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# ASSESSING CANOPY METRICS AS A NON-DESTRUCTIVE PROXY FOR BLUE CARBON ESTIMATION IN *ZOSTERA MARINA*

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## Abstract

*Seagrass standing stock biomass can be used to estimate organic carbon when preparing blue carbon evaluations for anthropogenic climate change mitigation potential and seagrass conservation priorities. Current sampling techniques destroy sampled plants and disturb fragile seagrass meadows. This study aimed to determine whether simple seagrass canopy metrics, capable of non-destructive measurement, were robust predictors of biomass in Jersey's *Zostera marina* seagrass meadows. Significant differences in mean biomass between two study sites 2km apart were observed. Older seagrass had a higher mean biomass in one site but a lower mean biomass in the other. Overall, the use of simple canopy metrics to inform predictor models was not supported although the age of seagrass was an important influential factor. *Z. marina* meadows in Jersey are subjected to significant environmental, ecological and anthropogenic disturbances, differing within meadow sites, which were not accounted for in this study. Jersey has committed to conserving and extending seagrass standing stock and this study concludes that non-destructive sampling techniques are consistent with this objective albeit recognising site- or meadow-specific calibrations will be required in future study.*

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## Introduction

Seagrass has long been prominent in research into mitigation of anthropogenic climate change. Seagrass stores carbon in its above-ground shoots and leaves and below-ground in its roots and rhizomes and seagrass biomass calculations underpin organic carbon storage estimates (Fourqurean *et al.*, 2012). Duarte *et al.* (2013) considered seagrass meadows amongst the most intense carbon sinks on Earth. Seagrass is gaining international recognition as a nature-based solution contributing to climate change mitigation and adaptation targets (Macreadie *et al.*, 2021). Seagrass also has many co-benefits including mitigating against sea level rise by raising the sea floor (Duarte *et al.*, 2013), improving water quality (Zhang *et al.*, 2023), and serving as permanent homes, nurseries, feeding grounds, and temporary shelters for various species (Jackson *et al.*, 2001).

The Island of Jersey is in the Normano-Breton Gulf, a shallow tidal area in the southwestern English Channel. Jersey's territorial waters extend to several offshore reefs and are used extensively for commercial fishing and recreation and have the third largest tidal range in the world (Government of Jersey *n.d.*). Two species of seagrass are found in Jersey, *Zostera marina* and *Zostera noltei*. Despite belonging to the same genus, they occupy different ecological niches and fulfil distinct roles within Jersey's coastal ecosystem (Chambers *et al.*, 2022).

This study considers *Z. marina*, a perennial marine angiosperm with worldwide distribution growing in intertidal and subtidal zones (ScienceDirect Topics, *n.d.*) and concentrated on Jersey's south coast from St Catherine's Breakwater along the east coast to Icho Tower

(Chamber *et al.*, 2022). The sites surveyed for this study are within this area. Davies *et al.* (2024) determined that seagrass habitat had the highest densities of carbon (both inorganic and organic) in Jersey's territorial waters.

Globally seagrass faces several challenges, Unsworth *et al.* (2019) considered lack of recognition of their importance as a coastal system as one of six main challenges. Therefore, quantifying the blue carbon contribution of seagrass is likely to ensure prioritisation for conservation. Jersey's Carbon Neutral Roadmap (*Government of Jersey*, 2022) recognises blue carbon's potential to absorb greenhouse gases and enhance biodiversity, with plans to expand seagrass meadows as part of its net-zero strategy.

Dunn (2024), in a comprehensive review of seagrass meadows in Jersey, highlighted several knowledge gaps in the assessment of seagrass organic carbon stock. These include inconsistent, non-standardised, mapping techniques (mainly through diver surveys and aerial imagery analysis) and failure to capture the full extent of seagrass especially in deeper or isolated meadows or where access is difficult (e.g. Les Minquiers and Les Écréhous reefs). Dunn (2024) noted that measurement studies to date provide only a starting point and further, long-term, study is required.

Previous studies in St Catherine's Bay (a site included in this study) have collected *Z. marina* samples using grabs (Davies *et al.*, 2024) or manually by scuba divers (Blampied, 2021). Both are destructive, removing living plants and creating disturbance to *Z. marina* meadows which are known to be sensitive to mechanical disturbance (Roca *et al.*, 2016). A predictive model to estimate biomass in seagrass meadows that does not require a destructive sampling method (e.g. underwater drones equipped with image capturing equipment analysed using AI) could offer a consistent, scalable and repeatable sampling basis across all seagrass meadows in Jersey. This study considers whether visible, measurable, seagrass canopy metrics (total blade length and total blade number) are a robust predictor of seagrass total plant biomass (above and below ground) which could be used to address current sampling limitations and knowledge gaps in the assessment of blue carbon in Jersey.

## **Materials and methods**

### *Study site*

Surveys were carried out between 31 July and 10 August 2021 in four seagrass meadow at two Locations: St Catherine's Bay ("St Catherines") and Anne Port, Jersey Channel Islands. One Old (>25 years) and one Young (4-9 years) meadow was surveyed at each Location (see Figure 1 and Table 1) as part of a postgraduate investigation (Blampied, 2021). St Catherines is considered to have a high potential for carbon storage due to favourable seagrass growing substrate and protection from strong wind and wave action by the 600m long breakwater to its north (Kuo, 2022) which also shelters a harbour in which seagrass loss has been recorded due to the use of block and chain moorings (*Island Research Repository*, 2023). Lying further south, Anne Port does not benefit from the breakwater although human activity and disturbance is lower (*Government of Jersey*, 2024). Both Locations have the most extensive seagrass meadows around Jersey's coast (*Government of Jersey*, 2024).

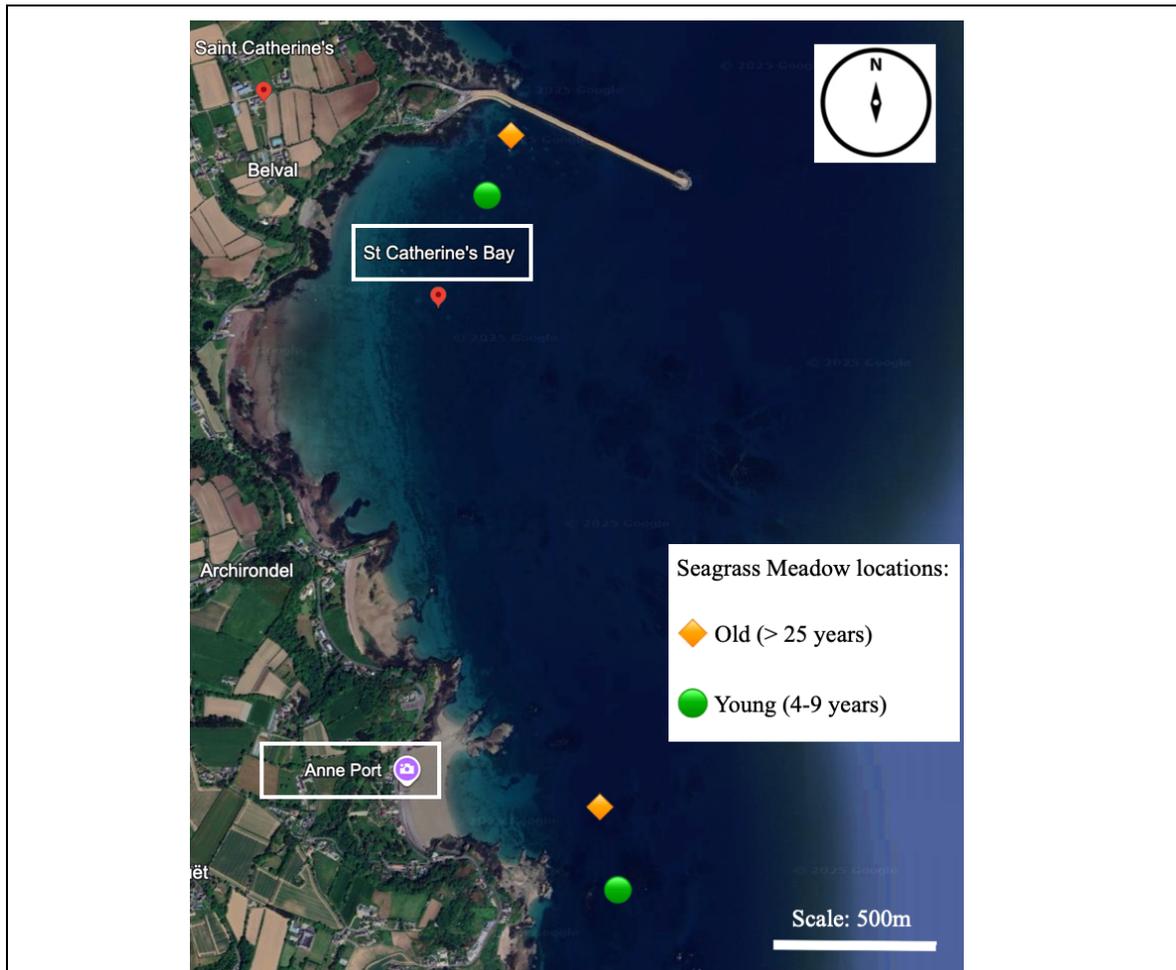


Figure 1. Approximate location of the four *Z. marina* meadows surveyed. Coloured shapes indicate Age as per legend. Exact meadow locations shown in Table Y. Map: Google Earth (2026), boxes, shapes, scale bar and legend added by student.

