Investigating the Impacts of Fish Farming on *Posidonia oceanica* Seagrass Meadow Health in Poros, Greece

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Abstract

Widespread loss of the Mediterranean's dominant seagrass species, *Posidonia oceanica*, have been documented over the past two decades and are expected to increase under imminent aquaculture expansion plans. This study aims to investigate historic and current impacts of fish farming on *P. oceanica* meadows around the island of Poros, Greece, aiming to quantify the magnitude, spatial extent, and persistence of potential health declines, to establish baseline conditions for future monitoring and to anticipate future impacts. Three key seagrass health indicators were measured: maximal leaf length (individual-level), meadow cover (population-level), and epiphyte cover (community-level). To establish the nature and spatio-temporal scale of *P. oceanica* responses, these metrics were compared between currently farmed sites, previously farmed sites and unfarmed control sites; at increasing distances from fish farms; and under increasing cumulative durations of fish farming activity. Overall, fish farming was associated with substantial declines in *P. oceanica* health. Health reductions persisted 14 years after farm removal, though there was some evidence for individual-level recovery with depth. Although negative effects diminished with distance, significant health reductions were maintained at long distances (900m) from farms. Unexpectedly, epiphyte cover was lowest near to active farms but elevated at the previously farmed location, suggesting potential complex community-level responses. *P. oceanica* health declined under increasing durations of cumulative fish farming activity, with significant reductions detectable at least 10 years after introduction. These findings underscore the need for spatially informed and time-aware aquaculture management strategies to prevent long-term and potentially irreversible seagrass degradation.

1. Introduction

Seagrass meadows are among the most productive (Panayotidis *et al.*, 2022) and economically valuable (Lima *et al.*, 2022) ecosystems on the planet. Seagrass ecosytems are globally significant carbon sinks, sequestering carbon at 35 times the rate of tropical rainforests (McLeod et al, 2011), and provide a wide range of ecosystem services, such as habitat maintenance, food provision, water purification and coastal protection (Lima *et al.*, 2022). However, seagrasses are increasingly under threat from both global pressures and local anthropogenic disturbances, particularly in the Mediterranean (Chefaoui *et al.*, 2018; Litsi-Mizan, 2023).

Posidonia oceanica (Linnaeus) *Delile*, is a marine angiosperm endemic to the Mediterranean and is the dominant seagrass species found in the region (Panaytotidis *et al.*, 2022). *P. oceanica* covers an estimated 2.79 million ha of the Mediterranean coasts (Pergent-Martini *et al.*, 2021), forming meadows comprised of a dense network of horizontal and vertical rhizomes termed 'matte' that can be thousands of years old (Larkum *et al.*, 2006). *P. oceanica* grows best in sheltered coastal zones between depths of 0-40m and good water clarity is critical for meadow formation (Panayotidis *et al.*, 2022). In optimal conditions, *P. oceanica* leaves can grow to 2m, but are relatively slow-growing, each leaf taking 50.68 days to grow on average. This growth rate is significantly lower than other seagrass species: for example, a leaf of *Zostera noltii* grows in approximately 13.71 days (Marba *et al.*, 2004). Thus, this species is especially vulnerable to pollution and losses in *P. oceanica* cover are deemed irreversible (Delgado *et al.*, 1999).

As coastal populations doubled across the second half of the 20th Century, anthropogenic stress on the Mediterranean coastal zone has rapidly increased (Benoit & Comeau, 2012). As a result, 25% of *P. oceanica* meadow area was lost between 1984 and 2014, and the remaining area is increasingly threatened by anthropogenic activity (Blanco-Murillo *et al.*, 2012). On a local scale, aquaculture is a rapidly increasing anthropogenic threat, with coastal fish farming being considered one of the most significant local stressors to seagrass ecosystems in the Mediterranean (Holmer *et al.* 2008; Apostolaki *et al.*, 2009).

Aquaculture refers to the practice of cultivating aquatic or marine species in tanks or enclosures in natural or pseudo-natural environments, typically to produce food (FAO, 2022). Fish-producing aquaculture is known as pisciculture or fish farming. Fish farming is an important and rapidly growing food sector across the globe (FAO, 2022). In 2020, more than 88 million tonnes of global aquatic animal production were accounted for by fish farming, making up half of total fisheries and aquaculture production (FAO, 2022). Aquaculture has also been the fastest-growing major food production sector over the past two decades, growing by 5.8% per annum between 2001 and 2016, exceeding the growth rate of capture fisheries (FAO, 2018).

Since the early 1990s, the Mediterranean region has been a hotspot for aquaculture production and in recent years has experienced a growing demand from consumers worldwide (FAO, 2018). One of the top-producing Mediterranean countries is Greece, with an estimated 144,595 mt of marine fish produced by fish farms each year (Taşkın *et al.*, 2024). In 2011, the Greek government established the Special Planning Framework for Aquaculture (Government Gazette 2505/B/4-11-2011). This framework has encouraged the further development and expansion of fish farms across Greece, defining 25 regions as Organised Aquaculture Development Areas (OADAs). The Ministry of Development has also declared a Multi-Year National Strategic Plan for the Development of Aquaculture (2021-2030), which will further boost the growth of the industry. Based on the 2011 framework, Greece aims to increase current production 24-fold across a significant portion of its mainland and island coastlines, including the focal site of this study: Poros (Greek Ministry of Environment and Energy, 2011).

Fish farming in this region is dominated by two main species, the European seabass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*) (Massa *et al.*, 2017). The typical farming strategy uses suspended cages placed in productive coastal zones. To maximise yield, growing fishes are fed with dry fodder and chemicals are applied to prevent disease and biofouling (Massa *et al.*, 2017). These inputs, and outputs such as fish faeces, escape into the surrounding natural environment. Fish farming discharges have contributed to significant environmental degradation, including increased sedimentation, altered nutrient flows and disease spread (Marino, 2011; Aguado-Gimenez *et al.* 2012). Due to the oligotrophic nature of the Mediterranean Sea, the nutrients introduced by intensive farming practices are especially destabilising to benthic ecosystems (Cancemi *et al.*, 2003; Holmer *et al.*, 2008).

The ideal habitat conditions for *P. oceanica* are also optimal for fish farming activity (Holmer *et al.*, 2008). As seagrass meadows are found over approximately 70% of the Greek coastline (Panayotidis *et al.*, 2022), *P. oceanica* frequently co-occurs with fish farms, and significant losses in meadow area have been attributed to fish farming practices (Pergent-Martini *et al.*, 2006). For example, Ruiz *et al.* (2001) reported 50% *P. oceanica* meadow loss five years after the onset of fish farming, extending to 7-fold the fish-farming area.

Various studies have reported significant impacts derived from fish farming on *P. oceanica* meadows across biochemical, physiological, morphological and ecological metrics (Ruiz *et al.*, 2001; Cancemi *et al.*, 2002; Holmer *et al.*, 2008; Apostolaki *et al.*, 2009). Impacts include reductions in shoot density and cover; abnormal (typically reduced) length and width of leaves; reduced rates of primary production; and lowered biodiversity. These changes have been observed as a function of distance from fish farms and are highly site-dependent, with significant impacts observed from 200m to 1.2 km from farms (Pergent-Martini *et al.*, 2006; Marba *et al.*, 2006). Organic loading from fish farm discharges is also transforming seagrass meadows under cages from sinks to sources of organic carbon, which may intensify the effects of global climate change (Apostolaki *et al.*, 2011). Based on these findings, it is likely that increasing fish farming activity in Greece will further threaten *P. oceanica* populations, especially when considering this species' particular vulnerability and low recovery rate.

As a designated OADA, Poros, Greece, is subject to government plans that will increase current fish farm production levels 6.7 times, from the current annual production of 1,147 tons across five farms to 8,831 tons (*Fig. 1b*). To support elevated production levels, the area designated to support farms will expand to 28 times its current size, occupying 25% of the island's coastline (*Fig. 1*) (Municipality of Poros, 2022). Therefore, a minimum of 25% of the coastal environment is set to be impacted by fish farms, with a greater area likely to be affected due to the leakage of aquaculture effluents.

However, data on the historical and current condition of the *P. oceanica* meadows surrounding Poros, remain scarce, limiting our ability to assess the extent to which existing fish farms may have already damaged these ecosystems. Furthermore, the relationship between the duration of fish farming activity and seagrass health remains poorly understood (Thomsen et al, 2020). As a result, the potential severity and scale of future impacts under proposed aquaculture expansion plans on Poros are difficult to anticipate.

Moreover, no quantitative baseline currently exists against which we can monitor long-term ecological changes on Poros, and seagrass ecosystem baselines across the eastern Mediterranean remain relatively limited (Taskin *et al.*, 2024). This is particularly concerning given the dual pressures of aquaculture pollution and climate change, which together may exacerbate stress on vulnerable seagrass ecosystems into the future (Litsi-Mizan *et al.*, 2023).

Therefore, this study aims to provide a current assessment of *P. oceanica* health in response to fish farming activity on the highly threatened coasts of Poros. Thus, offering a critical reference point for detecting future change and guiding adaptive fish farm management strategies on Poros and the wider Mediterranean region. Given plans to expand coastal aquaculture by 2030, if we are to protect the vital ecological and economic roles *P. oceanica* provides, understanding the spatial and temporal impact of fish farms in locations such as Poros is particularly urgent.

The main goal of this study is to:

Establish the nature and scale of fish farming impacts to *P. oceanica* meadow health on Poros

The sub-goals (followed by corresponding hypotheses) of this study are to:

1. **Establish whether *P. oceanica* health improves after fish farm removal, to determine the recovery potential of *P. oceanica***

H1: *P. oceanica* health will be significantly reduced even after fish farm removal.

2. **Identify the spatial extent of any observed changes to *P. oceanica* health, to derive estimated radiuses of fish farming impact**

H2: *P. oceanica* health will improve with increasing distance from fish farms.

3. **Evaluate changes to *P. oceanica* health under increasing cumulative fish farm activity durations**

H3: *P. oceanica* health will be significantly reduced with increasing duration of cumulative fish farm activity.

4. **Identify high-impact hotspots on Poros**

Hypothesis		Test Conditions	Maximal Leaf length	Meadow Cover	Epiphyte Cover	Overall Health
H1:	a)	Current farm vs. control	↓	↓	↑	↓
	b)	Previous farm vs. control	↓	↓	↑	↓
	c)	Previous farm vs. current farm	↑	↑	↓	↑
H2:		Increasing distance from fish farm	↑	↑	↓	↑
H3:		Increasing duration of cumulative fish farm activity	↓	↓	↑	↓

Table 1: Table of specific *P. oceanica* health responses in line with each hypothesis (H1-H3) and under different test conditions used in this study. *P. oceanica* health is generally hypothesised to decrease under current and previous fish farm pressure relative to controls; improve with distance from farms; and decrease under increasing durations of cumulative fish farm activity.

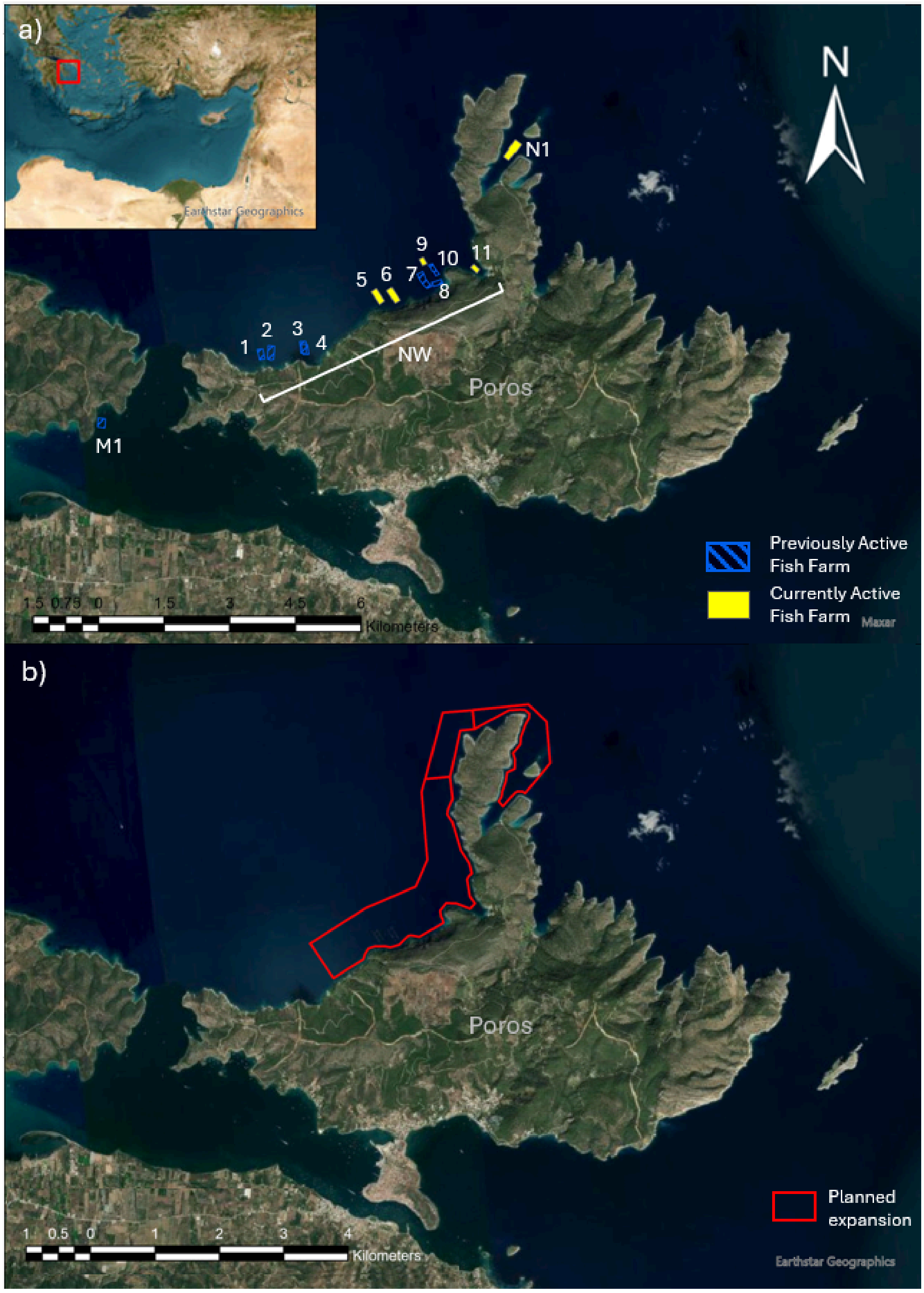
2. Methods

2.1 Study Area:

This study was conducted on the island of Poros, Greece (37.5206° N, 23.4717° E), over three weeks in October 2024. Poros is in the southern Saronic Gulf of the Aegean Sea, 29 nautical miles south of Piraeus port, Athens. This island supports a population of 3993, primarily exporting tourism (Hellenic Statistical Authority, 2011).

Poros's northern coasts have had a long and varied history of fish farming. The first farms arose in Poros in the early 1990s, being licensed under a now-illegal governing board. Since then, fish farms have been situated in over 12 locations along Poros's N and NW coasts. Currently, there are five active farms on Poros, which have been active for anywhere between 6-35 years (Table 1). The main species harvested on Poros are gilthead seabream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*).

Poros's 48km-coastline is dominated by monospecific (*P. oceanica*) seagrass meadows that are under increasing threat by fish farms (Fig. 1b).



2.2 Data Collection

2.2.1 Seagrass Sampling and Metrics

Data was collected across a significant portion of Poros's coastline, from sites that represented three increasing levels of fish farming activity: "control" (i.e. no activity), "previously farmed" and "currently farmed" (Fig. 2). Ten sites total were selected to attain replication for each farming level where possible within the limited fieldwork period. However, as current farming activity is relatively widespread, there was only one exclusively "previously farmed" site in the study area.

Five sites along the northern coasts were sampled to capture the impacts of active farms, excluding an inaccessible offshore fish farm (Fig. 2a). These sites formed the “currently farmed” level, where fish farming impacts were expected to be most severe due to sustained farming activity.

A mainland bay located 1km west of Poros (37°31'16.3"N, 23°24'40.0"E) was also included due to its comparable ecological conditions. This site hosted an active fish farm between 1989 and 2010 and therefore represented the "previously farmed" category (see 2.2.3). Given the 14-year cessation of farming activity and its distance from active farms, this site was expected to exhibit reduced aquaculture pressure and serve as a reference for long-term recovery dynamics.

Finally, four sites were selected on the southern coasts as the “control” level. However, data from potential control site CS1 (Fig. 2a) was discarded as meadows were heavily scarred by anchors, and potential sites with a northern aspect were not sampled due to weather. As Poros’s southern coast is hydrodynamically isolated from the northern coasts, it remains relatively unaffected by fish farms (Kontoyiannis, 2008). The distribution of the seagrass meadows at the control sites is also comparable to that of the meadows at the farmed sites in the 1970s (pre-fish farming) and has not appeared to change significantly since pre-fish farming times according to Athinaiou *et al.* (2024). Hence, these control sites are a reliable reference for healthy meadow conditions prior to fish farming activity.

At each site, two transects were completed by a pair of SCUBA divers (Fig. 3), starting at a fish farm, or pseudo-fish farm origin point at the previously farmed and control sites, in parallel with the coast to remain within the relatively shallow (0-40m) *P. oceanica* habitat zone. At the currently farmed sites, transects typically started ~150m from each active farm perimeter to reduce potential conflict with farm operators. Transects extended up to 1.2km, as this is the maximal fish farm impact radius reported for *P. oceanica* (Marba *et al.*, 2006). However, this distance was not always achieved due to time and weather constraints.

