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The Establishment of Macrobenthic Faunal Communities in Planted Mangroves of Increasing Age

Minor Field Study

Simon Jacobsen Ellerstrand

Division of Water Resources Engineering
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Abstract

The mangrove ecosystem is a tropical coastal community inhabited by organisms adapted to the stressful environment of the tidal zone. Mangrove ecosystems has in recent years been noted for the important ecosystem services they offer. Still, they are threatened by anthropogenic activities and it has been estimated that a third of the world's natural mangrove cover has already been lost. Efforts have been made to conserve and to introduce new mangrove areas in many countries.

The macrobenthic faunal community structure in the mangrove forest has a close association to environmental factors such as the age of the mangrove stand. It may therefore be used as an indicator of environmental quality.

For this study, I sampled and identified macrobenthic fauna in planted mangroves of ages 18, 31 and 54 years, as well as a bare mudflat site in the Jiulongjiang Estuary, Fujian Province China. The sampled organisms were used to investigate the establishment and potential increase of diversity in macrobenthic faunal communities as a planted mangrove area develops with age.

In total, 1871 individuals from 52 taxa belonging to 7 phyla were found, Crustacea and Polychaeta being the two most dominant groups. The species richness increased with mangrove age, and a linear regression on the Shannon-diversity based on biomass also showed a significant increase (p=0.0135, R^2=0.6059), while the Shannon-diversity based on abundance did not. Only the 54-year old mangrove showed any significant difference to the mudflat site, and only in terms of a higher abundance (p=0.0016) and barely in a higher Shannon-diversity based on biomass (p=0.0509).

The few replicates and sampled sites are probable explanations for the non-significant results. Future studies should increase the number of replicates, be conducted over several seasons, and include other physical parameters in the analysis, to get a better overview and comprehension of the macrobenthic faunal community.

Sammanfattning

Mangroveskogens ekosystem är ett tropiskt kustsamhälle bebott av organismer anpassade till den stressfulla miljön i tidvattenszonen. Mangroveskogen har på senare år blivit erkänd för de viktiga ekosystemtjänsterna den erbjuder. Trotts detta hotas den av mänskliga aktiviteter, och en tredjedel av världens naturliga mangroveskog uppskattas redan ha gått förlorad. Ansträngningar har gjorts för att bevara, samt introducera nya mangroveskogar i många länder.

Strukturen av det makrobentiska faunasamhället i mangroveskogen har en nära association med faktorer i den kringliggande miljön, så som åldern av mangroveskogen själv. Därmed kan den användas som en indikator av kvaliteten på miljön.

I denna studien identifierade jag makrobentisk fauna från prover jag tagit i planterade mangroveskogar av åldern 18, 31 och 54 år, samt i ett kalt strandområde, vid Jiulongjiangs flodmynning, Fujian provinsen, Kina. Organismerna funna i proverna användes för att undersöka etableringen av och den eventuella ökningen av diversitet i makrobentiska faunasamhällen efterhand som en planterad mangroveskog utvecklas med åldern.

Totalt 1871 individer från 52 taxa tillhörande 7 fyla fanns i proverna, varav Crustacea och Polychaeta var de två mest dominanta grupperna. Artrikedomen ökade med åldern av mangroveskogen, och en linjär regression med Shannon.diversiteten baserad på biomassa visade en signifikant ökning (p=0.0135, R^2=0.6059), medan Shannon.diversiteten baserad på individantal inte visade något signifikant resultat. Endast den 54 år gamla mangroveskogen visade någon signifikant skillnad från det kala strandområdet, och då endast när det kom till ett högre individantal (p=0.0016) och knappt när det kom till en högre Shannon-diversitet baserad på biomassa (p=0.0509).

De få replikaten och provtagna områdena är troliga förklaringar till de icke-signifikanta resultaten. Framtida studier bör öka antalet replikat, genomföras under flera årstider, samt inkludera andra fysiska parametrar i analysen för att få en bättre översikt och förståelse av det makrobentiska faunasamhället.

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1. Introduction

1.1 Background

The Mangrove forest is found in tropical coastal regions within the 20 °C isotherm and consists of vascular plants such as woody trees and shrubs. It is home to a community of organisms which together with the plants themselves all have adapted to the stressful conditions of the tidal zone, being characterized by cyclic desiccation and inundation, leading to varying salinity levels (Kaiser *et al.* 2011).

Mangrove plants are ecosystem engineers that directly affect their surrounding environment in a way that becomes facilitating for other organisms. By binding soil with their roots, and the canopy maintaining a shaded and moist environment during low tide, they provide a suitable habitat for benthic organisms that in turn raise the oxygen levels of the anoxic soil by bioturbation. The plant root system and the benthic macrofauna provides a nursing ground for other marine species such as fish that may find protection from predation and food during high tide (Gutiérrez *et al.* 2011).

Mangroves have in recent years been noted for the important ecosystem services they offer. Mangroves postpone coastal erosion, while deforested areas increase water turbidity, which may have unknown effects on adjacent habitats such as seagrass beds and coral reefs (Gutiérrez *et al.* 2011). Their function as a nursing ground for marine species have been shown to provide recruitment to local fisheries (Kaiser *et al.* 2011). They have shown to provide highly valued water purification services (Polidoro *et al.* 2010). They were even shown to have a marked protection against the tsunami of December 2004 compared to deforested areas (Kaiser *et al.* 2011). Globally mangrove forests may work as important carbon sinks, as they contribute significantly to coastal sediment carbon storage and export of particulate terrestrial carbon to the ocean (Alongi 2014).

The mangrove ecosystem is however threatened by several anthropogenic activities, including pollution from industry, nutrient over-enrichment from sewage and agricultural discharge, whereas the biggest threat is deforestation to make room for coastal luxury shrimp farm facilities (Stockholm University 2005). It has been estimated that a third of the world's mangroves has been lost during the past 50 years (Alongi 2002). In China, only a third of the historical mangrove cover remained in 2014 (Lunstrum and Chen 2014).

The loss of mangroves is a development issue relating to the temptation of short-lived great earnings causing a long-term negative effect on other important industries and biodiversity due to environmental ruin. In this case, deforestation of mangrove forests to make room for shrimp farms may leave a vast number of coastal resident impoverished as a consequence of reduced local fishing stock (Stockholm University 2005).

In later years however, conservation efforts such as rehabilitation and restoration projects have increased around the world (Alongi 2002). In the early 1990s, the Chinese government begun to invest in mangrove reforestation to regain the provided ecosystem services of the mangrove forest (Lunstrum and Chen 2014).

Macrobenthic fauna are defined as animals larger than 1 mm living on top of, or in, underwater sediment, mostly down to a depth of 20-30 cm of the surface layer (Tagliapietra and Sigovini

2010; Sánchez-Moyano *et al.* 2004). Macrobenthic fauna may act as bioturbators, affecting the chemistry of the soil by aeration, which in turn has positive effects on the mangrove plants (Kaiser *et al.* 2011). Macrobenthic fauna feeding on detritus or phytoplankton play an important role in the mangrove ecosystem. By feeding on primary producers, they transport energy to higher trophic levels such as fish and birds that may feed on the macrobenthic fauna (Chen *et al.* 2007; Herman *et al.* 1999; Tagliapietra and Sigovini 2010).

The macrobenthic faunal community structure in mangrove forests are strongly affected by factors such as salinity, nutrient enrichment and human disturbances (Liao *et al.* 2016), as well as the mangrove flora, the age of the mangrove stand, the rate of litterfall productivity, and crown size or shading (Pagliosa *et al.* 2016). Information on the macrobenthic faunal community in planted mangroves may therefore be used as an important indicator to assess the environmental quality of the ecosystem (Herman *et al.* 1999; Liao *et al.* 2016; Pagliosa *et al.* 2016), and reestablishment of macrobenthic faunal communities may be expected after plantation of mangroves (Pagliosa *et al.* 2016).

1.2 Purpose of Study

The purpose of this study is to (i) do a survey of the macrobenthic fauna in planted mangrove sites outside the town of Fugong in the Jiulongjiang Estuary for the Collage of the Environment and Ecology, Xiamen University, and (ii) investigate the establishment of macrobenthic faunal communities over time by studying planted mangroves of different ages. If the mangrove has had more time to grow and facilitate its surroundings, the macrobenthic fauna should have had an increased opportunity to immigrate and establish. I therefore hypothesize that (i) older mangroves will have an increased macrobenthic faunal diversity, and (ii) the planted mangroves will have a higher macrobenthic faunal diversity than the bare mudflats (non-mangrove site). This study is done in conjunction with Hultman (2017), having the same purposes and hypotheses, but focusing on meiobenthic fauna.

2. Methods

2.1 Study Area

Sampling was carried out outside the town of Fugong in the Jiulongjiang Estuary, Fujian Province, China. The climate of the region is a southern subtropical maritime, with low air temperatures during winter (December to February), wet season during spring (March to May), and heavy rainfall during hot seasons (summer from June to August and autumn from September to November) (Li and Ye 2014). The annual mean air temperature of the region is 21.0 °C, with an annual precipitation of 1284 mm, with summer typhoons contributing to most of the rainfall (Chen *et al.* 2007; Li and Ye 2014).

Within the study area, mangroves of the species *Kandelia obovata* has been planted during the last half century to protect the coastline from erosion (Hongyou, H., personal communication; Li and Ye 2014). The mean salinity of the water has previously been measured to 17.1 psu. The tides are semi-diurnal with an average range of 4 m. The mangrove sites have been observed to be inundated by high tides for 6-8 days every month (Chen *et al.* 2007; Li and Ye 2014). Inland of the mangroves sites, the area is covered by aquaculture, such as shrimp farms (Hongyou, H., personal communication; Rao, Y. Y., personal communication).

The sampling sites were chosen for being located relatively close to each other, being accessible, and for their age. Three sites of different mangrove ages were sampled; the low age site L was planted 1999 and is 18 years old (24°23'21.5"N, 117°54'6.5"E), the medium age site M was planted 1986 and is 31 years old (24°23'40"N, 117°54'36.5"E), the high age site H was planted 1963 and is 54 years old (24°23'37.5"N, 117°55'31"E). A control site C was also sampled on the nearby bare mudflats (24°23'39.5"N, 117°54'25"E) (Hongyou, H., personal communication; Fig 1).

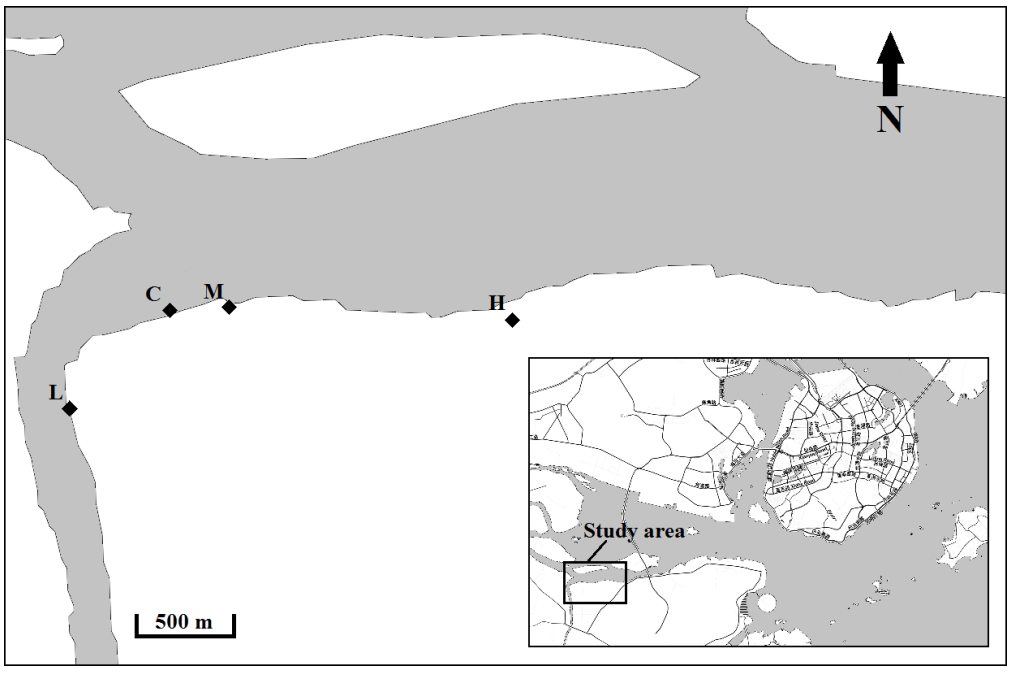

Figure 1 Location of the sampled sites along the Jiulongjiang. C: Control site (mudlfats); L: Low age mangrove (planted 1999, 18 years old); M: Medium age mangrove (planted 1986, 31 years old); H: High age mangrove (planted 1963, 54 years old).

The sampling of the mangrove sites was carried out on the 7th of March 2017. The sampling of the mudflat site was carried out on the 24th of March 2017. This time of the year is considered spring season in the Fujian Province (Hongyou, H., personal communication). Pictures of the study area can be found in the appendix (App 1).

2.2 Sampling Procedures

At each site, three sediment samples were taken. As the sites had not been visited before the day of sampling, the exact method of taking the samples had to be improvised on site. Within each site, the sample location was chosen by walking into the planted mangrove forest to find an area relatively free of twigs and trash brought in and by the high tide. To choose the sample locations as randomly as possible, a metal frame was thrown haphazardly on the ground within the chosen area.

The metal frame used for taking the samples were a square frame of 25x25 cm and 30 cm of depth. The frame was pushed down into the sediment as deep as possible, and the sediment within the frame was dug up to a depth of approximately 30 cm. The samples were taken in pairs with sediment samples for the study by Hultman (2017). To avoid flooding during this procedure, the sampling was carried out on a day without heavy rain and during low tide. Some pictures of the sampling procedures can be found in the appendix (App 1).

2.3 Treatment of the Samples

After the samples were taken, they were washed and sieved through a 1 mm mesh in the field. The samples were then brought back to the lab and were immediately fixed with 10 % formalin. The samples were also stained with rose bengal over night to simplify the extraction of the organisms from the samples. The samples were later washed again with the same sieve to remove excess particles. The samples were then searched for macrobenthic fauna which were removed with tweezers and put in 75 % ethanol.

The extracted organisms were then examined with a stereo microscope and identified with the help of Rao Yiyong and the use of relevant identification literature (Rao, Y. Y., personal communication; Zongguo and Lin 2012 (Vol.1, 2, 3, 6 and 8); Wu *et al.* 1997; Yang and Sun 1988; Sun and Yang 2014; Blake and Kudenov 1978; Okutani 2000; Aiyun *et al.* 1986; Lizhe 2015; Douwes *et al.* 1997; Bouchard 2004). Finally, the taxonomical details were corrected using the World Register of Marine Species (WoRMS Editorial Board 2017). As some organisms are more difficult than others to identify down to species level using only morphology, these were only identified to the lowest practical taxonomical level.

For each taxonomical group, the number of individuals were counted and weighed for the wet weight, hereby referred to as the “biomass”. Pictures were also taken of each taxonomical group (App 2). From the results, the species richness for each site was calculated. The Shannon-diversity index was also calculated for each replicate and for the site in total according to:

$$H' = -\sum_{i=1}^{n} p_i ln(p_i)$$

were p_i is the proportion of species i in the sample (Magurran 1988). The Shannon-diversity index was calculated in two versions, based on the number of individuals H'_{abu} or the biomass H'_{bio} of each species. The Shannon-diversity is usually calculated as one value per sampled site, henceforth referred to as the "total H'". Due to the few sampled sites, the Shannon-diversity was also calculated per individual sample, henceforth referred to as just "H'".

2.4 Data Analysis

The software R 3.4.0 for windows 64-bit with RStudio 1.0.143 was used for calculations and the statistical analysis of the data. The raw data and the R-script can be found in the appendix (App 3; App 4).

To examine if the properties of the macrobenthic faunal community change with the planted mangrove age, linear regressions were fitted with abundance, biomass, H'_{abu} and H'_{bio} as functions of the mangrove age. The same was also done for the species richness, H'_{abu} and H'_{bio} calculated for each site in total. In the analysis, regressions were fitted both excluding the mudflat site, and including it as an age 0 mangrove site.

