Copepods and pigmentation
a plastic response to light?

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Master of Science Thesis

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Abstract

In an environment without hiding places, pelagic copepods utilise several potential strategies to avoid predation and increase fitness. Many of the strategies are light dependent and responses to visual predation. Light conditions vary with depth in the pelagic realm and affect diel vertical migration (DVM), habitat choice and camouflage strategies. Many pelagic organisms, including copepods, undergo DVM, but the patterns are variable. Both non-migratory and migratory species display nocturnal feeding. Copepods accumulate pigments through feeding and can appear to look green, brown or transparent. Some of the pigments can further be converted to astaxanthin by the copepods, enabling them to appear blue, yellow or red. To hide from visual predators copepods can apply strategies such as counter pigmentation and transparency. How marine copepods express the different camouflage strategies may be size dependent and or governed by internal state or light conditions, but this is not well understood.

In my thesis, I first conducted a field study in Oslofjorden to investigate how apparent pigmentation relates to depth and size within and across different copepod genera by using photography and subsequent image analysis. Secondly, I extracted pigments from copepods to investigate how pigment content correlates with depth before I compared how apparent pigmentation correlate with pigment concentration. As a final part, I created a quantifiable, controlled light setup to test whether copepods plastically control their apparent pigmentation as a response to different light intensities and compositions.

The results indicate that there were some depth effects on apparent pigmentation, but these effects were dependent on genus and genus alone explained most of the variance in the data. Apparent pigmentation increased with depth in just a few genera. However, apparent pigmentation was also dependent on size. Transparency appeared as the primary strategy for epipelagic copepods in Oslofjorden. Pigment contents tended to correlate positively with increased depth in carnivore and omnivore genera, but more unstructured patterns appeared in herbivore genera. The effect of depth on pigment content also depended on copepod genera. Apparent pigmentation and pigment concentration were not correlated. The experimental results to test if the apparent pigmentation is a function of light intensity were inconclusive and need further testing. All in all, responses in both apparent pigmentation and pigment concentrations suggest that copepods can alter their appearance, but further experiments are necessary to understand this phenomenon better.

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This thesis and the work presented in it was accomplished at the section for Aquatic Biology and Toxicology (AQUA) at the Department of Biosciences at the University of Oslo. My main supervisor was Josefin Titelman (Professor, UiO), and co-supervisors were Tom Andersen (Professor, UiO) and Jan Heuschele (Post Doc, UiO). Now, I owe you all many thanks. Your doors have always been open, and I have been welcomed into the group with open arms in a way I did not think was possible. You have allowed me to take ownership in my thesis, make mistakes and to solve them to the best of my abilities along the way. All along with you guiding me in the right direction.

Josefin, thank you for not giving up on size measurements, Grammarly, and giving me the best feedback, I could ever ask for. You have given me the opportunity to be independent, and then when I needed you, you were always there and willing to help. Thank you! Tom, this all started when you tried to teach me statistics. I knew then that I wanted to work with you, and if it had not been for you, none of this would have happened as it did. Moreover, your supervision in R and the world of statistics has been all I needed and then some. Thank you! Jan, I mean, what would I have done without you? From discussing problems along the way, joining me in fieldwork, opening the world of 3D-design and print to me, R-coding and so much more. I cannot help to wonder if I have walked in and out of your office more times than you have done yourself these last years. I am immensely lucky to have had you as my supervisor. Thank you!

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Contents

Introduction

The pelagic realm is the most prominent habitat type on earth with the volume of the world's oceans estimated to be 1.3 billion km^3 and an average depth of ~3700 m (Charette and Smith 2010). The pelagic zone starts at the low tide mark and includes the entire free water column. We divide the pelagic waters into different zones according to depth. The epipelagic zone ranges from the surface down to 200 m, mesopelagic zone 200 – 1000 m, bathypelagic zone 1000 – 4000 m, abyssopelagic zone 4000 – 6000 m and hadalpelagic zone 6000+ m. Pelagic zones are distinctly different from each other in many ways, but especially in terms of light conditions.

Light in the ocean can come directly from the sun, stars, indirectly reflected by the moon, humanmade light sources or from bioluminescence. Solar radiation provides most of the energy input to the oceans. In the euphotic zone, where light levels are sufficient to support net production, i.e. >1% of surface irradiance, the photosynthetically active radiation (PAR, wavelength 350 – 700 nm) supports almost half of the primary production on earth (Duarte and Cebrián 1996; Field 1998). The primary production creates the foundation for the pelagic ecosystems and food webs (Not et al. 2012). During photosynthesis, light is getting absorbed by photosynthetically active pigments in the phytoplankton. The water itself absorbs light, and molecules dissolved in the seawater also absorb and scatter light. The combined effect of absorption and scattering of light are known as light attenuation. The amount of dissolved and suspended matter in the seawater, therefore, determines how the light gets attenuated through the water column (Woźniak and Dera 2007). Below the euphotic zone, in the disphotic zone, light levels are too low to support net production by the phytoplankton but still enough light to support visual predation (Didyk et al. 1978).

Oligotrophic seawater transmits light at wavelengths around 470 nm to depths well below 600 m (Duntley 1962). In coastal water, increasing amounts of dissolved organic matter, phytoplankton, particulate organic matter, suspended minerals, gas bubbles and oil droplets increase the attenuation coefficient, and the light regime and penetration in coastal seawater are distinctly different from the open ocean waters. Coastal water which often is more eutrophic transmits most light at wavelengths around 500 nm. Usually, 99 % of the surface irradiance gets attenuated within the first 20 – 40 m in coastal water. In estuarine waters where runoff from land and allochthonous substances has an even more significant effect on light attenuation, light transmits best at wavelengths around 580 nm (Cohen and Forward 2002). Since the physio-chemical composition of the seawater dictates the attenuation coefficients, the attenuation in coastal waters often varies seasonally due to precipitation, run-off from surrounding land masses and seasonal phytoplankton blooms (Duntley 1962; Woźniak and Dera 2007). Daily fluctuations in light in the sea also occur due to weather conditions and the seasonal changes in solar azimuth angle. The amount of light that penetrates the water surface influences many aspects of life in the pelagic systems.

Light has a bottom-up effect in pelagic systems via photosynthesis and productivity. Light also has a top-down effect as many predators in pelagic systems are visual predators and rely on light for prey detection (Aksnes et al. 2004; Kaartvedt 2008). A range of factors determines the visual range of predators; e.g. eye physiology, surface light, water turbidity, size and contrast of the prey, and the distance between predator and prey (Aksnes and Giske 1993; Aksnes and Utne 1997). In the epipelagic zone, visual predation is mainly dependent on sunlight. In the mesopelagic zone and more profound depth, the predators rely increasingly on bioluminescence. Approximately 90 % of mesopelagic species are bioluminescent (Vestheim and Kaartvedt 2006). The predator’s ability to detect prey visually is thereby closely related to turbidity and depth as well as the prey’s ability to avoid detection.

Copepods at different life stages can reside at, or migrate between, different depths to optimise their fitness (Holliland et al. 2012). Predation risk is related to visibility, which again is related to size. Thus the well-illuminated surface waters with abundant food supplies, higher temperature and low predation risk from bigger copepods due to pressure from visual predators, are selected for by nauplii stages, smaller copepodite stages and small copepods (Titelman and Fiksen 2004; Ohman and Romagnan 2016).

The pelagic waters are primarily devoid of structural components giving refuge to prey organisms. As a response to the lack of structural complexity, a variety of highly sophisticated strategies has evolved to avoid predation across different phyla. Three of the strategies are rare or absent in other habitats, transparency, mirroring and counterillumination (Johnsen 2014). In the epipelagic zone, transparency is widespread as a camouflage strategy as the organisms must match the ambient light to decrease their contrast. Transparency is the only camouflage that is equally successful from all viewing angles (Johnsen 2001). Cryptic colouration depends on production or accumulation of pigments matching the ambient colours and is sensitive to the viewpoint and ambient light conditions (Johnsen 2002). In pelagic systems, cryptic colouration towards the surface is blue, while animals in the mesopelagic zone are increasingly redder or blacker (fig. 1). Mirrored sides are well known, and an excellent form of camouflage at euphotic and upper disphotic zones as it reflects the ambient light conditions, but this strategy is highly dependent on the viewpoint. Counterillumination, where an organism simulates light conditions to become less conspicuous and obscure the outline of the animal, is only successful at disphotic depths or during moonlit nights and is also metabolically expensive (Johnsen 2001). With camouflage strategies being so complex and sophisticated, the predators have evolved traits to compensate for the camouflage. Visual sensory capabilities as UV and polarisation sensitivity and intraocular filters are thought to facilitate detection of camouflaged animals. If the organism is adequately small, size can be a camouflage strategy.

Detection by visual predators is highly dependent on prey size. Many copepods and their early developmental stages are small in size. Thus, small sizes may act as a form of camouflage to visual predators in itself (Aksnes and Giske 1993; Aksnes et al. 2004). Small copepods in the well-illuminated mixed surface layers can, therefore, afford to be heavily pigmented by, i.e. red carotenoid pigments like astaxanthin, when it might be expected to be more profitable to be transparent. Copepods are not able to synthesise pigments *de novo* (Holeton et al. 2009; Schneider et al. 2016). From phytoplankton, copepods obtain carotenoids and xanthophylls, which serves as the primary precursors for astaxanthin synthesis in zooplankton. Copepods further synthesise astaxanthin via xanthophylls such as zeaxanthin or lutein (Andersson et al. 2003; Holeton et al. 2009). Copepods can also convert red-coloured free astaxanthin to blue astaxanthin-protein complexes (Mojib et al. 2014). Omnivorous, and predatory copepods can subsequently accumulate astaxanthin from feeding on herbivorous copepods. The carotenoids are antioxidants and can be photoprotective against DNA damage from radical oxygen species created by ultraviolet radiation (UVR) (Schneider et al. 2016). Copepods can also accumulate non-pigmented mycosporine-like amino acids (MAAs) that absorbs UVR and protect against DNA damage (Hylander et al. 2012; Hylander et al. 2014). However, copepod species in the well-illuminated parts of the epipelagic zone tends to be transparent and slightly blue (Mojib et al. 2014), with increasing degrees of redness with increased depth. *Paraeucaheta norvegica* individuals caught in surface layers are for example less pigmented than individuals caught at deeper layers with less ambient light (Vestheim 2004; Vestheim and Kaartvedt 2006). Another way of avoiding predation is to migrate to a light level that contributes to the camouflage.

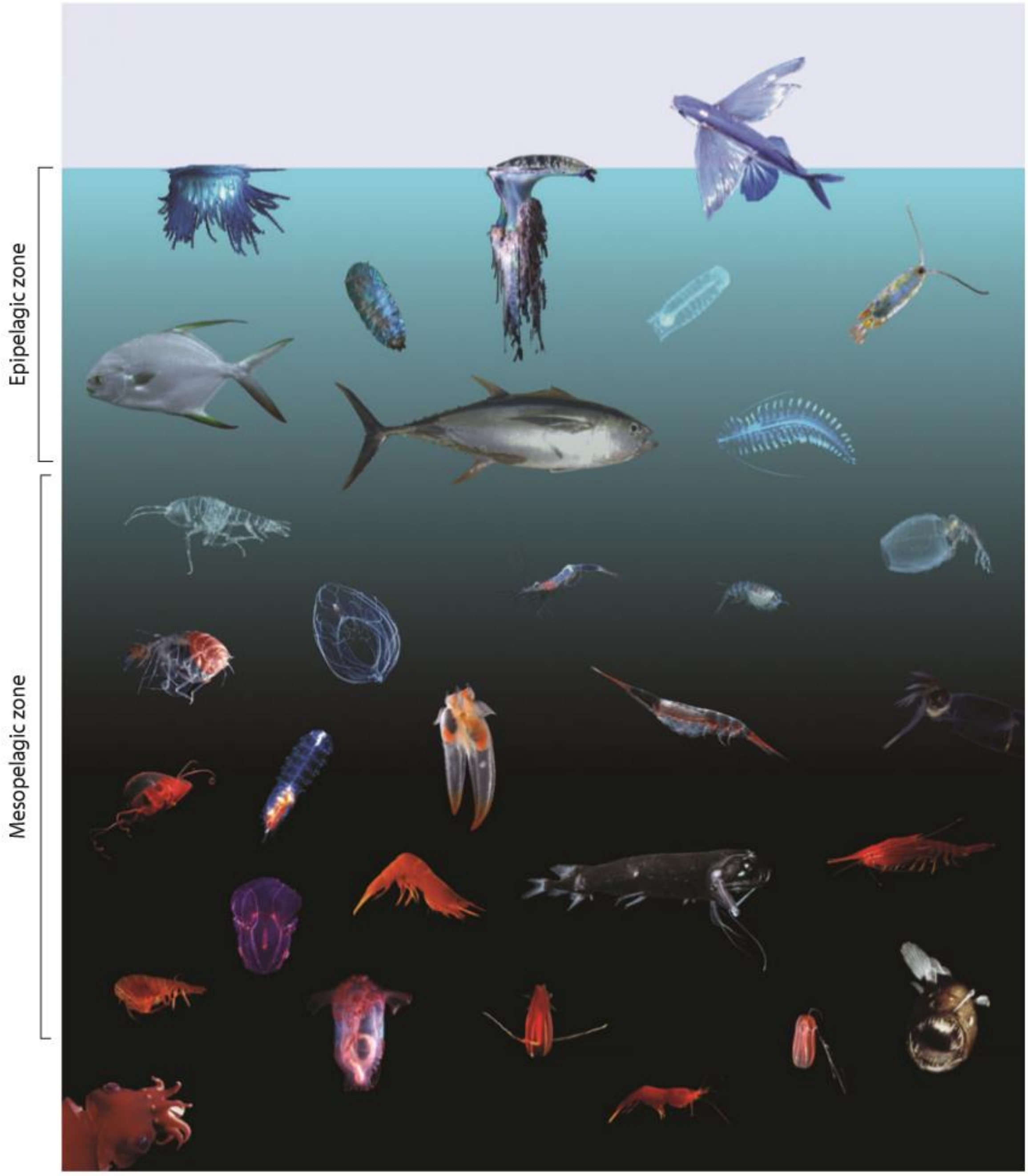

Figure 1 The increasing gradient of redness and blackness with depth in pelagic species as presented by Johnsen (2014). Images are not to scale. The figure illustrates three of the mentioned anti-predator strategies as a function of depth; mirroring, transparency and counter colouration. When focusing on counter colouration, invertebrates appear redder with increased depth, and in Crustacea, astaxanthin and its esters are the dominating pigments causing red colour. Vertebrates tend to appear blacker with increased depth due to the accumulation of melanin (Johnsen 2005).

Diel vertical migration (DVM) is widespread amongst organisms living in the pelagic realm, and copepods are no exception. DVM of zooplankton in the oceans and lakes of the world is arguably some of the most significant animal migrations on Earth (Williamson et al. 2011). Copepods display several different DVM patterns. Some species display nocturnal DVM where the animals migrate from a maximum depth to minimum depth at sunset and back down to a maximum depth at sunrise. Reversed DVM is the opposite of nocturnal, where animals migrate to a maximum depth at sunset and ascend to a minimum depth at sunrise. Twilight DVM is a third pattern characterised by an ascent to the surface at sunset, followed by a descend to deeper water at midnight. Subsequently, there is a second ascent to surface waters followed by a descend again at sunrise. Some species are also non-migratory, and there is variability in which strategy various species display at a different place, time or even ontogenetic stage (Sekino and Yamamura 1999; Cohen and Forward 2002; Titelman and Fiksen 2004; Holliland et al. 2012).

Predator avoidance is thought to be the ultimate evolutionary driver for nocturnal DVM in most literature (Zaret and Suffern 1976; Aksnes and Giske 1990; Hays et al. 1994; Fiksen and Carlotti 1998; Cohen and Forward 2002). Proximate physiological mechanisms for initiating DVM can be, i.e. light, state-dependencies or temperature. There is evidence that indicates that light triggers DVM in marine copepods (Stearns and Forward 1984). The spectral sensitivity in migrating copepods is in the wavelengths available in the ambient seawater at twilight (Cohen and Forward 2002). Light is also affecting the DVM amplitude, and increased water transparency leads to increasing DVM amplitudes (Williamson et al. 2011; Hylander and Hansson 2013). DVM in marine copepods may also be state-dependent as some findings show that some copepod species with high amounts of carotenoids more often display DVM than those containing lower amounts of carotenoids (Hays et al. 1994). The carnivore copepod *Paraeuchaeta norvegica* also display state-dependent DVM where visually conspicuous ovigerous females select greater depths than non-ovigerous which are less conspicuous to predators (Vestheim 2004).

While it is clear that much of the vertical structure and dynamics of copepod populations is related to size and pigmentation (De Robertis et al. 2000), if and how copepods alter their pigmentation remains unclear (Vestheim and Kaartvedt 2006). Epipelagic copepods are mainly transparent, obtaining their pigments from the diet, rendering the guts the most conspicuous part of the body (Tsuda et al. 1998). Feeding patterns due to, e.g. DVM and nocturnal feeding should alter the concentration of pigments and thus, apparent pigmentation (Tsuda et al. 1998). However, it is not necessarily as simple. Although the gut becomes visible when feeding, apparent pigmentation does not directly correlate with food intake. Astaxanthin and alloxanthin, which contribute to red and yellow colouration, are accumulated in body tissue and not in the gut (Antajan and Gasparini 2004; Hylander et al. 2012).

Vestheim et al. (2006) showed that *Paraeuchaeta norvegica*, when incubated for 24 hours in total darkness without any food supplies increased their apparent pigmentation. In decapod crustaceans, chromatophores are well known (Herring 1973). Chromatophores are specialised cells that contain pigments and the distribution of the pigments within the chromatophores cause changes in pigmentation (Fingerman 1985). The ability to alter pigmentation without increasing ingestion of pigments may point towards chromatophores in *P.norvegica* as well. Some of the photos of low-pigmented individuals analysed by Vestheim et al. (2006) had small asterisk-shaped red patches which could indicate the presence of chromatophores. If that is the case, and if plasticity in pigmentation is a more widespread phenomena in copepods (Byron 1982; Vestheim and Kaartvedt 2006; Hylander et al. 2009) it would be interesting to investigate how pigment concentration and apparent pigmentation correlate, and whether different light regimes absent of UVR can induce changes in pigmentation in copepods at large.

Aims for my thesis

In Oslofjorden, we can find a wide range of depths (0 – 200 m) and thereby different optical habitats. Additionally, the copepod diversity in terms of size and lifestyles is considerable, e.g. the average total body length of *Oithona nana* is 0,55 mm, while *Paraeuchaeta norvegica* average total length is 8.1 mm (Brun et al. 2017). My master thesis targets three specific aims, each with their specific questions, related to the overall hypothesis that pigmentation is a plastic trait in copepods (Vestheim and Kaartvedt 2006; Hylander et al. 2012).

First, I wanted to quantify visible differences in apparent pigmentation of copepods from different depth intervals in the water column. I approached this with a photographic method and subsequent photo analysis. Based on the theory of visual predation and camouflage (Aksnes and Giske 1993; Aksnes and Utne 1997; Johnsen 2001; Aksnes et al. 2004; Johnsen 2014), I predicted that copepods in the deeper layers appear more pigmented than the copepods in the upper layers. Therefore, I asked the question:

- Is apparent pigmentation in copepods correlated with depth? If so, how?

Secondly, I aimed to examine if differences in apparent pigmentation (aim 1) reflect differences in pigment contents. I approached this by spectrophotometric analysis of pigments extracted from copepods. The second question I aimed to answer is:

- Is pigment content in copepods related to depth? If so, how?

A third question combines the first and second aim of my thesis;

- When compared, do apparent pigmentation correlate with actual pigment concentrations in copepods?

Finally, I aimed at developing a setup where one can test if and how pigmentation in copepods changes as a plastic response to light in a controlled and quantifiable light environment, to address the question:

- Is pigmentation in copepods a plastic trait that can be manipulated by experimental light conditions?

Methods

Sampling copepods for image and pigment analysis

Sampling for all parts of my thesis was conducted with research vessels Bjørn Føyn and Trygve Braarud (fig. 2), at Elle (fig. 3), a deep water (~200 m) survey location outside of Drøbak in Oslofjorden, Norway. I measured light attenuation with TriOS RAMSES (TriOS Measurement and Data Technology GmbH, Germany) hyperspectral radiance and irradiance sensors for the ultraviolet (UV), visible (VIS) or UV/VIS range from 0 – 25 m. I collected hydrographical data with an SBE 9plus CTD (Sea-Bird Scientific, USA), which recorded conductivity (salinity), temperature and density as it descended from 0 – 200 m.

Figure 2 Image of research vessels Bjørn Føyn and Trygve Braarud (Bjoertvedt 2014).

I used a WP2 plankton net with an opening of 57 cm, mesh size 200µm, closing mechanism and a closed cod end for all sampling. The closing mechanism is operated with a messenger and allows for interval hauls. Targeted intervals were 200 – 160, 160 – 120, 120 – 80, 80 – 40, and 40 – 0 m, with an exception for the first sampling trip in March 2017, which targeted 200 – 100, 100 – 30 and 30 – 0 m.

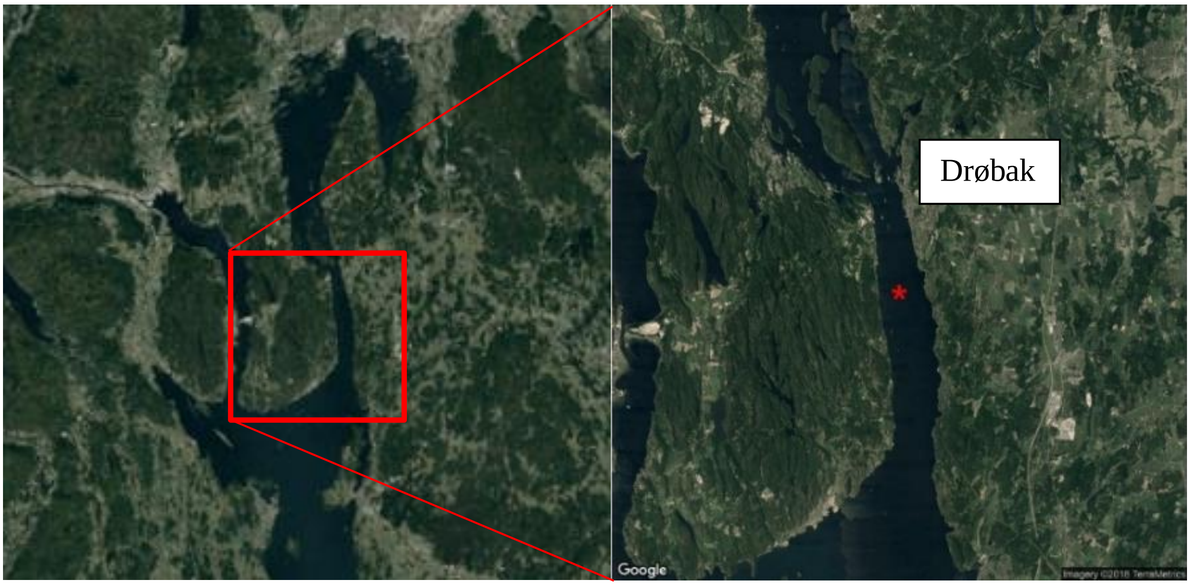

Figure 3 Map showing the Elle location (red star) outside Drøbak, Norway (made in R with RGoogleMaps).

I sampled in March 2017, June 2017, and March 2018 at a similar time of day and weather conditions (tab. 1). Sampling in 2017, separate interval hauls were conducted for image analysis and pigment extraction. In 2018, the interval hauls got separated into two halves using a separator. I photographed one half, and the other half was filtered and frozen for pigment extraction in the lab at the University of Oslo.

Table 1 Date and weather conditions for field sampling of copepods. Replicates state the number of hauls at each targeted depth interval.

Sampling date	Targeted depth interval (m)	Replicate	Thesis part	Weather conditions
28.03.2017	200-100, 100-30, 30-0	2	Image analysis	Slight overcast
27.06.2017	200-160, 160-120, 120-80, 80-40, 40-0	2	Pigment analysis	Sunny
28.06.2017	200-160, 160-120, 120-80, 80-40, 40-0	1	Pigment analysis	Sunny/slight overcast
13.03.2018	200-160, 160-120, 120-80, 80-40, 40-0	3	Image/pigment analysis	Sunny/slight overcast/cloudy

Photography and image analysis of apparent pigmentation

The camera setup consisted of, a Canon EOS 7D camera with a Canon MP-E 65mm macro lens with a ring flash, which was attached to an adjustable rack (fig. 4, left). I glued a 3d printed rack for holding the animals at the same height and focal plane relative to the base of the camera rack. The sample container consisted of a standard microscope slide with a 3d printed frame glued on top (fig. 4, right). The container ensured that the animals stayed inside the picture frame during photography.

Figure 4 Images of the camera setup (left) and object glass with 3d-printed frame laying on the rack (right).

When analysing images for apparent pigmentation, it is essential to have one or more grey references to calibrate the image (Troscianko and Stevens 2015). I, therefore, took a photo of a ColorChecker grey-scale reference of 3,1% reflectance (X-Rite© #MSCCPP) for each photo session (2018). I took all the photographs with the ring flash set to the same light intensity and duration. The ring flash functioned as the primary light source for the photos and ensured equal light conditions.

I randomly subsampled animals from the bulk hauls with pipettes, below deck. Immediately after, live animals from the subsamples got photographed on the ship. This procedure maintained the animals' true colours and ambient light as much as possible (Vestheim and Kaartvedt 2006). All photographs got saved as RAW files (fig. 5). I used the MICA-Toolbox in ImageJ for my analysis, which offers a range of photo analysis methods to examine colouration (Troscianko and Stevens, n.d., 2015). The RAW format ensures the maximum amount of information per pixel and yields as accurate reflectance data as possible with the applied methods.

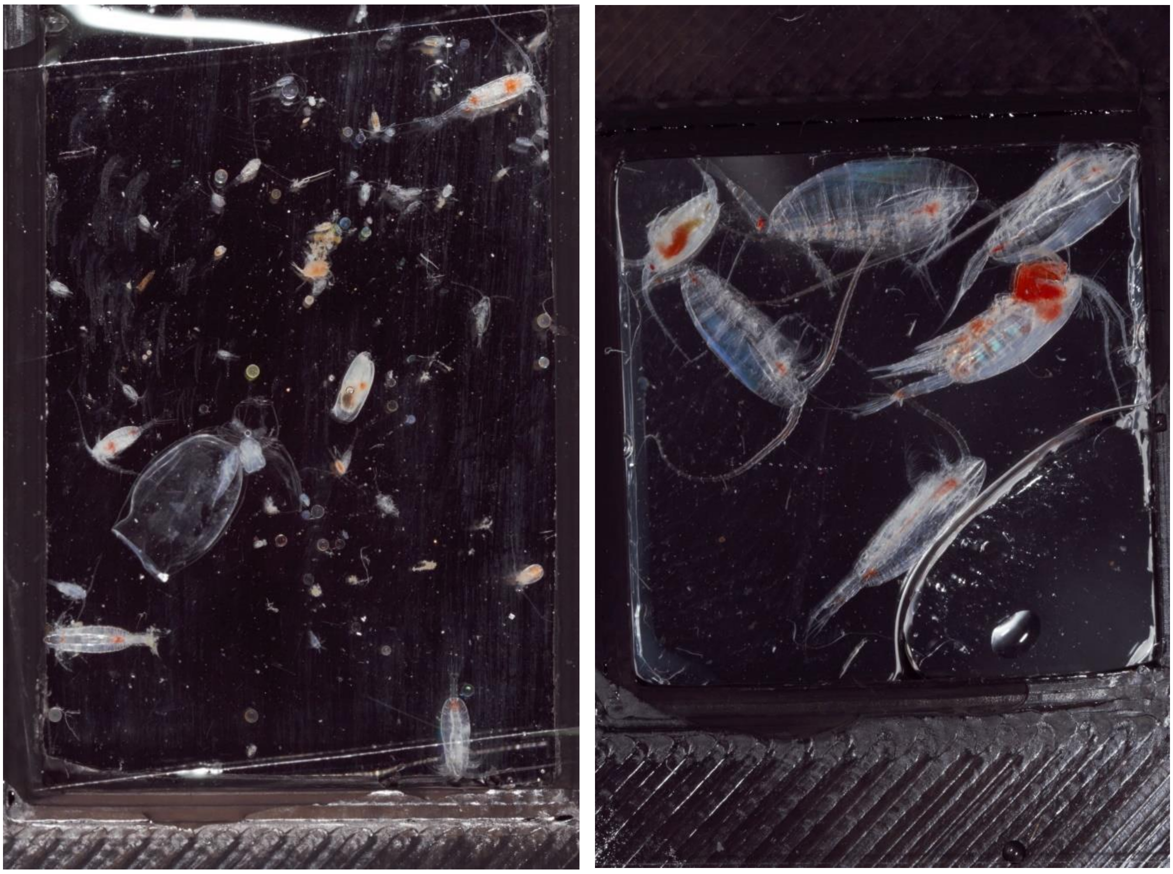

Figure 5 Typical images from 2017 (left) and 2018 (right) with differences in species composition and abundance between years.

Most cameras generate nonlinear images to produce clear, bright colours that correlate with human vision. To get as close to spectrometry as possible with digital photo analysis, the MICA-toolbox generates linear multispectral layered images from the nonlinear RAW-files before analysing the layers using a multispectral batch analysis function (Troscianko and Stevens 2015).

The multispectral images generated consists of three or more (e.g. red, green, blue and UV light) colour layers (spectra). In my thesis I only consider visible light, so only the red, green and blue layers are of interest. The multispectral images made therefor consisted of three layers. In the multispectral images, I selected regions of interest (ROIs) by outlining individual copepods prosome with a polygon.

The ROIs got identified to copepod genus or species and subsequently analysed using the multispectral batch analysis function in the MICA-toolbox plugin. This function analyses and calculates normalised pixel values (Vnorm) from the linear pixel values (Vlinear) of the multispectral images (visualised in fig. 6), with S being the grey standard reflectance value (3.1% in 2018), the maximum bit range value ($65535 = 2^{16} - 1$; the maximum value in a 16 bit image), and G being the mean pixel values for the grey standard (Troscianko and Stevens 2015):

$$V_{norm} = V_{linear} \frac{\left(\frac{S}{100}\right) 65{,}535}{G}$$

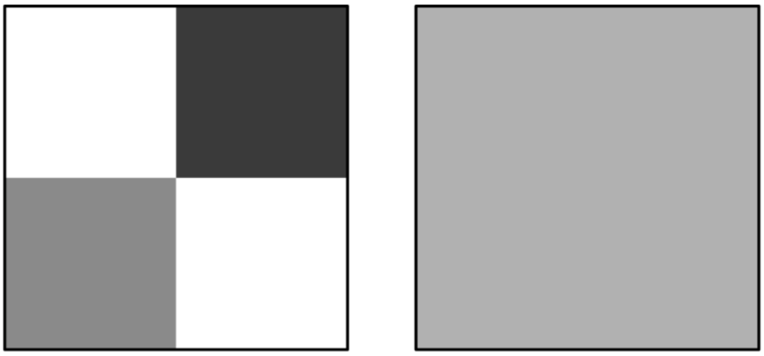

Figure 6 Visualisation of how the MICA-toolbox calculates the mean normalised pixel values. An imagined region of interest (ROI) of four pixels with different colour values (left). To the right, the same region of interest after analysis with the MICA toolbox (multispectral batch analysis) with normalised mean pixel values for all four pixels.

The multispectral batch analysis yields a data table with colour values, normalised mean pixel reflectance, for red, green and blue light in the ROIs. For ease of interpretation of my data, first consider how three hypothetical ROIs are predicted to appear in the photography, in well-illuminated surface layers, and light restricted deep water (fig. 7). The distribution of colour values is predicted for the three ROIs in the hypothetical boxplots (fig. 7). If an animal appears with a bright red colour in the photography, the reflectance values should be high in the red spectrum, but very low in the green and blue spectra (fig. 7). That animal may appear red in well-illuminated layers in the water column, but if red pigments absorb blue wavelengths, the animal may appear as black in the light restricted areas (fig. 7). A bright white animal in a photograph should have equally high reflectance values in all three colour spectra and appear bright white in surface layers (fig. 7). However, in deeper light restricted layers, it is unclear if the animal would appear as white or reflect the ambient light, making it more or less invisible (fig. 7). A transparent animal may appear white-looking in the photograph, but when using a calibrated grey-scale reference, it would have equally low reflectance values in all three colour spectra (fig. 7). In surface waters and more light restricted layers, transparent animals are predicted to be well camouflaged.

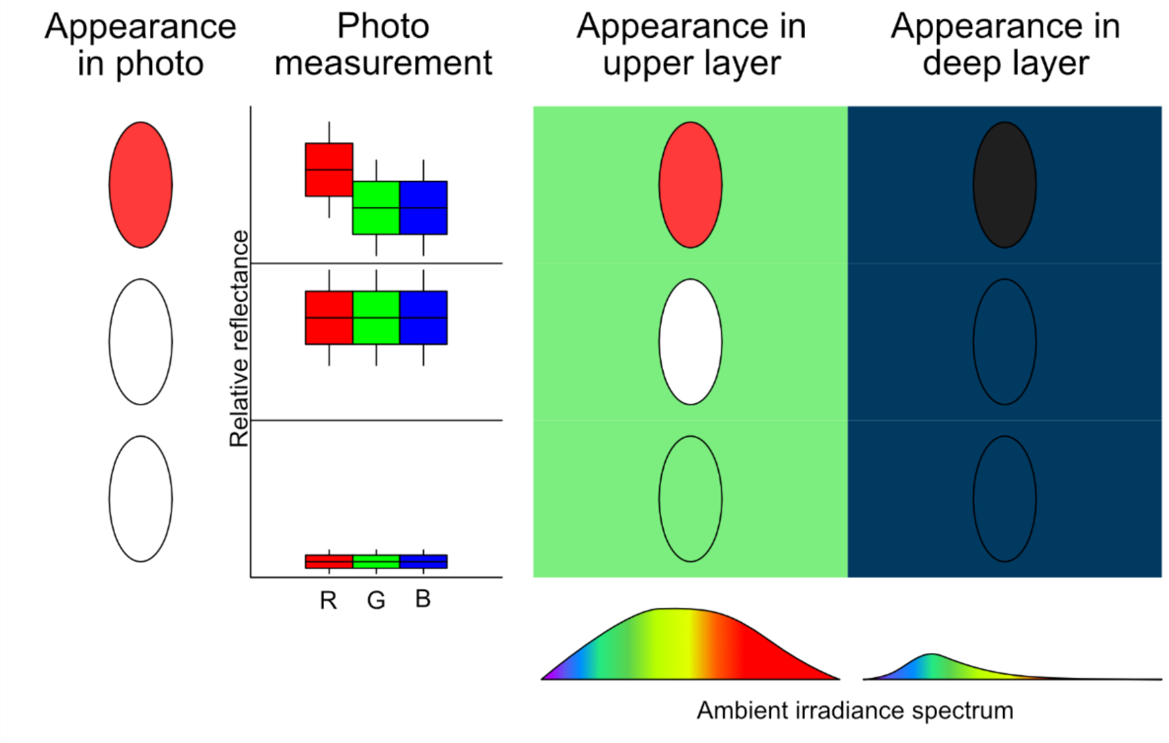

Figure 7 Visual presentation of how three different hypothetical ROIs are expected to appear in photos, in well-illuminated layers, and deeper light restricted layers. The imagined boxplots represent reflectance values for a red, a white and a transparent ROI.

I calculated the percentage of maximum pixel reflectance to make the values more understandable. First, the relative share of maximum pixel reflectance scaled to maximum bit range (65535):

$$\frac{Colour(R, G, B)NormalisedMean}{Maximum\ bitrange\ value(65535)} = Relative\ reflectance$$

Percentage of maximum pixel reflectance:

$$Relative\ reflectance\ (Scaled\ to\ maximum\ bitrange) \times 100 = \%\ reflected\ light$$

Total reflectance:

$$RNormalisedMean + \ GNormalisedMean + \ BNormalisedMean = Total\ Reflectance$$

Percentage total reflectance:

$$\frac{Total\ reflectance}{Max.bitrange\ \times 3} \times 100 = \ \%\ total\ reflectance(RGB)$$

Relative colour, scaled to 1:

$$\frac{Colour(R, G, B)NormalisedMean}{Total\ reflectance} = Relative\ colour.$$

Table 2 Number of analysed images with the number of individuals representing individual copepods (ROIs)

Sampling date	Targeted depth	Images	Individuals
28.03.2017	0 – 30	11	39
28.03.2017	30 – 100	9	61
28.03.2017	100 – 200	6	31
13.03.2018	0 – 40	21	40
13.03.2018	40 – 80	22	48
13.03.2018	80 – 120	22	64
13.03.2018	120 – 160	21	55
13.03.2018	160 – 200	20	45
	Total	132	383

I analysed the results from 2017 and 2018 separately, due to differences in depth intervals, exposure settings on the camera and grey-scale reference between years. I only present results from 2018 in my thesis. The sampling and results from 2017 thereby worked as a trial and can be found in Appendix A and B. All analysis was done using the open access software R-studio. A total of 383 ROIs in 132 images got analysed (tab. 2).

Pigment extraction and content analysis

The Gauss-peak Spectra (GPS) method uses known pigments spectra as weighted sums of Gaussian functions to model the absorbance spectra of unknown pigment mixtures (Küpper et al. 2007). Thrane et al. (2015) modified this method to be able to use plate readers to measure the absorbance spectra of multiple solutions in well-plates. After extracting the pigments from copepods, and removing remaining exoskeletons, the pigment mixtures could be analysed with the modified GPS-method. This method is cost-efficient and highly accurate when compared to the more traditional HPLC-method (Thrane et al. 2015; Küpper et al. 2007).

Astaxanthin is the primary pigment component in copepods (Byron 1982; Andersson et al. 2003). To be able to use the GPS method I incorporated astaxanthin to the core set of pigment references used by Thrane et al. (2015). I used 97% pure astaxanthin (Sigma-Aldrich #SML0982-50MG) extracted from *Haematococcus pluvialis* to create a stock solution of 10mg/L, astaxanthin/96%ethanol. From the stock solution, I created a linear dilution row from 10 mg/L → 0 mg/L in steps of 2 mg/L. I pipetted 200µL from each step of the dilution in the wells of a 96 well plate (Falcon® #353072), ten wells per dilution step. I measured the absorbance for each nm wavelength, between 400-750 nm, in each well with a SynergyMX plate reader (BioTek Instruments, Vermont, USA).

Astaxanthin changes absorbance spectra in different solvents (Buchwald and Jencks 1968). I, therefore, added a linear gradient of filtered seawater (FSW) to the dilution rows of astaxanthin solutions to see if the FSW gradient altered absorbance properties of the astaxanthin. By taking these measurements, I aimed to achieve that the reference spectra were as accurate as possible while being able to control for possible interactions between seawater, ethanol and the astaxanthin. From the linear dilutions, I made linear regression models and found the specific absorbance coefficients for astaxanthin and used them for the pigment core set for the GPS method. I further created a pigment subset of the core set consisting of astaxanthin, alloxanthin, myxoxanthin, canthaxanthin, peridinin and pheophytin a and b to use in my pigment analysis as these were the most abundant pigments in my samples.

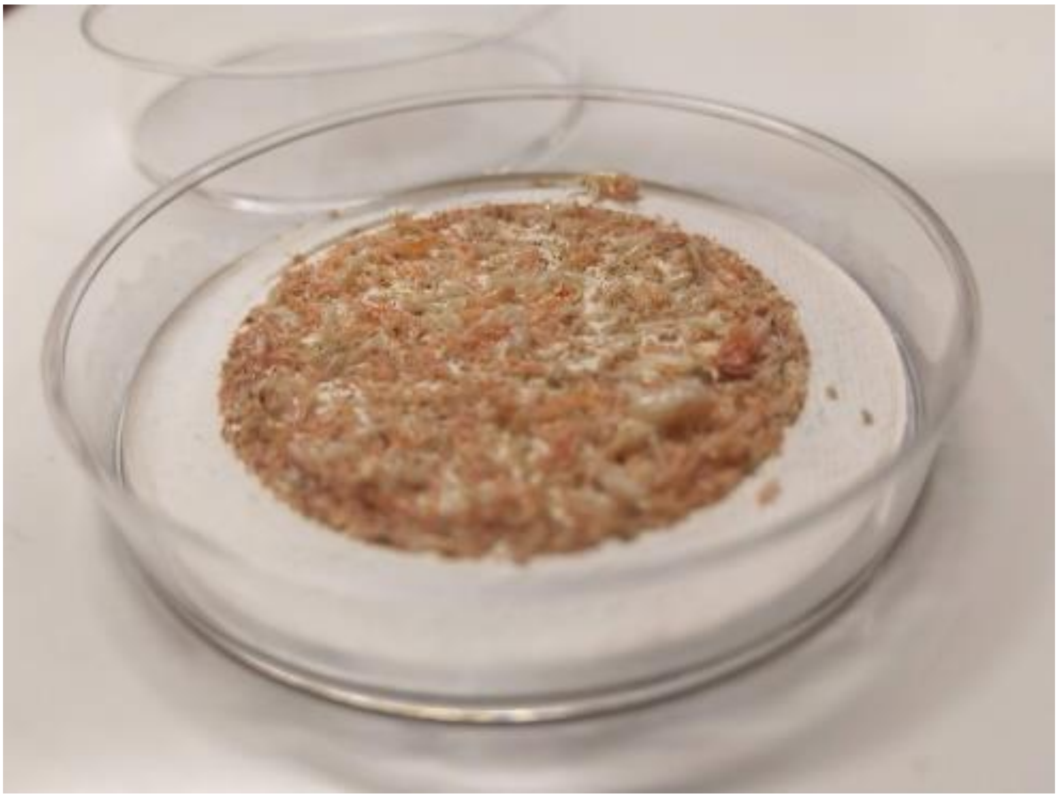

Figure 8 Frozen samples on filter ready for picking animals for pigment extraction

To be able to preserve the pigments, sampled copepods got filtered on to glass microfiber filters (Whatman® GF/F 47mm, #1825-047) and frozen with a cooling spray, as soon as they got hauled on to the ship. I stored the frozen samples (fig. 8) at -18°C at the University of Oslo until pigment extraction.

Table 3 Targeted depth intervals and the number of animals picked for and analysed by pigment extraction from 2017-18.

Target Depth	27.06.2017	13.03.2018	Total	Number of Plates
0 – 40	176	0	176	2
40 – 80	88	24	112	2
80 – 120	143	88	231	3
120 – 160	176	88	264	3
160 – 200	176	88	264	3
Total	759	288	1047	13

A total of 1047 frozen animals (tab. 3) got identified to genus, and picked for pigment extraction, under a stereomicroscope in the lab at the University of Oslo. I transferred animals from the filter to clear 96-well polystyrene plates with flat bottoms (Falcon® #353072) with one animal and 200µL 96 % ethanol added to each well. I also included blanks (i.e. wells containing only ethanol). I incubated the plates with the samples for 24 h at 4°C, and the absorbance read in the SynergyMX plate reader. The results from the plate reader got analysed in R and gave the pigment concentrations in mg/L. I further calculate pigment content as ng per animal by multiplying the concentration in ng/µL with the amount of ethanol in the extract, 200µL:

$$\frac{mg}{L} = \frac{\mu g}{mL} = \frac{ng}{\mu L}$$

$$\frac{ng}{\mu L} \times 200\mu L = \frac{ng}{animal}$$

Apparent pigmentation and actual pigment concentration

I wanted to compare the results from the photo analysis with the pigment extraction data. To make the comparison as accurate as possible I chose only to include data from the same sampling date, 13.03.2018. When sampling in 2018, as mentioned, the samples got separated into two halves. One half got photographed, while the other half was frozen and used for pigment extraction. Therefore, I assume that the animal sizes in the two analysis parts are similar. The photo analysis yields reflectance values normalised to size. To be able to compare the photo data with the extraction data I first had to calculate the specific pigment concentration (ng/mm^3) from the content per animal. In the data from the photo analysis, the area of the copepod is the number of pixels in the ROIs. One mm in my photos covers 176 pixels, measured by photographing a calibration line. From the area I calculate the size as mm^2 using the following equations:

$$1\ mm = 176\ Pixels$$

$$1\ mm^2 = 176 * 176\ Pixels$$

$$Size\ (mm^2) = \frac{Area\ (Pixels)}{176^2\ (\frac{Pixels}{mm^2})}$$

I further assume that the area (size) from the photo analysis is circular, and the animal shape to be spherical to be able to calculate the volume as an approximate measurement of animal volume. These assumptions make it possible to calculate the volume of the extracted animals from the size measurements from the photo data, to get a pigment concentration/volume ratio:

$$Area\ of\ a\ circle = \pi * r^2$$

$$Radius\ of\ my\ animals = \sqrt{\frac{Size\ (mm^2)}{\pi}}$$

$$Volume\ of\ a\ sphere = \frac{4}{3} * \pi * r^3$$

$$Volume\ of\ my\ samples\ (mm^3) = \frac{4}{3} * \pi * \left(\sqrt{\frac{Size\ (mm^2)}{\pi}}\right)^3$$

After making these calculations in R, I calculate the average reflectance and pigment concentration for each target depth and genus. I use those values to compare the results to see if there is a correlation between pigment concentration and apparent pigmentation by plotting the results and making a linear regression.

Creating the light setup

When developing the light setup, I made some initial decisions that would allow for a well-rounded final product where plasticity in pigmentation could be tested. The containers should not have sharp corners where aggregation of animals, food or other substances could occur. Also, the LED light source should be controllable and quantifiable and contain red, green and blue light that all together should give white light. The LEDs and associated connections should be waterproofed, allowing for the bottles to be submerged in water to maintain a stable temperature as well as the light to be in close contact with seawater. Air and food supply were assumed not to be necessary, as the experiments were predicted not to last longer than a few hours at a time. Finally, I wanted to contain the treatment light within the containers, and ambient light from outside the containers should be blocked out.

With those thoughts in mind, I chose to use glass bottles with a LED light source integrated into the bottle caps for the setup. Reagent bottles (VWR) with GL45 caps (fig. 9) come in several different volumes. The GL45 cap, however, is the same and fits all different bottle volumes, as well as old and new bottles. The flexibility of the GL45 cap greatly enhances the user-friendliness and usability of the light caps.

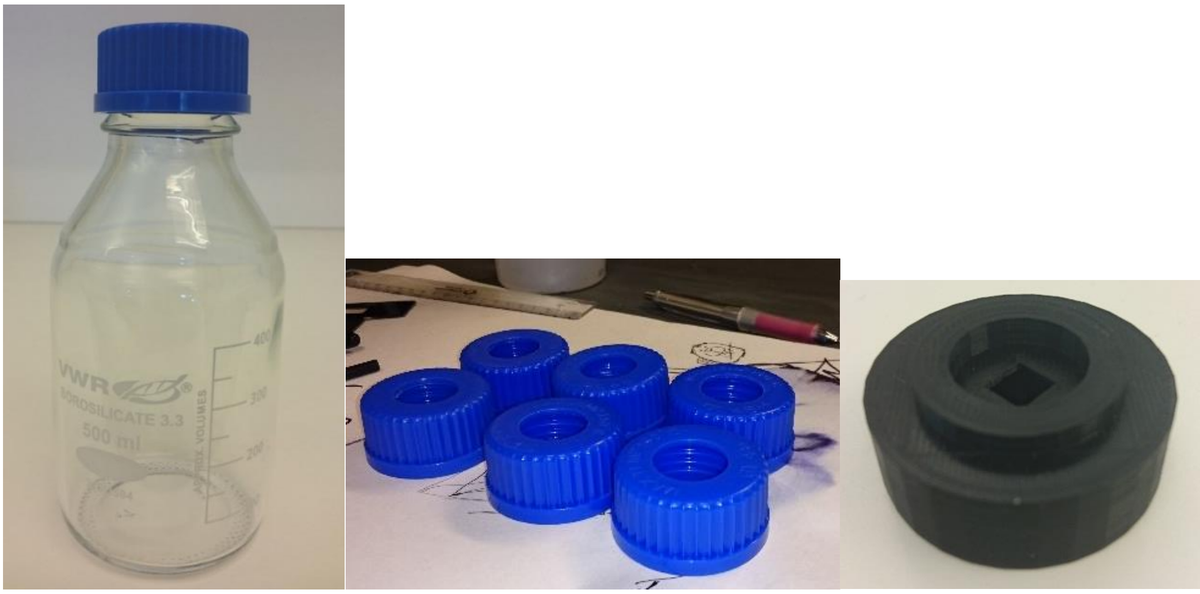

Figure 9 The medium bottle (right), with prefabricated GL45 caps (middle) and the insertable piece designed by Jan Heuschele (right)

I needed to develop the design of the light cap. The reagent bottles came with caps which had a premade hole in it, e.g. for air supply. We first made an insertable piece in 3D print, designed by my supervisor Jan Heuschele (fig. 9). I decided to use NeoPixel Flora v.2 LEDs (Adafruit® #1260) to be able to replicate levels of red green and blue light in different depths of Oslofjorden. The NeoPixels contains three LEDs per unit, red, green and blue. Each colour in each NeoPixel in a string can be programmed individually, which makes it possible to quantify and control the light conditions in each separate bottle.

I used an Arduino Uno (Adafruit® #discontinued[1]) to program the LEDs for calibration and test runs. Arduino Uno is a small computer that can control a variety of components including LEDs and is programmable via USB. The Arduino Uno has light intensity settings ranging from 0-

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[1] Replaced with Arduino Metro 328 (Adafruit® #2488)

255 for each LED in the NeoPixel. When set to 0 the LEDs are turned off, and when set to 255 the light source is set to maximum intensity. Arduino offers a free software, Arduino IDE, for programming the Arduino products available in app-stores. I used Arduino IDE to generate a script for testing and running the light setup (Appendix E).

Connecting the NeoPixels is a relatively straightforward operation with parallel connections of the positive and negative poles on each unit, and the controlling wire connected in series (fig. 10). I made all the connections with a soldering iron set to 300 °C under a fume hood. Wiring diagrams can be found on Adafruit.com [(“Basic Connections | Adafruit NeoPixel Überguide | Adafruit Learning System” n.d.)]

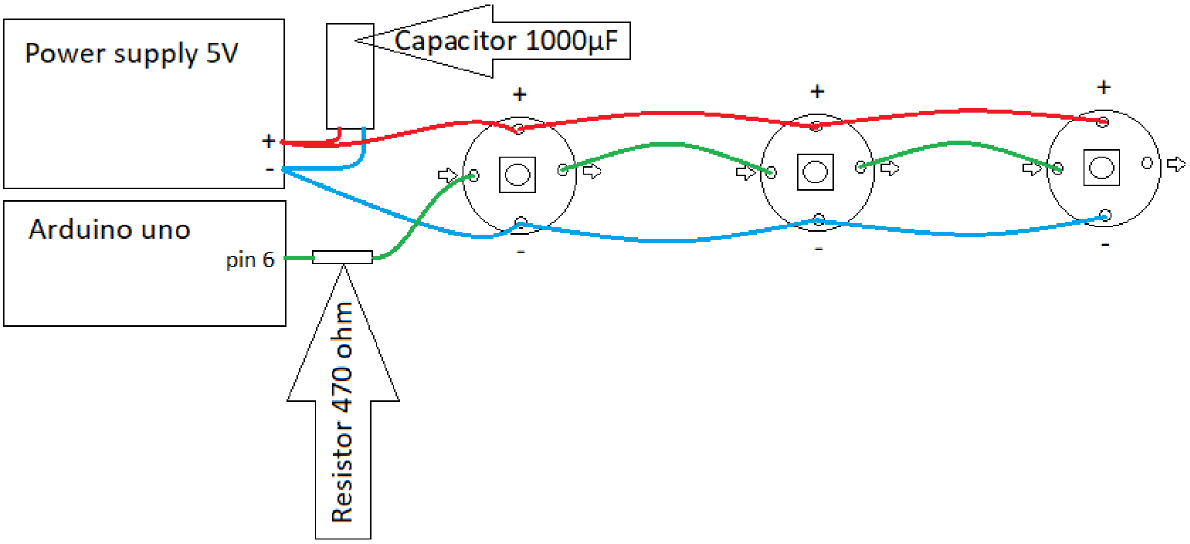

Figure 10 Wiring diagram for NeoPixel with 5 Volt power supply, Arduino Uno for programming, a capacitor to protect against irregular pulse amplitudes in the main power supply (+/-), and resistor on the green program wire.

A non-toxic silicone (TRANSCLEAR, TEC7) with a crystal clear finish was used as a sealant around all connections, wires, as well as providing a relatively flat and smooth lens and insulation between the connections, LEDs and the ambient seawater within and around the bottles.

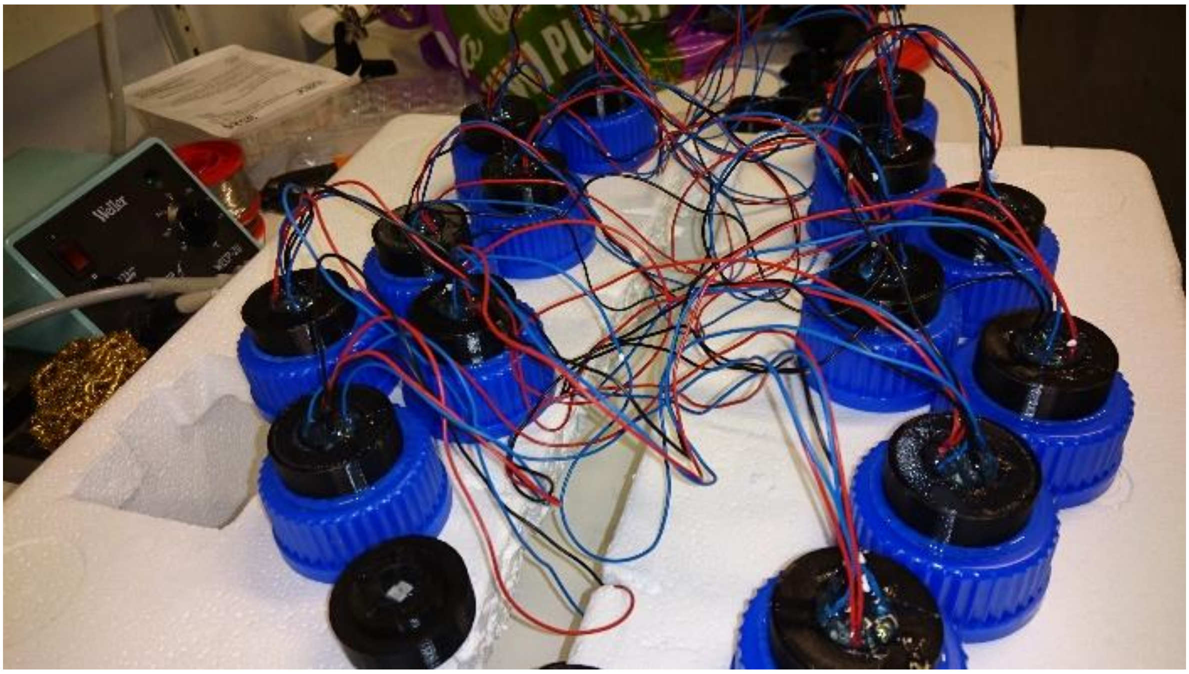

Figure 11 The first edition light caps with an insertable piece in original blue caps, ready for light calibration and testing.

The LED was positioned so high in the original blue plastic cap (fig. 11) so that the blue colour of the plastic in the original caps added colour to the emitted light. I, therefore, decided to create new black caps by 3D printing the cap as one piece with room for the LED already integrated. After measuring the opening and the threads of the reagent bottles, I created a 3D design of the final edition caps using Tinkercad (“Tinkercad | Create 3D Digital Designs with Online CAD” n.d.) (fig. 12).

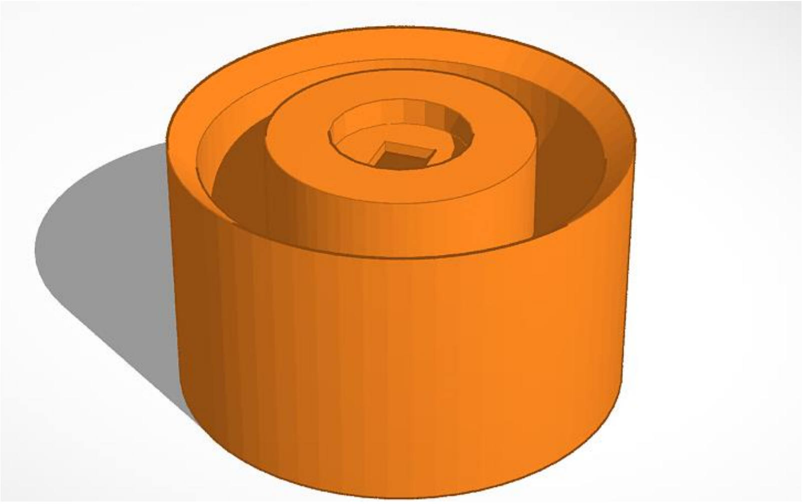

Figure 12 The final 3d design of the light caps created with Tinkercad

The insertable piece for the first edition caps and the final edition caps got printed on an Ultimaker 3+ 3Dprinter, with black Polylactic Acid (PLA) filaments with a diameter of 2,85mm (3DNet.no #1701222). The PLA filament is made from plant-based starch, and thereby it is non-toxic and biodegradable (3DNet n.d.). I did not include threads in the final edition light caps (fig. 13). No threads made it easier to open and close the bottles and ensure less handling time while starting up and finishing experiments. To contain internal light and block out ambient light in all the bottles, they should be wrapped in aluminium foil during experiments.

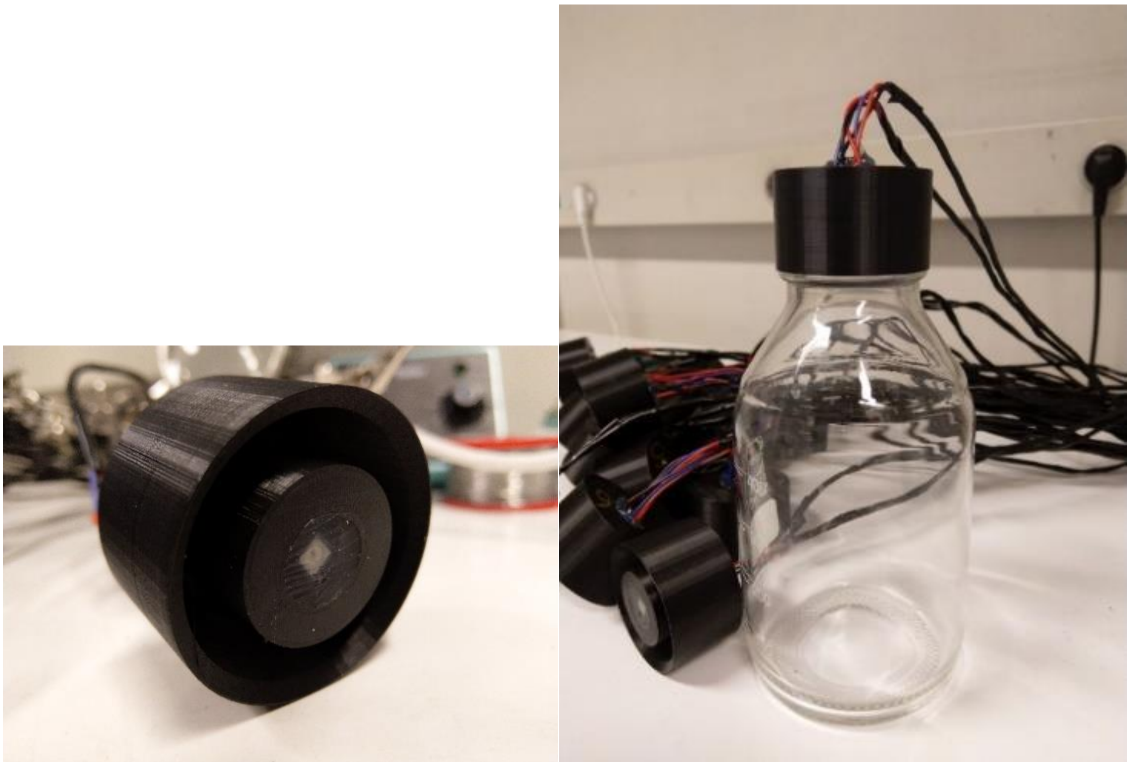

Figure 13 The final edition light cap with integrated light and silicone lens (left) fitted on a 500mL reagent bottle (VWR #215-1594) (right).

