Project Title: An investigation into the drivers of *Mytilus edulis* decline within Northumberland Marine Special Protection Area.

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Title: An investigation into the drivers of *Mytilus edulis* decline within Northumberland Marine Special Protection Area.

Executive Summary

M.edulis are important biogenic reef forming species that are currently declining at a rapid rate within Northumberland Marine Special Protection Area with a loss of 36.5% of percentage cover at Fenham flats over 16yrs and 31% of percentage cover at Holy Island Sands over 4yrs. The reasons for these declines are unknown, it is thought that biocontamination and water quality may be contributing to the decline of *M.edulis* populations, however, this has not been investigated previously. A variety of temporal environmental datasets taken from the Northumberland marine special protected area (SPA) at Lindisfarne was analysed, to explore how environmental variables such as water quality and biocontamination have affected *M.edulis* beds at Fenham Flats and Holy Island Sands to establish trends or links to the loss of percentage cover over the past 4-16yrs. Exploratory multivariate canonical correspondence analysis (CCA) was used initially to visualise any important explanatory variables affecting *M.edulis* percentage cover, before more in-depth analysis using Pearson's correlation coefficients to determine significance levels of those variables against percentage cover data. *M.edulis* shows a steady trend of decline in the SPA of around 30% for both *M.edulis* beds and the data suggests a significant negative correlation with PBDE154 a polybrominated diphenyl ether which (a banned flame retardant) and dieldrin and endrin (banned pesticides). These biocontaminants may be affecting *M.edulis* populations at Lindisfarne due to increased storm events, but it is unclear if this is seasonal. Monthly monitoring of *M.edulis* bed percentage cover in combination with PBDE154, dieldrin and endrin levels are recommended to further investigate these links and are justified given the rapid loss of these beds. The loss of *M.edulis* beds at Lindisfarne has long term ramifications for marine species and bird biodiversity in the SPA. Ensuring the protection and recovery of mussel beds here will contribute to Lindisfarne reaching good water quality and chemical status, in line with the Water Framework Directive.

Introduction

The Northumberland coast is an important habitat for the reef-building marine bivalve *Mytilus Edulis* (Blue Mussel). Blue mussel beds are protected under the Convention for the Protection of the Marine Environment of the North-East Atlantic (OSPAR) Annex V list of threatened and declining species habitats, and the Natural Environment and Rural Communities (NERC) Act 2006 - Habitats and Species of Principal Importance in England. *M.edulis* are important for regulating services such as carbon storage as well as supporting services they provide for biodiversity, nutrient cycles, and water cycling. *Mytilus edulis* is found on all coasts in the British Isles, in the intertidal and subtidal zones ranging from the lower littoral tidal zone to shallow subtidal zones up to 5m depth. In Northumberland, *M.edulis* beds were present in most estuaries in the early 19th century namely Berwick, Holy Island, Alnmouth, Amble, and Blyth (Lebour, 1906). There has been a gradual decline in the population of *M.edulis* beds since 2006 as documented by NIFCA in Holy Island at Fenham Flats and Holy Island Sands (Image 1), Blyth has suffered losses, and there was a total loss of a bed in Budle Bay due to smothering by storm events, however, at Holy Island and Blyth, the reason for declines is unknown (Coulson, 1999; Boon, 2020a; Boon, 2020b).

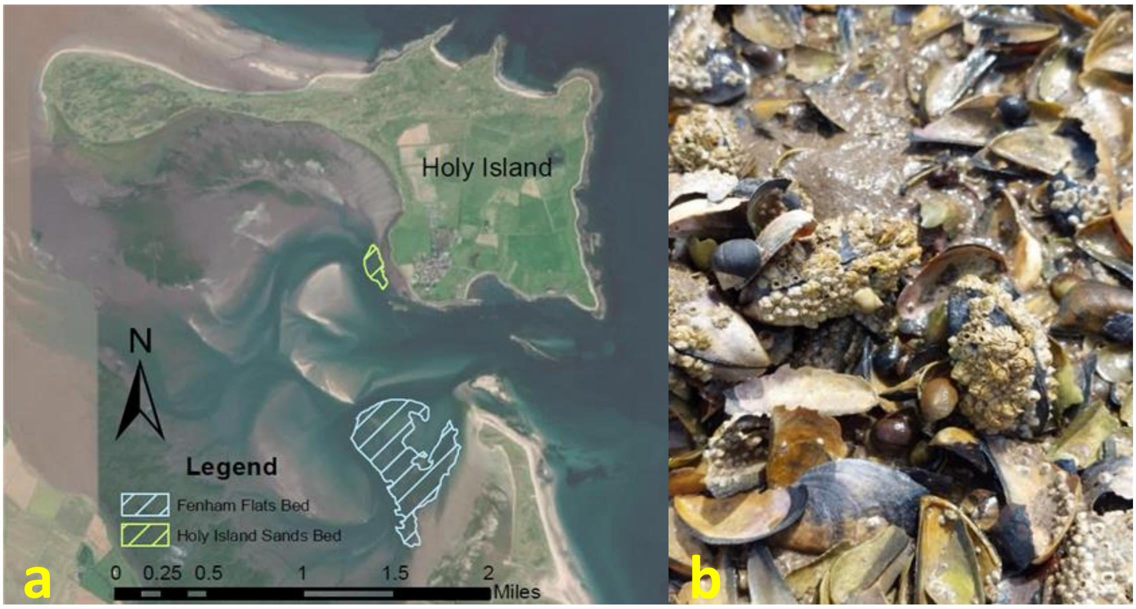

Image 1. (a) Location of Fenham Flats and Holy Island Sands *M.edulis* beds in 2020, created from data from DEFRA (DEFRA, 2019). (b) Mytilus edulis in situ on Holy Island Sands on June 28th, 2021, showing the biodiversity associated with these *M.edulis* beds.

M.edulis attach to hard substrata such as muddy sand using fibrous byssus threads (Tyler-Walters, 2008) they form biogenic reef structures (Image 2) composed of a single or multilayer matrix of living and dead mussels on a base of sediment, faeces, pseudo faeces, shells, and other detritus (Seed and Suchanek, 1992). Gaps and crevices in these beds increase the surface area available for other species to inhabit and these reefs can grow to a considerable size allowing them to support a wide range of biodiversity making them extremely important ecosystems, in Northumberland they provide a food source for protected bird species such as the resident Eider duck, Oystercatchers, and other overwintering birds, and crustaceans such as *Carcinus maenas* and *Cancer pagurus* (Briggs, 1982; Lintas and Seed, 1994; Coulson, 1999).

Image 2. Holy Island Sands *M.edulis* beds June 28th, 2021. Image showing the 3D structure and cover of the mussel beds at Lindisfarne located on the mixed and sandy sediments of the estuary at low tide.

Large populations of *M.edulis* are valuable for bioremediation within estuarine waterbodies like Holy Island (MMO, 2016) and are monitored in the UK by The Environment Agency as part of the Water Framework Directive requirements covered by the Environmental Quality Standards Directive (EQSD). They are sessile suspension feeders that process phytoplankton, bacteria, organic matter, and other detritus which makes them a valuable indicator species for terrestrial and marine contaminants as they filter and improve water quality (Seed and Suchanek, 1992; Holt, 1998; McIntosh, 2006; OSPAR, 2010).

M.edulis is tolerant of most environmental and water quality variables such as temperature fluctuations, salinity changes, and food availability according to the literature. A comprehensive water quality monitoring program is carried out by the Environment Agency and Table 1 summarises the factors that have been considered in this study and reviews the current literature available to summarise the trends and effects of water quality on *M.edulis* health.

Table 1. Summary of environmental and water quality factors that may be affecting *M.edulis* health at Lindisfarne including the trends seen from the literature and the effects upon mussel health.

Biotic Factor	Trends	Effect on *M.edulis* Health	Literature
Sea Surface Temperature (SST)	There has been an increase in SST of between 1-3°C in the North Sea since the 1980s, and warming is not uniform, the southern North Sea has been one of the areas most affected by the rise. These changes in ocean productivity due to temperature fluctuations may cause declines in regional biodiversity on a larger scale thus affecting *M.edulis* populations.	Community change of species has been linked to rises in water temperature and modelling shows a 1°C increase in sea temperature could decrease *M.edulis* aquaculture production by 50%. This could be extremely detrimental to intertidal *M.edulis* due to larger temperature fluctuations on exposure to air between tides, however, *M.edulis* has an upper sustained thermal limit of 29°C and it is thought that temperature alone is unlikely to affect *M.edulis* negatively.	(Holt, 1998; Smith, 2006; Callaway et al., 2013; Black, 2017; Singer et al., 2017)
Eutrophication (Water Quality)	Nitrogen in its 3 forms, Nitrate, Nitrite, Ammonia all make up the dissolved inorganic nitrogen value. *M.edulis* is capable of removal of nitrogen in water as it is readily available for removal and transfer via filtration from the pelagic realm to the benthos.	When nitrogen levels are high, and *M.edulis* are processing a lot of it then ammonia is excreted, metabolism of nitrogen causes high levels of ammonia excretion and has the potential to reduce the dissolved oxygen in the water inducing hypoxic conditions. These conditions may induce valve closure and cause mortality and reduced growth rates	(Pourmozaffar *et al.*, 2020)
Oxygen Content	Good levels of dissolved oxygen should be 80-120% or 6.5 – 8 mg/L. In most tidal estuaries the tides replenish oxygen levels frequently and *M.edulis* are generally very tolerant of changes in dissolved oxygen.	*M.edulis* mortality from hypoxia in experiments was higher in larger M.*edulis* and caused reduced growth rates. Hypoxia was classed as a dissolved oxygen concentration of less than 2.8mg/L.	(Altieri and Witman, 2006; Mainwaring, 2014)
Algal Blooms / Phytoplankton	High levels of chlorophyll indicate an increase in phytoplankton, this is attributed to increased nutrient inputs into water bodies and seasonal algal blooms. Diatoms bloom in spring followed by a smaller bloom in autumn, these algal populations tend to shift after spring as the silicate found in diatoms is consumed and *M.edulis* is a consumer of these diatoms	Studies show high chlorophyll levels benefit *M.edulis* by increasing food availability, healthy *M.edulis* populations are highly effective in controlling eutrophication by consuming phytoplankton, but extremely high levels of phytoplankton may cause mass mortalities due to anoxia or clogging of the animal's gills.	(Holt, 1998; Johns, 2001; Altieri and Witman, 2006; Wall *et al.*, 2011)
Salinity	Normal Salinity levels for the open ocean is 34.6 – 34.8ppt and 0.5-35ppt in estuaries and the normal range for *M.edulis* and salinity is 8.5-29.9ppt. Salinity will be affected by large inputs of rainfall from surrounding water bodies and may, in turn, affect estuaries for short periods during storm events and periods of unsettled weather or accidental inputs from industrial sources.	Salinity can be an extreme stressor to *M.edulis* living in estuaries and intertidal habitats, causing mortality and reduced growth. *M.edulis* closes its valves to protect its tissues, reducing respiration for the length of exposure. This may alter the distribution of *M.edulis.* In combination with Nitrogen and Oxygen level fluctuations, these factors can produce a positive feedback loop and ultimately lead to populations being unable to recover.	(Riisgard *et al.*, 2014; Pourmozaffar *et al.*, 2020)

