Fisheries New Zealand

Tini a Tangaroa

Environmental Health Measures for Open Ocean Salmon Aquaculture – Identifying thresholds for sub-lethal responses in the context of wider ecosystem change

R.M. McMullin
E.C. McGrath
J. Atalah

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PLAIN LANGUAGE SUMMARY

As finfish farming in Aotearoa New Zealand moves further offshore into more open and dynamic marine environments, there is growing concern about how waste from these farms affects the seafloor and the animals that live there. In particular, large shellfish and other seabed-dwelling animals (known as epifauna) play important roles in healthy marine ecosystems, but little is known about how they respond to waste from offshore salmon farms in Aotearoa New Zealand.

This report, part of the Environmental Health Measures for Open Ocean Aquaculture project, aims to better understand these effects by investigating how three key species – the horse mussel (*Atrina zelandica*), scallop (*Pecten novaezelandiae*), and a brachiopod or lamp shell (*Neothyris lenticularis*) – respond to increasing levels of organic waste from salmon farms. We measured a range of physiological responses in these species at different levels of seabed waste and looked at how their responses relate to one another and to current indicators used in salmon farm monitoring.

Key findings
- Statistical analyses show the levels of organic enrichment that cause changes in physiological functioning in the three key species – and possibly early signs of stress.
- Many of the measured responses were closely linked, suggesting that a smaller set of indicators could reliably detect wider changes in organism health.
- The study shows how the health of the key species can be used as an early-warning signal for broader ecosystem impacts.

The Environmental Health Measures for Open Ocean Aquaculture project is an important first step in developing reliable, ecologically meaningful tools to assess and manage the environmental effects of open ocean aquaculture. Future studies should test to see how well the indicators work with other species and at different farm sites with varying levels of enrichment.

EXECUTIVE SUMMARY

Finfish aquaculture is expanding rapidly on a global scale, with new developments moving from sheltered inshore areas into more dynamic offshore or 'open ocean' environments. This transition increases the potential for widespread dispersal of organic waste, with implications for diverse benthic habitats and the functionally distinct communities they support. Large epibiota play critical roles within food webs, and stress from salmon farming activities could trigger cascading ecological effects over large spatial scales, particularly when they involve ecosystem engineers such as bivalves. In Aotearoa New Zealand, the physiological responses of epifaunal species in offshore ecosystems to farm-derived waste remain poorly understood.

To support sustainable aquaculture in offshore environments, it is essential to address critical knowledge gaps around how benthic epifauna respond to organic enrichment and how these responses relate to ecosystem health. Identifying early species-level indicators of environmental change can help improve monitoring frameworks and inform adaptive management.

This project represents an initial step towards that goal by examining the physiological responses of three ecologically and culturally important species – horse mussel (*Atrina zelandica*), scallop (*Pecten novaezelandiae*), and a brachiopod or lamp shell (*Neothyris lenticularis*) – across a gradient of organic enrichment. A suite of analytical approaches was applied to assess a range of physiological traits, which were analysed in relation to one another and to standard indicators of organic enrichment currently used in routine salmon farm monitoring. Correlation analyses and generalised additive models were used to explore relationships and identify thresholds at which physiological functioning departs from baseline conditions.

Collinearity analysis revealed strong correlations among many physiological response variables, supporting the use of a representative sub-set as indicators of broader physiological change. Overall Enrichment Stage (ES) threshold values were calculated and were relatively consistent across variables and species, although some variation occurred between experimental systems. The baseline data generated through this study also provide important reference points for understanding physiological ranges in the absence of stressors, addressing a significant gap in current knowledge.

While the findings of this study are promising, additional data are needed to refine these indicators, validate their application across other species and extend their use across broader environmental contexts. Overall, this research provides a critical foundation for linking organism-level physiological responses to ecosystem-level indicators and contributes to the development of tools with strong ecological relevance and predictive capability to support environmental monitoring and management of offshore aquaculture in Aotearoa New Zealand.

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[1] All authors are affiliated with Cawthron Institute.

1.   INTRODUCTION

The global shift towards open ocean[2] finfish aquaculture (OOA) raises new questions about the interactions this activity will have with diverse and complex benthic habitats in these environments (Giles et al. 2021). While the ecological effects of farm-derived organic enrichment are well documented for inshore, soft sediment environments (Keeley et al. 2015; McMullin et al. 2021; Weitzman et al. 2019), offshore areas of interest for aquaculture development are often proximal to hard- and mixed-bottom epifaunal communities (Bennett et al. 2022; Elvines et al. 2021a). In contrast to benthic habitats surrounding inshore salmon farms, little is known about how communities in open ocean areas will respond to OOA-derived wastes.

While some similarities may exist between inshore and offshore effects on soft sediment infauna (Keeley et al. 2012; Macleod et al. 2008), epifaunal communities remain largely understudied in both environments. As such, the likely tolerance and physiological responses of epifaunal species to potential farm-derived organic material in these systems are largely unknown. Given the greater structural complexity and ecological significance of offshore benthic communities, understanding their structure, function and physiological response to organic enrichment is essential for assessing the potential impacts of OOA and guiding sustainable management practices.

The main seabed impact from finfish farm operations is the addition of nutrient-dense farm-derived organic material, primarily comprising faecal waste (Carvajalino-Fernandez et al. 2020), and to a lesser extent, uneaten feed pellets (Reid et al. 2009; Schumann & Brinker, 2020). The severity of enrichment is driven mainly by feed inputs[3] and the physical characteristics of the receiving environment. Deep, well-flushed locations (hereafter termed 'dispersive') have greater potential for dispersal and dilution of farm-derived organic waste compared to shallower sites with lower current speeds. As a result, organic enrichment beneath farm pens at dispersive sites is expected to be more diffuse than that at non-dispersive sites(Keeley, 2020; Giles et al. 2021). This difference is due to the increased current transport of fine particles and flocculent material into the far field from the primary depositional footprint, as well as increased particle resuspension from the seabed (Keeley et al. 2019).

The tolerance of marine organisms to ingestion and assimilation of farm-derived organic material tends to be site- and species-specific (Keeley et al. 2020; Laroche et al. 2022; McMullin, 2020; White et al. 2017). For some species, consuming this material can be beneficial, enhancing growth, body condition and population size (Bergvik et al. 2019; George & Parrish, 2015). However, for other organisms, ingestion or assimilation of farm-derived organic material may present risks of chronic or long-term physiological stress. These effects can stem from declines in seston or food quality (trophic subsidies), or from the increased energetic costs associated with selective feeding, depending on the duration and intensity of exposure (Ellis et al. 2017; Elvines et al. 2024; McMullin et al. 2022). Such subsidies may lead to sub-lethal impacts, including reduced growth, condition or reproductive output (Drolet et al. 2022; Sardenne et al. 2020; White et al. 2016). Beyond serving as a trophic subsidy, farm-derived organic material can also interfere with normal feeding and

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[2] Also referred to as offshore aquaculture.
[3] And, to a lesser extent, other farming characteristics, such as the type of pen structures used, fish stocking density, type of feed and feeding system, settling velocity of fish faeces, and severity of water flow reduction caused by the farm structures (Keeley et al. 2015).

physiological processes. It may clog feeding appendages of suspension feeders, affect respiration rates, or in some cases, smoother and kill organisms (Ellis et al. 2017; McGrath et al. 2017; Williams, 2022). Direct changes to physiological functioning can, in turn, trigger cascading ecological effects over large spatial scales, particularly when they involve functionally important species (Elvines et al. 2024; Sarà et al. 2021; White et al. 2018).

1.1   Project background

Fisheries New Zealand has identified open ocean finfish aquaculture as a key priority for expansion of the aquaculture industry (Giles et al. 2021), with the aim of reaching NZ$3 billion in annual sales by 2035. At present, there are few operational examples of OOA worldwide and environmental effects of these farms have not yet been well documented. In Aotearoa New Zealand, several OOA salmon farms have been proposed, the first of which (The New Zealand King Salmon Co. Ltd's [NZKS] Blue Endeavour farm in Cook Strait) was approved in 2022 (Bennett et al. 2022; Elvines et al. 2021a). Compared to many offshore operations overseas, the proposed sites here – including the Cook Strait location – are highly exposed, with diverse habitats close by.

Fisheries New Zealand engaged Cawthron Institute in a multi-stage research project with an overall aim to understand how organic enrichment at the levels that are anticipated for OOA will impact benthic species. The project stages are:
- Objective 1 (complete) – Literature review on 'Candidate species selection for the development of environment health measures for open ocean salmon aquaculture'.
- Objective 2 (complete) – Define the effects of organic enrichment on the health of key species identified in Specific Objective 1 through laboratory and field trials.
- Objective 3 (present study) – Assess the potential magnitude and effect of organic enrichment from OOA on ecosystem health using measured physiological responses from Objective 2.

1.2   Summary of project work to date

Objective 1: Rationale for species selection

In the first stage of this research project, Objective 1 (McMullin & McGrath, 2023), a literature review was conducted that delivered a framework for selecting key species for field and laboratory experiments. A comprehensive list of epifaunal taxa from proposed OOA sites and existing farm sites was compiled. Locations included northeast Marlborough Sounds, northern Stewart Island / Rakiura, eastern Foveaux Strait and Tory Channel / Kura Te Au. The ecological and cultural importance of these species and their responses to organic enrichment and sedimentation were evaluated. Using a scoring matrix based on ecological and cultural factors, and excluding species with logistical constraints, a list of suitable candidate taxa was created. The final species selected for laboratory and field trials in Objective 2 were the horse mussel (*Atrina zelandica*), scallop (*Pecten novaezelandiae*), and a brachiopod or lamp shell (*Neothyris lenticularis*).

Objective 2: Laboratory and field trials

A brief description of the experimental design used in Objective 2 is provided in Appendix 1. For more information, see the Objective 2 report (McMullin et al. 2025).

The Objective 2 component employed a two-pronged approach to demonstrate evidence of stress response in the target species when exposed to a range of organic enrichment levels. Laboratory conditions enabled isolation of the stressor of interest (farm-derived organic enrichment) and its associated responses, while the field trial captured responses to natural processes associated with salmon farming, such as farm waste resuspension, interactions with sediment microbes, physical effects of sedimentation and smothering on feeding, and stress. Times of exposures were approximately 3 months under laboratory conditions and 5 months under field conditions; therefore, results presented provide an indication of short- to medium-term responses.

The laboratory trial included four treatments: one algae-only control; and three levels of organic enrichment, which were informed by depositional modelling for potential OOA sites in Aotearoa New Zealand. By isolating a key stressor, the experiments provided clearer insight into organism-level responses compared to *in situ* studies, where multiple, interacting factors can confound physiological changes. Laboratory trials also enabled the identification of molecular or compound-specific biomarkers in organisms exposed to known enrichment levels. This approach is particularly valuable for detecting subtle physiological changes and early indicators of stress that may not be easily observed in field settings. Furthermore, these trials contributed to a more refined understanding of low-level organic enrichment effects on epifaunal health, including tolerance thresholds, providing context for field-based observations.

The field trial involved translocating horse mussel and brachiopod individuals[4] along an organic enrichment gradient adjacent to the Clay Point salmon farm operated by NZKS in the Marlborough Sounds. Horse mussel and brachiopods were mounted on frames and deployed across four sites: two sites within the footprint of the farm, and two unimpacted control sites. All sites were of a similar depth (approximately 30 m), and all were over soft sediment with low-density epifauna communities present.

Objective 3: Linking physiological change to wider ecosystem health

This report presents and discusses the findings of Objective 3 – linking physiological change to wider ecosystem health. In Objective 3, laboratory and field data collated in Objective 2 were used to establish empirical relationships between species-specific response indicators and environmental metrics associated with enrichment. Specifically, physiological responses were modelled against Enrichment Stage (ES), a metric currently used in routine salmon farm monitoring that integrates changes in microbial and infaunal communities, sediment chemistry and sediment organic content (Keeley et al. 2012). These physiological responses could also be modelled against individual enrichment indicators, such as eDNA-based metrics (e.g. bacterial Metabarcoding Index [b-MBI]), if adaptation is required to align with future consent conditions or monitoring approaches.

