Energy Reserves of the Cold-water Coral *Desmophyllum dianthus* at Different Water Depths in Comau Fjord, Chile

Energiereserven der Kaltwasserkoralle *Desmophyllum dianthus* in verschiedenen Wassertiefen im Comau Fjord (Chile)

Bachelorarbeit

zur Erlangung des akademischen Grades

Bachelor of Science (B. Sc.) im Fach Biologie

angefertigt in der Arbeitsgruppe Bentho-Pelagische Prozesse am

Alfred-Wegener-Institut Helmholtz Zentrum für Polar- und Meeresforschung

Eingereicht von Carina Krämer

Matrikelnummer: 4588863

1. Gutachter: Prof. Dr. Claudio Richter
2. Gutachter: Dr. Hans-Konrad Nettmann

Bremen, den 17.02.2023

1. Betreuerin: Dr. Marlene Wall
2. Betreuerin. Dr. Kristina Beck

Table of contents

Summary

Cold-water corals are abundant worldwide and important habitat builders. However, *in situ* physiological assessments are still scarce compared to tropical corals, because of the difficult accessibility of their natural deep habitat. Deep-water emergent cold-water corals, like *Desmophyllum dianthus* in Comau Fjord (Chile), therefore offer a great opportunity for *in situ* assessments as these corals are more easily accessible. Cold-water corals are influenced by several abiotic factors as well as food supply, and the variability of these factors. In this context, energy reserves can provide information about the coral's metabolism and how they cope with strenuous conditions and limited energy supply.

The purpose of this study was to determine if there are differences in the energy reserves of *D. dianthus* in different water depths in Comau Fjord (Chile). Previous work has provided first indications that corals at deeper water depth foster higher energy reserves as well as higher calcification rates, correlating with lower environmental variability and potentially higher food supply, as well as less competition.

For this study, coral samples were taken from six stations along the fjord (head to mouth) with three shallow stations (approx. 25 m) and three deep stations (100 - 200 m). Energy reserves (protein, carbohydrate and lipid content) were determined from grounded coral samples and normalised to biomass calculated from ash-free dry weight.

Unexpectedly and in contrast to the previous study, energy reserves of *D. dianthus* did not differ between water depths. Recalculating the energy content of the previous study by normalising to biomass revealed a similar result and no clear correlation with depth. The reason for these different results depending on the reference value was found in the strongly differing amount of biomass per surface area between depths. Approximate calculation of energy reserves per surface area for three stations of the present study using data collected at the same time and in the same population for different individuals showed the previously found higher energy reserves in deep corals.

Energy reserves did not differ along a horizontal gradient, but interestingly among the shallow stations highest energy reserves were found at the station in the centre of the fjord. However, they also had the smallest tissue covered surface area. The nearby salmon farm was identified as a possible cause for these mixed traits.

Zusammenfassung

Kaltwasserkorallen sind weltweit verbreitet und wichtige Lebensraumgestalter. Im Vergleich zu tropischen Korallen gibt es jedoch nur wenige physiologische *In-situ* Untersuchungen, da ihr natürlicher Lebensraum in der Tiefe schwer zugänglich ist. Kaltwasserkorallen, die auch in sehr geringer Tiefe vorkommen können wie *Desmophyllum dianthus* im Comau Fjord (Chile), bieten daher eine gute Gelegenheit für diese Korallen näher *in situ* zu untersuchen, da sie leichter zugänglich sind. Kaltwasserkorallen werden durch verschiedene abiotische Faktoren sowie durch das Nahrungsangebot und die Variabilität dieser Faktoren beeinflusst. In diesem Zusammenhang können die Energiereserven Aufschluss über den Stoffwechsel der Korallen geben und darüber, wie sie mit anstrengenden Bedingungen und begrenzter Energieversorgung zurechtkommen.

Ziel dieser Studie war es, festzustellen, ob es Unterschiede in den Energiereserven von *D. dianthus* in verschiedenen Wassertiefen im Comau Fjord (Chile) gibt. Frühere Arbeiten lieferten erste Hinweise darauf, dass Korallen in größerer Wassertiefe höhere Energiereserven sowie höhere Kalzifizierungsraten aufweisen, was mit geringeren Umweltschwankungen und einem potenziell höheren Nahrungsangebot sowie geringerer Konkurrenz korreliert sein könnte.
Für diese Studie wurden Korallenproben an sechs Stationen entlang des Fjords (vom Kopf bis zur Mündung) entnommen, davon drei in geringer Tiefe (ca. 25 m) und drei in großer Tiefe (100 - 200 m). Die Energiereserven (Protein-, Kohlenhydrat- und Lipidgehalt) wurden aus den gemahlenen Korallenproben bestimmt und auf die anhand des aschefreien Trockengewichts berechnete Biomasse normiert.
Unerwarteterweise und im Gegensatz zur vorherigen Studie unterschieden sich die Energiereserven von *D. dianthus* nicht zwischen den Wassertiefen. Die Neuberechnung des Energiegehalts der früheren Studie durch Normalisierung auf die Biomasse ergab ein ähnliches Ergebnis und keine eindeutige Korrelation mit der Tiefe. Der Grund für diese unterschiedlichen Ergebnisse je nach Referenzwert lag in der stark unterschiedlichen Menge an Biomasse pro Fläche in den verschiedenen Wassertiefen. Eine annähernde Berechnung der Energiereserven pro Oberfläche für drei Stationen der vorliegenden Studie unter Verwendung von Daten, die zur gleichen Zeit und in der gleichen Population für andere Individuen gesammelt wurden, zeigte die zuvor gefundenen höheren Energiereserven in tiefen Korallen.

Die Energiereserven unterschieden sich nicht entlang eines horizontalen Gradienten, aber interessanterweise wurden unter den flachen Stationen die höchsten Energiereserven an der Station in der Mitte des Fjords gefunden. Allerdings wiesen diese auch die kleinste mit Gewebe bedeckte Oberfläche auf. Die nahegelegene Lachsfarm wurde als mögliche Ursache für diese auf den ersten Blick leicht widersprüchlichen Merkmale ausgemacht.

1. Introduction

1.1 Cold-water corals

Cold-water corals (CWC) are widely distributed worldwide and inhabit a wide range of habitats, from tropical to polar and shallow to deep-waters (Freiwald et al. 2004, Roberts et al. 2006). Some species occur in very shallow water depth, a phenomenon called deep-water emergence (Häussermann et al., 2021), e.g. in fjord systems in New Zealand (Grange et al., 1981), Norway (Rapp & Sneli, 1999) and Chilean Patagonia (Försterra & Häussermann, 2003). They mainly occur along continental shelves, around offshore submarine banks and seamounts and in rugged topographies like fjords and canyons (Freiwald & Roberts, 2005). Their occurrence seems to be heavily influenced by highly productive surface waters, ambient current strength and elevated hard substrate for settlement (van Rooij et al. 2003; Kiriakoulakis et al., 2004). Several CWC species form large and complex reefs, comparable to tropical coral reefs in size, complexity and biodiversity (Henry & Roberts, 2017). These CWC reefs in the deep sea provide an important 3-dimensional habitat for various deep reef associated species, such as fish and benthic invertebrates (Freiwald et al., 2004; Roberts et al., 2006).

1.2 Influence of abiotic factors on CWC

CWCs are influenced by several abiotic factors like pH, temperature, oxygen content and salinity (e.g. Guinotte et al., 2006; Dullo et al., 2008; Hennige et al., 2014, Naumann et al., 2014; Gori et al., 2016; Chapron et al., 2021). This also means that they are affected by environmental changes like ocean acidification and global warming (e.g. Roberts et al., 2009; Roberts & Cairns, 2014; Mora et al., 2013; Doney et al., 2009). Several previous studies have already shown that CWCs are not as sensible to e.g. ocean acidification (Maier et al., 2013; Maier et al., 2012; Hennige et al., 2014; Wall et al., 2015; Beck et al., 2022) and temperature changes (Dodds et al., 2007; Naumann et al., 2014) as previously thought. However, compared to tropical corals, *in situ* physiological assessments of CWCs are still scarce, mostly due to the difficult accessibility of their natural habitat. Therefore, CWCs which occur in shallow waters like *Desmophyllum dianthus* in Comau Fjord offer a great opportunity to study their physiological traits and response to environmental parameters and changes.

So far, the physiological response of CWCs to environmental factors and changes has mostly been investigated in controlled laboratory experiments. However, *in situ* measurements showed that the environment of CWCs is far less uniform than previously thought as temperature, salinity, oxygen and pH vary seasonally and even daily in response to tides, internal waves and advection (Mienis et al., 2007, 2014; Findlay et al., 2013; Georgian et al., 2016; Juva et al., 2021). It has already been shown that the environmental variability affects the physiological response of phytoplankton and mussels (Bernhardt et al., 2018; Morash et al., 2018; Marshall et al., 2021), for example decreasing growth rates under variable compared to constant temperatures were found (Bernhardt et al., 2018; Marshall et al., 2021). Recently it was shown that the CWC *D. dianthus* seems to be negatively influenced by higher environmental variability as well (Beck et al., 2022, in prep.), highlighting the importance of considering environmental variability in future research.

1.3 Food supply

In contrast to tropical corals, CWCs do not have photosynthetic algal symbionts (zooxanthellae) and therefore completely rely on heterotrophy. They feed on a wide range of food sources, like zooplankton, bacteria, phytoplankton and even dissolved organic matter, with zooplankton being considered the most important food source (Naumann et al., 2011, Mueller et al., 2014, Maier et al., 2021). As sessile suspension feeders, they are dependent on external food supply that sinks down from surface waters or reaches them by current. This food supply can be very variable, as it depends on various factors like hydrodynamic conditions and seasonal cycles. Hydrodynamic processes like rapid downwelling surface water (Davies et al., 2009; Duineveld et al., 2012), internal waves (Mienis et al., 2007) and Ekman transport (Thiem et al., 2006) can locally and temporarily enhance food availability, as well as seasonal cycles like temporal mismatch between phytoplankton and zooplankton production during the spring bloom (Thiem et al., 2006; Duineveld et al., 2007; Davies et al., 2009). On the other hand, stronger stratification of the water column in summer and lower primary production in winter (Duineveld et al., 2004, 2007; Lavaleye et al., 2009) diminish food supply to the CWCs.

1.4 Energy reserves

Energy reserves may be an important factor enablinging CWCs to deal with environmental changes and enable them to deal with periods of low food availability (Anthony et al., 2009; Maier et al., 2013; Hennige et al., 2014). During periods of high food availability, corals can build up energy reserves from excess energy that is not needed for metabolic requirements and use these in times of reduced food availability (Maier et al., 2019). While some CWC species tolerate low food availability for several months (Larsson et al., 2013; Baussant et al., 2017; Maier et al., 2019), long-term or complete food deprivation will negatively affect their metabolism and calcification (Naumann et al., 2011; Larsson et al., 2013; Baussant et al. 2017). In order to deal with food deprivation, CWCs can either deplete their energy reserves (Chapron et al., 2021) or downregulate their metabolism to prevent the depletion of their energy reserves (Dodds et al., 2009). Additionally, as corals have to divide their metabolic energy between physiological processes like maintenance, growth, reproduction and competition, energy reserves may vary despite constant food supply (Leuzinger et al., 2012). Therefore, energy reserves can provide information about the coral's metabolism and how they cope with limited energy supply and strenuous conditions.

The total energy reserves consist of lipids, proteins and carbohydrates. Lipids are the major component and can make up up to 40% of the dry biomass and are used for long-time energy storage (Harland et al, 1993; Imbs 2013; Seemann et al., 2013). They provide about twice as much metabolic energy per gram than proteins or carbohydrates (Bureau et al., 2002).

Energy reserves in CWCs have not been studied as detailed yet, only recently studies on the total energy reserves of CWC were carried out (Chapron et al., 2021; Beck et al., in prep.), while studies before focused only on lipid analysis investigating total lipid content (Larsson et al., 2013; Movilla et al. 2014; Baussant et al., 2017) or lipid composition (Dodds et al., 2009; Naumann et al., 2015; Gori et al., 2018; Maier et al., 2019, 2020).

1.5 *Desmophyllum dianthus* in Comau Fjord

The study organism of the present study is the cosmopolitan, azooxanthellate scleractinian CWC *D. dianthus* (Esper 1794). It occurs mostly between 25-2500 m water depth (Cairns et al., 2005) and is a solitary coral that can also form pseudocolonies as a result of secondary recruitment, i.e. younger corals growing on the basal portion of the skeleton of older ones (Försterra & Häusermann, 2003). The polyps can be up to 40 cm in height with a growth rate of 2.2-10 mm per year (Jantzen et al., 2013). *D. dianthus* is the most abundant CWC species in Patagonian Fjords and regularly occurs in large assemblages of up to 1500 individuals/m^2 in depths below 25 m, with some individuals occurring as shallow as 8 m (Försterra & Häussermann, 2003; Häussermann & Försterra 2007). It mainly grows at vertical and overhanging walls with the calyx oriented downward, presumably to prevent negative effects of sedimentation (Försterra & Häussermann, 2003; Cairns et al., 2005; Försterra et al., 2005).