Both transects sampled a different depth range to ensure natural variation with depth and potential depth-dependent fish farm impacts were captured (Kletou *et al.*, 2018) (Fig 3a & 3b). Although, studied depth was limited to 25m to reduce dive-related risks (Appendix 2).

An estimate for each seagrass health metric, a photo quadrat and a depth reading were recorded approximately every 5m along each transect by the primary diver to ensure high-resolution meadow health changes were captured (Fig. 3c). All metrics were visually estimated in situ by the same diver or another diver calibrated against the original diver for consistency. Photo quadrats were taken at a fixed distance above the seafloor and calibrated to $1m^2$ to ensure consistent sampling unit area (Fig. 4).

Martinez-Crego *et al.* (2008) suggest indicators across different levels of biological organisation are needed to holistically monitor seagrass conditions. Therefore, three widely-used descriptors were used to assess seagrass health: maximal leaf length (individual-level), meadow cover (population-level) and epiphyte cover (community-level) (Pergent-Martini *et al.*, 2006; Martinez-Crego *et al.*, 2008). Martinez-Crego *et al.* (2008) posit that these descriptors are strong indicators of anthropogenic stress, and decreases in maximal blade length and meadow cover and epiphyte overgrowth are widely associated with aquaculture activity (Delgado *et al.*, 1999; Ruiz *et al.*, 2001; Holmer *et al.*, 2008). Due to *P. oceanica* protections in this region (WFD, 2000), only non-invasive methods of data collection were used, meaning visually-estimated epiphyte cover served as a proxy for epiphyte biomass, which is the preferred metric (Buia *et al.*, 2004). Seasonal variability was not a concern as sampling occurred at a fixed season.

Maximal leaf length was recorded per 1m^2-quadrat (Fig. 5a). Meadow cover was recorded as a percentage of the 1m^2-quadrat area covered in live seagrass (Fig. 5b) and estimates were verified using a 20-photo sample in *ImageJ* (Scheider *et al.*, 2012). Epiphyte cover was recorded as a percentage of the total meadow cover (i.e. live and dead) in each quadrat (Fig. 5c), then weighted by total meadow cover to account for size variation.

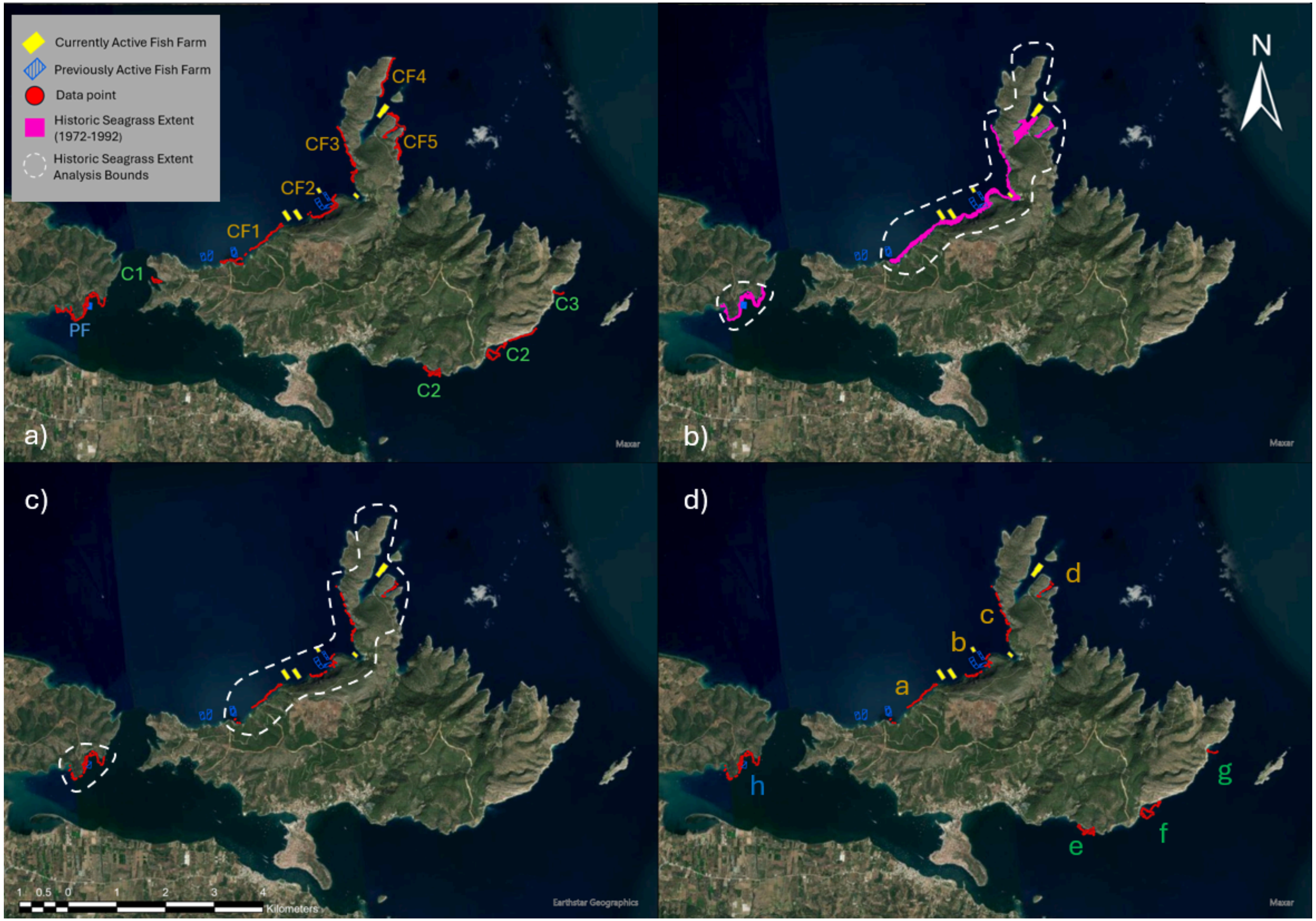


Figure 2: Maps of Poros outlining data filtering process. a) Map of original site selection. Data was collected from five currently farmed sites (CF1-CF5), one previously farmed site (PF) and four control sites (C1-C4). b) Map of historic seagrass extent. Derived from aerial imagery from the Greek National Archives (1972-1992). Historic extent has only been estimated for select areas (dashed line) near to fish farms where data was collected in the field. c) Map of data points filtered by historic seagrass extent. Only data which co-occurred with historic seagrass meadows was used in analyses. d) Map of final site selection. After filtering by historic seagrass extent, four currently farmed sites (a-d) and the previously farmed site (h) remained. Original site CF4 did not show evidence of having historic seagrass meadows. Three control sites (e-g) remained, as original site C1 showed strong evidence of anchoring scars.

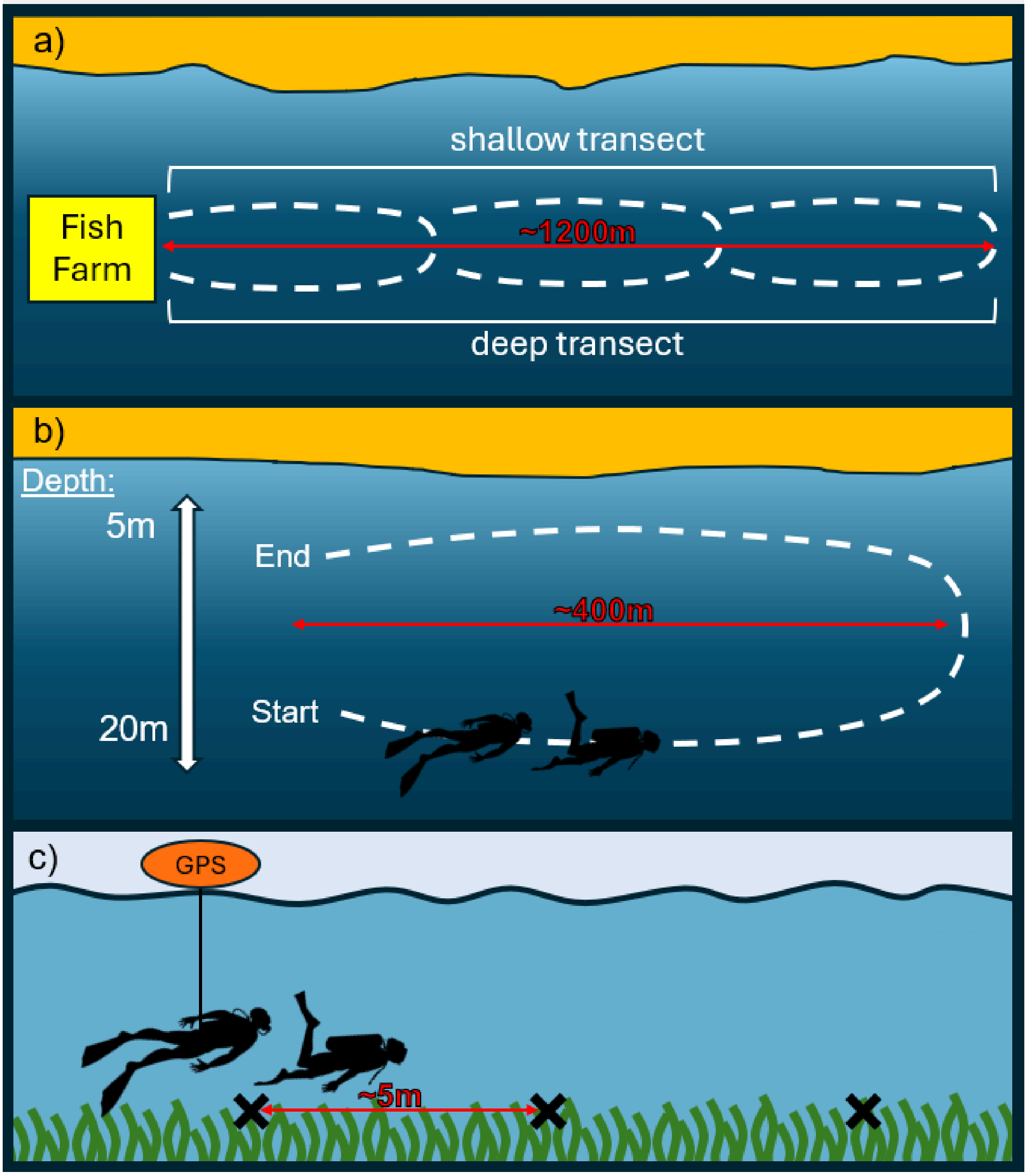


Figure 3: Schematic of sampling methodology in the field. a) Aerial view of a complete pair of transects. One transect captured deeper depths, while the other captured shallower depths. Both transects were completed simultaneously over multiple looped dives (dashed line). The first dive was started as close to the fish farm perimeter as possible then continued in parallel with the coast away from the fish farm. After each dive, the next dive along the transect began at the furthest point from the start point reached in the previous dive based on the recorded GPS track, and continued along the coast. Dives were carried out in succession in this way until a minimum 900m-long pair of transects was complete. This methodology was replicated at the previously farmed site and control sites using the centre of each bay as the transect origin. b) Aerial view of the dive route for each data collection dive. Each dive was looped, with divers following a compass bearing parallel to the coast along a deeper profile (15-20m) out from the start point, then a shallower profile (5-10m) on the return journey. Each loop covered approximately 400m of the coastline. c) Cross-sectional view of the sampling protocol. A pair of SCUBA divers carried out each data collection dive. The primary diver took samples (marked as an 'x') every 5m along the dive route. The accompanying diver held a reel attached to a surface buoy with a GPS tracker (orange) to record the geographic position of each sample, staying as close to the primary diver as possible to reduce tracking error.

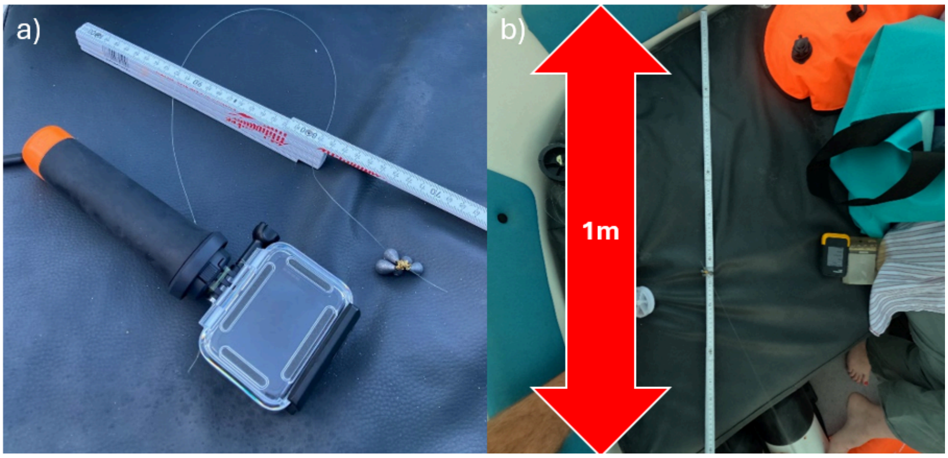


Figure 4: a) Photo quadrat calibration equipment: GoPro camera with line and weight attached. 1 metre ruler. b) $1m^2$ photo quadrat calibration: the height of the image is the exact length of the 1m ruler when the weighted line is directly beneath the camera and touching the ground, thus the image represents a 1m x 1m area.

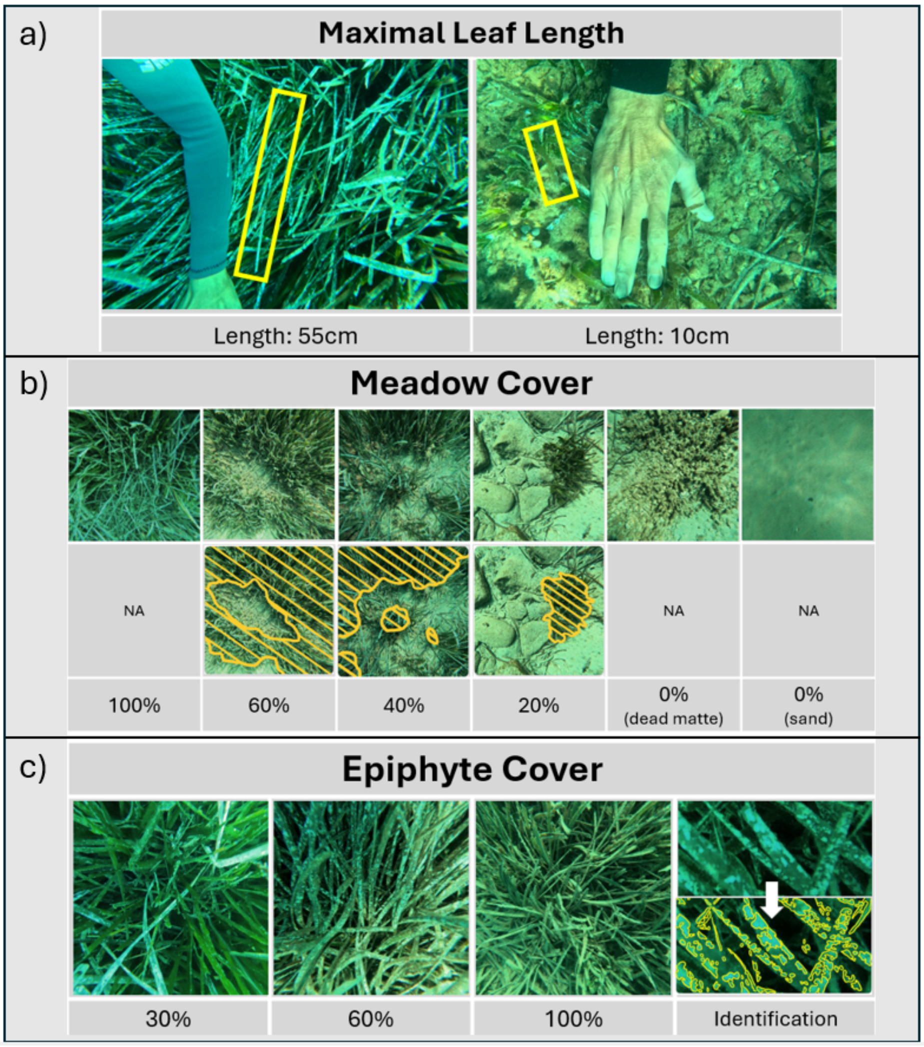


Figure 5: Seagrass health metric estimation methods. a) Maximal leaf (outlined in yellow) length was estimated for the longest leaf identified per $1m^2$ quadrat based on comparisons with divers' forearms and hands. b) Photo quadrat of estimated meadow cover based on visual assessments and ImageJ software. Meadow cover only quantified live seagrass cover. c) Photo quadrat of estimated epiphyte cover based on visual assessments. Epiphytes identified by white coating on seagrass leaf surface (outlined in yellow).

2.2.2 Post Processing Field Data:

Each photo quadrat was geotagged using *GeoSetter* (Version 3.5.3; Schmidt, 2024), which matched each photo to GPS coordinates recorded based on time. Geotagged photos were cross-referenced with seagrass health data, giving a position to each data record, and were visualised spatially in mapping software *ArcGIS Pro* (Version 3.4; Esri, 2024).

