Deliverable No. 1.3

Evaluation of Low Trophic Species Production Barriers

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AquaVitae

Project title:

New species, processes and products contributing to increased production and improved sustainability in emerging low trophic, and existing low and high trophic aquaculture value chains in the Atlantic

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Deliverable D1.3

Evaluation of Low Trophic Species Production Barriers

10/03/2021

Executive Summary

Deliverable 1.3 is an overview and evaluation of the barriers to the production of low trophic species at a commercial scale in the project area from the seven case studies linked with WP1. The information detailed in D1.3 was composed from interviews with CS leaders and participants, workplans and CS reports (scientific and technical D1.2 appendix 3). These summarise the biological, technical, legislative and economic barriers to hatchery production of LTS, as identified by relevant CS and WPs and form the basis of a framework to overcome the barriers to production. The identified barriers in this deliverable will be actively monitored for the duration of the AquaVitae project as the outputs related to industry barriers for the LTS production are completed. This and previous WP1 deliverables (D1.1 & 1.2) were developed to identify, evaluate and address barriers to production in the AquaVitae project, along with the development of new and novel hatchery protocols for LTS production across the five value chains incorporating seven out of 13 case studies (see following list for selected CSs):

- CS1 Macroalgae, new species production
- CS3 Land-based IMTA
- CS7 Sea cucumber species, site selection and key hatchery steps
- CS8 Improving seed availability and grow-out of native and non-native oysters
- CS9 Offshore production of blue mussels
- CS10 Optimisation of freshwater fish production in Brazil &
- CS11 Marine fish farming

In this deliverable, 24 key production barriers for the seven case studies have been identified and are listed under the subchapter for each relevant case study. Furthermore, the production barriers identified will be monitored through the CS reporting platform developed by WP1, 2 & 3 leaders and will form part of the good practice recommendations of T1.4 and T1.7. This will contribute to resolving the key production barriers which is a central task for the completion of D1.4 and T1.7.

An overview of the main findings of D1.3 are listed below. These are described in further detail in the case study subchapters. Similar barriers to production are experienced by operators in different regions of the project area:

- The production of LTS species (macroalgae, benthic invertebrates and bivalves) are suited to the development of a multi-species hatchery, which may be a more economically viable output for some operators. Especially if linked with IMTA production. This may help overcome the economic barriers to LTS production.
- Fundamental research is required to overcome technical barriers to production, specific are included in the CS subchapters.
- Licencing of land-based facilities is one of the main industry barriers for the production of LTS experienced by operators.
- Economic barriers to production are primarily the initial start-up costs for operators. These may be lessened when the technical and biological barriers are overcome.

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Introduction

Synopsis of AquaVitae

AquaVitae is a research and innovation project funded by the EU's Horizon 2020 programme. The project consortium consists of 35 partners, from 16 different countries, spread across four continents. In addition to Europe, partners are situated in countries bordering the Atlantic Ocean, including Brazil, South Africa, Namibia, as well as in North America. The AquaVitae projects' broad objective is to introduce new low trophic species, products and processes in marine aquaculture value chains across the Atlantic.

Scope and motivation of D1.3

The overall objectives for WP1 are to develop hatchery and seedling production of LTS (SO1 & SO2), specifically:

- Carry out hatchery/seedling innovation and exploitation activities in the specific CSs linked to WP1
- Coordinate WP1 CSs with work carried out on the same CSs in WP2 and 3
- Identify industry barriers to the optimisation of production of seed for low trophic and extractive species
- Develop robust new and novel hatchery protocols and production processes for new and emerging species in each of the five AquaVitae value chains, formulate a "Good Practice" recommendation based on these protocols, and publish this recommendation as a low level, voluntary industry standard
- Optimise and test wild collection methods for native and non-native LTS species

Specific CSs' involved in WP1 are listed in Table 1. Deliverable 1.3 will focus on Task 1.3; to identify barriers to the expansion of LTS production, evaluate industry barriers and overcome the technical challenges. To achieve this task, each CS has provided the specific tasks from D1.2 which identify key barriers relevant to their species(s) be it technical, biological, environmental, or economic. The industry barriers presented in this deliverable are aimed at the hatchery production of juveniles and seedlings. Information gathered in D1.3 will also form an outline for "Good Practice" recommendations for T1.4 (and D1.7) (Figure 1).

Table 1: Overview of the CSs' directly linked to WP1 and their lead partner organisation. These lead partner organisations are responsible for reporting to the WP1 on specific tasks and outputs.

VC1 Macroalgae	VC2 IMTA	VC3 Echinoderm	VC4 Shellfish	VC5 Finfish
CS1: Macroalgae, new species production CS Lead: CIIMAR	CS3: Land-based IMTA CS Lead: FCPCT	CS7: Sea cucumber species, site selection and key hatchery steps CS Lead: AWI	CS8: Improving seed availability and grow-out of native and non-native oysters CS Lead: IVL	CS10: Optimisation of freshwater fish production in Brazil CS Lead: EmBraPa
			CS9: Offshore production of blue mussels CS Lead: DTU	CS11: Marine fish farming CS Lead: FURG

Figure 1. Outlines the connectivity between previous deliverables and how D1.3 will link with T1.4. Figure 1 follows the time line of the AV project and the submission of deliverables (D1.1 M6 and D1.2 in M12). Initial barriers to production are identified in the CS work plans and in D1.1, these barrier identifications are further developed in the technical reports in M12 submitted as part of D1.2 and further evaluated here in D1.3.