Table 1. Geographic coordinates for surveyed *Z. marina* meadows. Blampied, 2021.

	Age: Old	Age: Young
Location: St Catherines	49°13'23.3"N 2°01'09.2"W	49°13'15.6"N 2°01'15.6"W
Location: Anne Port	49°12'16.6"N 2°00'59.8"W	49°12'05.4"N 2°00'58.7"W

### Experimental design

Scuba-divers laid 30 m transects out at each meadow and a 0.5 m<sup>2</sup> quadrat was placed at pre-determined random intervals along each transect. Sampling effort varied among Age–Location combinations, resulting in unequal numbers of quadrats per group. Within each quadrat all seagrass within a smaller 0.25 m<sup>2</sup> quadrat was collected and placed into a labelled bag (“sample”) preserving as much root matter as possible. On land, samples were washed of residual sediment and individual blade number, and length were recorded for each sample. Each sample was oven-dried at ~80°C and dried biomass weighed.

## Statistical analysis

All analyses were undertaken using R (version 4.5.2) and “tidyverse” suite, “car”, “MuMIn” and “lme4” packages. A p-value of  $< 0.05$  was considered statistically significant. Data frames were created (variables shown in Table 2) from two raw data sources by attributing a unique quadrat identifier (Quad\_ID) to each data row, merging of datasets by Quad\_ID, and subsequent removal of duplicate or non-matching Quad\_IDs. All analyses were conducted at the quadrat (unit-area) scale and inferences refer to variation among quadrats rather than individual plants.

Table 2. Variable name, description, and data type used in this study.

Name	Full Name	Description	Data type
TOTB	Total Biomass	Dry plant weight in grams per Quad_ID	Response variable
TBL	Total Blade Length	Length in mm of all blades per Quad_ID	Predictor variable
TBN	Total Blade Number	Number of blades per Quad_ID	Predictor variable
Age	Old or Young	Age of <i>Z. marina</i> meadows sampled	Factor
Location	St Catherines or Anne Port	Location of <i>Z. marina</i> meadows sampled	Factor

### Comparison of TOTB by Age and Location

TOTB data (n=45) were first summarised across all observations to inspect overall variability using descriptive statistics, supported by box plots grouped by Age by Location to visualise the distribution around the median and potential outlier data points. Duarte and Chiscano (1999) found a general tendency for a balanced distribution between above and below ground biomass in seagrass justifying the use of TOTB in this study.

TOTB grouping by Age and Location was then introduced for formal hypothesis testing of mean differences. Normality assumptions were inspected visually using Q-Q plots and formally tested within groups using the Shapiro-Wilk test, the appropriate test as sample  $n < 50$  (Mishra et al., 2019) to inform transformation choice. Homogeneity of variances across groups was tested with Levene’s test. Independent two-tailed t-tests were used to examine the differences in mean  $\log(\text{TOTB})$  by Age and by Location. The null hypotheses for these tests were (1) “there was no significant difference in mean  $\log(\text{TOTB})$  between Young and Old seagrass meadows ( $p \geq 0.05$ )”; and (2) “there was no significant difference in mean  $\log(\text{TOTB})$  between St Catherines and Anne Port seagrass meadows ( $p \geq 0.05$ )”. The alternative hypotheses in both cases were that a significant difference in mean  $\log(\text{TOTB})$  was observed ( $p < 0.05$ ).

## Linear regression modelling of TOTB as a function of TBL or TBN

Linear regression modelling was used to address the primary study question. The null hypothesis was that canopy metrics (TBL or TBN) do not predict TOTB (i.e. slope ( $\beta$ ) = 0) once Age or Location is accounted for; the alternative hypothesis was that a significant relationship exists between canopy metrics and TOTB after accounting for Age or Location.

Merging TOTB, TBL, and TBN data by Quad\_ID generated a subset for modelling (“TOTB Subset”) with  $n=31$ . A similar study by Thomson, Veracaemer and Wong (2025) pooled *Z. marina* seagrass data across 9 sites along the Atlantic coast of Nova Scotia before undertaking regression analysis on observed canopy cover and seagrass biomass justifying the use of regression modelling using the combined TOTB Subset in this study. Descriptive statistics were examined on the TOTB Subset. Normality of the untransformed TOTB Subset was assessed, rather than inferred from the full TOTB dataset ( $n = 45$ ), to inform transformation choice using a Q-Q plot and the Shapiro-Wilk test.

Separate linear regression models were fitted using log-transformed TOTB Subset (“log(TOTB Subset)”) as the response variable and each canopy metric, including either (i) Age or (ii) Location as a fixed effect factor.

Akaike’s Information Criterion was used to compare models based on both goodness of fit and parsimony, penalising unnecessary model complexity. The AICc function in R was used as it includes a correction for small sample sizes ( $n=31$ ) and provides a more conservative assessment of model complexity (Burnham and Anderson, 2004). The model with the lowest AICc was considered the most parsimonious and models with  $\Delta AICc \leq 2$  were considered equally supported by the data.

For the most parsimonious fitted models: (i) model assumptions, including residuals normality and homoscedasticity, were evaluated visually using the four standard diagnostic plots generated in R and formally tested using the Shapiro-Wilk test and the Breusch-Pagan test for homoscedasticity of residuals variances (Breusch and Pagan, 1979); (ii) model coefficients, standard errors, and significance of fixed effects were evaluated using summary statistics; and (iii) model predictions were visually presented on the log scale.

## **Results**

### *Differences in TOTB by Age and Location*

The descriptive statistics (Table 3), supported by visual inspection of the box plots (Figure 2), indicate substantial within-group variability in TOTB within Age-Location combinations (in particular, Old-St Catherines). Mean and Median TOTB were higher for Old and Young seagrass at St Catherines whereas the opposite was observed at Anne Port. Two high-value observations were noted in the Old-St Catherines group (Figure 2); however, residuals versus leverage plots (Appendix 5) indicated all observations lay within Cook’s distance thresholds. These values therefore fall within the observed biological variability of TOTB and were retained for analysis.

Table 3. Descriptive statistics for TOTB showing substantial variability in each of the four Age-Location combinations. All results (other than n) in grams.