To examine if the properties of the macrobenthic faunal community differ from the mudflat site, ANOVAs were used on the parameters; abundance, biomass, H'_{abu} and H'_{bio}. In case of a significant difference, post-hoc Tukey HSD tests were used to determine exactly which sites differ from each other.

To verify the normality of the data, Shapiro-Wilks Normality Tests combined with histograms and QQ-plots were used on the residuals of the data. To assess the equality of variance for the ANOVAs, the Levene's test for Equality of Variances was used.

3. Results

3.1 Macrofaunal Communities

In total, the macrobenthic fauna identified at the 4 sites totalled 1871 individuals from 52 taxa belonging to 7 phyla (App 2). The three mangrove sites totalled 1694 individuals, 46 taxa and 7 phyla. The numbers at the bare mudflat site was 177 individuals, 13 taxa and 4 phyla (Tab 1, Tab 2).

In total, the most dominant group found was Crustacea (46 %) in terms of individuals. The most dominant groups found at the individual sites were Gastropoda at the mudflats (56 %), Polychaeta at the 18-year old mangrove (58 %), Gastropoda at the 31-year old mangrove (49 %), and Crustacea at the 54-year old mangrove (65 %).

In terms of biomass, the most dominant groups found was Crustacea and Polychaeta (47 %, 29 %). The most dominant groups at the individual sites were Crustacea at the mudflats (88 %), Polychaeta at the 18-year old mangrove (65 %), Crustacea at the 31-year old mangrove (45 %), and Crustacea and Polychaeta at the 54-year old mangrove (48 %, 36 %; Tab 1, Fig 2).

Table 1 List of the species found at the sampled sites along the Jiulongjiang Estuary. The species are grouped into convenient higher taxa such as phylum, class or subclass. The abundance and biomass are given for each species as the sum from the replicates at each site.

		Site C - Mudflats		Site L - Mangrove 18		Site M - Mangrove 31		Site H - Mangrove 54	
Higher taxa	**Species**	**Abundance (ind.)**	**Biomass (g)**	**Abundance (ind.)**	**Biomass (g)**	**Abundance (ind.)**	**Biomass (g)**	**Abundance (ind.)**	**Biomass (g)**
Cnidaria	Actiniaria sp.1	1	0,0014						
	Actiniaria sp.2					2	0,0016		
Nemertea	*Procephalothrix* sp.					4	0,1416	3	0,4132
Polychaeta	*Nephtys* sp.	11	0,0467						
	Namalycastis abiuma					1	0,006		
	Nereididae sp.					9	0,0066		
	Sigambra hanaokai							4	0,0011
	Lepidonotus sp.					1	0,0088		
	Phyllodocidae sp.			1	0,0577				
	Polydora sp.	1	0,0000						
	Chaetozone setosa							32	0,0017
	Capitella capitata							4	0,0000
	Notomastus latericeus			69	4,8806	40	3,8034	53	3,3725
Oligochaeta	Oligochaeta spp.	9	0,0006	6	0,0015	16	0,0214	39	0,005
Sipuncula	*Phascolosoma arcuatum*			11	2,3438	47	1,1796		
	Phascolosoma sp.							2	0,005
Bivalvia	*Xenostrobus atratus*	2	0,0039						
	Placunidae sp.							1	0,0044
Gastropoda	*Cerithidea* sp.					1	0,7349		
	Pyramidellidae or Eulimidae sp.			2	0,0125	16	0,1309		
	Alvania sp.							8	0,0935
	Assiminea brevicula	46	0,7441	8	0,2022	12	0,0473	2	0,0428
	Assiminea sp.	44	0,2029	8	0,0272	113	0,9104		
	Stenothyra glabrata					1	0,0111		
	Ceratia sp.	2	0,0242						
	Wakauraia sakaguchii			1	0,0077	29	0,4152	2	0,0195
	Adeorbis plana	8	0,009					3	0,03
Crustacea	Copepoda sp.					1	0,0000		
	Byblis sp.							19	0,001
	Corophium sp.	1	0,0002			2	0,0053	705	1,2785
	Sesarma dehaani					1	0,0287		
	Sesarma sinensis					3	1,5735		
	Helice tridens pingi					2	1,7178		
	Metaplax elegans							1	0,3847
	Metaplax sp.	1	0,7158						
	Cleistostoma dilatatum							2	0,1779
	Ilyoplax ningpoensis	46	7,0072			1	0,0051	5	0,6376
	Uca arcuata			1	0,0046	7	3,6252		
	Alpheus sp.							2	0,3465
	Exopalaemon carinicauda			1	0,0115			10	0,1177
	Macrobrachium sp.							4	0,6942
	Laomedia astacina					3	0,2367	2	0,6388
	Apseudes sp.							40	0,2649
	Ostrocoda sp./spp.							8	0,0007
Insecta	Chironomidae sp. larvae							189	0,1367
	Dolichopodidae sp. larvae	5	0,0343	11	0,0269	36	0,1226	45	0,3102
	Diptera spp. pupae			2	0,001	2	0,0023	2	0,0017
	Naucoridae sp.							1	0,1492
	Corixidae sp.							2	0,0032
	Heteroptera sp. nymph							9	0,0071
	Zygoptera sp.							12	0,2159
Chordata	*Boleophthalmus pectinirostris*					1	1,214	11	0,0837

Table 2 Information on the macrobenthic faunal communities of the sampled sites along the Jiulongjiang Estuary. Total number of individuals, their biomass, the density of individuals and biomass, species richness S, the total Shannon-diversity H' of the replicates added together and the average Shannon-diversity of the individual replicates. The Shannon-diversity is calculated from both individuals and biomass.

						H' - individuals		H' - Biomass	
Site	Total individuals	Total biomass (g)	Density by individuals (ind. m^{-2})	Density by biomass (g m^{-2})	S	Total H'	Average H'	Total H'	Average H'
C - Mudflats	177	8,7903	236	11,7204	13	1,8296	1,7102	0,7594	0,6750
L - Mangrove 18	121	7,5772	161,3333	10,1029	12	1,5586	1,2959	0,8551	0,4876
M - Mangrove 31	351	15,9500	468	21,2667	25	2,2739	2,0951	2,1775	1,4167
H - Mangrove 54	1222	9,4389	1629,3333	12,5852	31	1,6845	1,5979	2,3075	1,8801

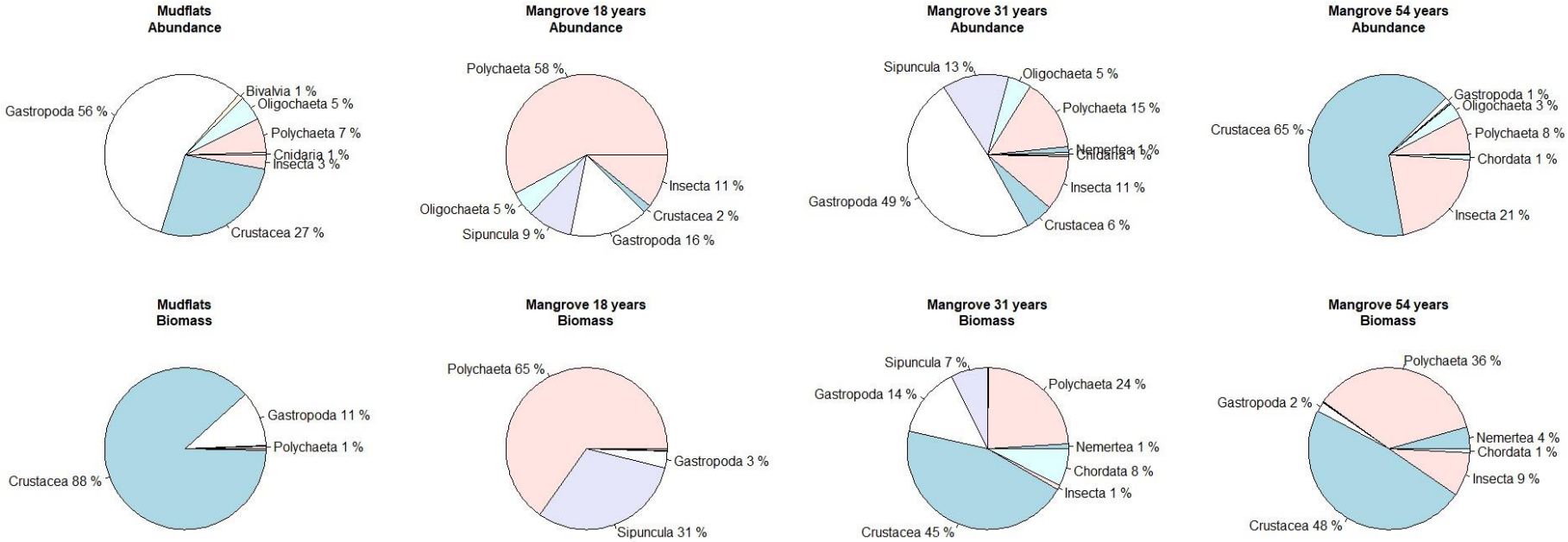

Figure 2 The ratio of the organism groups found at the sampled sites along the Jiulongjiang Estuary. The species are grouped into convenient higher taxa such as phylum, class or subclass. The top row illustrates the ratio of individuals and the bottom row illustrates the ratio of biomass. The names of groups rounded to 0.5 % or lower are not shown in the figure.

3.2 Increasing Diversity with Mangrove Age

Linear regressions were fitted to the data, including and excluding the mudflat site as a mangrove site of age 0. As the best fit was obtained excluding the mudflat site, the analysis including the mudflats was discarded. Further reasons for excluding the mudflat site in the linear regressions will be argued for in the discussion section.

The abundance of the macrobenthic fauna increased significantly with the mangrove age and had a good fit to the data (p=0.0007, R^2=0.8227; Fig 3A). The biomass did not increase significantly with the mangrove age and did not have a good fit to the data (p=0.9347, R^2=0.0010; Fig 3B). The species richness did not increase significantly with the mangrove age, but did have a good fit to the data (p=0.2347, R^2=0.8702; Fig 3C). The H'_{abu} did not increase significantly with the mangrove age and did not have a good fit with the data (p=0.6316, R^2=0.0346; Fig 3D). The same result was found for the total H'_{abu} (p=0.9958, R^2=4.275e-05; Fig 3E). The H'_{bio} increased significantly with the mangrove age and had a good fit to the data (p=0.0135, R^2=0.6059; Fig 3F). The total H'_{bio} did not increase significantly with the mangrove age, but did have a good fit to the data (p=0.3830, R^2=0.6796; Fig 3G).

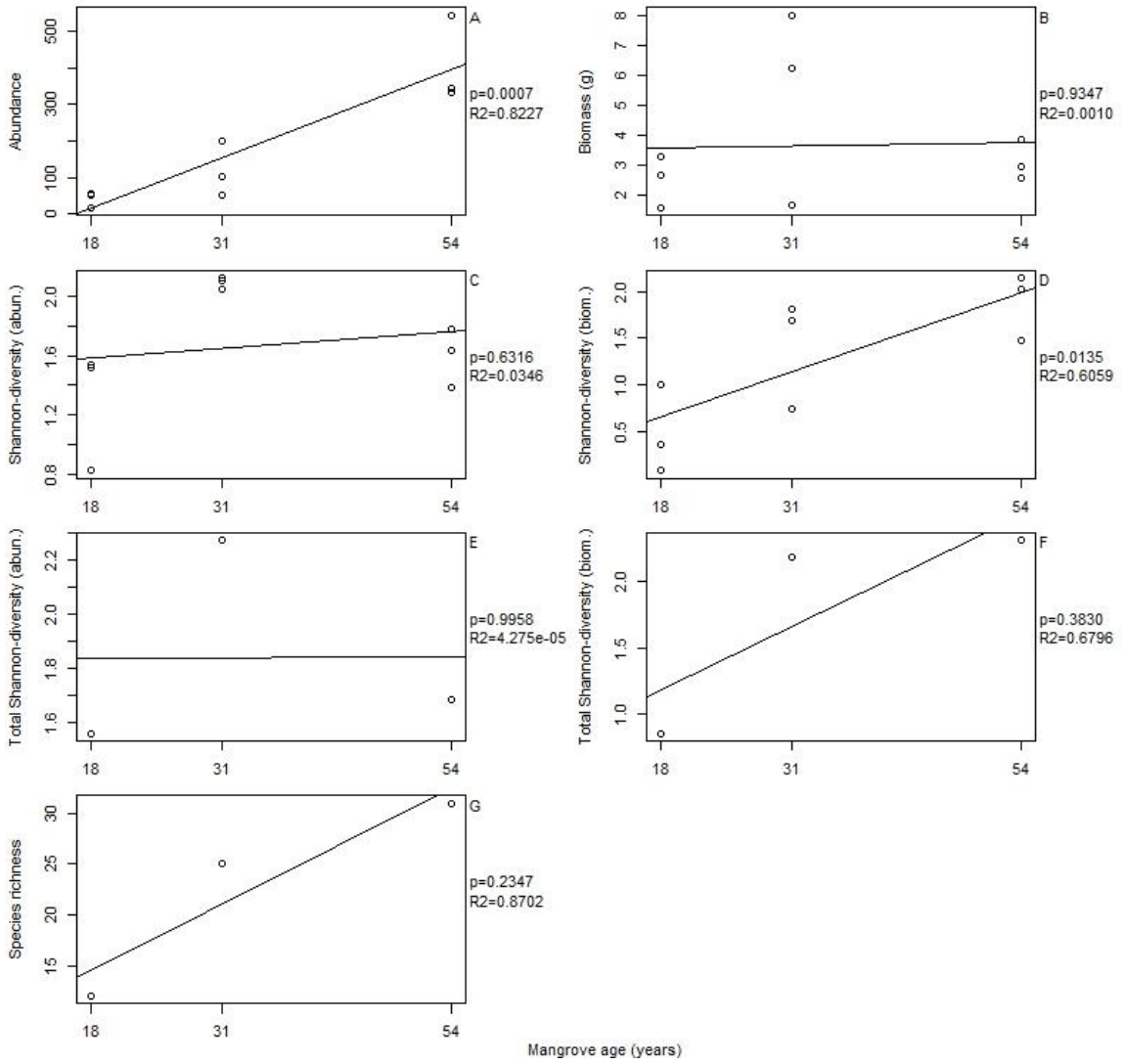

Figure 3 Scatterplots with regression lines fitted to the data; A) the total number of individuals, B) their biomass, C)D) the Shannon-diversity of the individual replicates, E)F) the total Shannon-diversity of each site and G) the species richness. All data is plotted against the mangrove age of the sampled sites along the Jiulongjiang Estuary.

3.3 Difference in Diversity between Sites

ANOVAs combined with post-hoc Tukey HSD tests were used to determine if there was any difference in the parameters between sites, and in that case if the difference was between the mudflat site and the mangrove sites.

The abundance of the macrobenthic fauna at the mudflat site only differed significantly to the 54-year old mangrove (p=0.0016). The 54-year old mangrove did however also differ significantly to the 18- and 31-year old mangrove sites (Fig 4A). The biomass did not differ significantly between any of the sites (Fig 4B). The H'_{abu} did not differ significantly between the mudflat site and any of the mangrove sites. The 18-year old mangrove site did however differ significantly to the 31-year old mangrove site (Fig 4C). The H'_{bio} at the mudflat site did barely differ significantly to the 54-year old mangrove site (p=0.0509). The 54-year old mangrove did however also differ significantly to the 18-year old mangrove site (Fig 4D).

