Investigating Small- and Large-Scale Drivers of Seaweed Biofouling Using Molecular eDNA Tools: Advancing Ecological Insights and Biofouling Management

Literature Review

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Seaweed Aquaculture

The emergence of macroalgae cultivation as a pivotal sector within the rapidly growing aquaculture industry is being driven by its significant potential economic and environmental benefits (Sultana et al., 2023). The seaweed farming industry shows high potential in advancing global food security, mitigating climate change via carbon sequestration and reduced carbon footprints (EU, 2022). Additionally, it may provide a novel source for a range of bioactive compounds with medical, textile and nutritional applications (Cotas et al., 2023). Seaweed shows further promise in increasing the sustainability of established mariculture projects, such as finfish farming, through innovative practices such as restorative aquaculture and integrated multitrophic aquaculture (IMTA; Veenhof et al., 2024). While the commercial market is dominated by Asian producers, accounting for 97% of global production, other regions such as the North-East Atlantic are showing gradual expansion (Veenhof et al., 2024).

A critical bottleneck in commercial profitability for the predominantly small-scale farms across UK and Europe is the inherent difficulties in scaling up operations (Kuech et al., 2023). Limitations in storage, processing, supply chains and maintenance of a consistent production quality are key among the factors hindering expansion and broader market access (Holland and Shapira, 2024). To help the seaweed sector establish in the North- East Atlantic coast, funding initiatives and policy frameworks, such as the European Union's Blue Economy strategy and Horizon Europe are currently driving development by supporting innovative farming technologies. Key challenges within for algae sector include high production costs, small-scale operations, limited market awareness and clear understanding of associated environmental impacts; all of which is governed by fragmented frameworks and directives. (EU, 2022). Future and current projects require focus on improving the sector by advancing production methods, boosting both food and non-food consumption, enhancing sustainability, and driving technological innovation.

Amongst the projects are infrastructure enhancement initiatives which are developing more stable storage and processing facilities alongside more robust distribution networks that will contribute to improved reliability and consistency of supply chains (Kuech et al., 2023). One such project is SeaMark funded by a Horizon Europe grant (€9 million) which aims upscale seaweed

production and identify market applications within Europe through breeding technologies to optimise yields and advance processing methods through means of biotransformation and fermentation (Voldnes et al., 2024). Another project that is focused on creating automated offshore seeding and harvesting systems is SeaGrown which received £32 million from the UK Biomass Feedstocks Innovation Programme. Using adapted deck machinery tailored to a novel submerged growing rig design the fully automated transportable system is projected to save capital and running costs by 45% and 60%, respectively (EU, 2022). Further efforts are directed at addressing production quality inconsistencies through food quality safety standardization and certification, a framework commonly requested by seaweed businesses (Cerca et al., 2023).

These significant investments and strategic initiatives underscore the region's recognition of the algal sector's vast economic and environmental potential. The policies not only align with global sustainability goals but also work to reinforce Europe's desired position as a global leader in aquaculture innovation and sustainable fisheries management. Successful implementation of these enterprises has strong potential in generating economic growth, creating jobs and contributing to sustainable food security.

The Problem

A significant constraint to scaling up efficient and sustainable seaweed production is the impact of biofouling species on cultivated seaweed lines (Bannister et al., 2019). The cold, nutrient-rich coastline conditions that make the North-East Atlantic region highly suited for commercial macroalgae cultivation also create favourable conditions for the seasonal proliferation of harmful epibionts (Forbord et al., 2020; Macias et al., 2025). The seaweed surface provides an optimal substrate for a range of epibiont groups including bryozoans, hydroids, tunicates, crustaceans, gastropods, bivalves and epiphytic algae (Matsson et al., 2019). Epibiont settlement on both wild and cultivated seaweed can result in three primary negative impacts; physical damage to the frond, interference with key physiological processes and competition for vital resources (Bannister et al., 2019). Fouling species can physically shade and block fronds inhibiting the transfer of light and nutrients. This negatively impacts photosynthesis, reproductive spore release, and can ultimately lead to tissue necrosis (Walls et al., 2017). Additionally, fouling organisms reduce the flexibility of seaweed fronds, particularly in high-wave energy environments, by heightening the risk of breakage and substrate detachment due to increased drag which result in loss of biomass yield (Krumhansl et al., 2011). The commercial value of seaweed decreases as fouling worsens due to increased waste, deterioration of frond quality and taste, and a heightened allergen risk to humans (Walls et al., 2017, Bannister et al., 2019). To

avoid biofouling, farmers face the dilemma of shortening the growing season, which avoids the onset of epibiont infestation, but restricts further yield growth, raising financial sustainability issues for the farms (Visch et al., 2020).