Biotic Factor	Trends	Effect on *M.edulis* Health	Literature
Turbidity	*M.edulis* generally inhabit areas where turbidity is naturally high, additionally, turbidity can be attributed to anthropogenic sources such as dredging, runoff from land, and storm events causing disturbance to the seabed. Turbid water generally reduces phytoplankton in turn decreasing food availability, but bivalves are not considered highly sensitive to turbidity.	Larger M.*edulis* are more resistant to turbidity, however, the effect on spat that may have already settled is not known. In river systems, large volumes of rainfall can transport sediments and nutrients downstream and have the potential to cause harmful algal blooms in farmed *M.edulis* therefore this is a possibility in intertidal estuarine wild *M.edulis*.	(Birkett *et al.*, 1998; Dare, 2004; Tyler-Walters, 2008; Callaway *et al.*, 2012; Mainwaring, 2014)
Wind Speed and Direction	Wind direction and forcing may influence *M.edulis* populations, as disruption by stormy weather affects the settlement of larvae after spawning in spring and late summer in Holy Island.	Data from The Wash shows that *M.edulis* larvae are affected by wind forcing and that successful recruitment is seen when easterly (onshore) winds prevail in June. Onshore winds are thought to improve settlement whereas offshore winds post-spawning will disperse larvae out to sea.	(Dare, 2004)

Holy Island has been monitored yearly since 2015 for biocontaminants, and from 2015-2018 Holy Island had moderate ecological status and good chemical status, then failed in 2019, this appears to have been attributed to agricultural pollution and contamination from wastewater (EA, 2020a; EA, 2020c). A comprehensive list of contaminants is regularly tested for in Holy Island and they have been summarised in Table 2 with a review of the current literature available and how these contaminants enter the marine environment and their effect on *M.edulis* health. The cumulative effects of these contaminants are not well understood in *M.edulis* and other marine biota, therefore a comparison of measured contaminant levels, and mussel bed percentage cover, structure and biodiversity are required to determine if any trends are emerging and whether causes of observed decline can be inferred.

Table 2. Summary of biocontaminants that are tested for in *M.edulis* by The Environment Agency as part of the Water Framework Directive, including possible sources of contamination, mode of transport to the location, uptake and accumulation in tissues, and the hypothesised effects upon *M.edulis* health from the literature.

Contaminant Group	Source and status	Contaminants (year banned)	Accumulation and Transport method	Scope for Growth	Supporting Literature
Polycyclic Aromatic Hydrocarbons (PAH's)	Natural and industrial sources, leachate from fisheries aggregation devices such as tyres. Combustion of organic compounds, sewage, slurry, and accidental waste releases.	Anthracene Benzo[b]fluoranthene Benz[g,h,i] perylene Benzo[a]pyrene benzo[a]anthracene Benzo[k]fluoranthene Chrysene Fluoranthene Naphthalene Indeno[1,2,3 -cd] -Pyrene	Particulate matter from the environment enters the water and settles into marine sediments, this causes bioaccumulation in Mytilus tissues during filter feeding.	90% decline in scope for growth at sustained high levels.	(Coles *et al.*, 1994; Widdows *et al.*, 1995; McIntosh, 2006; Widdows, 2006; Webster, 2018; Halsband *et al.*, 2020)
Metals	Many sources, atmospheric transport, landfill, agricultural runoff, ingredients in pesticides, insecticides, and fertilisers as well as legacy industrial pressures.	Hg Cu Zn Ag Ni Se Pb As Cr Cd	Metals can be present in sediments and resuspended during flood and storm events and assimilated by Mytilus edulis during feeding.	Metals can be toxic and must be at very high levels to inhibit scope for growth in M.*edulis*.	(Widdows *et al.*, 1995; Giusti *et al.*, 1999)
Organochloride Pesticides and fungicides	Often a direct source and persistent levels are seen in urban areas. In rural areas (such as Holy Island) contamination can be	Hexachlorocyclohexanes (α ,β ,γ,δ) Heptachlor (1981) Cis- heptachlor Endrin (1984) DDT (1972) Dieldrin and Aldrin (1989)	Accumulated in soil, and subsequently washed into marine sediments. Insoluble in water, therefore, storm events may	No scope for growth data is available	(Widdows *et al.*, 1995; Jorgenson, 2001; Olenycz *et al.*, 2015)

Contaminant Group	Source and status	Contaminants (year banned)	Accumulation and Transport method	Scope for Growth	Supporting Literature
	due to long-range atmospheric deposition.	PCB28,52,101,118,138,153,180 Quinoxyfen	resuspend pollutants in estuaries.		
Polybrominated diphenyl ethers (PBDEs)	Flame retardants are hazardous substances under the water framework directive (WFD) and OSPAR chemicals of priority concern that have been banned since 2007. They are found in many products such as upholstery, building materials, cars, and plastics/foams.	PBDE47 PBDE99 PBDE100 PBDE154 PBDE153 Hexabromocyclododecane (HBCDD) Hexachlorobutadiene (HCBD)	Long-range atmospheric transport and accumulated during filter feeding when resuspended in marine sediments.	Long-term exposure affects immune function and may influence community composition.	(Webster *et al.*, 2009; Jiang *et al.*, 2017; Apeti *et al.*, 2018; EA, 2019)

Holy Island is currently part of the Northumberland Coast Area of Outstanding National Beauty management plan for 2020 -2024. This states that the special qualities of the area should be conserved, and enhanced, protecting natural capital found here. There are opportunities here to fulfil the need for nature recovery networks as set out in the Lawton review for habitat management, restoration, and creation within the lifespan of this plan and the review of management plans for Lindisfarne itself are currently one of the priorities (Anon, 2020).

This study aims to investigate biocontamination trends in combination with other environmental and water quality data that may potentially affect *M.edulis* health within the SPA. Multivariate analysis was used to explore relationships in the data and to create hypotheses that surround this variation before moving on to more in-depth statistical techniques using Pearson's correlation coefficients. This will provide a basis for investigation of other sites in Northumberland when more data becomes available (ter Braak, 1995; Zuur, 2010).

Terms of reference

Appendix J

Methods

Data Sources

M.edulis percentage cover survey data was sourced from NIFCA for Fenham flats (16yrs), and Holy Island Sands (4yrs). Supporting water quality data and annual biocontamination data from The Environment Agency was provided for analysis. Data sources and modifications are summarised in Table 3.

Table 3. Summary of the data types analysed, units of measurement, data source and whether data modifications were carried out for statistical analysis and location of data sources.

Data Type	Data units	Data source and reference	Data modifications	Data Location
Mussel Health (% cover)	%	NIFCA (Northeast Inshore Fisheries and Conservation Authority)	None	Appendix K
Biocontamination	µg/kg	The Environment Agency	None	Appendix B
Rainfall	mm	The Met Office Hadley observation centre (Anon, 2021b)	None	Appendix N
Mean wind direction	degrees	The Met Office Midas system - Boulmer (Northumberland) weather station ('MIDAS,' 2021)	None	Appendix M
Mean wind speed	knots	The Met Office Midas system - Boulmer (Northumberland) weather station ('MIDAS,' 2021)	None	Appendix M
Storm Events	storms/month storms / year	The Met Office UK Storm Centre (Anon, 2021a)	None	Appendix L
Water Temperature	°C	The Environment agency	None	Appendix A
Salinity	ppt	The Environment agency	Mean used for missing values	Appendix A
pH	pH	The Environment agency	Mean used for missing values	Appendix A
Turbidity	ftu	The Environment agency	Mean used for missing values	Appendix A
Dissolved Oxygen %	%	The Environment agency	Mean used for missing values	Appendix A
Dissolved Oxygen	mg/l	The Environment agency	Mean used for missing values	Appendix A
Chlorophyll	µg/l	The Environment agency	Mean used for missing values	Appendix A
Nitrate	Mg/l	The Environment agency	Mean used for missing values	Appendix A
Nitrite	Mg/l	The Environment agency	Mean used for missing values	Appendix A
Nitrogen dissolved inorganic	Mg/l	The Environment agency	Mean used for missing values	Appendix A
Total Oxidised Nitrogen	Mg/l	The Environment agency	Mean used for missing values	Appendix A
NH3 (Ammoniac Nitrogen)	Mg/l	The Environment agency	Mean used for missing values	Appendix A
Silicate (Si02)	Mg/l	The Environment agency	Mean used for missing values	Appendix A
Orthophosphate	Mg/l	The Environment agency	Mean used for missing values	Appendix A

Data Analysis

RStudio version 4.0.2 was used for data analysis (RStudio, 2020). Multivariate analysis was carried out with the NES8010.R package provided by Newcastle University (Sanderson, 2021).

Data exploration

Data exploration was carried out in RStudio using the ggplot2 package (Wickham, 2016). Water quality, environmental factors, and contamination data were initially plotted against *M.edulis* bed percentage cover to identify any preliminary relationships, and all data was tested for normality. Percentage cover was chosen as the response variable, it is a commonly used figure for determining the proportion of bed covered by *M.edulis* and is derived from biomass estimations on commercial *M.edulis* beds (Walker, 1986; Dare, 2004). Percentage cover is calculated by walking in zigzags along straight transect lines and placing a 0.1m^2 quadrat at the end of each transect to record *M.edulis* density. Estimates of *M.edulis* cover are calculated by recording the number of footsteps that land on *M.edulis* and the density of these measurements are extracted from a volumetric determination of the random samples, this is carried out within the constraints of the *M.edulis* bed, covering all the bed at random. The percentage cover method is used by other UK IFCAs and would be replicable in those regions (JNCC, 2001; Dare, 2004; Jenkin, 2016; Jessop, 2018; Boon, 2020b).

Multivariate constrained ordination using Canonical Correspondence Analysis (CCA).

Multivariate canonical correspondence analysis (CCA) was carried for both *M.edulis* beds using the same water quality dataset (Appendix A). Fenham Flats is not routinely sampled for water quality or biocontamination but is geographically close and subject to similar conditions as Holy Island Sands due to its tidal nature. For environmental and contamination data the Vegan multivariate statistical analysis package was used in conjunction with the NES8010.R script (Oksanen, 2020; Sanderson, 2021). CCA was used to extract relationships between ecological and environmental variables and then visualised on an ordination diagram. CCA was chosen as it is commonly used in aquatic sciences, (including marine ecology) where environmental variables are implicated in the biological composition of communities such as *M.edulis*. CCA allows the comparison of environmental variables affecting *M.edulis* simultaneously. Colinear covariates were dropped from the analysis if their explanation was obvious, for example, collinearity between rainfall and salinity.

