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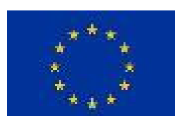

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Presence and containment of seaweed pathogens in hatcheries and farms

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Executive summary

This deliverable summarises the state-of-art regarding the detection and the containment of seaweed pathogens in hatcheries and seaweed farms. A survey of pathogens in wild European populations of seaweeds has been initiated. The work was mainly carried out at The Scottish Association for Marine Science facilities (SAMS, United Kingdom), as well in other seaweed farms (including GENIALG partners). As planned, monitoring of known pathogens has been performed on the cultivated kelp *Saccharina latissima.* In addition, the scope of the work has been extended to other kelps, *Alaria esculenta* and *Laminaria digitata*. Likewise, containment options have been explored for all the above species. Molecular tools have been developed to streamline the diagnosis of diseases and enable the robust, medium-throughput quantification of disease symptoms. Finally, a service opened not only to members of the consortium but also any other interested party has been set-up. It aims to detect diseases in cultivated and wild macroalgae (Phaeophyceae, Rhodophyta and Chlorophyta), and to accelerate the discovery and description of seaweed pathogens.

1. Introduction: currently known pathogens of cultivated seaweed

In algal cultivation, disease outbreaks can mean a high economic loss. For example, losses of 25-30% and even up to 64 %, of the crop have been reported in Japan and Korea (Gachon et al., 2010). The intensive and dense mariculture practices enable diseases to spread much faster than before and their impact is expected to increase (Gachon et al., 2010; Carney et al., 2014; Kim et al., 2014). For mass culture systems, it is important to monitor algal diseases for a better control and understanding of possible infections.

Marine ecosystems are no exception and seaweed pathogens are prevalent in nature and highly diverse. They include, amongst others, endophytes and epiphytes algae species, oomycetes, fungi (i.e. chytrids), algal-lysing bacteria, viruses etc. As an example, the Table 1 list all the known pathogens of the Kelp species *Saccharina latissima* which also infect other brown algae species.

Table 1.List of known pathogens of *Saccharina latissima* (and other brown algae host species of those pathogens).

Pathogens		Phaeophyceae Species including ***S. latissima***	Reference(s)
Taxonomic group	**Species**		
Phaeophyceae	*Laminarionema elsbetiae*	*Saccharina japonica* ***Saccharina latissima***	(1-5)
	Laminariocolax aecidioides	*Costaria costata* *Ecklonia maxima* *Fucus vesiculosus* *Himantothallus grandifolius* *Laminaria digitata* *Laminaria hyperborea* *Lessonia berteroana* *Lessonia nigrescens* *Macrocystis pyrifera* ***Saccharina latissima*** *Saccharina nigripes* *Saccharina sessilis* *Saccorhiza polyschides* *Undaria pinnatifida*	(5-7)
	Laminariocolax tomentosoides	*Alaria esculenta* *Himanthalia elongata* *Laminaria digitata* *Laminaria hyperborea* ***Saccharina latissima*** *Saccorhiza polyschides*	(5, 8, 9)
	Laminariocolax atlanticus	*Laminaria digitata* *Laminaria hyperborea* ***Saccharina latissima***	(5)
	Microspongium alariae	*Alaria esculenta* *Fucus* spp. ***Saccharina latissima***	(8, 10)
Chlorophyta	*Ulvella viridis* *(=Entocladia viridis)*	*Laminaria digitata* ***Saccharina latissima***	(11, 12)
Oomycota	*Eurychasma dicksonii*	*Pylaiella* sp. Ectocarpus siliculosus 45 species of brown seaweeds (including Laminariales)	(13) (14)
	Anisolpidium ectocarpii	*Ectocarpus* and other brown algae (including Laminariales)	(15-17)
Phytomyxean	*Maullinia ectocarpii*	*Ectocarpus* spp. and other brown algae (including Laminariales)	(18)
Fungi	*Chytridium polysiphoniae*	23 species of brown seaweeds (including Laminariales)	(14)
	Phycomelaina laminariae	*Alaria esculenta*, ***Saccharina latissima*** *Saccharina longicruris* *Laminaria digitata*	(11) and references therein
Bacteria	Proteobacteria Firmicutes Bacteroidetes Actinobacteria	***Saccharina latissima***	(19)
Virus	Phycodnaviridae	***Saccharina latissima***	

2. SAMS Hatchery setup and measurements

Since the beginning of the GENIALG project in 2017, a wet laboratory and the algal hatchery have been upgraded at SAMS. The work was carried out to meet the following specifications: 1) upscaling the quantity of biological material produced to meet the growing demand of biomass production (link with WP3); 2) developing protocols for quality control including detection methods of potential pathogen threats; 3) performing regular monitoring for pests in the hatchery, the seaweed farms but as well in wild populations; 4) to contain potential threats in the indoor facilities, for example through treatment of effluents.

2.1 Set-up of a wet laboratory and hatchery for microbiological containment

Overview of the hatchery process. The production process of seeded lines start with male and female kelp gametophyte cultures obtained with spores release of fertile sporophytes. The reader is referred to the review by Alsuwaiyan et *al.* (2019) for published protocols on the experimental release of kelp zoospores. aiming to identify commonalities and provide guidance on best practices (20). Spore release typically involves three main steps: i) a pre-treatment (physical and/or chemical) which included cleaning of the reproductive tissue to eliminate epiphytic organisms, ii) a desiccation of the reproductive material (sori) and iii) an immersion in a seawater medium to induce the spore release. In the SAMS wet laboratory, local fertile kelps are used to release spore and then gametophyte cultures are maintained in F/2 medium under red light (15-20 µmol·m^{-2}·s^{1} 12:12 L:D, 10°C) (21) to foster vegetative growth. Sexual reproduction is induced by gametophyte fragmentation below 70 µm, combined into a 2L bubbled culture of F/2 medium (21) and moved in white light (25-30 µmol· m^{-}

Figure 1. (A). Wet laboratory set-up used for spores release from gametophytes prior to the hatchery step, as well as the analysis and the quality control of seeded lines. Benches and an autoclave (visible at the back) were installed, with small equipment such as stereomicroscope (on the left). (B). Kelp gametophytes are kept under red light (12:12) at constant temperature (13°C) in incubators (two are visible in A). (C). A laminar flow has been installed to work under sterile conditions, in particular to avoid contamination of the samples by pests or potential pathogens.

^{2}·s^{1} 12:12 L:D, 10°C). Within two weeks, the gametophytes become fertile and juvenile sporophytes are evident. Twine spools are then seeded at a density of 10,000 sporophyte·m^{-1}. In the first week, the seawater is treated with a saturated germanium dioxide solution (22, 23) to prevent diatom growth. The tanks are drained, cleaned and refreshed each week. Environmental conditions are controlled and kept stable to ensure growth. Room temperature is set to 12.5 °C with a chiller unit (AK-RC101, Danfoss, UK), with lighting was provided by cool white LEDs (12:12 L:D,Figure 2). The physiochemical parameters of the tanks are monitored three times a week: dissolved oxygen and temperature are recorded using an Oxi3401 meter equipped with a DurOx 325 sensor (WTW, UK). Temperature-corrected free scale pH is recorded using a handheld meter (902P, SciQuip Ltd, UK) equipped with a pH probe (LE438, Mettler Toledo, USA) (Figure 3 and Figure 5).