Epibiont community composition differs greatly between locations, resulting in varied impacts on macroalgae. Two commonly cultivated species *Saccharina latissima* and *Alaria esculenta*, show highly similar fouling succession phases that align with Wahl's (1989) initial description of epibiosis (Forbord et al., 2020). Biofouling progresses from an initial rapid formation of a thin film of organic compounds which provides substrate for bacterial colonization, followed by diatoms which establish the first visible fouling layer (Forbord et al., 2020). These early stages of settlement are predominantly influenced by physical and chemical interactions between the macroalgal surface and the surrounding environment (Wahl, 1989). Succession continues with settlement by algal spores and invertebrate larvae including more complex organisms such as hydrozoans and bryozoans which commonly show high prevalence, if not dominance, within the seaweed fouling community (Ronowicz et al., 2008, Rolin et al., 2017). Alongside other biofoulers, hydrozoans and bryozoans add to the structural complexity of the epibiont layer. For example, bryozoans form extensive mat-like colonies made up of calcified zooids which significantly alter the ecological characteristics of the host surface (Førde et al., 2015). In some cases, hydrozoan colonies themselves can act as substrate for some crustacean, bryozoan and indeed other hydroid species (Tendal and Dinesen, 2005). This successional shift from microbial and diatom colonization to the establishment of more complex invertebrate communities highlights how dynamic and multifaceted the fouling process is.

Knowledge Gaps and Challenges

A critical stage in the life cycle of aquatic epibionts is the meroplanktonic stage that enables the organism to disperse larvae into the water column before its eventual settlement and colonisation of a suitable surface, i.e. plant/algal matter, hard substrate or man-made structure (Agostini and Ozorio, 2022). In the Northern Hemisphere, biofouling occurs predominantly between spring and autumn, driven by seasonal shifts in temperature, light availability and hydrodynamic flux (Visch et al., 2020). However, the relative influence of environmental conditions remains unclear. Timing, dominant taxa and infestation severity can vary significantly depending on the year, geographic location, and cultivation depth (Rolin et al., 2017, Matsson et al., 2019, Pratt et al., 2022) . This regional and ecotypic variation has been associated with a range of environmental drivers that may affect the prevalence and settlement risk of biofouling

meroplanktonic larvae, including temperature, light (photosynthetic active radiation (PAR)), salinity and wave action (Forbord et al., 2020, Handå et al., 2013, Bruhn et al., 2016).

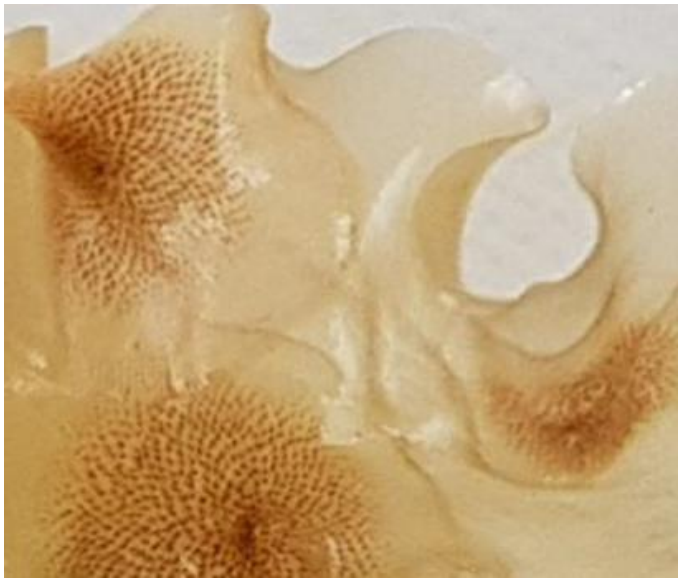


Figure 1. *Membranipora membranacea* colony infestation on *Saccharina latissima* (Forbord et al. 2020).

