseases and parasites (Novak, 2014).

Other example of invasive species on aquaculture is the Pacific oyster, *Crassostrea gigas*, native to Japan and coastal regions of northeast Asia (FAO, 2017b; Nehls and Büttger, 2007). Its widespread distribution was caused by its introduction to replenish areas with declining populations of native oysters, due to an inherent rapid growth and low maintenance costs: it was firstly introduced in Washington, USA, in 1902 and in Europe, around 1960, for aquaculture purposes, due to the declining of the native American, Olympia oysters (*Ostrea conchapila*), and native European species, such as the European oyster (*Ostrea edulis*) and Portuguese oyster (*Crassostrea angulata*) (Harris, 2008; Nehring, 1999, 2011). More recently, Ruesink et al. (2005) have concluded that the Pacific oyster has been identified as an invasive alien species in 17 of the 66 reviewed countries, mostly in temperate coastal ecosystems.

Even though the production of Pacific oyster can be a major cause for the economic development in regions where it is cultivated, it may also have a negative impact on the environment (Harris, 2008): as an ecosystem engineer, the Pacific oyster has the capacity to modify natural habitats to suit its biological needs, thus changing both the physical and chemical properties of the environment (Kochmann, 2007; Padilla, 2010). This, adding to its high filtration rate, which in certain conditions might cause food depletion in the water column and further impact autochthonous filter-feeding species and its resistance to stress conditions (Troost, 2010), makes it a troublesome invasive species to deal with, creating the need for sustainable management practices (e.g. Herbert et al., 2015).

The Manila clam, *Ruditapes philippinarum* is one of the most commercially relevant species introduced for aquaculture purposes (e.g. Nerlović et al., 2016; DFO, 1999), due to its habitat adaptability, fast growth, high productivity and disease resistance (e.g. Humphreys et al., 2015; Cordero et al., 2017). It is an endemic species in the Pacific coast of southeastern Asia (FAO, 2017c; Cordero et al., 2017), with wild populations occurring in the Philippines, Yellow Sea, Sea of Japan, Sea of Okhotsk, South China and East China Seas, and around the Southern Kuril Islands (FAO, 2017c).

Its expansion as an alien species is well documented (e.g. Çolakoğlu and Palaz, 2014; Mortensen and Strand, 2000; Jensen et al., 2004; Nerlovic et al., 2016; de Montaudouin et al., 2016;): since it was first unintentionally introduced in North America from Japan around the 1930s, mixed with Pacific oyster seeds, it expanded throughout all the Pacific coast, from California up until Vancouver (Flassch and Leborgne, 1992). Its European introduction was caused by a visible decline of the native European species (*Ruditapes decussatus*) (Nerlovic et al., 2016), starting in Arcachon Bay, France, between 1972 and 1974, where a commercial hatchery imported 500,000 spat and 1000 adults from Puget Sound (Flassch and Leborgne, 1992).

Later, the United Kingdom and Italy followed the same trend. Spain's introduction happened somewhere in the 1980s, by being brought alongside other invasive species seeds, and further expanded to different parts of the country, e.g. Galicia, Cantabria or Cataluña (Pérez Camacho and Cuña, 1985). Afterwards, it is hypothesized that somehow Manila clam spread to Portugal, where as stated in Chainho et al. (2015), it was probably introduced in an aquaculture context in 1984, in the Ria Formosa (SE Portugal). At present, it has colonized most of Portuguese lagoons (e.g. Ria de Aveiro) and estuaries (Sado and Tejo).

The ecological impacts related to the introduction and colonization of Manila clam are similar to those reported for the Pacific oyster: high density populations of Manila clam may cause food depletion, and negatively impact native species populations, by changing the biogeochemical cycles (e.g. Bartoli et al., 2001) and colonizing their native habitats (McKindsey et al., 2007). The relationship between *Ruditapes decussatus* and Manila clam has been extensively reported in

many areas (e.g. Tagus Estuary, Venice Lagoon, Bay of Santander, Arcachon Bay), because of the declining of the native species whenever it occupied the same habitat as Manila clam, due to the latter's higher environmental resilience and faster reproduction rate (e.g. Laruelle et al., 1994), as well as to the reduced fitness of the native species caused by the hybridization between both species (Bidegain and Juanes, 2013).

Another plausible justification is due to their burial depths differences: as reported in Bidegain and Juanes (2013), where the authors have compared Zaklan and Ydenberg (1997) findings with the respective burial depths of both species (2 – 4 cm, *R. philippinarum* and 10 – 12 cm, *R. decussatus*): according to Zaklan and Ydenberg (1997), the energetic efficiency of feeding with a siphon decreases with burial depth. This means that Manila clam can filter particles more quickly than *R. decussatus*, which could explain their growth differences, considering that it can filter particles more quickly and invest less in the development of its siphons, even though it is more exposed to predation.

2.5. Manila clam

The Manila clam *Ruditapes philippinarum* (Adams and Reeve, 1852) is an infaunal marine bivalve mollusc that lives in the higher parts of the intertidal (Lee, 1996) or subtidal areas, in sand, muddy gravel or clay substrates (Nerlovic et al., 2016), and this way, it is more exposed to tidal cycles and stressful conditions, such as extremes of temperature, low salinity and oxygen, food limitation and predation (Jones et al., 1993; WGS, 2005; Yin et al., 2016), as well as relative humidity (Ali and Nakamura, 1999) The fact that they are less burrowed within the sediments is linked to its shorter siphons, being mostly fused and only separated at the tips, hence the name “littleneck” clam (Bourne, 1982).

Understanding its innate characteristics and environmental adaptations is fundamental to why it keeps being introduced in aquaculture, and further spread and colonize each introduced region: i) it is euryhaline and can grow with salinities from 12 (larvae threshold) to 36 psu (Breber, 1996; Weber et al., 2008; Coughlan et al., 2009), albeit its optimal values range between 24 and 30 psu (Coughlan et al., 2009) or 32 (Chew, 1989). The lack of agreement concerning the highest threshold in the optimal range should not be detrimental to the clams growth or survival; ii) by burrowing into deeper sediments, clams find a “thermal refuge” (Macho et al., 2016), with temperature being a key factor in the gametogenic cycle; and iii) when in low oxygen environments, Manila clam close its shell or valve and start to use its anaerobic metabolism, which uses reserve energy and produces less energy (Weber et al., 2008).

The Manila clam is a dioecious animal, meaning it has distinct male and female genders (e.g. Gosling, 2004), becoming sexually mature between the first and the third year of age (Sladonja et al., 2011). It is strictly gonochoric (e.g. Milani et al., 2014), with the gametes being continuously released to the water column, where the fertilization afterwards occurs, as reported in DFO (2011). Even though other environmental conditions (e.g. food availability and salinity) are important for the gametogenic cycle (Choughlan et al., 2009; Zhao et al., 2013; Komorita et al., 2014; Velez et al., 2016), temperature is considered to be “the single exogenous factor” influencing gametogenesis (Gosling, 2004) and the most studied (e.g. Toba and Miyama, 1995; Kang et al., 2016; Paillard et al., 2004). As noted in Drummond et al. (2006), temperature varies in accordance with geographical locations, with its seasonal variations affecting the reproductive cycle (Delgado and Camacho, 2007a, b): the reported lower threshold for gonadal activity is 8 °C (Mann, 1979; Bourne and Farlinger, 1982), 12 °C for proper development and 14 °C for spawning (Mann, 1979), with the optimal temperature range for growth is between 20 and 22 °C (Weber et al., 2007).

2.6. Portuguese Context

Until the 1970s, the Tagus Estuary was known for being a significantly relevant economic area for Portuguese oyster production (Batista, 2007; Anjos, 2014), where the biggest population in Europe was located (APA, 2012). Despite the fact that Portuguese Oyster was economically relevant, with an annual production of 7.5 thousand t between 1962-1971 (APA, 2012). Most the production was exported to France, where it was appraised and therefore named “La Portugaise” (IAC, 1998). According to APA (2012), until 1954, 80% of the total area destined for its production was in the Tagus Estuary.

However, it is estimated that around 1966, Portuguese oyster populations started to decline (APA, 2012), which is hypothesized to have been caused by i) the spoiling of water quality in the Tagus Estuary, due to industrial discharges around Lisbon’s main area and Barreiro (APA, 2012); ii) the introduction of an anti-fouling agent, tributyltin (TBT) in dockyards within the estuary (Bettencourt et al., 1999); iii) Portuguese oysters also started to show signs of diseases, namely gill injuries (then called as “gill disease”) that reduced its filtering capacity (Bento, 2008), and an excessive thickening of the shells, that resulted in high mortalities (APA, 2012), and iv) overfishing and bad resource management, which almost led to its disappearance (Batista, 2007; Anjos, 2014). Since then, oyster cultures stopped in the estuary.

Until recently, before Manila clam introduction, Portuguese clam production was primarily based on two native species: *Venerupis corrugata* and *Ruditapes decussatus* (Chiesa et al., 2016). However, since 2009, production of the Manila clam has increased until reaching its peak in 2014 (Table 5) (EUROSTAT, 2017), which has led to the declining of the good clam populations, and consequently to new fishing locations, as to accommodate the more lucrative Manila clam (Chiesa et al., 2016). Table 5 values do not account for illegal fishing in e.g. the Tagus Estuary.

Table 5: *Ruditapes philippinarum* production (t) and economic revenue associated, between 2009 and 2015 (Eurostat, 2017)

| Year | 2009 | 2010 | 2011 | 2012 | 2013 | 2014 | 2015 |
|----------------------|-------|--------|----------|----------|----------|----------|----------|
| Production (t) | 9.5 | 105.3 | 567.14 | 889 | 772.5 | 1,036.1 | 930.1 |
| Economic value (k €) | 26.97 | 263.99 | 1,252.17 | 1,739.96 | 1,598.99 | 1,389.31 | 1,181.97 |

There are several studies that emphasize the relationship between both clams in the Tagus Estuary (Muehlbauer et al., 2014; Garaulet, 2011; Oliveira, 2012; Gaspar, 2010; Chainho et al., 2015; Ramajal et al., 2016). Garaulet (2011) reported that Manila clam Tagus colonization is coincidental in time with a significant decrease of the native clam population, which could be explained by either i) overfishing of native clams lead to the introduction of the Manila clam in the estuary and its high habitat adaptability stopped the natural restocking of the native clam or ii) the competition for the same habitats and resources eventually induced a declining of the good clam population.

2.7. Site Selection

Aquaculture is growing and being increasingly seen as a way to reduce the dependency on fisheries and sustainably produce food, as world per capita of fish and seafood consumption are reaching all-time highs (e.g. FAO, 2016; Seafood, 2016). Coastal waters, due to their proximity to urban areas, gather considerable attractivity for aquaculture siting (e.g. sources with urban aquaculture). However, as coastal waters are used by a multitude of different activities, sometimes problems may arise due to incompatible uses (e.g. Clark, 1992). Coastal aquaculture, if not properly sited, is going to compete for “economic, social, physical and ecological resources with other industries” (Ross et al., 2013), which can undermine its viability (e.g. Longdill et al., 2008). Hence, there is a need to pinpoint the best locations for aquaculture, that ensures its

success and minimizes the social and environmental negative effects, which is addressed by site selection studies.

Site selection comprises a wide array of tools and different thematic areas, as well as requires the communication between scientific community and stakeholders, whose goal is to identify the best locations for aquaculture practices in a particular area, in order to maximize production and economic profit, reduce environmental impacts associated to unsustainable aquaculture practices, provide quality products that respect human health safety concerns, avoid social conflicts that may arise due to competition for space (e.g. Aguilar-Manjarrez et al., 2017; Llorente and Luna, 2013; Pillay and Kutty, 2005; Wu, 1995) and increase aquaculture's social acceptability (Whitmarsh and Palmieri, 2009).

This is achieved by taking into account, e.g., i) the cultivated species' biological factors influencing its growth to a harvestable size, and even though the biological parameters should be approximately the same (e.g. temperature, salinity or food sources), different species should have different optimal values for growth within the same parameter; ii) possible sources of pollution that can reduce the harvested product's quality and therefore put the public health at risk; iii) societal acceptability, as to avoid visual impacts in an urban area context; iv) factors influencing production, namely distance to depuration units or roads, and v) assess possible legal and spatial constraints that will limit practices, as to avoid conflicting uses regarding other activities and/or stakeholders, as well as areas ecologically relevant.

Therefore, site selection is a relatively complex spatial decision problem, which can be seen as highly subjective and changeable: different initial choices will have different outputs, depending on both expert and stakeholders' preferences and interests (Malczewski, 2000), as the chosen inputs do not have a rigid structure that all site selection problems must respect, and can be further combined into several different criteria/categories.

In the last decades, the development of virtual tools and new technologies, such as GIS (Geographic Information System), has facilitated the spatial management of resources and coastal planning, and given the aquaculture context, also the ecosystem approach to aquaculture (EAA), which encompasses the best management practices regarding aquaculture (e.g. Aguilar-Manjarrez et al., 2017). The combination of Multi-Criteria Evaluation (MCE) principles and GIS tools, which Dapueto et al. (2015) also called "Spatial Multi-Criteria Evaluation", is a useful tool for aquaculture site selection, as it allows the spatial mapping and management of the identified criteria, according to the relative importance given by experts and/or decision-makers (e.g. Carver, 1991).

2.7.1. GIS based Multi-Criteria Evaluation

GIS is a useful tool to provide spatial analysis support to complex issues concerning aquaculture and coast planning (Nath et al., 2000), and balance conflicting uses and respective management (Sanchirico et al., 2013). It allows the storing, elaboration and analysis of data (e.g. external environmental factors, infrastructure facilities) relevant for aquaculture development in a certain location, allowing an antecedent study that evaluates whether or not an aquaculture project has conditions to be successful (Meaden and Aguilar-Manjarrez, 2013).

However, there are several limitations inherent to the use of GIS that might limit the final outputs (e.g. Griffin, 2013; Nath et al., 2000; Puniwai et al., 2014): i) the final output is dependent on the accuracy of the collected data; ii) high spatial resolution data can be limited by hardware and budget; iii) inputs are subjective and are opened to interpretation; iv) disregard of the real studied area, by focusing only on the GIS maps.

Relevant applications of GIS tools in an aquaculture site selection context can be further consulted in Buitrago et al. (2005), Ragbirsingh and Souza (2005), Pérez et al. (2005), Radiarta et al. (2008), Longdill et al. (2008).

Multi-Criteria Evaluation (MCE) is one of the most common methods for aquaculture site selection (Hossain et al., 2008; Silva et al., 2011; Radiarta et al., 2008; Falconer et al., 2016; Dapueto et al., 2015), since it allows the combination of several variables (e.g. temperature, food sources, distance to urban areas) in a structure, where each variable has a relative weight proportional to its importance (Nath et al., 2000). This allows an assessment of spatial variability of each criterion, namely environmental, socioeconomic, biological and spatial variables relevant to an aquaculture site selection study, considering each level of relative importance, and providing a qualitative and quantitative analysis, useful for decision makers (Baidya et al., 2014; Falconer et al., 2016).

Before comparisons can be made, all studied variables must be reclassified to a common scale (e.g. 0 – 1, 0 – 100, 0 – 255) (Eastman, 1999); afterwards weights can be assigned, either based on expert knowledge, through the literature in similar works or comparison between relative weights (i.e. Analytical Hierarchy Process).

As previously mentioned, GIS and MCE are connected, since data collected concerning the MCE's criteria can be stored and furthered analyzed in a GIS software (e.g. ArcGIS, QGIS): according to Eastman (1999), GIS based MCE has two different ways to approach:

- All criteria are converted to Boolean (logical true/false), according to which areas are suitable (1) or unsuitable (0). This is mostly applied to constraints for the site selection, which can be further combined by different degrees of intersection (e.g. logical AND) or union (logical OR);
- In the second most common procedure for MCE, quantitative criteria (typically defined as factors) are evaluated as continuous variables, each expressing different degrees of suitability, and then converted to a common scale (Voogd, 1983). Afterwards, each criterion is combined by means of a weighted linear combination (WLC) and then the result can be calculated by the product of the constraints, according to the following equation, Eq. (1):

$$S = \sum w_i X_i * \prod C_j \quad (1)$$

where S = Suitability

w_i = weighted assigned to factor i

X_i = criterion score of factor i

C_j = constraint j

However, as stated in Silva et al. (2011) and Griffin (2013), most applications of Multi-Criteria Evaluation to aquaculture site selection (e.g. Dapueto et al., 2015; Falconer et al., 2016; Hossain et al., 2008; Lin, 2010; Radiarta et al., 2008) do not include dynamic models for estimation of carrying capacity and temporal variability of environmental effects, which might be considered detrimental to differentiate successful and unsuccessful aquaculture practices.

2.8. Dynamic modelling

The Farm Aquaculture Resource Management (FARM) is a model targeted at farmers and managers, that combines physical and biogeochemical models, shellfish growth models, and eutrophication screening models, in order to determine shellfish production, providing a marginal analysis of farm production potential and profit maximization, as well as an adapted version for the eutrophication assessment, based on Bricker et al. (2003), accounting for nutrient trading credits according to carbon and nitrogen removal, Fig. 1 (Ferreira et al., 2007, 2009a).

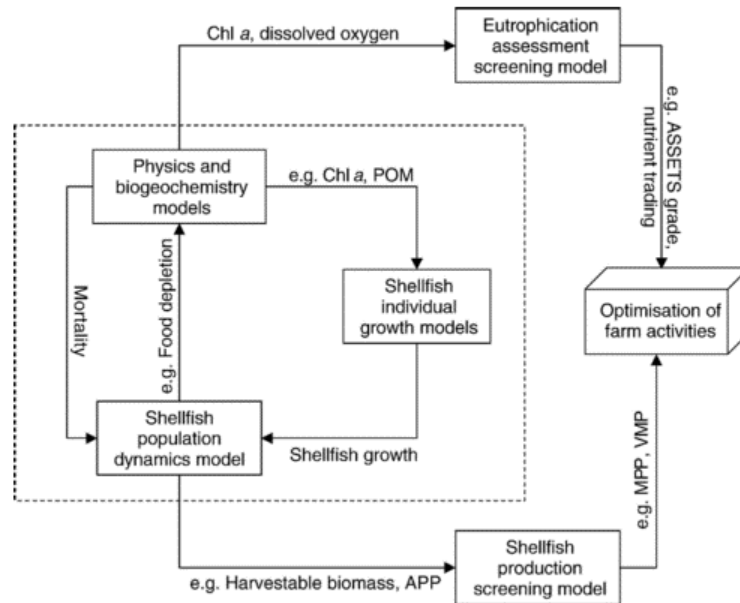


Fig. 1: Conceptual scheme of the model's components; in the dotted rectangle is displayed the core model (Ferreira et al., 2007)

In the present work, an adapted and simplified version of FARM was used, therefore only those outputs will be addressed. A more thorough description about FARM can be found in Ferreira et al. (2007), www.farmscale.org and Ferreira et al. (2009 a,b).

The input requirements of the model can be divided into three groups (Ferreira et al., 2007; Silva et al., 2011): i) time-series of drivers for environmental conditions, such as salinity, dissolved oxygen, suspended particle matter (TPM) and particulate organic matter (POM), chlorophyll a, current speed and tidal variations; ii) farm layout and respective dimensions, and iii) shellfish cultivation practices (cultivation density, mortality and culture period).

FARM can simulate the individual growth of several bivalve species (e.g. Pacific oyster *Crassostrea gigas*, blue mussel *Mytilus edulis*, Chinese scallop *Chlamys farreri*, cockle *Cerastoderma edule*), although only Manila clam *Ruditapes philippinarum* was used, whose model is based on Solidoro et al. (2000). Biomass production of market-size organisms is simulated, by integrating shellfish growth in a population dynamics framework using well-established equations

The general layout input is shown in Fig. 2 and can be applied to suspended culture from rafts or longlines, as well as bottom culture (Ferreira et al., 2007).

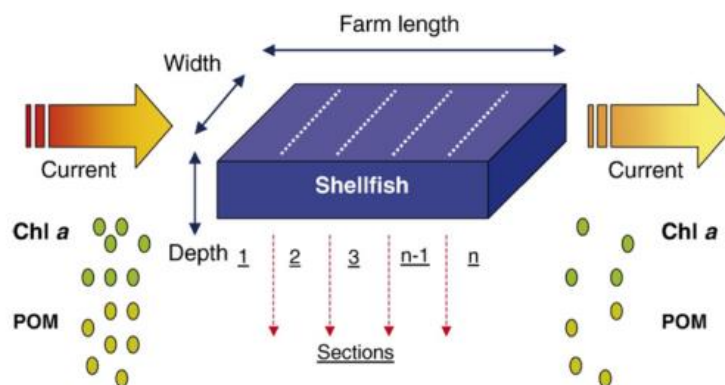


Fig. 2: Farm layout (rope and bottom culture) (Ferreira et al., 2007)

The outputs of the used version of the FARM model are only based on aquaculture production and associated economic value, and as defined in the original paper (Ferreira et al., 2007):

- FARM's aquaculture production is based on a Cobb-Douglas equation (McCausland et al., 2006), which establishes a relation between total physical product (TPP) and total fresh weight (TFW):

$$Y = f(x_1, x_2, x_3, \dots, x_n) \quad (2)$$

Where

Y – output of harvestable organisms

x_1 – initial stocking density of seed, considered the only variable input

x_2 - x_n – other inputs, considered as constant

FARM also calculates the average physical production (APP) after each run (Eq. (3)), as well as the equivalent expressed as individuals, providing an indicator of the capacity of the farm to produce harvestable animals.

$$APP_{x_1} = \frac{TPP}{x_1} \quad (3)$$

3. Methodology

3.1. Overview

This thesis applies Geographical Information Systems (GIS) methods for aquaculture site selection (e.g. Silva et al., 2011; Dapueto et al., 2015) and combines these with local-scale dynamic models for production and environmental effects (e.g. Ferreira et al., 2007). Different spatial factors and other categories are used to identify potential cultivation sites, and the aquaculture performance at those sites is assessed by means of the Farm Aquaculture Resource Management (FARM) model.

Therefore, it was developed a four-stage methodology approach (Fig. 3), combining both a Multi-Criteria Evaluation and Dynamic Modelling. The methodology used was adapted mainly from two different works: Silva et al. (2011) and Falconer et al. (2015).

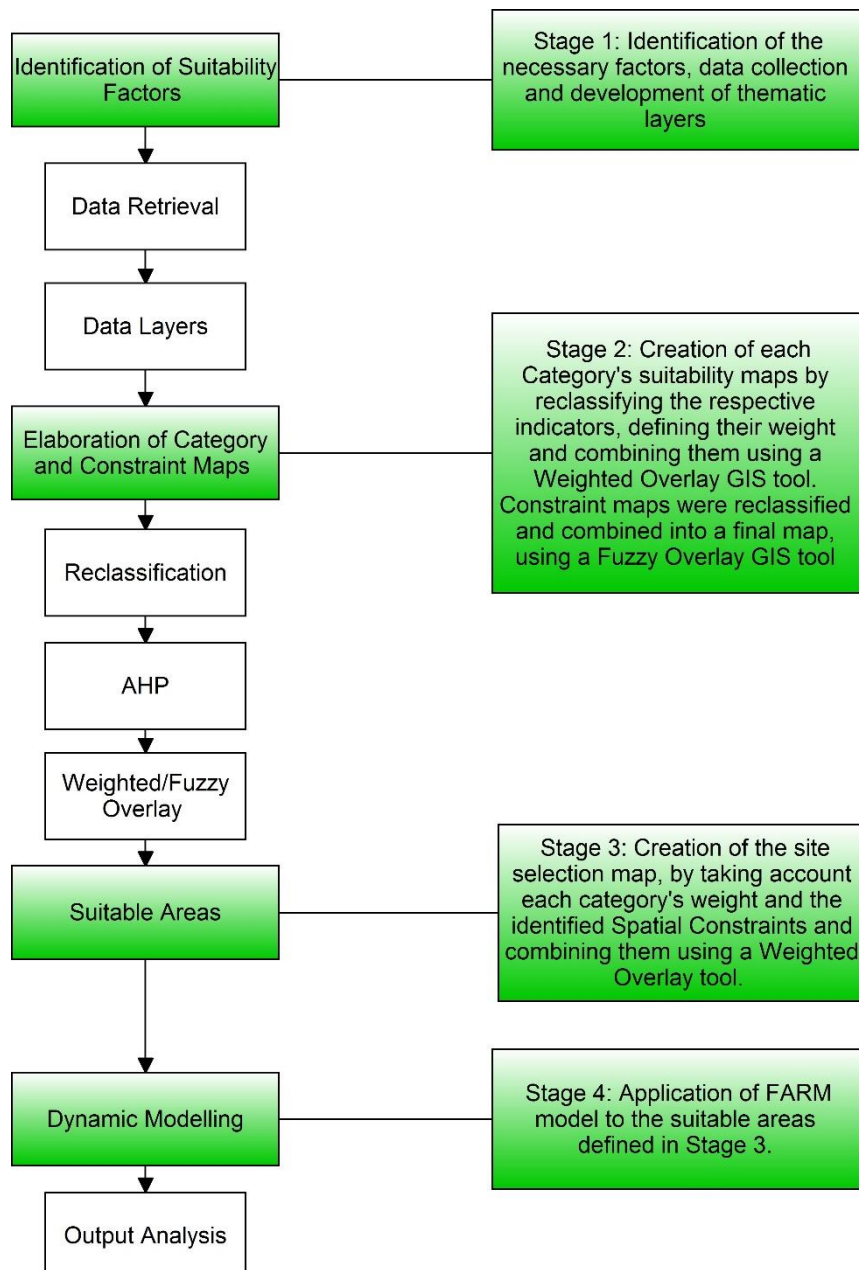


Fig. 3: Conceptual diagram of the methodology used (adapted from Falconer et al., 2015)

The main goal of the MCE is to achieve a final result of all suitable areas for shellfish aquaculture in the Tagus Estuary, while FARM's is to study possible economic and environmental impacts associated with shellfish farms. To achieve this, it was developed a three stepped MCE model, which consists of:

- 1) Identification, data retrieval and elaboration of Suitability Factors (in Dapueto et al., 2015), hereby defined as Categories and respective indicators, that might influence the suitability of a shellfish farming site, as well as the existing Spatial Constraints in the estuary.
- 2) The thematic maps are reclassified into a common scale, each indicator's weight is calculated by means of an AHP and then combined into three categories using GIS based Weighted Overlay technique; the final Spatial Constraints map was obtained using GIS based Fuzzy Overlay.
- 3) Weights are defined for each category and then a suitability equation is applied, by means of a Weighted Overlay, accounting for each category suitability maps and the final Spatial Constraints map. The outcome is defined as the site selection thematic map.
- 4) Application of the FARM model to suitable areas defined in Stage 3;

This methodology was tested for Manila clam aquaculture in a study area located in the Tagus Estuary, Portugal (38°40'N; 9°15'W).

3.2. Study area

The Tagus Estuary (38°44'N, 09°08'W) (Fig. 4) is one of the largest estuaries in Europe, covering nearly 320 km² (Monteiro et al., 2016), having an average depth of less than 10 m, as well as containing small islands, sand banks and an intertidal zone with a total area of 146 km², mainly composed of tidal flats (64%), salt marshes (13%) (Caçador et al., 1996) and dead oyster beds (Granadeiro et al., 2007) with an area close to 16 km² (Ferreira et al., 2003). Studies (Valentim et al., 2013; Fortunato et al., 1999; Dias and Valentim, 2011) refer the importance of the tidal flats, as they slow tidal propagation and highly dissipate tidal energy or, from an ecological approach, significantly contribute to primary production.

According to Pritchard (1989), the estuary can be classified as a coastal plain estuary, due to its morphological characteristics, such as its location in temperate latitudes, the maximum depth of about 45 m, its large width-depth ratio, the existence of intertidal flat, extensive mudflats and saltmarshes; although its shape does not fit with coastal plain estuaries, as it does not widen towards the mouth (Neves, 2010). Also, according to NOAA's classification (Duarte et al., 2013), it is considered a mesotidal estuary, with an average tide amplitude of 2.4 m, ranging between 1 and 4 m during neap and spring tides, respectively (Dias et al., 2008; Campuzano et al., 2012); the tidal excursion is also almost 50 km from the mouth, near Vila Franca de Xira (Guerreiro et al., 2015). The mean air temperature is 16.3 °C and the total annual precipitation of 700 mm (Gameiro et al., 2004).

The Tagus river is also the major source of freshwater, with a mean river flow of 400 m³ s⁻¹, variable both seasonally and interannually (França et al., 2005), with rivers Sorraia and Trancão (displayed in Appendix A) being comparatively smaller, with average annual discharges of 35 and 2.5 m³ s⁻¹, respectively (Neves, 2010). The water column is well mixed during spring tides, but it is partially stratified during neap tides (Vale and Sundby, 1987). In addition, strong stratification can occur in the upper estuary under large river flows and neap tides.

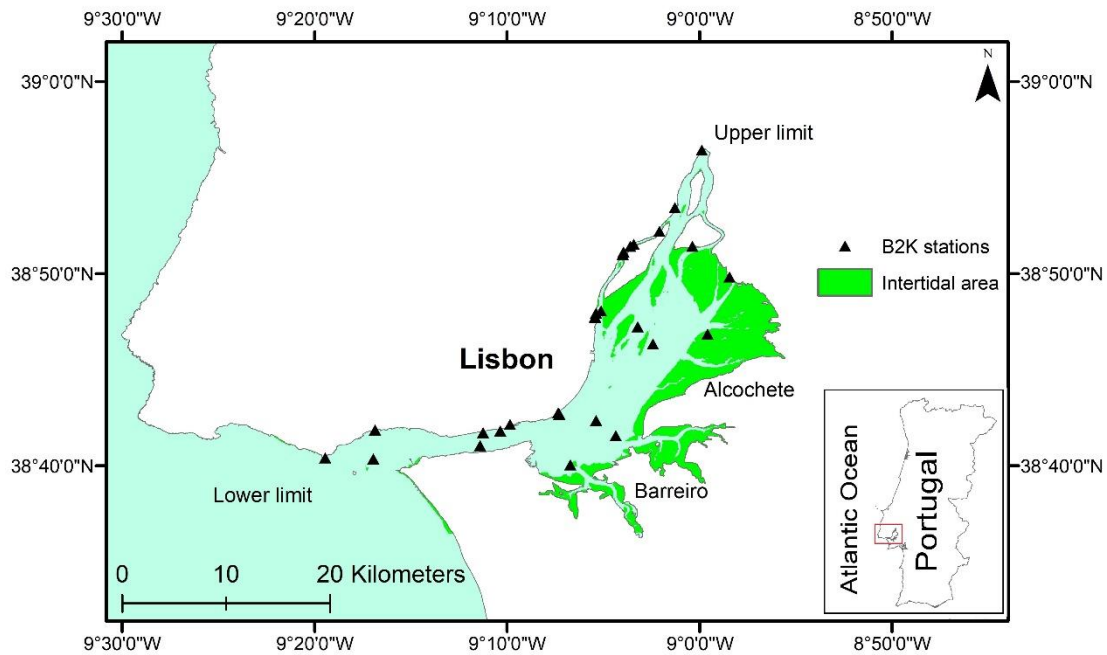


Fig. 4: Area in study, the Tagus Estuary, and respective lower and upper limits

The Tagus Estuary is also located in the most populated area of Portugal (Caçador et al., 2012a), the Lisbon Metropolitan Area, with 2.5 million people living in its vicinities (Campuzano et al., 2012). Because of this, it has been subjected to intense anthropogenic impacts over the years, related to fishing, industrial growth, urbanization, agriculture and port activities, that leads to heavy metal contaminations (Cabral et al., 1999; Carvalho et al., 2007; Fauconneau, 2001; Figuères et al., 1985; Vale, 1990; Araújo et al., 1998; Vale et al., 1998, 2008; Canário et al., 2003; Nogueira et al., 2003), which tend to be adsorbed on cohesive sediments and accumulated in saltmarshes (Caçador et al., 2009; Caçador and Duarte, 2012b).

França et al. (2005) also concluded that the estuary is contaminated, by studying heavy metal concentrations (Cd, Cu, Pb and Zn) in sediments, molluscs and fishes in three salt marshes. Studies also have shown high levels of Zn, Cu, Pb and Hg in sediments (Vale et al., 2008), suspended particle matter (Vale, 1990; Canário et al., 2008) and in tissues of several fish and crustacean species (Ferreira et al., 2003), and faecal contamination, as reported in Anacleto et al. (2013). The same author proved that *E. coli* levels are related to areas with industries, farming explorations and highly populated areas, with high levels of *E. coli* in clams harvested within the estuary. Those contaminated areas are usually where the shellfish harvesting is located (Cunha, 2012).

3.3. GIS based Multi-Criteria Evaluation for shellfish aquaculture site selection

The first three stages in the present methodology are based on a Multi-Criteria Evaluation by means of GIS tools. The application of the Multi-Criteria Evaluation aims to achieve the evaluation of the suitability for shellfish aquaculture in the Tagus Estuary.

Firstly, emphasis should be added to MCE's used terms, in order to achieve the final goal of the final site selection thematic map. Three different criteria will be used and defined as categories. Within each category there are several indicators that together are used to describe it. Categories can also be named "Suitability Factors", since each category will affect the overall suitability for shellfish aquaculture.

Each indicator will be mapped using a GIS tool, which will be defined as a thematic map. Thematic maps will then be grouped according to each category, forming a final Category suitability map, an adapted version of those used in Falconer et al. (2016). Afterwards, by

grouping each identified category, a final suitability thematic map will be produced, allowing an assessment of possible locations for shellfish aquaculture.

3.3.1. Stage 1: Identification of Suitability Factors

The first stage of the MCE is focused on the identification of Suitability Factors. Suitability Factors were chosen according to the literature and adapted to the existing conditions in the Tagus Estuary. The chosen Suitability Factors were divided into three main categories: Environmental Category, Production Category and Product Contamination Category. Each Category is subsequently divided into several different indicators (Fig. 5).

The next step was to search, retrieve and elaborate data to construct thematic maps corresponding to the selected indicators for each Category/Suitability Factor. This was achieved by searching for either scientific works in the Tagus Area or looking for GIS data, which will be further addressed.