Location	Age	n	mean	sd	min	max	median	IQR
St Catherines	Old	16	41.00	21.28	16.19	96.74	31.60	16.55
	Young	9	12.40	7.38	3.35	24.54	13.13	11.15
Anne Port	Old	10	10.27	4.62	2.93	18.08	9.87	6.54
	Young	10	12.83	7.53	3.13	26.85	11.08	10.26

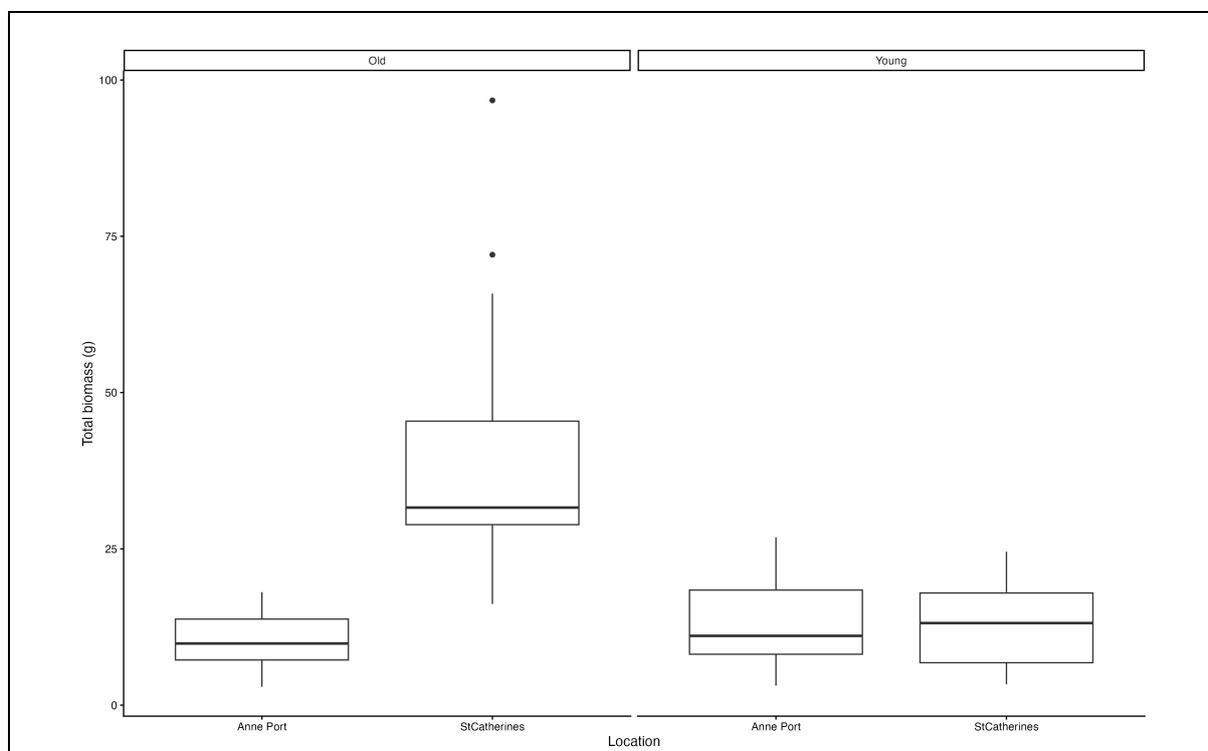


Figure 2. Distribution of TOTB per quadrat across Locations and Age classes. Boxes represent medians and interquartile ranges, with whiskers extending to  $1.5 \times IQR$ ; points indicate high-biomass quadrats. The figure illustrates contrasting Age-Location patterns and high within-group variability.

Inspection of Q-Q plots (see Appendix 1) suggested that TOTB data for St Catherines and Old groups were not normally distributed whereas data for Anne Port and Young groups were normally distributed. These observations were confirmed by the Shapiro-Wilk test (results in Appendix 2). Normality and homogeneity of variances assumptions were met for log-transformed TOTB (“log(TOTB)”) within and across all groups enabling use of parametric testing (see Appendix 2).

Mean log(TOTB) differed significantly between locations, with higher log(TOTB) values in St Catherines than Anne Port, and between age groups, with higher log(TOTB) values in Old compared to Young seagrass (see Table 4).

Table 4: *t*-test results for  $\log(\text{TOTB})$  as a function of Location and Age showing significant differences in means between both Location and Age groups. Positive mean differences shown in CI shows St Catherines and Old seagrass have higher  $\log(\text{TOTB})$  within respective groups.

Group	t-value	df	p	95% CI
Location	3.87	43	<0.01	0.41 to 1.31
Age	3.11	43	<0.01	0.26 to 1.20

*Linear regression results for TOTB Subset as a function of TBL or TBN*

Descriptive statistics (see Table 5) indicated substantial variability in the TOTB Subset data. Diagnostic plots of the untransformed TOTB Subset (see Appendix 3) indicated deviation from normality. A Shapiro–Wilk test on model residuals supported this observation ( $W = 0.85$ ,  $p < 0.01$ ).

Table 5. Descriptive statistics for TOTB Subset showing substantial variability. All results (other than  $n$ ) in grams.

Location	Age	n	mean	sd	min	max	median	IQR
All	All	31	27.32	21.30	3.13	96.74	25.23	18.37

Amongst competing models fitted with the  $\log(\text{TOTB Subset})$  data, AICc supported models with Age as a factor and both TBL (the most parsimonious) and TBN ( $\Delta\text{AICc} < 2$ ) as predictor variables (Appendix 4). Normality and homoscedasticity assumptions were met for both models (see Appendices 5 and 6) validating the use of linear regression models for inference on predictor relationships. However, the TBL-based model was promoted based on: (i) having the lowest AICc score and highest AICc weight; (ii) residual diagnostic tests and plots indicating better adherence to variance assumptions (i.e. no evidence of heteroscedasticity and more uniform residual dispersion); and (iii) having the simplest biological interpretation.

Visual inspection of Figure 3 indicated weak positive relationships between TBL and both Old and Young seagrass with Old seagrass. The estimated slope ( $\beta$ ) was the same for Old and Young seagrass (reflecting Age as a fixed effect in the model), with regression lines differing only in intercept. Across the observed range of TBL, Old seagrass exhibited higher  $\log$ -transformed total biomass than Young seagrass. These observations were supported by regression model outputs finding that TBL was not a statistically significant predictor of  $\log(\text{TOTB Subset})$  once Age was accounted for ( $\beta = 1.30 \times 10^{-4} \pm 1.16 \times 10^{-4} \text{ SE}$ ,  $p = 0.27$ ) whereas Age was a significant predictor with lower values in Young compared to Old seagrass ( $\beta = -1.02 \pm 0.27 \text{ SE}$ ,  $p < 0.01$ ). The Age-based regression model explained 44% (adjusted  $R^2 = 0.44$ ) of the variance in  $\log(\text{TOTB Subset})$ . Consequently, results are presented on the transformed scale in Figure 3 to avoid over-interpretation through back-transformation.

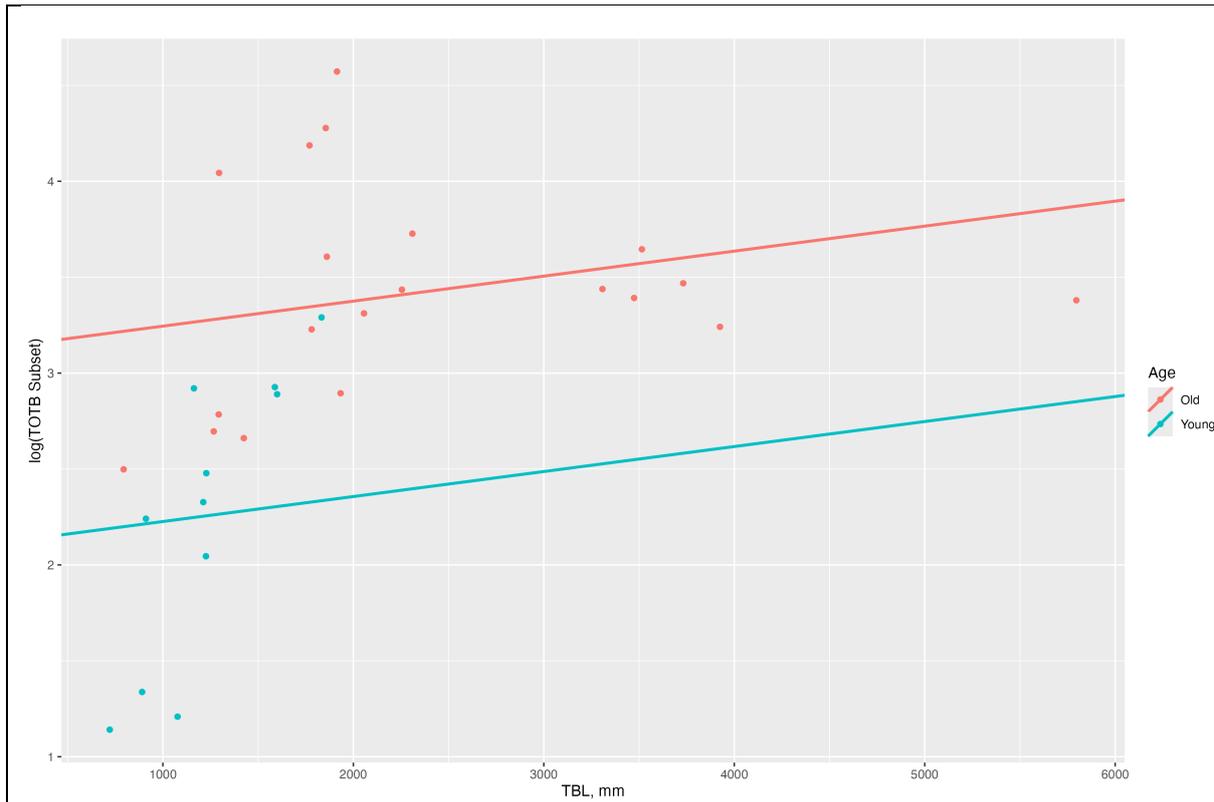


Figure 3. Scatterplot of  $\log(\text{TOTB Subset})$  against TBL for Old and Young seagrass. Points represent individual observations; lines show fitted linear regressions with parallel slopes and age-specific intercepts.