Methodology

The AquaVitae project implements a spiral model of innovation (Figure 1, D1.1). D1.3 is an integral part of WP1 deliverables as it contributes a detailed overview of the production barriers experienced by commercial enterprises, CS & WP leaders throughout the project area. Similar technical barriers are experienced by producers at each of the four corners of the Atlantic and these are identified in this deliverable.

To identify and establish the scope of the barriers to hatchery production experienced by LTS operators, interviews with CS leaders and the extraction of information from each work plan submitted as part of D1.1 and CS technical detailed reports submitted as part of D1.2 were carried out. Initial stakeholder surveys were carried out in M5 and this information was used to identify challenges and also make sure the CS work plans were in-line with the needs of the industry (i.e. what are the key bottleneck that need to be resolved, and do the work plans address these barriers).

The breakdown of the production barriers (outlined in Tables 2-8 in this deliverable) are also linked directly to CS tasks that are monitored and updated via the case study reporting platform which will provide technical information to T1.4. To overcome the relevant barriers to hatchery production, the AquaVitae project has identified and detailed solutions and these form part of the specific CS tasks which links to the overall WP1 deliverables (D1.3 & D1.4).

D1.3 has developed a framework to firstly identify the technical challenges and secondly to overcome the industry barriers to advance the commercial potential of LTS production (Figure 2). Through the identification of the production barriers, new and novel hatchery methods are developed and implemented. Technology transfer has also been implemented across all the seven case studies. The advancement of robust technology and approaches can overcome production barriers and by using the spiral model of innovation (Figure 1, D1.1) allows for these advancements to be mapped and evaluated in a repeated evaluation cycle using the case study reporting platform.

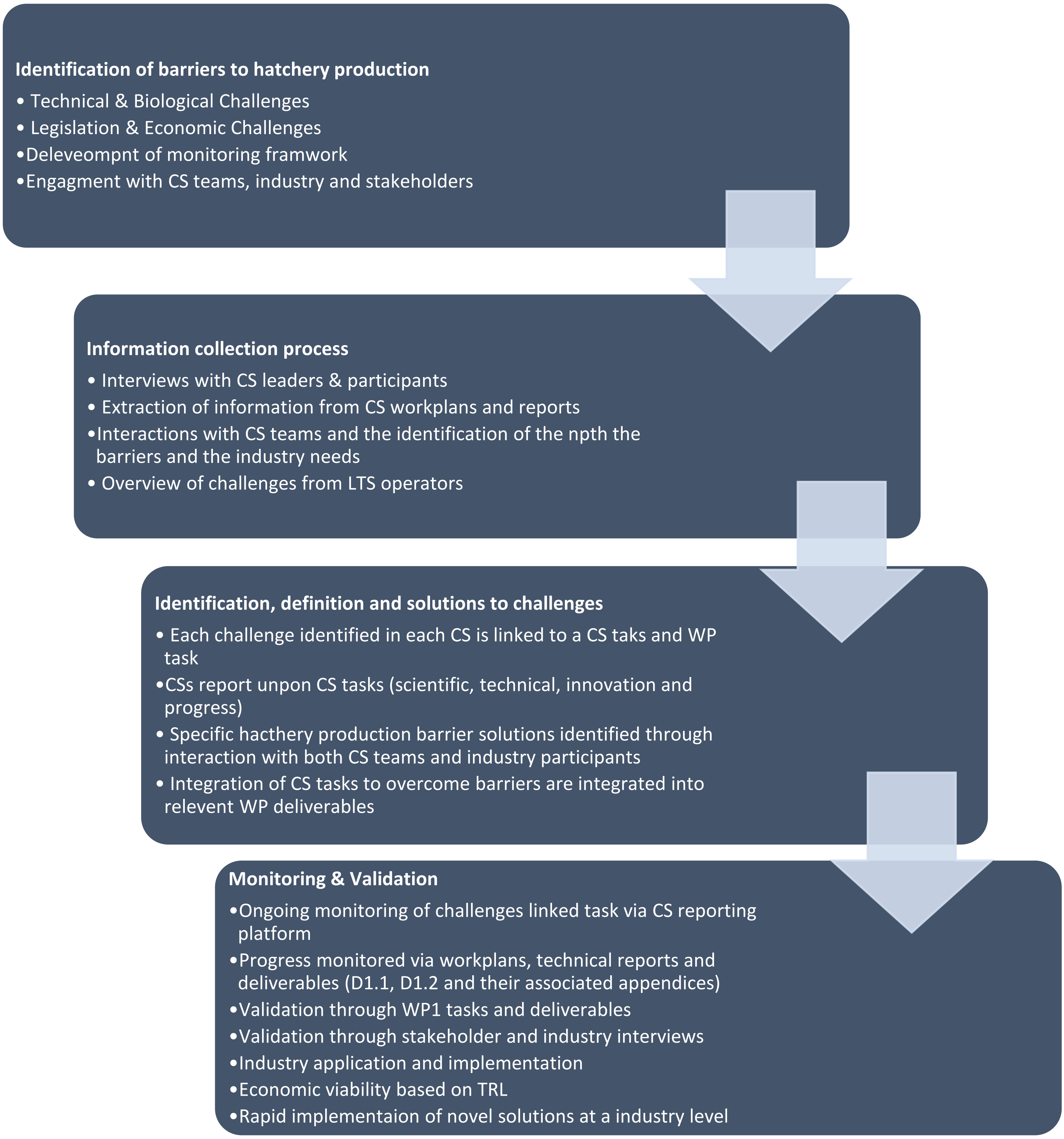

Figure 2. Framework and development process to overcome technical challenges for LTS hatchery production. The process is imbedded in the development of WP1 deliverables through the integration and contributions of CS tasks. All identified industry barriers are identified, evaluated and overcome through the advancement and of CS and WP tasks.

