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Priority environmental contaminants in seafood: safety assessment, impact and public perception

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1. **Summary**Erro! A origem da referência não foi encontrada.

Seafood has been recognized as a high quality and healthy food item. However, seafood can also be a source of environmental contaminants (e.g. insecticides) with potential impact on human health. To counteract this problem, a growing interest was acknowledged to use phycoremediation (a mitigation strategy), which involves the use of algae for the removal or biotransformation of contaminants from aquatic systems. The main aim of this report was to carry out a technical and economical sustainability evaluation of phycoremediation for contaminants for which an effective reduction was observed in the ECsafeSEAFOOD project (i.e. venlafaxine (VEN), diflubenzuron (DFB) and inorganic arsenic (iAs)) in a previous study with the Mediterranean mussel *Mytilus galloprovincialis* in the presence of *Laminaria digitata* (mature individuals). It was concluded that phycoremediation with *L. digitata* is not viable due to the high costs of production and maintenance of this algae species in an integrated recirculation aquaculture system (e.g. need for an expensive and potent refrigeration system, a very large installation and a supplementation of *L. digitata* with nutrients), compared to the low price per ton of the resulting biomass.

2. Introduction

Seafood[1] is one of the most important food commodities consumed worldwide. The health benefits of a diet rich in seafood have been extensively recognized. Namely, ω3 polyunsaturated fatty acids (ω3-PUFA) are associated with decreased mortality from several diseases (e.g. reduced risk of coronary heart disease, stroke and hypertension) (Simopoulos, 2002). The Food and Agriculture Organization (FAO) and the World Health Organization (WHO) recommend a regular seafood (i.e. finfish (vertebrates) and shellfish (invertebrates) of marine or freshwater origin, farmed or wild) consumption of 1–2 servings per week in order to provide an equivalent of 200-500 mg of ω3-PUFA eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids (FAO/WHO, 2003, 2011).

Nevertheless, it is known that organic pollutants and heavy metals are introduced in the aquatic ecosystems as a result of intensive anthropogenic activity (e.g. industrial discharges). The presence of these pollutants in aquatic systems can cause serious problems to the environment and organisms, negatively affecting the stability of many aquatic ecosystems and also causing adverse effects to human health (Chekroun et al., 2014). Seafood can also be a source of pernicious environmental contaminants, such as pharmaceuticals, residues of pesticides, toxic elements and other contaminants of emerging concern. To counteract this problem, the overall objective of the ECsafeSEAFOOD project is to assess food safety issues mainly related to priority contaminants present in seafood as a result of environmental contamination, including those associated with marine litter and those originating from harmful algal blooms, and evaluate their impact on public health, contributing to the improvement of seafood risk management and risk communication.

Recently, a growing interest was acknowledged to use algae to remove, degrade or render harmless contaminants in aquatic systems (Chekroun et al., 2014). Phycoremediation has emerged as a promising technology, as uses algae capacity to accumulate, translocate and concentrate high amounts of certain toxic elements. The ability of macroalgae to accumulate metals in their tissues has led to their widespread use as sentinel species of metals availability in marine systems. Additionally, the accumulation of heavy metals and

[1] **Seafood** comprises fishery products and live bivalve molluscs, and no distinction is made between products coming from the sea and other sources, nor between wild catch and aquaculture species (Regulation (EC) No 853/2004)

organic pollutants by macroalgae provides an advantage of phycoremediation over other methods that are more expensive and not environmentally friendly (Chekroun and Baghour, 2013).