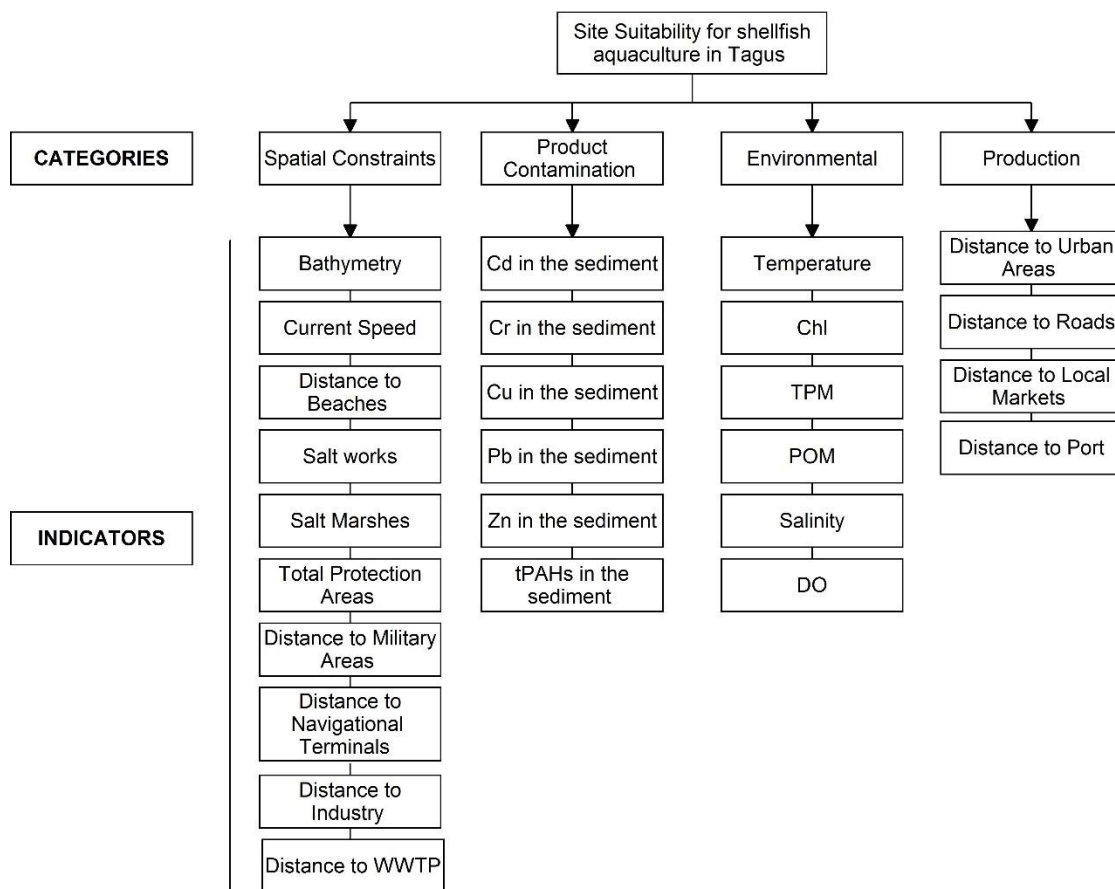


Fig. 5: Suitability Categories and respective indicators for shellfish aquaculture site selection in the Tagus Estuary

All GIS thematic maps were created under the same coordinate system (WGS 1984) and assigned a Tagus Estuary Analysis Mask so that processing only occurs on locations that fall within the mask, with locations outside of it assigned to NoData in the output (Silva et al., 2013a) – Appendix A.

3.3.1.1. Spatial Constraints

Spatial Constraints here are defined as all conditions and factors that screen out unsuitable areas for shellfish aquaculture in the study area, by delimiting possible legal and spatial obstacles, in contemplation of helping decision making. Spatial Constraints allow the mapping of the maximum possible area for shellfish aquaculture by studying possible limitations, such as sources of pollution (Industry and WWTP), possible human interferences (beaches and

navigational ports), as well as either forbidden areas (Military Areas), culture practices for shellfish aquaculture (Current Speed and bathymetry) or legal requirements (Natura Network 2000, Total Protection Areas and Salt marshes).

The Spatial Constraints considered relevant for the Tagus Estuary are identified in the table below (Table 6), as well as their corresponding source and map type.

Thematic maps created from point files (vector and table list) were either interpolated (Inverse Distance Weighted – IDW) within the used mask limits or safety buffers created around each point (e.g. Distance to WWTP); thematic maps based on polygon files were created by selecting the necessary features (e.g. selecting “industry” from CLC12). The only exception was Total Protection Areas, which was delimited manually in ArcGIS editor, since it did not recognize the used WFS, in order to digitize it with the Vectorization command from ArcScan extension.

Table 6: Relevant Spatial Constraints for shellfish site selection in Tagus, sources of data used in the MCE (GIS)

| Spatial Constraint | Data Sources | Map type |
|------------------------------------|--|-----------------|
| Bathymetry | Interpolated using data from Instituto Hidrográfico (2013) | Point |
| Current Speed | Interpolated using data from B2K | Point |
| Distance to military areas | OpenStreetMaps (2016) | Polygon |
| Salt works | DGT (2017); EEA (2014) | Polygon |
| Salt marshes | EEA (2014); RAMSAR (1992) | Polygon |
| Total Protection Areas | Contoured according to IGEO (2016) | Line |
| Distance to beaches | DGT (2017) | Polygon |
| Distance to navigational terminals | DGT (2017) | Polygon |
| Distance to industry | Copernicus (2012) | Polygon |
| Natura 2000 Network | EEA (2016) | Polygon |
| Distance to WWTP | Data obtained (coordinates) through Saraiva (2001) and CMLisboa (2014) | Point |

3.3.1.1.1. Indicators

The rationale behind each individual spatial constraint identified is briefly explained in Table 7.

Table 7: Brief description of each individual spatial constraint

| | |
|------------------------------|--|
| Bathymetry and current speed | <p>Included in Spatial Constraints are current speed and bathymetry. Both are fundamental aspects of the inherent type of applied culture system (Silva et al., 2011), since current speed has influence on food transport as well on dilution of waste, with an optimal water flow of $0.3 - 1 \text{ m s}^{-1}$ (Vincenzi et al., 2006): slower current speeds do not allow appropriate water circulation, whereas higher values might have negative impacts on the intertidal areas (Vincenzi et al., 2006).</p> <p>The same author reports that optimal sites for Manila clam located in water depths of less than 2 m; Toba et al. (1992) states that Manila clam are most abundant at 1-2m. Below that range they are vulnerable to various predators and may have to compete against other hard clam species; they are also easier to harvest. Above it, they are too exposed to the air and their growth rate declines (Toba et al., 1992). Hence, they are both essential for shellfish aquaculture practices, and because of this they are not part of either category.</p> |
| Natura 2000 | <p>Another selected Spatial Constraint with a special connotation is Natura 2000 Network, which aims to protect habitats and species of European interest that are rare or threatened, however it does not necessarily exclude all human activities. Its aim is to ensure that, within these Natura 2000 sites, human activities are undertaken in a way that still allows the site’s conservation objectives to be reached (EC, 2012a).</p> <p>Natura 2000 was briefly explained in table 3: aquaculture projects within the area included in Natura 2000 are required to assess possible environmental impacts, where afterwards the competent national agencies may approve it or not. In the present work, areas within the Tagus Estuary that are encompassed in Natura 2000 are only going to be analyzed after the creation of the site selection thematic map (Appendix B).</p> |

(cont.) Table 7: Brief description of each individual spatial constraint

| | |
|------------------------------------|--|
| Distance to Military Areas | <p>Land use areas corresponding to military areas are unsuitable for coastal aquaculture (Gibson et al., 1993; Coimbra, 1998; Puniwai et al., 2014). According to Gibson et al., (1993), military use of the coastal areas should present little environmental impact. However, a large military presence could pose a potential environmental problem, as could other unmanaged human activities.</p> <p>It was decided to try to contact some military facilities in the study area, namely Montijo's Air Base No. 6, and inquire whether a possible aquaculture project in their adjacent area would be authorized, which the answer was no, which is in accordance with Decree No. 42090/1959. Because of this, military areas within the estuary were considered as unsuitable, and a safety distance was created.</p> |
| Distance to Beach | <p>Beaches are here defined as a spatial constraint in contemplation of avoiding the perturbances that may be caused by beach users, as well as to avoid possible theft situations. The presence of beaches within estuaries is frequent (Freire et al., 2006): the area of the Tagus Estuary's mouth is influenced by ocean waves, with the estuary's interior being sheltered from the ocean's influence. They are formed exclusively by local wind driven waves (Jackson and Nordstrom, 1992).</p> <p>Freire et al. (2006) divided Tagus left margin in several sectors, each containing the main estuarine beaches: Alcochete-Samouco, Air Base (Samouco to Montijo); Montijo's inlet, Barreiro's inlet, Seixal's bay and Alfeite. Despite the fact that the Tagus interior beaches are not yet suitable for bathing season (APA, I.P/ARH), people still go there and therefore a safety distance must be established.</p> |
| Distance to Industry | <p>Anthropogenic impacts related to industrial growth are well known and studied in Tagus, with considerable environmental impacts, namely high concentrations of heavy metals in industrial areas in the riverbanks, such as lead, cadmium and zinc, in superficial waters, deep sediment layers and particulate matter (e.g. Vale et al., 2008). This way, an appropriate safety distance to industries is important to screen out areas negatively influenced by industry and thus, to the site selection itself.</p> |
| Salt works | <p>Salt works are physical structures created for salt extraction, that has tanks with different depths: the seawater coming from the estuary passes through each tank, increasing the concentration of dissolved salts and subsequent deposition. This, in addition to the sequence and disposition of the existing tanks enables the existence of some fish and shrimp species in the first tank (called Nursery), as well as insect larvae, small coleoptera (beetles) and crustaceans, such as <i>Artemia spp</i> on the remaining tank (ICNF, n/d): <i>Artemia</i> is very important in salt works as it feeds on the organic particles that would otherwise contaminate salt crystals, and convert them into cysts or faeces that do not impact salt production (Sorgeloos, 1983); in addition, <i>Artemia</i> can be also used in aquaculture (Sorgeloos, 1983), as it produces cysts that can be hatched on demand, in order to be used as live food to fish or crustacean larvae (Sorgeloos et al., 1977).</p> <p>Salt works are considered sheltered places because of their placement in salt marshes (e.g. Lindemans, 2010); this, adding to the existing food sources, make them important feeding and resting habitats in the winter high tides for many species, while in spring and summer they are a nesting place for waders (ICNF, n/d). The Samouco Saline Complex is included within Tagus Estuary Special Protection Areas (Birds Directive 2009/147/EC) (Araújo et al., 2006). Inactive facilities exist throughout the estuary, which adding to their ecological importance, made them unsuitable for shellfish aquaculture.</p> |
| Distance to Navigational Terminals | <p>Shellfish aquaculture sites should not interfere with navigational terminal, harbors or impede navigation within the estuary. In particular, areas inside and in the vicinities, where entry and exit maneuvers are regularly carried out (Maritime New Zealand, 2005). Also, proximity to the previously mentioned locations may be associated with sediment disturbances, as well as proximity to people. Therefore, areas close to those sites were regarded as unsuitable and in order to prevent possible negative interactions between shellfish aquaculture sites and navigational terminals, future studies should further investigate active all active terminals.</p> |
| Distance to WWTP | <p>There are many urban areas within or near the Tagus Estuary area, where the corresponding urban wastewaters are discharged into the estuary, making areas near Wastewater Treatment Plants (WWTP) discharges known for their pollution (Saraiva, 2001). According to the same author, Trancão river also is a meeting point to three different WWTP plants (Saraiva, 2001) and thus, it is also considered. A safety buffer was established, since it was not found any reference to WWTP in aquaculture site selections.</p> |

(cont.) Table 7: Brief description of each individual spatial constraint

| | |
|--|---|
| TPA | Total protection Areas (Hidroprojecto, 2007) are natural areas defined as having high environmental sensitivity, hence they are focal points concerning nature conservation. They consist of two different areas, namely Pancas salt marsh and intertidal zone associated, in order to properly manage their inherent natural processes, as those areas are considered as feeding, resting and nesting areas for aquatic birds. Because of this, the mentioned areas were classified as unsuitable. |
| Salt marshes – Partial Protect Area (type I) | Salt marshes are found in flat, protected waters usually within the protection of a barrier island, estuary, or along low-energy coastlines (e.g. Daly, 2013). According to Reis (2009), salt marshes have several ecosystem functions, such as i) habitat to many crustaceans and fish species, due to high levels of phytoplankton and other particles suspended in the water column; ii) nurseries for many ecological relevant species; iii) nesting and resting areas for migratory birds and iv) can act as a sink for pollution. Type I of Partial Protection Areas represent areas containing relevant scenic and natural resources, defined as having a moderate ecological sensitivity. The identified Type I areas are all salt marshes within the Tagus Estuary Nature Reserve, and although salt marshes are ideal places for aquaculture (Cloern, 1982; Roman and Tenore, 1984; Newton and Mudge, 2005), salt marshes within Ramsar convention wetland areas (Ramsar Convention Secretariat, 2013) were considered unsuitable. |

3.3.1.2. Category 1: Production

Production category assesses the distance between facilities (i.e. farms), such as piers (here defined as Port) and Local markets, as well as accessibilities, namely roads and work labour (Urban Areas). All the mentioned factors are important because they increase the efficiency of the access to shellfish farms, since farms must be at a reasonable distance from roads, and afterwards, the transportation of the shellfish from the site, directly to markets or relaying sites within the estuary, if possible. Farms should also be a suitable distance of the population, neither too close, as to avoid possible visual impacts, nor too far.

Hence, category 1 was named “Production”, since all identified indicators evaluate where the production can occur (where it can be sold; where people who can work it through are located; where it can be transported).

Distance to local markets was the only created thematic map based on point files and was created based on OSM’s maps. This was further improved by searching for markets in the studied area using Google Earth Pro and saving their coordinates (Appendix A). The remaining thematic maps were created by selecting the necessary features from the data sources displayed in Table 8.

Table 8: Relevant indicators concerning Production Category for shellfish site selection in Tagus, sources of data used in the MCE (GIS)

| Indicators | Data Sources | Map type |
|---------------------------|---|----------|
| Distance to urban areas | Copernicus (2012) | Polygon |
| Distance to local markets | OpenStreetMaps (2016) | Point |
| Distance to port | Open Street Maps (2016) | Line |
| Distance to major roads | Open Street Maps (2016) Infraestruturas de Portugal roads thematic map | Line |

3.3.1.2.1. Indicators

The indicators relevant for aquaculture production in the Tagus Estuary are represented in Table 9.

Table 9: Brief description of Production indicators

| | |
|---------------------------|--|
| Distance to urban areas | Urban areas in this context are characterized by being discontinuous urban fabric, which is a CLC nomenclature (Rojo, 2015). Urban areas consist of the existing labour within the area, as most existing urban areas are gathered around the estuary, which, in addition to fishing tradition in many more secluded areas outside of Lisbon (Alcochete, Seixal, Trafaria, Póvoa de Santa Iria, Barreiro and Samouco) (Cunha, 2012) and the existing illegal circuit, means that there is plenty of labour already with some degree of know-how. |
| Distance to local markets | Local markets, as the name implies, represent only local markets close to the estuary. Possible shellfish aquaculture areas must be as close to local markets, as it reduces transport costs: the closer the market is to the farm, the fewer intermediaries (Spliethoff, 1987). It was already mentioned that there is a known issue regarding illegal fishing of Manila clam: and as reported in Ramajal et al. (2016), there are middle-men who directly sell to markets, as well as restaurants and other food outlets. |
| Distance to port | The existence of piers (defined as port, in the present work) in the study area allows fishermen transportation within the estuary, which increases accessibility by enabling access to otherwise more difficult areas to go, by either foot or road. It may enable transportation of shellfish from more polluted areas to cleaner ones (relaying areas). This is linked to a Spatial Constraint identified as Distance to Navigational Terminals and Ports, meaning that areas near to the piers are unsuitable. |
| Distance to major roads | A GIS assessment of accessibilities in areas near to the estuary was needed in order to study the areas gathering the best conditions for shellfish aquaculture closer to roads. According to Perumal et al. (2015), accessibility is important for aquaculture practices as it is important for seed production and transportation of broodstock and juveniles. Proximity to roads is also important because by reducing transportation cost, it reduces product price determination (Spliethoff, 1987). It also facilitates transportation for relaying technique: change the harvested product in a polluted area (such as Class C) to a cleaner one (Class A or B). This will be further explained In Chapter 4. |

3.3.1.3. Category 2: Environmental

When selecting a site for commercial production of Manila clams, environmental indicators that influence clam survival and growth should be studied (Toba et al., 1992; Silva et al., 2011), such as temperature, salinity, food availability (Chlorophyll a and POM), dissolved oxygen and Total Particulate Matter (TPM). Optimal sites are characterized by gathering suitable conditions for growth and development of the clams, since growth and high quality shellfish are essential for the economic sustainability of the industry (Longdill et al., 2008).

Indicators like bathymetry and current speed could also be categorized as Environmental indicators. But as already explained, since both are fundamental for culture practices and will influence where the site selection is going to be, environmental indicators must be studied within those sites. Other factors such as substrate type (e.g. Toba et al., 1992) could also be listed below.

Ideal substrate type for clam cultures is characterized by a high sand percentage and the remaining fraction made up of silt and clay (20-30%) (Vincenzi et al., 2006; Toba et al., 1992). According to ICNB (2007), silt sediments prevail in the upper and middle part of the estuary, while the sand sediments are at a greater percentage in the lower estuary (Fig.6), which is in accordance with the optimal sediments type.

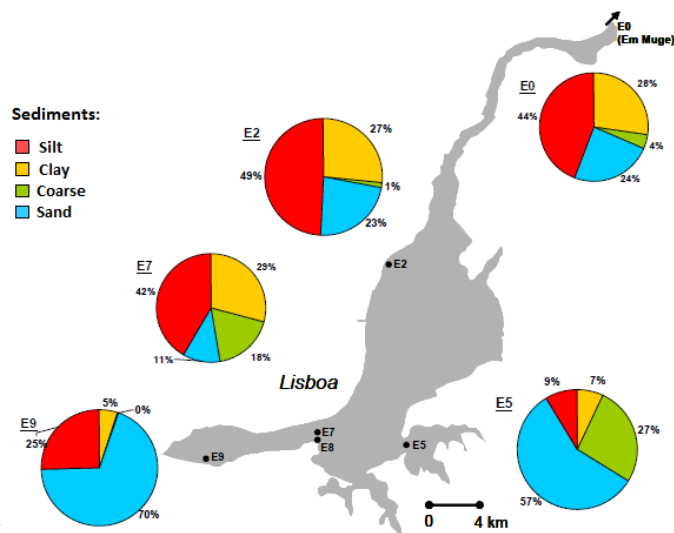


Fig. 6: Type of sediments in the estuary (Adapted from ICNB, 2007)

To study where the best environmental indicators are gathered in the estuary, it was used GIS to map the chosen category. In the table below (Table 10), it is represented each chosen indicator, along with its source and GIS map type. All the data for the creation of thematic maps concerning Environmental indicators is displayed in Appendix A.

All used indicators were based on data from B2K database (water quality data retrieved in the 1990s from the Tagus Estuary) and then interpolated in ArcGIS, according to the spatial limits defined.

Table 10: Relevant indicators concerning Environmental Category for shellfish site selection in Tagus, sources of data used in the MCE (GIS)

| Indicators | Data Sources | Map type |
|-------------|--|----------|
| DO | Interpolated using data obtained from B2K Database | Point |
| Temperature | | Point |
| Salinity | | Point |
| Chl | | Point |
| TPM | | Point |
| POM | POC to POM conversion: $POC = 0.38POM$ (Grant and Bacher, 1998). The data was then interpolated in ArcGIS. | Point |

3.3.1.3.1. Indicators

Several relevant environmental variables (i.e. Environmental indicators) were already briefly mentioned in Chapter 2.5 “Manila clam”, although they will be more thoroughly addressed in Table 11.

Table 11: Brief description of Environmental indicators

| | |
|----|--|
| DO | Dissolved Oxygen is essential to Manila clams' because if the oxygen concentration of the water becomes too low, the difference between the water and the circulatory system is lower, which may reduce or even stop the transfer of oxygen. At this point, the clam will close its valve or shell and begin to use its anaerobic metabolism, converting fuel reserves to energy without using oxygen (Weber et al., 2008). This produces far less energy (6% of the aerobic metabolism), as well as the fact that the clams are not feeding, growing or putting effort into reproduction (Weber et al., 2008; Swingle, 1969). Adult hard clams can maintain aerobic respiration down to DO levels of about 5 mg L^{-1} (Weber et al., 2008; Vincenzi et al., 2006). |
|----|--|

(cont.) Table 11: Brief description of Environmental indicators

| | |
|-------------|---|
| Temperature | <p>Clams are poikilothermic organisms; therefore, their metabolism is influenced by water temperature: increasing water temperature increases metabolic rate, and the opposite, affecting both growth and reproduction (Weber et al., 2007).</p> <p>Intertidal organisms such as the Manila clam are highly exposed to the effects of high temperatures above their biological threshold: Malouf and Bricelj (1989) reported thermal tolerances of 4-30 °C for adult Manila clams, with an optimum range of 16-27 °C (Weber et al., 2007; Macho et al., 2016) and 14-27 °C for larvae, while Chew (1989) reported temperature tolerance for larvae of 0-36 °C, whereas the optimum was between 23 and 24 °C -, especially during low tide in the summer months. This alters their performance, by limiting feeding and growth rates, activity, burrowing capacity and ultimately causing mortality (Verdelhos et al., 2015; Morley et al., 2012; Humphreys et al., 2007).</p> |
| Salinity | <p>The Manila clam is euryhaline and inhabits a wide range of areas (Gillespie et al., 2012), however salinities varying from 25 to 35 psu are considered the optimal physiological values (Baker et al., 2007; Dang et al., 2010; Coughlan et al., 2009; Vincenzi et al., 2006), as the feeding rates, growth and pumping rates are at their peak (Baker et al., 2007). According to Coughlan et al. (2009), clams can maintain normal metabolic activities in a wide range of salinity levels (15-35 psu) for long periods of time.</p> |
| Chl | <p>Most clams feed on organic particles (POM) and phytoplankton, which has a photosynthetic pigment named chlorophyll <i>a</i> (Chl) that mediates photosynthesis and consequently, primary production (Komorita et al., 2014). Studies have highlighted phytoplankton's importance regarding bivalve survival and growth (Grant et al., 1998; Zarnoch and Schreiberman, 2008); also, Chl is considered to be an indicator for food availability in bivalve growth modelling (e.g. Vincenzi et al., 2006; Hyun et al., 2006), where according to Toba et al. (1992), the Manila clam appears to grow better when a moderate food supply is consistently available over long periods than when a lot of food is available sporadically for shorter periods, even though it can change seasonally, as the metabolic demands increase with temperature (Zarnoch and Schreiberman, 2008; Komorita et al., 2009).</p> |
| POM | <p>Particulate organic matter includes the suspended organic particles in tidal flats, containing carbon from many different sources (e.g. phytoplankton, sewage effluents) (Müller-Solger et al., 2002), and are an important food source for benthic animals (Yoshino et al., 2012). Particles size influence the ability to efficiently filter, retain and ingest food, with smaller particles having lower retention efficiency (Secrist, 2013). The same author has also highlighted that both POM and Chl are used in the assessment of food availability, in order to account for alternative food sources, such as benthic microalgae and macroalgae detritus.</p> |
| TPM | <p>Total particulate matter comprises the organic and inorganic suspended particles in the water column, controlling the turbidity and transparency of the water, according to the type and concentration of the suspended particles (Hancock and Hewitt, 2004).</p> |

3.3.1.4. Category 3: Product Contamination

Heavy metals are widespread and persistent in aquatic ecosystems, potentially toxic (Suresh et al., 2012; Taweel et al., 2013; Tang et al., 2014), since they can be adsorbed by suspended solids, then strongly accumulated in sediments and biomagnified along aquatic food chains (Yi et al., 2011; Gumgum et al., 1994). Sediments act as sinks, and may in turn act as sources of heavy metals (Suresh et al., 2012).

Because of this, in order to evaluate heavy metal pollution in sediments and shellfish, studied heavy metals were grouped into Product Contamination Category. This denomination comes from the fact that heavy metal pollution is identified as a variable that influences the product quality of shellfish aquaculture (Silva et al., 2011; Laing and Spencer, 1997; Silva and Batista, 2008; Oliveira et al., 2013). Shellfish accumulate heavy metals in their tissues in proportion to the degree of environmental contamination, and therefore can raise public health concerns as they are consumed as seafood (Cardellicchio et al., 2010)

Heavy metals studied in the present work are Chromium, Cadmium, Copper, Zinc, Lead and total Polycyclic Aromatic Hydrocarbon (tPAH) and are represented in the table below (Table 12), as

well as their GIS sources and map type. The selected heavy metals were chosen according to their toxicity and availability in sources available online.

Vale (1986) collected superficial sediment samples to study the presence of the heavy metals already mentioned. Because it was only mapped out where the samples were taken, not their coordinates, the stations were visually pinpointed using Google Earth Pro. Even though this is not the best and most efficient method, the amount of stations made up for the lack of coordinates. Torre (2014) PAH values were also used, where EPA's sixteen were summed (tPAHs).

After being added to ArcGIS, each sampling point was interpolated according to the used mask limits. This was done for every indicator displayed in Table 12.

Table 12: Relevant indicators concerning Product Contamination category for shellfish site selection in the Tagus, sources of data used in the MCE (GIS)

| Indicators | Data Sources | Map type |
|------------|--|----------|
| Cr | Data obtained (Cr values, sample station site) in Vale (1986), coordinates were taken from Google Earth Pro and the data were interpolated afterwards. | Point |
| Cd | | Point |
| Cu | | Point |
| Zn | | Point |
| Pb | | Point |
| tPAHs | Data obtained (tPAH values, sample station site) in Torre (2014) | Point |

3.3.1.4.1. Indicators

An exhaustive investigation concerning the toxicity of the selected heavy metals has not been carried out for the purposes of this work, as such, the information presented will be kept short and simple, focusing on how each pollutant can enter and further contaminate marine environments, and shellfish by association (e.g. bioaccumulation and biomagnification), as well as possible effects on human health (Table 13).

Table 13: Brief description of Product Contamination indicators.

| Heavy metal | Marine environment sources | Bioaccumulation | Biomagnification | Effects |
|--------------------|---|-----------------|---------------------|---|
| Cr ^a | Wastewater and industrial discharges [1] | No [2] | No [2] | Lungs, kidney, renal and brain damage; gastrointestinal diseases [3] |
| Cd ^a | Atmospheric deposition and river discharges [4] | Yes [5,6] | Inconclusive [7] | Vomiting, diarrhea, bone damage, muscle pain (i.e. "tai tai" disease) [8,9] |
| Cu | e.g. Antifouling paints and coatings for ship hulls [10] | No [11,12] | No [11] Yes [12] | Liver and kidney damage [13] |
| Zn | Wastewater and industrial discharges; geological rock weathering [14] | Moderately [15] | No [16] | Abdominal pain, nausea and vomiting, pancreas damage [14, 17] |
| Pb ^a | Mining and manufacturing; atmospheric deposition [18] | Yes [19] | No [20] | Brain and kidney damage, gastrointestinal diseases [21] |
| tPAH ^{b*} | e.g. Wastewater discharges [22] | Yes [23] | Yes [24] | Kidney and liver damage, breathing problems or skin irritation. [25] |

(cont.) Table 13: Brief description of Product Contamination indicators

| | | | |
|-------------------------------|--|-----------------------------------|--------------------------------|
| [1] Berry et al. (2004) | [2] Pourahmad et al. (2005) | [3] ATSDR (2012) | [4] Kennish (1996) |
| [5] Frazier (1979) | [6] Wu et al. (2013) | [7] Croteau et al. (2005) | [8] Cravey (1995) |
| [9] Godt et al. (2006) | [10] Botton et al. (1998) | [11] Lewis and Cave (1982) | [12] Zeng et al. (2013) |
| [13] Kramárová et al. (2005) | [14] ATSDR (2005) | [15] Fukunaga and Anderson (2011) | [16] Gächter and Geiger (1979) |
| [17] Plum et al. (2010) | [18] Tchounwou et al. (2012) | [19] Pugazhvendan et al. (2012) | [20] Solomon (2008) |
| [21] ATSDR (2007) | [22] Cheung et al. (2007) | [23] Bouloubassi et al. (2006) | [24] Takeuchi et al. (2009) |
| [25] Rengarajan et al. (2015) | a) carcinogenic b) some compounds are carcinogenic * sum of the concentration of EPA's sixteen (Appendix A) | | |

3.3.2. Stage 2: Elaboration of Suitability and Constraint maps

The second stage of the MCE represents the elaboration of Category and Constraint Maps (here defined as Suitability Maps), based on the thematic maps previously constructed. The stage 2 stepwise methodology is represented below (Fig. 7).

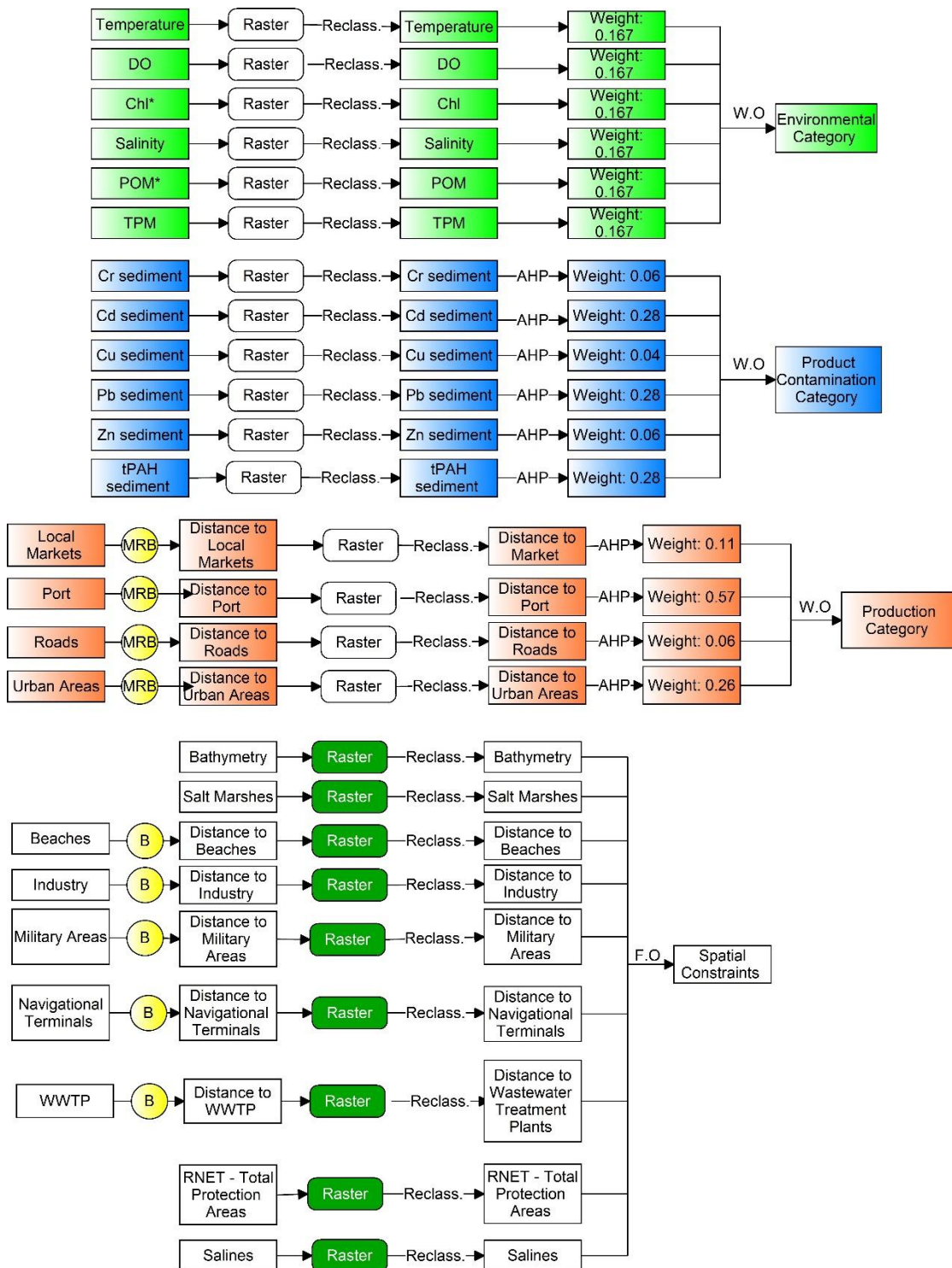


Fig. 7: GIS methodology used in Stage 2

AHP – Analytical Hierarchy Process; MRB – Multiple Ring Buffer; B – Buffer; F.O – Fuzzy Overlay; W.O – Weighted Overlay; * WinShell was used for the reclassification

First of all, Buffers and Multi-Ring Buffers were applied to several thematic maps, in order to define unsuitable areas (for Spatial Constraints) or to define distances with different importance concerning the site selection (Production Category). The next step was to convert all the thematic maps to raster format, and then reclassify each map to a common scale, based on literature.

Afterwards, relative weights for each indicator were calculated by means of an AHP and then grouped up by respective category using a Weighted Overlay GIS technique.

3.3.2.1. Application of Buffers and Multiple Ring Buffers

Buffers were applied only to indicators concerning of both Production Category and Spatial Constraints. Although this was briefly reported in Stage 1, it will be more thoroughly explained why several thematic maps were buffered (Table 14). All buffers and Multiple Ring Buffers were done in accordance to the reclassification values (1-4) found in literature, and are represented in Table 15 (Suitability Sources). All created buffers were afterwards converted to raster format.

Table 14: Justification for the buffering of some Spatial Constraints and Production Category

| Spatial Constraints | |
|------------------------------|--|
| | In the consulted bibliography, there were no references to suitable distances regarding industries in an aquaculture context. Therefore, it was chosen a distance from 1.5 km from the identified industries in the respective thematic map. |
| Industry | Future studies should assess the impact of industrial discharges in the estuary, in accordance to the type of pollutant, respective quantity and its interaction to either water or sediment, and hydrodynamic conditions throughout the year; also, it is recommended that the compliance of best management practices of the existing industries should be analyzed. |
| | To avoid human perturbances and possible theft situations, it was applied a buffer of 500 m to the identified coastal and interior beaches identified in the respective thematic map (Dapueto et al., 2015). |
| Beaches | This is a troublesome topic as the interior beaches are not identified as suitable for bathing season, which ideally would mean that fewer people would use them for recreative activities and thus those same beaches could be included in this study for shellfish aquaculture. However, this is not the case and so safety buffers were applied. |
| | Suitable sites for shellfish aquaculture should not impede access to any navigational terminal, mooring bay or impede navigation within the estuary. Therefore, the use of a safety buffer (200m) (Maritime New Zealand, 2005) was required, although, as defined by bathymetry, suitable areas should be within intertidal range, and so there should not be any conflict with existing navigational terminals. |
| Navigational terminals | |
| WWTP | A safety distance of 750m was placed, in order to avoid pollution from discharge points into the estuary itself. Suitable distances for Wastewater Treatment Plants were not identified in the consulted bibliography, although Dapueto et al. (2015) have established a buffering function as function of sewage pipes: something similar could be done in the Tagus, according to the size of each WWTP and population it serves. |
| Production indicators | |
| | Local Markets should be sufficiently close to shellfish farms, to reduce transport costs. Markets buffer values were taken from Giap et al. (2005) and Hossain et al. (2008). |
| Local markets | Because of the proximity to Lisbon and its suburbs and their placement within the coastline, a distance until 4 km was deemed as suitable (less than 2 km being the optimum). An unsuitable reclassification value was not attributed to this factor since it was not considered as having any relevant impact in the site selection. |
| | Piers or Port are important infrastructures since they enable boat transportation of both product and people, allowing the access to otherwise unreachable areas. Used buffer values were based on Lin (2010), although they were adapted according to the used suitability scale and studied areas (this will be more talked in more detail further in this work). Less than 4 km was considered as the optimum for the estuary, although for a smaller scale studies a lower distance can be used. |
| Port | |

(cont.) Table 14: Justification for the buffering of some Spatial Constraints and Production Category

| Production indicators | |
|-----------------------|---|
| Port | More than 8 km was considered as unsuitable, because crossing between both margins was not considered. |
| Roads | Buffers related to Distance to major roads were adapted from Hossain et al. (2008), Falconer et al. (2016) and Giap et al. (2008). Road buffering was used in the urban areas context, as long distances and bad roads make e.g. rural aquaculture difficult (Edwards et al., 2012), and may limit the transportation of the harvested product by car, either to be sold at local markets or for relaying areas. |
| Urban Areas | Urban areas correspond to possible human labour, namely to existing fishing communities in areas near the estuary, such as Barreiro, Alcochete, Trafaria, Póvoa de Santa Iria and Montijo (Cunha, 2012). Because of this, ideally, shellfish farms should be as close as possible to urban areas, albeit far enough to not cause visual impacts and human perturbances (Aguilar-Manjarrez et al., 2017). Urban areas reclassification values were obtained from Falconer et al. (2016). |

3.3.2.2. Reclassification

Before proceeding to the next step, all thematic maps were converted to raster format: this means all the buffered and non-buffered layers. The reclassification of each Category and Spatial Constraint (Table 15), was obtained from the literature. Indicators were reclassified to a common scale of 1 to 4 (1 – Unsuitable; 2 – Moderately Suitable; 3 – Suitable; 4 – Highly Suitable) (FAO, 1976):

- Unsuitable (1): It is either greater or lesser than the identified thresholds in literature, which deems unsuitable either the growth of the clams, the quality of seafood inherent or even the environment itself.
- Moderately Suitable (2): The reclassified values are within the threshold scale, although far from optimum. Requires costly investments.
- Suitable (3): Provides good conditions for shellfish aquaculture in the study area, meaning that the reclassified values are within a range considered good for growth (Production), are at a reasonably good distance from needed infrastructures and labour, even though heavy metal pollution is not confirmed to exist.
- Highly Suitable (4): Ideal for the establishment of shellfish aquaculture, gathering the best conditions for growth, with heavy metal concentration having next to minimal environmental impact (and hence, on the shellfish) and are at an enough distance to be near facilities and labour, but not close enough to allow human disturbances.