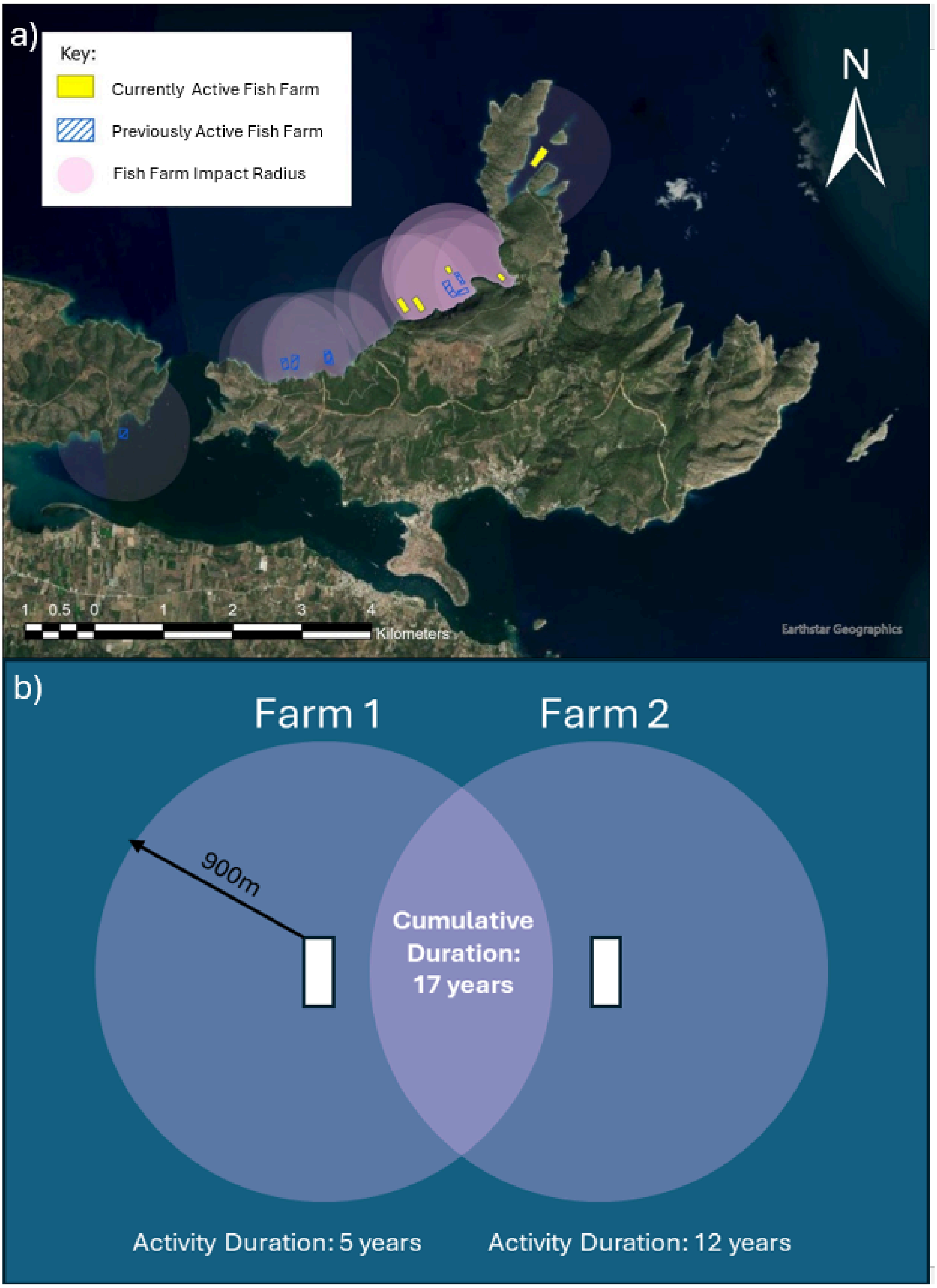
2.2.3 Fish Farm Records:

Historic satellite imagery was used to complete a record of each fish farm on Poros with guidance from local experts. Images were examined across *Google Earth Pro* (Version 7.3.6.10201; Google, 2025), *Copernicus Open Access Hub* (European Space Agency, 2025) and *World Imagery Wayback* (Esri, 2025), to estimate farm introductions and removals to the highest precision (nearest 6 months). Whenever a fish farm was introduced to a new location, a polygon marking its area and location was created on *Google Earth Pro* (Fig. 1) and key temporal information was recorded (Table 2).

Due to extensive farming history, Poros's coasts likely experience the combined effects of multiple farms. Thus, the maximal 900m fish farm impact radius estimated in Analysis 3.2.2 was applied to each farm in *ArcGIS Pro* ('Buffer' tool), excluding land ('Clip' tool). Data points in each radius intersection zone were assigned a cumulative duration value based on the total of the overlapping farms (Fig. 6), assuming the effect of time since a farm was removed had relatively insignificant effect on seagrass health based on Analysis 3.1.1 and 3.1.2, and health within 900m was relatively constant and below control levels (Analysis 3.2.1, 3.2.2). Each farm was assumed to be active (i.e. supporting fish stocks) when visible and have similar annual discharge levels, as farm size did not vary significantly and no data detailing farm-specific practices (e.g. fodder type, yield) was available.

Fish Farm Identity	Duration (years)	Time Since Removed (years)	Longitude	Latitude
M1	21	14	37°31'13.30"N	23°24'38.10"E
NW1	0.5	11.5	37°32'10.74"N	23°28'22.26"E
NW2	4.5	6	37°31'35.83"N	23°26'15.55"E
NW3	0.5	11.5	37°31'36.35"N	23°26'21.55"E
NW4	4.5	6	37°31'36.15"N	23°26'41.72"E
NW5	6	NA	37°31'58.04"N	23°27'27.46"E
NW6	6	NA	37°31'58.66"N	23°27'36.17"E
NW7	9	13	37°32'3.35"N	23°27'55.39"E
NW8	5.5	6.5	37°31'37.34"N	23°26'40.60"E
NW9	6.5	NA	37°32'12.66"N	23°27'55.80"E
NW10	10	13	37°32'8.24"N	23°28'1.75"E
NW11	32	NA	37°32'7.01"N	23°28'26.05"E
N1	35	NA	37°33'1.27"N	23°28'54.43"E

Table 2: Record of fish farms on Poros as of Dec. 2024. Active farms are highlighted (see Fig. 1).



Distances were generated from each spatially referenced data point (n= ~2000) to each fish farm perimeter (n=13) using the 'Euclidean Distance' and 'Optimal Path to Line' analysis tools in *ArcGIS Pro* with 2m cell size (Fig. 7). Land was masked so estimations were through water only. Within the bays adjacent to CF3 and CF4 (Fig. 2a), the farms changed shape and placement slightly over time. The most recent placements could be seen to the highest resolution and so were used to generate distance estimates for those sites.

Figure 7: Distance estimation method on ArcGIS Pro. Optimal distance lines (light pink) extended from the perimeter of the fish farm (bright pink) to each data point (light blue circles), calculating the minimum distance through water between these points.

2.2.4 Historic Meadow Extent

Subsequent to field survey, aerial imagery was obtained from 1972-1992 to determine historic seagrass meadow extent at the studied sites pre-fish farming (General State Archives of Greece, 2025). This data was not accessible prior to March 2025 and thus could not inform sampling site selection. Coastline sections in each image were identified then matched and overlaid on a 2025 Airbus satellite map (Airbus Defence and Space, 2025) on *Google Earth Pro*. Patches of visible seagrass were outlined, forming polygons with spatial references that were used to filter the original dataset via the ‘Select By Location’ tool in *ArcGIS Pro* (Fig 2b & 2c). Only data points within the estimated historic meadow area were included in analyses. As a result, the observed ‘0’s in meadow cover in the filtered dataset are inferred to represent losses in seagrass cover or small-scale natural within-meadow cover variability. Meadow areas were assumed to be *P. oceanica*, as it is the primary species on Poros and forms monospecific meadows (Larkum *et al.*, 2006).

2.3 Data Analysis

As seagrass conditions have widely been shown to vary with depth (Martinez-Crego *et al.*, 2008; Rountos *et al.*, 2012; Kletou *et al.*, 2018), depth was included as a covariate in all models and was included as an interaction term for comparisons between farming levels to determine depth-dependent recovery. For all models, $\alpha=0.05$.

2.3.1 Effect of Farming Level on *P. oceanica* Health and Recovery Potential

To determine the relative impact of current and previous farming activity and whether fish farm impacts improved after activity cessation, seagrass health (meadow cover, maximal leaf length and epiphyte cover) was compared between each fish farming level (currently farmed, previously farmed and control). To ensure consistency of fish farm impacts at each farming level, seagrass health data was compared among the constituent sampling sites at each farming level, before being pooled into each farming level (Fig. 9-11).

Meadow cover and maximal leaf length data in both farmed categories was filtered to within 650m of a fish farm, an intermediate and somewhat conservative spatial estimate for farm impact based on the 400m maximal impact extent reported by Holmer *et al.* (2008) and the 1.2km estimate by Marba *et al.* (2006), and the impacted distances observed in Analysis 3.2.1 and 3.2.2. Epiphyte cover data was filtered to 450m from farms based on the aforementioned 400m estimate and the 450m impact range observed in Analysis 3.2.3.

A beta regression with "logit" link on *R* (Version 4.5.0; R Core Team, 2025) was used for meadow cover and epiphyte cover analyses, due to the bounded nature of these variables (Zuur *et al.*, 2007), using the betareg package (Cribari-Neto & Zeileis, 2010). To accommodate the requirements of the beta distribution, percentage estimates were converted to proportions then compressed using the transformation:

$$\text{Proportion Meadow Cover} = \text{Percentage Meadow Cover} \times 0.999 + 0.005$$

The beta regression models were as follows:

<u>Among Sampling Sites:</u>

$$\text{Proportion Meadow Cover} \sim \text{Sampling Site Identity} + \text{Depth}$$

$$\text{Proportion Epiphyte Cover} \sim \text{Sampling Site Identity} + \text{Depth}$$

<u>Between Farming Levels:</u>

$$\text{Proportion Meadow Cover} \sim \text{Farming Level} + \text{Depth} + \text{Farming Level*Depth}$$

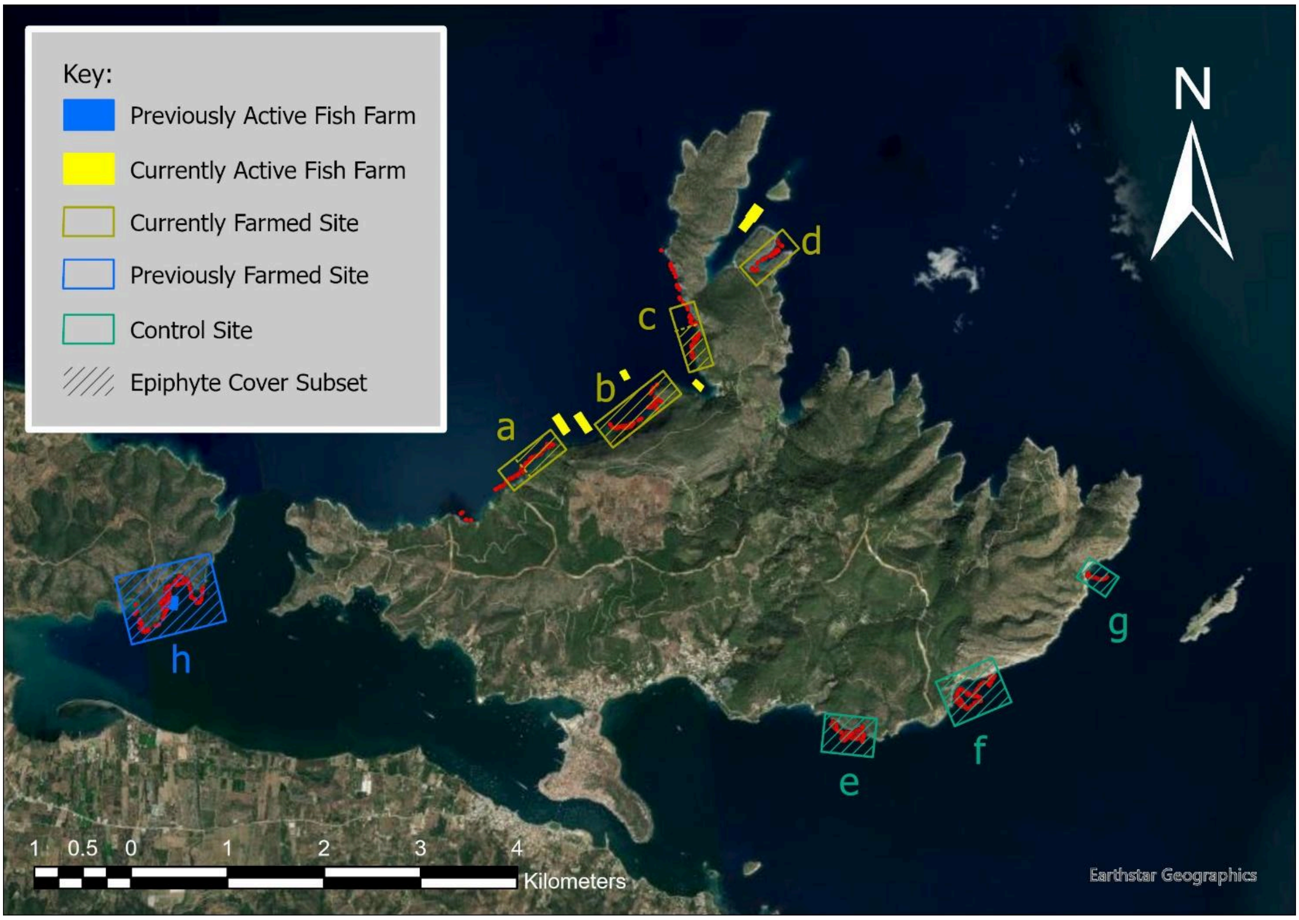
$$\text{Proportion Epiphyte Cover} \sim \text{Farming Level} + \text{Depth} + \text{Farming Level*Depth}$$

As maximal leaf length had unequal variance of residuals, a linear model with robust standard errors was used for comparisons among sampling sites and between farming levels (White, 1980), from the sandwich package (Zeileis, 2004).

$$\text{Maximal Leaf Length} \sim \text{Sampling Site Identity} + \text{Depth}$$

$$\text{Maximal Leaf Length} \sim \text{Farming Level} + \text{Depth} + \text{Farming Level*Depth}$$

To identify significant differences between specific sampling sites and each farming level in each of the above models, Tukey-adjusted pairwise comparisons of estimated marginal means were conducted (emmeans package; Lenth, 2024).



2.3.2 Spatial Extent of Current Farming Impacts to *P. oceanica* Health

To assess the spatial extent of fish farming impacts, the health of *P. oceanica* at increasing distances up to 900m from fish farm perimeters were compared to reference values from control sites. Data from Site *b* was excluded as multiple fish farms have been present here (Fig. 2d), obscuring health-distance relationships. Thus, only data from zones minimally affected by historic farming activity (a, c, d in Fig. 2d) were selected. Distances from sampling sites to nearest current fish farms (derived in 2.2.3) were discretised into 150m bins to allow for comparison with controls. The 0-150m category was omitted due to lack of data (n=8).

Meadow cover and epiphyte cover were statistically compared across distance categories, respectively, using beta regression with “logit” link.

$$\text{Proportion Meadow Cover} \sim \text{'Distance to Nearest Farm' Category} + \text{Depth}$$

$$\text{Proportion Epiphyte Cover} \sim \text{'Distance to Nearest Farm' Category} + \text{Depth}$$

A linear model with robust standard errors was used for maximal leaf length.

$$\text{Maximal Leaf Length} \sim \text{‘Distance to Nearest Farm’ Category} + \text{Depth}$$

To identify significant differences between distance categories in each model, Tukey-adjusted pairwise comparisons of estimated marginal means were conducted.

2.3.3 Effect of Cumulative Fish Farm Activity Duration on *P. oceanica* Health

To assess the relationship between *P. oceanica* health and cumulative fish farming duration, a beta regression was fitted to mean proportions of meadow cover, and maximal leaf length, across 10-year duration bins between 10-70 years (derived from 2.2.3). No sites were subject to <10 years cumulative farm activity. Epiphyte cover was omitted due to its varied response with site history (see 3.1.3).

$$\text{Proportion Meadow Cover} \sim \text{Duration Category} + \text{Depth}$$

$$\text{Maximal Leaf Length} \sim \text{Duration Category} + \text{Depth}$$

To identify significant differences between the duration groups in each model, Tukey-adjusted pairwise comparisons of estimated marginal means were conducted.

2.3.4 Hotspots of Fish Farming Impacts

To identify geographic hotspots of fish farming impacts on Poros, 900m impact radius derived from Analysis 3.2 was plotted around each farm on a map in *ArcGIS Pro*. As impacts to *P. oceanica* can be sustained long-term (Section 3.1; Delgado *et al.*, 1999), these impact radii were applied to both currently and previously active farms. To identify future impacted locations, an impact zone around planned expansion zones was estimated using a 900m buffer.

