

# **Investigating biofouling in seaweed aquaculture using eDNA diagnostics**

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### **Abstract**

The North-East Atlantic seaweed aquaculture industry is significantly threatened by biofouling organisms. Seasonal proliferation of epibionts, including Bryozoans and Hydrozoans, limits sustainability by impacting seaweed yield and quality. This study investigates biofouling dynamics through development of species-specific primers which target high-copy number genomic regions of key epibiont taxa. Integration of novel molecular diagnostic tools with conventional survey techniques, alongside environmental and physical monitoring, enabled assessment of biofouling presence, timing, and driving factors. By sampling 11 farms across Scotland, England, Norway, and Sweden throughout the growing season, this research captured broad geographic and temporal variation in biofouling dynamics. A preliminary study found eDNA signals often preceded physical settlement, and colonisation was most severe on older, distal blade segments, highlighting the diagnostic value of early molecular techniques. These findings aim to advance the use of molecular tools in biofouling monitoring frameworks by providing early warning systems that support informed management strategies.

**Keywords:** Biofouling, Epibionts, Seaweed Aquaculture, Environmental (eDNA), Species-specific primers, Biomonitoring

### **Introduction**

Macroalgae cultivation offers significant potential as a sustainable marine bioresource, capable of advancing global food security, mitigating climate change and delivering high-value products across medical, agricultural and industrial sectors (Sultana et al., 2023, Jagtap and Meena, 2022, Duarte et al., 2021). Although Asian producers have long dominated global seaweed markets, emerging expansion within regions like the North-East Atlantic demonstrates the rising international interest for macroalgae production (Veenhof et al., 2024, Zhang et al., 2022). Seaweed farming is critical to the EU's sustainable blue economy which aims to increase production to 8 million tonnes by 2030, creating 85,000 jobs and generating an estimated 9 billion Euros of revenue (Jueterbock et al., 2025). However, there are significant barriers to commercial viability for Europe's seaweed industry which is predominantly made up of small-scale startups (Addamo et al., 2022). Limitations in infrastructure, high production costs and inconsistent biomass qualities that meet market demand hinder industry expansion and broader market access (Holland and Shapira, 2024).

Another major constraint to commercial profitability is the significant and destructive impact of proliferating biofouling organisms upon farmed seaweed (Bannister et al., 2019). The same cold, mesotrophic conditions that make North-East Atlantic coastlines suitable for seaweed mariculture also favour rapid seasonal growth of harmful epibionts which colonise seaweed blades (Forbord et al., 2020). Biofouling taxa include bryozoans, hydrozoans, gastropods, amphipods, gastropods and bivalves exert varying impacts to both wild and cultivated seaweed populations (Matsson et al., 2019). Physical damage to fronds, physiological disruptions and competition for keys resources by biofouling organisms collectively impact afflicted seaweed (Walls et al., 2017). Fouling colonies such as encrusting bryozoans shade and block fronds; impairing photosynthesis and reproductive spore release and can ultimately result in tissue

necrosis (Bannister et al., 2019). Epibiont coverage reduces blade flexibility and subsequent increases hydrodynamic drag which increases susceptibility to breakage, dislodgement and reduced overall yield (Krumhansl et al., 2011). As biofouling intensifies, the commercial value of seaweed declines due to deterioration in frond quality and taste, increased biomass waste, and heightened allergen risk consumers (Walls et al., 2017, Bannister et al., 2019). To mitigate impacts, farmers often shorten cultivation seasons to avoid onset of epibiont infestation. Although effective, this strategy limits potential maximum yields which raises concerns over the long-term financial sustainability of European operations (Visch et al., 2020).

In the Northern Hemisphere, biofouling predominantly occurs between spring and autumn, as seasonal shifts in environmental conditions are believed to drive increased prevalence and settlement of meroplanktonic stages of epibionts dispersed within the water column (Visch et al., 2020). Regional and ecotypic variability has been linked to a range of environmental drivers that may influence meroplanktonic larvae dynamics, including temperature, light (photosynthetically active radiation (PAR)), salinity and wave action (Handå et al., 2013, Bruhn et al., 2016, Forbord et al., 2020). The relative influence of individual parameters remains poorly understood, with dominant fouling taxa, infestation severity and timing varying considerably between species, year, geographic location and cultivation depths (Rolin et al., 2017, Pratt et al., 2022, Matsson et al., 2019). Advancing understanding of seasonal interactions between environmental conditions and biofouling communities may enable farmers to optimize cultivation strategies and harvesting schedules, ultimately enhancing biomass productivity while minimising fouling-associated damage. To achieve this, it is important to (a) identify the temporal window when biofouling epibionts are most prevalent in the water column and (b) determine whether environmental conditions during that period are conducive to the settlement of epibionts on seaweed blades.

Molecular techniques such as quantitative (qPCR) and digital droplet (ddPCR) offer quantitative assessment and identification of aquatic communities from environmental DNA (eDNA – genetic material sourced from urine, waste, mucus and sloughed cells) (Senapati et al., 2018). However, species detection sensitivity and accuracy are highly dependent on the utilised targeting strategy. Species-specific primers offer improved accuracy when detecting cryptic or low-abundance taxa relative to universal primers used in metabarcoding, which although can provide broader community-level insights it is often at the sacrifice of quantitative resolution (Wood et al., 2019). This is resultant from detection inconsistencies and amplification biases caused by the primer selectivity, particularly when targeting low-concentration DNA in complex environments such as those within biofouling communities (Borrell et al., 2017).