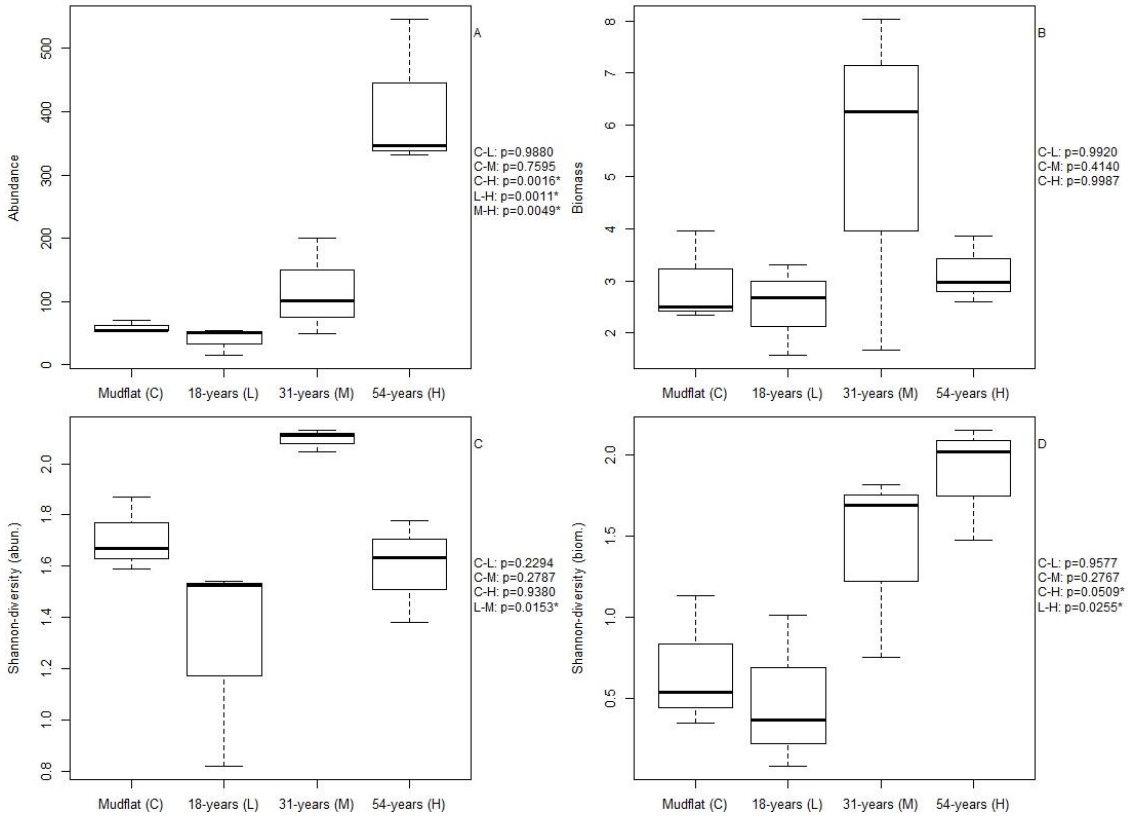

Figure 4 Boxplots of the sampled sites along the Jiulongjiang Estuary with A) the total number of individuals, B) their biomass and the Shannon-diversity based on C) abundance and D) biomass. On the right-hand side, the p-values for the mudflats versus the mangrove sites of different ages are shown, together with other significant p-valuess. Significant p-values are marked with "".*

3.4 Tests for Normality and Equality of Variance

All the data passed the Shapiro-Wilks Normality Test but without much power in some of the cases. The shortcomings in normality could also be seen in the histograms and the QQ-plots. This was probably caused by the small sampling size. Therefore, transformation of the data was not deemed necessary. All data passed the Levene's test for Equality of Variances. The results from the tests for normality and equality of variance can be found in the appendix (App 5).

4. Discussion

4.1 Different Habitat Types

For the linear regressions, I decided to exclude the mudflat site, rather than including it as a representation of an age 0 mangrove site. Excluding it did show better results than including the mudflat site, but more importantly it is doubtful that the bare mudflats are a fair representation of a very young mangrove habitat. It would probably be more accurate to consider it a different habitat entirely. This was also indicated by the similar species richness, but very different species composition between the mudflats and the 18-year old mangrove site.

For instance, *Nephtys* sp. was found in all the mudflat samples but in none of the mangrove samples. 46 individuals of the crab *Ilyoplax ningpoensis* was found at the mudflat site, but only 6 of them in all of the mangrove sites taken together. It is likely that a change in the physical properties occur during the initial years after plantation, suiting species associated with mangrove habitats better. The mangrove species succeeds and replaces some of the mudflat species, which results in a relatively constant diversity rather than an increase.

4.2 Increasing Diversity with Mangrove Age

In this study I used linear regressions to analyse several parameters to find support for my hypothesis (i) older mangroves will have an increased macrobenthic faunal diversity. There was a significant increase in abundance with a good fit, which indicate that older mangrove areas can sustain an increased number of individuals. The biomass did however not show any significant increase or decrease. Even though not significant, the species richness did increase notably between the three sites. Species richness is by itself an indication of diversity, and the increase does support my hypothesis. As only one value of the species richness was retrieved per site, I could only use 3 data points in the regression. This was probably the main reason for the non-significant result.

My interpretation of these results is that the amount of biomass sustained in the sediment does not increase with the age of the mangrove, but the older mangroves tend to harbour an increased number of smaller individuals from an increased number of species. This suggests that the macrobenthic faunal community changes as the mangrove develops and that new species establish themselves by succession.

A common indicator used to study diversity of macrobenthic fauna is the Shannon-diversity index based on abundance. This did not show any significant results for neither the H'_{abu} nor the total H'_{abu}. When calculating the Shannon-diversity based on the biomass, the linear regressions did however show a significant increased for the H'_{bio},. The total H'_{bio} being non-significant was probably due to the few data points (as with the species richness).

I haven't found any study on macrobenthic fauna using the Shannon-diversity based on biomass. This is, however. commonly practised in other areas of ecology such as in plant communities, where the biomass is considered a better indicator of resource use and might provide a more meaningful comparison between different taxonomic levels of organisms than abundance (Magurran 1988). Even though macrobenthic fauna is easy to measure by counting

the number of individuals, the community usually consists of small organisms in large numbers, many feeding on the lowest trophic levels of detritus and phytoplankton. For this reason, I suggest that the Shannon-diversity based on biomass could also be used in the future to increase our understanding of macrobenthic faunal communities.

It is highly unlikely that an increase in diversity would be fully linear, but rather decelerate towards a maximum value when approaching very high aged mangroves. As a transformation in habitat type might occur in newly planted mangroves, as discussed above, it is even probable that the curve is S-shaped. In this study, while only sampling three sites of different ages, it was only of interest to find an increase in diversity. This was done assuming that the sampled ages were within the linear part of a hypothetical S-shaped curve. However, a possibility is that the H'_{abu} had already reached its maximum diversity at the sampled mangrove ages.

I believe that my results strongly indicate that the diversity of macrobenthic fauna does increase with increasing mangrove age. However, due to the few replicates coupled with the inconsistencies in the Shannon-diversity based on abundance, I cannot reject the null hypothesis.

4.3 Higher Diversity in Mangroves than Mudflats

In this study I used ANOVAs with post-hoc Tukey HSD tests to find support for my hypothesis (ii) the planted mangroves will have a higher macrobenthic faunal diversity than the bare mudflats. There was no significant difference between the mudflat site relative to the 18- or the 31-year old mangrove sites in any of the analysed parameters. The abundance in the 54-year old mangrove did show a significant higher abundance to all the other sites, which is probably due to the 705 individuals of *Corophium* sp. and the 189 Chironomidae larvae found at that site. The biomass and the H'_{abu} did not show any interesting results and is consistent with the linear regressions. The H'_{bio} did however show a significant higher value at the 54-year old mangrove site compared to the mudflats and the 18-year old mangrove, which is probably due to the higher species richness, as the biomass didn't differ significantly between sites.

The non-significant results between the mudflats and the 18- and 31-year old mangrove in most of the tests, but the significant difference in the 54-year old mangrove compared to the other sites, supports the above discussion. There is a transformation in habitat type when a mangrove is newly planted, and the increase in diversity is first seen in mangroves of higher age.

The reason for the 31-year-old mangrove not having any significant difference to the mudflat site is, again, highly likely due to the few replicates. There is a visible variation in the data, and more replicates would probably have reduced the variation, maybe enough for significant results in some of the tests.

In this case I cannot reject the null hypothesis. Not all mangrove ages have a higher diversity than the bare mudflat site. However, the results further support my first hypothesis that the diversity increases with mangrove age, and that the difference might not be seen until many years after plantation due to a transformation in habitat type.

4.4 Method Flaws and Future Studies

The sampling sites were chosen firstly for their age and their relative closeness to each other. However, being placed along the coastline of an estuary, there was a difference in distance from the sea that could result in a gradual change of physical factors between the sites. A change in a factor such as the salinity could influence the structure of the macrobenthic faunal community. As seen in figure 1, site L, C and M are placed much closer than site H to the outlet of an adjoining river, which might have led to a much lower salinity level due to the inflow of freshwater. This could have had a heavy influence on the larger abundance and diversity seen at site H.

The placement of the replicates relative to the distance from the waterline could also have affected the results. There should be a gradual decrease in inundation time further from the waterline. This could in turn have had an influence on the macrobenthic community structure, which might differ within each site. This was hard to account for, as the waterline would change during the time spent sampling and walking between each site. Instead, the replicates were placed haphazardly.

The method for treating the samples was not very efficient, with the extraction and identification of the organisms being very time consuming. Previous experience with the local macrobenthic fauna and literature in a for me, known language, would probably have reduced the time spent on identification. Some organisms are nearly impossible to identify down to species level without the use of DNA analyses. The identification to higher taxonomical groups will result in a lower estimation of diversity.

There was also a clear difference in the time demand of organism extraction between sites. Samples from increasing mangrove ages showed a notable increase in organic matter, such as leaves and small sticks, whereas the mudflat samples showed barely any. The large amount of organic material left in the samples after washing made it harder to skim through it for organisms. Where a 54-year old mangrove sample could take me 10 hours to examine, all 3 mudflat samples were examined within 1 hour.

It is however hard for me to figure out some alternative and more efficient method that would provide the sought results. As this method requires mostly basic equipment, it is very cost-efficient. As argued, the few replicates per site is likely the reason for the low significance shown in the statistical analyses. More replicates could have reduced the variation, thereby strengthening the test. More replicates would however have been highly impractical due to the high time demand for each sample. To reduce time demand, it may be considered if smaller sample volumes would be sufficient to represent the macrobenthic faunal community, as used in the study by Pagliosa *et al.* (2016).

Except the noted increase in organic material with mangrove age mentioned above, there was also a visual increase in canopy height and decrease in tree density with higher mangrove ages. The canopy height and the tree density parameters were measured, but were not included in any analysis due to time limitation. These two parameters would probably have correlated with the mangrove age, but for a better understanding of the mangrove community structure, other parameters should also have been measured such as salinity, pH, canopy and grain size. These could be used to determine if any found difference in diversity was actually due to the

mangrove age, or rather being influenced by one of these parameters. This could be done by using an appropriate statistical method such as a PCA.

If time demand wouldn't have been a problem, it might also have been useful to study the diversity in regard of each organism's function in the ecosystem. Examining the diversity of functional groups might be more appropriate to study the diversity and community structure in an ecosystem than the actual species themselves. This would, however, have demanded a lot of time spent on literature search on each of the found species.

For a full coverage of the diversity in the macrobenthic faunal community, replicates should also have been taken during all seasons of the year. Due to the life cycle of these organisms, there could be significant changes in diversity between seasons, and some species might not even be detected during some seasons. For example, if some species would be more active during summer than during the sampling in spring, one site showing the lowest diversity during spring could show a higher diversity than other sites during summer. Therefore, all seasons should be considered while studying the diversity.

5. Conclusion

A survey of the sampled sites in the Jiulongjiang Estuary was successfully done, with 52 taxa from 7 different phyla found. Even though some support for the hypotheses was found, there was not enough significant results to reject the null hypotheses. There were, however, indications that the diversity of macrofaunal communities does increase with the mangrove age, but not until many years after plantation. The few sites and replicates are probable explanations for the few significant results, which couldn't yield enough power to the tests. Therefore, this should only be regarded as a pilot study.

Further studies should increase the number of sites and/or replicates, which were beyond the possibilities of this study. For a better overview and understanding of how macrofaunal communities establish over time in the planted mangroves, there are several factors that should be considered. Future studies should measure and correct for other physical parameters that might have a higher impact on the diversity than the age of the mangrove area by itself. Also, more focus should be put in the role and function played by the found species themselves, rather than only the diversity index, as some species affect and are affected more by disturbances in their environment. Future studies should also sample the sites during all seasons of the year to get a more extensive overview of the macrofaunal community structure in the planted mangrove sites along the Jiulongjiang Estuary

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Appendix 1 - Pictures of the study area and the methods

Here follow some pictures taken at the study site and of the methods used for this study.

The sampling sites

The 18-year old mangrove site.

The 31-year old mangrove site.

The 54-year old mangrove site.

The bare mudflats.

The surrounding area

Buildings and aquaculture.

Aquaculture.

Trash washed in by the high tide.

Unknown sewage disposed directly into the mangroves and the Jiulongjiang Estuary.

The mangroves and the coastline.

Sprouts of the mangrove species *Kandelia obovate*.

The sampling procedures

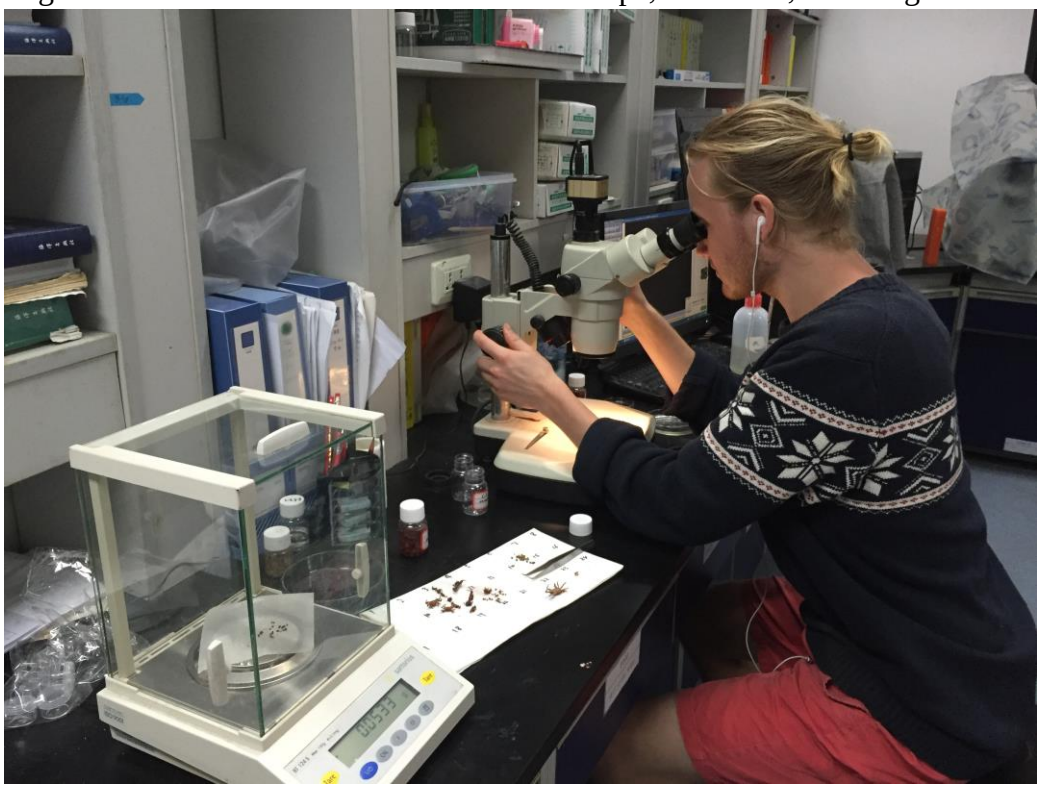
Metal frame placed haphazardly in the sediment.

Sample being dug up and placed in a plastic bag.

The treatment of samples

Samples are washed through a 1 mm sieve in the field.

Organisms are examined with a stereo microscope, identified, and weighed.