Wet lab improvements. To increase capacity, various pieces of equipment were purchased and installed to allow the production of spores in microbiologically controlled conditions. This includes several incubators, a laminar flow hood, an autoclave (Fig. 1). Likewise, a culture room was renovated to ensure constant, controllable cultivation conditions. A sand- and UV-filtration system was installed to purify inflow seawater (Fig. 2).

Figure 2. Hatchery setup with a capacity of 45km of twine lines (8000L), as off 2019. The principal components are the seawater supply with a sand-filtration system at 50, 20 and 1 µm (Fileder Filter Systems Ltd, UK) followed by 110W UV sterilisation (P2, Tropical Marine Centre Ltd, UK; see the blue structure on the rear wall). The tanks are independent from each other and easily washable, with waterproof LED lighting. The room was air-conditioned to maintain a constant temperature, a constant oxygenation intake for each tank.

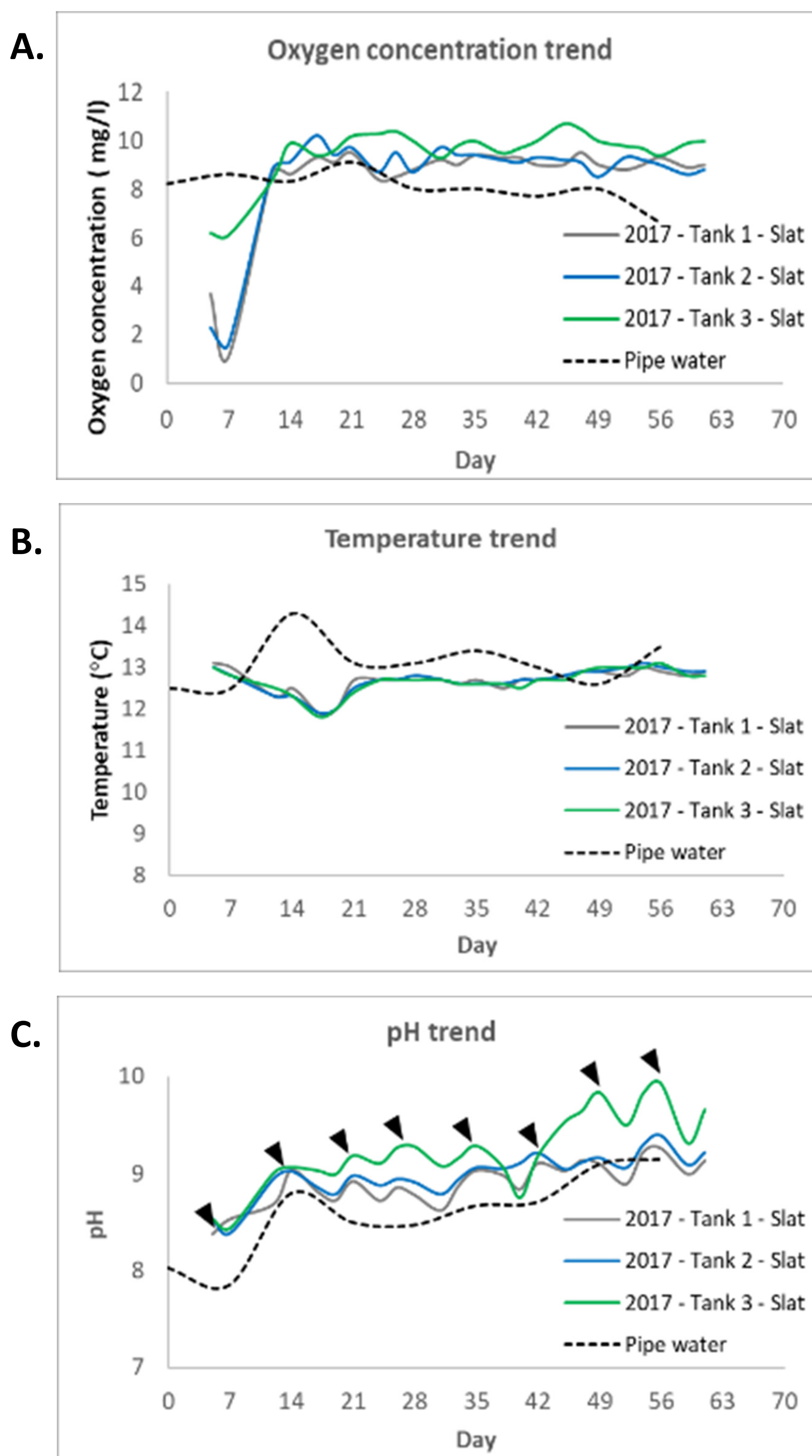
Set-up of microbiological containment and waste disposal. A 3-step custom process was designed and implemented at SAMS to ensure the microbiological safety of all hatchery effluents. First, the seawater from the drain and from the hatchery tanks is filtered to remove the organic matter. Then, a concentrated Stabilised Hydrogen Peroxide (EndoSan, Endo Enterprises, UK) solution is mix with the

seawater in a hazardous waste container. According to the manufacturer: "EndoSan is a solution of Hydrogen Peroxide (H2O2) which is stabilised using a proprietary ionic silver based chemistry. When correctly applied to water, air or any surface, EndoSan will disinfect (Gram Positive and Gram Negative bacteria, Viruses, Fungi, Spore, Algae, Mycobacteria and Protozoa) through an oxidisation process and continue to safely provide unrivalled residual performance. EndoSan is chlorine and alcohol free, with no corrosive effects on usual materials of construction during application. After use it simply degrades into harmless water and oxygen as one of the safest forms of disinfection. EndoSan is a highly effective water treatment solution which is a truly credible alternative to traditional chlorine disinfection methods. It will physically remove biofilms whilst being non-toxic, non-corrosive and will maintain its efficacy at a wide range of temperatures and pH values. Uniquely, EndoSan is also the only product of its kind to obtain NSF/ANSI Standard drinking water disinfection approval (http://www.star-international.co.uk/)".

The typical disinfection level is between 100 and 200ppm EndoSan (i.e. 50 and 100ppm peroxide) for 24 hours depending if the system is clean or dirty. The disinfection level of 200ppm EndoSan is safe for washing and drinking. Preliminary degradation trial in standing water shows that targeting for 100ppm H202, 94-96ppm were measured immediately, down to 93-94 ppm after 24 hours and to 85ppm after a week making the seawater environmentally safe.