However, the reclassification scale considered for Spatial Constraints uses a Boolean logic (0 – Unsuitable or 1 – Suitable) (Eastman, 1999), in order to map the maximum possible area that does not have human, legal or pollution disturbances. Hence:

- Unsuitable (0): A thematic map is identified as unsuitable if its use might negatively impact shellfish aquaculture, because any of the reasons given in the paragraph above and therefore screening out all the selected unsuitable areas. Concerning the combined constraint map, it uses a fuzzy overlay technique, so an unsuitable area in this map means that at least one of the individual constraint maps are deemed unsuitable.
- Suitable (1): Suitable areas in this context correspond to the maximum possible area on which shellfish aquaculture practices are possible and no impacts were identified.

Reclassification of environmental and production indicators was based on values found in the literature, with the goal of studying the sites with sufficient conditions for the optimal growth. The Winshell software was used to reclassify both chlorophyll and POM. Winshell is a shellfish model to determine the individual shellfish growth for oysters, clams and mussels and it is designed for shellfish farmers and water managers, in order to determine how an animal will

grow in a certain area, on the basis of food availability and environmental conditions in coastal and estuarine areas.

Table 15: Reclassification table of the Categories and respective indicators. as well as the Spatial Constraints.

| Variable | Unit | Data Range | Data Source | Suitability Values | | | | Suitability Source |
|------------------------------------|--------------------|---------------------|-------------|--------------------|------------------------|-----------|--------------|--------------------|
| | | | | 1 | 2 | 3 | 4 | |
| Environmental | | | | | | | | |
| Temperature | °C | 14 – 21.2 | 1 | < 14 | - | 14 – 20 | 20 - 22 | 5, 6, 7 |
| DO | mg L ⁻¹ | 5.9 – 7.8 | 1 | < 5 | - | 5 – 7 | 7 – 9.5 | 8, 9 |
| Salinity | mg L ⁻¹ | 9.9 - 36 | 1 | < 20 | 20 – 24 | 30 – 36 | 24 – 30 | 8, 10 |
| TPM | mg L ⁻¹ | 5 - 117 | 1 | <10; > 350 | 10 – 100; 200 – 350 | - | 100 – 200 | 11 |
| Chl | µg L ⁻¹ | 1.1 - 15 | 1 | < 4.35 | 4.35 – 5.71 | 5.71 – 7 | > 7 | Calculated |
| POM | mg L ⁻¹ | 0.22 – 7.8 | 1 | < 2.64 | 2.64 – 4.5 | 4.5 – 5.1 | > 5.1 | |
| Production | | | | | | | | |
| Distance to major roads | km | - - - | - | > 1.5 | 1.5 – 1 | 1 – 0.5 | < 0.5 | 12, 13, 14 |
| Distance to urban areas | km | - - - | - | < 0.5; > 8 | 4 – 8 | 2 – 4 | < 2 | 12, 15 |
| Distance to port | km | - - - | - | > 8 | 6 – 8 | 4 – 6 | 0 – 4 | 15 |
| Distance to local markets | km | - - - | - | - | > 4 | 2 – 4 | < 2 | 13, 14 |
| Product Contamination | | | | | | | | |
| Cu in the sediment | ppm | 5 - 291 | 2 | > 108 | 18.7 – 108 | - | < 18,7 | 16, 17 |
| Cd in the sediment | ppm | 0.2 – 5.4 | 2 | > 4.21 | 0.68 – 4.21 | - | < 0,68 | 16, 17 |
| Cr in the sediment | ppm | 7 - 148 | 2 | > 160 | 52.3 – 160 | - | < 52,3 | 16, 17 |
| Pb in the sediment | ppm | 32 - 2478 | 2 | > 112 | 30.2 – 112 | - | < 30,2 | 16, 17 |
| Zn in the sediment | ppm | 26 - 1310 | 2 | > 271 | 124 – 271 | - | < 124 | 16, 17 |
| TPAHs in the sediment | ppb | 17 - 1270 | 3, 4 | > 650 | 150 – 650 | - | < 150 | 16, 17, 18 |
| Spatial Constraints | | | | | | | | |
| Salt works | - | Exclusion | 19 | | | | | |
| Distance to beaches | m | < 500 | 20 | | | | | |
| Military areas | m | < 150 | n/a | | | | | |
| Salt marshes | - | Exclusion | 19 | | | | | |
| TPA | - | Exclusion | 19 | | | | | |
| Natura 2000 | - | * | 19 | | | | | |
| Distance to Industry | km | < 1.5 | n/a | | | | | |
| Distance to Navigational terminals | m | < 200 | 21 | | | | | |
| Distance to WWTP | m | < 750 | n/a | | | | | |
| Bathymetry | m | < - 2 & > - 0.83 | 22 | | | | | |
| Current speed | m s ⁻¹ | < 0.3 & > 1 | 22 | | | | | |

*Not considered for the Spatial Constraints final thematic map

[1] BarcaWin2000 Database
 [5] Sladonja et al. (2011)
 [9] Best et al. (2007)
 [13] Giap et al. (2005)
 [17] Long and Morgan (1991)
 [21] Maritime Safety Authority of New Zealand (2005)

[2] Vale (1989)
 [6] Malouf and Bricelk (1989)
 [10] Chew (1989)
 [14] Hossain et al. (2008)
 [18] Torre (2014)
 [22] Vincenzi et al. (2006)

[3] Serafim et al. (2014)
 [7] Shpigel and Fridman (1989)
 [11] Kang et al. (2016)
 [15] Lin (2010)
 [19] Hidroprojecto (2007)

[4] Torre (2014)
 [8] Weber et al. (2008)
 [12] Falconer et al. (2016)
 [16] Dimitrakakis et al. (2014)
 [20] Dapuetto et al. (2015)

3.3.2.2.1. Spatial Constraints

Spatial Constraints reclassification can be divided into unsuitable areas by exclusion, by creating a safety buffer or due to best practice management. Excluded areas, such as salt works, salt marshes or total protection areas are all highly sensitive areas in which aquaculture could have a negative impact and are restricted by law. Not all safety buffers were found in the literature, namely for WWTP and industry discharges (see table 14). Culture management practices were reclassified according to the type of used aquaculture (extensive) and its applied area, in the intertidal areas.

Bathymetry reclassification suitable range (0.83 to 2m) was adapted from the literature, which was between 0 and 1 (Vincenzi et al., 2006; Toba et al., 1992), by incorporating the calculated monthly average for the registered lowest low tide of 2016 (Porto de Lisboa, 2016). This was done to ensure that cultivated clams are not exposed to the air for long periods of time, as low tides were only equal or superior to 1 m in two months in 2016 (Appendix A).

3.3.2.2.2. Production Category

It was found reclassification values for all of Production indicators, besides Distance to Port. Distance to Port reclassification values were adapted from Lin (2010) “GIS-based Multi-Criteria Analysis for Aquaculture Site Selection”, on which it is selected a different suitability level than in the present work: 8 vs 4 levels, subsequently affecting the distance of each level. Therefore, there was a need to adapt Lin (2010) values.

3.3.2.2.3. Environmental Category

There are plenty of sources concerning optimal values and survival thresholds for Manila clams growth for temperature (Shean, 2011; Malouf and Bricelj, 1989; Chew, 1988; Shpigel and Fridman, 1990; Mann, 1979; Sladonja et al., 2011), salinity (Chew, 1989; Weber et al., 2008), dissolved oxygen (Weber et al., 200): temperature reclassification value of 1 (unsuitable) is due to 14 °C being the lower threshold of larvae’s thermal tolerance (Malouf and Bricelj, 1989) and It is within the range of values for good growth, spawning and low mortality (13 – 23 °C) (Shpigel and Fridman, 1990). Optimal temperature, as reported by Sladonja et al. (2011) is between 20 and 22 °C. Salinity and Dissolved Oxygen reclassification values were given by Prof. João Gomes Ferreira.

Total suspended matter (TPM) was more difficult to find and generally seems less studied, however, Kang et al., (2016) reported an optimal growth between TPM values of 100 – 200 mg L⁻¹. Food availability (Chlorophyll a and POM) information was also very scarce and hard to find. Chlorophyll *a* (Chl) optimal values for Manila clam were not clear in any work found, although Barillari et al. (1990) as reported in Vincenzi et al. (2006), assumed an optimal concentration of 2 to 11 mg L⁻¹ of Chl for clam farming. Barillari et al. (1990) could not be found, in order to understand their work. POM values were not found specifically for Manila clam nor any species of hard clam.

So, to understand how Chl and POM affect growth, it was used Winshell software. Winshell is a shellfish model to determine the individual shellfish growth for oysters, clams and mussels and it is designed for shellfish farmers and water managers, in order to determine how an animal will grow in a certain area, on the basis of food availability and environmental conditions in coastal and estuarine areas (Longline, 2017a). Winshell was applied to Manila clam.

3.3.2.2.4. Food Sources (Chl and POM reclassification)

Winshell’s initial drivers were temperature, salinity, Chl, POM (and TPM were the default values were used for temperature, salinity and TPM. Default model specs were used (Day 1; Runtime 365 days; 1 animal; 1 m³), as well as seed size (0.65 TFW g).

Winshell gives as output live weight (g) and length (cm) at harvest. Length size is useful for the reclassification, as according to Regulation (EC) No. 850/98, 40 mm is the minimal harvest size for the Manila clam. Only Chl and POM values obtained for the estuary were used, ranging from 0.2 to 7.8 mg L⁻¹ and 1 to 14 µg L⁻¹, respectively.

This specific methodology can be divided into two different steps: live weight study concerning each variable and definition of reclassification scales.

3.3.2.2.4.1. Part One: Live Weight study

The predefined values for both variables are here defined as “sensitivity scale”, because in order to study one variable, each selected value for the other one is predefined.

POM’s sensitivity scale consists of 0.2, 1, 3, 5, 7 and 7.8 mg L⁻¹, while Chl’s 1, 3, 5, 7, 9, 11, 13 and 14 µg L⁻¹. After both sensitivity scales were defined, the next step was to study each variable. The first one to be studied was POM: for each Chl value was studied every POM value and respective outputs (live weight). Fig. 8 exemplifies what was previously mentioned for only 3 POM values (0.2, 1 and 3 mg L⁻¹), although it continued until it reached 7.8 mg L⁻¹. Afterwards, Chl’s next value defined in the sensitivity scale (3 µg L⁻¹) was studied until it has reached 13 µg L⁻¹. The same was also done for Chl.

All results were plotted as xy graphs, where x was either POM or Chl (depending on which one was being studied) and y was the live weight (g) (Fig. 9, 10). The graphics will be used for the reclassification, which will be based primarily on the live weight.

| | A | B | C | D | E | F |
|---|------------|------------------|--------------|------------------------|--------------|--------------|
| 1 | Julian day | Temperature (oC) | Salinity (-) | Chlorophyll a (ug L-1) | POM (mg L-1) | TPM (mg L-1) |
| 2 | | | | | | |
| 3 | 15 | 7 | 35 | 1 | 0,2 | 15 |
| 4 | 75 | 12 | 35 | 1 | 0,2 | 12 |
| 5 | 135 | 16 | 35 | 1 | 0,2 | 16 |
| 6 | 195 | 20 | 35 | 1 | 0,2 | 20 |
| 7 | 255 | 14 | 35 | 1 | 0,2 | 25 |
| 8 | 305 | 10 | 35 | 1 | 0,2 | 15 |
| 9 | | | | | | |

Fig. 8: Winshell’s setup, in order to study POM and live weight to a predefined Chl value (1 µg L⁻¹)

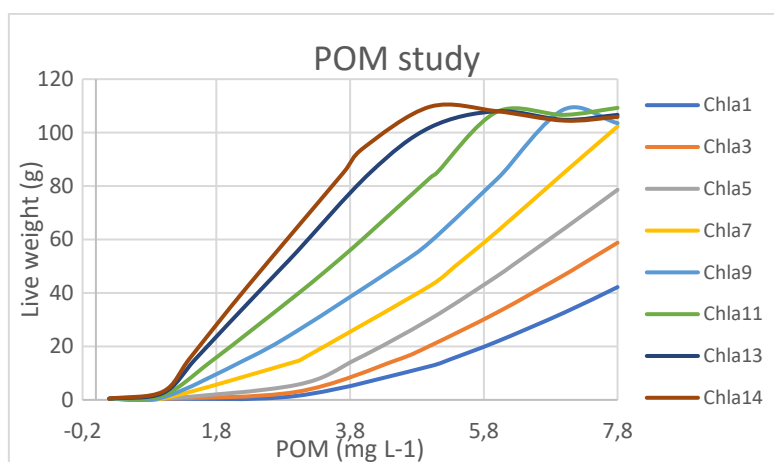


Fig. 9: POM sensitivity study

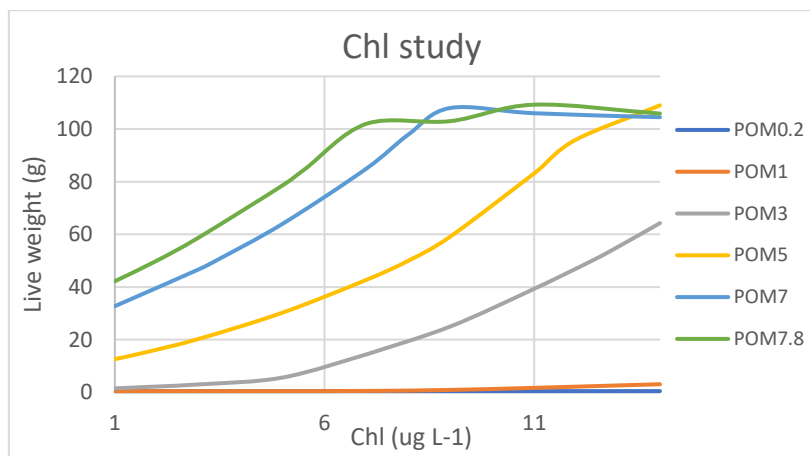


Fig. 10: Chl sensitivity study

3.3.2.2.4.2. Part Two: Reclassification

The reclassification was based on the same scale already used: 1 (unsuitable) to 4 (Highly Suitable) (Table 16). Shell length is also a Winshell output, given with live weight and therefore was experimentally obtained. The live weights related to a length of 4 cm were used for the reclassification (LWS4).

Afterwards, it was assigned two live weights for suitability levels 2 and 3: 50 and 85g, respectively. Both values were also obtained experimentally using Winshell for each variable and sensitivity scale values.

Both live weights were assigned by the author, since no studies were found, thus utilization of the said values in other works should be done with cautious. The table below (Table 16) represents the suitability scale applied according to live weight, and subsequently to POM and Chl.

Table 16: Used suitability scale, based on Winshell's live weight output, for POM and Chl

| Scale | Live Weight (g) | POM (mg L ⁻¹) | Chl (µg L ⁻¹) |
|-------|-----------------|---------------------------|---------------------------|
| 1 | LWS4 | < 2.64 | < 4.35 |
| 2 | LWS4 – 50 | 4.50 | 5.71 |
| 3 | 50 – 85 | 5.10 | 7 |
| 4 | > 85 | > 5.10 | >7 |

3.3.2.2.5. Product Contamination Category

Heavy metals were reclassified according to Canadian Council of Ministers of the Environment (2001) and Dimitrakakis et al. (2014). The use of Sediment Quality Guidelines (SQG) is a common practice for evaluating the sediment contamination of an area (e.g. Violintzis et al., 2009). Canadian Guideline sets two different values: TEL (Threshold Effect Level and PEL (Probable Effect Level), with TEL representing the lower range values, below which adverse effects upon living organisms are not likely to occur. PEL represents concentrations which are strongly affiliated with the appearance of adverse effects to aquatic organisms (Long et al., 1995; MacDonald et al., 1996). TEL and PEL indicators use the geometric mean of both the 10 and 50 % of concentration values that create or not adverse biological effects (Violintzis et al., 2009).

Canadian SQGs were chosen instead of USA's (e.g Dimitrakakis et al., 2014): ERL/ERM indicators use the 10 and 50 % of metal concentrations that create adverse effects to benthic organisms; since when compared, they represent lower threshold values (Long and Morgan, 1991).

Since the reclassification is based only on two different values, TEL and PEL, it was done in a different way (Table 17).

Table 17: Justification for the chosen reclassification values for TEL. TEL-PEL and PEL range of values

| Range of Values (TEL and PEL) | Reclass. value | Justification |
|-------------------------------|----------------|---|
| < TEL | 4 | Below TEL, adverse effects on living organisms are not likely to occur (e.g. Long and Morgan, 1991). Hence, it was given a reclassification value of 4. |
| TEL – PEL | 2 | The chosen reclassification values can be the most controversial, since it is the middle range of both TEL and PEL. And while below TEL is 4 and above PEL is 1, TEL-PEL could be either 3 (suitable) or 2 (moderately suitable). So, based on only the reclassification values and the uncertainty associated, TEL-PEL will be reclassified as Moderately Suitable, in order to avoid possible contamination points otherwise thought as safe. |
| > PEL | 1 | PEL represents concentrations are strongly linked with the appearance of adverse effects to aquatic organisms. For that reason, it was given a reclassification value of 1, as such areas are unsuitable for shellfish production, since are most likely contaminated. |

3.3.2.3. Analytic Hierarchy Process (AHP)

In this work, the AHP is divided in two different parts, although both use the principles inherent to AHP. The first part focused on the Category and respective indicators and their weight. Part Two, presented in Stage 3 of this MCE is going to focus only on the three categories and their relative weight as to create a final suitability map, which combines the three category maps, as well as the Constraint Map. In the AHP methodology, criteria can be applied to both categories and indicators.

In short, AHP is a useful tool to decompose a problem into several different criteria, making it easier to understand and evaluate. To achieve this, AHP uses a series of pairwise comparisons between criteria and subcriteria, allowing the calculation of the respective relative weights, in accordance to the importance given by the decision-maker: a higher weight means a higher relative importance. Afterwards, the AHP also allows the validation of the consistency of the used evaluations, reducing the bias in the process (Bhusan and Rai, 2004).

The methodology developed for AHP can be thoroughly consulted in the bibliography (Saaty, 1980, 2008) and a brief version is displayed in Appendix B, taken from Bhusan and Rai (2004), that gives a step-to-step methodology.

3.3.2.3.1. Production indicators

The pairwise comparison between production indicators was based on their relative importance: Distance to port was considered the most important factor, seeing that it provides fishermen accessibility and transportation to and from the estuary. Urban areas were then used to represent fishing communities and labour. Local markets were considered as the third most important factor, as a distribution point: closer markets means lower transportation costs. The final factor was major roads: although roads are important for transportation of either people and product, the reasoning for being considered the less important was the fact that the estuary is located near Lisbon Metropolitan Area and its suburbs, which are near the estuarine coastline and thus, road access is available. Distance to major roads focuses mostly on the accessibility for fishermen to go to the farms and then retrieve some of the harvested product by car, either for selling or relaying.

The AHP was then filled and it is represented below (Table 18). The achieved Consistency Ratio was lower than 10% (3.69).

Table 18: AHP concerning Production indicators

| | Distance to Port | Distance to Urban Areas | Distance to Local markets | Distance to Roads | Weight |
|------------------------------|------------------|-------------------------|---------------------------|-------------------|--------|
| Distance to Port | 1 | 3 | 5 | 7 | 0.57 |
| Distance to Urban Areas | 1/3 | 1 | 3 | 9/2 | 0.26 |
| Distance to Local Markets | 1/5 | 1/3 | 1 | 2 | 0.11 |
| Distance to Major Roads | 1/7 | 2/9 | 1/2 | 1 | 0.06 |
| Consistency Ratio (CR) = 3.7 | | | | | |

3.3.2.3.2. Environmental indicators

Environmental indicators were considered as having equal importance (16.67% each) because all can influence the quality of the product and its growth: i) if DO levels are below 5 mg L⁻¹, adult clams can not maintain aerobic respiration, using the anaerobic metabolism that converts fuel reserves to energy with a much reduced efficiency (Weber et al., 2008); ii) exterior temperature regulates clams body temperature: decreasing water temperature reduces the metabolic rate, affecting both growth and reproduction (Weber et al., 2008); iii) below salinity levels of 15 psu, studies have revealed mortalities for both juvenile and adult clams (e.g. Elston et al., 2003; Coughlan et al., 2009); iv) POM and Chl are considered as food sources, therefore they can regulate growth and TPM encompasses both organic and inorganic matter, ultimately influencing available food sources (Secrist, 2013).

Food availability may also be affected by macroalgal growth, as dense blooms can occur on predator exclusion nets in hard clam aquaculture settings (Secrist, 2013), although no toxic blooms have been observed in the estuary, over 20 years (Ferreira et al., 2003).

Because of this, AHP was not used for the calculated weights of Environmental indicators weights.

3.3.2.3.3. Product Contamination indicators

The AHP concerning Product Contamination indicators was based off Priority List of Hazardous Substances (ATSDR, 2015), developed by ATSDR (Agency for Toxic Substances and Disease Registry Division of Toxicology and Human Health Sciences) and EPA (Environmental Protection Agency). A Substance Priority List consists of arranging, in order of priority, substances that are most commonly found at facilities on the National Priorities List (NPL) and which are determined to pose the most significant potential threat to human health due to their known or suspected toxicity and potential for human exposure at these NPL sites (ATSDR, 2015).

The methodology used to define each pairwise comparison is explained below:

- 1- Each heavy metal studied in NPL was identified: Pb was the most dangerous at no. 2, and Cu was the least dangerous, at no. 118;
- 2- A working range of 118 was established, and was divided by the number of AHP's maximum scale (9), giving nine equal ranges of values (Table 19) – Normalized scale. Each range of values corresponding to a normalized scale number has a fixed importance, meaning that: since Pb (2), Cd (7) and tPAHs (9) are within the normalized value of 1, in AHP they all have the same importance;

Table 19: Normalized scale for AHP for each of the heavy metals used

| Normalized scale (NC) | SPL range of values | Heavy Metals on SPP |
|-----------------------|---------------------|-------------------------|
| 1 | 1 – 13 | Pb, Cd and tPAHs |
| 2 | 14 – 27 | |
| 3 | 28 – 41 | |
| 4 | 42 – 55 | |
| 5 | 56 – 69 | |
| 6 | 70 – 83 | Zn and Cr |
| 7 | 84 – 97 | |
| 8 | 97 – 110 | Cu |
| 9 | 110 – 118 | |

3- Normalized scale was then used in the pairwise comparison, following the criterion example (J and K) (Annex B).

$$\Delta PC_{JK} = NC_J - NC_K \quad (7)$$

4- Then, according to ΔPC_{JK} principle, the matrix was filled (represented in the table below, Table 20). If both NC values are equal then the difference is set to 1.

Table 20: AHP concerning Product Contamination's indicators

| | Pb | Cd | tPAHs | Zn | Cr | Cu | Weight |
|-------|-----|-----|-------|-----|-----|----|--------|
| Pb | 1 | 1 | 1 | 5 | 5 | 8 | 0.28 |
| Cd | 1 | 1 | 1 | 5 | 5 | 8 | 0.28 |
| tPAHs | 1 | 1 | 1 | 5 | 5 | 8 | 0.28 |
| Zn | 1/5 | 1/5 | 1/5 | 1 | 1 | 3 | 0.06 |
| Cr | 1/5 | 1/5 | 1/5 | 1 | 1 | 3 | 0.06 |
| Cu | 1/8 | 1/8 | 1/8 | 1/3 | 1/3 | 1 | 0.04 |

Consistency Ratio (CR) = 1.5

Finally, Consistency Ratio of 1.55 % was calculated, which is below Saaty's 10% recommended Consistency Ratio (Saaty, 1980).

3.3.2.4. Weighted and Fuzzy Overlaying Techniques

After Part One of the AHP was done and weights were obtained, each final category suitability map was created using a GIS-based weighted linear combination (WLC); meanwhile, final Spatial Constraints thematic map was obtained by applying a fuzzy overlay technique ('AND' operator).

The weighted linear combination for suitability calculation for each indicator was already explained in Chapter 2.7.2 (Multi-Criteria Evaluation), although the equation used for this step is different, based on Malczewski (2006), since the obtained suitability corresponds to each indicator and not the final suitability map:

$$s = \sum_j^n w_j r_{ij} \quad (4)$$

Where according to Malczewski (2006):

s = suitability for each indicator

w_j = normalized weight for each indicator, such that $\sum w_j = 1$, calculated by means of AHP

r_{ij} = category transformed into the used suitability scale (1,....., 4), expressed in the reclassification table

This was done using Spatial Analyst Tool, Weighted Overlay, in ArcGIS, for all indicators within each category, in order to create the respective final thematic maps.

For Spatial Constraints final thematic map, it was used Boolean logic principles with Fuzzy Overlay. After their reclassification to Boolean logic (either Suitable or Unsuitable) (Eastman, 1999; Mesgari et al., 2008), each spatial constraint thematic map was combined using Fuzzy Overlay Tool, using ‘And’ operator, which returns the minimum value of the sets the cell location belongs to.

3.3.3. Stage 3: Site Selection based on Suitability map

Stage 3 of this MCE focuses on obtaining the weight for each category, by means of an AHP (Part II). Afterwards, a GIS-based Weighted Overlay was applied, as well as a constraint map related to the Spatial Constraints, on which shellfish aquaculture may be implemented.

3.3.3.1. AHP – Part Two

Part two of the AHP is based on the three categories and has similar principles to Part One’s: pairwise comparison between the categories, in order to calculate their respective relative weight (Fig. 11; Table 21).

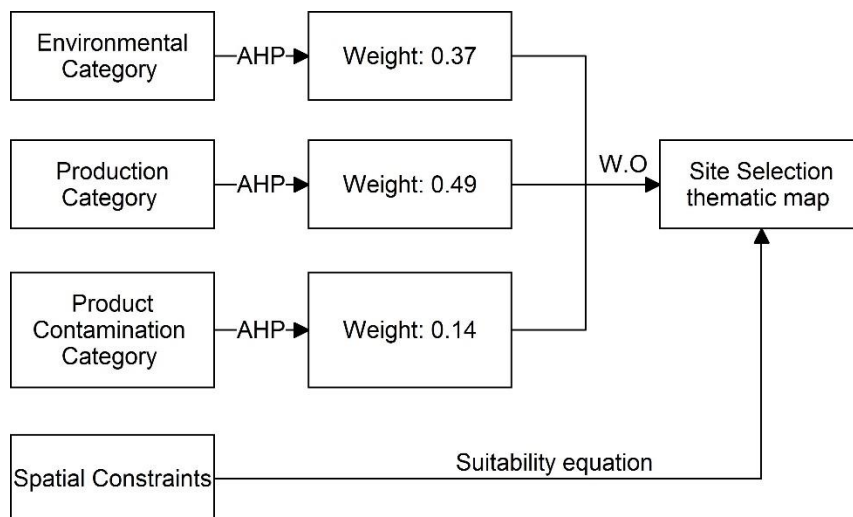


Fig. 11: Stage 3 used methodology for obtaining the final site selection map for shellfish aquaculture; W.O – Weighted Overlay

Product Contamination category evaluates anthropogenic points of pollution within the Tagus Estuary that influence the quality of the shellfish harvesting and production areas (Oliveira et al., 2013; Laing and Spencer, 1997; Silva and Batista, 2008), and was considered to be the less important, as it is the easiest one to avoid or minimize: shellfish aquaculture siting just has to avoid contaminated areas. Environmental category is essential to extensive aquaculture, as no inputs are required (e.g. food sources), thus clams only depend on external variables.

Production category was identified as the most important category, as it assesses where are the infrastructures upon which aquaculture practices depend, such as roads or piers. Both are very important for an efficient transportation of fishermen and harvested products, as well as to reduce transportation costs; local markets, where the harvested product can be sold and urban areas, which evaluates where possible human labour lives. The present AHP is valid according to Saaty (1980) Consistency Ratio constraint.

Table 21: AHP concerning the all identified categories

| | Production | Environmental | Product Contamination | Weight |
|------------------------------|------------|---------------|-----------------------|--------|
| Production | 1 | 2 | 3 | 0.49 |
| Environmental | 1/2 | 1 | 3/2 | 0.37 |
| Product Contamination | 1/3 | 2/3 | 1 | 0.14 |
| Consistency Ratio (CR) = 1.8 | | | | |

3.3.3.2. Final Site Selection thematic map

The final step involving GIS tools is the application of a weighted linear combination (WLC) equation, otherwise define as Weighted Overlay, and then the result can be intersected by the product of the Boolean constraints (Eastman, 1999), by following Eq. (5):

$$S = \sum w_i X_i * \prod C_j \quad (5)$$

where S = Suitability

w_i = weight assigned to factor i

X_i = category score of factor i

C_j = constraint j

However, Eq. (5) can be divided into two different parts, where the first one focus on the multiplication of each category to its relative weight and the second one adds the Boolean multiplication (0 to the unsuitable areas and 1 to the suitable ones).

Since all Spatial Constraints were mixed together using a Fuzzy Overlay, the second part is not exactly as defined in the equation, and can be rewritten as, Eq. (6):

$$S = \sum w_i X_i * SC \quad (6)$$

Where SC = Fuzzy Overlay of all identified Spatial Constraints

By applying this by means of GIS tools, S is equivalent to the final site selection map to ArcGIS output.

Equation 1 can be divided in two different parts, as already mentioned:

$$\left\{ \begin{array}{ll} s = \sum w_i X_i & , \text{ gives as output } s \text{ (final suitability thematic map)} \\ S = s.SC & , \text{ gives as output } S \text{ (site selection thematic map)} \end{array} \right.$$

3.3.4. Stage 4: Dynamic modelling using a farm scale model (FARM)

Stage 4 focuses on the application of the FARM model (see Chapter 2.8) on the site selection thematic map obtained by means of a GIS based MCE (Steps 1-3).

FARM could be applied to any area within the site selection thematic map with a suitability score of either 3 (Suitable) or 4 (Highly Suitable), since those are associated with less polluted areas (i.e. heavy metal pollution), gather the best environmental conditions for growth and generally are less resource intensive (i.e. require the least investment when compared to unsuitable or moderately suitable areas).

This way, the first step was to identify possible areas to establish as conceptual for farms. Shumway (2011) defines a farm as an integrated production unit, subjected to specific pressures and associated impacts. The dimensions for each farm and the number of sections were selected, although the total area should not exceed 5000 m² per farm (Ferreira et al., 2007).

In order to gain access to environmental required for modelling Manila clam growth in FARM, two different B2K stations (#2.0 and #3.0, Fig. 12) were selected: DO, salinity, temperature, Chl, POM, TPM and dissolved inorganic nitrogen (DIN). Monthly averages were calculated for each station, and modelled for a 395 days culture period. Only superficial samples taken from a depth of 0.5 m were considered and used in the calculations, due to the type of culture (intertidal).

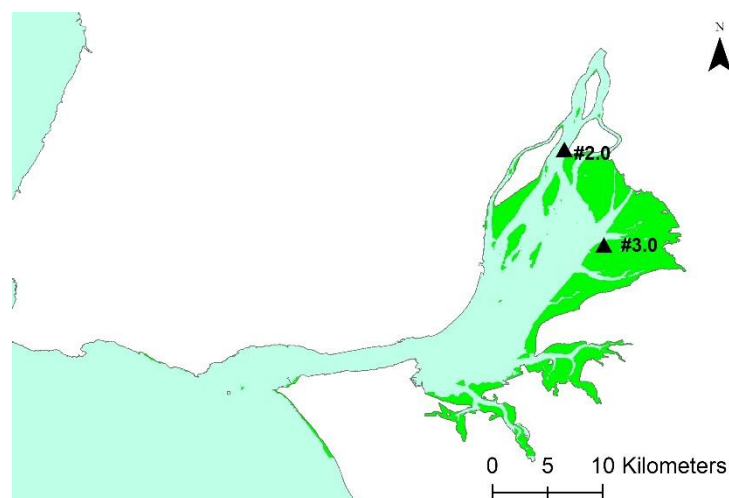


Fig. 12: Location of both B2K stations

In addition, each station was divided according to both types of tides: neap and spring tides, to evaluate their potential impact when compared to the median values for both concept farms. Environmental data for both concept farms are displayed in Appendix D. Average values calculated for each station do not add to the ones calculated from spring and neap tides, since some samples did not have the proper identification concerning the type of tide.

Other data was also required (Table 22), namely peak current at both spring and neap tide; spring and neap tidal ranges. Mortality was assumed to be 35%. Semi-diurnal tide was also used. Seed cost per kg and sale price per kg values were the default ones, as well as for seed and harvest weight. Biodeposition was not considered.