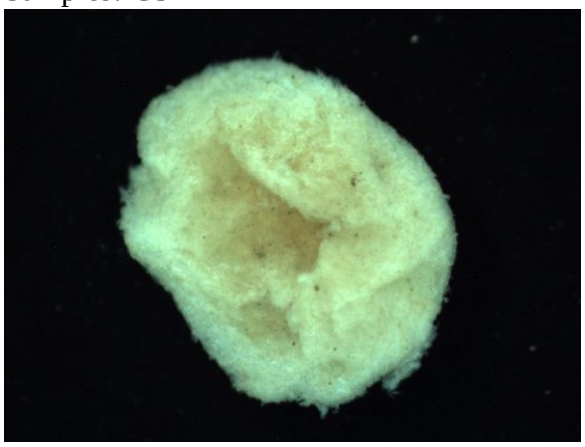


Appendix 2 – Species list

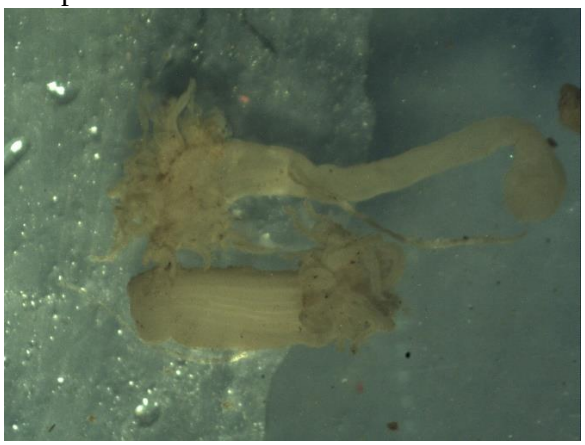
This is a list sorted by phylum of the species identified in the samples. The taxonomical details follow the World Register of Marine Species (WoRMS Editorial Board 2017). All pictures are taken by the author during the time of the project unless otherwise is stated.

Cnidaria

Class: Anthozoa, Order: Actiniaria (Sea anemones), Species: **Unknown sp.1**
Samples**: C3**

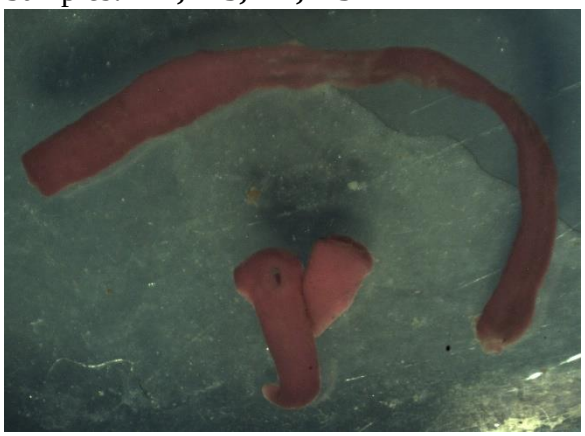


Class: Anthozoa, Order: Actiniaria (Sea anemones), Species: **Unknown sp.2**
Samples**: M1**



Nemertea

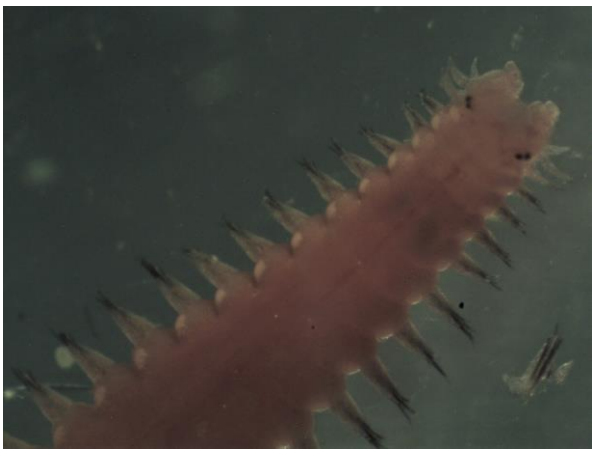
Class: Palaeonemertea, Order: *Incertae sedis*, Family: Cephalothricidae,
Species: ***Procephalothrix* sp.**
Samples**: M2, M3, H1, H3**



Annelida - Class Polychaeta

Order: Phyllodocida, Family: Nephtyidae, Species: ***Nephtys* sp.**
Samples**: C1, C2, C3 (present in all mudflat samples)**

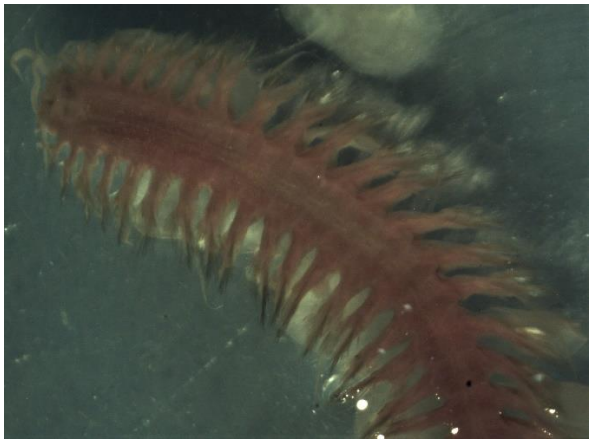

Order: Phyllodocida, Family: Nereididae, Species: ***Namalycastis abiuma***
Samples**: M2**

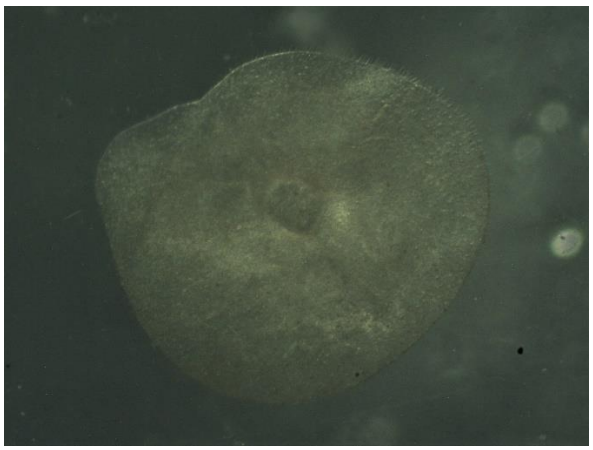



Order: Phyllodocida, Family: Nereididae, Species: **Unknown sp.**
Samples**: M1, M2, M3 (present in all medium age mangrove samples)**

Order: Phyllodocida, Family: Pilargidae, Species: ***Sigambra hanaokai***
Samples**: H1, H3**
*Photos provided by Rao Yiyong from a separate sample.


Order: Phyllodocida, Family: Polynoidae, Species: ***Lepidonotus* sp.**
Samples**: M2**

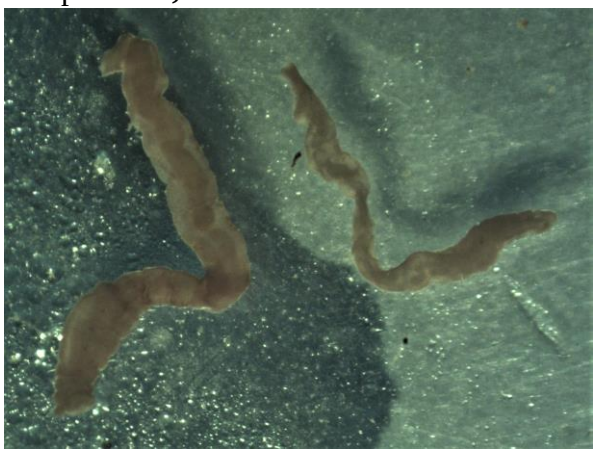


Order: Phyllodocida, Family: Phyllodocidae, Species: **Unknown sp.**
Samples**: L2**

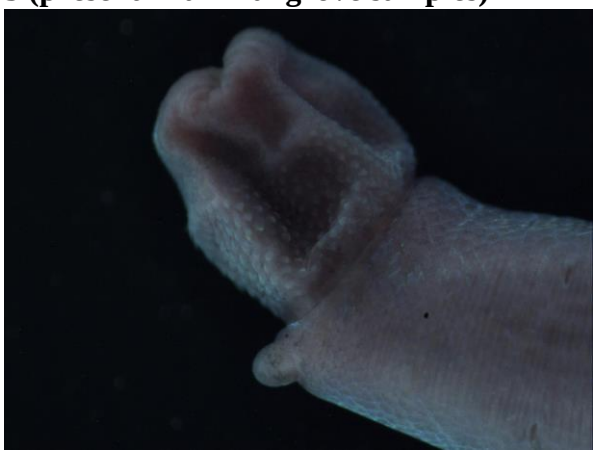

Order: Spionida, Family: Spionidae, Species: ***Polydora* sp.**
Samples**: C1**
*Photos provided by Rao Yiyong from a separate sample. Possible another species within the same genus.

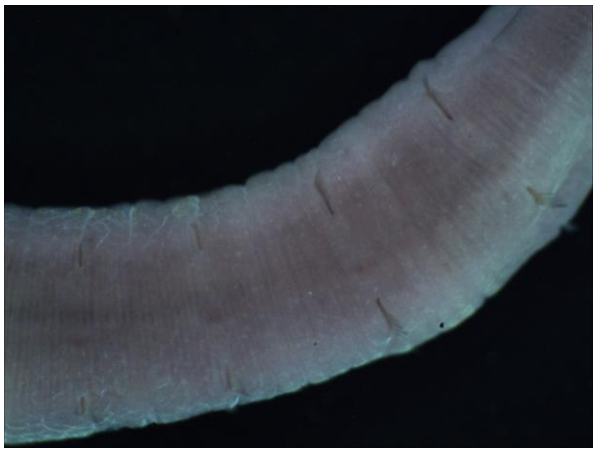

Order: Terebellida, Family: Cirratulidae, Species: ***Chaetozone setosa***
Samples**: H1, H2, H3 (present in all high age mangrove samples)**

Order: *Incertae sedis*, Family: Capitellidae, Species: ***Capitella capitata***
Samples**: H2, H3**



Order: *Incertae sedis*, Family: Capitellidae, Species: ***Notomastus latericeus***
Samples**: L1, L2, L3, M1, M2, M3, H1, H2, H3 (present in all mangrove samples)**





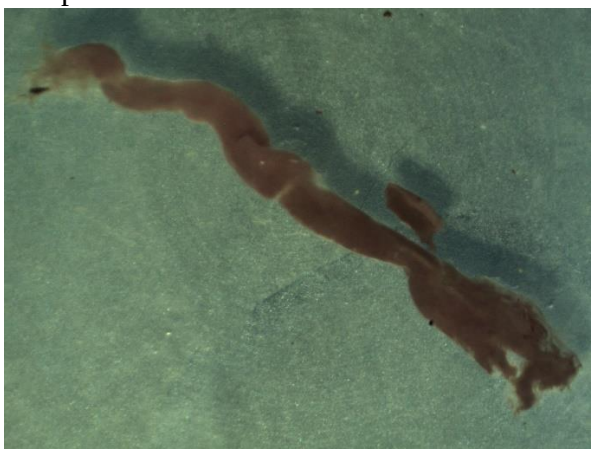
Annelida - Class: Clitellata

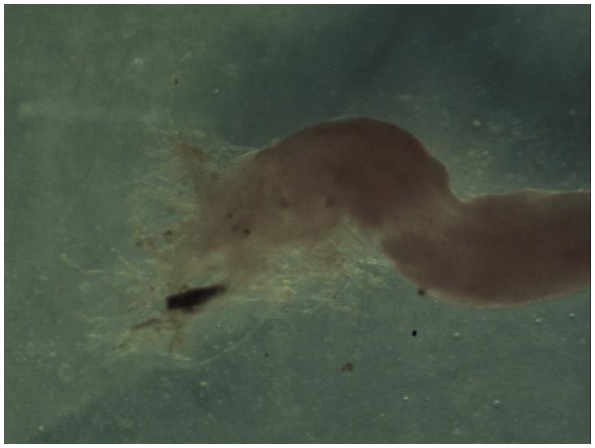
Subclass: Oligochaeta, Species: **Unknown spp.**
Samples**: C1, C2, C3, L1, M2, M3, H1, H2, H3**

Sipuncula

Class: Phascolosomatidea, Order: Phascolosomatida, Family: Phascolosomatidae,
Species: ***Phascolosoma arcuatum***
Samples**: L1, L2, L3, M1, M2, M3 (present in all low and medium age mangrove samples)**

Class: Phascolosomatidea, Order: Phascolosomatida, Family: Phascolosomatidae,
Species: ***Phascolosoma*** **sp. from H1**
Samples**: H1**

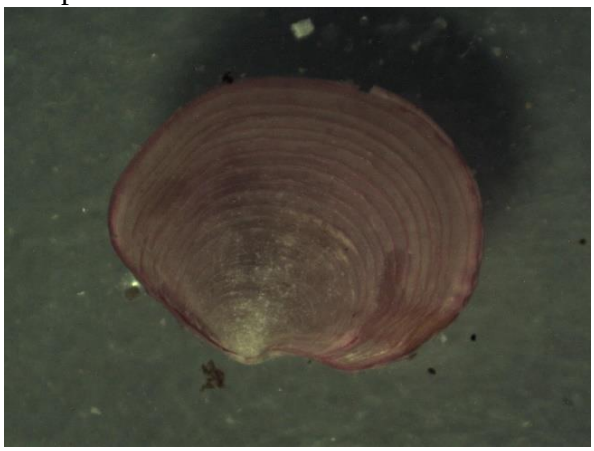




Mollusca - Class: Bivalvia

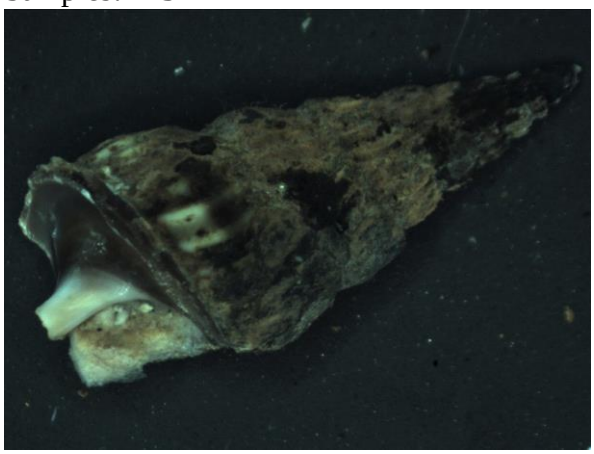
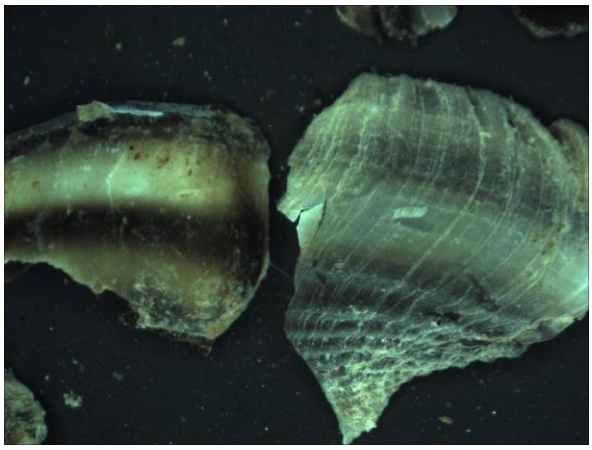
Order: Mytilida, Family: Mytilidae, Species: ***Xenostrobus atratus***
Samples**: C1, C3**

Order: Pectinida, Family: Plancunidae, Species: **Unknown sp.**
Samples**: H3**

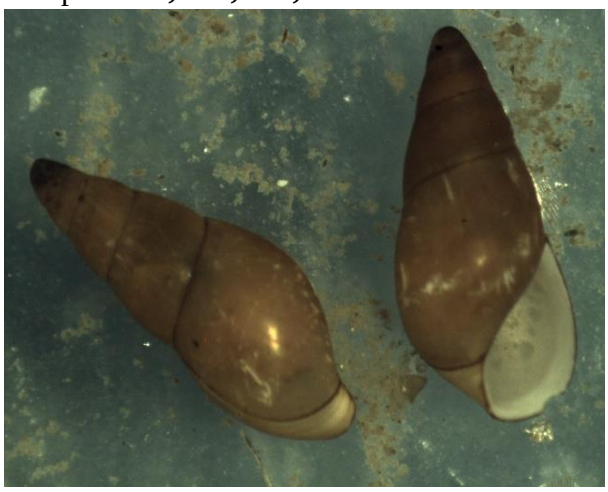


Mollusca - Class: Gastropoda

Order: Caenogastropoda, Family: Potamididae, Species: ***Cerithidea* sp.**
Samples**: M3**