Detection sensitivity in eDNA biomonitoring may be improved by designing primers that target highly repetitive genomic regions. These regions are in greater abundance in environmental samples thus improving likelihood of detection relative to the single-copy mitochondrial genes commonly used in conventional assays (Marshall et al., 2022). Moreover, primers can be tailored to target repetitive sequences conserved across related species or broader taxonomic groups. This approach allows for detection of multiple species through clade-specific assays with the benefit of broadening taxonomic surveillance coverage while still maintaining high sensitivity through high-copy targeting. This approach could prove highly effective for seaweed aquaculture biomonitoring where multiple fouling taxa can occur in combination by enhancing early warning capabilities and management responses.

Integrated analysis of molecular data alongside physical observations enables investigation into the temporal lags between meroplanktonic larvae within the water column and their

subsequent physical settlement upon the seaweed substrate. While metabarcoding has proven effective in community composition studies, its potential as a tool for early detection is increasingly evident as it offers broad taxonomic resolution and ability to detect often overlooked or cryptic species. However, with metabarcoding, challenges remain in detection inconsistencies, particularly in low abundance species due to amplification biases caused by primer selectivity (Algueró-Muñiz et al., 2024). By aligning molecular reads with visual observations such as planktonic counts and blade colony assessments, it becomes possible to generate a more holistic understanding of biofouling dynamics. Collectively, this can improve the accuracy and reliability in using molecular read data within biofouling monitoring frameworks.

The current study aims to advance biofouling detection capabilities through the development of species-specific molecular markers which target high-copy repetitive genomic regions. Tailored primers with enhanced sensitivity will be applied to eDNA samples from seaweed farms located throughout the North-East Atlantic to assess species distribution, seasonal abundance and key ecological drivers. When integrated with physical observations, this approach will enable the development of a robust understanding of early-stage biofouling dynamics. The resultant insights will contribute to building a scalable diagnostic tool to inform a predictive biofouling model capable of supporting proactive farm management and long-term sustainability of bio-economies.

## Methods

### *Sampling Sites*

Sampling was carried out at 11 seaweed farms located across Scotland, England, Sweden and Norway. Between 4 and 5 sampling stations were designated at each farm where eDNA, phytoplankton, zooplankton, nutrients and chlorophyll-a were collected on a monthly (November to February) and bimonthly (March to August) schedule. Bimonthly sampling was implemented to improve data granularity during peak biofouling activity.

**Table 1.** North-East Atlantic Seaweed farm locations

Farm Name	Location
The Kilchoan Estate	Loch Melfort, Scotland
Atlantic mariculture	Loch Sunart, Scotland
Aird Fada	Isla of Mull, Scotland
SAMS	Oban, Scotland
Jurassic sea farms	Portland Harbour, England
Cornish Seaweed Company (x2)	Porthallow Bay and St. Austell, England
Algapelago	North Devon, England
Koastal	Gothenburg, Sweden
NTNU in collaboration w/Seaweed Solutions (x2)	Frøya Island, Norway

### *Environmental DNA*

Seawater was collected at seaweed farms using a Buerkle Vampire Sampler from 5 stations located throughout the cultivation area. 1 litre of seawater from a depth of 1.5m was passed through a sterile Sterivex™ filter following flush through before and after each station to prevent cross-contamination. Filtrate was discarded and air passed through the filter to remove residual seawater which could interfere with subsequent processing. Filters were stored in labelled 50ml

centrifuge tubes preloaded with silica beads to improve desiccation and immediately stored at -20°C. Filters were kept frozen until laboratory extraction could be performed.

Filters were thawed overnight at 4°C before the smaller outlet was individually capped. 500µl of Buffer ATL and 50µl of Proteinase K (Qiagen DNeasy® Blood and Tissue Kit) was then pipetted into the larger inlet and subsequently capped. Filters were loaded into a custom-built rotator rack and incubated whilst tumbling at 56°C for a minimum of 16 hours. A secondary stage rack was placed on the incubator rack which positioned a 2ml Eppendorf under the filter outlet. The combined apparatus was centrifuged at 1700g for 3 minutes to recover filter lysate into each Eppendorf. DNA extraction was performed as per the DNeasy® protocol with minor modifications. Extracted DNA was stored at -20°C prior to further purification and subsequent use molecular diagnostics (specific protocol to be determined).

#### *Environmental Parameters*

Abiotic environmental parameters including temperature, salinity and light intensity were continuously monitored using a smart sensor buoy system (SoftSeaweed). Buoys were deployed at each seaweed farm and recorded data daily. The high-resolution abiotic data set-up captured both spatial and temporal variability in seaweed farm conditions throughout the growing season.

#### *Zooplankton*

Zooplankton was sampled via standard vertical zooplankton net pulled from a depth of 10m. Samples were transferred to a plastic bottle pre-filled with 40% formol to yield a final preservative concentration of 4%. Residual organisms were rinsed from the net and collector using a freshwater squeeze bottle and stored at room temperature until analysis.

Samples were sieved through 50µm mesh and rinsed with artificial seawater (35ppt) to remove excess preservative. Zooplankton were then resuspended and homogenised in 50ml of artificial seawater from which a 2ml aliquot was taken and diluted to 10ml for counting. Target taxa were subsequently counted and identified using a Bogorov counting chamber under a stereomicroscope. Important meroplanktonic taxa include *Membranipora membranacea* (Bryozoa), *Electra pilosa* (Bryozoa) and *Obelia* sp. (Hydrozoa).