R code used for this study is included in Appendix 7.

## Discussion

### *Study Aim*

This study evaluated whether visible canopy metrics (total blade length (TBL) and total blade number (TBN)) could act as proxies for total *Zostera marina* plant biomass (TOTB) in Jersey to support the replacement of destructive sampling methods currently used to estimate seagrass biomass and, by extension, blue carbon standing stock.

### *Variability in Biomass Across Age and Location*

Substantial within-group variability in TOTB was observed across all Age–Location combinations, particularly within the Old seagrass meadows at St Catherines. This variability was evident in both the magnitude and spread of values and in the presence of mixed normal and non-normal distributions prior to transformation. Such patterns probably reflect a combination of small and unequal sample sizes, biological heterogeneity, and unmeasured environmental (including hydrological) influences. Although unequal sampling limited statistical power for subgroup inference, it was an unavoidable constraint when working with the legacy dataset provided.

Contrasting Age-specific patterns between Locations were apparent. At St Catherines, Old seagrass meadows exhibited the highest mean and median TOTB both in St Catherines and

compared to Anne Port. On the other hand, the Old seagrass meadows at Anne Port had lower biomass than the Young seagrass meadows at that Location. Although t-tests identified significant overall differences in mean log(TOTB) by Age and by Location, these opposing patterns indicate that the effect of meadow age on biomass is not consistent across sites. Rather than representing a uniform age effect, the results suggest that age-related biomass patterns are strongly context-dependent. This interpretation is supported by previous work in Jersey, which demonstrated that age effects on seagrass biomass differed in both magnitude and direction among locations (*Blampied, 2021*).

St Catherines benefits from shelter provided by the breakwater, potentially reducing hydrodynamic stress and supporting biomass accumulation in older meadows. However, the site is also subject to localised anthropogenic disturbance, including damage from moorings (*Island Research Repository, 2023*), which may contribute to the high within-meadow variability observed. In contrast, Anne Port is more exposed but experiences lower direct human activity. Together, these factors highlight that coarse-level variables such as Age or Location alone cannot capture the ecological complexity influencing seagrass biomass (even when meadows are separated by short distances, 2km in this study) and a more nuanced context-specific approach is needed (*Collier et al. 2021*).

#### *Canopy Metrics as Predictors of Biomass*

Linear regression modelling provided limited support for canopy metrics as predictors of total biomass. When included alongside Age as a fixed effect, neither TBL nor TBN was a statistically significant predictor of log(TOTB Subset). Model selection based on AICc consistently favoured models including Age; however, differences among candidate models were small, suggesting similar relative parsimony rather than strong explanatory separation among models.

The most parsimonious model ( $\log(\text{TOTB Subset}) \sim \text{TBL} + \text{Age}$ ) explained 44% of the variance in log(TOTB Subset). Differences between Age classes were expressed as higher intercepts for Old relative to Young seagrass (Figure 3), indicating consistently higher biomass across the range of TBL. The slope of TBL was small and non-significant, demonstrating that TBL contributed little predictive value once age-related differences were accounted for. The clear separation of fitted values between age groups (Appendix 5) further supports Age as a major structural determinant of biomass, consistent with the findings from the linear regression modelling. The contrasting observations reported with Old seagrass having higher mean biomass results in St Catherines and Young seagrass having higher mean biomass in Anne Port suggests caution is needed to avoid over-interpreting the effect of Age.

These findings indicate that TBL alone does not address the study question. While inclusion of Age as a fixed effect improved model fit, the modest explanatory power achieved suggests that a TBL+Age model would not provide a robust or generalisable predictor of biomass across meadows. The weak canopy-biomass relationships and large residual dispersion indicate that biomass variation is driven by additional biological, ecological, and environmental factors not captured by simple canopy metrics (even when accounting for Age). This is consistent with findings in other studies that site-specific characteristics, such as sediment processes or biological disturbance, may exert stronger controls on carbon-related variables than seagrass structural attributes alone (*Pogson, 2024*).

## *Implications for Biomass and Carbon Estimation*

The results suggest that a single predictor model applicable across all *Z. marina* meadows in Jersey is unlikely to be supported. Instead, canopy-biomass relationships appear to be site- and potentially meadow-specific. While this limits immediate transferability, it does not preclude future application. A more realistic framework is a site- or meadow-specific calibrated approach, whereby limited destructive sampling is initially used to establish local relationships, followed by non-destructive monitoring thereafter. If validated, such calibrated models could reduce future disturbance while enabling repeatable biomass estimation.

While seagrass biomass is commonly linked to organic carbon standing stock (*Fourqurean et al., 2012*), the present study was explicitly concerned with variation in living biomass and its relationship with canopy metrics and age structure. Consequently, the results are interpreted in terms of biomass patterns rather than whole-ecosystem carbon storage.

## *Limitations and Future Research*

The primary limitation of this study was the small and uneven sample size, particularly for the subset used in regression modelling. This constrained statistical power, precluded testing of interaction effects, and limited detection of potentially weak but biologically meaningful relationships. Quadrats sampled along transects may also exhibit spatial clustering, meaning observations may not be fully independent.

Future research should prioritise more balanced sampling designs, informed by power analysis, allowing robust testing of Age-Location interactions and whether the relationship between canopy metrics and biomass differs among groups. Incorporating additional predictors, such as sediment characteristics, hydrodynamic exposure, or disturbance intensity, may improve explanatory power although they might prove challenging to quantify. Thomson, Veracaemer and Wong (2025) studied *Z. marina* in Atlantic Canada and found their regression relationships were improved by including a site covariate group based on principal component analysis of site-level environmental data. The techniques employed in their study could be tested in Jersey *Z. marina* seagrass meadows. In addition, establishing non-destructive approaches for estimating sedimentary organic carbon would further advance blue carbon assessment while reducing reliance on core sampling.

## *Conclusion*

This study demonstrates that simple canopy metrics are insufficient as universal proxies for seagrass biomass estimation across heterogeneous meadows in Jersey. Biomass patterns appear strongly context-dependent and shaped by interacting biological and environmental factors. While this limits generalisation, it supports site- or meadow-specific calibrated approaches for future non-destructive monitoring of seagrass biomass and blue carbon stock assessment in Jersey.

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Appendix 1. Q-Q plots of untransformed TOTB data (n=45) by Location and Age.