Ryland and Stebbing (1971) suggested that before settling, meroplanktonic larvae of the highly problematic bryozoan *Membranipora membranacea* (fig. 1) can remain in the water column for several weeks to months. Colonies have been shown to survive cold winter conditions and may provide source for the spring larvae (Menon, 1972). Førde et al. (2015) also recorded year-round presence of same cyphonautes larvae in North-Atlantic waters although abundances were low until late June before quickly proliferating. The authors suggest that colony settlement appeared to occur when water column larvae abundance was at its peak; potentially due to the increased temperature that may have stimulated spawning from established colonies or indeed because of a general increase in seasonal plankton (Førde et al., 2015)

In Nova Scotia, where *M. membranacea* is highly invasive in kelp beds, Scheibling and Gagnon (2009) conducted a ten-year study on its infestation dynamics. They findings suggest that warm sea temperatures in late spring and autumn drive infestation severity by accelerating colony growth on the kelp blades. In contrast, despite winter and spring temperatures showing influence on recruitment timing and rates, the lower temperatures are less critical in determining outbreak severity (Scheibling and Gagnon, 2009). For ectothermic organisms, including invertebrate epibionts, warmer water temperatures directly influence metabolic rates, resulting in accelerated development and increased growth rates (Atkinson, 1994). It is therefore feasible that seasonally elevated temperatures may promote colony growth and enable to expanded

coverage of seaweed substrates. Overall, temperature appears to play a crucial role in epibiont dynamics; by influencing larval settlement patterns, triggering spawning events, or driving colony growth through metabolic regulation. However, the relative impact of each response, as well as their interaction with other variables remains largely unknown, further highlighting epibiont meroplankton dynamic complexities (Scheibling and Gagnon, 2009).

In a study comparing seaweed site locations with varying salinities, Forbord et al. (2020) recorded epibiont community dominance by *M. membranacea* at most sites. However, in areas with higher freshwater influence, the euryhaline bivalve *Mytilus edulis* and oligohaline hydroid *Obelia geniculata* were also prevalent on the *S. latissima* fronds (Forbord et al., 2020). The authors suggests that one of *M. membranacea* life history stages may be more sensitive to lower salinities than *M. edulis* or *O. geniculata*, explaining its reduced presence in areas with higher freshwater input (Forbord et al., 2020). Given that larval *M. edulis* cells have been observed to rupture under reduced salinity conditions, *M. membranacea* may exhibit even lower tolerance (Saranchova and Flyachinskaya, 2001). This is interesting as although lower salinities may help mitigate *M. membranacea* infestations, it may also promote a more diverse and potentially more resilient epibiont community.

Meroplankton, including epibionts, are generally considered to be poor ion regulators which makes them vulnerable to internal ion balance disruption in low-salinity waters (Sameoto and Metaxas, 2008). Ion loss through cell membranes, coupled with increased osmotic pressure from freshwater influx can disrupt osmolarity and subsequently cell functionality (Sameoto and Metaxas, 2008). The influence of salinity should be considered an important driver epibiont distribution and its implications considering during location selection for seaweed farm.

Stronger seasonal currents and increased wave action have been observed to limit biofouling by dislodging epibionts and preventing settlement, whereas calmer conditions appear to promote accumulation (Førde et al., 2015). Along Swedish coastal sites, biofouling on *S. latissima* was reduced at sites with lower wave exposure (Visch et al., 2020). However, these findings are inconsistent, a Norwegian study by Matsson et al. (2019) reported higher fouling at exposed sites while Bruhn et al. (2016) found no correlation in Danish estuaries.

A study of Welsh seaweed farms also showed significantly lower biofouling coverage in sheltered sites with strong tidal currents, relative to more exposed, weaker current areas (Berger et al., 2024). Despite wave exposure being commonly linked with biofouling, Berger et al. (2024) states biofouling was limited at one site that experiences consistently high current speeds from tidal straits. They suggest that this current exposure is a key driver of biofouling by regulating

temperatures and reduce light penetration through sedimentation during summer growing periods (Berger et al., 2024).