#### *Phytoplankton*

Phytoplankton was collected using the Vampire Sampler from a depth of 1.5 and stored in a 300ml amber glass sampling bottle and immediately preserved using Lugol's iodine solution (5% concentration). Bottles were stored upright at room temperature.

Phytoplankton samples were vacuum filtered under low pressure through cellulose nitrate paper ensuring samples were homogeneous via gentle inversion and rotation of the bottle. During high biomass events (e.g. blooms), when full volumes could not be filtered, achieved overall filtrate was noted.

Filter papers were transferred to labelled petri dishes with lids ajar and dried at 40°C for 60 minutes. Dried filters were mounted to glass slides using immersion oil applied above and below the paper. A coverslip was then placed on top ensuring filter grids aligned horizontally with the slide edges.

Phytoplankton taxa were identified and counted at 400x magnification with counts conducted on four diagonally arranged central squares per slide, avoiding duplicate counts. The relative

abundance of cells was calculated and standardised to cells per litre (cells/L) as per the formula:

$$\text{Species abundance per cell} = (\text{no. cells across squares counted} \times 1000\text{ml}) \div (\text{no. squares counted} \times \text{sample volume per square})$$

### *Hydrodynamics*

At each farm site, a HOBO® Pendant® G Data Logger (UA-004-64) was tethered to a cultivation support line at the same depth as the seaweed line using strong fishing braid (3-4cm) and a cable-tie. The sensor was mounted to maintain a vertical orientation within the water column under calm conditions. The pendant was configured to record X-Axis acceleration at 2-minute intervals. As water movements increased, the logger would deviate further from an upright angle from which a proxy for turbulence could be determined. Previous studies have utilised the same device to measure tidal flow, but this represents a novel application in a fully submerged environment (Ladd et al., 2024). To validate reliability, data was cross-referenced against hydrodynamic measurements from external sources (e.g. Copernicus / EMODnet). This setup allowed for data to be collected over a 90-day period before data had to be downloaded, and the logger reset for continued monitoring. Acceleration data collected throughout the growing season was processed through a bespoke coding pipeline to infer relative current velocity.

### *Primer design - Plan*

To improve species-specific sensitivity within eDNA assays, primers were designed which targeted high-copy number repeat within regions within the genome of focal epibiont taxa. DNA was extracted from biofouling species using Qiagen DNeasy® Blood and Tissue Kit. Once purified and quality checked, whole genome sequencing was performed on extracted DNA using nanopore methodology (Oxford Nanopore Technologies, ONT). Repeat regions were identified through a developed bioinformatic pipeline that located regions with high copy abundances. Selected repeat regions were then used to design primers using optimised criteria for GC content, melting temperature and amplicon size. This approach follows previous studies which have demonstrated that primers which target multicopy genomic regions have significantly improved detection sensitivities in eDNA diagnostic assays (Marshall et al., 2022).

## **Methods – Kelpcrofting**

### *DNA Extraction and Species Identification*

A total of 24 epibiont specimens were collected from Kelpcrofters Seaweed Farm (Isle of Skye) between June 2021 and January 22. Samples were preserved in 95% ethanol and stored at -20°C until processing. Genomic DNA was extracted using Qiagen DNeasy® Blood and Tissue Kit according to manufacturer's protocol. Extracted DNA was quantified with a Qubit™ Fluorometer and diluted to a concentration of 1.3ng/µl prior to submission for Sanger sequencing (University of Dundee) Resulting sequences were trimmed and aligned using BioEdit Sequence Alignment Editor. Taxonomic identification was performed through BLAST searches within the NCBI GenBank database, using criteria of ≥97% sequence similarity, amplicon lengths >80 bp, and the lowest E-value for species assignment. Sequences that failed to meet these criteria were omitted. Identified taxa were subsequently compared with results from visual surveys conducted using a stereomicroscope.

## *Data Analysis*

A given dataset sourced from a previous project was worked upon which comprised of eDNA sequences data, plankton microscopy counts and epibiont data from visual surveys of seaweed blades. Descriptive and comparative analysis was carried out to fulfil three key objectives: 1) Evaluate molecular vs. microscopy methods for identifying epibionts on blades and in plankton. 2) Assess time lags between eDNA, planktonic presence and blade settlement of epibionts. 3) Test depth effects on epibiont distribution on seaweed blades.