2.2 Quality control of the hatchery process

A procedure for regular monitoring has been optimised and is now carried out routinely in the hatchery. The exemplary data shown in Figs. 3-4 were acquired in 2017 by an MSc student during an ERASMUS exchange. They illustrate some optimisations that have been introduced to the hatchery process. Fig. 5-6 illustrate similar data in 2019, where cultivation was conducted at a larger scale, on several kelps species, and when conditions were more stable.



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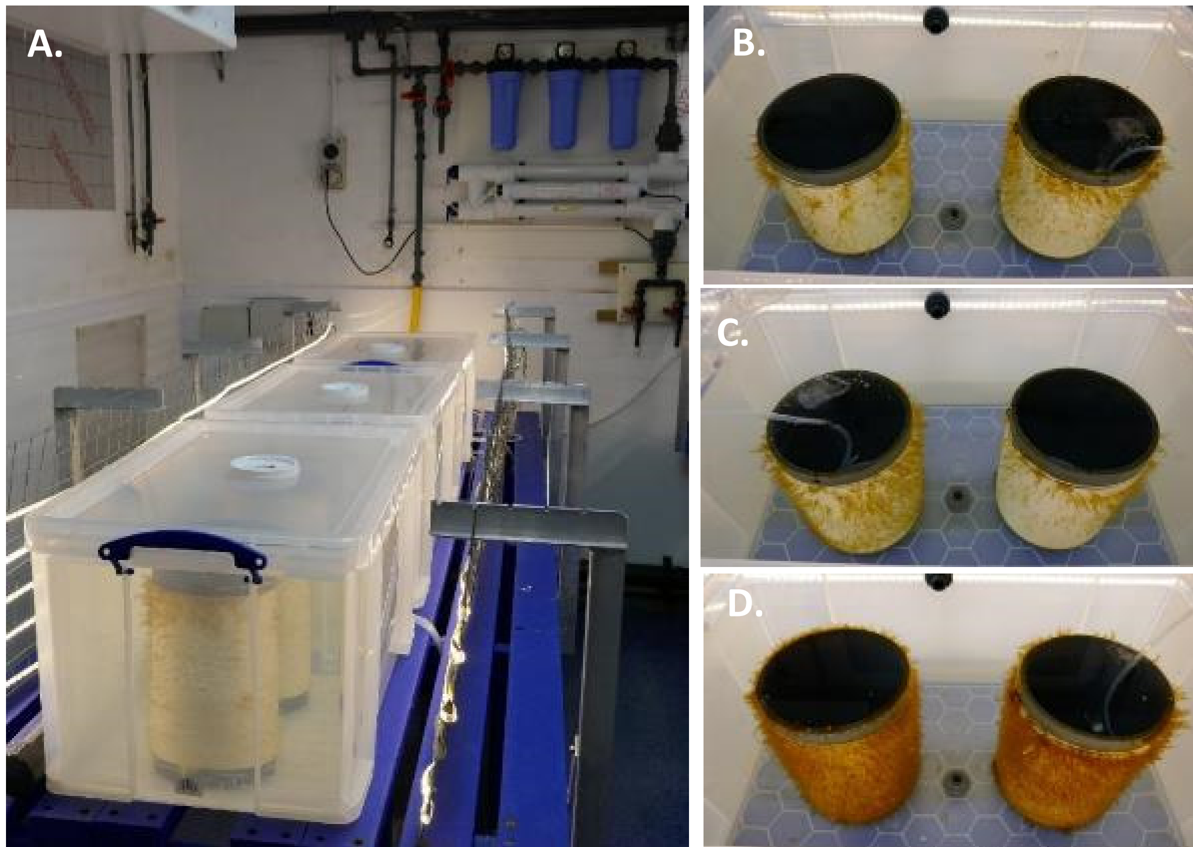

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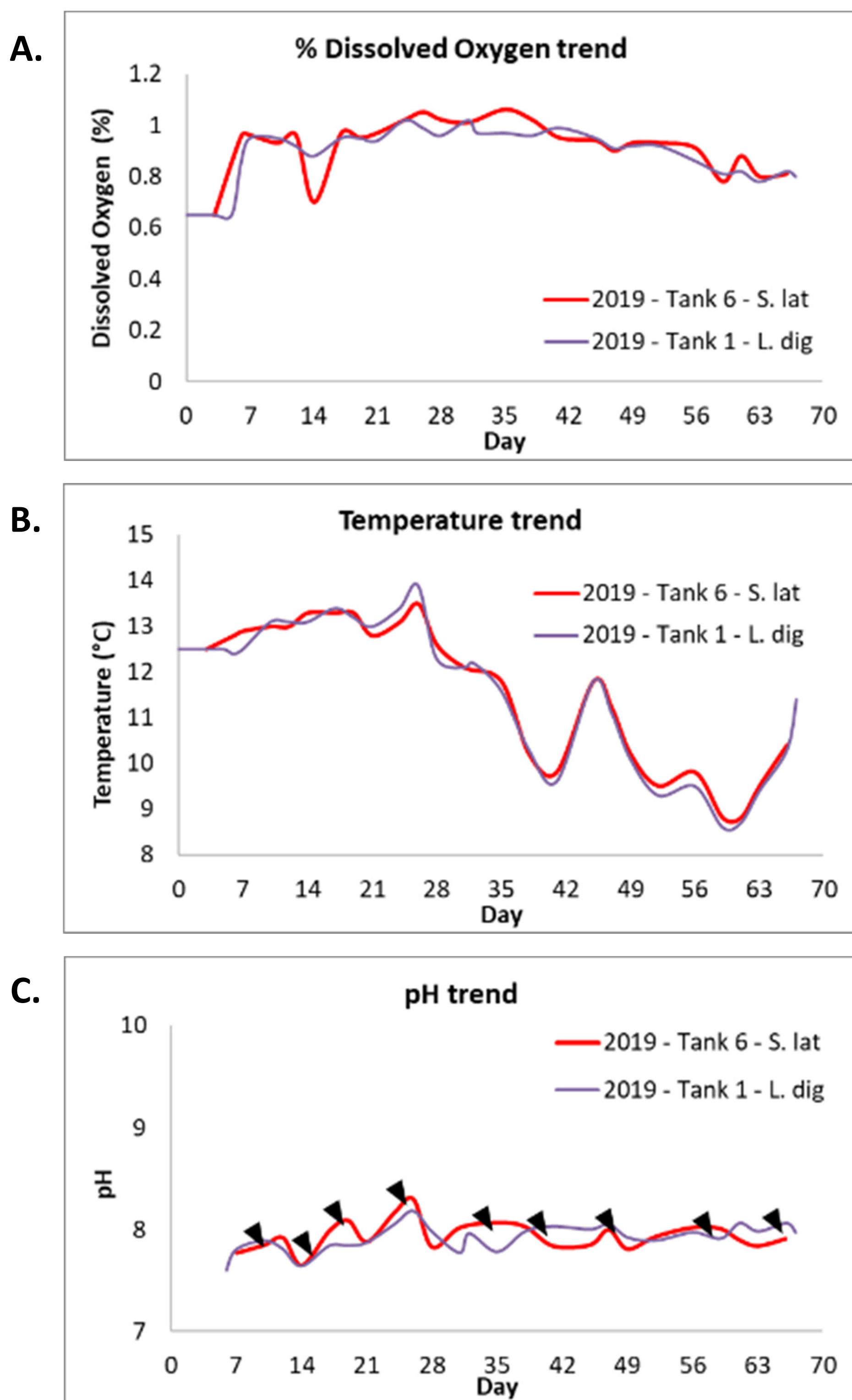