Production barrier identification

In conjunction with CS leaders and participants, D1.3 has identified technical, biological, legislative and economic barriers to hatchery production through the framework development process. Solutions have been developed to overcome the biological and technological barriers and implemented through the AquaVitae project for each specific barrier, and the efficacy of the solutions will be monitored and validated through implementation at a LTS producer level. In this section, D1.3 introduces the specific barriers for each of the seven case studies and outlines the solution.

Case Study 1 Macroalgae new species production

CS Lead: Centro Interdisciplinar De Investigação Marinha E Ambiental (CIIMAR)

Overcoming the challenges of producing macroalgae species (hatchery production) for commercial production is still a key industry barrier for specific macroalgae species. CS1 has identified three species that require strategic research activities (Table 2). The three species *Codium tomentosum*, *Ulva rigida*, and *Ulva ohnoi* require three key tasks to be addressed (see details in Table 2). CS1 will develop a robust production process for both vegetative propagation and sexual reproduction for the three species in two regions of the Atlantic at the industrial producer level. Upscaling of the production process to allow for increased on-growing activities in earthen ponds will also be established using novel settlement substrates.

CS1 Main Barriers:

1. Hatchery reproduction of *Codium tomentosum.* This species of dichotomously branched spongy algae still requires key hatchery steps to be developed to close the reproductive cycle as new species for upscaling for commercial production. For upscaling of this species to large scale land-based production suitable settlement substrates are required to allow on-growing in earthen ponds.
2. Reproduction of *Ulva ohnoi* in Brazil. This species of macroalgae is not typically cultured in Southern Brazil. CS1 will develop protocols for vegetative propagation and sexual reproductive of *Ulva ohnoi*, which has the potential to boost the commercial cultivation of local *Ulva* species.
3. Sexual reproduction of *Ulva rigida* in Portugal. Control over the sporulation process is required to establish optimized protocols for the sexual reproduction of the species. The protocols developed for *U. ohnoi* in Brazil will be transferred and applied to the genetically related *U. rigida* in Portugal.
4. Cultivation of *Ulva rigida* in large scale earthen ponds. Development and validation of large-scale cultivation of *Ulva* in earthen ponds has been identified as an opportunity for lower cost of production. This process will be validated as part of CS1 and will enhance the opportunity for the industry to uptake the processes in a cost-effective manner.

Legislation and economic barriers to hatchery production

The main legislative barriers to the production of macroalgae in Europe and Brazil is the licencing of both aquaculture facilities and sites on land and at sea. Licencing policies and legislation are not up to date with the current production potential which has also been identified and outlined in the AquaVitae Deliverable 8.2 (Report on industry perceptions on current policy frameworks). There are no specific economic barriers to the production of macroalgae in the hatchery as it is relatively low cost. However, the overall cost of developing a standalone hatchery for macroalgae production can be prohibitive. Implementing a production process into a pre-existing hatchery as a multi-species output may be a solution for some producers where facilities, legislative regulations and space allow.

Table 2: Overview of the CS1 hatchery related production barriers for new macroalgae species. CS leaders have identified the production barriers related to specific species.

Ident.	Output type	LTS Production Barrier	Addressing the Production Barrier	Current TRL	WP task
1.1.1	Process	• Limited genetic variability and quality of the biobank • Lack of optimised protocols for reproduction • Lack of efficient procedure for seeding into substrates (e.g. cultivation line)	A novel reproduction method for *C. tomentosum* will allow the improvement of the genetic diversity of the bio-bank and the development of efficient procedures for seeding on substrates.	3	T1.2
1.3.1	Process/ Technology Transfer	• Limited genetic variability and quality of the biobank • Lack of optimised protocols for reproduction	A new protocol will allow to stablish efficient vegetative and sexual reproduction methods for the local *Ulva ohnoi*, in order to boost the cultivation of the species in Southern Brazil (and potentially *U. rigida* in Portugal)	4	T1.2
1.4.1	Process	• Lack of efficient procedure for seeding into substrates (e.g. cultivation line). • Large non- or underutilised potential cultivation areas (earthen ponds) in the farm.	A new method for cultivation of *Ulva rigida* in substrates will improve the deployment and harvest of biomass, and will allow upscale its production in underutilised, low cost, earthen pounds. This will be done in an organic certified land-based IMTA.	4	T1.2, T2.2

Case Study 3 Land-based IMTA

CS Lead: Fundación Canaria Parque Científico Tecnológico de La Universidad de Las Palmas de Gran Canaria (FCPCT-ULPGC)

The production of invertebrates and their early life stages of development and metamorphosis have been proven to be the most critically impacted by environmental and climatic conditions, especially for calcifying species of invertebrates including abalone. Consequently, there is a need to improve the reliability of hatchery and nursery production while ensuring the sustainability of the production processes in order to reduce the inconsistency between production batches and ensure a reliable production of seed/germlings for further production in land-based Integrated multi-trophic aquaculture (IMTA) systems. The integration of various species in IMTA is relevant to increase future sustainable development of aquaculture. However, one of the challenges of shifting from species monoculture to polyculture systems lies in the need to obtain a reliable source of spat or germlings for various species. These can be sourced from hatchery production or from the wild. Consequently, it is of interest to develop hatchery production processes for the different animals and vegetal cultured species to be included in the IMTA system to reduce the need of capture from the wild. Depending on the different geographic locations, different species are to be integrated in the systems. For some species the hatchery production techniques have been established and research and development activities need to be furthered in order to improve the production process while in other regions and for other new species the hatchery production processes are still in their infancy and to be developed.