In a previous study on phycoremediation (within WP3 of the ECsafeSEAFOOD project, task T3.4.1. Mitigation strategies involving industrial processes: Phycoremediation), the capacity of seaweeds to uptake organic (venlafaxine (VEN), diflubenzuron (DFB) and lindane) and inorganic contaminants (inorganic arsenic (iAs), copper (Cu) and cadmium (Cd)) from seawater in the presence of the mussel *Mytilus galloprovincialis* (farmed bivalve) was assessed in an integrated recirculation aquaculture system, in order to evaluate the reduction of the contaminants in mussels. This work showed that despite the great ability of *Laminaria digitata* to uptake different contaminants from seawater, the levels in mussels were only slightly reduced when mussels and algae were simultaneously exposed to contaminated water (D3.3). The best results were obtained for VEN, iAs and diflubenzuron, with decreases of 25%, 84% and 70%, respectively. It was demonstrated that *L. digitata* is able to biotransform VEN, iAs and diflubenzuron present in the medium, thereby decreasing the uptake of these contaminants by the mussels over 25%. Through these results, it was also concluded that phycoremediation by macroalgae represent a good mitigation technology to effectively reduce some contaminant levels in bivalves and, consequently, to decrease the risks for human health and to strength human confidence in seafood consumption. For this reason, the aim of this report is to carry out a technical and economical sustainability evaluation of phycoremediation for contaminants revealing an effective reduction in the presence of *L. digitata*. However, this evaluation cannot be very detailed due to the unavailability of economic data, thus constituting a preliminary study.

This work was performed by **ICETA-REQUIMTE** (Faculty of Pharmacy, University of Porto, Portugal) and **Hortimare Projects and Consultancy B.V.,** in collaboration with **DTU** (Technical University of Denmark), **ICRA** (Catalan Institute for Water Research, Spain) and **IPMA, I.P.** (Portuguese Institute for the Sea and Atmosphere, I.P.).

3. Seaweed and bivalve species analysed

Taking into account the results obtained in the previous study on phycoremediation (D3.3), the target bivalve and seaweed species analysed in this technical and economical report were, respectively:

- the Mediterranean mussel (***Mytilus galloprovincialis***) belonging to the Mollusca phylum and Bivalvia class;

- the Oarweed (***Laminaria digitata***) (**mature individuals**), i.e. a brown seaweed of the Heterokontophyta phylum within the Phaeophyceae class.

3.1. Reproductive cycle of *Laminaria digitata*

Kelps are some of the largest species of seaweeds that grow on rocky shores (Edwards et al., 2011). *Laminaria digitata* is an abundant shallow water kelp species, being found in coastal areas of the North Atlantic (Kregting et al., 2016). Their reproductive cycle, or life history (figure 1), alternates between a large and structurally complex phase with a holdfast, stipe and frond, to the development of microscopic filaments that settle on rocks and other hard surfaces. The large plant is known as a **"sporophyte"**, while small filaments are known as **"gametophytes"** (Edwards et al., 2011).