The coral samples for this study were collected in Comau Fjord, which is located in Northern Chilean Patagonia. This fjord is generally very remote with little civilization, however in the early 2000s the region was discovered for salmon aquaculture. Currently four salmon farms are active within the fjord (M. Wall, pers. comm.), but many more farms are present (23 salmon farms and 9 mussel farms in 2012; Häussermann et al., 2013). This fjord is about 41 km long, 2-8 km wide, about max. 480 m deep and has a high tidal range of up to 7.5 m (Häussermann & Försterra, 2009). It is a naturally strongly stratified fjord with a low salinity surface layer in the upper 7-8 m, due to high precipitation and freshwater runoff from rivers (Häussermann & Försterra 2009, Fillinger & Richter 2013). The water column is also stratified with respect to pH, which is highest in the upper 12-20 m, where the maximum pH value of 8.42 was measured, then sharply decreases between 22 and 50 m and reaches a minimum pH value of 7.71 below 300 m (Fillinger & Richter, 2013; Beck et al., 2022). Compared to this relatively strong vertical gradient, there is a weaker horizontal gradient of decreasing pH from fjord mouth to head (Fillinger & Richter, 2013; Beck et al., 2022). Above the thermocline (20-50 m), the temperature reaches up to 17 °C in summer and 10-12 °C below the thermocline (Fillinger & Richter, 2013; Beck et al., 2022). Environmental parameters fluctuate more in shallow (20 m) than deep water (300 m), with highest variability in summer and autumn and daily temperature fluctuations of up to 3.7 °C (Beck et al., 2022).

Environmental variability is also higher at the head of the fjord than at the mouth (Beck et al., 2022). The calcification rate and energy reserves of *D. dianthus* in Comau Fjord seems to be negatively affected by this higher environmental variability in shallow waters in terms of calcification rate and energy reserves, showing decreased values in shallow waters and at the head of the fjord (Beck et al. 2022; in prep).

A recent study showed that the zooplankton abundance and biomass are higher in surface waters than in deep waters of the fjord (Garcia-Herrera et al., 2022). However, the growth rates and energy reserves of *D. dianthus* indicate a higher food availability in deep waters of the fjord (Beck et al., 2022, in prep). This may be because large zooplankton, mysids and krill were not quantitatively assessed in the study by Garcia-Herrera et al. (2022).

1.6 Aim

Due to its natural environmental gradients and the ubiquitous occurrence of *D. dianthus* in Comau Fjord, this fjord offers a unique opportunity to examine the impact of contrasting physico-chemical environmental and feeding conditions on CWCs. Previous work provided the first indication that deeper sites foster higher energy reserves and thus healthier corals. However, only one deep site could be implemented in this previous study. Therefore, the aim of this thesis was to investigate the energy reserves of *D. dianthus* at more deep stations to further investigate the previous results.

2. Objectives and hypotheses

Objective 1:

Determination of energy reserves of *D. dianthus* at different water depths in Comau Fjord. Are there differences in the amount of energy reserves of *D. dianthus* between deep and shallow stations?

H1: *D. dianthus* has a higher amount of energy reserves at deep stations in Comau Fjord.

H0: *D. dianthus* does not have a higher amount of energy reserves at deep stations in Comau Fjord.

Objective 2:

Determination of energy reserves of *D. dianthus* along the fjord. Do energy reserves of *D. dianthus* at the same depth differ along a horizontal gradient?

H1: Total energy reserves of *D. dianthus* differ between stations at the same depth along a horizontal gradient.

H0: Total energy reserves *D. dianthus* do not differ between stations at the same depth along a horizontal gradient.

3. Material and Methods

3.1. Coral sampling

The samples of *D. dianthus* were collected in Comau Fjord (Chile, at approx. 42-56°S and along 72°28'W) in October/November 2021 as part of the DACCOR project. The corals were sampled at five stations from fjord mouth to head: Lilihuapi (42°09.6610'S 72°36.0430'W) at 24 m depth, Punta Llonco (42°23.2130'S 72°27.7720'W) at 118 m, Punta Huinay (42°22.27'S 72°25.42'W) at 27 m depth, Punta Gruesa (42°24.6210'S 72°25.4460' W) at 25 m and 190 m depth and Rio Bodudahue (42°27.3600'S 72°24.8260'W) at 165 m depth (Fig. 1). The stations will be referred to as A to E from fjord mouth to head. At station D, the shallow sampling station will be referred to as station Ds and the deep sampling station as Dd.

Figure 1: Coral sampling stations in Comau Fjord, Chile. *Desmophyllum dianthus* was sampled along the fjord from head to mouth at 6 stations at water depths between 24 and 190 m. The research station is located in Huinay.

The coral samples in shallower water depths (< 30 m) were collected by scientific SCUBA divers. The corals were carefully removed from the fjord wall using a hammer and chisel and placed in closed Kautex vials to protect the coral samples from the low salinity surface layer during the ascent to prevent an osmotic shock. Corals at deeper water depths (> 100 m) were collected by a remotely operated vehicle (BlueROV2, BlueRobotics), which was equipped with a wire frame to scrape the corals from the wall and collect them in a plastic bag attached to it. The plastic bag was closed for the ascend to also protect the corals from the low salinity surface layer. On the boat, the corals were transferred to a cool box filled with seawater. The corals were then transported to the research station and frozen in liquid nitrogen on the same day. Only the samples from Punta Gruesa from 25 m depth were kept in a flow-through aquarium with seawater from the fjord (pumped from 20 m depth in front of the research station) for about two weeks before freezing. The coral samples were transported to the Alfred Wegener Institute in a liquid nitrogen container (dry shipper) and stored in a - 80°C freezer until processing.

3.2. Processing of coral samples

The processing of coral samples followed established protocols (McLachlan et al., 2020) with slight modification. First, the coral samples were weighed with a high precision balance (CPA225D-0CE, Sartorius weighing technology, precision: 0.01 mg) and then grounded in a liquid nitrogen mortar with a cooled pestle. To prevent the samples from defrosting and degrading, it was made sure that there was always enough liquid nitrogen in the mortar and that one coral sample was completely processed within 30 min. After grinding the coral samples into small pieces, a homogeneous powder was produced with circular movements. Every ground sample was then partitioned and weighed in four pre-weighed containers: one pre-baked (in a muffle furnace for four hours at 450°C) aluminium pan for the quantification of ash-free dry weight and three cryo vials: one for genetic analysis and two for determination of energy reserves (one for proteins and carbohydrates and one for lipids).

3.3. Determination of ash-free dry weight

The ash-free-dry-weight (AFDW) determination followed the same protocol (Mclachlan et al., 2020) as for the processing of the coral samples. The ground coral samples in the pre-baked aluminium pans were placed in a drying oven at 60 °C

overnight. On the next day, after cooling down for about half an hour in a desiccator with silica gel, they were all weighed (dry weight) and then placed in a muffle furnace at 450°C for four hours. After cooling down for about 12 hours in the desiccator, they were weighed again to determine the AFDW using the following equation:

AFDW (g)= Weight of dry sample – Weight of combusted sample

To calculate the biomass per coral sample the following equation was used:

$$\textit{Biomass (g dry weight)} = \frac{AFDW(g)}{Subsample\ wet\ weight\ (g)\ /\ Total\ sample\ wet\ weight\ (g)}$$

3.4. Biomass data

The total amount of biomass per coral sample and the percentage of biomass per total wet weight of the coral samples were calculated and discussed in my PM4 report. A figure of the percentage of biomass per total wet weight and a table with the mean ± standard deviation can be found in the supplements (Supplementary Figure 1 and Supplementary Table 3).

3.5. Preparation of the subsamples for protein and carbohydrate analysis

The preparation of the subsamples followed the preparation steps of a protocol for quantification of soluble protein in ground coral samples (Mclachlan et al., 2020) with slight adaptations. The method of freeze-thaw lysis was used to disrupt the cells and solubilise the proteins and carbohydrates. The pre-weighed (0.153-1.473 g) coral samples were taken from the -80°C freezer and placed in a water bath at room temperature for 4 min until they were completely defrosted. Afterwards, they were vortexed, placed in a dewar of liquid nitrogen for 10 sec and then again in the water bath for 4 min. 1.5-3.5 ml of MilliQ water (conductivity: 18.0 MΩcm; Sartorius arium pro, Sartorius Corporate Administration GmbH, Germany) were added to the samples depending on their weight and vortexed again afterwards. Then the samples were centrifuged at 4°C and 4200 rpm for 20 min (Multifuge X3 FR, Thermo Fisher Scientific). 1-2 ml of the middle phase (upper phase: thin whitish or sometimes red matrix, lower phase: particulate matter) were then transferred into a 2 ml Eppendorf cup.

3.6. Protein analysis

The soluble protein concentration was determined after Lowry et al. (1951) using the BioRad Standard Protein Assay kit (DC Protein Assay kit, Bio-Rad Laboratories) with bovine serum albumin (BSA) as standard. First, the reagent S and reagent A from the protein assay kit were mixed at a ratio of 1:50, resulting in reagent A‘. As reagent A‘ can only be used for one week, a new calibration needs to be done for each new reagent A’. For the standard curve, a dilution series from 0.01-1.5 mg/ml of the protein standard was used, which was diluted with MilliQ. From each standard and sample, 25 µl was transferred in a 2 ml Eppendorf cup. Then 125 µl from Reagent I was added, then vortexed and afterwards incubated for 1 min. Next, 125 µl Reagenz II were added, vortexed and centrifuged at 15.000 g for 5 min (Espresso centrifuge, Thermo Electron Corporation). After that, the supernatant from the Eppendorf cup was completely removed by tipping the liquid onto a laboratory tissue. Next, 127µl of Reagenz A‘ was added, vortexed and incubated for 5 min. Afterwards, the tube was vortexed again to make sure the protein pellet completely dissolved, then 1 ml of Reagenz B was added, vortexed and incubated for 15 min. The protein concentration was measured within one hour at a photometer (UV-1800 spectrophotometer, Shimadzu Corporation) at 750 nm and the protein content was calculated using the standard curve.

3.7. Carbohydrate analysis

For the carbohydrate analysis an existing protocol (Bove and Baumann, 2021) that was already modified for CWCs (Beck, 2021) was used, using phenol and sulfuric acid for the colorimetric detection after Dubois et al. (1956).

The standard dilution series (0-200 µg/ml) was prepared using a 2000 µg/ml d-glucose solution and MilliQ water. 200 µl of each standard were then transferred into test tubes. The same coral samples as for the protein analysis were used for the carbohydrate measurements. From each sample, 100 µl were pipetted into a test tube and mixed with 100 µl MilliQ. Then, 5 % phenol and 1 ml of 98 % sulfuric acid were added to each standard and subsample. Next, they were incubated for 10 min and then put in a 30°C water bath for 20 min. From each tube, 200 µl were pipetted in triplicates in wells of the microplate. The absorbance was measured on a microplate reader (TriStar LB941 Multimode Reader, Berthold Technologies) at 485 nm and the carbohydrate content calculated using the standard curve.

3.8. Lipid analysis

Existing protocols for coral lipid analyses on the microplate reader were used (Bove & Baumann, 2021) that were already adapted for CWCs (Kayser, 2022) and here further modified for whole grounded coral samples.

First, all solutions were prepared: 0.05 M NaCl, 2:1 Chloroform:Methanol solution, vanillin reagent and stock solution. Afterwards, standards were prepared from the 15 mg/ml stock solution using corn oil as standard (245 µl corn oiled diluted in 14755 µl chloroform) and serially diluted from 1750 to 250 µg/ml concentration. The zero concentration standard only contained chloroform and no corn oil. From all coral samples, three 100 mg subsamples were taken and then prepared for the analysis with two freeze-thaw cycles to disrupt the cells and solubilise the lipids. To each thawed subsample, 600 µl 2:1 chloroform:methanol solution was added and then vortexed for 20 min. Then, 160 µl 0.05 M NaCl were added and the tubes were inverted two times, shortly opened to let possible gases escape and centrifuged for 5 min at 3000 rpm. From the second lowest (which contained the soluble lipids and chloroform) of the four phases, 300 µl were pipetted into a new safe lock tube. To each standard and new subsample, 150 µl methanol were added and then vortexed for 30 sec. The tubes were then placed in a water bath at 90 °C for 20 min with open lids to let all the liquid evaporate. Next, 300 µl sulfuric acid was added to each standard and subsample, then vortexed for 30 sec and placed again in the water bath for 20 min. Afterwards, the tubes were cooled on ice for 2 min. From each tube, 75 ul (in triplicates) were pipetted into wells of the microplate. Subsequently, the absorbance was measured at 530 nm on a microplate reader (TriStar LB941 Multimode Reader, Berthold Technologies) to obtain the background values before 34.6 µl of vanillin was added to each well. The plate was shaken for 10 sec in the microplate reader, incubated for 10 min and shaken again before the absorbance was measured again at 530 nm. To calculate the total lipid content, the background values were subtracted from the second absorbance values. The mean of each triplicate sample was calculated and the mean of means for each sample. Other than in the original adapted protocol for CWCs, the equation resulting from the standard dilution series was a quadratic equation.