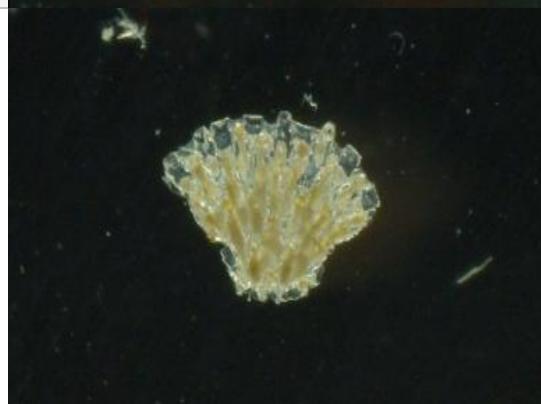
## **Results and Discussion**

### *Molecular vs. microscopy methods for identifying epibionts on blades and in plankton*

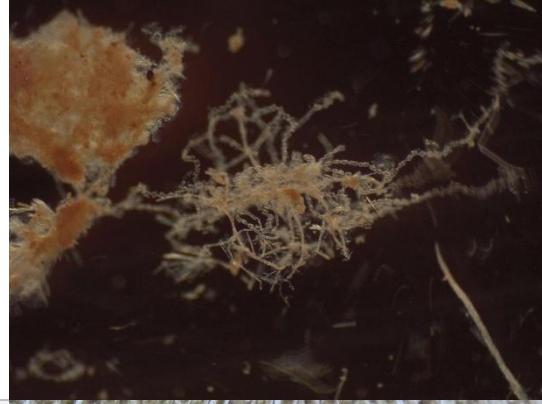
The provided dataset details several epibiont species associated with seaweed cultivation (*Table 2*). In most cases, identification of epibiont taxa was achieved only to the resolution level of order (e.g. Amphipoda/Hydrozoa). Several of the epibionts were identified on the blades via rope scrubbing, a method that involved scraping biofouling organisms from cultivation ropes that are submerged in the water column prior to macroalgal seeding. This temporal relationship indicates that epibionts may colonise farm ropes and remain there until blades have grown to a suitable size for colonisation. Farming operations may benefit from cleaning ropes or removing lines from the sea altogether prior to seeding to mitigate their reservoir/vector effect.

Planktonic identification of species is always difficult due to the often-fragmented form of samples as well as the strong morphological similarities among species and life-stages. However, the integrated use of DNA barcoding and BLAST sequence analysis provided considerably enhanced taxonomic resolution. For example, *Hiatella arctica* and *Celleporella hyalina* were confidently identified from high-percentage barcode matches, despite limited plankton identification. This molecular approach not only corroborated physical detection methods but also provided insight into species that may otherwise go unnoticed using microscopy alone. Overall, integrating visual data with barcoding reveals key species affecting seaweed farms and ultimately advances the understanding of biofouling dynamics.

**Table 2.** Summary of epibiont taxa identified and the highest taxonomic resolution achieved by each method: visual identification from blades, planktonic microscopy surveys, and DNA barcoding. Percentage value (%) denotes percentage identity match of sequence with GenBank database. Rope scrub presence (Y/N) denotes taxa identification from scrubbing of farm ropes prior to seaweed seeding.

Epibiont ID	Plankton ID	Barcode ID	Rope Scrub Presence	Photo
Amphipoda (Caprellidae)	NA	<i>Caprella mutica</i> (100%)	Y	
Amphipoda (Caprellidae)	NA	<i>Jassa herdmani</i> (100%)	Y	
Amphipoda (Jassa)	NA	<i>Jassa herdmani</i> (100%)	Y	
Bryozoans (cf Celleporella hyalina)	Cyphonaute	<i>Celleporella hyalina</i> (98.79%)	N	

Epibiont ID	Plankton ID	Barcode ID	Rope Scrub Presence	Photo
Clam juvenile	Bivalvia	<i>Hiatella arctica</i> (97.7%)	Y	
Dendronotid sea slug (Doto)	Gastropod	<i>Doto coronata</i> (99.62%)	Y	
Electra pilosa	Cyphonaute	<i>Electra pilosa</i> (97.3%)	Y	
Hydroid (Tubulariidae)	NA	<i>Ectopleura larynx</i> (100%)	Y	

Epibiont ID	Plankton ID	Barcode ID	Rope Scrub Presence	Photo
Hydroid (with Ligmophora attached)	NA	<i>Bougainvillia muscus</i> (99.41%)	Y	
Hydroid (with Ligmophora attached)	Clytia sp.	<i>Clytia hemisphaerica</i> (99.59%)	Y	
Membranipora membranacea	Cyphonaute	<i>Membranipora membranacea</i> (99.60%)	N	
NA	Balanoid nauplii	<i>Amphibalanus improvisus</i> (100%)	N	

Epibiont ID	Plankton ID	Barcode ID	Rope Scrub Presence	Photo
Obelia sp.	Obelia sp.	Obelia dichotoma (99.67%)	N	A photograph of a yellowish, branching Obelia dichotoma colony against a dark background. The colony has many fine, hair-like tentacles extending from the main branches.
Rhodophyta (Pterosiphonia spinifera)	NA	Pterothamnion plumula (98.86%)	Y	A photograph of a reddish-brown, branching Pterothamnion plumula colony against a dark background. The colony has a more rounded, bushy appearance compared to Obelia.
Unidentified	NA	Laminaria digitata (99.6%)	Y	A photograph of a large, brown, fan-shaped Laminaria digitata colony against a dark background. The colony has a distinctively lobed or digitate shape.
Unidentified algae (with Licmophora attached)	Hydroid	Bougainvillia muscus (99.41%)	Y	A photograph of a yellowish, branching Bougainvillia muscus colony against a dark background. The colony has a delicate, branching structure with small, purple, finger-like projections (Licmophora) attached.