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Figure 6. (A) SAMS hatchery tanks prior to the lines deployment at SAMS seaweed farm in 2019. The tank 1 and 6 (B, C) show respectively a good development of *Laminaria digitata* and *Saccharina latissima* sporophytes (biomass and pigmentation) at 20, 27, 49 and 66 days post spores release.

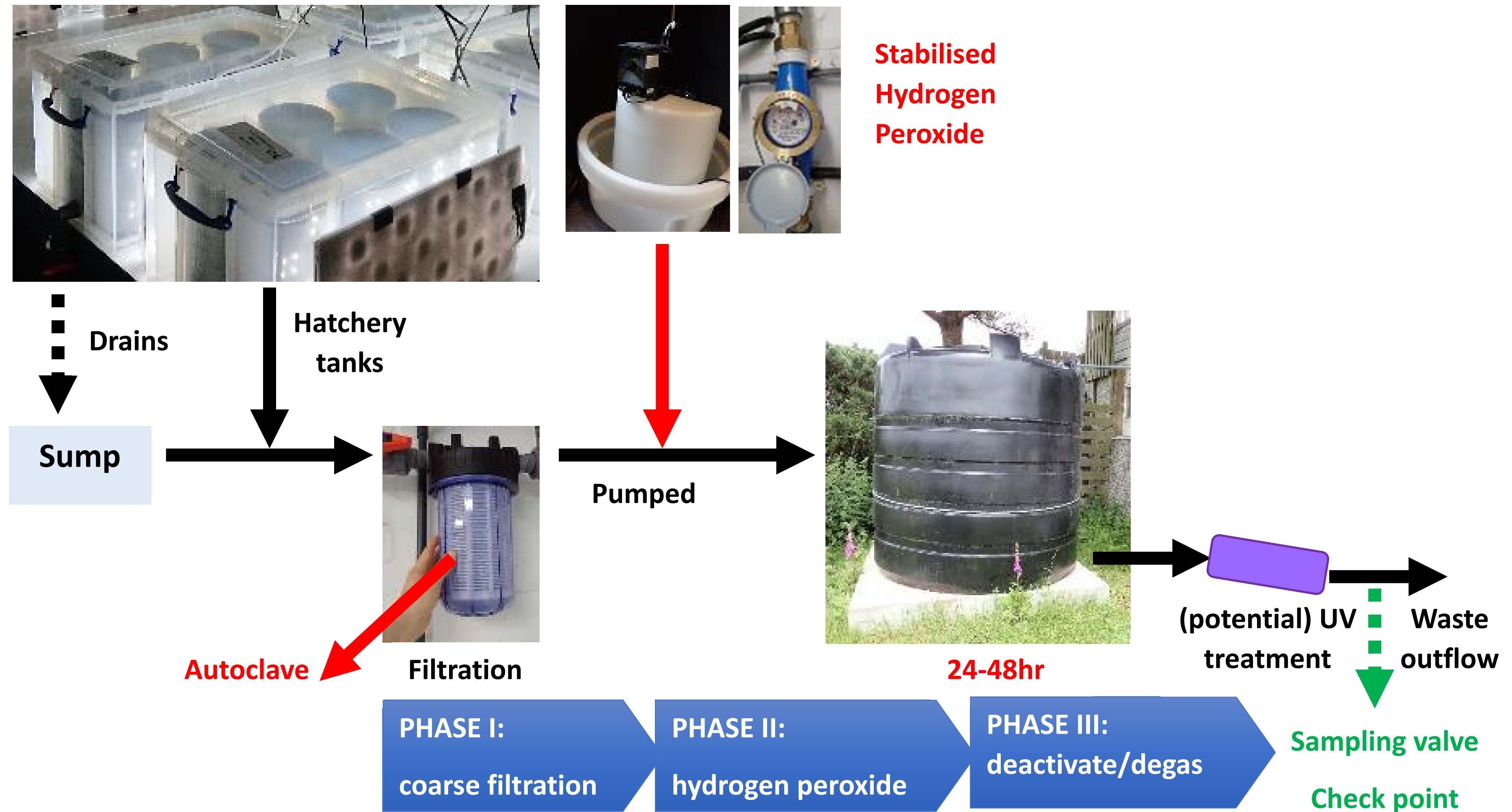


Figure 7. Containment and treatment of the seawater waste from the hatchery before disposal. This process takes place in 3 phases. Phase I: The seawater from the drain and from the hatchery tanks are filtered to remove the main organic matter after which the filter are autoclave to sterilised them. Phase II: Stabilised Hydrogen Peroxide "EndoSan" is mixed with the seawater to kill living brown algae and other potential organisms such as Gram Positive and Gram Negative bacteria, Viruses, Fungi, Spore, Algae, Mycobacteria and Protozoa (http://www.star-international.co.uk/images/PDFs/endosan_brochure_v_7.pdf).

3. New diagnostic methods for algal pathogens

A first step of disease diagnostic is typically the histological examination of symptoms. Depending on the algal species analysed, its morphology, and the fixation methods used for the samples (fresh material, dry, wet in different type of buffers) the preparation of the samples for histology will vary. Preparation methods may involve tissue fixation (for example in 4% Paraformaldehyde), embedding (for example in paraffin), washing and/or rehydration. Thick tissues (e.g. sporophytes of kelps) are cut in fine sections with a microtome. Histology slides are prepared by mounting either prepared sections of algae or directly more simple structure as single cells and filamentous algae. To help diagnostic, samples may be stained with DNA stains such as DAPI or Sybr-Green, or more specific strain such as calcofluor white which stain cellulose i.e. oomycete pathogen cell walls (*26*). Microscopic observation is usually performed with differential interference contrast (DIC) microscopy and/or by fluorescence microscopy (Chlorophyll autofluorescence λext = 485 nm, λem = 655 nm). Existing protocols have been continuously applied and refined during the project.

In addition, effort along two complementary line: development of molecular diagnostic tools for a number of pathogens found in seaweed hatcheries and in the open sea, and of a cost-effective, quantitative, medium throughput method for laboratory measurement of disease resistance, applicable for quantitative genetics (link WP2).