3.9. Conversion into kJ gdw^{-1}

The protein, carbohydrate and total lipid concentrations (g gdw^{-1}) were converted to kilojoules (kJ; protein: 23.9 kJ g^{-1}, carbohydrate: 17.5 kJ g^{-1}, lipids: 39.5 kJ g^{-1}; Gnaiger & Bitterlich, 1984) and standardised to the biomass of the corals.

3.10.Statistical analysis

All statistical analyses were performed using the software R (version 4.1.0, R Core Team, 2021). The Shapiro-Wilk test was used to test for normality and the Leneve test to test homogeneity of variances.

The Rosner's test and Grubb's test were used to identify outliers. The result was then also visually checked through a histogram and qqnorm. For all further analyses and graphics, the data without significant outliers were used.

First, the data were tested for normality and homogeneity of variance. If the data met these assumptions a linear model was applied (protein data), and a general linear model if not (all other data). In case of the latter, gamma and log distribution were used and the performance package applied to identify the best model. This showed the best result for the log-transformed data of the Gamma distribution. Post-hoc comparisons of significant effects were tested using the *lsmeans* function of the package *lsmeans*.

4. Results

4.1 Proteins

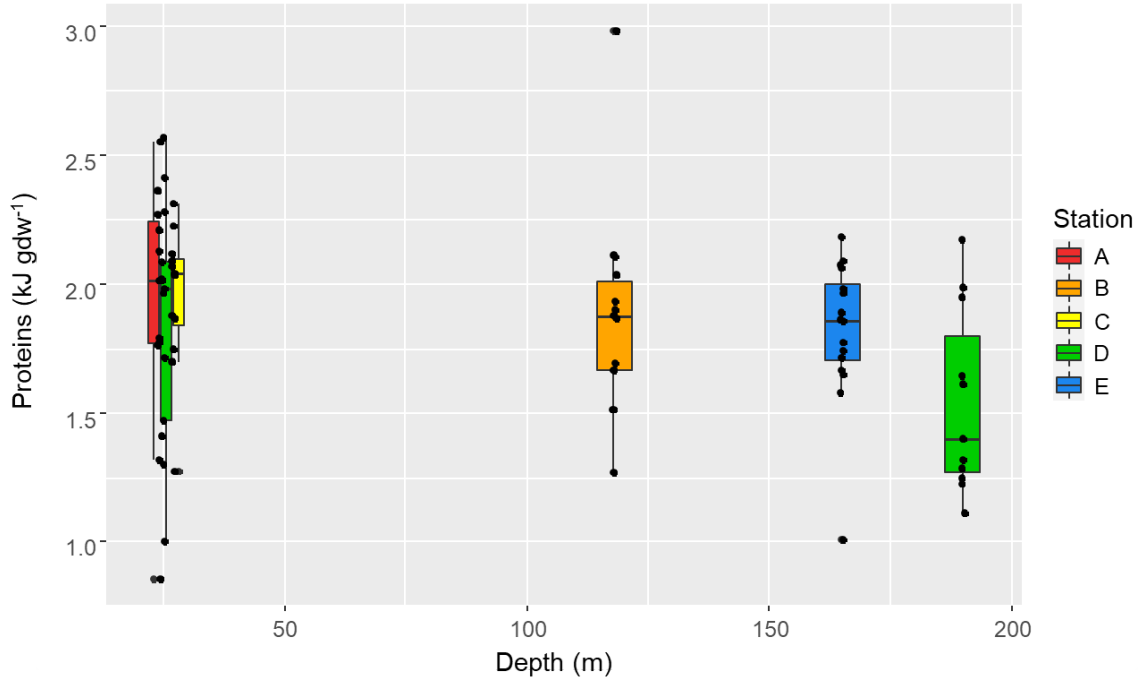


Figure 2: Protein concentration of *Desmophyllum dianthus* in Comau Fjord. The protein energy content is shown in kJ gdw^{-1} at 6 stations along the fjord in relation to the water depths of the stations. The colours correspond to the stations along the fjord, from fjord mouth (A, red) to fjord head (E, blue).

The protein concentration of *D. dianthus* was similar between all stations in Comau Fjord ($p > 0.05$; Fig.2). Generally, there was a slight trend of higher protein concentration per biomass at shallow stations, which was not statistically significant. There was no discernible trend of protein concentration along the fjord.

4.2 Carbohydrates

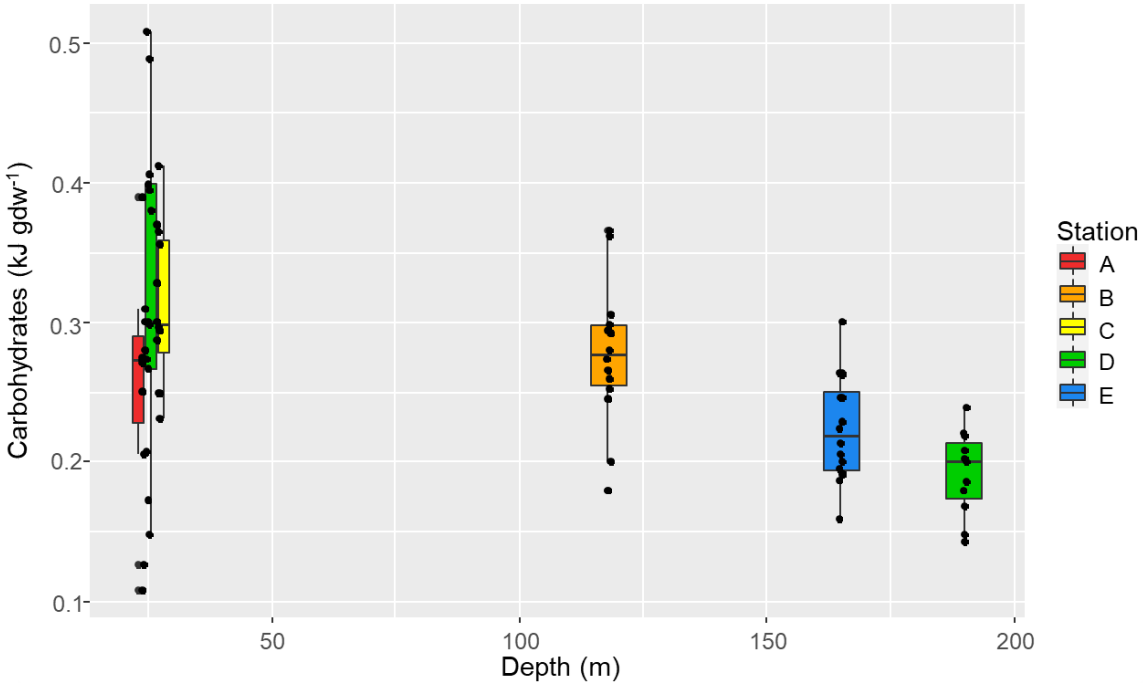


Figure 3: Carbohydrate concentration of *Desmophyllum dianthus* in Comau Fjord.
The carbohydrate concentration is shown in kJ gdw^{-1} at 6 stations along the fjord in relation to the water depths of the stations. The colours correspond to the stations along the fjord, from fjord mouth (A, red) to fjord head (E, blue).

The carbohydrate concentration of *D. dianthus* was significantly lower at the deep stations compared to the shallow stations C and Ds and the middle station B (B-Dd: p=0.0036, C-Dd: p=0.0001, Ds-Dd: p<0.0001, C-E: p=0.0058, Ds-E: p=0.0008; Fig. 3). Additionally, the carbohydrate concentration was slightly higher in shallow waters at the head of the fjord than at the mouth.

4.3 Lipids

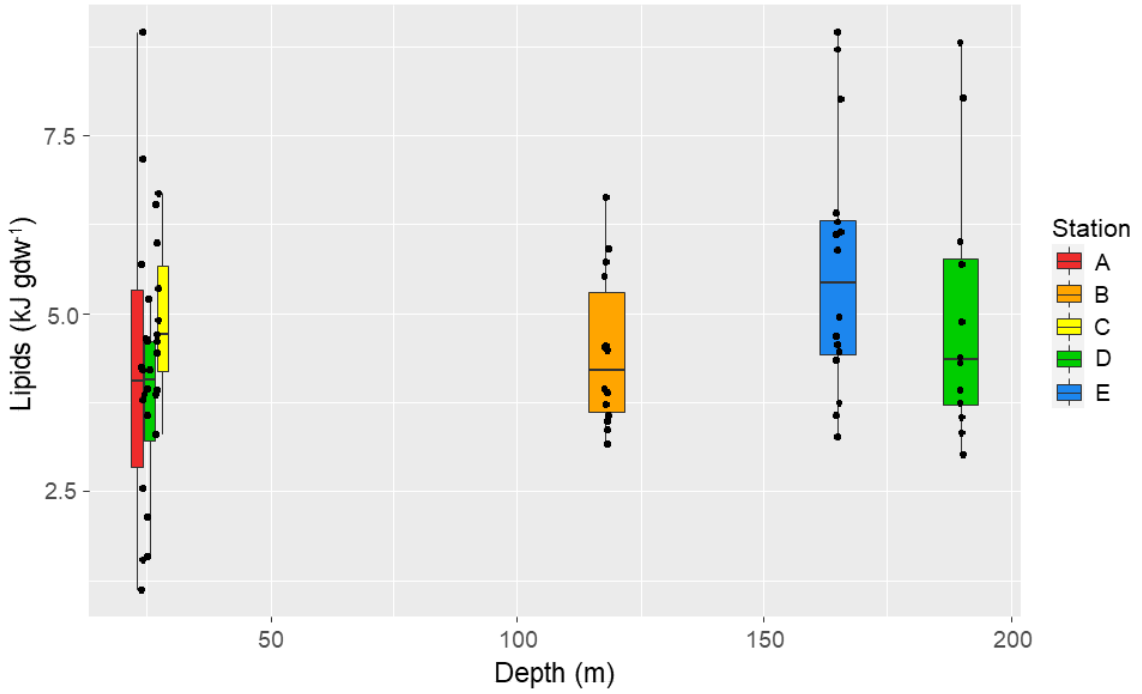


Figure 4: Lipid concentration of *Desmophyllum dianthus* in Comau Fjord
The lipid energy content is shown in kJ gdw^{-1} at 6 stations along the fjord in relation to the water depths of the stations. The colours correspond to the stations along the fjord, from fjord mouth (A, red) to fjord head (E, blue).

The lipid concentration of *D. dianthus* was similar between all stations in Comau Fjord ($p > 0.05$; Fig. 4). However, corals at deep stations tended to have slightly higher, but not significantly, lipid concentrations, but similar to station C.

4.4 Total energy reserves

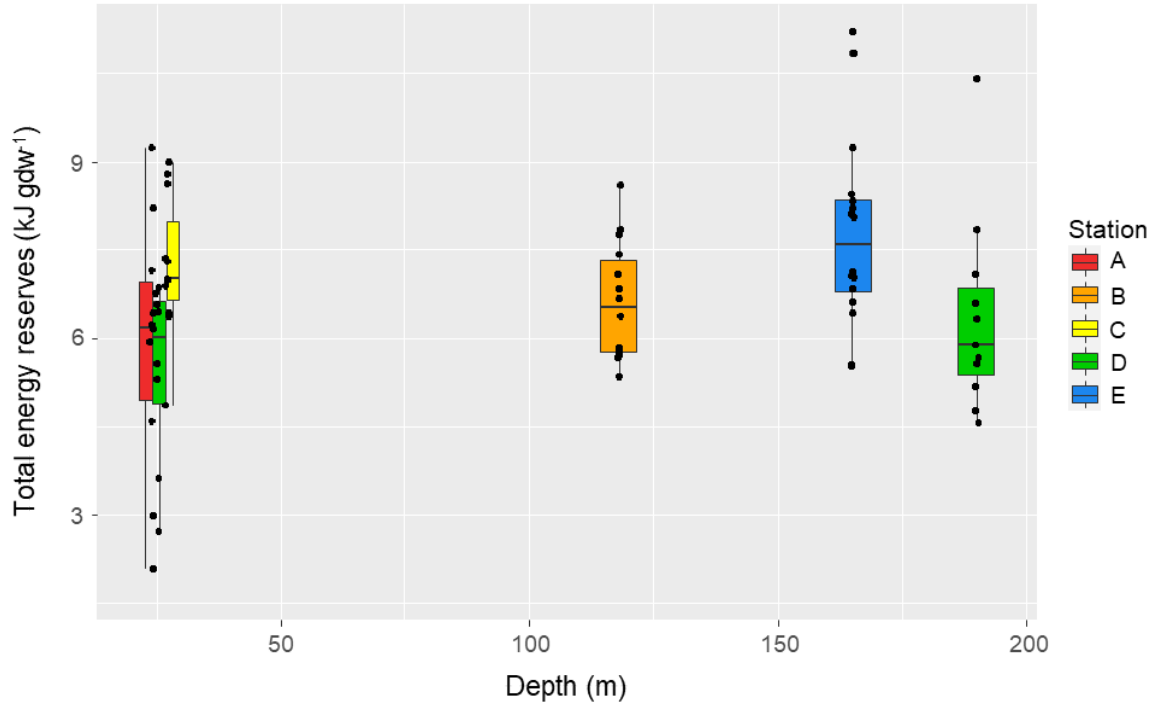


Figure 5: Total energy reserves of *Desmophyllum dianthus* in Comau Fjord. The total energy reserves are shown in kJ gdw^{-1} at 6 stations along the fjord in relation to the water depths of the stations. The colours correspond to the stations along the fjord, from fjord mouth (A, red) to fjord head (E, blue).