Table 22: FARM drivers for each concept farm and respective type of tide

| FARM drivers | Concept farm A | | | Concept farm B | | |
|--|----------------|-------|---------|----------------|-------|---------|
| | Spring | Neap | Average | Spring | Neap | Average |
| Length (m) | | 160 | | | 180 | |
| Width (m) | | 25 | | | 25 | |
| Total area (m ²) | | 4,000 | | | 4,500 | |
| depth (m) | 1.4 | 1.4 | 1.4 | 1.5 | 1.5 | 1.5 |
| No. sections | | 2 | | | 3 | |
| Peak current at spring tide (m s ⁻¹) * | | 1.25 | | | 1.48 | |
| Peak current at neap tide (m s ⁻¹) * | | 0.35 | | | 0.42 | |
| Spring tidal range (m) * | 3.9 | 3.5 | 3.7 | 4.2 | 3.8 | 4.0 |
| Neap tidal range (m) * | 0.9 | 1.3 | 1.1 | 0.9 | 1.2 | 1.0 |
| Mid-tide height above datum (m) | 1.4 | 1.3 | 1.3 | 1.1 | 1.1 | 1.1 |

* Adapted from Neves (2010)

The model was simulated for bottom (intertidal) culture for a stocking density of 100 and 300 ind m⁻², in contemplation of assessing the influence on the final economic outputs, and applied to the Manila clam.

FARM outputs were highlighted in Chapter 2.8 (see also Ferreira et al., 2007). In this work, an adapted version of FARM was used to estimate production and return on investment of the

cultivated population, expressed as Total Physical Product (TPP) and Average Physical Product (APP), a proxy for return on investment (Ferreira et al., 2007) and evaluates environmental externalities by calculating carbon removal for food assimilated by the clams, from the energy balance; Carbon is then converted to nitrogen according to Redfield ratio and then to populations equivalent (PEQ), which according to EPA's water quality trading (EPA, 2013), may also have an economic value associated.

In order to upscale production, a simple equation was developed to calculate the Gross Economic Value (GEV) based on each concept farm's harvesting production, Eq. (7):

$$GEV = \frac{0.005 TA}{F_A} \cdot F_{ev} \quad (7)$$

where:

TA – Location total area (should be > 5 km²)

F_A – Concept farm total area (should be < 5000 m²)

F_{EV} – Concept farm associated production economic value

The rationale behind FARM's upscale production is that a small percentage of a studied location's total area is selected (0.5%); afterwards, the calculated area is divided according to the concept farm's area (farm equivalent) and then multiplied by the harvest economic value related. Gev is merely indicative, as most likely it is an overestimation from the actual values of real shellfish cultures.

4. Results and discussion

Thematic maps and respective GIS data processing are shown in the following order:

- 1) Stage 1 + Stage 2: Thematic maps created in stage 1; creating of buffers and the reclassification was done in stage 2, according to the sequence: Spatial Constraints, Production, Environmental and Product Contamination;
- 2) Stage 3: Site selection thematic map, calculated from weighting each category and masked afterwards, using the final Spatial Constraints map;
- 3) Stage 4: FARM outputs obtained for each selected site;

In this Chapter, every thematic map used thought to be able to influence the site selection for shellfish aquaculture is displayed and commented, in order to answer Chapter 1 “Introduction” questions relative to the feasibility of shellfish aquaculture in the Tagus Estuary

All GIS-based thematic maps were exported with a resolution of 600 dpi and color mode of 24-bit True Color. All the data used for thematic map creation are displayed in “Appendix A” (Stage 1).

4.1. Stage 1 + Stage 2

In Stage 1 + Stage 2, the created maps for each category and spatial constraints are displayed in the following order: all left images correspond to thematic maps, with all the right images representing the respective reclassification maps. Also, only the most relevant individual spatial constraints thematic maps are displayed (i.e. bathymetry, current speed, TPA, salt marshes, distance to industry and WWTP), and were selected based on, i) total area occupied, and ii) relevance, i.e. culture practices, pollution sources and legal requirements were prioritized. The remaining maps are displayed in Appendix D.

4.1.1. Spatial Constraints

4.1.1.1. Bathymetry

Bathymetry thematic map (Fig. 13) shows the navigational channels that allow fishermen to navigate in the estuary using boats, to areas otherwise impossible to access (either by foot or road). This is essential to shellfish aquaculture in Tagus, especially in the eastern part, above Alcochete (1,2 and 3), which is less accessible in general and the area is mostly intertidal.

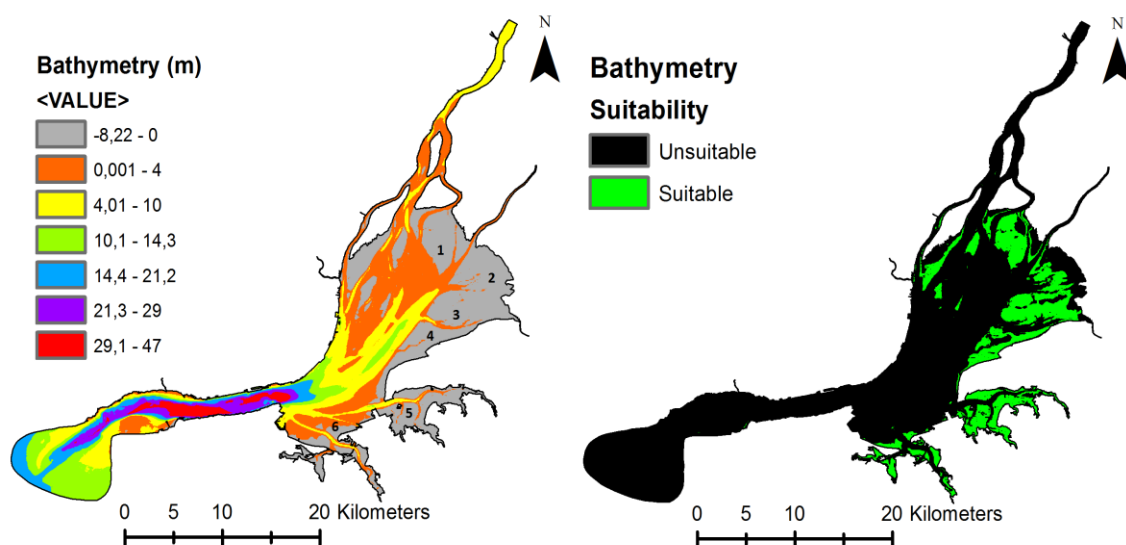


Fig. 13: Bathymetry thematic map (left pane) and its reclassification map (right pane). Numbers 1-5 represent the identified navigational channels with a maximum depth of 4m (orange)

Bathymetry reclassification was based on the type of culture practiced (intertidal), where suitable areas must be between -0.83 and -2 m according to the scale used in the bathymetry thematic map. A different type of culture used would mean different reclassification values, which would influence differently the final outcome. Therefore, bathymetry, as well as current speed, are considered as being key factors in the definition of culture practices.

Bathymetry is also identified as a major spatial constraint in the estuary, as the only considered suitable areas were the intertidal flats. Because of the type of Fuzzy Overlay used (“AND” operator) for the creation of the final Spatial Constraints thematic map, if one area is deemed as unsuitable at least by one thematic map, then shellfish aquaculture is not possible in that particular area.

Because of this, only intertidal areas will be considered for the suitability analysis. The remaining identified spatial constraints will then only have influence within intertidal areas. A more detailed analysis will be done in the final Spatial Constraints thematic map.

4.1.1.2. Current speed

Current speed thematic map shows a small variation of current velocity in the estuary. A major limitation highlighted is the fact that only four stations were available for the median calculations, which can have profound effects on the current speed values. According to MARETEC (2000), the average current speed is approximately 1 m s^{-1} : the highest values are near the main channel (1) and can reach 2.5 m s^{-1} in neap tides.

Since the values used for the reclassification were between $0.3 - 1 \text{ m s}^{-1}$, with more stations the obtained values would probably be higher and unsuitable areas would be found near the main channel (1). This ultimately would have no effect on the final Spatial Constraints thematic map since bathymetry already identified those areas as unsuitable.

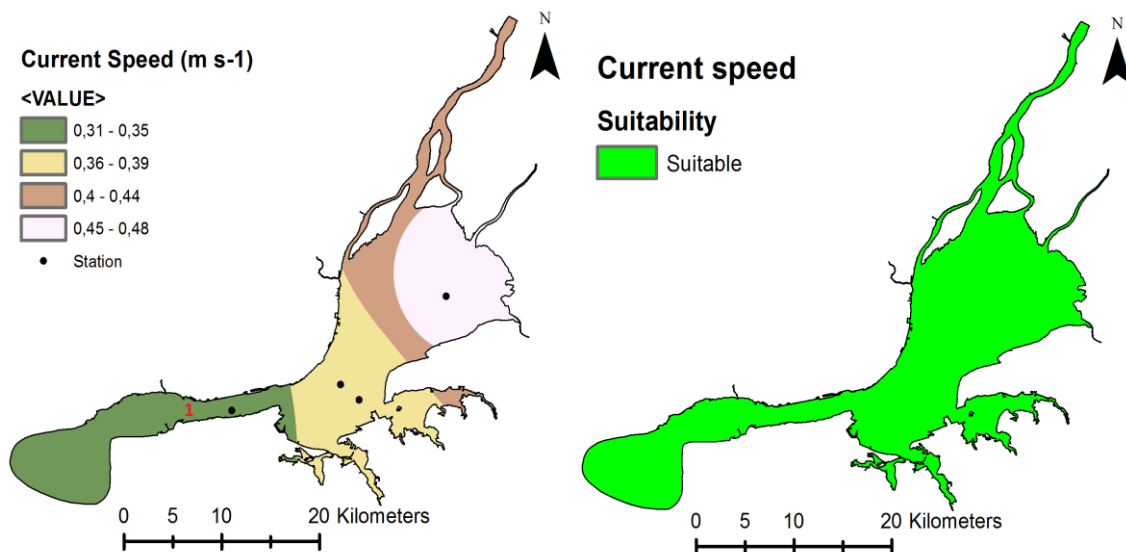


Fig. 14: Current speed thematic map (left pane) and its reclassification map (right pane). 1 – Main channel

4.1.1.3. Military areas

Military areas were considered as unsuitable and it was established a safety distance of 150 m, as to avoid possible human disturbances, which was confirmed afterwards by a spokesman’s of Montijo’s Air Base No. 6 (4) to be enough. The decision to try to contact the mentioned Air Base was to investigate whether nearby areas would be safe for aquaculture practices, since according to Cunha (2012) clam samples, that area had clam densities higher than 100 individuals/m².

Identified military areas in the right margin (Fig. 15), near Lisbon (3), were considered as having no impact whatsoever, since they are very close to the estuary's mouth, which it is a very used navigational channel. The military area near Trancão river (2) was not considered as having physical impact on itself, although it might have impacts on the discharges to the river, which will be accounted for in the Distance to Industry thematic map. The same could be said to military areas near Póvoa de Stª Iria and Vila Franca de Xira (1), since those are known to be heavily industrialized areas and are deemed as unsuitable in Distance to Industry.

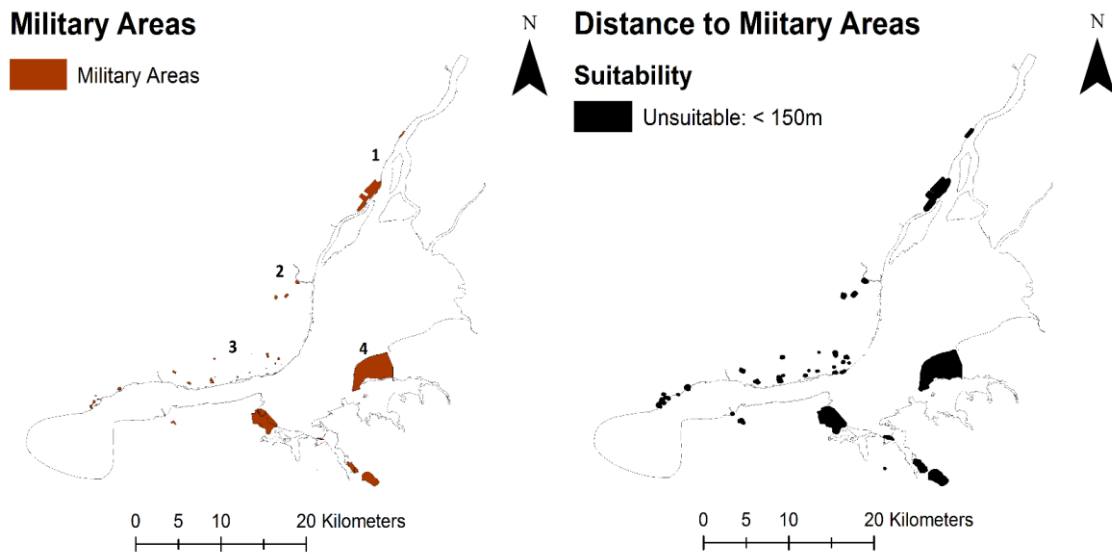


Fig. 15: Identified military areas (left pane) and their reclassification map (Distance to Military Areas) (right pane).

1 – Póvoa de Stª Iria and Vila Franca de Xira; 2 – Trancão river; 3 – Lisbon; 4 - Montijo's Air Base No. 6

4.1.1.4. Distance to WWTP

The area near Trancão river (1) receives external inputs from three different Wastewater Treatment Plans: Beirolas, S. João da Talha and Frielas (Saraiva, 2001). Of all identified WWTP, further studies regarding Alcochete, Montijo and Barreiro (2, 3 and 4, respectively) must be done to ensure that they do not contaminate (e.g. faecal coliforms) possible shellfish farming areas

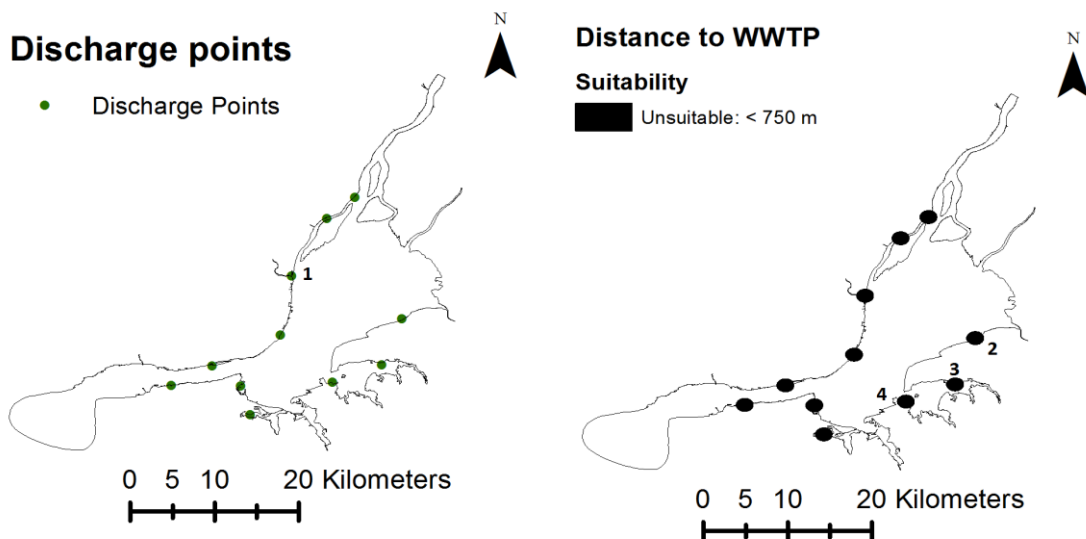


Fig. 16: Identified WWTP's discharge points in the estuary (left pane) and their reclassification map (right pane)

1 – Trancão river; 2 – Alcochete; 3 – Montijo; 4 - Barreiro

4.1.1.5. Distance to Industry

Industries were a major concern and considered as a key aspect in the definition of the final Spatial Constraints thematic map, since they can contaminate the shellfish and thus, worsen the quality of the product.

As previously mentioned, the estuary is heavily industrialized, namely in Barreiro (1) and Póvoa de St^a Iria-Vila Franca de Xira section (2), even though both Seixal (3) and Montijo's (4) bay also have shown significant areas concerning industry uses. The right margin was already deemed as unsuitable by bathymetry, apart from the area below Mouchão da Póvoa (5), which is now also undermined. Aquaculture practices within the vicinities of the Seixal bay were also impeded; the same happened to most of the Montijo's bay.

Future studies need to: i) properly establish a safety distance from industry areas that ensures the product's quality; ii) identify the type of pollutants associated to the existing industries, and iii) identify inactive industries throughout the estuary.

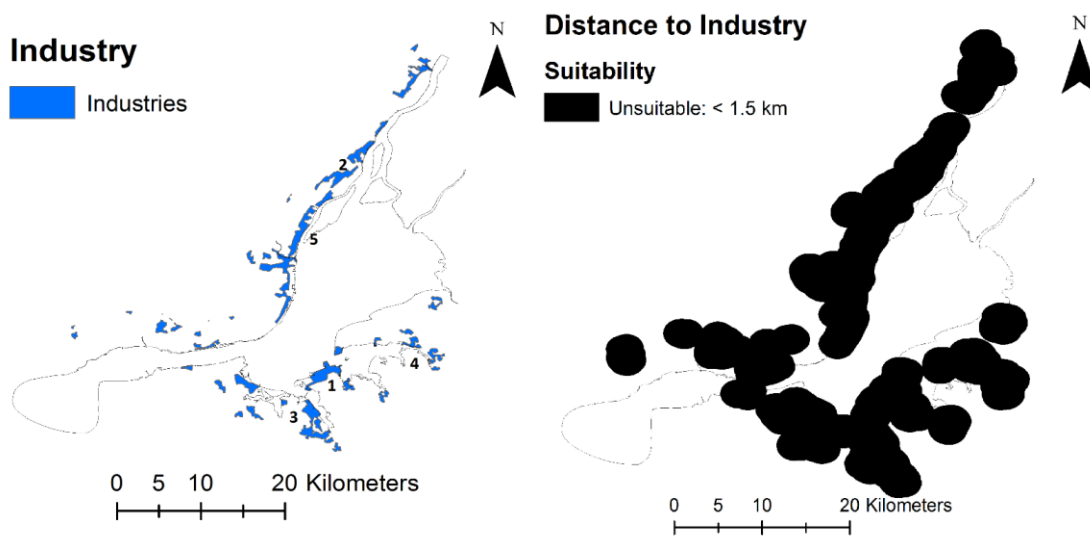


Fig. 17: Identified industry areas in the estuary (left pane) and their reclassification map (right pane). 1 – Barreiro; 2 – Póvoa de St^a Iria - Vila Franca de Xira section; 3 – Seixal bay; 4 – Montijo bay; 5 – Mouchão da Póvoa

4.1.1.6. Total Protection Areas

TPA, as defined in PORNET, must be protected from any type of anthropogenic impacts: the unsuitable area displayed in the figure below (Fig. 18) represents the Pancas salt marsh. Although only the specified area is defined as unsuitable and anthropogenic impacts prohibited, a safety distance can be recommended if further implementations are to be made, to ensure that shellfish aquaculture does not negatively impact the protected area (e.g. harvesting techniques, boat navigation).

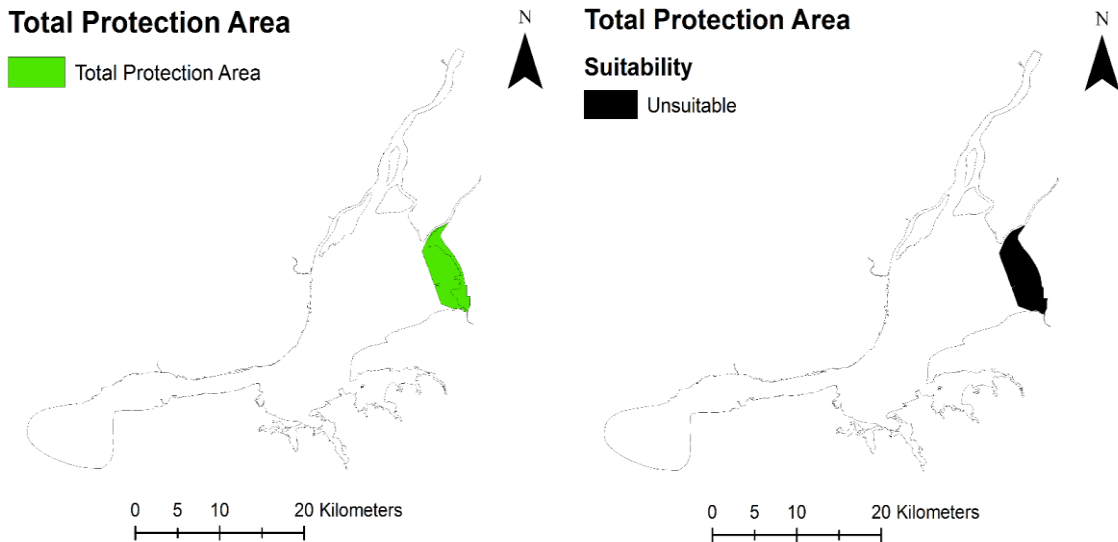


Fig. 18: Area defined as Total Protection Area according to PORTNET (left Image) and its reclassification map (right image)

4.1.1.7. Salt marshes

Only salt marshes within RAMSAR areas were deemed as unsuitable, which are coincidental to those of PORNET's, since they were identified with the most ecological sensitivity. They were identified three different salt marshes concerning the ones protected by PORNET: Mouchão da Póvoa (1), Mouchão do Lombo do Tejo (2) and Pancas (3).

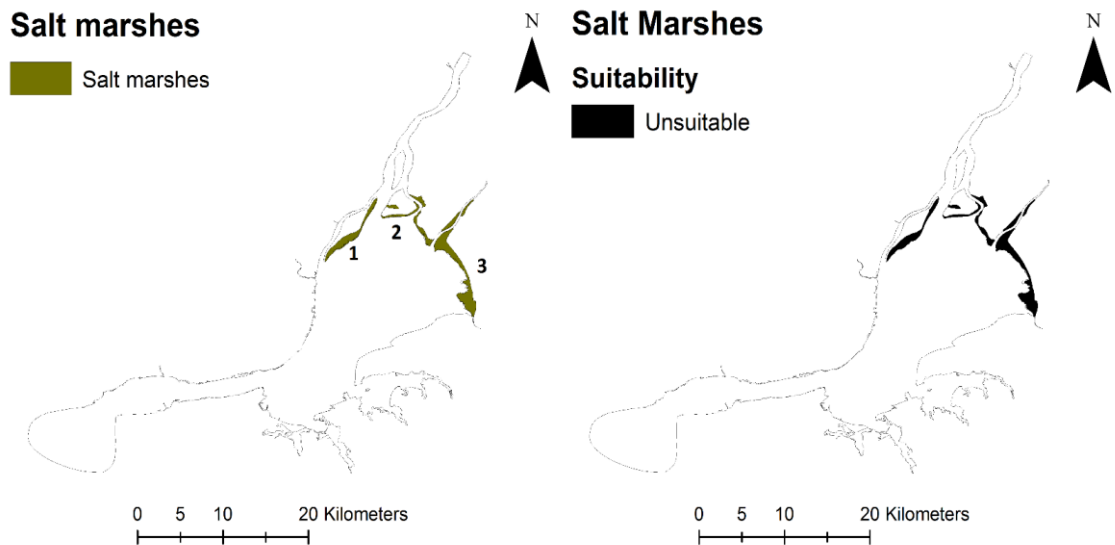


Fig. 19: Identified salt marshes with the most ecological sensitivity (left pane) and their reclassification map (right pane); 1 – Mouchão da Póvoa; 2 – Mouchão do Lombo do Tejo; 3 - Pancas

4.1.2. Production Category

The displaying of Production indicators thematic maps follows the same logic as previously applied to Spatial Constraints. However, since the created buffers around the area were too large to allow an easy graphic display, it was chosen to show the thematic maps with a Tagus mask applied.

4.1.2.1. Distance to Urban Areas

Lisbon (3), being Portugal’s biggest and most populated city, has the best overall suitability score, as well as its suburbs (e.g. Parque das Nações – 4; 5 – Póvoa de Stª Iria). The left margin corresponded to a good suitability score as well, from Montijo (1) to Trafaria (2), while area 6 scored the worst value, meaning it is the less populated area.

A “safety” distance of 500 from all identified urban areas was established, due to possible landscape impacts (Dumbauld et al., 2009; Scottish Natural Heritage, 2011), which may cause public problems, as well as to avoid possible human disturbances and theft situations.

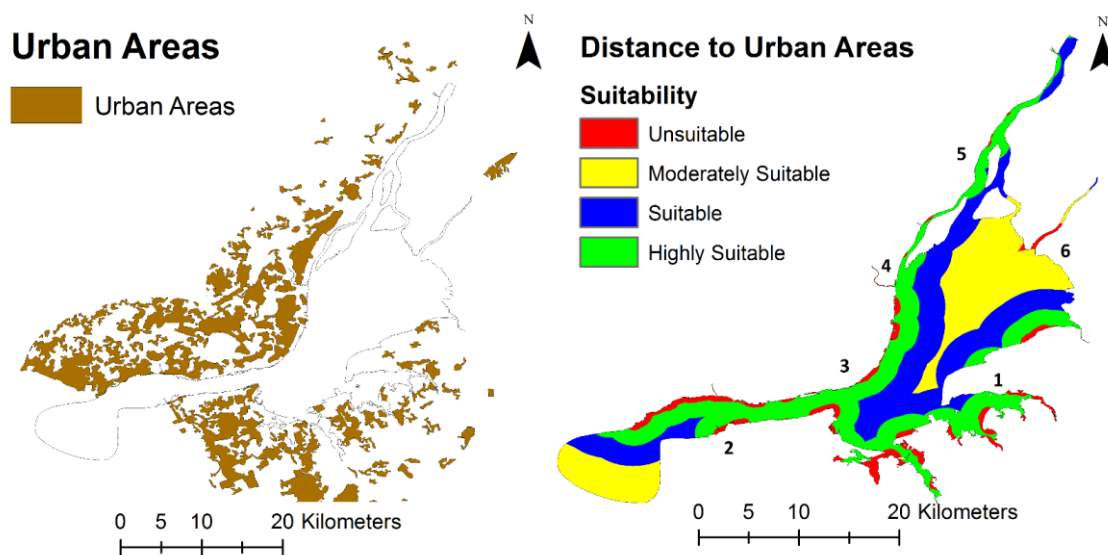


Fig. 20: Identified urban areas near the estuary (left image) and their reclassification map (Distance to Urban Areas)
1 – Montijo; 2 – Trafaria; 3 – Lisbon; 4 – Parque das Nações; 5 – Póvoa de Stª Iria; 6 – Pancas salt marsh

4.1.2.2. Distance to Roads

Distance to Roads thematic map serves a number of purposes: it is useful to understand possible locations for product transportation (by car) after its harvesting, as well as for the people participating in the respective trade chain.

To achieve this, concerning OSM’s road data, it was selected primary, secondary and tertiary: primary roads correspond to major traffic movement between centers of population and economic activity on a regional and national levels, also linking strategic roads to residential streets or industrial roads; secondary roads provide access to properties within a residential area, while tertiary roads are not usable by motor vehicles and may include footways, footpaths or cycleways (Designing Buildings, 2017).

Infraestructuras de Portugal (I.P) gave access to major highways within the studied area, in order to evaluate access between different major population aggregates and possible farm areas, although this was not considered relevant in the reclassification map, as residential roads are much closer to the estuary, as seen in Fig. 21.

Area 1 was assigned to the worst suitability score area, Pancas salt marsh. Although this particular area was deemed as unsuitable by Total Protection Area thematic map (within PORNET’s scope), the nearby areas (which as already seen, are mostly intertidal areas) should only be accessed by boat.

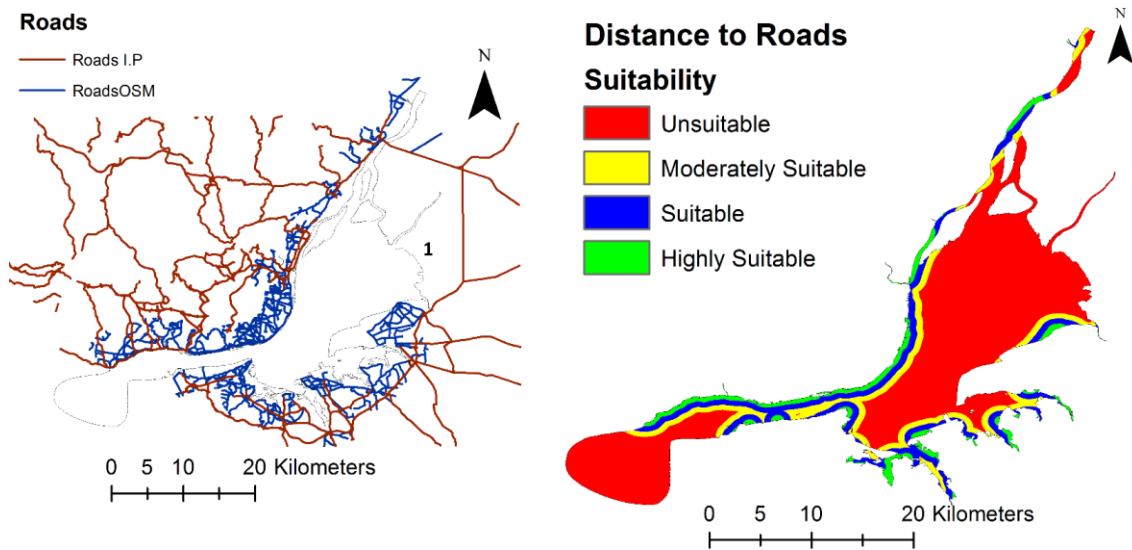


Fig. 21: Identified roads within the estuary (left pane) and their reclassification map (right pane). 1 – Pancas saltmarsh

4.1.2.3. Distance to Port

Piers are a major factor for boat transportation, either people or even the harvested product. Hence, the study of their location is a key factor for the identified Production factors, and consequently to the whole MCE, since they dictate where people will be unloading the product.

Most of the estuarine area obtained a good suitability score, apart from the Pancas salt marsh (1), which mostly encompasses intertidal areas and consequently is harder to navigate, depending on low tides. The other area (2) is identified as the Atlantic Ocean and is unaccounted for in the MCE

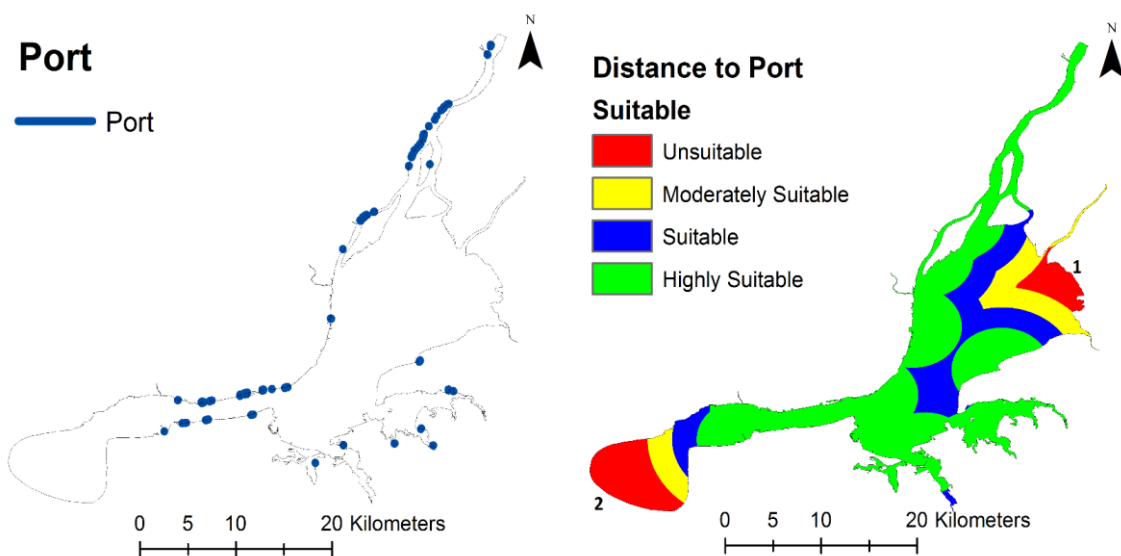


Fig. 22: Identified Ports within the estuary (left pane) and their reclassification map (Distance to Port) (right pane)
1 – Pancas salt marsh; 2 – Atlantic Ocean

4.1.2.4. Distance to Local Markets

Since Portugal is one of the biggest seafood consumers in the world (Almeida, 2014), with a per capita consumption of about 55 kg in 2014 (EUMOFA, 2016), this indicator focusses on the consumption at a local and regional scale, hence the assessment of local markets.

Highly Suitable areas were all identified as being closer to urban areas. No unsuitable areas were found in the estuary, although it should be highlighted that this requires further studies and/or confirmation from each municipality within the area, since all the identified local markets were based on OSM's files or by independent research using Google Earth Pro software. It is possible that some selected local markets are either: i) closed; ii) do not sell fresh seafood (namely clams), as intended in this indicator.

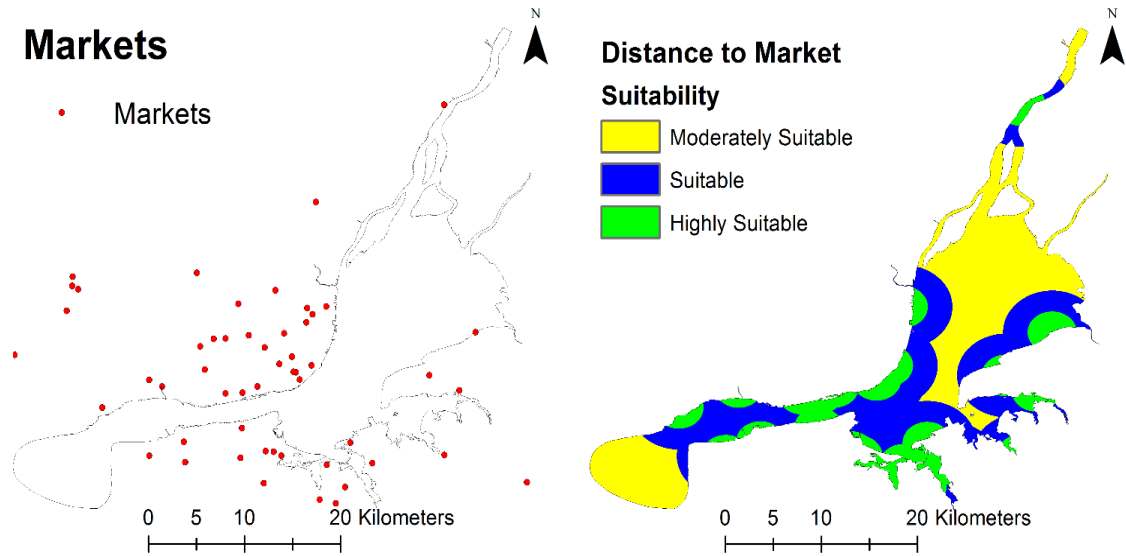


Fig. 23: Identified local markets near the estuary (left pane) and their reclassification map (right pane)

4.1.3. Environmental Category

All the thematic maps created for each Environmental indicator are represented below: maps on the left pane correspond to the Stage 1 and have both the used stations for the interpolation, whereas the right pane images represent the respective reclassification maps.

4.1.3.1. Dissolved Oxygen (DO)

It was not found any constraint on the reclassification map concerning DO, where the lowest suitability score was 3 (Suitability). Dissolved oxygen levels were typically higher near the estuary mouth (1) and its whole corridor, until approximately 25 de Abril Bridge (2); in most of the middle estuary (3), as well as the upper, from Mouchão da Póvoa (4) to Castanheira do Ribatejo (5). DO levels should not limit clams' growth in the Tagus Estuary.