3. Results

3.1 Effect of Farming Level on *P. oceanica* Health (Recovery Potential)

Full outputs of models: Appendices 3-5

3.1.1 Maximal Leaf Length (Individual-Level Recovery)

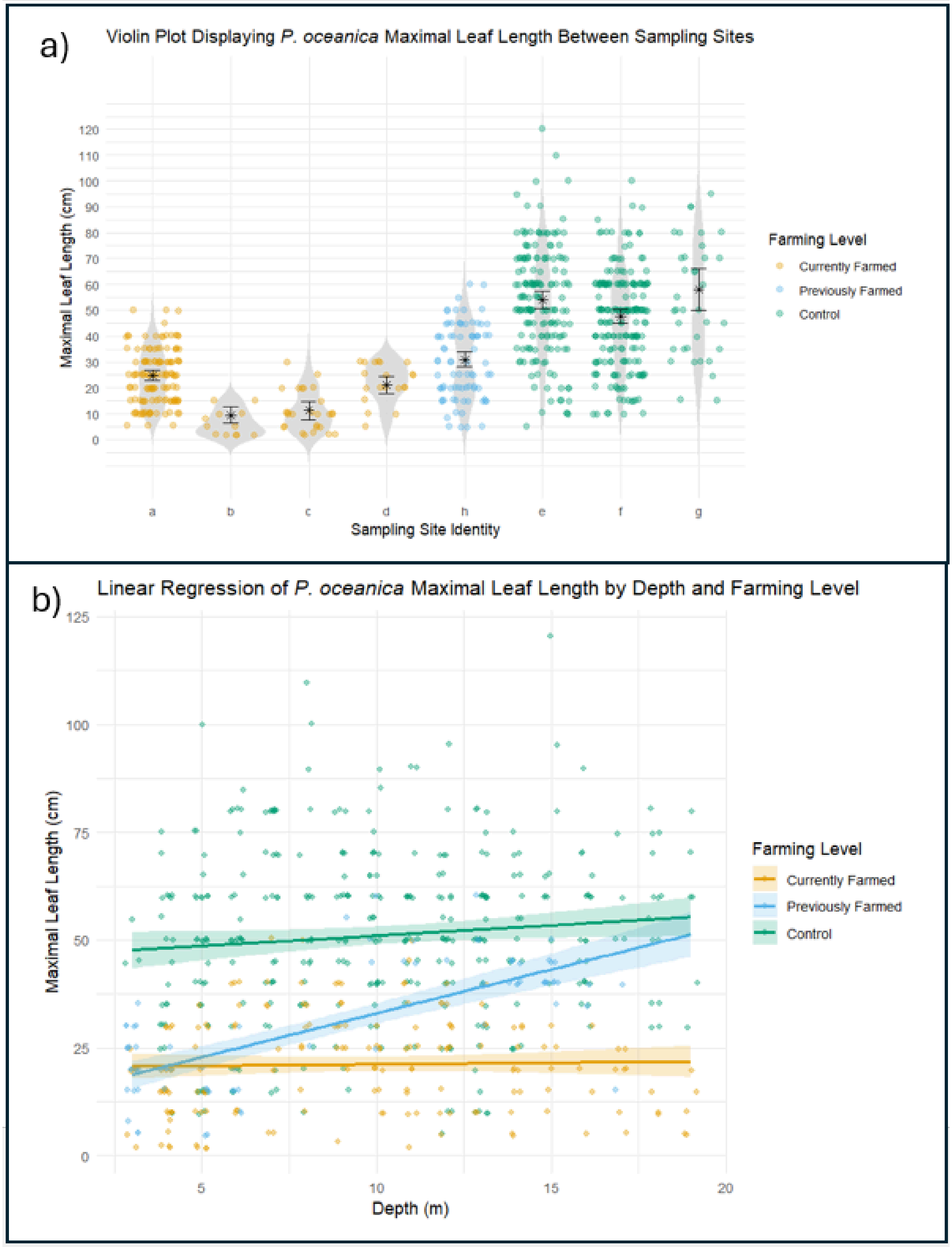
Pairwise comparisons of model-estimated marginal means indicated maximal leaf length was generally not significantly different among control sites, nor among currently farmed sites (Fig. 9a). However, maximal leaf length was marginally lower at control site *f* vs. *e* (β=-6.38, p=0.0121) and significantly higher at currently farmed site *a* relative to site *c* (β=13.5, p=0.0033).

Compared to the control level, leaves were significantly shorter at the previously farmed level (β=-18.4, p<.0001) and currently farmed level (β=-29.7, p<.0001) (Fig. 9b). However, maximal leaf length at the previously farmed level was significantly improved compared to the currently farmed level (β=11.3, p<.0001). Maximal leaf lengths were 58% lower when currently farmed and 36% lower when previously farmed, relative to controls (Table 3).

The beta regression showed that depth did not significantly influence maximal leaf length. However, significant interaction effects were observed with depth at the previously farmed level (β=1.96, p<.0001), with leaf lengths increasing with depth and approaching control levels by 17m.

Farming Level	**Maximal Leaf Length Estimated Marginal Mean (cm)**	**Standard Error of Mean**	**% Decrease vs Control**
Currently Farmed	21.2	1.25	58.30%
Previously Farmed	32.6	1.93	36.00%
Control	50.9	0.89	-

Table 3: Table of estimated marginal means, standard error of means and the percentage decrease relative to the control level for *P. oceanica* maximal leaf length at each farming level (currently farmed, previously farmed, control).



3.1.2 Meadow Cover (Population-Level Recovery)

Pairwise comparisons of model-estimated marginal means revealed meadow cover did not significantly differ between control sites (*e, f; f, g*), nor currently farmed sites (*b–d*). However, meadow cover at control site *e* was significantly lower than at *f* (β=-0.0957, p=0.0419) and significantly higher at currently farmed site *a* vs. *b* and *c* (p<0.01). Overall, the results among sites in each farming level were relatively homogeneous and meadow cover across the farmed sites was significantly lower than at the control level (p<0.05) (Fig. 10a).

The beta regression model found that depth was not significantly associated with meadow cover and there was no significant interaction between farming level and depth. Pairwise comparisons of model-estimated marginal means showed that meadow cover was significantly lower at the currently farmed level and previously farmed level compared to controls (β=-0.252, p< .0001; β=-0.246, p< .0001) (Fig. 10b), with losses in cover estimated at 46% and 45%, respectively (Table 4).

There was no significant difference between the currently farmed and previously farmed levels.

Farming Level	**Proportional Meadow Cover Estimated Marginal Mean**	**Standard Error of mean**	**% Decrease vs. Control**
Currently farmed	0.296	0.0151	45.90%
Previously farmed	0.302	0.0184	44.80%
Control	0.547	0.0152	–

Table 4: Table of estimated marginal means, standard error of means and the percentage decrease relative to the control level for proportional *P. oceanica* meadow cover at each farming level (currently farmed, previously farmed, control).

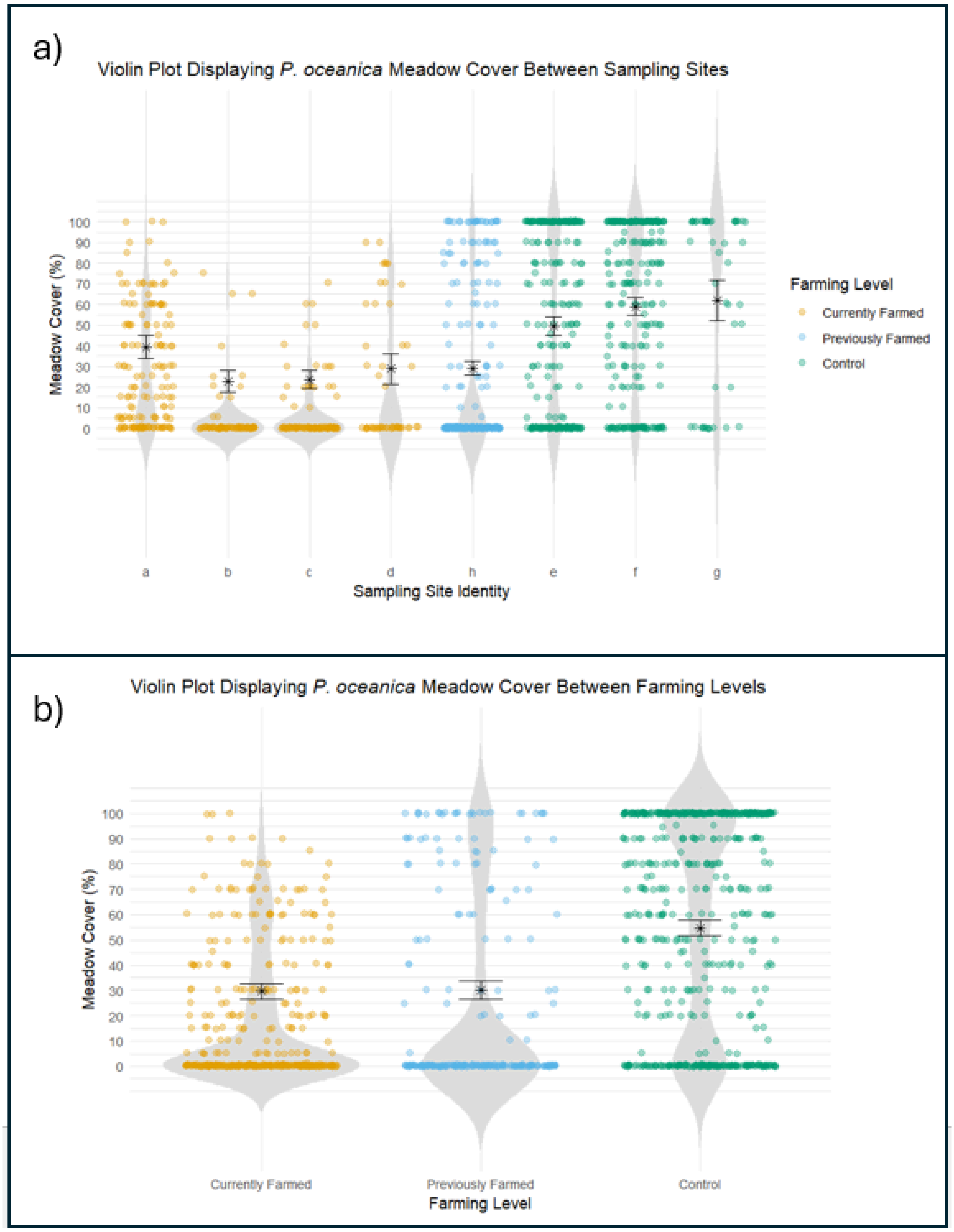


Figure 10: Meadow Cover a) was similarly depressed across all farmed sites except site a, though all farmed sites remained significantly lower than the controls. Original data points are shown. Stars represent model-estimated marginal means and intervals represent the 95% confidence interval for these means. b) Meadow cover was significantly lower at both the currently (gold) and previously farmed level (blue) relative to healthy reference meadows at the control level (green).

3.1.3 Epiphyte Cover (Community-Level Recovery)

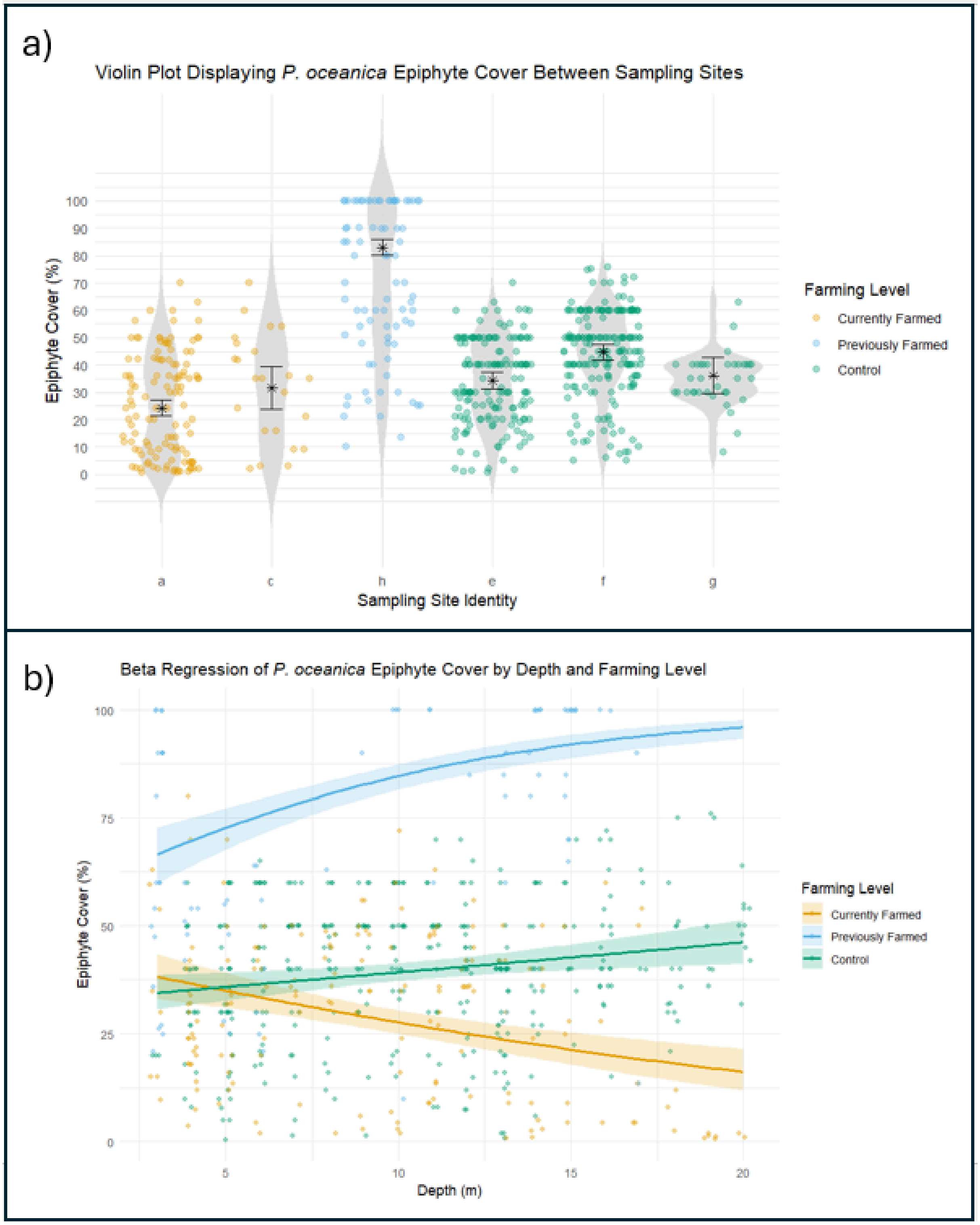
Tukey-adjusted pairwise comparisons of model-estimated marginal means showed that epiphyte cover did not vary significantly among the currently farmed sites (*a, c*) nor between control sites *e* vs. *g* and *f* vs. *g*. Although, control site *f* exhibited significantly lower epiphyte cover than control site *e* (β=-0.10, p<0.001). Therefore, epiphyte cover was relatively consistent among sites within each farming level (Fig. 11a).

The beta regression showed the previously farmed level had significantly higher epiphyte cover compared to both other levels, at approximately 2-fold that of the control level (β=0.973, p<.0001) and 3-fold that of the currently farmed level (Fig. 11b; Table 5) The currently farmed level was significantly lower than the control level (β=-0.446, p=0.0183).

Depth was significantly associated with each farming level (p<.001) and there were strong and opposing interaction effects between farming level and depth. In the previously farmed level, epiphyte cover increased more steeply with depth than the control level (β=0.117, p<.0001). Whereas, in the currently farmed level, epiphyte cover decreased with depth (Fig. 11b).

Farming Level	Proportional Epiphyte Cover Estimated Marginal Mean	Standard Error of Means	% Difference vs Previous Farm	% Difference vs Control
Currently farmed	0.278	0.0138	–67.1%	-29.1%
Previously farmed	0.845	0.0149	-	+116%
Control	0.392	0.0106	–53.6%	-

Table 5: Table of estimated marginal means, standard error of means and the percentage decrease relative to the previously farmed level for proportional *P. oceanica* epiphyte cover at each farming level (currently farmed, previously farmed, control).



3.2 Spatial Impacts of Fish Farming to *P. oceanica* Health

Full outputs of models: Appendices 6-8

3.2.1 Maximal Leaf Length

Pairwise comparisons of model-estimated marginal means showed that maximal leaf length at all distance categories within 900m of fish farms was significantly lower than the control group (Fig

12a). Within 600m of farms, maximal leaf length did not significantly differ between distance categories and reached a minimum estimated marginal mean of 18.8cm (SE=2.72), representing a 63% decrease relative to controls (EMM= 50.4, SE=0.89). Even at 750-900m, mean maximal leaf length was 32% lower (EMM=34.4, SE=4.83) than at controls.

The relative proportion of higher-length leaves vs. lower-length leaves gradually increased with distance (Fig. 12b) and 0% of samples within 900m of farms exceeded 60cm.

3.2.2 Meadow Cover

Fig. 13 shows that meadow cover slightly increased up to 900m from farms. Pairwise comparisons of model-estimated marginal means indicated each distance category up to 750m had significantly reduced meadow cover relative to the control sites ($p<.05$). Meadow cover was lowest at 300-450m (β=-0.291, p <.0001), representing a 53% decrease relative to controls (EMM=0.263, SE=0.0235; EMM=0.554, SE=0.0159). Mean meadow cover between 750-900m was still 30% lower than control levels, though this difference was not found to be statistically significant (EMM=0.388, SE=0.0696, β=-0.166, p=0.186). Notably, meadow cover in the 150-300m group was significantly higher than the 300-450m group (β=0.151, p<.0001).

Fig. 13b shows a positive association between proportion of high meadow cover values (80-100%) with distance. 0% of samples reached high cover within 150-300m from farms, rising to 10% high-cover samples at 750-900m, and 17% high-cover values at control sites.