This work bridges the gap between physiological response and ecosystem-level indicators, with the goal of enhancing both the ecological relevance and predictive power of enrichment monitoring tools. By identifying relationships between organism-level stress responses and broader ecological shifts, monitoring programmes can move beyond reactive assessments to

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[4] Scallops were not included in the field trial due to concern over their chance of survival following collection, transfer, and housing in cages. High mortality rates were observed in the laboratory trial for scallops due to their sensitivity to handling and transport.

anticipatory frameworks. Physiological stress indicators, or bioindicators, identified as having a clear, statistically supported relationship to environmental predictors (e.g. ES or b-MBI) can serve as sentinel indicators, offering early warnings of ecosystem changes before they become pronounced or irreversible. This approach is particularly valuable for benthic species that are difficult to assess using conventional monitoring tools such as video surveys, providing a more sensitive and integrative measure of farm impact. Furthermore, the approach not only improves responsiveness, but it also supports proactive management strategies aimed at preventing degradation rather than capturing impacts only after they manifest.

Specific aims for Objective 3
- Use laboratory and field data collected in Objective 2 to identify and model how specific physiological responses (e.g. respiration, lipid composition, gene expression, histological pathologies) change along a gradient of environmental enrichment.
- Establish empirical relationships between physiological indicators and ES to help interpret ecological condition based on biological function (early, sub-lethal responses), rather than relying on community-level indices, which may reflect changes only after more substantial impacts.
- Evaluate which physiological indicators show consistent responses across species and environments, identifying those that may serve as robust or early-warning tools for ecosystem health monitoring.
- Develop a pipeline through which a response indicator may be refined (tested and validated across species, environments, enrichment levels) and implemented in environmental monitoring or for validation of modelled predictions.

2. METHODS

2.1 Data analysis

Data used for analyses

Predictive variable

ES is a composite metric that characterises the ecological response of the seabed to organic enrichment from aquaculture (primarily salmon farming; Keeley et al. 2012; Fletcher et al. 2022). It integrates measures of sediment organic content, sediment chemistry and infaunal or microbial biodiversity and abundance[5]. ES is also commonly used in the context of depositional modelling outputs, providing a meaningful link between predicted organic deposition and expected ecological effects.

Given that ES is a composite of multiple environmental metrics, its strong correlation with those inputs is expected (as shown in Figure 1). However, its ability to capture variation across physical, chemical, and biological parameters highlights its potential as a useful integrative indicator of environmental change. Accordingly, we have selected ES as the primary predictor variable in our assessment of relationships between environmental drivers and physiological response variables.

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[5] Overall Enrichment Stage calculation can be based on macrofauna community data or bacterial environmental DNA (eDNA). We refer the reader to the best management practice guidelines for salmon farms in the Marlborough Sounds for more information (Fletcher et al. 2022).
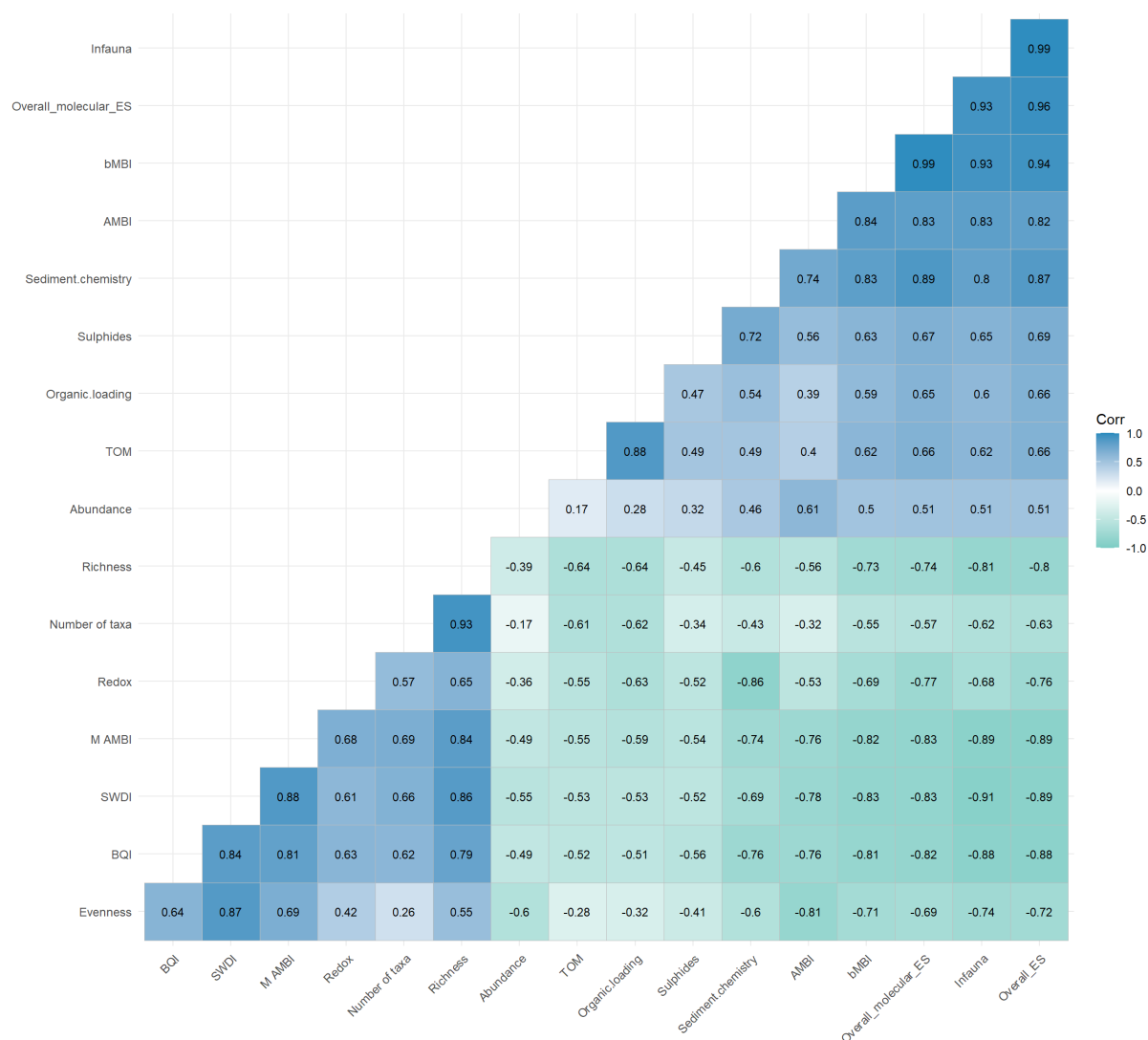
Figure 1: Correlation matrix showing correlation coefficients between environmental variables and indices sampled along salmon farm enrichment gradients (from Pochon et al. 2021).

Field ES values were derived using benthic monitoring data collected at NZKS's Clay Point salmon farm located in Tory Channel / Kura Te Au, Marlborough Sounds, over a 6-year period[6] (McGrath et al. 2020, 2021, 2022, 2023; Fletcher et al. 2024; Millar et al. 2025). These data included a suite of environmental variables used to calculate an ES score for each sampling site, based on a weighted combination of sediment chemistry, organic content, and infaunal community composition or microbial metrics.

Laboratory ES values were estimated by converting the measured depositional flux of salmon faeces (kg solids m^{-2} yr^{-1}) into ES scores using an empirically derived equation from Keeley et al. (2013):

$$\ln ES = 1.0875 + 0.156 \ln(x + 0.01) + 0.01225 ln(x + 0.01)^2$$

where x is the value for depositional flux in kg solids m^{-2} yr^{-1}, and ES is the Enrichment Stage score.

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[6] Data courtesy of The New Zealand King Salmon Co Ltd.

This conversion enabled laboratory dosing treatments to be aligned with the field-based ES scale, allowing for direct comparison of physiological responses across environments and enrichment levels.

Physiological response variables

Physiological response variables describe the parameters measured for organisms during the Objective 2 laboratory and field trials (McMullin et al. 2025). These include condition indices, fatty acid contents, metabolic / respiration rates, gene expression and histological pathologies collected from horse mussels, scallops and brachiopods.

Data compilation and integration for analysis

Multiple datasets containing information on physiological response variables from different analytical approaches were collated in R (R Core Team, 2025). Key identifiers such as site (for field data) or treatment group (for laboratory data), species, animal ID and time point were used to align records across datasets.

Due to incomplete data coverage at the individual level (i.e. not all analysis types were available for every animal), aggregated values were calculated to support comparative analysis.

Binary columns indicating presence (1) or absence (0) of features (e.g. histological or fatty acid traits) were automatically identified. A sub-set of rare or irrelevant binary variables was excluded from further analysis. Numeric columns of continuous data were also identified. For each combination of site and species, or site and treatment group, median values were calculated for continuous variables, and percentage prevalence was calculated for presence / absence variables.

Correlations among physiological response variables

To reduce multicollinearity between physiological response variables, correlation analysis was performed separately for each species within the laboratory and field datasets. Numeric variables were extracted, and pairwise Pearson correlation coefficients were calculated using the *cor()* function in R, based on pairwise complete observations. Highly correlated variables were identified using a network-based approach. The *igraph* package (Csárdi et al. 2025) was used to construct a network graph in which nodes represented individual variables and edges connected variable pairs with absolute correlation coefficients ($|r|$) greater than 0.9. Connected components of the graph, representing clusters of highly correlated variables, were identified using the *components()* function. Cluster membership was exported for further interpretation and analysis.

From each correlation cluster, a sub-set of representative variables was selected for subsequent modelling of relationships between environmental predictors and physiological response variables. Variable selection was guided by best professional judgement, supported by two quantitative criteria: (1) variables with the lowest average correlation to variables outside their cluster, and (2) variables with the highest within-cluster variance. Best professional judgement also considered factors such as the strength of trends identified in Objective 2, representation across analytical approaches, and practical considerations such as sampling feasibility, time and cost efficiency.

Testing correlations for variable refinement

A second iteration of the correlation analysis was run to further refine the sub-set of physiological response variables. The reduced variable sets identified in the initial multicollinearity screening were used to generate new correlation matrices for each species, separately for the laboratory and field datasets. The resulting matrices were visualised using the *ggcorrplot* package in R. This allowed for easy identification of any remaining highly correlated variable pairs. This process was followed for each species dataset from the laboratory and field.

Assessing relationships between environmental predictors and physiological response variables

Species responses to organic enrichment (ES) were modelled using generalised additive models (GAMs; Wood, 2017). Separate GAMs were fitted for each species–variable combination, selected based on prior correlation analyses. The physiological response variables were Box–Cox transformed, to meet modelling assumptions. ES was included as a smooth term using a cubic regression spline with shrinkage. To minimise overfitting, the basis dimension for each smooth was limited to $k = 3$. Models were fitted using the *mgcv* package (Wood, 2004, 2011) in R (R Core Team, 2025).

To identify significant changes in species responses along the ES gradient, first derivatives of the smooth terms were estimated using the *gratia* package (Simpson, 2024). A response was considered significant where the 95% confidence interval of the derivative did not include zero (Simpson, 2018). For non-linear relationships, ecological thresholds were identified as ES values corresponding to significant rates of change

Results were visualised with partial dependence plots showing fitted smooths and associated confidence intervals, overlaid with derivative plots highlighting significant trends. For models exhibiting linear relationships, the slope direction was first determined. Subsequently, baseline variability was characterised by extracting the upper and lower bounds of the 90% confidence interval (CI) from control groups (baseline ES levels). The ES threshold was then defined as the point along the fitted linear response where predicted values crossed at either the lower 90% CI (for negative slopes) or upper 90% CI (for positive slopes) of the control group. In cases where derivative-based thresholds from non-linear models fell below the upper ES range observed in controls, the same control-based method as used for linear models was applied to ensure consistent interpretation of baseline departures. This adjustment was necessary only for variables from the field dataset.