3.1. A non-invasive, cost-effective, quantitative and parallelisable assay for algal disease resistance

Non-destructive methods (unlike histology and qPCR assays) are necessary to follow-up on the evolution of pathogen infection over time. In collaboration between SAMS and CNRS-SBR, a non-invasive, parallelisable method to measure disease resistance has been developed. The method relies on the combination of biomass measurement with nephelometry and the labelling of the pathogen with a fluorochrome. Its proof of concept has been published, alongside other possible applications for growth and fertility measurement. The assay is now used to quantify the degree of infection of the oomycete *Anisolpidium ectocarpii* in laboratory cultures of the model brown filamentous alga *Ectocarpus*.

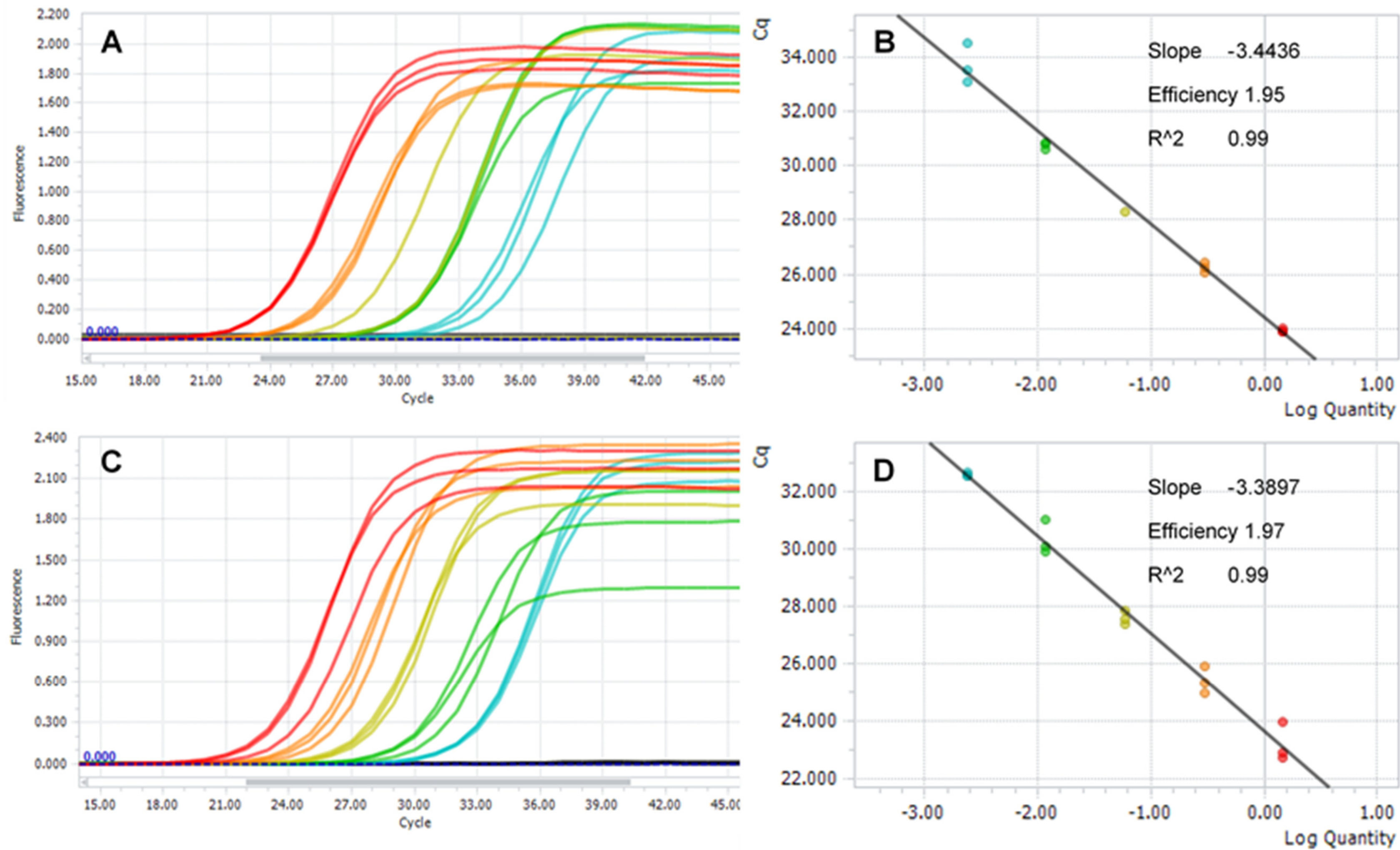
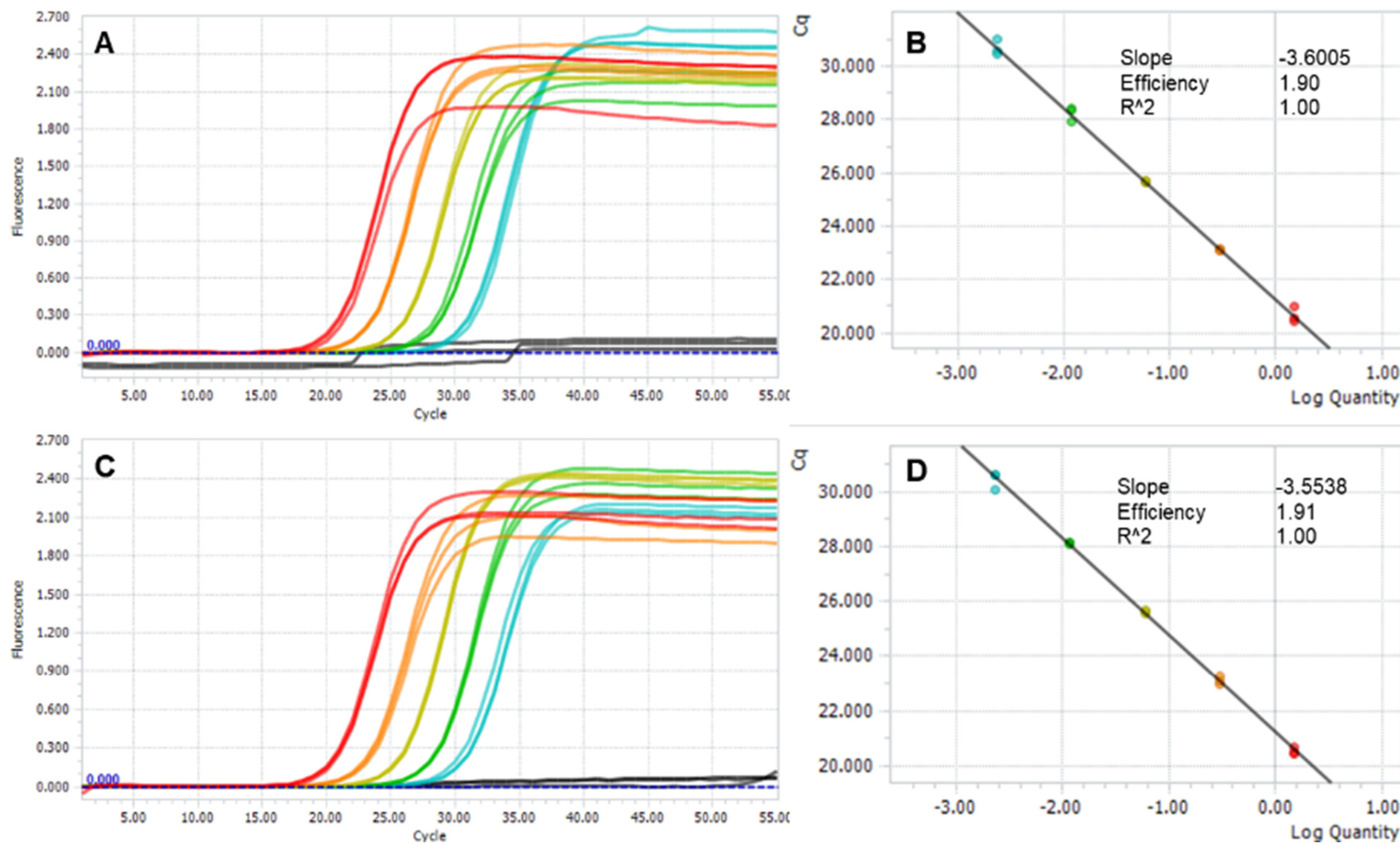