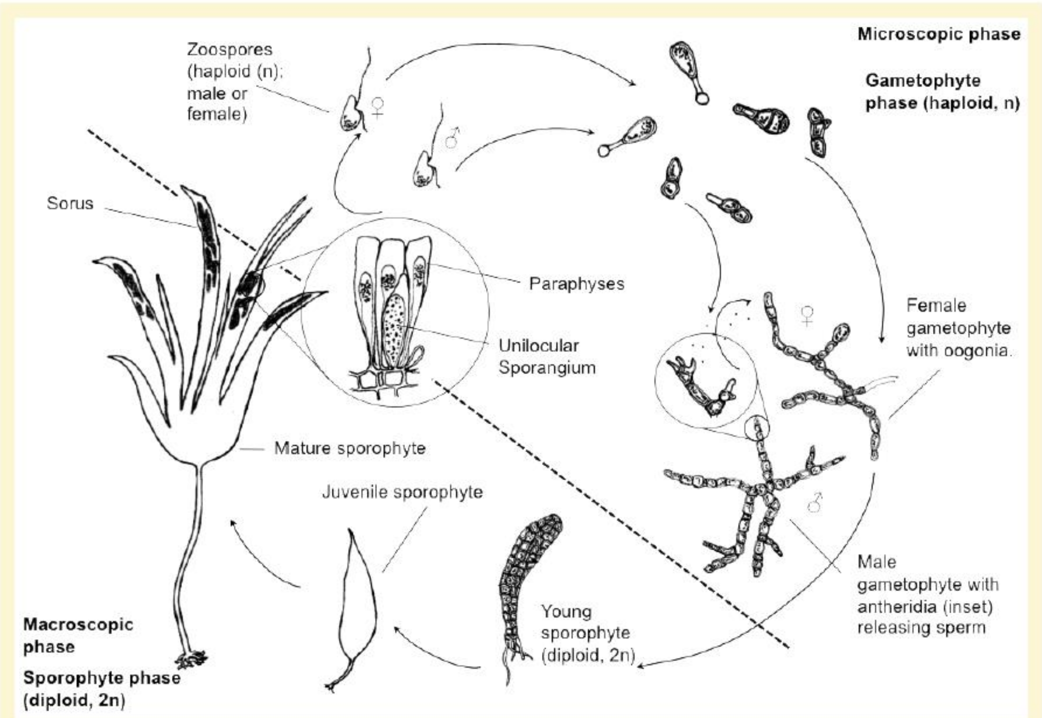

Figure 1 - Life history of *Laminaria digitata* (adapted from Edwards et al., 2011).

Laminaria digitata life cycle starts when reproductive patches ("sori") develop on the distal end of the blades. The timing of sorus development can change, depending on location. The sori appear as dark, slightly raised patches on the blade. Within these sori, reproductive cells (unilocular sporangia) develop, and zoospores are released when they mature. Each cell can contain/release 32 zoospores (Kain, 1979). These zoospores are equipped with two flagella, enabling the cell to have some control over the settlement. After a period in the water column, the motile zoospore loses its motility and settle passively onto a suitable substratum (e.g. under the algal canopy, where low light levels are suitable for germination) (Kain, 1979; Roleda et al., 2010). Once the zoospore germinates, cellular division begins. This is now called the **gametophyte phase**. Gametophytes will bear either female or male reproductive structures (oogonia and antheridia, respectively). Under appropriate cultivation conditions, gametophyte cellular division continues unabated, and individuals can become quite large (Edwards et al., 2011). Once the environmental cues change (including changes in light quality and intensity, or quantum dose), the development of reproductive structures is triggered. The availability of **blue light** is important for this process. In natural conditions, there is usually sufficient blue light available and gametophytes become reproductive almost immediately (Lüning, 1980). Female gametophytes produce ova/eggs, while male gametophytes produce spermatozoids. The eggs remain attached to the gametophyte, and emit a pheromone that attracts male sperm (Maier et al., 1988). Once fertilisation takes place, a zygote develops, followed by cellular division as the new sporophyte develops. These cells of the juvenile sporophyte are differentiated at an early stage into frond cells, and rhizoid-like cells become the holdfast. The sporophyte also attaches to a suitable substrate, and in the first year grows to approximately 50-60 cm (Edwards et al., 2011). Kelp, such as *L. digitata*, have the ability to take up and store phosphorus, which can be limiting at certain times of the year. Once established, *L. digitata* **start growing during the winter**, thus being considered as "season anticipators". This gives them a selective advantage, as they can start growing earlier than other algal species (Bartsch et al., 2008). The life-cycle of this algae species is around 5 to 7 months (Edwards et al., 2011).

3.2. Reproductive cycle of *Mytilus galloprovincialis*

M. galloprovincialis are gonochoristic broadcast spawners that release millions of gametes (eggs or sperm) into the water column when they are reproductively mature at around one year of age (Branch and Steffani, 2004; Picker and Griffiths, 2011). The maturation of eggs and sperm takes place in the gonad tissue that develops within the folds of the mantle (immediately in contact with the interior of the shell) (Beaumont et al., 2007; FAO, 2016). Thus, gonads extend throughout the body, being cream coloured in males and pink in females (Picker and Griffiths, 2011). Additionally, this species may spawn just once or several times each year depending on **environmental temperature and food availability** (Beaumont et al., 2007). For example, Cáceres-Martínez and Figueras (1998) showed that the peak of the spawning season occurs in spring, coinciding with an increase in temperature and chlorophyll-a concentration in Ria de Vigo (NW Spain) (favourable conditions for larval growth). Seed and Suchanek (1992) also mentioned that the onset and duration of both gametogenesis and spawning in *Mytilus* can exhibit considerable temporal and spatial variation. Thus, the reproductive studies of *Mytilus galloprovincialis* from around the world have shown a wide range of reproductive patterns both between and within populations (Villalba, 1995; Bhaby et al., 2014).