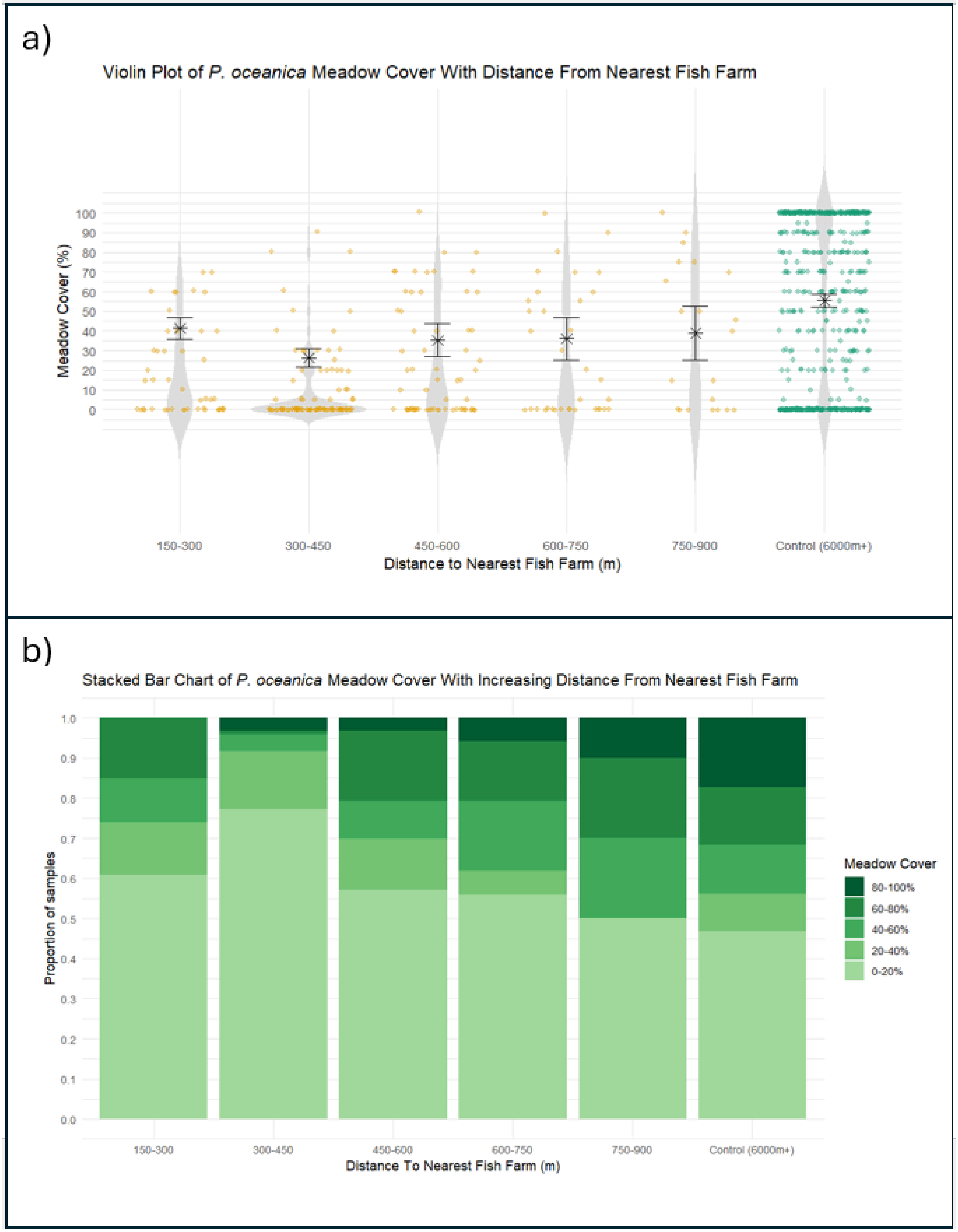
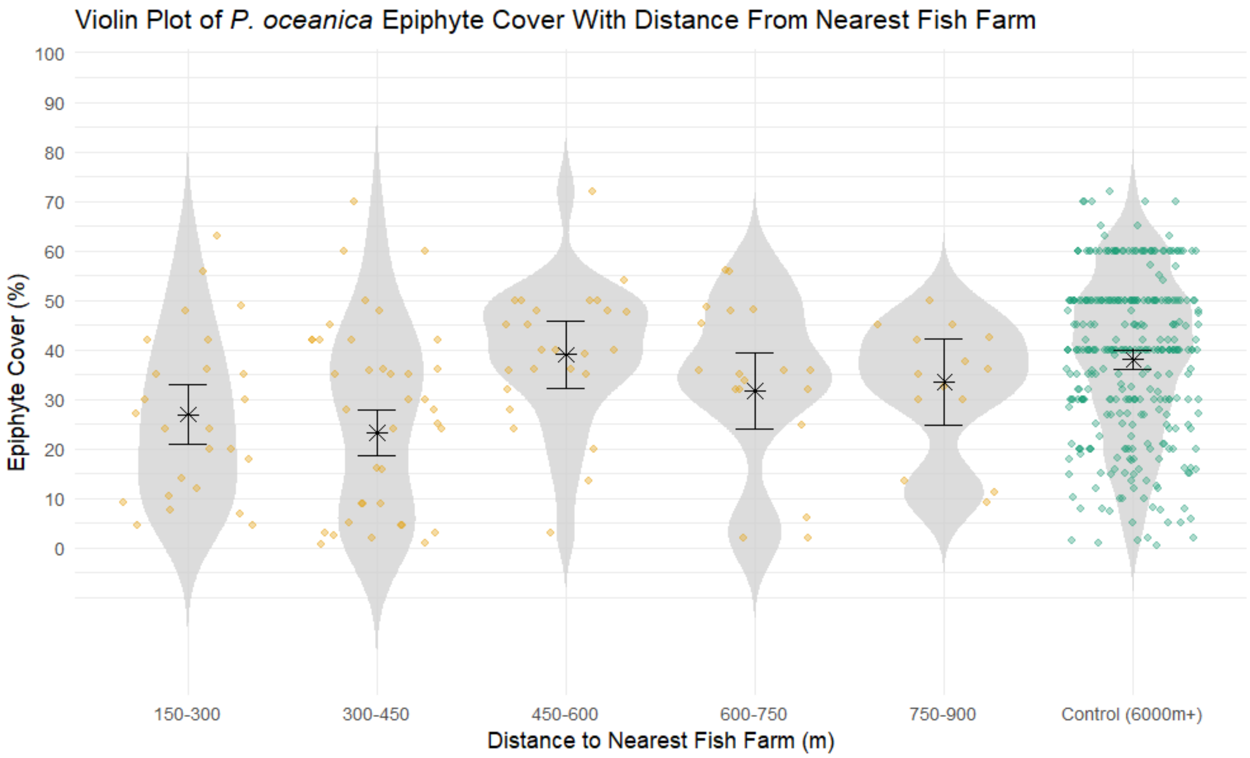


Figure 13: a) Meadow cover was significantly reduced up to 750m from its nearest fish farm. Original data points are shown. Stars represent model-estimated marginal means and intervals represent the 95% confidence interval for these means. b) The relative proportion of higher-cover samples vs. lower-cover samples gradually increased with distance but still represented significantly lower maximal meadow cover (80-100%) compared to controls.

3.2.3 Epiphyte Cover

Pairwise comparisons of model-estimated marginal means revealed significantly reduced epiphyte cover up to 450m from fish farms relative to the control group (p<.01) (Fig. 14). Beyond 450m, there was no significant difference in epiphyte cover relative to the control group.



3.3 Effect of Cumulative Fish Farming Duration on *P. oceanica* Health

Full outputs of models: Appendices 9-10

3.3.1 Maximal Leaf Length

Cumulative fish farming duration had a strong negative effect on maximal leaf length, with pairwise comparisons of model-estimated marginal means showing significant losses after 10-20 years of cumulative fish farm activity relative to the control group ($p<.0001$) (Fig. 15). Within 20 years, maximal leaf length decreased by 46% (EMM=27.36, SE=1.85), reaching an 85% decrease after 50 years (EMM=7.87, SE=6.86), relative to the control group (EMM=50.65, SE=0.915).

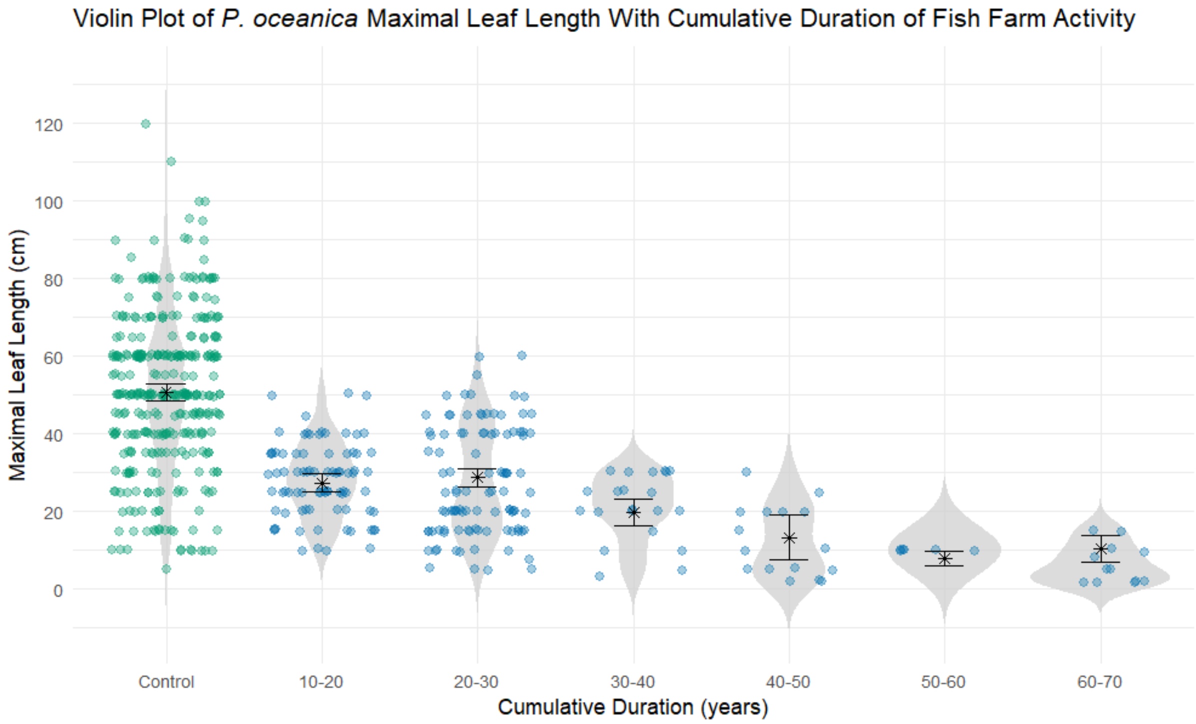
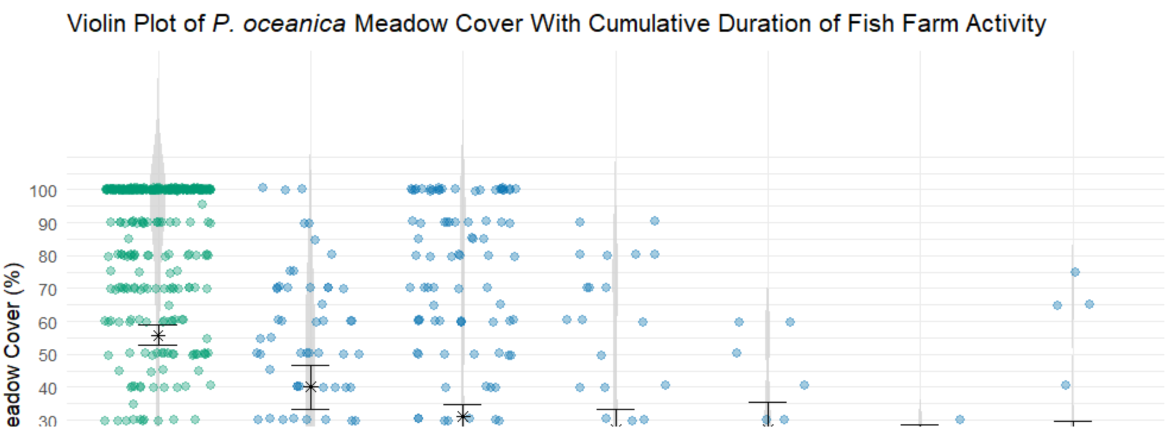


Figure 15: *P. oceanica* maximal leaf length decreases significantly as the cumulative duration of fish farm impact increases. Original data points are shown. Stars represent model-estimated marginal means and intervals represent the 95% confidence interval for these means.

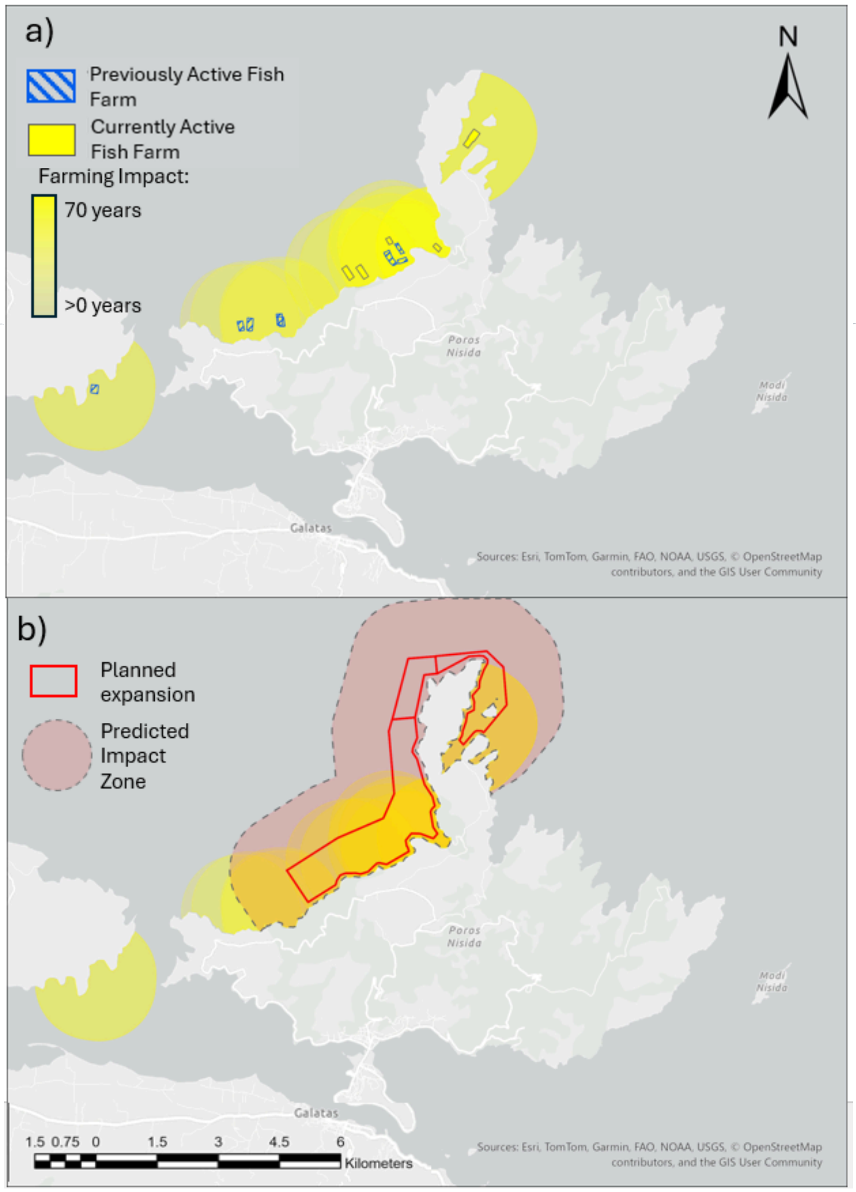
3.3.2 Meadow Cover

Cumulative fish farming duration was associated with significant losses in meadow cover (Fig 16). Pairwise comparisons of model-estimated marginal means indicated significant losses over 10-70 years of farming relative to the control group ($p<.001$). Within 20 years of fish farm introduction, 28% meadow cover was lost (EMM=0.40, SE=0.003), increasing to 60% meadow cover loss after 50 years (EMM=21.9, SE=0.034), relative to the control group (EMM=0.557, SE=0.016).



3.4 Hotspots of Fish Farming Impacts

Historic and current fish farm impact zones (Fig. 17) extend over the N and NW coast of Poros and mainland site, covering an area of 8.53km^2 and 17km of coastline. A particularly high intensity of impact can be seen in the central northern zone, where 70 years of cumulative farming activity has occurred. The estimated impact zone from future farming activity may increase the spatial extent of impact by 83%, to 15.6km^2, potentially intensifying current farming impacts and affecting seagrass meadows along an additional 3.2km of relatively undisturbed coastline.



4. Discussion

One key finding of this study is that fish farming activity is associated with long-term reduced seagrass health, particularly in shallow waters. The results in 3.1 revealed meadow cover declined by 46% near active farms and remained similarly depressed 14 years after fish farm removal relative to healthy meadows (Fig. 10b). Maximal leaf length was also reduced by 36% at previously farmed sites, though approached near-healthy levels in deep waters (~17m depth) (Fig. 9b). These results align with Hypothesis 1a, and 2a at the individual-level (Table 1). This suggests that nutrient loading from aquaculture practices may negatively and irreversibly impact *P. oceanica* health. This aligns with findings by Sanz-Lázaro and Marín (2006), which suggest that aquaculture effluent can lead to persistent anoxic conditions in the sediment. Furthermore, Delgado *et al.* (1999) reported depressed *P. oceanica* health three years after farm removal, hypothesising that nutrient-rich sediments are easily resuspended in denuded meadows, thus increasing dissolved nutrient availability and increasing light competition with epiphytes. Coupled with *P. oceanica*'s low growth rate, this may explain the long-term nature of health declines (Marba *et al.*, 2004). Previous research also suggests that water exchange is limited in shallow zones (Simonetti *et al.*, 2022). Thus, the relatively quicker removal of nutrient stress at depth may underlie the individual-level depth-dependent recovery pattern, and which may not yet be detected on a population level due to the relatively slower response rate of meadow cover to environmental change (Larkum *et al.*, 2006; Martinez-Crego *et al.*, 2008). Thus, this study provides evidence for sustained fish farming impacts over four-times longer than the historic literature, reinforcing the irreversibility of damage to *P. oceanica* meadow environments on human timescales.