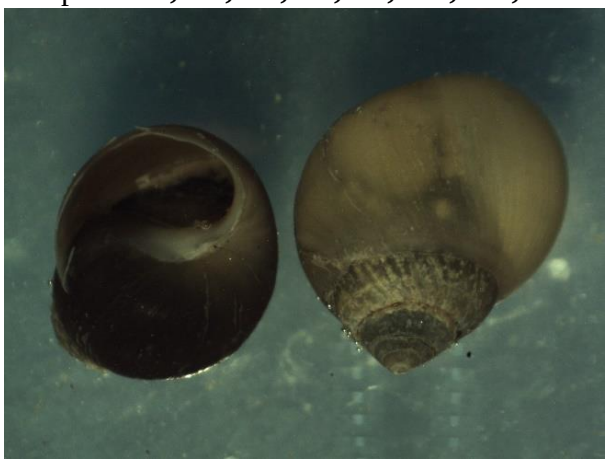



Order: *Incertae sedis*, Family: Pyramidellidae, or Order: Littorinimorpha, Family: Eulimidae, Species: **Unknown sp.**
Samples**: L3, M1, M2, M3**

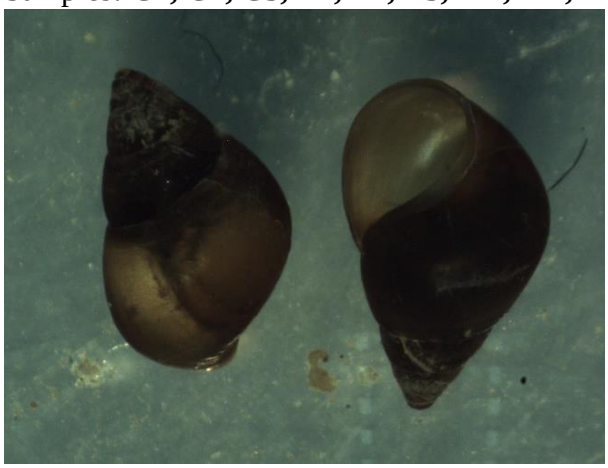


Order: Littorinimorpha, Family: Rissoidae, Species: ***Alvania* sp.**
Samples**: H1, H2**

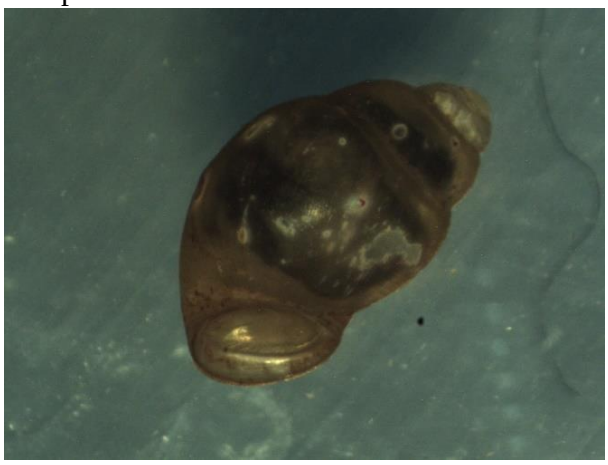
Order: Littorinimorpha, Family: Assimineidae, Species: ***Assiminea brevicula***
Samples**: C1, C2, C3, L2, L3, M2, M3, H2**



Order: Littorinimorpha, Family: Assimineidae, Species: ***Assiminea* sp.**
Samples**: C1, C2, C3, L1, L2, L3, M1, M2, M3 (absent in all high age mangrove samples)**



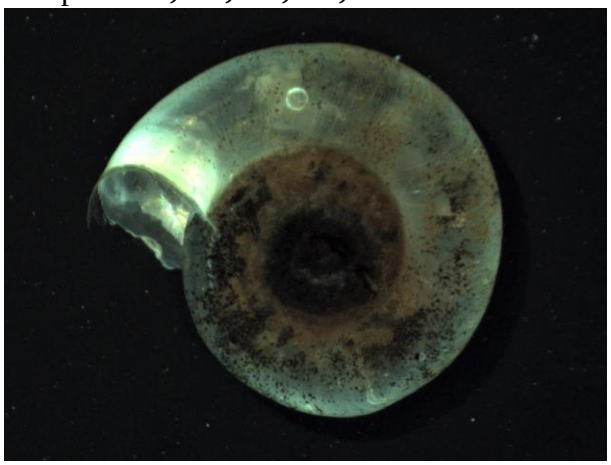
Order: Littorinimorpha, Family: Stenothyridae, Species: ***Stenothyra glabrata***
Samples**: M2**



Order: Littorinimorpha, Family: Iravadiidae, Species: ***Ceratia* sp.**
Samples**: C2**

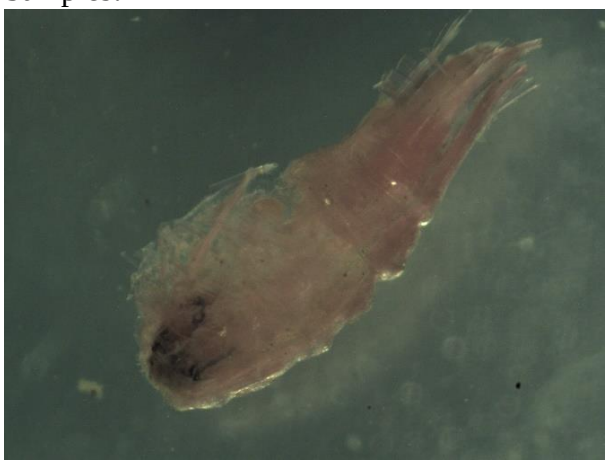
Order: Littorinimorpha, Family: Iravadiidae, Species: ***Wakauraia sakaguchii***
Samples**: L3, M1, M2, M3, H2**

Order: Littorinimorpha, Family: Tornidae, Species: ***Adeorbis plana***
Samples**: C1, C2, H1, H2, H3**



Arthropoda – Subphylum: Crustacea

Class: Hexanauplia, Subclass: Copepoda, Species: **Unknown sp.**
Samples**: M2**

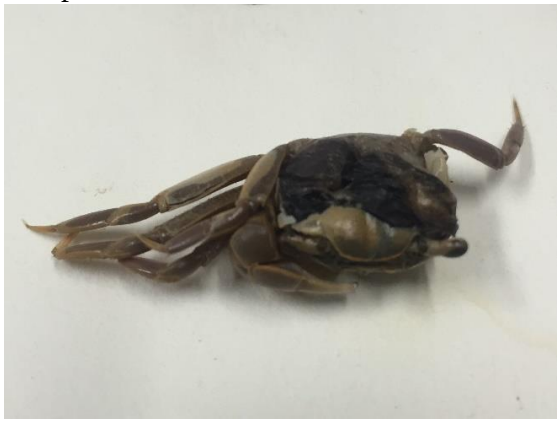


Class: Malacostraca, Order: Amphipoda, Family: Ampeliscidae, Species: ***Byblis*** **sp.**
Samples**: H1, H2, H3 (present in all high age mangrove samples)**

Class: Malacostraca, Order: Amphipoda, Family: Corophiidae, Species: ***Corophium*** **sp.**
Samples**: C2, M1, H1, H2, H3 (dominant in all high age mangrove samples)**

Class: Malacostraca, Order: Decapoda, Family: Sesarmidae, Species: ***Sesarma dehaani***
Samples**: M1**


Class: Malacostraca, Order: Decapoda, Family: Sesarmidae, Species: ***Sesarma sinensis***
Samples**: M1, M2**




Class: Malacostraca, Order: Decapoda, Family: Varunidae, Species: ***Helice tridens pingi***
Samples**: M3**


Class: Malacostraca, Order: Decapoda, Family: Varunidae, Species: ***Metaplax elegans***
Samples**: H2**

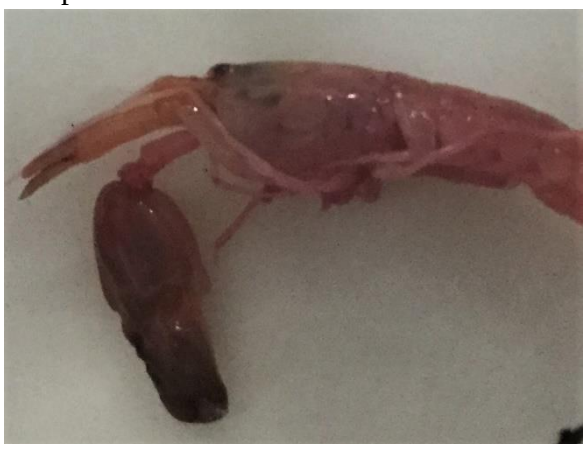
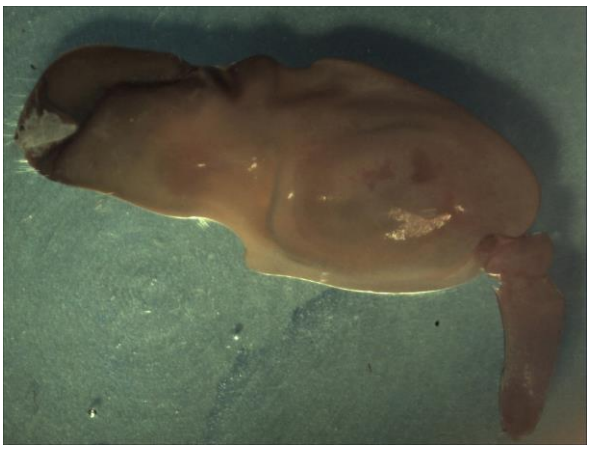
Class: Malacostraca, Order: Decapoda, Family: Varunidae, Species: ***Metaplax* sp.**
Samples**: C2**

Class: Malacostraca, Order: Decapoda, Family: Camptandriidae,
Species: ***Cleistostoma dilatatum***
Samples**: H1**

Class: Malacostraca, Order: Decapoda, Family: Dotillidae, Species: ***Ilyoplax ningpoensis***
Samples**: C1, C2, C3, M3, H1, H2**


Class: Malacostraca, Order: Decapoda, Family: Ocypodidae, Species: ***Uca arcuata***
Samples**: L2, M1, M2, M3**

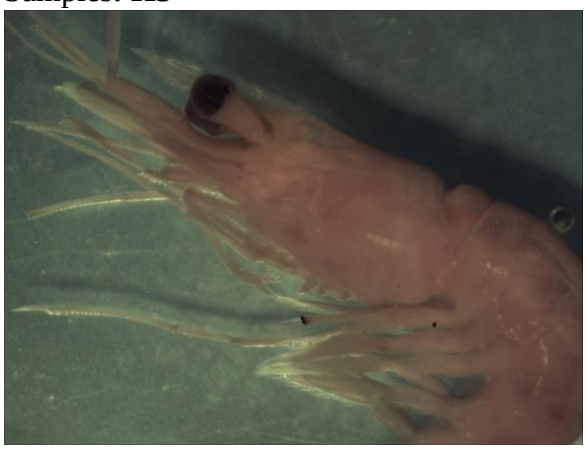

Class: Malacostraca, Order: Decapoda, Family: Alpheidae, Species: ***Alpheus* sp.**
Samples**: H1**

Class: Malacostraca, Order: Decapoda, Family: Palaemonidae,
Species: ***Exopalaemon carinicauda***

Samples**: L2, H1, H2**

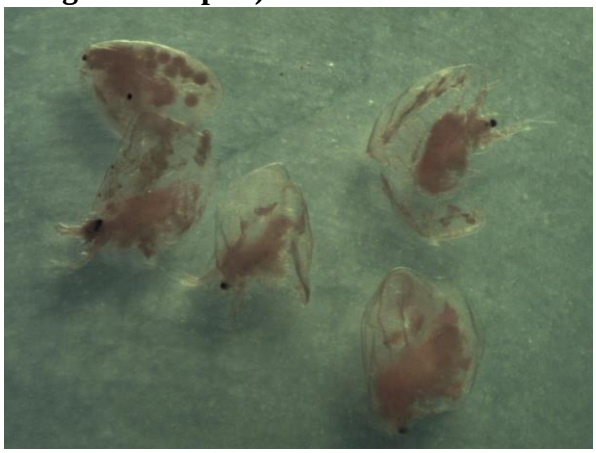
Class: Malacostraca, Order: Decapoda, Family: Palaemonidae, Species: ***Macrobrachium* sp.**
Samples**: H3**



Class: Malacostraca, Order: Decapoda, Family: Laomediidae, Species: ***Laomedia astacina***
Samples**: M1, M2, M3, H1, H2**

Class: Malacostraca, Order: Tanaidacea, Family: Apseudidae, Species: ***Apseudes* sp.**
Samples**: H1, H2**

Class: Ostracoda, Species: **Unknown sp/spp.**
Samples**: H1, H2, H3 (present in all high age mangrove samples)**



Arthropoda – Subphylum: Hexapoda

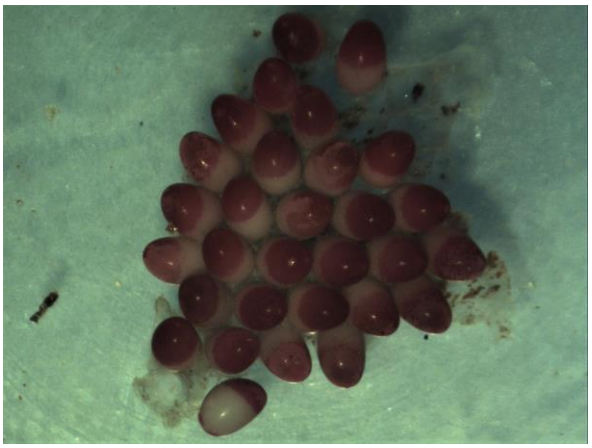
Class: Insecta, Order: Diptera, Family: Chironomidae,
Species: **Unknown sp. (mosquito larvae)**
Samples**: H1, H2, H3 (present and dominant after *Corophium* sp. in all high age mangrove samples)**

Class: Insecta, Order: Diptera, Family: Dolichopodidae, Species: **Unknown sp. (larvae)**
Samples**: C1, C2, C3, L1, L2, L3, M1, M2, M3, H1, H2, H3 (present in all samples)**

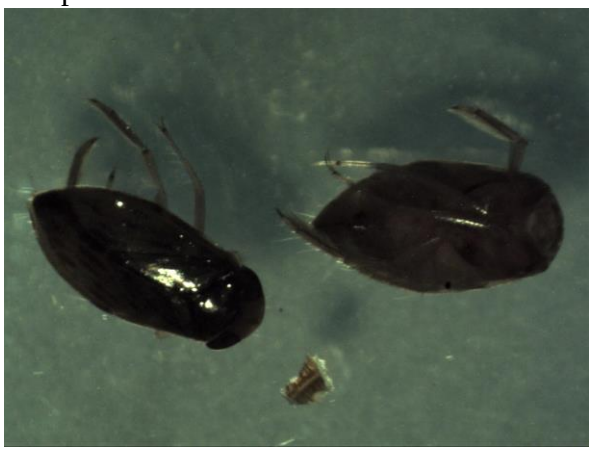
Class: Insecta, Order: Diptera, Species: **Unknown spp. (pupae)**
Samples**: L2, M3, H3**

Class: Insecta, Order: Hemiptera, Suborder: Heteroptera, Family: Naucoridae,
Species: **Unknown sp.**
Samples**: H2**



Class: Insecta, Order: Hemiptera, Suborder: Heteroptera, Family: Corixidae,
Species: **Unknown sp.1**
Samples**: H1**



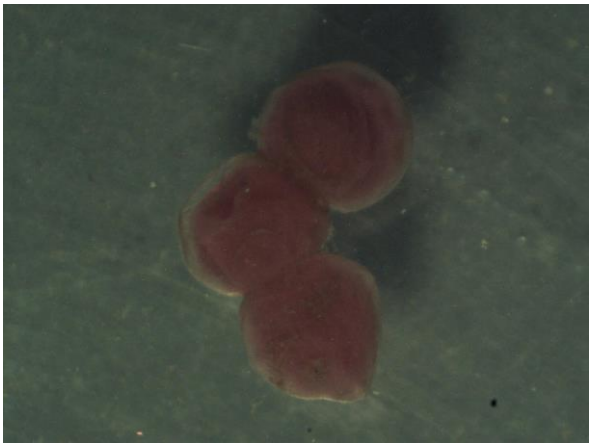
Class: Insecta, Order: Hemiptera, Suborder: Heteroptera, Species: **Unknown sp. (nymph)**
Samples**: H1, H3**