3.   RESULTS

3.1   Correlation assessment

The network visualisations revealed strong correlations among numerous physiological, biochemical, and molecular variables across all species in both laboratory (Figures 2–4) and field (Figures 5–6) datasets. The presence of densely connected clusters indicates a high degree of redundancy among variables, supporting the selection of a reduced set of representative metrics for inclusion in GAMs (Tables 1–5). These representative variables serve as effective indicators of broader physiological processes, enabling more streamlined and interpretable modelling of organismal responses to environmental change.

Figure 2: Network diagram showing highly correlated variables for horse mussels from the laboratory experiment. Each node represents a measured variable, and edges (links) represent strong Pearson correlations (|*r*| > 0.95) between variables.

Table 1: Sub-set of variables selected from network clusters generated for horse mussels from the laboratory trial. For the analysis types, FA = fatty acid, H = histology, OS = oxidative stress, R = respiration, T = transcriptomics.

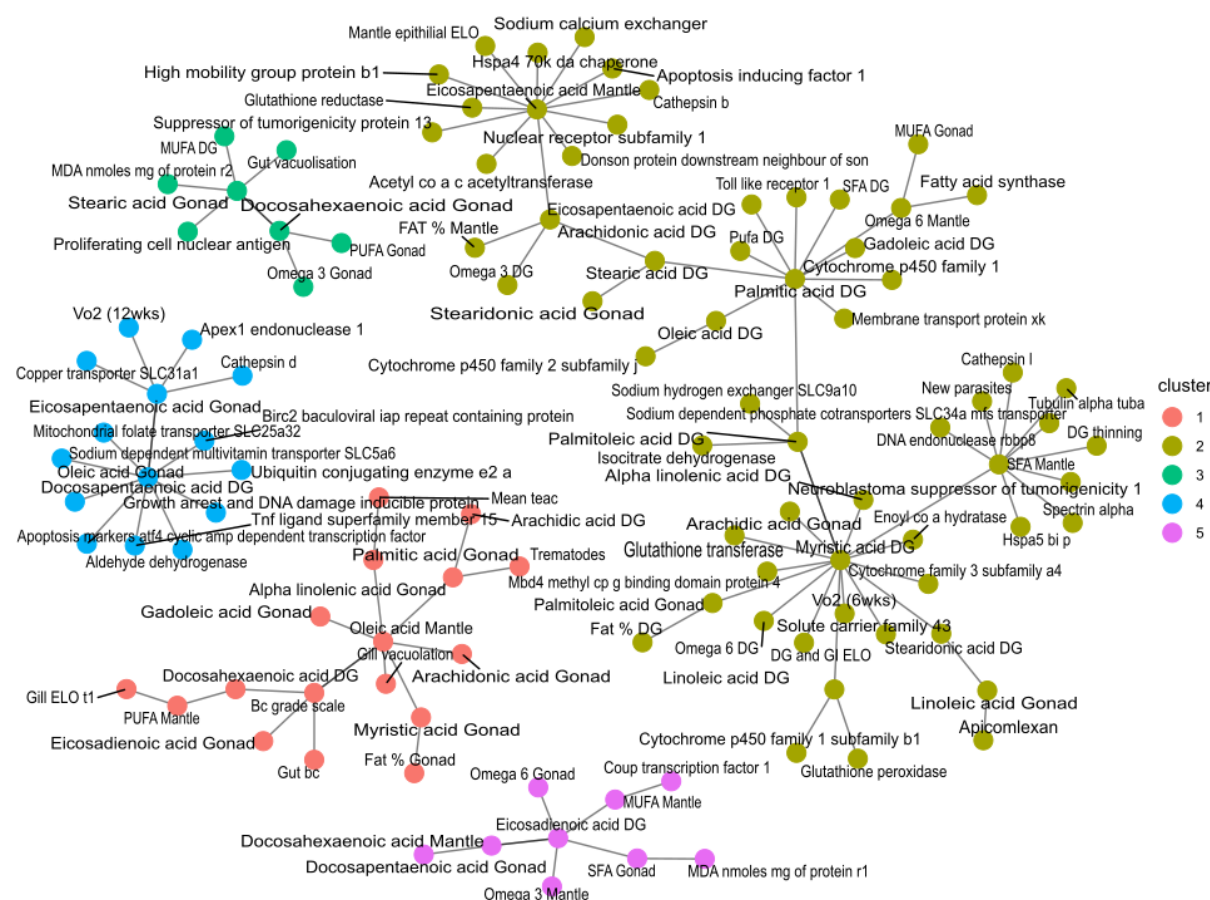| Selection based on best professional judgement (horse mussels)	Analysis	Cluster	Selection based on best professional judgement (horse mussels)	Analysis	Cluster
Oleic acid Mantle	FA	1	Glutathione reductase	T	2
Fat % Gonad	FA	1	Hspa5 bi p	T	2
Gill elo t1	H	1	Sodium calcium exchanger slc8a	T	2
Trematodes	H	1	PUFA Gonad	FA	3
Mean teac	OS	1	Gut vacuolisation	H	3
Oleic acid DG	FA	2	MDA nmoles mg of protein r2	OS	3
MUFA Gonad	FA	2	Proliferating cell nuclear antigen	T	3
Fat % DG	FA	2	Oleic acid Gonad	FA	4
PUFA DG	FA	2	Vo2(12wks)	R	4
SFA DG	FA	2	Cathepsin d	T	4
Dg and gi elo	H	2	Copper transporter slc31a1	T	4
Mantle epithelial elo	H	2	Growth arrest and DNA Damage inducible protein gadd45	T	4
Vo2 (6wks)	R	2	Sodium-dependent multivitamin transporter slc5a6	T	4
Cytochrome p450 family 1	T	2	SFA Gonad	FA	5
Donson protein downstream neighbour of son	T	2	MDA nmoles mg of protein r1	OS	5
Fatty acid synthase	T	2	Coup transcription factor 1	T	5

Figure 3: Network diagram showing highly correlated variables for scallops from the laboratory experiment. Each node represents a measured variable, and edges (links) represent strong Pearson correlations (|_r_| > 0.95) between variables.

Table 2: Sub-set of variables selected from network clusters generated for scallops from the laboratory trial. For the analysis types, FA = fatty acid, H = histology, OS = oxidative stress, R = respiration.

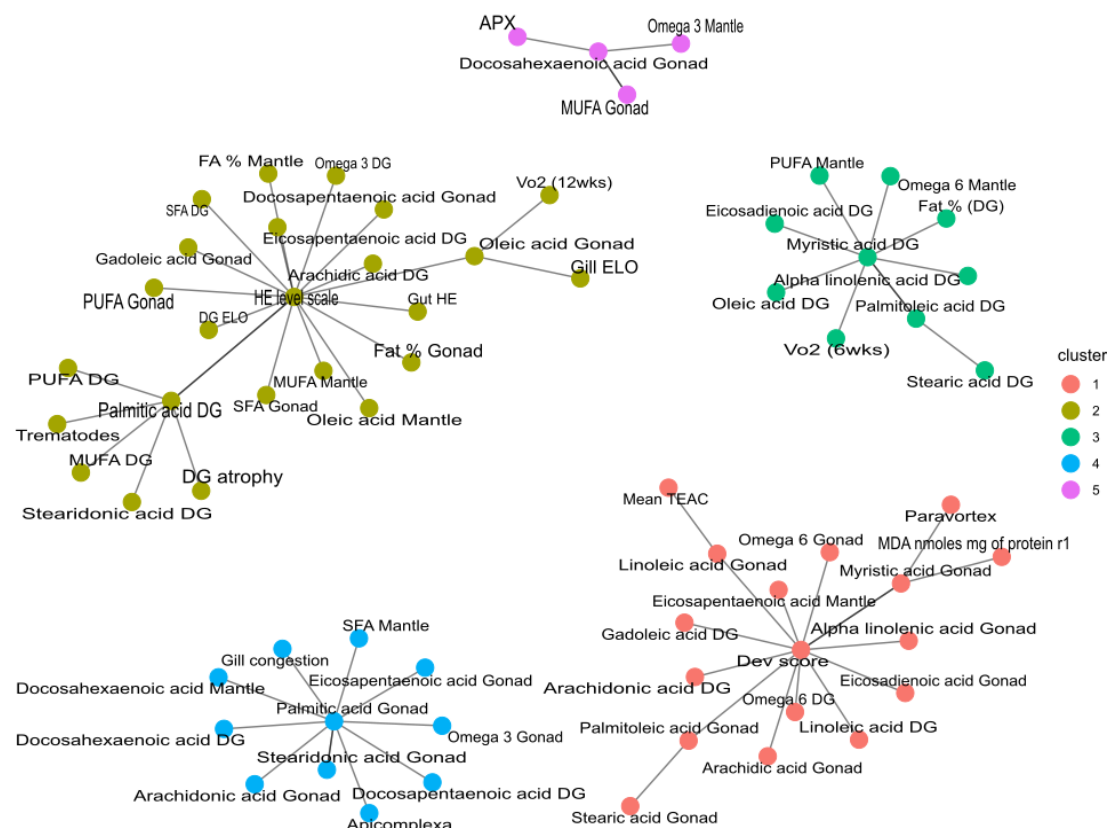| Selection based on best professional judgement (scallops)	Analysis	Cluster
Omega 6 DG	FA	1
Omega 6 gonad	FA	1
Dev score	H	1
MDA nmoles mg of protein r1	OS	1
Mean teac	OS	1
Oleic acid gonad	FA	2
Omega 3 DG	FA	2
Fat % gonad	FA	2
PUFA gonad	FA	2
DG atrophy	H	2
DG elo	H	2
Gill elo	H	2
Trematodes	H	2
Vo2 (12wks)	R	2
Oleic acid DG	FA	3
Fat % DG	FA	3
Vo2 (6wks)	R	3
Omega 3 gonad	FA	4
Gill congestion	H	4
MUFA gonad	FA	5
APX	H	5

Figure 4: Network diagram showing highly correlated variables for brachiopods from the laboratory experiment. Each node represents a measured variable, and edges (links) represent strong Pearson correlations (|*r*| > 0.9) between variables.

Table 3: Sub-set of variables selected from network clusters generated for brachiopods from the laboratory trial. For the analysis types, FA = fatty acid, H = histology, OS = oxidative stress, R = respiration.

Best professional judgement (brachiopods)	Analysis	Cluster
Omega 3 gonad	FA	1
Omega 3 non-gonadal	FA	1
Percent fat gonad	FA	1
DG atrophy	H	1
MDA nmoles mg of protein r1	OS	1
Vo2 (6wks)	R	1
Alpha linolenic acid gonad	FA	2
SFA gonad	FA	2
PUFA non-gonadal	FA	3
DG luminal space open	H	3
Mean teac	OS	3
MUFA non-gonadal	FA	4
Vo2 (12wks)	R	4
Omega 6 gonad	FA	5
Omega 6 non-gonadal	FA	5
MDA nmoles mg of protein r2	OS	5
Arachidic acid non-gonadal	FA	6
Gut epi atrophy	H	6

Figure 5: Network diagram showing highly correlated variables for horse mussels from the field experiment. Each node represents a measured variable, and edges (links) represent strong Pearson correlations (|*r*| > 0.9) between variables.

Table 4: Sub-set of variables selected from network clusters generated for horse mussels from the field experiment. For the analysis types, C = condition, FA = fatty acid, H = histology, T = transcriptomics.