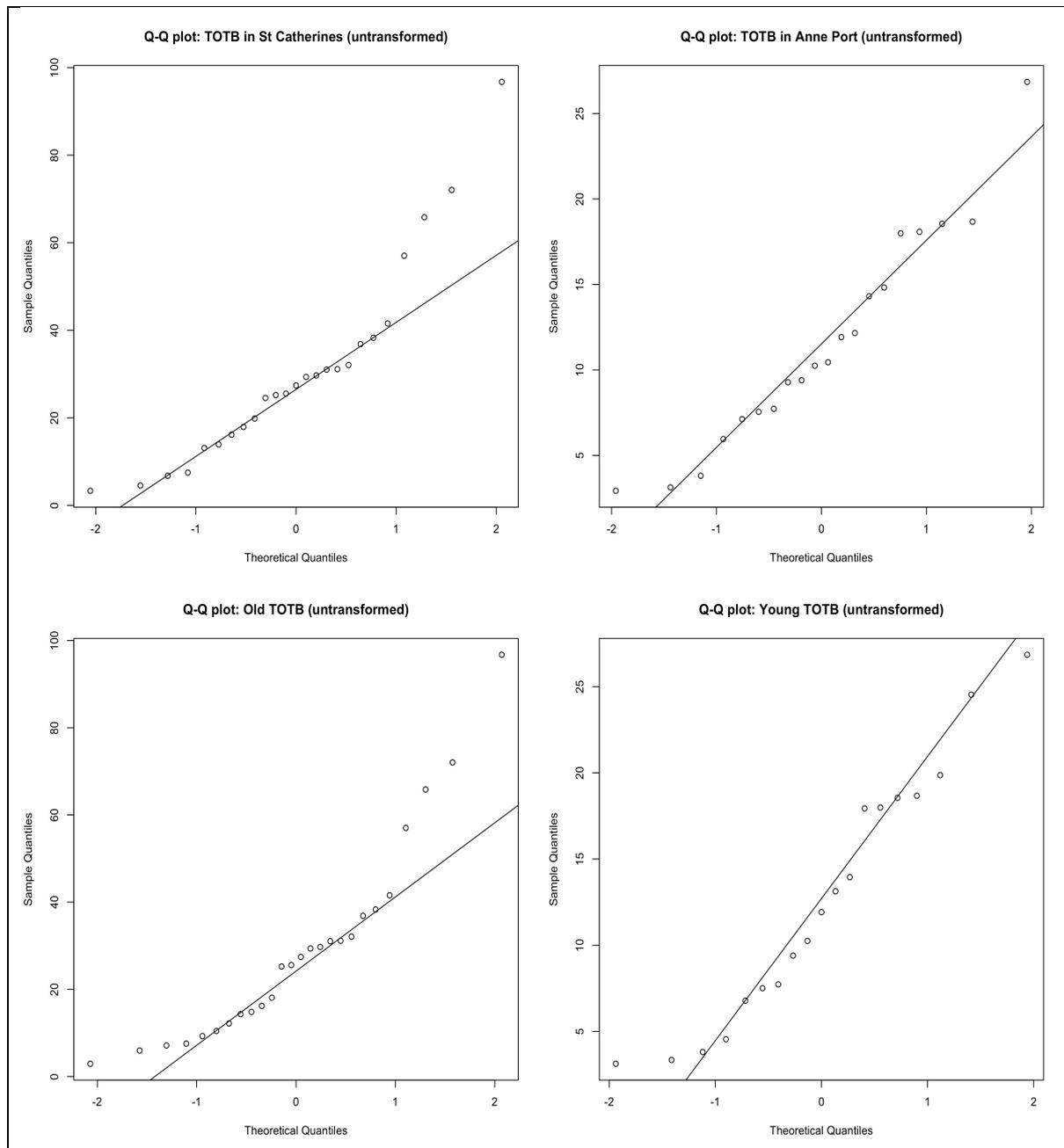


Figure A1. Q-Q diagnostic plots with fitted line of best fit for untransformed TOTB Location and Age groups indicating that data for St Catherines and Old groups are not normally distributed whereas data for Anne Port and Young groups are normally distributed.

Appendix 2. Normality and homogeneity assumption test results for Location and Age combinations

Table A1. Shapiro-Wilk outputs for untransformed TOTB as a function of Location and Age confirming data for St Catherines and Old groups are not normally distributed whereas data for Anne Port and Young groups are normally distributed.

<b>Grouping</b>		<b>W</b>	<b>p</b>
Location	St Catherines	0.88	0.01
	Anne Port	0.95	0.33
Age	Old	0.87	<0.01
	Young	0.94	0.27

Table A2. Shapiro-Wilk outputs for log(TOTB) as a function of Location and Age. All Age-Location combinations were normally distributed.

<b>Group</b>		<b>W</b>	<b>p</b>
Location	St Catherines	0.95	0.25
	Anne Port	0.95	0.34
Age	Old	0.98	0.78
	Young	0.93	0.17

Table A3. Levene's test of homogeneity of variances results confirming homogeneity assumptions were met for log(TOTB) as a function of Location and Age.

<b>Group</b>	<b>F-value</b>	<b>df<sub>1</sub></b>	<b>df<sub>2</sub></b>	<b>p</b>
Location	0.71	1	43	0.40
Age	0.46	1	43	0.50

Appendix 3. Diagnostic plot for untransformed TOTB Subset.

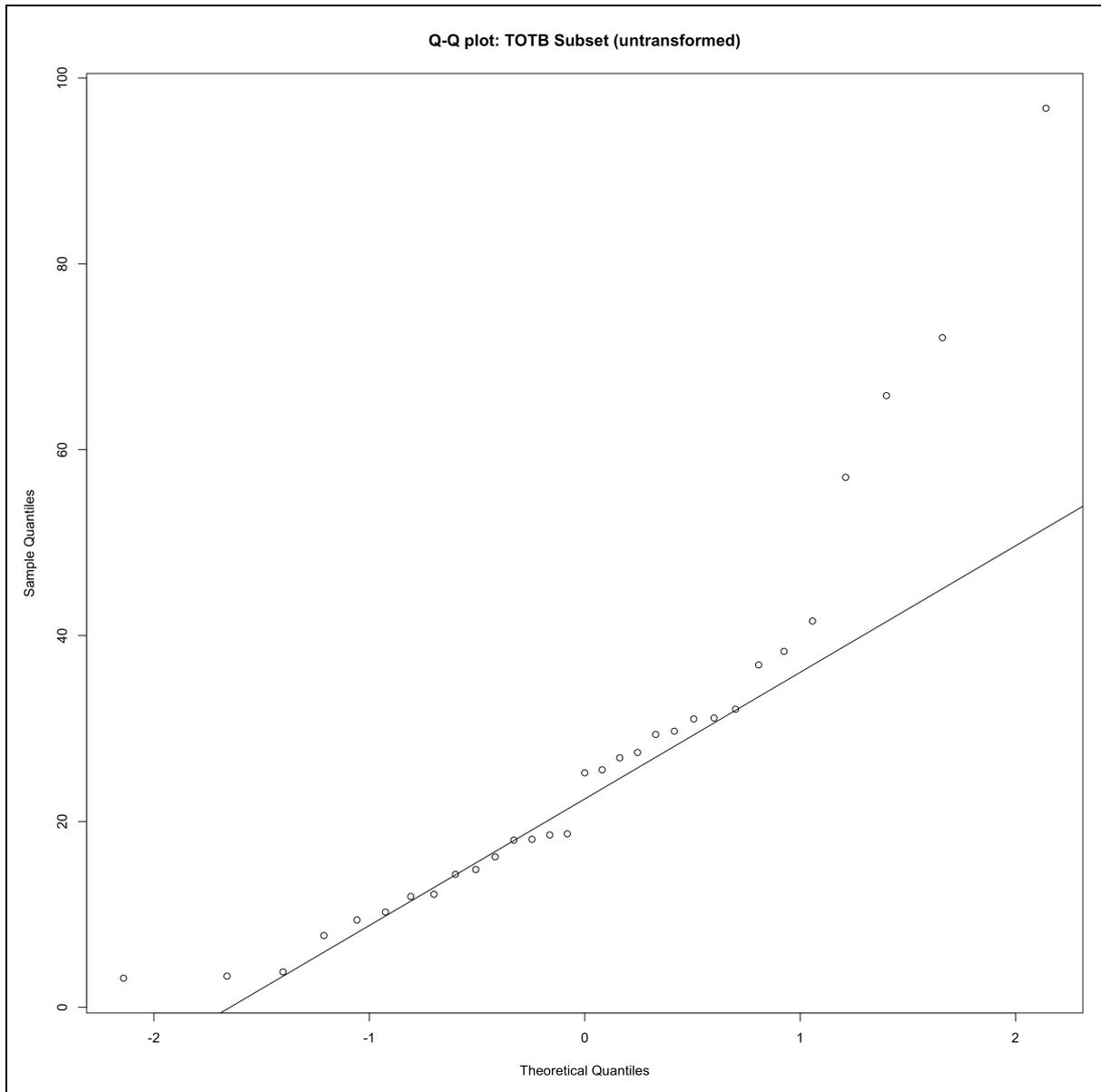


Figure A2. Q-Q diagnostic plot for untransformed TOTB Subset indicating data are not normally distributed.

Appendix 4. Results from Akaike's Information Criterion (AICc) analysis of log(TOTB Subset).