Another key finding is that proximity to fish farms is associated with significantly reduced *P. oceanica* health, confirming Hypothesis 2. The results in section 3.2.2 showed, compared to healthy reference meadows located over 6000m away, meadow cover was up to 53% lower within 450m of farms and up to 900m maximal leaf length was significantly lower (32%) (Fig 12). Elevated health at 150-300m is likely attributable to overrepresentation of data from site *a*, which has experienced relatively shorter farm activity (NW5; Table 2). These results suggest fish farming impacts are most severe near to farms and can impact *P. oceanica* at a population- and individual-level over large distances beyond farm boundaries. These findings are consistent with previous research indicating that nutrient inputs (e.g. phosphorus, nitrogen) are highest directly under fish farm cages, with multi-study assessments by Pergent-Martini *et al.* (2006) and Holmer *et al.* (2008), recommending siting of fish farms beyond 200m and 400m from *P. oceanica* meadows, respectively, to avoid toxic effects. By comparison, the 900m radius identified in this study represents a large area of potential impact. Highly variable reports of spatial aquaculture impact on *P. oceanica* are often attributed to local hydrodynamism and discharge levels (Holmer *et al.*, 2007; Ruiz *et al.*, 2010). As Poros is relatively sheltered within the Saronic Gulf (Kontoyiannis, 2010), water circulation is reduced, hindering the movement of fish farm effluents away from seagrass meadows. The farms on Poros are also relatively close (within 100m) to shore (Fig. 2), limiting dissipation of nutrients in deep-water currents (Simonetti *et al.*, 2012). As limited data are available regarding farm-specific practices (e.g. annual fodder input, chemical usage) for Poros, and the relationship between seagrass health and specific aquaculture practices remains poorly understood, the significance of this variable is difficult to interpret and requires further investigation. Overall, these results provide evidence for aquaculture-driven health declines over a larger spatial scale than typically reported, necessitating updated management recommendations for farms situated in shallow, sheltered zones like on Poros. Although, as sampling depth was limited, future research incorporating deeper sampling (30-40m) could enhance understanding of the full spatial extent over which aquaculture activities impact *P. oceanica*.

Notably, this study identified reduced epiphyte cover near to currently active farms, contrary to H1a and H2 (Table 1). Within 450m of active farms, epiphyte cover was significantly reduced (29%) relative to healthy reference meadows. This finding is surprising as nutrient elevation in the water column and reductions in meadow cover and leaf length, as seen in this study, have been frequently linked to epiphyte overgrowth (Pergent-Martini *et al.*, 2006). However, Pitta *et al.* (2006) posit that nutrient transfer up the food chain is particularly efficient in the oligotrophic Mediterranean. This means that high epiphyte growth stimulated by fish farm introduction may be rapidly consumed by grazing organisms (Ruiz *et al.*, 2001). Due to the abundant food source (epiphytes), herbivory levels also often exceed natural levels and have resulted in overgrazing (Ruiz *et al.*, 2001), explaining the low epiphyte levels recorded.

In contrast, epiphyte cover was elevated after farm removal, contradicting Hypothesis 1c. Section 3.1.3 showed epiphyte cover was 2-times and 3-times higher after previous farming activity compared to control and currently farmed meadows, respectively. As nutrients are transient in the water column and epiphyte growth requires water-borne nutrients, fish farm discharges do not explain epiphyte elevation long after farm removal (Perez *et al.*, 2008). However, no specific natural or anthropogenic sources of nutrients unique to this area have been identified, primarily due to lack of local environmental data. Consequently, the potential influence of site-specific nutrient inputs on elevated epiphyte cover cannot be ruled out. Alternatively, as epiphyte cover results at the previously farmed site contradict most existing literature and challenge other findings in this study, it may be the case that visual estimation does not reliably reflect epiphyte loads on seagrass (Martinez-Crego *et al.*, 2008) and likely bias estimations towards epiphyte loads at the leaf tips. Hence, the typical method of epiphyte extraction and biomass quantification (Buia *et al.*, 2008) should be favoured in seagrass monitoring studies where invasive methods are possible.

This study also identified a negative relationship between long-term cumulative duration of fish farming activity and *P. oceanica* health, in agreement with Hypothesis 3, with most severe declines observed at the individual-level. Maximal leaf length nearly halved (46%) and 28% meadow loss occurred after 10-20 years of fish farming activity. This suggests that *P. oceanica* populations have limited resistance to fish farming impacts and the cumulative effects from multiple farms can worsen health outcomes. These findings are consistent with previous research indicating that even after a year of fish farming activity, *P. oceanica* vertical growth is stunted (Marba *et al.*, 2006), while Taskin *et al.* (2024) reported total loss after a decade. As Martinez-Crego *et al.* (2008) suggest environmental change first presents through plant physiology and morphology, the relatively high maximal leaf length reductions over the studied durations thus emphasise the magnitude of long-term environmental changes induced by farms. Additionally, this study's results conservatively estimate the magnitude of health declines as a large spatial footprint of farming activity (900m) was used for cumulative duration estimations and did not account for proximity-intensified health declines nor potential recovery. Thus, future long-term health assessments are critical to establish the adaptive potential of *P. oceanica* and identify temporal tipping points at which losses may become irreversible. Overall, these findings contribute important new evidence by quantifying fish farming impacts over long timescales, highlighting the vulnerability of *P. oceanica* to short- and long-term disturbance.

This study has also identified the northern coasts of Poros as hotspots for seagrass loss and degradation (Fig. 14), currently and into the future. These zones have experienced up to 70 years of cumulative farming activity, resulting in up to 85% lower maximal leaf length and 60% loss in meadow cover (see 3.3). Under expansion plans, the impacted zone may increase by 83%, affecting 3.2km of Poros's remaining coastline. Hence, fish farm expansions in this region may increase pressure on currently impacted zones and initiate health declines in relatively unaffected meadow areas. Importantly, these are preliminary estimates of future impacts, the magnitude and spatio-temporal scale of potential declines depending on the size, number, inputs and siting of future farms (Ruiz *et al.*, 2010; Kalantzi *et al.*, 2021). Climate change has also been found to interact synergistically with eutrophication impacts (Krishna *et al.*, 2025), so in combination with farm expansion plans, may result in even more extreme health declines in future. Thus, it is imperative that multi-stressor interactions are further investigated in the warming Mediterranean (Chefaoui *et al.*, 2018). Overall, the fate of *P. oceanica* on Poros depends on conservation-conscious decision-making, particularly regarding fish farm management.

Limitations

As Poros's eastern side is subject to relatively destructive hydrodynamic conditions (Kontoyiannis, 2010), the southeastern control sites may experience increased water circulation and nutrient turnover, potentially altering *P. oceanica* growth relative to currently farmed sites in the north-west (Fig. 3; Panayotidis *et al.*, 2022). However, as all sites were located near Poros and followed the same sampling methodology, most significant environmental factors influencing seagrass health (Ticina *et al.*, 2020) were controlled for, such as sediment type and depth. Health among currently farmed sites on different coasts (N vs. NW) was also relatively homogeneous (3.1). Thus, the relative contribution of coast-dependent hydrodynamics to differences observed between farming levels is likely low.

Furthermore, only a single replicate for the "previously farmed" level was available (Fig. 2) and may experience additional anthropogenic stress due to its proximity (~500m) to the daily Poros-Piraeus ferry route (Boudouresque *et al.*, 2009). Therefore, the results of this study may underestimate the recovery potential of *P. oceanica*.

Pollution and mechanical damage can also reduce seagrass health (Boudouresque *et al.*, 2009). A sewage discharge point was identified 1.3km from site *a* (Fig. 2). This point's entering load (383 tons) represented 1/3 annual fish farm discharge on Poros (European Environment Agency, 2023; Municipality of Poros, 2025). Considering this and the maximal 900m farm impact radius (3.2), the mobilisation of sewage pollution into site *a* is unlikely, evidenced by this site's relative health among currently farmed sites (see 3.1), but must be considered. Additionally, no sampled sites showed signs of anchoring damage, and minimal agricultural activity is recorded on Poros (Hellenic Statistical Authority, 2011).

Overall, some consideration for natural variability and other anthropogenic stressors should be made when interpreting the results of this study, but are not likely to underlie the key trends identified. The spatio-temporal associations of seagrass health with aquaculture infrastructure further imply that fish farming is the primary driver of health declines reported in this study.

Conclusion

Overall, this study provides clear evidence of the negative effects of fish farming on the seagrass *Posidonia oceanica*, demonstrating significant reductions in individual-level and population-level meadow health, as well as changes to community-level dynamics, in areas influenced by both current and historical aquaculture activity. *P. oceanica* health generally did not improve after 14 years, though showed some individual-level recovery with depth. Significant health reductions extended over a large spatial scale (900m) from fish farms. Health continued to decline under increasing cumulative fish farm duration and significant health reductions were detectable after 10 years. As northern Poros has experienced maximal fish farming intensity and is targeted by expansion plans, *P. oceanica* meadows in this region are particularly threatened.

These findings underscore the need for improved fish farm management: future expansion of aquaculture should avoid *P. oceanica* habitats, particularly in shallow, sheltered coastal zones, to maximise conservation outcomes.

5. Acknowledgements

I'd like to thank Dr Gwilym Rowlands and Nancy Burrell for their support and advice on this project. It has been brilliant working with you, and I am very grateful to have had such kind mentors who have remained faithful to this project despite major career changes. I would also like to thank Dr Katrina Davis for adopting me into her wonderful lab group, all of whom were extremely welcoming and shared a passion for all things marine. I would also like to thank the Argosaronic Environment

Foundation, in particular Nancy Burrell and Daphne Mantziou, for financially supporting this project and making it logistically possible. I am also very grateful for the support from Katheti while on Poros, particularly from Fay Orfanidou and Tasos Rodis, who made me feel very at home and gave me the opportunity to take part in exciting environmental outreach activities. A very special thanks to Joe Boyle, the best fieldwork companion, whose research diving experience was invaluable to the success of this project and who worked tirelessly to make each dive count and keep me safe and comfortable during the fieldwork period. Thank you to Dive in Blue for providing such an excellent dive team (Giorgos, Yiannis, Theo and Franki) who were wonderful company in the field and kept procedures safe, efficient and fun. The wonderful Ilektra Athinaiou from iSea also provided invaluable help towards fieldwork planning and data sourcing. Thank you also to the Rauch Foundation, Oxford Deep Blue Society and the other organisations above, for being such strong supporters of this project and seagrass conservation and giving me a platform to talk about my work; it has been so exciting being surrounding by such passionate and environmentally conscious people. Back in Oxford, I would also like to thank the lovely Dr Ellie Bath for her statistical advice and reassurance. Finally, a massive thank you to my very patient friends and family for believing in me and providing moral support.

6. Management Report

Supervised by Dr. Gwilym Rowlands and Nancy Burrell, I led the design and delivery of a Master's research project investigating the response of *Posidonia oceanica* seagrass to aquaculture impacts in the Argosaronic region of Greece, particularly around the island of Poros. The project was primarily funded by the Argosaronic Environment Foundation, with additional support from a £1,000 University of Oxford grant. I was responsible for overall project management, including the development of the sampling methodology, coordination of all travel logistics, sourcing of field equipment, direct liaison with the dive team, and management of a project budget of approximately £13,000.

Due to seasonal constraints on dive safety, fieldwork was limited to a three-week window in October 2024. Despite time pressures and limited access to historical records—partly due to language barriers—I developed a robust, non-invasive methodology suitable for visual data collection.

As a non-qualified research diver, I led the field operations from the surface and via snorkel, instructing a local dive team and Oxford-based research diver Joe Boyle on the survey protocols and ensuring accurate implementation of data collection methods across each transect.

Although weather disruptions and sea conditions restricted access to some planned control and farm sites—particularly around the neighbouring island of Methana and the NE—we successfully sampled across the four active inshore fish farms and four control sites on Poros. Crucially, weather limitations prevented us from collecting data from control sites on more sheltered coastlines. The dive team, was also limited to three dives per day due to safety and logistical considerations, preventing further data collection. Divers collected data on leaf length, live and dead meadow cover, and epiphyte abundance across 1 m^2 quadrats.

Following fieldwork, I processed over 5,000 photographs and 2,500+ individual data entries. Each image was geotagged using GPS tracks and manually matched with corresponding data using GeoSetter and Google Earth Pro. This task required significant manual effort, including filtering duplicates, organising dive logs, and ensuring all data were correctly spatially referenced. I also managed all financial tracking and receipts, and prepared interim reports and outreach materials for stakeholders.

Between November 2024 and March 2025, I conducted spatial analyses using ArcGIS Pro to map current and historic fish farm locations, supported by satellite imagery and archival research. Given the lack of centralised historic farm data, I manually reviewed satellite images from Google Earth Pro and Copernicus Hub to reconstruct farm operation timelines. With limited supervisor familiarity with newer ArcGIS tools, I self-taught relevant spatial methods via tutorials and applied them to estimate farm proximities and generate key figures.

In the final phase (March–May 2025), I performed statistical analyses in R, adapting techniques suitable for bounded ecological data and hierarchical sampling designs. Some metrics, such as dead meadow cover, were excluded based on methodological reliability identified in the literature. Attempts to incorporate environmental covariates such as temperature and salinity were limited by data availability for the region. The project concluded with the completion of a dissertation and first full draft in early May 2025.

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8. Appendices

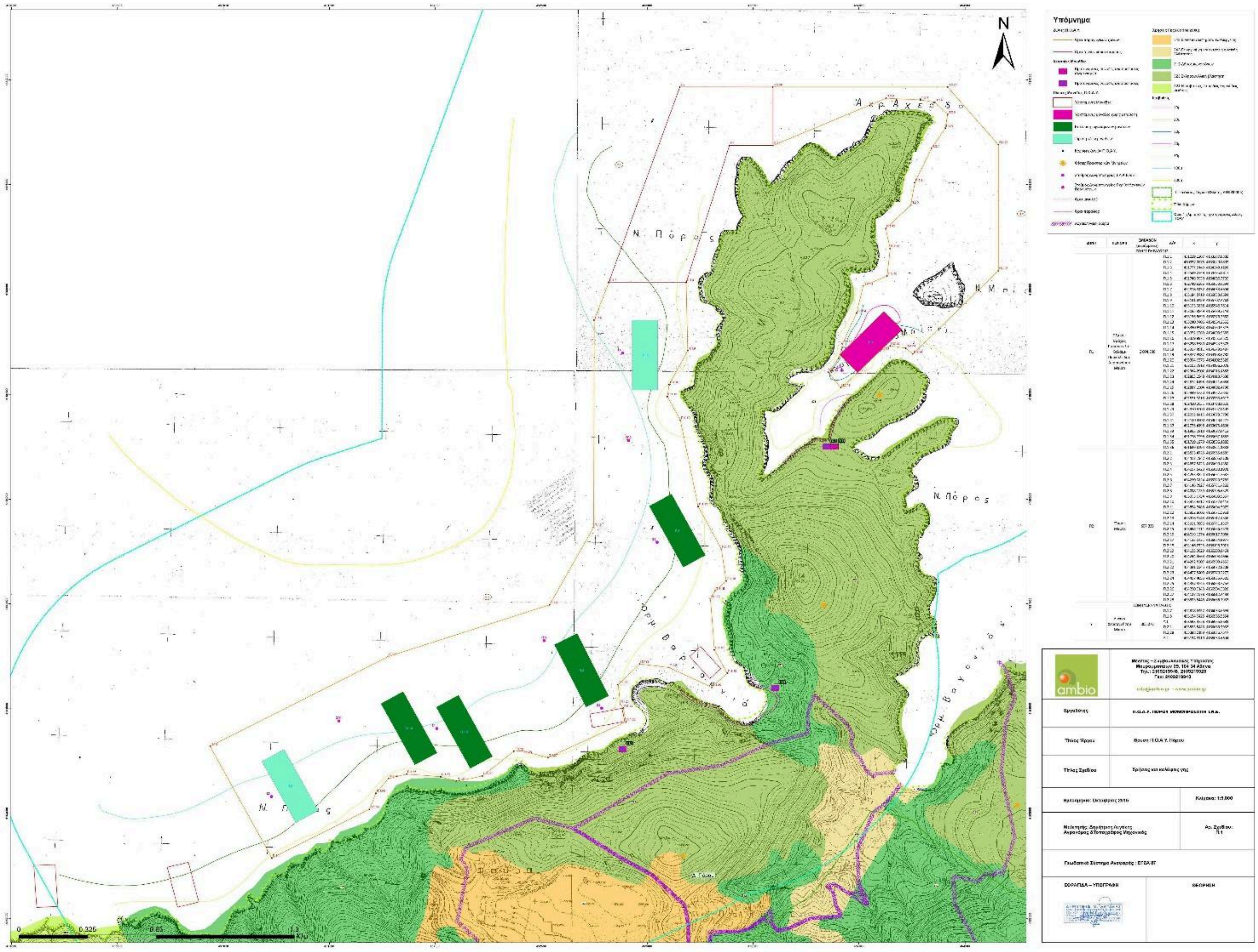
Appendix 1: SCUBA Dive Protocol Excerpt

'Dive Operations

- Dive operations will follow those of the vessel operator, as discussed in daily briefing and vessel orientation.
- All diving and boat related activity to be completed within daylight hours.
- No solo diving. All diving shall be conducted using the buddy system. Divers may be paired or in buddy groups of not more than 3 divers. Buddy groupings will be assigned even if two or more groups are operating in close proximity as part of a larger team.
- No diving in overhead environments. Overhead environments are defined as underwater environments where there is a physical obstacle that prevents a direct ascent to the water's surface. These environments include caves, wrecks, culverts, under ships, nets and other structures.
- A dynamic safety assessment shall be made at each site to ensure the proposed dive site is free of fishing nets or other in-water dangers.
- Pre-dive equipment and safety checks will be conducted with their buddy following standard diving protocols (e.g. Buoyancy, Weights, Releases, Air, and Final Check), and recorded into dive log by surface support officer.
- One diver in each buddy grouping must tow a Surface Marker Buoy (SMB) throughout the dive as required by the survey protocol. Any static operations such as sediment cores, vegetation sampling or measurement, will be similarly marked with a surface marker buoy.
- Diver entry and exit will follow the protocols of the vessel operator. Divers will receive a briefing in these protocols before the dive. Divers will enter the water together in buddy groupings, convene at the surface, before descending together to start the dive.
- Ascent and decent shall be made directly under the SMB. Divers may carry a delayed SMB (dSMB) to deploy in case of loss of the towed SMB during a dive and deploy this to mark their location to surface support and to ascend under.
- Divers are responsible for dismantling and securely stowing personal equipment.
- Divers are expected to carry suitable exposure suits for the diving conditions
- Divers will be adequately weighted for diving in their equipment configuration to avoid loss of buoyancy towards the end of the dive.
- The maximum depth of all diving operations shall be 25 m or within the limits of each diver's qualifications and experience.