In order to address these challenges CS3 has identified the need for reliable hatchery and nursery protocols as they still remain key barriers to production and the need to develop an integrated production processes that will contribute to consistent production, survival and growth of the early life stages while promoting sustainable practices (Table 3).

There is a need to develop consistent integrated production techniques for abalone species that can be tested and used between geographical locations. The initial exchange of technical knowledge and the standardisation of hatchery processes will facilitate their successful tech-transfer to other invertebrate species (LTS species), including hatchery production of abalone, mussels (CS9) and sea cucumbers (CS7). Each of these are likely to be also integrated in land based IMTA grow out production processes. Initial production barriers of live algae settlement substrates will be overcome in through implementation of robust production procedures and the implementation of prime live algal settlement substrates.

To improve the hatchery and nursery production processes and strengthen the capacity for adaptation to climate change for hatchery and nursery production through the adoption of integrated and organic methods for these production cycles. Specific key production barriers have been outlined (Table 3).

More specifically the challenges identified include the need to:

1. Identification of induction cues for settlement and survival of abalone post-larvae and juveniles to optimise the production processes. Inconsistent settlement and survival rates in abalone. This is a key industry barrier in Europe and South Africa, identification and implementation of a prime settlement cue that enhances postlarval survival is an industry need and a key bottleneck. CS3 will identify suitable prime settlement cues for use in a multi species hatchery model. Benthic invertebrates require similar settlement cues and CS3 will investigate their efficacy for both abalone and sea cucumbers.
2. Understanding the effect of temperature on growth and survival of juvenile abalone and the effects of temperature and pH on the settlement rates of blue mussels (*Mytilus edulis*) in the hatchery with an aim of assessing impacts of ocean warming and pH on performances. Hatchery production of juveniles and seed is a controlled process that can be impacted directly by local conditions and water quality. By understanding the impact in the hatchery will allow the industry to stay ahead of environmental changes and mitigate their direct impact on juvenile and seed production.
3. Identify and establish hatchery protocols for sea cucumber production. Sea cucumber production in Europe and South Africa is of growing interest to hatcheries. However, procedures and protocols for juvenile production are not well understood or established. CS3 and CS7 will develop shared outputs to address the lack of methodologies for juvenile production.

Legislation and economic barriers to hatchery production

There is no existing legislation in the EU for land based IMTA. However, there are developments around the inclusion of polyculture and IMTA in commercial enterprises. In South Africa IMTA is under active development at abalone farm enterprises, however the bureaucratic process involved in obtaining a research permit is prohibitive. As with Europe there are no specific food safety controls for products coming from systems that are for human consumption (D8.2). Licencing is still the main overall barrier to production from a legislation standpoint across the project area.

With regards to the hatchery, there are no specific economic barriers other than the cost of seed production and nursery on-growing. If an enterprise is starting the process of constructing a facility, then the costs may be prohibitive. IMTA may be a solution for hatchery producers with the opportunity of producing more than one species at different times of the year to offset the costs of purchasing seed or juveniles across the whole system.

Table 3: Overview of the CS3: Land based IMTA, hatchery related production barriers for abalone species including their live algal settlement substrates are identified. CS leaders have identified the production barriers related to specific species within CS3.

Ident.	Output type	LTS Production Barrier	Addressing the Production Barrier	Current TRL	WP task
3.1.1	Process/ Technology Transfer	Use of *Ulvella lens* for settlement under organic	The output of the task will provide protocols including data for integrated hatchery production to be implemented at a	4-5	T1.1, T1.2, T1.3

		certification standard.	industry/commercial level under organic certification standard		
3.1.2	Process/ Technology Transfer	Land based abalone hatchery system.	The output of the task will provide protocols including procedures for abalone hatchery systems to standardise settlement induction protocols for tech-transfer throughout the project area.	4-5	T1.1, T1.2, T1.3
3.1.3	Process/ Technology Transfer	Method to ensure consistent settlement of South African abalone using species specific cues (abalone mucous) were developed for instances when the natural diatom cultures were inconsistent.	Standardise the commercial production method and provide relevant settlement protocols and data for abalone hatchery systems to standardise settlement induction protocols across the project area.	4-5	T1.1, T1.2, T1.3
3.2.1	Process/ Technology Transfer	Understanding the changes associated with global warming.	The output will investigate abalone nursery production under a global warming scenario and provide protocols to mitigate direct impact.	5	T1.2
3.3.1	Process/ Technology Transfer	Optimised nursery system for European abalone.	EU abalone production is in a state of flux and this output will compile available technologies including robust protocols to aid the advancement of the current industry standard	3-4	T1.2