CCA tri plots were created (Figures 3,4,6 & 7) to visualise variables and gain a clearer overall picture than the examination of variables independently. CCA axis scores were reported for both sites. Important explanatory variables from the CCA triplots were taken forward for further statistical analysis and contaminants that exceeded environmental quality standards (EQS) thresholds. All biocontamination determinants were included in the multivariate analysis even if within EQS standards as for some determinants the threshold levels in *M.edulis* are not known, as the values published are for fish only. There is a lack of literature citing the EQS threshold levels in *M.edulis* which are filter feeders and have a different trophic level to fish, therefore they will be affected differently by these contaminants.

Univariate Analysis

Pearson's Correlation Coefficient and Regression

Pearson's correlation coefficients were then used for explanatory variables that were significant in the CCA, or those biocontaminants that exceed the recommended EQS thresholds from the preliminary data exploration. It was not possible to combine the water quality and biocontamination data to complete an overall multivariate analysis as the biocontamination data was annual and spans only 4 years, however, water quality data was monthly and spans 16yrs. Mussel Health data was available for 16yrs for Fenham Flats and 5 years for Holy Island Sands, therefore this required separate analysis. Mussel percentage cover was assumed to be the same value annually and monthly because no monthly percentage cover data was available from NIFCA.

Results

Mussel Health Data

Data Exploration and Trends

Percentage cover data collected throughout the survey period showed an overall trend of decline with decreasing percentage cover for both *M.edulis* beds, in some years percentage cover increased briefly but never reaching the peak of 80% cover in 2007 (Figure 1). Both *M.edulis* beds have seen a major decline, Fenham Flats has declined by 36.5% over the 16-year monitoring period whilst Holy Island sands has declined 31% over only 4 years and its status before 2018 is unknown.

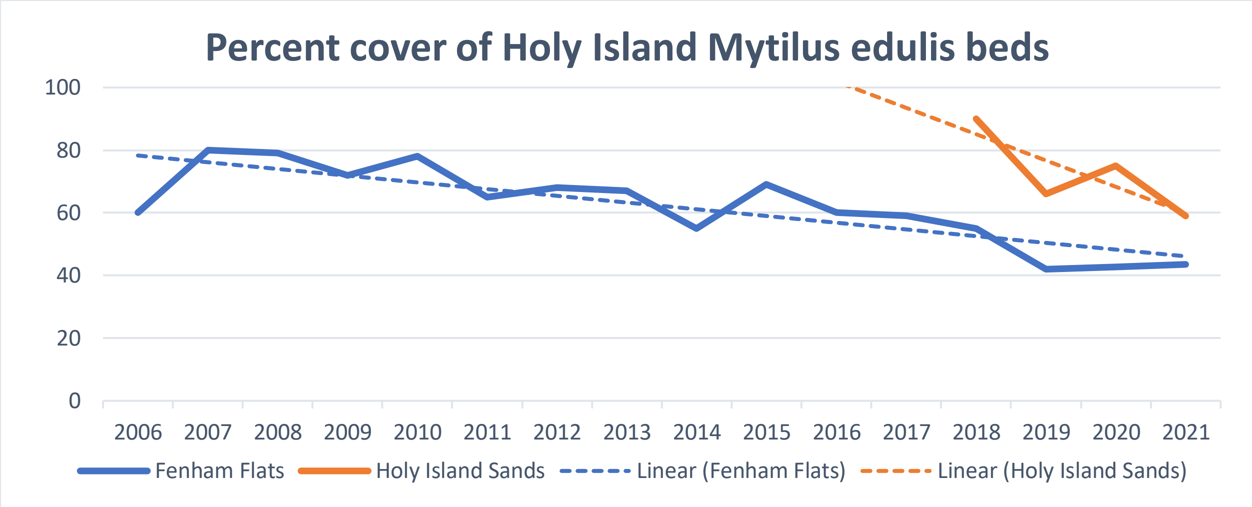

Figure 1. The decline of % cover of Mytilus edulis beds at Fenham flats in blue and Holy Island Sands in orange, and trend lines for both sites. Holy island sands mussel bed is declining at a more rapid rate over a shorter time period.

Water Quality Data

Data Exploration and trends

Water quality data collected by the Environment Agency monitoring program at Lindisfarne have been summarised in Figure 2 and they appear to be relatively stable over the past 16yrs with the expected seasonal fluctuations of water temperature, chlorophyll, silicate, and nitrogen. The estuary has had moderate ecological status since 2015 (month 107 onwards) that reflects these trends (EA, 2020c).

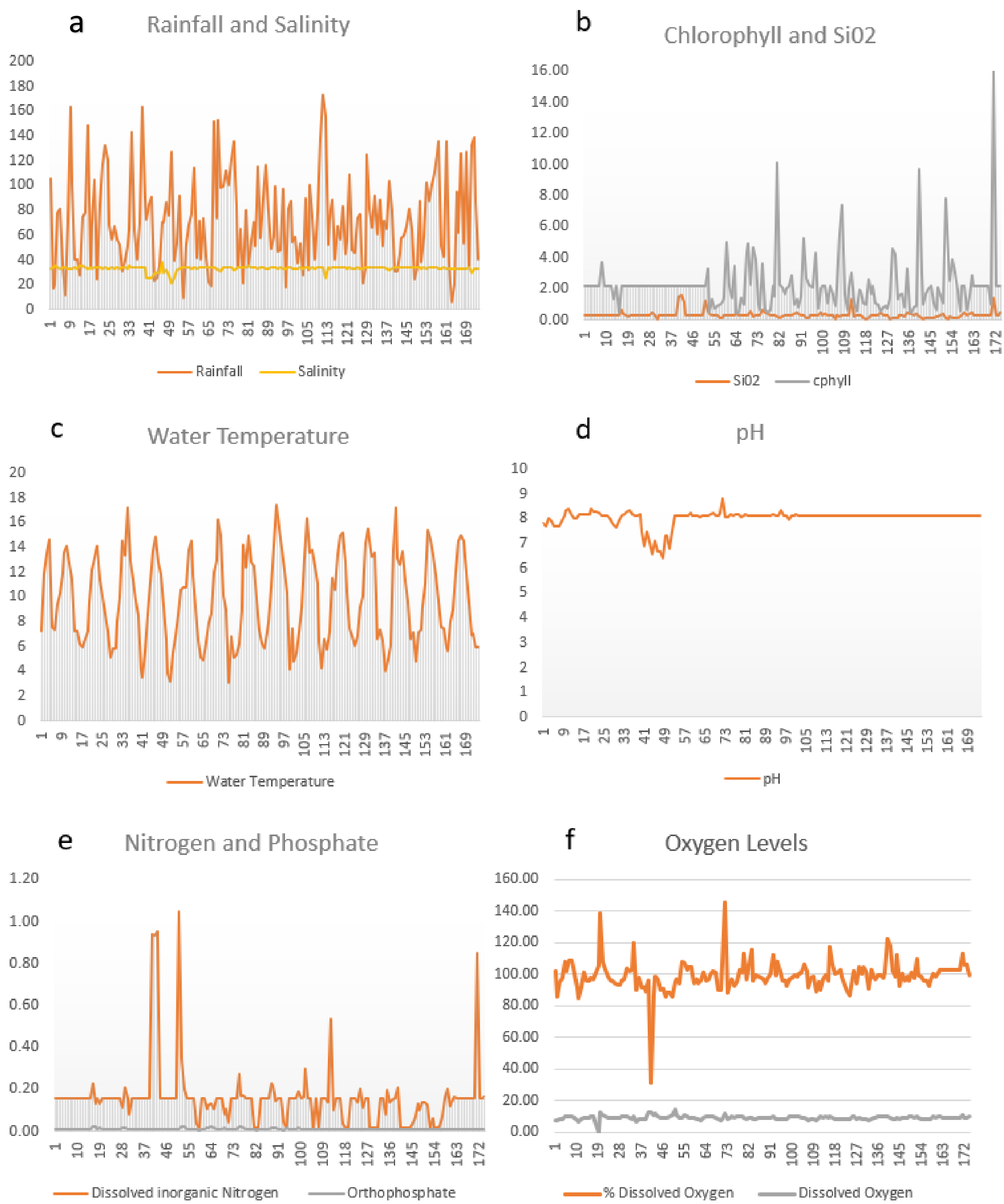

Figure 2 . Graphs showing the trends in environmental and water quality data monitored by the environment agency over the past 16yrs x-axis representing the number of months since January 2006. (a) Rainfall and Salinity, (b) Chlorophyll and SiO2, (c) Water Temperature, (d) pH, (e) Nitrogen and Phosphate, (f) Oxygen levels.

Water Quality - Exploratory Multivariate Analysis

Fenham Flats

CCA plots (Figure 3) show that the most important explanatory variables (those with the longest arrows) are storms, pH, and dissolved oxygen percentage. Closely correlated variables are close together (e.g., rainfall, water temperature, and turbidity), and unrelated variables will have arrows opposite each other or at 90 degrees to one another (e.g., water temperature and pH).

CCA axis 1 for Fenham Flats explained 99.7% of the total variance in mussel bed percentage cover based on the water quality dataset (Table 4) and that pH was the main factor contributing to variation in CCA1 ($p = 0.001$). Storm frequency appeared to be an important factor in the plot but was not found to be statistically significant when the multivariate statistical analysis was carried out (Table 5).