Light setup trial

Due to time constraints, the final modified black cap version of the setup could not be tested in an experimental setting. The first edition blue caps with the insertable piece got tested during a pelagic ecology field course (UiO) in 2018. One group of students attending the course got to do the test. *Calanus hyperboreus* was sampled at Elle and used directly at the biological station in Drøbak. They used 20 bottles with six individuals of *C. hyperboreus* per bottle.

Pictures for multispectral batch analysis were taken at the start, immediately as the animals got transferred from the original samples to the bottles, and directly after the duration of the experiment. The students chose to use four different intensities, 31, 63, 127, 255, of white (R+G+B) light as the treatment, with bottles with no light as a negative control.

All bottles got wrapped in aluminium foil to contain the light within each bottle as well as blocking out ambient light. The exposure time was 2-5 hours per treatment, as we expected a change to occur relatively fast if apparent pigmentation is a plastic response to light conditions. The subsequent photo analysis was conducted as described in the section on photography and image analysis.

Results

Light and hydrographical data

Light measurements show that light attenuation (fig. 14) at the sampling location coincides with the theory of how light behaves in temperate coastal waters. The shortest and longest wavelengths attenuated rapidly in the first meters of the water column. Mid-range wavelengths penetrated the deepest both before and after sampling for animals. The surface irradiance varied slightly between morning and mid-day measurements. However, the composition of wavelengths and their penetration depth was quite similar from 5 m down to ~25 m.

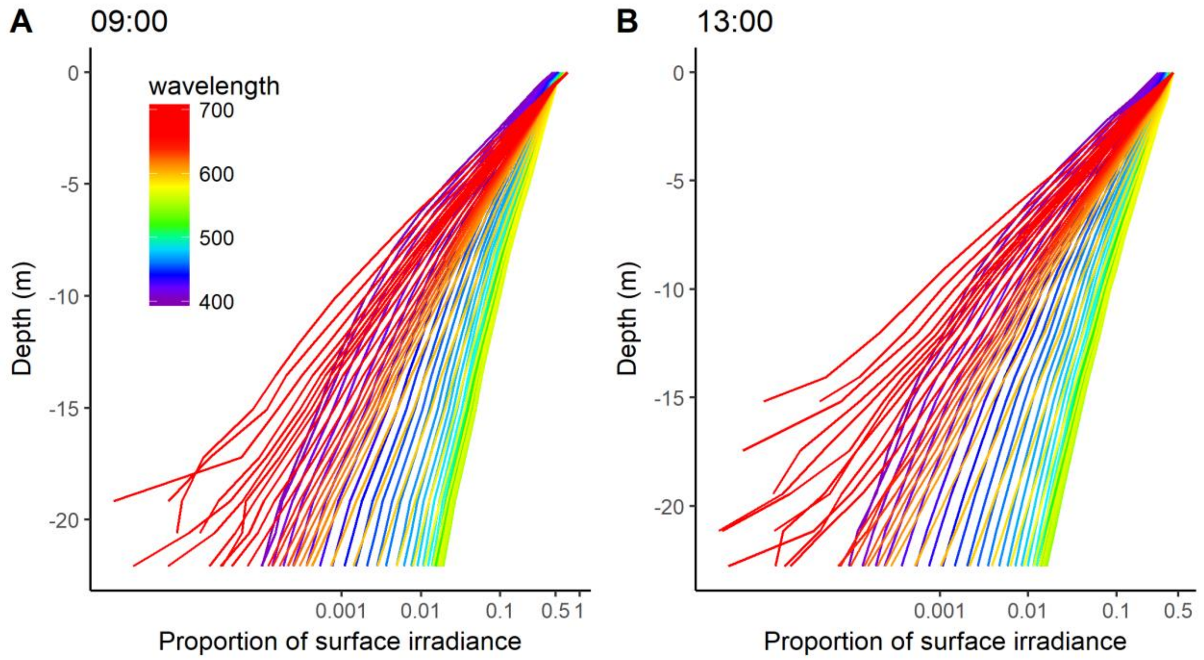

Figure 24 Light attenuation over increasing depth presented as the average proportion of surface irradiance (log-scaled). Measurements were done 13.03.2018 before (09:00, A), and after (13:00, B) sampling for animals. Colours represent the wavelengths measured (400-700 nm) The shortest and longest wavelengths attenuated rapidly in the first 5-10 m, while the mid-range wavelengths penetrated the furthest down in the water column. Measurements stop at 20-25 m due to the length of the cables for the TriOS RAMSES.

The temperature reached a maximum of 7.5 °C at 20 – 25 m (dbar), stabilising at 7 °C around 50 m (fig. 15). The data also showed one pycnocline at a few m and one at approximately 20 m. Salinity and water density increased rapidly in the upper 20 – 25 m and stabilised at a maximum of just around 50 m. The T-S plot indicated that the water column was relatively stable with little mixing except for in the uppermost parts.

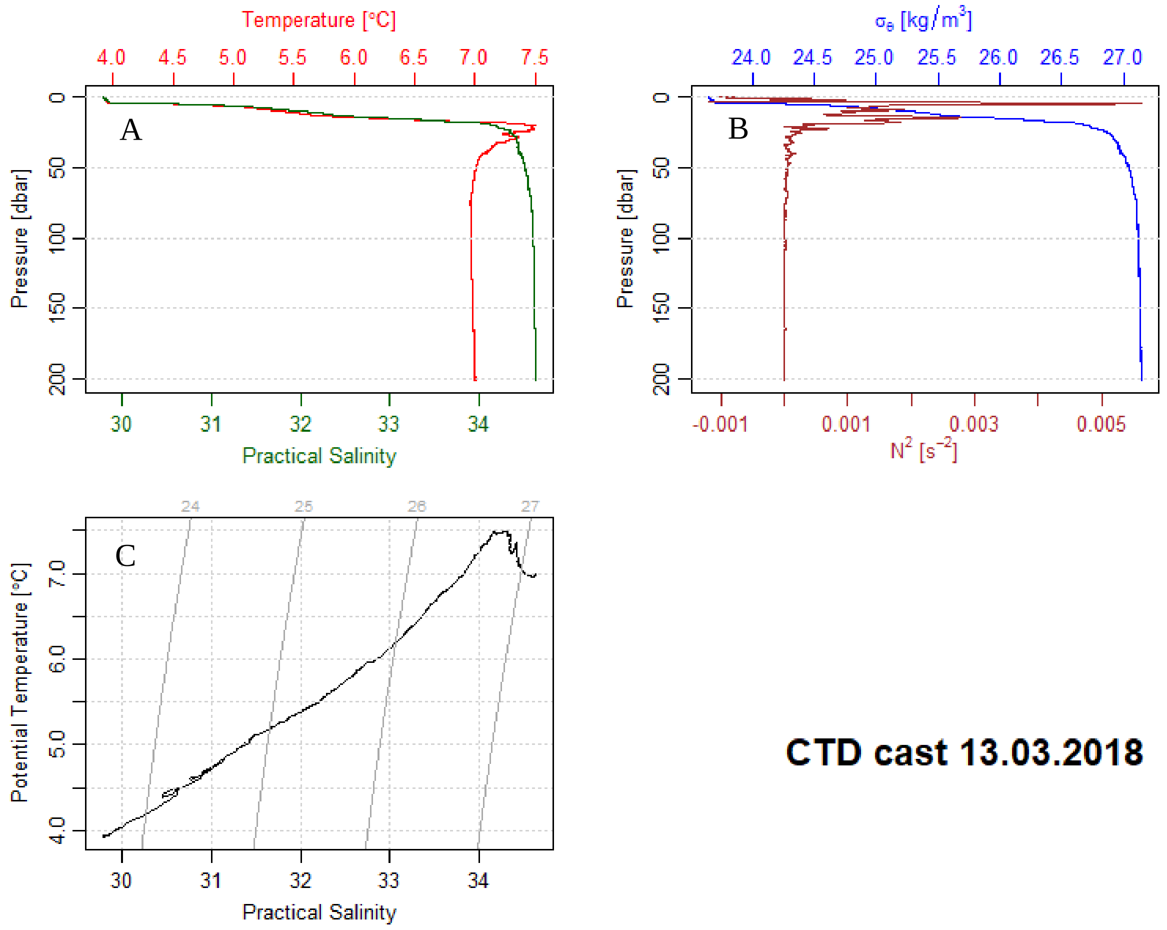

Figure 15 CTD cast, A: Temperature (red) with a peak at 7.5 °C at a depth of around 25 m (1 dbar ≈ 1 m seawater), stabilised at 7 °C at around 50 m, and salinity (PSU, green) stabilised at 34-35 PSU around 50 m depth. B: The blue line shows that the density stabilised, as the salinity, at around 50 m, and the Brunt-Väisälä frequency (burgundy/dark red) shows two pycnoclines. One strong pycnocline is visible at just a few m, and a less pronounced pycnocline closer to 20-25 m. C: TS plot indicates a stable water column with little to no mixing.

Apparent pigmentation analysis

I first plotted the overall % reflectance of the 252 animals (R+G+B reflectance, scaled to the maximum obtainable pixel value 3*65535) over increasing depth (fig. 16). I plotted the raw data as filled, semi-transparent circles so that overlaying points indicate similarities by making them look darker (blacker). The animals displayed variability within genera ~3 % - ~12.5 %, especially *Calanus* which was the most abundant genus in my samples. *Calanus* also displayed relatively high variability within the different target depths. Across depth, there were no apparent trends in the plots from the raw data.

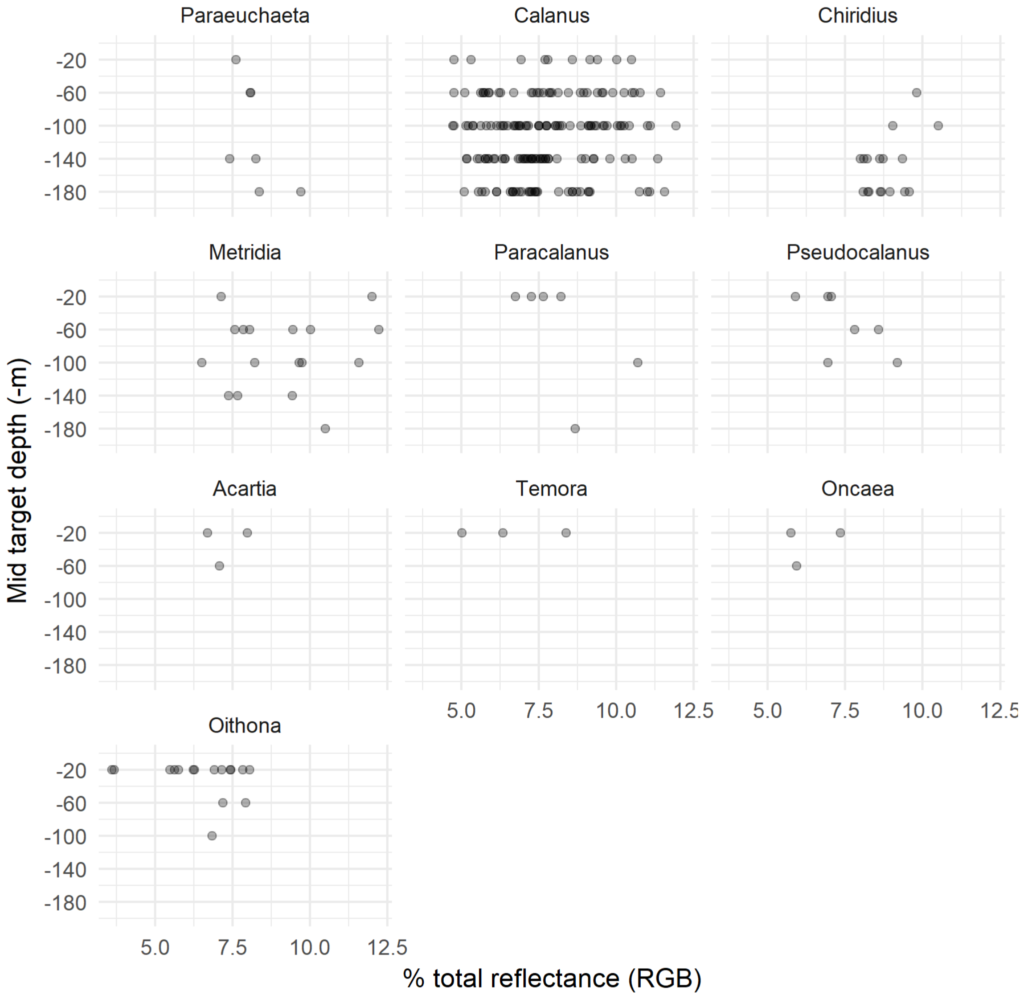

Figure 16 % total reflectance (scaled to maximum obtainable pixel value = 65535 * 3) in all three bands (R+G+B) over increased depth. Mid target depth (y-axis) indicates the middle depth of the targeted depth interval. By plotting the points as open circles, the overlaying points look darker than single observations indicating similarity. No apparent patterns are observable within genera.

The overall reflectance varied mostly within depths. *Paraeuchaeta* caught in the deepest layer had a higher reflectance than in the shallower depths, with the highest reflectance in the red spectrum for all depths (fig. 17). *Chiridius* showed an opposite trend with decreasing reflectance with increased depth along with *Metridia*, all varied within a 2 – 3 % range. It looks as if the composition of red, green and blue in most animals were evenly distributed (fig. 17). However, some genera displayed the highest reflectance values in the blue spectrum such as *Calanus*, *Metridia*, *Acartia* and *Oithona*, while others such as *Paracalanus*, *Temora* and *Oncaea* displayed the highest reflectance in the red spectrum. Green reflectance values were generally low in the data set.

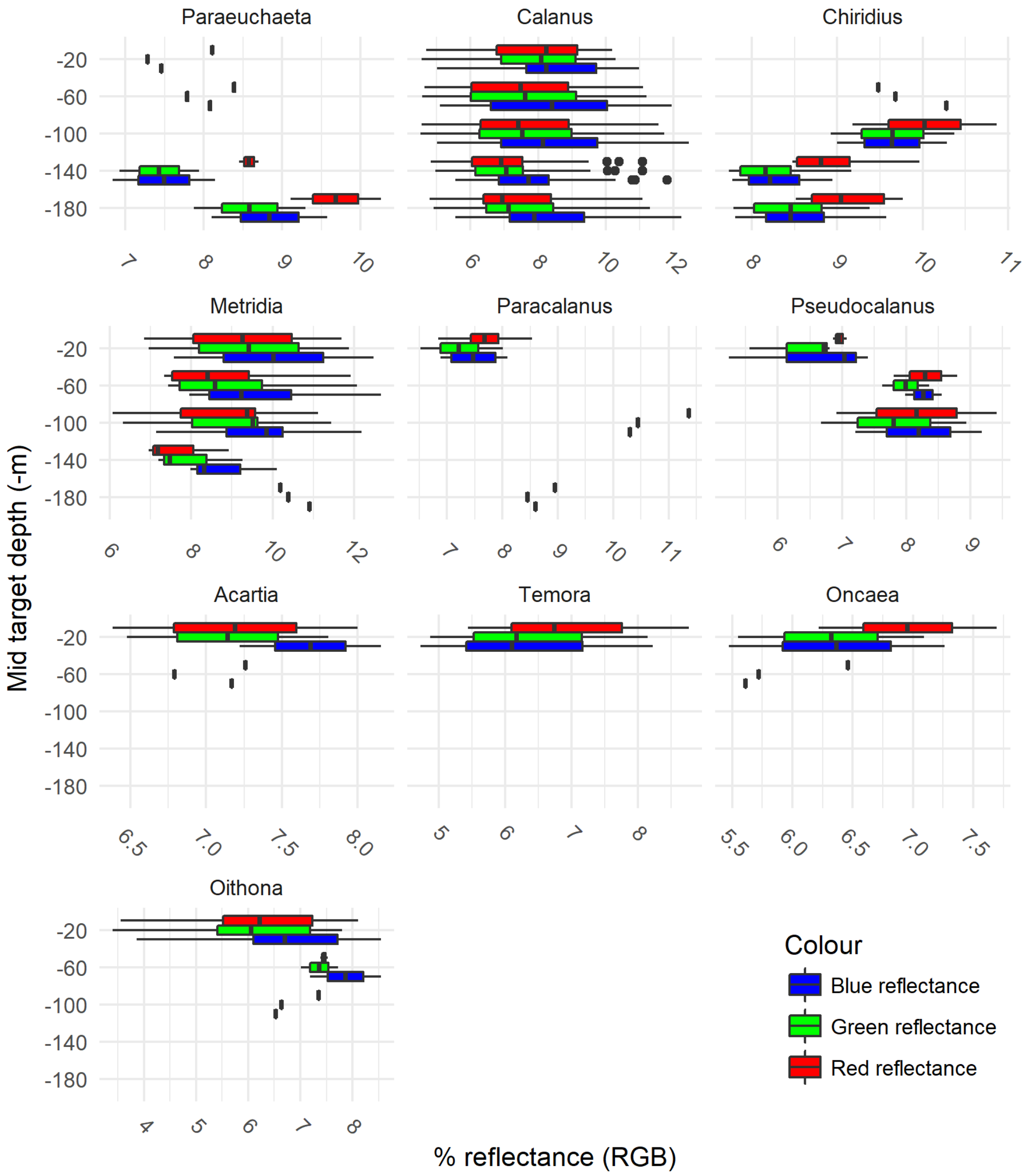

Figure 17 Boxplot with % reflectance (scaled to maximum obtainable pixel value = 65535) for all three bands (RGB) over increasing mid target depth. Boxes indicate 1st and 3rd quartile and the median. The whiskers stretch to the max (upper whisker), and min (lower whisker) value within the range of ± 1.5 * box length (3rd quartile – 1st quartile), with any data points outside the range of the whiskers plotted as outliers. Within some genera, there are observable differences in % light reflected and the colour composition of the reflected light with depth. Both the amount of reflected light, and the composition seems to vary with depth in genera sampled at different depths. The x-axis is scaled to each facet plot.

To take a closer look at the composition of the reflectance, I calculated the relative amount of reflectance in the red, green and blue spectra. Scaling the data to total reflectance, R+G+B = 100 %, made it possible to see how colour composition appeared at each target depth (fig. 18). If the relative reflectance of each colour band is situated at, or close to the dashed grey line which indicates 33 % (fig. 18), the animals are assumed to be white or transparent. Hence, the further the points and added lines are apart, the more, or less, pronounced the colour of the animal is, depending on which side of the 33 % line the relative reflectance is situated (see conceptual figure, fig. 7).

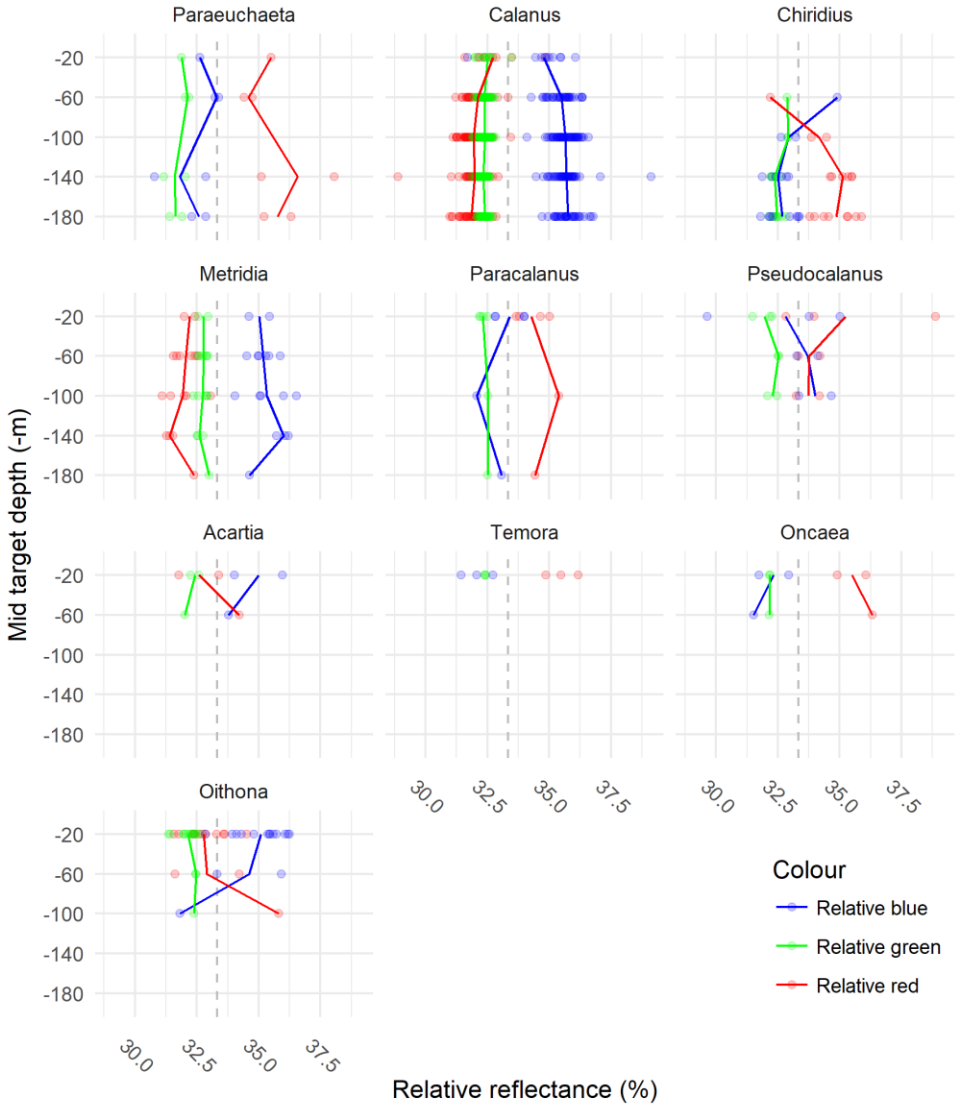

Figure 18 Relative reflection (scaled to 100 %) in % over mid target depth. Filled, semi-transparent show the raw data and overlapping points indicate where most observations are located by appearing stronger in colour. Lines are plotted as relative reflectance averaged by depth and genus to indicate changes in the relative composition of reflected light over depth. The vertical line (grey, dashed) intercepts the x-axis at 33 % and indicates where the three coloured lines should be oriented if the animals are colourless (transparent or white).

Temora was only caught in the upper layer, so there is no pattern to be shown. *Chiridius* and *Oithona* appeared to go from a higher reflectance in the blue band to a higher share in the red reflectance with increasing depth. *Paraeuchaeta* and *Paracalanus* displayed a higher red reflectance than green and blue throughout the sampling range. *Calanus* and *Metridia* appeared to be bluer according to the relative reflectance. However, most of the genera had relative reflectance values situated close to the 33% line, indicating that they are colourless (white or transparent).

Figure 19 shows predictions from a linear mixed effect model of red reflectance (log-scaled) over depth with genus as a random effect for both intercept and slope, fitted with the nlme-package in R. The open blue circles represent each animal from a given target depth. The blue lines indicate a non-significant positive fixed effect of red reflectance over depth (p = 0.0656) across all genera. The pink lines indicate the individual genus effect. The random effects (~Depth | Genus) capture some variance (0. $0.108^2 + 0.001^2$) but most is unexplained (0. 209^2). In the samples from 2018, red reflectance generally increased slightly with increased depth according to this model. I made four linear models allowing for full interactions, additive interactions and no interactions with genus and depth (tab. 4).

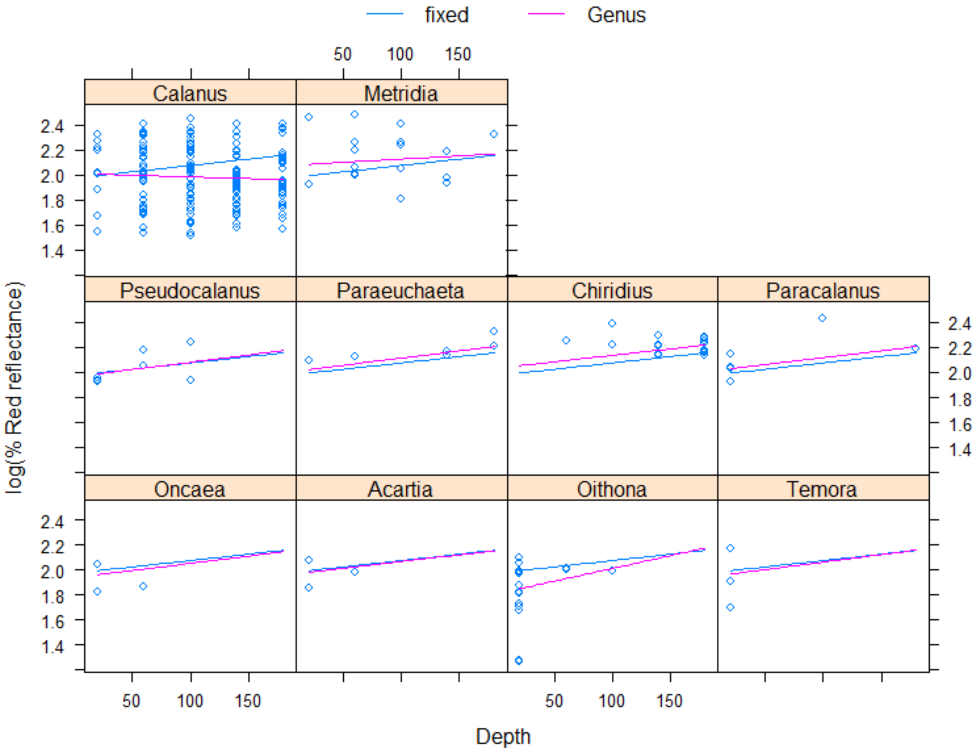

Figure 19 Linear mixed effect model (lme(log(Red reflectance) ~ Depth | Genus, data=d18), p-value = 0,07). X-axis = mid-depth interval, y-axis = log scaled % reflectance of red colour (scaled to maximum obtainable pixel value 65535). The blue line indicates the non-significant positive fixed effect (log(Red reflectance) ~ Depth), pink lines indicate individual genus effect.

The linear models indicate that there may be some interaction between depth and genus. Models that allow for both full interaction and additive interaction have p-values lower than 0.05 for the interaction term. However, the model with red reflectance modelled by genus (log(Red reflectance) ~ Genus) has the highest F-ratio (5.198), the lowest p-value (1.836e-06) and the lowest AICc-value (-58.94) and is decided to be the model that best describes the variation in red reflectance. The decision is backed up by visually checking the distribution of the residuals in q-q-plots and histograms of residuals for the various models (Appendix A). Models for all colours and total reflectance are available in Appendix A. The distribution of red reflectance for each genus is presented in a boxplot (fig. 20).

Table 4 Linear models used for analysing red reflectance over depth in the data from the photo analysis of my samples. R^2 explain how much of the variance in the dependent variable that is predictable by the independent variables and ranges from 0 to 1 with 1 indicating a perfect fit of the model. The F-ratio is a measure for the strength of the regression, and a high F-ratio indicates a strong relationship between the dependent and the independent variable. The higher the F-ratio, the stronger the relationship between variables is. The p-value indicates the significance level of the model. A p-value lower than 0.05 indicates significance and discarding of H_0. Degrees of freedom used by the model is denoted as df. The column with AICc-values shows the corrected Akaike information criterion for each model. AICc is used as an estimate of the relative quality of the models. The lowest AICc value indicates the best model. In this case log(Red reflectance) ~ Genus is the best model (AICc = -58.94) describing 13.08 % of the variance in the data using 11 degrees of freedom.

Linear models, red reflectance and depth Data = 2018	**R^2**	**F-ratio**	**P-value**	**df**	**AICc-value**
lm(log(Red reflectance) ~ Depth * Genus)	0.1198	2.897	0.0001209	20	-44.77
lm(log(Red reflectance) ~ Depth + Genus)	0.1278	4.679	4.121e-06	12	-56.91
lm(log(Red reflectance) ~ Depth)	0.0025	1.637	0.2019	3	-33.05
lm(log(Red reflectance) ~ Genus)	0.1308	5.198	1.836e-06	11	-58.94

In figure 21, the prosome area is plotted over increased depth and varies from 0.042 mm^2 to 10.25 mm^2. The average sizes of *Pareuchaeta*, *Chiridius* and *Paracalanus* indicate a clear increase with increased depth. *Calanus* shows a slight increase in average size over increased depth. *Metridia* has a similar average size in the top layer and the bottom layer, with slightly smaller sizes between the top and bottom. *Pseudocalanus*, *Acartia*, *Oithona* and *Oncaea* varies around 0.1 mm^2 in average size between depths. *Temora* was only found in the top layer with an average size just below 0.1 mm^2.

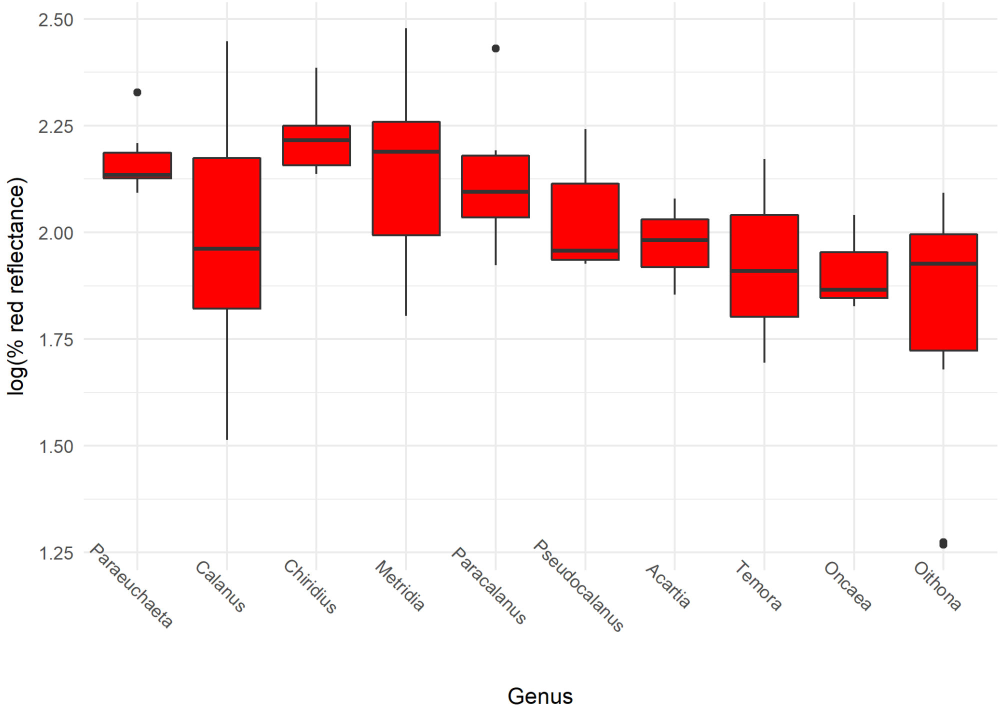

Figure 20 Distribution of red reflectance of 2018 samples (log scaled, % scaled to max obtainable pixel value 65535) over genus. The linear model (log(Red reflectance) ~ Genus) show significant p-values for *Paraeuchaeta*, *Calanus* and *Oithona* when compared with the others.

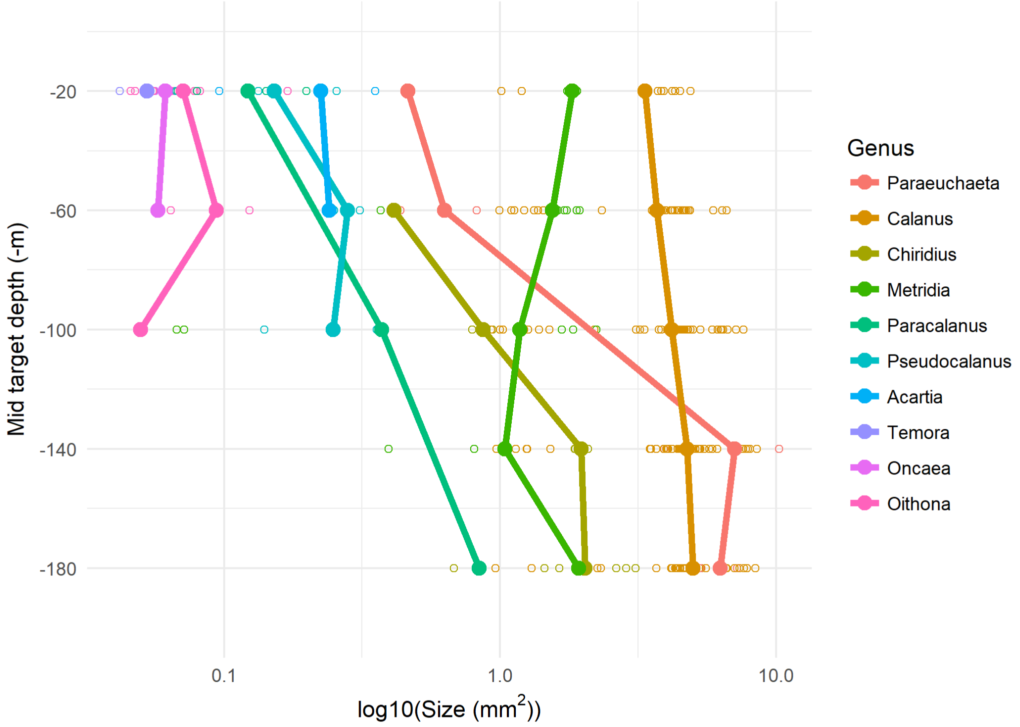

Figure 21 Prosome area in samples from 2018 plotted as size (mm^2, log10 scale) over mid target depth. The open circles (points) shows the raw data, while the filled points and associated lines indicate the trends of average size, within genera, over increasing depth.

It looks as if the smaller copepods, *Paracalanus*, *Pseudocalanus*, *Acartia*, *Oncaea* and *Oithona*, had lower reflectance values than the ones with sizes around one mm^2 (fig. 22). However, the cluster with *Calanus* had lower reflectance than those slightly smaller. Except for the big sized-low reflectance cluster of *Calanus*, reflectance values increased with increased prosome size in my data.

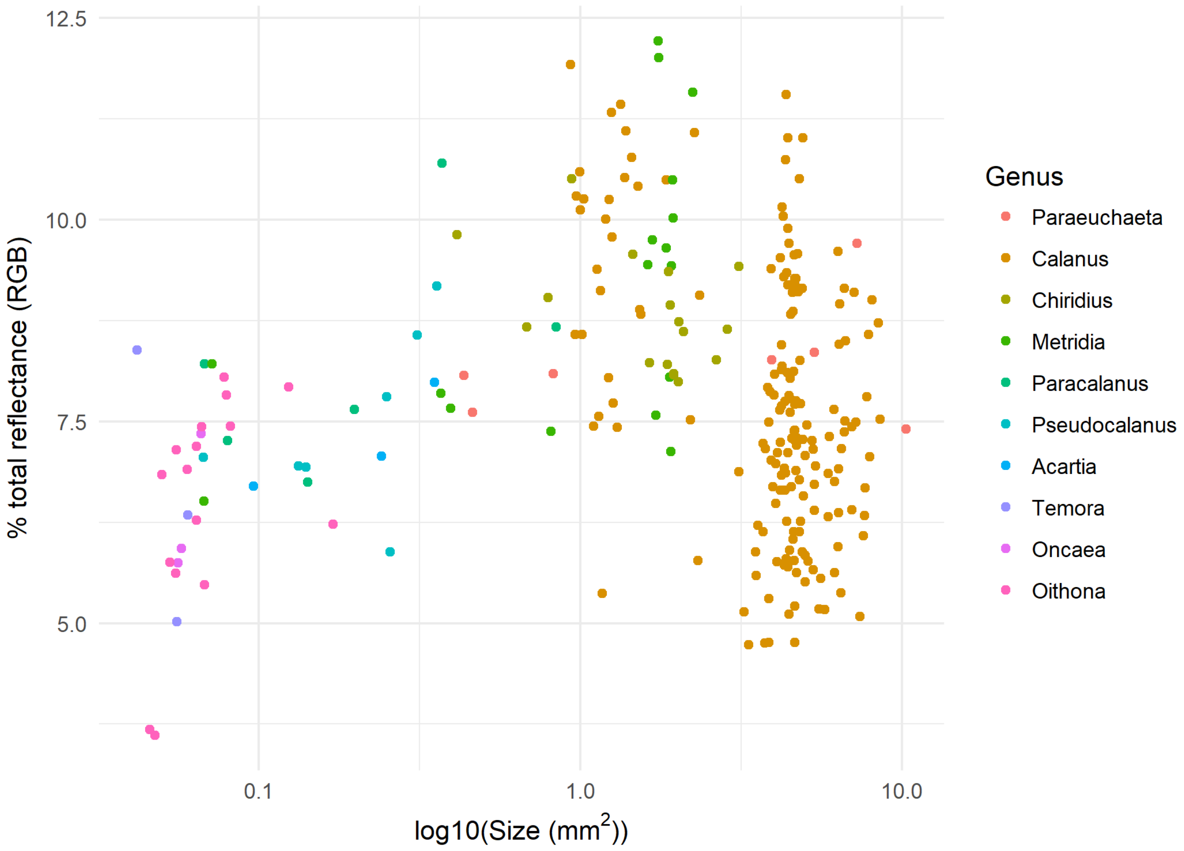

Figure 22 % reflectance (scaled to the maximum obtainable pixel value = 65535 * 3) in all three bands (R+G+B) over prosome area plotted as size (mm^2, log10 scale), samples from 2018. Reflectance values increased with increased prosome size across most genera, except for the cluster of *Calanus* which strongly deviated from the pattern.

The linear mixed effect model for red reflectance with size and genus (fig. 23) indicates a non-significant positive fixed effect of prosome size across genera (blue lines, $p = 0.07$). That indicate that increased prosome size increase reflectance as a general trend. The random effects (~ log(Size) | Genus) capture very little of the variance ($0.0009^2 + 0.08^2$), and most is unexplained (0.19^2). Thus, I made linear models to investigate the relations further (tab. 5).

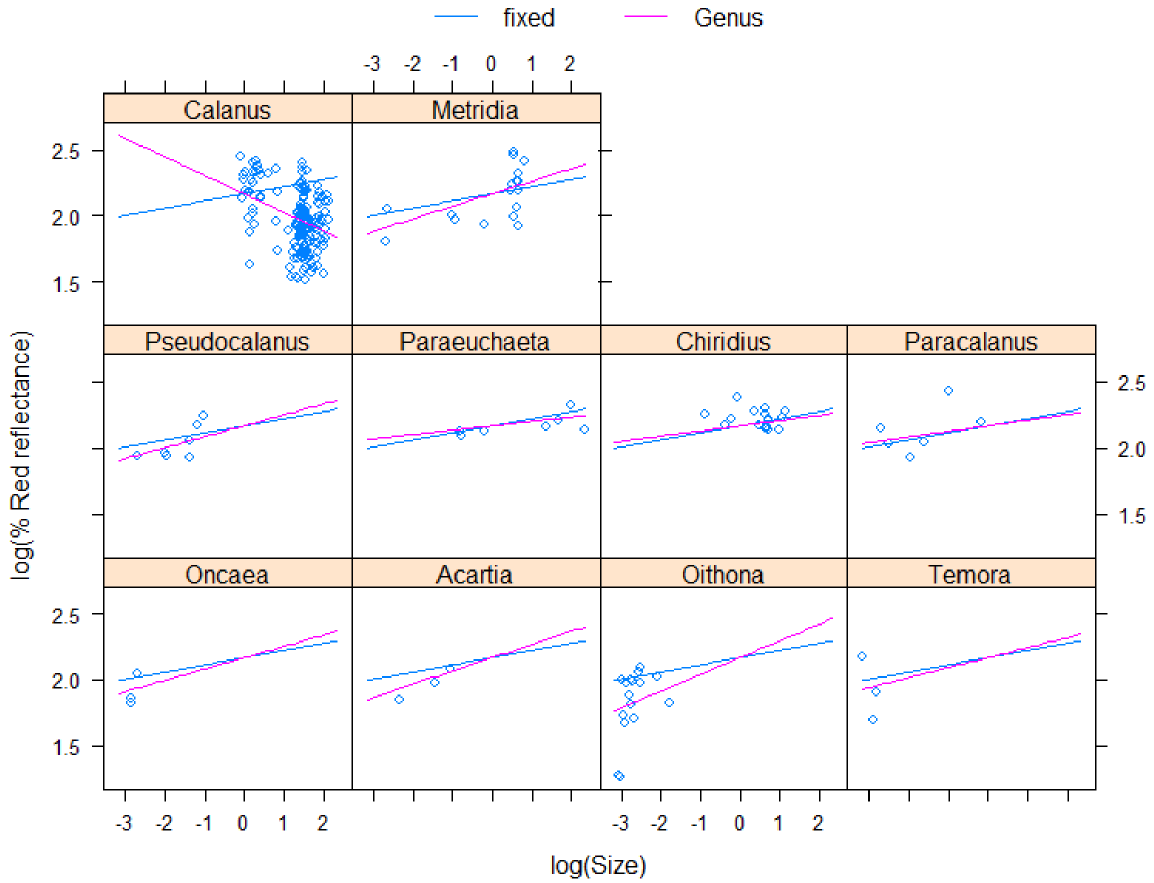

Figure 23 2018 samples, linear mixed effect model (lme(log(Red reflectance) ~ log(Size) | Genus, data=d18), p-value = 0.0711). x-axis = log(Size), y-axis = log scaled % reflectance of red colour (scaled to maximum obtainable pixel value (65535)). Blue line indicates the non-significant positive fixed effect (log(Red reflectance) ~ log(Size)), pink lines indicate random genus effects.

Table 5 Linear models used for analysing red reflectance and size in the data from the photo analysis. Log(Red reflectance) ~ log(Size) * Genus allowing for full interactions with size and genus is the best model. The model uses 21 degrees of freedom explaining 26.2 % of the variance with a F-ratio of 5.69, p-value = 1.547e-11 and AICc = -87.88.

Linear models, red reflectance and size Data = 2018	**R^2**	**F-ratio**	**P-value**	**df**	**AICc-value**
lm(log(Red reflectance) ~ log(Size) * Genus)	0.262	5.690	1.547e-11	21	-87.88
lm(log(Red reflectance) ~ log(Size) + Genus)	0.144	5.223	6.096e-07	12	-61.63
lm(log(Red reflectance) ~ log(Size))	-0.004	0.011	0.9163	3	-31.42
lm(log(Red reflectance) ~ Genus)	0.131	5.198	1.836e-06	11	-58.94

The best linear model allows for full interactions between size and genus (log(Red reflectance) ~ log(Size) * Genus). The model uses 21 degrees of freedom explaining 26.2 % of the variance with a F-ratio of 5.69, p-value = 1.55e-11 and AICc = -87.88. I verified the best model decision by visual interpretation of residual distribution in q-q-plot and histogram of residuals. Models for all colours with associated residual plots for both 2017 and 2018 is available in Appendix B.

Pigment content analysis

Astaxanthin was the most abundant pigment in my samples (fig. 24). Other relatively abundant pigments in the extracts were alloxanthin, pheophytin a, myxoxanthin, peridinin, canthaxanthin and pheophytin b.

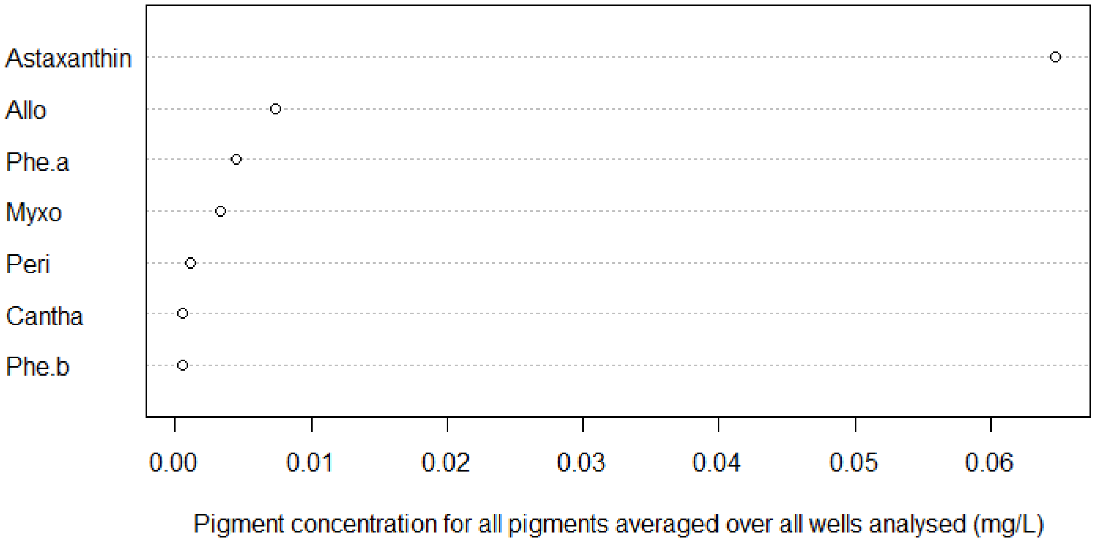

Figure 24 Dot chart showing the concentration of the most abundant pigments averaged over all 1047 samples in mg/L. Astaxanthin was the most abundant pigment with an average of ~ 0.6 mg/L.

Astaxanthin dominated in *Paraeuchaeta*, *Calanus*, *Chiridius* and *Centropages* (fig. 25). For *Metridia*, *Paracalanus* and *Temora*, with a few exceptions for all three, alloxanthin was the dominating pigment. Both pigments may contribute to red, yellow and blue colouration depending on their protein matrix (Buchwald and Jencks 1968; Li et al. 2013; Khalili et al. 2016).

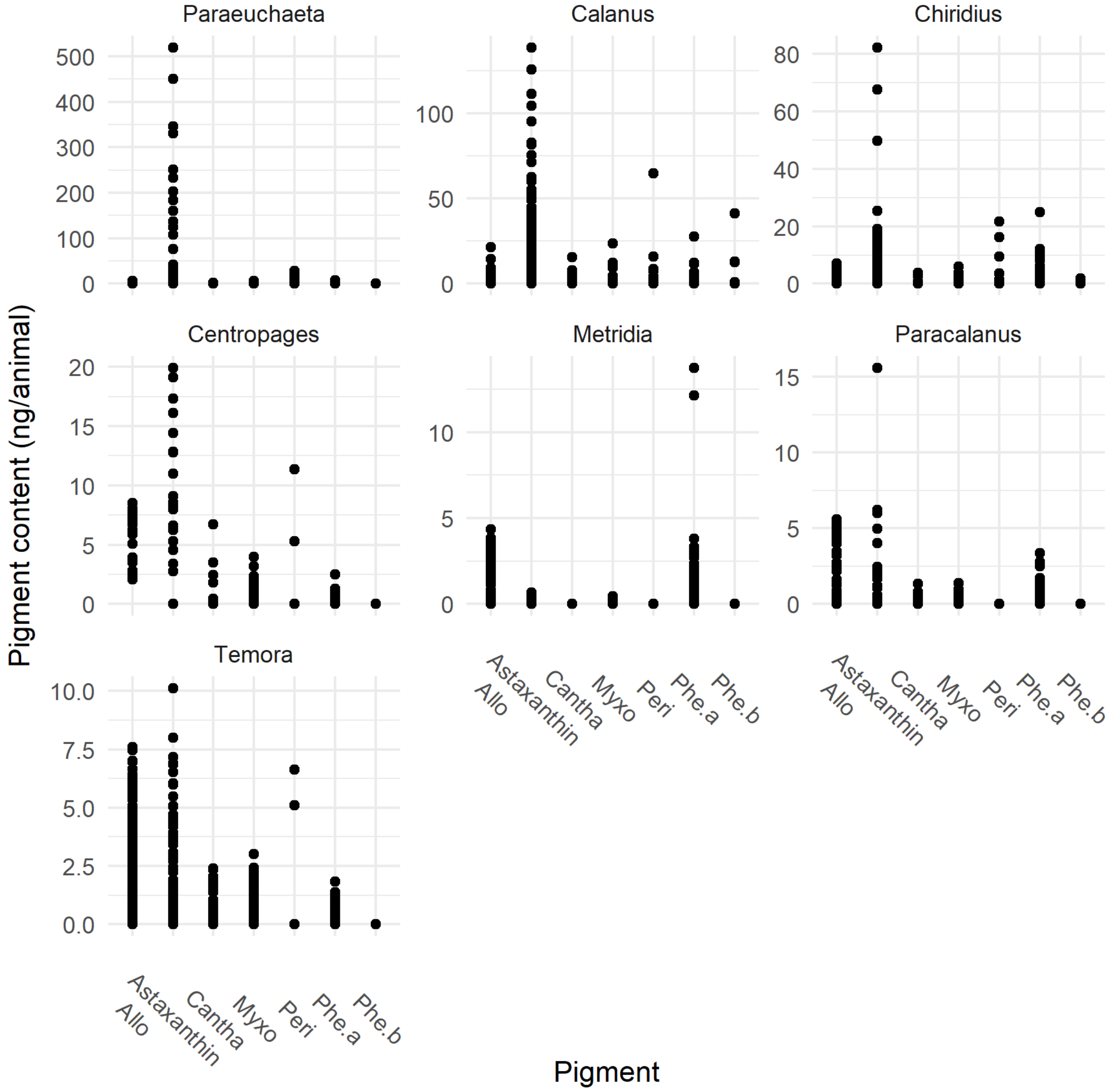

Figure 25 Pigment distribution and content plotted for each genus with scaled y-axis for each facet plot. Apparent differences in content and distribution of the analysed pigments between species. There is also evident that there is a relatively high variation within genera.

Most genera were found at more than one target depth. In *Paraeuchaeta*, *Calanus* and *Chiridius*, specimens with the highest total pigment content were caught at deeper depths than specimens with lower contents (fig. 26). *Centropages* was only caught at one target depth and contained slightly less pigments than, e.g. *Chiridius*. *Metridia*, *Paracalanus* and *Temora*. For the three latter genera, there is no apparent pattern (fig. 26).

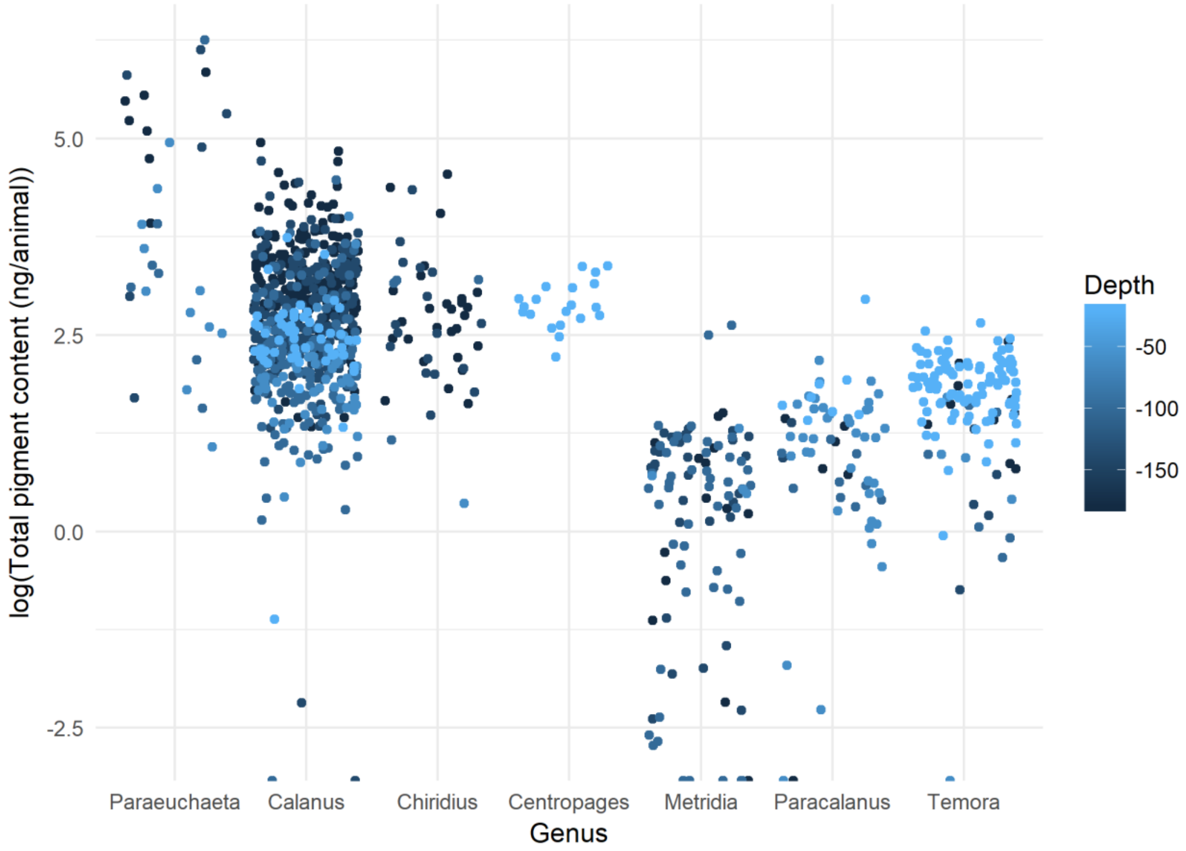

Figure 26 Jitterplot showing total pigment content (log-scaled) per genus with points colour scaled to sampling depth. The darker the points, the deeper the animal is sampled. In *Paraeuchaeta* and *Calanus* it looks as if higher pigment content is correlated with depth as we see the lighter blue points has lower contents than the darker blue points.

The boxplots (fig. 27) showing the total pigment content over depth indicate that pigment content in the copepods in this study varied with depth in most genera. *Paraeuchaeta*, *Calanus* and *Chiridius* tended to have increased content with increased depth, while *Paracalanus* seemed to display an opposite trend. *Centropages* was only caught at the shallowest depth, so no trend is observable. *Metridia* displayed a relatively steady content throughout the sampling range, while *Temora* contained the highest contents at the most shallow and deepest depth with relatively stable content levels in between. I plotted the averaged content for each pigment per target depth for each genus to examine if there are different trends for the different pigments (fig. 28).

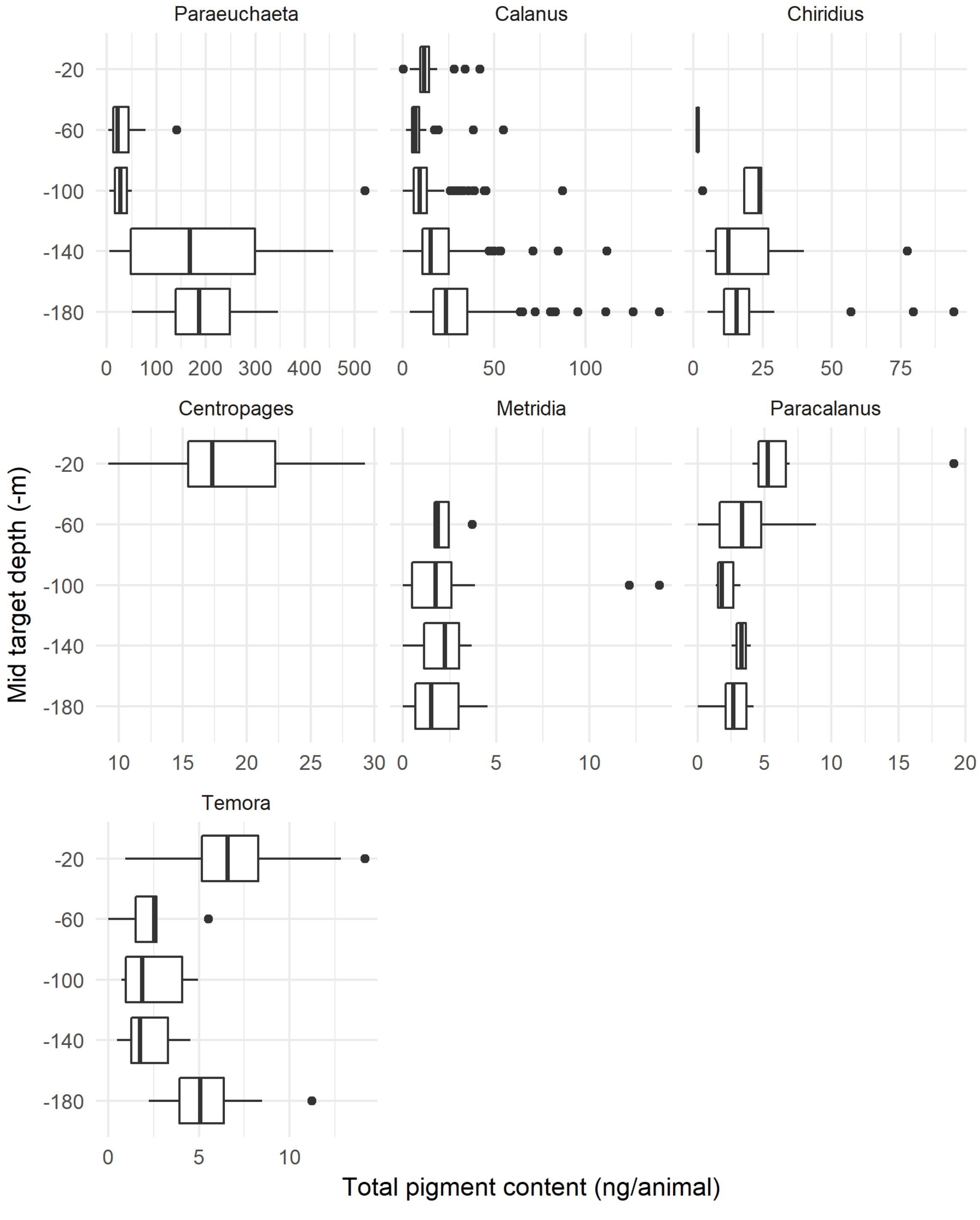

Figure 27 Boxplot with total pigment content per animal (x-axis) over increased depth (y-axis). *Paraeuchaeta*, *Calanus* and *Chiridius* had an increase in pigment content with depth. *Metridia* displayed similar content at all depths. A slight decrease with depth was displayed in *Paracalanus*, while *Temora* had the highest content in the most shallow and deepest layer.

The different genera contain different amounts and compositions of the pigments tested in this study. Compared to the other pigments, *Paraeuchaeta* contains high amounts of astaxanthin, with the average increasing from just over 25 ng to over 175 ng with increasing depth. In *Calanus* and *Chiridius* there is also an apparent increase in astaxanthin with increased depth.