Case Study 7 Sea cucumber species, site selection and key hatchery steps

CS Lead: Alfred Wegener Institute (AWI)

Sea cucumbers are well suited for integration into IMTA and polyculture (Zamora et al 2018). The culture of sea cucumbers in Europe remains novel and is poorly investigated. This is primarily due to the prior lack of fisheries pressure, which has driven the aquaculture research on the other species in other areas of the world, primarily Asia (Purcell 2014). Production research is in its infancy in Brazil and South Africa, where the majority of CS7 specific tasks are taking place. The fundamental biological information required to establish a species in the hatchery in some cases is not fully understood. CS7 is investigating the reproductive cycle of sea cucumber species in South Africa to fully understand the yearly cycle and enable induction in the hatchery. A suitable endemic species firstly needs to be identified followed by broodstock collection. In this case *Neostichopus grammatus*, in South Africa and *Holothuria grisea* in Brazil. The first main production barrier in CS7 is primary spawning induction and fertilisation followed by larval rearing and settlement. The participants in CS7 have extensive experience in rearing echinoderm larvae along with the closure of two European sea cucumber species life cycles. The juveniles reared in CS7 will be integrated into IMTA systems as part of CS3. The initial hatchery testing can provide insights into suitability for future production and closure of the value chain. Table 4 contains the specific current barriers to production, which will be addressed through the AquaVitae project. This specific case study will benefit from clustering activities with the BlueBio funded InEVal project which is focusing on new value chains for echinoderms (BlueBio ERA-NET Co-fund H2020 Project number 817992).

Main barriers

1. Lack of robust hatchery protocols for sea cucumber species in the project area. CS7 in conjunction with CS3 will develop and implement hatchery production methods for sea cucumbers of commercial interest. As sea cucumbers are well suited to IMTA system integration, development and implementation of protocols will enhance LTS seed production.
2. Suitable settlement cues and settlement substrates (Linked with CS3). Identification of live algal settlement cues will be utilised which will increase settlement and post larval survival.

Legislation and economic barriers to hatchery production

The main legislative hurdles for invertebrate hatcheries across the project area are water access (pumping sites are difficult to establish/obtain) and the terms of water discharge related to biosecurity concerns. These limitations are not evenly distributed throughout Europe, with e.g. French and Spanish hatcheries facing less scrutiny / being established as industries due to the greater recognition of aquaculture production. These limitations can of course be partially avoided using closed or semi-closed RAS hatchery systems. This is probably the most legislatively viable approach for sea cucumbers.

Economic viability seems to depend on the market strength of the product in terms of end-consumer demand. Where there is a demand for the end product this creates demand down the value chain from the producers all the way back to the hatcheries. This leaves limited space for novel species or novel hatcheries However, large existing hatcheries (in the case of bivalves) have the capacity to experiment with alternative species may be the best way to establish economically viable sea cucumber hatchery production as a diversification of species in their hatchery. However, this is contingent on the relatively rapid establishment / accessing of end-consumer market for sea cucumbers.

Table 4: Overview of the CS7: Sea cucumber species, site selection and key hatchery steps. CS leaders have identified the production barriers related to specific species within CS7.

Ident.	Output type	LTS Production Barrier	Addressing the Production Barrier	Current TRL	WP task
7.3.1	Process	Hatchery protocols for sea cucumber larvae production in Brazil and South Africa.	These protocols will include details of the application of adapted methods for broodstock collection, broodstock spawning, fertilisation, larval rearing and larval settlement to first artificial feeding of settled juveniles. These methods will be of significant value to the farmers interested in the integration of sea cucumbers in South Africa and Brazil in Abalone and Oyster farms respectively.	4	T1.2, T1.4

Case Study 8 Improving seed availability and grow-out of native and non-native oysters

CS Lead: Swedish Environmental Research Institute (IVL)

The development and expansion of the oyster sector in the Atlantic area is hampered by oyster seed availability. Key bottlenecks have been identified by the partners and participants in CS8 (see table 5). The primary and main barrier to increased production is the lack of protocols for production of seed of native oyster species. CS8 will compile the different approaches to native oyster seed production, i.e. hatchery production, spatting pond technology and sea-based seed collection, in an aim to best aid producers to overcome these inconsistencies. The production of *Ostrea edulis* in Europe has fallen due to the lack of reliable land-based hatchery production. In AV, commercial procedures are therefore being developed utilising large scale spatting ponds to provide a robust protocol for the European industry. Production of the native *Crassostrea gasar* in Brazil is at its first stages of hatchery development, and in AV activities to support this production includes development of live algae feeds, brood stock conditioning and water quality improvements for enhanced hatchery production of the species. Additionally, as similar inconsistent settlement rates and a need to optimize target species settlement during sea based seed collection are experienced (although for different species) at each partner location through the Atlantic, AV works with development of protocols and evaluation of settlement substrates for improves seed collection.

Specific CS8 production barriers:

1. Need for tech-transfer and optimisation of hatchery production of native oysters. In Europe the native flat oyster *O. edulis*, and in Brazil the native oyster *C. gasar*, are of increasing interest for oyster producers both in regard to hatchery production and spat collection using sea-based collectors. Suitable tech-transfer and procedures need to be implemented to address knowledge and technology gaps in the production process, and new procedures and protocols must be developed for emerging species.
2. Lack of technical information for producers to produce tetraploid and triploid pacific oysters in areas where oysters are underutilised. A road map will be developed to allow producers to overcome the challenge of ploidy manipulation. This will allow for great expansion of species production.
3. Industry requirement for the evaluation of alternative techniques and development of new protocols for collection of wild settled oyster spat targeting native oyster species in Scandinavia and Brazil.
4. Economic costs of hatchery production and wild spat collection. Data for cost-benefit analysis of hatchery production of oyster spat and collection of wild spat to identify the most economically valid technique in regions with emerging oyster production.