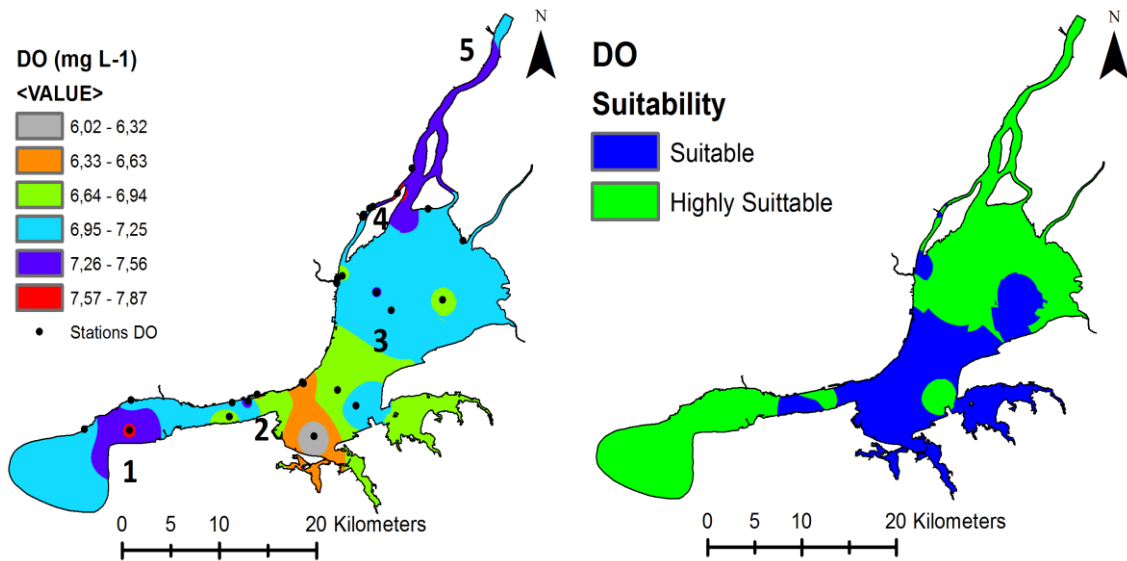


Fig. 24: DO values (left pane) and their reclassification map (right pane). 1 – Estuary mouth; 2 – 25 de Abril Bridge; 3 – Middle Estuary; 4 – Mouchão da Póvoa; 5 – Castanheira do Ribatejo

4.1.3.2. Temperature

Most of the estuary obtained a good suitability score: the areas from Alhandra (2) to Castanheira do Ribatejo (3) and near Trancão river (1) were the best ones, with a suitability score of 4 (Highly Suitable).

It was not found any constraint concerning temperature levels (should always be above 14 °C), which can be considered as a good indicator for Manila clam reproduction in the estuary, as temperature is one of the most important external variables for Manila clams' reproduction cycle and growth.

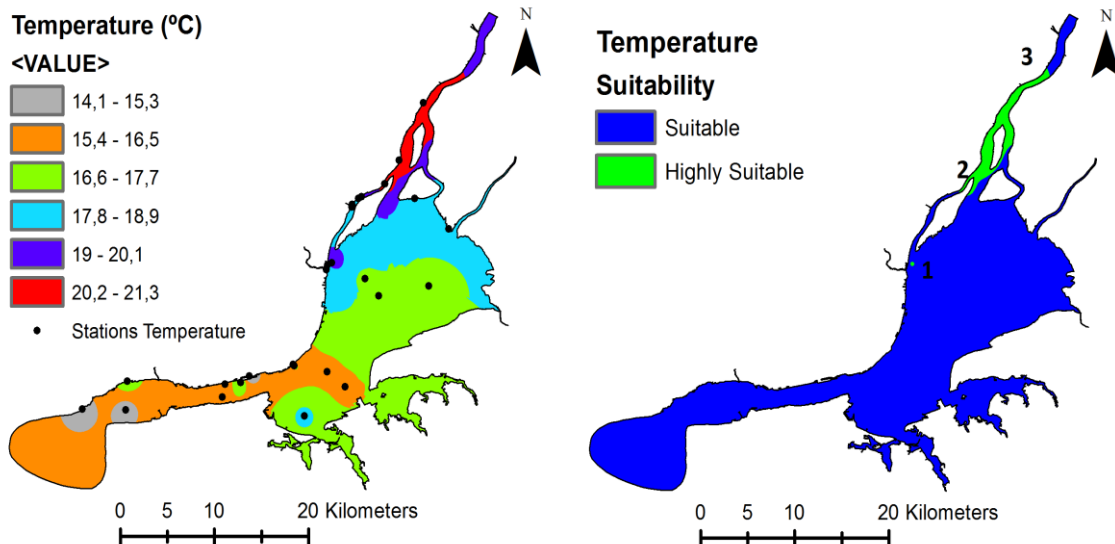


Fig. 25: Temperature levels in the estuary (left pane) and their reclassification map (right pane). 1 – Trancão river; 2 – Alhandra; 3 – Castanheira do Ribatejo

4.1.3.3. Salinity

Salinity reclassification map encompasses all four suitability values: unsuitable areas were found near Póvoa de Stª Iria (2) and to an area in-between Seixal bay and Barreiro, where can also be found a sharp decline of salinity values (from highly suitable to unsuitable) (1); moderately suitable areas are in the upper part of the estuary. The lower and middle part of the estuary mostly includes Suitable and Highly Suitable areas

The clams' growth might not be possible in areas classified as Unsuitable and Moderately Suitable, since as previously mentioned, salinity is a key factor. Therefore, those areas are not recommended for shellfish aquaculture siting.

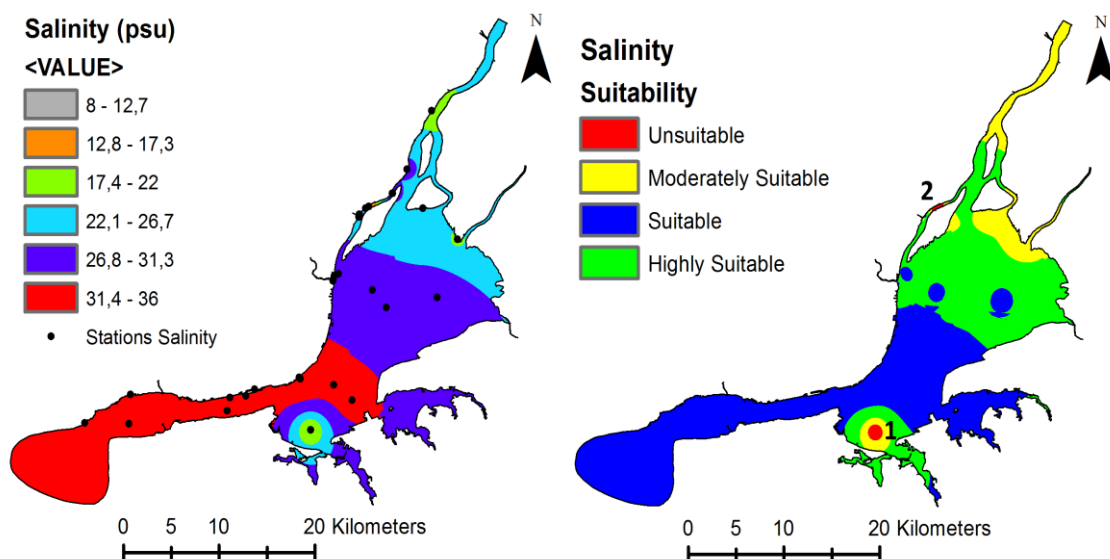


Fig. 26: Salinity values in the estuary (left pane) and their reclassification map (right pane). 1 – Area in-between Seixal and Barreiro; 2 – Póvoa de Stª Iria

4.1.3.4. TPM

Most of the estuary obtained a suitable score, excepted four different areas (two are unsuitable, with the other two being identified as highly suitable): i) the areas in-between Seixal and Barreiro (1) (similar to salinity's area No. 2) and Lisbon's Port (2) were considered unsuitable; and ii) Sorraia's river mouth and an area in the eastern part of the estuary (4) scored a Highly Suitable value. Generally, the highest TPM values can be seen near the identified Highly Suitable areas, from Alcochete (5) to an area nearby Mouchão do Lombo do Tejo (6). Meanwhile, the lowest levels correspond to area 2, to Vila Franca de Xira (7) and the estuary's mouth (8).

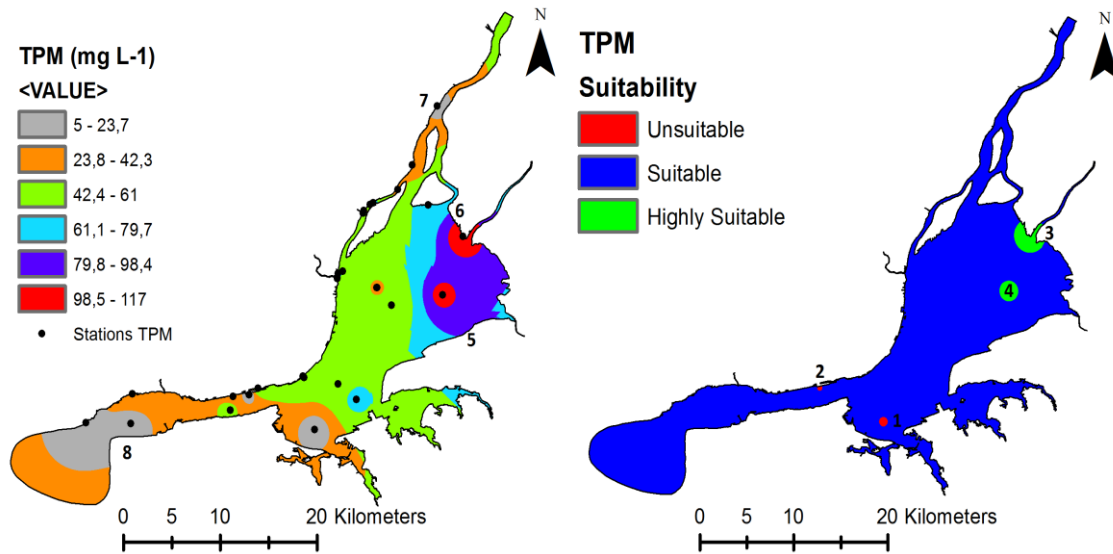


Fig. 27: TPM values in the estuary (left pane) and their reclassification map (right pane). 1 – Area in-between Seixal and Barreiro; 2 – Lisbon's Port; 3 – Sorraia river; 4 – Highly Suitable spot within the middle estuary; 5 – Alcochete; 6 – Mouchão do Lombo do Tejo; 7 – Vila Franca de Xira; 8 – Estuary's mouth

4.1.3.5. POM

Areas identified as unsuitable are found near the estuary's mouth (1) and in the upper channel (2): clams might not have sufficient food to properly grow and reach harvest size. Meanwhile, the highest values are located mostly in the middle part of the estuary (3), which is coincidental,

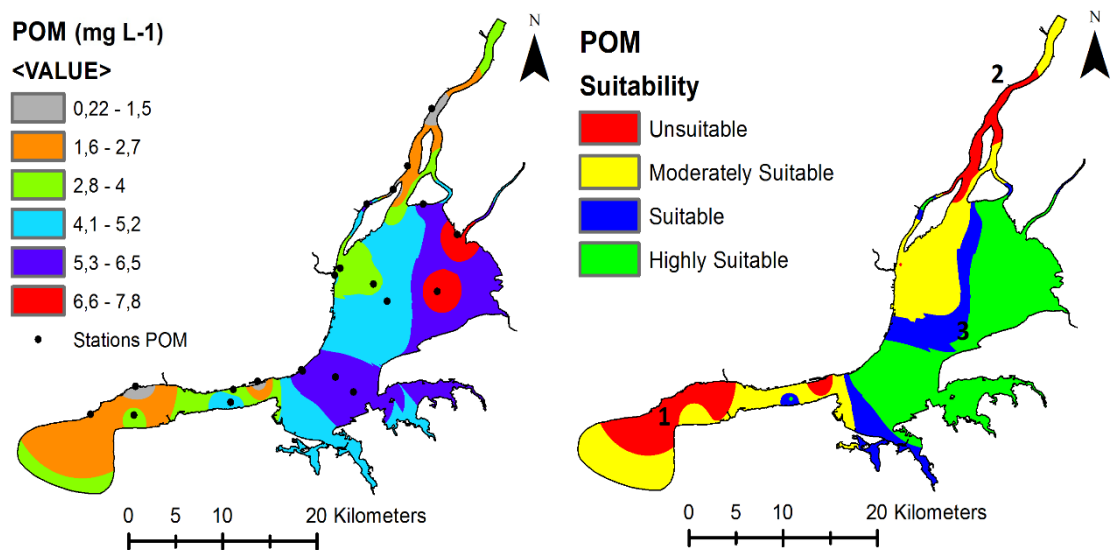


Fig. 28: POM values in the estuary (left pane) and their reclassification map (right pane). 1 – Estuary's mouth; 2 – Upper part of the estuary; 3 – Middle part of the estuary

for the most part, to areas reported to have the highest densities of Manila clam (Garaulet, 2011).

Moderately suitable areas are dispersed throughout the estuary: near the main channel, as well as middle and upper parts. The unsuitable areas may restrict growth, due to the low values.

4.1.3.6. Chl

Chl values show a declining trend from the upper part to the estuary's mouth: unsuitable areas encompass all the lower part and most of the middle estuary, as well as the areas near Póvoa de Stª Iria (1), meaning that those areas should restrict growth to a certain degree.

Suitable and Highly Suitable areas are located in the upper part of the estuary, in addition to the vicinities of Trancão river (2).

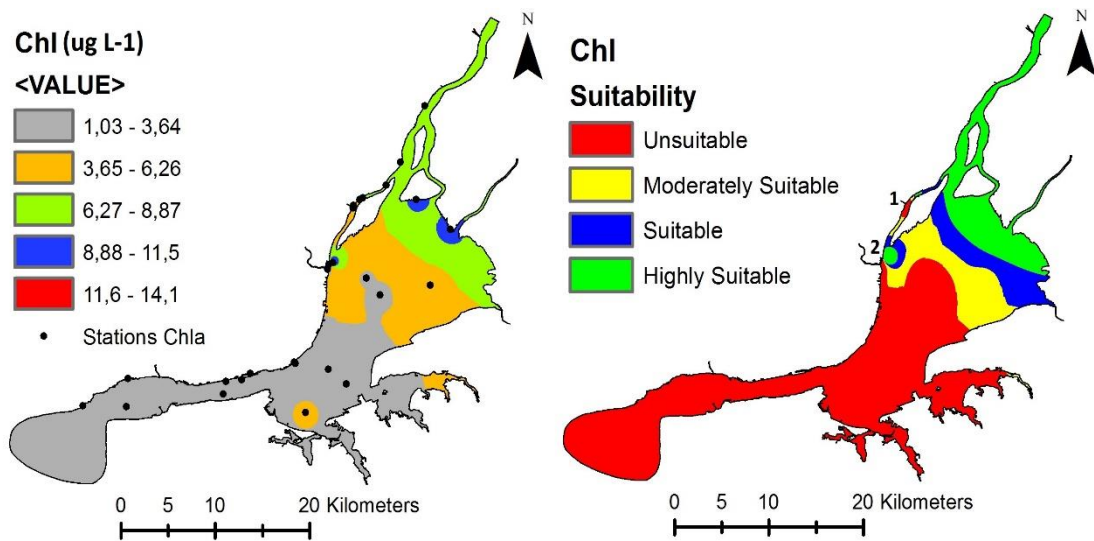


Fig. 29: Chl values in the estuary (left pane) and their reclassification map (right pane). 1 – Póvoa de Stª Iria; 2 – Trancão river

4.1.4. Product Contamination Category

Product Contamination follows the same logic applied to Environmental Category. It also should be highlighted again that the use of PEL-TEL into the reclassification, which can be considered as a limitation, since the four suitability values are reduced to only three.

The value corresponding to less polluted areas (Highly Suitable – 4) and to the more polluted ones (Unsuitable – 1) are well defined by either being lower or higher than PEL or TEL, respectively. In-between both indicators, it was chosen to choose the suitability level of 2 (Moderately Suitable), in order to play safe and avoid possible food contaminations by thinking the concentrations were not high enough.

4.1.4.1. Chromium (Cr)

The reclassification map concerning Cr does not show any obvious contaminated location (i.e. area scoring a value of 1, unsuitable). Most of the lower estuary scored a value of 2 (Moderately Suitable), apart from an area close to 25 de Abril Bridge (1), with the upper end of the estuary being considered as Highly Suitable. The middle section of the estuary scored a value of 4 in area 2, which encompasses Alcochete, Sorraia's river mouth up until the area below Mouchão do Lombo do Tejo. Area 3 is mostly considered as Moderately Suitable, apart from four spots that have scored a higher value (Highly Suitable).

Concerning the areas defined as Moderately Suitable: areas displayed with the color orange (52.4 – 70) are closer to PEL (52.3) and are less contaminated, while blue or red areas are the ones with higher values and thus more prone to shellfish contamination. This way, areas near Barreiro should be avoided (signaled by a red rectangle in the left image).

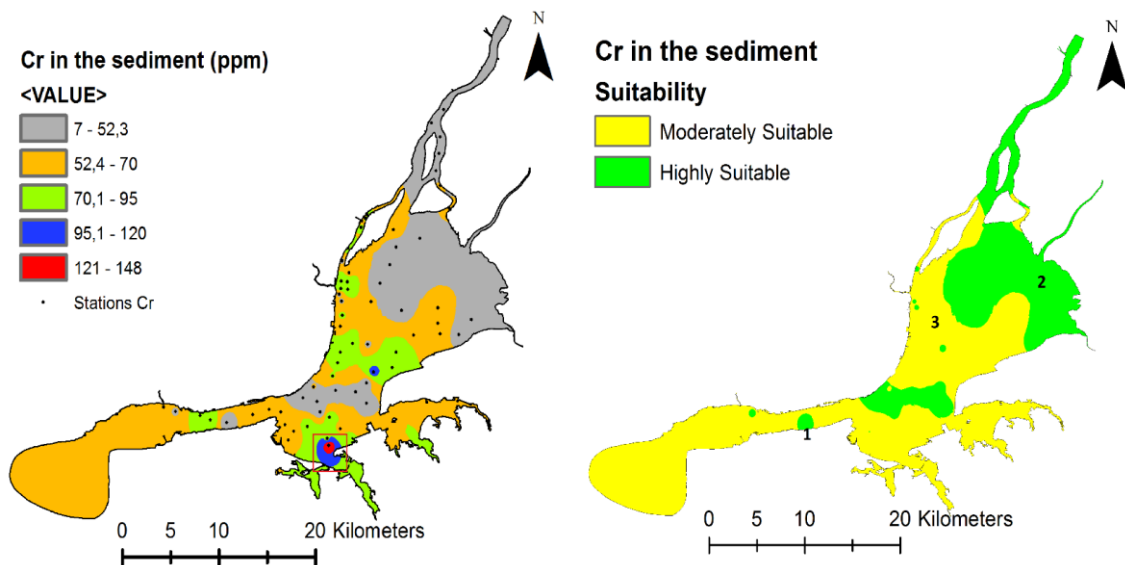


Fig. 30: Chromium (Cr) values in the estuarine sediments (left pane) and their reclassification map (right pane). 1 – 25 de Abril Bridge; 2 – Highly Suitable area that encompasses Alcochete, up until Mouchão do Lombo do Tejo; 3 – Middle part of the estuary

4.1.4.2. Cadmium (Cd)

Cadmium values in sediments were mostly considered as Moderately Suitable. This can also be good or bad, depending on each side of the thresholds (e.g. closer to PEL than TEL) the value is, and thus there is a lot of uncertainty associated.

In-between PEL and TEL (0.68 – 4.21, respectively), orange areas are the less contaminated and are well distributed within the estuary: near the estuary’s mouth (1) and in the upper end of the estuary (2), near Sorraia’s river mouth (3) and Montijo’s bay (4). Green and light and dark blue areas can be classified as having higher probability of food contamination and are distributed near Barreiro (5), Trancão river (6) and near Póvoa de Stª Iria (7).

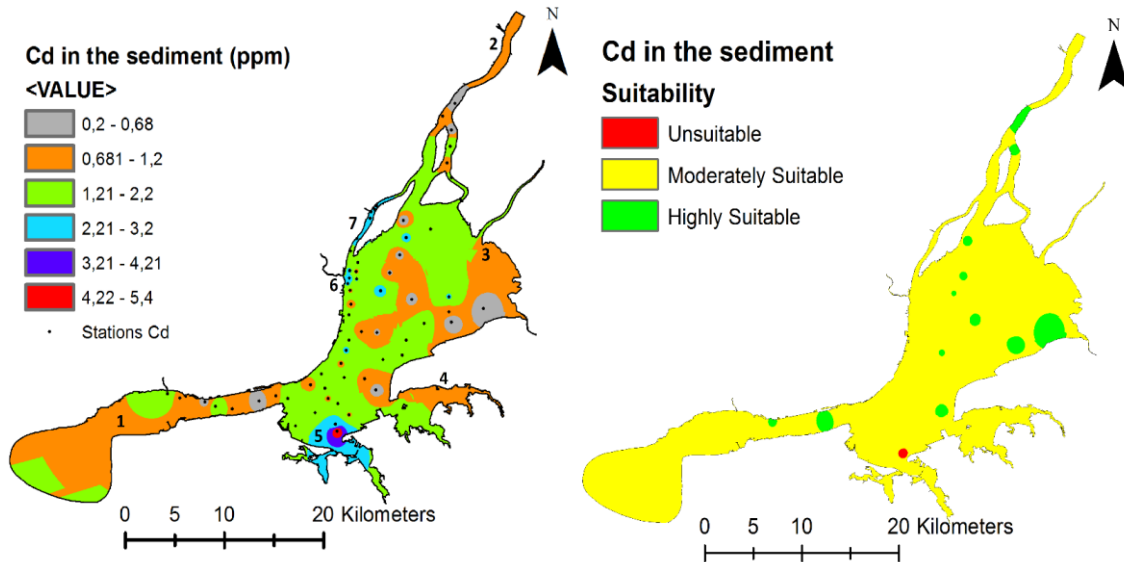


Fig. 31: Cadmium (Cd) concentration in the estuarine sediments (left pane) and their reclassification map (right pane). 1 – Estuary’s mouth; 2 – Upper end of the estuary; 3 – Sorraia river; 4 – Montijo; 5 – Barreiro; 6 – Trancão river; 7 – Póvoa de Stª Iria

4.1.4.3. Copper (Cu)

Copper sediment values are considered as unsuitable in the area in-between Seixal and Barreiro (1), as well as having concentration values close to the higher threshold of PEL-TEL (18.7 – 108), displayed as blue areas. Most of the estuary scored a value of 2 concerning the suitability scale (Moderately Suitable), where both the upper end and most of the middle part of the estuary are close to the minimum threshold of PEL-TEL range, being less contaminated (orange) than those in the vicinities of area 1 and estuary’s mouth.

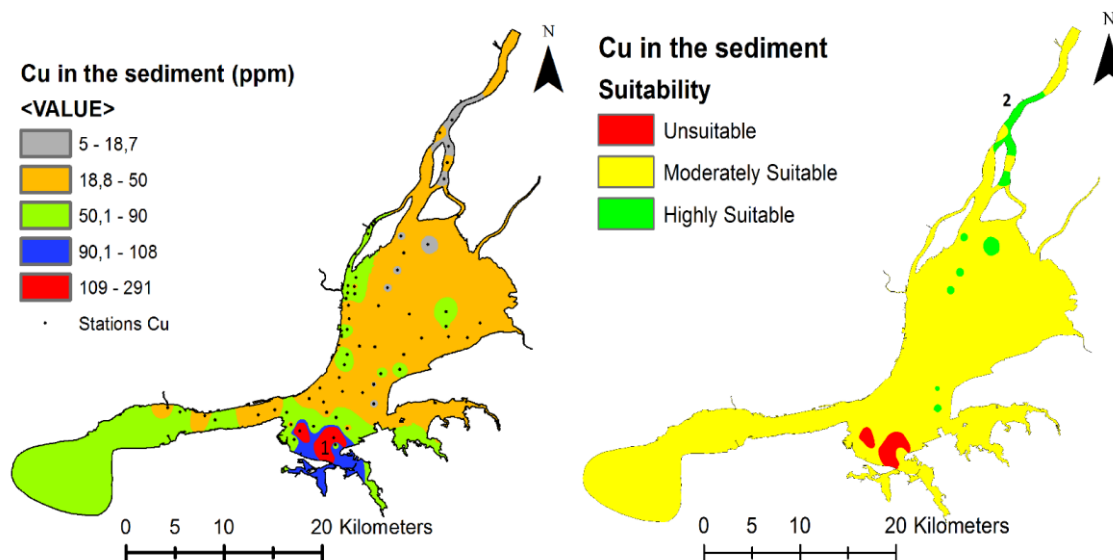


Fig. 32: Copper (Cu) concentration in the estuarine sediments (left pane) and their reclassification map (right pane); 1 - Seixal and Barreiro; 2 – Castanheira do Ribatejo

The areas near Alhandra up to Castanheira do Ribatejo (2), as well as minor spots on the middle part of the estuary scored the best values (Highly Suitable).

4.1.4.4 Zinc (Zn)

According to the suitability scale used, most of the estuary scored a value of 1 (unsuitable). Highly Suitable areas were distributed near Vila Franca de Xira and Castanheira do Ribatejo (1), as well as minor spots near Lisbon's port (2) and Montijo's Air base (3). Moderately Suitable areas exist near areas 2 and 3 and the remaining part of the upper estuary: lower threshold values concerning Moderately Suitable areas are displayed in orange in areas 2, 3, near Mouchão do Lombo do Tejo (4) and in the vicinities of Mouchão da Póvoa (5).

Hence, it is possible to conclude that most of the estuary is contaminated by Zn, corroborated by França et al. (2005).

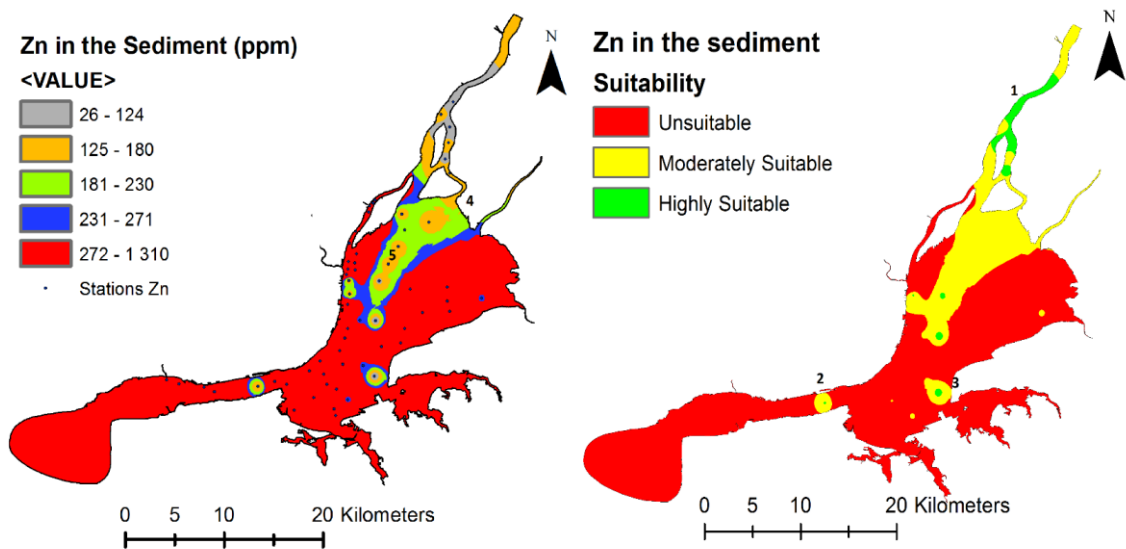


Fig. 33: Zinc (Zn) concentrations in the estuarine sediments (left pane) and their reclassification map (right pane).

1 – Vila Franca de Xira-Castanheira do Ribatejo section; 2 – Lisbon's Port; 3 – Montijo's Air Base No. 6; 4 – Mouchão do Lombo do Tejo; 5 – Mouchão da Póvoa

4.1.4.4. Lead (Pb)

Areas near the estuary's mouth (1), Mouchão da Póvoa (2) and most of the middle part of the estuary were deemed as unsuitable, according the used reclassification scale. The remaining parts of the estuary scored a value of 2 (Moderately Suitable): between Trafaria and 25 de Abril bridge (3), near Montijo's Air Base No. 6 (4), all the upper part of the estuary, as well as part of Alcochete (5), Parque das Nações (6). The lower thresholds of Moderately Suitable areas were mostly located in the upper part of the estuary (7). Besides Zn, the estuary is also contaminated by lead, also supported by França et al. (2005).

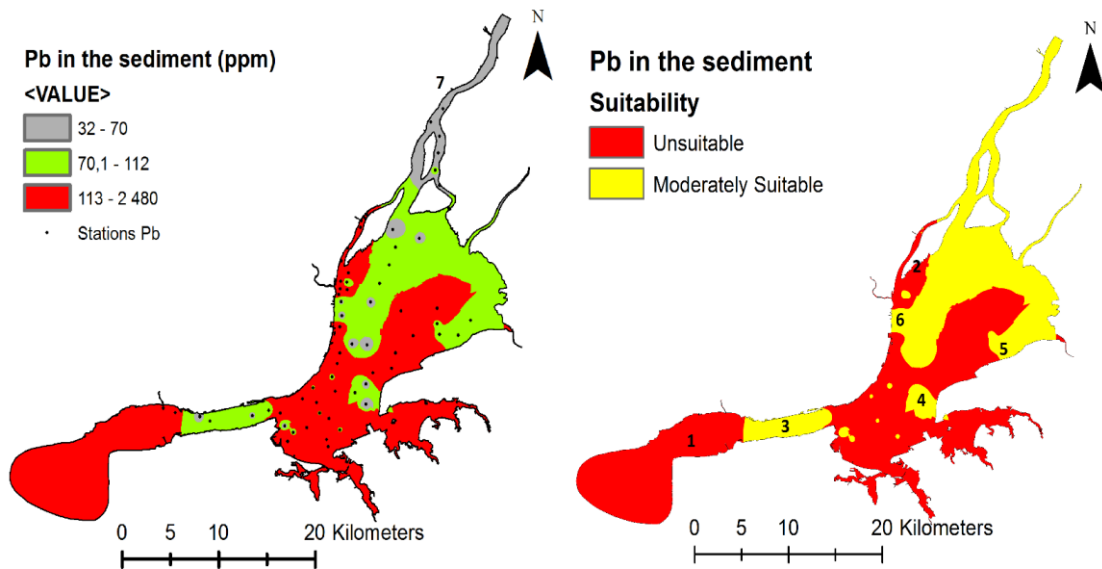


Fig. 34: Lead (Pb) concentrations in the estuarine sediments (left pane) and their reclassification map (right pane) 1 – Estuary’s mouth; 2 – Mouchão da Póvoa; 3 – Trafaria – 25 de abril bridge section; 4 – Montijo’s Air Base No. 6; 5 – Alcochete; 6 – Parque das Nações; 7 – upper part of the estuary

4.1.4.5. Total Polycyclic Aromatic Hydrocarbons (tPAH)

TPAH’s reclassification map shows that suitability values are dispersed throughout the estuary: Highly Suitable areas are located in the middle and upper parts of the estuary, apart from a small area near Seixal (1); Moderately Suitable areas are also dispersed through the lower part of the estuary, dominating both the middle and upper parts.

Most of the lower estuary was classified as being unsuitable due to high tPAH levels in sediments, namely Barreiro (2) and Montijo (3)

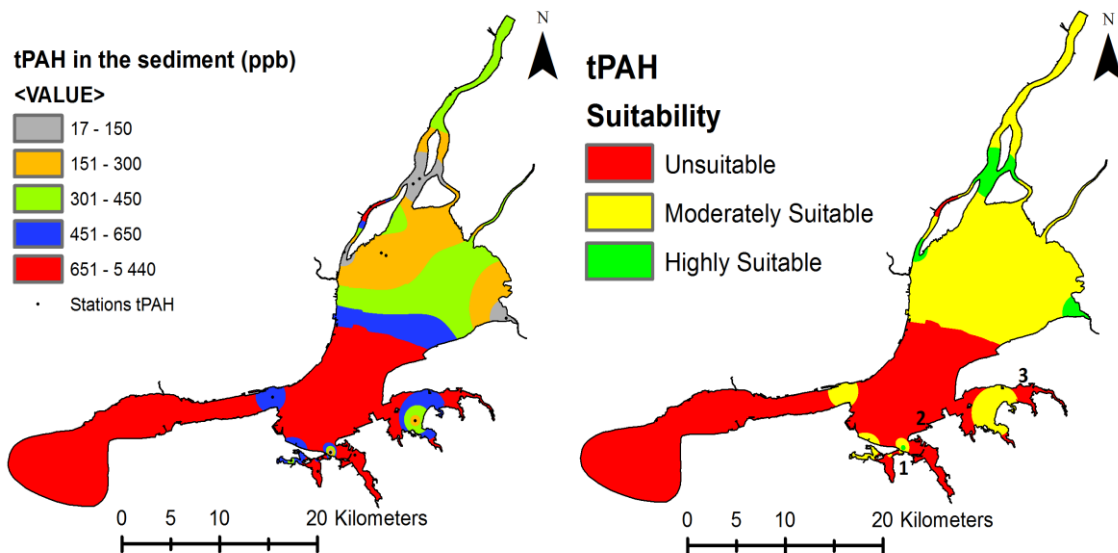


Fig. 35: tPAH concentrations in the estuarine sediments (left pane) and their reclassification map (right pane) 1 – Seixal; 2 – Barreiro; 3 - Montijo

4.1.5. Final thematic maps

Spatial Constraints final thematic map was created by means of a Fuzzy Overlay, accounting for every identified spatial constraint and the process of screening out areas deemed as unsuitable by at least one thematic map.

Each Category's suitability map was obtained by means of a Weighted Overlay, based on the calculation of relative weights in the AHP. Total areas were calculated for every suitability score, in order to understand where each Category gathers the best conditions for shellfish aquaculture.

4.1.5.1. Spatial Constraints

The final Spatial Constraints thematic map is displayed in the figure below (Figure 36).