Figure 8. Preliminary results of the molecular quantification test of the brown algae endophyte *Laminariocolax* which is currently under development. The DNA amplification curves (A, C) of two different pairs of primers show a linear correlation between the logarithm of the DNA quantity and the cycle quantification value (Cq) (respectively B and D).



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3.2. Molecular detection of pathogens

3.2.1 Brown algae endophytes

The main endophytes pathogens of *Saccharina latissima* are brown algae from the Ectocarpales order: *Laminarionema elsbetiae (1, 3), Laminariocolax aecidioides, L. tomentosoides, L. atlanticus* (5). They induce symptoms including warts, dark spots, galls or twists in the thallus (which could also be asymptomatic). The prevalence in the wild of those endophytes varies from one population to another (5). For example, Bernard et *al*. 2018 reported that 33% of sporophytes from Southern Brittany contained detectable amounts of *L. elsbetiae*, compared with 85-93% for wild *S. latissima* from Northern Brittany and Western Scotland.

Prior to the start of GENIALG, CNRS Roscoff developed a qPCR protocol for the quantitative detection of *L. elsbetiae* in brown algae thallus (2). This protocol can be used on laboratory-grown samples and on samples from seaweed farms. In order to be able to detect the second main type of *Saccharina latissima* endophytes, SAMS has been developing a similar test for *Laminariocolax* genus (Figure 8). Preliminary results suggest that it will be possible to quantify the degree of endophyte infection in brown algae and we are hopeful to validate this test on wild and farm *S. latissima* samples. Ultimately, these results should be published in a peer-reviewed journal (cf. §7) and the qPCR *Laminariocolax* and *L. elsbetiae* detection tests could be used for kelp pathogen diagnostic and survey.

3.2.2 Oomycetes

Marine oomycetes are also important algae pathogens and are the cause of serious economic losses every year (27). For example, *Olpidiopsis* is one of the most common disease of *Pyropia* (Rhodophyta) and the total economic loss caused by it may be more than US $10 million per year just in Korea (28). Gametophyte stage and young sporophytes of cultivated kelp species, such as *Saccharina latissima*, can be infected by two different species of oomycetes: *Anisolpidium ectocarpii (16)* and *Eurychasma dicksonii* (14).

Prior to the start of GENIALG, a qPCR test already existed to quantify *Eurychasma dicksonii* in brown algae (29). Here, SAMS has developed a similar assay for the oomycete *Anisolpidium ectocarpii* in brown algae cultures (Figure 9). This test is now used to quantify the degree of disease resistance of this pathogens within members of the same *Ectocarpus* family and will shortly be used for the GWAS experiment planned in WP2.

Serendipitously, during field sampling targeted at kelps, pathogens of epiphytic diatoms were observed. Their molecular characterisation was initiated, in collaboration with a PhD student funded externally. This revealed that these pathogens belong to entirely novel groups of oomycetes, closely related to *Olpidiopsis* spp., some of which are economically relevant pathogens of red algae (30). Whilst not directly aligned with the initial objectives of the grant, this study provides an important, unexpected insight into the diversity of oomycete pathogens of marine algae and also allowed us to pilot novel technologies relevant to GENIALG. In particular, the draft genome sequencing of pathogen using single infected cells proved very useful to acquire quickly enough sequence information for phylogenetic analysis.

4. Monitoring of *Saccharina latissima* pathogens in hatcheries, farms, and wild populations

At SAMS, monitoring of the hatchery (prior to the lines deployment) and of the seaweed farm (after deployment) has been carried on *Saccharina latissima* lines every 6 weeks from September 2017 to December 2019 (Figure 10). In parallel, CNRS Roscoff performed monitoring with a similar protocol and applied the *L. elsbetiae* qPCR assay in cultivation conditions. They showed respectively no and a low infection (<12%) rate 6 months post transfer (2). On the other hand, wild populations of *S. latissima* shown a seasonal and geographic variation of higher infection rates (2).

Figure 10. Diagram representing the regular morning of SAMS hatchery during two seaweed farming seasons (Sept 2017-Dec 2018 and Sept 2018-Dec 2019) as well as prior to the lines deployment (Sept 2017). When possible 5 different pieces of tissue were taken from the holdfast, the stipe, at the beginning, the middle and the tip of the blade and stored for further analysis.

5. Towards an Open Access resources on algal diseases, with a worldwide remit.

To further extend the scope of the work performed under GENIALG, we have seized the opportunity to mutualise efforts with a project funded by the UKRI Global Challenges Research Fund. The latter aims to characterise and manage diseases of farmed seaweeds in tropical countries, with a particular focus on the hydrocolloid-producing genera *Kappaphycus* and *Eucheuma*. Taken together, the GENIALG and GlobalSeaweed-STAR consortia undertake experimental and field work on the diseases of kelp and red algae, across Europe and tropical countries. To complement the data acquired by both consortia, we have developed an online platform called “My Seaweed Looks Weird” Figure 11 (https://www.globalseaweed.org/?page_id=889) aiming to:

- Offer our expertise in disease diagnostic to seaweed producers (including, but not restricted to GENIALG partners)
- Encourage reporting of diseases in a participative way, even on algal species that are not worked on by consortium members
- Accelerate the discovery and description of pathogens in seaweed farm and wild populations

My seaweed looks weird

About the project

Our aim is to accelerate the description of algal diseases worldwide, by screening samples submitted by scientists, seaweed professionals or members of the public. We will use the data to identify and map diseases, as well as describe novel or emerging issues. With time, we will make the results of this work available for free, through Open Access publications and a (yet-to-be-built) online disease atlas.