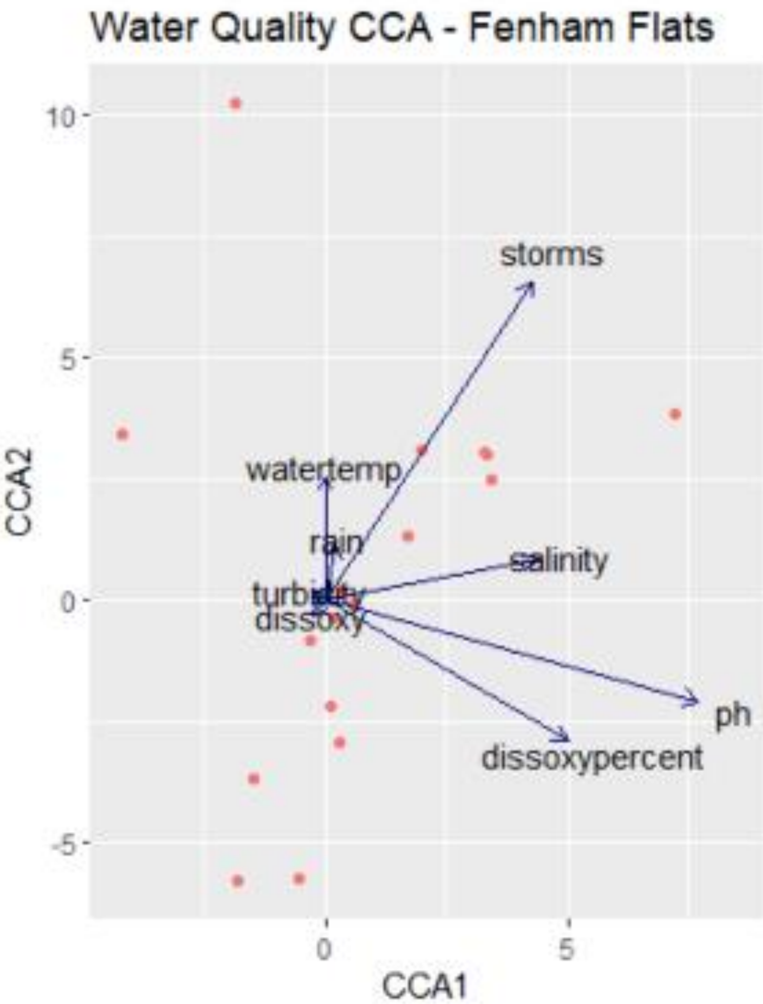

Figure 3. CCA plot for Fenham Flats water quality multivariate data analysis from 2006 to 2021, showing all water quality variables were analysed against mussel bed percentage cover, showing storms and pH to be the most important.

Table 4. Biplot scores for constraining variables for Fenham Flats water quality data and proportion of variance explained for CCA axis including eigen values.

Explanatory	CCA1	CCA2	Accumulated constrained eigenvalues, importance of components:		
Turbidity	0.01481	-0.299093		CCA1	CCA2
Salinity	0.41408	0.280756	Eigenvalue	0.0009399	0.011
Dissolved Oxygen %	0.38787	-0.273714	Proportion Explained	0.9976610	0.087
Dissolved Oxygen	-0.12972	-0.310821	Cumulative Proportion	0.9976610	0.10
Water Temperature	0.01270	0.273027			
Rain	0.07052	0.001611			
Storms	0.27025	0.504928			
pH	0.85071	-0.045837			
Chlorophyll	0.10147	-0.157998			
Nitrogen dissolved inorganic	-0.34616	-0.267034			
Phosphate	-0.03073	-0.621458			
Silicate (sio2)	-0.25206	-0.252929			

Table 5. Statistical significance including P-values of all explanatory variables from the Fenham Flats CCA water quality data analysis, showing pH to be significant from the CCA analysis.

Explanatory variable	Df	Chi-square	F	Pr(>F)
pH	1	0.0004502	17.25037	0.001***
Turbidity	1	0.0000916	3.508957	0.070
Water temperature	1	0.0000575	2.202063	0.124
Storm frequency	1	0.0000449	1.721114	0.153
% Dissolved Oxygen	1	0.0000405	1.550605	0.181
Salinity	1	0.0000320	1.22606	0.229
Chlorophyll	1	0.0000198	0.758825	0.371
Silicate (sio2)	1	0.0000093	0.358065	0.560
Rainfall	1	0.0000061	0.234002	0.632
Dissolved Oxygen	1	0.0000021	0.079189	0.748
Orthophosphate	1	0.0000008	0.028786	0.895
Dissolved inorganic Nitrogen	1	0.0000001	0.003724	0.980
Residual	161	0.0042014	NA	NA

Holy Island Sands

Canonical correlation analysis (CCA) using the water quality data (Figure 4) for Holy Island Sands explained 99.2% of the total variance (Table 6). Factors contributing to variation in CCA1 were predominantly salinity and dissolved oxygen, p-value = 0.001 and 0.026, respectively (Table 7.)

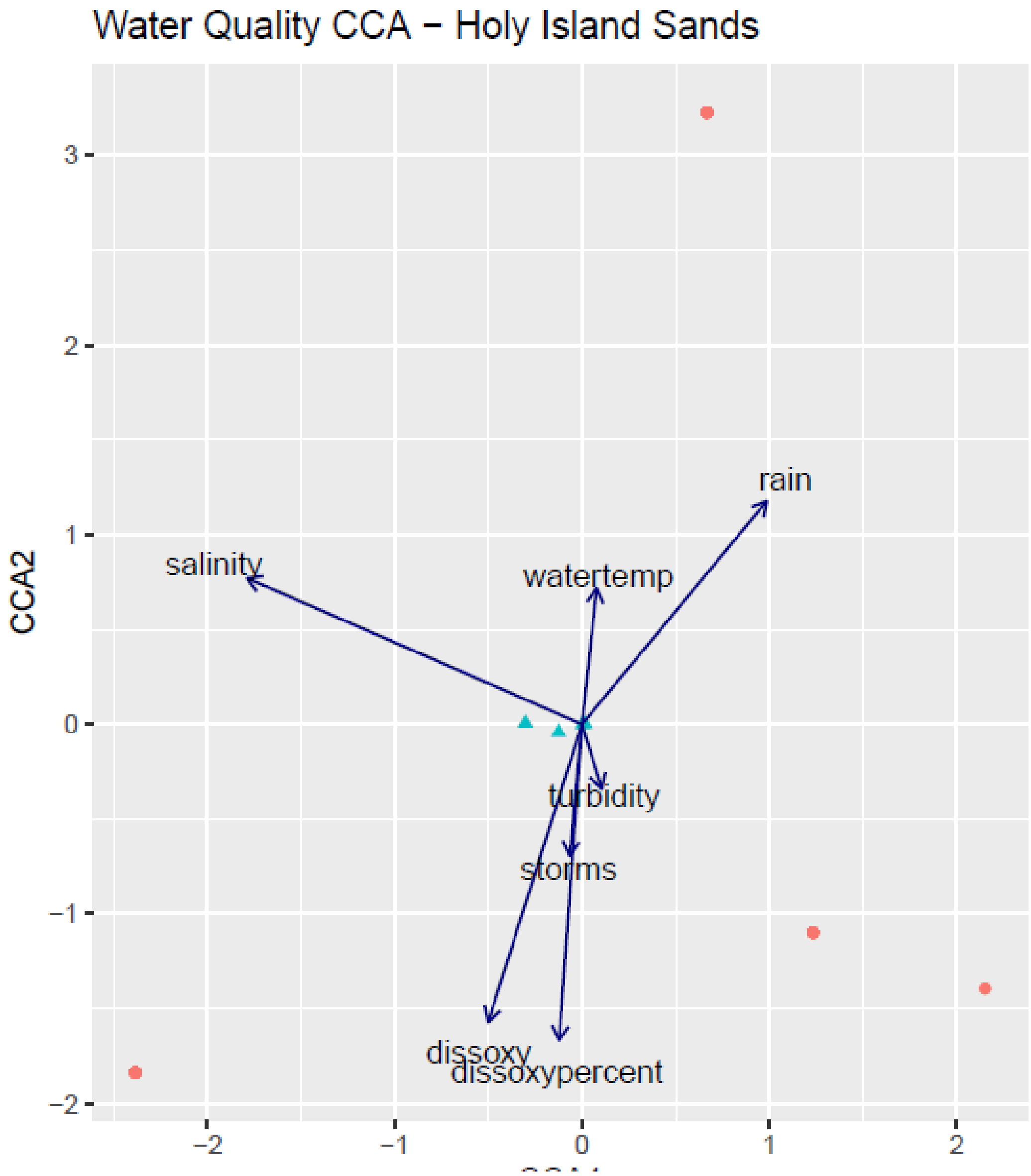

Figure 4. CCA triplot for Holy Island Sands water quality data multivariate analysis.

Table 6. Biplot scores for constraining variables Holy Island Sands water quality data and proportion of variance explained for CCA axis including eigenvalues.

Biplot scores for constraining variables				Accumulated constrained eigenvalues, importance of components:			
	CCA1	**CCA2**	**CCA3**		**CCA1**	**CCA2**	**CCA3**
Turbidity	0.04446	-0.1427	0.450933	**Eigenvalue**	0.00056	0.00000	0.00000
Salinity	-0.75195	0.3241	0.133514	**Proportion Explained**	0.99292	0.00699	0.00008
Dissolved Oxygen %	-0.05136	-0.7009	-0.128138	**Cumulativ e Proportion**	0.99292	0.99990	1.00000
Dissolved Oxygen	-0.21060	-0.6603	0.006827				
Water Temperature	0.03306	0.3016	-0.514784				
Rainfall	0.41487	0.4948	-0.194961				
Storms	-0.02758	-0.2918	-0.186059				

Table 7. Significance of explanatory variables from Holy Island Sands water quality data CCA analysis, salinity, and % dissolved oxygen showing significant p-values.

Explanatory variable	Df	Chi Square	F	Pr(>F)
Turbidity	1	0.00000117	0.0398	0.852
Salinity	1	0.00038388	13.0298	0.001 ***
% Dissolved Oxygen	1	0.00001914	0.6497	0.457
Dissolved Oxygen	1	0.00015275	5.1846	0.026 *
Water temperature	1	0.00002098	0.7120	0.399
Rainfall	1	0.00000178	0.0603	0.826
Storm frequency	1	0.00000733	0.2489	0.617
pH	0	0.00000000	0	-Inf
Residual	31	0.00091332	NA	NA

Explanatory variables taken forward for univariate analysis from the water quality data results were pH, salinity, and dissolved oxygen.

Biocontamination Data exploration and Trends

106 contaminants were monitored by the Environment Agency over the study period but not all were analysed. Contaminants that exceeded levels in *M.edulis* as set by the EQSD (environmental quality standards directive) from The Environment Agency monitoring program or that were steadily increasing over the monitoring period were selected for multivariate analysis (Appendix B). Peaks are seen in 2019 for endrin, dieldrin, PCBs (polychlorinated biphenyls), arsenic, zinc, and PBDEs (polybrominated diphenyl ethers). Mercury and heptachlor though flat are consistently over the recommended EQS levels and quinoxyfen fluctuates from 2017 onwards (Figure 5). Data for 2021 was assumed to be the same as 2020 as these results were not yet available.