After spawning, the fertilization (external) of an egg occurs (IUCN/SSC Invasive Species Specialist Group (ISSG), 2009; FAO, 2016). Since mussels produce millions of eggs, they lose a considerable amount of their glycogen reserve (FAO, 2016). Fertilized eggs develop into a trochophore larvae, and then into a planktonic veliger larvae (figure 2) that is carried by tides and currents (Beaumont et al., 2007; FAO, 2016). Microscopic larvae can drift with water currents for several weeks before settling down in the benthos (bottom of the sea bed) (Boersma et al., 2006; Green, 2014). Depending upon water movement, plankton may drift more than 200 km (large distances) (Suchanek et al., 1997). After settling, larvae begin to metamorphose into their adult form and secrete byssal threads to attach to hard substrate (IUCN/SSC Invasive Species Specialist Group (ISSG), 2009; Green, 2014) (figure 2 and 3). According to FAO (2016), the pediveligers (larvae) attach themselves with their byssus threads to filamentous substrates when mussels reach a shell length of 0.25 mm. They are able to detach themselves and reattach to other non-specific substrates (FAO, 2016). Mussels often grow into beds of individuals upon settling, coating any hard surface area. Colony sizes can reach millions of individuals (Green, 2014).

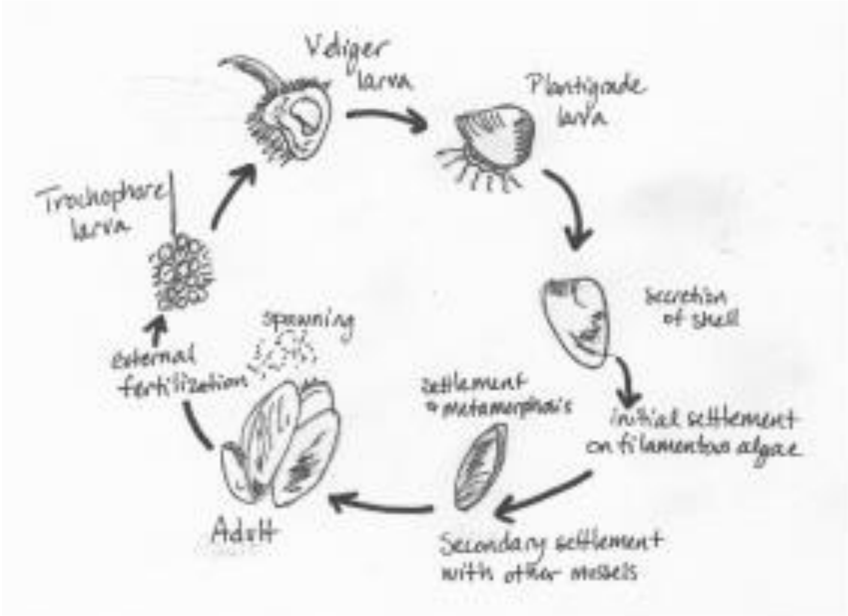

Figure 2 - Life cycle of *Mytilus* from trochophore larvae to spawning adults. After about one year, mussels are reproductively mature, releasing millions of gametes into the water (Branch and Steffani, 2004) (adapted from Green, 2014).