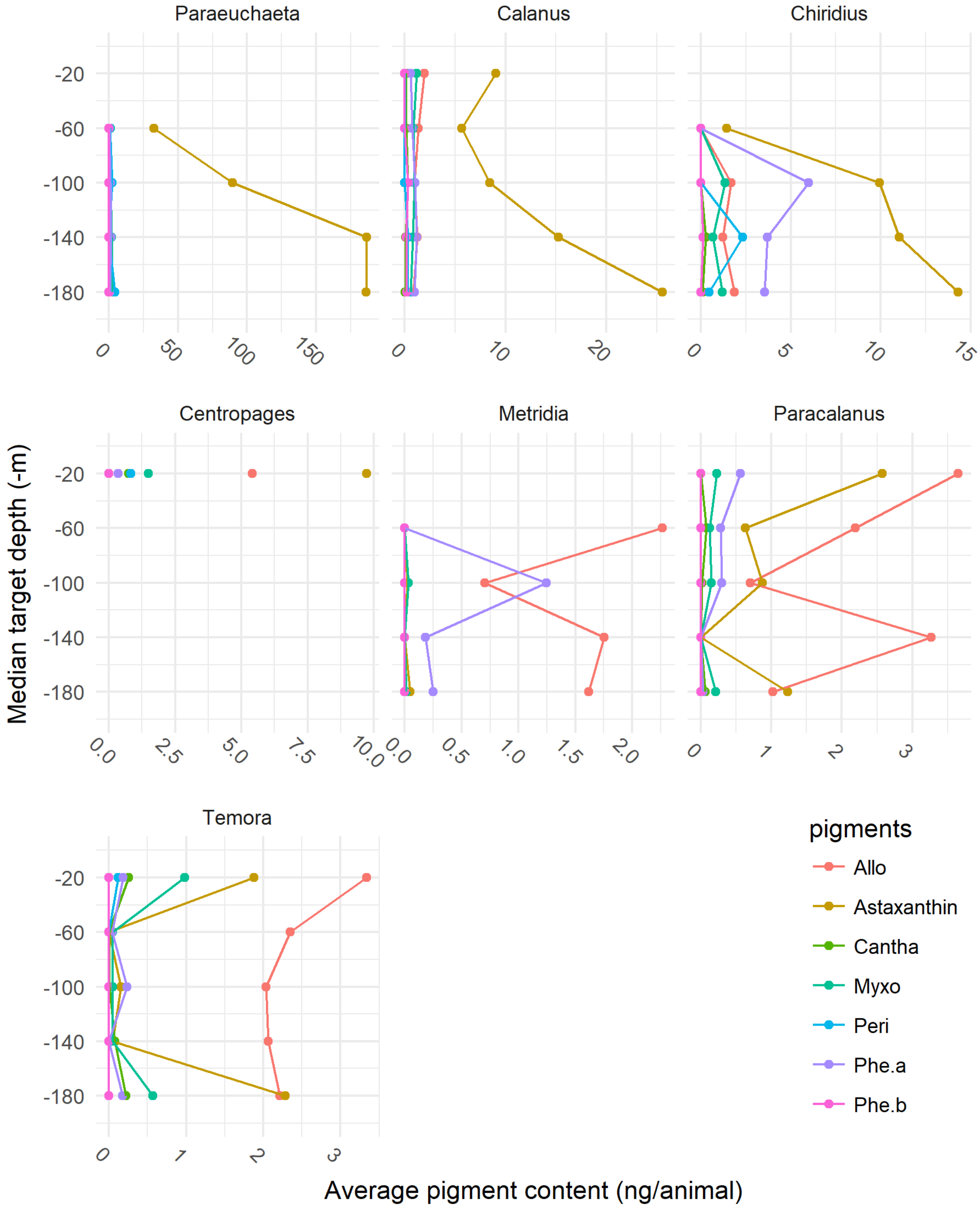

Figure 28 Average pigment content per animal (x-axis, adjusted for each facet plot, averaged for each target depth and genus) over increased depth (y-axis) with lines to indicate trends. A similar increase in pigment content (astaxanthin) with increased depth in *Paraeuchaeta*, *Calanus* and *Chiridius*. The other genera do not show as clear trends.

Metridia, *Paracalanus* and *Temora* contain on average higher amounts of alloxanthin than other pigments with a few exceptions at some target depths were the content lines cross. This result is also visible when comparing total pigment content over depth with astaxanthin contents over depth (fig. 29 and 30). There are marginal differences in the averaged lines in *Paraeuchaeta*, *Calanus* and *Chiridius*.

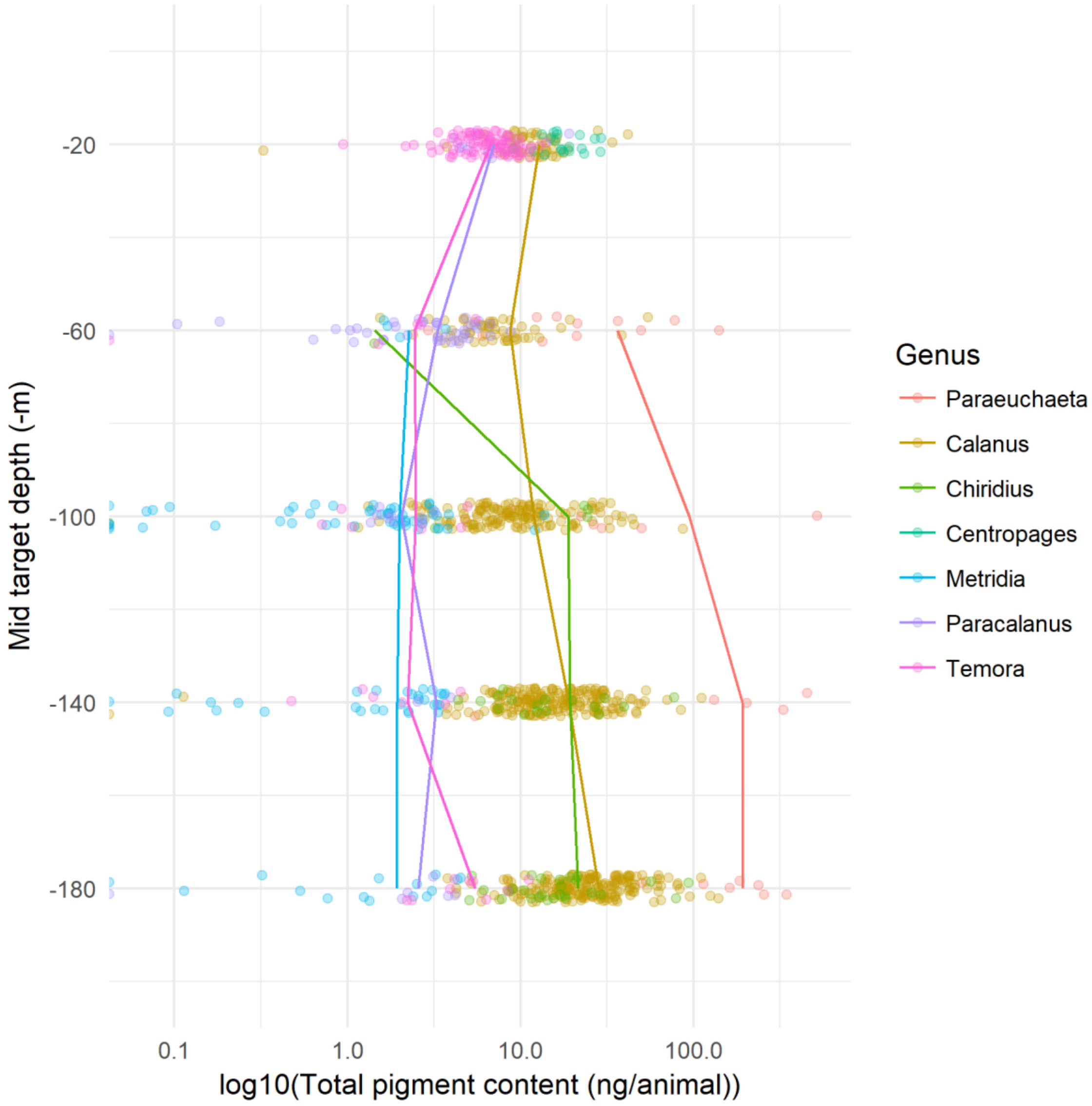

Figure 29 Total pigment content per animal over increasing depth, jitterplotted with the log10-scaled x-axis. Lines indicate averaged pigment content for each genus per depth. *Paraeuchaeta*, *Calanus* and *Chiridius* show an increase of total pigment content with increased depth. *Temora* and *Paracalanus* show clear indications of containing the most pigments in the upper layers and decreasing content with depth. *Metridia* also shows indications of decreasing contents with depth. *Centropages* was only present at the shallowest sampling depth.

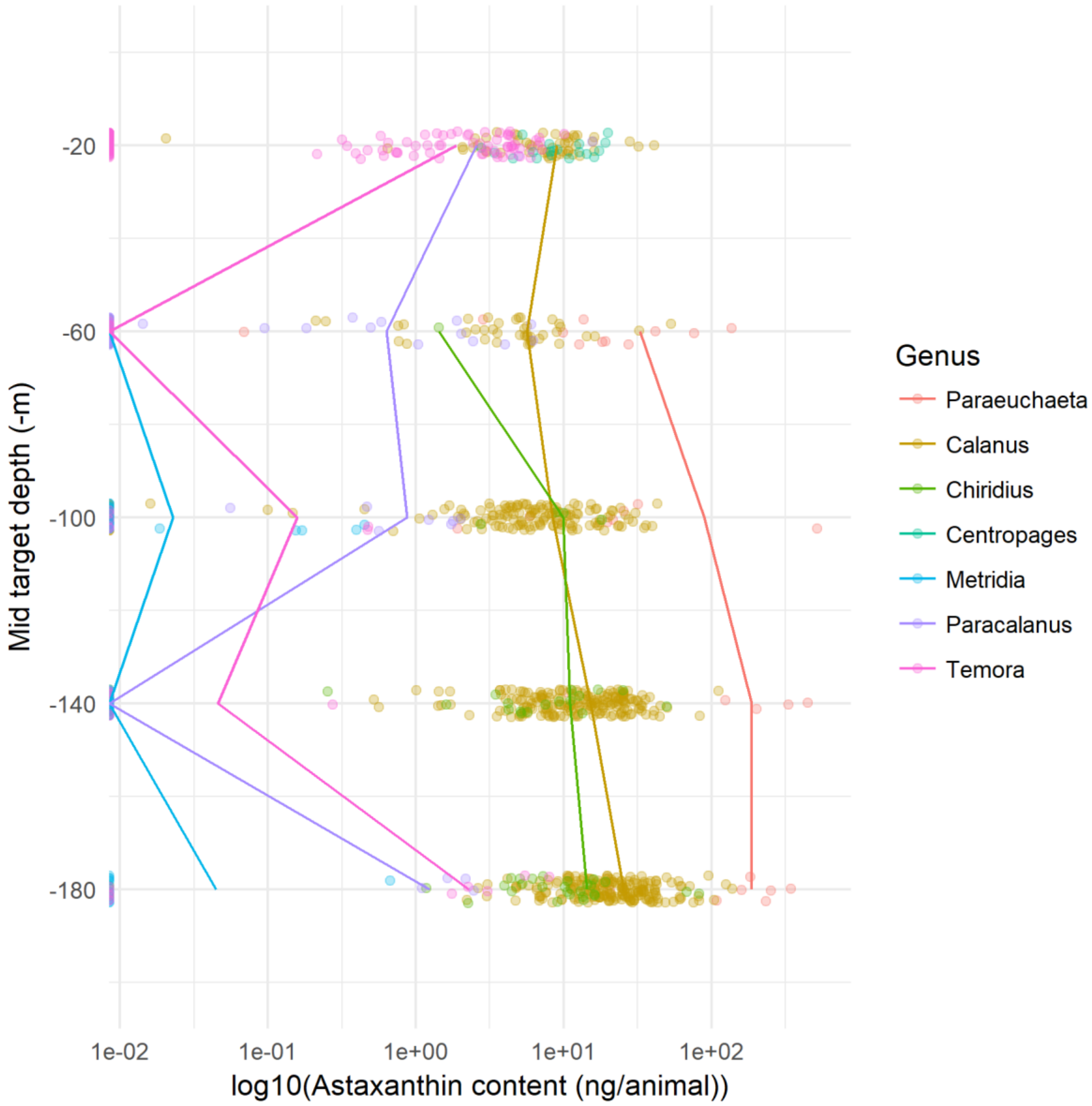

Figure 30 Astaxanthin content per animal over increasing depth, jitterplotted with the log10-scaled x-axis. Lines indicate averaged astaxanthin content for each genus per depth. *Paraeuchaeta*, *Calanus* and *Chiridius* show an increase of astaxanthin content with increased depth. *Temora* and *Paracalanus* show clear indications of containing the most astaxanthin in the upper layers and decreasing content with depth. *Metridia* also shows indications of decreasing contents with depth. *Centropages* was only present at the shallowest sampling depth.

In *Metridia*, *Paracalanus* and *Temora*, the lines change quite dramatically when comparing contents including pigments obtained through feeding (i.e. alloxanthin, myxoxanthin, canthaxanthin, peridinin and pheophytin a and b) with only astaxanthin. At some target depths, the content of astaxanthin is zero for *Metridia*, *Paracalanus* and *Temora*, shifting the lines to the left in the plot. The linear mixed effect model indicates a non-significant (p-value = 0.20) positive fixed effect of pigment content over depth (fig. 31). Individual predictions by genera indicate a positive correlation between pigment content per animal with increased depth in *Paraeuchaeta*, *Calanus*, *Chiridius* and *Centropages* though the latter was only found at one target depth. A negative correlation is observable in the other three genera. The random effects (~ Depth | Genus) captures some variance in the data ($0.649^2 + 0.009^2$) but also in this model, most of the variance is unexplained (0.764^2). I made four additional linear models examining further how the pigment content correlate with depth in my samples (tab. 6).

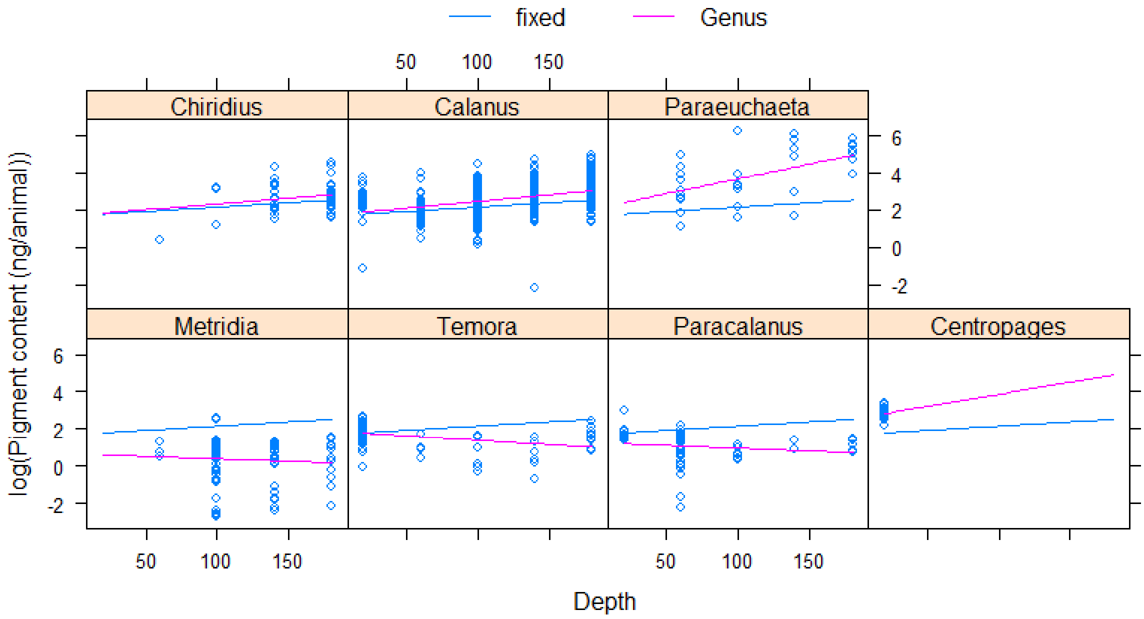

Figure 31 Linear mixed effect model (lme(log(Pigment content) ~ Depth | Genus, data=d22), p-value = 0.20). X-axis = mid-depth interval, y-axis = log scaled pigment content (ng/well). The blue line indicates the non-significant positive fixed effect (log(Pigment content) ~ Depth), pink lines indicate individual Genus effect.

Table 6 Linear models used to analyse pigment content over depth in the pigment extracts. Log(Content) ~ Depth * Genus explains the most of the variance in the residuals ($R^2 = 0.57$) and has the lowest AICc value (2393.81) indicating that this is the best model of the four even though it uses the highest amount of degrees of freedom (df = 14).

Linear models, pigment content and depth **Data = 2017, 2018**	**R^2**	**F-ratio**	**P-value**	**df**	**AICc-value**
lm(log(Content) ~ Depth * Genus)	0.57	115.0	< 2.2e-16	14	2393.81
lm(log(Content) ~ Depth + Genus)	0.53	167.2	< 2.2e-16	9	2480.84
lm(log(Content) ~ Depth)	0.09	103.9	< 2.2e-16	3	3156.45
lm(log(Content) ~ Genus)	0.49	167.9	< 2.2e-16	8	2558.93

All four models have a p-value <2.2e-16 and indicate that both depth and genus are significant variables determining the pigment content in my samples. However, the model allowing for full interactions between depth and genus (lm(log(Pigment content) ~ Depth * Genus)), have the best fit ($R^2 = 0.57$) and the lowest AICc-value (2393.81) although it uses the most degrees of freedom (14). In the summary of the model (Appendix C) levels of significance is found for depth and all depth-genus interactions.

Apparent pigmentation and actual pigment concentration

The average total reflectance correlated negatively with the averaged pigment content per animal ($p = 0.027$) (fig. 32). The negative correlation indicates that increased pigment content leads to a decrease in reflectance values. In other words, the animals appear less colourful with more pigments. The *Calanus* points formed a cluster while the other genera seemed more dispersed indicating that the variation between depths within *Calanus* was low compared to the others. By normalising the pigment contents to animal size, I further investigate this relationship.

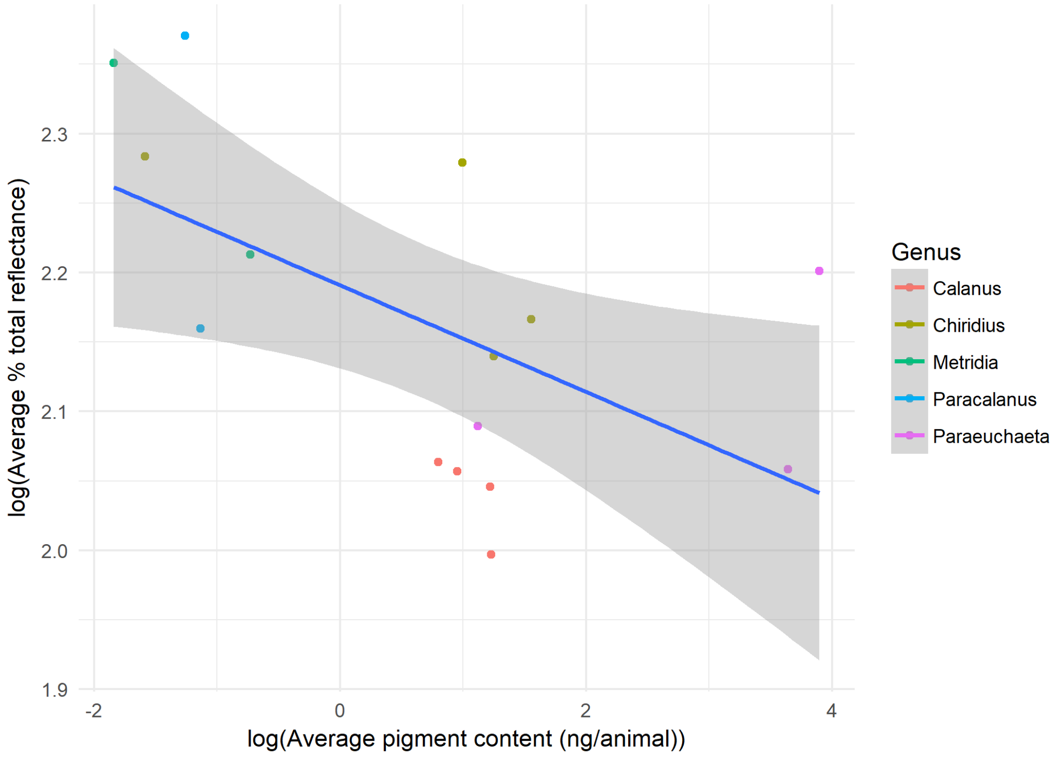

Figure 32 Averaged % total reflectance plotted as a function of averaged pigment content per animal, both log-scaled and averaged to each depth for each genus. Points are coloured by and represent the different genera. The blue line indicates the significant negative correlation between reflectance and pigment content, with the grey areas showing the standard error.

The comparison of reflectance and pigment concentration normalised to body volume reveals that there is no correlation (fig. 33). Increasing or decreasing the concentration of pigments does not affect apparent pigmentation at all. The linear model further verifies this non-existing correlation (tab. 7). The points representing *Calanus*, *Chiridius* and *Paracalanus* are even more clustered together with their genera. *Metridia* and *Paracalanus*, represented by two points each, are more dispersed when plotting the normalised concentration values. The way some genera such as *Calanus*, *Chiridius* and *Paracalanus* forms clusters indicates that there were similarities in pigment concentration per volume unit within several genera across depths. Increased dispersion that was the case for *Metridia* and *Paracalanus*, on the other hand, indicates greater variation between depths.

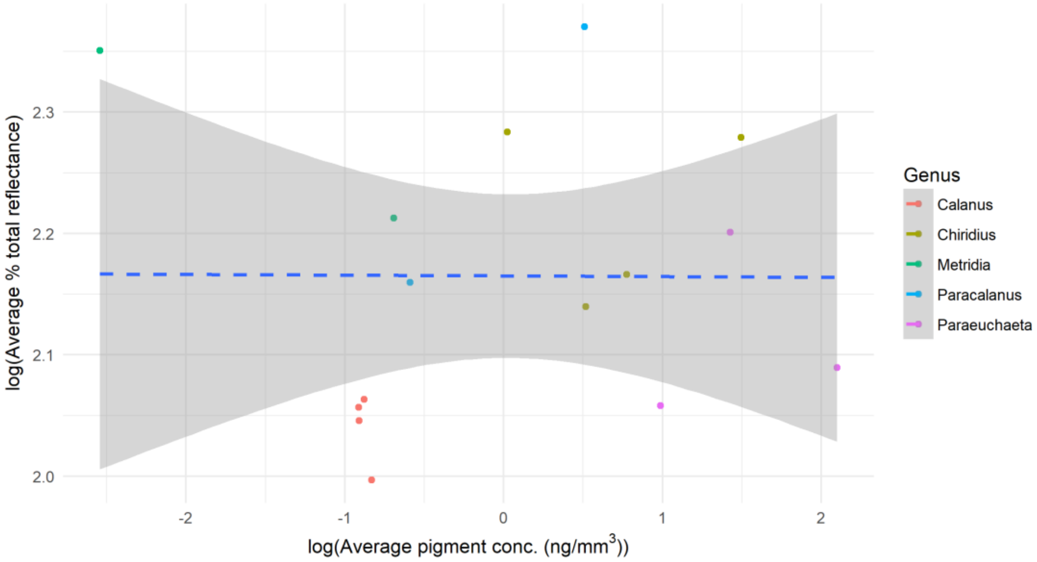

Figure 33 Averaged % total reflectance plotted as a function of averaged pigment concentration per mm^3, both log-scaled and averaged to each depth for each genus. Points are coloured by and represent the different genera. The dashed blue line indicates the non-significant non-existing correlation between reflectance and pigment concentration, with the grey areas showing the standard error.

Table 7 The linear models used to analyse the relationship between pigment content and concentration and total reflectance. The first model seems to be the best model, describing some of the variances in the residuals (27 %) with a good F-ratio (6.238) and significant p-value (0.027). However, when using the normalised pigment concentration values, the second model may describe the relationship more accurate, indicating no correlation between reflectance and pigment concentration with normalised values to size or volume.

Linear models, reflectance and pigment concentration **Data = photo 2018, pigments 2018**	**R^2**	**F-ratio**	**P-value**	**df**
lm(log(Averaged Reflectance) ~ log(Averaged Content (ng/animal)))	0.27	6.238	0.027	3
lm(log(Averaged Reflectance) ~ log(Averaged Concentration (ng/mm^3)))	-0.08	0.0005	0.98	3

Light setup trial results

The results from the light setup test were very inconclusive indicated by the lines pointing in all directions over the treatment (fig. 33). The experiment ran 12th of March indicate that all treatments led to a decrease of reflectance in all three colour bands. On the 13th of March, only the control treatment led to a decrease, while the "31", "63" and "127" treatments led to both increased and decreased reflectance with an increase in reflectance in the "255" treatment. The 15th of March all treatments led to both increasing and decreasing reflectance.

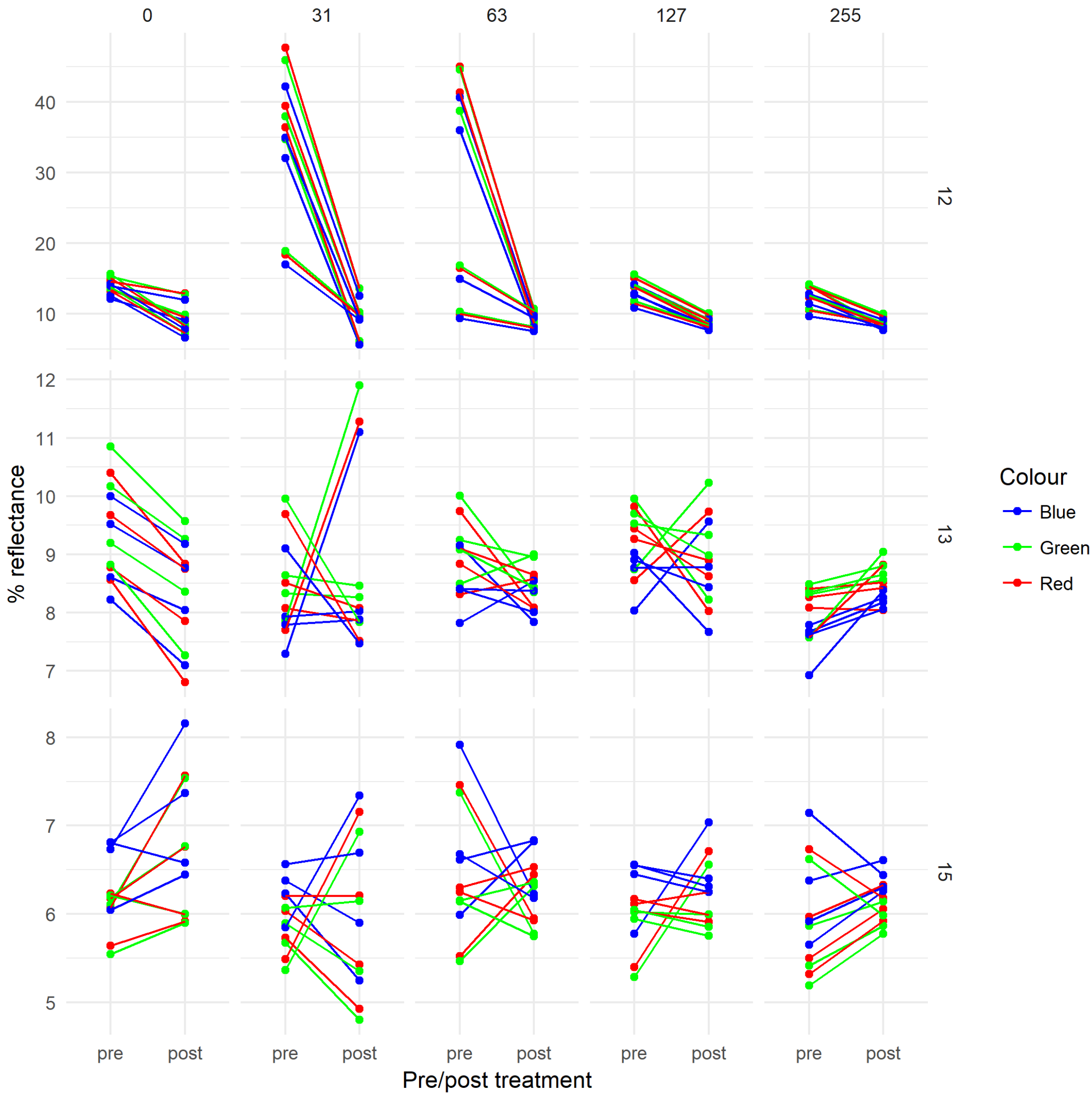

Figure 33 Results from photo analysis of *C. hyperboreus* before and after treatment ran at three different days (12th, 13th, and 15th of March 2018, labelled to the right in the plot) in 4 different light intensities and no light (light intensities labelled at the top of the plot). Y-axis shows % of full reflectance, x-axis shows before and after treatment, facet plotted by light intensity (treatment) and date. No apparent patterns emerge, and the results seem inconclusive.

The results appear more organised when looking at the relative reflectance of the three colour bands (fig. 34). The results indicate that the amount of green reflectance was relatively stable across treatments and was the highest relative share of total reflectance except for the 15th. On the 15th, the relative shares seemed to be opposite from the other two days with the blue reflectance being the strongest. On the 13th, all treatments appeared to increase the relative blue reflectance and decrease the relative red reflectance.

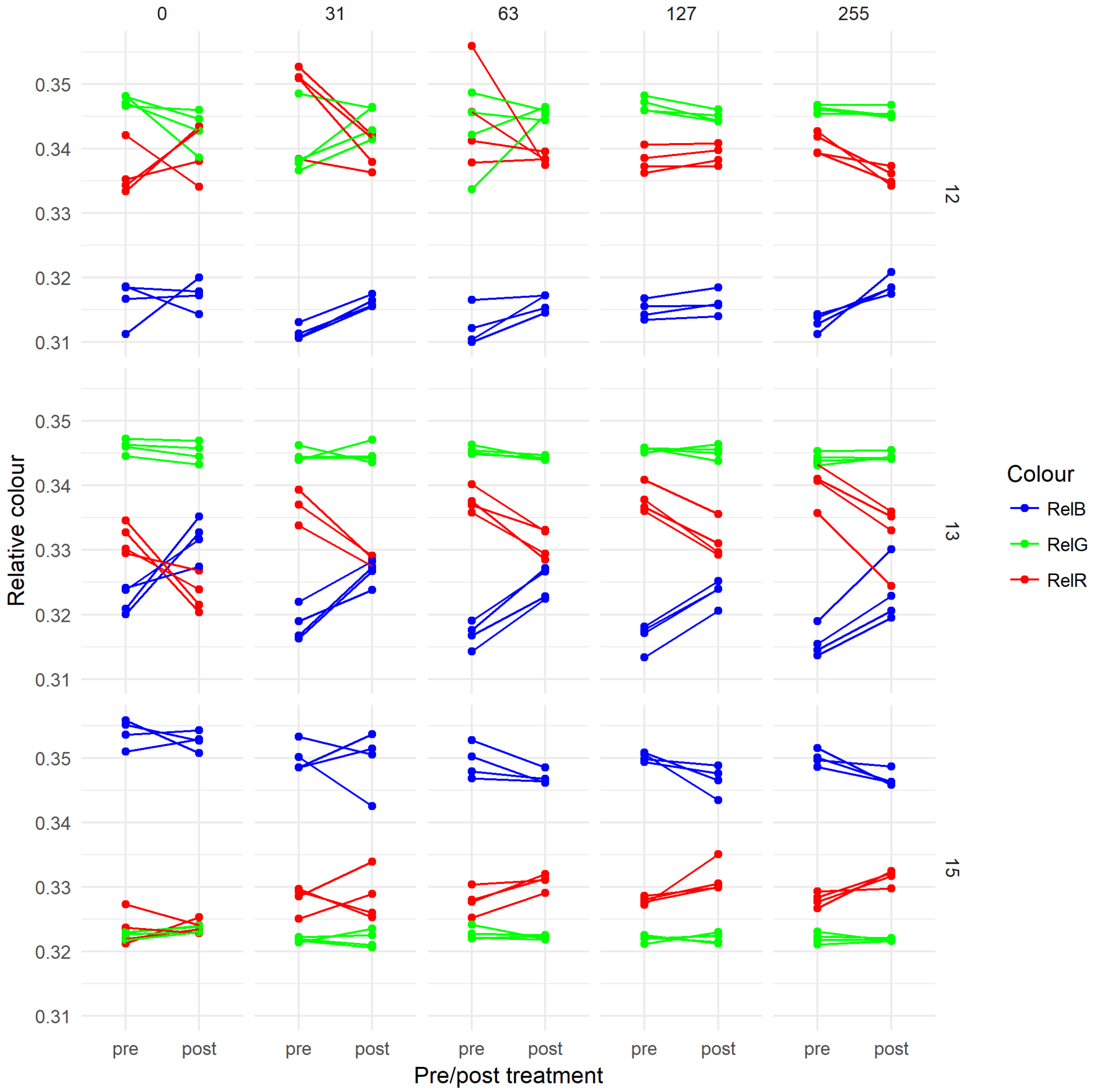

Figure 34 Relative reflectance (scaled to total reflectance, max = 1) before and after light treatment ran at three different days (12th, 13th, and 15th of March 2018, labelled to the right in the plot) in 4 different light intensities and no light (light intensities labelled at the top of the plot). In the results from the experiment conducted the 13th, it looks as if the relative redness decrease and the relative blue reflectance increase throughout the experiment. Otherwise, no apparent patterns are emerging from the results.

Discussion

Apparent pigmentation

My results indicate that there are some depth effects on apparent pigmentation. However, apparent pigmentation was highly dependent on the genus and size. Transparency appeared as the primary camouflage strategy. Transparency is an excellent camouflage strategy applied by many epipelagic species residing in well-illuminated parts of the epipelagic zone (Johnsen 2001).

The transparency strategy is also a reasonable explanation for the low reflectance values in my results (fig. 16, fig. 17). The MICA-toolbox calculates the normalised mean pixel value in the ROIs, and the maximum pixel value is 65535. If an animal would yield reflectance values close to maximum in all or some of the three colour bands, all the pixels in the ROI of that animal appears either bright white or colourful. Hence, the combination of low reflectance values and relative shares of red, green and blue close to 33 % for all three colour bands indicate transparency (fig. 18), corresponding well with the findings of Johnsen (2001, 2014).

The relative levels of blue (fig. 17, fig. 18) reflectance in *Calanus*, *Metridia* and surface dwelling *Acartia* and *Oithona* are consistent with the notion that some epipelagic copepods are transparent-blue (Mojib et al. 2014). *Paraeuchaeta* had the highest reflectance in the red colour spectrum, and reflectance increased with increased depth (fig. 17), as expected from the works of Vestheim and Kaartvedt (2006). The generally low reflectance values in green colour in the herbivorous and omnivorous genera might indicate low feeding rates during the day and indirectly suggest nocturnal feeding (Peterson et al. 1990; Tsuda et al. 1998). Figure 19 and the model with full interactions (tab. 4) further indicate that the depth influence on red reflectance is highly dependent on the genus. This influence, however, is weak as the model with genus as the only describing factor performed better (fig. 20). It is likely that other, non-observed, factors like size or ontogenetic stage also contribute to red reflectance in my data. Based on my analysis, I can say that apparent pigmentation in marine copepods varies with depth dependent on genus or species.

Size and ontogenetic stage of copepods also influence habitat choice with younger stages residing in the surface and older stages at depth (Titelman and Fiksen 2004; Ohman and

Romagnan 2016). Visual predation is highly dependent on the ambient light conditions and the size of the prey, as this determines the contrast and visibility of the prey in its surroundings (Aksnes and Giske 1993; Aksnes and Utne 1997). The size distributions over depth results in my thesis (fig. 21) are consistent with those studies, as smaller copepods within a genus and across genera resided in the upper layers where light is not a limiting factor for visual predation. However, although residing in the well-illuminated, food abundant surface layers may promote growth and secondary production by small copepod species, they face substantial predation by ctenophores and chaetognaths (Davis 1984).

Total reflectance appears to increase with prosome size except for the large size, low reflectance cluster of *Calanus* which appears to reflect less light than many of the smaller genera (fig. 22). The linear mixed model (fig. 23) further indicate that size influence on red reflectance depends on the genus although the effect is small. When including prosome size in the linear models (tab. 5), size and genus-size interactions both affect reflectance. Keeping in mind that size tended to increase with depth, these findings coincide with habitat choice and profitability according to life stages and maximising fitness (Aksnes and Giske 1990; Fiksen and Carlotti 1998).

Copepods typically go through six naupliar and five copepodid stages before maturing into adults (Mauchline 1998). Optimum prey size typically scales with copepod size, and different life stages may, therefore, feed on different food resources (Hansen et al.1994). In the comparison of apparent pigmentation, the contribution of different copepodite stages is probably a source to the inconclusive results of the model (fig. 21, 23). E.g. small *Paraeuchaeta* residing in the upper layers are probably earlier copepodite stages than the larger individuals found at greater depths (fig. 21). In my data, the reflectance values were higher in the deep-dwelling *Paraeuchaeta*, which may indicate differences in the allocation of food recourses. For example, early copepodite stages need the energy to grow, and adult individuals allocate energy and maybe also pigments towards reproduction (Aksnes and Giske 1990; Aksnes 1996; Schneider et al. 2016).

The applicability of image analysis

Digital photography is cheaper and faster than, i.e. spectrometry (Stevens et al. 2007), and I considered it to be an excellent tool for the photography part of my thesis. However, there are a few things that are important to clarify with this method. The photoreceptors in digital cameras are not calibrated to recreate the exact image. Most cameras are made to create images that please the human eye. That does not mean the images represent the actual colours, so the images must be calibrated before analysis. With good grey-scale references, calibration is obtained through the procedures using the MICA-toolbox in image-j (Troscianko and Stevens 2015).

Using photography and image analysis to investigate copepod pigmentation taught me a lot, and especially about the potential pitfalls in the method. The grey-scale reference used during the trial in 2017 was a noncalibrated cardboard reference (DANES-PICTA #BST13). In 2018 we acquired the professional ColorChecker grey-scale reference, which rendered the analysis more reliable. Along with light settings, the exposure settings of the camera are some of the most important to keep consistent, when using digital photography for analysing animal pigmentation (Stevens et al. 2007). It is thereby inferable that the inconsistency in grey-scale reference and exposure settings across the years leads to the incomparability of the results from 2017 and 2018. Difficulties in the comparison of the two years also related to the different target depths between sampling years.

Targeted depth intervals in 2017 may have contributed to the opposite trends in the model predictions between the two years (Appendix A). An animal caught in the 100 – 200 m interval may have resided either at 100 m or 200 m. If depth influences expressed pigmentation in copepods and the critical depth is within that depth interval, it would be impossible to distinguish. Although the 40 m intervals targeted in 2018 may also be prone to the same issues, the higher depth-resolution enhance the likelihood of detecting vertical differences.

During the 2017 trial, it became clear that it was essential to align the camera perpendicular to the object to avoid reflections of the copepods in the microscope slide. The copepod reflections added noise to the images and led to some uncertainties in the reflectance values. There was also some issues with most of the images taken in the upper layers of the water column (30 – 0 m). Phytoplankton saturated many of the images from the surface layer. Thus, phytoplankton overlapped the zooplankton, and whether we measured phytoplankton colours or zooplankton colours was somewhat unclear. The phytoplankton issue could be solved quite easily by filtering the samples with a mesh size that sorts out only the copepods from the original samples. In the images from 2018, this was not an issue as the spring bloom of phytoplankton did not occur at the same time as the copepod sampling, rendering the results more trustworthy.

Pigment content

Astaxanthin proved to be the most dominant pigment in my samples (fig. 24), as expected from other studies showing that astaxanthin is the most abundant pigment in crustaceans (Byron 1982; Matsuno 2001; Andersson et al. 2003; Khalili et al. 2016). However, it is evident in my results that pigment content and compositions also vary with genus and depth in my data (fig. 25 and fig. 26). The differences in total pigment content between genera are likely linked to size differences, as bigger bodies can contain higher contents of pigments per animal merely because they have more room for it. Hays et al. (1994) analysed DVM behaviour in 41 copepod taxa from the North Atlantic. Their findings indicate that large-sized taxa showed significantly stronger DVM than smaller taxa (Hays et al. 1994). They also show that increased carotenoid concentration (µg g^{-1}) in small taxa (<1 mm prosome width) induced increased DVM performance (Hays et al. 1994). Such a correlation was not present in larger taxa (>1 mm prosome width) (Hays et al. 1994). My pigment extraction data (2017-18) lack size measurements and weight specific pigment concentration, hampering a direct comparison with the results of Hays et al. (1994). However, the larger copepods like *Paraeuchaeta*, *Calanus* and *Chiridius* contained higher pigment contents per animal in the deeper layers (fig. 27). The smaller *Paracalanus* and *Temora* contained the highest pigment contents per animal in the surface layers (fig. 27). My results are in line with the theory of size dependencies for prey detection in visual predators (Aksnes and Giske 1993). Small-sized genera can be pigmented in well-illuminated parts of the water column and still obtain camouflage by their small size, while bigger genera relay on transparency (Johnsen 2001).

Copepods display a wide variety of feeding habits ranging from more or less strict herbivory, via omnivory to strict carnivory (Mauchline 1998). With the exception of *Metridia*, carnivorous and omnivorous genera contained the highest contents of astaxanthin per animal (fig. 28). In those genera, *Paraeuchaeta, Calanus* and *Chiridius*, astaxanthin contents increase with increasing depth. Comparing those findings with apparent pigmentation in the photo results is interesting as *Paraeuchaeta* has increased reflectance with depth. *Calanus,* however, have similar reflectance values throughout the sampling range, and *Chiridius* appear to have decreased reflectance with depth. If red pigmentation is a direct function of astaxanthin contents, one would expect apparent pigmentation to increase with astaxanthin contents, but that is only the case for *Paraeuchaeta* in my data. In *Metridia* and the smaller herbivorous *Paracalanus* and *Temora*, alloxanthin and other food pigments, e.g. pheophytin a, are more dominant than astaxanthin. As copepods obtain the pigments through the diet, results like these might indicate direct effects of trophic niches (Andersson et al. 2003; Antajan and Gasparini 2004).

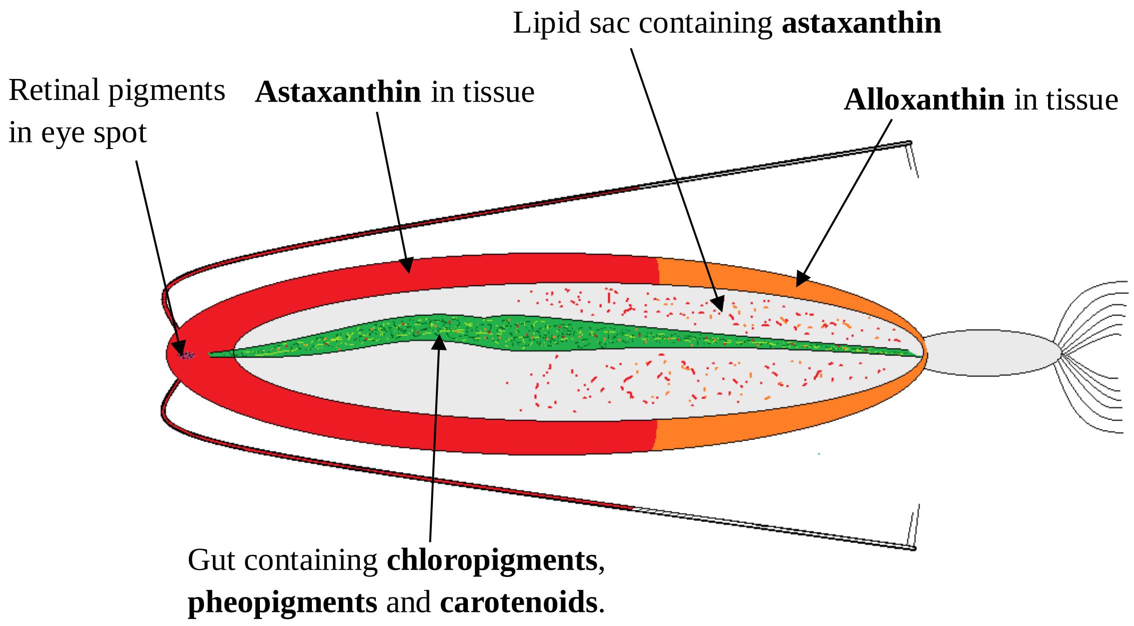

Figure 35 Schematic presentation of where different pigments may accumulate in copepods. Astaxanthin and alloxanthin are known to accumulate in body tissue in copepods (Antajan and Gasparini 2004; Schneider et al. 2016), however, not necessarily in different parts of the body as in this presentation. Chloropigments, pheopigments and carotenoids are found in the gut of the copepods (Penry and Frost 1991). In strictly carnivorous copepods, astaxanthin and possibly alloxanthin will be taken up by eating other copepods containing the pigments, as it is for other organisms in marine systems (Andersson et al. 2003). Astaxanthin is also associated with lipids in the lipid sac of calanoid copepods (Schneider et al. 2016). If alloxanthin is associated with lipids in the same way as astaxanthin, it would possibly also accumulate in the lipid sac.

Alloxanthin is a pigment characteristic for phytoplankton belonging to Cryptophyceae, e.g. *Rhodomonas* (Guisande et al. 2002; Not et al. 2012). Alloxanthin accumulates in body tissue of *Temora longicornis* and *Centropages hamatus* and is not necessarily a good indicator for determining copepod grazing rates on Cryptophyceae (Antajan and Gasparini 2004). However, it is a possible explanation for the content of alloxanthin in my data. On the other hand, Alloxanthin may be similarly produced by the copepods as astaxanthin, and may also act as a precursor for astaxanthin production (Antajan and Gasparini 2004). Many zooplankton species conduct nocturnal feeding, in addition to, or independent of DVM (Tsuda et al. 1998). *Calanus pacificus* have been documented to migrate to the food-rich surface layers just before sunset and feed at low rates until after sunset. Then they stop feeding before sunrise but do not descend until after sunrise (Dagg et al. 1989).

In large migratory copepods (*Centropages brachiatus* and *Calanus australis*), gut pigment content is generally low and constant during the daytime (Peterson et al. 1990). The gut pigment content increases rapidly the first hours after sunset, and night contents reported to be 2-6 times higher than day contents (Peterson et al. 1990). They also show that gut pigment content varies less between night and day in the small *Paracalanus parvus* (Peterson et al. 1990). Similarly, there was generally low contents of gut-associated pigments (fig. 35) in my samples from daytime. However, the gut-associated pigment contents contributed more to the total pigment content in the smaller genera (compare fig.29 and fig. 30). The differences in how pigment contents are related to depth in different genera are apparent in figure 31. The linear models confirmed that the relationship between pigment content and depth is highly dependent on the specific genus (tab. 6). This indicates, similar to the photo analysis that smaller copepods can allow themselves to be more pigmented and still be less conspicuous than bigger copepods in well-illuminated parts of the epipelagic realm. It also fits quite elegantly with transparency being a camouflage strategy used mainly by bigger copepods (Johnsen 2001).

In the photo part of my thesis, results show that *Calanus*, *Metridia* and *Acartia* had higher reflectance values in the blue end of the spectrum which may be caused by blue coloured protein-bound astaxanthin. The GPS-method may have failed to detect this as the absorption spectrum of the blue astaxanthin-protein complex will be different from the GPS-modelled spectrum for free astaxanthin. Earl R. Byron reported that alcohol extraction of pigments denatures carotenoproteins, making blue astaxanthin-proteins into red astaxanthin (Byron 1982). The pigment references I used for my analysis was modified by Thrane et al. (2015) to analyse algal communities in freshwater systems. It could therefore probably benefit from modification, including other pigments to better fit pigment analysis of marine copepods. A problem with including more carotenoids, for example, is that they may have similar absorption spectra leading to aliasing (Küpper et al. 2007; Thrane et al. 2015). The alloxanthin dominance in some of the genera that were identified by the GPS-method should, therefore, be investigated further, e.g. with HPLC analysis and perhaps also transcriptomics as done by Mojib et al. (2014). Another factor I would include in future studies would be size measurements. Bigger animals can, as mentioned above carry more pigments than smaller animals. However, in the perspective of visual predation and predator avoidance, and the notion that smaller animals are camouflaged mostly by their small size, the pigment concentration per bodyweight is likely to be higher in smaller animals.

Apparent pigmentation and actual pigment concentrations – size effects?

The plots of reflectance as a function of content per animal (fig. 32) indicated a significant negative correlation between the two, which is supported by the linear model (tab. 7). This result implies that the more pigments a copepod accumulate either in its guts or tissue, the less pigmented the copepod appear. However, several experiments show that copepods with high levels of gut and tissue pigments are more conspicuous and preferred by visual predators than copepods containing low levels of carotenoids and other food pigments (Tsuda et al. 1998; Hylander et al. 2014). The initial results may only tell part of the story, however, since the reflectance values are normalised to size (pixels) and the pigment content is not. It is apparent in my pigment extraction results that bigger copepods can contain much more pigments than smaller ones, and this is likely a direct effect of animal size.

When I incorporate the size data from the photo analysis and calculate the actual concentration per volume unit, the significant negative correlation gets replaced by an insignificant non-correlated relationship (fig. 33) supported by the linear model (tab. 7). This result is still quite puzzling, as it indicates that a change in pigment concentration will not affect apparent pigmentation in the copepods in my samples. If this is true, nocturnal feeding as an anti-predator strategy (Tsuda et al. 1998) and increased DVM activity in heavily pigmented copepods as shown by Hays et al. (1994) appears redundant. However, as visual predators can detect other aspects of colouration compared to a camera, e.g. ocular filters, polarisation sensitivity (Johnsen 2014), this is highly unlikely. An alternative and more likely explanation may be that copepods can alter their apparent pigmentation by other means than merely adjusting their pigment content. One way of doing so may be the use of chromatophores as suggested by Vestheim and Kaartvedt (2006). Many decapod crustaceans have chromatophores (Herring 1973; Fingerman 1985) but are to my knowledge not described in copepods.

Also, photo analysis and pigment extractions were not conducted on the same animals. If I were to do this research again, I would first photograph the animals, and then directly extract their pigments. Including precise measurements of size and the ontogenetic stage would allow for a direct comparison of reflectance values to a corresponding specific pigment concentration. I imagine that such improvements could make the results more accurate. As mentioned above, there might be significant differences between different ontogenetic stages in how they utilise pigments from their diet. Including ontogenetic stages would allow for resolving those patterns. There might also be species differences that are not picked up by the methods, and this would be interesting to investigate further.

Light setup trial

The light test project yielded inconclusive results (fig. 33) as each day yielded utterly different results. On the first experimental run (12th), all treatments resulted in a decrease in apparent pigmentation. The results from the other two days points in every direction. The results from the third experimental run (15th), show that the reflectance composition is opposite from the other two days. This switch of reflectance composition is very apparent in the relative composition of the reflectance (fig. 34). The green reflectance that is most dominant the first two days may reflect green pigments in the gut like chlorophyll a or pheophytin a. The third day, however, the green reflectance is the least contributor to the overall reflectance.

In total, the light setup trial raised more questions than it gave answers. There is, however, still a lot to learn from these results. For instance, the differences in before treatment reflectance values between treatments (fig. 33, 34) may be a direct effect of how the students conducted the experiment. E.g. they did not randomise the treatments and the replicates when initiating the experiments. The duration of the different experiments was not standardised and varied from two to five hours. At what time of day the experiments was conducted also varied from day to day. These inconsistencies may be part of the explanation for how the results differ between the days. It could be that the measurements reflect diel colouration patterns such as the dial variations found in *P. norvegica* (Vestheim and Kaartvedt 2006). It could also be that *C. hyperboreus* do not respond to light at all in apparent pigmentation. Alternatively, the light setting may not match the light conditions that *C. hyperboreus* responds to.

In future experiments like this, I would like to acclimatise the animals to total darkness long enough to exclude diel fluctuations in pigmentations. I want to stress the importance of randomisation in the initiation of the different treatments and replicates, as well as consistency in experiment duration. Whether apparent pigmentation in *C. hyperboreus* is a plastic trait that response to different light intensities remains a mystery.

The light setup

The light system works, but it needs further calibration. Initially, I wanted to calibrate the LEDs to simulate light conditions found in Oslofjorden. The first edition blue caps contaminated the light to the degree that light calibration was difficult. That is why I initiated the production of the black caps, and the final edition seems very promising. Sadly the production of the black light caps was very time-consuming as I met some complications with the 3D-printer. Together with my supervisor Jan D. Heuschele, we started the calibration with the aim to simulate different light conditions, e.g. during sunset, sunrise, at shallow depths and depths with limited light. Copepods have been shown to have spectral sensitivities that match their ambient light conditions at different wavelengths dependant on whether they migrate vertically or not (Cohen and Forward 2002). It is likely that this also affects pigmentation control if this is a plastic trait in copepods, triggered by light. Calibrating the light from the LEDs must, therefore, include measuring the light emitted directly from the LEDs to be able to replicate the light intensities found in Oslofjorden, e.g. as $\mu E\ m^{-2}\ s^{-1}$. Also to make sure that all the LEDs in a string emits the same light when programmed with the same settings. The light conditions in the container should be as homogenous as possible. The light should be measured within the containers while the containers are wrapped in aluminium foil as the reflection by the aluminium foil is predicted to contribute to the dispersion of the light. It is essential to make sure that all the animals in the treatment are exposed to the same light composition no matter where they reside in the container.

Conclusions

Digital photography appears to be a fast, reliable method to use for investigating apparent pigmentation in copepods. By using calibrated grey-scale references, maintaining equal light conditions and exposure settings, the method detects subtle variations in pigmentation. The GPS-method also proved to be a functionally sound method in analysing copepod pigment content. However, the method may benefit from improvements in the pigment references. That said, the blue pigmentation that is detectable with the photo method may be difficult to relate to the carotenoproteins due to the extraction methods. The biggest improvement overall would probably be to use the same animals for analysis of both apparent and actual pigmentation, which would subsequently improve the reliability of the comparison between them.

The light system for testing the plasticity of apparent pigmentation in copepods is operational, but need further calibration. My results, however, are in line with many other findings in the science of copepods, pigmentation, camouflage and predator avoidance.

In my aims (page 6) I asked four questions that I have tried to find the answers to:

- Is apparent pigmentation in copepods correlated with depth? If so, how?

Both yes and no. Within some of the analysed copepod genera, it appears as apparent pigmentation may correlate with depth. This correlation is not verified by any of my models as there is generally no significant effect of depth on apparent pigmentation. However, my results indicate that apparent pigmentation is dependent on size, but the effect of size is highly dependent on the genus.

- Is pigment content in copepods related to depth? If so, how?

Again, both yes and no. Pigment content correlates with depth in my data, but the effect of depth is highly dependent on genus, and there might be hidden size and stage effects not detected in my thesis.

- When compared, do apparent pigmentation correlate with pigment concentrations in copepods?

When comparing normalised values for both apparent pigmentation and pigment concentration, no correlation is detected.

- Is pigmentation in copepods a plastic trait regulated by ambient light conditions?

No such plasticity is apparent in the result of the test project.

All in all, responses in both apparent pigmentation and pigment concentrations suggested that copepods may be able to alter their appearance, but further experiments are necessary to understand this phenomenon better.