## Assessment of time lags between eDNA, plankton and blade settlement of epibionts

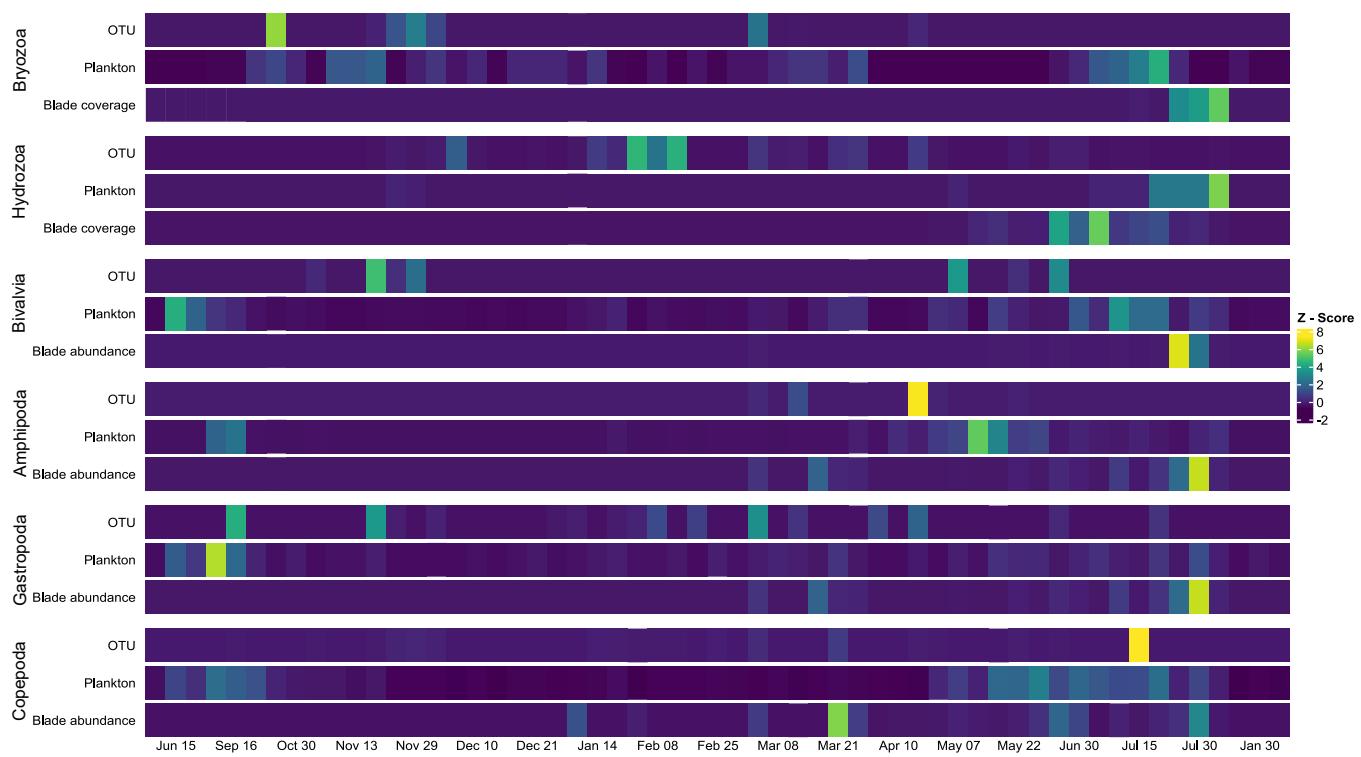


Figure 1. Heatmap shows Z-score standardized temporal dynamics of biofouling taxa detected via three observation methods carried out on samples from Isle of Skye coastlines: OTU (Operational Taxonomic Unit) = Planktonic eDNA metabarcoding, Plankton abundance (cells/L), and blade coverage (%) or blade abundance (individuals/cm<sup>2</sup>).

The heatmap (Fig 1.) indicates distinct seasonal patterns in detection, a majority of taxa show elevated planktonic presence in late spring / summer prior to their detection on seaweed blades e.g. Bryozoa, Bivalvia and Amphipoda. This indicates planktonic presence is a strong indicator of forthcoming blade colonization highlighting its value in forecasting biofouling events. The early-season correlation reveals critical temporal periods for monitoring and potential intervention strategies. Nevertheless, conventional plankton monitoring is labour-intensive and requires specialist taxonomic expertise; a challenge to implement at scale and in a time effective manner.

Planktonic eDNA (OTU) detections often preceded or aligned with visual microscopic abundance counts. This shows eDNA metabarcoding is capable of detecting early signs of biofouling before their physical observation in visual surveys. The loss of eDNA signal is concerning. Expectation would be that once epibiont DNA is detectable in the water column, its presence should persist and increase. Over time, as colonies establish and proliferate on seaweed fronds more genetic material is shed and should there be detectable. This inconsistency suggests potential limitations of eDNA metabarcoding sensitivity within the context of biomonitoring.

By integrating molecular techniques into biomonitoring frameworks, enhanced temporal, and particularly taxonomic, resolution can support more proactive and sustainable farm management. This approach may allow growers to mitigate biofouling damage prior to settlement and subsequent impacts to seaweed yield and quality.