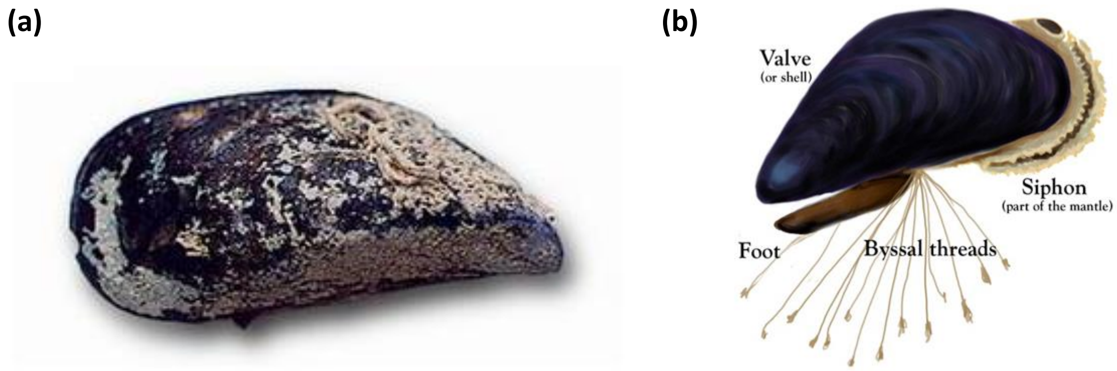

Figure 3 – a) *Mytilus galloprovincialis* - Lamarck, 1819 (adapted from FAO, 2016); b) External parts of *Mytilus*, including valve, foot, byssal threads and siphon. Byssal threads are used to attach to hard substrates, the valve houses the internal soft body, and the siphon is used for feeding. (http://shipfoulingchem409group2.wikispaces.com/file/view/MusselAnatomy.jpg/474397934/MusselAnatomy.jpg) (adapted from Green, 2014).

This species lives between one to two years, but can live up to 20 years (Boersma et al., 2006). They are larger than other bivalve species, growing up to 15 cm, although typically only 5-8 cm (IUCN/SSC Invasive Species Specialist Group, 2009). They also grow rapidly, reaching 70 mm length in the first year in favourable sites (Picker and Griffiths, 2011).

4. Target emergent contaminants evaluated

As referred above, in a previous phycoremediation study, the best results were obtained for inorganic arsenic (an inorganic element), venlafaxine and diflubenzuron (two organic compounds). *Laminaria digitata* was able to accumulate these contaminants from the environment to a certain degree. For this reason, these environmental contaminants were selected for this technical and economical sustainability evaluation.

Arsenic is a widely distributed metalloid, occurring in rock, soil, water and air. Water concentrations are usually <10 µg/l, although higher concentrations may occur near anthropogenic sources (e.g. agricultural and industrial sources, such as the manufacture and use of arsenic pesticides and wood preservatives) (WHO, 2001; Järup, 2003). Arsenic compounds cause short-term and long-term effects (e.g. inhibition of growth, photosynthesis and reproduction, death, and behavioural effects) in individuals, populations and communities of organisms. These effects are evident, for example, in aquatic species at concentrations ranging from a few micrograms to milligrams per litre. The nature of the toxic effects depends on the species and time of exposure. Environments contaminated with arsenic contain few species and individuals (Green Facts, 2016). On the other hand, the most important source of exposure to arsenic in human populations is via food intake (Järup, 2003). In addition to skin cancer, long-term exposure to arsenic may also cause cancer of the bladder and lungs. The International Agency for Research on Cancer (IARC) has classified arsenic and its compounds as carcinogenic to humans (WHO, 2016).

Venlafaxine (a pharmaceutical compound marketed as Effexor) is included in the class of drugs called selective serotonin/norepinephrine reuptake inhibitors (SNRIs). It is used to treat depressions, anxiety, and other mood disorders (FDA, 2015). However, this anti depressive compound has been detected in parts per billion levels in tertiary-treated municipal wastewater effluent (Best et al., 2014). This means that venlafaxine can enter in the aquatic environment and affect aquatic organisms in different ways (e.g. may disrupt the normal endocrine function of fish; induce spawning, glochidia release and parturition in bivalves) when treated wastewater is discharged into rivers or streams (Fong and Hoy, 2012;

MDH, 2015). The occurrence of venlafaxine in surface waters has been reported in concentrations ranging from some ng L^{-1} up to 1000 ng L^{-1} (Feito et al., 2013).