There is discussion that lowering seaweed cultivation lines to lower depths may minimise epibiont coverage as it limits photoautotrophic food availability for suspension feeding epibionts (Førde et al., 2015). Trials with this strategy remain inconclusive and the overall effects of depth have been shown to be location-specific with freshwater-induced sites showing greater influence from deeper submersion (Matsson et al., 2019). Stratification in the water column both in terms of salinity and temperature may be important factor in epibiont distribution by restricting meroplankton movement within specific water layers (Saunders and Metaxas, 2007).

Another suggestion is that increased light intensity may facilitate seaweed surface metabolite upregulation and influence epibiont establishment (Rickert et al., 2016, Pavia and Toth, 2000). In summer months a significant temperature- and light- driven up-regulation of saccharides and hydroxy acid molecules was observed in *Fucus vesiculosus* and *Fucus serratus* (Rickert et al., 2016). In this report the examined seasonal shift in the surface metabolome did not appear to influence the fouling directly but the observed changed in seaweed surface chemistry does warrant further exploration considering its direct association with the biofilm community and subsequent epibiont succession (Rickert et al., 2016).

A study on *S. latissima* cultured along the Norwegian coastline reported contrasting results, where biofouling decreased with increasing light availability (Forbord et al., 2020). This was suggested as being a resultant from lower light levels coinciding with higher concentrations of food particles, a factor previously linked to epibiont coverage (Saunders and Metaxas, 2009, Forbord et al., 2020). This finding contradicts conventional expectations as greater food availability is typical of enhanced growth, particularly for a species like *M. membranacea*, a suspended particle filter feeder (Saunders and Metaxas, 2009). It is possible that these results may be influenced by the absence of flowing seawater in the experimental design which could have limited feeding success and subsequent colony growth, an important link previously observed in encrusting bryozoans (Arkema, 2009). Colonies of encrusting biofoulers have been shown to increase feeding activity and growth rates in gentle currents relative to stronger currents (Pratt, 2008, Visch et al., 2020).

An improved understanding of the seasonal interactions between the discussed environmental conditions and meroplankton community will enable farmers to optimize cultivation and harvesting schedules, ultimately maximizing high-quality biomass production whilst minimizing fouling impacts. To achieve that, it is important to a) identify the time window when biofouling

epibionts are most likely to be prevalent in the water column and b) to assess whether, during that time window, conditions are optimal for the settlement of epibionts on the blades. Obtaining a thorough understand of these two key points will allow established farmers to extend their growing season while minimising biofouling risks. This will establish a more stability in the supply and demand that will protect the interests of both seaweed sellers and buyers respectively, thus fostering a sustainable seaweed market. Moreover, combining this information into a biofouling risk index will inform strategic decisions for future farm locations. Developing such an approach requires comprehensive understanding of the dynamics of biofouling meroplankton taxa, their interaction with environmental conditions, and the actual observed outcomes of biofouling observed on the farmed seaweed. Finally, it requires development of a casual model that uses this system data to make robust predictions on biofouling risk that will inform stakeholders and regulatory bodies during initial site planning phases at the level of business, local coastal authority or state.

Broad-Scale and Longitudinal Monitoring

Traditionally, studies on biofouling of natural and/or farmed seaweed have been carried out with a focus on a single geographical location either on a single season or through a whole annual cycle (i.e. longitudinal studies). Single-site studies can provide beneficial calibration adjustments to enhance cultivation practices within established farms such as growing depth, line seeding position and substrate type (Khan et al., 2024, Boderskov et al., 2021).However, the limited spatial and temporal extent of longitudinal studies means that any predictive models are lacking sufficient contrasts in environmental conditions, such as temperature and nutrient differences, as well as taxa types to meaningfully inform a predictive model. It is therefore imperative for coastal monitoring to be covering geographical scales that span a broad range of physicochemical conditions and represent various hydrodynamic regimes (high energy, sheltered, tidal currents etc). Second, it is also imperative to develop monitoring systems that are fast, cost-effective, sensitive and less subject to human error. This dual approach covering both broad spatial coverage and long-term temporal data collection will be valuable for providing detailed, region-specific insights that can facilitate industry up-scaling, enhance sustainability and improve biomass yields though more effective management practices (Forbord et al., 2020).