Best professional judgement	Analysis	Cluster	Best professional judgement	Analysis	Cluster
Condition	C	1	Hspa5 bi p	T	3
Oleic acid DG	FA	1	PUFA DG	FA	4
Fat % DG	FA	1	SFA gonad	FA	4
Fat % gonad	FA	1	Gut bc	H	4
Omega 6 PUFA DG	FA	1	Copper transporter slc31a1	T	4
SFA DG	FA	1	Coup transcription factor 1	T	4
Mantle epithelial elo	H	1	Arachidonic acid aa gonad	FA	5
Fatty acid synthase	T	1	Docosapentaenoic acid gonad	FA	5
Sodium-dependent multivitamin transporter slc5a6	T	1	DG and Gi elo	H	5
PUFA gonad	FA	2	DG atrophy	H	5
Gut vacuolisation	H	2	Tubulin alpha tuba	T	5
Cytochrome p450 family 1 subfamily a1	T	2	Stearic acid gonad	FA	6
Growth arrest and DNA damage inducible protein gadd45	T	2	Sodium hydrogen exchanger slc9a10	T	6
Gut f he	H	3	Gill elo t1	H	7
Cathepsin d	T	3	Donson protein downstream neighbour of son	T	7
Glutathione reductase	T	3	Proliferating cell nuclear antigen	T	7

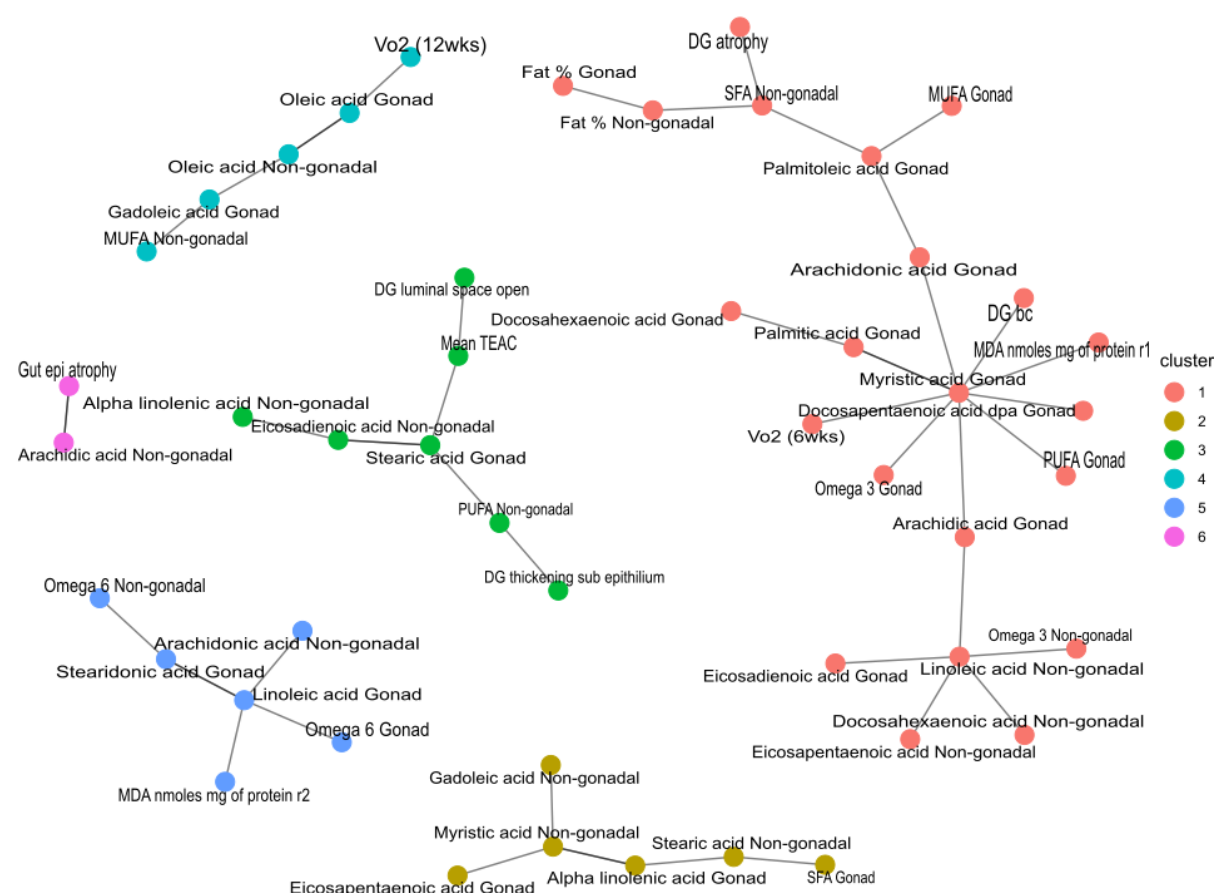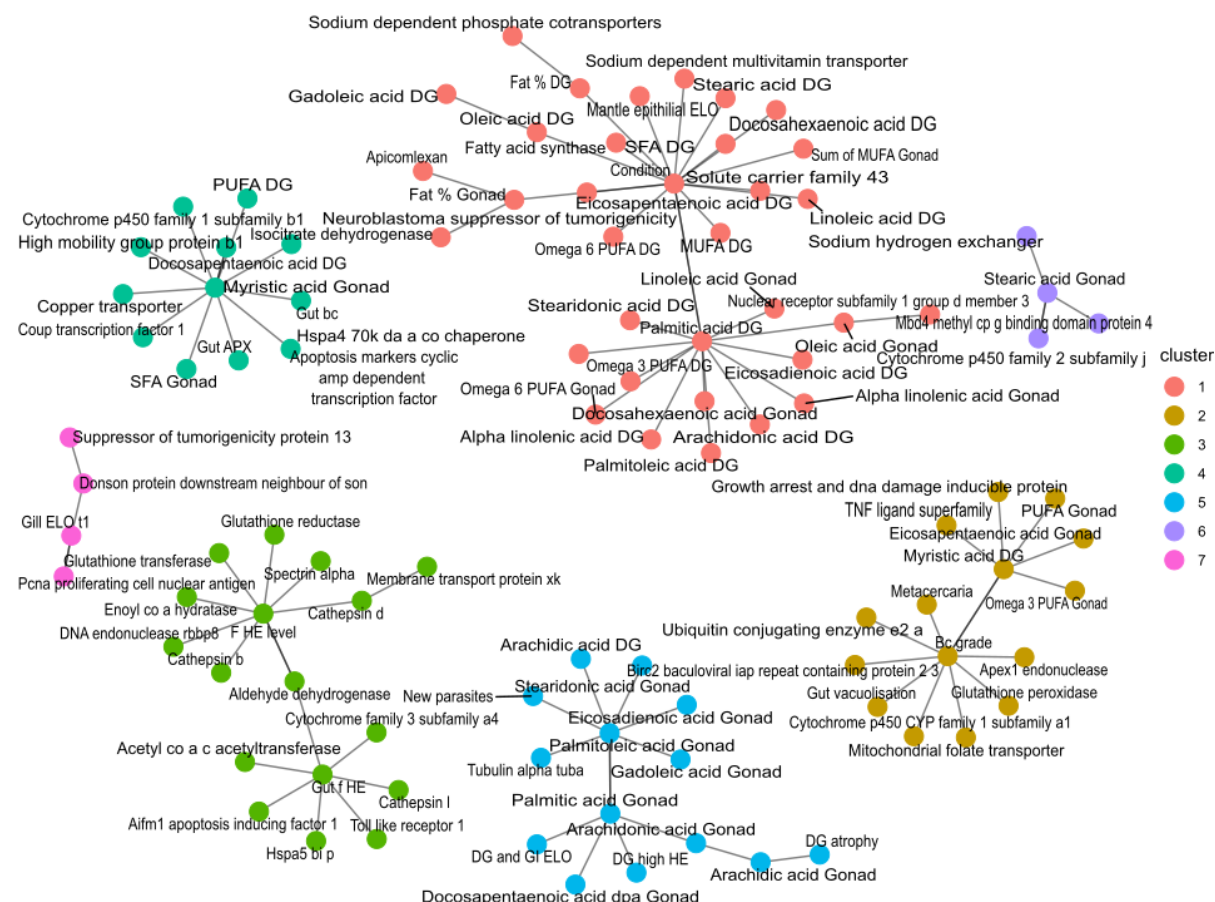**Figure 6: Network diagram showing highly correlated variables for brachiopods from the field experiment. Each node represents a measured variable, and edges (links) represent strong Pearson correlations ($|r| > 0.9$) between variables.**

Table 5: Sub-set of variables selected from network clusters generated for brachiopods from the field experiment. For the analysis types, FA = fatty acid, H = histology.

Best professional judgement	Analysis type	Cluster
Fat % gonad	FA	1
Fat % non-gonadal	FA	1
SFA gonad	FA	1
DG atrophy	H	1
DG bc	H	1
Oleic acid gonad	FA	2
MUFA gonad	FA	2
Omega 3 PUFA non-gonadal	FA	2
Pufa non-gonadal	FA	2
Omega 3 PUFA gonad	FA	3
Omega 6 PUFA gonad	FA	3
PUFA gonad	FA	3

3.2   Generalised additive models

Laboratory experiments

Of the 83 GAMs fitted for the selected physiological response variables, 23 demonstrated significant relationships with ES, including 4 for brachiopods, 17 for horse mussels and 2 for scallops (Figure 7; see Appendix 2 for a summary of GAM results). The proportion of deviance explained by these significant models varied by species, with median values of 57.3% for brachiopods, 53.0% for horse mussels and 58.5% for scallops. Overall model fit ranged widely, from 10.2% to 84.9%, indicating species- and variable-specific differences in response strength (Figure 7).

Eight of the significant models had estimated degrees of freedom equal to 1, indicating linear relationships between ES and the response variable. For these linear models, ES thresholds ranged from 2.7 to 4.1, with a median threshold of 3.34 (interquartile range [IQR]: 3.00–3.72; see Appendix 2 for threshold ES values identified). The remaining models exhibited non-linear relationships, suggesting more complex response patterns. These non-linear relationships revealed threshold responses to enrichment, with significant changes typically occurring around moderate to high ES values (ES 3.0–4.1; median ES = 3.49; IQR: 3.40–3.64).
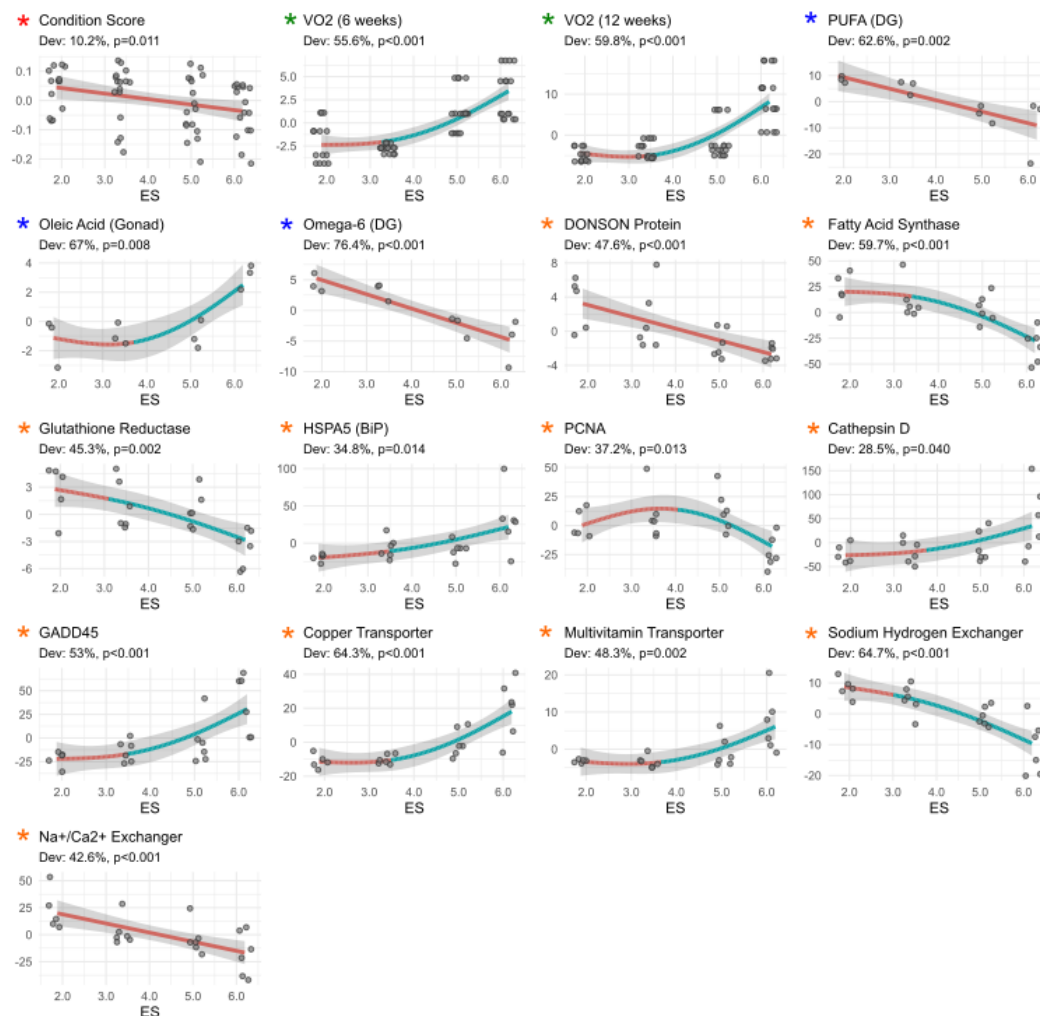
Figure 7: Partial dependence plots from generalised additive models showing the relationship between Enrichment Stage (ES) and variables with significant responses from the laboratory experiments, for (A) horse mussels, (B) scallops and (C) brachiopods. Lines represent fitted smooths, with blue shading indicating areas of significant change based on the first derivative of the smooth. The y-axis is unitless and reflects the contribution of the smooth term to the linear predictor. Variables are from analysis types: condition (red asterisks), respiration (green asterisks), fatty acids (blue asterisks) and transcriptomics (orange asterisks).