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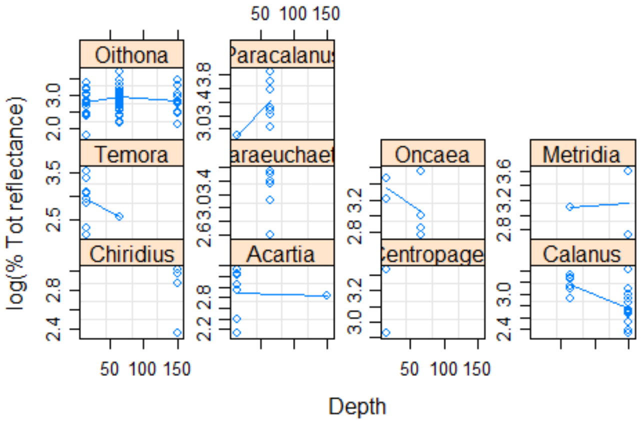
Appendix A

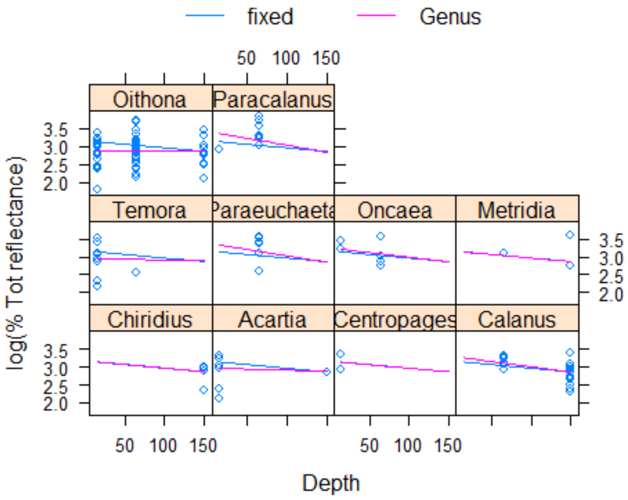
Additional models and plots, reflectance over depth

Total reflectance

2017

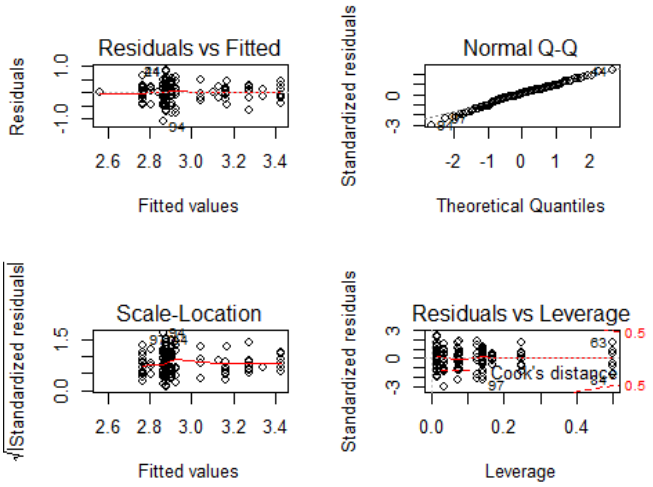


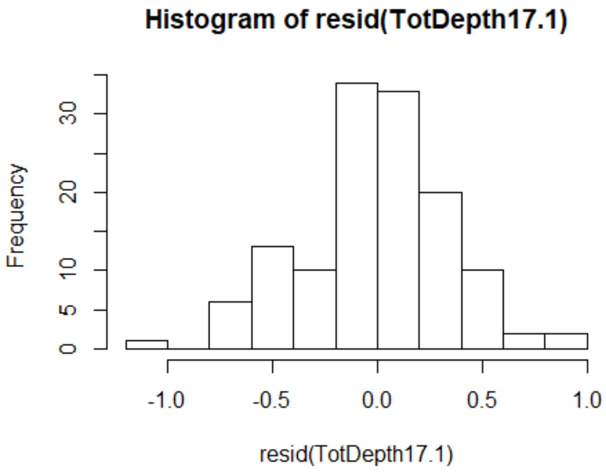
```
summary(m17 <- lme(g17))
## Linear mixed-effects model fit by REML
##  Data: g17
##       AIC      BIC    logLik
##   140.864 158.0229 -64.43201
##
## Random effects:
##  Formula: ~Depth | Genus
##  Structure: General positive-definite
##             StdDev      Corr
## (Intercept) 0.258793212 (Intr)
## Depth       0.001853069 -1
## Residual    0.361499752
##
## Fixed effects: log.Tot ~ Depth
##                Value  Std.Error  DF  t-value p-value
## (Intercept)  3.170954 0.11498548 120 27.57700  0.0000
## Depth       -0.002012 0.00096744 120 -2.07931  0.0397
##  Correlation:
##       (Intr)
## Depth -0.915
##
## Standardized Within-Group Residuals:
##         Min          Q1         Med          Q3         Max
## -3.00637133 -0.50875517  0.08826288  0.58551266  2.30168916
##
## Number of Observations: 131
## Number of Groups: 10
plot(augPred(m17, level=0:1), ylab = "log(% Tot reflectance)")
```



```
anova(m17)
##             numDF denDF  F-value p-value
## (Intercept)     1   120 4044.654  <.0001
## Depth           1   120    4.324  0.0397

TotDepth17.1 = lm(log.Tot ~ Depth * Genus, data=d17)
TotDepth17.2 = lm(log.Tot ~ Depth + Genus, data=d17)
TotDepth17.3 = lm(log.Tot ~ Depth, data=d17)
TotDepth17.4 = lm(log.Tot ~ Genus, data=d17)

AICc(TotDepth17.1, TotDepth17.2, TotDepth17.3, TotDepth17.4)

##              df     AICc
## TotDepth17.1 18 129.8310
## TotDepth17.2 12 123.2022
## TotDepth17.3  3 124.9215
## TotDepth17.4 11 121.4079

summary(TotDepth17.1)
## Call:
## lm(formula = log.Tot ~ Depth * Genus, data = d17)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -1.06487 -0.17580  0.01583  0.20492  0.84124
##
## Coefficients: (3 not defined because of singularities)
##                         Estimate Std. Error t value Pr(>|t|)
## (Intercept)            3.2559535  0.1521520  21.399  < 2e-16 ***
## Depth                  0.0002760  0.0010174   0.271  0.78665
## GenusCalanus           0.2088162  0.3119012   0.669  0.50453
## GenusChiridius        -0.4925615  0.2431356  -2.026  0.04511 *
## GenusCentropages      -0.1312613  0.2950981  -0.445  0.65730
## GenusMetridia         -0.1995244  0.6862208  -0.291  0.77176
## GenusParacalanus      -0.5013041  0.4969588  -1.009  0.31523
## GenusAcartia          -0.3471234  0.2189840  -1.585  0.11570
## GenusTemora           -0.2214255  0.2504906  -0.884  0.37857
## GenusOncaea            0.1699023  0.3703668   0.459  0.64729
## GenusOithona          -0.3946228  0.1442180  -2.736  0.00721 **
## Depth:GenusCalanus    -0.0049296  0.0023381  -2.108  0.03719 *
## Depth:GenusChiridius          NA         NA      NA       NA
## Depth:GenusCentropages        NA         NA      NA       NA
## Depth:GenusMetridia    0.0004665  0.0053219   0.088  0.93030
## Depth:GenusParacalanus  0.0100498  0.0078180   1.285  0.20123
## Depth:GenusAcartia    -0.0007311  0.0030459  -0.240  0.81073
## Depth:GenusTemora     -0.0075488  0.0077577  -0.973  0.33258
## Depth:GenusOncaea     -0.0061247  0.0063613  -0.963  0.33768
## Depth:GenusOithona            NA         NA      NA       NA
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.3625 on 114 degrees of freedom
```

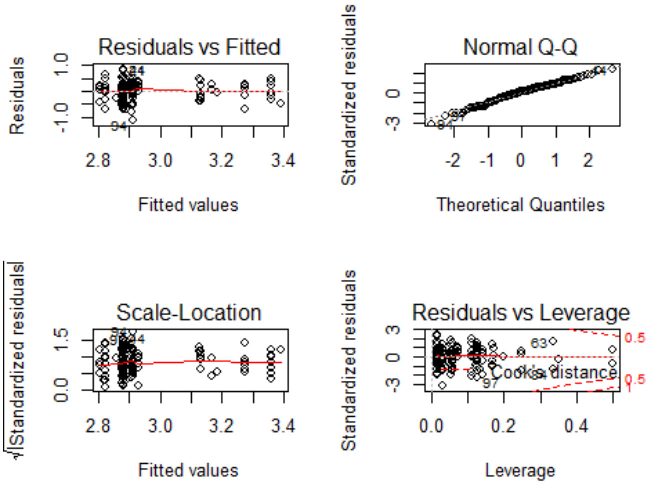
```
## Multiple R-squared:  0.2169, Adjusted R-squared:  0.107
## F-statistic: 1.974 on 16 and 114 DF,  p-value: 0.02058
```

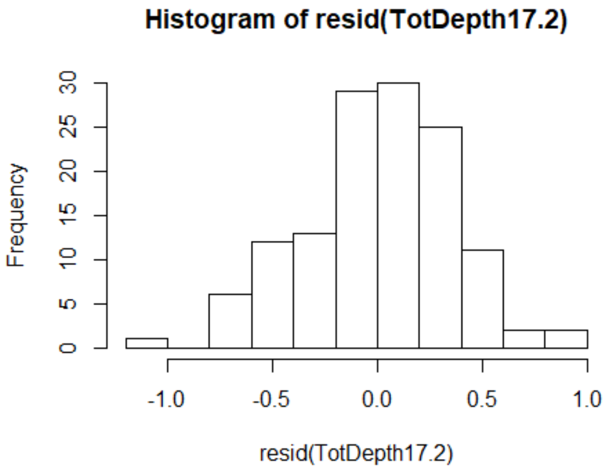




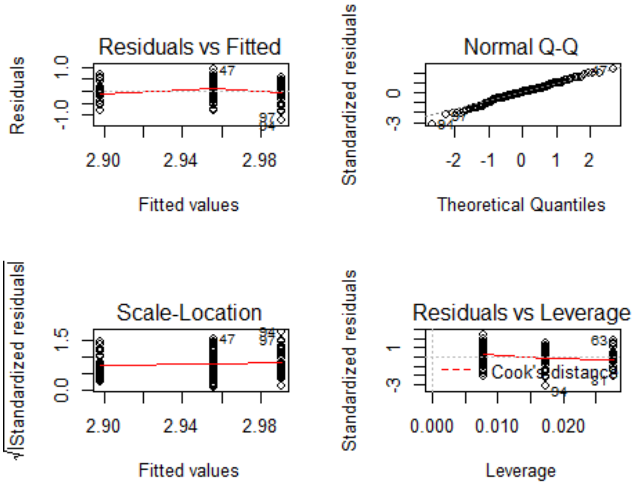
```
summary(TotDepth17.2)
## Call:
## lm(formula = log.Tot ~ Depth + Genus, data = d17)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -1.11079 -0.19557  0.04683  0.21557  0.84145
##
## Coefficients:
##                    Estimate Std. Error t value Pr(>|t|)
## (Intercept)       3.3159308  0.1487634  22.290  < 2e-16 ***
## Depth            -0.0006467  0.0008494  -0.761  0.44794
## GenusCalanus     -0.3446353  0.1689760  -2.040  0.04359 *
## GenusChiridius   -0.4141296  0.2401853  -1.724  0.08725 .
## GenusCentropages -0.1773977  0.2960978  -0.599  0.55022
## GenusMetridia    -0.0904770  0.2567573  -0.352  0.72517
## GenusParacalanus  0.0833558  0.1892285   0.441  0.66036
## GenusAcartia     -0.4009937  0.1912352  -2.097  0.03811 *
## GenusTemora      -0.4176057  0.1880138  -2.221  0.02822 *
## GenusOncaea      -0.1415028  0.2038267  -0.694  0.48888
## GenusOithona     -0.3948357  0.1453868  -2.716  0.00759 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.3655 on 120 degrees of freedom
```

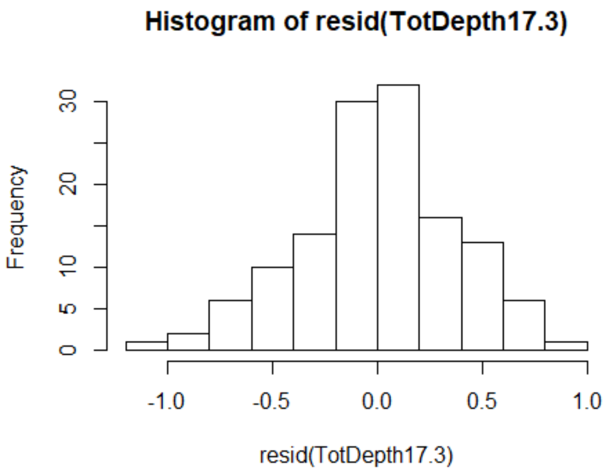
```
## Multiple R-squared:  0.1623, Adjusted R-squared:  0.0925
## F-statistic: 2.325 on 10 and 120 DF,  p-value: 0.01539
```



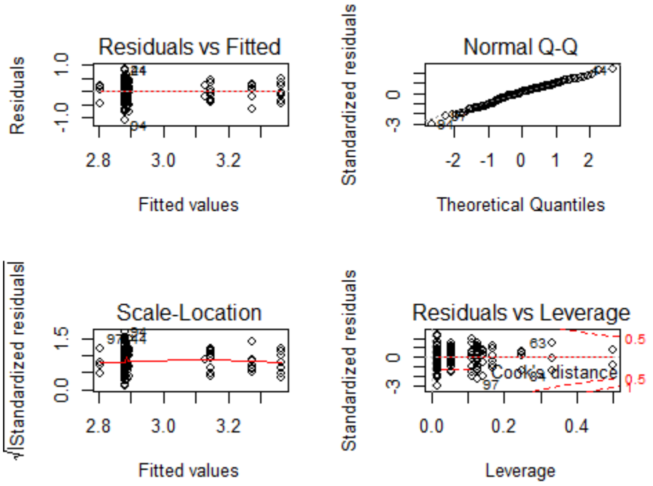


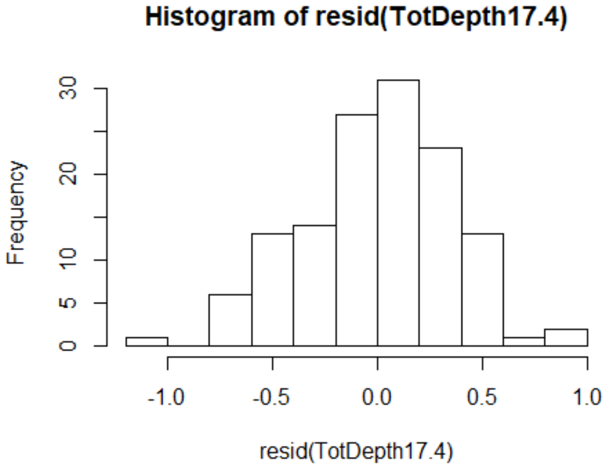
```
summary(TotDepth17.3)
## Call:
## lm(formula = log.Tot ~ Depth, data = d17)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -1.18988 -0.19637  0.00509  0.23908  0.89452
##
## Coefficients:
##               Estimate Std. Error t value Pr(>|t|)
## (Intercept)  3.0007575  0.0583685  51.411   <2e-16 ***
## Depth       -0.0006854  0.0006804  -1.007    0.316
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.3836 on 129 degrees of freedom
## Multiple R-squared:  0.007804,   Adjusted R-squared:  0.0001129
## F-statistic: 1.015 on 1 and 129 DF,  p-value: 0.3157
```

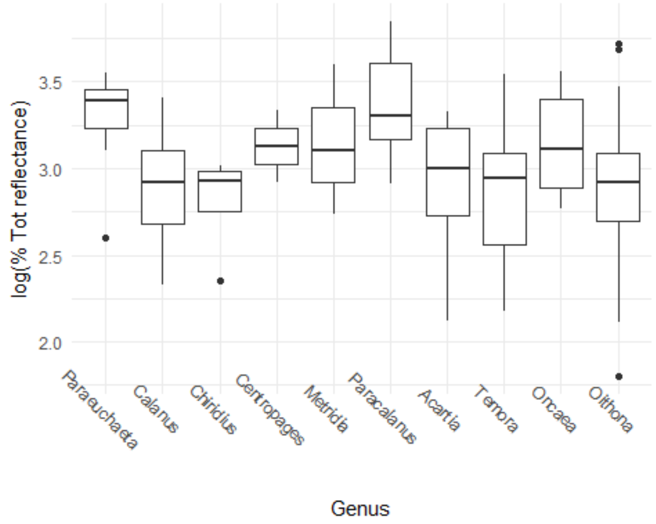




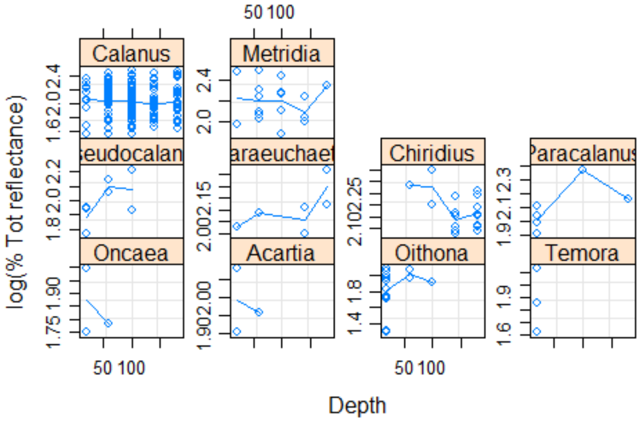
```
summary(TotDepth17.4)
## Call:
## lm(formula = log.Tot ~ Genus, data = d17)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -1.07861 -0.20527  0.04668  0.22509  0.84130
##
## Coefficients:
##                   Estimate Std. Error t value Pr(>|t|)
## (Intercept)         3.2739     0.1379  23.741   <2e-16 ***
## GenusCalanus       -0.3822     0.1613  -2.370   0.0194 *
## GenusChiridius     -0.4691     0.2287  -2.051   0.0424 *
## GenusCentropages   -0.1451     0.2925  -0.496   0.6209
## GenusMetridia      -0.1271     0.2518  -0.505   0.6145
## GenusParacalanus    0.0874     0.1888   0.463   0.6443
## GenusAcartia       -0.3796     0.1888  -2.010   0.0466 *
## GenusTemora        -0.3889     0.1839  -2.115   0.0365 *
## GenusOncaea        -0.1307     0.2030  -0.644   0.5208
## GenusOithona       -0.3947     0.1451  -2.719   0.0075 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.3648 on 121 degrees of freedom
## Multiple R-squared:  0.1583, Adjusted R-squared:  0.09565
## F-statistic: 2.528 on 9 and 121 DF,  p-value: 0.01085
```


Residuals vs Fitted
Residuals
1.0
-1.0
24
94
2.8
3.0
3.2
Fitted values
Normal Q-Q
Standardized residuals
0
-3
94
-2
-1
0
1
2
Theoretical Quantiles
Scale-Location
√|Standardized residuals|
1.5
0.0
97
94
24
2.8
3.0
3.2
Fitted values
Residuals vs Leverage
Standardized residuals
0
-3
63
97
Cook's distance
0.5
0.5
0.0
0.2
0.4
Leverage

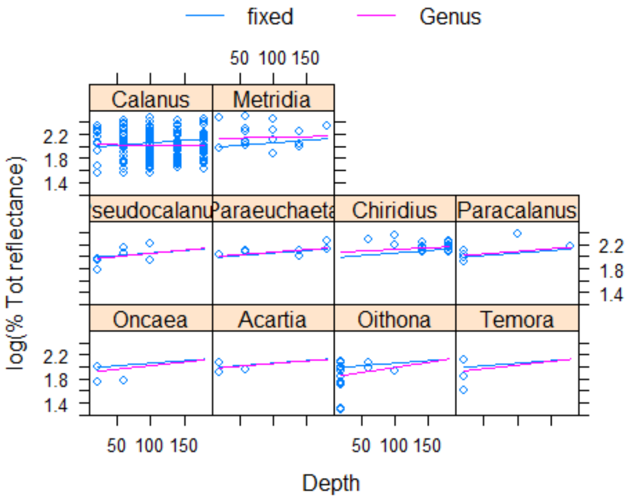

Histogram of resid(TotDepth17.4)
Frequency
0
5
10
20
30
-1.0
-0.5
0.0
0.5
1.0
resid(TotDepth17.4)


log(% Tot reflectance)
2.0
2.5
3.0
3.5
Paraeuchaeta
Calanus
Chiridius
Centropages
Metridia
Paracalanus
Acartia
Temora
Oncaea
Oithona
Genus

2018



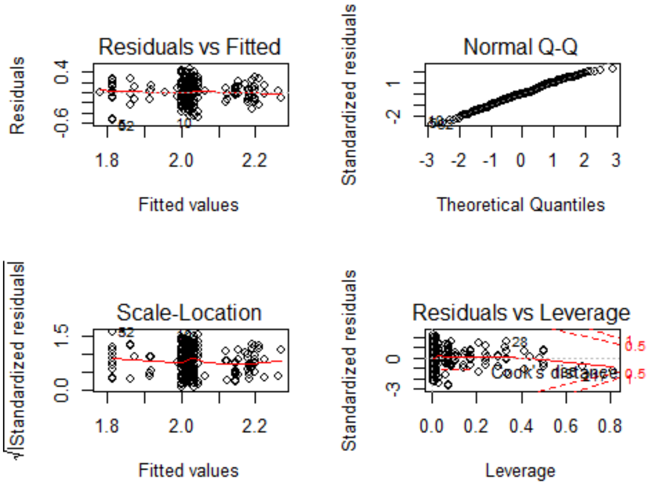
```
summary(m18 <- lme(g18))
## Linear mixed-effects model fit by REML
##  Data: g18
##         AIC       BIC   logLik
##   -36.94775 -15.81899 24.47388
##
## Random effects:
##  Formula: ~Depth | Genus
##  Structure: General positive-definite
##             StdDev      Corr
## (Intercept) 0.124886583 (Intr)
## Depth       0.000880208 -0.821
## Residual    0.206477130
##
## Fixed effects: log.Tot ~ Depth
##                 Value  Std.Error  DF  t-value p-value
## (Intercept) 1.9774304 0.05530510 241 35.75493  0.0000
## Depth       0.0008408 0.00048361 241  1.73853  0.0834
##  Correlation:
##       (Intr)
## Depth -0.789
##
## Standardized Within-Group Residuals:
##         Min          Q1         Med          Q3         Max
## -2.75996916 -0.57487796 -0.03476131  0.73868214  2.20385325
##
## Number of Observations: 252
## Number of Groups: 10
```

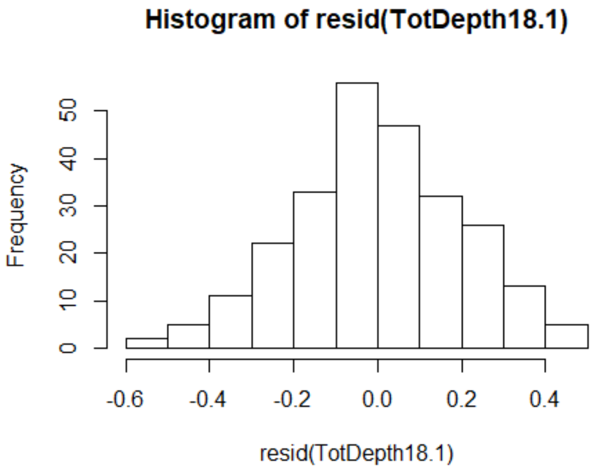


```
TotDepth18.1 = lm(log.Tot ~ Depth * Genus, data=d18)
TotDepth18.2 = lm(log.Tot ~ Depth + Genus, data=d18)
TotDepth18.3 = lm(log.Tot ~ Depth, data=d18)
TotDepth18.4 = lm(log.Tot ~ Genus, data=d18)
```

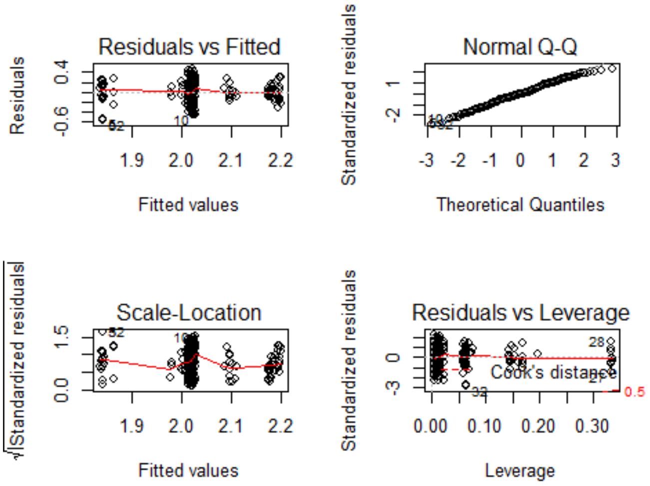
```
AICc(TotDepth18.1, TotDepth18.2, TotDepth18.3, TotDepth18.4)
```

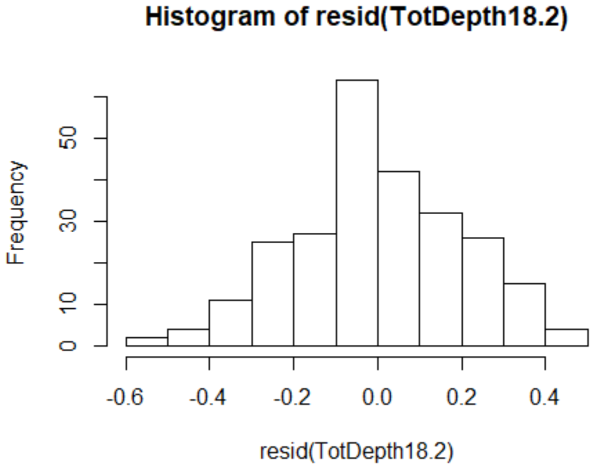
```
##              df      AICc
## TotDepth18.1 20 -50.88755
## TotDepth18.2 12 -63.85281
## TotDepth18.3  3 -48.44006
## TotDepth18.4 11 -65.98224

summary(TotDepth18.1)
## Call:
## lm(formula = log.Tot ~ Depth * Genus, data = d18)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.53113 -0.11518 -0.00072  0.14795  0.45571
##
## Coefficients: (1 not defined because of singularities)
##                            Estimate Std. Error t value Pr(>|t|)
## (Intercept)               2.0193843  0.1676465  12.045   <2e-16 ***
## Depth                     0.0007478  0.0013277   0.563    0.574
## GenusCalanus              0.0301417  0.1728039   0.174    0.862
## GenusChiridius            0.3006125  0.2777788   1.082    0.280
## GenusMetridia             0.1936213  0.2037354   0.950    0.343
## GenusParacalanus         -0.0133484  0.2057968  -0.065    0.948
## GenusPseudocalanus       -0.1539388  0.2254424  -0.683    0.495
## GenusAcartia             -0.0132002  0.2965511  -0.045    0.965
## GenusTemora              -0.1724283  0.1883058  -0.916    0.361
## GenusOncaea              -0.1019611  0.2965511  -0.344    0.731
## GenusOithona             -0.2576735  0.1890000  -1.363    0.174
## Depth:GenusCalanus       -0.0010173  0.0013711  -0.742    0.459
## Depth:GenusChiridius     -0.0017147  0.0019610  -0.874    0.383
## Depth:GenusMetridia      -0.0009781  0.0017764  -0.551    0.582
## Depth:GenusParacalanus    0.0007127  0.0019250   0.370    0.712
## Depth:GenusPseudocalanus  0.0017858  0.0027134   0.658    0.511
## Depth:GenusAcartia       -0.0015809  0.0065239  -0.242    0.809
## Depth:GenusTemora                NA         NA      NA       NA
## Depth:GenusOncaea        -0.0030388  0.0065239  -0.466    0.642
## Depth:GenusOithona        0.0018818  0.0026838   0.701    0.484
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.2086 on 233 degrees of freedom
## Multiple R-squared:  0.1573, Adjusted R-squared:  0.09218
## F-statistic: 2.416 on 18 and 233 DF,  p-value: 0.001423
```



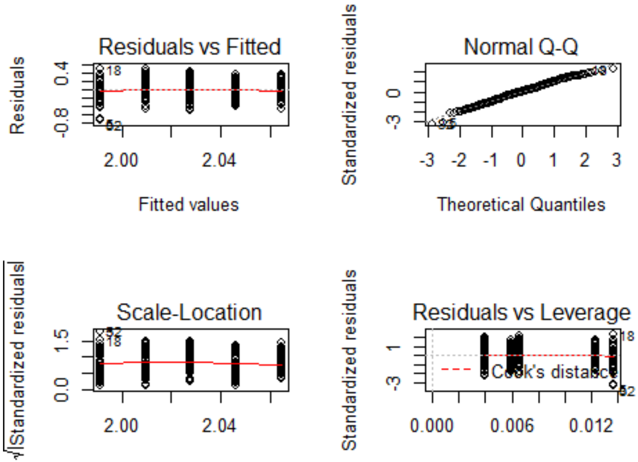


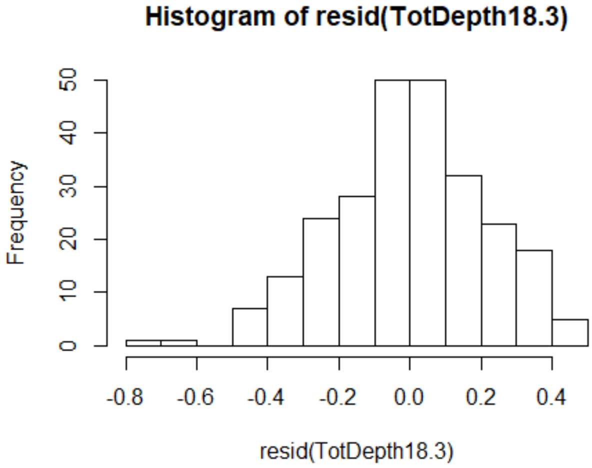
```
summary(TotDepth18.2)
## Call:
## lm(formula = log.Tot ~ Depth + Genus, data = d18)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.55822 -0.11503 -0.00898  0.15670  0.45825
##
## Coefficients:
##                       Estimate Std. Error t value Pr(>|t|)
## (Intercept)          2.112e+00  8.503e-02  24.833  < 2e-16 ***
## Depth               -7.992e-05  2.964e-04  -0.270  0.78767
## GenusCalanus        -8.359e-02  7.993e-02  -1.046  0.29670
## GenusChiridius       7.586e-02  9.378e-02   0.809  0.41936
## GenusMetridia        8.812e-02  9.336e-02   0.944  0.34616
## GenusParacalanus    -1.316e-02  1.163e-01  -0.113  0.91005
## GenusPseudocalanus -1.043e-01  1.121e-01  -0.930  0.35311
## GenusAcartia        -1.305e-01  1.449e-01  -0.901  0.36861
## GenusTemora         -2.481e-01  1.456e-01  -1.704  0.08967 .
## GenusOncaea         -2.679e-01  1.449e-01  -1.849  0.06574 .
## GenusOithona        -2.686e-01  9.700e-02  -2.769  0.00605 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.2073 on 241 degrees of freedom
## Multiple R-squared:  0.1391, Adjusted R-squared:  0.1034
## F-statistic: 3.896 on 10 and 241 DF,  p-value: 6.38e-05
```



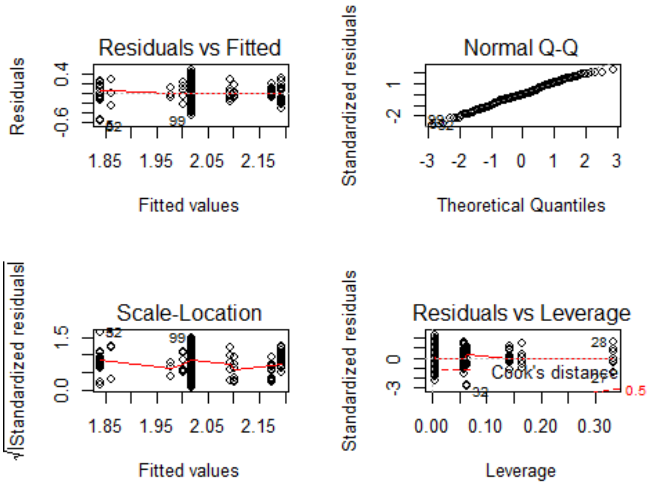


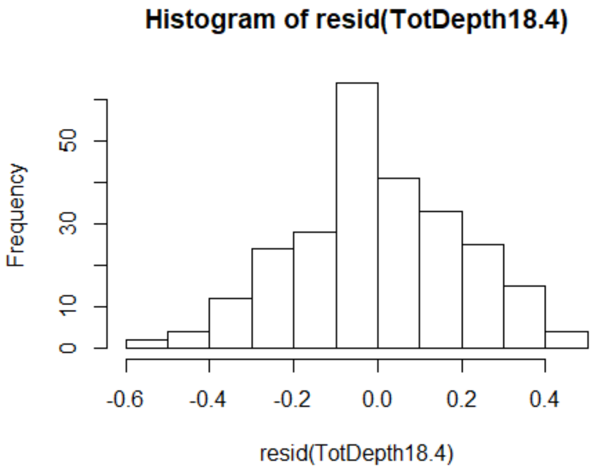
```
summary(TotDepth18.3)
## Call:
## lm(formula = log.Tot ~ Depth, data = d18)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.70805 -0.13945  0.00746  0.15215  0.49459
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept) 1.9820722  0.0299228  66.240   <2e-16 ***
## Depth       0.0004573  0.0002585   1.769    0.078 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.218 on 250 degrees of freedom
## Multiple R-squared:  0.01237,    Adjusted R-squared:  0.008418
## F-statistic: 3.131 on 1 and 250 DF,  p-value: 0.07804
```

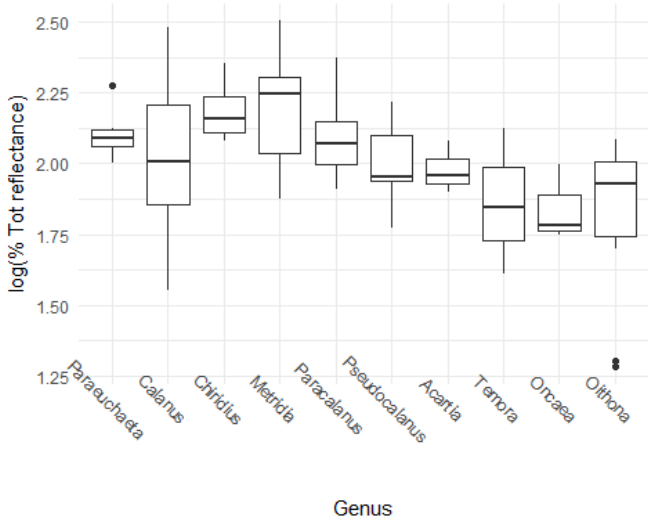




```
summary(TotDepth18.4)
## Call:
## lm(formula = log.Tot ~ Genus, data = d18)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.55743 -0.11515 -0.01076  0.15842  0.45932
##
## Coefficients:
##                    Estimate Std. Error t value Pr(>|t|)
## (Intercept)         2.102712   0.078207  26.887   <2e-16 ***
## GenusCalanus       -0.083748   0.079773  -1.050   0.2948
## GenusChiridius      0.072825   0.092924   0.784   0.4340
## GenusMetridia       0.089974   0.092924   0.968   0.3339
## GenusParacalanus   -0.009048   0.115117  -0.079   0.9374
## GenusPseudocalanus -0.099727   0.110601  -0.902   0.3681
## GenusAcartia       -0.124297   0.142785  -0.871   0.3849
## GenusTemora        -0.240800   0.142785  -1.686   0.0930 .
## GenusOncaea        -0.261654   0.142785  -1.832   0.0681 .
## GenusOithona       -0.262113   0.093767  -2.795   0.0056 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.2069 on 242 degrees of freedom
## Multiple R-squared:  0.1389, Adjusted R-squared:  0.1069
## F-statistic: 4.337 on 9 and 242 DF,  p-value: 2.975e-05
```

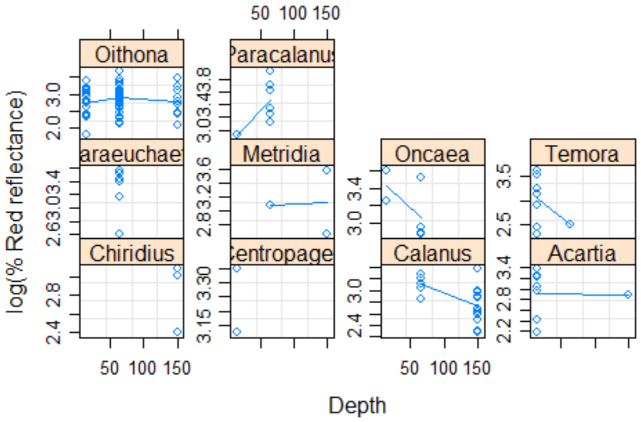

Residuals vs Fitted
Residuals
0.4
-0.6
52
99
1.85 1.95 2.05 2.15
Fitted values
Normal Q-Q
Standardized residuals
1
-2
-3 -2 -1 0 1 2 3
Theoretical Quantiles
Scale-Location
√|Standardized residuals|
1.5
0.0
52
99
1.85 1.95 2.05 2.15
Fitted values
Residuals vs Leverage
Standardized residuals
0
-3
28
27
32
Cook's distance
0.5
0.00 0.10 0.20 0.30
Leverage


Histogram of resid(TotDepth18.4)
Frequency
0
10
30
50
-0.6
-0.4
-0.2
0.0
0.2
0.4
resid(TotDepth18.4)

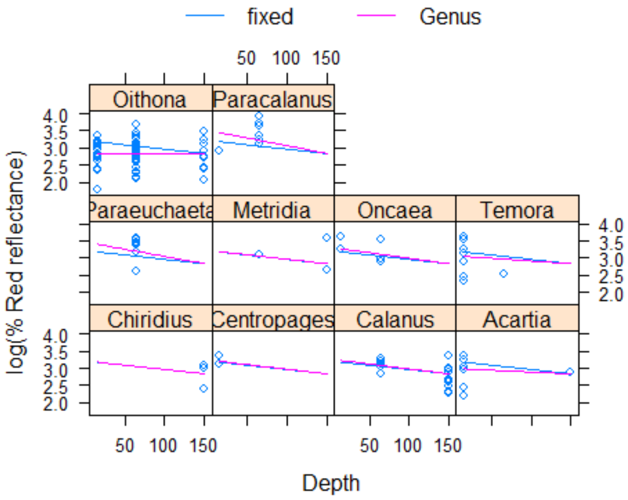

log(% Tot reflectance)
2.50
2.25
2.00
1.75
1.50
1.25
Paraeuchaeta
Calanus
Chiridius
Metridia
Paracalanus
Pseudocalanus
Acartia
Temora
Oncaea
Oithona
Genus

Red reflectance

2017

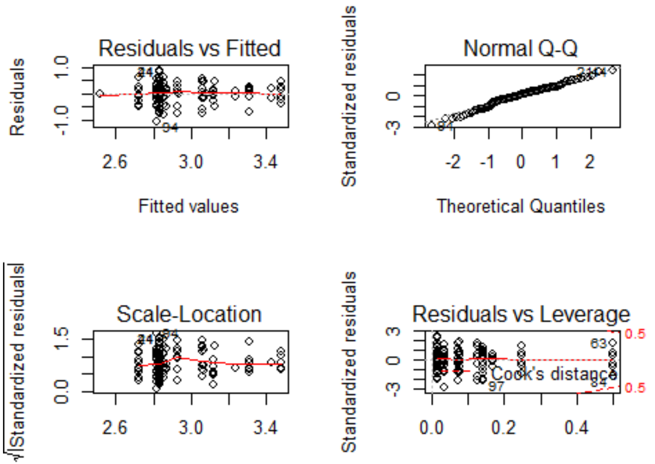


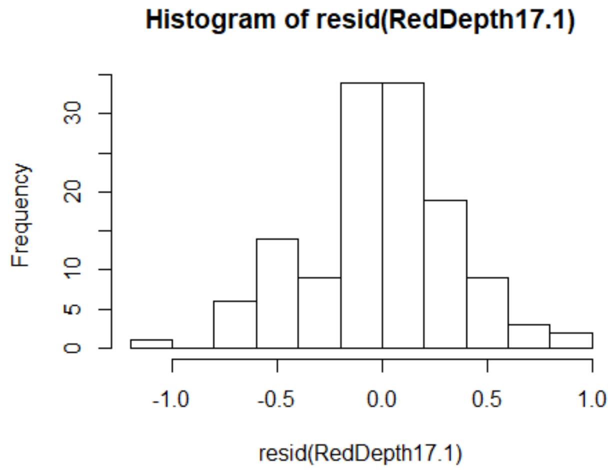
```
summary(m17 <- lme(g17))
## Linear mixed-effects model fit by REML
##  Data: g17
##       AIC      BIC    logLik
##   145.788 162.9469 -66.89402
##
## Random effects:
##  Formula: ~Depth | Genus
##  Structure: General positive-definite
##             StdDev      Corr
## (Intercept) 0.279409779 (Intr)
## Depth       0.001843334 -1
## Residual    0.367256860
##
## Fixed effects: log.Red ~ Depth
##                 Value  Std.Error  DF   t-value p-value
## (Intercept)  3.221851 0.12167639 120 26.478849  0.0000
## Depth       -0.002539 0.00097876 120 -2.593971  0.0107
##  Correlation:
##       (Intr)
## Depth -0.91
##
## Standardized Within-Group Residuals:
##         Min          Q1         Med          Q3         Max
## -2.87757690 -0.52625138  0.06810646  0.63012771  2.31299699
##
## Number of Observations: 131
## Number of Groups: 10
```



```
anova(m17)

##             numDF denDF  F-value p-value
## (Intercept)     1   120 3378.141  <.0001
## Depth           1   120    6.729  0.0107

RedDepth17.1 = lm(log.Red ~ Depth * Genus, data=d17)
RedDepth17.2 = lm(log.Red ~ Depth + Genus, data=d17)
RedDepth17.3 = lm(log.Red ~ Depth, data=d17)
RedDepth17.4 = lm(log.Red ~ Genus, data=d17)

AICc(RedDepth17.1, RedDepth17.2, RedDepth17.3, RedDepth17.4)

##              df     AICc
## RedDepth17.1 18 132.5908
## RedDepth17.2 12 127.3713
## RedDepth17.3  3 135.7321
## RedDepth17.4 11 125.7012

summary(RedDepth17.1)
## Call:
## lm(formula = log.Red ~ Depth * Genus, data = d17)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -1.03108 -0.16676  0.02504  0.20144  0.86010
##
## Coefficients: (3 not defined because of singularities)
##                          Estimate Std. Error t value Pr(>|t|)
## (Intercept)             3.2911404  0.1537632  21.404  < 2e-16 ***
## Depth                   0.0002499  0.0010282   0.243  0.80838
## GenusCalanus            0.1281037  0.3152039   0.406  0.68520
## GenusChiridius         -0.4570674  0.2457102  -1.860  0.06544 .
## GenusCentropages       -0.0599310  0.2982230  -0.201  0.84109
## GenusMetridia          -0.2413527  0.6934873  -0.348  0.72846
## GenusParacalanus       -0.5221167  0.5022212  -1.040  0.30072
## GenusAcartia           -0.3602119  0.2213028  -1.628  0.10635
## GenusTemora            -0.0724733  0.2531431  -0.286  0.77517
## GenusOncaea             0.2467104  0.3742886   0.659  0.51113
## GenusOithona           -0.4765165  0.1457451  -3.270  0.00142 **
## Depth:GenusCalanus     -0.0048780  0.0023628  -2.064  0.04124 *
## Depth:GenusChiridius           NA         NA      NA       NA
## Depth:GenusCentropages         NA         NA      NA       NA
## Depth:GenusMetridia     0.0002559  0.0053783   0.048  0.96214
## Depth:GenusParacalanus  0.0106211  0.0079007   1.344  0.18151
## Depth:GenusAcartia     -0.0005969  0.0030781  -0.194  0.84658
## Depth:GenusTemora      -0.0110308  0.0078398  -1.407  0.16214
## Depth:GenusOncaea      -0.0075400  0.0064287  -1.173  0.24329
## Depth:GenusOithona             NA         NA      NA       NA
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
```

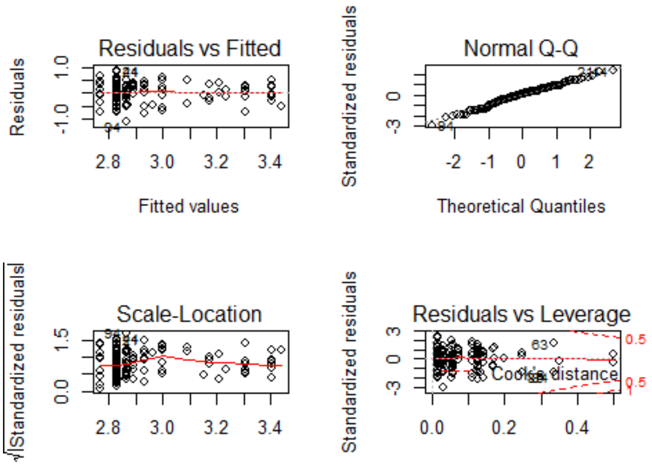
```
## Residual standard error: 0.3664 on 114 degrees of freedom
## Multiple R-squared:  0.2693, Adjusted R-squared:  0.1668
## F-statistic: 2.627 on 16 and 114 DF,  p-value: 0.00157
```

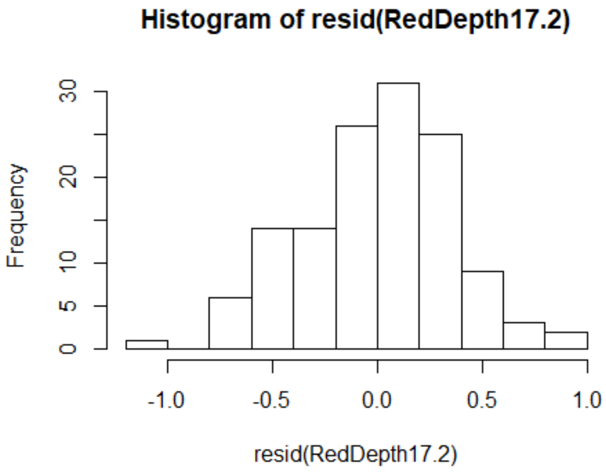




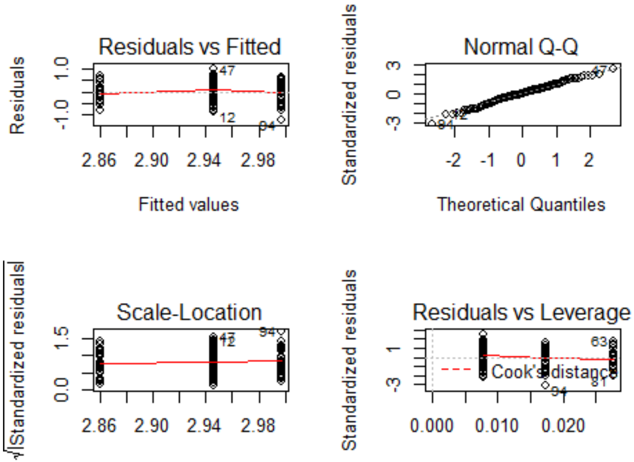
```
summary(RedDepth17.2)
## Call:
## lm(formula = log.Red ~ Depth + Genus, data = d17)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -1.07931 -0.21261  0.03989  0.24079  0.86032
##
## Coefficients:
##                   Estimate Std. Error t value Pr(>|t|)
## (Intercept)       3.354120   0.151149  22.191  < 2e-16 ***
## Depth            -0.000719   0.000863  -0.833  0.40645
## GenusCalanus     -0.416311   0.171686  -2.425  0.01681 *
## GenusChiridius   -0.374710   0.244038  -1.535  0.12730
## GenusCentropages -0.108377   0.300847  -0.360  0.71930
## GenusMetridia    -0.155318   0.260876  -0.595  0.55272
## GenusParacalanus  0.095818   0.192264   0.498  0.61914
## GenusAcartia     -0.411334   0.194302  -2.117  0.03633 *
## GenusTemora      -0.342281   0.191029  -1.792  0.07569 .
## GenusOncaea      -0.133873   0.207096  -0.646  0.51923
## GenusOithona     -0.476740   0.147719  -3.227  0.00161 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.3713 on 120 degrees of freedom
```

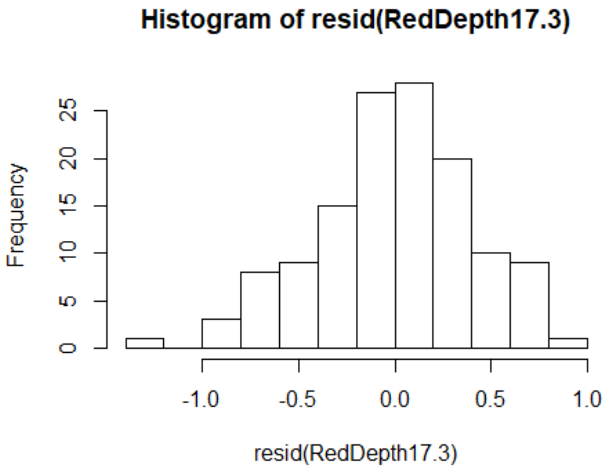
```
## Multiple R-squared:  0.2099, Adjusted R-squared:  0.1441
## F-statistic: 3.188 on 10 and 120 DF,  p-value: 0.00116
```



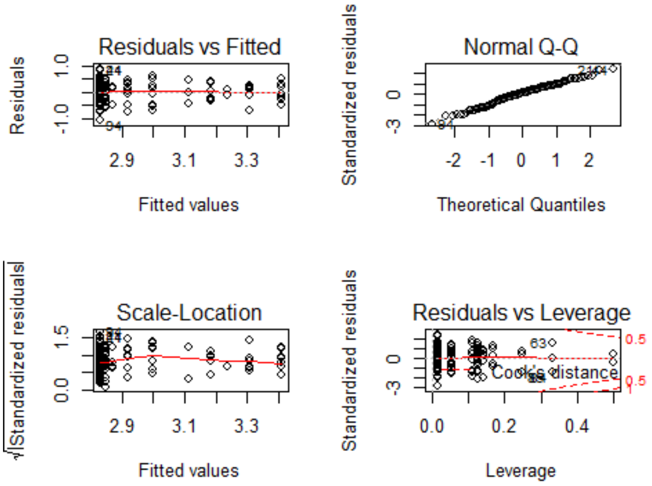


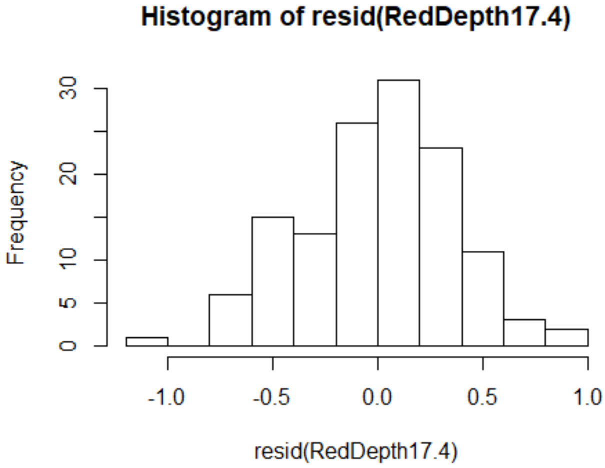
```
summary(RedDepth17.3)
## Call:
## lm(formula = log.Red ~ Depth, data = d17)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -1.20971 -0.23413  0.01845  0.24592  0.97902
##
## Coefficients:
##               Estimate Std. Error t value Pr(>|t|)
## (Intercept)  3.0121690  0.0608273  49.520   <2e-16 ***
## Depth       -0.0010116  0.0007091  -1.427    0.156
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.3998 on 129 degrees of freedom
## Multiple R-squared:  0.01553,    Adjusted R-squared:  0.0079
## F-statistic: 2.035 on 1 and 129 DF,  p-value: 0.1561
```

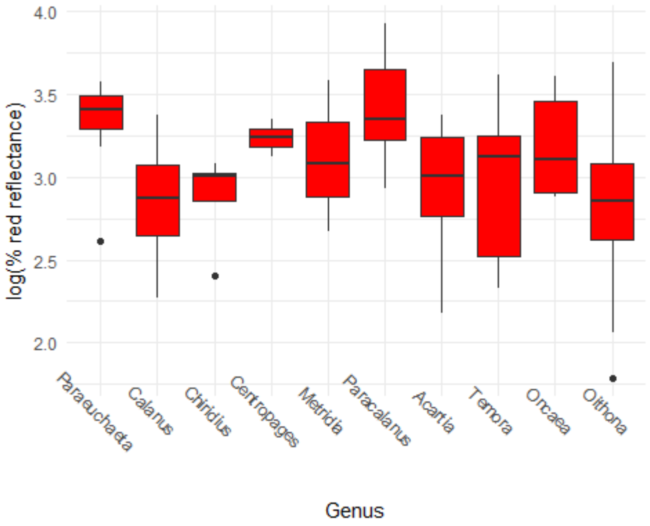




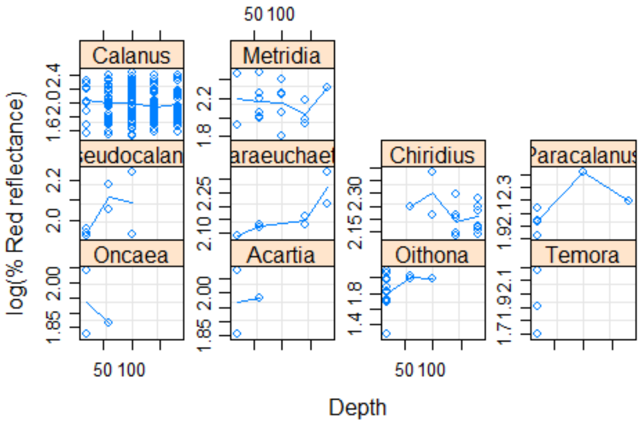
```
summary(RedDepth17.4)
## Call:
## lm(formula = log.Red ~ Genus, data = d17)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -1.0435 -0.2189  0.0357  0.2465  0.8602
##
## Coefficients:
##                  Estimate Std. Error t value Pr(>|t|)
## (Intercept)       3.30739    0.14018  23.594  < 2e-16 ***
## GenusCalanus     -0.45812    0.16398  -2.794  0.00606 **
## GenusChiridius   -0.43582    0.23246  -1.875  0.06322 .
## GenusCentropages -0.07243    0.29736  -0.244  0.80798
## GenusMetridia    -0.19606    0.25593  -0.766  0.44512
## GenusParacalanus  0.10031    0.19194   0.523  0.60220
## GenusAcartia     -0.38752    0.19194  -2.019  0.04571 *
## GenusTemora      -0.31033    0.18690  -1.660  0.09943 .
## GenusOncaea      -0.12189    0.20633  -0.591  0.55580
## GenusOithona     -0.47657    0.14753  -3.230  0.00159 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.3709 on 121 degrees of freedom
## Multiple R-squared:  0.2054, Adjusted R-squared:  0.1462
## F-statistic: 3.474 on 9 and 121 DF,  p-value: 0.000758
```


Residuals vs Fitted
Residuals
1.0
-1.0
2.9
3.1
3.3
Fitted values
Normal Q-Q
Standardized residuals
0
-3
-2
-1
0
1
2
Theoretical Quantiles
Scale-Location
√|Standardized residuals|
1.5
0.0
2.9
3.1
3.3
Fitted values
Residuals vs Leverage
Standardized residuals
0
-3
63
0.5
Cook's distance
0.5
0.0
0.2
0.4
Leverage

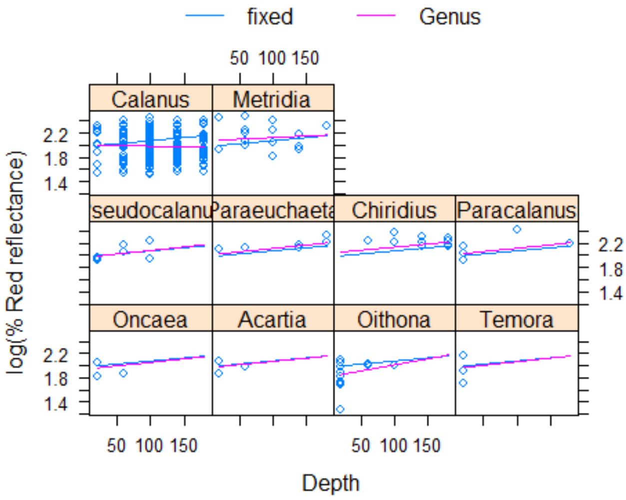

Histogram of resid(RedDepth17.4)
Frequency
30
20
10
5
0
-1.0
-0.5
0.0
0.5
1.0
resid(RedDepth17.4)


log(% red reflectance)
4.0
3.5
3.0
2.5
2.0
Paraeuchaeta
Calanus
Chiridius
Centropages
Metridia
Paracalanus
Acartia
Temora
Oncaea
Oithona
Genus

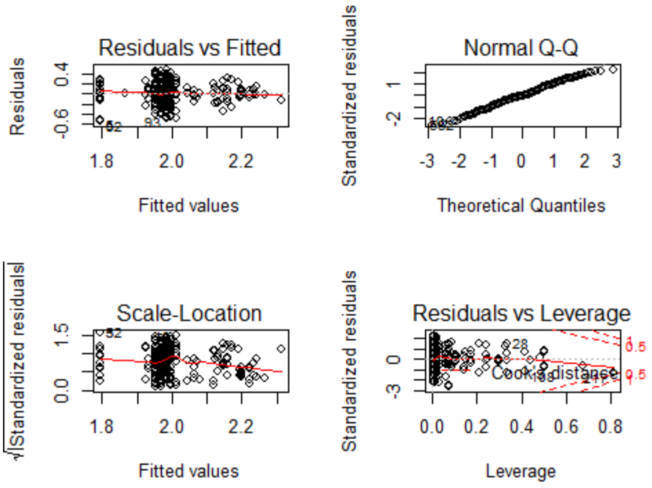
2018

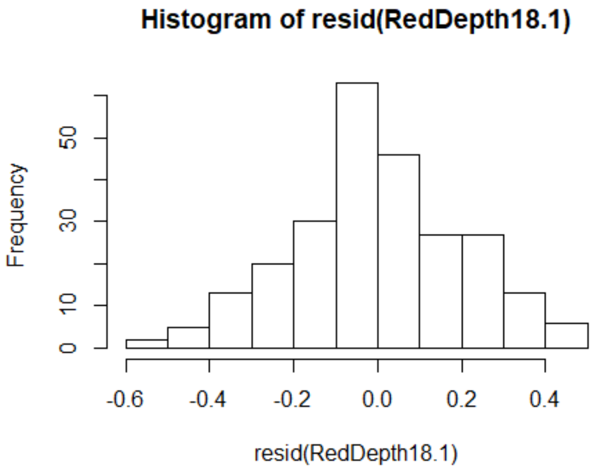


```
summary(m18 <- lme(g18))
## Linear mixed-effects model fit by REML
##  Data: g18
##        AIC       BIC   logLik
##   -30.9127 -9.783932 21.45635
##
## Random effects:
##  Formula: ~Depth | Genus
##  Structure: General positive-definite
##             StdDev      Corr
## (Intercept) 0.108406943 (Intr)
## Depth       0.001018649 -0.722
## Residual    0.208927321
##
## Fixed effects: log.Red ~ Depth
##                 Value  Std.Error  DF  t-value p-value
## (Intercept) 1.9755950 0.05180469 241 38.13545  0.0000
## Depth       0.0010121 0.00054715 241  1.84980  0.0656
##  Correlation:
##       (Intr)
## Depth -0.746
##
## Standardized Within-Group Residuals:
##         Min          Q1         Med          Q3         Max
## -2.78804790 -0.60715022 -0.02709793  0.71106311  2.21519862
##
## Number of Observations: 252
## Number of Groups: 10
```

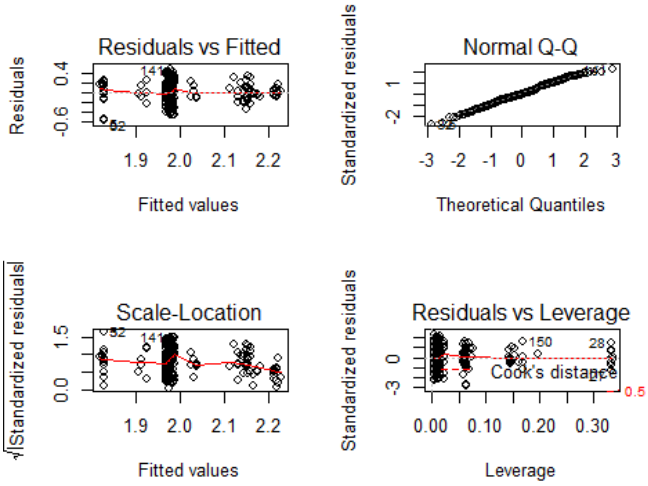


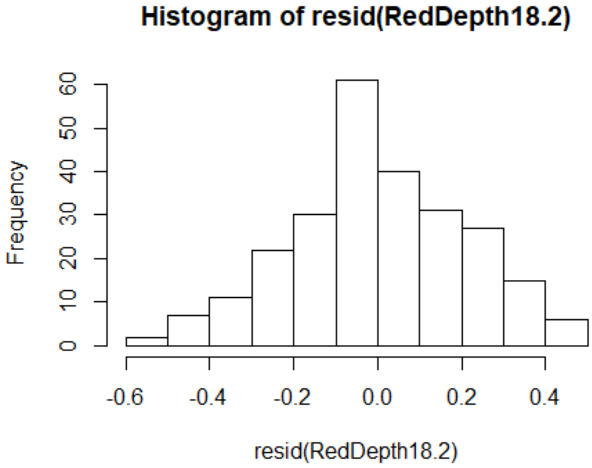
```
RedDepth18.1 = lm(log.Red ~ Depth * Genus, data=d18)
RedDepth18.2 = lm(log.Red ~ Depth + Genus, data=d18)
RedDepth18.3 = lm(log.Red ~ Depth, data=d18)
RedDepth18.4 = lm(log.Red ~ Genus, data=d18)

AICc(RedDepth18.1, RedDepth18.2, RedDepth18.3, RedDepth18.4)

##              df      AICc
## RedDepth18.1 20 -44.76960
## RedDepth18.2 12 -56.91185
## RedDepth18.3  3 -33.05345
## RedDepth18.4 11 -58.94480

summary(RedDepth18.1)
## Call:
## lm(formula = log.Red ~ Depth * Genus, data = d18)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.52636 -0.11777 -0.00803  0.14688  0.46430
##
## Coefficients: (1 not defined because of singularities)
##                           Estimate Std. Error t value Pr(>|t|)
## (Intercept)              2.0609884  0.1696939  12.145   <2e-16 ***
## Depth                    0.0009653  0.0013440   0.718   0.4733
## GenusCalanus            -0.0406212  0.1749143  -0.232   0.8166
## GenusChiridius           0.2379109  0.2811713   0.846   0.3983
## GenusMetridia            0.1180400  0.2062235   0.572   0.5676
## GenusParacalanus        -0.0258431  0.2083101  -0.124   0.9014
## GenusPseudocalanus      -0.1366552  0.2281957  -0.599   0.5499
## GenusAcartia            -0.1023793  0.3001728  -0.341   0.7334
## GenusTemora             -0.1553981  0.1906056  -0.815   0.4157
## GenusOncaea             -0.0928175  0.3001728  -0.309   0.7574
## GenusOithona            -0.3345556  0.1913082  -1.749   0.0816 .
## Depth:GenusCalanus      -0.0013353  0.0013878  -0.962   0.3370
## Depth:GenusChiridius    -0.0015205  0.0019850  -0.766   0.4444
## Depth:GenusMetridia     -0.0012962  0.0017981  -0.721   0.4717
## Depth:GenusParacalanus   0.0005762  0.0019485   0.296   0.7677
## Depth:GenusPseudocalanus 0.0010256  0.0027466   0.373   0.7092
## Depth:GenusAcartia      -0.0005705  0.0066036  -0.086   0.9312
## Depth:GenusTemora               NA         NA      NA       NA
## Depth:GenusOncaea       -0.0026736  0.0066036  -0.405   0.6859
## Depth:GenusOithona       0.0024708  0.0027165   0.910   0.3640
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.2112 on 233 degrees of freedom
## Multiple R-squared:  0.1829, Adjusted R-squared:  0.1198
## F-statistic: 2.897 on 18 and 233 DF,  p-value: 0.0001209
```