*Table A4. Results from Akaike's Information Criterion (AICc) analysis of log(TOTB Subset) models accounting for Age and Location as factors. For each model the table presents degrees of freedom, AICc result, difference in AICc score from most parsimonious model ( $\Delta AICc$ ) and weight of model in the AICc calculation. Models accounting for Age are: (i) the most parsimonious (having the lowest AIC scores); (ii) have no substantial difference between them ( $\Delta AICc < 2$ ), and (iii) collectively accounted for 91.2% of the total AICc weight across the candidate models, indicating strong relative support for including Age.*

<b>Factor</b>	<b>Predictor</b>	<b>df</b>	<b>AICc</b>	<b><math>\Delta AICc</math></b>	<b>weight</b>
Age	TBL	4	65.67	0.00	0.602
	TBN	4	67.00	1.33	0.310
Location	TBL	4	70.43	4.76	0.056
	TBN	4	71.52	5.85	0.320

Appendix 5. Diagnostic plots of the linear model residuals for AICc supported models.

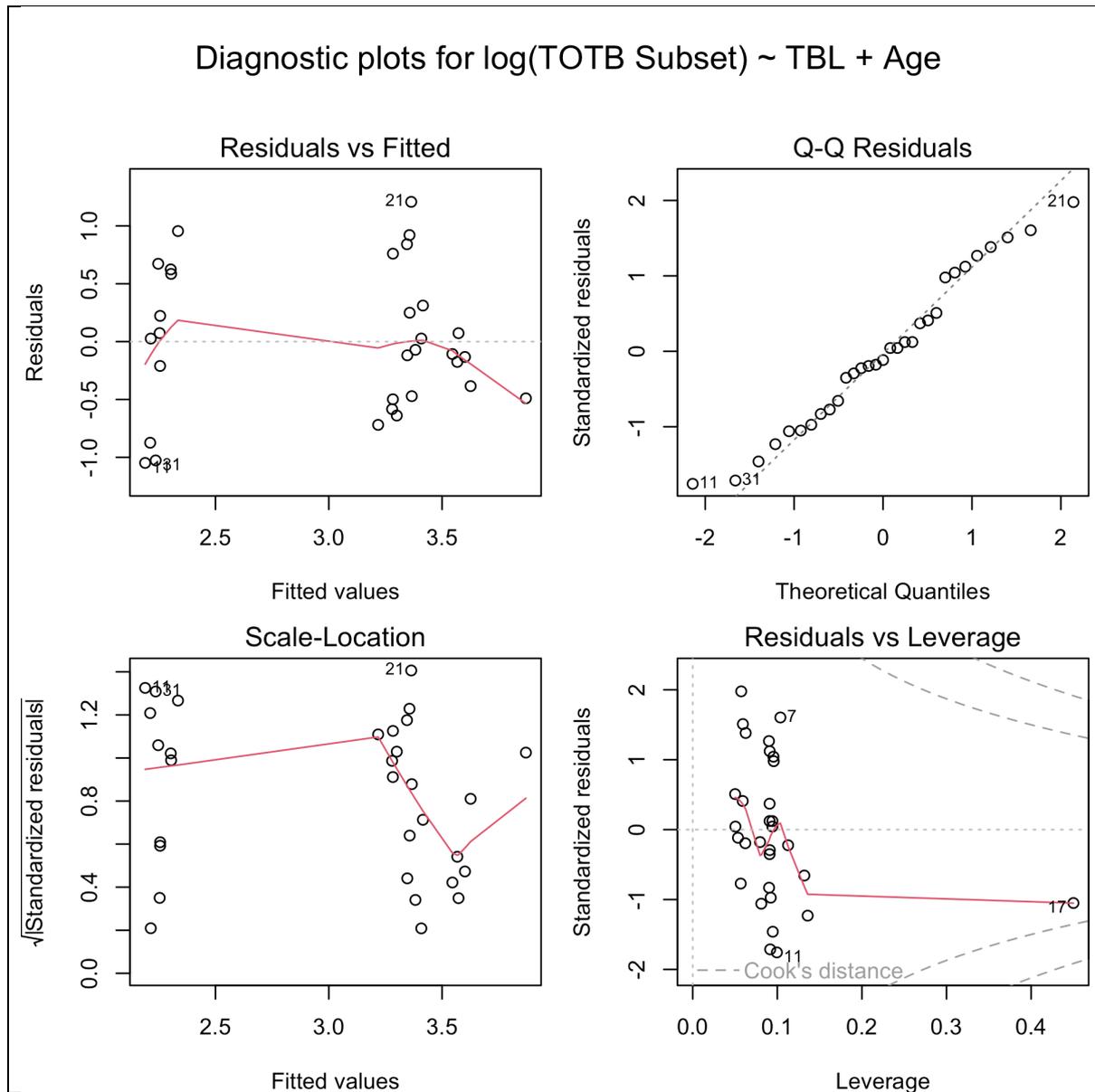


Figure A3. Diagnostic plots of the residuals of AICc supported models using  $\log(\text{TOTB Subset})$ , Age as a factor and TBL as predictor variable. Plots show (i) residuals vs fitted values, (ii) Q-Q plot, (iii) scale-location, and (iv) residuals vs leverage. Linearity and homoscedasticity were evaluated using residuals vs. fitted and scale-location plots, respectively. The Q-Q plot indicates the residuals are approximately normal. Heteroscedasticity is not evident. The clear separation of fitted values between age groups suggests Age is a major structural determinant of biomass.

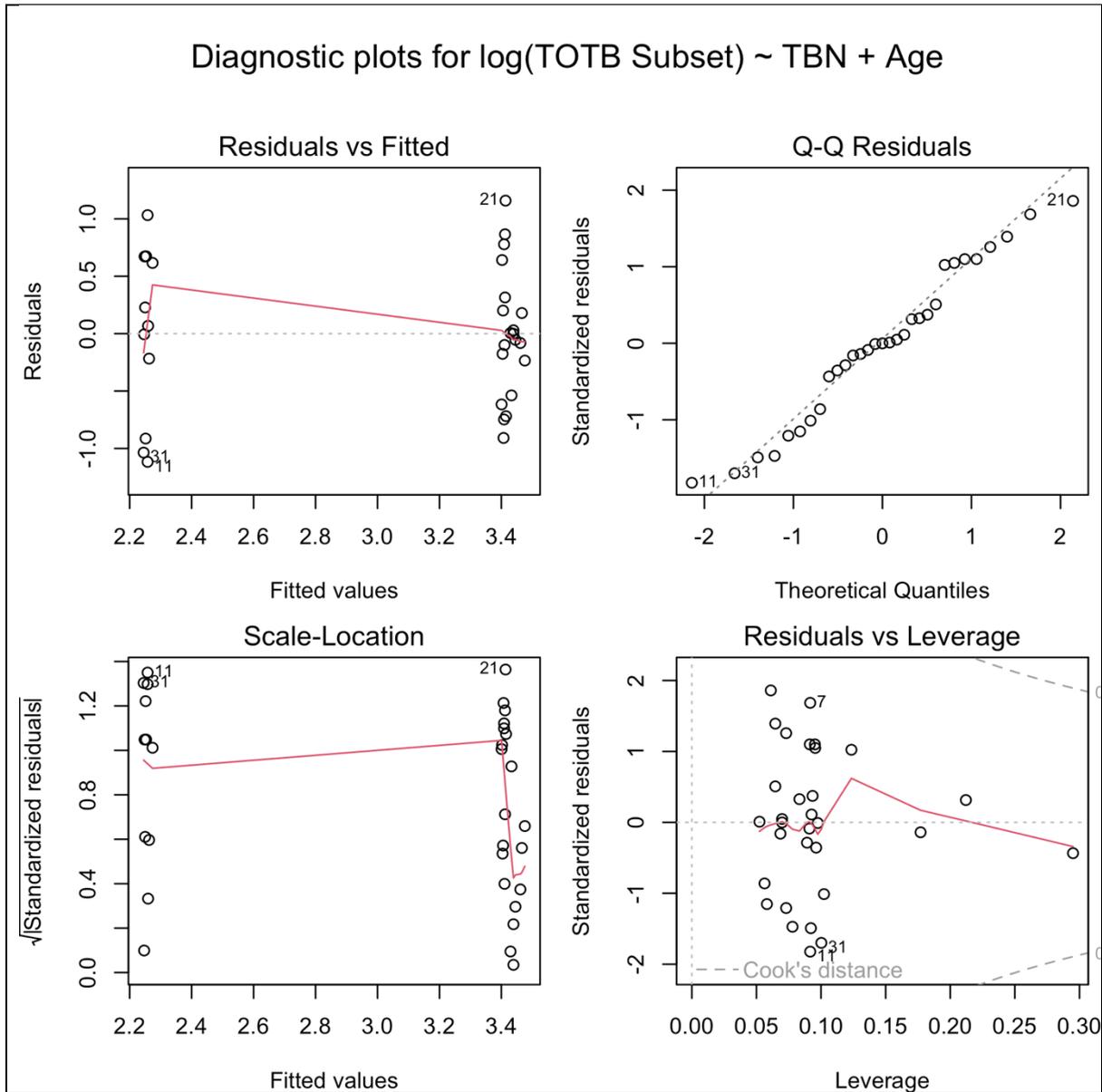


Figure A4. Diagnostic plots of the residuals of AICc supported models using  $\log(\text{TOTB Subset})$ , Age as a factor and TBN as predictor variable. Plots show (i) residuals vs fitted values, (ii) Q-Q plot, (iii) scale-location, and (iv) residuals vs leverage. Linearity and homoscedasticity were evaluated using residuals vs. fitted and scale-location plots, respectively. The Q-Q plot indicates the residuals are approximately normal. Some evidence of heteroscedasticity is observed. The clear separation of fitted values between age groups suggests Age is a major structural determinant of biomass.