The total energy reserves of *D. dianthus* in Comau Fjord were only significantly higher at station E than at station Ds (p: 0.0159; Fig. 5). As lipids made up most of the total energy reserves (see Fig. 6, ranging from 38.65 to 78.69 %), the observed non-significant trends are the same as noted for lipid concentrations: a slight trend to higher total energy reserves in deep waters, which where however similar to station C.

4.5 Relative content of energy reserves

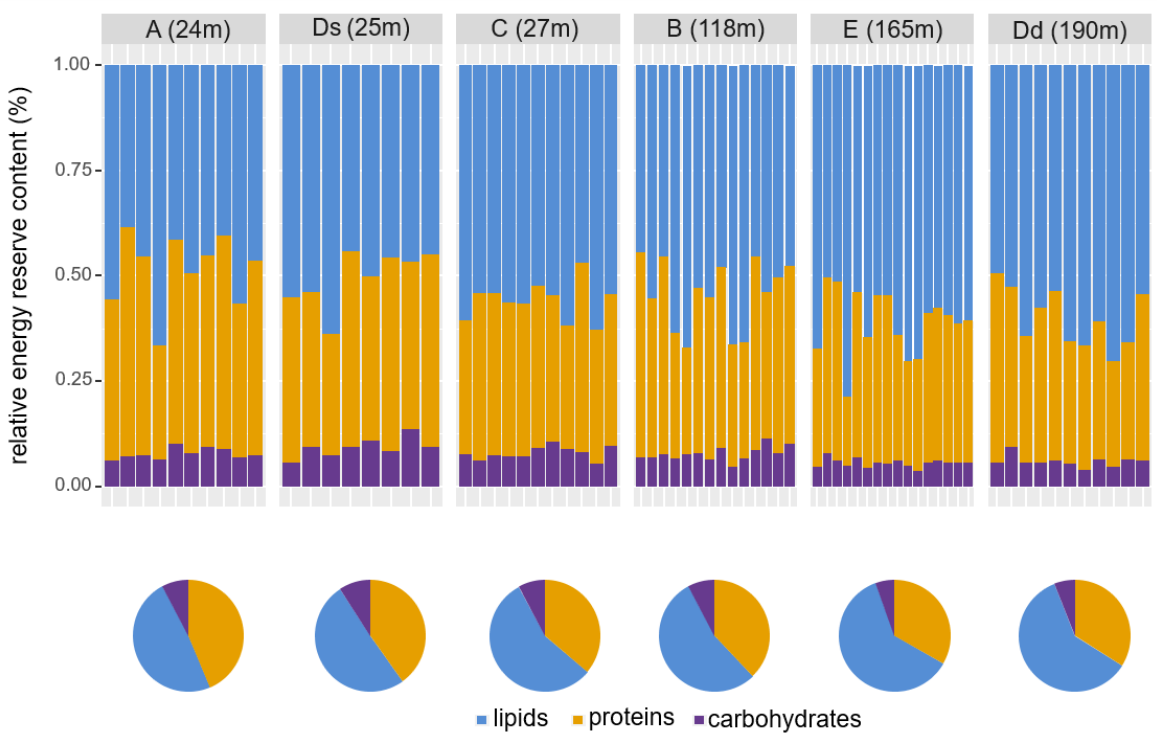


Figure 6: Relative content of energy reserves of *Desmophyllum dianthus* in Comau Fjord. The relative content of energy reserves is shown in % at 6 stations sorted according to the depth from shallowest (A) to deepest (Dd). Each bar represents one individual. Each circle shows the mean relative energy reserves contents. The colours correspond to the energy reserves.

The relative energy reserve composition revealed a significantly higher relative lipid content in the two deep stations E and Dd compared to the two shallow stations As and Ds (A-E: p=0.0010, A-Dd: p=0.0071, Ds-E: p=0.0220, Ds-Dd: p=0.0476; Fig. 6). In turn, this results in lower relative protein and carbohydrate contents at these stations. Relative protein content was significantly lower at station Dd and E compared to the station A (A-Dd: p=0.0281; A-E: p=0.0061). Relative carbohydrate content was significantly lower at station Dd and E compared to all other stations (A-Dd: p=0.0452, A-E: p=0.0024, B-Dd: p=0.0264, B-E: p=0.0007, C-Dd: p=0.0207, C-E: p=0.0007, Ds-Dd: p=0.0003, Ds-E: p<0.0001). No trend along the fjord is noticeable.

5. Discussion

This is only the second *in situ* study which measured the energy reserves of *D. dianthus* and the first that incorporated several deep stations. Unexpectedly and different to the previous study, no correlation between water depth and the amount of energy reserves was found. However, the two deepest stations (Dd and E) had a significantly higher relative lipid content than two of the shallow stations (A and Ds). No differences in energy reserves along a horizontal gradient in shallow or deep stations were found, but higher energy reserves were found at the centre station C compared to the other shallow stations.

5.1 Comparison of energy reserves with other corals

So far, most studies on energy reserves were conducted on tropical corals (e.g. Rodrigues & Grottoli, 2007; Schoepf et al., 2013; Wall et al., 2019; Keister et al., 2023). The total energy reserves of the present study (mean: 6.58 ± 1.68 kJ gdw^{-1}) are within the range observed so far in these previous studies (mean values: 6.51 to 21.35 kJ gdw^{-1}), but at the lower end. Only a few studies investigated total energy reserves of CWCs (Chapron et al., 2021; Beck et al., in prep.), some more total lipid content (Larsson et al., 2013; Movilla et al., 2014; Baussant et al., 2017) and lipid composition (Dodds et al., 2009; Naumann et al., 2015; Maier et al, 2019, 2020). Comparable total energy reserves in these studies ranged from an average of 7.09 to 12.9 kJ gdw^{-1} (Chapron et al., 2021; K. Beck, unpub. data[1]) and the comparable lipid content ranged from 2.37 to 9.48 kJ gdw^{-1} (Dodds et al., 2009; Larsson et al., 2013; Naumann et al., 2015; Chapron et al., 2021; K. Beck, unpub. data). The values of the present study (mean total energy reserves: 6.58 ± 1.68 kJ gdw^{-1}; mean lipids: 4.54 ± 0.73 kJ gdw^{-1}) for *D. dianthus* are slightly lower in terms of total energy reserves than in the previous studies on other CWC species, but still similar to the previously measured values for *D. dianthus*. In terms of lipid content the values were in the previously measured range.

The relative lipid content (based on mass concentration) was a little higher than in other CWC taxa, with an average of 55.83 ± 8.35 % (ranging from 38.65% to

[1] In the study by Beck et al. (in prep.) energy reserves were calculated per surface area. Since in the present study energy reserves were calculated per biomass, the values of the previous study were transformed into per biomass, possible because biomass was measured in that study as well. However, it was not included in the manuscript, therefore all transformed data will be cited as unpublished data. Additionally, here and in all following only data from non-transplanted individuals from that study will be considered.

78.69%) as the lipid content generally makes up up to 40% of the total energy reserves (Harland et al., 1993; Imbs, 2013). However, Chapron et al. (2021) observed a similar range for *Lophelia pertusa* and *Madrepora oculata* with an average of 55% and 61%, respectively.

The average total energy reserves in this study are similar, while a bit lower, to the ones measured in the previous study in Comau Fjord in August 2017 (7.09 ± 1.60 kJ g^{-1}; K. Beck, unpub. data; Supplementary Table 2), which is the closest sampling time to this study (October & November 2021). They are lower than the ones derived for May (9.05 ± 2.82 kJ g^{-1}; K. Beck, unpub. data; Supplementary Table 2). This suggests that the sampling time of this study in austral spring was still before or at the start of the spring phytoplankton bloom and the corals could not yet increase their energy reserves. In May, following the zooplankton bloom during austral summer (Garcia-Herrera et al., 2022) corals benefited from the energy rich zooplankton availability and were able to accumulate higher energy stores that likely peak in May (Beck et al., in prep).

5.2 Energy reserves at different water depths

It was expected that *D. dianthus* has a higher amount of energy reserves at greater water depth than in shallow waters of Comau Fjord, based on the results of Beck et al. (in prep). Additionally, higher calcification rates were measured at the deep station (Beck et al., 2022), which supports the hypothesis that CWCs at deeper depths of this fjord are generally fitter. However, contrary to the hypothesis, the results of this study showed that *D. dianthus* did not generally have a higher amount of total nor individual energy reserves at the deep stations compared to shallow stations, but rather a similar or lower amount (Fig. 2-5). However, corals at the two deepest stations have a significantly higher relative lipid content than the two shallow stations A and Ds (Fig. 6), which provides a first hint towards improved fitness, as it is indicative of an elevated ability to accumulate storage lipids.

Differences in the energy reserves at deep and shallow water depth of this study and Beck et al. (in prep) can be explained by the different reference values used to normalise energy reserves. Beck et al. (in prep.) calculated energy reserves per tissue covered surface area, while in this study energy reserves were calculated per biomass. Most studies investigating energy reserves or lipids in tropical corals and

CWCs normalised them to biomass (e.g. Grottoli et al., 2004; Dodds et al., 2009; Movilla et al., 2014; Chapron et al., 2021). Even though many studies also measured the amount of biomass per surface area (e.g. Rodrigues & Grottoli, 2007; Schoepf et al., 2013; Wall et al., 2019), except for the study by Beck et al. (in prep.) no studies calculated energy reserves or lipids per surface area. As the biomass of *D. dianthus* was measured as well in the previous study (K. Beck, unpublished data), it was possible to convert the data of Beck et al. (in prep) into energy reserves per biomass to compare them to this study. This converted data showed no clear correlation with depth (Fig. 7 a; Supplementary Table 2) as well. This means that the results and therefore interpretation of the data changes depending on the reference value that is used.

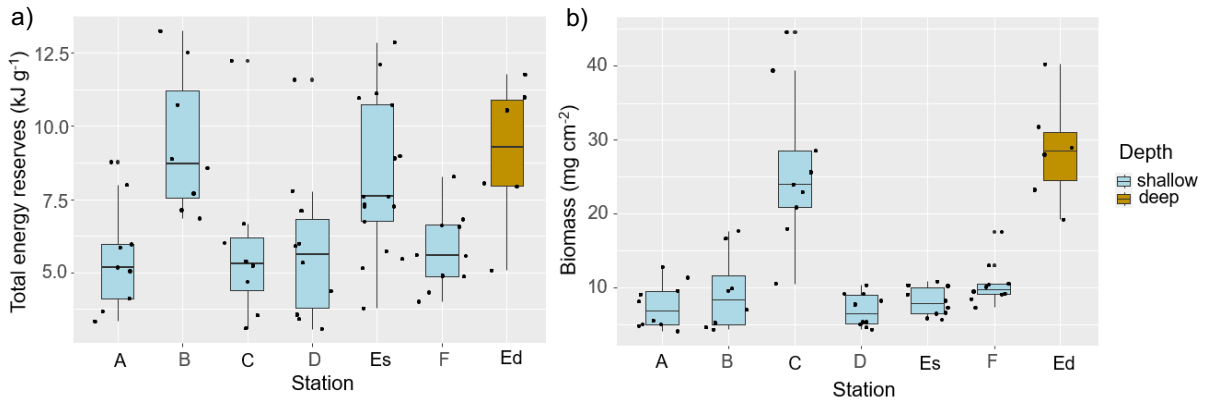


Figure 7: a) Total energy reserves and b) biomass of *Desmophyllum dianthus* in Comau Fjord in August 2017. Total energy reserves (kJ g^{-1}) and biomass per tissue covered surface area (mg cm^{-2}) at 7 stations along the fjord. The colours correspond to the water depth: shallow = blue, deep = yellow. The stations are not the same as in the present study as station A is located at the head of the fjord and station F at its mouth. Stations A-F are located at 20 m water depth, whereas station Ed is located at 300 m depth. Data are from Beck et al. (2022; in prep) and K. Beck, unpub. Data, excluding transplanted individuals from these studies.