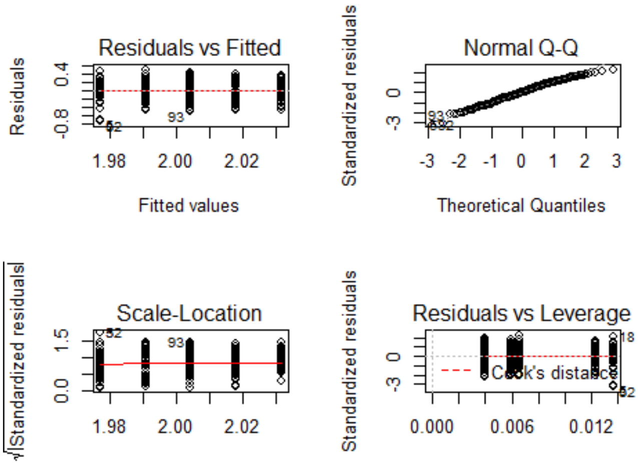


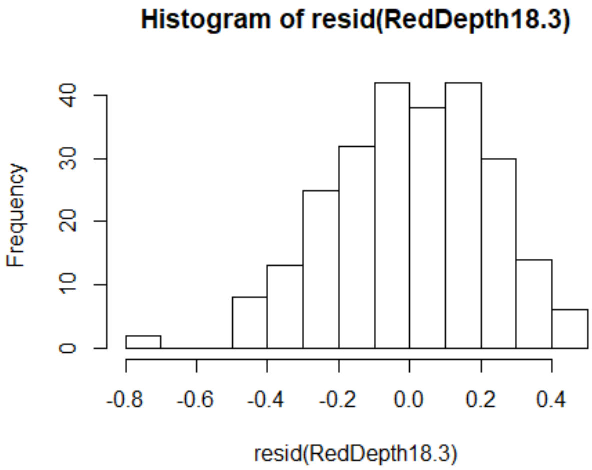
```
summary(RedDepth18.2)
## Call:
## lm(formula = log.Red ~ Depth + Genus, data = d18)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.56194 -0.11786 -0.01167  0.15893  0.46762
##
## Coefficients:
##                      Estimate Std. Error t value Pr(>|t|)
## (Intercept)         2.1821492  0.0862123  25.311  < 2e-16 ***
## Depth              -0.0001221  0.0003005  -0.406 0.684939
## GenusCalanus       -0.1899062  0.0810372  -2.343 0.019920 *
## GenusChiridius      0.0520278  0.0950808   0.547 0.584751
## GenusMetridia      -0.0215529  0.0946501  -0.228 0.820063
## GenusParacalanus   -0.0471902  0.1179548  -0.400 0.689459
## GenusPseudocalanus -0.1431122  0.1136550  -1.259 0.209184
## GenusAcartia       -0.2063125  0.1469300  -1.404 0.161560
## GenusTemora        -0.2548121  0.1476229  -1.726 0.085611 .
## GenusOncaea        -0.2668546  0.1469300  -1.816 0.070582 .
## GenusOithona       -0.3489728  0.0983426  -3.549 0.000466 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.2102 on 241 degrees of freedom
## Multiple R-squared:  0.1626, Adjusted R-squared:  0.1278
## F-statistic: 4.679 on 10 and 241 DF,  p-value: 4.121e-06
```



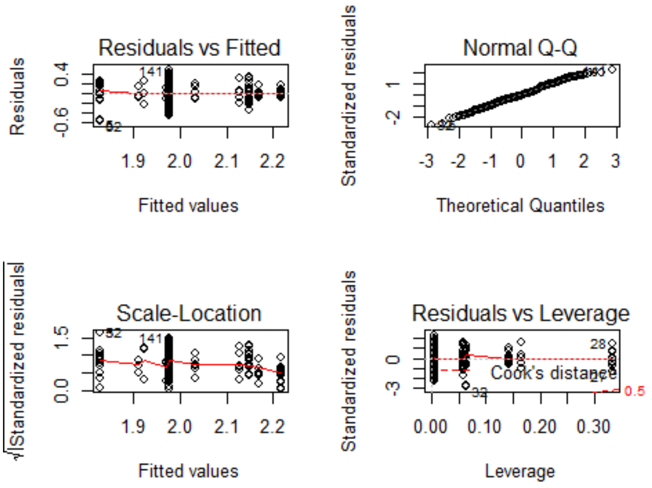


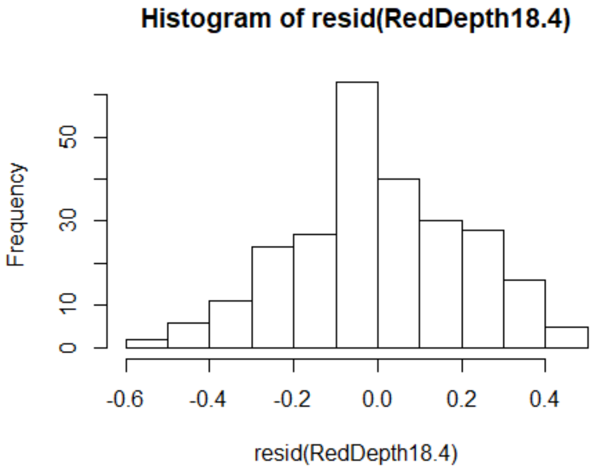
```
summary(RedDepth18.3)
## Call:
## lm(formula = log.Red ~ Depth, data = d18)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.70832 -0.14776  0.00601  0.17524  0.48752
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept) 1.9702880  0.0308504  63.866   <2e-16 ***
## Depth       0.0003409  0.0002665   1.279    0.202
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.2248 on 250 degrees of freedom
## Multiple R-squared:  0.006505,   Adjusted R-squared:  0.002531
## F-statistic: 1.637 on 1 and 250 DF,  p-value: 0.2019
```

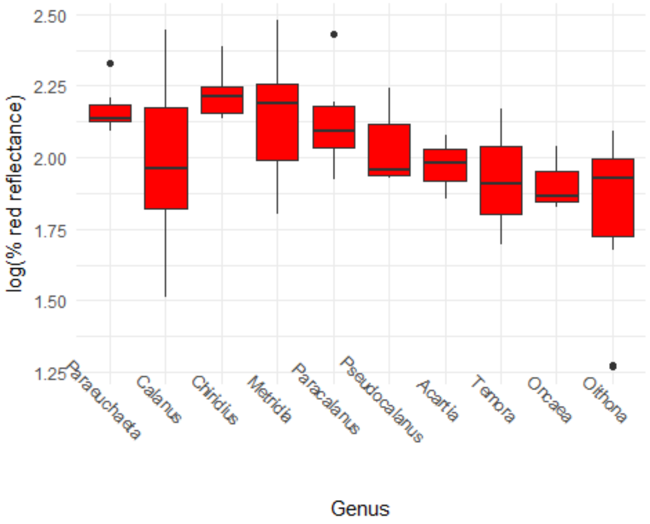




```
summary(RedDepth18.4)
## Call:
## lm(formula = log.Red ~ Genus, data = d18)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.56072 -0.12174 -0.01308  0.15908  0.46926
##
## Coefficients:
##                    Estimate Std. Error t value Pr(>|t|)
## (Intercept)         2.16855    0.07931  27.344  < 2e-16 ***
## GenusCalanus       -0.19015    0.08090  -2.351 0.019549 *
## GenusChiridius      0.04739    0.09423   0.503 0.615472
## GenusMetridia      -0.01872    0.09423  -0.199 0.842680
## GenusParacalanus   -0.04091    0.11674  -0.350 0.726293
## GenusPseudocalanus -0.13614    0.11216  -1.214 0.226003
## GenusAcartia       -0.19678    0.14479  -1.359 0.175400
## GenusTemora        -0.24365    0.14479  -1.683 0.093713 .
## GenusOncaea        -0.25732    0.14479  -1.777 0.076797 .
## GenusOithona       -0.33903    0.09509  -3.566 0.000437 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.2098 on 242 degrees of freedom
## Multiple R-squared:  0.162,  Adjusted R-squared:  0.1308
## F-statistic: 5.198 on 9 and 242 DF,  p-value: 1.836e-06
```

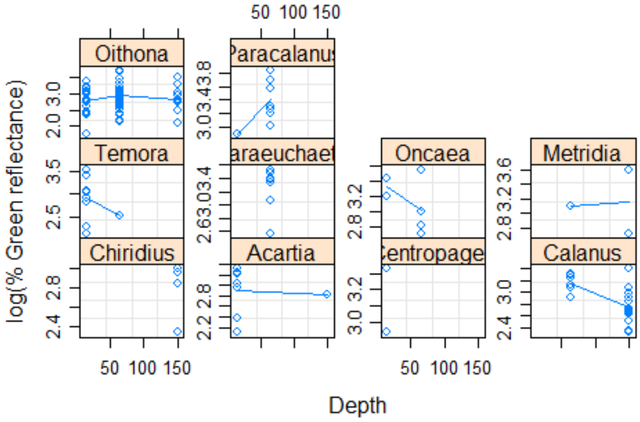

Residuals vs Fitted
Residuals
0.4
-0.6
141
52
1.9
2.0
2.1
2.2
Fitted values
Normal Q-Q
Standardized residuals
1
-2
32
-3
-2
-1
0
1
2
3
Theoretical Quantiles
Scale-Location
√|Standardized residuals|
1.5
0.0
52
141
1.9
2.0
2.1
2.2
Fitted values
Residuals vs Leverage
Standardized residuals
0
-3
28
27
32
Cook's distance
0.5
0.00
0.10
0.20
0.30
Leverage


Histogram of resid(RedDepth18.4)
Frequency
0
10
30
50
-0.6
-0.4
-0.2
0.0
0.2
0.4
resid(RedDepth18.4)

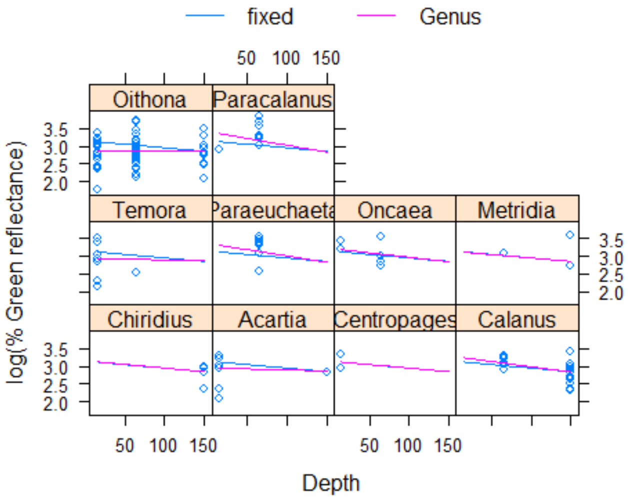

log(% red reflectance)
2.50
2.25
2.00
1.75
1.50
1.25
Paraeuchaeta
Calanus
Chiridius
Metridia
Paracalanus
Pseudocalanus
Acartia
Temora
Oncaea
Oithona
Genus

Green reflectance

2017

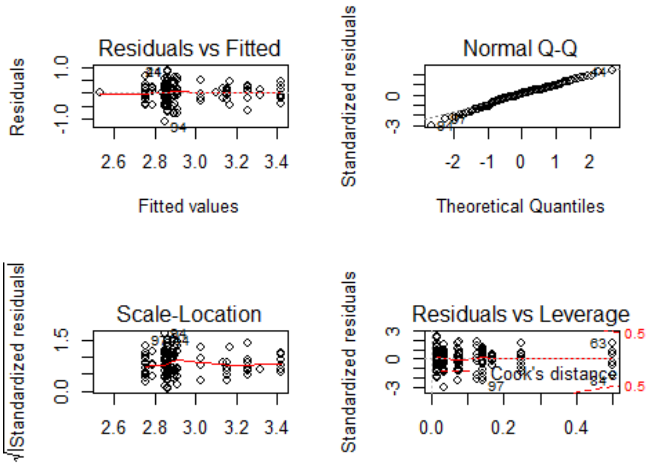


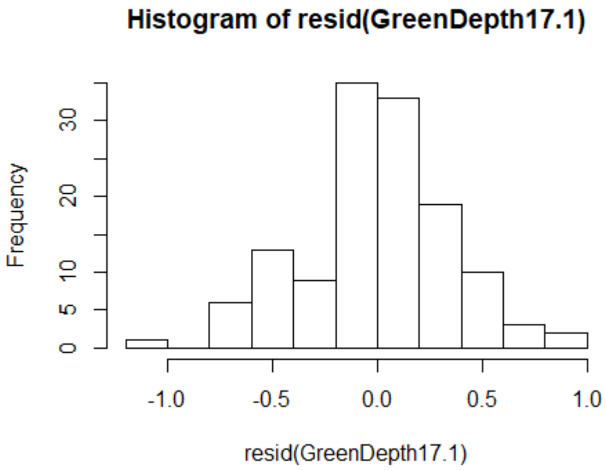
```
summary(m17 <- lme(g17))
## Linear mixed-effects model fit by REML
##  Data: g17
##        AIC      BIC    logLik
##   144.1689 161.3278 -66.08447
##
## Random effects:
##  Formula: ~Depth | Genus
##  Structure: General positive-definite
##             StdDev     Corr
## (Intercept) 0.26012826 (Intr)
## Depth       0.00188532 -1
## Residual    0.36631309
##
## Fixed effects: log.Green ~ Depth
##                 Value  Std.Error  DF   t-value p-value
## (Intercept)  3.1571136 0.11587919 120 27.244871  0.0000
## Depth       -0.0019995 0.00098157 120 -2.037087  0.0438
##  Correlation:
##       (Intr)
## Depth -0.916
##
## Standardized Within-Group Residuals:
##         Min          Q1         Med          Q3         Max
## -3.00659389 -0.51544963  0.08418567  0.59193992  2.29741718
##
## Number of Observations: 131
## Number of Groups: 10
```



```
anova(m17)

##             numDF denDF  F-value p-value
## (Intercept)     1   120 3981.242  <.0001
## Depth           1   120    4.150  0.0438

GreenDepth17.1 = lm(log.Green ~ Depth * Genus, data=d17)
GreenDepth17.2 = lm(log.Green ~ Depth + Genus, data=d17)
GreenDepth17.3 = lm(log.Green ~ Depth, data=d17)
GreenDepth17.4 = lm(log.Green ~ Genus, data=d17)

AICc(GreenDepth17.1, GreenDepth17.2, GreenDepth17.3, GreenDepth17.4)

##                df     AICc
## GreenDepth17.1 18 133.1958
## GreenDepth17.2 12 126.7746
## GreenDepth17.3  3 128.0836
## GreenDepth17.4 11 124.9029

summary(GreenDepth17.1)
## Call:
## lm(formula = log.Green ~ Depth * Genus, data = d17)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -1.0789 -0.1810  0.0102  0.2052  0.8512
##
## Coefficients: (3 not defined because of singularities)
##                          Estimate Std. Error t value Pr(>|t|)
## (Intercept)             3.2306306  0.1541187  20.962  < 2e-16 ***
## Depth                   0.0003426  0.0010306   0.332  0.74015
## GenusCalanus            0.2329953  0.3159327   0.737  0.46234
## GenusChiridius         -0.4941446  0.2462783  -2.006  0.04718 *
## GenusCentropages       -0.1019914  0.2989125  -0.341  0.73357
## GenusMetridia          -0.1808751  0.6950906  -0.260  0.79517
## GenusParacalanus       -0.4907416  0.5033823  -0.975  0.33168
## GenusAcartia           -0.3340477  0.2218145  -1.506  0.13484
## GenusTemora            -0.2083028  0.2537283  -0.821  0.41338
## GenusOncaea             0.1763430  0.3751540   0.470  0.63921
## GenusOithona           -0.3875102  0.1460821  -2.653  0.00912 **
## Depth:GenusCalanus     -0.0050871  0.0023683  -2.148  0.03383 *
## Depth:GenusChiridius           NA         NA      NA       NA
## Depth:GenusCentropages         NA         NA      NA       NA
## Depth:GenusMetridia     0.0004369  0.0053907   0.081  0.93555
## Depth:GenusParacalanus  0.0100821  0.0079190   1.273  0.20555
## Depth:GenusAcartia     -0.0007569  0.0030852  -0.245  0.80664
## Depth:GenusTemora      -0.0079104  0.0078580  -1.007  0.31622
## Depth:GenusOncaea      -0.0062205  0.0064435  -0.965  0.33639
## Depth:GenusOithona             NA         NA      NA       NA
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
```

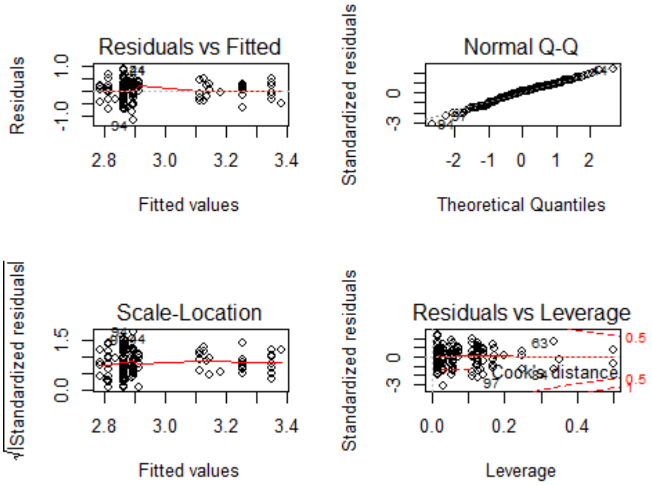
```
## Residual standard error: 0.3672 on 114 degrees of freedom
## Multiple R-squared:  0.2151, Adjusted R-squared:  0.105
## F-statistic: 1.953 on 16 and 114 DF,  p-value: 0.02226
```

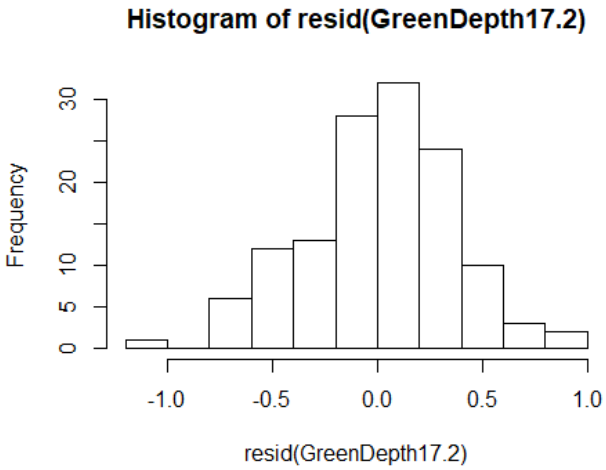




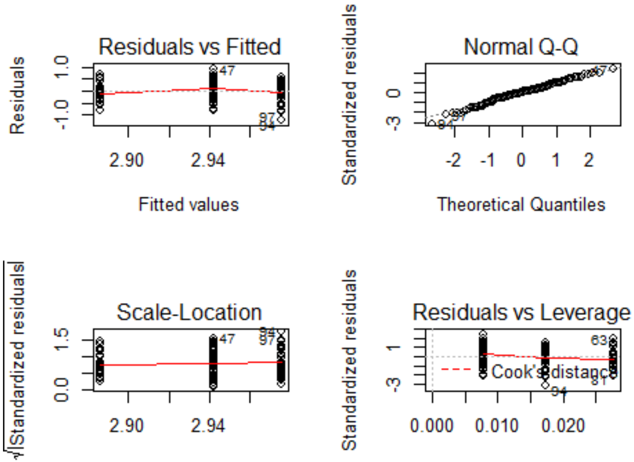
```
summary(GreenDepth17.2)
## Call:
## lm(formula = log.Green ~ Depth + Genus, data = d17)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -1.12648 -0.19437  0.04844  0.21442  0.85141
##
## Coefficients:
##                   Estimate Std. Error t value Pr(>|t|)
## (Intercept)       3.2928121  0.1508057  21.835  < 2e-16 ***
## Depth            -0.0006140  0.0008611  -0.713  0.47718
## GenusCalanus     -0.3378834  0.1712958  -1.973  0.05085 .
## GenusChiridius   -0.4128303  0.2434826  -1.696  0.09257 .
## GenusCentropages -0.1498233  0.3001627  -0.499  0.61859
## GenusMetridia    -0.0735122  0.2602821  -0.282  0.77810
## GenusParacalanus  0.0956048  0.1918263   0.498  0.61912
## GenusAcartia     -0.3898629  0.1938605  -2.011  0.04656 *
## GenusTemora      -0.4134231  0.1905949  -2.169  0.03205 *
## GenusOncaea      -0.1402604  0.2066248  -0.679  0.49856
## GenusOithona     -0.3877309  0.1473827  -2.631  0.00964 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.3705 on 120 degrees of freedom
```

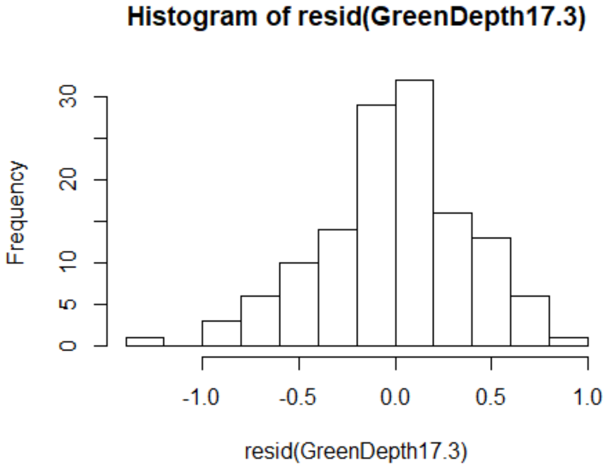
```
## Multiple R-squared:  0.1591, Adjusted R-squared:  0.08898
## F-statistic:  2.27 on 10 and 120 DF,  p-value: 0.01807
```



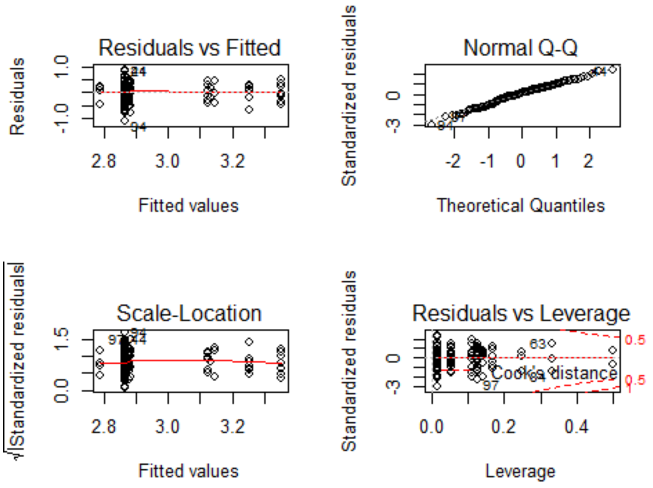


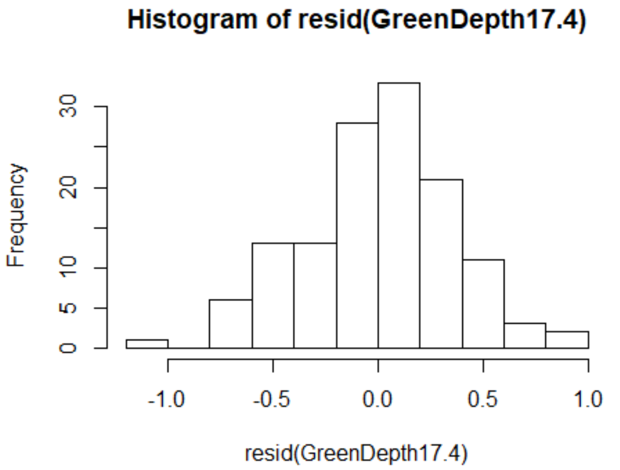
```
summary(GreenDepth17.3)
## Call:
## lm(formula = log.Green ~ Depth, data = d17)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -1.20599 -0.20706  0.01059  0.24713  0.90486
##
## Coefficients:
##               Estimate Std. Error t value Pr(>|t|)
## (Intercept)  2.9852767  0.0590772  50.532   <2e-16 ***
## Depth       -0.0006600  0.0006887  -0.958     0.34
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.3883 on 129 degrees of freedom
## Multiple R-squared:  0.007069,   Adjusted R-squared:  -0.0006277
## F-statistic: 0.9185 on 1 and 129 DF,  p-value: 0.3397
```

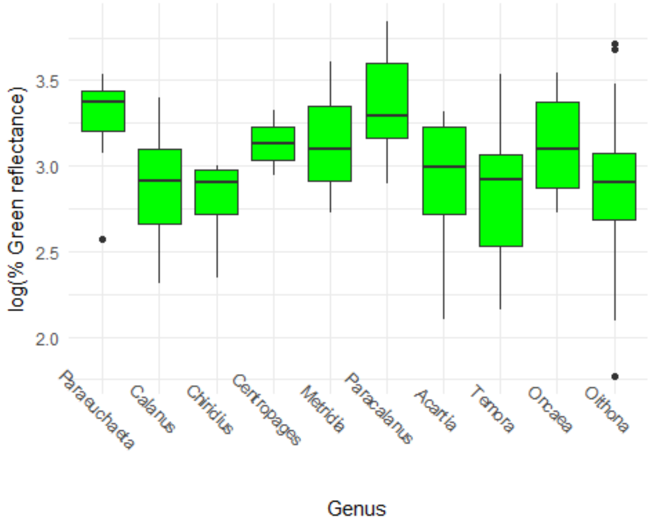




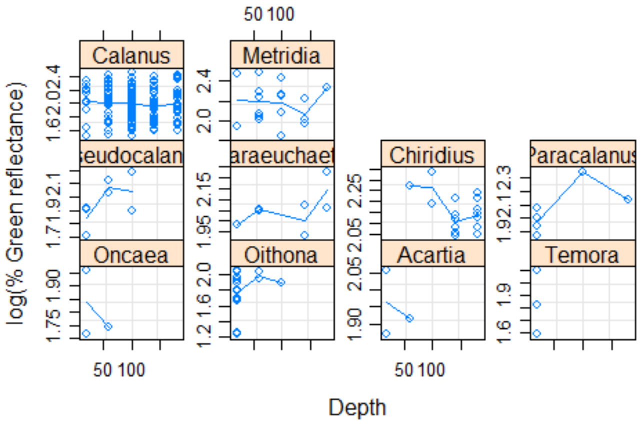
```
summary(GreenDepth17.4)
## Call:
## lm(formula = log.Green ~ Genus, data = d17)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -1.09593 -0.20210  0.04838  0.22457  0.85127
##
## Coefficients:
##                   Estimate Std. Error t value Pr(>|t|)
## (Intercept)        3.25290    0.13975  23.277  < 2e-16 ***
## GenusCalanus      -0.37359    0.16348  -2.285  0.02404 *
## GenusChiridius    -0.46502    0.23175  -2.007  0.04703 *
## GenusCentropages -0.11912    0.29646  -0.402  0.68852
## GenusMetridia     -0.10831    0.25515  -0.424  0.67197
## GenusParacalanus  0.09944    0.19136   0.520  0.60425
## GenusAcartia      -0.36952    0.19136  -1.931  0.05582 .
## GenusTemora       -0.38613    0.18633  -2.072  0.04036 *
## GenusOncaea       -0.13003    0.20571  -0.632  0.52852
## GenusOithona      -0.38759    0.14708  -2.635  0.00951 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.3697 on 121 degrees of freedom
## Multiple R-squared:  0.1555, Adjusted R-squared:  0.09268
## F-statistic: 2.475 on 9 and 121 DF,  p-value: 0.01253
```


Residuals vs Fitted
Residuals
1.0
-1.0
2.8
3.0
3.2
Fitted values
Normal Q-Q
Standardized residuals
0
-3
-2
-1
0
1
2
Theoretical Quantiles
Scale-Location
√|Standardized residuals|
1.5
0.0
2.8
3.0
3.2
Fitted values
Residuals vs Leverage
Standardized residuals
0
-3
Cook's distance
0.5
0.5
0.0
0.2
0.4
Leverage

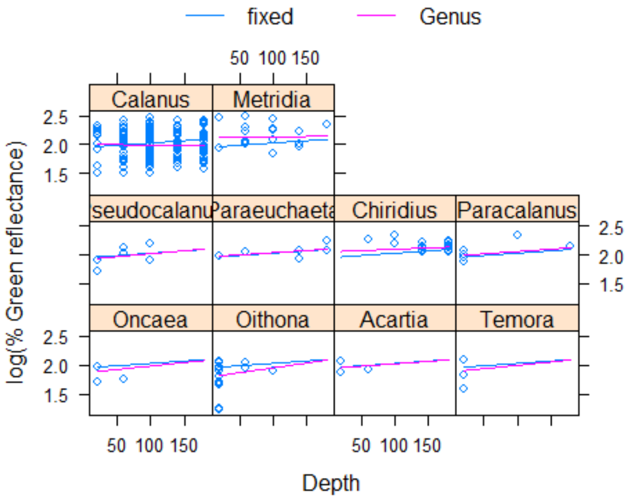

Histogram of resid(GreenDepth17.4)
Frequency
0
5
10
20
30
-1.0
-0.5
0.0
0.5
1.0
resid(GreenDepth17.4)


log(% Green reflectance)
3.5
3.0
2.5
2.0
Paraeuchaeta
Calanus
Chiridius
Centropages
Metridia
Paracalanus
Acartia
Temora
Oncaea
Oithona
Genus

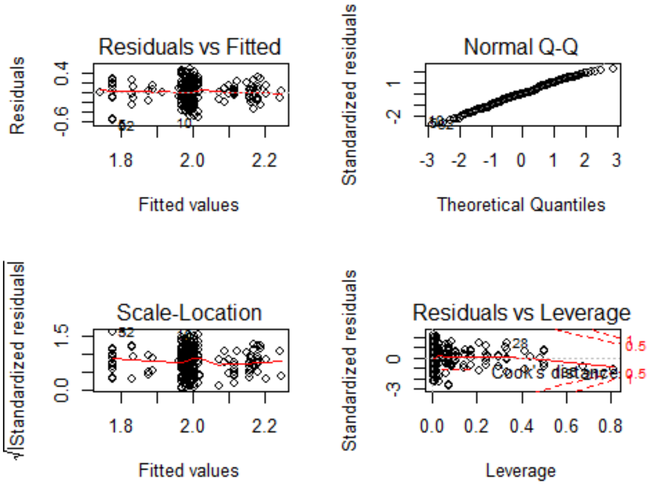
2018

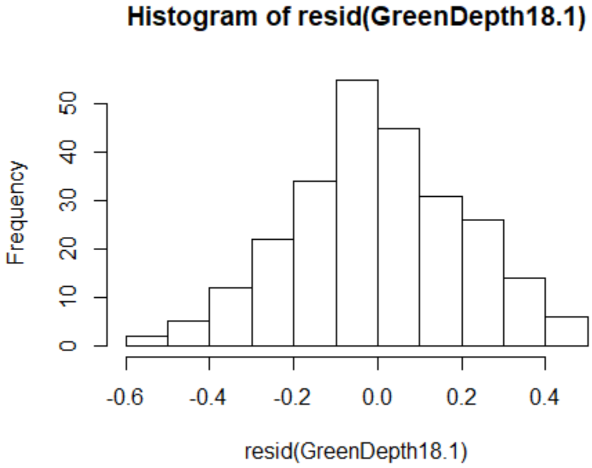


```
summary(m18 <- lme(g18))
## Linear mixed-effects model fit by REML
##  Data: g18
##        AIC       BIC   logLik
##   -22.97658 -1.847817 17.48829
##
## Random effects:
##  Formula: ~Depth | Genus
##  Structure: General positive-definite
##             StdDev       Corr
## (Intercept) 0.1311893259 (Intr)
## Depth       0.0008783059 -0.818
## Residual    0.2122306497
##
## Fixed effects: log.Green ~ Depth
##                Value  Std.Error  DF  t-value p-value
## (Intercept) 1.949805 0.05750949 241 33.90406  0.0000
## Depth       0.000801 0.00048973 241  1.63563  0.1032
##  Correlation:
##       (Intr)
## Depth -0.784
##
## Standardized Within-Group Residuals:
##         Min          Q1         Med          Q3         Max
## -2.79638311 -0.56952286 -0.03973622  0.73446257  2.20786530
##
## Number of Observations: 252
## Number of Groups: 10
```

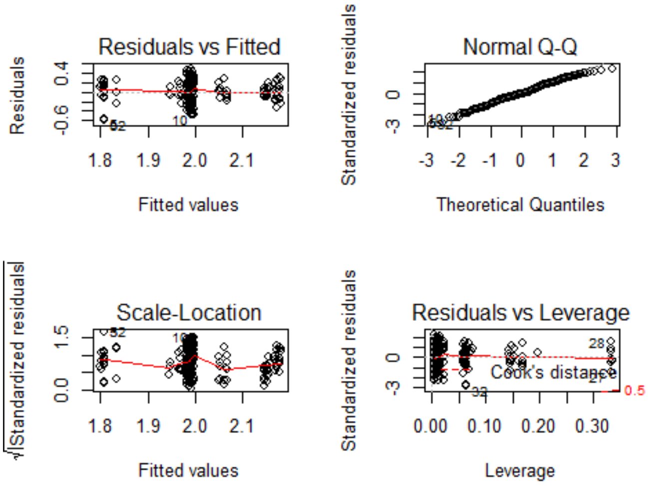


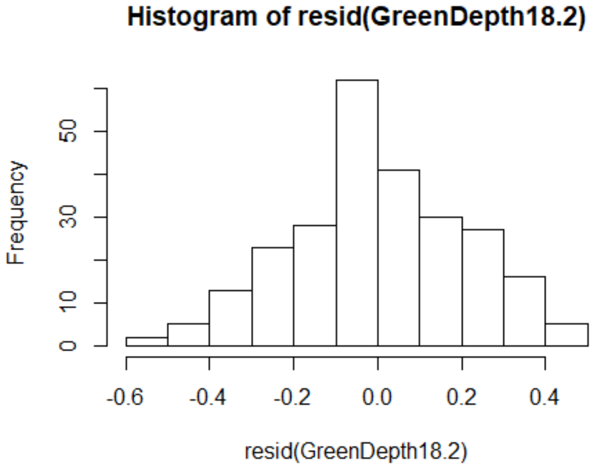
```
GreenDepth18.1 = lm(log.Green ~ Depth * Genus, data=d18)
GreenDepth18.2 = lm(log.Green ~ Depth + Genus, data=d18)
GreenDepth18.3 = lm(log.Green ~ Depth, data=d18)
GreenDepth18.4 = lm(log.Green ~ Genus, data=d18)

AICc(GreenDepth18.1, GreenDepth18.2, GreenDepth18.3, GreenDepth18.4)

##                df      AICc
## GreenDepth18.1 20 -37.11317
## GreenDepth18.2 12 -49.92547
## GreenDepth18.3  3 -34.06901
## GreenDepth18.4 11 -52.03011

summary(GreenDepth18.1)
## Call:
## lm(formula = log.Green ~ Depth * Genus, data = d18)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -0.5537 -0.1190 -0.0008  0.1509  0.4690
##
## Coefficients: (1 not defined because of singularities)
##                           Estimate Std. Error t value Pr(>|t|)
## (Intercept)              1.9823398  0.1722915  11.506   <2e-16 ***
## Depth                    0.0006574  0.0013645   0.482    0.630
## GenusCalanus             0.0399953  0.1775918   0.225    0.822
## GenusChiridius           0.3294611  0.2854753   1.154    0.250
## GenusMetridia            0.2132136  0.2093803   1.018    0.310
## GenusParacalanus        -0.0080234  0.2114988  -0.038    0.970
## GenusPseudocalanus      -0.1586010  0.2316888  -0.685    0.494
## GenusAcartia             0.0025759  0.3047677   0.008    0.993
## GenusTemora             -0.1614132  0.1935232  -0.834    0.405
## GenusOncaea             -0.0999023  0.3047677  -0.328    0.743
## GenusOithona            -0.2596964  0.1942366  -1.337    0.183
## Depth:GenusCalanus      -0.0009409  0.0014091  -0.668    0.505
## Depth:GenusChiridius    -0.0017379  0.0020154  -0.862    0.389
## Depth:GenusMetridia     -0.0008944  0.0018256  -0.490    0.625
## Depth:GenusParacalanus   0.0008465  0.0019783   0.428    0.669
## Depth:GenusPseudocalanus 0.0020168  0.0027886   0.723    0.470
## Depth:GenusAcartia      -0.0018131  0.0067046  -0.270    0.787
## Depth:GenusTemora               NA         NA      NA       NA
## Depth:GenusOncaea       -0.0029639  0.0067046  -0.442    0.659
## Depth:GenusOithona       0.0021137  0.0027581   0.766    0.444
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.2144 on 233 degrees of freedom
## Multiple R-squared:  0.1589, Adjusted R-squared:  0.09388
## F-statistic: 2.445 on 18 and 233 DF,  p-value: 0.001232
```



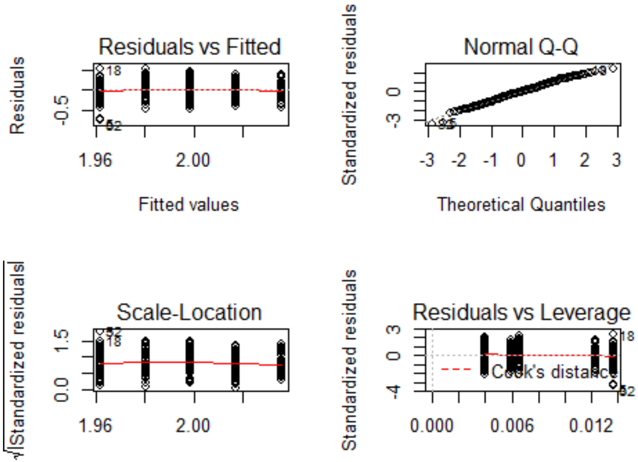


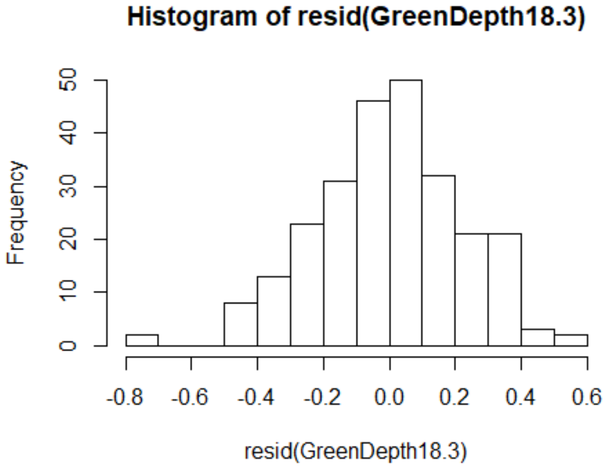
```
summary(GreenDepth18.2)
## Call:
## lm(formula = log.Green ~ Depth + Genus, data = d18)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.58239 -0.11941 -0.00626  0.16076  0.47158
##
## Coefficients:
##                     Estimate Std. Error t value Pr(>|t|)
## (Intercept)        2.066e+00  8.742e-02  23.636   <2e-16 ***
## Depth             -9.461e-05  3.047e-04  -0.311   0.7564
## GenusCalanus      -6.522e-02  8.217e-02  -0.794   0.4281
## GenusChiridius     9.836e-02  9.641e-02   1.020   0.3086
## GenusMetridia      1.169e-01  9.597e-02   1.218   0.2246
## GenusParacalanus   4.091e-03  1.196e-01   0.034   0.9727
## GenusPseudocalanus -9.209e-02  1.152e-01  -0.799   0.4250
## GenusAcartia      -1.166e-01  1.490e-01  -0.783   0.4346
## GenusTemora       -2.302e-01  1.497e-01  -1.538   0.1254
## GenusOncaea       -2.574e-01  1.490e-01  -1.728   0.0853 .
## GenusOithona      -2.575e-01  9.972e-02  -2.583   0.0104 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.2131 on 241 degrees of freedom
## Multiple R-squared:  0.1402, Adjusted R-squared:  0.1046
## F-statistic: 3.931 on 10 and 241 DF,  p-value: 5.638e-05
```



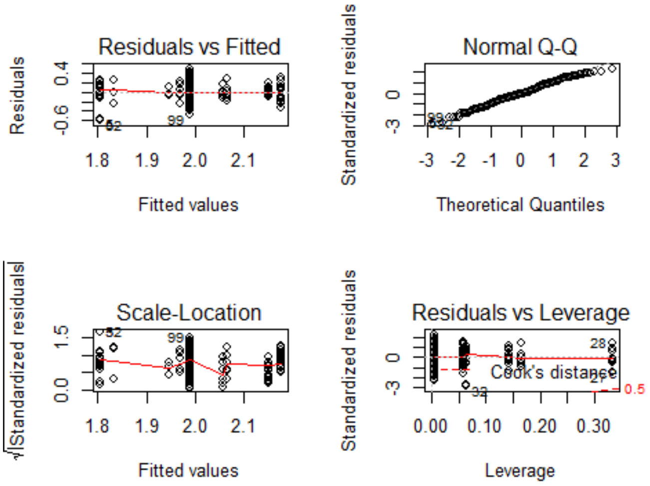


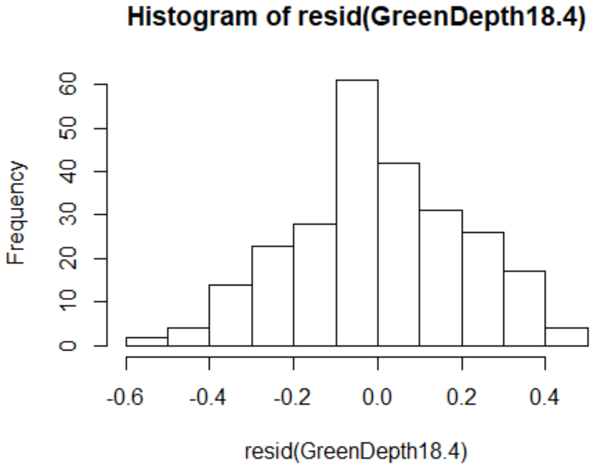
```
summary(GreenDepth18.3)
## Call:
## lm(formula = log.Green ~ Depth, data = d18)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -0.7376 -0.1394  0.0096  0.1564  0.5122
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept) 1.9527191  0.0307883  63.424   <2e-16 ***
## Depth       0.0004611  0.0002659   1.734   0.0842 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.2243 on 250 degrees of freedom
## Multiple R-squared:  0.01188,    Adjusted R-squared:  0.007929
## F-statistic: 3.006 on 1 and 250 DF,  p-value: 0.08419
```

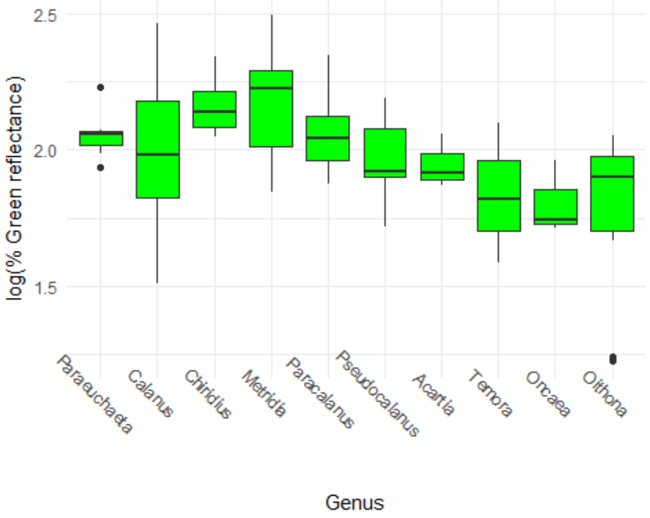




```
summary(GreenDepth18.4)
## Call:
## lm(formula = log.Green ~ Genus, data = d18)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.58144 -0.12044 -0.00795  0.16450  0.47285
##
## Coefficients:
##                    Estimate Std. Error t value Pr(>|t|)
## (Intercept)         2.055595   0.080402  25.566   <2e-16 ***
## GenusCalanus       -0.065411   0.082013  -0.798   0.4259
## GenusChiridius      0.094767   0.095532   0.992   0.3222
## GenusMetridia       0.119049   0.095532   1.246   0.2139
## GenusParacalanus    0.008957   0.118349   0.076   0.9397
## GenusPseudocalanus -0.086686   0.113706  -0.762   0.4466
## GenusAcartia       -0.109203   0.146793  -0.744   0.4576
## GenusTemora        -0.221520   0.146793  -1.509   0.1326
## GenusOncaea        -0.250041   0.146793  -1.703   0.0898 .
## GenusOithona       -0.249818   0.096399  -2.592   0.0101 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.2127 on 242 degrees of freedom
## Multiple R-squared:  0.1399, Adjusted R-squared:  0.1079
## F-statistic: 4.373 on 9 and 242 DF,  p-value: 2.645e-05
```

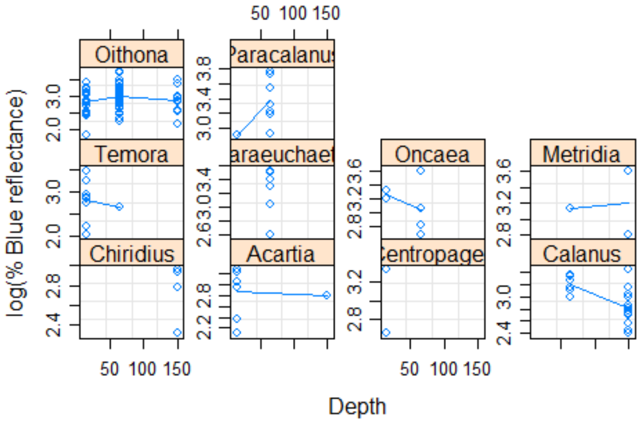

Residuals vs Fitted
Residuals
0.4
-0.6
52
99
1.8
1.9
2.0
2.1
Fitted values
Normal Q-Q
Standardized residuals
0
-3
99
52
-3
-2
-1
0
1
2
3
Theoretical Quantiles
Scale-Location
√|Standardized residuals|
1.5
0.0
52
99
1.8
1.9
2.0
2.1
Fitted values
Residuals vs Leverage
Standardized residuals
0
-3
28
27
32
Cook's distance
0.5
0.00
0.10
0.20
0.30
Leverage


Histogram of resid(GreenDepth18.4)
Frequency
0
10
20
30
40
50
60
-0.6
-0.4
-0.2
0.0
0.2
0.4
resid(GreenDepth18.4)

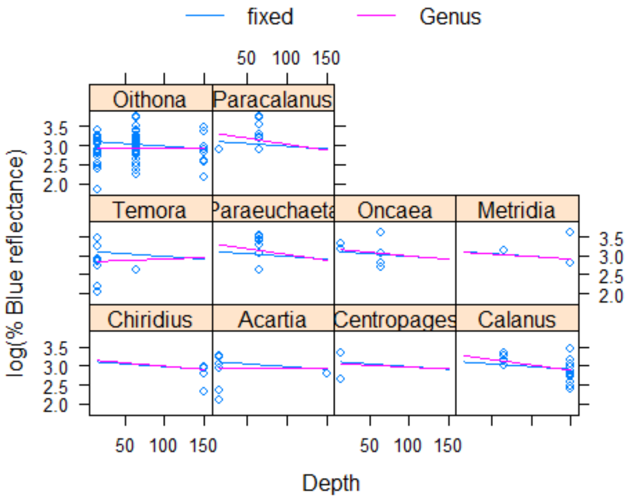

log(% Green reflectance)
2.5
2.0
1.5
Paraeuchaeta
Calanus
Chiridius
Metridia
Paracalanus
Pseudocalanus
Acartia
Temora
Oncaea
Oithona
Genus

Blue reflectance

2017

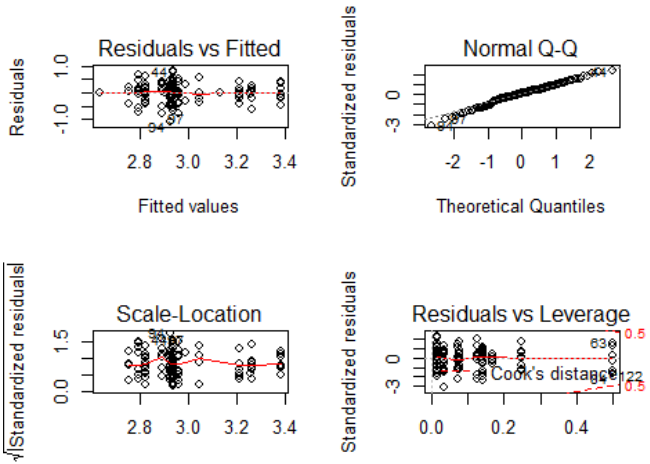


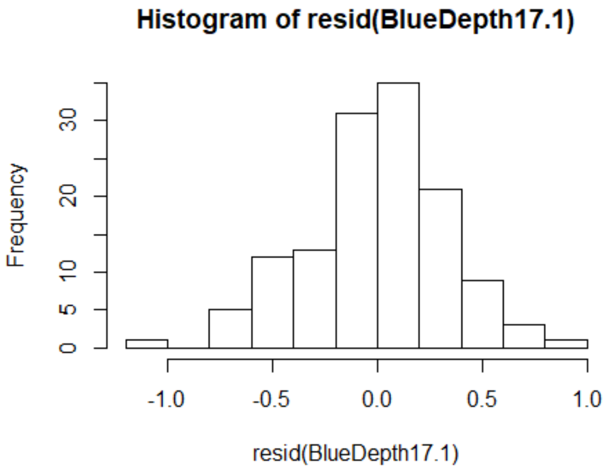
```
summary(m17 <- lme(g17))
## Linear mixed-effects model fit by REML
##  Data: g17
##       AIC      BIC    logLik
##   137.653 154.8119 -62.82651
##
## Random effects:
##  Formula: ~Depth | Genus
##  Structure: General positive-definite
##             StdDev      Corr
## (Intercept) 0.255673614 (Intr)
## Depth       0.001951256 -1
## Residual    0.357260525
##
## Fixed effects: log.Blue ~ Depth
##                  Value Std.Error  DF   t-value p-value
## (Intercept)  3.1297601 0.1133471 120 27.612176   0.000
## Depth       -0.0014275 0.0009828 120 -1.452497   0.149
##  Correlation:
##       (Intr)
## Depth -0.921
##
## Standardized Within-Group Residuals:
##         Min          Q1         Med          Q3         Max
## -3.07371305 -0.42725154  0.08281826  0.63882834  2.26966744
##
## Number of Observations: 131
## Number of Groups: 10
```



```
anova(m17)

##             numDF denDF  F-value p-value
## (Intercept)     1   120 4548.833  <.0001
## Depth           1   120    2.110   0.149

BlueDepth17.1 = lm(log.Blue ~ Depth * Genus, data=d17)
BlueDepth17.2 = lm(log.Blue ~ Depth + Genus, data=d17)
BlueDepth17.3 = lm(log.Blue ~ Depth, data=d17)
BlueDepth17.4 = lm(log.Blue ~ Genus, data=d17)

AICc(BlueDepth17.1, BlueDepth17.2, BlueDepth17.3, BlueDepth17.4)

##               df     AICc
## BlueDepth17.1 18 127.4017
## BlueDepth17.2 12 119.3694
## BlueDepth17.3  3 118.7981
## BlueDepth17.4 11 117.5178

summary(BlueDepth17.1)
## Call:
## lm(formula = log.Blue ~ Depth * Genus, data = d17)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -1.08246 -0.17425  0.01928  0.21167  0.81548
##
## Coefficients: (3 not defined because of singularities)
##                         Estimate Std. Error t value Pr(>|t|)
## (Intercept)            3.2446801  0.1507478  21.524   <2e-16 ***
## Depth                  0.0002291  0.0010080   0.227   0.8206
## GenusCalanus           0.2646402  0.3090225   0.856   0.3936
## GenusChiridius        -0.5289738  0.2408916  -2.196   0.0301 *
## GenusCentropages      -0.2580779  0.2923746  -0.883   0.3793
## GenusMetridia         -0.1741219  0.6798874  -0.256   0.7983
## GenusParacalanus      -0.4890746  0.4923722  -0.993   0.3227
## GenusAcartia          -0.3464952  0.2169629  -1.597   0.1130
## GenusTemora           -0.4071331  0.2481787  -1.640   0.1037
## GenusOncaea            0.0749584  0.3669485   0.204   0.8385
## GenusOithona          -0.3220351  0.1428869  -2.254   0.0261 *
## Depth:GenusCalanus    -0.0048228  0.0023165  -2.082   0.0396 *
## Depth:GenusChiridius          NA         NA      NA       NA
## Depth:GenusCentropages        NA         NA      NA       NA
## Depth:GenusMetridia    0.0006918  0.0052728   0.131   0.8959
## Depth:GenusParacalanus 0.0093886  0.0077458   1.212   0.2280
## Depth:GenusAcartia    -0.0008366  0.0030178  -0.277   0.7821
## Depth:GenusTemora     -0.0033755  0.0076861  -0.439   0.6614
## Depth:GenusOncaea     -0.0044689  0.0063026  -0.709   0.4797
## Depth:GenusOithona            NA         NA      NA       NA
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
```

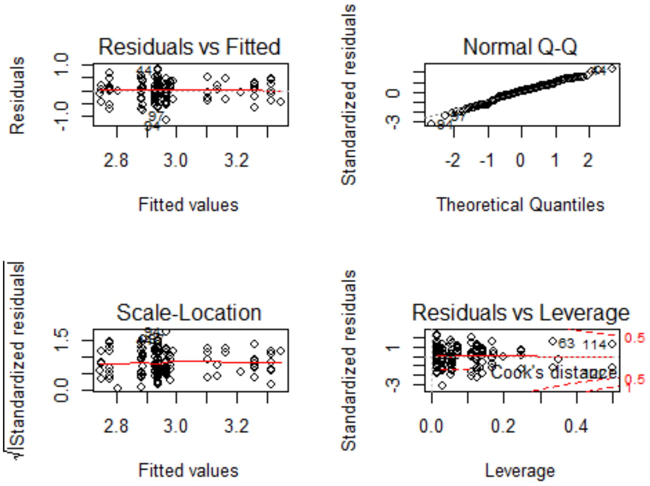
```
## Residual standard error: 0.3592 on 114 degrees of freedom
## Multiple R-squared:   0.19,  Adjusted R-squared:  0.07631
## F-statistic: 1.671 on 16 and 114 DF,  p-value: 0.06224
```

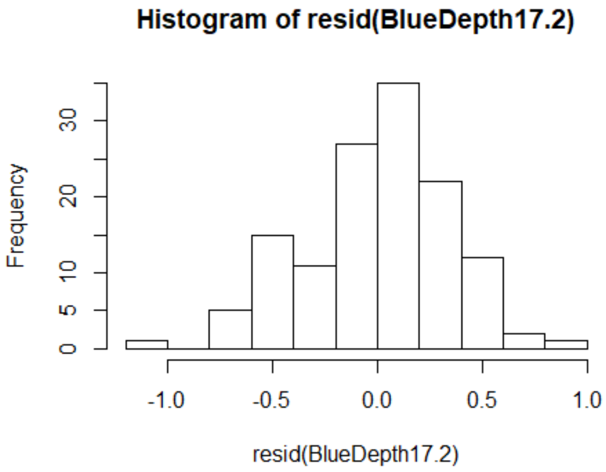




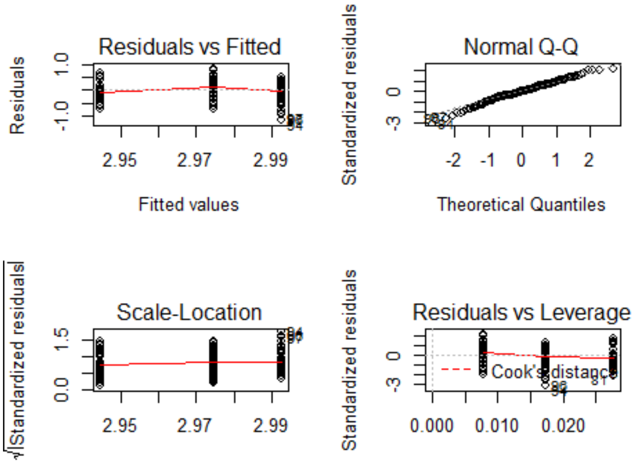
```
summary(BlueDepth17.2)
## Call:
## lm(formula = log.Blue ~ Depth + Genus, data = d17)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -1.1241 -0.1881  0.0498  0.2290  0.8157
##
## Coefficients:
##                   Estimate Std. Error t value Pr(>|t|)
## (Intercept)       3.2990688  0.1466030  22.503  < 2e-16 ***
## Depth            -0.0006077  0.0008371  -0.726  0.46928
## GenusCalanus     -0.2806581  0.1665220  -1.685  0.09451 .
## GenusChiridius   -0.4578501  0.2366971  -1.934  0.05543 .
## GenusCentropages -0.2999154  0.2917976  -1.028  0.30610
## GenusMetridia    -0.0425410  0.2530285  -0.168  0.86677
## GenusParacalanus  0.0572773  0.1864803   0.307  0.75926
## GenusAcartia     -0.4008789  0.1884579  -2.127  0.03545 *
## GenusTemora      -0.5137077  0.1852833  -2.773  0.00645 **
## GenusOncaea      -0.1549839  0.2008665  -0.772  0.44188
## GenusOithona     -0.3222282  0.1432753  -2.249  0.02634 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.3602 on 120 degrees of freedom
```

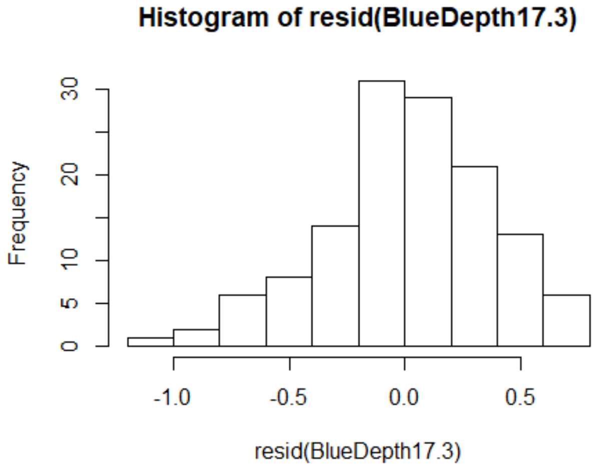
```
## Multiple R-squared:  0.1427, Adjusted R-squared:  0.07128
## F-statistic: 1.998 on 10 and 120 DF,  p-value: 0.03923
```



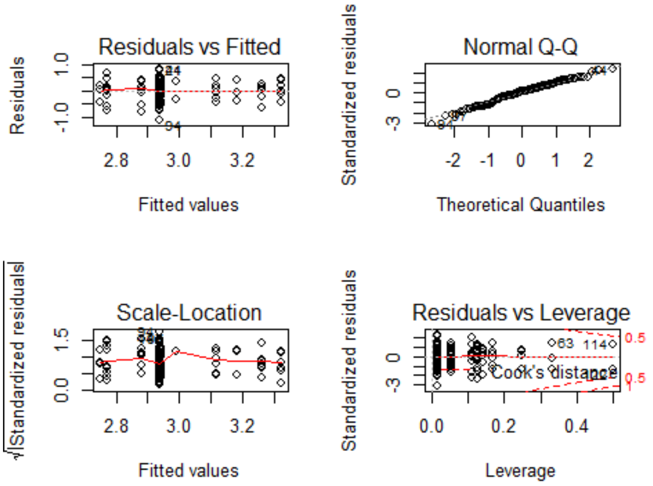


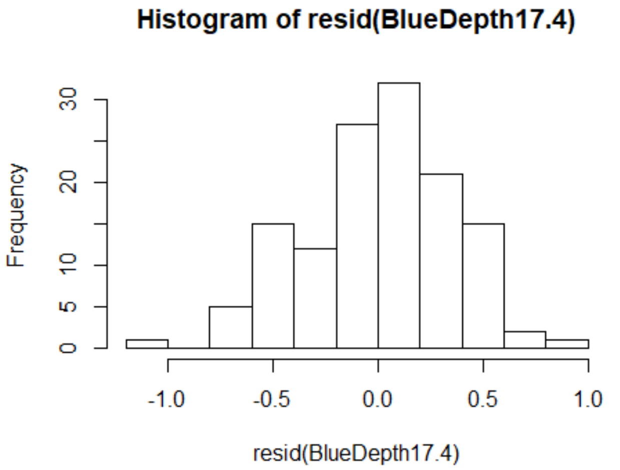
```
summary(BlueDepth17.3)
## Call:
## lm(formula = log.Blue ~ Depth, data = d17)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -1.14888 -0.18431  0.02484  0.24583  0.79900
##
## Coefficients:
##               Estimate Std. Error t value Pr(>|t|)
## (Intercept)  2.9978210  0.0570201  52.575   <2e-16 ***
## Depth       -0.0003549  0.0006647  -0.534    0.594
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.3748 on 129 degrees of freedom
## Multiple R-squared:  0.002205,   Adjusted R-squared:  -0.005529
## F-statistic: 0.2851 on 1 and 129 DF,  p-value: 0.5943
```