Molecular Ecology Techniques

The use of rapidly advancing molecular techniques for identifying biofouling species from environmental samples has shown promise over traditional visual surveys, offering notable advantages in efficiency, sensitivity, and cost-effectiveness (Bae et al., 2023, Zaiko et al., 2016, Ammon et al., 2018). eDNA analysis offers a powerful non-invasive method that sequences specific genetic barcode regions from DNA extracted from diverse environmental samples, including water, sediment, and air (Djurhuus et al., 2018). Molecular detection of eDNA is achieved through two means, the first is through metabarcoding which combines gene barcoding with high-throughput (HT) sequencing and has proven useful in community-wide biodiversity assessments particularly with presence/absence data of rare and cryptic eukaryotic groups (Zaiko et al., 2016, Senapati et al., 2018, MacAulay et al., 2022). The second method utilises real-time quantitative PCR (qPCR) or digital drop PCR (ddPCR) for specific targeting of single-species and are generally considered to have better sensitivity and improved quantitative power over metabarcoding (LeBlanc et al., 2020). Utilisation of either metabarcoding or species-specific PCR techniques within a broad spatial monitoring framework throughout multiple seasons has significant potential in elucidating the key environmental drivers of biofouling species dynamics. Successful identification of the conditional changes that precede peaks in epibiont larval dispersal, coupled with continuous monitoring of these factors, could provide farmers with an early warning system for predicting the likelihood of settlement events. This proactive approach could allow for extension of the growing period to extend a new maximum, eliminating premature harvesting and ultimately enhance the farmers' yield.

The identification of specific species from collected eDNA involves two main approaches: 1) marker discovery through untargeted metagenomics or metabarcoding multispecies) and 2) targeted sequencing using tailored primers (species-specific) (Garlapati et al., 2019, Kim et al., 2021). Metagenomics involves sequencing all genetic material within an environmental sample to generate a reconstructed whole genome and comprehensive view of the community, although this is dependent on the size of genome and the sequencing depth (Setubal, 2021). In contrast, metabarcoding targets only a single genetic locus, a process that is considerably less expensive than metagenomics but generates far less detailed community information (Garlapati et al., 2019). Within this study, genome sequencing of individual organisms will be utilised to identify high copy number regions within their respective genomes (Dorant et al., 2020). Focusing on these high copy number regions can enhance sensitivity of later processing stages because marker regions are present in multiple copies within the genome. Designed primers that amplify these regions have improved specificity from the greater resolution of genomic information

provided thus reducing cross-reactivity with non-target species. Isolating and amplifying these marker regions is the foundation for developing of highly sensitive diagnostic assays, namely qPCR and ddPCR. Collectively, this comprehensive approach will enable deployment of a robust detection framework for monitoring aquatic species (Uthicke et al., 2018, Hernandez et al., 2020, Marques et al., 2024).

Validation and testing of species-specific primers will be an important step in evaluating their efficacy and sensitivity. Deep sequencing via metabarcoding, conducted in silico or invitro can assesses primer accuracy in targeting desired species within complex environmental samples (Ammon et al., 2018). This complements the generated biodiversity profile whilst also revealing amplification biases or off-target sequences that have appeared during processing. The second approach entails DNA extraction directly from an organism from which specific sequences can be identified, amplified and used for the design of targeting primers (LeBlanc et al., 2020, Ammon et al., 2018). This targeted approach is generally considered more time and cost efficient as it only detects and amplifies DNA from species of interest. In contrast although the untargeted protocol generates a more comprehensive biodiversity structure and can detect previously unseen species, it has a higher economic expense and requires more complex data interpretation.