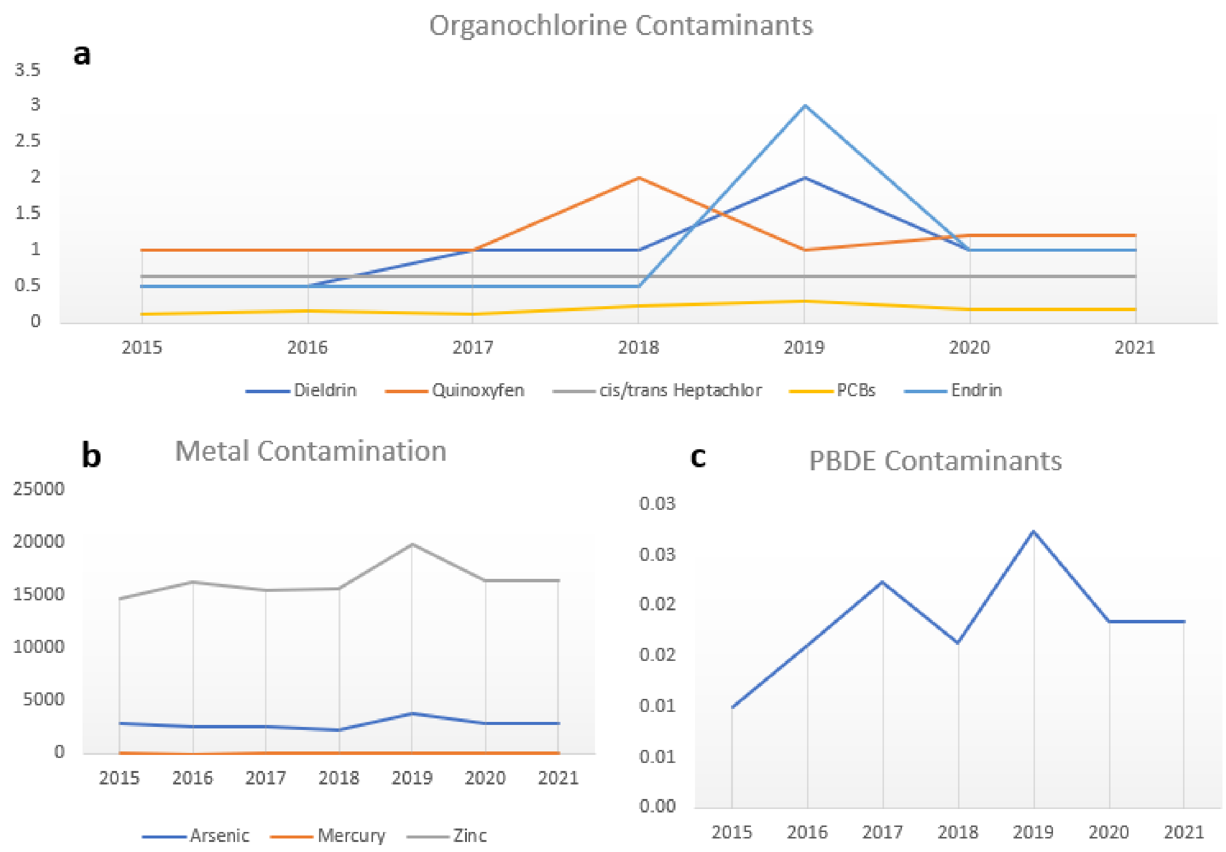

Figure 5 – Trends from 2015 to 2021 for biocontaminants at Fenham Flats and Holy Island sands, showing contaminants over EQS standards (a) organochlorine contaminants (pesticides and fungicides), (b) metal contamination, (c) Polybrominated diphenyl ethers (flame retardants).

Biocontamination Exploratory Multivariate Analysis

Fenham Flats

The CCA plot showed heptachlor, endrin, dieldrin, and PCB101 and PCB108 were the most important explanatory variables (Figure 6). CCA axis 1 explained 99.4% of the total variance of the dataset with dieldrin contributing the most to the variance in the dataset (Table 8). Further analysis for biocontamination data was carried out using Pearson's correlation coefficients with the reduced dataset.

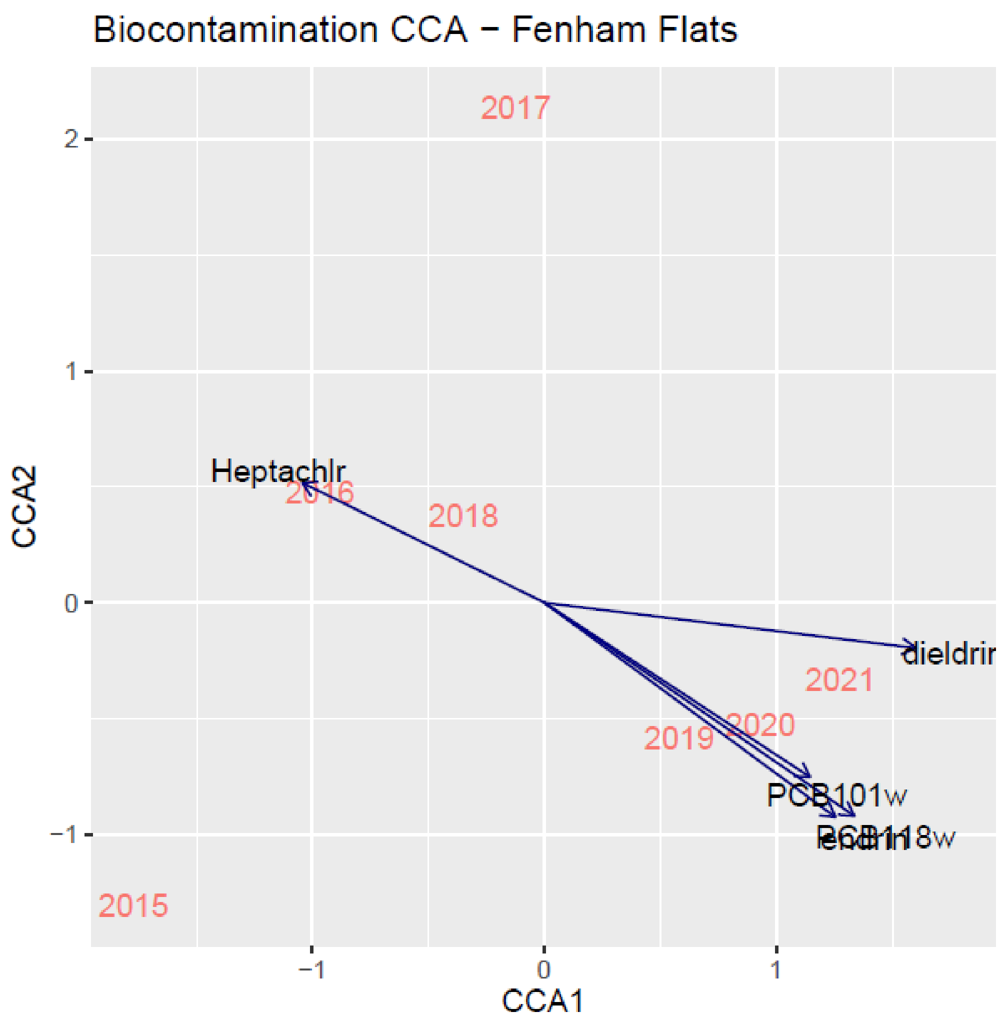

Figure 6. CCA triplot for Fenham Flats biocontamination data multivariate analysis 2015 to 2021.

Table 8. Biplot scores for constraining variables at Fenham Flats biocontamination data and proportion of variance explained for CCA axis including eigenvalues.

Biplot scores for constraining variables			
	CCA1	CCA2	CCA3
Dieldrin	0.5837	-0.07141	-0.79305
Heptachlor	-0.3805	0.18840	0.07145
Endrin	0.4570	-0.33664	-0.80024
PCB118w	0.4867	-0.33483	-0.79391
PCB101w	0.4172	-0.27371	-0.81127

Accumulated constrained eigenvalues, Importance of components:	**CCA1**	**CCA2**	**CCA3**
Eigenvalue	0.000289	0.000001	0.000001
Proportion Explained	0.994333	0.003648	0.002020
Cumulative Proportion	0.994333	0.998000	1.000000

Holy Island Sands

CCA of biocontamination data for Holy Island Sands (Figure 7) explained 98.1% of the total variation and no explanatory variables were found to be significant for this data (Table 9).

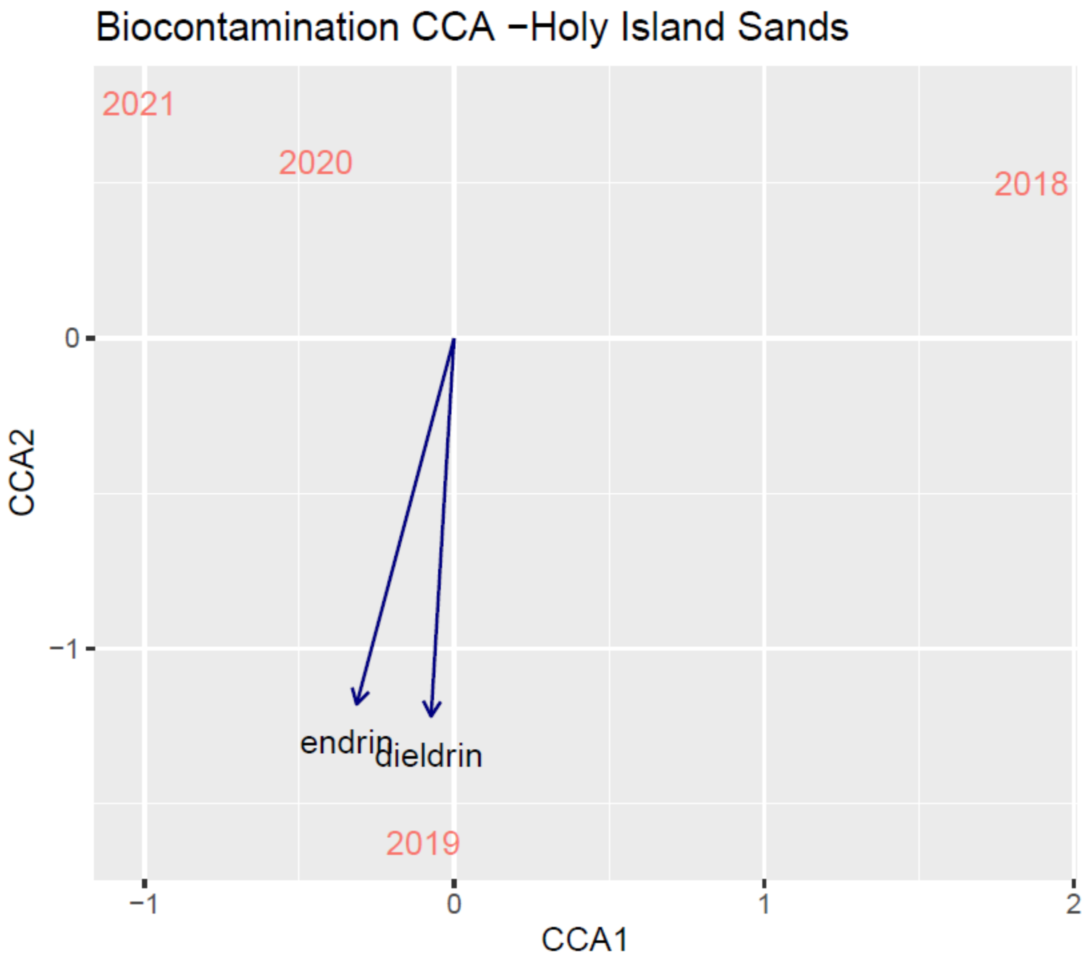

Figure 7. CCA triplot of Holy Island Sands biocontamination data multivariate analysis from 2015 – 2021, with endrin and dieldrin showing the most important explanatory variables that are closely correlated.