Class: Insecta, Order: Odonata, Suborder: Zygoptera, Species: **Unknown sp.**
Samples**: H1, H2, H3 (present in all high age mangrove species)**

Chordata

Class: Actinoterygii, Order: Perciformes, Family: Gobiidae,
Species: ***Boleophtalmus pectinirostris*** **(mudskipper)**
Samples**: M2, H1, H2, H3 (also visually observed at the mudflats in site C)**



group	species	c1	c1ww	c2	c2ww	c3	c3ww	l1	l1ww	l2	l2ww	l3	l3ww	m1	m1ww	m2	m2ww	m3	m3ww	h1	h1ww	h2	h2ww	h3	h3ww
Cnidaria	Actiniaria sp.1					1	0,0014																		
Cnidaria	Actiniaria sp.2													2	0,0016										
Nemertea	*Procephalothrix* sp.															2	0,0764	2	0,0652	1	0,3914			2	0,0218
Polychaeta	*Nephtys* sp.	4	0	1	0,0166	6	0,0301																		
Polychaeta	*Namalycastis abiuma*															1	0,006								
Polychaeta	Nereididae sp.													3	0,0005	5	0,0061	1	0						
Polychaeta	*Sigambra hanaokai*																			1	0,0005			3	0,0006
Polychaeta	*Lepidonotus* sp.															1	0,0088								
Polychaeta	Phyllodocidae sp.									1	0,0577														
Polychaeta	*Polydora* sp.	1	0																						
Polychaeta	*Chaetozone setosa*																			3	0	8	0	21	0,0017
Polychaeta	*Capitella capitata*																					2	0	2	0
Polychaeta	*Notomastus latericeus*							41	3,2683	28	1,5745	0	0,0378	9	0,0542	14	1,3532	17	2,396	16	0,9519	22	1,2011	15	1,2195
Oligochaeta	Oligochaeta spp.	6	0,0006	2	0	1	0	6	0,0015							9	0,0043	7	0,0171	6	0,0007	20	0,0017	13	0,0026
Sipuncula	*Phascolosoma arcuatum*							1	0,0273	3	0,8557	7	1,4608	8	0,0584	19	0,5673	20	0,5539						
Sipuncula	*Phascolosoma* sp.																			2	0,005				
Bivalvia	*Xenostrobus atratus*	1	0,0019			1	0,002																		
Bivalvia	Placunidae sp.																							1	0,0044
Gastropoda	*Cerithidea* sp.																	1	0,7349						
Gastropoda	Pyramidellidae or Eulimidae sp.											2	0,0125	2	0,0173	11	0,0883	3	0,0253						
Gastropoda	*Alvania* sp.																			2	0,0266	6	0,0669		
Gastropoda	*Assiminea brevicula*	16	0,2223	19	0,3181	11	0,2037			6	0,1543	2	0,0479			8	0,038	4	0,0093			2	0,0428		
Gastropoda	*Assiminea* sp.	15	0,0722	13	0,0534	16	0,0773	1	0,0024	6	0,0234	1	0,0014	3	0,0084	76	0,7299	34	0,1721						
Gastropoda	*Stenothyra glabrata*															1	0,0111								
Gastropoda	*Ceratia* sp.			2	0,0242																				
Gastropoda	*Wakauraia sakaguchii*											1	0,0077	2	0,0235	26	0,3779	1	0,0138			2	0,0195		
Gastropoda	*Adeorbis plana*	7	0,0077	1	0,0013															1	0,0154	1	0,014	1	0,0006
Crustacea	Copepoda sp.															1	0								
Crustacea	*Byblis* sp.																			1	0	13	0,001	5	0
Crustacea	*Corophium* sp.			1	0,0002									2	0,0053					210	0,3693	347	0,6558	148	0,2534
Crustacea	*Sesarma dehaani*													1	0,0287										
Crustacea	*Sesarma sinensis*													1	1,4022	2	0,1713								
Crustacea	*Helice tridens pingi*																	2	1,7178						
Crustacea	*Metaplax elegans*																					1	0,3847		
Crustacea	*Metaplax* sp.			1	0,7158																				
Crustacea	*Cleistostoma dilatatum*																			2	0,1779				
Crustacea	*Ilyoplax ningpoensis*	17	2,0024	12	1,3632	17	3,6416											1	0,0051	2	0,0946	3	0,543		
Crustacea	*Uca arcuata*									1	0,0046			1	0,0123	4	3,2414	2	0,3715						
Crustacea	*Alpheus* sp.																			2	0,3465				
Crustacea	*Exopalaemon carinicauda*									1	0,0115									8	0,0952	2	0,0225		
Crustacea	*Macrobrachium* sp.																							4	0,6942
Crustacea	*Laomedia astacina*													1	0,0059	1	0,0752	1	0,1556	1	0,0945	1	0,5443		
Crustacea	*Apseudes* sp.																			39	0,2597	1	0,0052		
Crustacea	Ostrocoda sp./spp.																			5	0,0007	1	0	2	0
Insecta	Chironomidae sp. larvae																			20	0,0135	91	0,0587	78	0,0645
Insecta	Dolichopodidae sp. larvae	3	0,0246	1	0,0083	1	0,0014	5	0,0153	3	0,0029	3	0,0087	15	0,0406	18	0,057	3	0,025	9	0,0664	11	0,0497	25	0,1941
Insecta	Diptera spp. pupae									2	0,001							2	0,0023					2	0,0017
Insecta	Naucoridae sp.																					1	0,1492		
Insecta	Corixidae sp.																			2	0,0032				
Insecta	Heteroptera sp. nymph																			7	0,0064			2	0,0007
Insecta	Zygoptera sp.																			2	0,0579	4	0,0539	6	0,1041
Chordata	*Boleophthalmus pectinirostris*															1	1,214			4	0,0021	6	0,0537	1	0,0279

Appendix 4 – R-script

This is the R-script written by the author and used for calculations, the analysis of data and generating plots. Figures and results from the tests for normality and equality of variances can be found in Appendix 5.

```
########## Macrobenthic fauna ##########
rm(list=ls())

### Import data
setwd('C:/Users/Simon/Documents/macrobenthos')
data1 <- read.csv('benthosspecies.csv', sep = ";", dec=",")
data2 <- read.csv('benthosspeciestot.csv', sep = ";", dec=",")

### Calculations
### Abundance, biomass and shannon-diversity
abundance3 <- rep(0, 12)
biomass3 <- rep(0, 12)
shannon.abu3 <- rep(0, 12)
shannon.bio3 <- rep(0, 12)
for(j in 1:12) {
  abundance3[j] <- sum(data1[1:52,j*2+1], na.rm=T)
  biomass3[j] <- sum(data1[1:52,j*2+2], na.rm=T)
  for(i in 1:52) {
    if(!is.na(data1[i,j*2+1])) {
      if(data1[i,j*2+1] > 0) {
        shannon.abu3[j] <- shannon.abu3[j] -
(data1[i,j*2+1]/abundance3[j])*log(data1[i,j*2+1]/abundance3[j])
      }
    }
    if(!is.na(data1[i,j*2+2])) {
      if(data1[i,j*2+2] > 0) {
        shannon.bio3[j] <- shannon.bio3[j] -
(data1[i,j*2+2]/biomass3[j])*log(data1[i,j*2+2]/biomass3[j])
      }
    }
  }
}

# Total abundance, biomass and shannon-diversity
tot.abu <- rep(0, 4)
tot.bio <- rep(0, 4)
shannon.abu.tot <- rep(0, 4)
shannon.bio.tot <- rep(0, 4)
for(j in 1:4) {
  tot.abu[j] <- sum(data2[1:52,j*2+1], na.rm=T)
  tot.bio[j] <- sum(data2[1:52,j*2+2], na.rm=T)
  for(i in 1:52) {
    if(!is.na(data2[i,j*2+1])) {
      if(data2[i,j*2+1] > 0) {
        shannon.abu.tot[j] <- shannon.abu.tot[j] -
(data2[i,j*2+1]/tot.abu[j])*log(data2[i,j*2+1]/tot.abu[j])
      }
    }
    if(!is.na(data2[i,j*2+2])) {
      if(data2[i,j*2+2] > 0) {
        shannon.bio.tot[j] <- shannon.bio.tot[j] -
(data2[i,j*2+2]/tot.bio[j])*log(data2[i,j*2+2]/tot.bio[j])
      }
    }
  }
}

# Prepare data
age1 <- c(18, 18, 18, 31, 31, 31, 54, 54, 54)
abundance1 <- abundance3[4:12]
```

```
biomass1 <- biomass3[4:12]
shannon.abu1 <- shannon.abu3[4:12]
shannon.bio1 <- shannon.bio3[4:12]
age2 <- c(18, 31, 54)
shannon.abu2 <- shannon.abu.tot[2:4]
shannon.bio2 <- shannon.bio.tot[2:4]
richness2 <- c(12, 25, 31)
age3 <- factor(c(0, 0, 0, 18, 18, 18, 31, 31, 31, 54, 54, 54))

### Linear regression
# Abundance
plot(age1, abundance1)
abundance1.lm <- lm(abundance1~age1)
summary(abundance1.lm)
abline(abundance1.lm)

# Biomass
plot(age1, biomass1)
biomass1.lm <- lm(biomass1~age1)
summary(biomass1.lm)
abline(biomass1.lm)

# Species richness
plot(age2, richness2)
richness2.lm <- lm(richness2~age2)
summary(richness2.lm)
abline(richness2.lm)

# Shannon Abundance
plot(age1, shannon.abu1)
shannon.abu1.lm <- lm(shannon.abu1~age1)
summary(shannon.abu1.lm)
abline(shannon.abu1.lm)

# Total Shannon Abundance
plot(age2, shannon.abu2)
shannon.abu2.lm <- lm(shannon.abu2~age2)
summary(shannon.abu2.lm)
abline(shannon.abu2.lm)

# Shannon Biomass
plot(age1, shannon.bio1)
shannon.bio1.lm <- lm(shannon.bio1~age1)
summary(shannon.bio1.lm)
abline(shannon.bio1.lm)

# Total Shannon Biomass
plot(age2, shannon.bio2)
shannon.bio2.lm <- lm(shannon.bio2~age2)
summary(shannon.bio2.lm)
abline(shannon.bio2.lm)

### ANOVA
# Abundance
aov.abundance3 <- aov(abundance3~age3)
summary(aov.abundance3)
TukeyHSD(aov.abundance3)
boxplot(abundance3~age3)

# Biomass
aov.biomass3 <- aov(biomass3~age3)
summary(aov.biomass3)
TukeyHSD(aov.biomass3)
boxplot(biomass3~age3)

# Shannon Abundance
aov.shannon.abu3 <- aov(shannon.abu3~age3)
summary(aov.shannon.abu3)
TukeyHSD(aov.shannon.abu3)
```

```
boxplot(shannon.abu3~age3)

# Shannon Biomass
aov.shannon.bio3 <- aov(shannon.bio3~age3)
summary(aov.shannon.bio3)
TukeyHSD(aov.shannon.bio3)
boxplot(shannon.bio3~age3)

### Tests for normality and equality of variance
op <- par(mfrow=c(1,2))
## Linear regressions
# Abundance
hist(residuals(abundance1.lm), breaks=10)
shapiro.test(residuals(abundance1.lm))
qqnorm(residuals(abundance1.lm))
qqline(residuals(abundance1.lm))

# Biomass
hist(residuals(biomass1.lm), breaks=10)
shapiro.test(residuals(biomass1.lm))
qqnorm(residuals(biomass1.lm))
qqline(residuals(biomass1.lm))

# Species richness
hist(residuals(richness2.lm), breaks=10)
shapiro.test(residuals(richness2.lm))
qqnorm(residuals(richness2.lm))
qqline(residuals(richness2.lm))

# Shannon abundance
hist(residuals(shannon.abu1.lm), breaks=10)
shapiro.test(residuals(shannon.abu1.lm))
qqnorm(residuals(shannon.abu1.lm))
qqline(residuals(shannon.abu1.lm))

# Total Shannon abundance
hist(residuals(shannon.abu2.lm), breaks=10)
shapiro.test(residuals(shannon.abu2.lm))
qqnorm(residuals(shannon.abu2.lm))
qqline(residuals(shannon.abu2.lm))

# Shannon biomass
hist(residuals(shannon.bio1.lm), breaks=10)
shapiro.test(residuals(shannon.bio1.lm))
qqnorm(residuals(shannon.bio1.lm))
qqline(residuals(shannon.bio1.lm))

# Total Shannon biomass
hist(residuals(shannon.bio2.lm), breaks=10)
shapiro.test(residuals(shannon.bio2.lm))
qqnorm(residuals(shannon.bio2.lm))
qqline(residuals(shannon.bio2.lm))

## ANOVAs
library(car)
# Abundance
hist(residuals(aov.abundance3), breaks=10)
shapiro.test(residuals(aov.abundance3))
qqnorm(residuals(aov.abundance3))
qqline(residuals(aov.abundance3))
leveneTest(abundance3, age3)

# Biomass
hist(residuals(aov.biomass3), breaks=10)
shapiro.test(residuals(aov.biomass3))
qqnorm(residuals(aov.biomass3))
qqline(residuals(aov.biomass3))
leveneTest(biomass3, age3)
```

```
# Shannon abundance
hist(residuals(aov.shannon.abu3), breaks=10)
shapiro.test(residuals(aov.shannon.abu3))
qqnorm(residuals(aov.shannon.abu3))
qqline(residuals(aov.shannon.abu3))
leveneTest(shannon.abu3, age3)

# Shannon biomass
hist(residuals(aov.shannon.bio3), breaks=10)
shapiro.test(residuals(aov.shannon.bio3))
qqnorm(residuals(aov.shannon.bio3))
qqline(residuals(aov.shannon.bio3))
leveneTest(shannon.bio3, age3)

# Export with width=1000 and height=559
par(op)

### Plots
## Piecharts
data3 <- read.csv('benthosspeciesgroups.csv', sep = ";", dec=",") # Total
for each site and group, summarized with excel
op <- par(mfrow=c(2, 4))

# Mudflats Abundance
pct <- round(data3[,2]/sum(data3[,2])*100)
lbls <- paste(data3[,1], pct, "%")
for(k in 1:10) {
  if(pct[k] < 0.5) {
    lbls[k] <- NA
  }
}
pie(data3[,2], labels=lbls, main="", cex=1.5)
mtext("Mudflats\nAbundance", font=2, line=-4)

# Mangrove 18 years Abundance
pct <- round(data3[,4]/sum(data3[,4])*100)
lbls <- paste(data3[,1], pct, "%")
for(k in 1:10) {
  if(pct[k] < 0.5) {
    lbls[k] <- NA
  }
}
pie(data3[,4], labels=lbls, main="", cex=1.5)
mtext("Mangrove 18 years\nAbundance", font=2, line=-4)

# Mangrove 31 years Abundance
pct <- round(data3[,6]/sum(data3[,6])*100)
lbls <- paste(data3[,1], pct, "%")
for(k in 1:10) {
  if(pct[k] < 0.5) {
    lbls[k] <- NA
  }
}
pie(data3[,6], labels=lbls, main="", cex=1.5)
mtext("Mangrove 31 years\nAbundance", font=2, line=-4)

# Mangrove 54 years Abundance
pct <- round(data3[,8]/sum(data3[,8])*100)
lbls <- paste(data3[,1], pct, "%")
for(k in 1:10) {
  if(pct[k] < 0.5) {
    lbls[k] <- NA
  }
}
pie(data3[,8], labels=lbls, main="", cex=1.5)
mtext("Mangrove 54 years\nAbundance", font=2, line=-4)

# Mudflats Biomass
pct <- round(data3[,3]/sum(data3[,3])*100)
```

```
lbls <- paste(data3[,1], pct, "%")
for(k in 1:10) {
  if(pct[k] < 0.5) {
    lbls[k] <- NA
  }
}
pie(data3[,3], labels=lbls, main="", cex=1.5)
mtext("Mudflats\nBiomass", font=2, line=-4)

# Mangrove 18 years Biomass
pct <- round(data3[,5]/sum(data3[,5])*100)
lbls <- paste(data3[,1], pct, "%")
for(k in 1:10) {
  if(pct[k] < 0.5) {
    lbls[k] <- NA
  }
}
pie(data3[,5], labels=lbls, main="", cex=1.5)
mtext("Mangrove 18 years\nBiomass", font=2, line=-4)

# Mangrove 31 years Biomass
pct <- round(data3[,7]/sum(data3[,7])*100)
lbls <- paste(data3[,1], pct, "%")
for(k in 1:10) {
  if(pct[k] < 0.5) {
    lbls[k] <- NA
  }
}
pie(data3[,7], labels=lbls, main="", cex=1.5)
mtext("Mangrove 31 years\nBiomass", font=2, line=-4)

# Mangrove 54 years Biomass
pct <- round(data3[,9]/sum(data3[,9])*100)
lbls <- paste(data3[,1], pct, "%")
for(k in 1:10) {
  if(pct[k] < 0.5) {
    lbls[k] <- NA
  }
}
pie(data3[,9], labels=lbls, main="", cex=1.5)
mtext("Mangrove 54 years\nBiomass", font=2, line=-4)

# Export from Zoom-version and crop with paint
par(op)

## Scatter plots from linear regressions
op <- par(mfrow=c(4,2), mar=c(2, 4, 1, 6), oma=c(1, 0, 0, 0))

# Abundance
plot(age1, abundance1, ylab="Abundance", xaxt="n", mtext(" A\n\n\n\n
p=0.0007\n R2=0.8227\n\n\n\n\n\n", side=4, outer=F, line=0, cex=0.7, las=2))
axis(1, at=c(18, 31, 54))
abline(abundance1.lm)

# Biomass
plot(age1, biomass1, ylab="Biomass (g)", xaxt="n", mtext(" B\n\n\n\n
p=0.9347\n R2=0.0010\n\n\n\n\n\n", side=4, outer=F, line=0, cex=0.7, las=2))
axis(1, at=c(18, 31, 54))
abline(biomass1.lm)

# Shannon Abundance
plot(age1, shannon.abu1, ylab="Shannon-diversity (abun.)", xaxt="n",
mtext(" C\n\n\n\n p=0.6316\n R2=0.0346\n\n\n\n\n\n", side=4, outer=F,
line=0, cex=0.7, las=2))
axis(1, at=c(18, 31, 54))
abline(shannon.abu1.lm)

# Shannon Biomass
```