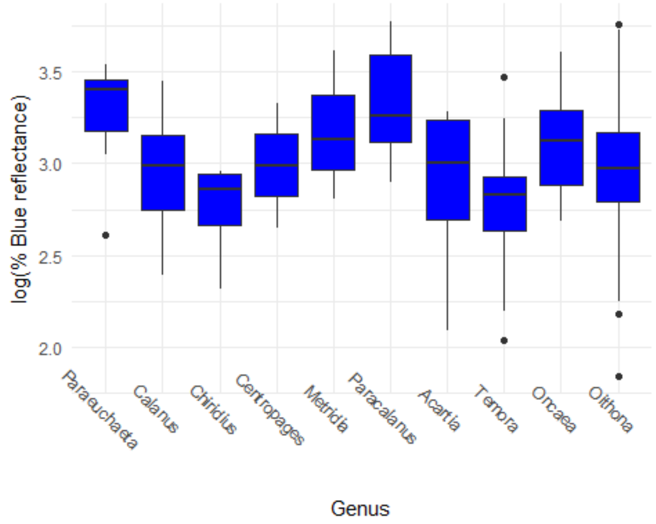




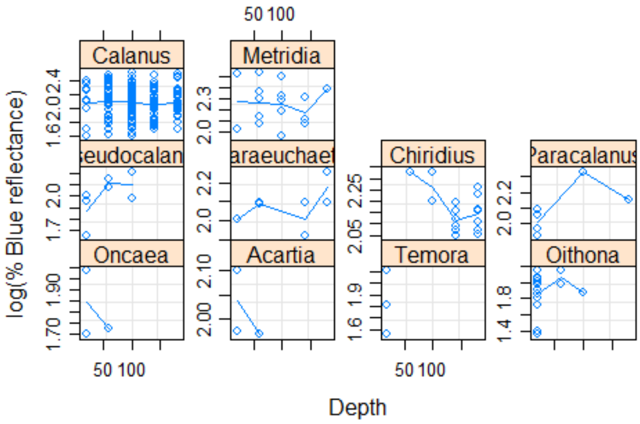
```
summary(BlueDepth17.4)
## Call:
## lm(formula = log.Blue ~ Genus, data = d17)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -1.09386 -0.19570  0.04149  0.22723  0.81554
##
## Coefficients:
##                  Estimate Std. Error t value Pr(>|t|)
## (Intercept)       3.25957    0.13587  23.991  < 2e-16 ***
## GenusCalanus     -0.31600    0.15894  -1.988  0.04904 *
## GenusChiridius   -0.50950    0.22531  -2.261  0.02552 *
## GenusCentropages -0.26953    0.28822  -0.935  0.35156
## GenusMetridia    -0.07698    0.24806  -0.310  0.75686
## GenusParacalanus  0.06108    0.18604   0.328  0.74326
## GenusAcartia     -0.38075    0.18604  -2.047  0.04287 *
## GenusTemora      -0.48670    0.18115  -2.687  0.00823 **
## GenusOncaea      -0.14486    0.19999  -0.724  0.47027
## GenusOithona     -0.32209    0.14299  -2.252  0.02610 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.3595 on 121 degrees of freedom
## Multiple R-squared:  0.139,  Adjusted R-squared:  0.07491
## F-statistic:  2.17 on 9 and 121 DF,  p-value: 0.02866
```


Residuals vs Fitted
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles
Scale-Location
√|Standardized residuals|
Fitted values
Residuals vs Leverage
Standardized residuals
Cook's distance
Leverage

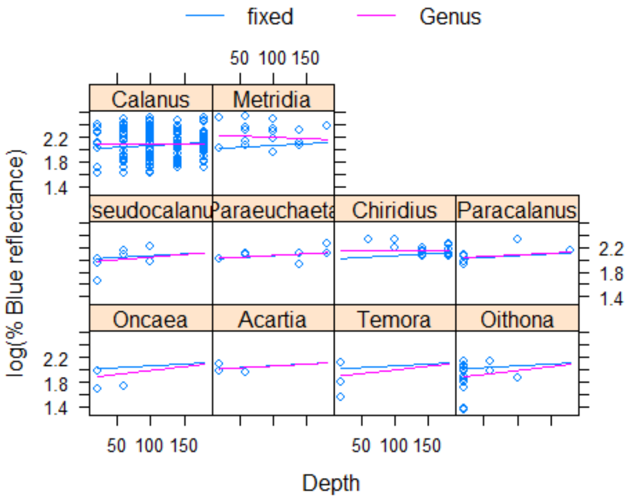

Histogram of resid(BlueDepth17.4)
Frequency
0
5
10
20
30
-1.0
-0.5
0.0
0.5
1.0
resid(BlueDepth17.4)


log(% Blue reflectance)
2.0
2.5
3.0
3.5
Paraeuchaeta
Calanus
Chiridius
Centropages
Metridia
Paracalanus
Acartia
Temora
Oncaea
Oithona
Genus

2018

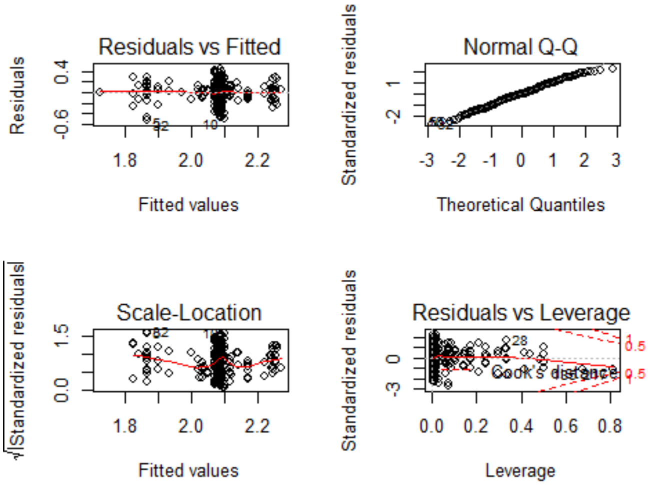


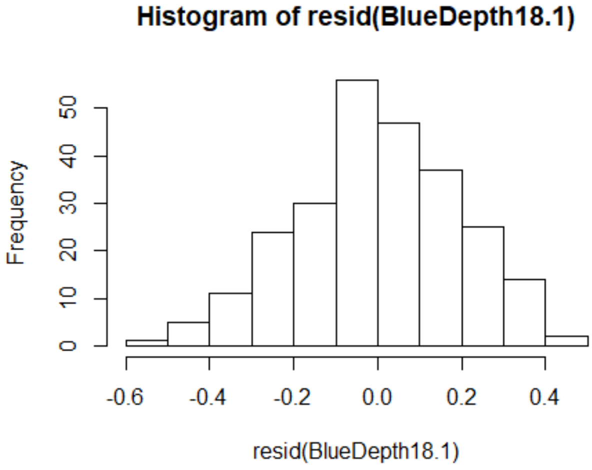
```
summary(m18 <- lme(g18))
## Linear mixed-effects model fit by REML
##  Data: g18
##        AIC       BIC  logLik
##   -48.9714 -27.84263 30.4857
##
## Random effects:
##  Formula: ~Depth | Genus
##  Structure: General positive-definite
##             StdDev       Corr
## (Intercept) 0.1531491832 (Intr)
## Depth       0.0007788006 -0.951
## Residual    0.2012431294
##
## Fixed effects: log.Blue ~ Depth
##                 Value  Std.Error  DF  t-value p-value
## (Intercept) 2.0077501 0.06122068 241 32.79530  0.0000
## Depth       0.0005817 0.00040471 241  1.43737  0.1519
##  Correlation:
##       (Intr)
## Depth -0.858
##
## Standardized Within-Group Residuals:
##         Min          Q1         Med          Q3         Max
## -2.66281803 -0.53829288 -0.00363598  0.77177476  2.16472273
##
## Number of Observations: 252
## Number of Groups: 10
```



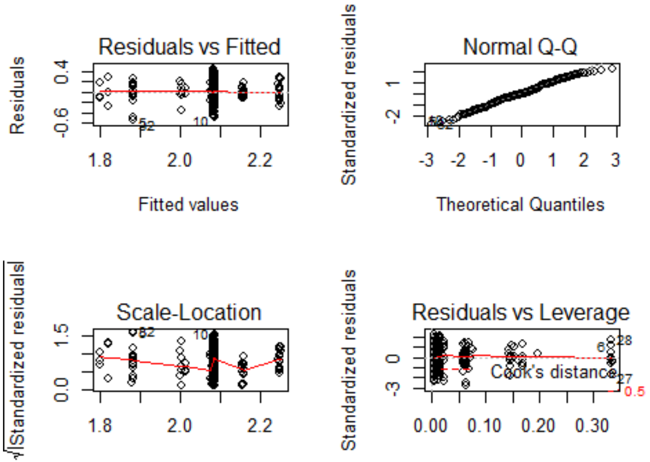
```
BlueDepth18.1 = lm(log.Blue ~ Depth * Genus, data=d18)
BlueDepth18.2 = lm(log.Blue ~ Depth + Genus, data=d18)
BlueDepth18.3 = lm(log.Blue ~ Depth, data=d18)
BlueDepth18.4 = lm(log.Blue ~ Genus, data=d18)

AICc(BlueDepth18.1, BlueDepth18.2, BlueDepth18.3, BlueDepth18.4)
```

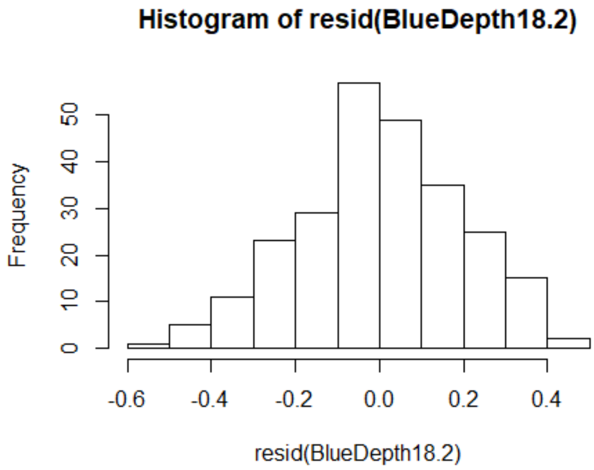
```
##               df      AICc
## BlueDepth18.1 20 -64.31126
## BlueDepth18.2 12 -77.49830
## BlueDepth18.3  3 -61.39133
## BlueDepth18.4 11 -79.69165

summary(BlueDepth18.1)
## Call:
## lm(formula = log.Blue ~ Depth * Genus, data = d18)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -0.5147 -0.1131  0.0000  0.1424  0.4358
##
## Coefficients: (1 not defined because of singularities)
##                            Estimate Std. Error t value Pr(>|t|)
## (Intercept)               2.0131908  0.1632403  12.333   <2e-16 ***
## Depth                     0.0005953  0.0012928   0.460    0.646
## GenusCalanus              0.0899388  0.1682621   0.535    0.593
## GenusChiridius            0.3352262  0.2704780   1.239    0.216
## GenusMetridia             0.2492157  0.1983806   1.256    0.210
## GenusParacalanus         -0.0058693  0.2003879  -0.029    0.977
## GenusPseudocalanus       -0.1736450  0.2195172  -0.791    0.430
## GenusAcartia              0.0586660  0.2887569   0.203    0.839
## GenusTemora              -0.2017224  0.1833566  -1.100    0.272
## GenusOncaea              -0.1136196  0.2887569  -0.393    0.694
## GenusOithona             -0.1797818  0.1840326  -0.977    0.330
## Depth:GenusCalanus       -0.0007591  0.0013350  -0.569    0.570
## Depth:GenusChiridius     -0.0018670  0.0019095  -0.978    0.329
## Depth:GenusMetridia      -0.0007303  0.0017297  -0.422    0.673
## Depth:GenusParacalanus    0.0007393  0.0018744   0.394    0.694
## Depth:GenusPseudocalanus  0.0024305  0.0026421   0.920    0.359
## Depth:GenusAcartia       -0.0023015  0.0063524  -0.362    0.717
## Depth:GenusTemora                NA         NA      NA       NA
## Depth:GenusOncaea        -0.0035176  0.0063524  -0.554    0.580
## Depth:GenusOithona        0.0010771  0.0026132   0.412    0.681
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.2031 on 233 degrees of freedom
## Multiple R-squared:  0.165,  Adjusted R-squared:  0.1005
## F-statistic: 2.559 on 18 and 233 DF,  p-value: 0.0006931
```



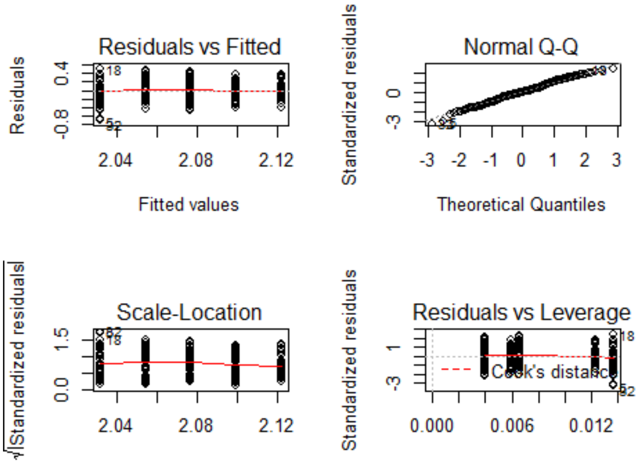


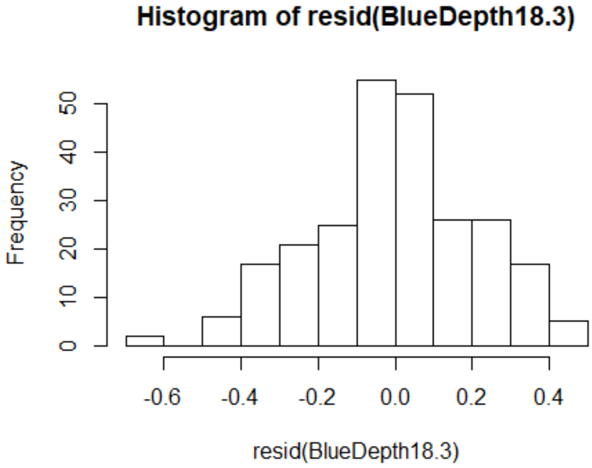
```
summary(BlueDepth18.2)
## Call:
## lm(formula = log.Blue ~ Depth + Genus, data = d18)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.53174 -0.11485 -0.00044  0.16009  0.43762
##
## Coefficients:
##                     Estimate Std. Error t value Pr(>|t|)
## (Intercept)        2.083e+00  8.276e-02  25.168   <2e-16 ***
## Depth             -3.101e-05  2.885e-04  -0.108   0.9145
## GenusCalanus       5.095e-03  7.779e-02   0.065   0.9478
## GenusChiridius     8.006e-02  9.128e-02   0.877   0.3813
## GenusMetridia      1.702e-01  9.086e-02   1.874   0.0622 .
## GenusParacalanus   6.277e-03  1.132e-01   0.055   0.9558
## GenusPseudocalanus -7.749e-02  1.091e-01  -0.710   0.4782
## GenusAcartia      -6.696e-02  1.410e-01  -0.475   0.6354
## GenusTemora       -2.590e-01  1.417e-01  -1.828   0.0689 .
## GenusOncaea       -2.798e-01  1.410e-01  -1.984   0.0484 *
## GenusOithona      -1.985e-01  9.441e-02  -2.102   0.0366 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.2018 on 241 degrees of freedom
## Multiple R-squared:  0.1478, Adjusted R-squared:  0.1125
## F-statistic: 4.181 on 10 and 241 DF,  p-value: 2.359e-05
```



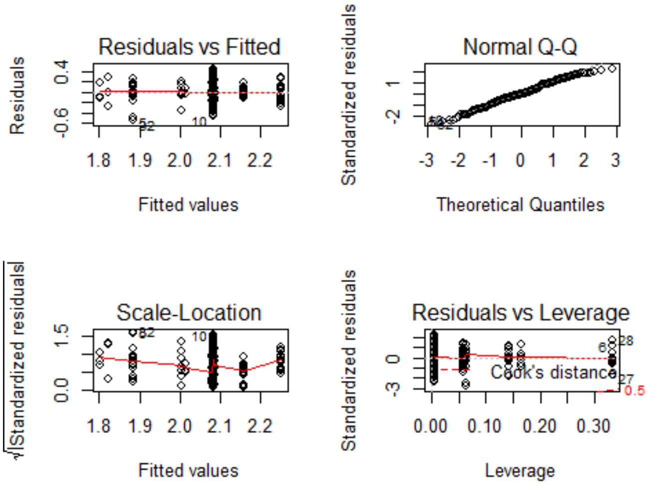


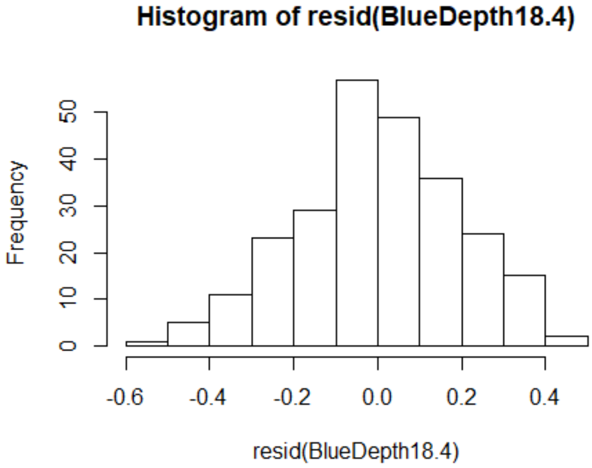
```
summary(BlueDepth18.3)
## Call:
## lm(formula = log.Blue ~ Depth, data = d18)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.67950 -0.12894  0.00061  0.13818  0.49195
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept) 2.0203827  0.0291637  69.277   <2e-16 ***
## Depth       0.0005637  0.0002519   2.238   0.0261 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.2125 on 250 degrees of freedom
## Multiple R-squared:  0.01963,    Adjusted R-squared:  0.01571
## F-statistic: 5.007 on 1 and 250 DF,  p-value: 0.02613
```

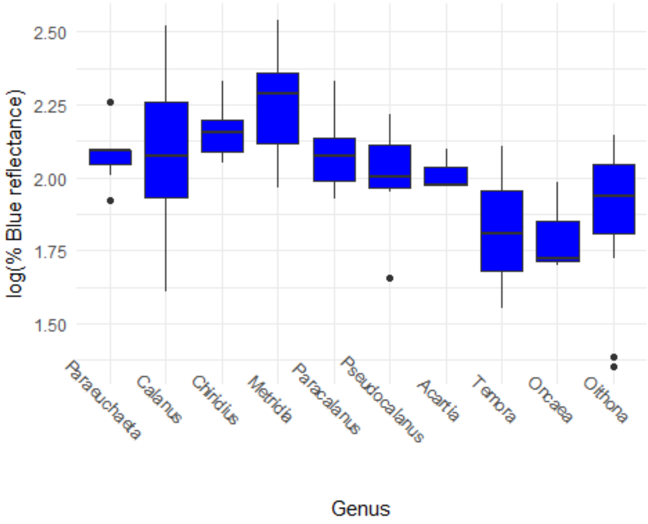




```
summary(BlueDepth18.4)
## Call:
## lm(formula = log.Blue ~ Genus, data = d18)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.53143 -0.11432 -0.00038  0.16019  0.43803
##
## Coefficients:
##                   Estimate Std. Error t value Pr(>|t|)
## (Intercept)        2.079525   0.076108  27.323   <2e-16 ***
## GenusCalanus       0.005034   0.077633   0.065   0.9484
## GenusChiridius     0.078884   0.090430   0.872   0.3839
## GenusMetridia      0.170968   0.090430   1.891   0.0599 .
## GenusParacalanus   0.007872   0.112028   0.070   0.9440
## GenusPseudocalanus -0.075722   0.107633  -0.704   0.4824
## GenusAcartia      -0.064542   0.138954  -0.464   0.6427
## GenusTemora       -0.256150   0.138954  -1.843   0.0665 .
## GenusOncaea       -0.277362   0.138954  -1.996   0.0470 *
## GenusOithona      -0.195943   0.091250  -2.147   0.0328 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.2014 on 242 degrees of freedom
## Multiple R-squared:  0.1478, Adjusted R-squared:  0.1161
## F-statistic: 4.663 on 9 and 242 DF,  p-value: 1.038e-05
```


Residuals vs Fitted
Residuals
0.4
-0.6
52
10
1.8 1.9 2.0 2.1 2.2
Fitted values
Normal Q-Q
Standardized residuals
1
-2
-3 -2 -1 0 1 2 3
Theoretical Quantiles
Scale-Location
√|Standardized residuals|
1.5
0.0
82
10
1.8 1.9 2.0 2.1 2.2
Fitted values
Residuals vs Leverage
Standardized residuals
0
-3
28
6
27
Cook's distance
0.5
0.00 0.10 0.20 0.30
Leverage


Histogram of resid(BlueDepth18.4)
Frequency
0
10
20
30
40
50
-0.6
-0.4
-0.2
0.0
0.2
0.4
resid(BlueDepth18.4)

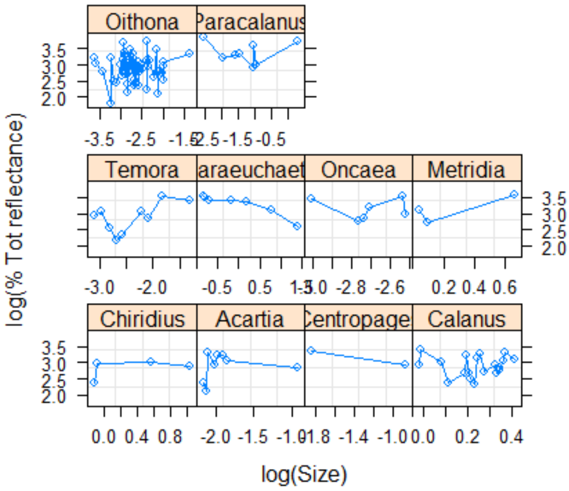

log(% Blue reflectance)
2.50
2.25
2.00
1.75
1.50
Paraeuchaeta
Calanus
Chiridius
Metridia
Paracalanus
Pseudocalanus
Acartia
Temora
Oncaea
Oithona
Genus

Appendix B

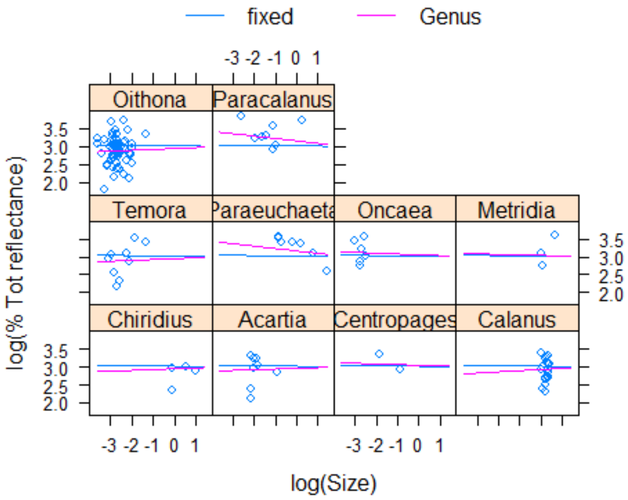
Additional models and plots, reflectance over size

Total reflectance

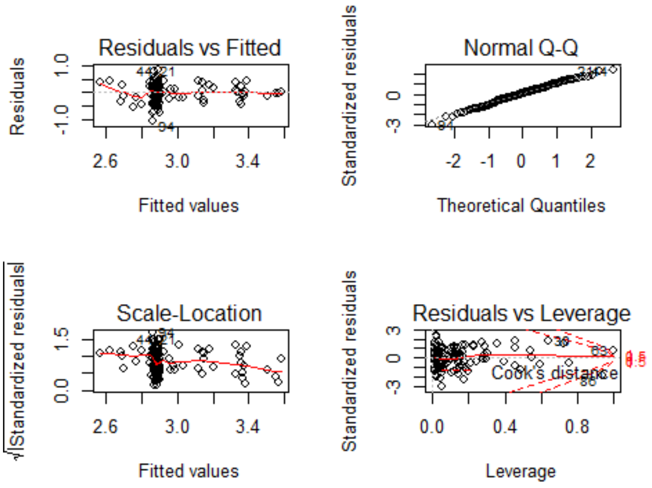
2017



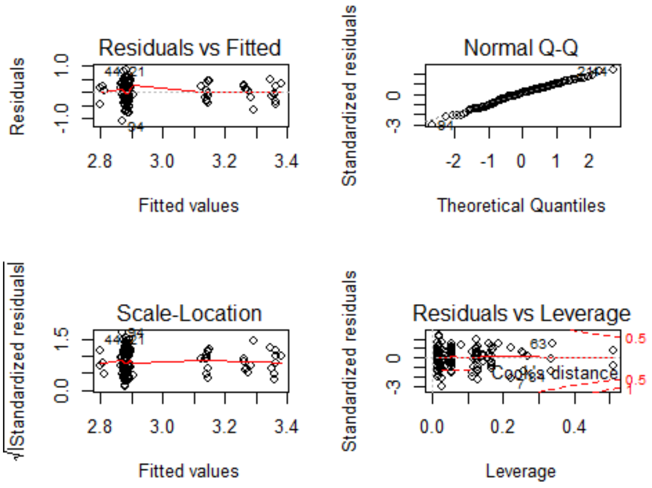
```
summary(m17 <- lme(g17))
## Linear mixed-effects model fit by REML
##  Data: g17
##        AIC      BIC    logLik
##   134.4652 151.6241 -61.23261
##
## Random effects:
##  Formula: ~log.size | Genus
##  Structure: General positive-definite
##             StdDev     Corr
## (Intercept) 0.12026595 (Intr)
## log.size    0.05137047 -1
## Residual    0.36129122
##
## Fixed effects: log.Tot ~ log.size
##                  Value  Std.Error  DF  t-value p-value
## (Intercept)  3.0240174 0.07347829 120 41.15525   0.000
## log.size    -0.0064213 0.04949045 120 -0.12975   0.897
##  Correlation:
##          (Intr)
## log.size 0.379
##
## Standardized Within-Group Residuals:
##         Min          Q1         Med          Q3         Max
## -2.95722387 -0.58975641  0.09289369  0.66857147  2.29565094
##
## Number of Observations: 131
## Number of Groups: 10
```



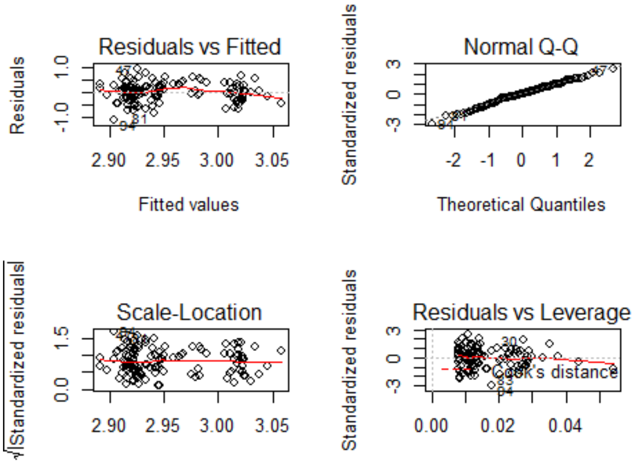
```
TotSize17.1 = lm(log.Tot ~ log.size * Genus, data = d17)
TotSize17.2 = lm(log.Tot ~ log.size + Genus, data = d17)
TotSize17.3 = lm(log.Tot ~ log.size, data = d17)
TotSize17.4 = lm(log.Tot ~ Genus, data = d17)

AICc(TotSize17.1, TotSize17.2, TotSize17.3, TotSize17.4)

##             df     AICc
## TotSize17.1 21 133.9403
## TotSize17.2 12 123.8008
## TotSize17.3  3 124.3143
## TotSize17.4 11 121.4079

summary(TotSize17.1)
## Call:
## lm(formula = log.Tot ~ log.size * Genus, data = d17)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -1.05856 -0.17365  0.00928  0.21786  0.83118
##
## Coefficients:
##                            Estimate Std. Error t value Pr(>|t|)
## (Intercept)                 3.26544    0.13669  23.890  < 2e-16 ***
## log.size                   -0.35531    0.17072  -2.081  0.03971 *
## GenusCalanus               -0.37674    0.22751  -1.656  0.10057
## GenusChiridius             -0.54933    0.26196  -2.097  0.03827 *
## GenusCentropages           -0.69870    0.75606  -0.924  0.35742
## GenusMetridia              -0.40978    0.31803  -1.288  0.20026
## GenusParacalanus            0.06716    0.28297   0.237  0.81284
## GenusAcartia               -0.14141    0.66163  -0.214  0.83115
## GenusTemora                 0.71230    0.55265   1.289  0.20012
## GenusOncaea                -0.48919    2.48057  -0.197  0.84402
## GenusOithona               -0.29514    0.33504  -0.881  0.38027
## log.size:GenusCalanus       0.36783    0.70864   0.519  0.60474
## log.size:GenusChiridius     0.60934    0.41337   1.474  0.14329
## log.size:GenusCentropages  -0.05967    0.54306  -0.110  0.91270
## log.size:GenusMetridia      1.45066    0.76154   1.905  0.05938 .
## log.size:GenusParacalanus   0.33325    0.23615   1.411  0.16099
## log.size:GenusAcartia       0.47748    0.37825   1.262  0.20947
## log.size:GenusTemora        0.80921    0.27589   2.933  0.00408 **
## log.size:GenusOncaea        0.21999    0.92762   0.237  0.81297
## log.size:GenusOithona       0.38948    0.20501   1.900  0.06005 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.3615 on 111 degrees of freedom
## Multiple R-squared:  0.242,  Adjusted R-squared:  0.1122
## F-statistic: 1.865 on 19 and 111 DF,  p-value: 0.02391
```



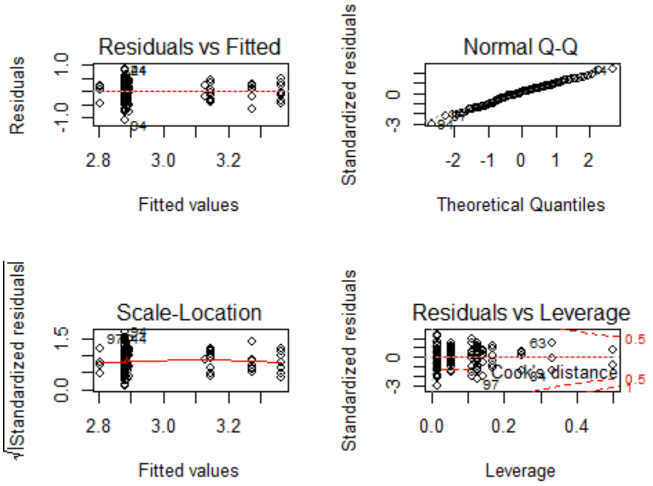
```
summary(TotSize17.2)
## Call:
## lm(formula = log.Tot ~ log.size + Genus, data = d17)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -1.07125 -0.20898  0.04947  0.22636  0.83759
##
## Coefficients:
##                  Estimate Std. Error t value Pr(>|t|)
## (Intercept)       3.27419    0.13847  23.646   <2e-16 ***
## log.size          0.01254    0.07248   0.173   0.8630
## GenusCalanus     -0.38549    0.16305  -2.364   0.0197 *
## GenusChiridius   -0.47377    0.23119  -2.049   0.0426 *
## GenusCentropages -0.12838    0.30914  -0.415   0.6787
## GenusMetridia    -0.13075    0.25365  -0.515   0.6072
## GenusParacalanus  0.10341    0.21097   0.490   0.6249
## GenusAcartia     -0.35630    0.23247  -1.533   0.1280
## GenusTemora      -0.35898    0.25283  -1.420   0.1582
## GenusOncaea      -0.09703    0.28192  -0.344   0.7313
## GenusOithona     -0.36156    0.24062  -1.503   0.1356
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.3663 on 120 degrees of freedom
## Multiple R-squared:  0.1585, Adjusted R-squared:  0.08834
## F-statistic:  2.26 on 10 and 120 DF,  p-value: 0.0186
```



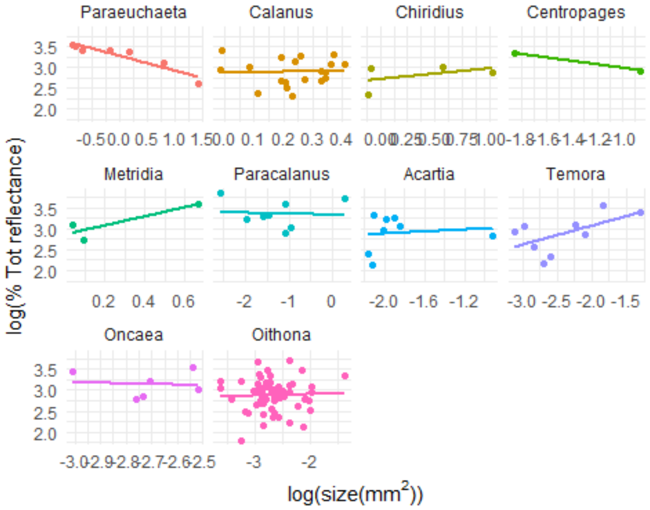
```
summary(TotSize17.3)
## Call:
## lm(formula = log.Tot ~ log.size, data = d17)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -1.10327 -0.19876  0.02087  0.27150  0.92447
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept)  3.01138    0.05702  52.813   <2e-16 ***
## log.size     0.03306    0.02598   1.272    0.206
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.3827 on 129 degrees of freedom
## Multiple R-squared:  0.01239,    Adjusted R-squared:  0.004737
## F-statistic: 1.619 on 1 and 129 DF,  p-value: 0.2055
```



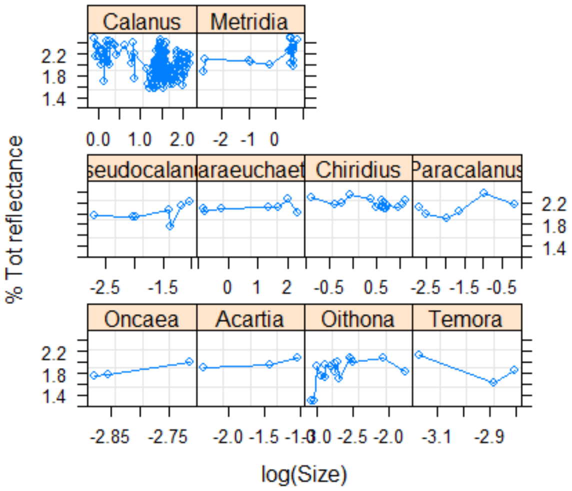
```
summary(TotSize17.4)
## Call:
## lm(formula = log.Tot ~ Genus, data = d17)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -1.07861 -0.20527  0.04668  0.22509  0.84130
##
## Coefficients:
##                 Estimate Std. Error t value Pr(>|t|)
## (Intercept)       3.2739     0.1379  23.741   <2e-16 ***
## GenusCalanus     -0.3822     0.1613  -2.370   0.0194 *
## GenusChiridius   -0.4691     0.2287  -2.051   0.0424 *
## GenusCentropages -0.1451     0.2925  -0.496   0.6209
## GenusMetridia    -0.1271     0.2518  -0.505   0.6145
## GenusParacalanus  0.0874     0.1888   0.463   0.6443
## GenusAcartia     -0.3796     0.1888  -2.010   0.0466 *
## GenusTemora      -0.3889     0.1839  -2.115   0.0365 *
## GenusOncaea      -0.1307     0.2030  -0.644   0.5208
## GenusOithona     -0.3947     0.1451  -2.719   0.0075 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.3648 on 121 degrees of freedom
## Multiple R-squared:  0.1583, Adjusted R-squared:  0.09565
## F-statistic: 2.528 on 9 and 121 DF,  p-value: 0.01085
```


Residuals vs Fitted
Residuals
1.0
-1.0
24
94
2.8
3.0
3.2
Fitted values
Normal Q-Q
Standardized residuals
0
-3
-2
-1
0
1
2
Theoretical Quantiles
Scale-Location
√|Standardized residuals|
1.5
0.0
97
94
44
2.8
3.0
3.2
Fitted values
Residuals vs Leverage
Standardized residuals
0
-3
63
97
0.5
0.5
Cook's distance
0.0
0.2
0.4
Leverage

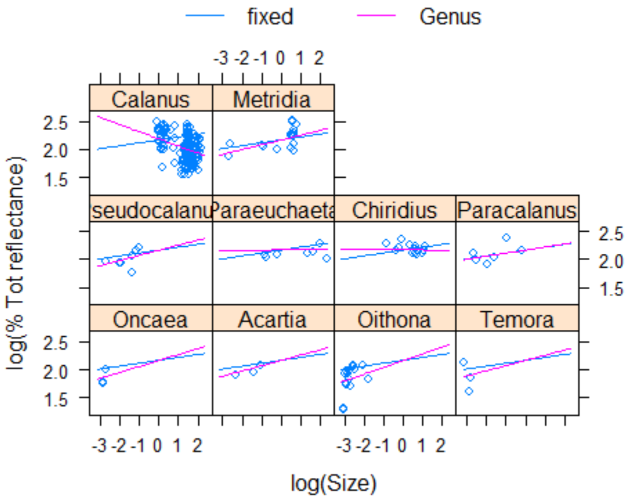

Histogram of resid(TotSize17.4)
Frequency
0
5
10
20
30
-1.0
-0.5
0.0
0.5
1.0
resid(TotSize17.4)


Paraeuchaeta
Calanus
Chiridius
Centropages
Metridia
Paracalanus
Acartia
Temora
Oncaea
Oithona
log(% Tot reflectance)
log(size(mm²))
3.5
3.0
2.5
2.0
-0.5
0.0
0.5
1.0
1.5
0.0
0.1
0.2
0.3
0.4
0.00
0.25
0.50
0.75
1.00
-1.8
-1.6
-1.4
-1.2
-1.0
0.2
0.4
0.6
-2
-1
0
-2.0
-1.6
-1.2
-3.0
-2.5
-2.0
-1.5
-3.0
-2.9
-2.8
-2.7
-2.6
-2.5
-3
-2

2018

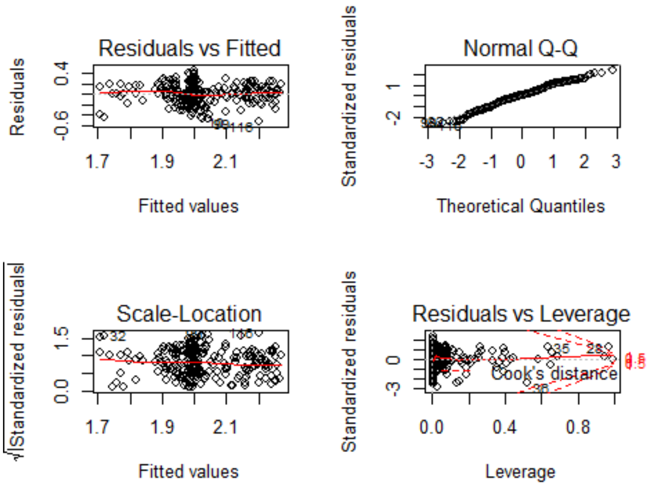


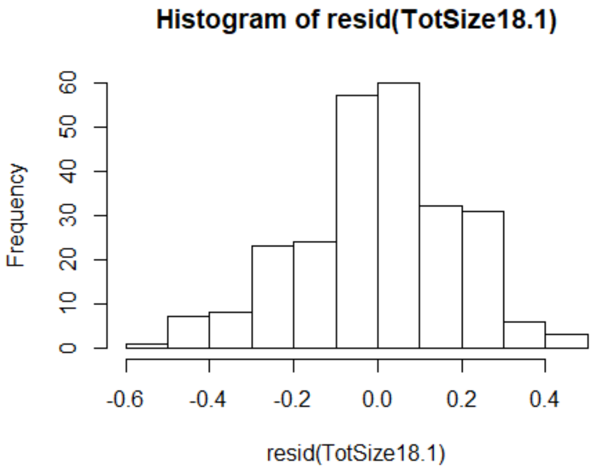
```
summary(m18 <- lme(g18))
## Linear mixed-effects model fit by REML
##  Data: g18
##         AIC       BIC   logLik
##   -78.56903 -57.44027 45.28452
##
## Random effects:
##  Formula: ~log.size | Genus
##  Structure: General positive-definite
##             StdDev      Corr
## (Intercept) 0.008534895 (Intr)
## log.size    0.084353470 -1
## Residual    0.191128960
##
## Fixed effects: log.Tot ~ log.size
##                 Value  Std.Error  DF  t-value p-value
## (Intercept) 2.1702009 0.02231068 241 97.27185  0.0000
## log.size    0.0517905 0.02983461 241  1.73592  0.0839
##  Correlation:
##          (Intr)
## log.size -0.07
##
## Standardized Within-Group Residuals:
##          Min           Q1          Med           Q3          Max
## -2.688057957 -0.571132279 -0.006050883  0.671070196  2.329375976
##
## Number of Observations: 252
## Number of Groups: 10
```



```
anova(m18)

##             numDF denDF  F-value p-value
## (Intercept)     1   241 9532.209  <.0001
## log.size        1   241    3.013  0.0839

TotSize18.1 = lm(log.Tot ~ log.size * Genus, data = d18)
TotSize18.2 = lm(log.Tot ~ log.size + Genus, data = d18)
TotSize18.3 = lm(log.Tot ~ log.size, data = d18)
TotSize18.4 = lm(log.Tot ~ Genus, data = d18)

AICc(TotSize18.1, TotSize18.2, TotSize18.3, TotSize18.4)

##             df      AICc
## TotSize18.1 21 -91.99212
## TotSize18.2 12 -69.48917
## TotSize18.3  3 -46.49202
## TotSize18.4 11 -65.98224

summary(TotSize18.1)
## Call:
## lm(formula = log.Tot ~ log.size * Genus, data = d18)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.52089 -0.10095  0.00768  0.12141  0.44636
##
## Coefficients:
##                             Estimate Std. Error t value Pr(>|t|)
## (Intercept)                  2.08678    0.08586  24.305  < 2e-16 ***
## log.size                     0.02008    0.05801   0.346  0.72956
## GenusCalanus                 0.13808    0.09416   1.466  0.14390
## GenusChiridius               0.11961    0.10581   1.130  0.25944
## GenusMetridia                0.10666    0.09765   1.092  0.27584
## GenusParacalanus             0.15961    0.18898   0.845  0.39922
## GenusPseudocalanus           0.11140    0.24672   0.452  0.65203
## GenusAcartia                 0.08700    0.35486   0.245  0.80655
## GenusTemora                 -3.30908    2.10617  -1.571  0.11752
## GenusOncaea                  4.00086    4.29636   0.931  0.35271
## GenusOithona                 0.65129    0.39453   1.651  0.10013
## log.size:GenusCalanus       -0.17249    0.06378  -2.705  0.00735 **
## log.size:GenusChiridius     -0.08699    0.10568  -0.823  0.41127
## log.size:GenusMetridia       0.07527    0.07172   1.049  0.29506
## log.size:GenusParacalanus    0.07205    0.10700   0.673  0.50135
## log.size:GenusPseudocalanus  0.09745    0.14441   0.675  0.50047
## log.size:GenusAcartia        0.10180    0.21151   0.481  0.63074
## log.size:GenusTemora        -1.06361    0.71340  -1.491  0.13735
## log.size:GenusOncaea         1.48783    1.52587   0.975  0.33054
## log.size:GenusOithona        0.31389    0.15356   2.044  0.04207 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
```

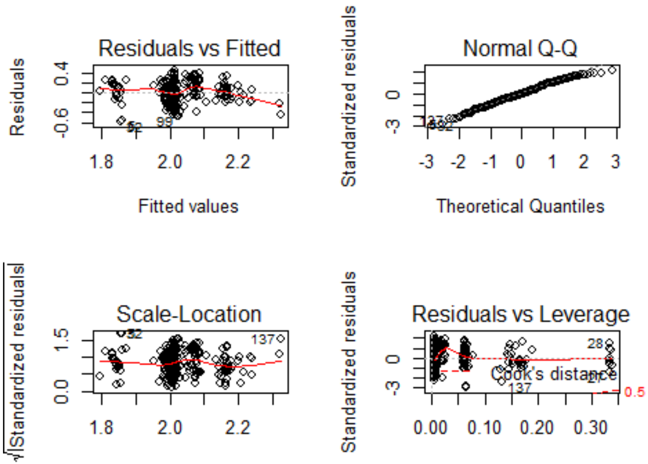
```
## Residual standard error: 0.1918 on 232 degrees of freedom
## Multiple R-squared:  0.2908, Adjusted R-squared:  0.2328
## F-statistic: 5.008 on 19 and 232 DF,  p-value: 6.728e-10
```

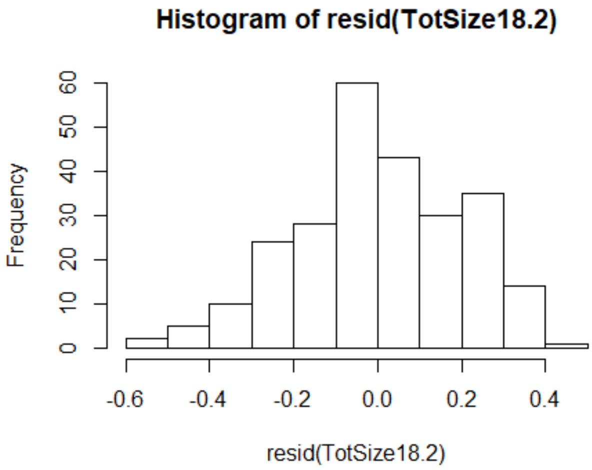




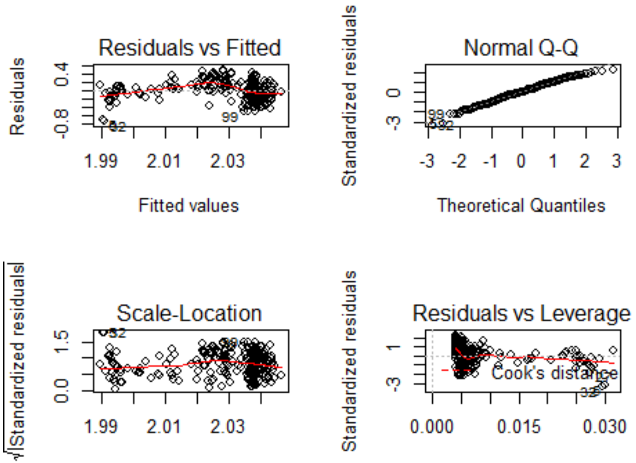
```
summary(TotSize18.2)
## Call:
## lm(formula = log.Tot ~ log.size + Genus, data = d18)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.57474 -0.11927 -0.00786  0.15916  0.43376
##
## Coefficients:
##                     Estimate Std. Error t value Pr(>|t|)
## (Intercept)          2.14131    0.07921  27.035  < 2e-16 ***
## log.size            -0.04865    0.02070  -2.351 0.019549 *
## GenusCalanus        -0.05662    0.07988  -0.709 0.479115
## GenusChiridius       0.05667    0.09232   0.614 0.539940
## GenusMetridia        0.05099    0.09355   0.545 0.586205
## GenusParacalanus    -0.12829    0.12483  -1.028 0.305102
## GenusPseudocalanus -0.21913    0.12078  -1.814 0.070885 .
## GenusAcartia        -0.24087    0.14991  -1.607 0.109410
## GenusTemora         -0.42318    0.16135  -2.623 0.009278 **
## GenusOncaea         -0.43726    0.15998  -2.733 0.006738 **
## GenusOithona        -0.43145    0.11756  -3.670 0.000299 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.205 on 241 degrees of freedom
```

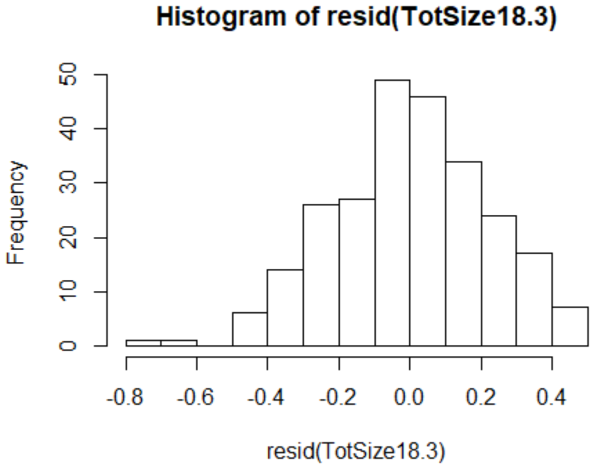
```
## Multiple R-squared:  0.1582, Adjusted R-squared:  0.1233
## F-statistic: 4.529 on 10 and 241 DF,  p-value: 6.977e-06
```



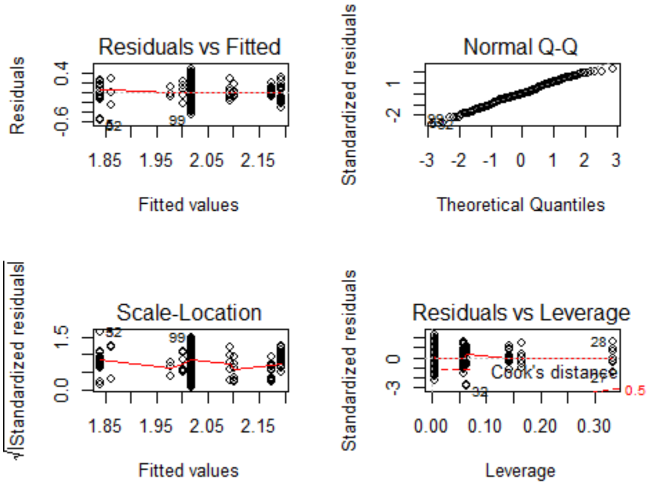


```
summary(TotSize18.3)
## Call:
## lm(formula = log.Tot ~ log.size, data = d18)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.70788 -0.13523  0.00378  0.15830  0.47471
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept) 2.022533   0.015057 134.323   <2e-16 ***
## log.size    0.010344   0.009516   1.087    0.278
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.2189 on 250 degrees of freedom
## Multiple R-squared:  0.004704,   Adjusted R-squared:  0.000723
## F-statistic: 1.182 on 1 and 250 DF,  p-value: 0.2781
```

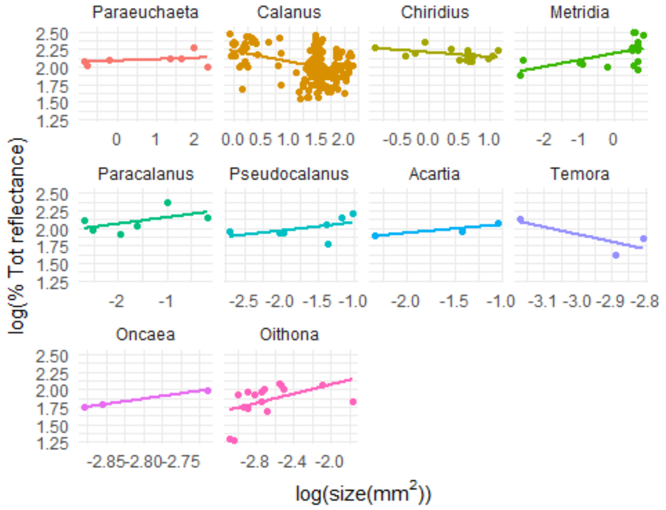




```
summary(TotSize18.4)
## Call:
## lm(formula = log.Tot ~ Genus, data = d18)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.55743 -0.11515 -0.01076  0.15842  0.45932
##
## Coefficients:
##                    Estimate Std. Error t value Pr(>|t|)
## (Intercept)         2.102712   0.078207  26.887   <2e-16 ***
## GenusCalanus       -0.083748   0.079773  -1.050   0.2948
## GenusChiridius      0.072825   0.092924   0.784   0.4340
## GenusMetridia       0.089974   0.092924   0.968   0.3339
## GenusParacalanus   -0.009048   0.115117  -0.079   0.9374
## GenusPseudocalanus -0.099727   0.110601  -0.902   0.3681
## GenusAcartia       -0.124297   0.142785  -0.871   0.3849
## GenusTemora        -0.240800   0.142785  -1.686   0.0930 .
## GenusOncaea        -0.261654   0.142785  -1.832   0.0681 .
## GenusOithona       -0.262113   0.093767  -2.795   0.0056 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.2069 on 242 degrees of freedom
## Multiple R-squared:  0.1389, Adjusted R-squared:  0.1069
## F-statistic: 4.337 on 9 and 242 DF,  p-value: 2.975e-05
```

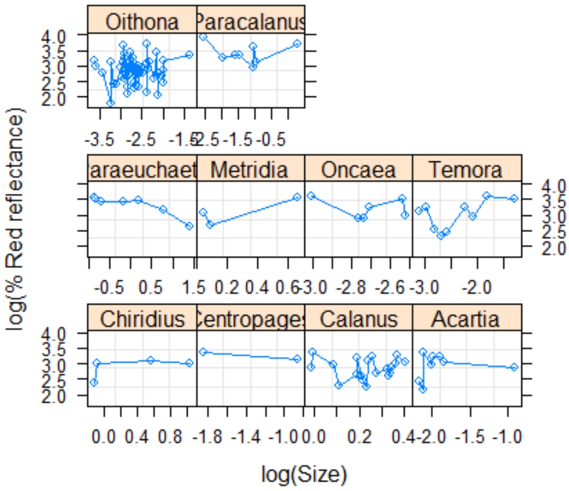

Residuals vs Fitted
Residuals
0.4
-0.6
52
99
1.85 1.95 2.05 2.15
Fitted values
Normal Q-Q
Standardized residuals
1
-2
-3 -2 -1 0 1 2 3
Theoretical Quantiles
Scale-Location
√|Standardized residuals|
1.5
0.0
52
99
1.85 1.95 2.05 2.15
Fitted values
Residuals vs Leverage
Standardized residuals
0
-3
28
27
32
Cook's distance
0.5
0.00 0.10 0.20 0.30
Leverage


Histogram of resid(TotSize18.4)
Frequency
0
10
30
50
-0.6 -0.4 -0.2 0.0 0.2 0.4
resid(TotSize18.4)

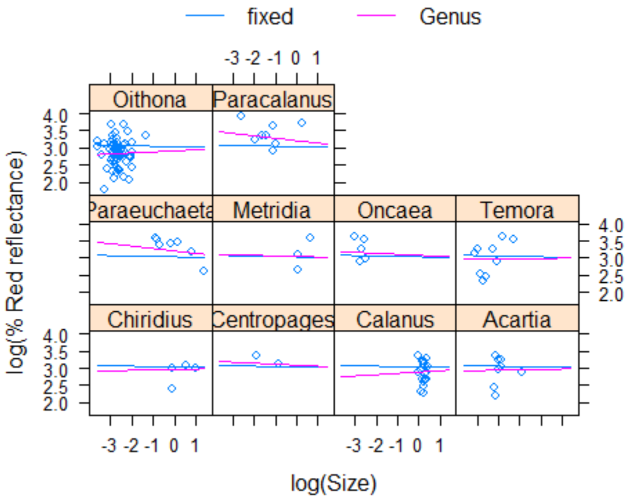

Paraeuchaeta
Calanus
Chiridius
Metridia
2.50
2.25
2.00
1.75
1.50
1.25
0 1 2
0.0 0.5 1.0 1.5 2.0
-0.5 0.0 0.5 1.0
-2 -1 0
Paracalanus
Pseudocalanus
Acartia
Temora
-2 -1
-2.5 -2.0 -1.5 -1.0
-2.0 -1.5 -1.0
-3.1 -3.0 -2.9 -2.8
Oncaea
Oithona
-2.85-2.80-2.75
-2.8 -2.4 -2.0
log(% Tot reflectance)
log(size(mm²))

Red reflectance

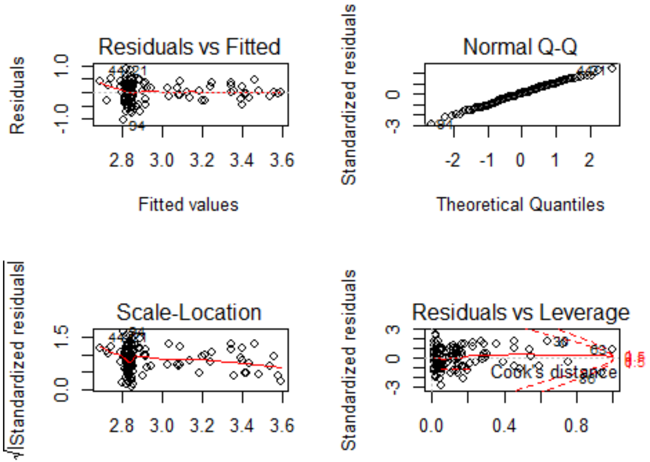
2017



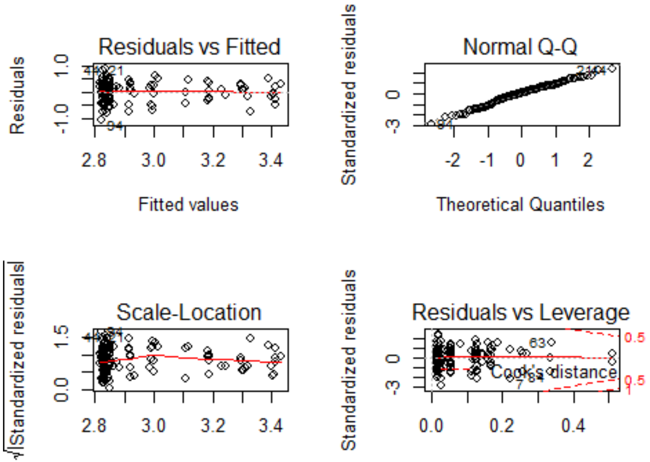
```
summary(m17 <- lme(g17))
## Linear mixed-effects model fit by REML
##  Data: g17
##        AIC      BIC    logLik
##   139.8525 157.0113 -63.92624
##
## Random effects:
##  Formula: ~log.size | Genus
##  Structure: General positive-definite
##             StdDev    Corr
## (Intercept) 0.1465349 (Intr)
## log.size    0.0498786 -1
## Residual    0.3673308
##
## Fixed effects: log.Red ~ log.size
##                  Value  Std.Error  DF  t-value p-value
## (Intercept)  3.0407780 0.08106815 120 37.50891  0.0000
## log.size    -0.0121106 0.05228445 120 -0.23163  0.8172
##  Correlation:
##          (Intr)
## log.size 0.377
##
## Standardized Within-Group Residuals:
##        Min         Q1        Med         Q3        Max
## -2.8092873 -0.5987439  0.0565306  0.7052549  2.3020593
##
## Number of Observations: 131
## Number of Groups: 10
```



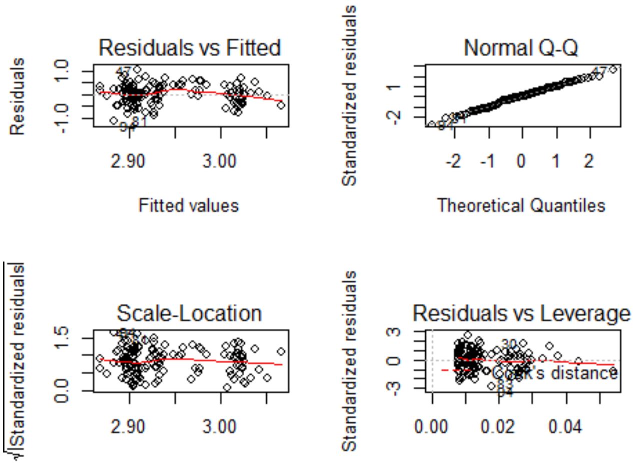
```
RedSize17.1 = lm(log.Red ~ log.size * Genus, data = d17)
RedSize17.2 = lm(log.Red ~ log.size + Genus, data = d17)
RedSize17.3 = lm(log.Red ~ log.size, data = d17)
RedSize17.4 = lm(log.Red ~ Genus, data = d17)

AICc(RedSize17.1, RedSize17.2, RedSize17.3, RedSize17.4)

##             df     AICc
## RedSize17.1 21 138.8866
## RedSize17.2 12 128.0865
## RedSize17.3  3 135.6720
## RedSize17.4 11 125.7012

summary(RedSize17.1)
## Call:
## lm(formula = log.Red ~ log.size * Genus, data = d17)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -1.02097 -0.20448  0.00905  0.23945  0.85412
##
## Coefficients:
##                            Estimate Std. Error t value Pr(>|t|)
## (Intercept)                 3.29917    0.13929  23.685  < 2e-16 ***
## log.size                   -0.34539    0.17397  -1.985  0.04957 *
## GenusCalanus               -0.44834    0.23185  -1.934  0.05569 .
## GenusChiridius             -0.53891    0.26696  -2.019  0.04593 *
## GenusCentropages           -0.37141    0.77047  -0.482  0.63071
## GenusMetridia              -0.48829    0.32409  -1.507  0.13475
## GenusParacalanus            0.05494    0.28836   0.191  0.84925
## GenusAcartia               -0.15036    0.67424  -0.223  0.82394
## GenusTemora                 0.75429    0.56319   1.339  0.18320
## GenusOncaea                -1.60646    2.52784  -0.636  0.52641
## GenusOithona               -0.36590    0.34142  -1.072  0.28618
## log.size:GenusCalanus       0.33873    0.72214   0.469  0.63995
## log.size:GenusChiridius     0.66423    0.42125   1.577  0.11769
## log.size:GenusCentropages   0.11859    0.55341   0.214  0.83071
## log.size:GenusMetridia      1.47587    0.77606   1.902  0.05980 .
## log.size:GenusParacalanus   0.30420    0.24065   1.264  0.20886
## log.size:GenusAcartia       0.46716    0.38546   1.212  0.22811
## log.size:GenusTemora        0.78421    0.28115   2.789  0.00622 **
## log.size:GenusOncaea       -0.20512    0.94530  -0.217  0.82861
## log.size:GenusOithona       0.38383    0.20891   1.837  0.06885 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.3684 on 111 degrees of freedom
## Multiple R-squared:  0.2808, Adjusted R-squared:  0.1577
## F-statistic: 2.281 on 19 and 111 DF,  p-value: 0.004069
```