Table 9. Biplot scores for constraining variables Holy Island Sands biocontamination data and proportion of variance explained for CCA axis including eigenvalues.

Accumulated constrained eigenvalues, Importance of components:		
Axis	**CCA1**	**CCA2**
Eigenvalue	0.00170	0.00003
Proportion Explained	0.98110	0.01887
Cumulative Proportion	0.98110	1.00000

Biplot scores for constraining variables		
	CCA1	**CCA2**
Dieldrin	-0.06075	-0.9982
Endrin	-0.25750	-0.9663

Variables taken forward for univariate analysis of biocontamination data were endrin, dieldrin, heptachlor, PCB118, and other contaminants that exceed the threshold EQS standard, which was arsenic, heptachlor, quinoxyfen, all PBDEs, and PCBs.

Univariate Analysis of data for Fenham Flats and Holy Island Sands

Pearson Correlation Coefficient

Correlograms were used to visualise the data, red circles indicate negative relationships with *M.edulis* percentage cover and the size of the circle indicates the strength of the correlation from weak to strong. Blue circles indicate positive correlations, showing variables that have a positive effect on percentage cover, each correlation was checked for significance in R and only significant correlations were reported in the results (Simko, 2021).

Water Quality Data

Fenham Flats

Frequent storm events had a significant negative correlation with percentage cover of *M.edulis* at Fenham Flats, r(df 14) = -0.50, p = 0.04 (Figure 8).

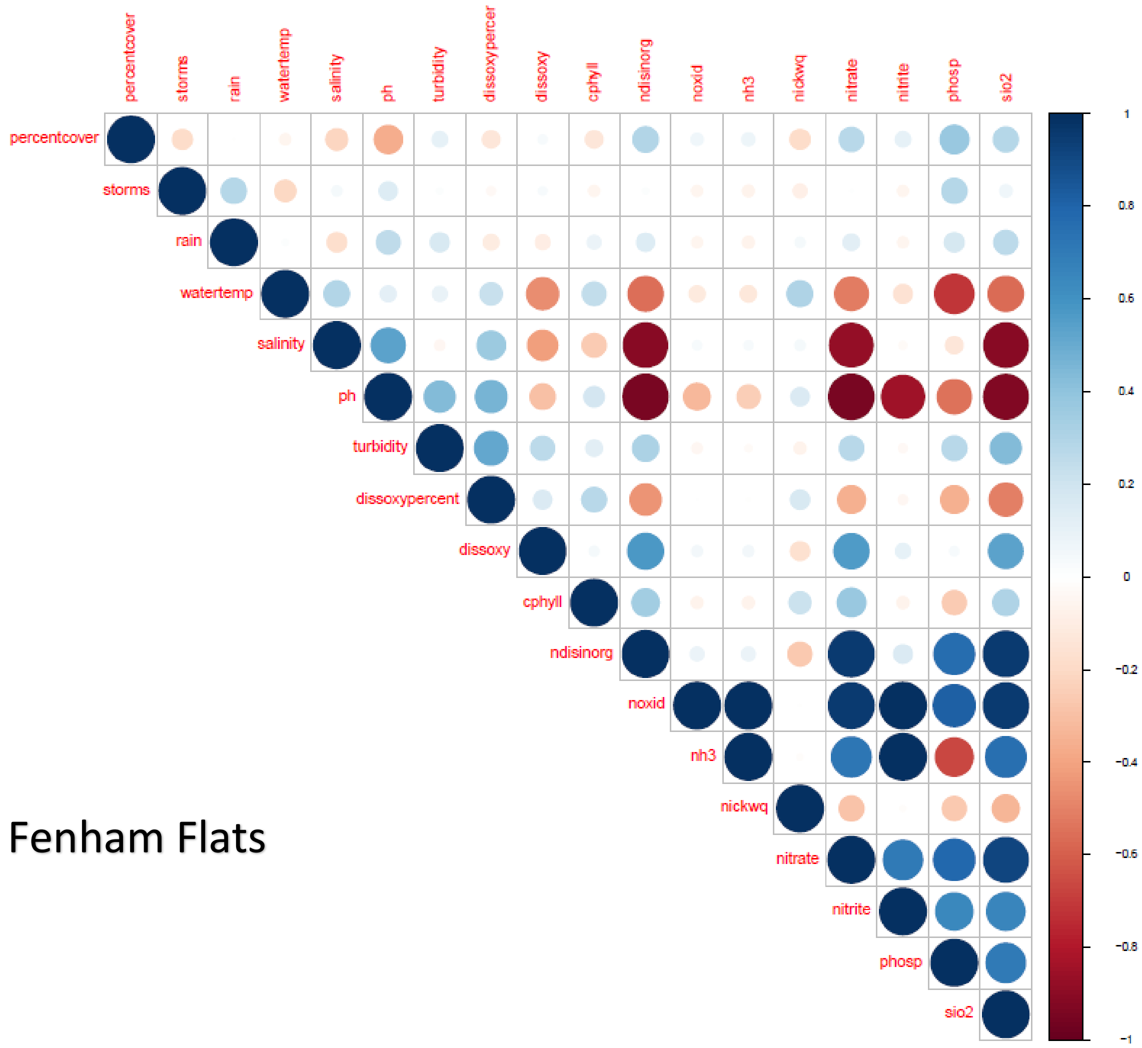

Figure 8. Correlograms displaying the correlation matrix for Fenham Flats EA water quality data and the positive and negative correlations versus *M.edulis* percentage cover. The first line shows percentage cover correlations to each variable.

Holy Island Sands

At Holy Island Sands no significant correlations were found for all water quality data including storms and percentage cover, r(df 2) = -0.09, p = 0.9 (Figure.9); however, it is worth noting that only 4 years of data were available for Holy Island Sands.

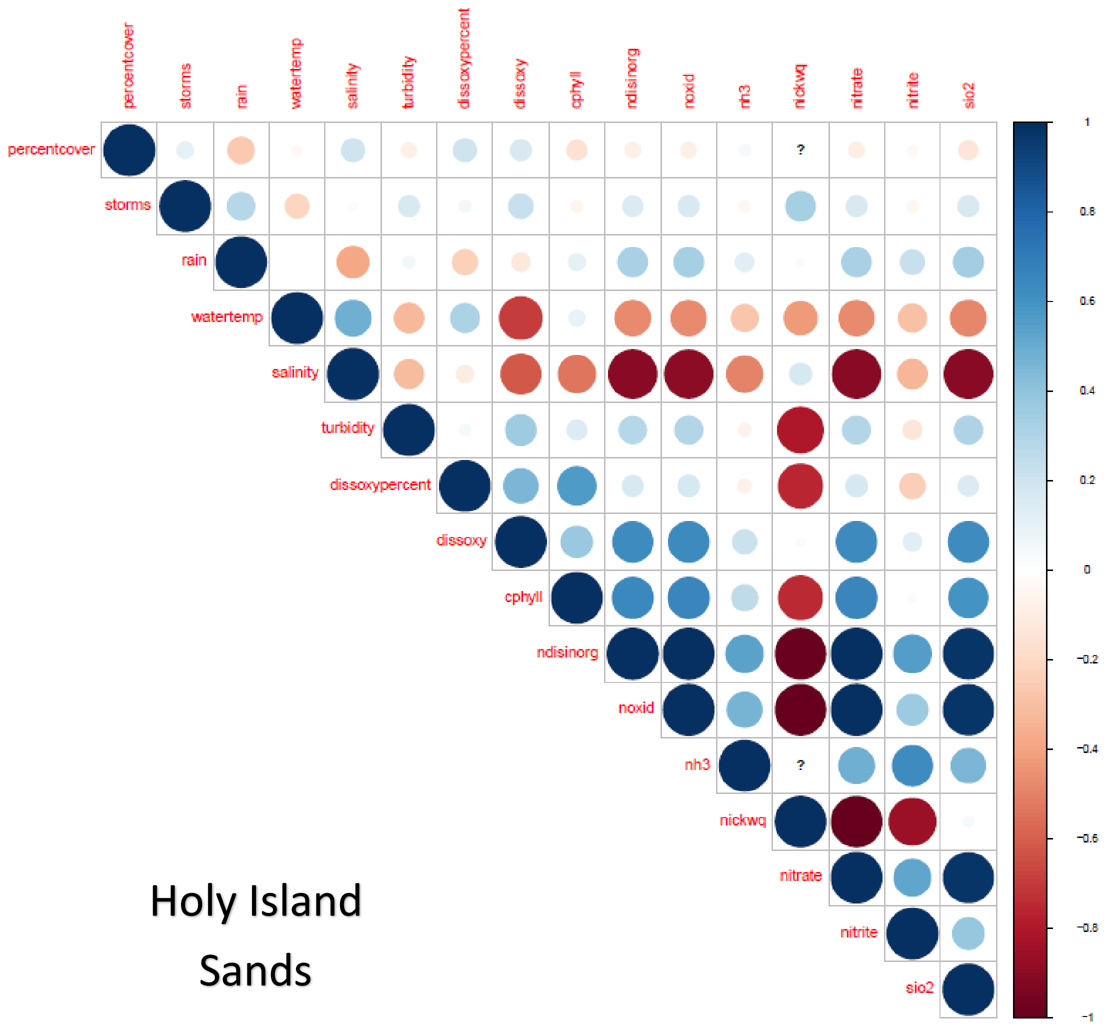

Figure 9. Correlograms displaying the correlation matrix for Holy Island Sands EA water quality data and the positive and negative correlations versus *M.edulis* percentage cover. The first line shows percentage cover correlations to each variable.

Environmental and water quality data (Appendix L) sourced from the same spatial and temporal location showed the possibility of storm events (p =.04), and fluctuations in pH (p=.006) being linked to bed loss at Fenham Flats (Table 10).

Table 10. Table showing univariate statistical analysis of water quality and environmental data on mussel percentage cover for Fenham Flats and Holy Island Sands, variables with p values of <0.05 and negative correlations on M.edulis are denoted with *.