Despite the growing popularity and numerous recorded successes of eDNA, particularly through metabarcoding, in detecting rare and cryptic species, concerns have arisen in the quantitative limitations of the technique (Rishan et al., 2023, Duarte et al., 2023). A key challenge lies in the type of DNA being sampled. eDNA is shed from a living organism into its surroundings (e.g. urine, faeces, skin) but also encompasses community DNA (cDNA) which includes microbial DNA and free DNA from dead organisms (Xiong et al., 2024). This distinction is problematic as it can result in false-positives and overrepresentation of a species currently inactive in the water (Marinchel et al., 2023). Overall, these limitations generate noisy compositional data that undermines the reliability of biomass abundance estimates (Xiong et al., 2024, Rishan et al., 2023, Algueró-Muñiz et al., 2024). Keck et al. (2022) highlights these limitations, particularly for plankton, noting that the short DNA fragments utilised in metabarcoding are inefficient in size and variability to accurately distinguish separate morpho-taxonomic species. Further, reference databases for many microeukaryotic communities remain incomplete to a species level emphasizing the need for coordinated improvement of libraries that will benefit future metabarcoding studies (Keck et al., 2022).Metabarcoding is limited by the lack of a universal marker across phyla, this results in detection inconsistencies from amplification biases caused by selectivity of the used primer (Borrell et al., 2017). Numerous studies have subsequently used multiple barcode regions including the mitochondrial Cytochrome Oxidase I (COI) gene which

improves distinction of metazoan species, or the more conserved 18S rRNA gene which has a broader range of taxa detection (Ammon et al., 2018, He et al., 2023). Furthermore, there is broad consensus that expansion of the genetic database with more species is needed to heighten taxonomic resolution and improve species identification in metabarcoding studies (Bucklin et al., 2021). In the case of zooplankton for example, many species are absent or have incomplete data which restricts their exact identification with eDNA (Bucklin et al., 2021). Having a more robust database would allow for a more accurate interpretation of communities, their environmental drivers and their role within an ecosystem.

Nevertheless, Rourke et al. (2021) and Klymus et al. (2015) supports its use and have recorded linear relationships between eDNA concentrations and biomass and density. These contrasting findings highlight the need for better standardization and validation of the method, especially so considering its discussed use within future policy making and regulatory framework (Fonseca et al., 2023, Hinz et al., 2022).

An established strategy for enhancing detection sensitivity and reducing false positive and false negatives is to combine metabarcoding analyses with conventional morpho-taxonomic surveys through quantitative microscopy (Ammon et al., 2018, Chen et al., 2023). When combined, the distinct types of data generated by each method contributes to building a more comprehensive biodiversity profile. eDNA analyses typically yield qualitative or low level semi-quantitative data revealing species presence or absence within an environmental sample (Zaiko et al., 2016). In this respect, eDNA performs well; however, results using DNA concentration as a proxy for species biomass or density remains contentious. Hansen et al. (2018) and Danziger et al. (2022) bring into question its reliability due to confounding factors like DNA shedding rates, environmental degradation and even species' breeding cycle and seasonality.

A recent study examining planktonic threats in salmon aquaculture revealed poor correlation between microscopic and molecular approaches (Algueró-Muñiz et al., 2024). Microscopy provides direct quantitative data revealing precise biomass estimates but is highly dependent on the observer's taxonomic expertise and is both highly labour intensive and time consuming (Chen et al., 2023). The molecular method (eDNA metabarcoding) demonstrated limited predictive power for organism abundance and considerable variation in detection sensitivities was also observed between the methods, with each approach identifying certain species that the other failed to detect (Algueró-Muñiz et al., 2024). These findings highlight that no single method is perfect, while metabarcoding clearly has potential in monitoring, its current limitations must be considered. Key challenges in disentangling true signals from high biological noise when using

universal markers hinders accurate species identification (Gold et al., 2023). Additionally, failures to detect other species due to processing biases or reference database gaps must also be considered. More targeted approaches therefore seem better suited for specific applications but do come at the expense of limiting the scope for uncovering unknown biodiversity (Fonseca et al., 2023). As such integration of the two methods can balance the limitations of each approach to provide a more accurate depiction of the community dynamics.

Accurate isolation of biofouling-derived DNA (eDNA) from environmental samples can be facilitated by differential filtration techniques that target DNA fragments of interest by excluding non-target cellular or microbial debris (Djurhuus et al., 2017). For example, sequential filtration has been utilised to determine the optimal pore size for enhanced material separation, resulting in improved sample purity (Bowers et al., 2021). A significant caveat that should be addressed is the near impossibility of distinguishing planktonic juveniles from fragmented adult forms. The lack of molecular variation between these stages results in even the most advanced detection techniques failure in reliably differentiating them. This challenge underscores the need for an integrated approach that combines detailed microscopic with genetic data to improve the community assessment reliability.