Field experiments

Of the 47 GAMs fitted for the selected species-response variable combinations, 18 showed significant relationships with ES, comprising 10 models for horse mussels and 8 for brachiopods (Figure 8). Among the significant models, the proportion of deviance explained ranged from 35.0% to 86.0% for brachiopods (median: 59.8%, IQR: 21.9%), and from 26.6% to 94.5% for horse mussels (median: 47.9%, IQR: 52.0%; Figure 8).

Ten of the significant models had estimated degrees of freedom equal to 1, indicating linear relationships. ES thresholds in these cases ranged from 2.3 to 2.8, with a median of 2.63 (IQR: 2.55–2.69). The remaining eight models displayed non-linear relationships, with threshold responses to enrichment occurring at low to moderate ES values (ES: 2.2–3.6; median ES = 2.34; IQR: 2.25–2.53).
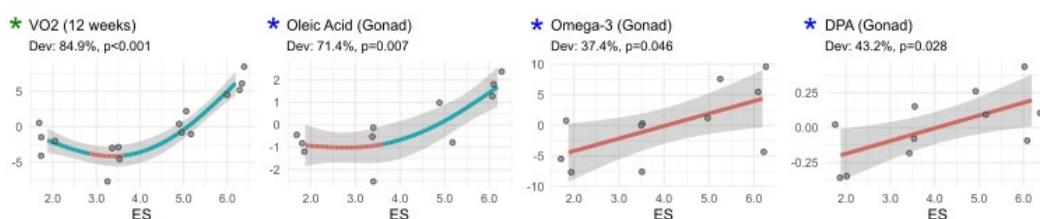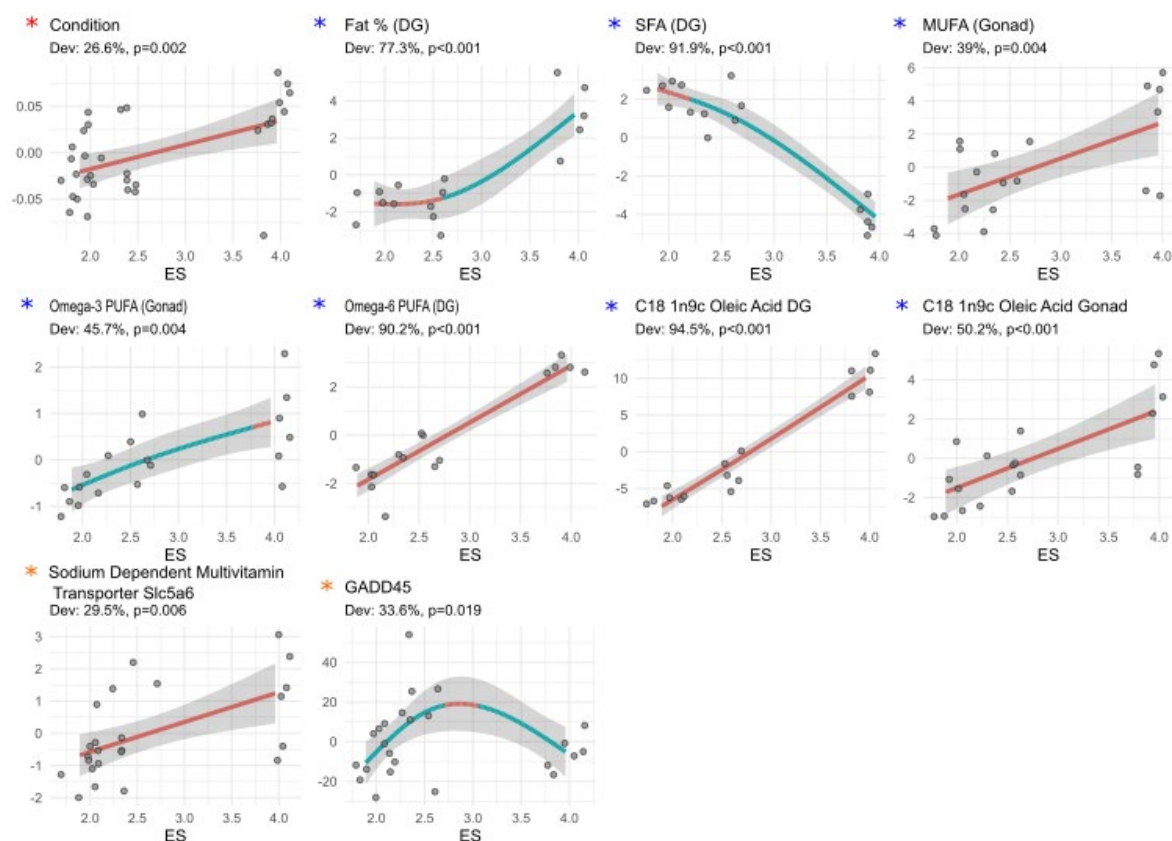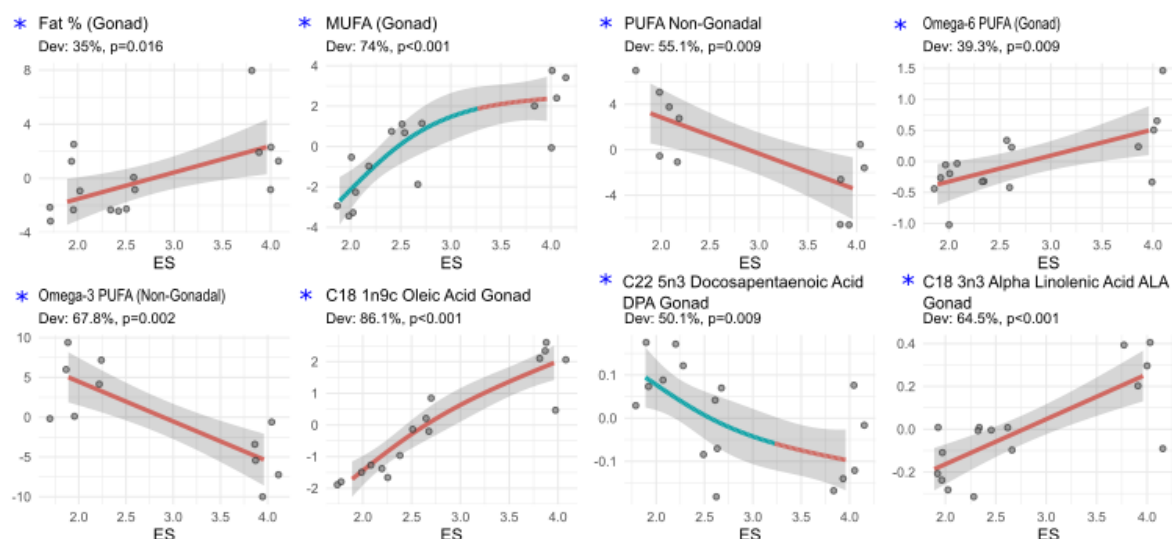
Figure 8: Partial dependence plots from generalised additive models showing the relationship between Enrichment Stage (ES) and variables with significant responses from the field experiments, for (A) horse mussel and (B) brachiopods. Lines represent fitted smooths, with blue shading indicating areas of significant change based on the first derivative of the smooth. The y-axis is unitless and reflects the contribution of the smooth term to the linear predictor. Variables are from analysis types: condition (red asterisks), fatty acids (blue asterisks) and transcriptomics (orange asterisks).

4. DISCUSSION

The key objectives of this study were to assess the relationships between physiological response variables and organic enrichment in the form of ES, identify thresholds associated with physiological change, assess which physiological response variables show potential as indicators for future research and ecosystem health monitoring, and develop a framework for refining and applying these indicators in environmental assessment contexts. This work aims to bridge the gap between organism-level physiological response and ecosystem-level indicators, ultimately enhancing the ecological relevance and predictive utility of enrichment monitoring tools.

Detecting early signs of stress in ecologically important and potentially sensitive taxa is critical for the design of effective monitoring programmes (Foley et al. 2015). Early identification of sub-lethal effects can enable timely management interventions, helping to maintain ecosystem function and resilience.

4.1 Thresholds

Thresholds were calculated to identify departures from normal physiological functioning (Monaco & Helmuth, 2011). In both linear and non-linear models, these thresholds represent points at which response values move beyond baseline variation, signalling the onset of physiological stress that may affect organismal health and survival (Harley et al. 2017).

Threshold values were relatively consistent across a range of physiological indicators in both the laboratory and field datasets (see Section 3.2), typically clustering around similar ES values (Figures 7 & 8, Tables 8 & 10). This convergence suggests the presence of a shared tipping point at which organic enrichment begins to elicit marked physiological responses (Munson et al. 2018). The alignment of thresholds across diverse indicators, including molecular, metabolic and condition-based measures, suggests a coordinated biological response rather than isolated or artefactual effects, strengthening confidence in the ecological relevance of the identified thresholds.

The emergence of consistent thresholds highlights their potential utility as early-warning indicators in environmental monitoring programmes (Foley et al. 2015). Once ES values approach these critical values, physiological stress responses are likely to be underway. Detecting such responses at the individual level can enable proactive management, potentially averting declines in population-level indicators such as survivorship or density.

Thresholds identified in the field study (median ES = 2.78) were generally lower than those from the laboratory trial (median ES = 3.49). This discrepancy may reflect the influence of additional and more variable stressors in natural environments, such as fluctuations in temperature, dissolved oxygen, sedimentation and biotic interactions, which lead to an earlier onset of stress responses (Carrier-Belleau et al. 2023). Furthermore, a gap in field data coverage between ES ~2.5 and ~4 may have influenced this pattern. Threshold detection is sensitive to the distribution of data across the enrichment gradient; limited observations in this critical mid-range may have caused thresholds to cluster towards the lower end of the observed ES spectrum, potentially underestimating the true point of departure.

Further research is needed to examine how thresholds vary across seasons, sites, species and exposure durations. Future studies should prioritise comprehensive sampling across the full

enrichment gradient, particularly within transitional zones, to improve the precision of threshold estimates and better inform adaptive management strategies (Foley et al. 2015).