	Fenham Flats			Holy Island Sands		
Explanatory variable	p-value	df	95% CI	p-value	df	95% CI
Storms (monthly)	0.04*	14	-0.8, 0.01	0.9	2	-0.97, 0.95
pH	0.006*	52	-0.57, -0.1	NA	NA	NA
Rainfall	0.97	172	-0.25,0.14	0.77	2	-0.93,0.97
Water Temperature	0.5	149	-0.21,0.1	0.19	2	-0.99,0.69
Turbidity	0.2	106	-0.08,0.2	0.08	2	-0.99,0.40
Dissolved Oxygen %	0.9	149	-0.28,0.02	0.91	2	-0.96,0.95
Chlorophyll	0.15	107	-0.31,0.05	0.14	2	-0.99,0.60
Nitrogen Dissolved Organic	0.007	77	0.08,0.48	0.76	2	-0.97,0.93
Nitrogen	0.54	80	-0.15,0.28	0.79	2	-0.97,0.94
NH3	0.50	79	-0.14,0.28	0.65	2	-0.98,0.92
Nickel	0.2	34	-0.48,0.15	NA	NA	NA
Nitrate	0.01	76	0.05,0.46	0.77	2	-0.97.0.93
Nitrite	0.35	78	-0.11,0.31	0.7	2	-0.93,0.97
Orthophosphate	0.04	27	0.008,0.65	NA	NA	NA
Si02	0.01	77	0.068,0.47	0.52	2	-0.98,0.89

Biocontamination Data

Fenham Flats

At Fenham flats 2,2′,4,4′,5,6′-hexa-bromodiphenyl ether (PBDE154) and percentage cover of *M.edulis* are strongly negatively correlated, $r(5) = -0.95$, $p = 0.009$, as is dieldrin and percentage cover of *M.edulis,* $r(5) = -0.75$, $p = 0.04$ (Figure 10). Both relationships are statistically significant

(Table 11).

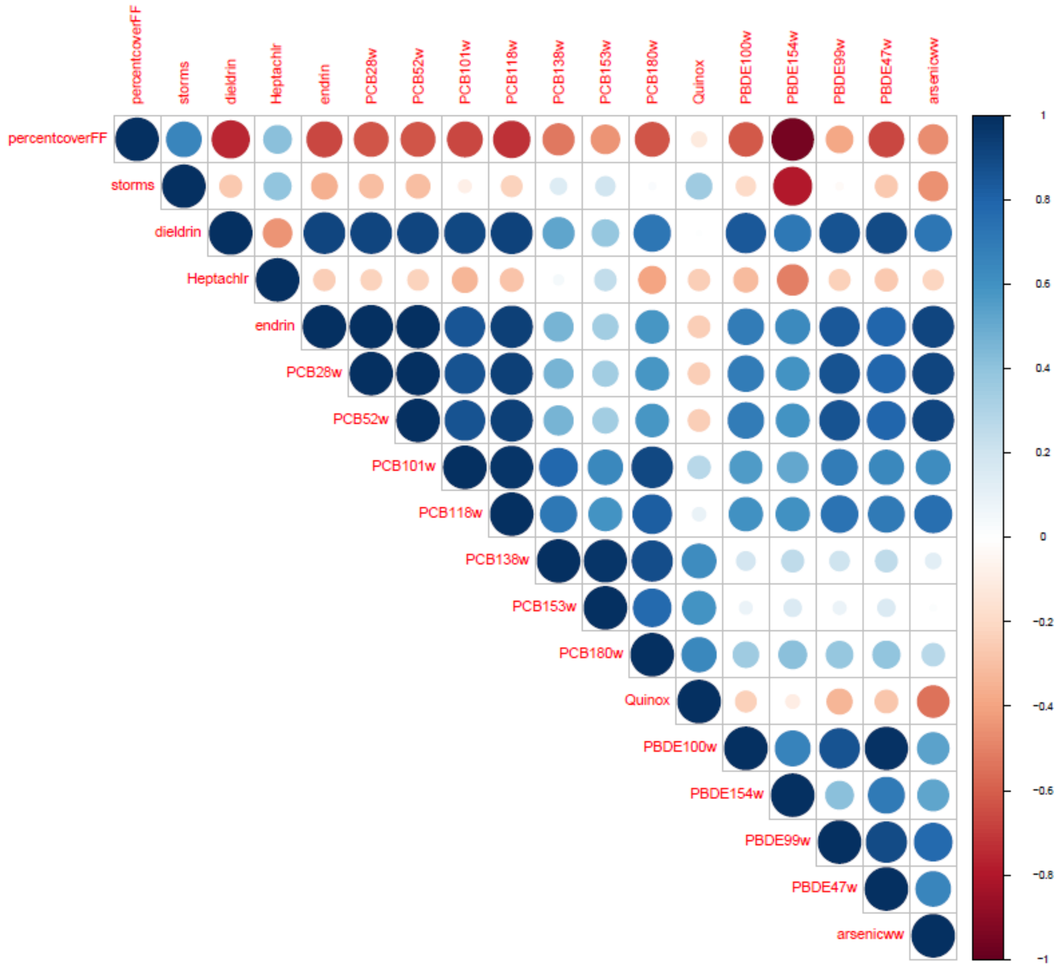

Figure 10. Correlogram showing correlations of all data from multivariate analysis or over EQS levels against percentage cover of *M.edulis* at Fenham flats note the majority of biocontamination determinants have a negative correlation with percentage cover as shown by dark red circles.

Holy Island Sands

PBDE154 and endrin versus percentage cover at Holy Island sands is negatively correlated (Figure 11), r(5) = -0.87, p = 0.01, r(5) = -0.75. p = 0.04 respectively, and both relationships are statistically significant (Table 11).

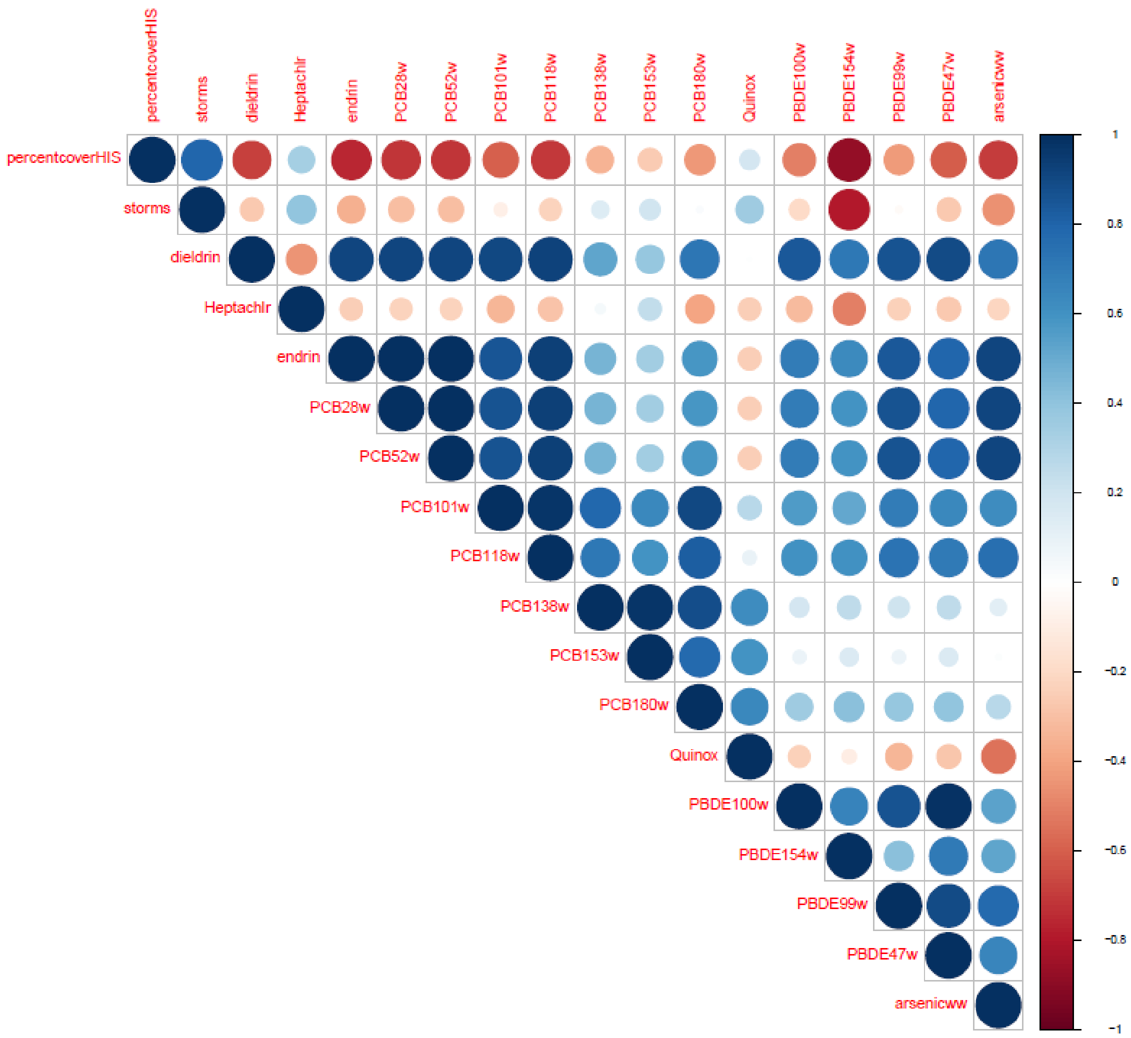

Figure 11. Correlogram showing correlations of all data from multivariate analysis or over EQS levels against percentage cover of *M.edulis* at Holy Island, note the majority of biocontamination determinants have a negative correlation with percentage cover as shown by dark red circles.

Table 11. Table showing univariate statistical analysis of Fenham Flats and Holy Island Sands biocontamination data.