Furthermore, the influence on downstream DNA amplification (false negatives / decreased sensitivity) by inhibitors introduced during field sampling, or laboratory processing and preservation can be removed by chemical or enzymatic treatments (Schrader et al., 2012). Collectively, these protocols aid in preserving DNA integrity while maximising recovery rates and may improve detection sensitivities, particularly of rare and low abundance species (Sanches and Schreier, 2020).

Techniques such as qPCR and ddPCR can provide quantitative evaluation and aid in the identification of seasonal shifts and regional hotspots in epibiont communities (Audrezet et al., 2021). qPCR offers real-time amplification of species-specific generic markers generating sensing qualitative abundance data and has proven effective in studies investigating biofouling species (Kim et al., 2021, Revilla-Castellanos et al., 2015). When combined with environmental driver data, strategies can be developed by farmers that mitigate epibiont colonization by dictating harvesting periods or dictating when antifouling measures should be deployed.

Project Summary

This project aims to address the critical challenges posed by biofouling in seaweed aquaculture across the North-East Atlantic. Through a unique collaborative effort, the protocol will sample 11 farms spanning Scotland, England, Sweden, and Norway to provide an unparalleled opportunity to examine the dynamics of biofouling species across a vast spatial scale. The project will be complemented by continuous sampling across multiple seasons, providing a comprehensive understanding epibiont community variance both temporally and spatially.

Collected eDNA samples will undergo processing through qPCR and ddPCR to assess epibiont presence and abundance. Key problem species will be isolated, and their genomes sequenced. Primers will then be designed for high copy number regions to enable highly sensitive community analyses of biofouling organisms. This step will also contribute to the expansion of current genetic databases, assisting future biofouling research. Subsequently, targeted markers will be benchmarked against integrated metabarcoding data to ensure their effectiveness for long-term monitoring within the project.

Molecular data will then be integrated with detailed environmental metrics of salinity, temperature and PAR recorded via state-of-the-art smart sensor buoys deployed at each farm. When combined, it will enable the identification of the key environmental drivers of epibiont communities. Planktonic epibiont eDNA abundance will then be compared with microscopic surveys to evaluate their respective prediction capacities and ascertain if environmental drivers are consistent across the method groups.