4.2   What makes a good bioindicator?

Selecting appropriate bioindicators is critical for effective environmental monitoring and impact assessment (Holt & Miller, 2011; Moraitis et al. 2018), particularly in relation to organic enrichment. A robust bioindicator should possess the following key characteristics to ensure scientific reliability and practical utility in assessing ecosystem health:

- **Strong and predictable relationship to enrichment metrics**
  An effective bioindicator exhibits a clear and statistically supported relationship with ES or other relevant environmental predictors. This allows for reliable interpretation of ecological condition and potential stressor effects.

- **Specificity to the target stressor**
  Indicators should be primarily responsive to organic enrichment, rather than confounded by unrelated environmental variables. High specificity ensures that observed changes reflect the stressor of interest, supporting accurate attribution and management decisions.

- **Consistency across species and environments**
  Indicators that demonstrate consistent responses across multiple species and locations are more likely to reflect system-wide processes. Such consistency supports broader applicability and enhances integration into regional or national monitoring frameworks.

- **Feasibility and cost-effectiveness**
  Practical indicators are those that can be measured effectively, with minimal sample processing and without requiring large numbers of replications. While some approaches (e.g. molecular markers) may currently be resource-intensive, they should show potential for standardisation and streamlining to reduce cost and improve throughput over time.

- **Ecological relevance and proxy potential**
  Ideal indicators are not only sensitive to change but also ecologically meaningful. They provide insight into underlying biological processes, such a metabolic function, reproductive status or resilience, and can serve as proxies for broader ecosystem condition.

4.3   Promising indicators for future development

In this study, several physiological and biochemical indicators demonstrated consistent responses across species and between laboratory and field experiments, suggesting their potential as robust indicators of enrichment-related stress at the ecosystem level.

In the laboratory trial, respiration rate (VO_2) increased consistently with ES across all species, indicating elevated metabolic demand under increasing organic enrichment. However, measuring respiration rates *in situ* is challenging due to logistics, costs and the time-consuming nature of the approach (Cheng et al. 2025). Therefore, while respiration is well suited to initial assessments of physiological responses to a stressor/s, it is unlikely to become a widely adopted tool for environmental health assessments.

Fatty acid composition also showed strong and consistent responses. In the laboratory trial, oleic acid levels in gonad tissues increased with ES, while shifts in fatty acid profiles, such as declining omega-3 and increasing omega-6 fatty acids, suggested altered energy storage or diet quality under stress (Pilecky et al. 2021; Simopoulos & Cleland, 2003).

In the field trial, total fatty acid content and monounsaturated fatty acid (MUFA) levels in gonad tissues increased with ES across the two species studied. Trends in oleic acid closely mirrored those observed in the laboratory trial, reinforcing the consistency of fatty acid responses across systems. These findings highlight fatty acid profiles, particularly oleic acid, as promising integrative indicators of nutritional and physiological stress associated with organic enrichment.

Transcriptomic data were available only for horse mussels, but the gene *SLC5a6* (sodium-dependent multivitamin transporter) showed similar enrichment-related trends in both the laboratory and field datasets, suggesting its potential as a molecular biomarker (Dutt Vadlapudi et al. 2012). The stress-response gene *GADD45* also responded to enrichment, although only at high ES levels, indicating its possible utility in detecting more advanced or severe stress states (Grassi et al. 2017).

Together, these indicators demonstrate strong potential for application in environmental monitoring due to their consistency across species and systems and their ecological relevance. However, further validation across a wide range of taxa, environmental conditions and enrichment levels is needed to confirm their robustness and support their integration into monitoring and management frameworks.

4.4   Implications for planned offshore aquaculture operations in Aotearoa New Zealand

The physiological thresholds identified in this study overlap with the upper range of modelled ES values predicted for proposed and planned OOA sites in Aotearoa New Zealand (Table 6; Elvines et al. 2021b). This indicates that sensitive epifaunal species located in areas of highest predicted depositional flux may be at risk of sub-lethal physiological impacts. However, these zones are generally characterised by low-density, soft sediment epifaunal communities rather than biogenic habitats of high ecological value. As a result, while localised exceedance of physiological thresholds may occur, the broader ecological risk is likely to be reduced by the spatial separation of high-enrichment zones from more sensitive habitat types, achieved through careful site selection and farm layout.

It is also important to note that the highest modelled ES values are based on peak production scenarios, which may not reflect actual exposure conditions. In practice, feed volumes fluctuate throughout production cycles, typically increasing as fish grow and tapering off towards harvest. While peak scenarios are useful for precautionary planning, they often overestimate seabed exposure, particularly in offshore environments where physical processes such as sediment resuspension and dispersion further reduce organic accumulation (Keeley, 2020; Elvines et al. 2021b).

Further refinement of depositional modelling and *in situ* validation will be critical for assessing the long-term impact of OOA on benthic ecosystems. Nonetheless, these findings highlight the importance of incorporating physiological indicators into monitoring frameworks and support the continued development of such models to inform consent

conditions and define appropriate buffer zones for future OOA developments in Aotearoa
New Zealand.

Table 6: Depositional flux (DF) and Enrichment Stage (ES) values for Blue Endeavour under a maximum permissible periodic discharge scenario, and physiologically important thresholds defined through laboratory and field experiments. IQR = interquartile range.

Site	DF (kg m^{-2} yr^{-1})	ES relating to DF
Blue Endeavour	0–4.6	< 2–3.87
ES thresholds – lab trial	Median DF = 2.62; IQR: 2.26–3.30	Median ES = 3.49; IQR: 3.40–3.64
ES thresholds – field trial	Median DF = 2.78; IQR: 0.18–1.80	Median ES = 2.78; IQR: 2.37–3.27

4.5 Limitations and future work

Experimental limitations

This study provides valuable insights into the physiological responses of benthic invertebrates to organic enrichment; however, several limitations should be acknowledged. First, the results are specific to selected benthic species and may not be generalisable to other functional groups, life stages (Leung & McAfee, 2020) or habitat types (Dubuc et al. 2021). The field component was also limited to a single site, which may not capture the full range of environmental variability typical of high-flow open ocean systems.

Temporal scale represents another constraint. The experimental period of 3–5 months may not reflect the cumulative or chronic effects of long-term exposure over multiple production cycles (Keeley et al. 2015). Laboratory trials, while useful for isolating enrichment effects, did not account for the interaction of multiple co-occurring stressors (e.g. temperature fluctuations, hypoxia), which are important drivers of ecological response (Turschwell et al. 2022).

Moreover, the range of organic enrichment achieved in the field deployments did not fully overlap with that used in the laboratory, limiting the strength of direct comparisons between the two systems. Lastly, the absence of seasonal replication restricts our ability to assess how physiological responses may vary across different times of year, underscoring the importance of future studies that incorporate seasonal dynamics and interannual variability.

Future Research

While the species selected for this study are ecologically and culturally important[7], they do not represent the full range of functional groups or life history strategies that may be sensitive to organic enrichment. To improve the ecological relevance and applicability of the physiological indicators identified here, future research could assess their performance across a broader range of taxa. This includes expanding testing to multiple farm locations and sites spanning a wider enrichment gradient, to evaluate indicator robustness and generality under different environmental conditions (Keeley et al. 2012, 2018).

There is also a need to develop simplified, cost-effective methodologies, such as rapid assays for fatty acid profiles or gene expression markers, to support wider implementation in field-

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[7] See the Objective 1 report (McMullin & McGrath, 2023) for an in-depth examination of species likely to be present in OOA developments in Aotearoa New Zealand, along with their cultural importance, functional roles and known sensitivity to organic enrichment.

based monitoring programmes. Investigating indicator responses under multiple, interacting stressors (e.g. enrichment, temperature, hypoxia) will further improve ecological interpretation and help assess the indicators' utility in wider environmental contexts beyond aquaculture, such as seabed mining or dredging operations (Turschwell et al. 2022; Nodder et al. 2025).

Once key indicators, or a composite ecosystem health index, are sufficiently validated, the next step would involve engagement with regulatory agencies and industry partners to support integration into environmental monitoring frameworks. This includes the development of trigger values and guidelines for operational or consent-related decision-making.

Case study: transcriptomics

Benthic habitat data used in site scoping and farm operations are typically collected via seabed video surveys. While useful for broad habitat classification, this method often lacks the resolution needed to assess organism health or detect sub-lethal stress effects associated with aquaculture activities. Results from Objective 3 highlight the potential of physiological response variables, including molecular biomarkers, to serve as early-warning indicators of sub-lethal stress. However, further development would be required to refine and implement response indicators for application in open ocean environmental assessments.

Figure 9 outlines a potential workflow for integrating gene expression markers into routine ecological assessments. The development of targeted digital droplet PCD (ddPCR) assays for candidate stress-response genes offers a scalable and cost-effective method for quantifying molecular responses. This approach supports rapid, high-throughput analysis compared to broader transcriptomic methods.

A transcriptomics stress index, which integrates expression data from multiple genes into a composite score, can provide a standardised and interpretable measure of organismal stress (Cecchetto et al. 2023). This index could be incorporated into environmental monitoring frameworks by linking it with other indicators such as the Bacterial Metabarcoding Biotic Index (b-MBI), allowing for threshold-setting and integration into consent conditions or adaptive management strategies.

The approach demonstrated in Objective 3 could be adapted to establish relationships between transcriptomic responses and broader ecological indicators, enabling the identification of threshold values and supporting a more responsive and biologically meaningful monitoring strategy in offshore aquaculture contexts.
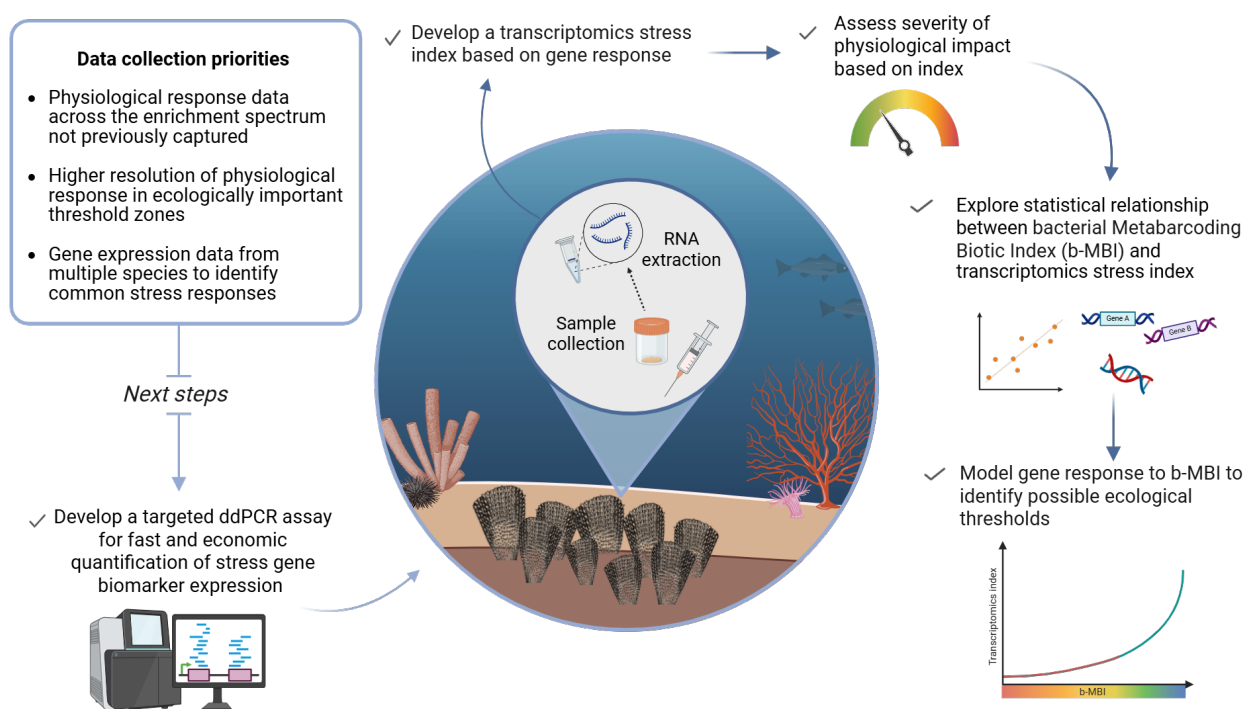
Figure 9: Example of a theoretical pipeline for ecological assessments using gene expression (Created in BioRender. Cawthron Institute (2025) https://BioRender.com/l52w638).