	Fenham Flats			Holy Island Sands		
Explanatory variable	**p-value**	**df**	**95% CI**	**p-value**	**df**	**95% CI**
Dieldrin	0.04*	5	-0.96,-0.01	0.08	5	-0.94,0.13
Heptachlor	0.3	5	-0.49,0.88	0.46	5	-0.56,0.86
Endrin	0.1	5	-0.94,0.10	0.04*	5	-0.96,-0.006
PCB28	0.12	5	-0.93,0.23	0.06	5	-0.95,0.07
PCB52	0.1	5	-0.93,0.23	0.06	5	-0.95,0.07
PCB101	0.1	5	-0.94,0.18	0.1	5	-0.93,0.2
PCB118	0.06	5	-0.95,0.06	0.07	5	-0.95,0.09
PCB138	O.22	5	-0.91,0.37	0.44	5	-0.87,0.55
PCB153	0.32	5	-0.89,0.4	0.57	5	-0.84,0.6
PCB180	0.13	5	-0.93,0.23	0.32	5	-0.89,0.47
Quinoxyfen	0.8	5	-0.80, 0.69	0.68	5	-0.65,0.82
PBDE100	0.14	5	-0.93,0.26	0.24	5	-0.91,0.4
PBDE154	0.0009***	5	-0.99,-0.7	0.01*	5	-0.98,-0.34

	Fenham Flats			Holy Island Sands		
PBDE99	0.39	5	-0.88,0.51	0.33	5	-0.89,0.47
PBDE47	0.1	5	-0.94,0.17	0.15	5	-0.93,0.27
Arsenic	0.2	5	-0.90,0.44	0.08	5	-0.95,0.12

Wind Data

Annual wind direction data shows that southerly and south westerly winds that dominate the Northumberland Coast are positively correlated with percentage cover for Fenham Flats and Holy Island Sands but there are no significant relationships found in the data (Appendix M).

Discussion

Mussel Declines

Data strongly suggests accelerating declines in *M.edulis* percentage cover at Fenham Flats and Holy Island Sands with the loss of 36.5% and 31% of percentage cover at Fenham Flats and Holy Island Sands in recent years (Dent, 2019; Boon, 2020a). This decline is not unique to the area, it is seen in Blyth where 15% of *M.edulis* percentage cover has been lost since 2015, and further afield in the wider North Sea region, affecting other bivalve species such as cockles (Dare, 2004; Callaway *et al.*, 2013; Harvey, 2020). *M.edulis* percentage cover is influenced by a combination of environmental variables, including some that have not been investigated as part of this study and there is evidence that more work is required to understand the reasons for their declines. Under suboptimal conditions in the intertidal zone where *M.edulis* is exposed to multiple environmental stressors, it may take 15-20 years for *M.edulis* to reach 20-30mm and *M.edulis* in Northumberland marine SPA rarely grow more than 75mm in length at Fenham Flats and 67mm at Holy Island Sands this suggests that recovery could take many years if stressors are not minimised (Seed and Suchanek, 1992; Dent, 2019; Boon, 2020a).

Water Quality

Percentage cover at Holy Island Sands and Fenham flats does not appear to be negatively impacted by water quality based on the 14 water quality indicators analysed (Appendix O), no indicators gave significant results when analysed using Pearson's correlation coefficients except for pH, however, this was disregarded as the mean was used from 2015 after the EA ceased testing here so it is not possible to draw any accurate conclusions from this data. All water quality indicators were within normal parameters for a tidal estuary, except for some short periods of heavy rainfall causing drops in salinity; however, it is not known how long these events persisted due to the monthly nature of the data. Some summer periods showed above-average water temperatures peaking at 17.2-17.4°C in June 2008, July 2014, and August 2008, whereas mean summer water temperature was 13.76°C. Despite these fluctuations, this is within the temperature tolerance range of *M.edulis* according to the literature (Bayne *et al.*, 1976). The results suggest that factors such as fluctuations in salinity, rainfall, and oxygen content are not harming *M.edulis* health here, this is positive considering the water quality failure identified in 2019 but it should be interpreted with caution as monthly data may not be sufficient to pick up smaller changes (EA, 2020c).

Storms

Monthly storm frequency and the loss of percentage cover of *M.edulis* at Fenham Flats supports the contamination findings, $r(14) = -0.50$. $p = 0.04$. The data shows a significant increase in storm events correlating with a reduction in bed cover for Fenham Flats, however, this was not the case for Holy Island sands, possibly due to the bed being in a more sheltered position compared to Fenham Flats which is more exposed to bad weather. *M.edulis* are intolerant of smothering and displacement caused by stormy weather, however, they do have the ability to re-emerge if covered in small amounts of sediment <2cm and it is thought that *M.Edulis* with a higher percentage cover has an advantage as they may be able to withstand much greater force from storms than sparser areas of the bed, this further supports the need to protect these beds to ensure the ecosystem services they provide are not lost, this is especially important due to the current climate challenges, healthy, well-structured mussel beds can be valuable buffers against coastal erosion in the face of rising sea levels (Katwijk, 2001; Tyler-Walters, 2008; Hutchison *et al.*, 2016).

Biocontamination

This study appears to show no significant effects upon percentage cover for most biocontaminants tested by the Environment Agency. The exceptions are, PBDE154 (2,2,4,4,5,6 Hexabromodiphenyl Ether) and dieldrin, which were strongly negatively correlated to the loss of *M.edulis* percentage cover at Fenham Flats ($p = 0.0009$ and 0.04) and PBDE154 and endrin at Holy Island Sands ($p = 0.01$ and 0.04). The data strongly suggests that PBDE154 is having a negative effect upon *M.edulis* percentage cover at both sites along with dieldrin.

PBDE154

Annually PBDE154 is increasing and consistently over the EQS level set at 0.0085µg/kg ranging from 0.01-0.014µg/kg and is significantly correlated with loss of percentage cover at Fenham Flats and Holy Island Sands. This EQS value is set for fish at a lower trophic level than filter feeders such as *M.edulis*, and EA sampling occurs annually in March, it is possible levels of PBDE154 fluctuate seasonally, however, a study by (Webster et al., 2009) found this not to be the case. Sampling frequently follows periods of unsettled winter weather and despite the smaller dataset for Holy Island Sands PBDE154 was still found to be significant and negatively correlated which suggests that PBDE154 is affecting both *M.edulis* beds. There was a water quality failure identified in 2013 for PBDEs at Lindisfarne of unknown origin that may have contributed to the build-up of PBDEs in *M.edulis* as identified by the EQS testing from 2015 onwards (EA, 2020b). PBDEs are persistent organic pollutants and have been heavily restricted and banned in the UK, therefore the question remains how these contaminants are accumulating in *M.edulis* tissues and whether this is a consistent pollutant that is present year-round in sediment, linked to storms events or being transferred by atmospheric transport and this may have some effect on community composition of *M.edulis*. The consequences of long-term exposure to these manmade flame retardants are not clear, but there appears to be a link that would warrant further investigation (Jiang *et al.*, 2017; EA, 2019; Ruus, 2021).

Dieldrin and Endrin

Dieldrin is a restricted pesticide and persistent organic pollutant that was banned in 1989, it is a product of aldrin and endrin degradation, and in rural areas such as Holy Island, dieldrin may be coming from contaminated soil that has been washed into marine sediments after heavy rainfall, or resuspended in storm events (McIntosh, 2006). At Holy Island Sands the higher levels of endrin suggest that they come from a recent exposure, and the EQS results support this showing a peak in endrin concentration in 2019 to 3µg/kg, dropping back to 1µg/kg in 2020. Dieldrin has no agreed EQS standard in *M.edulis* and its levels have fluctuated from 0.5µg/kg in 2018 up to 2µg/kg in 2019 then dropped back to 1µg/kg in 2020. This year's data (2021) was unfortunately not available for comparison, so it is difficult to conclude if there is a definite trend here, but the mussel beds are suffering a continual reduction in percentage cover year on year. The location of both *M.edulis* beds may have some effect as there is no significant farming on Holy Island in comparison to the mainland, therefore Fenham Flats may be more exposed to agricultural runoff, and this could explain the link with the data at this site. Dieldrin is known to be particularly toxic to birds and has been implicated in bird mortality from exposure through diet, this may be a major concern for bird species within the SPA that feed on these *M.edulis* beds and have consequences for the management of the SPA (Jorgenson, 2001; Kimbrough, 2008).

Wind

Wind Direction and Speed

Wind direction data ('MIDAS,' 2021) show that the southerly winds that dominate the Northumberland coast and speed data positively correlate with increased percentage cover on Fenham Flats. Direction may influence *M.edulis* as south-westerly winds could keep larvae near-shore allowing more successful settlement, this is important around spawning times in the spring and autumn however, no significant correlations were found for wind data at Lindisfarne, but this data set may be too limited to confirm any accurate relationships.

Limitations

Data for this study was sourced from many different locations, therefore assumptions were necessary to enable a dataset to be produced that could be easily analysed. The lack of data for *M.edulis* health at Holy Island Sands was a particular issue and perhaps does not give an accurate indication of what is happening there in the long term. *M.edulis* health at Fenham Flats has been studied for many years, however, the water quality data came from a location closer to Holy Island Sands mussel beds as only 1 year of data was available for Fenham Flats. This 2018 data was incorporated into the other data by using the mean for that year in the final dataset. Frequency of sampling is a major issue, despite NIFCA and EA data being well aligned with annual sampling taking place in March, the lack of finer-scale data for biocontamination and *M.edulis* health makes it difficult to see if percentage cover fluctuates seasonally. Water quality indicators are tested monthly, but since 2016 pH was not tested by the EA, this extra data would have been useful given the significance of pH from the multivariate analysis.

Conclusions and Recommendations

From the literature review and this study, the effects of the contaminants tested by The Environment Agency are not well understood at a population level in marine biota especially in *M.edulis.* Of course, this is not the intention of their testing program, but it highlights further studies would be required to investigate the interactions between these contaminants and *M.edulis* populations both here and in the rest of the UK. *M.edulis* is a resilient species used to wide fluctuations in a tidal estuary and the other abiotic factors that were investigated do not appear to negatively influence *M.edulis* health, therefore, can probably be excluded from any further investigations at Lindisfarne.

At a local level, monthly monitoring of PBDE154, dieldrin, and endrin levels alongside *M.edulis* percentage cover surveys and water quality data that covers the current test site above the mussel beds at Holy Island Sands and in addition at Fenham Flats, may provide some benefit to assess if contamination levels fluctuate between sites, seasonally or coincide with stormy weather and whether these levels of PBDE's are linked to the water quality failures back in 2013. The combination of this sampling would give a good indication of the rate of *M.edulis* bed loss at each site and considering the decline in recent years, monitoring would be justified to avoid total loss of beds as seen in other parts of the Northeast of England. Current *M.edulis* health monitoring methods are damaging to *M.edulis* beds, therefore alternative methods such as monitoring by a drone (which are currently being investigated) could be an alternative option to measure percentage cover data if monitoring was carried out monthly. Considering the rapid loss of the *M.edulis* beds, particularly at Holy Island Sands, prompt management is required to preserve and protect these beds from future decline to ensure the species that rely on them for food and shelter are protected and to reduce biodiversity loss in the SPA. Targeted monitoring and a joined-up approach from NIFCA and The Environment Agency will provide a basis for future management of these beds and aim to promote recovery in the long term, this will contribute to the SPA reaching good water quality and chemical status, in line with the Environment Agency water framework directive and fulfil in part the requirements set out in the current Northumberland Coast AONB management plan to conserve and protect an important special feature of the SPA for the future.

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Appendices Index