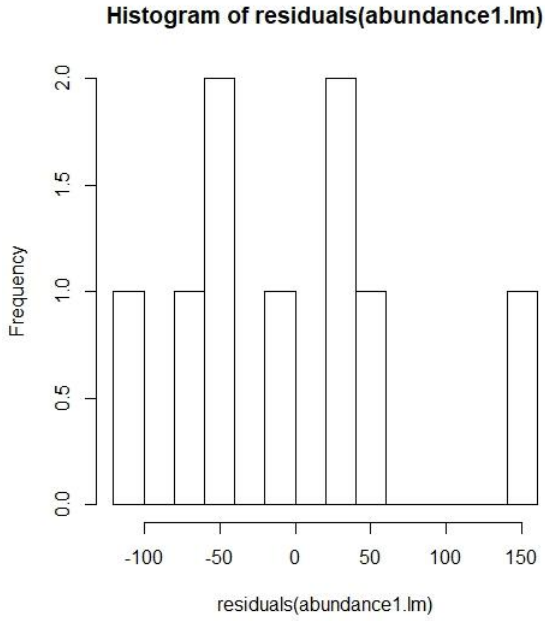
```
plot(age1, shannon.bio1, ylab="Shannon-diversity (biom.)", xaxt="n",
mtext(" D\n\n\n\n\n p=0.0135\n R2=0.6059\n\n\n\n\n\n", side=4, outer=F,
line=0, cex=0.7, las=2))
axis(1, at=c(18, 31, 54))
abline(shannon.bio1.lm)

# Tot Shannon Abundance
plot(age2, shannon.abu2, ylab="Total Shannon-diversity (abun.)", xaxt="n",
mtext(" E\n\n\n\n\n p=0.9958\n R2=4.275e-05\n\n\n\n\n\n", side=4, outer=F,
line=0, cex=0.7, las=2))
axis(1, at=c(18, 31, 54))
abline(shannon.abu2.lm)

# Tot Shannon Biomass
plot(age2, shannon.bio2, ylab="Total Shannon-diversity (biom.)", xaxt="n",
mtext(" F\n\n\n\n\n p=0.3830\n R2=0.6796\n\n\n\n\n\n", side=4, outer=F,
line=0, cex=0.7, las=2))
axis(1, at=c(18, 31, 54))
abline(shannon.bio2.lm)

# Species richness
plot(age2, richness2, ylab="Species richness", xaxt="n", mtext(" G\n\n\n\n\n
p=0.2347\n R2=0.8702\n\n\n\n\n\n", side=4, outer=F, line=0, cex=0.7, las=2))
axis(1, at=c(18, 31, 54))
abline(richness2.lm)

mtext('                Mangrove age (years)', side=1, outer=T, line=0,
cex=0.7)
# Export with width=800 and height=750
par(op)

## Box-plots from ANOVAs
op1 <- par(mfrow=c(2,2), mar = c(2, 4, 1, 6))

# Abundance
boxplot(abundance3~age3, ylab="Abundance", names=c("Mudflat (C)", "18-
years (L)", "31-years (M)", "54-years (H)"))
mtext(" A\n\n\n\n\n\n\n\n\n C-L: p=0.9880\n C-M: p=0.7595\n C-H: p=0.0016*\n
L-H: p=0.0011*\n M-H: p=0.0049*\n\n\n\n\n\n\n\n\n", side=4, outer=F,
line=0, cex=0.75, las=2)

# Biomass
boxplot(biomass3~age3, ylab="Biomass", names=c("Mudflat (C)", "18-years
(L)", "31-years (M)", "54-years (H)"))
mtext(" B\n\n\n\n\n\n\n\n\n C-L: p=0.9920\n C-M: p=0.4140\n C-H:
p=0.9987\n\n\n\n\n\n\n\n\n\n\n", side=4, outer=F, line=0, cex=0.75, las=2)

# Shannon Abundance
boxplot(shannon.abu3~age3, ylab="Shannon-diversity (abun.)",
names=c("Mudflat (C)", "18-years (L)", "31-years (M)", "54-years (H)"))
mtext(" C\n\n\n\n\n\n\n\n\n C-L: p=0.2294\n C-M: p=0.2787\n C-H: p=0.9380\n
L-M: p=0.0153*\n\n\n\n\n\n\n\n\n\n", side=4, outer=F, line=0, cex=0.75,
las=2)

# Shannon Biomass
boxplot(shannon.bio3~age3, ylab="Shannon-diversity (biom.)",
names=c("Mudflat (C)", "18-years (L)", "31-years (M)", "54-years (H)"))

mtext(" D\n\n\n\n\n\n\n\n\n C-L: p=0.9577\n C-M: p=0.2767\n C-H: p=0.0509*\n
L-H: p=0.0255*\n\n\n\n\n\n\n\n\n\n", side=4, outer=F, line=0, cex=0.75,
las=2)
# Export with width=1100 and height=800
par(op)
```

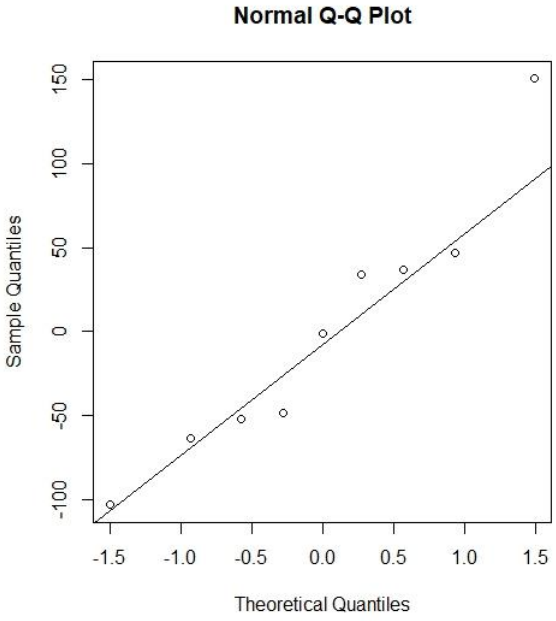
Appendix 5 – Tests for Normality and Equality of Variance

Linear Regressions

Abundance

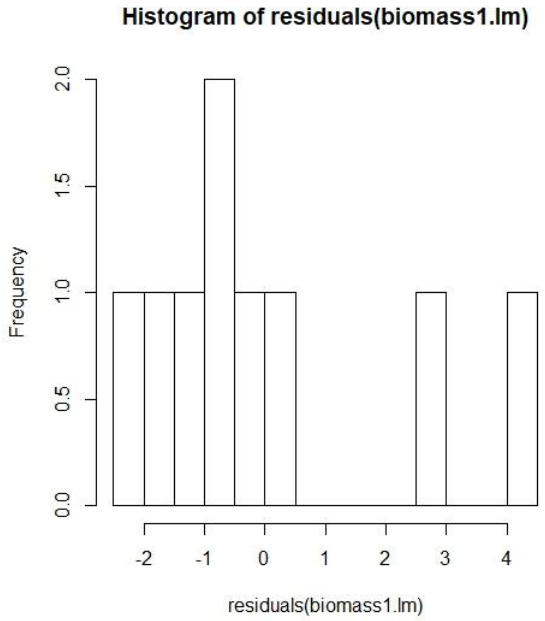
Shapiro-Wilks Normality Test: p=0.5972.

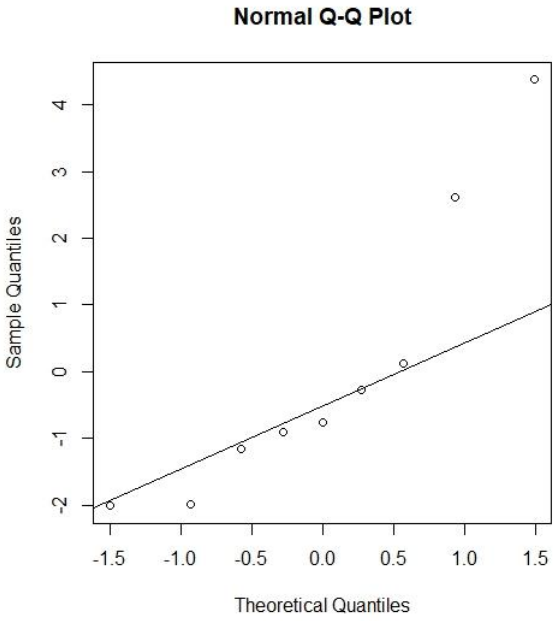




Biomass

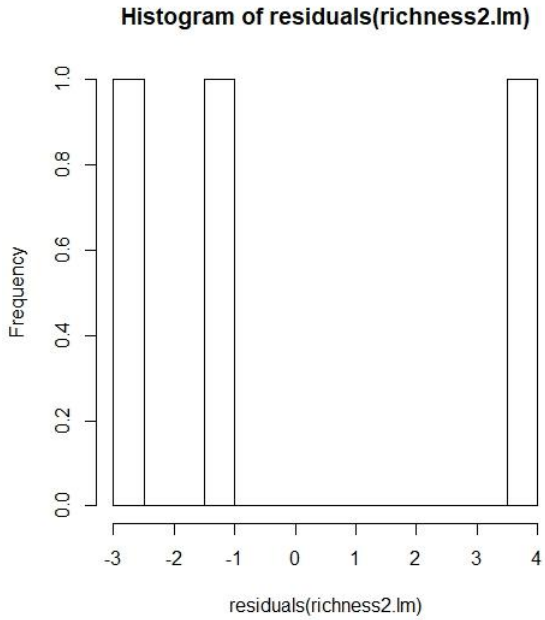
Shapiro-Wilks Normality Test: p=0.0.0597.

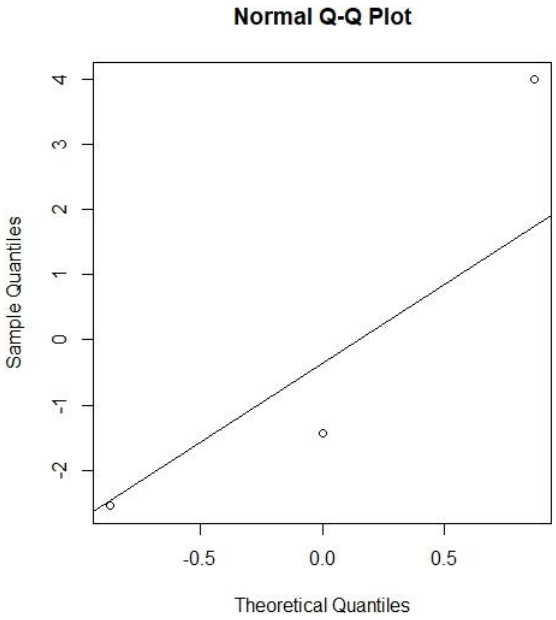




Species richness

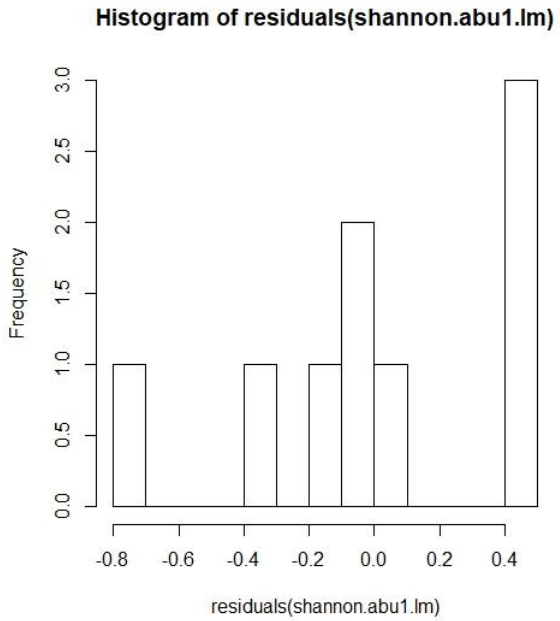
Shapiro-Wilks Normality Test: p=0.3037.

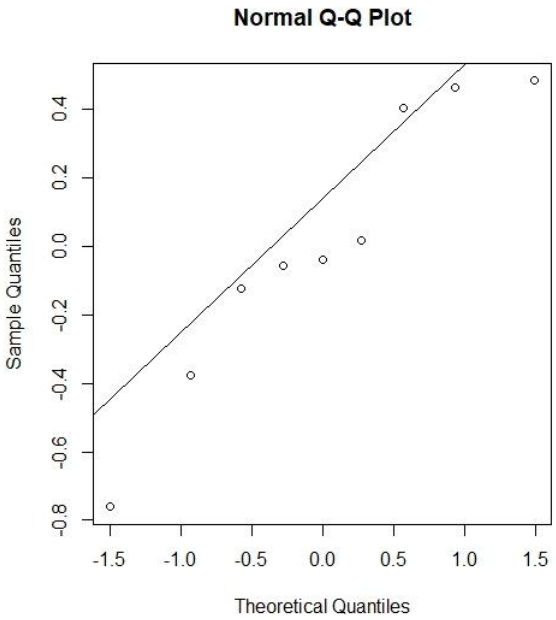




H'abu

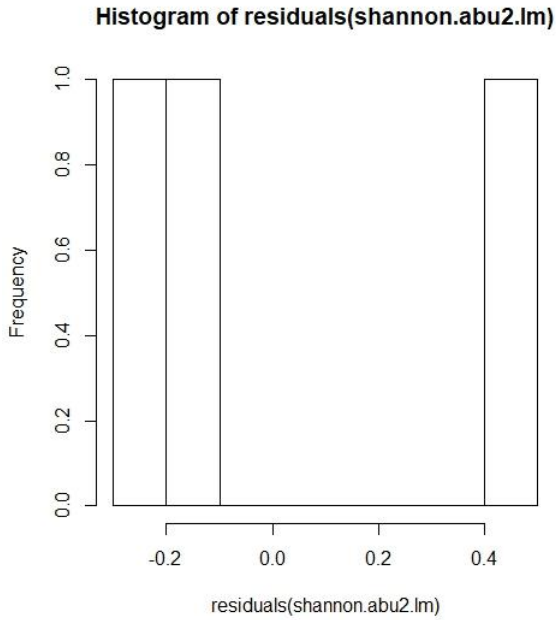
Shapiro-Wilks Normality Test: p=0.4049.