- The standard maximum dive time shall be 60 minutes or less, as determined by the dive profile, or air consumption. If dives are expected to be shallow (<10m) then dive time may be extended to 90 minutes with prior agreement during the dive briefing. Maximum dive time describes the time from descending to time to resurface. Dive tables will be available on the boat in the event of computer failures (see last page).
- Surface interval between dives shall be a minimum of 1 hour.
- Within buddy groupings, divers shall dive within the limits described by their computer. If a diver is not using a dive computer, they will use an approved set of diving tables (e.g. the PADI Recreational Dive Planner) and an underwater timing device to plan and monitor their dive. The most conservative dive profile of each buddy grouping shall be followed.
- If a diver's computer fails, they should switch to their (i) back up computer or (ii) their timing device and pre-determined dive table profiles to continue the dive. If these options are unavailable the dive should be aborted, and the time noted for subsequent dives to be possible.
- Buddy groupings should complete their dive and return to the surface when (i) the first diver in a buddy team nears the stipulated air reserve (divers should return to the surface with at least 50 bar/500 psi in their tank), (ii) the maximum dive time is reached, or (iii) the survey is complete.
- Divers will conduct a safety stop at 5 metres for 3 minutes on all dives, and any additional safety stops if required by their dive computer.
- On reaching the surface divers should reconnect in their buddy grouping, inflate their BCD to ensure positive buoyancy, signal to the vessel, and await pickup.
- Emergency decompression. Should a diver accidentally dive longer that the no decompression limits defined by their computer, they should follow the instructions of their computer to decompress. If using dive tables for planning, the diver should follow the standard emergency decompression guidelines e.g. <5 minutes over, - extend the safety stop to 8 minutes (no diving for 6 hours), >5 minutes over - extend the safety stop to 15 minutes, air permitting (no diving for 24 hours).'

Appendix 2: Poros P.O.A.Y (O.A.D.A) Fish Farm Expansion Plans



Appendix 3: Results of Analysis 3.1.1

<u>Maximal Leaf Length Farming Level Comparisons- linear model with robust standard errors</u>

Predictor	Estimate	Std. Error	t value	p-value	Significance
(Intercept)	20.5428	1.968562	10.435	< 2.2e-16	***
SiteType: Previously Farmed	-7.87206	2.894077	-2.72	0.006714	**
SiteType: Control	25.71357	3.42269	7.513	2.08E-13	***
Depth	0.069217	0.179976	0.385	0.700675	n.s.
Previously Farmed × Depth	1.96409	0.283611	6.925	1.11E-11	***
Control × Depth	0.408362	0.303576	1.345	0.179072	n.s.

<u>Model Summary</u>

Metric	Value
Residual Standard Error	16.68
Degrees of Freedom (Residuals)	608
Multiple R-squared	0.4233
Adjusted R-squared	(not shown)

Number of Samples

Farming Level	n
Control	357
Previous farm	78
Current farm	179

Estimated Marginal Means

Site Type	Estimated marginal mean (cm)	SE	df	Lower CL	Upper CL	% Decrease vs Control
Currently Farmed	21.2	1.25	608	18.8	23.7	58.30%
Previously Farmed	32.6	1.93	608	28.8	36.3	36.00%
Control	50.9	0.89	608	49.2	52.7	0.00%

Tukey-adjusted pairwise comparisons of estimated marginal means

Contrast	Estimate (cm)	SE	df	t-ratio	p-value
Currently Farmed – Previously Farmed	–11.3	2.3	608	–4.931	<.0001
Currently Farmed – Control	–29.7	1.53	608	–19.374	<.0001
Previously Farmed – Control	–18.4	2.12	608	–8.652	<.0001

Maximal Leaf Length Sampling Site Comparisons- linear model with robust standard errors

Sampling Site	Estimate	Std. Error	t value	Pr(>|t|)	Significance
(Intercept)|	19.6242	2.1424	9.16	< 2e-16	***
b	-15.3077	5.06	-3.025	0.00259	**
c	-13.4936	3.5064	-3.848	0.000132	***
d	-3.6666	3.9815	-0.921	0.357459	n.s.
e	29.0832	2.0372	14.276	< 2e-16	***

f	22.7016	1.9262	11.786	< 2e-16	***
g	33.1777	3.5106	9.451	< 2e-16	***
h	6.1303	2.3983	2.556	0.01083	*
Depth	0.5347	0.1568	3.41	0.000693	***

Model Summary

Metric	Value
Residual Std. Error	16.45
Degrees of Freedom	605
Multiple R^2	0.4416
Adjusted R^2	0.4342
F-statistic	59.81
Model DF	8 and 605
Model p-value	< 2.2e-16

Number of Samples

Sampling Site	n
a	120
b	12
c	27
d	20
e	143
f	187
g	27
h	78

Estimated marginal means

Transect	Emmean	SE	df	Lower CL	Upper CL
a	24.85	1.5	605	21.9	27.8
b	9.55	4.83	605	0.05	19
c	11.36	3.17	605	5.14	17.6
d	21.19	3.69	605	13.95	28.4
e	53.94	1.38	605	51.23	56.6
f	47.56	1.21	605	45.19	49.9
g	58.03	3.17	605	51.8	64.3
h	30.98	1.87	605	27.31	34.7

Tukey-adjusted pairwise comparisons of estimated marginal means

Contrast	Estimate	SE	df	t.ratio	p-value
a - b	15.31	5.06	605	3.025	0.0524
a - c	13.49	3.51	605	3.848	0.0033
a - d	3.67	3.98	605	0.921	0.984

a - e	–29.08	2.04	605	–14.276	<0.0001
a - f	–22.70	1.93	605	–11.786	<0.0001
a - g	–33.18	3.51	605	–9.451	<0.0001
a - h	–6.13	2.4	605	–2.556	0.1744
b - c	–1.81	5.76	605	–0.315	1
b - d	–11.64	6.11	605	–1.904	0.5486
b - e	–44.39	5.03	605	–8.826	<0.0001
b - f	–38.01	4.99	605	–7.610	<0.0001
b - g	–48.49	5.81	605	–8.343	<0.0001
b - h	–21.44	5.15	605	–4.160	0.0009
c - d	–9.83	4.87	605	–2.019	0.4698
c - e	–42.58	3.46	605	–12.322	<0.0001
c - f	–36.20	3.39	605	–10.668	<0.0001
c - g	–46.67	4.49	605	–10.397	<0.0001
c - h	–19.62	3.67	605	–5.341	<0.0001
d - e	–32.75	3.93	605	–8.325	<0.0001
d - f	–26.37	3.87	605	–6.806	<0.0001
d - g	–36.84	4.85	605	–7.590	<0.0001
d - h	–9.80	4.14	605	–2.364	0.261
e - f	6.38	1.83	605	3.49	0.0121
e - g	–4.09	3.46	605	–1.184	0.9362
e - h	22.95	2.32	605	9.877	<0.0001
f - g	–10.48	3.39	605	–3.091	0.0433
f - h	16.57	2.23	605	7.429	<0.0001
g - h	27.05	3.69	605	7.327	<0.0001

Appendix 4: Results of Analysis 3.1.2

Meadow Cover Farming Level Comparisons- beta regression

Predictor	**Estimate**	**Std. Error**	**z value**	***p* value**	**Significance**
(Intercept)	0.211	0.155	1.364	0.173	n.s.
Current farm	–0.897	0.225	–3.985	<0.001	***
Previous farm	–1.189	0.23	–5.161	<0.001	***
Depth	–0.002	0.014	–0.159	0.873	n.s.
Current farm × Depth	–0.017	0.021	–0.806	0.42	n.s.
Previous farm × Depth	0.017	0.022	0.752	0.452	n.s.

Model Summary

Statistic	**Value**
Log-likelihood	2493 (on 7 DF)
Pseudo R^2	0.188
Estimation method	ML (BFGS + Fisher)

Number of iterations	24 (BFGS) + 1 (Fisher)

Number of Samples

Farming Level	n
Control	570
Previous Farm	244
Current Farm	359

Estimated marginal means

Site Type	Estimated Meadow Cover (Mean)	SE	95% CI	Site Type	Estimated Meadow Cover (Mean)	SE	95% CI	% Loss Relative to Control
Control	0.547	0.0152	[0.517, 0.577]	Control	0.547	0.0152	[0.517, 0.577]	–
Current farm	0.296	0.0151	[0.266, 0.325]	Current farm	0.296	0.0151	[0.266, 0.325]	45.90%
Previous farm	0.302	0.0184	[0.266, 0.338]	Previous farm	0.302	0.0184	[0.266, 0.338]	44.80%

Tukey-adjusted pairwise comparisons

Contrast	Estimate	SE	z-ratio	*p*-value	Significance
Control – Current farm	0.252	0.0217	11.57	< 0.0001	***
Control – Previous farm	0.246	0.0242	10.16	< 0.0001	***
Current farm – Previous farm	–0.006	0.023	–0.27	0.962	n.s.

Meadow Cover Sampling Site Comparisons- beta regression

Sampling Site	Estimate	Std. Error	z value	*p*-value	Significance
(Intercept)	–0.333	0.147	–2.273	0.023	*
b	–0.792	0.192	–4.123	<0.001	***
c	–0.741	0.173	–4.281	<0.001	***
d	–0.473	0.214	–2.214	0.027	*
e	0.408	0.146	2.799	0.005	**
f	0.795	0.146	5.451	<0.001	***
g	0.918	0.242	3.801	<0.001	***
h	–0.457	0.142	–3.220	0.001	**
Depth	–0.010	0.0089	–1.140	0.254	n.s.
Reference level for Sampling Site is Transect a.					

Model summary

Metric	**Value**
Phi (precision)	0.503 (SE = 0.017)
z value (phi)	29.14
p-value (phi)	< 0.001 (*** significant)
Log-likelihood	2510 (on 10 DF)
Pseudo R^2	0.231
Iterations	19 (BFGS) + 2 (Fisher scoring)

Number of Samples

Sampling Site	**n**
a	142
b	65
c	100
d	52
e	263
f	259
g	48
h	244

Estimated marginal means

Sampling Site	**Estimated marginal mean**	**SE**	**95% CI (Lower)**	**95% CI (Upper)**
a	0.394	0.0275	0.34	0.448
b	0.227	0.0273	0.174	0.281
c	0.237	0.0237	0.19	0.283
d	0.288	0.0373	0.215	0.361
e	0.494	0.0225	0.45	0.538
f	0.59	0.0213	0.548	0.632
g	0.619	0.0499	0.522	0.717
h	0.292	0.0177	0.257	0.326

Tukey-adjusted pairwise comparison of estimated marginal means

Contrast	**Estimate**	**SE**	**z-ratio**	**p-value**	**Significance**
a - b	0.16643	0.0385	4.327	0.0004	***
a - c	0.15732	0.036	4.374	0.0003	***
a - d	0.10562	0.046	2.298	0.2947	n.s.
a - e	-0.10041	0.0354	-2.833	0.087	

a - f	-0.19609	0.035	-5.605	<0.0001	***
a - g	-0.22555	0.0571	-3.949	0.002	**
a - h	0.10232	0.0323	3.164	0.0334	*
b - c	-0.00911	0.0353	-0.258	1	n.s.
b - d	-0.06081	0.0458	-1.327	0.8888	n.s.
b - e	-0.26684	0.0355	-7.521	<0.0001	***
b - f	-0.36252	0.0351	-10.331	<0.0001	***
b - g	-0.39198	0.0573	-6.839	<0.0001	***
b - h	-0.06411	0.0317	-2.025	0.4648	n.s.
c - d	-0.0517	0.0437	-1.182	0.9373	n.s.
c - e	-0.25773	0.0328	-7.869	<0.0001	***
c - f	-0.35341	0.0323	-10.927	<0.0001	***
c - g	-0.38287	0.0557	-6.879	<0.0001	***
c - h	-0.055	0.0287	-1.919	0.5374	n.s.
d - e	-0.20602	0.0434	-4.744	0.0001	***
d - f	-0.3017	0.0432	-6.989	<0.0001	***
d - g	-0.33117	0.0625	-5.301	<0.0001	***
d - h	-0.00329	0.0408	-0.081	1	n.s.
e - f	-0.09568	0.031	-3.091	0.0419	.
e - g	-0.12514	0.0547	-2.288	0.3	n.s.
e - h	0.20273	0.0287	7.065	<0.0001	***
f - g	-0.02947	0.0541	-0.545	0.9994	n.s.
f - h	0.29841	0.0282	10.577	<0.0001	***
g - h	0.32788	0.0534	6.145	<0.0001	***

Appendix 5: Results of Analysis 3.1.3

Epiphyte Cover Farming Level Comparisons- beta regression

Term	Estimate	Std. Error	z value	Pr(>|z|)	Significance
(Intercept)	-0.27586	0.15091	-1.828	0.0676	n.s.
SiteTypePreviously Farmed	0.52673	0.2497	2.109	0.0349	*
SiteTypeControl	-0.44648	0.18925	-2.359	0.0183	*
Depth	-0.0683	0.015	-4.553	0.000005	***
SiteTypePreviously Farmed:Depth	0.21403	0.02592	8.256	< 2e-16	***
SiteTypeControl:Depth	0.09688	0.01807	5.361	8.29E-08	***
(phi)	4.7617	0.2549	18.68	< 2e-16	***

Predictor	**Estimate**	**Std. Error**	**t value**	**p-value**	**Significance**
(Intercept)	–0.72233	0.11437	–6.316	2.69E-10	***
SiteType: Current farm	0.44648	0.18925	2.359	0.01831	*

SiteType: Previous farm	0.97321	0.22951	4.24	2.23E-05	***
Depth	0.02858	0.01004	2.845	0.00444	**
Current farm × Depth	–0.09688	0.01807	–5.361	8.29E-08	***
Previous farm × Depth	0.11716	0.02328	5.032	4.85E-07	***

<u>Number of Samples</u>

Farming Level	**n**
Control	365
Previous farm	77
Current farm	171

<u>Estimated marginal means</u>

Site Type	**Emmean**	**SE**	**Lower CL**	**Upper CL**	**% Difference vs Previous Farm**
Control	0.392	0.0106	0.371	0.413	–53.6%
Current farm	0.278	0.0138	0.251	0.305	–67.1%
Previous farm	0.845	0.0149	0.816	0.874	Reference

<u>Tukey-adjusted pairwise comparison of means</u>

Contrast	**Estimate**	**SE**	**z-ratio**	**p-value**
Control – Current farm	0.114	0.0172	6.61	<.0001
Control – Previous farm	–0.453	0.0185	–24.553	<.0001
Current farm – Previous farm	–0.567	0.0207	–27.443	<.0001

<u>Epiphyte Cover Sampling Site Comparisons- beta regression</u>

Term	Estimate	Std. Error	z value	Pr(>|z|)	Significance
(Intercept)	-1.25786	0.106132	-11.852	< 2e-16	***
C	0.362159	0.201233	1.8	0.071908	n.s.
E	0.491148	0.106335	4.619	3.86E-06	***
F	0.928375	0.102391	9.067	< 2e-16	***
G	0.57237	0.167801	3.411	0.000647	***

H	2.731803	0.139471	19.587	< 2e-16	***
Depth	0.010774	0.006321	1.705	0.088255	n.s.
(phi)	4.4672	0.2347	19.03	< 2e-16	***

Number of samples

Sampling Site	**n**
a	122
c	23
e	170
f	201
g	36
h	80

Estimated marginal means

Sampling Site Identity	Estimated marginal mean	SE	Lower CI	Upper CI
a	0.242	0.0153	0.212	0.272
c	0.315	0.0397	0.237	0.392
e	0.343	0.0152	0.313	0.373
f	0.447	0.0148	0.418	0.476
g	0.362	0.0336	0.296	0.427
h	0.831	0.0153	0.801	0.861

Tukey-adjusted pairwise comparison of estimated marginal means

Contrast	Estimate	SE	df	z.ratio	p.value	Significance
a - c	-0.0724	0.0424	Inf	-1.709	0.5256	n.s.
a - e	-0.1009	0.0214	Inf	-4.721	<.0001	***
a - f	-0.2049	0.0212	Inf	-9.657	<.0001	***
a - g	-0.1194	0.0369	Inf	-3.234	0.0155	*
a - h	-0.5886	0.022	Inf	-26.724	<.0001	***
c - e	-0.0285	0.0426	Inf	-0.669	0.9854	n.s.
c - f	-0.1325	0.0424	Inf	-3.127	0.0218	*
c - g	-0.047	0.0524	Inf	-0.897	0.9474	n.s.
c - h	-0.5161	0.0425	Inf	-12.132	<.0001	***
e - f	-0.104	0.0211	Inf	-4.929	<.0001	***
e - g	-0.0185	0.0366	Inf	-0.507	0.9959	n.s.
e - h	-0.4877	0.022	Inf	-22.195	<.0001	***
f - g	0.0855	0.0367	Inf	2.331	0.1816	n.s.
f - h	-0.3837	0.0214	Inf	-17.921	<.0001	***
g - h	-0.4692	0.0374	Inf	-12.536	<.0001	***

Appendix 6: Results of Analysis 3.2.1

Maximal leaf length with distance from nearest fish farm- linear model with robust standard errors

Predictor	Estimate	Std. Error	t value	p-value	Significance
(Intercept)	24.289	2.642	9.192	< 2e-16	***
300–450 m	–4.263	2.85	–1.496	0.135	n.s.
450–600 m	–2.532	3.004	–0.843	0.4	n.s.
600–750 m	3.73	3.035	1.229	0.22	n.s.
750–900 m	11.252	3.724	3.022	0.00263	**
Control (6000+ m)	27.34	2.668	10.248	< 2e-16	***
Depth	–0.104	0.154	–0.676	0.499	n.s.