Appendix 6. Linear model residuals normality and homoscedasticity assumption test results.

Table A5. Shapiro-Wilk outputs for AICc supported model residuals using  $\log(\text{TOTB Subset})$ , Age as a factor and TBL and TBN as predictor variables. Residuals for both models were normally distributed.

<b>Predictor</b>	<b>W</b>	<b>p</b>
TBL	0.97	0.59
TBN	0.97	0.44

Table A6. Breusch-Pagan outputs for AICc supported model residuals using  $\log(\text{TOTB Subset})$ , Age as a factor and TBL and TBN as predictor variables. Homoscedasticity assumptions were met for both models.

<b>Predictor</b>	<b>BP</b>	<b>df</b>	<b>p</b>
TBL	3.64	2	0.16
TBN	5.44	2	0.07

## Appendix 7. R Code used in this study.

```
##### Code for creating my dataframes Raw-Weight and SQDF_all to include TBL, TBN,  
Location, Age, and TOTB (adding AGB and BGB) #####
```

```
# Preparatory step –tidyverse package loads dplyr, tidyr all needed for this assignment
```

```
library(tidyverse)
```

```
# Read in raw blade length data file and convert to dataframe for testing
```

```
Raw_Blade_Length <- read_csv("Data_Input/Seagrass_Blade_Lengths.csv") %>%  
  select(-Date) %>% # removes the Date column  
  rename(Blade_length = `Blade length`) %>% # tidier and removes need to use backticks
```

```
# replaces Petit Port with Anne Port so only have 2 locations St Catherines and Anne Port  
mutate(Location = if_else(Location=="Petit Port", "Anne Port", Location)) %>%
```

```
# case_when() is preferred way to do multiple conditional recodings  
mutate(Age = case_when(Age == 20 ~ "Old", Age == 9 ~ "Young", TRUE ~  
as.character(Age))) %>%
```

```
# pastes together shorthand location and age with transect and quadrat number to  
# create a unique identifier for each quadrat in a new column
```

```
mutate(  
  Quad_ID = paste0(case_when(  
    Location == "St Catherines" ~ "SC",  
    Location == "Anne Port" ~ "AP",  
    TRUE ~ NA_character_),  
  case_when(  
    Age == "Old" ~ "O",  
    Age == "Young" ~ "Y",  
    TRUE ~ NA_character_),  
  Transect, Quadrat))
```

```
names(Raw_Blade_Length) # checks column names
```

```
# Creates my Seagrass Quadrat Data Frame (SQDF) and TBL and TBN data
```

```
SQDF_Raw <- Raw_Blade_Length %>%  
  group_by(Quad_ID) %>% # uses Quad_ID as the identifier for pipe actions  
  summarise(# summarise () collapses multiple blade length rows per quadrat into a single  
quadrat-level dataset  
    Location = first(Location), # first() returns the value in the first row of the specified  
variable  
    Age = first(Age),  
    TBL = sum(Blade_length, na.rm = TRUE), # sums all the blade lengths per Quad_ID  
ignoring missing ones (na.rm = TRUE)  
    TBN = sum(!is.na(Blade_length)), # sum(!is.na()) returns total number of blades by  
calculating number of rows in Quadrat_ID with a blade length in
```

```

.groups = "drop") %>% # returns a normal ungrouped dataframe at the end, so future
operations don't accidentally keep grouping
mutate(Location = factor(Location), Age = factor(Age)) # these must be factors for
subsequent tests

```

```

# Read in raw quadrat weight data file and convert to dataframe for testing

```

```

Raw_Weight <- read_csv("Data_Input/Seagrass_Weight.csv") %>%
select(-Date, -Collector, -`Sample bag number`) %>%
mutate(Location = if_else(Location=="Petit Port", "Anne Port", Location)) %>%
mutate(Location = if_else(Location=="Anneport", "Anne Port", Location)) %>%
mutate(Age = case_when(Age == 20 ~ "Old", Age == 25 ~ "Old", Age == 9 ~ "Young",
TRUE ~ as.character(Age))) %>%
mutate(Quad_ID = paste0(case_when(Location == "StCatherines" ~ "SC", Location ==
"Anne Port" ~ "AP", TRUE ~ NA_character_), case_when(Age == "Old" ~ "O", Age ==
"Young" ~ "Y", TRUE ~ NA_character_), Transect, Quadrat)) %>%
rename(BGB = `Roots g`, AGB = `Blades g`) %>%
mutate(
  BGB = na_if(as.character(BGB), "N/A"), # converts "N/A" to NA
  BGB = as.numeric(BGB), # to be safe convert the rest of the column back to numeric
  AGB = na_if(as.character(AGB), "N/A"),
  AGB = as.numeric(AGB)
) %>%
mutate(TOTB = AGB + BGB) %>% # calculates total biomass in g
drop_na(Quad_ID, Location, Age, Transect, Quadrat, AGB, BGB, TOTB) # drop_na()
removes rows that are missing any critical information needed for modelling

```

```

# Any Quad_IDs in blades but not in weights? expect the answer 0 if there are none

```

```

SQDF_Raw %>%
anti_join(Raw_Weight, by = "Quad_ID") %>%
count()

```

```

# join the BGB data into SQDF using Quad_ID

```

```

SQDF_join <- SQDF_Raw %>%
left_join(Raw_Weight %>% select(Quad_ID, AGB, BGB, TOTB), by = "Quad_ID")

```

```

# Tidy up of SQDF_join to remove blank cells due to identified Quad_ID mismatches and
cells with "N/A"

```

```

SQDF_all <- SQDF_join %>%
group_by(Quad_ID) %>%
filter(n() == 1) %>% # keeps only groups with exactly one row
ungroup() %>%
drop_na(Quad_ID, Location, Age, TBL, TBN, AGB, BGB, TOTB) # drop_na() removes
rows that are missing any critical information needed for modelling

```

```

# double check no duplicates in final SQDF

```

```

SQDF_all %>% count(Quad_ID) %>% filter(n > 1)