5. CONCLUSIONS

- A range of relationships were observed between ES and physiological response variables, with several showing consistent patterns across species and experimental systems.
- Threshold ES values were identified for multiple physiological response variables. While generally consistent across species and indicators, these thresholds varied between laboratory and field systems.
- Collinearity analysis revealed strong correlations among many physiological response variables, supporting the use of a reduced, representative sub-set as indicators of broader physiological change.
- Baseline data captured the physiological range of key species in the absence of enrichment stress, providing valuable reference points for future impact assessments and addressing critical knowledge gaps for these ecologically and culturally important taxa.
- Several indicators, such as fatty acid profiles and gene expression markers, show promise for further development, but additional data across a broader range of species and contexts are needed to confirm their robustness and general applicability.
- This research represents an important step in linking organism-level physiological response to ecosystem-scale indicators. It contributes to the development of monitoring tools with strong ecological relevance and predictive power. However, further refinement and validation are necessary before a reliable, widely applicable ecosystem health index can be established.

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APPENDIX 1
Experimental design for laboratory and field trials

Animals were collected from the Marlborough Sounds during April and May 2023 and were acclimated at the Cawthron Aquaculture Park for at least 3 weeks (24 days for *Pecten novaezelandiae* and *Neothyris lenticularis*, and 56 days for *Atrina zelandica*) in tanks with aerated, flow-through seawater and continuous, drip-fed algae. Four treatments were used in the experiments (Figure 10): an algae-only control treatment, and three different concentrations of salmon faeces (organic enrichment), with the same algae supply as for the control – low, moderate and high. Depositional flux (kg m^{-2} yr^{-1}) for levels of the factor 'Treatment' were: control = 0, low = 2.81, moderate = 18.48, high = 40.85 (Figure 10).
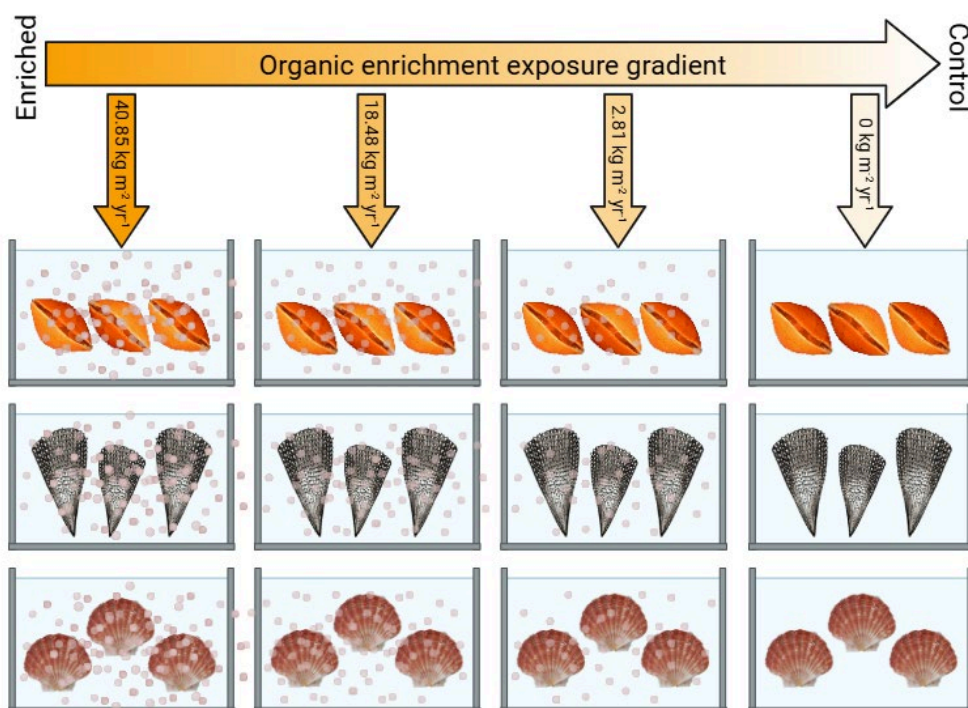
Figure 10: Set-up for laboratory enrichment trials, where all treatments received algae and the low, moderate and high treatments received a dose of salmon faeces equivalent to 2.81, 18.48 and 40.85 kg m^{-2} yr^{-1}, respectively.

The field trial involved translocating *A. zelandica* and *N. lenticularis* individuals[8] along an organic enrichment gradient adjacent to an operational salmon farm in a high-current area of the Marlborough Sounds from December 2023 to April 2024. The selected site, Clay Point (Figure 11A), experiences relatively strong mid-water currents (average 19.6 cm s^{-1}), making it a reasonably suitable analogue for proposed OOA sites in Aotearoa New Zealand, where average mid-water current speeds range between 38 cm s^{-1} and 44 cm s^{-1} (Newcombe et al. 2020; Bennett et al. 2022). *Atrina zelandica* and *N. lenticularis* individuals were mounted on frames built from concrete reinforcing bars (Figure 11B). Four translocation sites were selected: immediately adjacent to the farm pens (Pen station), 300 m to the east of the farm cage (300 m E station), and two control sites (Tory Channel Control – 1, Ctl-1; and Tory

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[8] *Pecten novaezelandiae* individuals were not included in the field trial as they had the highest mortality rates in the laboratory trials.

Channel Control – 3, Ctl-3). The depth at each site was approximately 30 m, and all were over soft sediment habitats with low densities of epifauna.
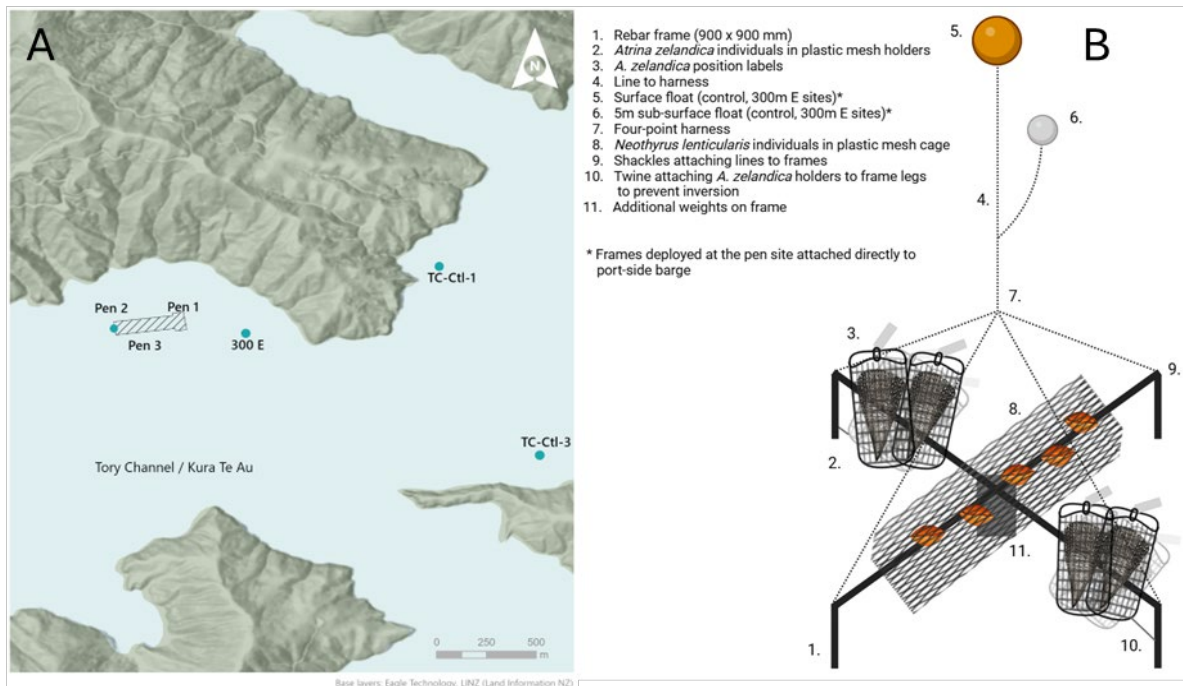
Figure 11: (A) Map of deployment sites for the field trial, carried out in Tory Channel / Kura Te Au in the Marlborough Sounds, Aotearoa New Zealand. The shaded polygon marks the salmon farm's location, while blue dots indicate frame deployment sites. (B) Frame design with representative illustrations and labels. Three replicate frames were deployed at each of the four study sites.

For the laboratory experiments, tissue samples were collected at three time points:

1. **Field baseline** – at the time of field collections to establish natural conditions.
2. **Post-acclimation** – immediately following the acclimation period to assess potential laboratory effects.
3. **Post-experiment** – immediately following the experimental period to evaluate the effects of organic enrichment.

For the translocation experiments, tissue samples were collected at initial collection (field baseline), and at retrieval following the deployment period. All sampling was destructive and tissue types varied according to species and response indicator (see McMullin et al. (2025) for further detail).

A range of molecular, histological, biochemical and whole-organism-level approaches were selected to assess the response and / or tolerance of key species to salmon farm enrichment under laboratory and field conditions. Measurements taken included mortality, condition, respiration, fatty acid profiles, gene expression, histological assessments and oxidative stress (see McMullin et al. (2025) for detailed indicator descriptions and methodology).

APPENDIX 2

Results from generalised additive models (GAMs), and Enrichment Stage (ES) thresholds derived from linear and non-linear GAMs

Table 7: Summary of generalised additive model (GAM) results for each species and variable from the laboratory experiment. The table includes the *p*-value for the smooth term, deviance explained (%), estimated degrees of freedom (edf), and an indicator of whether the relationship was statistically significant (*p* < 0.05).