Model summary

Metric	Value
Residual Standard Error	18
Degrees of Freedom (Residuals)	547
Multiple R-squared	0.3261
Adjusted R-squared	0.3187
F-statistic	44.11 (on 6 and 547 DF)
p-value (overall model)	< 2.2e-16

Number of Samples

Distance (m)	n
150-300	85
300-450	45
450-600	34
600-750	18
750-900	14
Control	336

Estimated marginal means

Distance to Farm (m)	Emmean	SE	df	Lower CL	Upper CL	% Loss vs Control
150–300	23.1	3.56	547	16.1	30.1	54.20%
300–450	18.8	2.72	547	13.5	24.2	62.70%
450–600	20.6	2.74	547	15.2	26	59.10%
600–750	26.8	4.27	547	18.4	35.2	46.80%
750–900	34.4	4.83	547	24.9	43.8	31.70%

Control (6000+)	50.4	0.89	547	48.7	52.2	0.00%

Tukey-adjusted pairwise comparison of estimated marginal means

Contrast	Estimate	SE	df	t-ratio	p-value
(150–300) – (300–450)	4.26	4.51	547	0.946	0.9343
(150–300) – (450–600)	2.53	4.5	547	0.563	0.9933
(150–300) – (600–750)	–3.73	5.52	547	–0.676	0.9845
(150–300) – (750–900)	–11.25	5.97	547	–1.886	0.4119
(150–300) – Control (6000+)	–27.34	3.68	547	–7.435	<0.0001
(300–450) – (450–600)	–1.73	3.86	547	–0.448	0.9977
(300–450) – (600–750)	–7.99	5.09	547	–1.570	0.6185
(300–450) – (750–900)	–15.52	5.56	547	–2.793	0.0601
(300–450) – Control (6000+)	–31.60	2.86	547	–11.047	<0.0001
(450–600) – (600–750)	–6.26	5.08	547	–1.232	0.8209
(450–600) – (750–900)	–13.78	5.55	547	–2.482	0.1311
(450–600) – Control (6000+)	–29.87	2.89	547	–10.354	<0.0001
(600–750) – (750–900)	–7.52	6.41	547	–1.173	0.8498
(600–750) – Control (6000+)	–23.61	4.37	547	–5.402	<0.0001
(750–900) – Control (6000+)	–16.09	4.91	547	–3.276	0.0142

Appendix 7: Results of Analysis 3.2.2

Meadow cover with distance from nearest fish farm- beta regression

Predictor	Estimate	Std. Error	z value	*p*-value	Significance
(Intercept)	–0.483	0.157	–3.076	0.0021	**
300–450 m	–0.682	0.166	–4.112	<0.0001	***
450–600 m	–0.260	0.22	–1.182	0.2373	
600–750 m	–0.225	0.262	–0.858	0.3908	
750–900 m	–0.105	0.315	–0.335	0.7376	

Control (6000+ m, ref)	0.566	0.133	4.255	<0.0001	***
Depth	0.0149	0.012	1.234	0.2171	
Reference group: 0–300 m (baseline category for distance).					

Model summary

Statistic	Value
Phi (dispersion)	0.467 (SE = 0.0179)
Log-likelihood	1696 (on 8 DF)
Pseudo R^2	0.161
Residuals (Quantile)	Min: –1.578, 1Q: –0.808, Median: 0.007, 3Q: 1.085, Max: 1.820
Iterations	18 (BFGS) + 2 (Fisher scoring)

Number of samples

Distance (m)	n
150-300	138
300-450	113
450-600	50
600-750	32
750-900	21
Control	457

Estimated marginal means

Distance Category	Estimated marginal means	SE	95% CI (Lower)	95% CI (Upper)	% Loss Relative to Control
150–300 m	0.414	0.0281	0.359	0.469	25.30%
300–450 m	0.263	0.0235	0.217	0.309	52.50%
450–600 m	0.352	0.0429	0.268	0.436	36.40%
600–750 m	0.36	0.0545	0.254	0.467	35.00%
750–900 m	0.388	0.0696	0.252	0.525	30.00%
Control (6000+ m)	0.554	0.0159	0.523	0.585	–

Tukey-adjusted pairwise comparison of estimated marginal mean

Contrast	Estimate	SE	z-ratio	p-value
(150–300) – (300–450)	0.15066	0.0362	4.161	0.0005
(150–300) – (450–600)	0.06125	0.051	1.201	0.8367
(150–300) – (600–750)	0.05328	0.061	0.873	0.9529

(150–300) – (750–900)	0.02531	0.0749	0.338	0.9994
(150–300) – Control (6000+)	-0.1405	0.0324	-4.333	0.0002
(300–450) – (450–600)	-0.08941	0.0484	-1.846	0.4361
(300–450) – (600–750)	-0.09738	0.0589	-1.654	0.5626
(300–450) – (750–900)	-0.12536	0.0732	-1.711	0.5241
(300–450) – Control (6000+)	-0.29116	0.0288	-10.126	<0.0001
(450–600) – (600–750)	-0.00797	0.069	-0.116	1
(450–600) – (750–900)	-0.03594	0.0816	-0.441	0.9979
(450–600) – Control (6000+)	-0.20175	0.0459	-4.392	0.0002
(600–750) – (750–900)	-0.02797	0.0882	-0.317	0.9996
(600–750) – Control (6000+)	-0.19378	0.057	-3.4	0.0088
(750–900) – Control (6000+)	-0.1658	0.0715	-2.318	0.1866

Appendix 8: Results of Analysis 3.2.3

Epiphyte Cover with distance from nearest fish farm- beta regression

Term	Estimate	Std. Error	z value	Pr(>|z|)	Significance
(Intercept)	-1.11118	0.17617	-6.307	2.84E-10	***
300-450	-0.1983	0.20583	-0.963	0.33533	n.s.
450-600	0.55115	0.21559	2.556	0.01057	*
600-750	0.23034	0.23835	0.966	0.33384	n.s.
750-900	0.31271	0.2553	1.225	0.22063	n.s.
Control (6000m+)	0.51008	0.16265	3.136	0.00171	**
Depth	0.0121	0.00984	1.229	0.21898	n.s.

Number of samples

Distance (m)	**n**
150-300	25
300-450	38
450-600	25
600-750	18
750-900	14
Control	334

Estimated marginal means

Distance	emmean	SE	asymp.LCL	asymp.UCL	% Change vs Control
150-300	0.27	0.0311	0.209	0.33	-29.13
300-450	0.232	0.0235	0.186	0.278	-39.11
450-600	0.39	0.0351	0.322	0.459	2.36
600-750	0.317	0.0391	0.241	0.394	-16.8
750-900	0.335	0.0449	0.247	0.423	-12.07
Control (6000m+)	0.381	0.00961	0.362	0.399	0

Tukey-adjusted pairwise comparison of estimated marginal means

Contrast	Estimate	SE	df	z.ratio	p.value	Significance
(150-300) - (300-450)	0.03722	0.039	Inf	0.954	0.9321	n.s.
(150-300) - (450-600)	-0.12082	0.0468	Inf	-2.582	0.1016	n.s.
(150-300) - (600-750)	-0.04767	0.0496	Inf	-0.96	0.9304	n.s.
(150-300) - (750-900)	-0.06577	0.0545	Inf	-1.207	0.8336	n.s.
(150-300) - (Control)	-0.11109	0.0324	Inf	-3.424	0.0081	**
(300-450) - (450-600)	-0.15804	0.0422	Inf	-3.743	0.0025	**
(300-450) - (600-750)	-0.08489	0.0458	Inf	-1.856	0.43	n.s.
(300-450) - (750-900)	-0.10299	0.0507	Inf	-2.03	0.3252	n.s.
(300-450) - (Control)	-0.14831	0.0253	Inf	-5.872	<.0001	***
(450-600) - (600-750)	0.07314	0.0525	Inf	1.394	0.7307	n.s.
(450-600) - (750-900)	0.05504	0.057	Inf	0.966	0.9287	n.s.
(450-600) - (Control)	0.00973	0.0364	Inf	0.268	0.9998	n.s.
(600-750) - (750-900)	-0.0181	0.0594	Inf	-0.305	0.9997	n.s.

(600-750) - (Control)	-0.06342	0.0402	Inf	-1.576	0.6142	n.s.
(750-900) - (Control)	-0.04531	0.0459	Inf	-0.987	0.9224	n.s.

Appendix 9: Results of Analysis 3.3.1

Maximal leaf length by cumulative duration of fish farming activity- linear model with robust standard errors

Cumulative Duration	Estimate	Std. Error	t value	Pr(>|t|)	Significance
(Intercept)	20.5333	2.07183	9.9107	< 2.2e-16	***
20-30	1.38189	1.7116	0.8074	0.4198	n.s.
30-40	-7.5731	2.13219	-3.5518	0.0004	***
40-50	-14.0536	3.18695	-4.4097	1.24E-05	***
50-60	-19.4992	1.4704	-13.2611	< 2.2e-16	***
60-70	-17.0017	2.11789	-8.0277	5.74E-15	***
Control	23.28817	1.58709	14.6735	< 2.2e-16	***
Depth	0.72696	0.17126	4.2447	2.56E-05	***

Model summary

Metric	Value
Residual standard error	16.76
Degrees of freedom	568
Multiple R-squared	0.4087
Adjusted R-squared	0.4015
F-statistic	56.1 on 7 and 568 DF
p-value	< 2.2e-16

Number of Samples

Cumulative Duration (years)	**n**
10-20	83
20-30	101
30-40	21
40-50	16
50-60	16
60-70	12
Control	337

Estimated marginal means

Cumulative Duration	emmean	SE	df	lower.CL	upper.CL	% Change vs Control
10-20	27.36	1.85	568	23.741	31	-45.98
20-30	28.75	1.69	568	25.436	32.1	-43.24

30-40	19.79	3.67	568	12.581	27	-60.93
40-50	13.31	4.2	568	5.066	21.6	-73.72
50-60	7.87	6.86	568	-5.612	21.3	-84.46
60-70	10.36	4.93	568	0.675	20.1	-79.54
Control	50.65	0.915	568	48.857	52.4	0

<u>Tukey-adjusted pairwise comparisons of estimated marginal means</u>

Contrast	Estimate	SE	df	t.ratio	p.value	Significance
(10-20) - (20-30)	-1.38	2.51	568	-0.55	0.998	n.s.
(10-20) - (30-40)	7.57	4.1	568	1.848	0.5158	n.s.
(10-20) - (40-50)	14.05	4.59	568	3.059	0.0374	*
(10-20) - (50-60)	19.5	7.09	568	2.748	0.0885	n.s.
(10-20) - (60-70)	17	5.29	568	3.212	0.0234	*
(10-20) - Control	-23.29	2.06	568	-11.33	0.0001	***
(20-30) - (30-40)	8.96	4.06	568	2.206	0.2939	n.s.
(20-30) - (40-50)	15.44	4.51	568	3.423	0.0117	*
(20-30) - (50-60)	20.88	7.08	568	2.947	0.0517	n.s.
(20-30) - (60-70)	18.38	5.17	568	3.558	0.0074	**
(20-30) - Control	-21.91	1.93	568	-11.38	0.0001	***
(30-40) - (40-50)	6.48	5.59	568	1.159	0.9091	n.s.
(30-40) - (50-60)	11.93	7.76	568	1.537	0.7224	n.s.
(30-40) - (60-70)	9.43	6.2	568	1.521	0.7322	n.s.
(30-40) - Control	-30.86	3.78	568	-8.167	0.0001	***
(40-50) - (50-60)	5.45	8.06	568	0.676	0.9939	n.s.
(40-50) - (60-70)	2.95	6.44	568	0.458	0.9993	n.s.
(40-50) - Control	-37.34	4.3	568	-8.685	0.0001	***
(50-60) - (60-70)	-2.5	8.51	568	-0.293	0.9999	n.s.
(50-60) - Control	-42.79	6.92	568	-6.185	0.0001	***
(60-70) - Control	-40.29	5.03	568	-8.014	0.0001	***

Appendix 10: Results of Analysis 3.3.2

<u>Meadow cover with cumulative duration of fish farming activity- beta regression</u>

Predictor	**Estimate**	**Std. Error**	**z value**	***p*-value**	**Significance**
(Intercept)	–0.455	0.177	–2.572	0.0101	*

Duration: 20–30 yrs	–0.394	0.162	–2.431	0.0151	*
Duration: 30–40 yrs	–0.589	0.211	–2.784	0.0054	**
Duration: 40–50 yrs	–0.587	0.259	–2.265	0.0235	*
Duration: 50–60 yrs	–0.865	0.242	–3.575	0.0004	***
Duration: 60–70 yrs	–0.763	0.216	–3.538	0.0004	***
Control (no farming)	0.634	0.154	4.122	<0.0001	***
Depth	0.0055	0.0107	0.516	0.6062	

Model summary

Metric	**Value**
Phi (precision)	0.494 (SE = 0.0176)
Log-likelihood	2305 (on 9 DF)
Pseudo R^2	0.2244
Quantile residuals	Min: –1.597, 1Q: –0.725, Median: –0.207, 3Q: 0.697, Max: 1.985
Iterations	20 (BFGS) + 3 (Fisher scoring)

Number of Samples

Cumulative Duration (years)	**n**
10-20	117
20-30	272
30-40	73
40-50	43
50-60	40
60-70	66
Control	570

Estimated marginal means

Duration Category	**Emmean**	**SE**	**Lower CL**	**Upper CL**	**% Loss vs Control**
10–20	0.4	0.0335	0.335	0.466	28.20%
20–30	0.31	0.018	0.275	0.346	44.30%
30–40	0.27	0.0319	0.208	0.333	51.50%
40–50	0.271	0.0431	0.186	0.355	51.40%
50–60	0.219	0.0342	0.152	0.286	60.70%
60–70	0.237	0.0299	0.179	0.296	57.50%
Control	0.557	0.0158	0.526	0.588	0.00%

Tukey-adjusted pairwise comparisons of estimated marginal means

Contrast	**Estimate**	**SE**	**z-ratio**	**p-value**	**Significance**

(10–20) – (20–30)	0.08988	0.0378	2.375	0.2092	n.s.
(10–20) – (30–40)	0.12993	0.0458	2.838	0.0682	n.s.
(10–20) – (40–50)	0.12963	0.0546	2.374	0.2095	n.s.
(10–20) – (50–60)	0.1809	0.0475	3.805	0.0027	**
(10–20) – (60–70)	0.16288	0.0448	3.637	0.0051	**
(10–20) – Control	–0.15689	0.0371	–4.228	0.0005	***
(20–30) – (30–40)	0.04005	0.0361	1.109	0.9257	n.s.
(20–30) – (40–50)	0.03975	0.046	0.863	0.9779	n.s.
(20–30) – (50–60)	0.09102	0.0381	2.391	0.202	n.s.
(20–30) – (60–70)	0.073	0.034	2.147	0.3247	n.s.
(20–30) – Control	–0.24677	0.0244	–10.130	<0.0001	***
(30–40) – (40–50)	–0.00030	0.0534	–0.006	1	n.s.
(30–40) – (50–60)	0.05097	0.0462	1.104	0.9271	n.s.
(30–40) – (60–70)	0.03296	0.0433	0.761	0.9885	n.s.
(30–40) – Control	–0.28682	0.0357	–8.030	<0.0001	***
(40–50) – (50–60)	0.05127	0.0547	0.938	0.9664	n.s.
(40–50) – (60–70)	0.03325	0.0517	0.643	0.9954	n.s.
(40–50) – Control	–0.28652	0.0462	–6.197	<0.0001	***
(50–60) – (60–70)	–0.01802	0.0449	–0.401	0.9997	n.s.
(50–60) – Control	–0.33779	0.0379	–8.924	<0.0001	***
(60–70) – Control	–0.31977	0.0341	–9.365	<0.0001	***

Appendix 11: GitHub Link: R Script for Analyses and Figures

https://github.com/etjkong5/Masters-2024