Read More ›

How to add your data

You can report an algal disease using this short form. We welcome your information and your samples, even if you are not sure whether they are relevant to us.

Start here ›

What we will do with your samples

Using a combination of microscopy and potentially DNA analysis, we shall endeavour to send you back an in-kind diagnosis for all samples received.

I want to know more ›

Figure 11. Screenshot of the “My Seaweed Looks Weird” online portal.

6. We are using our histology and molecular methods

(cf. §3 New diagnostic methods for algal pathogens).

A first step of disease diagnostic is typically the histological examination of symptoms. Depending on the algal species analysed, its morphology, and the fixation methods used for the samples (fresh material, dry, wet in different type of buffers) the preparation of the samples for histology will vary. Preparation methods may involve tissue fixation (for example in 4% Paraformaldehyde), embedding (for example in paraffin), washing and/or rehydration. Thick tissues (e.g. sporophytes of kelps) are cut in fine sections with a microtome. Histology slides are prepared by mounting either prepared sections of algae or directly more simple structure as single cells and filamentous algae. To help diagnostic, samples may be stained with DNA stains such as DAPI or Sybr-Green, or more specific strain such as calcofluor white which stain cellulose i.e. oomycete pathogen cell walls (26). Microscopic observation is usually performed with differential interference contrast (DIC) microscopy and/or by fluorescence microscopy (Chlorophyll autofluorescence λext = 485 nm, λem = 655 nm). Existing protocols have been continuously applied and refined during the project.

In addition, effort along two complementary line: development of molecular diagnostic tools for a number of pathogens found in seaweed hatcheries and in the open sea, and of a cost-effective, quantitative, medium throughput method for laboratory measurement of disease resistance, applicable for quantitative genetics (link WP2).

Since the inception of the web portal, we have dealt with several cases, such as quality control of seeded lines before their deployment at sea, or different cultivated kelp species on farms. The service is set-up in a way privileging information exchange and collaboration. However, bearing in mind the commercial sensitivity of some information, it also allows for submitters to request the anonymization of cases and samples. Already, this approach has enabled us to discover novel pathogens, which we are currently working on describing, in collaboration with the submitters. Our objective is to create a database of reports, from which we will generate a fully Open Access, online atlas of seaweed diseases. Particular care is being taken to develop this platform with a long-term view, especially to make it sustainable after the end of GENIALG. As a first step, it is now hosted on the globalseaweed.org domain, which has been purchased for 10 years (2018-27).

7. Outputs

Three peer-reviewed manuscripts have already been published under the umbrella of this deliverable. An exemplary list of oral communications in conferences is also given here to illustrate the breadth of our activities (an exhaustive list of talks and posters has been included in the GENIALG reporting on dissemination activities).

7.1 A highly prevalent filamentous algal endophyte in natural populations of the sugar kelp *Saccharina latissima* is not detected during cultivation in Northern Brittany (GENIALG partner: CNRS)

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Abstract.

The sugar kelp *Saccharina latissima* is cultivated in Europe for food, feed and ultimately the production of chemical commodities and bioenergy. Being cultivated in the open sea, *S. latissima* is exposed to potentially harmful organisms, such as *Laminarionema elsbetiae*, a filamentous brown algal endophyte with a very high prevalence in wild populations of European *S. latissima*. As it was shown previously that S. latissima sporophytes get infected by *L. elsbetiae* very early in their life, seeding the spores on collectors and keeping them under controlled conditions during the critical time of a possible infection with filamentous endophytes could be advantageous over direct seeding techniques, where the ropes are deployed within days after seeding. We used a qPCR-assay to assess the prevalence of the endophyte *L. elsbetiae* in *S. latissima* cultivated during winter in Northern Brittany, comparing individuals from direct-seeded ropes and collector seeded lines that were kept in laboratory conditions for different time spans. No DNA of the endophyte was detected in the samples, suggesting that either the kelps were not infected or the amount of endophytic filaments were below the detection rate of the qPCR assay. Furthermore, *L. elsbetiae* could not be detected in the seawater surrounding the kelp farm, indicating that *L. elsbetiae* is not fertile or disperses at a very small scale in Northern Brittany during the deployment time of young kelps. Our results suggest that infections of cultivated *S. latissima* with the endophyte *L. elsbetiae* might be a minor problem in kelp farms in Northern Brittany if the seeding production is kept under controlled conditions without external contamination.

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7.2 Parallelisable non-invasive biomass, fitness and growth measurement of macroalgae and other protists with nephelometry (GENIALG partners: SAMS and CNRS)

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Abstract.

With the exponential development of algal aquaculture and blue biotechnology, there is a strong demand for simple, inexpensive, high-throughput, quantitative phenotyping assays to measure the biomass, growth and fertility of algae and other marine protists. Here, we validate nephelometry, a method that relies on measuring the scattering of light by particles in suspension, as a non-invasive tool to measure in real-time the biomass of aquatic micro-organisms, such as microalgae, filamentous algae, as well as non-photosynthetic protists. Nephelometry is equally applicable to optic density and chlorophyll fluorescence measurements for the quantification of some microalgae, but outperforms other spectroscopy methods to quantify the biomass of biofilm forming and filamentous algae, highly pigmented species and non-photosynthetic eukaryotes. Thanks to its insensitivity to the sample's pigmentation, nephelometry is also the method of choice when chlorophyll content varies between

samples or time points, for example due to abiotic stress or pathogen infection. As examples, we illustrate how nephelometry can be combined with fluorometry or image analysis to monitor the quantity and time-course of spore release in fertile kelps or the progression of symptoms in diseased algal cultures.

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7.3 "*Ectrogella*" Parasitoids of the Diatom *Licmophora* sp. are Polyphyletic (GENIALG partner: CNRS)

Published in Journal of Eukaryotic Microbiology Volume67, Issue1 (18-27) (*30*)
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Abstract.

The diatom genera *Licmophora* and *Fragilaria* are frequent epiphytes on marine macroalgae and can be infected by intracellular parasitoids traditionally assigned to the oomycete genus *Ectrogella*. Much debate and uncertainty remains about the taxonomy of these oomycetes, not least due to their morphological and developmental plasticity. Here, we used single-cell techniques to obtain partial sequences of the parasitoids 18S and cox2 genes. The former falls into two recently identified clades of *Pseudo-nitzschia* parasites temporarily named OOM_1_2 and OOM_2, closely related to the genera of brown and red algal pathogens *Anisolpidium* and *Olpidiopsis*. A third group of sequences falls at the base of the red algal parasites assigned to *Olpidiopsis*. In one instance, two oomycete parasitoids seemed to co-exist in a single diatom cell; this co-occurrence of distinct parasitoid taxa not only within a population of diatom epiphytes, but also within the same host cell, possibly explains the ongoing confusion in the taxonomy of these parasitoids. We demonstrate the polyphyly of *Licmophora* parasitoids previously assigned to *Ectrogella* (sensu Sparrow, 1960) and show that parasites of red algae assigned to the genus *Olpidiopsis* are most likely not monophyletic. We conclude that combining single-cell microscopy and molecular methods is necessary for their full characterisation.