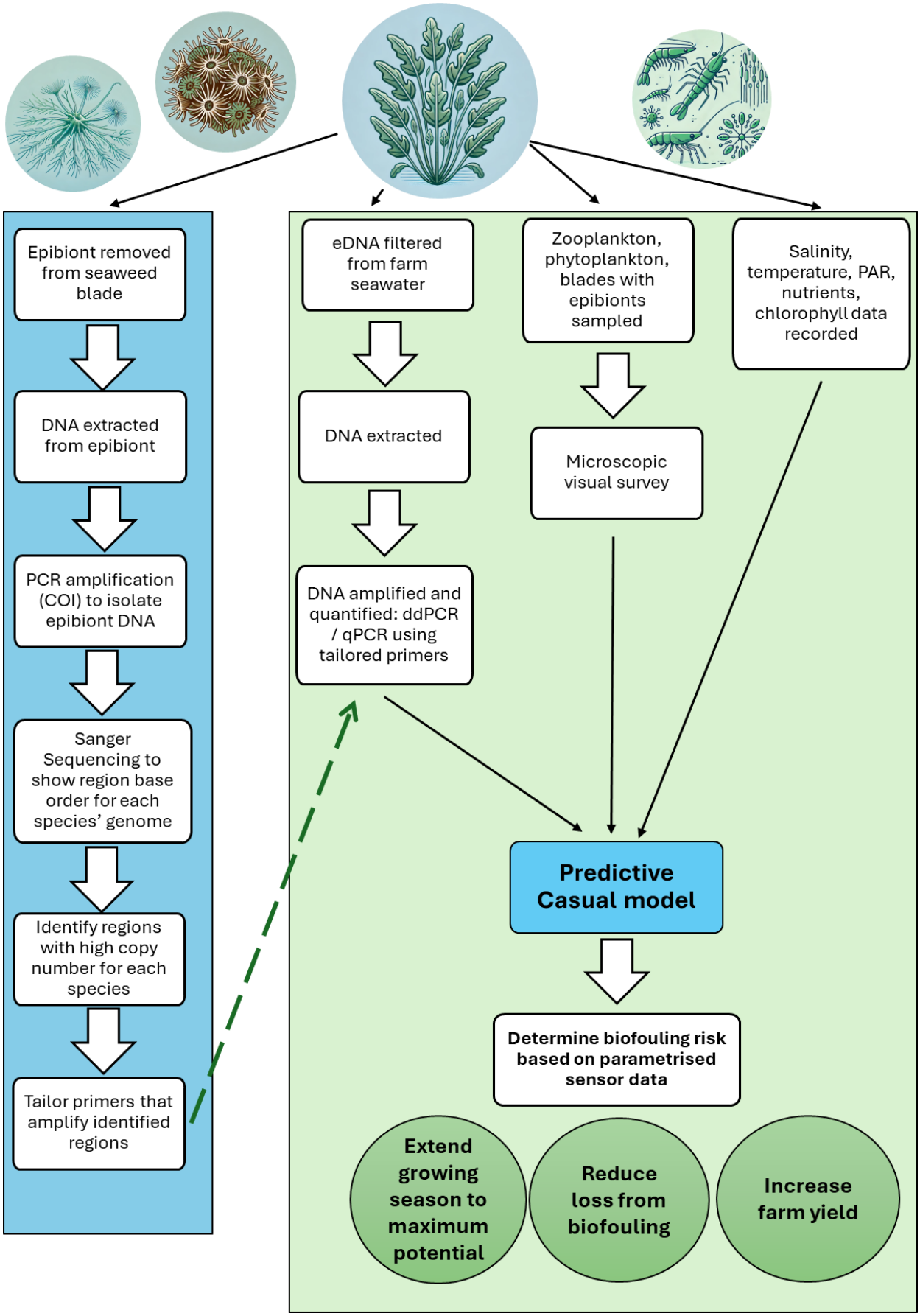
Ultimately, this project aims to provide insight into the temporal and spatial patterns of epibiont colonization, revealing critical environmental thresholds that precede their emergence. By identifying these drivers, the project will offer aquaculture practitioners predictive tools to optimize farming schedules, enhance biomass yield, and mitigate biofouling impacts through proactive antifouling strategies. This innovative approach has great potential in supporting the expansion of sustainable seaweed across the North-East Atlantic region. Insights gained form this project will provide farmers with data-driven strategies to improve productivity, enhance scalability and reduce waste, overall promoting sustainable growth of a highly promising and economically prospective industry.

Research Questions and Approaches

1. Detection: Can we identify problematic epibionts (e.g., hydrozoans, bryozoans) in plankton eDNA samples?
    - Approach: Design specific primers for high copy number regions identified by whole genome sequencing of seaweed epibionts.
2. Prediction: How predictive is planktonic DNA of biofouling abundance compared to microscopy?
    - Approach: Correlate genetic data reads with microscope counts and refine primers for individual species / groups.
3. Environmental Drivers: What are the main environmental drivers for biofouling presence/abundance, and how do these vary seasonally?
    - Approach:
        i. Assess environmental conditions factors across farms spanning a broad spatial area, incorporating continuous sampling throughout multiple seasons.
        ii. Analyze temporal shifts in environmental conditions such as temperature, salinity, current velocity, nutrient levels, and photosynthetically active radiation (PAR) to identify critical seasonal thresholds that influence biofouling dynamics.

Year 1 Timeline

- Months 1–3: Literature review, initial fieldwork planning, and equipment setup.
- Months 1–12: Sampling at multiple farms; eDNA extraction.
- Months 4–9: Primer design and testing for problematic species; data analysis.
- Months 10–12: Correlation studies, refine method, and prepare for model development



Simplified flow diagram plan to for biofouling project. Blue section denotes pre-processing stage wherein novel primers are developed for biofouling species. Green section denotes field sampling, molecular assays and model developed supported by visual survey results.

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