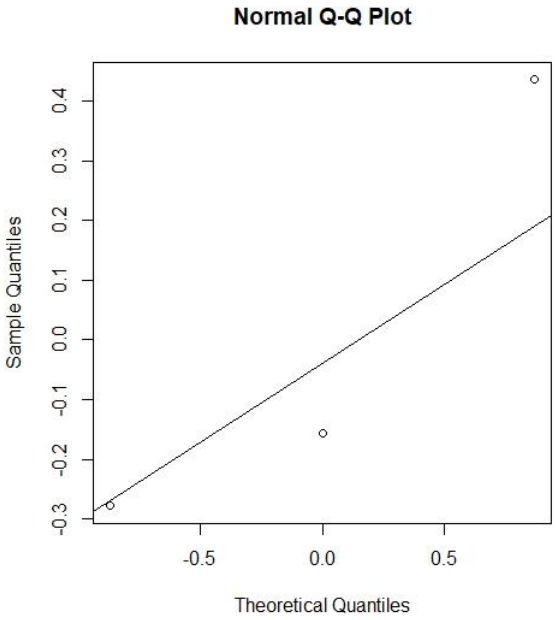




Total H'_{abu}

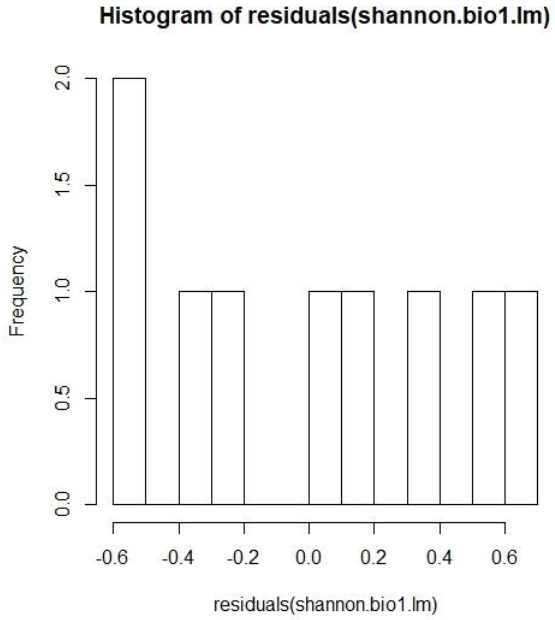
Shapiro-Wilks Normality Test: p=0.3037.

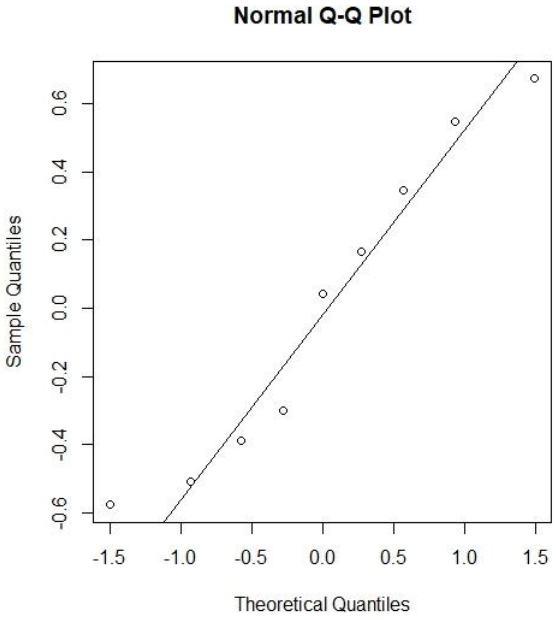




***H'*$_{bio}$**

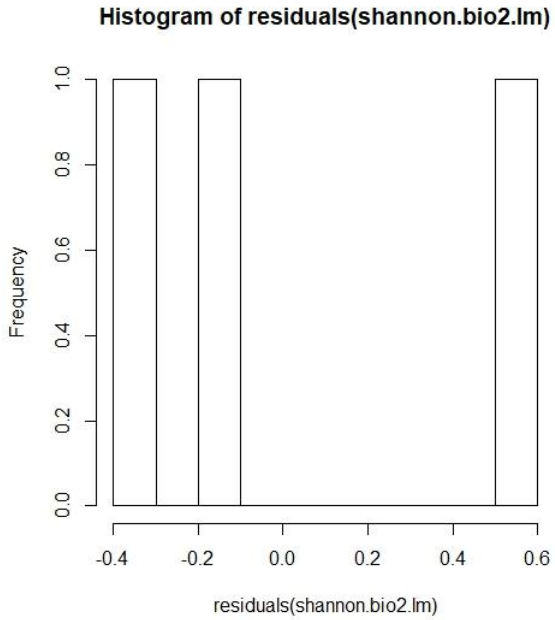
Shapiro-Wilks Normality Test: p=0.4626.

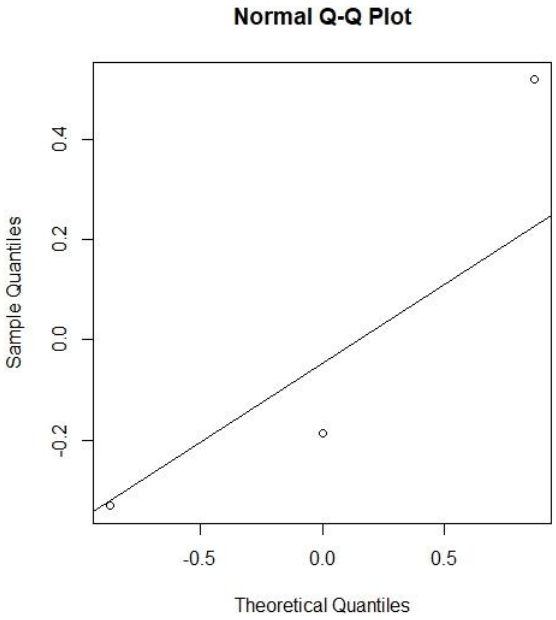




Total *H'*$_{bio}$

Shapiro-Wilks Normality Test: p=0.3037.

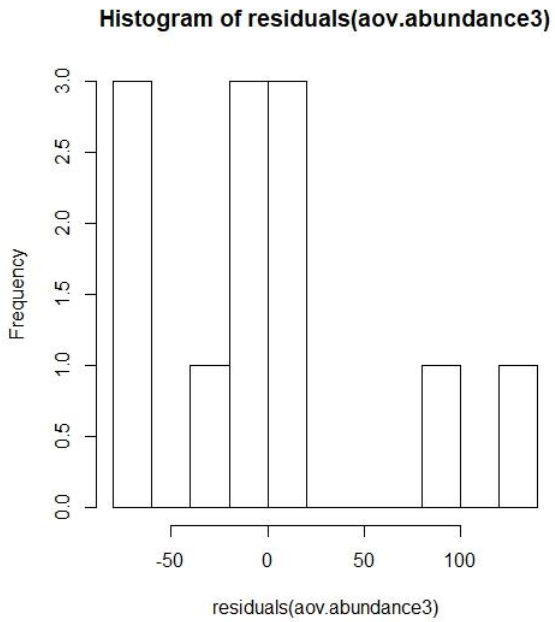


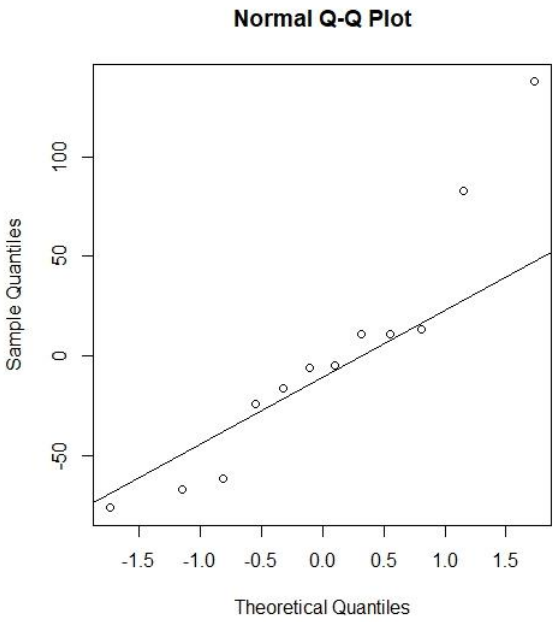


ANOVAs

Abundance

Shapiro-Wilks Normality Test: p=0.1612.

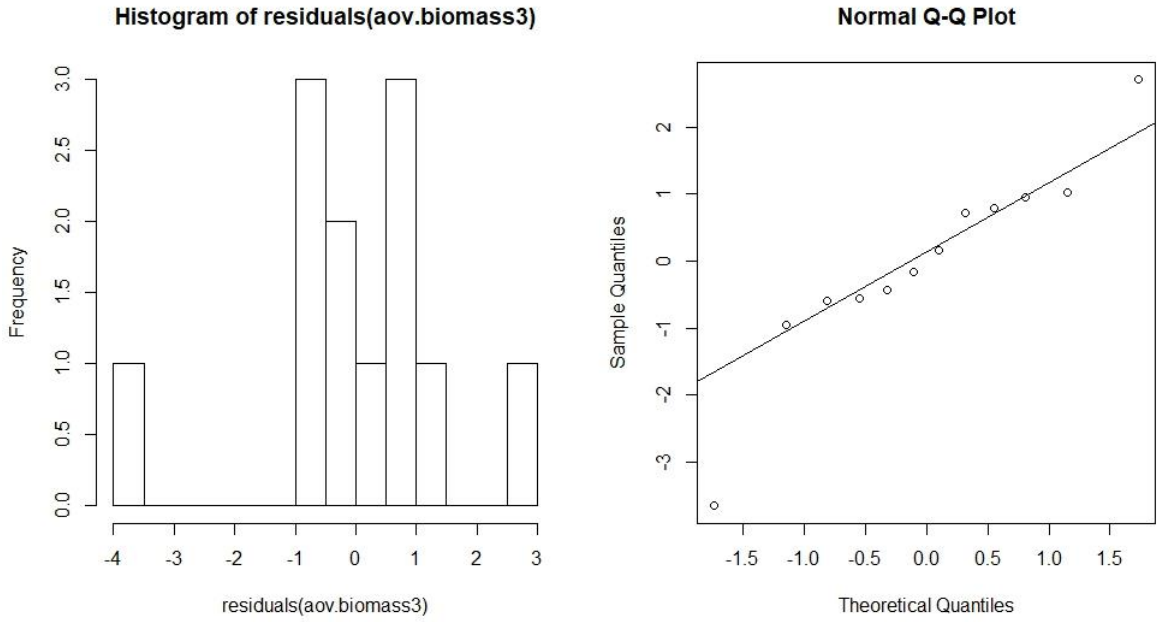




Levene's test for Equality of Variances: p=0.5449.

Biomass

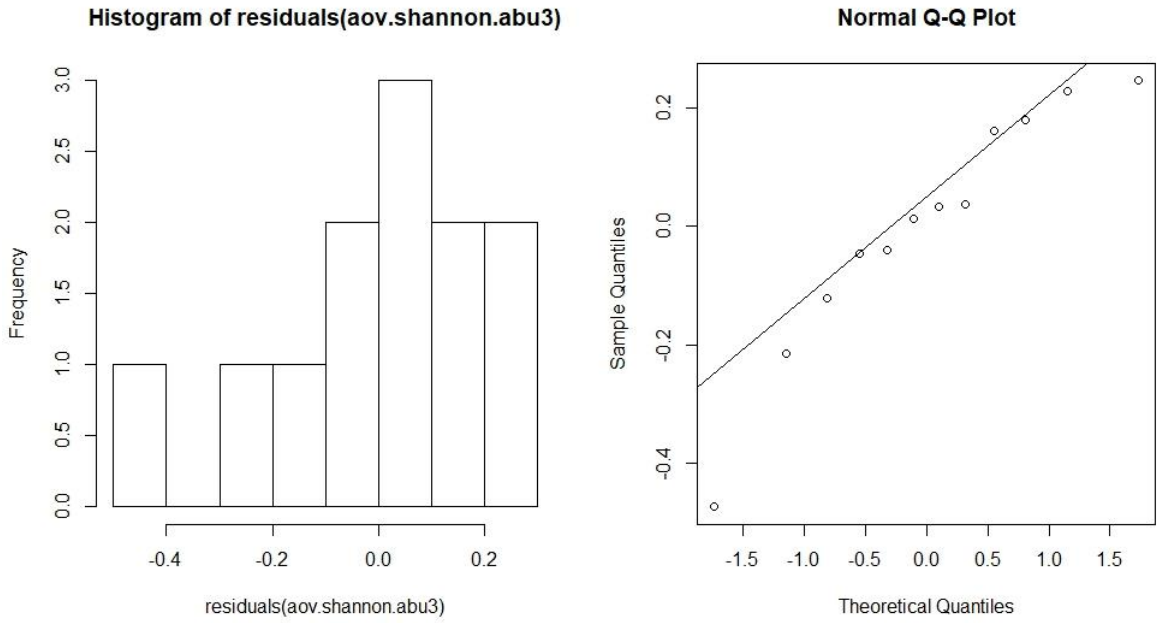
Shapiro-Wilks Normality Test: p=0.2299.



Levene's test for Equality of Variances: p=0.3727.

H'abu

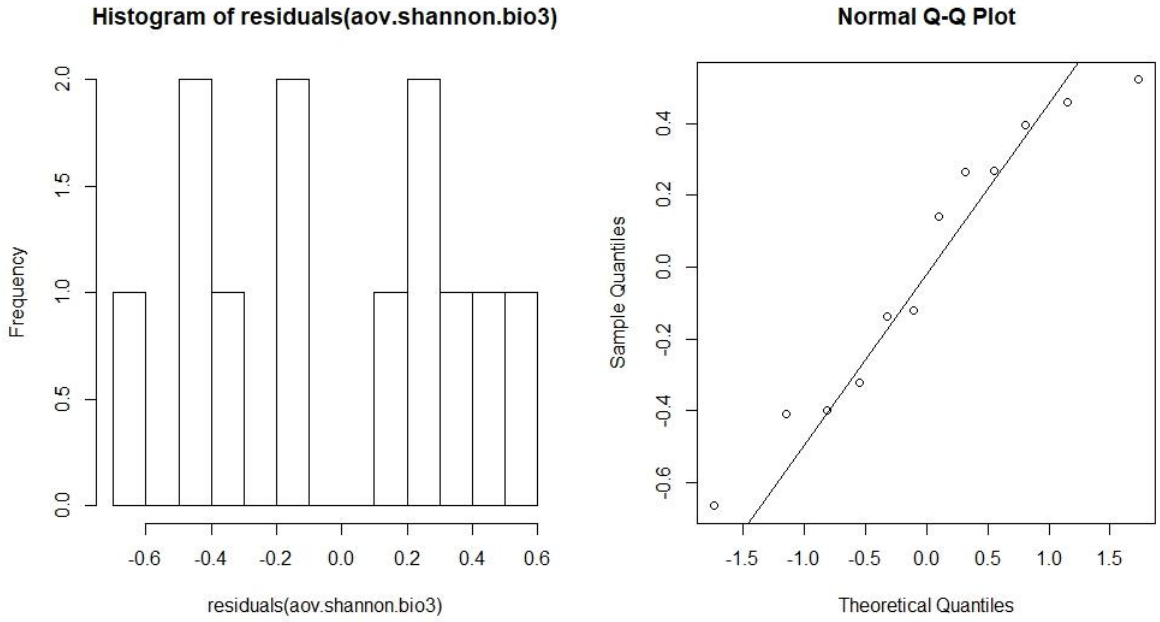
Shapiro-Wilks Normality Test: p=0.2922.



Levene's test for Equality of Variances: p=0.6877.

H'abu

Shapiro-Wilks Normality Test: p=0.4326.



Levene's test for Equality of Variances: p=0.9742.