Legislation and economic barriers to hatchery production

Oysters, both native and non-native, across the project area experience similar biological, technical, legislative and economic barriers. One of the specific legislative barriers to oyster production is the culture of non-native species in new regions. This can be mitigated through the production of triploid oysters, however there is a licencing cost for the use of this technology and a general lack of knowledge about the efficiency of the production methods. The overall main legislative and economic barriers to oyster production is the licencing and start-up costs for new entrants to the industry. The economic cost for seed production can be prohibitive if there are unsuccessful seasons as a result of dependency on the success of the production system. Moreover, insecurity in seed supply, and consequently in production plans and return on investment, reduces the possibilities and incentives for farmers to invest in oyster aquaculture.

Table 5: Overview of the CS8: Improving seed availability and grow-out of native and non-native oysters. CS leaders have identified the production barriers related to specific species within CS8.

Ident.	Output type	LTS Production Barrier	Addressing the Production Barrier	Current TRL	WP task
8.1.1	Product	A new diet for *C. gasar* larvae in hatchery production	*Crassostrea gasar* is a new species in Brazilian aquaculture and hatchery protocols for the species are not well developed. The food requirements of *C. gasar* larvae is not well known and survival in hatchery production using traditional microalgae species is low. Native microalgae species may improve hatchery production of the species.	3	T1.2, T1.3, T1.4, T1.5
8.1.2	Report	A new conditioning protocol for *C. gasar*	To enable control of the reproductive cycle in hatchery conditions, a condition protocol must be developed for *C. gasar.*	3	T1.2, T1.3, T1.4, T1.5
8.1.3	Report	A new protocol for water improvements in small-scale oyster hatcheries using estuarine water	To enhance survival of *C. gasar* larvae in hatchery production, the importance of water quality and means to improve water quality must be evaluated.	3	T1.2, T1.3, T1.4, T1.5
8.1.4	Report	A new protocol for enhanced survival of flat oyster seed using small-scale, low-tech nursery systems	The production of *O. edulis*, the European flat oyster, is hampered by low seed availability. One of the major bottlenecks for small scale industries is transfer of seed from hatchery systems to sea based grow out systems as large scale industry nursery systems are often lacking. Alternative systems for small scale farmers must therefore be developed.	4	T1.2, T1.3, T1.4, T1.5

8.1.5	Report	A new production protocol for flat oyster spat pond production	Hatchery production is only economically viable in larger industries and less cost intensive seed production techniques suitable for small scale industries must therefore be developed. Spatting ponds is a promising technique but there are no protocols enabling implementation of this technique in new areas.	4	T1.2, T1.3, T1.4, T1.5
8.2.1	Report	A new protocol for sea based native oyster spat production including recommendations on new seed collector materials and new protocols adapted to local species	Seed production using sea-based collectors is a common strategy for extensive seed production. However, existing techniques are adapted to large scale industries and alternatives for small scale industries must be developed. Moreover, in areas where more than one oyster species exists a mixture of seed from different oyster species will be obtained on the collectors. Protocols to optimise capture of target species must therefore be developed.	4	T1.2, T1.3, T1.5, (T3.2)
8.2.2	Product	A new software for automatic identification of oyster species	Seed production using sea-based collectors is a common strategy for extensive seed production. In areas where more than one oyster species exists a mixture of seed from different oyster species will be obtained on the collectors. Automated methods to separate oyster seed by species must be developed.	4	T1.2, T1.3, T1.5, T3.2

Case Study 9 Offshore production of blue mussels

CS Lead: Danish Technical University (DTU)

The European production of blue mussels *Mytilus edulis* grown in rope culture is a sustainable form of bivalve production, however the industry is completely reliant on wild spat settlement or spat fall, which is fully dependent on regional ecological conditions inshore (Smaal, 2002). For decades growers through Europe have not invested in hatchery research for this species due to the industry production methods been interlinked directly with the wild availability of spat. For the further development of the mussel industry hatchery production techniques will be developed for use in inshore and offshore waters, CS9 participants have identified the main barriers to production (see table 6). Offshore or high energy sites may not have adequate recruitment or spat fall; therefore, a reliable hatchery methodology must be implemented. Along with the development of production techniques, these also need to be in line with requirements for organic certification, but likely ASC-certification should be possible. CS9 which is developing the offshore production of mussels needs a guaranteed supply of mussel seed and the main obstacles identified by the project participants in CS9 are:

1. Need for specific hatchery production techniques for *M. edulis* seed for on growing at inshore and offshore/exposed sites. CS9 will produce mussel larvae, settle them on to long lines and on-grow them in the hatchery. The size of seed with developed byssal threads and how to transfer them to the growing system will be identified. CS9 will identify/modify technologies to produce blue mussels in high-energy offshore areas and to ensure stable and cost-efficient supply of larvae/spat from the hatchery. Techniques will be developed to produce seed and transfer protocols for on growing at sea post hatchery.