### Depth effect on epibiont distribution on seaweed blades

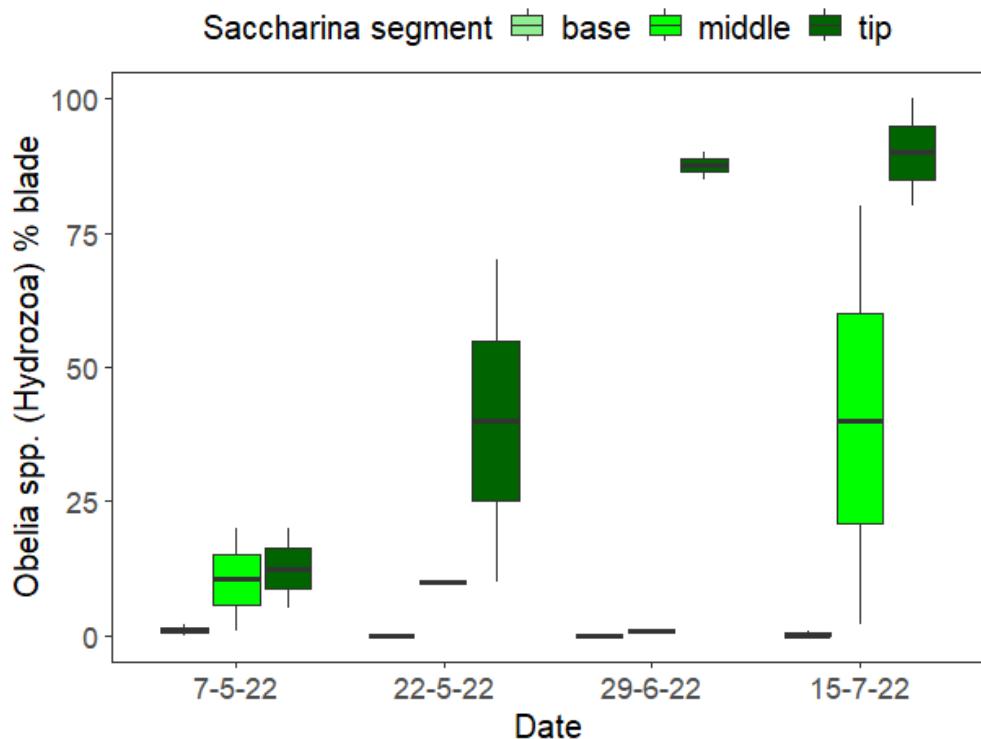


Figure 2. Temporal and vertical variation in Hydrozoan (*Obelia* spp.) coverage (%) on cultivated *Saccharina latissima* blades

The boxplot (Figure 3.) shows how the percentage of blade colonisation by hydrozoans varies among three blade segments – base (shallowest), middle, and tip (deepest) across four sampling dates. Overall, Hydrozoan coverage was lowest in early May but rapidly increased by mid-July. Tip segments were most afflicted by colonisation throughout the growing season. In contrast, base segments remained uncolonized throughout.

Two-way ANOVA showed that hydrozoan coverage was significantly influenced by blade segment depth ( $F_{2,19} = 12.93, p < 0.001$ ) and also varied significantly across sampling dates ( $F_{3,19} = 3.44, p < 0.05$ ). This suggests that sugar kelp biofouling activity by *Obelia* sp. is driven by depth and time. This may be due to colonisation being favoured by a more stable water column microenvironment. For example, reduced light penetration, turbulence and potentially higher nutrient availability may contribute to hydrozoan proliferation. Segments deeper in the water column would have less mechanical disturbance from wave action further enabling sustained polyp growth.

Furthermore, seaweed growth occurs as the base/meristem while older tissue at the tips continually sheds (Mann, 1973). The distal blade segment represents the oldest, most established surface. The increased colonisation at the tip may be resultant from the tissue being more withered or structurally compromised due to longer exposure to environmental stressors thus heightening susceptibility to infestation. More simply, the tip section of the frond

has existed the longest and therefore has had the greatest exposure time, providing more opportunities for settlement events and colony expansion. These combined physical and biological factors offer a plausible explanation for the consistently higher levels of biofouling observed at the blade tips. However, further targeted investigation is needed to disentangle the relative contribution of each factor and determine whether a single dominant driver or a synergistic combination is primarily responsible for hydrozoan colonisation patterns.

### **Conclusion**

The preliminary results described here illustrate the value of integrating molecular techniques with conventional microscopic methods for biofouling detection strategies. Barcoding of epibiont DNA greatly enhanced identifying taxonomic resolution relative to traditional microscopy alone. Temporal patterns revealed via heatmap analyses showed eDNA signals often precede physical settlement on blades. This indicates strong potential for molecular tool use in early warning systems, but further refinement of techniques is required for consistent detection patterns. Depth and temporal effects on blade colonisation showed distinct vertical trends, with older, distal blade segments showing more severe hydrozoan infestation. Overall, these findings highlight the importance of combining molecular diagnostics with ecological context to improve monitoring accuracy and better understand biofouling dynamics within seaweed aquaculture.

## **Thesis Overview, Future Work and Progress**

### **Chapter 1**

#### *Comparative Study of Molecular and Microscopic Diagnostics in Seaweed Biofouling*

Continue analysis of Kelpcrofting dataset investigating biofouling detections. Describe seasonal patterns and dominant taxa. Explore seeding influence of farm gear on later season infestation and dynamics of allergen risk taxa (Bivalvia).

Good progress has been made on generating relevant figures, completion of manuscript draft is expected in coming months (1-2).

### **Chapter 2**

#### *Development of Species-Specific Primers with High-Copy Genomic Repeats for Seaweed Epibiont Detection*

This chapter will require Whole-Genome Sequencing (Oxford Nanopore) of key epibiont taxa to provide accurate genomic data from which high-copy genomic regions can be identified. There will also be focus on generating high-quality long-read sequencing data to improve the accuracy and completeness of the genome assembly, increasing precision and reliability of downstream assays. This will allow for the development of species-specific primers and sensitive biofouling molecular diagnostic assays. In collaboration with lab group colleagues, bioinformatic pipelines will be developed which are capable of detecting these repetitive regions within genomes. Primers will be optimised and validated to confirm specificity.

At present, training has begun on pipeline development and high-quality DNA extractions with WGS expected to start as soon as possible.

### **Chapter 3**

#### *Early Detection and Ecological Drivers of Seaweed Epibionts in eDNA Across the Northeast Atlantic*

This chapter will apply the newly developed, species-specific primers to eDNA samples collected from NE Atlantic sites. Using both ddPCR and qPCR, the aim is to detect and quantitatively track seasonal variation in important biofouling taxa in seaweed farms with high sensitivity and accuracy. The broad latitudinal scope of the study will inform wide spatial and temporal patterns in epibiont dynamics. This chapter will further evaluate primer performance in samples from differing regions. Molecular data will be integrated with visual and environmental parameter data to identify ecological drivers which influence epibiont occurrence, settlement and prevalence: an area of key concern for seaweed farm sustainability.