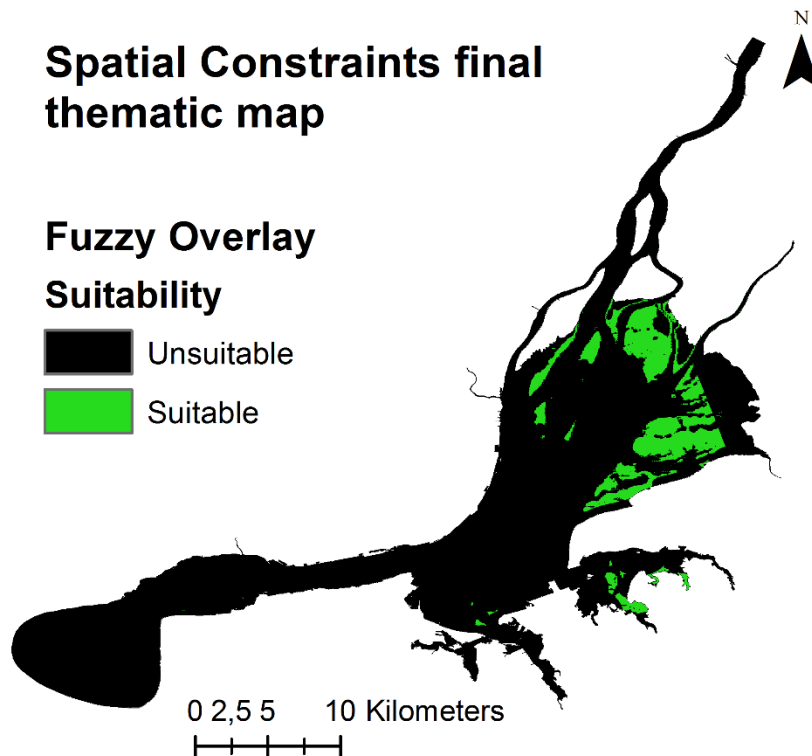


Fig. 36: Spatial Constraints final thematic map

The final Spatial Constraints thematic map accounted for approximately 314.18 km² of unsuitable areas, with the remaining suitable areas totaling 33.4 km² (Table 23). This shows that the estuary has a wide variety of incompatible uses for shellfish aquaculture (e.g. Rilo et al., 2013), since more than 90% of its area was considered as unsuitable.

Table 23: Total area for each suitability score

| Suitability | Total area (km ²) |
|-------------|-------------------------------|
| 0 | 314.18 |
| 1 | 33.4 |

Bathymetry was already considered the most fundamental spatial constraint since it restricted the existing areas to those suitable for intertidal aquaculture. Bathymetry's total unsuitable areas accounted for 287.89 km², which is approximately 91% of the unsuitable areas within the final Spatial Constraints map.

Because bathymetry was reclassified in accordance with the existing intertidal flats, which are mostly gathered in the left margin of the Estuary, from Seixal to Sorraia river, as well as in the vicinities of Mouchão da Póvoa and Mouchão do Lombo do Tejo. It should be emphasized that bathymetry's total unsuitable areas encompass most of the other spatial constraints (e.g.

Distance to Navigational Terminals). Different aquaculture practices, namely suspended aquaculture, would need different bathymetry values for their reclassification and therefore, different importance.

The other individual Spatial Constraints considered as relevant (in terms of area) were Distance to Beach and Distance to Industry: Distance to Beach restricted the studied area in about 23.77 km² (7.9% of the total area) and Distance to Industry, 83.78 km² (27.89%).

Some beaches and respective safety distances restricted possible intertidal flats for aquaculture practices, such as in the vicinities of Barreiro and Seixal bay (7.5 km²) and near Alcochete's coastline (1.38 km²).

Distance to Industry deemed as unsuitable the areas from Almada up to Montijo, totaling 39.53 km². Most of the right margin was also classified as unsuitable, on which two sections can be defined: the middle section of the Tagus main channel, from Lisbon's Port up to Porto Brandão (9.67 km²); and a large portion of the right margin, from approximately St^a Apolónia to the upper end of the estuary, near Castanheira do Ribatejo (28.44 km²).

When no reclassification values or safety distances were found in the literature related to Distance to WWTP and Industry, the strictness of the values used (higher values for making sure no contamination would occur) were considered of medium range. This is because they were considered as "introductory values", even though the established distances should avoid major contaminations.

Legal constraints, such as TPA and the salt marshes defined in PORNET (areas near Mouchão da Póvoa and Mouchão do Lombo do Tejo and Pancas), were considered as Spatial Constraints, as they are considered areas with high ecological sensitivity, due to their importance concerning ecosystems functions and nesting and resting sites for migratory birds. Areas included in Natura 2000 are displayed in Appendix B.

No information about navigational charts in the estuary was found, so to understand whether the identified suitable areas may influence nautical navigation, Lisbon's Port was contacted, to which Fig. 36 was shown and it seems no identified suitable areas may disturb ship's navigation routes. Besides Lisbon's Ports, Port Authority should be further contacted in case of the development of any type of aquaculture projects, since they ultimately have the jurisdiction of the Tagus Estuary area.

4.1.5.2. Product Contamination suitability map

Product Contamination category scored the worst values between all the studied categories (Fig. 37): no Highly Suitable areas can be found. 39.7% of all estuarine area is classified as "Unsuitable", meaning that they are most likely contaminated by heavy metals, particularly in the lower and middle parts of the estuary.

Areas scoring a value of 3 (Suitable) are distributed throughout the estuary, focusing on the upper part of the estuary (5.9%), with most of the estuary being classified as either Moderately Suitable or Unsuitable. Moderately Suitable correspond to 54.4% of the total area and its meaning can be dubious, for the same reasons already mentioned: most of the middle part of the estuary, the estuary's mouth and Montijo's bay are the most important areas that scored a suitability value of 2.

Table 24: Total areas concerning each suitability level scored in Product Contamination

| Suitability | Total area (km ²) |
|-------------|-------------------------------|
| 1 | 137.94 |
| 2 | 189.46 |
| 3 | 20.36 |

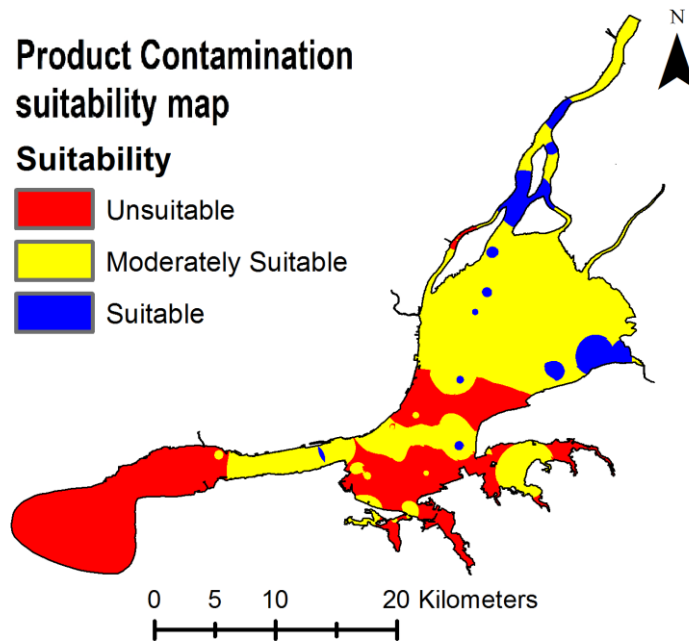


Fig. 37: Product Contamination suitability map

The main constraints identified for the suitability of the Product Contamination final map were lead and zinc concentrations, where most of the estuary was deemed as unsuitable. The remaining studied heavy metals are mostly composed of Moderately Suitable areas, although copper has an unsuitable area. Also, Product Contamination suitability map can be deceiving, as it can give the illusion that “suitable” areas are pollution free, which is not the case. Since weights were given for each heavy metal, suitable areas are merely indicative of which areas are less contaminated, e.g. an area can be heavily contaminated by copper and still have an overall suitability score of 4, even though copper would reduce the product’s quality. More trustworthy comparisons and conclusions can not be made, due to the larger scope of this work and the author’s expertise, as the final suitability map should only be seen as an overall state.

Although all Product Contamination and respective indicators are related to heavy metal pollution, faecal coliforms (FC) could also be considered as a factor influencing product’s final quality (e.g. Silva et al., 2011) and because of this, it will be emphasized ways to overcome significant levels of FC and respective European legal requirements that are used to regulate them.

The Tagus Estuary is subjected to strong anthropogenic pressures that affect water quality and result in high levels of faecal microorganisms (e.g. Lemarchand et al., 2004), and considering that it could not be gathered recent data, it was chosen not to present out of date data; some studies evaluate them locally (Anacleto et al., 2013; Brondani, 2015).

Problems associated with the consumption of shellfish grown in waters contaminated with microbiological pathogens are mainly due to food-borne diseases, including heavy metal and PAH contaminations, toxins from harmful algal blooms or contamination from pathogenic microorganisms (e.g. Anacleto, 2014; Watkins et al., 2008; Lipp and Rose, 1997; Gholami et al., 1998). Thus, they represent a significant human health risk (Lees et al., 2010), which can be reduced by depurating the harvested shellfish in tanks, or transfer them to a cleaner area (process called relaying).

According to Lee et al. (2008), depuration is a “technique used to reduce microbial contaminant from light to moderately contaminated bivalve molluscs, by placing them in tanks of clean water, in order to resume normal pumping activity and further expel contaminants from their gills and

intestinal, over a period of time that can range from several hours to days”. Depuration techniques are not suitable to apply in shellfish harvested from heavily polluted areas (e.g. heavy metal contamination) (Lees et al., 2010; Hickey et al., 2015). El-Shenawy (2004) studied the effects of depuration regarding Manila clam (Table 25).

Table 25: Effects of depuration on the elimination of microbiological and chemical contaminants from bivalves (Adapted from Anacleto, 2014; El-Shenawy, 2004)

| Contaminant | Faecal Coliforms | <i>Vibrio spp.</i> | faecal <i>Streptococcus</i> | coliphage | Fe | Ni | Co | Cu |
|---------------|------------------|--------------------|-----------------------------|-----------|----|----|----|----|
| Reduction (%) | 85 | 50 | 41 | 69 | 47 | 20 | 28 | 36 |
| Time (h) | | 96 | | | | | 48 | |

Cunha (2012) studied the effects of depuration in Manila clam in the estuary, where mortality in the transition of the animals to the depuration tanks was close to 0%. It was concluded that depuration was effective in the reduction of Pb levels; Cd depuration time should be longer than the used one (16 days), in order to reach proper conclusions; Hg levels were not considered a limiting factor, although still considered as high; reduction of Fe levels was similar to those displayed in the table above (47%).

In the process of “relaying”, the shellfish harvested from polluted areas can be replaced in areas free of microbiological contamination, allowing shellfish to cleanse or purge themselves by continuation of their normal filter-feeding activities (Lees et al., 2010; Rees et al., 2010).

When used as treatment processes to reduce microbial contamination, commercial depuration and relaying are subjected to legal control in several countries, including those in the EU (Lees et al., 2010), according to the requirements expressed in Regulation (EC) No. 853/2004; whereas production, harvesting and the commercialization of bivalves, as well as the classification of the overlying waters are regulated by the Regulation (EC) No. 854/2004 and 1441/2007, which also establishes microbiological criteria (Table 26). Lees et al. (2010) have stated that “if an area only meets the standards for certain periods because of predictable pollution events, authorities may classify them for a restricted period”.

Table 26: Microbiological criteria applied to areas where production, harvesting and commercialization occurs, as well as to possible relaying areas and respective relaying periods

| EU Class | Description | Microbiological standard * |
|------------|---|----------------------------|
| A | Class A areas are considered the cleanest growing areas from which shellfish can be harvested, and safe for direct human consumption without further processing. | ≤ 230 |
| B | Class B areas on which live bivalve molluscs may be collected, but placed on the market for human consumption only after treatment in a purification center or after relaying in a Class A area, as to meet the health standards defined in Regulation (EC) No. 842/2004. | ≤ 4,600 |
| C | Class C areas from which live bivalve molluscs may be collected but placed on the market only after relaying in Class A or B areas, over a long period (minimum of two months) so as to meet the health standards defined in Regulation (EC) No. 805/2004. | ≤ 46,000 |
| Prohibited | Areas from which bivalve molluscs cannot be harvested for human consumption, e.g. close to untreated sewage discharges (Cefas, 2006). | > 46,000 |

* *E. coli*/100 g fresh and intravalvular liquid;

According to Order No. 15264/2013 and later Order No. 4022/2015, both being included in the scope of Regulation (EC) No. 854/2004 and Ordinance No. 1421/2006, after three years of monitoring and microbiological quality control of bivalve molluscs, production zones should be reclassified. Tagus Estuary was classified as class C by IPMA, I. P (Portuguese Institute for the Ocean and Atmosphere).

The illegal fishing that is still happening (as of 2017) in the estuary can lead to public health problems, since many shellfish are harvested and then sold without being properly treated (e.g. in depuration units) and might be contaminated by high levels of FC or heavy metals (Público, 2016). Hence, the need for creating proper settings, either spatially and legally, within the Tagus Estuary.

This way, both Product Contamination and Production Categories are intimately linked, making possible to harvest shellfish in contaminated areas: Product Contamination (including a proper evaluation of microbiological sources) assess which areas are the most polluted, being from heavy metal or faecal contamination. Areas can then be divided according to their microbiological standard values (Class A, B, C or prohibited), in order to quantify them and thus, understanding i) where shellfish cannot be harvested; ii) where shellfish can be relayed to (Class A or B areas) and iii) where shellfish is mostly harvested nowadays and to which class those areas correspond. It should be mentioned that despite being from Class A areas, there many cases (e.g. in the United Kingdom) of many businesses holding harvested products in depuration units, “as a matter of due diligence” (Lee et al., 2008; Seafish, 2010).

Production Category, by taking into account where are the possible relaying areas and where shellfish is mostly harvested, evaluate all possible infrastructures in order to allow product transportation in the most efficient ways.

4.1.5.3. Production Category suitability map

Production Category suitability map (Fig. 38) shows that the closer to urban areas, facilities and populations, the better for shellfish production: 47.5 % of the estuary scored a value of 3 on the suitability scale, with 28.9 % being highly suitable for shellfish aquaculture. The unsuitable values (only 10.3%) are focused near Sorraia river and the Atlantic Ocean.

The suitability score of the area near Sorraia river can be explained by the lack of accessibilities, having neither piers or roads nearby, as well as no close urban areas. The area is only accessible by boat and since there are almost none human interferences, can make it ideal for shellfish aquaculture. According to the reclassification values, the area in the vicinities of the Atlantic Ocean (end of the estuary) is considered too isolated for shellfish cultures to be sited there.

Table 27: Areas concerning each suitability score for Production Category

| Suitability | Total area (km ²) |
|-------------|-------------------------------|
| 1 | 35.69 |
| 2 | 46.07 |
| 3 | 165.39 |
| 4 | 100.50 |

Spatial Constraints final thematic map already classified that area as unsuitable, and because it is closer to the ocean, ideal physical conditions for shellfish aquaculture (bathymetry and current speed) are not gathered.

Overall, and according to the reclassification map, the estuary seems to gather sufficient conditions to comprise aquaculture practices, even considering that a “trade chain of live bivalves” (Bettencourt et al., 2012) is lacking, or is extremely disorganized. The trade chain of live bivalves can be summarized as (Bettencourt et al., 2012; Anacleto, 2014): after the bivalves are harvested, they are transported to i) depuration center, ii) relaying area, or iii) industrial unit, according to the classification of the harvesting areas and respective levels of pollution; afterwards, they go to a dispatch center, where they can be transported nationally to wholesalers/retailers/local markets, or internationally, where they are to be sold to consumers.

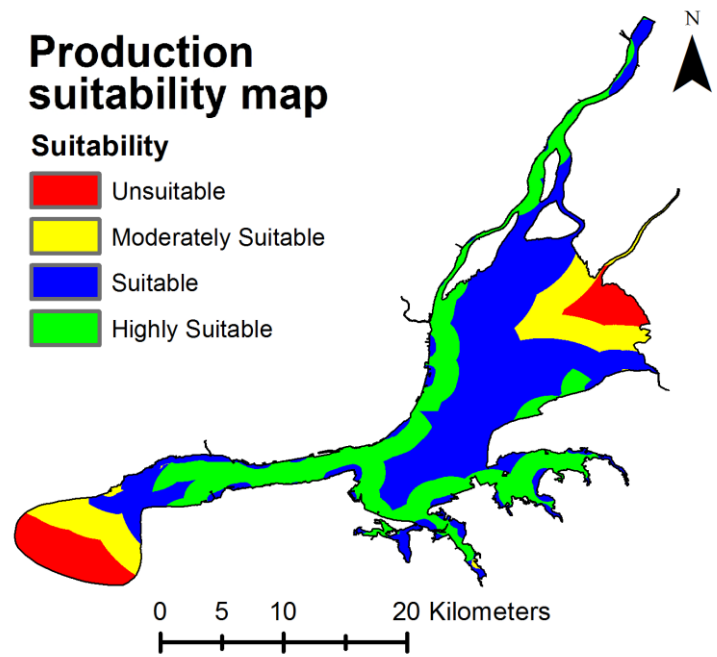


Fig. 38: Production suitability map

This work focuses mainly on the existing facilities that allow fishermen transportation to harvesting/production locations (e.g. roads or piers) and their trip back, with the harvested product, as well as the existing local markets in the vicinities: ideally, as the populations of clams are still growing, adding to the high seafood consumption per capita in Portugal, the harvested product would be sold locally, with the remaining clams being sold to other locations (i.e. imported/exported).

The transportation of the harvested products should follow strict salubrious hygiene conditions (Bettencourt et al., 2012), as to avoid the death of the animals and subsequently a possible (re)contamination.

Possible methods for growing and/or harvesting techniques were not assessed, although Ramajal et al. (2016) described different harvesting methods in the Tagus Estuary regarding the Manila clam, and concluded that most clams are harvested manually by foot, for a total amount of 1,111 catchers (in 1,724 case studies).

4.1.5.4. Environmental Category suitability map

Since clams are known to be growing in the estuary, environmental variables were expected to be significantly good, which the Environmental Category suitability map (Fig. 39; Table 28) proved: most of the estuary is classified as Suitable (93.8%), with the vicinities of Sorraia river scoring a value of 4 (Highly Suitable), corresponding to 5.1 % of the total area.

Table 28: Total areas concerning each suitability level scored in Environmental category

| Suitability | Total area (km ²) |
|-------------|-------------------------------|
| 2 | 3.70 |
| 3 | 326.69 |
| 4 | 17.81 |

Although classified as Suitable, areas near the main and upper channels were identified as being unsuitable for both food sources, which might severely restrict clam populations there. Salinity reclassification has also shown lower values for the upper part of the estuary. Therefore, even though the suitability map is considered suitable for those areas, clam populations might not reach harvest size there.

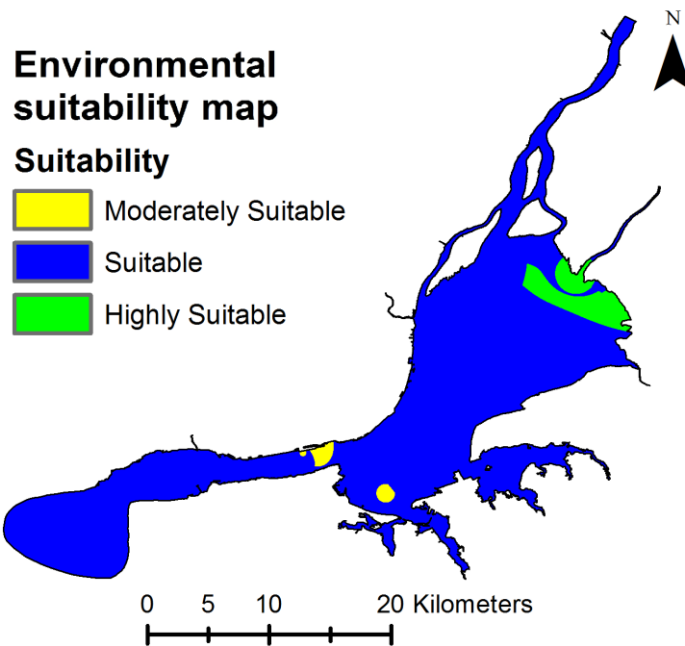


Fig. 39: Environmental suitability map

Salinity and food sources (Chl and POM) were identified as possible restrictions for both reproduction and growth. Salinity, albeit not considered unsuitable in any place, was classified as being “Moderately Suitable” in several areas, with values ranging from 20 to 24 psu, which are still far from the reported survival threshold of 14/15 psu (e.g. Coughlan et al., 2009). Still, it should be here highlighted that as larvae are less resilient if salinity levels are low enough (i.e. less than the survival threshold) in the beginning of the reproduction cycle, it might influence the growth and survival of juveniles and have repercussions in the establishment of populations.

According to the reclassification maps, most of the lower estuary was classified as having low food availability (both Chl and POM), whereas the middle estuary only scored low values for Chl. As most of the middle estuary was reported to have the biggest populations of the Manila clam (Garaulet, 2011), such as Seixal or Montijo, this could mean that either: i) the reclassification methodology carried for Chl and POM was not sensitive enough and the reclassification values do not reflect the real importance on growth, and/or ii) areas reported to have growing populations were classified as “unsuitable” concerning Chl, which can be attributed to POM’s higher importance for the clams feeding processes.

Even though the lower part of the estuary gathers suitable conditions for the growth of Manila clam, Garaulet (2011) has reported clam populations with lower densities there. The same author also reported that clams are starting to expand to the main channel. Ramajal et al. (2016) have estimated a total of 4 to 17 thousand t year⁻¹ of harvested Manila clam in the Tagus Estuary, with an associated economic value of 10 to 23 million €.

For a term of comparison, as no legal aquaculture settings or management practices are being ensured, the production reflects only the environmental factors (i.e. no site selection was done). The table below (Table 29) represents Manila clam production in major growing areas worldwide. Neither production or economic values were found for any Chinese area (e.g. estuary, lagoon), as China is the biggest producer in the world. By comparing table 29 values with Ramajal et al. (2016) lower thresholds of 4 thousand t and 10 million €, it is possible to conclude that the Tagus Estuary still has both significant production and economic potential for Manila clam aquaculture, gathering sufficient conditions for its growth and further development.

Table 29: Manila clam production in known growing areas worldwide and estimated economic value

| Location | Reported year | Production (thousand t) | Estimated economic value (million €) | Source |
|---|---------------|-------------------------|--------------------------------------|----------------------------|
| North Puget Sound, Washington, USA | 2013 | 2 (approx.) | 9.9 | Washington Sea Grant, 2015 |
| Galician Rias, Spain | 2009 | 1.9 | 7.8* | Xunta de Galicia, 2008 |
| Arcachon Bay, France | 2007 | 1 | 4* | Caill-Milly et al., 2008 |
| Venice Lagoon, Italy | 2013 | 5 (approx.) | 20* | Bartoli, 2016 |
| Venice Lagoon, Italy (highest value reported) | 1999 | 40 | 160* | Canu et al., 2011 |
| Northern Chiba, Tokyo Bay, Japan | 1999 | 0.8 | 3.2* | Toba, 2004 |

* assuming a price kg⁻¹ of € 4.0 (Público, 2016)

Irrespective of all the possible benefits from shellfish aquaculture, there is still a lack of relevant studies concerning Manila clam environmental impact in the estuary, apart from its competition with the native clam. As such, the areas reported to have the biggest populations must be assessed, in order to understand whether they are negatively influencing benthic habitats and the inherent communities. Higher density populations could be influencing food depletion in the water column, which could explain the reduction of the native species population, as *R. decussatus* is a less efficient filter-feeder (e.g. Garaulet, 2011). Local studies should also be made in the scope of Natura 2000's AA, namely an assessment of bird feeding and roosting behavior, due to their overall ecological importance and possible impacts relative to the colonization of the Manila clam.

4.2. Stage 3

Stage 3 is based on the creation of the final site selection thematic map, which followed the general methodology in stage 1: Weighted Overlay, although with a particular point, which consisted of incorporating Spatial Constraints, to screen out unsuitable areas, either because of pollution, legal hindrance or direct anthropogenic disturbances. This way, weights were calculated by means of an AHP and then a Weighted Overlay tool was applied to the three categories (Fig. 40).

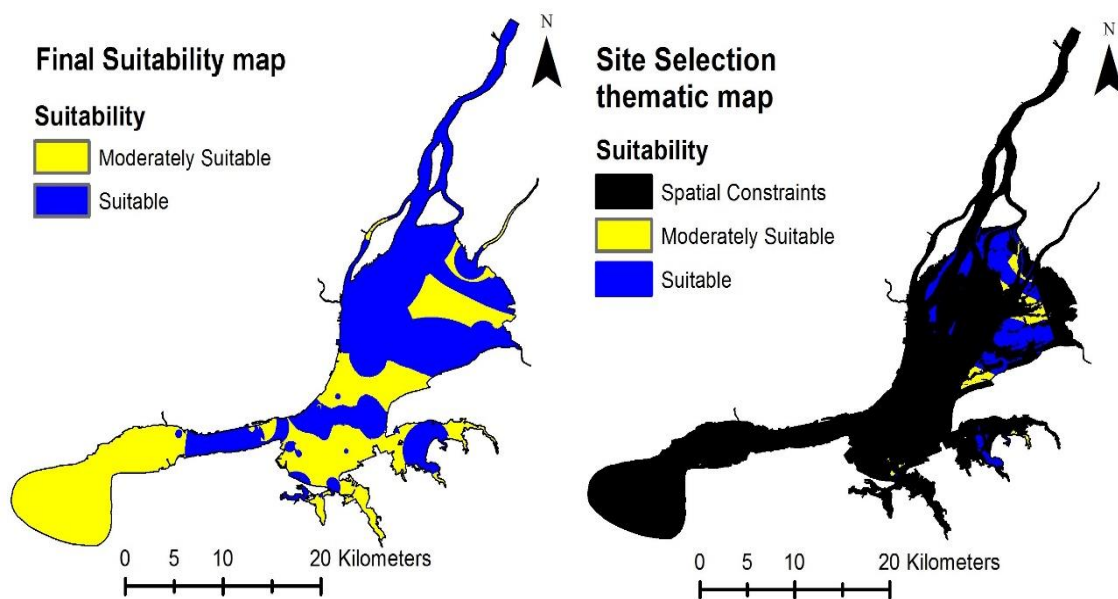


Fig. 40: Final Suitability map (left pane) and Site Selection thematic map (right pane)

The next step was creating the final site selection thematic map, by using the Suitability equation, Eq. (6), combining both Spatial Constraints and the final Suitability maps. Another way to describe and do this is by merely applying the Spatial Constraint map as a mask to the final Suitability map. Table 30 shows the respective areas to each suitability level.

Table 30: Obtained areas for each suitability level for both the Final Suitability and Site Selection maps; n/a – not available

| Areas (km ²) | | |
|--------------------------|-----------------------|--------------------|
| Suitability | Final Suitability map | Site Selection Map |
| Spatial Constraints | n/a | 313.39 |
| 2 | 161.83 | 6.56 (-95%) |
| 3 | 185.40 | 26.82 (-85%) |

Suitable sites are displayed in Fig. 41: by combining each category (suitability values between 1 and 4), a final Suitability map consisting of only two different suitability values was obtained. Areas classified as Moderately Suitable occupy 46.6 % of the total area, with Suitable areas the remaining 54.4%. A possibly constraint to the final results can be the Product Contamination suitability map, where 39.7% of the total area was classified as Unsuitable, meaning that there are areas being negatively influenced by heavy metal pollution.

By combining the Spatial Constraints thematic map and the final suitability map, Moderately Suitable areas were down to 6.56 km² (1.8% of the total area) and Suitable to 26.82 km² (7.8% of the total area).

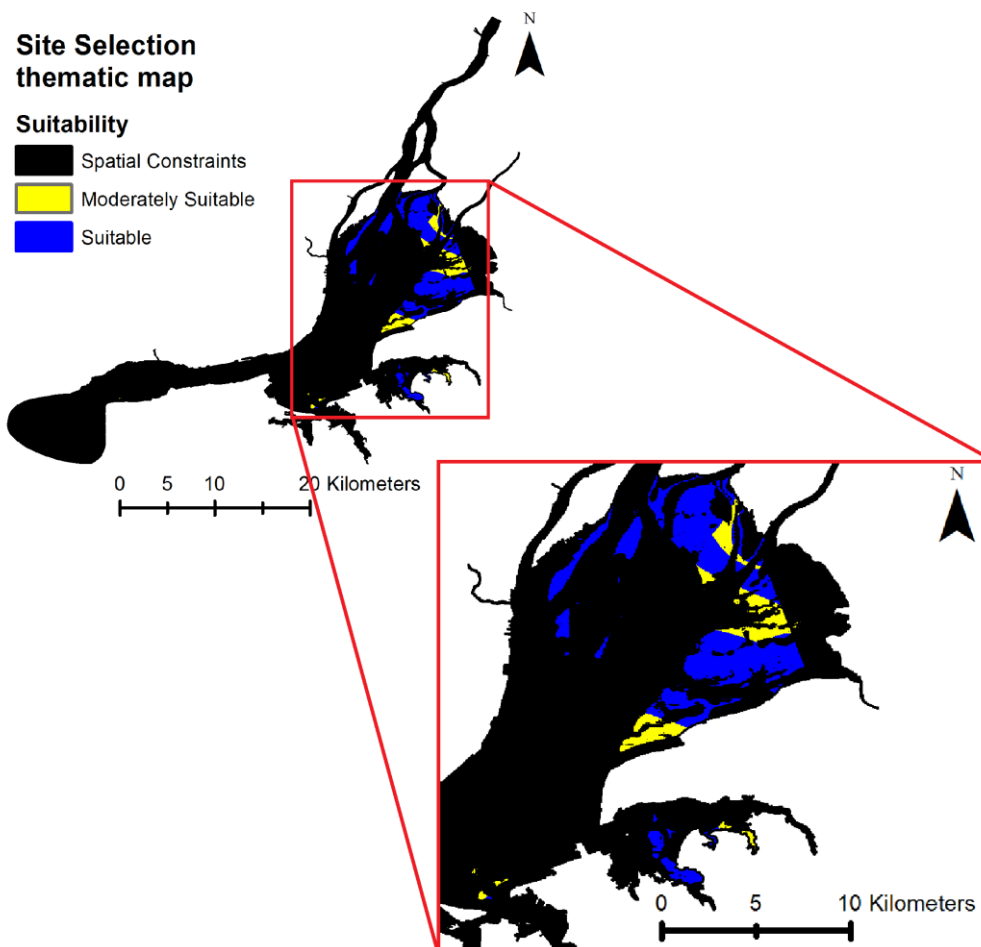


Fig. 41: Suitable areas according to the site selection thematic map

By superimposing the Spatial Constraints thematic map, Moderately Suitable and Suitable areas were reduced by 96% and 86 %, respectively. This further validates that estuaries have conflicting uses, such as fisheries, shipping, recreation, contaminated areas and urban growth (e.g. deFur and Rader, 1995).

In contemplation of what was highlighted in each of the final suitability maps:

- The Tagus Estuary gathers optimal environmental conditions for the growth of the Manila clam, which is supported by studies that have reported its colonization (e.g. Garaulet, 2011)
- Several areas, namely Barreiro, Seixal, Póvoa de St^a Iria were confirmed to be contaminated by heavy metals (corroborated by e.g. Vale et al., 2008)
- The Tagus Estuary has enough infrastructures and labour to support the legalization and further development of shellfish aquaculture;

The Tagus Estuary is in need of a proper management plan that regulates legal fishing and necessary treatment to avoid consumption of contaminated shellfish and its enforcement. This in turn would minimize or even stop the illegal transportation to Spain. Ramajal et al. (2016), in what could be called the first study concerning the trade chain of Manila clam in the Tagus Estuary, addressed all that was previously talked, quantifying total harvested clams in between 4 and 17 thousand t year⁻¹, with an associated economic value of 10 to 23 million € year⁻¹ (with 1.6 and 3.22 million € from hand harvesting). 90% of the total captures were illegally transported to Spain, accounting for approximately 9 thousand t year⁻¹ (Ramajal et al., 2016).

The next step will be based upon the defined areas for the application of FARM model. Those areas were selected as the best suitable for shellfish aquaculture, according to those obtained in the final site selection thematic map.

4.3. Stage 4

A site selection thematic map was created and displayed in Stage 3, where the whole estuary scored suitability values of 2 and 3, Moderately Suitable and Suitable, respectively. In the scope of this work, FARM will only be applied and studied in “Suitable” areas, because those gather the best conditions for individual farm siting, as the overall suitability value is the highest. The chosen individual farms will be hereby called as “concept farms”.

This way, two concept farms were sited in two different suitable locations, A and B (Fig. 42): farm A’s location was selected since it is in the vicinities of shipping routes, albeit far enough to not disturb nor be disturbed by ships (APL, n/d); farm B was sited in a location defined as having large clam populations (e.g. Garaulet, 2011). Each concept farm is within the areas influenced by B2K stations that will be used as environmental drivers for the FARM application: farm A is influenced by B2K station #2.0, whereas farm B by station #3.0. Concept farms are located in intertidal zones, where bottom culture with FARM’s standard stocking density of 100 ind m⁻² was modeled for a culture period of 395 days. It should be highlighted that both concept farms are within Natura 2000 areas.

A higher stocking density as input would increase the total yield of clams within the same culture period. Garaulet (2011) has reported a maximum population density concerning Manila clam of 1500 ind m⁻². This way, it was chosen to use an underestimated comparative density value of 300 ind⁻² for the same drivers, to compare its influence concerning FARM’s outputs, focusing on harvest profit.

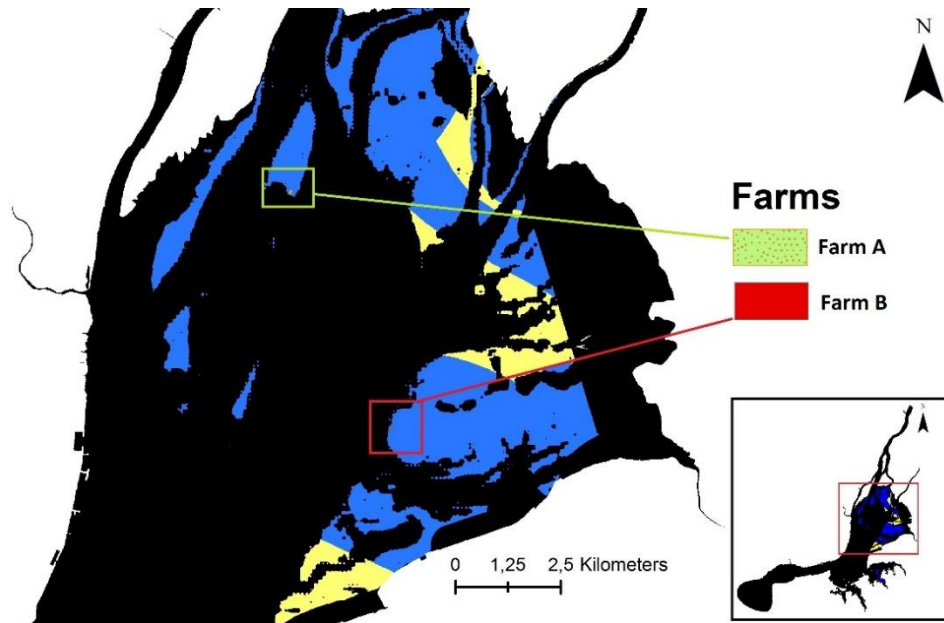


Fig. 42: Location of ‘concept’ farms

FARM’s environmental drivers (temperature, salinity, Chl, POM, TPM, DO, DIN) were used to model individual Manila clam growth in a 395 days’ culture period. Monthly averages were calculated for each driver and used to evaluate potential production, economic performance and environmental externalities. Since B2K values had also information concerning the type of tide when sampling occurred, it was decided to also calculate their monthly medians and see how FARM’s economic outputs are influenced by spring and neap tides. All main environmental driver’s value ranges are represented in Table 31, with the full used data displayed in Appendix D.