Legislation and economic barriers to hatchery production

The main legislative barriers to hatchery production of mussel is the licencing of a land-based pump ashore facility and the subsequent economic costs. The direct economic cost of producing mussel seed and seeding them onto lines in the hatchery is unknow, this work is ongoing. European growers have not invested in a breeding programme like the New Zealand green lipped mussel industry, so we are unable to make comparisons. The economics of a failed wild settlement would be detrimental to an SME so it is the aim of CS9 to develop the technology required to overcome this issue in a viable timeframe.

Table 6: Overview of the CS9: Offshore production of blue mussels. CS leaders have identified the production barriers related to specific species within CS9.

Ident.	Output type	LTS Production Barrier	Addressing the Production Barrier	Current TRL	WP task
9.1.1	Process	Production protocol for hatchery produced blue mussel seed.	Development of a standard protocol for hatchery-based production of mussel seeds for industry scale production. Due to the feeding of the mussels with microalgae it might not be possible to get the final mussel product organic certified but could most likely be ASC certified.	4	T1.4, T1.5
9.2.1	Process	Method for grow-out of hatchery produced blue mussel spat.	Testing on-growing of hatchery produced mussel seed by different deployment methods and monitoring the biomass growth and biofouling. Due to the feeding of the mussels with microalgae it might not be possible to get the final mussel product organic certified but could most likely be ASC certified.	4	T1.4, T1.5

Case Study 10 Optimisation of freshwater fish production in Brazil

CS Lead: Empresa Brasileira de Pesquisa Agropecuaria (EmBraPa)

CS10 has identified hatchery production barriers for two species of freshwater fish of commercial interest *Arapaima gigas* and *Colossoma macropomum*, both of these species have their own specific culture challenges (see table 7). For *A. gigas* there are available tools for sex identification of broodstock (vitellogenin assay, cannulation, among others) but there is a need for technology transfer of these tools so these can reach farmers around the amazon region. Such tools are important to allow farmers to select and isolate pairs of *A. gigas* in the earth pond systems used to increase rates of reproduction success. Along with identification of the sex of *A. gigas,* tools or methods to monitor gonad maturation for each sex are needed as they are poorly understood for the male of the species. Other challenges such as egg collection, artificial fertilisation and larval rearing are also in need of protocol development. This information can be directly transferred to farmers and producers.

Colossoma macropomum is the second of the freshwater species in CS10 and the case study partners have identified the following main commercial barriers that need resolving. Production of sterile or triploid fish which will reduce the amount of early maturation in males and increase the overall product yield and protect genetic lines used by farmers. The musculoskeletal construction of the fish is of interest to understand the development and variation in intermuscular bones. Also, it is important to reduce the impact of the bones on fillet yield. With the two mentioned challenges the species requires a predictive model for animal selection (IBs) and a dedicated genetic programme to strengthen the current linages to expand production and reach new niche markets. CS10 will develop a predictive model for IBs however during the lifetime of AV it may not be possible to establish a dedicated breading programme.

Specific identified CS10 challenges are:

1 Reproduction protocols for *A. gigas* including larval rearing. Suitable protocols for extraction of sperm and eggs is a limiting factor for *A. gigas*, along with fertilisation and larval rearing in the hatchery. This fundamental research is required for further tech transfer to the wider industry in Brazil.
2. Sex identification in *A. gigas* broodstock along with monitoring of gonad maturation. Fundamental industry research needs to be carried out to address industry challenges for upscaling production in Brazil. Although there are available tools for sex identification of broodstock (vitellogenin assay, cannulation, among others), there is a need for technology transfer of these tools so these can reach farmers around the amazon region. Such tools are important to allow farmers select and isolate pairs of *A. gigas* in earth pond systems used to increase rates of reproduction success.
3. Ploidy manipulation for *Colossoma macropomum.* Sterility in farmed fish is still a challenge for the industry because early maturation of males causes growth reduction in the species. In addition, triploid fish culture could be beneficial for several reasons, including the potential for higher growth rates, increased carcass yield and meat quality. Use of this methodology will also help to protect farmers genetic lines for production.
4 intermuscular bones (IBs) and genetic breading programme. there is limited information about development and variation of IBs in tambaqui. The development of predictive models to

identify the types, number and length variation of intermuscular bones could be included used/applied in the development of breeding programs of the species. The development of breeding programs and genetically improved lineages is now the current challenge for these species to reach a new level of production

Legislation and economic barriers to hatchery production

As experienced in other regions of the project area the supportive legislation is also lacking in regard to the licencing of facilities for the production of freshwater fish in Brazil. D8.1 reported that environmental licensing is still the most important bottleneck for aquaculture development in Brazil, and to overcome this challenge, there is an urgent need for investments and qualified staff with aquaculture background not only at the federal level but also at the state and cities government levels. Start-up costs for the development of a production facility is one of the main economic barriers to production. Once the specific technical and biological challenges are overcome it will lead to an overall reduction of the initial hatchery start-up costs, where investors in the industry will have more confidence and less risk associated with production.

Table 7: Overview of the CS10: Optimisation of freshwater fish production in Brazil. CS leaders have identified the production barriers related to specific species within CS10.