The reason why the results differ depending on the used reference value is the substantial difference in the amount of biomass per tissue covered surface area between corals from deep and most shallow stations, which was on average 2.6 times higher in deep corals (Fig. 7 b; Supplementary Table 2; Beck et al., 2022 (not considering transplanted individuals)), leading to higher energy reserves per surface area in deep corals (Beck et al., in prep.). In other studies that measured energy reserves and used biomass as the reference value, the biomass amount per surface area did not change significantly in response to treatment conditions (e.g. Rodrigues

& Grottoli, 2007; Schoepf et al., 2013), therefore causing no effect in calculation or interpretation, in contrast to what is seen here. This shows that it is important to know and take into account for the interpretation if the reference value differs, and if so to consider if not a different reference value might help to interpret the data more clearly. It is also important to match the reference value to the research hypothesis and the dependent variable (Edmunds & Gates, 2002). Based on this I would propose that for this study to calculate the energy reserves per tissue covered surface area would have been better, as the differences in biomass per surface area are then directly shown in the amount of energy reserves, which they are not if energy reserves are calculated per biomass, giving a more complete picture of the coral's condition in different environments.

For the present study, it can be assumed that the corals from the deep station had more biomass per tissue covered surface area as was observed in the previous study, which would have resulted in higher energy reserves calculated for tissue covered surface area. This can be approximated using data collected for another study at the same time and in the same populations as the coral samples were collected for this study (stations A, C and Dd; M. Wall and K. Beck, unpub. data). Total wet weight and tissue covered surface area were measured (Fig. 8 a and b; Supplementary Table 3), making it possible to approximate the energy reserves per surface area for the present study. The calculated %-biomass per coral wet weight for corals from the present study could be used to calculate the total amount of biomass per coral for these additional individuals. This allowed to convert the total biomass of the corals to biomass per surface area. These values (mean of deep corals: 30.16 ± 18.06 mg cm^{-2}, mean of shallow corals: 19.56 ± 8.74 mg cm^{-2}; Fig. 8 a; Supplementary table 4) are in line with the previous study of Beck et al. (in prep.) in August (mean of deep corals: 28.59 ± 7.22 mg cm^{-2}, mean of shallow corals: 11.02 ± 5.82 mg cm^{-2}; Supplementary Table 2). If the total energy reserves of the corals from the present study are now calculated per surface area, they are higher at the deep station, (however only significantly to station A; mean deep station Dd: 0.23 ± 0.14 kJ cm^{-2}; mean shallow stations: A: 0.10 ± 0.06 kJ cm^{-2}, C: 0.16 ± 0.05 kJ cm^{-2}), as has been hypothesised (Fig. 8 d; Supplementary Table 4).

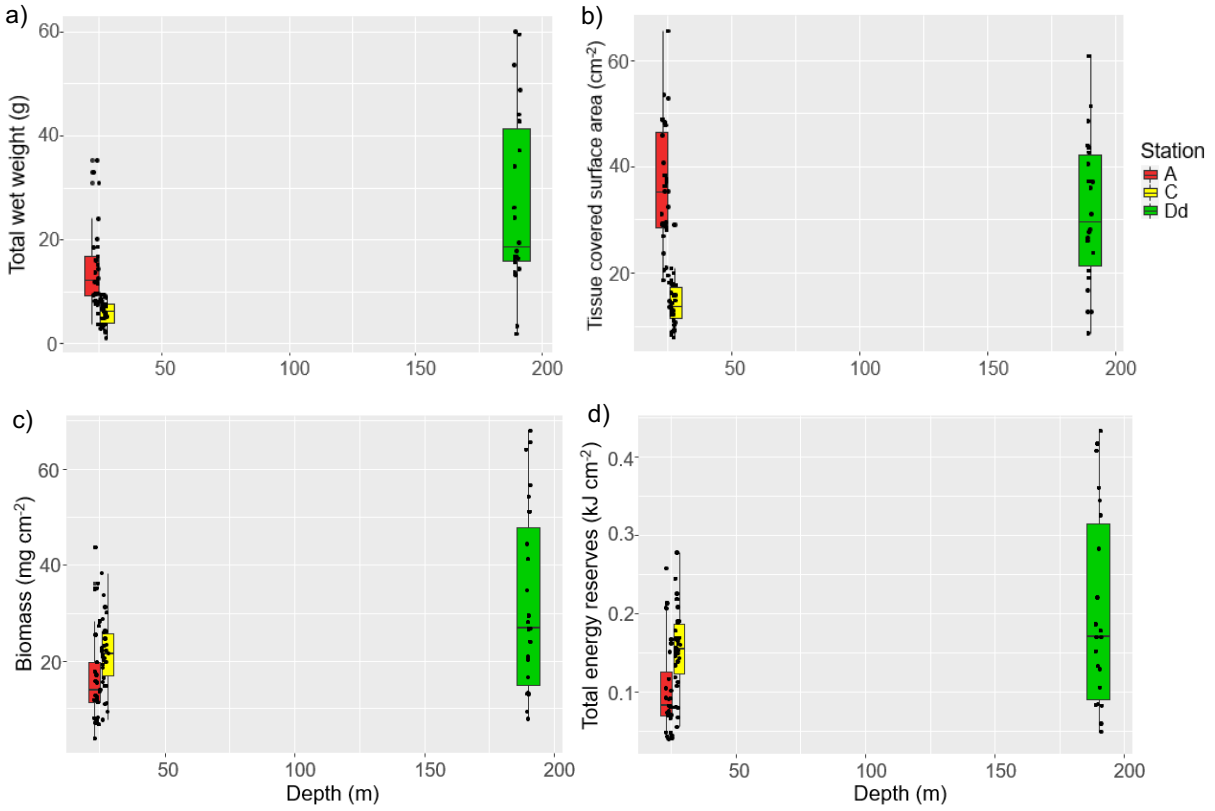


Figure 8: Total wet weight a), tissue covered surface area b), biomass c) and total energy reserves d) of *Desmophyllum dianthus.* Total wet weight (g) and tissue covered surface area (cm^2) were measured for different individuals than in the present study, but from the same populations and the same sampling time (M. Wall and K. Beck, unpub. data; Supplementary Table 3). Biomass (mg cm^{-2}) and total energy reserves (kJ cm^{-2}) were calculated for the corals of the present study, using the previous data from a) and b) and percentage of biomass per wet weight and the amount of energy reserves per biomass previously calculated for individuals from the present study (Supplementary Table 1 & 3). The colours correspond to the stations A (red), C (yellow) and Dd (green) of the present study.

These results would confirm the results of the previous study (Beck et al., in prep.). To explain the higher energy reserves in deep corals three explanations were proposed in the previous study: higher environmental variability in shallow waters, higher competition in shallow waters and possibly more food or better food quality in deep waters (Beck et al., in prep.).

Higher environmental variability was measured in shallow waters which could explain the lower energy reserves as it negatively affects *D. dianthus*, indicated by lower calcification rates (Beck et al., 2022). Higher mean annual temperatures and stronger temperature fluctuations in shallow waters could reduce the feeding time of the corals due to tentacle retraction and polyp inactivity at elevated temperatures (Chapron et al., 2021). Further, the high tidal range affects the water chemistry in shallow waters

by causing rapid salinity fluctuations, which may have an effect on the zooplankton abundance and composition, by causing osmotic stress, which leads to increased mortality in some taxa (Wells et al., 2022). Less competition was mentioned as another factor potentially contributing to higher energy reserves in deep corals (Beck et al., in prep.), as in the shallow regions of Comau Fjord a diverse benthic community of both sessile and mobile species can be found (Försterra et al., 2017), which decreases with depth (Försterra et al., 2005), possibly leading to less competition for food.

Even though higher zooplankton abundance and biomass were measured in shallow waters of Comau Fjord (0 - 50 m; Garcia-Herrera et al., 2022), this might not display the actual food availability. Large zooplankton, mysids and krill as mobile taxa are able to avoid plankton nets (Brinton 1962) and were therefore not quantitatively assessed in that study. Especially krill was not caught efficiently (Garcia-Herrera et al., 2022), but is known to be abundant in Comau Fjord (Palma & Silva, 2004; Sanchez et al., 2011) and provides a crucial energy supply for *D. dianthus* (Maier et al., 2021). Krill and other mobile taxa could be more abundant in deep waters, contributing to higher energy reserves in corals from deep stations.

5.3 Energy reserves along the fjord

Energy reserves per biomass did not differ along a horizontal gradient in shallow nor deep waters, and it is unknown in this study if the same result would have been obtained if energy reserves were calculated per surface area as this data is not available for all stations from this study.

Interestingly, corals from station C had higher energy reserves per biomass than corals at the other shallow stations, even though this was not significant (Fig. 5). They also had highest energy reserves of the shallow stations calculated per surface area in the study by Beck et al. (in prep.), and the same could be seen here in the approximate calculation in comparison to the shallow station A (Fig. 8d; Supplementary Table 4). Corals from station C also had the smallest tissue covered surface area (Fig. 8b; Supplementary Table 3), which was also the case in the study by Beck et al. (in prep.). As the characteristic of highest energy reserves and smallest surface area were conspicuous, energy reserves were calculated per polyp. If calculated per polyp, lowest energy reserves were found at station C as they had the

least amount of biomass per polyp (Fig. 9; Supplementary Table 4). This shows once again that it is important to take into account the different traits of the studied individuals, as the comparison between the data normalised to different reference values might lead to different results and might help to get a better and fuller understanding of the received results.

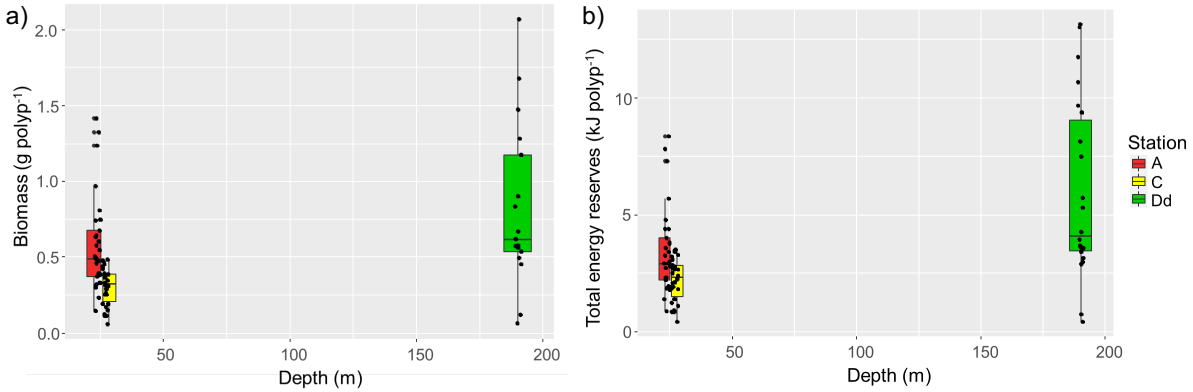


Figure 9: Biomass a) and total energy reserves b) per polyp of *Desmophyllum dianthus* in Comau Fjord. Biomass (mg polyp^{-1}) and total energy reserves (kJ polyp^{-1}) were calculated using total weight of the polyp from different individuals, but from the same populations at the same sampling time (M. Wall and K. Beck, unpub. data; Figure 8 a; Supplementary Table 3) and the percentage of biomass per wet weight and amount of energy reserves per biomass previously calculated for individuals of the present study (Supplementary Table 1 & 3). The colours correspond to the stations A (red), C (yellow) and Dd (green) of the present study.

The previously assessed physico-chemical conditions (e.g. temperature, pH) showed a gradient from fjord head to mouth (Fillinger & Richter, 2013; Beck et al., 2022) and thus, cannot explain the change in the amount of energy reserves and tissue covered surface area at the centre of the fjord. However, these may not be the only important drivers for coral performance in this fjord and other environmental parameters that have not been assessed yet may account for this deviation. Station C for instance is located directly next to an actively used salmon farm. Salmon farms can affect the ecosystem in a number of different ways, e.g. lost nets and ropes can get entangled in coral banks, water flow and food availability may be reduced (Häussermann et al., 2013) or the amount of sedimentation and nutrients may increase, which are the greatest risks from these farms for benthic organisms (Hargrave, 2010). It was also shown that *L. pertusa* reduces its metabolic rates and lipid stores by up to 70 % near salmon farms (Kutti et al., 2022). In order to deal with increased sedimentation,

CWCs produce larger amounts of cleaning mucus, which requires more energy (Häussermann et al., 2013). However, *D. dianthus* grows on vertical and overhanging walls with the calix oriented downwards, which minimises the influence of sedimentation (Försterra & Häussermann, 2003; Cairns et al., 2005; Försterra et al., 2005), but this must not mean that they are not affected at all. The higher energy reserves and higher amount of biomass per surface area could be a protection mechanism to make the tissue thicker to fight against sedimentation and other external influencing factors caused by the salmon farm not assessed so far in detail, e.g. pharmaceutical and anti-fouling substances (Bustamante, 2009; Försterra et al., 2014, 2016). The consequence is that there is less energy left, which leads to a smaller surface area (Beck et al., 2022) and lower calcification rate per biomass (K. Beck, unpub. data). In total, *D. dianthus* seems to be influenced negatively by the salmon farm, despite the first impression of higher energy reserves, which could have lead to the interpretation of a positive influence of the salmon on *D. dianthus* here. However, more research is needed to explore the effect of aquaculture, in particular salmon farms, on the performance of CWCs.