Diflubenzuron (DFB) is an acaricide/insecticide (insect growth regulator) used to control many leaf eating larvae of insects feeding on agricultural, ornamental and forest plants (e.g. mosquito larvae, gypsy moths, rust mites) (EPA, 1997). DFB is also commonly used to control sea lice (mainly *Lepeophtheirus salmonis*), a parasitic crustacean that strongly affects the production of fish farming in e.g. Norway (salmon and cod) , where this parasite has been detected in seawater near farmed aquaculture facilities. However, DFB is potentially toxic to nontarget biota because it can enter aquatic systems through aerial application or runoff after precipitation events (Fischer and Hall, 1992). For example, the quantity of diflubenzuron discharged in Norwegian seawaters has increased in the last years (from 1413 kg in 2009 to 5016 kg in 2014) (Grave and Horsberg, 2015). According to EPA (1997), diflubenzuron is highly toxic to freshwater, marine and estuarine invertebrates, including crustaceans and molluscs. Therefore, the use of diflubenzuron is expected to cause adverse acute and chronic effects to freshwater, estuarine and marine invertebrates, including endangered species. It has also been stated that diflubenzuron affects, for example, the reproduction, growth and survival of freshwater invertebrates, as well as the reproduction of several marine and estuarine invertebrates. Additionally, humans may be exposed to residues of diflubenzuron through the diet. Despite there is no evidence of carcinogenicity for diflubenzuron, some metabolites of diflubenzuron, such as p-chloroaniline (PCA) and pchlorophenylurea (CPU), are probable human carcinogen according to the Environmental Protection Agency (EPA, 1997).

5. Technical viability

The cultivation of seaweeds at sea currently uses different structures depending on their location. In general, at rougher seas vertical dropper/riser systems are used on which the seaweed grows, while in areas subjected to low hydro dynamism, such as fjords, simpler longline structures are used. The structure of a simple longline construction suitable for the cultivation of seaweed consists of a method of anchorage, connected to a header rope at (or near) the water surface, which is supported by buoys (Edwards et al., 2011). Therefore, an

integrated recirculation aquaculture system was simulated, combining mussels (*M. galloprovincialis*) and macroalgae (*L. digitata*) cultivated at sea (offshore) in longlines.
According to figure 4, there is a mismatch between growth period of both species (Gomez and Lüning, 2001; Kopp et al., 2005).

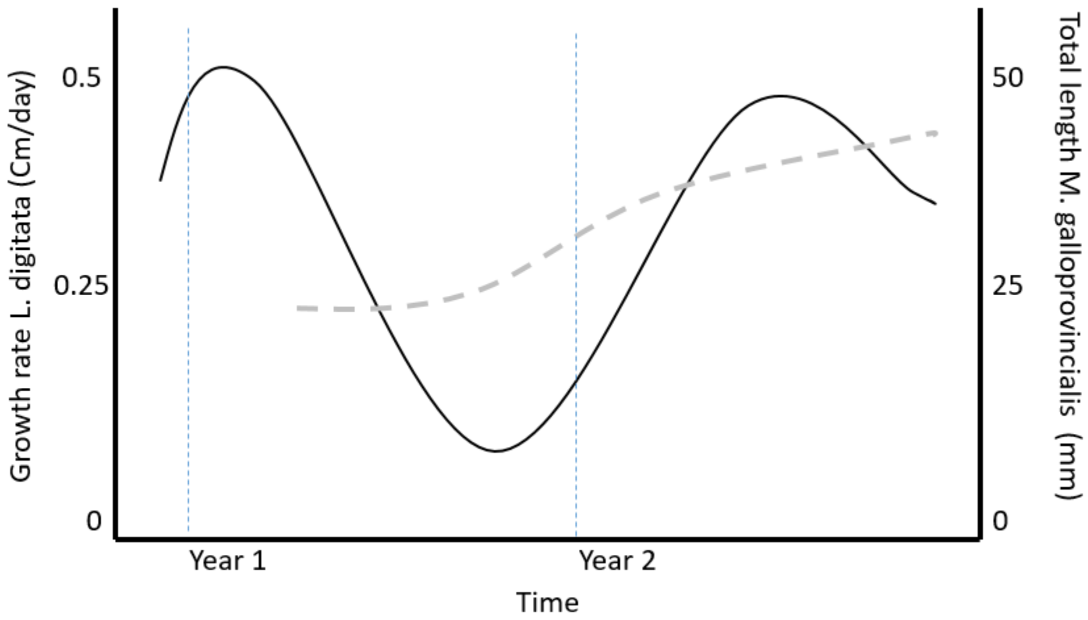

Figure 4 - Growth rate of experimental individuals of *Laminaria digitata* cultivated in outdoor tanks in constant short days (8 h light per day) (filled line); and growth of *Mytilus galloprovincialis* in shell length during an experimental period (1999-2000) (dotted line). Figure simplified and adapted from Gomez and Lüning (2001) and Kopp et al. (2005).