Ident.	Output type	LTS Production Barrier	Addressing the Production Barrier	Current TRL	WP task
10.1.1	Process/ Technology Transfer	Optimized protocol for captive reproduction of pairs of *Arapaima gigas* in earth ponds.	Protocol designed to increase spawning rates of *A. gigas* using synthetic hormonal inducers for pairs in earth ponds.	3	T1.2, T2.2
10.1.2	Process/ Technology Transfer	Novel method for milt collection in *Arapaima gigas*.	Method to allow the collection of milt in *A. gigas*, needed to develop artificial fertilisation in the species and thus increase control of captive reproduction. Method should include a description of the collection process (fish handling, hormonal manipulation, sample collection).	3	T1.2
10.1.3	Process/ Technology Transfer	Novel method for egg collection in *Arapaima gigas*.	Method to allow the collection of eggs in *A. gigas*, needed to develop artificial fertilisation in the species and thus increase control of captive reproduction. Method should include a description of the collection process (appropriate timing for fish handling, hormonal manipulations, sample collection). Where possible this	3	T1.2

			challenge will be address during the lifetime of AV.		
10.2.1	Process	Protocol of triploid induction in *tambaqui* Spp. with minimum of 70% induction success.	A protocol to induce triploidy in *tambaqui* Spp. using pressure shock onto fertilised eggs.	3	T1.2
10.2.2	Process	Predictive model for IBs in *tambaqui* Spp.	Development of a predictive model for intermuscular bone in *tambaqui* Spp.	3	T1.2

Case Study 11 Marine fish farming

CS Lead: FURG

Marine fish farming in warmer waters is a relatively new industry in Brazil and commercial production is low. A few small-scale fish farms are involved in the grow-out of Brazilian flounder but they are considered to have significant potential. The first attempts to rear Brazilian flounder *Paralichthys orbignyanus* date from early 2000s'. Juvenile flounder are now routinely produced at the experimental scale at the FURG hatchery using standard larviculture protocols and experimental indoor on-growing of flounder has been achieved. Developing a standardised species-specific protocol which is utilised for warmer waters urgently need for the Brazilian flounder producers. CS11 will lead the development of reproduction protocols with partners and implement them in RAS systems. The CS11 partner are developing a fully integrated protocol for reproduction, larval rearing and on-growing in RAS.

Bottlenecks for industry development include:

1. Optimising reproduction and larviculture protocols for *Paralichthys orbignyanus*. Bottlenecks in larval production still remains an industry barrier for this species commercial production. CS11 will develop and implement robust production protocols for *P. orbignyanus* in a RAS system.

Legislation and economic barriers to hatchery production

Developing land based flow through marine facilities in Brazil have both legislative and economic barriers similar to previous case studies Licencing is one of the main barriers however the use of RAS may aid the future development due the reduced environmental impact. The economic barrier is mainly the cost of scaling up the production to commercial levels in RAS, this can be accomplished through the successful implementation of protocols developed in CS11.

Table 8: Overview of the CS11: Marine fish farming. CS leaders have identified the production barriers related to specific species within CS11.

Ident.	Output type	LTS Production Barrier	Addressing the Production Barrier	Current TRL	WP task
11.1.1	Process	Optimised process for larviculture in RAS	Flounder larviculture has been usually carried out in static (first few days after hatching) or semi-static systems (up to complete metamorphosis). CS11 is developing a complete larviculture cycle in a recirculating aquaculture system. We have done it this past breeding season, and juvenile output has been positive. We are looking forward to upscale it, in order to make it appropriate for the industry.	4	T1.2, T1.3, T1.4
11.1.2	Process	Optimised process for grow out in RAS	Despite successful spawning and juvenile production in captivity, production of flounder up to commercial size has not been reliable so far. Some attempts were carried out previously, CS11 is optimising the process, including the application of results from several experiments carried out in the last few years, and a new RAS system, using raceways instead of circular tanks.	4	T1.2, T1.3, T1.4

Conclusion

Specific species and hatchery production barriers have been identified through engagement with WP1, CS leaders and AquaVitae partners to develop a road map (specific to CS tasks) to resolve the production barriers to hatchery production of LTS utilised in the AquaVitae project. D1.3 along with D1.2 have identified the key task and challenges needed to be overcome barriers to the commercial production and advancement of LTS in the project area.

For each CS, the specific biological and technical barriers have been identified through participation of CS participants along with the general legislative and economic barriers experienced by operators as part of the AquaVitae project. D1.3 contains a roadmap to resolve barriers to production. The start of the road map are the detailed CS workplans followed by the stakeholder engagement and interviews, the development of a task and progress monitoring platform which culminates with the development of D1.2 and the technical appendices contained within this deliverable which report upon the technical and scientific progress for the first 12-months of the AV project.

Furthermore, the production barriers identified will be monitored through the CS reporting platform developed by WP1, WP2 & WP3 leaders and will form a significant part of the good practice recommendations in T1.4. Resolving key production barriers is central to the completion of the of D1.4.

The main legislative and economic barriers experienced by LTS operators across the project area are the licencing and scaling up of hatchery production in a timeframe that is economically viable. For this to be achievable the defined technical challenges need to be overcome.

Validating the production processes at an industry level is a priority of the AquaViate project and an integral part of the previous and future deliverables, addressing production challenges at an industry level can be used to advise local and regional authorities the benefits and opportunities of LTS production.

Monitoring of the challenges or barriers to production is an ongoing process and will be updated at each reporting period at a CS and WP level. This will allow mapping of the framework process to overcoming the technical, biological, legislative and economic barriers to LTS production.

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