Table 31: Main environmental drivers for each concept farm, with the respective minimum and maximum values

| Environmental Drivers | Concept farm A | | | Concept farm B | | |
|------------------------------|----------------|-------------|--------------|----------------|-------------|--------------|
| | Neap | Spring | Farm A total | Neap | Spring | Farm B total |
| Temperature (°C) | 11.0 – 24.2 | 12.5 – 23.7 | 12.2 – 24.0 | 12.0 – 22.2 | 12.0 – 20.7 | 12.0 – 21.5 |
| Chl ($\mu\text{g L}^{-1}$) | 4.2 – 28.2 | 0.9 – 32.3 | 2.5 – 28.4 | 3.2 – 27.4 | 0.9 – 15.8 | 1.9 – 17.1 |
| Salinity (psu) | 0.5 – 20.8 | 2.1 – 7.8 | 6.5 – 26.8 | 18.5 – 31.3 | 12.8 – 33.1 | 16.6 – 32.0 |
| POM (mg L^{-1}) | 2.4 – 17.4 | 1.2 – 26.4 | 2.6 – 8.8 | 5.8 – 27.4 | 2.9 – 10.9 | 2.9 – 14.6 |
| TPM (mg L^{-1}) | 34.0 – 212.2 | 10.0 – 82.8 | 19.4 – 86.7 | 59.5 – 262.7 | 10.4 – 81.2 | 21.4 – 149.3 |
| DO (mg L^{-1}) | 6.1 – 10.2 | 5.9 – 9.1 | 6.0 – 9.3 | 6.3 – 8.3 | 6.3 – 8.1 | 6.4 – 8.1 |

Each concept farm and respective tides were modelled according to the data displayed in Table 31, in order to obtain and further compare the production of both farms, with nitrogen removal being also an output FARM provides: nitrogen removal was assessed, even though the main focus of FARM’s application in the scope of this work is to conclude whether shellfish production in the Tagus Estuary is economical viable. In addition, there is no market in the Tagus Estuary for nutrient trading (as of March 2017), and no farmer will cultivate shellfish only for nutrient removal as it is not cost-effective, unless subsidized (see tables 32 and 33 for comparison between nutrient treatment and harvest profit).

In the scope of the Millenium Ecosystem Assessment, and according to Brumbaugh and Toropova (2008), shellfish can provide different types of services: Provisioning (e.g., aquaculture), Cultural (tourism and recreation), Supporting (cycling of nutrients) and Regulating (water quality maintenance by reducing eutrophication symptoms), of which the latter is of special relevance in the environmental evaluation carried by FARM.

By removing nitrogen from the waterbodies, shellfish aquaculture may provide a similar service such as WWTPs and reduce eutrophication symptoms (e.g., Petersen et al., 2008; see Chapter 2.3 of the present work), thus improving water quality in estuaries. In short, FARM assesses the mass balance of carbon and nitrogen that measures the difference between inputs, such as phytoplankton and detritus, measured in carbon units, kg C (Rose et al., 2015), further converted to nitrogen based on the Redfield ratio (Ferreira et al., 2007); and outputs, namely excretion and faeces that are deposited to the seabed, transferring the organic nitrogen present in the inputs to the sediments (benthic-pelagic coupling) (Dame et al., 1989; Rice, 2008). The difference is negative as shellfish remove nitrogen and can be further converted into Population equivalent (PEQ).

After PEQ conversion, the potential economic return is then evaluated, according to each farm's nitrogen removal by taking in consideration nitrogen credits (Ferreira and Bricker, 2015), within the scope of EPA's water quality trading (EPA, 2013)/nutrient trading: one source (CBF, n/d) that has achieved nutrient removal value higher than required to generate "credits" that can be traded to other sources that cannot as easily, or cost-effectively, to reduce its nutrient loadings. A credit is defined as a unit of pollutant reduction (e.g. nitrogen) (EPA, 2017). More information about nutrient trading can be consulted in e.g., Newell and Mann, 2012; Newell, 2004; Reitsma et al., 2017; Ferreira and Bricker, 2015; Rose et al., 2014.

The shellfish production associated to the concept farms within suitable areas defined in the GIS site selection is the focus of FARM's application, evaluated by TPP, APP, expenditures and profit. Profit is calculated by accounting for TPP and sale price per kg (P_i), as well as total quantity of seeds used and its cost per kg (P_o). Used values for both P_i and P_o were FARM's default ones (4.6 € and 0.9 €, respectively), as they tend to fluctuate (e.g. Jones et al., 1993).

Considering that farms only account for a small area (< 5000 m²), in order to upscale production to larger areas, it should be noted that (in Rose et al., 2015): i) FARM model is only intended for local simulations and does not account for potential interactions among neighboring farms; ii) a large number of farms within a small waterbody, or if a farm is sited upstream of the modeled farms, may result in depletion of local food sources. The same authors also have emphasized that multi-farm interactions must be addressed by system-scale models, as FARM uses in these cases may result in an overestimation of actual production; direct comparison of nutrient removal among species and between the same species in different locations must be done with cautious, as nitrogen removal depends on a combination of environmental characteristics of the site, physiological characteristics of the species studied, and cultivation practice employed (Rose et al., 2015).

The following subtopics will address individually each concept farm and respective outputs, and afterwards a comparison will be made.

4.3.1. Concept farm A

Despite being in an area identified as suitable by the GIS site selection thematic map, concept farm A model outputs for a standard density were not favorable for shellfish aquaculture (Table 32), as after the end of the 395-day cycle, no clams were harvestable (TPP = 0) and harvest profits were negative, as seed cost is approximately 0.9 € kg⁻¹. This might be attributed to both temperature and salinities from January to March (see Appendix D), which were below 14 °C and 8 psu, respectively. Concerning neap tide, FARM did not register any change on total harvest (kg) throughout the cultured cycle (no clams grew); spring tide gathered the best economic

output, albeit still far from being economically viable, with a TPP of 20 kg worth of harvested clams after the cultured cycle.

Nutrient removal was not considered for neap tide, as no clams were registered to have grown; nutrient treatment in spring tide was positive, amounting 900 €, whereas the total values for concept farm A totaled 650 €, with total incomes of 740 and 390 €, respectively.

Not only higher stocking densities did not improve harvest profits, but also, they increase the expenditure in seeds, as more seeds were needed, aggravating the economic deficit in all three contexts. Again, neap tide values were the lowest, with farm also not registering any clam growing, although since there is an associated nutrient treatment, it is possible to conclude that clams did in fact grow, not reaching a total harvest of 1 kg.

Concerning nutrient removal, both spring tide and farm A accumulated higher income associated to nutrient treatment, due to a peak of the total harvest in March (60 and 16 kg, respectively), with a marginally better total income.

Table 32: FARM outputs for concept farm A, concerning a standard stocking density of 100 ind m⁻² and 300 ind m⁻²

| Studied outputs | Standard density of 100 ind m ⁻² | | | Stocking density of 300 ind m ⁻² | | |
|-------------------------------------|---|--------|--------------|---|--------|--------------|
| | Neap | Spring | Farm A total | Neap | Spring | Farm A total |
| Seed (t TFW) | 0.22 | 0.28 | 0.28 | 0.67 | 0.84 | 0.84 |
| TPP (t TFW) | 0.00 | 0.02 | 0.00* | 0.00 | 0.06 | 0.00 |
| APP | 0.00 | 0.08 | 0.00* | 0.00 | 0.07 | 0.00 |
| Harvest profit (k €) | -0.20 | -0.16 | -0.26 | -0.62 | -0.54 | -0.79 |
| PEQ year ⁻¹ | 1 | 21 | 18 | 2 | 63 | 54 |
| Nutrient treatment (k €) | 0.00 | 0.9 | 0.65 | 0.09 | 2.33 | 2.05 |
| Total income (k € y ⁻¹) | -0.20 | 0.74 | 0.39 | -0.53 | 1.79 | 1.26 |

*Values close to 0.00

4.3.2. Concept farm B

Total values concerning concept farm B are represented in table 33. For a standard stocking density of 100 ind m⁻², farm B obtained a TPP of 7.1 t TFW and an APP of 22.5 after the cultivation period, with a harvest profit of 32.75 thousand euros, for only 4500 m². Neap tide has achieved the highest TPP (7.30 t TFW) and an APP of 22.3, with a harvest profit of 33.7 thousand €, whereas spring tide obtained a TPP of 2.43 t TFW, an APP of 7.76 and a harvest profit of 11.2 thousand €. The APP roughly reflects the output generated by the seed inputs, e.g. 0.32 t TFW of seeds have generated 7.3 t of total harvested product.

PEQ and therefore, nutrient treatment, were proportional to the amount of harvested product, with neap and spring tides totaling 4.3 and 2.2 thousand €, respectively, and by association, total incomes of 38 and 13.4 thousand €. Total values for farm B achieved a nutrient treatment service worth of 3.25 thousand €, with a total income of 36 thousand €.

For a stocking density of 300 ind m⁻², all the outputs were roughly 3 times of those regarding standard density.

The main reason cited for the difference between the economic outputs for neap and spring tides is due to the difference of the low tides: by definition, spring tides low tides levels are a little lower, whereas neap tides are a little higher than the average values (NOAA, 2017); in turn, this affects the amount of time shellfish are exposed to external conditions in the intertidal areas (e.g., temperature, predators, DO levels).

This way, shellfish are less exposed during neap tides (higher low tide levels), and thus, can maintain their usual behaviors and burrowing depths in the sediments (i.e., higher energetic efficiency of feeding, metabolic rate – see Table 11) for longer when compared to spring tides. During spring tides, clams put more effort in survival than in growth, as they are more exposed to predation, have less access to food and must move to deeper sediments to avoid direct exposure to air. In short, an assessment of the different levels of the low tides throughout the year, within an aquaculture site selection context, is very important to understand both the survival and growth of a specific clam population.

In addition, it could also be hypothesized that the output difference could have been influenced by the i) difference in salinity, as both maximum and minimum were registered in spring tides (32), which is supported by Neves (2010), and might increase stress conditions for clams development; ii) food sources were higher in neap tides, meaning there is more available food for clams to feed on: this is further corroborated by Vaz et al. (2011), where it was concluded that within the estuary, Chl concentrations decreased during spring tides, while during neaps the concentration increased; even though TPM values do not add up to the same authors conclusions, as it is higher in neap tides.

Table 33: FARM outputs for concept farm B, concerning a standard stocking density of 100 ind m⁻² and 300 ind m⁻²

| Studied outputs | Standard density of 100 ind m ⁻² | | | Stocking density of 300 ind m ⁻² | | |
|-------------------------------------|---|--------|--------------|---|--------|--------------|
| | Neap | Spring | Farm B total | Neap | Spring | Farm B total |
| Seed (t TFW) | 0.32 | 0.32 | 0.32 | 0.95 | 0.95 | 0.95 |
| TPP (t TFW) | 7.30 | 2.43 | 7.10 | 22.0 | 7.15 | 21.25 |
| APP | 22.3 | 7.76 | 22.5 | 23.3 | 7.57 | 22.50 |
| Harvest profit (k €) | 33.7 | 11.2 | 32.76 | 101.7 | 33.0 | 98.2 |
| PEQ year ⁻¹ | 107 | 55 | 86 | 322 | 164 | 258 |
| Nutrient treatment (k €) | 4.3 | 2.2 | 3.25 | 12.9 | 6.6 | 10.3 |
| Total income (k € y ⁻¹) | 38.0 | 13.4 | 36.01 | 114.6 | 39.6 | 108.5 |

4.3.3. Discussion

According to FARM analysis, both concept farms have achieved different levels of economic relevance, despite both being within suitable areas as defined by the GIS site selection. Whereas harvest profit was restricted by low salinity levels in farm A, producing a financial loss, farm B has proven that shellfish aquaculture may be economically viable in a farm-scale level.

FARM model should not be applied in a multi-farm context, hence the need for more complex ecological models to estimate accurately the harvest production for the Manila clam. In the methodology, it was proposed the application of a simple equation to project possible harvest profit to a larger area than farm-scale (upscale production).

Upscale production will only be done assuming harvest profit values for concept farm B. Taking into consideration its location, it was decided to calculate an estimate of gross economic value for all the suitable areas in its vicinities, as defined in the GIS site selection, with a total area of approximately 11 km² (Fig. 43).

Nutrient treatment economic output was not considered in the upscale production, as there is no existing nutrient credit trading market (such as in Maryland or Virginia, USA) and fisheries are still illegal. Despite this, its viability could be further evaluated in the case of the development of shellfish aquaculture in the estuary and the possibility of the creation of a management plan that includes all relevant stakeholders within the vicinities of the estuary. This

would allow the creation of proper settings for the implements for a local nutrient credits market.

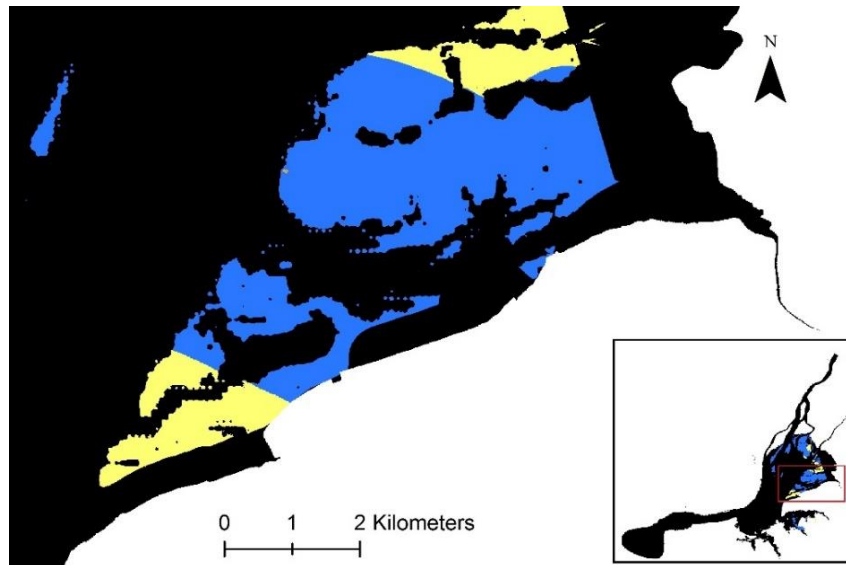


Fig. 43: Upscaled production area (in blue)

By applying the equation for the upscaled production's area, it was calculated an estimated gross economic value of approximately 0.4 million € for FARM's standard stocking density, and 1.2 million € for a stocking density of 300 ind m⁻². TPP in both cases would be close to 86 and 260 t TFW, respectively.

The goal of GEV's calculation was to assess whether shellfish aquaculture would be economically viable in a larger scale than farm-scale. It should be added emphasis to the fact that this value is merely indicative and probably an overestimation for the real values for the studied stocking densities. Higher stocking densities should be found in other areas, representing even a higher economic value, although in this case, ecological carrying capacity should be given the appropriate consideration, to avoid possible ecological impacts associated with higher density of infaunal bivalves.

In short, gross economic values for 42% of suitable defined by GIS site selection were estimated to be 0.4 and 1.2 million €, for a standard stocking density of 100 and 300 ind m⁻², respectively. Even though the remaining suitable areas must be evaluated, in order to do a more reliable comparison, according to the values reported in Ramajal et al. (2016), it is possible to compare the gross economic values to those concerning hand harvesting for the whole estuary, whose production is estimated to be around 836 to 1,673 t (Ramajal et al., 2016). Assuming the same price per kg (4.6 €), the associated economic value is around 3.3 to 7.7 million € year⁻¹; in addition, Ramajal et al. (2016) have estimated the hand harvesting production in all the areas where it does occur, while in the present work only the suitable areas are being considered. This being said, the gross economic values calculated are within the acceptable threshold for those highlighted in Ramajal et al. (2016).

More trustworthy values should be obtained by using more complex and reliable ecological models, even though the calculated gross economic values for 42% of the suitable areas defined in the GIS selection (Fig. 40) show a considerable potential for the implementation of shellfish aquaculture in the Tagus Estuary.

5. Conclusions

This work is based on the trustworthiness of the GIS sources used. Only the latest files concerning each source were used, although it was not possible for everything (e.g. 2007 Portugal's Land Use Map). Hence, some areas defined as existing and consequently accounted for the present study, might not exist in the present, where industries, piers and local markets should be highlighted.

Industries, being identified as a major constraint in the estuary and in this study, can have a significant impact if i.e. inactive industries as of yet (2017) were accounted for. This might be especially relevant in the Barreiro and Póvoa de St^a Iria areas. Piers were also considered key infrastructures for transportation of fishermen to more inaccessible areas and harvested products. It was not found any way to confirm and cross-reference the defined piers in the used OSM file, so the possibility of fishermen to be unable to use some piers exists; also, some piers might not exist. Current speed analysis was based only on four stations, so their interpolation was not as good as it could if i.e. 9 stations would be available. And since current speed is a key factor for the definition of aquaculture practices, more data would mean more reliable results. This was seen in FARM application, since the used current speeds were above the higher threshold of the optimal reclassification value, even though they are periodically and its median would probably be lower than 1 m s^{-1} .

Although a very important matter in urban aquaculture, the establishment of safety distances from industries and WWTP seem to be lacking on peer reviewed studies, which might be attributed to i) polluted areas are disregarded by common sense, due to health and hygiene problems (mostly on developed countries) or ii) lack of space and spatial management (i.e., China), as well as contaminated water by sewage and industrial waste, which then might have a synergy effect with the existing aquaculture farms' wastewaters (Barboza, 2007; Mao, 2016). Future aquaculture projects in the estuary are recommended to avoid siting in uncertain areas (near pollution sources) or by assessing each area's sanitary and health risks by developing a sanitary program to the estuary.

The data used for Environmental and Product Contamination (besides tPAH values) was collected in the 1980s and nowadays some variables might be significantly different, namely heavy metal concentrations, albeit this is not a major limitation in the present work. In case this work is used for any project that aims to develop Tagus Estuary aquaculture, it is recommended to use more recent data and if possible collect new samples.

Product Contamination lacks faecal coliform data, which would have proved very useful in screening out more contaminated areas. B2K database had data concerning faecal coliforms, although it was not used since values were considered to have changed meanwhile. This can be minimized with the application of relaying principles or even depuration units. The use of TEL-PEL reclassification method was also highlighted as not optimal, in consideration of the used range for reclassifying heavy metal concentrations as Moderately Suitable, which was as higher than it should: in some cases, lower thresholds of the TEL-PEL range could not have any visible or relevant effect on shellfish, while in others it could mean that shellfish are contaminated. This may change according to the studied species. Therefore, it is recommended further studies and experts participation.

Product Contamination suitability map has also confirmed what several studies already concluded: that the Tagus Estuary is contaminated by heavy metals, which influenced the overall suitability of the final suitability map.

Production Category was considered as the most important, because i) in the last years, Manila clam has been expanding and growing within the Tagus Estuary, being in the middle of illegal circuit, either the harvesting itself and the selling to restaurants or the transportation to Spain,

where it is accounted as a Spanish product; ii) legal requirements have been increasingly more demanding to restrict environmental pollution in marine habitats and iii) needed economic inputs (i.e. construction of depuration centers or new piers) were tried to be minimized, taking advantage of what currently is available. The worst areas irrespective of production were identified as being the same ones that scored the highest suitability values for the Environmental Category. This might be attributed to that area being in the vicinities of the Pancas saltmarsh, on which anthropogenic impact should be minimal.

The existing environmental conditions can and are supporting the establishment of Manila clam populations, even though there are two aspects that need proper attention: i) Manila clam survival thresholds must be respected always, to avoid situations such as concept farm A; and ii) the existing competition between the exotic and native clam species, and the declining of the latter, can lead to its extinction and should be properly managed (see 5.1 for recommendations).

The created site selection thematic map has merely mirrored what the author have considered as the most important aspects to assess where aquaculture practices might be located. Different experts and/or different teams, with different goals would have reached a totally different map. FARM application has exacerbated the need of including experts of different areas, such as heavy metal contaminations, biological conditions for shellfish growth and aquaculture (e.g., harvest techniques and hatchery settings), as well as that GIS tools, albeit useful for aquaculture siting and decision-making, should always be complemented by ecological models: in this context, despite being located in an area identified as suitable, concept farm A did not gather enough conditions for shellfish aquaculture. On the opposite, farm B has shown that extensive aquaculture in only a small intertidal area is able to yield a significant profit, with the associated environmental benefits.

The application of FARM model, although not considered a limitation, should be only seen as indicative of what benefits and impacts may lie in the application of shellfish aquaculture within the estuary. If further developments are seen in Portuguese aquaculture, more specifically in the Tagus estuary, more efficient and complex models must be applied, in order to properly evaluate the environmental and socioeconomic effects (e.g. EWN, Nunes et al., 2011; Ferreira et al., 2012) and hydrodynamic variables (e.g. MOHID, MARETEC, 2002; Leitão et al., 2003).

In conclusion, and considering the questions established in Chapter 1, Introduction:

- The major spatial constraints for shellfish aquaculture identified in the Tagus Estuary are bathymetry, distance to industry and distance to beach. As already pinpointed, current speed would also be considered a major spatial constraint, but the used data restricted it.
- In general, the estuary gathered suitable environmental conditions for shellfish aquaculture to prosper, which was already expected since clams are still expanding and growing in the estuary.
- The vicinities of urban areas scored the highest suitability values for production (visual impact must be assessed), with the suitability values being progressively lower as urban areas were getting more distant.
- The areas identified as being more contaminated where the same ones already emphasized by several studies: Barreiro, Lisbon and Póvoa de St^a Iria.
- Most areas identified in the site selection map as suitable for aquaculture need an impact assessment, since they are within Natura 2000 areas.

If properly managed, shellfish aquaculture in the estuary would provide several ecosystem services that would be beneficial to both local and national economy: by developing the existing local fishing communities, through the creation of a service mark for the Tagus Estuary, as

previously done with the Portuguese oyster (*Cultural*) and the improvement or even the restatement of the trade chain, aquaculture production levels would increase, thereby reducing the difference of Portugal's balance between fisheries and aquaculture (*Provisioning*). In addition, the positive environmental effects brought by levelled populations would reduce the amount of the limiting nutrient in phytoplankton production, nitrogen (*Supporting*), and thus, the reduction of eutrophication symptoms, as well as of the erosion of the ecologically relevant salt marshes (*Regulatory*).

Therefore, it is possible to conclude that shellfish aquaculture is viable in the Tagus Estuary, showing good prospects concerning both environmental and production conditions.

5.1. Recommendations

In order to create and enforce an eventual management plan concerning shellfish aquaculture in the Tagus Estuary, the author gives the following recommendations:

- Information about stakeholders should be gathered, such as the existing fishermen's associations, middlemen that enable the transportation of the harvested product to retailers and/or markets, and possibly companies interested in the development of shellfish aquaculture in the estuary.
- It is recommended that national entities that encompass aquaculture practices (i.e. IPMA, APL, DGRM, APA, ICNF) join efforts to overcome the illegal fishing, as it is an interdisciplinary problem: hygiene and sanitary, heavy metal contamination, ecological and socioeconomic.
- A more thorough GIS-based site selection should be made, by establishing a team with different experts (e.g. GIS, marine pollution and biology) and the use of recent data, as to increase the trustworthiness of shellfish aquaculture siting. It is advised to evaluate only the areas that respect the survival thresholds of the Manila clam, since it would reduce the odds of an inferior siting, even though the suitable areas are expected to be much smaller than in the present work.
- Relaying areas should be defined, according to the latest data concerning faecal coliforms. In case the division according to faecal concentration proves to be feasible, clams located in a lower EU class area would be transported to a cleaner area. If not, depuration units must be created, in order to purify the clams before being sold to the public. Areas contaminated by heavy metals must be avoided, as depuration/relaying are less efficient.
- The creation of small Aquaculture Management Areas (AMA) in the scope of an integrated coastal management initiative could be done in a first stage, in order to fully assess possible difficulties in the trade chain and spatial management, as well as the possible profit that it would have and how communities would react: would illegal fishing still persist?
- An appropriate economic analysis for larger areas should be conducted in the scope of an ecosystem approach to aquaculture, by taking into account the different types of carrying capacity (Inglis et al., 2000; McKindsey et al., 2006). The author further recommends an assessment on the ecological carrying capacity, as this study only addressed modelling at a farm scale level; as well as the regulatory carrying capacity (Ferreira et al., 2013), in order to create a management plan and enforce its compliance.
- We must learn from previous mistakes, of which the overfishing and bad resource management that eventually led to the decline of the Portuguese oyster, are a good example. Hence, the declining of the native clam species must be properly addressed and managed.

Before deciding how to proceed, this new problematic in the estuary must be seen from all the possible perspectives: Should Portuguese authorities treat the invasion and expansion of the Manila clam as an IAS and protect the native clam at all costs? Or should they take advantage of its economic value? The answer seems very ambiguous, and as a serious problem in the estuary, it should not be taken lightly. As such, adding to the lack of data concerning both species' populations in the estuary, more studies should be done in this regard, in order to support as best as possible, the decision-makers. For this, the author gives the following recommendations (Caddy and Defeo, 2003):

- Monitor both species' populations to evaluate their respective numbers and which areas have the highest densities.
- Control of predators, as Manila clams are more exposed to predators, due to the burrow depth difference. This aims to level the difference between both species' populations.
- Creation of a native species hatchery in the vicinities of the estuary that would help to maintain its wild stock.
- Enhancement of the native clams' wild stocks with seeds raised on hatchery or laboratory-based larvae spawning and rearing (genetic implications should be considered).

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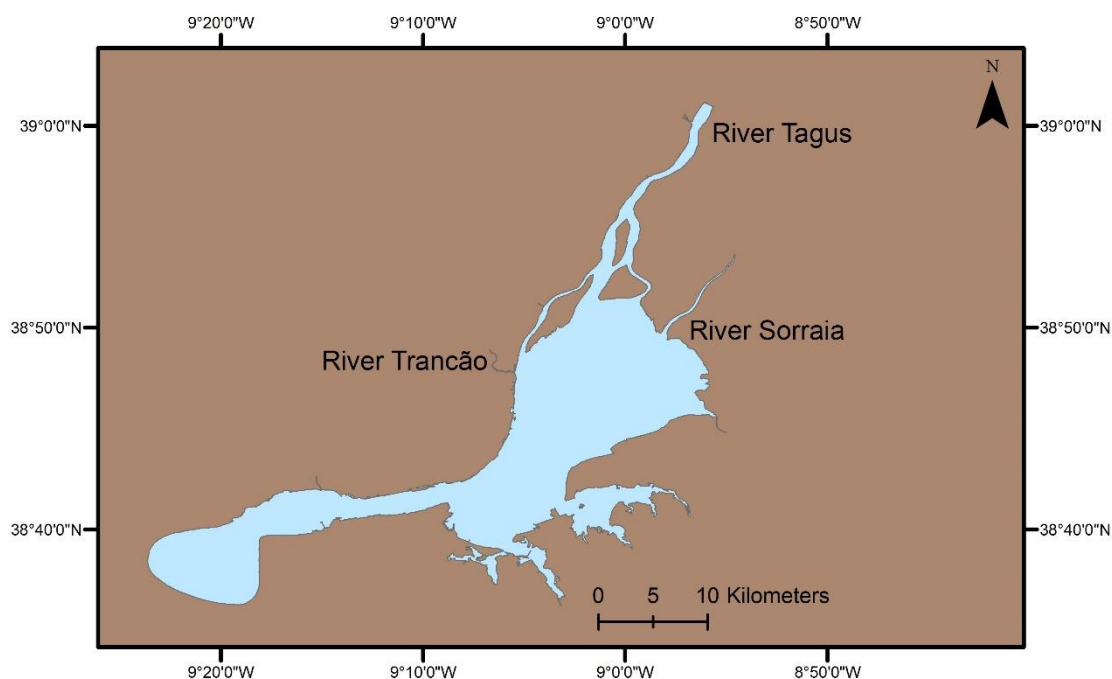
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7. Appendix

7.1. Appendix A

In the present appendix, all used data for the creation of each Category's thematic maps is displayed. The tables follow the same sequence as the one used and mentioned in the methodology: Spatial Constraints, Production, Environmental and Product Contamination.

Used mask for spatial delimitation concerning the Tagus Estuary; all the displayed rivers were shortened, in order to focus the estuarine area



Tide table for 2016, used for bathymetry reclassification it was used the lowest value, 0.82 m

| Month | Low tide average (m) | High tide average (m) |
|---------|----------------------|-----------------------|
| 1 | 0.82 | 3.32 |
| 2 | 0.90 | 3.26 |
| 3 | 0.92 | 3.36 |
| 4 | 0.97 | 3.53 |
| 5 | 0.98 | 3.50 |
| 6 | 0.92 | 3.40 |
| 7 | 0.89 | 3.30 |
| 8 | 0.91 | 3.38 |
| 9 | 0.91 | 3.56 |
| 10 | 1.01 | 2.78 |
| 11 | 1.00 | 3.53 |
| 12 | 0.92 | 3.42 |
| Average | 0.93 | 3.36 |

Used discharge points and respective coordinates. Taken from Saraiva (2001) and CMLisboa (2014) (Spatial Constraints – Distance to WWTP)

| Longitude | Latitude | WWTP discharge | Source |
|--------------------|-----------|---------------------|--------|
| -9.220243 | 38.675622 | Portinho da Costa | 1 |
| -9.176632 | 38.697242 | ETAR Alcantara | 1, 2 |
| -9.145651 | 38.675184 | ETAR Mutela | 1 |
| -9.135535 | 38.643083 | Quinta da Bomba | 1 |
| -8.973538 | 38.74947 | Etar Alcochete | 1 |
| -8.995117 | 38.698216 | ETAR Seixalinho | 1 |
| -9.091316 | 38.796319 | Trancão | 1 |
| -9.103106 | 38.731134 | Etar Chelas | 2 |
| -9.02352 | 38.883219 | Etar Alverca | 2 |
| -9.047706 | 38.679348 | Etar Barreiro | 1 |
| -9.053377 | 38.859779 | Etar Vila Franca | 1 |
| [1] Saraiva (2001) | | [2] CMLisboa (2014) | |

Obtained data for current speed (m s⁻¹), average was used and median was done to see if it would fit in the reclassification values. From Barcawin2000 (Spatial Constraints – Current Speed)

| Station | Number of samples | Average (m s ⁻¹) | Median (m s ⁻¹) |
|---------|-------------------|------------------------------|-----------------------------|
| 3.0 | 144 | 0.417 | 0.35 |
| 4.0 | 110 | 0.426 | 0.35 |
| 4.7 | 120 | 0.337 | 0.24 |
| 5.0 | 67 | 0.396 | 0.33 |

Local Markets in the Tagus Estuary vicinities (Production indicator – Distance to Local Markets)

| Longitude | Latitude | Description | Source |
|-----------|-------------|---|-------------------------------------|
| -9.078125 | 38.66126667 | Mercado 1º Maio | |
| -9.179494 | 38.673702 | Mercado Municipal Abastecedor de Almada | |
| -9.310194 | 38.691445 | Mercado Municipal de Oeiras | |
| -9.178852 | 38.704177 | Mercado Municipal Rosa Agulhas | |
| -9.10056 | 38.778463 | Mercado Municipal de Moscavide | |
| -8.976203 | 38.706214 | Mercado Municipal do Montijo | |
| -9.144448 | 38.729145 | Mercado 31 de Janeiro | |
| -9.15747 | 38.653831 | Mercado Municipal do Feijó | |
| -8.990272 | 38.952542 | Mercado Municipal Vila Franca Xira | Searched using Google Earth Pro [1] |
| -9.232536 | 38.644238 | Mercado Municipal Costa Caparica | |
| -9.218545 | 38.744081 | Mercado Municipal da Damaia | |
| -9.205943 | 38.75064 | Mercado Municipal de Benfica | |
| -9.110437 | 38.868676 | MARL | |
| -9.158228 | 38.743304 | Mercado Bairro de Santos | |
| -9.1732 | 38.75378333 | Mercado Bairro S. João | |
| -9.128962 | 38.721721 | Mercado de Sapadores | |
| -9.113448 | 38.77193 | Mercado Encarnação Sul | |

(cont.) Local markets

| Longitude | Latitude | Description |
|-----------|-----------|------------------------------------|
| -9.194838 | 38.703656 | Mercado da Ajuda |
| -9.148174 | 38.792518 | Mercado das Galinheiras |
| -9.13115 | 38.722223 | Mercado do Forno do Tijolo |
| -9.11859 | 38.777105 | Mercado Encarnação Norte |
| -9.004142 | 38.719349 | Mercado Samouco |
| -8.9611 | 38.75641 | Mercado Alcochete |
| -8.990022 | 38.650548 | Mercado Municipal da Moita |
| -9.119289 | 38.764699 | Mercado Olivais B. |
| -9.100193 | 38.642121 | Mercado Municipal Seixal |
| -9.149785 | 38.653225 | Mercado Municipal Laranjeiro |
| -9.091757 | 38.608808 | Mercado Municipal Casal do marco |
| -9.057463 | 38.643526 | Mercado Municipal Sto Andre |
| -9.424287 | 38.704581 | Mercado Municipal Cascais |
| -9.010261 | 39.047224 | Mercado Municipal Alenquer |
| -9.23393 | 38.661751 | Mercado Municipal Trafaria |
| -9.106708 | 38.612005 | Mercado Municipal Torre da Marinha |
| -9.180948 | 38.648172 | Mercado Municipal Sobreda |
| -8.91302 | 38.626995 | Mercado Municipal Pinhal Novo |
| -9.332757 | 38.793319 | Mercado Municipal #2 |
| -9.221636 | 38.807596 | Mercado Municipal #3 |
| -9.159176 | 38.626162 | Mercado Levante Corroios |
| -9.114328 | 38.727811 | Mercado de Xabregas |
| -9.194867 | 38.751084 | Mercado de São Domingos Benfica |
| -9.343584 | 38.774879 | Mercado São Carlos |
| -9.12546 | 38.715582 | Mercado de Santa Clara |
| -9.266251 | 38.715392 | Mercado de Queijas |
| -9.082973 | 38.622889 | Mercado de Paio Pires |
| -9.18297 | 38.780827 | Mercado de Odivelas |
| -9.142845 | 38.649785 | Mercado de Miratejo |
| -9.338422 | 38.796391 | Mercado de Fanares |
| -9.254354 | 38.709443 | Mercado de Carnaxide |
| -9.165124 | 38.709443 | Mercado de Campo de Ourique |
| -9.13266 | 38.735243 | Mercado de Arroios |
| -9.140171 | 38.7555 | Mercado de Alvalade Norte |
| -9.33788 | 38.804242 | Mercado de Algueirão |
| -9.266251 | 38.649957 | Mercado 25 abril 1974 |
| -8.985356 | 39.023413 | Mercado local |
| -9.214177 | 38.724172 | Makro Alfragide |
| -9.39211 | 38.736913 | Makro #1 |
| -8.998205 | 38.571792 | Makro #2 |

OSM [2]