The mussels grow steadily during the entire growing period (in this case from June, year 1 to October, year 2) (Kopp et al. 2005). However, *Laminaria digitata* has a decreased growth during summer/early autumn (Gomez and Lüning, 2001) (figure 4). Combined with the fact that during this period the degradation of the leaf occurs due to biofouling and herbivory, there is little biomass left of *Laminaria digitata* during this time (gross biomass gain is lower than gross biomass loss, resulting in net biomass loss). This result in a mismatch as almost no Oarweed biomass is available during the summer months for phycoremediation, which will in turn causes almost no contaminants removal from the water, being accumulated in the mussels.
The culture conditions simulated for *Laminaria digitata* were as follows:

- **water** temperature **below 15 ºC**;

- water salinity: 30-32 ppt (full saline and unpolluted coastal waters);
- currents with mid to high flow rates (between 5 and 10 cm s^{-1}) as it is considered relevant for seaweed cultivation (e.g. high flows will improve nutrient exchange; Edwards et al., 2011);
- seawater pH in the range of 7.8 to 8.2, since kelp grows better at pH ranging between 7.0 and 9.0 (Flavin et al., 2013);
- supplementation with macronutrients according to F/2 medium concentration (Guillard, 1975).

Water temperature is the main limiting factor for growth and development of *L. digitata*. Ideally, average maximum temperatures should not go above 15 ºC during spring/early summer (Edwards et al., 2011). For example, Broch et al. (2013) mentioned that when water temperatures, light intensity and consequently photosynthetic activity increases, the relative growth rate of *Saccharina latissima* decreases due to nutrient depletion. These authors observed that the concentration of nutrients remain low during summer, increase in October and winter due to mixing and light-limited phytoplankton growth (no nutrient consumption) (Broch et al., 2013). Other authors also observed a **typical pattern of nutrient limitation (e.g. nitrate and phosphate) in summer and luxury consumption in winter** in *L. digitata* (Kregting et al., 2016), as well as in other macroalgae species (Chapman and Craigie, 1977; Wheeler and North, 1981; Conolly and Drew, 1985; Martínez and Rico, 2002). Flavin and collaborators (2013) also stated that kelp grows better at chilled seawater (approximately 10 ºC) in nutrient rich waters. Thus, potent refrigeration systems are essential to maintain low water temperature in integrated recirculation aquaculture systems.

On the other hand, the growth of seaweed benefits from the addition of nitrogen, phosphorus and potassium in specific ratios (N:P:K). These elements are generally delivered in the form of nitrates and phosphates. Trace metals and vitamins are also required for normal algal development (Edwards et al., 2011). Therefore, an optimal production and maintenance of *L. digitata* in integrated recirculation aquaculture systems implies a supplementation with nutrients, thus representing additional costs.