Species	Variable	*p*-value	dev_explain	edf
brachiopod	vo2_per_g_12wks	p<0.001	84.9	1.9
brachiopod	c18_1n9c_oleic_acid_gonad	0.007	71.4	1.7
brachiopod	omega_3_gonad	0.046	37.4	1.0
brachiopod	mda_nmoles_mg_of_protein_r1	0.077	28.7	1.7
brachiopod	vo2_per_g_6wks	0.098	19.7	1.0
brachiopod	mufa_non_gonadal	0.098	46.5	1.6
brachiopod	c18_3n3_alpha_linolenic_acid_ala_gonad	0.142	22.3	1.0
brachiopod	pufa_gonad	0.236	15.2	1.0
brachiopod	mda_nmoles_mg_of_protein_r2	0.260	7.0	1.0
brachiopod	mean_teac	0.281	44.0	1.7
brachiopod	sfa_gonad	0.334	10.4	1.0
brachiopod	condition_score	0.346	1.4	1.0
brachiopod	mufa_gonad	0.468	22.2	1.6
brachiopod	percent_fat_gonad	0.480	19.0	1.4
brachiopod	omega_6_non_gonadal	0.633	2.6	1.0
brachiopod	c20_0_arachidic_acid_non_gonadal	0.760	1.1	1.0
brachiopod	pufa_non_gonadal	0.773	1.0	1.0
brachiopod	omega_6_gonad	0.913	0.1	1.0
brachiopod	omega_3_non_gonadal	0.933	0.1	1.0
horse_mussel	vo2_per_g_6wks	p<0.001	55.6	1.9
horse_mussel	vo2_per_g_12wks	p<0.001	59.8	1.9
horse_mussel	copper_transporter_slc31a1	p<0.001	64.3	1.8
horse_mussel	fatty_acid_synthase_fas	p<0.001	59.7	1.7
horse_mussel	omega_6_dg	p<0.001	76.4	1.0
horse_mussel	donson_protein_downstream_neighbour_of_son	p<0.001	47.6	1.0
horse_mussel	sodium_calcium_exchanger_slc8a	0.001	42.6	1.0
horse_mussel	growth_arrest_and_dna_damage_inducible_protein_g	0.001	53.0	1.7
horse_mussel	sodium_dependent_multivitamin_transporter_slc5a6	0.002	48.3	1.8
horse_mussel	glutathione_reductase	0.002	45.3	1.4
horse_mussel	pufa_dg	0.002	62.6	1.0
horse_mussel	c18_1n9c_oleic_acid_gonad	0.008	67.0	1.8
horse_mussel	condition_score	0.011	10.2	1.0
horse_mussel	pcna_proliferating_cell_nuclear_antigen	0.013	37.2	1.8
horse_mussel	hspa5_bi_p	0.014	34.8	1.4
horse_mussel	cathepsin_d	0.040	28.5	1.5
horse_mussel	omega_3_dg	0.059	31.3	1.0
horse_mussel	percent_fat_dg	0.083	27.0	1.0
horse_mussel	c18_1n9c_oleic_acid_dg	0.087	26.4	1.0
horse_mussel	mufa_gonad	0.203	33.4	1.6
horse_mussel	sfa_gonad	0.238	13.6	1.0
horse_mussel	c18_3n3_alpha_linolenic_acid_ala_gonad	0.271	11.9	1.0
horse_mussel	c18_1n9c_oleic_acid_mantle	0.279	39.0	1.7
horse_mussel	pufa_gonad	0.301	10.6	1.0
horse_mussel	omega_3_gonad	0.395	7.3	1.0
horse_mussel	omega_6_gonad	0.419	6.6	1.0
horse_mussel	coup_transcription_factor_1	0.711	0.7	1.0
horse_mussel	percent_fat_gonad	0.720	1.3	1.0
horse_mussel	mda_nmoles_mg_of_protein_r1	0.862	0.1	1.0
horse_mussel	mda_nmoles_mg_of_protein_r2	0.874	2.0	1.1

Species	Variable	*p*-value	dev_explain	edf
horse_mussel	mean_teac	0.911	0.1	1.0
horse_mussel	sfa_dg	0.980	0.3	1.0
scallop	vo2_per_g_6wks	p<0.001	83.8	1.9
scallop	vo2_per_g_12wks	0.050	33.3	1.0
scallop	c18_1n9c_oleic_acid_dg	0.061	56.9	1.4
scallop	pufa_gonad	0.106	32.9	1.0
scallop	c18_1n9c_oleic_acid_gonad	0.117	31.3	1.0
scallop	omega_3_gonad	0.120	30.9	1.0
scallop	omega_6_gonad	0.124	53.0	1.8
scallop	percent_fat_dg	0.216	26.9	1.1
scallop	percent_fat_gonad	0.216	20.9	1.0
scallop	c18_3n3_alpha_linolenic_acid_ala_gonad	0.228	20.0	1.0
scallop	sfa_gonad	0.265	17.4	1.0
scallop	omega_3_dg	0.438	8.8	1.0
scallop	pufa_dg	0.497	6.8	1.0
scallop	mufa_gonad	0.537	5.7	1.0
scallop	mean_teac	0.593	8.3	1.5
scallop	mda_nmoles_mg_of_protein_r1	0.634	1.0	1.0
scallop	c18_1n9c_oleic_acid_mantle	0.648	3.2	1.0
scallop	omega_6_dg	0.814	10.4	1.3
scallop	sfa_dg	0.844	0.6	1.0
scallop	condition_score	0.845	0.1	1.0

Table 8: Threshold Enrichment Stage (ES) values identified from significant generalised additive models (GAMs) for each species and variable from the laboratory experiment. For non-linear relationships (model type = 'non_linear'), thresholds represent the lowest ES value where a significant change in the derivative occurs. For linear relationships (model type = 'linear'), thresholds are defined as the ES value where the predicted response crosses the extreme (minimum or maximum) of the baseline range (ES = 1.88), depending on the direction of the relationship.

Species	Variable	threshold_es	model_type
brachiopod	c22_5n3_docosapentaenoic_acid_dpa_gonad	3.6	linear
brachiopod	omega_3_gonad	3.8	linear
horse_mussel	condition_score	3.7	linear
horse_mussel	donson_protein_downstream_neighbour_of_son	3.0	linear
horse_mussel	omega_6_dg	2.7	linear
horse_mussel	pufa_dg	3.0	linear
horse_mussel	sodium_calcium_exchanger_slc8a	3.1	linear
scallop	vo2_per_g_12wks	4.1	linear
brachiopod	c18_1n9c_oleic_acid_gonad	3.6	non_linear
brachiopod	vo2_per_g_12wks	3.6	non_linear
horse_mussel	c18_1n9c_oleic_acid_gonad	3.7	non_linear
horse_mussel	cathepsin_d	3.8	non_linear
horse_mussel	copper_transporter_slc31a1	3.5	non_linear
horse_mussel	fatty_acid_synthase_fas	3.4	non_linear
horse_mussel	glutathione_reductase	3.1	non_linear
horse_mussel	growth_arrest_and_dna_damage_inducible_protein_ga	3.5	non_linear
horse_mussel	hspa5_bi_p	3.5	non_linear
horse_mussel	pcna_proliferating_cell_nuclear_antigen	4.1	non_linear
horse_mussel	sodium_dependent_multivitamin_transporter_slc5a6	3.7	non_linear
horse_mussel	sodium_hydrogen_exchanger_slc9a10	3.0	non_linear
horse_mussel	vo2_per_g_12wks	3.4	non_linear
horse_mussel	vo2_per_g_6wks	3.3	non_linear
scallop	vo2_per_g_6wks	3.6	non_linear

Table 9: Summary of generalised additive model (GAM) results for each species and variable from the field experiment. The table includes the *p*-value for the smooth term, deviance explained (%), estimated degrees of freedom (edf), and an indicator of whether the relationship was statistically significant (*p* < 0.05).

Species	Variable	*p*-value	dev_expl	edf
Brachiopod	c18_1n9c_oleic_acid_gonad	p<0.001	86.1	1.6
Brachiopod	c18_3n3_alpha_linolenic_acid_ala_gonad	p<0.001	64.5	1.0
Brachiopod	sum_of_mufa_gonad	p<0.001	74.0	1.8
Brachiopod	sum_of_n_3_pufa_non_gonadal	0.002	67.8	1.0
Brachiopod	sum_of_pufa_non_gonadal	0.009	55.1	1.0
Brachiopod	c22_5n3_docosapentaenoic_acid_dpa_gonad	0.009	50.1	1.5
Brachiopod	sum_of_n_6_pufa_gonad	0.009	39.3	1.0
Brachiopod	fat_percent_gonad	0.016	35.0	1.0
Brachiopod	fat_percent_non_gonadal	0.124	43.6	1.7
Brachiopod	sum_of_sfa_gonad	0.127	29.8	1.6
Brachiopod	sum_of_n_3_pufa_gonad	0.130	15.6	1.0
Brachiopod	c20_0_arachidic_acid_non_gonadal	0.182	18.8	1.0
Brachiopod	c20_4n6_arachidonic_acid_aa_gonad	0.380	5.5	1.0
Brachiopod	sum_of_pufa_gonad	0.431	4.5	1.0
Brachiopod	c18_0_stearic_acid_gonad	0.534	2.8	1.0
Brachiopod	dg_luminal_space_open	0.787	0.5	1.0
Horse mussel	c18_1n9c_oleic_acid_dg	p<0.001	94.5	1.1
Horse mussel	sum_of_n_6_pufa_dg	p<0.001	90.2	1.0
Horse mussel	sum_of_sfa_dg	p<0.001	91.9	1.7
Horse mussel	fat_percent_dg	p<0.001	77.3	1.8
Horse mussel	c18_1n9c_oleic_acid_gonad	p<0.001	50.2	1.0
Horse mussel	condition	0.002	26.6	1.0
Horse mussel	sum_of_n_6_pufa_gonad	0.004	45.7	1.2
Horse mussel	sum_of_mufa_gonad	0.004	39.0	1.0
Horse mussel	sodium_dependent_multivitamin_transporter_sl	0.006	29.5	1.0
Horse mussel	growth_arrest_and_dna_damage_inducible_prot	0.019	33.6	1.9
Horse mussel	copper_transporter_slc31a1	0.057	23.7	1.4
Horse mussel	sum_of_sfa_gonad	0.058	31.0	1.5
Horse mussel	fatty_acid_synthase_fas	0.060	15.9	1.0
Horse mussel	glutathione_reductase	0.064	14.8	1.0
Horse mussel	tubulin_alpha_tuba	0.092	12.4	1.0
Horse mussel	c18_3n3_alpha_linolenic_acid_ala_gonad	0.103	14.9	1.0
Horse mussel	fat_percent_gonad	0.108	27.0	1.7
Horse mussel	c18_0_stearic_acid_gonad	0.109	27.2	1.8
Horse mussel	sum_of_n_3_pufa_gonad	0.154	24.0	1.8
Horse mussel	pcna_proliferating_cell_nuclear_antigen	0.170	8.4	1.0
Horse mussel	sodium_hydrogen_exchanger_slc9a10	0.185	7.8	1.0
Horse mussel	sum_of_pufa_dg	0.201	14.6	1.1
Horse mussel	cathepsin_d	0.203	7.3	1.0
Horse mussel	sum_of_pufa_gonad	0.271	18.4	1.7
Horse mussel	coup_transcription_factor_1	0.327	12.9	1.7
Horse mussel	gut_apx	0.433	2.4	1.0
Horse mussel	hspa5_bi_p	0.549	1.7	1.0
Horse mussel	c20_4n6_arachidonic_acid_aa_gonad	0.643	8.3	1.5
Horse mussel	cytochrome_p450_cyp_family_1_subfamily_a1	0.792	0.3	1.0
Horse mussel	c22_5n3_docosapentaenoic_acid_dpa_gonad	0.915	0.1	1.0
Horse mussel	donson_protein_downstream_neighbour_of_son	0.999	0.0	1.0

Table 10: Threshold Enrichment Stage (ES) values identified from significant generalised additive models (GAMs) for each species and variable from the field experiment. For non-linear relationships (model type = 'non_linear'), thresholds represent the lowest ES value where a significant change in the derivative occurs. For linear relationships (model type = 'linear'), thresholds are defined as the ES value where the predicted response crosses the extreme (minimum or maximum) of the baseline range (ES = 1.89–2.12), depending on the direction of the relationship.

Species	Variable	threshold_es	model_type
brachiopod	c18_3n3_alpha_linolenic_acid_ala_gonad	2.5	linear
brachiopod	fat_percent_gonad	2.8	linear
brachiopod	sum_of_n_3_pufa_non_gonadal	2.6	linear
brachiopod	sum_of_n_6_pufa_gonad	2.7	linear
brachiopod	sum_of_pufa_non_gonadal	2.7	linear
horse mussel	c18_1n9c_oleic_acid_gonad	2.5	linear
horse mussel	condition	2.6	linear
horse mussel	sodium_dependent_multivitamin_transporter_slc5a6	2.6	linear
horse mussel	sum_of_mufa_gonad	2.6	linear
horse mussel	sum_of_n_6_pufa_dg	2.3	linear
brachiopod	c18_1n9c_oleic_acid_gonad	2.3	non-linear
brachiopod	c22_5n3_docosapentaenoic_acid_dpa_gonad	2.4	non-linear
brachiopod	sum_of_mufa_gonad	2.3	non-linear
horse mussel	c18_1n9c_oleic_acid_dg	2.2	non-linear
horse mussel	fat_percent_dg	2.6	non-linear
horse mussel	growth_arrest_and_dna_damage_inducible_protein_gadd	3.6	non-linear
horse mussel	sum_of_n_6_pufa_gonad	2.5	non-linear
horse mussel	sum_of_sfa_dg	2.2	non-linear