5.4 Methodical discussion

For this study, the coral fragments were ground into a paste consisting of coral skeleton and tissue. This is a crucial difference to the previously used method, where the tissue was airblasted from the skeleton with a tissue slurry as the end product (e.g. in the study of Beck et al. in prep.). Therefore, it was very important that the ground paste was homogeneous to ensure that all sub-samples contained the same tissue to skeleton ratio for the down-stream analyses. In the original protocol, the coral fragments were ground on ice, which means that there was a very limited time to grind the corals until they thawed. In the adapted protocol, the coral fragments were ground in a liquid nitrogen dewar, which allowed a much longer time to properly grind the coral fragments. Still, sometimes larger pieces of skeleton could not be broken down further, which is linked to cementation processes of the coral skeleton due to tissue retraction. Where the tissue retracts, the bare skeleton becomes exposed and cementation processes take place, this can consolidate the skeleton making it stone hard in older parts. It could be considered that these cemented parts should be excluded from further processing in the next study. To summarise, although grinding the coral fragments required more effort for *D. dianthus* than

indicated for the tropical corals in the original protocol, it was overall easy to follow and resulted in a sufficiently homogeneous paste in most cases.

For the protein and carbohydrate analysis, variable amounts of the ground corals were used, contrary to the original protocol where always the same amount is used. This was because the coral samples were very different in size, ranging from 0.3 g to over 10 g and due to protocol procedure. Therefore, the calculations of the previous protocol could not be used, which made the new calculations more difficult. For further studies, it is therefore recommended to use exact amounts as in the original protocol.

In contrast to the variable amount of ground coral sample used for the protein and carbohydrate analyses, always the same amount was used for the lipid analysis by taking a subset of subsamples out of the allocated subsample for lipid analysis and leaving out too small samples, thereby standardising the analysis. Even though the adapted protocol for CWCs was still intended only for tissue slurry, it worked well with the ground coral samples.

6. Conclusion

In contrast to the previous study, no significant differences in energy reserves of *D. dianthus* were found between different water depths of Comau Fjord. The different reference values that were used for the normalisation of energy reserves, were identified as the reason for this. Based on previous and additional data, it was possible to conclude that if energy reserves had been normalised to tissue covered surface area, a correlation between depth and energy reserves would have been found. This supports the results from the previous study of Beck et al. (in prep) and can be explained by differences in environmental variability, competition and food supply.

No trends in energy reserves of *D. dianthus* were found along a horizontal gradient, but energy reserves per biomass were conspicuously higher at station C in the middle of the fjord compared to the other shallow stations. The same result was received if energy reserves were calculated per tissue covered surface area, but not if calculated per coral polyp as corals from station C also had the least amount of tissue covered surface area. Aquaculture was identified as a possible influencing factor for this and higher energy reserves per biomass could be a stress response or adaptation mechanism.

Overall, this study showed the influential effect of different reference values for the interpretation of energy reserve data. Future studies on energy reserves of CWCs should determine both biomass and tissue covered surface area of the corals as well as size in order to be able to better interpret observed physiological characteristics and responses.

7. Literature

Anthony, K. R. N., Hoogenboom, M. O., Maynard, J. A., Grottoli, A. G., & Middlebrook, R. (2009). Energetics approach to predicting mortality risk from environmental stress: A case study of coral bleaching. Functional Ecology, 23, 539–550.

Baussant, T., Nilsen, M., Ravagnan, E., Westerlund, S., & Ramanand, S. (2017). Physiological responses and lipid storage of the coral Lophelia pertusa at varying food density. Journal of Toxicology and Environmental Health, 80, 266–284.

Beck, K. K., Schmidt-Grieb, G. M., Laudien, J., Försterra, G., Häussermannn, V., González, H., Espinoza, J. P., Richter, C., & Wall, M. (2022). Environmental stability and phenotypic plasticity benefit the cold-water coral Desmophyllum dianthus in an acidified fjord. Communications Biology.

Beck, K., Schmidt-Grieb, G., Kayser, A., Wendels, J., Lago, A., Meyer, S. Woll, M., Graeve, M., Laudien, J., Richter, C., Wall, M. (in prep.). Seasonal energy reserves of the cold-water coral Desmophyllum dianthus from Comau Fjord, Chile

Bernhardt, J. R., Sunday, J. M., Thompson, P. L., & O'Connor, M. I. (2018). Nonlinear averaging of thermal experience predicts population growth rates in a thermally variable environment. Proceedings of the Royal Society B: Biological Sciences, 285, 20181076.

Bove, C. B., Baumann, J. (2021). Coral Lipid Assay for 96-well plates. Protocols.io

Bove, C. B., Baumann, J. (2021). Coral Carbohydrate Assay for 96-well plates. Protocols.io

Brinton, E. (1962). Variable factors affecting the apparent range and estimated concentration of euphausiids in the North Pacific.

Bureau, D., Kaushik, S., & Cho, C. Y. (2002). Bioenergetics. In J. E. Halver & R. W. Hardy (Eds.), Fish Nutrition. Academic Press, San Diego.

Bustamante, M. S. (2009). *The Southern Chilean Fjord Region: Oceanographic Aspects. Marine Benthic Fauna of Chilean Patagonia.* Santiago: Nature In Focus, 53–60.

Cairns, S. D., Häussermann, V., & Försterra, G. (2005). A review of the Scleractinian (Cnidaria: Anthozoa) of Chile, with the description of two new species. Zootaxa, 1018, 15–46.

Cairns, S.D. (2007): Deep-water corals: an overview with special reference to diversity and distribution of deep-water scleractinian corals. Bulletin of Marine Science 81: 311-322.

Chapron, L., Galand, P. E., Pruski, A. M., Vétion, G., Robin, S., & Lartaud, F. (2021). Resilience of coldwater coral holobionts to thermal stress. Proceedings of the Royal Society B, 288, 20212117.

Davies, A. J., Duineveld, G. C. A., Lavaleye, M. S. S., Bergman, M. J. N., & Van Haren, H. (2009). Downwelling and deep-water bottom currents as food supply mechanisms to the cold-water coral Lophelia pertusa (Scleractinia) at the Mingulay Reef complex. Limnology and Oceanography, 54, 620–629.

Dodds, L. A., Black, K. D., Orr, H., & Roberts, J. M. (2009). Lipid biomarkers reveal geographical differences in food supply to the cold-water coral Lophelia pertusa (Scleractinia). Marine Ecology Progress Series, 397, 113–124.

Dodds, L. A., Roberts, J. M., Taylor, A. C., & Marubini, F. (2007). Metabolic tolerance of the cold-water coral Lophelia pertusa (Scleractinia) to temperature and dissolved oxygen change. Journal of Experimental Marine Biology and Ecology, 349, 205–214.

Doney, S. C., Balch, W. M., Fabry, V. J., & Feely, R. A. (2009). Ocean acidification: a critical emerging problem for the ocean sciences. Oceanography, 22(4), 16-25

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. T., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. Analytical chemistry, 28(3), 350-356.

Duineveld, G. C. A., Jeffreys, R. M., Lavaleye, M. S. S., Davies, A. J., Bergman, M. J. N., Watmough, T., & Witbaard, R. (2012). Spatial and tidal variation in food supply to shallow cold-water coral reefs of the Mingulay Reef complex (Outer Hebrides, Scotland). Marine Ecology Progress Series, 444, 97– 115.

Duineveld, G. C. A., Lavaleye, M. S. S., & Berghuis, E. M. (2004). Particle flux and food supply to a seamount cold-water coral community (Galicia Bank, NW Spain). Marine Ecology Progress Series, 277, 13–23.

Dullo, W.-C., Flögel, S., & Rüggeberg, A. (2008). Cold-water coral growth in relation to the hydrography of the Celtic and Nordic European continental margin. Marine Ecology Progress Series, 371, 165– 176.

Edmunds, Peter & Gates, Ruth. (2002). Normalizing physiological data for scleractinian corals. Coral Reefs - CORAL REEF. 21.

Fillinger, L., & Richter, C. (2013). Vertical and horizontal distribution of Desmophyllum dianthus in Comau Fjord, Chile: A cold-water coral thriving at low pH. PeerJ, 1, e194.

Findlay, H. S., Artioli, Y., Moreno Navas, J., Hennige, S. J., Wicks, L. C., Huvenne, V. A. I., … Roberts, J. M. (2013). Tidal downwelling and implications for the carbon biogeochemistry of cold-water corals in relation to future ocean acidification and warming. Global Change Biology, 19, 2708– 2719.

Försterra, G., & Häussermann, V. (2003). First report on large scleractinian (Cnidaria: Anthozoa) accumulations in cold-temperate shallow water of south Chilean fjords. Zoologische Verhandelingen, 345, 117–128.

Försterra, G., Beuck, L., Häussermann, V., & Freiwald, A. (2005). Shallow water Desmophyllum dianthus (Scleractinia) from Chile: characteristics of the biocoenoses, the bioeroding community, heterotrophic interactions and (paleo)-bathymetric

implications. In A. Freiwald & M. J. Roberts (Eds.), Cold-water Corals and Ecosystems (pp. 937–977). Springer Berlin Heidelberg.

Försterra, G., Häussermann, V., Laudien, J., Jantzen, C., Sellanes, J., and Muñoz, P. (2014). Mass die-off of the cold-water coral *Desmophyllum dianthus* in the Chilean Patagonian fjord region. *Bull. Mar. Sci.* 90, 895–899.

Försterra, G., Häussermann, V., Laudien, J. (2016). Animal forests in the chilean fjords: discoveries, perspectives, and threats in shallow and deep waters. Marine Animal Forests.

Freiwald, A., Fossa, J. H., Grehan, A., Koslow, T., & Roberts, J. M. (2004). Cold-water coral reefs: Out of sight – no longer out of mind. Cambridge, UK: UNEP-WCMC.

Garcia-Herrera, N., Cornils, A., Laudien, J., Niehoff, B., Höfer, J., Försterra, G., ... & Richter, C. (2022). Seasonal and diel variations in the vertical distribution, composition, abundance and biomass of zooplankton in a deep Chilean Patagonian Fjord. PeerJ, 10, e12823.

Georgian, S. E., Deleo, D., Durkin, A., Gómez, C. E., Kurman, M., Lunden, J. J., & Cordes, E. E. (2016). Oceanographic patterns and carbonate chemistry in the vicinity of cold-water coral reefs in the Gulf of Mexico: Implications for resilience in a changing ocean. Limnology and Oceanography, 61, 648–665.

Gnaiger, E., & Bitterlich, G. (1984). Proximate biochemical composition and caloric content calculated from elemental CHN analysis: a stoichiometric concept. Oecologia, 62, 289–298.

Gori, A., Ferrier-Pagès, C., Hennige, S. J., Murray, F., Rottier, C., Wicks, L. C., & Roberts, J. M. (2016). Physiological response of the cold-water coral Desmophyllum dianthus to thermal stress and ocean acidification. PeerJ, 4, e1606.

Gori, A., Tolosa, I., Orejas, C., Rueda, L., Viladrich, N., Grinyó, J., Flögel, S., Grover, R., & Ferrier-Pagès, C. (2018). Biochemical composition of the cold-water coral Dendrophyllia cornigera under contrasting productivity regimes: Insights from lipid biomarkers and compound-specific isotopes. Deep Sea Research Part I: Oceanographic Research Papers, 141, 106–117.

Grange, K. R., Singleton, R. I., Richardson, J. R., Hill, P. J., & Main, W. D. (1981). Shallow rock-wall biological associations of some southern fiords of New Zealand. New Zealand journal of zoology, 8(2), 209-227.

Guinotte, J. M., Orr, J., Cairns, S., Freiwald, A., Morgan, L., & George, R. (2006). Will human-induced changes in seawater chemistry alter the distribution of deep-sea scleractinian corals? Frontiers in Ecology and the Environment, 4, 141–146.

Hargrave, B. T. (2010). Empirical relationships describing benthic impacts of salmon aquaculture. Aquaculture Environment Interactions, 1(1), 33-46.

Harland, A. D., Navarro, J. C., Spencer Davies, P., & Fixter, L. M. (1993). Lipids of some Caribbean and Red Sea corals: total lipid, wax esters, triglycerides and fatty acids. Marine Biology, 117(1), 113-117.

Häussermann, V., & Försterra, G. (2007). Large assemblages of cold-water corals in Chile: a summary of recent findings and potential impacts. Bulletin of Marine Science, 81, 195–207.

Häussermann, V., & Försterra, G. (2009). Marine Benthic Fauna of Chilean Patagonia. Santiago: Nature in Focus.

Häussermann, V., Försterra, G., Melzer, R. R., & Meyer, R. (2013). Gradual changes of benthic biodiversity in Comau Fjord, Chilean Patagonia–lateral observations over a decade of taxonomic research. Spixiana, 36(2), 161-171.