6. Economic viability

Currently, the production of 1 ton of Oarweed (with state of the art equipment and labour) costs approximately 4000 €. This includes labour (3 persons fulltime), boat use and maintenance (1 small fishing vessel), seedling production, and seeding and harvesting of the seaweed, but does not include installation and operating costs of the farm at which the seaweed is grown. In the future, with further improvement of cultivation techniques, systems and breeding of seaweed to higher biomass yields, the price is expected to drop towards 150-200 euro per ton seaweed. Moreover, a representative investment cost for the establishment of a typical single longline floating mussel farm (1-4 ha) is still quite high. For example, this cost ranges from €270,000-360,000 (average cost, €296,600) in Greece. However, this amount varies depending on location (distance from land-based facilities), farm size, equipment availability, and prevailing weather conditions in the area (Theodorou et al., 2011). Thus, the production of both *Mytilus galloprovincialis* and *Laminaria digitata* at sea (offshore) on longlines is extremely expensive. Additionally, a large quantity of seaweed biomass is required to have any significant effect on the quality of large batches of mussels. However, this biomass is currently unavailable from aquaculture production alone. This means that the natural stocks need to be harvested to achieve the required quantity of seaweed, which is not sustainable in long term for the environment (natural populations will be destroyed). Furthermore, wild harvested seaweed is of varying quality, which means potentially different uptake rates. On the other hand, the seaweed cannot be resold due to the added contaminants in the biomass. So, seaweed biomass would be unsuitable for food and feed and if it can be sold to bio-refinery, the cost price is too low to make it economically sustainable. Therefore, by adding contaminants to biomass in phycoremediation, the value of the biomass will become even lower as extra steps are needed to refine it for further use. Price for bio-refinery at the moment is 1000 € per ton dry (=100 € per ton wet).

A Strengths, Weaknesses, Opportunities and Threats (SWOT) analysis of this phycoremediation technique is presented in Table 1. This table highlights the internal and external factors that are favourable and unfavourable to its implementation.

Table 1- SWOT analysis for phytoremediation

Strengths	Weaknesses
Improvement of ecosystem quality by increased biodiversity	Limited practical experience; not applicable for all compounds
Environmental friendly activity	Limited production of Oarweed biomass during the summer months
Diversification of production and target-species	High cost of Oarweed biomass production
Opportunities	**Threats**
Opportunities for new products	Poor quality of seaweed biomass after phycoremediation to be used in biodiesel
Improvement of the economy of the fishery sector	Competition of other countries with cheaper production
Research opportunities	

Phytoremediation may represent an environmental–friendly alternative for the reduction of some contaminants in mussels. However, the use of phytoremediation in mussel production is constrained by the high cost of the biomass and the seasonality of algae life cycle that is associated with low water temperature.

7. New product acceptability by consumers

The EU food industry is a dynamic arena affected by wider socio-economic processes. To remain competitive in the modern world, food manufacturers must develop capacity to innovate quickly and effectively as reliance on a stable range of traditional foods can no longer ensure business success (Grunert et al., 1997). The following discussion start with a brief review of the factors deemed important for the success or failure of new seafood products.

Economic factors, such as price is often cited among the main barrier to consumption of fish and seafood (Birch et al., 2012; Liu et al. 2013), as a decrease in their prices relatively to other sources of proteins can act as a driver for consumption and overall expansion of the market. A wide array of non-economic factors also plays an important role in determining the trends in food consumption. Increasingly, these relate to 'intangible' aspects of the product, such as ethical and sustainable sourcing. As the populations of many industrialized countries are becoming older, richer, more educated and more health conscious, the demand for food that promotes health and well-being is growing (FAO, 2014). The actual life style determines the consumer´s demand towards the use of healthy/safe seafood products that retain their intrinsic sensory attributes and natural profile (Cardoso et al., 2013; Heide and Altintzouglou, 2015).

New products can merely constitute improved existing food items. In this study the resulting mussels (product with low levels of contaminants obtained after phycoremediation) could be well accepted by consumers. However, the success of new products implies a 'market oriented' strategy by the companies. They need to identify unsatisfied consumer needs, targeted barriers to seafood consumption, or exploited growing market trends.

8. General conclusions

Technically, phycoremediation is possible using the current technology. However, this study concludes that phycoremediation is economically unfeasible at the moment with the state-of-the-art production systems employed, since the production and maintenance of *L. digitata* biomass in an integrated closed aquaculture system is very expensive (e.g. need an expensive refrigeration system, a very large installation and a supplementation of *L. digitata* with nutrients), and the resulting biomass has a very low price per ton. Furthermore, despite the reduction of approximately 30 % for VEN in mussels (as observed in the previous study on phycoremediation) the levels of this contaminant in the environment are still not a concern for humans. Thus, this mitigation strategy does not compensate.

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