[1] Google Earth Pro

[2] OpenStreetMaps

Environmental indicators based on Barcawin2000 data

| ID | median Chl a ($\mu\text{g L}^{-1}$) | median DO (mg L ⁻¹) | median salinity (psu) | median temperature ($^{\circ}\text{C}$) | median TPM (mg L ⁻¹) |
|-------|---------------------------------------|---------------------------------|-----------------------|---|----------------------------------|
| 2.5 | 9.70 | 7.05 | 21.67 | 18 | 117.1 |
| 2.6 | 9.75 | 7.20 | 21.99 | 17.75 | 77.1 |
| 2.7 | 5.20 | 6.80 | 24.26 | 18.1 | 61 |
| 3.0 | 5.40 | 6.90 | 30.80 | 16.5 | 105.55 |
| 3.7 | 2.65 | 7.30 | 31.59 | 17.3 | 43.765 |
| 3.8 | 3.30 | 7.28 | 30.48 | 17.4 | 39.27 |
| 3.9 | 3.10 | 7.00 | 29.76 | 17.25 | 44.7 |
| 4.0 | 2.40 | 6.69 | 32.54 | 16.3 | 54.1 |
| 4.6 | 2.40 | 6.70 | 32.90 | 17.6 | 75.2 |
| 4.7 | 2.50 | 7.27 | 33.00 | 16.3 | 67.2 |
| 5.0 | 1.80 | 6.77 | 34.10 | 16.5 | 48 |
| 5.2 | 2.00 | 7.20 | 33.57 | 16 | 39 |
| 8.0 | 1.60 | 7.22 | 35.02 | 15.05 | 17 |
| 8.1 | 1.65 | 7.60 | 35.73 | 14.9 | 12.8 |
| ECOT1 | 8.57 | 7.50 | 8 | 20 | 65 |
| ECOT2 | 3.64 | 6.57 | 22 | 19.5 | 55 |
| ECOT3 | 2.34 | 6.70 | 26.5 | 18.75 | 75.5 |
| ECOT4 | 2.37 | 7.00 | 28 | 18 | 68.5 |
| R4 | 1.62 | 7.46 | 32.812 | 17.6 | 5 |
| R6 | 2.26 | 7.87 | 35.614 | 16.5 | 24 |
| R7 | 4.30 | 6.02 | 18.161 | 18 | 8 |
| T1 | 1.51 | 7.17 | 35.78 | 16.98 | 41.4 |
| T2 | 1.03 | 6.49 | 35.98 | 14.07 | 62.37 |
| T3 | 1.66 | 6.44 | 36 | 14.33 | 23.72 |
| T4 | 14.09 | 6.90 | 32.42 | 20.45 | 49.08 |
| T5 | 6.99 | 7.74 | 28.94 | 20.72 | 37.61 |
| T6 | 8.85 | 7.48 | 27.50 | 21.31 | 28.98 |
| T7 | 8.05 | 7.05 | 20.19 | 21.24 | 18.28 |

POM transformation: POC values were multiplied to TPM (for each station), then converted to mg L⁻¹ by dividing by 10000 and finally POC to POM conversion ratio was used, 0.38

| Stations | Median POC ($\mu\text{g mg}^{-1}$ TPM) | Median POM (mg L ⁻¹) |
|----------|---|----------------------------------|
| 2.5 | 25.20 | 7.77 |
| 2.6 | 28.69 | 5.82 |
| 2.7 | 34.90 | 5.60 |
| 3.0 | 27.00 | 7.50 |
| 3.7 | 37.57 | 4.33 |
| 3.8 | 36.70 | 3.79 |
| 3.9 | 36.00 | 4.24 |
| 4.0 | 39.08 | 5.56 |
| 4.6 | 39.34 | 7.76 |
| 4.7 | 33.37 | 5.90 |
| 5.0 | 40.84 | 5.16 |
| 5.2 | 32.83 | 3.37 |
| 8.0 | 57.35 | 2.57 |
| 8.1 | 89.88 | 3.03 |

| (cont.) POM transformation | | |
|-----------------------------------|--|---------------------------------------|
| Stations | Median POC ($\mu\text{g mg}^{-1}$ TPM) | Median POM (mg L⁻¹) |
| T1 | 1.30 | 0.22 |
| T2 | 4.20 | 0.61 |
| T3 | 19.05 | 3.78 |
| T4 | 14.35 | 2.59 |
| T5 | 25.50 | 0.34 |
| T6 | 30.30 | 1.91 |
| T7 | 41.45 | 0.87 |

Product Contamination based on Vale (1989)

| Longitude | Latitude | Zn ($\mu\text{g g}^{-1}$) | Pb ($\mu\text{g g}^{-1}$) | Cd ($\mu\text{g g}^{-1}$) | Cu ($\mu\text{g g}^{-1}$) | Cr ($\mu\text{g g}^{-1}$) |
|------------------|-----------------|---|---|---|---|---|
| -8.990174 | 38.94156 | 26 | 55 | 0.2 | 11 | 16 |
| -9.001578 | 38.930753 | 169 | 55 | 1.2 | 24 | 40 |
| -8.993514 | 38.919373 | 32 | 35 | 0.4 | 5 | 7 |
| -8.994609 | 38.905623 | 132 | 35 | 2.2 | 26 | 42 |
| -8.997677 | 38.891153 | 120 | 73 | 0.9 | 11 | 12 |
| -8.993508 | 38.878954 | 134 | 55 | 1.3 | 32 | 58 |
| -9.012239 | 38.835441 | 128 | 87 | 1.3 | 39 | 60 |
| -9.036893 | 38.842655 | 125 | 68 | 1.7 | 12 | 22 |
| -9.034629 | 38.82836 | 218 | 32 | 0.2 | 14 | 68 |
| -9.04043 | 38.813842 | 142 | 89 | 2.5 | 39 | 39 |
| -9.04947 | 38.7985 | 130 | 72 | 0.5 | 16 | 24 |
| -9.057502 | 38.783469 | 108 | 90 | 0.6 | 15 | 18 |
| -9.061548 | 38.855404 | 444 | 61 | 2.6 | 28 | 54 |
| -9.060858 | 38.855005 | 424 | 97 | 2.6 | 58 | 66 |
| -9.06395 | 38.853283 | 372 | 135 | 2.7 | 62 | 80 |
| -9.062849 | 38.852267 | 452 | 157 | 2.4 | 52 | 45 |
| -9.065977 | 38.849915 | 350 | 105 | 2.3 | 51 | 88 |
| -9.066905 | 38.844602 | 665 | 113 | 2.9 | 53 | 60 |
| -9.078653 | 38.827499 | 413 | 149 | 2.5 | 66 | 75 |
| -9.084669 | 38.816868 | 361 | 152 | 3.1 | 74 | 77 |
| -9.077935 | 38.807376 | 396 | 144 | 1.5 | 52 | 49 |
| -9.085255 | 38.800429 | 566 | 161 | 1.5 | 55 | 60 |
| -9.079458 | 38.799556 | 322 | 147 | 2.4 | 69 | 81 |
| -9.085604 | 38.794064 | 468 | 65 | 0.8 | 44 | 98 |
| -9.07902 | 38.793458 | 402 | 188 | 2.5 | 91 | 80 |
| -9.086891 | 38.789346 | 367 | 125 | 2.3 | 57 | 90 |
| -9.084792 | 38.783765 | 112 | 120 | 2.4 | 52 | 46 |
| -9.084297 | 38.772119 | 140 | 57 | 1 | 26 | 48 |
| -9.085999 | 38.763069 | 414 | 54 | 1 | 43 | 72 |
| -9.060537 | 38.748226 | 76 | 152 | 1.5 | 55 | 64 |
| -9.074635 | 38.748909 | 258 | 43 | 0.6 | 20 | 48 |
| -9.086007 | 38.741558 | 503 | 50 | 0.7 | 38 | 83 |
| -9.088379 | 38.733051 | 442 | 193 | 1.8 | 54 | 85 |

| (cont.) Product Contamination | | | | | | |
|--------------------------------------|-----------------|--------------------|--------------------|--------------------|--------------------|--------------------|
| Longitude | Latitude | Zn (µg g-1) | Pb (µg g-1) | Cd (µg g-1) | Cu (µg g-1) | Cr (µg g-1) |
| -9.093217 | 38.721879 | 329 | 152 | 2.4 | 64 | 78 |
| -9.090221 | 38.709922 | 338 | 107 | 1.1 | 42 | 72 |
| -9.07174 | 38.708883 | 263 | 159 | 1.2 | 23 | 41 |
| -9.061524 | 38.699414 | 88 | 107 | 1.1 | 36 | 43 |
| -9.055074 | 38.725463 | 503 | 45 | 0.4 | 15 | 37 |
| -9.038181 | 38.728837 | 698 | 46 | 1.4 | 16 | 41 |
| -9.034359 | 38.741474 | 538 | 227 | 1.6 | 57 | 109 |
| -9.018137 | 38.755801 | 564 | 184 | 1.3 | 56 | 92 |
| -8.995754 | 38.778273 | 492 | 154 | 1.6 | 46 | 85 |
| -9.085844 | 38.678709 | 223 | 222 | 2.3 | 47 | 68 |
| -9.029575 | 38.776205 | 318 | 162 | 1.1 | 66 | 67 |
| -8.995872 | 38.765621 | 632 | 49 | 0.5 | 42 | 64 |
| -8.993397 | 38.756869 | 288 | 132 | 0.8 | 30 | 34 |
| -8.964639 | 38.768277 | 265 | 107 | 0.3 | 52 | 66 |
| -9.110824 | 38.712975 | 472 | 105 | 0.3 | 39 | 57 |
| -9.107858 | 38.701165 | 366 | 74 | 1.5 | 29 | 44 |
| -9.120709 | 38.70402 | 366 | 105 | 2 | 20 | 34 |
| -9.104834 | 38.692203 | 262 | 109 | 0.9 | 41 | 53 |
| -9.096038 | 38.687866 | 482 | 178 | 1 | 19 | 28 |
| -9.098421 | 38.67041 | 1307 | 146 | 2.1 | 26 | 30 |
| -9.096806 | 38.664701 | 1037 | 87 | 2.2 | 34 | 36 |
| -9.11674 | 38.680477 | 597 | 184 | 5.4 | 63 | 84 |
| -9.134715 | 38.669293 | 823 | 1834 | 1.8 | 291 | 90 |
| -9.141534 | 38.692332 | 538 | 2478 | 2.2 | 64 | 148 |
| -9.152208 | 38.694675 | 426 | 198 | 1.2 | 64 | 89 |
| -9.167426 | 38.690252 | 120 | 493 | 1.2 | 71 | 65 |
| -9.207058 | 38.685829 | 729 | 46 | 0.2 | 208 | 52 |
| -9.216294 | 38.68957 | 362 | 42 | 1.2 | 41 | 71 |
| -9.239415 | 38.692547 | 542 | 187 | 1.5 | 52 | 53 |
| -9.250438 | 38.696158 | 604 | 91 | 0.5 | 40 | 53 |

tPAH values obtained from Torre (2014) (Product Contamination)

| Longitude | Latitude | TPAH (ppb) |
|------------------|-----------------|-------------------|
| -9.015324 | 38.881139 | 17 |
| -9.084002 | 38.81925 | 70 |
| -9.046083 | 38.816474 | 163 |
| -9.096767 | 38.649984 | 25 |
| -9.104429 | 38.646178 | 900 |
| -9.108627 | 38.644282 | 570 |
| -9.108547 | 38.633891 | 1270 |
| -9.125909 | 38.650337 | 500 |
| -9.133033 | 38.643126 | 415 |

| (cont.) tPAH values | | |
|---------------------|-----------|------------|
| Longitude | Latitude | TPAH (ppb) |
| -8.935635 | 38.763316 | 94 |

Priority PAHs, 16 EPA's PAHs (Serafim et al., 2014)

| |
|------------------------|
| Naphthalene |
| Acenaphthylene |
| Acenaphthene |
| Fluorene |
| Phenanthrene |
| Anthracene |
| (cont.) 16 EPA's PAHs |
| Fluoranthene |
| Pyrene |
| Benz[a]anthracene |
| Chrysene |
| Benzo[b]fluoranthene |
| Benzo[k]fluoranthene |
| Benzo[a]pyrene |
| Dibenz[a,h]anthracene |
| Benzo[ghi]perylene |
| Indeno[1,2,3-cd]pyrene |

7.2. Appendix B

In Part One of the AHP, the general methodology was applied to each category (Production, Environmental and Product Contamination and respective indicators). In the general methodology explanation, it is mentioned only each category. However, in Part One, each category's indicator were used in order to evaluate their weight. The step to step methodology explained here is based on Bhushan and Rai (2004), with only a few minor adaptations. Criterion can be applied to either categories or indicators:

- 1) A hierarchy should be established, consisting of goal, criteria, sub criteria and alternatives: this is a fundamental step to the process of AHP. Hierarchy indicates a relationship between elements of one level with those of the level immediately below. This relationship percolates down to the lowest levels of the hierarchy and in this manner every element is connected to every other one, at least in an indirect manner. A hierarchy is a more orderly form of a network. An inverted tree structure is similar to a hierarchy. Saaty (1980) suggests that a useful way to structure the hierarchy is to work down from the goal as far as one can and then work up from the alternatives until the levels of the two processes are linked in such a way as to make comparisons possible (pairwise comparison).
- 2) After a proper hierarchy is defined, data are collected from experts or decision-makers corresponding to the hierarchic structure, in the pairwise comparison of alternatives on a quantitative scale: if the relative score in a comparison between criterion J and K is 7, that means that J is much more important than K. The comparisons are made for each criterion.

Table of relative scores between pairs (Criterion J and Criterion K)

| Pairwise comparison relative scores | Interpretation |
|-------------------------------------|---|
| 1 | Criterion J and K are equally important |
| 3 | Criterion J is slightly more important than K |
| 5 | Criterion J is more important than K |
| 7 | Criterion J is strongly more important than K |
| 9 | Criterion J is absolutely more important than K |
| 2, 4, 6, 8 | Intermediate values |

- 3) The pairwise comparison of all the criteria generated in step 2 are organized into a square matrix ($m \times m$, where m is the number of criteria). The diagonal elements of the matrix are 1. The criterion in the j th row is better than criterion in the k th column if the value of element (j, k) is more than 1; otherwise the criterion in the k th column is better than that in the j th row. Both (j, k) element of the matrix and the (k, j) element must satisfy the following constraint

$$(j, k) \cdot (k, j) = 1$$

- 4) Priority vector is obtained by adding each column of the square matrix already created; afterwards, each element of the matrix is divided by the sum of its column (normalized relative weight), where the sum of each column is 1. The normalized principal Eigen vector can be obtained by averaging across the rows.
- 5) The consistency of the matrix of order n (number of evaluated criterion) is evaluated. Comparisons made by this method are subjective and the AHP tolerates inconsistency through the amount of redundancy in the approach. If this consistency index fails to reach a required level then answers to comparisons may be re-examined. The consistency index, CI, is calculated as:

$$CI = \frac{\lambda_{\max} - n}{n - 1}$$

where λ_{\max} is the maximum eigenvalue of the judgement matrix. In the next step, CI can be compared with that of a random matrix, RI.

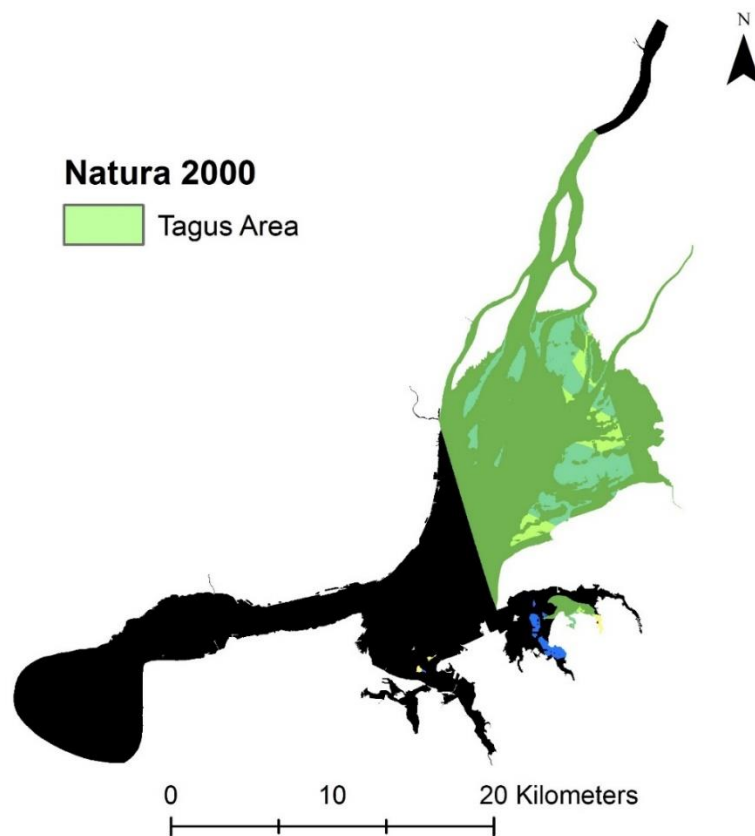
Random Consistency Index (RI)

| n | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|----|---|---|------|-----|------|------|------|------|------|------|
| RI | 0 | 0 | 0.58 | 0.9 | 1.12 | 1.24 | 1.32 | 1.41 | 1.45 | 1.49 |

The ratio between CI and RI is called the Consistency Ratio (CR), which is demonstrated below. Saaty (1980, 2008) suggests that the value of CR should be less than 10% (or 0.01).

$$CR = \frac{CI}{RI}$$

GIS site selection areas within the scope of Natura 2000



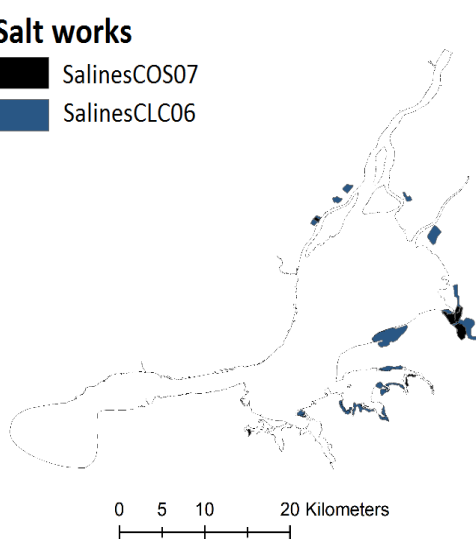
7.3. Appendix C

Appendix C displays the remaining Spatial Constraints thematic maps that were not considered and analyzed in Chapter 4 “Results and Discussion”.

Salt works

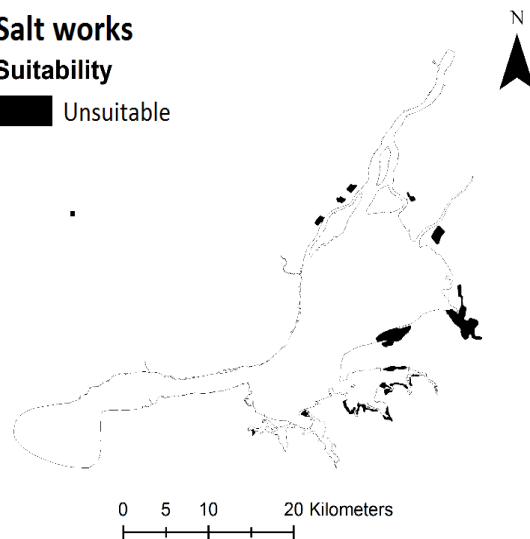
Salt works

- SalinesCOS07
- SalinesCLC06

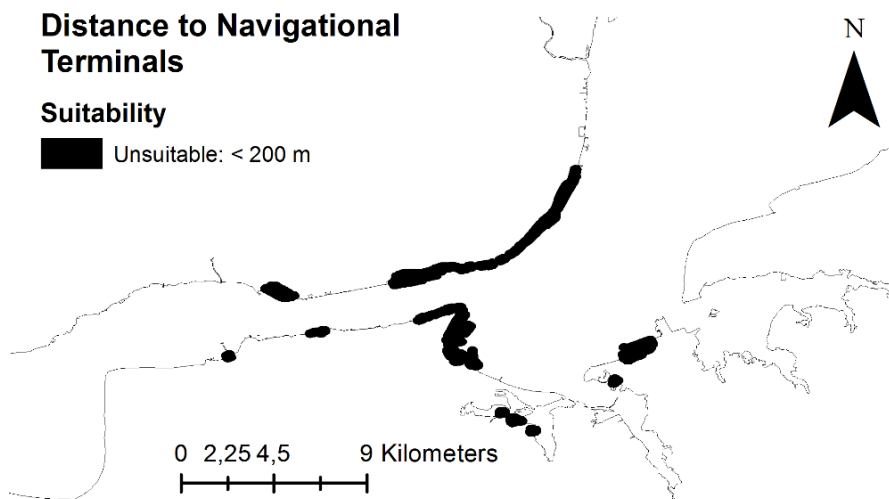
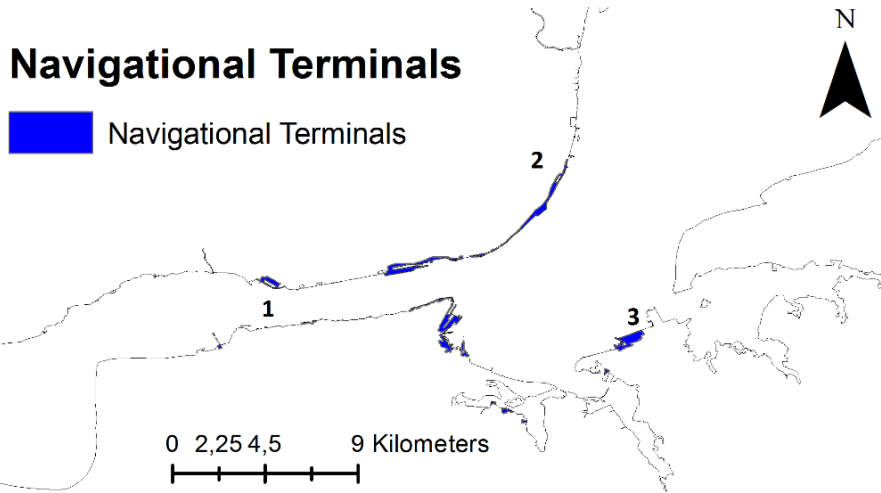


Salt works Suitability

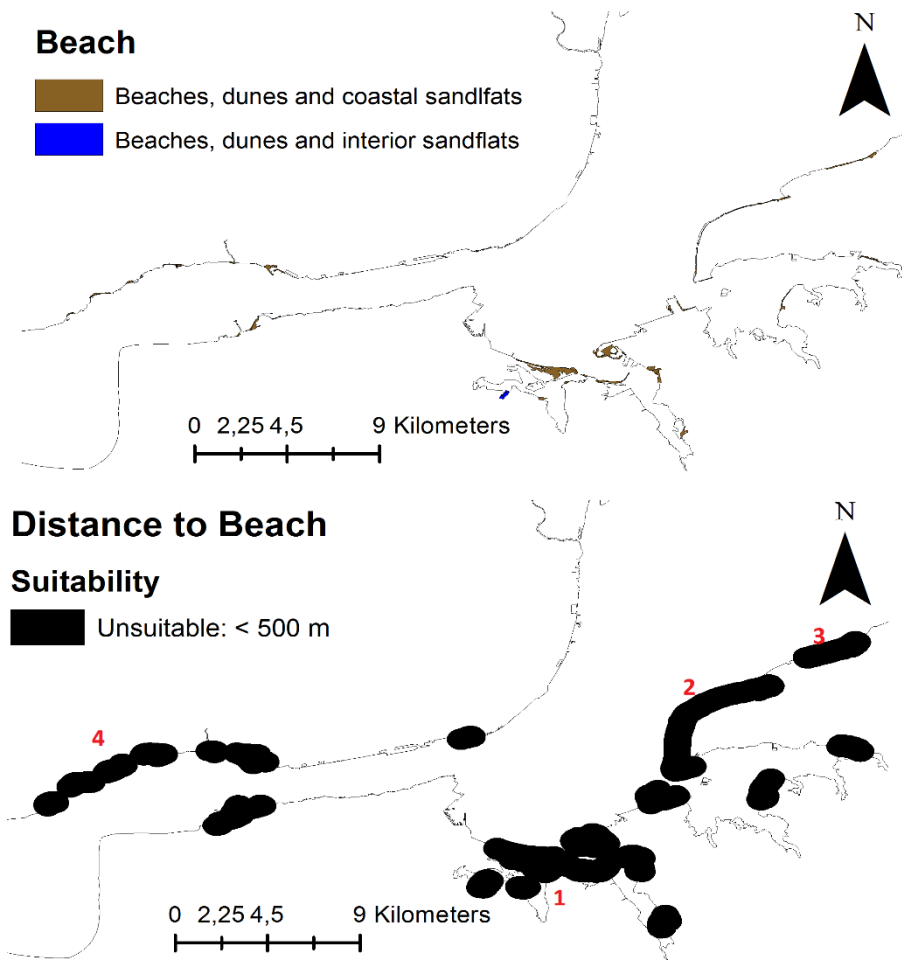
- Unsuitable



Distance to navigational terminals. 1 – Estuary's mouth; 2 – St^a Apolónia; 3 - Barreiro



Distance to beach. 1 – Area in-between Seixal and Barreiro; 2 – Montijo's Air Base no. 6; 3 – Alcochete; 4 – Linha de Cascais beaches



7.4. Appendix D

Appendix D displays FARM's environmental drivers for both concept farms (A and B).

Station 2.0 average values (Farm A)

| Month | Temp (°C) | Chla (ug L-1) | Salinity (psu) | POM (mg L-1) | DIN (umol L-1) | DO (mg L-1) | TPM (mg L-1) |
|-------|-----------|---------------|----------------|--------------|----------------|-------------|--------------|
| 1 | 12.2 | 4.2 | 6.468005 | 5.368421 | 57.2 | 9.3 | 30 |
| 2 | 12.5 | 2.5 | 25.89248 | 3.64569 | 49.88 | 7.025473 | 19.4042 |
| 3 | 14.6 | 2.95 | 14.34759 | 7.198943 | 63.75 | 8.9 | 54.475 |
| 4 | 16.9666 | 28.43333 | 13.74132 | 6.464922 | 58.29 | 7.787994 | 74.3413 |
| 5 | 18.4 | 32.25 | 22.295 | 7.122763 | 45.42 | 7.705 | 82.75 |
| 6 | 19.75 | 13.85 | 21.631 | 6.219038 | 32.67333 | 6.229955 | 63.8033 |
| 7 | 21.3 | 11.7 | 20.0002 | 8.841019 | 37.77333 | 6.533333 | 97.4 |
| 8 | 23.95 | 10.05 | 26.80102 | 2.619745 | 23.13 | 6.02278 | 43.3806 |
| 10 | 18.1 | 6.033333 | 11.41518 | 5.044342 | 40.98333 | 7.275937 | 47.5 |
| 11 | 14 | 6 | 12.70204 | 5.984526 | 45.1 | 7.731602 | 86.65 |
| 12 | 13.6 | 19.36667 | 7.004295 | 5.101316 | 38.02333 | 8.566667 | 49.3 |

Spring tide average values for station 2.0 (Farm A)

| Month | Temp (°C) | Chla (ug L-1) | Salinity (psu) | POM (mg L-1) | DIN (umol L-1) | DO (mg L-1) | TPM (mg L-1) |
|-------|-----------|---------------|----------------|--------------|----------------|-------------|--------------|
| 1 | 12.5 | 0.9 | 2.052632 | 10 | 51 | 9.1 | 1.150065 |
| 2 | 12.5 | 2.5 | 3.64569 | 19.4042 | 49.88 | 7.025473 | 25.89248 |
| 3 | 14.76667 | 2.533333 | 7.758591 | 47.3 | 55.93333 | 8.9 | 18.96437 |
| 4 | 16.85 | 28.55 | 5.595363 | 57.3275 | 52.45 | 7.603228 | 18.84921 |
| 5 | 18.4 | 32.25 | 7.122763 | 82.75 | 45.42 | 7.705 | 22.295 |
| 6 | 19.9 | 15.93333 | 5.882318 | 46.0318 | 32.2 | 6.562505 | 26.37557 |
| 8 | 23.7 | 5.9 | 3.652578 | 32.5053 | 20.55 | 5.930297 | 32.7633 |
| 10 | 17.2 | 3.2 | 3.647368 | 36 | 44.5 | 6.956268 | 21.37617 |
| 11 | 14.4 | 3.4 | 2.263579 | 33.6 | 44 | 8.114852 | 19.68247 |
| 12 | 15.1 | 6.8 | 6.3 | 57 | 35.3 | 7.2 | 15.52231 |

Neap tide average values for station 2.0 (Farm A)

| Month | Temp (°C) | Chla (ug L-1) | Salinity (psu) | POM (mg L-1) | DIN (umol L-1) | DO (mg L-1) | TPM (mg L-1) |
|-------|-----------|---------------|----------------|--------------|----------------|-------------|--------------|
| 1 | 11 | 7.4 | 2.73427 | 34 | 10.2 | 53.05 | 2.4157 |
| 3 | 14.1 | 4.2 | 0.49727 | 76 | 8.9 | 87.2 | 5.52 |
| 4 | 17.2 | 28.2 | 3.52556 | 108.369 | 8.157527 | 69.97 | 8.2040 |
| 6 | 23.4 | 16.5 | 12.0271 | 104.362 | 7.146108 | 33.02 | 7.55995 |
| 7 | 22.7 | 16.5 | 8.5 | 212.2 | 6.2 | 47.92 | 17.4283 |
| 8 | 24.2 | 14.2 | 20.8387 | 54.2558 | 6.115264 | 25.71 | 1.58691 |
| 10 | 18.55 | 7.45 | 6.43469 | 53.25 | 7.435772 | 39.225 | 5.74282 |
| 11 | 13.6 | 8.6 | 5.72160 | 139.7 | 7.348353 | 46.2 | 9.70547 |
| 12 | 12.85 | 25.65 | 2.74529 | 45.45 | 9.25 | 39.385 | 4.50197 |

Station 3.0 average values (Farm B)

| Month | Temp (°C) | Chla (ug L-1) | Salinity (psu) | POM (mg L-1) | DIN (umol L-1) | DO (mg L-1) | TPM (mg L-1) |
|-------|-----------|---------------|----------------|--------------|----------------|-------------|--------------|
| 1 | 12.166 | 2.76666 | 16.6284 | 9.93684 | 7.4 | 54.1 | 45 |
| 2 | 12 | 1.9 | 32.0291 | 2.92278 | 8.132331 | 35.65 | 21.3833 |
| 3 | 14.575 | 2.675 | 28.1318 | 14.575 | 7.225 | 32.55 | 113.6 |
| 4 | 16.875 | 15.2 | 25.3498 | 6.48122 | 8.128371 | 40.68 | 78.6538 |
| 5 | 18.15 | 11.9166 | 28.4294 | 8.10456 | 7.170451 | 31.15667 | 95.5596 |
| 6 | 20.35 | 10.4 | 30.1146 | 6.16833 | 6.35 | 38.3025 | 88.65 |
| 7 | 21.25 | 17.05 | 29.85 | 11.0718 | 6.35 | 22.09 | 122.6 |
| 8 | 21.533 | 8.66666 | 31.2527 | 7.61693 | 6.93487 | 23.55 | 77.1626 |
| 9 | 19.8 | 12.9 | 29.4 | 10.6560 | 6.542126 | 27.585 | 149.25 |
| 10 | 18.4 | 8.23333 | 25.8055 | 4.81114 | 6.64018 | 34.96667 | 53.6666 |
| 11 | 15 | 6.45 | 27.2420 | 6.88603 | 6.827622 | 39.9 | 122.7 |
| 12 | 14.166 | 8.36666 | 22.8448 | 5.50205 | 7.566667 | 37.21333 | 73.3 |

Spring tide average values for station 3.0 (Farm B)

| Month | Temp (°C) | Chla (ug L-1) | Salinity (psu) | POM (mg L-1) | DIN (umol L-1) | DO (mg L-1) | TPM (mg L-1) |
|-------|-----------|---------------|----------------|--------------|----------------|-------------|--------------|
| 1 | 12.5 | 0.9 | 12.80301 | 10.9136 | 16 | 73.7 | 7.9 |
| 2 | 12 | 1.9 | 32.02914 | 2.92278 | 21.383333 | 35.65 | 8.13233 |
| 3 | 14.966 | 2.5 | 29.35073 | 6.92452 | 70.4666 | 28.8 | 7.3 |
| 4 | 16.8 | 15.8 | 30.13292 | 5.63420 | 61.03 | 32.505 | 7.97337 |
| 5 | 17.7 | 13.9 | 29.28625 | 8.56829 | 81.2475 | 29.765 | 7.09958 |
| 6 | 19.95 | 9.2 | 33.14071 | 5.03202 | 55.8 | 40.095 | 6.3 |
| 7 | 20.7 | 6.7 | 33 | 5.57309 | 45.3 | 21.55 | 6.3 |
| 8 | 20.3 | 6.7 | 31.1 | 4.33789 | 10.4 | 23.22 | 7.6 |
| 9 | 19.6 | 7.8 | 32.3 | 3.89089 | 35.8 | 23.17 | 6.78741 |
| 10 | 18.6 | 3.6 | 26.81911 | 2.88 | 16 | 30.7 | 6.8 |
| 11 | 15.5 | 2.7 | 30.48438 | 3.58731 | 63.7 | 32.7 | 6.78119 |
| 12 | 15.8 | 2.5 | 28.32503 | 3.47368 | 22 | 43.7 | 6.9 |

Neap tide average values for station 3.0 (Farm B)

| Month | Temp (°C) | Chla (ug L-1) | Salinity (psu) | POM (mg L-1) | DIN (umol L-1) | DO (mg L-1) | TPM (mg L-1) |
|-------|-----------|---------------|----------------|--------------|----------------|-------------|--------------|
| 1 | 12 | 3.7 | 18.54114 | 9.448421 | 59.5 | 44.3 | 7.15 |
| 3 | 13.4 | 3.2 | 24.47529 | 10.74316 | 243 | 43.8 | 7 |
| 4 | 16.95 | 14.6 | 20.5668 | 7.328258 | 96.27778 | 48.855 | 8.283369 |
| 5 | 19.05 | 7.95 | 26.71586 | 7.176639 | 124.1839 | 33.94 | 7.312179 |
| 6 | 20.75 | 11.6 | 27.08857 | 7.304632 | 121.5 | 36.51 | 6.4 |
| 7 | 21.8 | 27.4 | 26.7 | 16.57066 | 199.9 | 22.63 | 6.4 |
| 8 | 22.15 | 9.65 | 31.32908 | 9.256458 | 110.544 | 23.715 | 6.602305 |
| 9 | 20 | 18 | 26.5 | 17.42116 | 262.7 | 32 | 6.296841 |
| 10 | 18.3 | 10.55 | 25.29876 | 5.776711 | 72.5 | 37.1 | 6.560269 |
| 11 | 14.5 | 10.2 | 23.99964 | 10.18476 | 181.7 | 47.1 | 6.874052 |
| 12 | 13.35 | 11.3 | 20.1048 | 6.516237 | 98.95 | 33.97 | 7.9 |