Häussermann, V., Ballyram, S. A., Försterra, G., Cornejo, C., Ibáñez, C. M., Sellanes, J., … Beaujot, F. (2021). Species That Fly at a Higher Game: Patterns of Deep–Water Emergence Along the Chilean Coast, Including a Global Review of the Phenomenon. Frontiers in Marine Science, 8, 1101.

Hennige, S. J., Wicks, L. C., Kamenos, N. A., Bakker, D. C., Findlay, H. S., Dumousseaud, C., & Roberts, J. M. (2014). Short-term metabolic and growth responses of the cold-water coral Lophelia pertusa to ocean acidification. Deep Sea Research Part II: Topical Studies in Oceanography, 99, 27-35.

Henry, Lea-Anne & Roberts, J.. (2017). Global Biodiversity in Cold-Water Coral Reef Ecosystems. Marine Animal Forests, 235-256.

Imbs, A. B. (2013). Fatty acids and other lipids of corals: composition, distribution, and biosynthesis. Russian Journal of Marine Biology, 39(3), 153-168.

Jantzen, C., Laudien, J., Sokol, S., Försterra, G., Häussermann, V., Kupprat, F., & Richter, C. (2013). In situ short-term growth rates of a cold-water coral. Marine and Freshwater Research, 64, 631.

Juva, K., Kutti, T., Chierici, M., Dullo, W.-C., & Flögel, S. (2021). Cold-Water Coral Reefs in the Langenuen Fjord, Southwestern Norway - A Window into Future Environmental Change. Oceans, 2, 583–610.

Keister, E.F., Gantt, S.E., Reich, H.G., Turnham, K. E., Bateman, T. G., Todd, C., Warner, M.E., Kemp, D.W. Similarities in biomass and energy reserves among coral colonies from contrasting reef environments. *Sci Rep* 13, 1355 (2023).

Kiriakoulakis, K., Bett, B.J., White, M., Wolff, G.A., (2004): Organic biogeochemistry of the Darwin Mounds, a deep-water coral ecosystem, of the NE Atlantic. Deep-Sea Research I 51: 1937-1954.

Kutti T, Legrand E, Husa V, Olsen SA, Gjelsvik Ø, Carvajalino-Fernandez M, Johnsen IA (2022) Fish farm effluents cause metabolic depression, reducing energy stores and growth in the reef-forming coral Lophelia pertusa. Aquacult Environ Interact 14:279-293.

Larsson, A. I., Lundälv, T., & Van Oevelen, D. (2013). Skeletal growth, respiration rate and fatty acid composition in the cold-water coral Lophelia pertusa under varying food conditions. Marine Ecology Progress Series, 483, 169–184.

Lavaleye, M., G. Duineveld, T. Lundälv, M. White, D. Guihen, K. Kiriakoulakis, and G. A. Wolff. 2009. Cold-water corals on the Tisler reef. *Oceanography* 22: 76–84.

Leuzinger, S., Willis, B. L., & Anthony, K. R. N. (2012). Energy allocation in a reef coral under varying resource availability. Marine Biology, 159, 177–186.

Lowry OH, Rosebrough NJ, Lewis FA, Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem. 1951;193:265–75.

Maier, C., Schubert, A., Berzunza Sànchez, M. M., Weinbauer, M. G., Watremez, P., & Gattuso, J. P. (2013a). End of the century pCO2 levels do not impact calcification in Mediterranean cold-water corals. PloS one, 8(4), e62655.

Maier, C., Bils, F., Weinbauer, M. G., Watremez, P., Peck, M. A., & Gattuso, J.-P. (2013b). Respiration of Mediterranean cold-water corals is not affected by ocean acidification as projected for the end of the century. Biogeosciences, 10, 5671–5680.

Maier, C., Watremez, P., Taviani, M., Weinbauer, M. G., & Gattuso, J. P. (2012). Calcification rates and the effect of ocean acidification on Mediterranean cold-water corals. Proceedings of the Royal Society B: Biological Sciences, 279, 1716–1723.

Maier, C., Popp, P., Sollfrank, N., Weinbauer, M. G., Wild, C., & Gattuso, J.-P. (2016). Effects of elevated pCO2 and feeding on net calcification and energy budget of the Mediterranean cold-water coral Madrepora oculata. Journal of Experimental Biology, 219, 3208–3217.

Maier, S. R., Bannister, R. J., van Oevelen, D., & Kutti, T. (2020). Seasonal controls on the diet, metabolic activity, tissue reserves and growth of the cold-water coral Lophelia pertusa. Coral Reefs, 39, 173– 187.

Maier, S. R., Jantzen, C., Laudien, J., Häussermann, V., Försterra, G., Cornils, A., … Richter, C. (2021). The carbon and nitrogen budget of Desmophyllum dianthus - a voracious cold-water coral thriving in an acidified Patagonian fjord. PeerJ, 9, e12609.

Maier, S. R., Kutti, T., Bannister, R. J., van Breugel, P., van Rijswijk, P., & van Oevelen, D. (2019). Survival under conditions of variable food availability: Resource utilization and storage in the cold-water coral Lophelia pertusa. Limnology and Oceanography, 64, 1651–1671.

Marshall, K. E., Anderson, K. M., Brown, N. E. M., Dytnerski, J. K., Flynn, K. L., Bernhardt, J. R., … Harley, C. D. G. (2021). Whole-organism responses to constant temperatures do not predict responses to variable temperatures in the ecosystem engineer Mytilus trossulus. Proceedings of the Royal Society B: Biological Sciences, 288, 20202968.

McCulloch, M., Falter, J., Trotter, J., & Montagna, P. (2012a). Coral resilience to ocean acidification and global warming through pH up-regulation. Nature Climate Change, 2, 623–627.

McCulloch, M., Trotter, J., Montagna, P., Falter, J., Dunbar, R., Freiwald, A., … Taviani, M. (2012b). Resilience of cold-water scleractinian corals to ocean acidification: Boron isotopic systematics of pH and saturation state up-regulation. Geochimica et Cosmochimica Acta, 87, 21–34.

Mclachlan, R., Dobson, K., Grottoli, A.G. (2020). Quantification of Total Biomass in Ground Coral Samples.

Mclachlan, R., Price, J., Dobson, K., Weisleder, N., Grottoli, A.G. (2020). Microplate Assay for Quantification of Soluble Protein in Ground Coral Samples.

Mienis, F., de Stigter, H. C., White, M., Duineveld, G., de Haas, H., & van Weering, T. C. E. (2007). Hydrodynamic controls on cold-water coral growth and carbonate-mound development at the SW and SE Rockall Trough Margin, NE Atlantic Ocean. Deep-Sea Research Part I: Oceanographic Research Papers, 54, 1655–1674.

Mienis, F., Duineveld, G. C. A., Davies, A. J., Lavaleye, M. M. S., Ross, S. W., Seim, H., … Van Haren, H. (2014). Cold-water coral growth under extreme environmental conditions, the Cape Lookout area, NW Atlantic. Biogeosciences, 11, 2543–2560.

Mora, C., Wei, C. L., Rollo, A., Amaro, T., Baco, A. R., Billett, D., ... & Yasuhara, M. (2013). Biotic and human vulnerability to projected changes in ocean biogeochemistry over the 21st century. PLoS biology, 11(10), e1001682.

Morash, A. J., Neufeld, C., MacCormack, T. J., & Currie, S. (2018). The importance of incorporating natural thermal variation when evaluating physiological performance in wild species. Journal of Experimental Biology, 221, 164673.

Movilla, J., Orejas, C., Calvo, E., Gori, A., López-Sanz, À., Grinyó, J., … Pelejero, C. (2014). Differential response of two Mediterranean cold-water coral species to ocean acidification. Coral Reefs, 33, 675–686.

Mueller, C. E., Larsson, A. I., Veuger, B., Middelburg, J. J., & van Oevelen, D. (2014). Opportunistic feeding on various organic food sources by the cold-water coral Lophelia pertusa. Biogeosciences, 11, 123–133.

Naumann, M. S., Orejas, C., Wild, C., & Ferrier-Pagès, C. (2011). First evidence for zooplankton feeding sustaining key physiological processes in a scleractinian cold-water coral. Journal of Experimental Biology, 214, 3570–3576.

Naumann, M. S., Orejas, C., & Ferrier-Pagès, C. (2014). Species-specific physiological response by the cold-water corals Lophelia pertusa and Madrepora oculata to variations within their natural

Naumann, M. S., Tolosa, I., Taviani, M., Grover, R., & Ferrier-Pagès, C. (2015). Trophic ecology of two cold-water coral species from the Mediterranean Sea

revealed by lipid biomarkers and compound-specific isotope analyses. Coral Reefs, 34, 1165–1175.

Palma, S., & Silva, N. (2004). Distribution of siphonophores, chaetognaths, euphausiids and oceanographic conditions in the fjords and channels of southern Chile. Deep Sea Research Part II: Topical Studies in Oceanography, 51(6-9), 513-535.

Rapp, H. T., & Sneli, J. A. (1999). Lophelia pertusa–myths and reality (abstract only). 2nd Nord. Mar. Sci. Meet., Hirtshals, Denmark, 2-4.

R Core Team. (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. Retrieved from https://www.r-project.org/

Roberts, J. M., Wheeler, A. J., & Freiwald, A. (2006). Reefs of the Deep: The Biology and Geology of Cold-Water Coral Ecosystems. Science, 312, 543–547.

Roberts, J. M., & Cairns, S. D. (2014). Cold-water corals in a changing ocean. Current Opinion in Environmental Sustainability, 7, 118-126.

Rodrigues, Lisa & Grottoli, Andrea. (2007). Energy Reserves and Metabolism as Indicators of Coral Recovery from Bleaching. Limnology and Oceanography. 52 (5)

Sanchez, N., González, H. E., & Iriarte, J. L. (2011). Trophic interactions of pelagic crustaceans in Comau Fjord (Chile): their role in the food web structure. Journal of Plankton Research, 33(8), 1212-1229.

Schoepf, V., Grottoli, A. G., Warner, M. E., Cai, W. J., Melman, T. F., Hoadley, K. D., … Baumann, J. H. (2013). Coral Energy Reserves and Calcification in a High-CO2 World at Two Temperatures. PLoS ONE, 8, e75049.

Seemann, J., Sawall, Y., Auel, H., & Richter, C. (2013). The use of lipids and fatty acids to measure the trophic plasticity of the coral Stylophora subseriata. Lipids, 48(3), 275-286.

Thiem, O., Ravagnan, E., Fossa, J. H., & Berntsen, J. (2006). Food supply mechanisms for cold-water corals along a continental shelf edge. Journal of Marine Systems, 60, 207–219.

Van Rooij, D., De Mol, B., Huvenne, V., Ivanov, M., Henriet, J.P. (2003): Seismic evidence of current-controlled sedimentation in the Belgica Mound province, upper Porcupine slope, southwest of Ireland. Marine Geology 195: 31-53.

Wall, M., Ragazzola, F., Foster, L. C., Form, A., & Schmidt, D. N. (2015). pH up-regulation as a potential mechanism for the cold-water coral Lophelia pertusa to sustain growth in aragonite undersaturated conditions. Biogeosciences, 12, 6869–6880.

Wall., C. B., Ritson-Williams, R., Popp, B. N., Gates, R. D. (2019). Spatial variation in the biochemical and isotopic composition of corals during bleaching and recovery. Limnology and Oceanography, 64 (5), 2011-2028

Wells, S. R., Bresnan, E., Cook, K., Eerkes-Medrano, D., Machairopoulou, M., Mayor, D. J., ... & Wright, P. J. (2022). Environmental drivers of a decline in a coastal zooplankton community. ICES Journal of Marine Science, 79(3), 844-854.

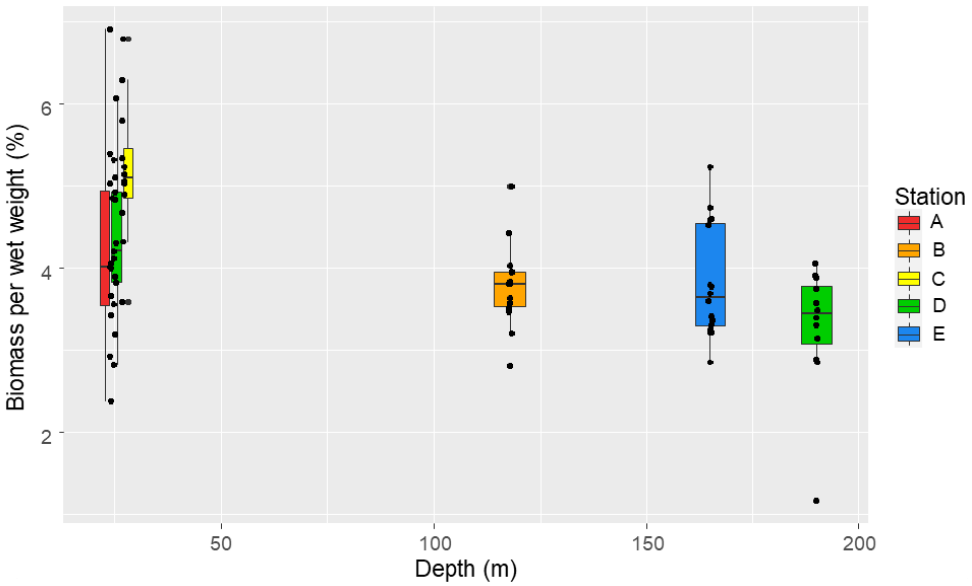
Acknowledgments

Ich möchte mich besonders bedanken bei meinen beiden Betreuerinnen Krissi und Marlene für die Hilfe im Labor, mit der Auswertung der Daten und der Unterstützung beim Schreiben der Bachelorarbeit. Ich bin sehr froh euch als Betreuerinnen gehabt zu haben.