This chapter has been continually worked on since the project started with monthly sampling trips to Kilchoan Estate. Laboratory work has been ongoing and extraction of DNA from filters has commenced. This process has been streamlined through development of a custom-built multi-filter rotator.

### **Chapter 4**

#### *Hydrodynamic Influence on Epibiont Settlement: Evaluation from Field and Mesocosm Experiments*

This chapter aims to assess the role of hydrodynamic conditions in settlement patterns and colonisation of epibionts on cultivated seaweed. This aims to isolate how flow conditions

influence settlement of meroplankton from the water column onto the seaweed frond and how colonisation progresses.

A pilot study was carried out at Kilchoan and determined HOBO pendants to be a viable option to infer water flow and turbidity data with an improved degree of granularity. Sensors were distributed to farms and have been collecting data since March. Flow data will be integrated with epibiont eDNA data to determine hydrodynamic influence on seasonality, settlement and colony growth. Concurrently, regular visits have been scheduled to a controlled mesocosm which is already operational. The set-up includes 6 tanks containing established seaweed frond within high or low water flow treatments and will allow for blade assessment of fouling under controlled conditions.

## **Chapter 5**

### *Using eDNA and Genomic Tools in Aquaculture Pathogen Surveillance (Opinion Piece)*

Opinion piece on integrating molecular diagnostics into biofouling monitoring frameworks to improve seaweed farm sustainability.

## Stage 1 : Epibiont sequencing and primer design

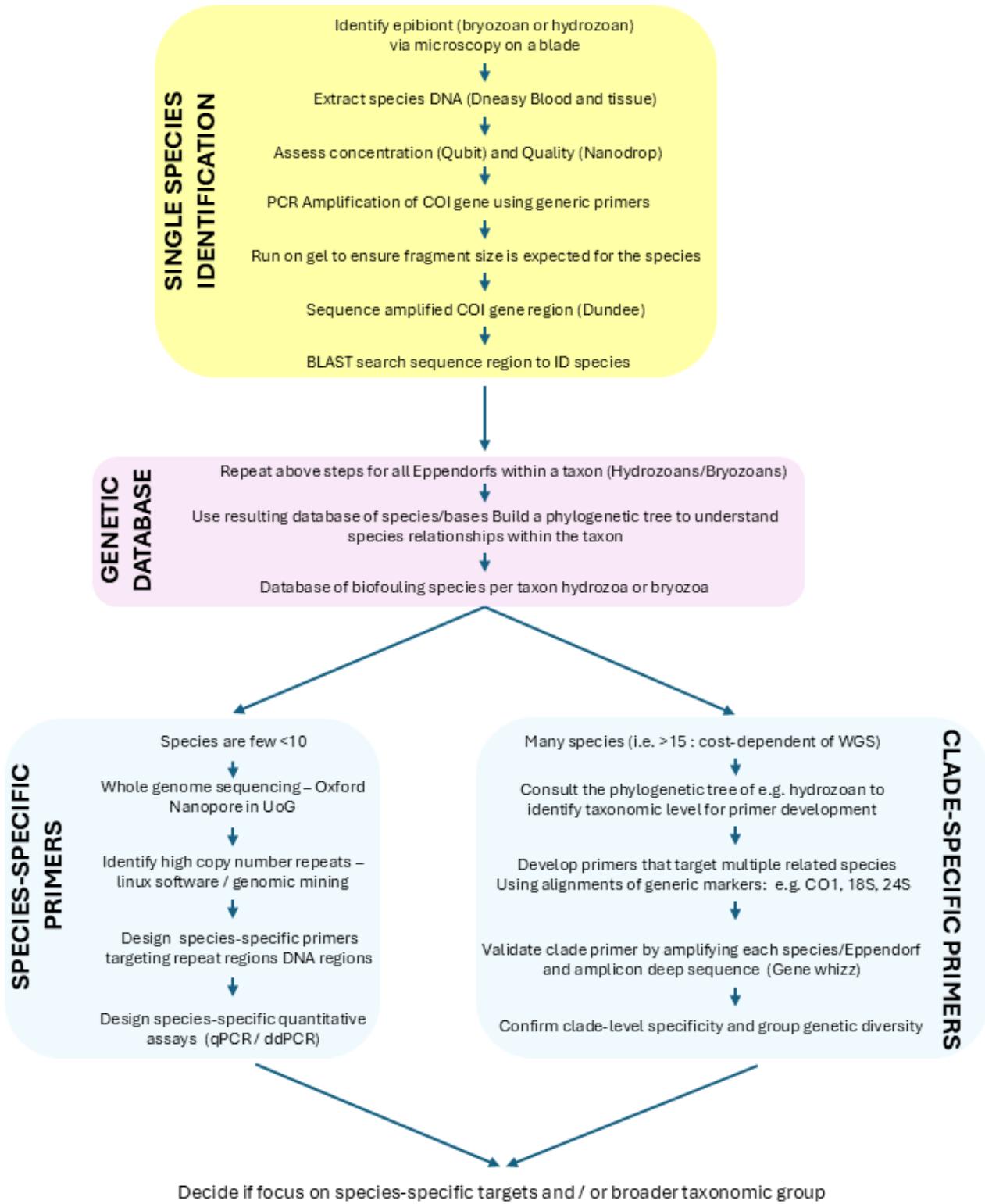
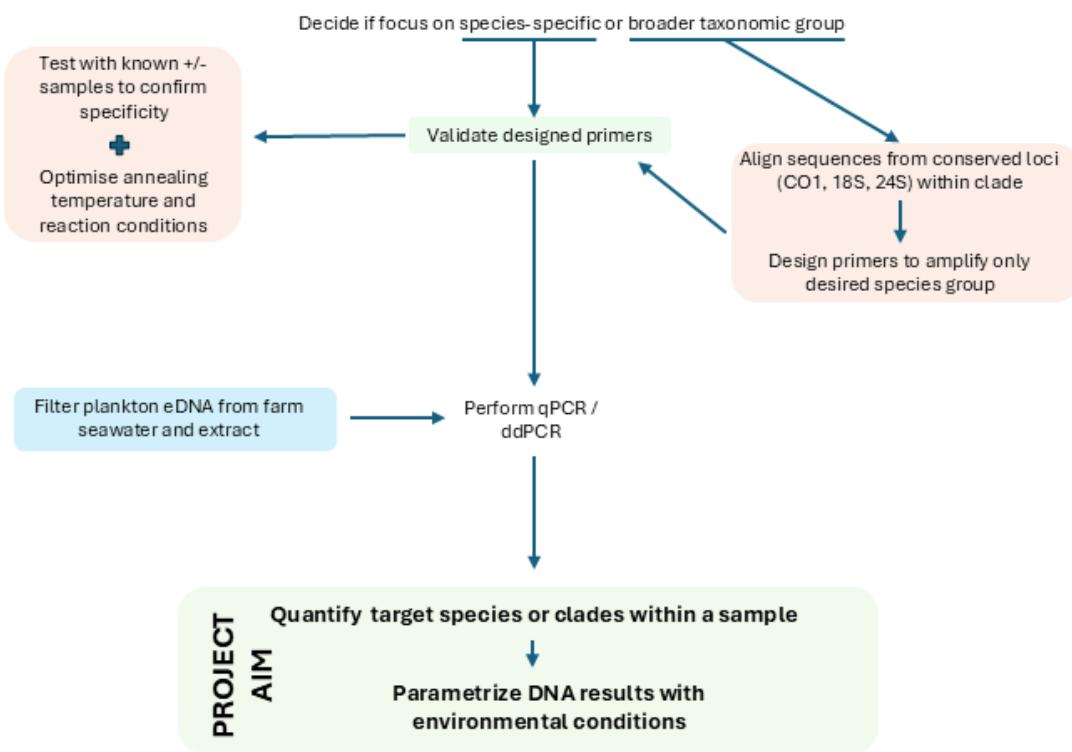