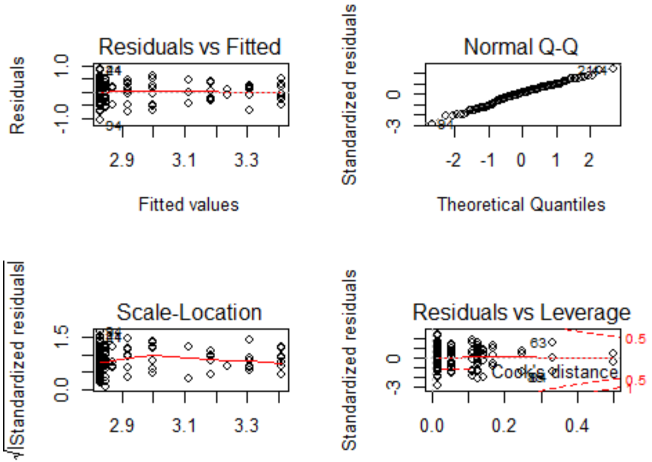
```
summary(RedSize17.2)
## Call:
## lm(formula = log.Red ~ log.size + Genus, data = d17)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -1.0352 -0.2174  0.0379  0.2487  0.8560
##
## Coefficients:
##                  Estimate Std. Error t value Pr(>|t|)
## (Intercept)       3.30772    0.14075  23.501   <2e-16 ***
## log.size          0.01416    0.07367   0.192   0.8479
## GenusCalanus     -0.46179    0.16574  -2.786   0.0062 **
## GenusChiridius   -0.44110    0.23500  -1.877   0.0629 .
## GenusCentropages -0.05359    0.31423  -0.171   0.8649
## GenusMetridia    -0.20016    0.25784  -0.776   0.4391
## GenusParacalanus  0.11839    0.21445   0.552   0.5819
## GenusAcartia     -0.36124    0.23631  -1.529   0.1290
## GenusTemora      -0.27658    0.25700  -1.076   0.2840
## GenusOncaea      -0.08384    0.28657  -0.293   0.7704
## GenusOithona     -0.43917    0.24459  -1.796   0.0751 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.3724 on 120 degrees of freedom
## Multiple R-squared:  0.2056, Adjusted R-squared:  0.1394
## F-statistic: 3.106 on 10 and 120 DF,  p-value: 0.001492
```



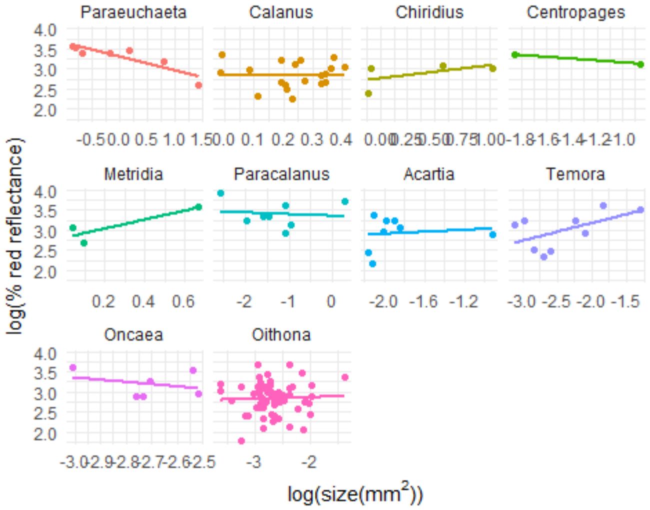
```
summary(RedSize17.3)
## Call:
## lm(formula = log.Red ~ log.size, data = d17)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -1.09591 -0.25586 -0.00355  0.28355  1.01564
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept)  3.01094    0.05955  50.565   <2e-16 ***
## log.size     0.03928    0.02713   1.448     0.15
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.3997 on 129 degrees of freedom
## Multiple R-squared:  0.01598,    Adjusted R-squared:  0.008355
## F-statistic: 2.095 on 1 and 129 DF,  p-value: 0.1502
```



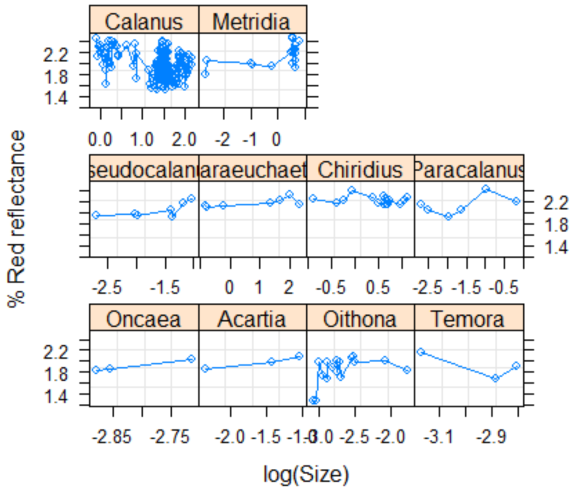
```
summary(RedSize17.4)
## Call:
## lm(formula = log.Red ~ Genus, data = d17)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -1.0435 -0.2189  0.0357  0.2465  0.8602
##
## Coefficients:
##                   Estimate Std. Error t value Pr(>|t|)
## (Intercept)        3.30739    0.14018  23.594  < 2e-16 ***
## GenusCalanus      -0.45812    0.16398  -2.794  0.00606 **
## GenusChiridius    -0.43582    0.23246  -1.875  0.06322 .
## GenusCentropages -0.07243    0.29736  -0.244  0.80798
## GenusMetridia     -0.19606    0.25593  -0.766  0.44512
## GenusParacalanus  0.10031    0.19194   0.523  0.60220
## GenusAcartia      -0.38752    0.19194  -2.019  0.04571 *
## GenusTemora       -0.31033    0.18690  -1.660  0.09943 .
## GenusOncaea       -0.12189    0.20633  -0.591  0.55580
## GenusOithona      -0.47657    0.14753  -3.230  0.00159 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.3709 on 121 degrees of freedom
## Multiple R-squared:  0.2054, Adjusted R-squared:  0.1462
## F-statistic: 3.474 on 9 and 121 DF,  p-value: 0.000758
```


Residuals vs Fitted
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles
Scale-Location
√|Standardized residuals|
Residuals vs Leverage
Cook's distance
Leverage

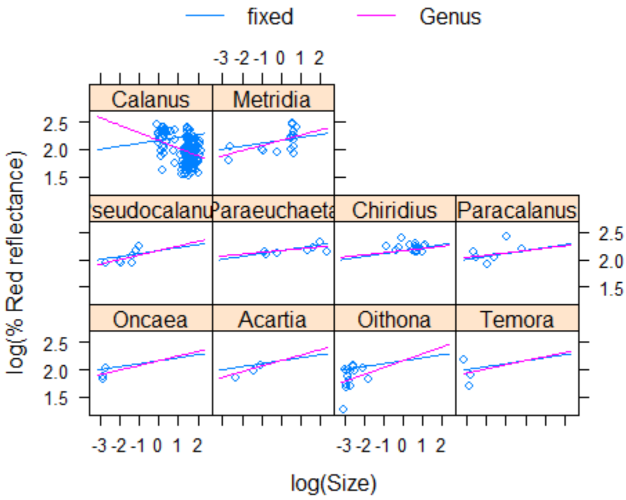

Histogram of resid(RedSize17.4)
Frequency
0
5
10
20
30
-1.0
-0.5
0.0
0.5
1.0
resid(RedSize17.4)


Paraeuchaeta
Calanus
Chiridius
Centropages
Metridia
Paracalanus
Acartia
Temora
Oncaea
Oithona
log(% red reflectance)
log(size(mm²))

2018

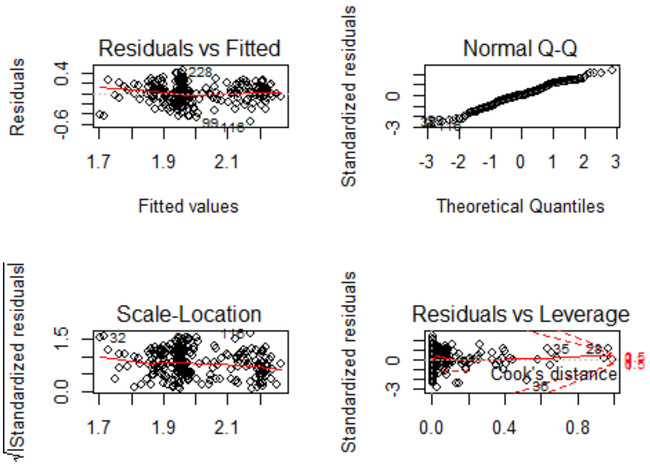


```
summary(m18 <- lme(g18))
## Linear mixed-effects model fit by REML
##  Data: g18
##        AIC       BIC   logLik
##   -76.70652 -55.57776 44.35326
##
## Random effects:
##  Formula: ~log.size | Genus
##  Structure: General positive-definite
##             StdDev       Corr
## (Intercept) 0.0009122274 (Intr)
## log.size    0.0836739648 1
## Residual    0.1919960843
##
## Fixed effects: log.Red ~ log.size
##                 Value  Std.Error  DF  t-value p-value
## (Intercept) 2.1683160 0.02323208 241 93.33284  0.0000
## log.size    0.0538206 0.02969306 241  1.81256  0.0711
##  Correlation:
##          (Intr)
## log.size 0.074
##
## Standardized Within-Group Residuals:
##        Min         Q1        Med         Q3        Max
## -2.69540637 -0.57799345  0.01389943  0.65599485  2.31848394
##
## Number of Observations: 252
## Number of Groups: 10
```



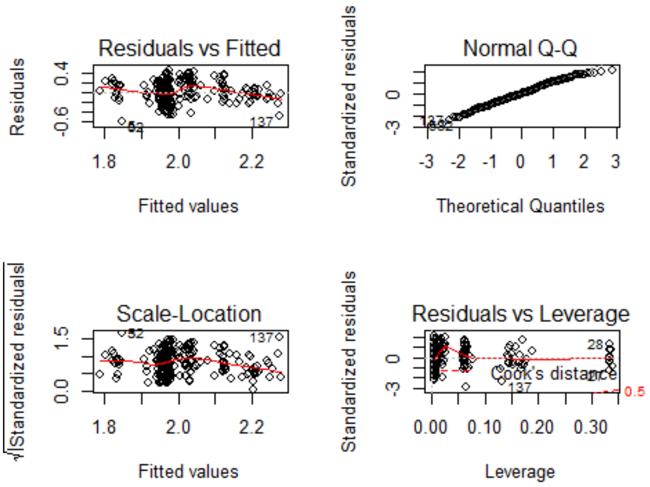
```
anova(m18)

##             numDF denDF  F-value p-value
## (Intercept)     1   241 8733.449  <.0001
## log.size        1   241    3.285  0.0711

RedSize18.1 = lm(log.Red ~ log.size * Genus, data = d18)
RedSize18.2 = lm(log.Red ~ log.size + Genus, data = d18)
RedSize18.3 = lm(log.Red ~ log.size, data = d18)
RedSize18.4 = lm(log.Red ~ Genus, data = d18)

AICc(RedSize18.1, RedSize18.2, RedSize18.3, RedSize18.4)

##             df      AICc
## RedSize18.1 21 -87.87934
## RedSize18.2 12 -61.63226
## RedSize18.3  3 -31.42007
## RedSize18.4 11 -58.94480

summary(RedSize18.1)
## Call:
## lm(formula = log.Red ~ log.size * Genus, data = d18)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.54217 -0.10461  0.00052  0.11677  0.44739
##
## Coefficients:
##                            Estimate Std. Error t value Pr(>|t|)
## (Intercept)                 2.13984    0.08656  24.720  < 2e-16 ***
## log.size                    0.03619    0.05848   0.619  0.53664
## GenusCalanus                0.05424    0.09493   0.571  0.56829
## GenusChiridius              0.08908    0.10667   0.835  0.40456
## GenusMetridia               0.01083    0.09845   0.110  0.91253
## GenusParacalanus            0.14121    0.19053   0.741  0.45936
## GenusPseudocalanus          0.15449    0.24874   0.621  0.53514
## GenusAcartia                0.10047    0.35777   0.281  0.77909
## GenusTemora                -3.09773    2.12343  -1.459  0.14596
## GenusOncaea                 3.37207    4.33156   0.778  0.43707
## GenusOithona                0.55621    0.39776   1.398  0.16334
## log.size:GenusCalanus      -0.19584    0.06430  -3.046  0.00259 **
## log.size:GenusChiridius    -0.06432    0.10654  -0.604  0.54662
## log.size:GenusMetridia      0.06897    0.07231   0.954  0.34116
## log.size:GenusParacalanus   0.05635    0.10787   0.522  0.60189
## log.size:GenusPseudocalanus 0.12151    0.14559   0.835  0.40482
## log.size:GenusAcartia       0.13135    0.21325   0.616  0.53854
## log.size:GenusTemora       -1.01157    0.71925  -1.406  0.16093
## log.size:GenusOncaea        1.24236    1.53838   0.808  0.42016
## log.size:GenusOithona       0.28627    0.15482   1.849  0.06572 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
```

```
## Residual standard error: 0.1933 on 232 degrees of freedom
## Multiple R-squared:  0.3178, Adjusted R-squared:  0.262
## F-statistic:  5.69 on 19 and 232 DF,  p-value: 1.547e-11
```

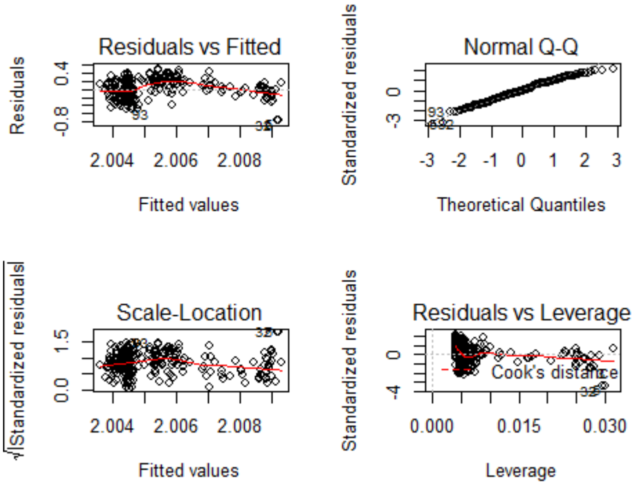


```
summary(RedSize18.2)
## Call:
## lm(formula = log.Red ~ log.size + Genus, data = d18)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -0.5770 -0.1278 -0.0103  0.1547  0.4335
##
## Coefficients:
##                    Estimate Std. Error t value Pr(>|t|)
## (Intercept)         2.20480    0.08045  27.406  < 2e-16 ***
## log.size           -0.04570    0.02102  -2.174  0.03070 *
## GenusCalanus       -0.16467    0.08113  -2.030  0.04349 *
## GenusChiridius      0.03221    0.09377   0.344  0.73150
## GenusMetridia      -0.05534    0.09502  -0.582  0.56085
## GenusParacalanus   -0.15291    0.12679  -1.206  0.22898
## GenusPseudocalanus -0.24828    0.12268  -2.024  0.04409 *
## GenusAcartia       -0.30628    0.15227  -2.011  0.04539 *
## GenusTemora        -0.41496    0.16388  -2.532  0.01198 *
## GenusOncaea        -0.42226    0.16250  -2.599  0.00994 **
## GenusOithona       -0.49808    0.11941  -4.171 4.23e-05 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.2082 on 241 degrees of freedom
```

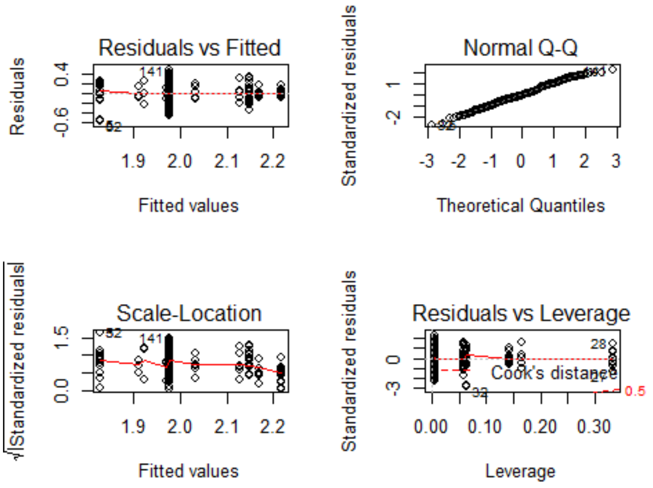
```
## Multiple R-squared:  0.1781, Adjusted R-squared:  0.144
## F-statistic: 5.223 on 10 and 241 DF,  p-value: 6.096e-07
```



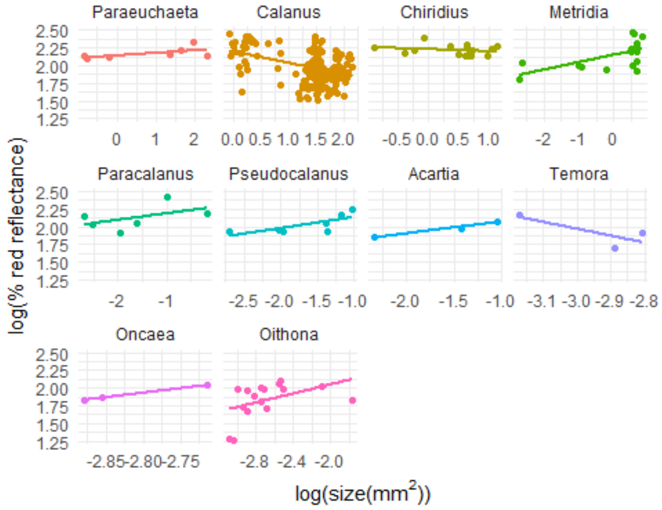
```
summary(RedSize18.3)
## Call:
## lm(formula = log.Red ~ log.size, data = d18)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.74036 -0.14619 -0.00152  0.17532  0.47283
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)  2.006011   0.015514 129.300   <2e-16 ***
## log.size    -0.001032   0.009805  -0.105    0.916
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.2255 on 250 degrees of freedom
## Multiple R-squared:  4.429e-05,  Adjusted R-squared:  -0.003956
## F-statistic: 0.01107 on 1 and 250 DF,  p-value: 0.9163
```



```
summary(RedSize18.4)
## Call:
## lm(formula = log.Red ~ Genus, data = d18)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.56072 -0.12174 -0.01308  0.15908  0.46926
##
## Coefficients:
##                    Estimate Std. Error t value Pr(>|t|)
## (Intercept)         2.16855    0.07931  27.344  < 2e-16 ***
## GenusCalanus       -0.19015    0.08090  -2.351 0.019549 *
## GenusChiridius      0.04739    0.09423   0.503 0.615472
## GenusMetridia      -0.01872    0.09423  -0.199 0.842680
## GenusParacalanus   -0.04091    0.11674  -0.350 0.726293
## GenusPseudocalanus -0.13614    0.11216  -1.214 0.226003
## GenusAcartia       -0.19678    0.14479  -1.359 0.175400
## GenusTemora        -0.24365    0.14479  -1.683 0.093713 .
## GenusOncaea        -0.25732    0.14479  -1.777 0.076797 .
## GenusOithona       -0.33903    0.09509  -3.566 0.000437 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.2098 on 242 degrees of freedom
## Multiple R-squared:  0.162,  Adjusted R-squared:  0.1308
## F-statistic: 5.198 on 9 and 242 DF,  p-value: 1.836e-06
```

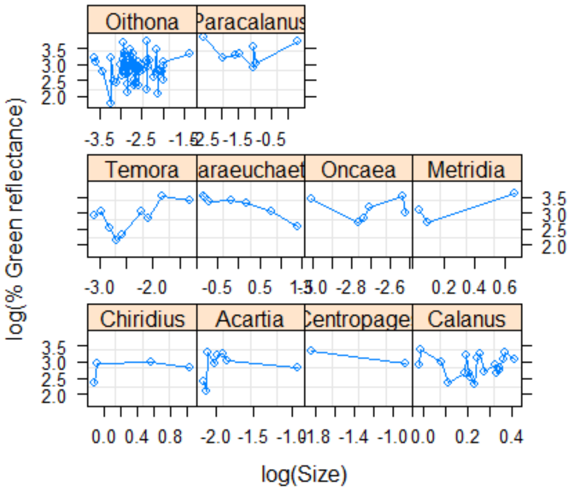

Residuals vs Fitted
Residuals
0.4
-0.6
141
52
1.9
2.0
2.1
2.2
Fitted values
Normal Q-Q
Standardized residuals
1
-2
32
-3
-2
-1
0
1
2
3
Theoretical Quantiles
Scale-Location
√|Standardized residuals|
1.5
0.0
52
141
1.9
2.0
2.1
2.2
Fitted values
Residuals vs Leverage
Standardized residuals
0
-3
28
27
32
Cook's distance
0.5
0.00
0.10
0.20
0.30
Leverage


Histogram of resid(RedSize18.4)
Frequency
50
30
10
0
-0.6
-0.4
-0.2
0.0
0.2
0.4
resid(RedSize18.4)

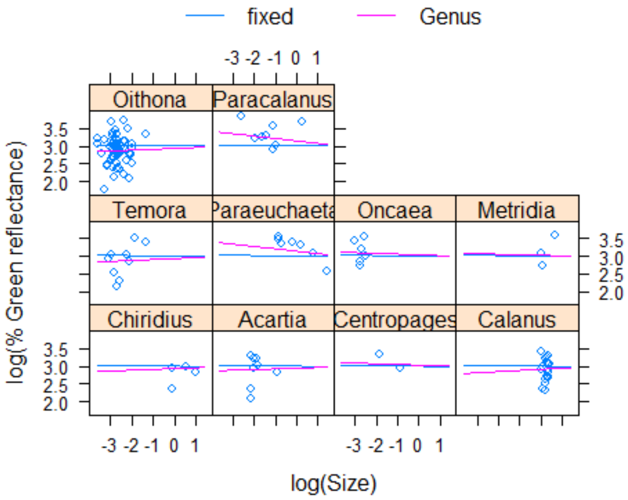

Paraeuchaeta
Calanus
Chiridius
Metridia
Paracalanus
Pseudocalanus
Acartia
Temora
Oncaea
Oithona
log(% red reflectance)
2.50
2.25
2.00
1.75
1.50
1.25
0
1
2
0.0 0.5 1.0 1.5 2.0
-0.5 0.0 0.5 1.0
-2
-1
0
-2
-1
-2.5 -2.0 -1.5 -1.0
-2.0
-1.5
-1.0
-3.1 -3.0 -2.9 -2.8
-2.85 -2.80 -2.75
-2.8 -2.4 -2.0
log(size(mm^2))

Green reflectance

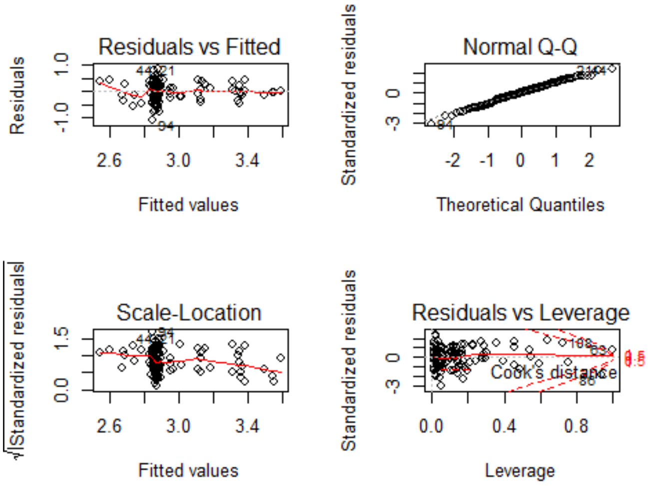
2017

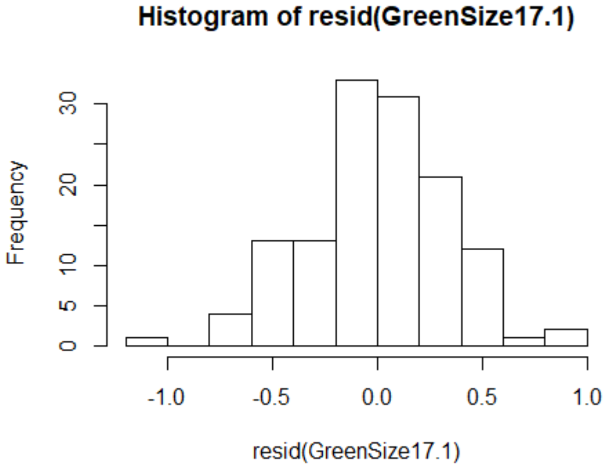


```
summary(m17 <- lme(g17))
## Linear mixed-effects model fit by REML
##  Data: g17
##        AIC     BIC    logLik
##   137.7801 154.939 -62.89004
##
## Random effects:
##  Formula: ~log.size | Genus
##  Structure: General positive-definite
##             StdDev     Corr
## (Intercept) 0.11842438 (Intr)
## log.size    0.05208671 -1
## Residual    0.36621914
##
## Fixed effects: log.Green ~ log.size
##                 Value  Std.Error  DF  t-value p-value
## (Intercept)  3.010748 0.07359825 120 40.90787  0.0000
## log.size    -0.006499 0.04979434 120 -0.13052  0.8964
##  Correlation:
##          (Intr)
## log.size 0.381
##
## Standardized Within-Group Residuals:
##        Min         Q1        Med         Q3        Max
## -2.9641696 -0.5990294  0.1109628  0.6763226  2.2912984
##
## Number of Observations: 131
## Number of Groups: 10
```

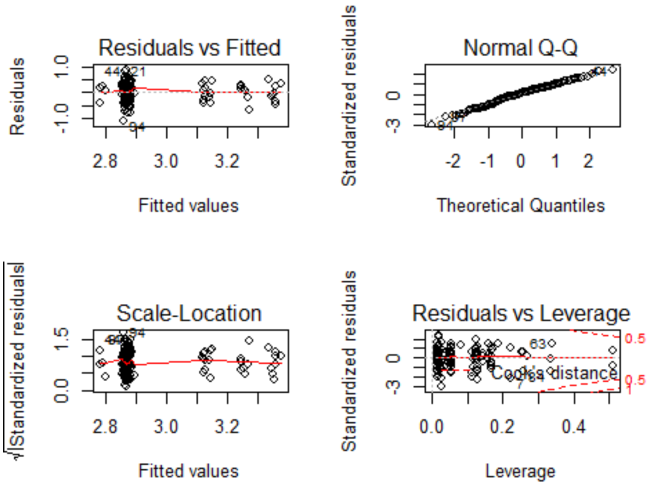


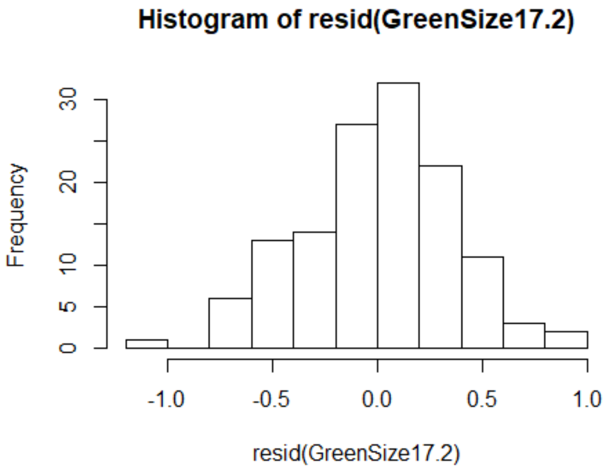
```
GreenSize17.1 = lm(log.Green ~ log.size * Genus, data = d17)
GreenSize17.2 = lm(log.Green ~ log.size + Genus, data = d17)
GreenSize17.3 = lm(log.Green ~ log.size, data = d17)
GreenSize17.4 = lm(log.Green ~ Genus, data = d17)

AICc(GreenSize17.1, GreenSize17.2, GreenSize17.3, GreenSize17.4)

##               df     AICc
## GreenSize17.1 21 137.5036
## GreenSize17.2 12 127.2907
## GreenSize17.3  3 127.3538
## GreenSize17.4 11 124.9029

summary(GreenSize17.1)
## Call:
## lm(formula = log.Green ~ log.size * Genus, data = d17)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -1.07459 -0.17873  0.01156  0.22205  0.84050
##
## Coefficients:
##                            Estimate Std. Error t value Pr(>|t|)
## (Intercept)                 3.24433    0.13856  23.415  < 2e-16 ***
## log.size                   -0.36029    0.17305  -2.082  0.03965 *
## GenusCalanus               -0.36990    0.23063  -1.604  0.11159
## GenusChiridius             -0.53755    0.26555  -2.024  0.04534 *
## GenusCentropages           -0.63859    0.76641  -0.833  0.40651
## GenusMetridia              -0.39660    0.32239  -1.230  0.22122
## GenusParacalanus            0.07567    0.28684   0.264  0.79241
## GenusAcartia               -0.11828    0.67069  -0.176  0.86034
## GenusTemora                 0.73904    0.56022   1.319  0.18982
## GenusOncaea                -0.35487    2.51454  -0.141  0.88803
## GenusOithona               -0.28210    0.33963  -0.831  0.40798
## log.size:GenusCalanus       0.38101    0.71834   0.530  0.59689
## log.size:GenusChiridius     0.59261    0.41903   1.414  0.16009
## log.size:GenusCentropages  -0.02955    0.55049  -0.054  0.95728
## log.size:GenusMetridia      1.47730    0.77197   1.914  0.05824 .
## log.size:GenusParacalanus   0.33543    0.23938   1.401  0.16394
## log.size:GenusAcartia       0.48935    0.38343   1.276  0.20453
## log.size:GenusTemora        0.82411    0.27967   2.947  0.00391 **
## log.size:GenusOncaea        0.27421    0.94032   0.292  0.77113
## log.size:GenusOithona       0.39664    0.20781   1.909  0.05889 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.3664 on 111 degrees of freedom
## Multiple R-squared:  0.2391, Adjusted R-squared:  0.1089
## F-statistic: 1.836 on 19 and 111 DF,  p-value: 0.0269
```



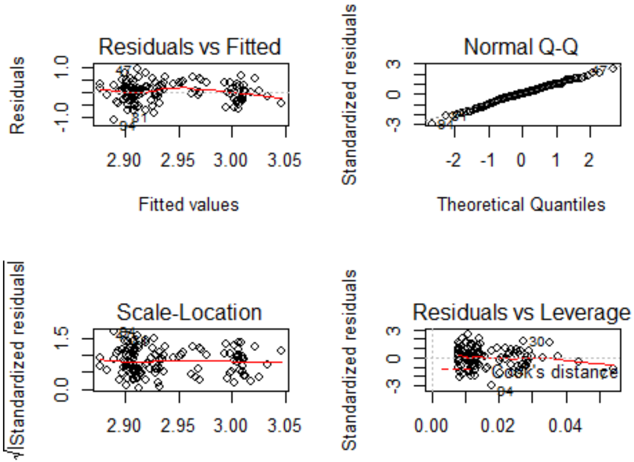


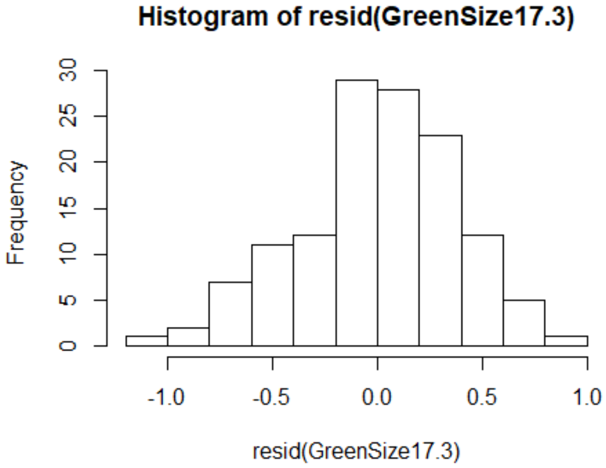
```
summary(GreenSize17.2)
## Call:
## lm(formula = log.Green ~ log.size + Genus, data = d17)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -1.08790 -0.20499  0.04664  0.22650  0.84722
##
## Coefficients:
##                   Estimate Std. Error t value Pr(>|t|)
## (Intercept)        3.25323    0.14032  23.184   <2e-16 ***
## log.size           0.01368    0.07345   0.186   0.8526
## GenusCalanus      -0.37714    0.16524  -2.282   0.0242 *
## GenusChiridius    -0.47012    0.23429  -2.007   0.0470 *
## GenusCentropages -0.10092    0.31328  -0.322   0.7479
## GenusMetridia     -0.11227    0.25705  -0.437   0.6631
## GenusParacalanus  0.11691    0.21380   0.547   0.5855
## GenusAcartia      -0.34414    0.23559  -1.461   0.1467
## GenusTemora       -0.35354    0.25622  -1.380   0.1702
## GenusOncaea       -0.09327    0.28571  -0.326   0.7446
## GenusOithona      -0.35146    0.24384  -1.441   0.1521
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.3712 on 120 degrees of freedom
## Multiple R-squared:  0.1557, Adjusted R-squared:  0.08538
## F-statistic: 2.214 on 10 and 120 DF,  p-value: 0.02125
```



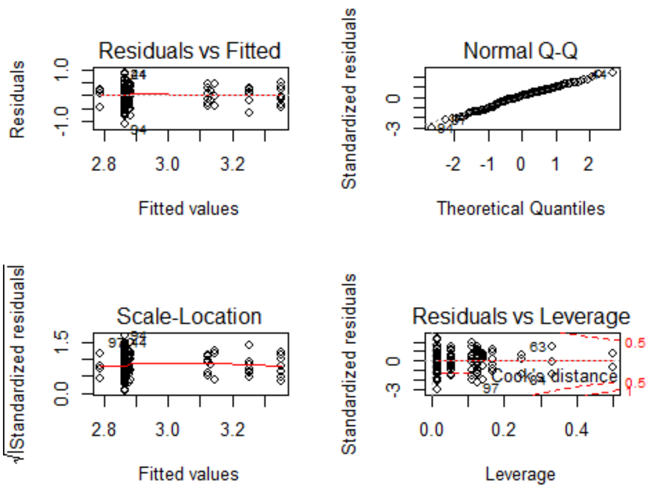


```
summary(GreenSize17.3)
## Call:
## lm(formula = log.Green ~ log.size, data = d17)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -1.11983 -0.20395  0.02486  0.28081  0.93520
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept)  2.99884    0.05769  51.986   <2e-16 ***
## log.size     0.03370    0.02628   1.282    0.202
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.3872 on 129 degrees of freedom
## Multiple R-squared:  0.01259,    Adjusted R-squared:  0.004931
## F-statistic: 1.644 on 1 and 129 DF,  p-value: 0.202
```

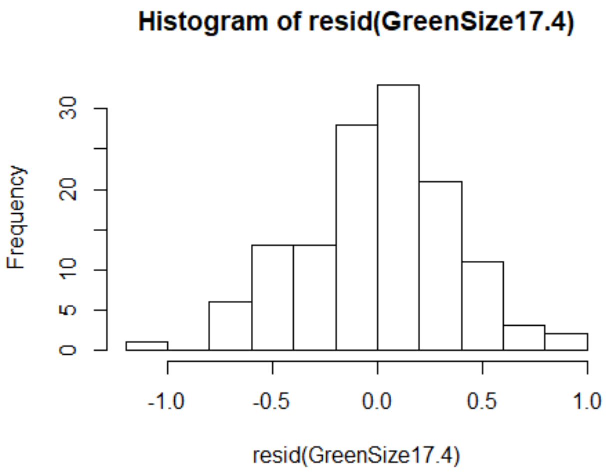




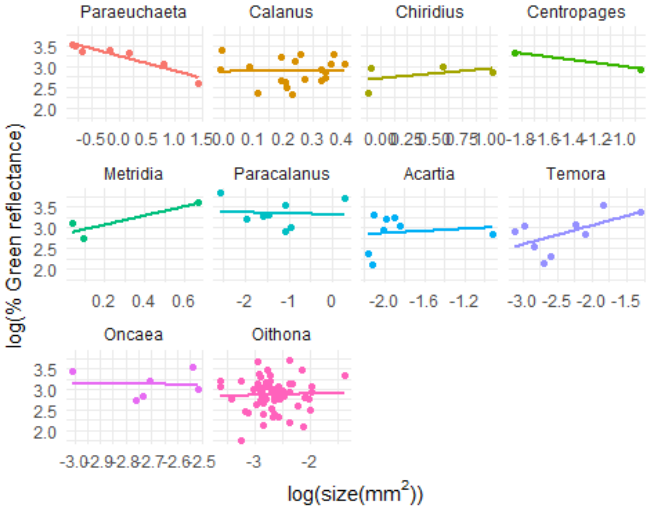
```
summary(GreenSize17.4)
## Call:
## lm(formula = log.Green ~ Genus, data = d17)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -1.09593 -0.20210  0.04838  0.22457  0.85127
##
## Coefficients:
##                   Estimate Std. Error t value Pr(>|t|)
## (Intercept)        3.25290    0.13975  23.277  < 2e-16 ***
## GenusCalanus      -0.37359    0.16348  -2.285  0.02404 *
## GenusChiridius    -0.46502    0.23175  -2.007  0.04703 *
## GenusCentropages -0.11912    0.29646  -0.402  0.68852
## GenusMetridia     -0.10831    0.25515  -0.424  0.67197
## GenusParacalanus  0.09944    0.19136   0.520  0.60425
## GenusAcartia      -0.36952    0.19136  -1.931  0.05582 .
## GenusTemora       -0.38613    0.18633  -2.072  0.04036 *
## GenusOncaea       -0.13003    0.20571  -0.632  0.52852
## GenusOithona      -0.38759    0.14708  -2.635  0.00951 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.3697 on 121 degrees of freedom
## Multiple R-squared:  0.1555, Adjusted R-squared:  0.09268
## F-statistic: 2.475 on 9 and 121 DF,  p-value: 0.01253
```



Residuals vs Fitted
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles
Scale-Location
√|Standardized residuals|
Fitted values
Residuals vs Leverage
Standardized residuals
Cook's distance
Leverage

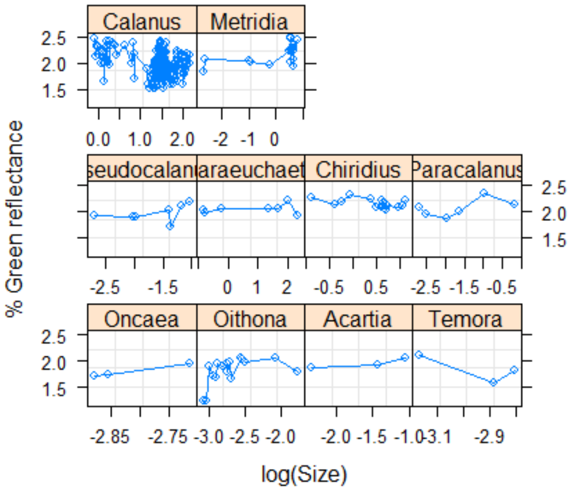


Histogram of resid(GreenSize17.4)
Frequency
0
5
10
20
30
-1.0
-0.5
0.0
0.5
1.0
resid(GreenSize17.4)

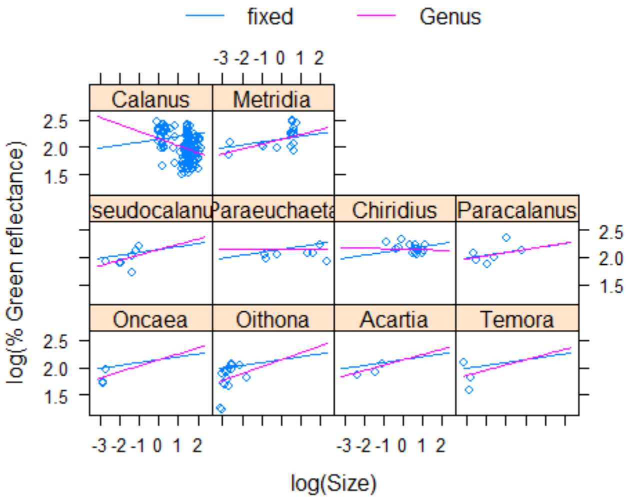


Paraeuchaeta
Calanus
Chiridius
Centropages
Metridia
Paracalanus
Acartia
Temora
Oncaea
Oithona
log(% Green reflectance)
log(size(mm²))

2018

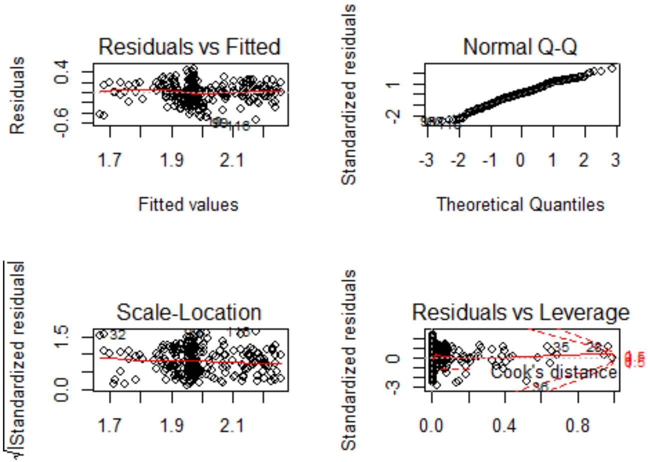


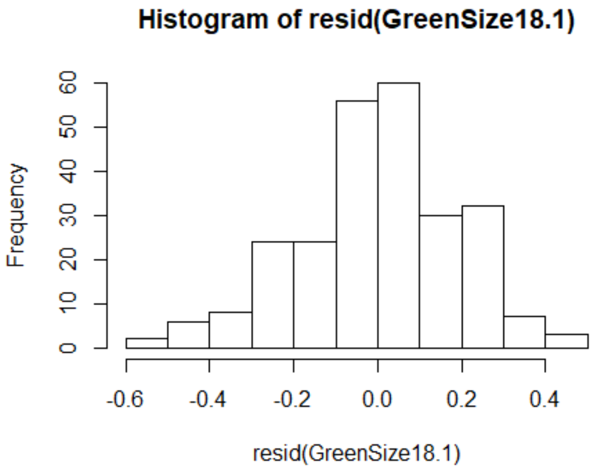
```
summary(m18 <- lme(g18))
## Linear mixed-effects model fit by REML
##  Data: g18
##        AIC       BIC   logLik
##   -63.86752 -42.73876 37.93376
##
## Random effects:
##  Formula: ~log.size | Genus
##  Structure: General positive-definite
##             StdDev      Corr
## (Intercept) 0.006557165 (Intr)
## log.size    0.088177689 -1
## Residual    0.196768379
##
## Fixed effects: log.Green ~ log.size
##                 Value  Std.Error  DF  t-value p-value
## (Intercept) 2.1479121 0.02317260 241 92.69188   0.000
## log.size    0.0531169 0.03110656 241  1.70758   0.089
##  Correlation:
##          (Intr)
## log.size -0.037
##
## Standardized Within-Group Residuals:
##         Min          Q1         Med          Q3         Max
## -2.72207891 -0.55886724 -0.01084472  0.68550135  2.30779039
##
## Number of Observations: 252
## Number of Groups: 10
```



```
anova(m18)

##             numDF denDF  F-value p-value
## (Intercept)     1   241 8615.251  <.0001
## log.size        1   241    2.916   0.089

GreenSize18.1 = lm(log.Green ~ log.size * Genus, data = d18)
GreenSize18.2 = lm(log.Green ~ log.size + Genus, data = d18)
GreenSize18.3 = lm(log.Green ~ log.size, data = d18)
GreenSize18.4 = lm(log.Green ~ Genus, data = d18)


AICc(GreenSize18.1, GreenSize18.2, GreenSize18.3, GreenSize18.4)

##               df      AICc
## GreenSize18.1 21 -78.00494
## GreenSize18.2 12 -55.61900
## GreenSize18.3  3 -32.25300
## GreenSize18.4 11 -52.03011

summary(GreenSize18.1)
## Call:
## lm(formula = log.Green ~ log.size * Genus, data = d18)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.53512 -0.10380  0.00833  0.12507  0.45503
##
## Coefficients:
##                            Estimate Std. Error t value Pr(>|t|)
## (Intercept)                 2.04413    0.08828  23.156  < 2e-16 ***
## log.size                    0.01445    0.05964   0.242  0.80878
## GenusCalanus                0.15669    0.09681   1.618  0.10692
## GenusChiridius              0.14247    0.10878   1.310  0.19159
## GenusMetridia               0.13130    0.10040   1.308  0.19223
## GenusParacalanus            0.17724    0.19430   0.912  0.36263
## GenusPseudocalanus          0.12581    0.25366   0.496  0.62038
## GenusAcartia                0.10522    0.36485   0.288  0.77331
## GenusTemora                -3.28556    2.16544  -1.517  0.13056
## GenusOncaea                 4.00207    4.41726   0.906  0.36587
## GenusOithona                0.70393    0.40563   1.735  0.08400 .
## log.size:GenusCalanus      -0.17036    0.06557  -2.598  0.00997 **
## log.size:GenusChiridius    -0.09303    0.10865  -0.856  0.39274
## log.size:GenusMetridia      0.08468    0.07374   1.148  0.25200
## log.size:GenusParacalanus   0.08015    0.11001   0.729  0.46701
## log.size:GenusPseudocalanus 0.10659    0.14847   0.718  0.47354
## log.size:GenusAcartia       0.11217    0.21746   0.516  0.60648
## log.size:GenusTemora       -1.05504    0.73348  -1.438  0.15167
## log.size:GenusOncaea        1.49135    1.56881   0.951  0.34279
## log.size:GenusOithona       0.33620    0.15788   2.129  0.03427 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

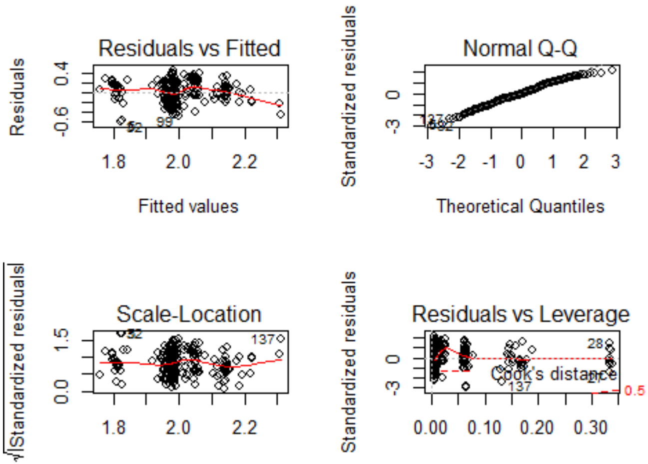
```
##
## Residual standard error: 0.1972 on 232 degrees of freedom
## Multiple R-squared:  0.2916, Adjusted R-squared:  0.2336
## F-statistic: 5.026 on 19 and 232 DF,  p-value: 6.094e-10
```

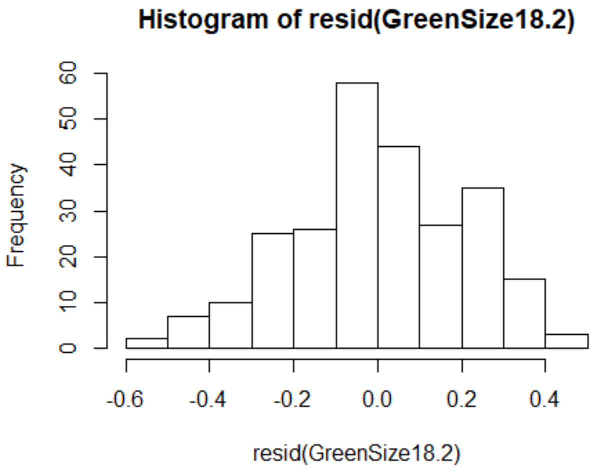




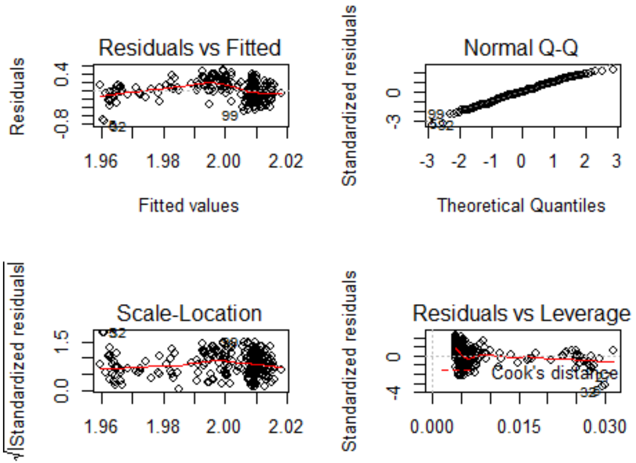
```
summary(GreenSize18.2)
## Call:
## lm(formula = log.Green ~ log.size + Genus, data = d18)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.59937 -0.12222 -0.00794  0.15981  0.44221
##
## Coefficients:
##                    Estimate Std. Error t value Pr(>|t|)
## (Intercept)         2.09555    0.08142  25.739  < 2e-16 ***
## log.size           -0.05037    0.02127  -2.368 0.018694 *
## GenusCalanus       -0.03732    0.08210  -0.455 0.649821
## GenusChiridius      0.07804    0.09490   0.822 0.411704
## GenusMetridia       0.07869    0.09616   0.818 0.413983
## GenusParacalanus   -0.11450    0.12831  -0.892 0.373090
## GenusPseudocalanus -0.21030    0.12415  -1.694 0.091571 .
## GenusAcartia       -0.22990    0.15409  -1.492 0.137020
## GenusTemora        -0.41035    0.16585  -2.474 0.014043 *
## GenusOncaea        -0.43185    0.16445  -2.626 0.009190 **
## GenusOithona       -0.42513    0.12084  -3.518 0.000519 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
```

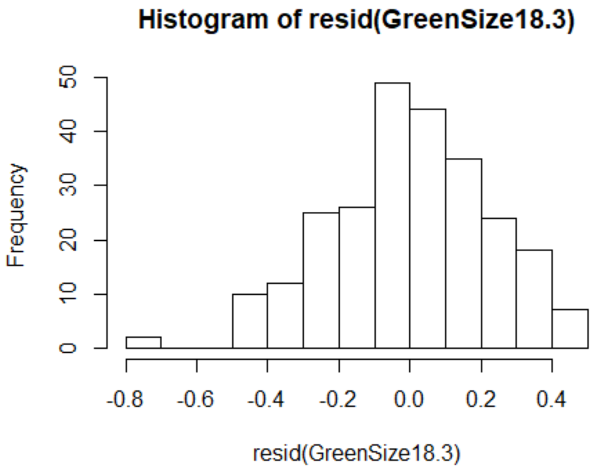
```
## Residual standard error: 0.2107 on 241 degrees of freedom
## Multiple R-squared:  0.1594, Adjusted R-squared:  0.1246
## F-statistic: 4.572 on 10 and 241 DF,  p-value: 6.004e-06
```



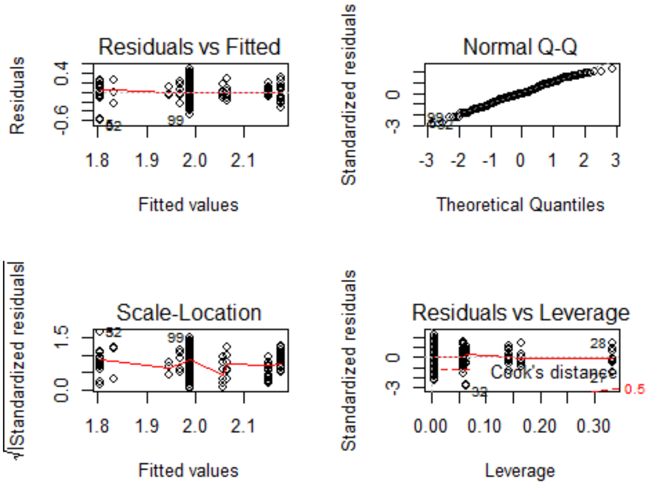


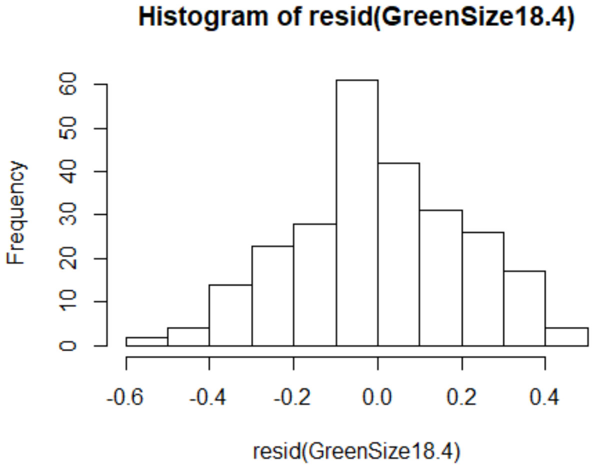
```
summary(GreenSize18.3)
## Call:
## lm(formula = log.Green ~ log.size, data = d18)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.73653 -0.13730  0.00273  0.16089  0.49212
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept) 1.993355   0.015489 128.697   <2e-16 ***
## log.size    0.010675   0.009789   1.091    0.277
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.2251 on 250 degrees of freedom
## Multiple R-squared:  0.004735,   Adjusted R-squared:  0.0007537
## F-statistic: 1.189 on 1 and 250 DF,  p-value: 0.2765
```

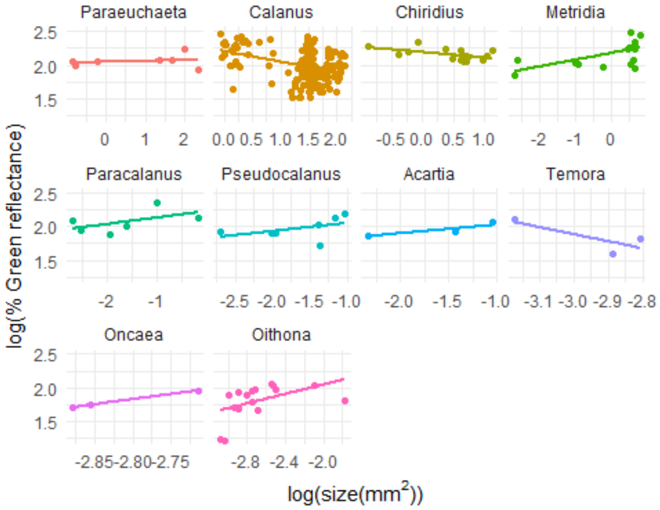




```
summary(GreenSize18.4)
## Call:
## lm(formula = log.Green ~ Genus, data = d18)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.58144 -0.12044 -0.00795  0.16450  0.47285
##
## Coefficients:
##                     Estimate Std. Error t value Pr(>|t|)
## (Intercept)         2.055595   0.080402  25.566   <2e-16 ***
## GenusCalanus       -0.065411   0.082013  -0.798   0.4259
## GenusChiridius      0.094767   0.095532   0.992   0.3222
## GenusMetridia       0.119049   0.095532   1.246   0.2139
## GenusParacalanus    0.008957   0.118349   0.076   0.9397
## GenusPseudocalanus -0.086686   0.113706  -0.762   0.4466
## GenusAcartia       -0.109203   0.146793  -0.744   0.4576
## GenusTemora        -0.221520   0.146793  -1.509   0.1326
## GenusOncaea        -0.250041   0.146793  -1.703   0.0898 .
## GenusOithona       -0.249818   0.096399  -2.592   0.0101 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.2127 on 242 degrees of freedom
## Multiple R-squared:  0.1399, Adjusted R-squared:  0.1079
## F-statistic: 4.373 on 9 and 242 DF,  p-value: 2.645e-05
```

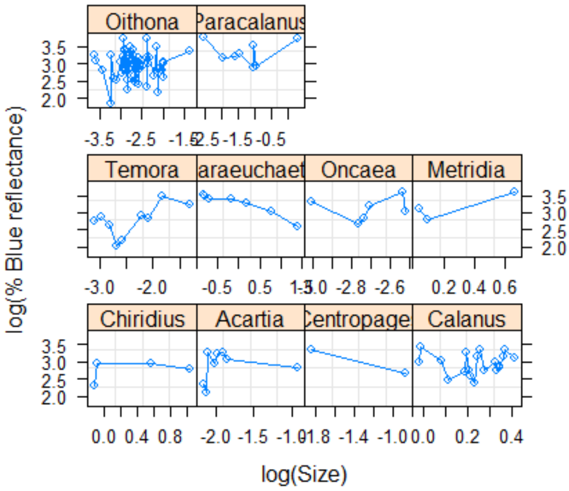

Residuals vs Fitted
Residuals
0.4
-0.6
52
99
1.8
1.9
2.0
2.1
Fitted values
Normal Q-Q
Standardized residuals
0
-3
99
52
-3
-2
-1
0
1
2
3
Theoretical Quantiles
Scale-Location
√|Standardized residuals|
1.5
0.0
52
99
1.8
1.9
2.0
2.1
Fitted values
Residuals vs Leverage
Standardized residuals
0
-3
28
27
32
Cook's distance
0.5
0.00
0.10
0.20
0.30
Leverage


Histogram of resid(GreenSize18.4)
Frequency
0
10
20
30
40
50
60
-0.6
-0.4
-0.2
0.0
0.2
0.4
resid(GreenSize18.4)

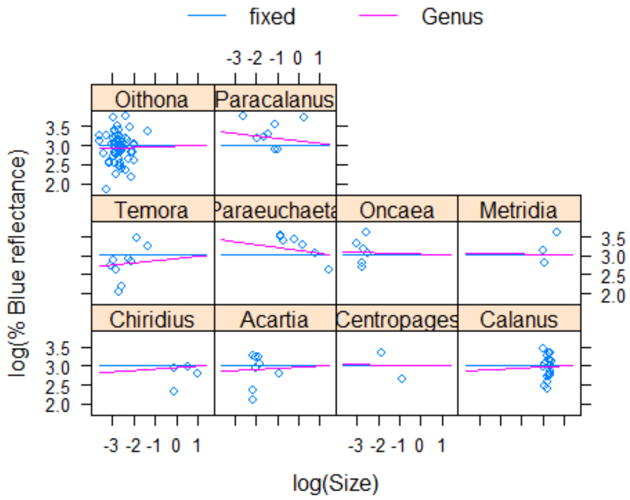

Paraeuchaeta
Calanus
Chiridius
Metridia
Paracalanus
Pseudocalanus
Acartia
Temora
Oncaea
Oithona
2.5
2.0
1.5
0
1
2
0.0 0.5 1.0 1.5 2.0
-0.5 0.0 0.5 1.0
-2
-1
0
-2
-1
-2.5 -2.0 -1.5 -1.0
-2.0
-1.5
-1.0
-3.1 -3.0 -2.9 -2.8
-2.85-2.80-2.75
-2.8 -2.4 -2.0
log(% Green reflectance)
log(size(mm²))

Blue reflectance

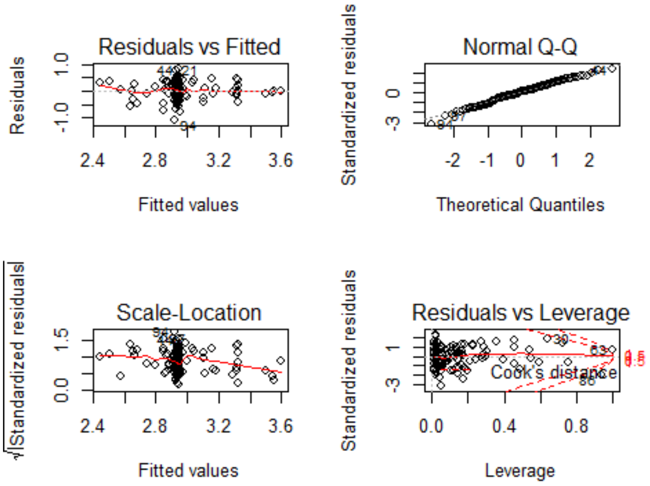
2017



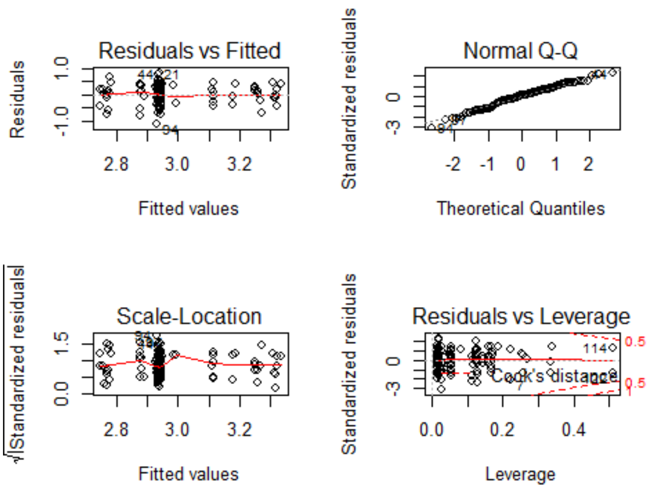
```
summary(m17 <- lme(g17))
## Linear mixed-effects model fit by REML
##  Data: g17
##        AIC      BIC    logLik
##   130.3803 147.5392 -59.19015
##
## Random effects:
##  Formula: ~log.size | Genus
##  Structure: General positive-definite
##             StdDev     Corr
## (Intercept) 0.09938903 (Intr)
## log.size    0.06096636 -1
## Residual    0.35605186
##
## Fixed effects: log.Blue ~ log.size
##                 Value  Std.Error  DF  t-value p-value
## (Intercept) 3.0202615 0.06723461 120 44.92123  0.0000
## log.size    0.0002748 0.04914857 120  0.00559  0.9955
##  Correlation:
##          (Intr)
## log.size 0.336
##
## Standardized Within-Group Residuals:
##         Min          Q1         Med          Q3         Max
## -3.04651815 -0.61304450  0.05830731  0.66550712  2.26926749
##
## Number of Observations: 131
## Number of Groups: 10
```



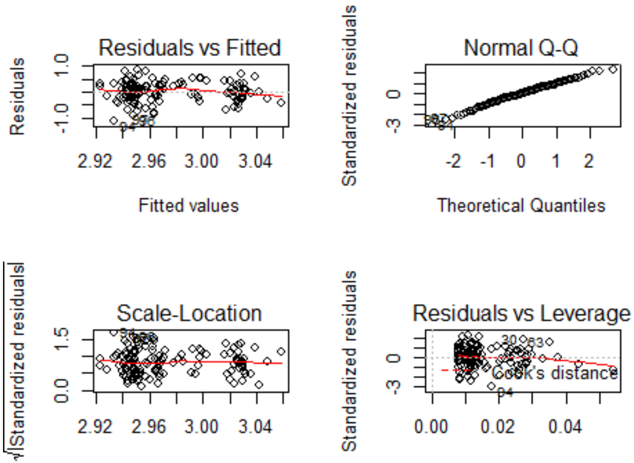
```
BlueSize17.1 = lm(log.Blue ~ log.size * Genus, data = d17)
BlueSize17.2 = lm(log.Blue ~ log.size + Genus, data = d17)
BlueSize17.3 = lm(log.Blue ~ log.size, data = d17)
BlueSize17.4 = lm(log.Blue ~ Genus, data = d17)