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7.4 Oral presentation: Towards marker-assisted selection for resistance to Oomycetes in Brown Algae (GENIALG partner: SAMS)

Oomycete Molecular Genetics Networks – 20th Annual Meeting (July 10-12th 2019, SAMS, Oban)

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Abstract.

The GENIALG project (Horizon 2020) aims to understand the natural diversity of the sugar kelp *Saccharina latissima* and to pursue a selective breeding program in order to improve the productivity and the composition of strains used in aquaculture. Building on more than 15 years experience, the brown algal model *Ectocarpus* is a perfect example to illustrate what could be achieved in terms of algae-variety improvement and what are the steps leading to it. Aiming to detect quantitative trait loci (QTLs) correlated with adaptation to biotic stress, we will focus on bioassays currently under development designed to phenotype, on the short term, the resistance of an *Ectocarpus* segregating population against an Oomycete pathogen (*Anisolpidium ectocarpii*) and, on the long term, to allow us to identified disease resistance genes of any brown algae species, including *S. latissima*. Acting in a complementary way to the QTLs analysis and based on quite similar analysis but on wild populations, Genome-Wide Association Study (GWAS) highlights the correlation between specific allele and a quantitative trait. To this end, the GENIALG project aims to biobank the broadest genetic diversity of *S. latissima* throughout its biogeographic range, to genotype this diversity using double digested RAD sequencing technique (ddRADseq) and to quantify the resistance of *S. latissima* gametophytes to pathogens, using on the bioassay developed on *Ectocarpus*. Hopefully, these approaches will allow us to identify in *S. latissima* some loci associated to for biotic stress tolerance.

7.5 Conference - Poster: Development of quantitative phenotyping techniques for disease resistance in brown algae with the model pathosystem *Ectocarpus* – *Anisolpidium* (GENIALG partner: SAMS)

Oomycete Molecular Genetics Networks – 20th Annual Meeting (July 10-12th 2019, SAMS, Oban)

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Abstract.

Seaweed aquaculture is growing exponentially worldwide, yet the genetic determinism and hereditability of interest traits (including disease tolerance) are virtually unknown. We used the model brown alga *Ectocarpus* to develop quantitative and parallelisable assays for disease resistance, against the oomycete *Anisolpidium ectocarpii*. Overall, we combined different techniques based on host's PAM fluorometry and chlorophyll autofluorescence, pathogen's chitin fluorescence (WGA-FITC) and DNA relative concentration (qPCR) in order to check detectable changes during the infection course. We tested two strains, Ec568f and Ec32m, which previously showed to have contrasting resistance against *A. ectocarpii* AnQU67-5. Preliminary results show that PAM fluorometry proxies normally used in physiology (e.g. quantum yield) are not very resolutive to capture subtle differences on infection progress, although sigma values (estimated cross section of the PSII) are promising because of their strong variation in *Anisolpidium*-challenged *Ectocarpus*. Contrarily, we found chlorophyll and chitin stained fluorescence are excellent to track infection progress, as long as corrections for biomass (i.e. nephelometry) are performed. In a similar way, the pathogen DNA quantification needs to be weighted with the host DNA to obtain a sensitive relative abundance. In total, four of the tested proxies are applicable to the same set up in different time points, proxies that are extrapolable to other algal pathosystems. The next steps using these phenotyping tools will include the characterization the ca. 90 individuals of a Ec568f x Ec32m progeny and correlate their outcome with their genetic maps, in order to identify potential loci conferring resistance against *A. ectocarpii*.

7.6 Conference - Oral presentation: Gene flow assessment and disease monitoring between farms and wild populations of *Saccharina latissima* (GENIALG partner: SAMS)

Algal Research for Policy-Making and Biotech Symposium (May 14-18th 2018, SAMS, Oban)

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Abstract.

The recent development of the seaweed industry in Europe comes with new some challenges and poses some risks. This offer is indeed a great opportunity to ensure sustainable exploitation of seaweed aquaculture. The GENIALG project (GENetic diversity exploitation for Innovative Macro-ALGal biorefinery) aims to increase the production of the brown alga *Saccharina latissima* at the European scale by many aspects like breading, reduced costs, up-scaling and biomass quality

improved, and social acceptability… Higher algae density could affect the environmental conditions including modifying local biodiversity and facilitate inadvertent introductions or disease outbreaks. Therefore, biosecurity is also an essential aspect of the seaweed industry development. Here, we will present the objectives, strategies and preliminary results of the GENIALG project about biosecurity focusing on two aspects. First, the assessment of *Saccharina latissima* biodiversity and the detection of potential genetic exchange between cultivated populations and natural populations surrounding seaweed farms. Secondly, the disease monitoring at all the different step of the production of *Saccharina latissima* (from the hatchery to the harvest) but also the identifying the most common pathogens in natural population. The long-term objective of this study is to contribute to the knowledge of Seaweed aquaculture industry in term of policy guideline for a sustainable exploitation.

7.7 Conference - Poster: Description of an endophytic Rhodophyte infecting *Saccharina latissima* (GENIALG partner: SAMS)

Algal Research for Policy-Making and Biotech Symposium (May 14-18th 2018, SAMS, Oban)

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Abstract.

European kelps are frequently infected by fungi and algal endophytes causing deformation and other disease symptoms. However, so far only few algal endophyte species have been described and their interaction with their host algae remains mostly obscure. Here, we describe an unidentified endophytic Rhodophyte infecting *Saccharina latissima,* collected from the west coast of Scotland. The *S. latissima* stipe showed dark patches without clear borders, patches from which the endophyte was isolated and cultivated. Based on preliminary microscopic observations and molecular analysis, it appears that this endophyte does not match any species already characterized molecularly. The 18S rDNA sequence indicates that this alga belongs to the group I of the Acrochaetiales (i.e. Acrochaetiaceae), which includes the genera *Acrochaetium*, *Audouinella* and *Rhodochorton*.
This study aims to describe the endophytic Rhodophyte of *Saccharina latissima*. Here, we will present a literature review of the morphological features characteristic of the Acrochaetiaceae and of the species that compose it. The ongoing morphological characteristic and the life cycle of the endophytic Rhodophyte will be compared to other known species. Likewise, we will also present the ongoing results of phylogenetic analysis based on different several markers (28S rDNA, *cox1* and *rbcL*) in order to confirm the preliminary results obtained with the 18S rDNA. Finally, the life cycle and molecular description of this Rhodophyte will contribute to filling the knowledge gap on the endophytic pathogens of kelps, and will allow further investigations, such as detection of their prevalence in European kelp populations.

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