Figure 3. Schematic representation of project plan, with workflow stages contingent upon the results: Stage 1. Epibiont sequencing and primer design

## Step 2: Primer validation and use in environmental samples



## Step 3: Additional Validation (Optional)

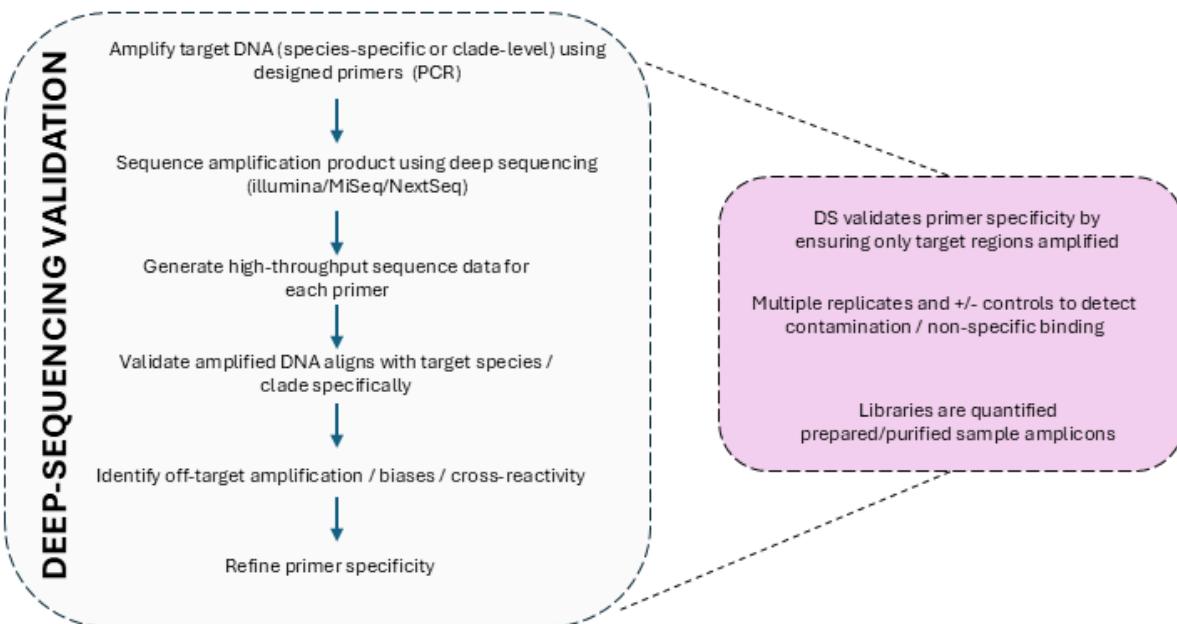


Figure 4. Schematic representation of project plan, with workflow stages contingent upon the results: Stage 2. Primer validation and application to eDNA samples.

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