AICc(BlueSize17.1, BlueSize17.2, BlueSize17.3, BlueSize17.4)

##              df     AICc
## BlueSize17.1 21 128.1811
## BlueSize17.2 12 119.9234
## BlueSize17.3  3 117.9503
## BlueSize17.4 11 117.5178

summary(BlueSize17.1)
## Call:
## lm(formula = log.Blue ~ log.size * Genus, data = d17)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -1.0776 -0.1788  0.0243  0.2232  0.8073
##
## Coefficients:
##                           Estimate Std. Error t value Pr(>|t|)
## (Intercept)                3.25097    0.13372  24.313  < 2e-16 ***
## log.size                  -0.36124    0.16700  -2.163  0.03268 *
## GenusCalanus              -0.31243    0.22257  -1.404  0.16319
## GenusChiridius            -0.57141    0.25627  -2.230  0.02778 *
## GenusCentropages          -1.18735    0.73962  -1.605  0.11126
## GenusMetridia             -0.34529    0.31112  -1.110  0.26947
## GenusParacalanus           0.07082    0.27682   0.256  0.79856
## GenusAcartia              -0.15441    0.64725  -0.239  0.81188
## GenusTemora                0.64908    0.54064   1.201  0.23247
## GenusOncaea                0.59370    2.42664   0.245  0.80717
## GenusOithona              -0.23948    0.32775  -0.731  0.46653
## log.size:GenusCalanus      0.38259    0.69323   0.552  0.58213
## log.size:GenusChiridius    0.56320    0.40438   1.393  0.16649
## log.size:GenusCentropages -0.32271    0.53125  -0.607  0.54479
## log.size:GenusMetridia     1.40316    0.74498   1.883  0.06225 .
## log.size:GenusParacalanus  0.36212    0.23102   1.568  0.11984
## log.size:GenusAcartia      0.47705    0.37003   1.289  0.20000
## log.size:GenusTemora       0.82946    0.26989   3.073  0.00266 **
## log.size:GenusOncaea       0.63044    0.90745   0.695  0.48868
## log.size:GenusOithona      0.38901    0.20055   1.940  0.05495 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.3536 on 111 degrees of freedom
## Multiple R-squared:  0.2356, Adjusted R-squared:  0.1048
## F-statistic: 1.801 on 19 and 111 DF,  p-value: 0.03109
```



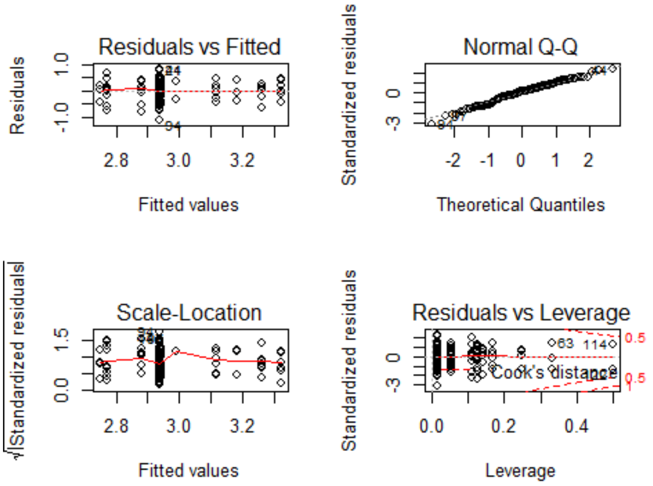
```
**summary**(BlueSize17.2)
## Call:
## lm(formula = log.Blue ~ log.size + Genus, data = d17)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -1.08819 -0.19829  0.04392  0.22735  0.81267
##
## Coefficients:
##                   Estimate Std. Error t value Pr(>|t|)
## (Intercept)       3.259800   0.136431  23.893   <2e-16 ***
## log.size          0.009666   0.071410   0.135   0.8926
## GenusCalanus     -0.318504   0.160654  -1.983   0.0497 *
## GenusChiridius   -0.513106   0.227790  -2.253   0.0261 *
## GenusCentropages -0.256670   0.304594  -0.843   0.4011
## GenusMetridia    -0.079774   0.249926  -0.319   0.7501
## GenusParacalanus  0.073419   0.207875   0.353   0.7246
## GenusAcartia     -0.362807   0.229059  -1.584   0.1158
## GenusTemora      -0.463662   0.249114  -1.861   0.0652 .
## GenusOncaea      -0.118877   0.277783  -0.428   0.6695
## GenusOithona     -0.296552   0.237083  -1.251   0.2134
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.3609 on 120 degrees of freedom
## Multiple R-squared:  0.1391, Adjusted R-squared:  0.06735
## F-statistic: 1.939 on 10 and 120 DF,  p-value: 0.04624
```



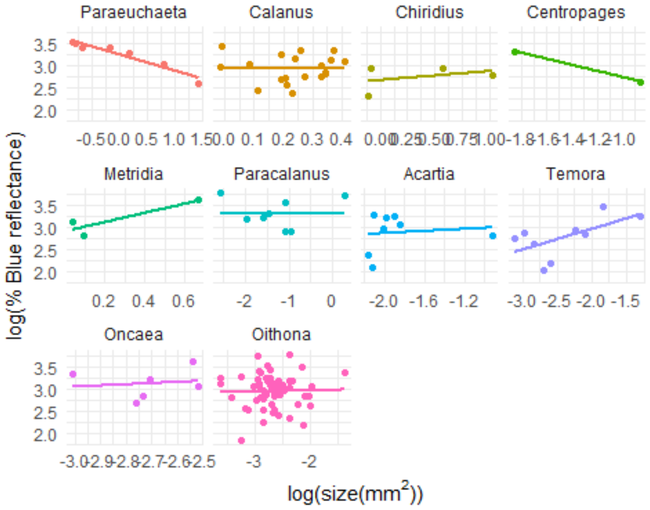
```
summary(BlueSize17.3)
## Call:
## lm(formula = log.Blue ~ log.size, data = d17)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -1.08961 -0.21818  0.01103  0.25975  0.82231
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept)  3.02070    0.05565   54.28   <2e-16 ***
## log.size     0.02689    0.02536    1.06    0.291
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.3736 on 129 degrees of freedom
## Multiple R-squared:  0.008642,   Adjusted R-squared:  0.0009575
## F-statistic: 1.125 on 1 and 129 DF,  p-value: 0.2909
```



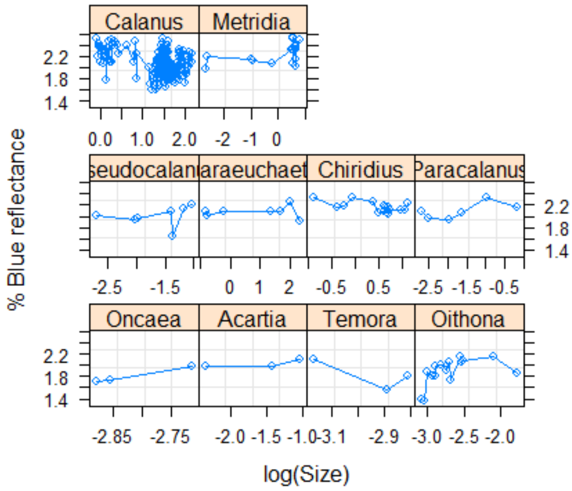
```
summary(BlueSize17.4)
## Call:
## lm(formula = log.Blue ~ Genus, data = d17)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -1.09386 -0.19570  0.04149  0.22723  0.81554
##
## Coefficients:
##                 Estimate Std. Error t value Pr(>|t|)
## (Intercept)      3.25957    0.13587  23.991  < 2e-16 ***
## GenusCalanus    -0.31600    0.15894  -1.988  0.04904 *
## GenusChiridius  -0.50950    0.22531  -2.261  0.02552 *
## GenusCentropages -0.26953    0.28822  -0.935  0.35156
## GenusMetridia   -0.07698    0.24806  -0.310  0.75686
## GenusParacalanus  0.06108    0.18604   0.328  0.74326
## GenusAcartia    -0.38075    0.18604  -2.047  0.04287 *
## GenusTemora     -0.48670    0.18115  -2.687  0.00823 **
## GenusOncaea     -0.14486    0.19999  -0.724  0.47027
## GenusOithona    -0.32209    0.14299  -2.252  0.02610 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.3595 on 121 degrees of freedom
## Multiple R-squared:  0.139,  Adjusted R-squared:  0.07491
## F-statistic:  2.17 on 9 and 121 DF,  p-value: 0.02866
```


Residuals vs Fitted
Residuals
1.0
-1.0
2.8
3.0
3.2
Fitted values
Normal Q-Q
Standardized residuals
0
-3
-2
-1
0
1
2
Theoretical Quantiles
Scale-Location
√|Standardized residuals|
1.5
0.0
2.8
3.0
3.2
Fitted values
Residuals vs Leverage
Standardized residuals
0
-3
63
114
0.5
Cook's distance
0.5
0.0
0.2
0.4
Leverage

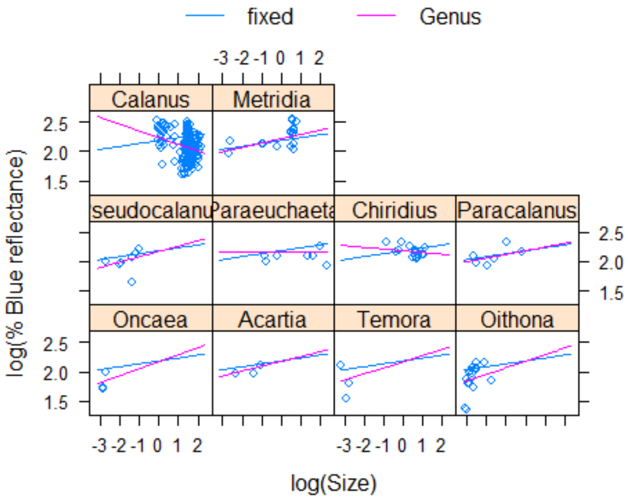

Histogram of resid(BlueSize17.4)
Frequency
30
20
10
5
0
-1.0
-0.5
0.0
0.5
1.0
resid(BlueSize17.4)


Paraeuchaeta
Calanus
Chiridius
Centropages
Metridia
Paracalanus
Acartia
Temora
Oncaea
Oithona
3.5
3.0
2.5
2.0
-0.5 0.0 0.5 1.0 1.5
0.0 0.1 0.2 0.3 0.4
0.00 0.25 0.50 0.75 1.00
-1.8 -1.6 -1.4 -1.2 -1.0
0.2 0.4 0.6
-2 -1 0
-2.0 -1.6 -1.2
-3.0 -2.5 -2.0 -1.5
-3.0 -2.9 -2.8 -2.7 -2.6 -2.5
-3 -2
log(% Blue reflectance)
log(size(mm²))

2018

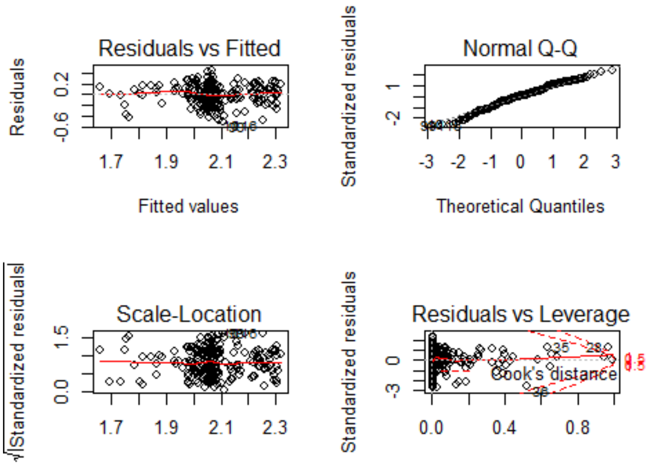


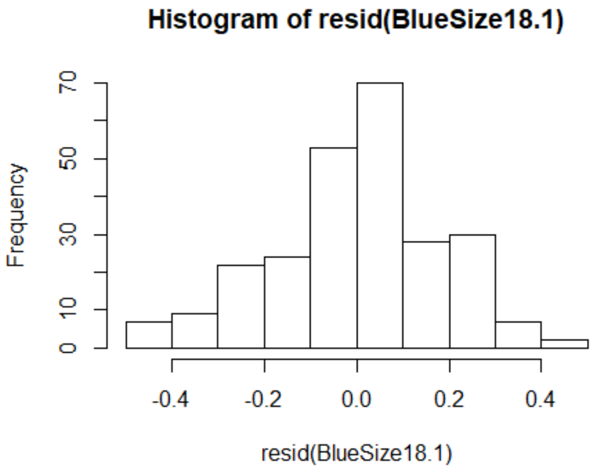
```
summary(m18 <- lme(g18))
## Linear mixed-effects model fit by REML
##  Data: g18
##         AIC       BIC   logLik
##   -83.13684 -62.00808 47.56842
##
## Random effects:
##  Formula: ~log.size | Genus
##  Structure: General positive-definite
##             StdDev     Corr
## (Intercept) 0.04501750 (Intr)
## log.size    0.08553671 -0.234
## Residual    0.18842393
##
## Fixed effects: log.Blue ~ log.size
##                 Value  Std.Error  DF  t-value p-value
## (Intercept) 2.1858615 0.03019899 241 72.38194  0.0000
## log.size    0.0497373 0.03093987 241  1.60755  0.1092
##  Correlation:
##          (Intr)
## log.size 0.035
##
## Standardized Within-Group Residuals:
##           Min            Q1           Med            Q3           Max
## -2.5983786721 -0.5773684543  0.0002728975  0.6780806108  2.3217560703
##
## Number of Observations: 252
## Number of Groups: 10
```



```
anova(m18)

##             numDF denDF  F-value p-value
## (Intercept)     1   241 5237.364  <.0001
## log.size        1   241    2.584  0.1092

BlueSize18.1 = lm(log.Blue ~ log.size * Genus, data = d18)
BlueSize18.2 = lm(log.Blue ~ log.size + Genus, data = d18)
BlueSize18.3 = lm(log.Blue ~ log.size, data = d18)
BlueSize18.4 = lm(log.Blue ~ Genus, data = d18)

AICc(BlueSize18.1, BlueSize18.2, BlueSize18.3, BlueSize18.4)

##              df       AICc
## BlueSize18.1 21 -102.69605
## BlueSize18.2 12  -83.89408
## BlueSize18.3  3  -61.63283
## BlueSize18.4 11  -79.69165

summary(BlueSize18.1)
## Call:
## lm(formula = log.Blue ~ log.size * Genus, data = d18)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.49291 -0.09322  0.00909  0.10838  0.43772
##
## Coefficients:
##                           Estimate Std. Error t value Pr(>|t|)
## (Intercept)                2.073581   0.084055  24.669   <2e-16 ***
## log.size                   0.007492   0.056789   0.132   0.8952
## GenusCalanus               0.202956   0.092184   2.202   0.0287 *
## GenusChiridius             0.128661   0.103582   1.242   0.2154
## GenusMetridia              0.177573   0.095597   1.858   0.0645 .
## GenusParacalanus           0.161754   0.185013   0.874   0.3829
## GenusPseudocalanus         0.049469   0.241532   0.205   0.8379
## GenusAcartia               0.061218   0.347406   0.176   0.8603
## GenusTemora               -3.563943   2.061915  -1.728   0.0852 .
## GenusOncaea                4.691932   4.206075   1.116   0.2658
## GenusOithona               0.698705   0.386241   1.809   0.0717 .
## log.size:GenusCalanus     -0.149598   0.062437  -2.396   0.0174 *
## log.size:GenusChiridius   -0.102537   0.103457  -0.991   0.3227
## log.size:GenusMetridia     0.075591   0.070216   1.077   0.2828
## log.size:GenusParacalanus  0.081750   0.104749   0.780   0.4359
## log.size:GenusPseudocalanus 0.064305  0.141373   0.455   0.6496
## log.size:GenusAcartia      0.067258   0.207068   0.325   0.7456
## log.size:GenusTemora      -1.128684   0.698410  -1.616   0.1074
## log.size:GenusOncaea       1.754930   1.493809   1.175   0.2413
## log.size:GenusOithona      0.323213   0.150331   2.150   0.0326 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
```

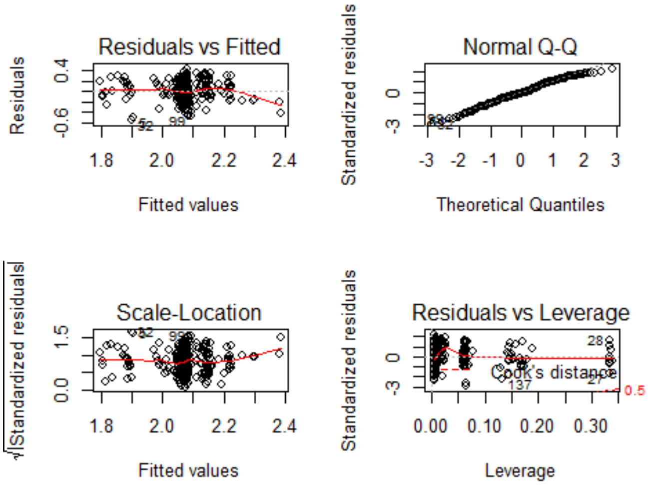
```
## Residual standard error: 0.1877 on 232 degrees of freedom
## Multiple R-squared:  0.2898, Adjusted R-squared:  0.2316
## F-statistic: 4.982 on 19 and 232 DF,  p-value: 7.795e-10
```



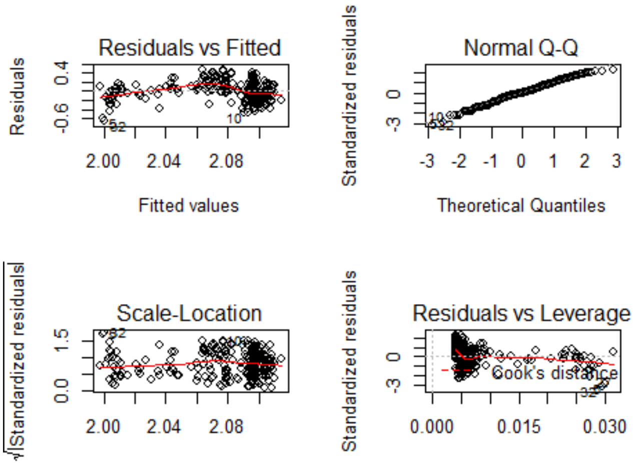


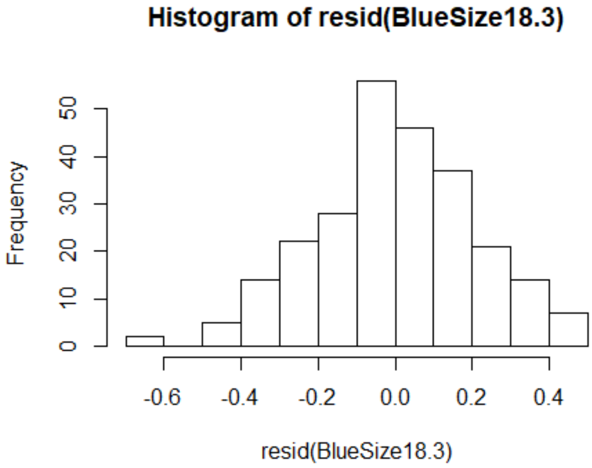
```
summary(BlueSize18.2)
## Call:
## lm(formula = log.Blue ~ log.size + Genus, data = d18)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -0.5493 -0.1071 -0.0058  0.1524  0.4265
##
## Coefficients:
##                     Estimate Std. Error t value Pr(>|t|)
## (Intercept)          2.11928    0.07697  27.532  < 2e-16 ***
## log.size            -0.05011    0.02011  -2.491  0.01340 *
## GenusCalanus         0.03298    0.07763   0.425  0.67135
## GenusChiridius       0.06224    0.08972   0.694  0.48853
## GenusMetridia        0.13082    0.09091   1.439  0.15147
## GenusParacalanus    -0.11495    0.12131  -0.948  0.34431
## GenusPseudocalanus -0.19870    0.11738  -1.693  0.09178 .
## GenusAcartia        -0.18462    0.14569  -1.267  0.20630
## GenusTemora         -0.44401    0.15680  -2.832  0.00502 **
## GenusOncaea         -0.45823    0.15547  -2.947  0.00352 **
## GenusOithona        -0.37036    0.11425  -3.242  0.00136 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.1992 on 241 degrees of freedom
```

```
## Multiple R-squared:  0.1692, Adjusted R-squared:  0.1347
## F-statistic: 4.908 on 10 and 241 DF,  p-value: 1.844e-06
```

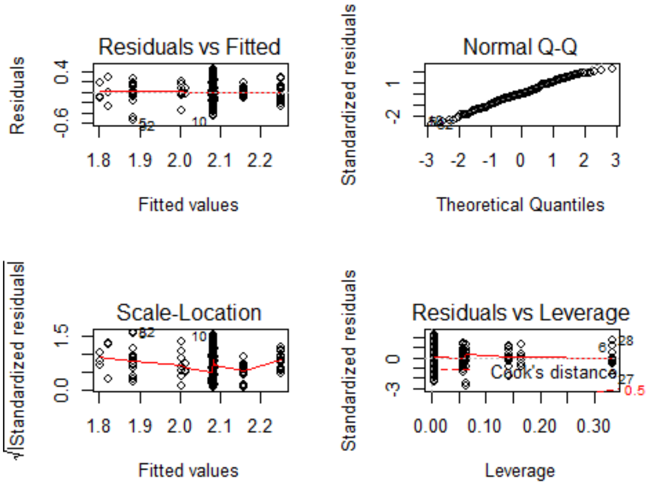


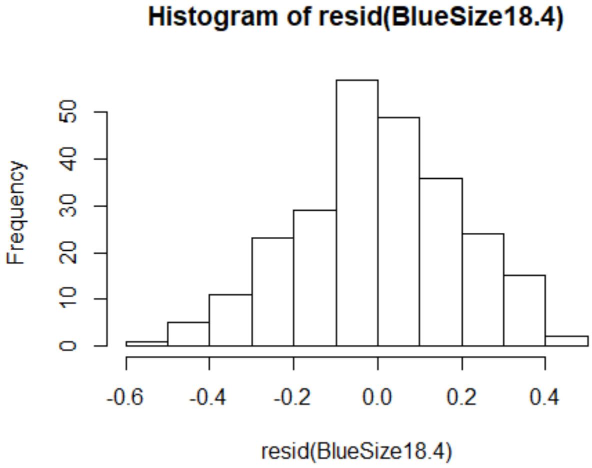
```
summary(BlueSize18.3)
## Call:
## lm(formula = log.Blue ~ log.size, data = d18)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.64835 -0.11959 -0.00142  0.15192  0.46164
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept) 2.064900   0.014612 141.319   <2e-16 ***
## log.size    0.021161   0.009234   2.292   0.0228 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.2124 on 250 degrees of freedom
## Multiple R-squared:  0.02057,    Adjusted R-squared:  0.01666
## F-statistic: 5.251 on 1 and 250 DF,  p-value: 0.02276
```

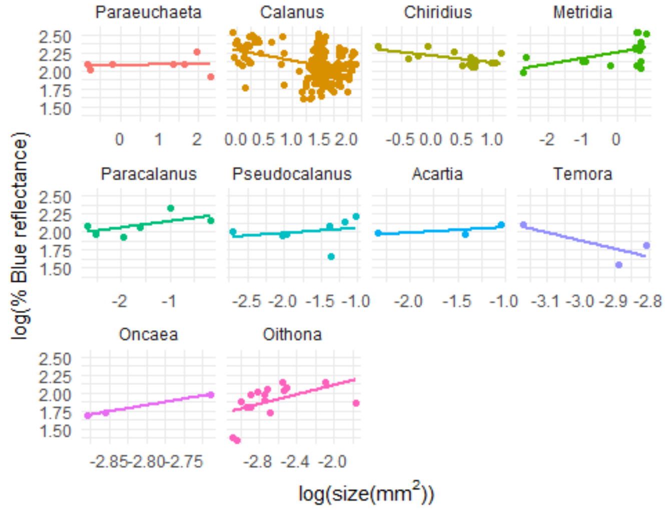




```
summary(BlueSize18.4)
## Call:
## lm(formula = log.Blue ~ Genus, data = d18)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.53143 -0.11432 -0.00038  0.16019  0.43803
##
## Coefficients:
##                   Estimate Std. Error t value Pr(>|t|)
## (Intercept)        2.079525   0.076108  27.323   <2e-16 ***
## GenusCalanus       0.005034   0.077633   0.065   0.9484
## GenusChiridius     0.078884   0.090430   0.872   0.3839
## GenusMetridia      0.170968   0.090430   1.891   0.0599 .
## GenusParacalanus   0.007872   0.112028   0.070   0.9440
## GenusPseudocalanus -0.075722   0.107633  -0.704   0.4824
## GenusAcartia      -0.064542   0.138954  -0.464   0.6427
## GenusTemora       -0.256150   0.138954  -1.843   0.0665 .
## GenusOncaea       -0.277362   0.138954  -1.996   0.0470 *
## GenusOithona      -0.195943   0.091250  -2.147   0.0328 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.2014 on 242 degrees of freedom
## Multiple R-squared:  0.1478, Adjusted R-squared:  0.1161
## F-statistic: 4.663 on 9 and 242 DF,  p-value: 1.038e-05
```

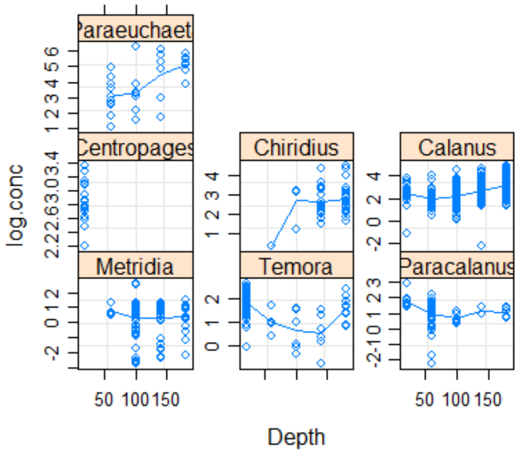

Residuals vs Fitted
Residuals
0.4
-0.6
1.8 1.9 2.0 2.1 2.2
Fitted values
52
10
Normal Q-Q
Standardized residuals
1
-2
-3 -2 -1 0 1 2 3
Theoretical Quantiles
Scale-Location
√|Standardized residuals|
1.5
0.0
1.8 1.9 2.0 2.1 2.2
Fitted values
52
10
Residuals vs Leverage
Standardized residuals
0
-3
0.00 0.10 0.20 0.30
Leverage
Cook's distance
28
6
27
0.5


Histogram of resid(BlueSize18.4)
Frequency
0
10
20
30
40
50
-0.6 -0.4 -0.2 0.0 0.2 0.4
resid(BlueSize18.4)

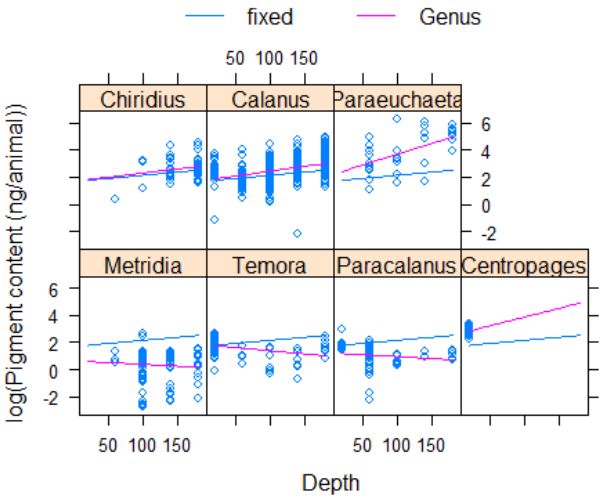

Paraeuchaeta
Calanus
Chiridius
Metridia
2.50
2.25
2.00
1.75
1.50
0 1 2
0.0 0.5 1.0 1.5 2.0
-0.5 0.0 0.5 1.0
-2 -1 0
Paracalanus
Pseudocalanus
Acartia
Temora
-2 -1
-2.5 -2.0 -1.5 -1.0
-2.0 -1.5 -1.0
-3.1 -3.0 -2.9 -2.8
Oncaea
Oithona
-2.85-2.80-2.75
-2.8 -2.4 -2.0
log(% Blue reflectance)
log(size(mm²))

Appendix C

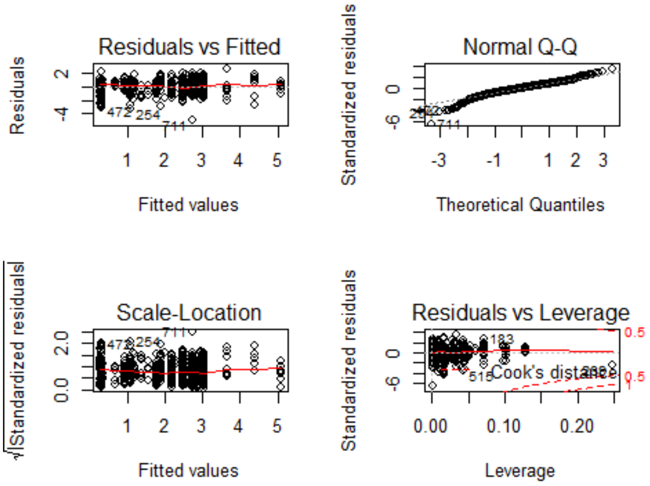
Additional models and plots, pigment content over depth

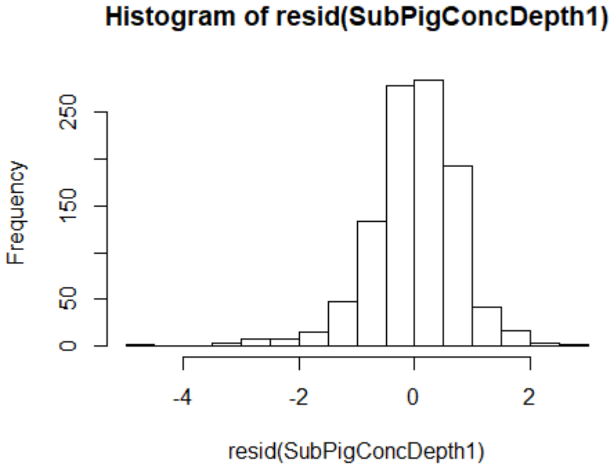


```
summary(m2 <- lme(g2))
## Linear mixed-effects model fit by REML
##  Data: g2
##       AIC      BIC   logLik
##   2445.32 2474.955 -1216.66
##
## Random effects:
##  Formula: ~Depth | Genus
##  Structure: General positive-definite
##             StdDev      Corr
## (Intercept) 0.649177802 (Intr)
## Depth       0.008785049 0.557
## Residual    0.764140875
##
## Fixed effects: log.conc ~ Depth
##                 Value  Std.Error   DF  t-value p-value
## (Intercept) 1.6962239 0.26524466 1026 6.394941  0.0000
## Depth       0.0046042 0.00359521 1026 1.280657  0.2006
##  Correlation:
##       (Intr)
## Depth 0.369
##
## Standardized Within-Group Residuals:
##         Min          Q1         Med          Q3         Max
## -6.45264883 -0.50554851  0.06981485  0.65999869  3.35470830
##
## Number of Observations: 1034
## Number of Groups: 7
```

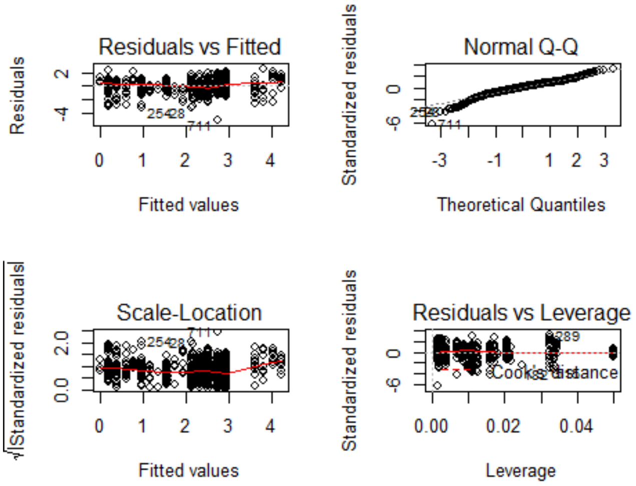


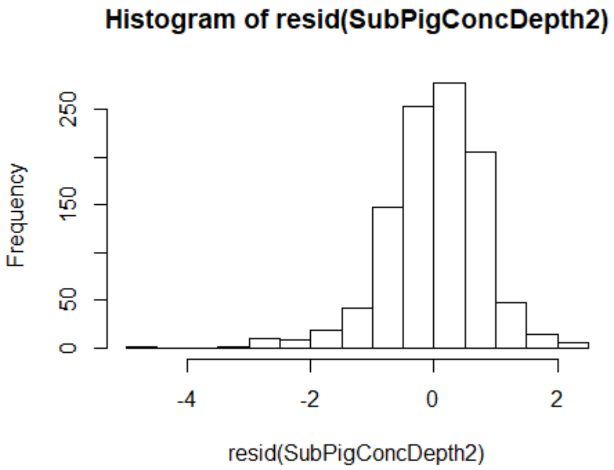
```
SubPigConcDepth1 = lm(log.conc ~ Depth * Genus, data = d22)
SubPigConcDepth2 = lm(log.conc ~ Depth + Genus, data = d22)
SubPigConcDepth3 = lm(log.conc ~ Depth, data = d22)
SubPigConcDepth4 = lm(log.conc ~ Genus, data = d22)

AICc(SubPigConcDepth1, SubPigConcDepth2, SubPigConcDepth3, SubPigConcDepth4)

##                  df     AICc
## SubPigConcDepth1 14 2393.807
## SubPigConcDepth2  9 2480.839
## SubPigConcDepth3  3 3156.449
## SubPigConcDepth4  8 2558.925

summary(SubPigConcDepth1)
## Call:
## lm(formula = log.conc ~ Depth * Genus, data = d22)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -4.9309 -0.3900  0.0536  0.4945  2.5873
##
## Coefficients: (1 not defined because of singularities)
##                          Estimate Std. Error t value Pr(>|t|)
## (Intercept)              1.904072   0.355165   5.361 1.02e-07 ***
## Depth                    0.017645   0.002935   6.012 2.55e-09 ***
## GenusCalanus            -0.158610   0.364904  -0.435  0.66390
## GenusChiridius          -0.310081   0.696285  -0.445  0.65617
## GenusCentropages         0.622850   0.346901   1.795  0.07287 .
## GenusMetridia           -1.524788   0.474459  -3.214  0.00135 **
## GenusParacalanus        -0.636202   0.407097  -1.563  0.11842
## GenusTemora             -0.014095   0.367032  -0.038  0.96937
## Depth:GenusCalanus      -0.010470   0.002999  -3.492  0.00050 ***
## Depth:GenusChiridius    -0.010740   0.004760  -2.257  0.02425 *
## Depth:GenusCentropages         NA         NA      NA       NA
## Depth:GenusMetridia     -0.018189   0.003846  -4.730 2.56e-06 ***
## Depth:GenusParacalanus  -0.020853   0.003788  -5.505 4.67e-08 ***
## Depth:GenusTemora       -0.022836   0.003253  -7.019 4.07e-12 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.7643 on 1021 degrees of freedom
## Multiple R-squared:  0.5748, Adjusted R-squared:  0.5698
## F-statistic:   115 on 12 and 1021 DF,  p-value: < 2.2e-16
```



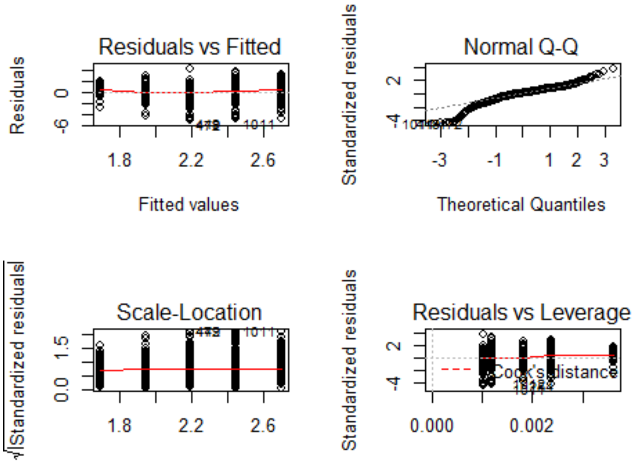


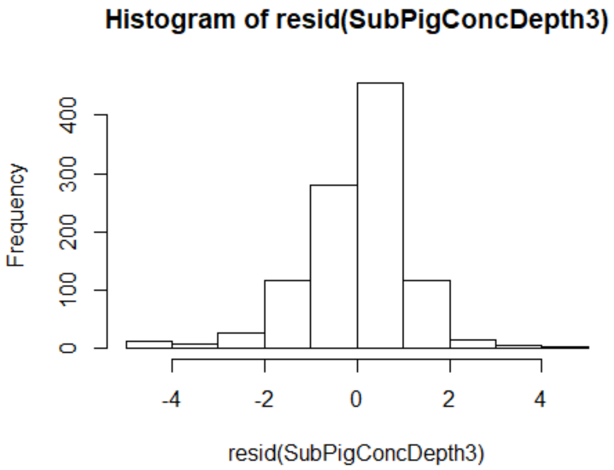
```
summary(SubPigConcDepth2)
## Call:
## lm(formula = log.conc ~ Depth + Genus, data = d22)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -4.9026 -0.4307  0.0500  0.5257  2.4400
##
## Coefficients:
##                   Estimate Std. Error t value Pr(>|t|)
## (Intercept)       3.3198652  0.1559117  21.293  < 2e-16 ***
## Depth             0.0049599  0.0005455   9.092  < 2e-16 ***
## GenusCalanus     -1.2926926  0.1471243  -8.786  < 2e-16 ***
## GenusChiridius   -1.4203317  0.1850712  -7.675 3.85e-14 ***
## GenusCentropages -0.5392456  0.2345859  -2.299   0.0217 *
## GenusMetridia    -3.6151284  0.1656221 -21.828  < 2e-16 ***
## GenusParacalanus -2.6458140  0.1766035 -14.982  < 2e-16 ***
## GenusTemora      -1.8697583  0.1652589 -11.314  < 2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.7991 on 1026 degrees of freedom
## Multiple R-squared:  0.5328, Adjusted R-squared:  0.5296
## F-statistic: 167.2 on 7 and 1026 DF,  p-value: < 2.2e-16
```



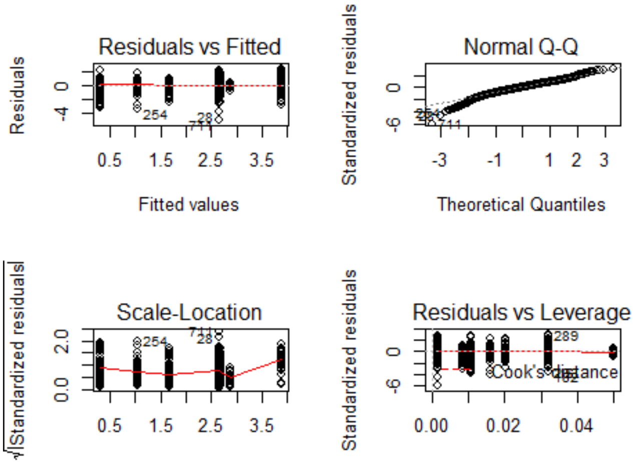


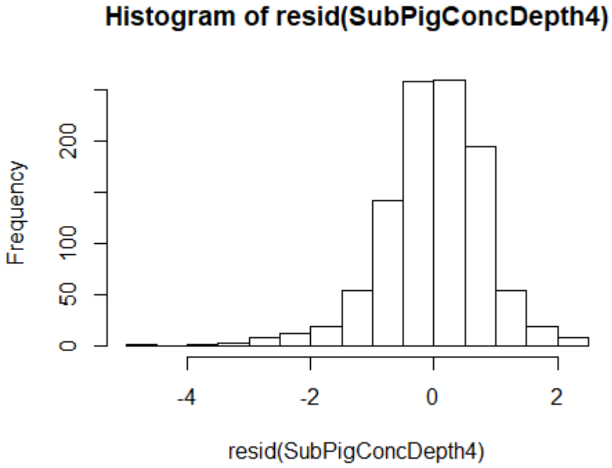
```
summary(SubPigConcDepth3)
## Call:
## lm(formula = log.conc ~ Depth, data = d22)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -4.9174 -0.4430  0.1454  0.6503  4.0627
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept) 1.5639400  0.0776309   20.15   <2e-16 ***
## Depth       0.0062917  0.0006173   10.19   <2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 1.111 on 1032 degrees of freedom
## Multiple R-squared:  0.09145,    Adjusted R-squared:  0.09057
## F-statistic: 103.9 on 1 and 1032 DF,  p-value: < 2.2e-16
```

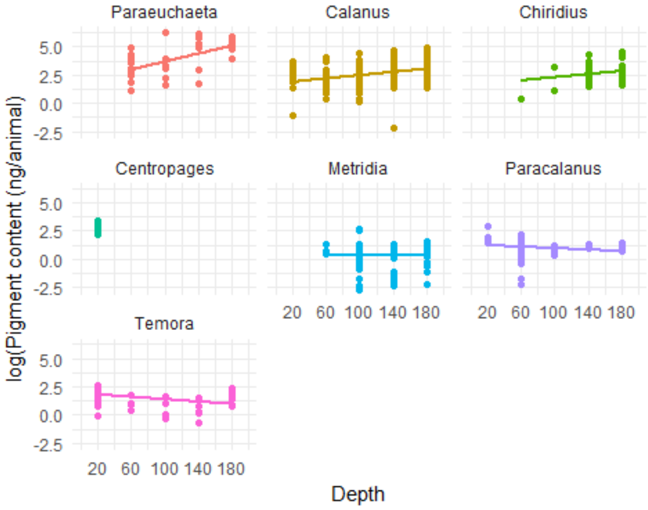




```
summary(SubPigConcDepth4)
## Call:
## lm(formula = log.conc ~ Genus, data = d22)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -4.8392 -0.4570  0.0459  0.5351  2.3824
##
## Coefficients:
##                  Estimate Std. Error t value Pr(>|t|)
## (Intercept)        3.8735     0.1491  25.974  < 2e-16 ***
## GenusCalanus      -1.2153     0.1526  -7.964 4.41e-15 ***
## GenusChiridius    -1.1945     0.1905  -6.269 5.35e-10 ***
## GenusCentropages  -0.9936     0.2381  -4.173 3.27e-05 ***
## GenusMetridia     -3.5609     0.1720 -20.706  < 2e-16 ***
## GenusParacalanus  -2.8388     0.1822 -15.584  < 2e-16 ***
## GenusTemora       -2.2084     0.1673 -13.202  < 2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.8303 on 1027 degrees of freedom
## Multiple R-squared:  0.4952, Adjusted R-squared:  0.4922
## F-statistic: 167.9 on 6 and 1027 DF,  p-value: < 2.2e-16
```

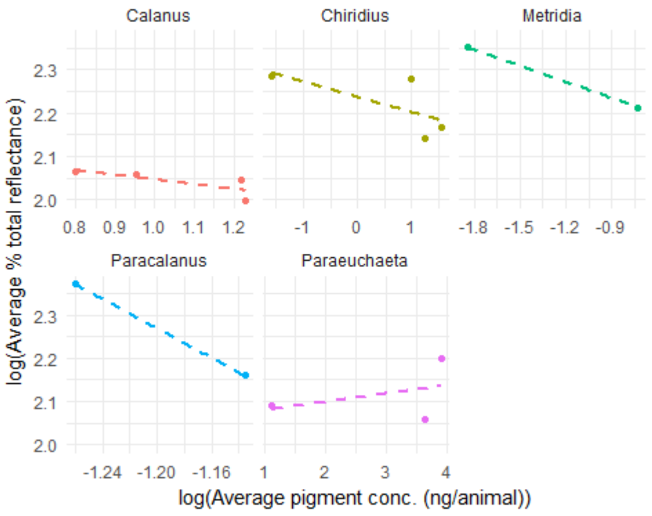

Residuals vs Fitted
Residuals
0
-4
254
28
711
0.5
1.5
2.5
3.5
Fitted values
Normal Q-Q
Standardized residuals
0
-6
254
711
-3
-1
1
2
3
Theoretical Quantiles
Scale-Location
√|Standardized residuals|
2.0
0.0
254
711
28
0.5
1.5
2.5
3.5
Fitted values
Residuals vs Leverage
Standardized residuals
0
-6
289
Cook's distance
162
0.00
0.02
0.04
Leverage

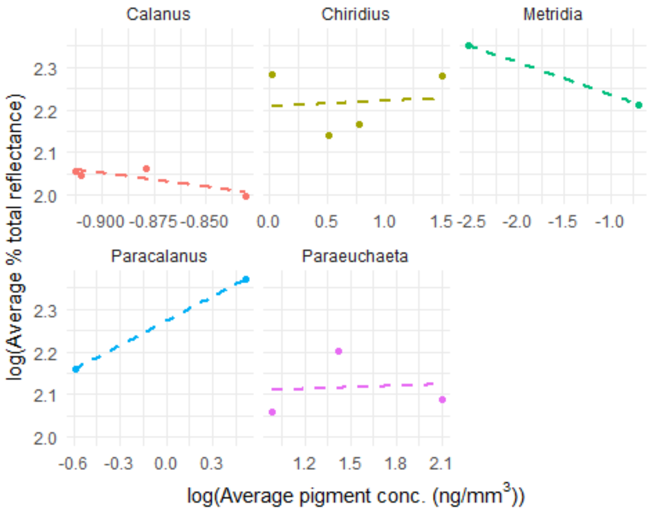

Histogram of resid(SubPigConcDepth4)
Frequency
0
50
100
200
-4
-2
0
2
resid(SubPigConcDepth4)


Paraeuchaeta
Calanus
Chiridius
Centropages
Metridia
Paracalanus
Temora
log(Pigment content (ng/animal))
5.0
2.5
0.0
-2.5
20
60
100
140
180
Depth

Appendix D

Additional models and plots, apparent pigmentation and actual pigment concentrations



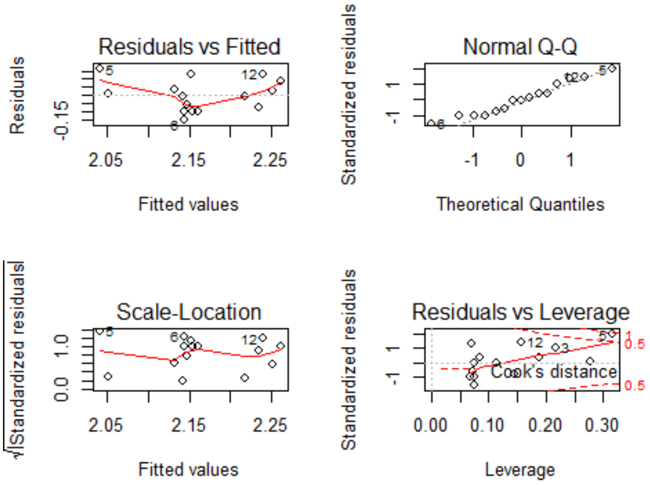


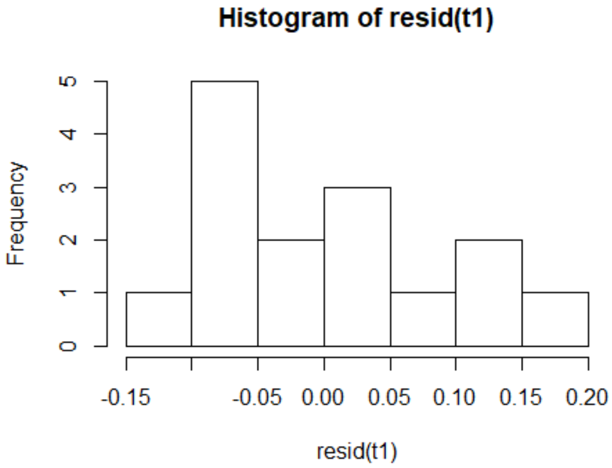
```
t1 = lm(log(avg_Total_Ref_Pct) ~ log(avg_ng_pig), data = CombinedDf)

summary(t1)
## Call:
## lm(formula = log(avg_Total_Ref_Pct) ~ log(avg_ng_pig), data = CombinedDf)
##
## Residuals:
##       Min        1Q    Median        3Q       Max
## -0.146863 -0.085751 -0.002992  0.062251  0.159880
##
## Coefficients:
##                 Estimate Std. Error t value Pr(>|t|)
```

```
## (Intercept)      2.19085    0.02765  79.231   <2e-16 ***
## log(avg_ng_pig) -0.03840    0.01538  -2.498   0.0267 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.0993 on 13 degrees of freedom
## Multiple R-squared:  0.3243, Adjusted R-squared:  0.2723
## F-statistic: 6.238 on 1 and 13 DF,  p-value: 0.02671
```

```
anova(t1)
```

```
## Analysis of Variance Table
##
## Response: log(avg_Total_Ref_Pct)
##                 Df   Sum Sq  Mean Sq F value  Pr(>F)
## log(avg_ng_pig)  1 0.061512 0.061512  6.2384 0.02671 *
## Residuals       13 0.128183 0.009860
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```



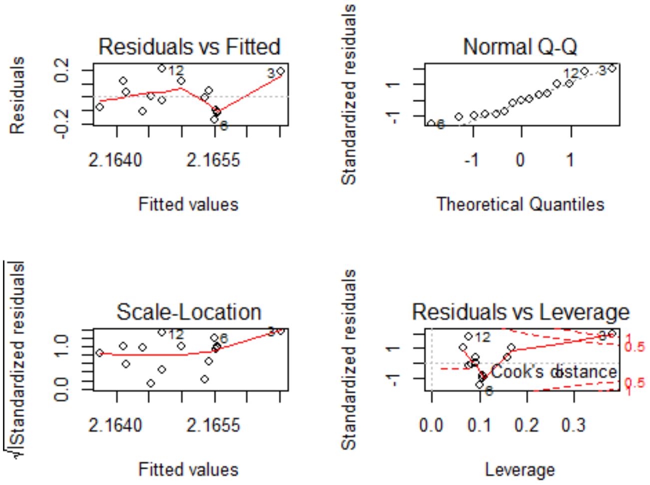


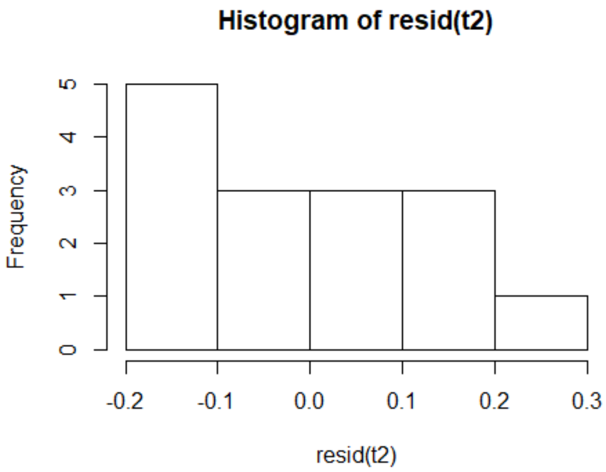
```
t2 = lm(log(avg_Total_Ref_Pct) ~ log(avg_ng_pig_spec), data = CombinedDf)

summary(t2)
## Call:
## lm(formula = log(avg_Total_Ref_Pct) ~ log(avg_ng_pig_spec), data = CombinedDf)
##
## Residuals:
##       Min        1Q    Median        3Q       Max
## -0.168662 -0.104179 -0.005621  0.081278  0.205754
```

```
##
## Coefficients:
##                      Estimate Std. Error t value Pr(>|t|)
## (Intercept)          2.165005   0.031200  69.391   <2e-16 ***
## log(avg_ng_pig_spec) -0.000597   0.026254  -0.023    0.982
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.1208 on 13 degrees of freedom
## Multiple R-squared:  3.978e-05,  Adjusted R-squared:  -0.07688
## F-statistic: 0.0005171 on 1 and 13 DF,  p-value: 0.9822
```

```
anova(t2)
```

```
## Analysis of Variance Table
##
## Response: log(avg_Total_Ref_Pct)
##                      Df   Sum Sq   Mean Sq F value Pr(>F)
## log(avg_ng_pig_spec)  1 0.000008 0.0000075   5e-04 0.9822
## Residuals            13 0.189687 0.0145913
```





Appendix E

Arduino scripts

Calibration scripts for the light caps

```
#include <Adafruit_NeoPixel.h>
#include <YesNoIO_rgbl.h>
#ifdef __AVR__
#include <avr/power.h>
#endif
#define PIN 6
#define NUMPIX 20
Adafruit_NeoPixel strip = Adafruit_NeoPixel(NUMPIX, PIN, NEO_GRB + NEO_KHZ800);

//Here I define the different colour intensities as colour codes, ranging from 5-255 in steps of 50.
#define OFF 0,0,0
#define White 255,255,255
#define Red 255,0,0
#define Red205 205,0,0
#define Red155 155,0,0
#define Red105 105,0,0
#define Red55 55,0,0
#define Red5 5,0,0
#define Green 0,255,0
#define Green205 0,205,0
#define Green155 0,155,0
#define Green105 0,105,0
#define Green55 0,55,0
#define Green5 0,5,0
#define Blue 0,0,255
#define Blue205 0,0,205
#define Blue155 0,0,155
#define Blue105 0,0,105
#define Blue55 0,0,55
#define Blue5 0,0,5

//This command resets the NeoPixels.
void setup() {
  // put your setup code here, to run once:
strip.begin();
strip.show();
}

//I tested five randomised light caps. I tested all colour intensities in combination with overall intensity settings ranging as the colour intensities ranging between 5-255 in steps of 50.
void loop() {
  // put your main code here, to run repeatedly:
strip.setPixelColor(NeoPixel number, rgbl(Colour code,Overall intensity)); //sets the colour and intensity of a specific Neopixel
strip.show(); //starts the colour command
delay(20000); //runtime in on mode (milliseconds)
strip.setPixelColor(14, rgbl(OFF,0)); //turns off the NeoPixel
strip.show(); //starts the off command
delay(10000); //runtime in off mode (milliseconds)
}
```

Light setup trial run

```
#include <Adafruit_NeoPixel.h>
#include <YesNoIO_rgbl.h>
#ifdef __AVR__
#include <avr/power.h>
#endif

#define PIN 6
#define NUMPIX 20
Adafruit_NeoPixel strip = Adafruit_NeoPixel(NUMPIX, PIN, NEO_GRB + NEO_KHZ800);

#define OFF 0,0,0
#define White 255,255,255

void setup() {
  // put your setup code here, to run once:
strip.begin();
strip.show();
}

void loop() {
  // put your main code here, to run repeatedly:
strip.setPixelColor(0, rgbl(White,0));
strip.show();

strip.setPixelColor(1, rgbl(White,0));
strip.show();

strip.setPixelColor(2, rgbl(White,0));
strip.show();

strip.setPixelColor(3, rgbl(White,0));
strip.show();

strip.setPixelColor(4, rgbl(White,31));
strip.show();

strip.setPixelColor(5, rgbl(White,31));
strip.show();

strip.setPixelColor(6, rgbl(White,31));
strip.show();

strip.setPixelColor(7, rgbl(White,31));
strip.show();

strip.setPixelColor(8, rgbl(White,63));
strip.show();

strip.setPixelColor(9, rgbl(White,63));
strip.show();

strip.setPixelColor(10, rgbl(White,63));
strip.show();

strip.setPixelColor(11, rgbl(White,63));
strip.show();

strip.setPixelColor(12, rgbl(White,127));
strip.show();

strip.setPixelColor(13, rgbl(White,127));
strip.show();

strip.setPixelColor(14, rgbl(White,127));
```

```
strip.show();

strip.setPixelColor(15, rgbl(White,127));
strip.show();

strip.setPixelColor(16, rgbl(White,255));
strip.show();

strip.setPixelColor(17, rgbl(White,255));
strip.show();

strip.setPixelColor(18, rgbl(White,255));
strip.show();

strip.setPixelColor(19, rgbl(White,255));
strip.show();
}
```