```

```
##### Code for descriptive stats and box plots for variable TOTB by each grouping factor,  
Age and Location #####
```

```
# Grouped summaries
```

```
TOTB_summary_group <- Raw_Weight %>%  
  group_by(Age, Location) %>%  
  summarise(  
    n = n(),  
    mean = mean(TOTB),  
    sd = sd(TOTB),  
    min = min(TOTB),  
    max = max(TOTB),  
    median = median(TOTB),  
    IQR = IQR(TOTB),  
    .groups = "drop"  
  )
```

```
write_csv(TOTB_summary_group, "Data_Output/TOTB_summary_group.csv")
```

```
# composite box plot of Age data by Location
```

```
library(ggplot2)  
TOTB_composite_box <- ggplot(Raw_Weight, aes(x = Location, y = TOTB)) +  
  geom_boxplot() +  
  facet_wrap(~ Age) +  
  labs(  
    x = "Location",  
    y = "Total biomass (g)"  
  ) +  
  theme_classic()
```

```
# Save box plot
```

```
ggsave("Data_Output/TOTB_composite_box.png", TOTB_composite_box, width = 30,  
height = 20, units = "cm")
```

```
##### TOTB COMPARISONS USING Raw_Weight #####
```

```
# Q-Q plots for Location and Age and save #
```

```
png("Data_Output/QQplots_TOTB_all.png",  
width = 4000, height = 4000, res = 300)  
par(mfrow = c(2, 2)) # 2 x 2 grid
```

```
# 1. St Catherines
```

```
qqnorm(Raw_Weight$TOTB[Raw_Weight$Location == "StCatherines"], main = "Q-Q plot:  
TOTB in St Catherines (untransformed)")  
qqline(Raw_Weight$TOTB[Raw_Weight$Location == "StCatherines"])
```

```
# 2. Anne Port
```

```
qqnorm(Raw_Weight$TOTB[Raw_Weight$Location == "Anne Port"], main = "Q-Q plot:  
TOTB in Anne Port (untransformed)")  
qqline(Raw_Weight$TOTB[Raw_Weight$Location == "Anne Port"])
```

```
# 3. Old
```

```
qqnorm(Raw_Weight$TOTB[Raw_Weight$Age == "Old"], main = "Q-Q plot: Old TOTB  
(untransformed)")  
qqline(Raw_Weight$TOTB[Raw_Weight$Age == "Old"])
```

```
# 4. Young
```

```
qqnorm(Raw_Weight$TOTB[Raw_Weight$Age == "Young"], main = "Q-Q plot: Young  
TOTB (untransformed)")  
qqline(Raw_Weight$TOTB[Raw_Weight$Age == "Young"])
```

```
dev.off()
```

```
# Comparisons by Location
```

```
# Run Shapiro-Wilk test for normality by Location
```

```
shapiro.test(Raw_Weight$TOTB[Raw_Weight$Location == "StCatherines"])  
shapiro.test(Raw_Weight$TOTB[Raw_Weight$Location == "Anne Port"])
```

```
# St Catherines not normally distributed. Re run Shapiro-Wilk on log transformed data
```

```
shapiro.test(log(Raw_Weight$TOTB)[Raw_Weight$Location == "StCatherines"])  
shapiro.test(log(Raw_Weight$TOTB)[Raw_Weight$Location == "Anne Port"])
```

```
# Levene test for homogeneity of variances (log transformed data is approximately normal)
```

```
library(car) # needed to run Levene's test  
leveneTest(log(TOTB) ~ Location, data = Raw_Weight)
```

```
# independent t-test assumptions have been met
```

```
# Relevel Location so St Catherines is the reference group for interpretability
```

```
# this affects coefficient signs only, not model fit or inference
```

```
t.test(log(TOTB) ~ relevel(factor(Location), ref = "StCatherines"), data = Raw_Weight,  
var.equal = TRUE)
```

```
# Comparisons by Age
```

```
# Run Shapiro-Wilk test for normality by Age
```

```
shapiro.test(Raw_Weight$TOTB[Raw_Weight$Age == "Young"])  
shapiro.test(Raw_Weight$TOTB[Raw_Weight$Age == "Old"])
```

```
# Both data sets not normally distributed. Re run Shapiro-Wilk on log transformed data
```

```
shapiro.test(log(Raw_Weight$TOTB)[Raw_Weight$Age == "Young"])  
shapiro.test(log(Raw_Weight$TOTB)[Raw_Weight$Age == "Old"])
```

```
# Levene test for homogeneity of variances (log transformed data is approximately normal)
```

```

leveneTest(log(TOTB) ~ Age, data = Raw_Weight)

# independent t-test assumptions have been met
t.test(log(TOTB) ~ Age, data = Raw_Weight, var.equal = TRUE)

##### Linear Regression modelling #####

# Code for descriptive stats for variable TOTB in subset of merged raw data files #

TOTB_summary_subset <- SQDF_all %>%
  summarise(
    n = n(),
    mean = mean(TOTB),
    sd = sd(TOTB),
    min = min(TOTB),
    max = max(TOTB),
    median = median(TOTB),
    IQR = IQR(TOTB)
  )

write_csv(TOTB_summary_subset, "Data_Output/TOTB_summary_subset.csv")

TOTB_summary_subset_split <- SQDF_all %>%
  group_by(Age, Location) %>%
  summarise(
    n = n(),
    mean = mean(TOTB),
    sd = sd(TOTB),
    min = min(TOTB),
    max = max(TOTB),
    median = median(TOTB),
    IQR = IQR(TOTB),
    .groups = "drop"
  )

write_csv(TOTB_summary_subset_split, "Data_Output/TOTB_summary_subset_split.csv")

# Q-Q plot on TOTB Subset

png("Data_Output/QQplot_TOTB_Subset.png",
     width = 4000, height = 4000, res = 300)
qqnorm(SQDF_all$TOTB, main = "Q-Q plot: TOTB Subset (untransformed)")
qqline(SQDF_all$TOTB)
dev.off()

shapiro.test(SQDF_all$TOTB)

```

```
# create core predictive linear regression models with log(TOTB Subset) as response variable Age and Location as factors
```

```
m_TBL_log_Age <- lm(log(TOTB) ~ TBL + Age, data = SQDF_all)
m_TBL_log_Loc <- lm(log(TOTB) ~ TBL + Location, data = SQDF_all)
m_TBN_log_Age <- lm(log(TOTB) ~ TBN + Age, data = SQDF_all)
m_TBN_log_Loc <- lm(log(TOTB) ~ TBN + Location, data = SQDF_all)
```

```
# use Akaike's Information Criterion (AICc) as a quantitative measure of model fit and parsimony
```

```
install(MuMIn)
library(MuMIn)
model.sel(m_TBL_log_Age, m_TBN_log_Age, m_TBL_log_Loc, m_TBN_log_Loc)
```

```
# Standard diagnostic plots for residuals v fitted on Age models on most parsimonious models
```

```
png("Data_Output/m_TBL_log_Age_diagnostics.png",
    width = 2000, height = 2000, res = 300)
par(mfrow = c(2, 2),
    oma = c(0, 0, 4, 0),
    mar = c(4, 4, 2, 1))
plot(m_TBL_log_Age, which = 1, sub.caption = "", main = "")
plot(m_TBL_log_Age, which = 2, sub.caption = "", main = "")
plot(m_TBL_log_Age, which = 3, sub.caption = "", main = "")
plot(m_TBL_log_Age, which = 5, sub.caption = "", main = "")
mtext("Diagnostic plots for log(TOTB Subset) ~ TBL + Age",
      outer = TRUE, line = 1.5, cex = 1.2)
dev.off()
```

```
png("Data_Output/m_TBN_log_Age_diagnostics.png",
    width = 2000, height = 2000, res = 300)
par(mfrow = c(2, 2),
    oma = c(0, 0, 4, 0),
    mar = c(4, 4, 2, 1))
plot(m_TBN_log_Age, which = 1, sub.caption = "", main = "")
plot(m_TBN_log_Age, which = 2, sub.caption = "", main = "")
plot(m_TBN_log_Age, which = 3, sub.caption = "", main = "")
plot(m_TBN_log_Age, which = 5, sub.caption = "", main = "")
mtext("Diagnostic plots for log(TOTB Subset) ~ TBN + Age",
      outer = TRUE, line = 1.5, cex = 1.2)
dev.off()
```

```
# Residuals diagnostics for normality testing of log(TOTB Subset) models with Age
```

```
shapiro.test(residuals(m_TBL_log_Age))
shapiro.test(residuals(m_TBN_log_Age))
```

```
# Homoscedasticity testing using the Breusch-Pagan test
```

```

install.packages("lmtest")
library(lmtest)

bptest(m_TBL_log_Age)
bptest(m_TBN_log_Age)

# model coefficients, standard errors and significance

summary(m_TBL_log_Age)

# plot TBL line differentiating by Age

b <- coef(m_TBL_log_Age)

line_dat <- data.frame(
  Age = factor(c("Old", "Young"), levels = levels(SQDF_all$Age)),
  intercept = c(b[1], b[1] + b["AgeYoung"]),
  slope = c(b["TBL"], b["TBL"])
)

TBL_log_Age_scat <-
ggplot(SQDF_all, aes(TBL, log(TOTB), colour = Age)) +
  geom_point() +
  geom_abline(
    data = line_dat,
    aes(intercept = intercept, slope = slope, colour = Age),
    linewidth = 1
  ) +
  labs(
    title = "",
    x = "TBL, mm",
    y = "log(TOTB Subset)"
  )

ggsave("Data_Output/TBL_log_Age_scat.png", TBL_log_Age_scat, width=30, height=20,
units="cm")

```