Weiter möchte ich mich bei Claudio bedanken, dafür dass ich am Alfred-Wegener-Institut meine Bachelorarbeit schreiben konnte, für die anfängliche Organisation und für die Bewertung meiner Bachelorarbeit. Dann möchte ich Hakon dafür danken, dass er als zweiter Gutachter meine Bachelorarbeit bewertet.

Danke auch an Esther, Antonia und Steffi für die Hilfe im Labor.

Supplements



Supplementary Figure 1: Percentage of biomass per wet weight of *Desmophyllum dianthus* in Comau Fjord. The biomass per wet weight is shown in % at 6 stations along the fjord in relation to the water depths of the stations. The colours correspond to the stations along the fjord, from fjord mouth (A, red) to fjord head (E, blue).

The percentage of biomass per total wet weight of *D. dianthus* was significantly higher at station C compared to all deep stations (C-B: p=0.0032, C-Dd: p<0.0001, C-E: p= 0.0032) and significantly higher at station A and Ds than at station Dd (A-Dd: p=0.0443, Ds-Dd: p=0.0165). The highest mean biomass per wet weight was found at station C and the lowest at station Dd.

Supplementary Table 1: Energy reserves of *Desmophyllum dianthus* in Comau Fjord. Proteins, carbohydrates, lipids and total energy reserves (all in kJ gdw^{-1}) of *D. dianthus* (mean ± standard deviation) at 6 stations ranging from 24 m to 190 m depth.

Station	N	Depth (m)	Proteins (kJ/gdw)	Carbohydrates (kJ/gdw)	Lipids (kJ/gdw)	Total energy reserves (kJ/gdw)
A	9	24	1.91 ± 0.47	0.25 ± 0.08	3.78 ± 1.71	5.91 ± 2.08
B	13	118	1.87 ± 0.39	0.28 ± 0.05	4.49 ± 1.03	6.64 ± 0.96
C	10	27	1.95 ± 0.27	0.31 ± 0.05	5.01 ± 1.01	7.25 ± 1.16
Ds	7	25	1.86 ± 0.44	0.28 ± 0.11	3.57 ± 1.14	5.50 ± 1.45
Dd	11	190	1.54 ± 0.34	0.19 ± 0.03	4.63 ± 1.40	6.36 ± 1.58
E	15	165	1.82 ± 0.27	0.22 ± 0.04	5.76 ± 1.60	7.80 ± 1.58

Supplementary Table 2: Energy reserves and biomass per surface area of *Desmophyllum dianthus* in Comau Fjord in 2017. Total energy reserves (kJ g^{-1}) and biomass (mg cm^{-2}) of *D. dianthus* at 7 stations (mean ± standard deviation). The stations are not the same as in the present study as station A is located at the head of the fjord and station F at its mouth. Stations A-F are located at 20 m water depth, whereas station Ed is located at 300 m depth. Data are from Beck et al. (2022; in prep) and K. Beck, unpub. Data, excluding transplanted corals.

Station	N	Depth	Total energy reserves (kJ g^{-1})		Biomass (mg cm^{-2})
			May	August	
A	7	20	9.45 ± 2.06	5.55 ± 1.74	7.52 ± 3.06
B	8	20	6.85 ± 1.83	9.45 ± 2.28	10.07 ± 5.23
C	8	20	8.60 ± 2.22	5.86 ± 2.65	26.05 ± 10.46
D	10	20	7.46 ± 3.16	5.82 ± 2.43	6.90 ± 2.21
Es	7	20	10.44 ± 3.22	8.21 ± 2.52	8.85 ± 1.15
Ed	6	300	-	9.06 ± 2.28	28.59 ± 7.22
F	10	20	11.52 ± 2.42	5.76 ± 1.25	10.50 ± 2.90

Supplementary Table 3: Total weight, tissue covered surface area and biomass amount per total wet weight of *Desmophyllum dianthus* in Comau Fjord. Total wet weight (g) and surface area (cm^2) were measured for another study for additional individuals of the same population and at the same sampling time as for the present study. The biomass amount per total wet weight (%) was calculated for the individuals of the present study. All values are represented as mean ± standard deviation.

Station	N	Depth (m)	Total wet weight (g)	Tissue covered surface area (cm^{-2})	biomass amount per total wet weight (%)
A	28	24	14.79 ± 7.78	36.10 ± 11.59	4.24 ± 1.20
C	30	27	6.02 ± 2.34	14.38 ± 4.43	5.18 ± 0.81
Dd	21	190	26.81 ± 15.45	30.23 ± 12.17	3.28 ± 0.74

Supplementary Table 4: Calculated biomass and total energy reserves for *Desmophyllum dianthus* in Comau Fjord. Biomass amount (g cm^{-2} and g $polyp^{-1}$), as well as total energy reserves (kJ cm^{-2} and kJ $polyp^{-1}$) were calculated using the mentioned values in Supplementary Table 3. All values are represented as mean ± standard deviation.

Station	N	Depth (m)	Biomass (mg cm^{-2})	Total energy reserves (kJ cm^{-2})	Biomass (g $polyp^{-1}$)	Total energy reserves (kJ $polyp^{-1}$)
A	28	24	17.58 ± 9.93	0.10 ± 0.06	0.59 ± 0.31	3.50 ± 1.84
C	30	27	21.40 ± 6.97	0.16 ± 0.05	0.31 ± 0.12	2.23 ± 0.87
Dd	21	190	30.16 ± 18.06	0.23 ± 0.14	0.88 ± 0.55	5.61 ± 3.50

Supplementary Table 5: Post-hoc tests for linear model of protein energy content of *Desmophyllum dianthus* in Comau Fjord. Statistically significant results are highlighted in bold.

contrast	estimate	SE	df	t.ratio	p.value
A-B	0.00198	0.00645	71	0.306	0.9996
A-C	-0.00139	0.00668	71	-0.208	0.9999
A-Ds	0.00211	0.00656	71	0.321	0.9995
A-Dd	0.01563	0.00683	71	2.289	0.2121
A-E	0.00396	0.00627	71	0.632	0.9882
B-C	-0.00337	0.0063	71	-0.534	0.9946
B-Ds	0.00013	0.00617	71	0.021	1
B-Dd	0.01365	0.00645	71	2.116	0.2909
B-E	0.00199	0.00586	71	0.339	0.9994
C-Ds	0.0035	0.00641	71	0.545	0.994
C-Dd	0.01702	0.00668	71	2.546	0.1247
C-E	0.00535	0.00611	71	0.875	0.9512
Ds-Dd	0.01352	0.00656	71	2.062	0.3191
Ds-E	0.00186	0.00598	71	0.311	0.9996
Dd-E	-0.01167	0.00627	71	-1.86	0.4348

Supplementary Table 6: Post hoc test for generalised linear model of carbohydrate energy content of *Desmophyllum dianthus* in Comau Fjord. Statistically significant results are highlighted in bold.

contrast	estimate	SE	df	t.ratio	p.value
A-B	-0.0875	0.0948	71	-0.923	0.9393
A-C	-0.2056	0.0982	71	-2.094	0.3023
A-Ds	-0.2518	0.0964	71	-2.613	0.1074
A-Dd	0.2754	0.1003	71	2.746	0.0788
A-E	0.1251	0.0921	71	1.357	0.7519
B-C	-0.1181	0.0925	71	-1.276	0.7969
B-Ds	-0.1644	0.0906	71	-1.814	0.4635
B-Dd	0.3629	0.0948	71	3.829	**0.0036**
B-E	0.2125	0.0861	71	2.469	0.1474
C-Ds	-0.0463	0.0942	71	-0.491	0.9963
C-Dd	0.481	0.0982	71	4.899	**0.0001**
C-E	0.3306	0.0898	71	3.681	**0.0058**
Ds-Dd	0.5272	0.0964	71	5.471	**<.0001**
Ds-E	0.3769	0.0878	71	4.291	**0.0008**
Dd-E	-0.1503	0.0921	71	-1.632	0.5806

Supplementary Table 7: Post hoc test for generalised linear model of lipid energy content of *Desmophyllum dianthus* in Comau Fjord. Statistically significant results are highlighted in bold

contrast	estimate	SE	df	t.ratio	p.value
A-B	-0.03435	0.144	65	-0.238	0.9999
A-C	-0.1352	0.152	65	-0.888	0.9481
A-Ds	0.14324	0.165	65	0.867	0.9531
A-Dd	-0.14303	0.149	65	-0.959	0.9291
A-E	-0.26668	0.14	65	-1.899	0.4123
B-C	-0.10085	0.14	65	-0.718	0.979
B-Ds	0.17759	0.154	65	1.15	0.8584
B-Dd	-0.10868	0.137	65	-0.793	0.9678
B-E	-0.23233	0.128	65	-1.822	0.4592
C-Ds	0.27844	0.162	65	1.72	0.524
C-Dd	-0.00783	0.145	65	-0.054	1
C-E	-0.13148	0.136	65	-0.963	0.9277
Ds-Dd	-0.28627	0.159	65	-1.8	0.4729
Ds-E	-0.40992	0.151	65	-2.717	0.0856
Dd-E	-0.12365	0.133	65	-0.929	0.9375

Supplementary Table 8: Post hoc test for generalised linear model of total energy reserves of *Desmophyllum dianthus* in Comau Fjord. Statistically significant results are highlighted in bold.

contrast	estimate	SE	df	t.ratio	p.value
A-B	-0.0821	0.118	64	-0.693	0.8494
A-C	-0.4004	0.125	64	-3.202	0.3825
A-Ds	0.092	0.136	64	0.678	0.9882
A-Dd	0.0232	0.125	64	0.185	0.9801
A-E	-0.2829	0.115	64	-2.453	0.06
B-C	-0.3183	0.115	64	-2.761	0.942
B-Ds	0.1741	0.127	64	1.373	0.4928
B-Dd	0.1052	0.115	64	0.913	0.998
B-E	-0.2008	0.105	64	-1.918	0.4526
C-Ds	0.4924	0.133	64	3.703	0.1461
C-Dd	0.4235	0.122	64	3.471	0.7999
C-E	0.1174	0.112	64	1.048	0.9709
Ds-Dd	-0.0689	0.133	64	-0.518	0.7772
Ds-E	-0.3749	0.124	64	-3.026	**0.0159**
Dd-E	-0.3061	0.112	64	-2.731	0.2722

Supplementary Table 9: Post hoc test for generalised linear model of relative lipid content of *Desmophyllum dianthus* in Comau Fjord. Statistically significant results are highlighted in bold.

contrast	estimate	SE	df	t.ratio	p.value
A-B	-0.1116	0.0554	64	-2.013	0.3465
A-C	-0.1397	0.0585	64	-2.388	0.1761
A-Ds	-0.0409	0.0635	64	-0.643	0.9872
A-Dd	-0.2126	0.0585	64	-3.633	**0.0071**
A-E	-0.229	0.054	64	-4.243	**0.001**
B-C	-0.0281	0.0539	64	-0.52	0.9952
B-Ds	0.0707	0.0593	64	1.192	0.839
B-Dd	-0.1009	0.0539	64	-1.871	0.429
B-E	-0.1174	0.049	64	-2.396	0.1732
C-Ds	0.0988	0.0622	64	1.588	0.6092
C-Dd	-0.0729	0.0571	64	-1.276	0.7966
C-E	-0.0893	0.0524	64	-1.703	0.5347
Ds-Dd	-0.1717	0.0622	64	-2.76	**0.0776**
Ds-E	-0.1881	0.058	64	-3.245	**0.022**
Dd-E	-0.0165	0.0524	64	-0.314	0.9996

Supplementary Table 10: Post hoc test for generalised linear model of relative protein content of *Desmophyllum dianthus* in Comau Fjord. Statistically significant results are highlighted in bold.

contrast	estimate	SE	df	t.ratio	p.value
A-B	0.1403	0.0753	64	1.862	0.4343
A-C	0.187	0.0795	64	2.352	0.189
A-Ds	0.0835	0.0863	64	0.967	0.9266
A-Dd	0.251	0.0795	64	3.157	**0.0281**
A-E	0.2699	0.0733	64	3.68	**0.0061**
B-C	0.0467	0.0733	64	0.637	0.9877
B-Ds	-0.0568	0.0806	64	-0.705	0.9807
B-Dd	0.1107	0.0733	64	1.51	0.6594
B-E	0.1296	0.0666	64	1.947	0.3841
C-Ds	-0.1035	0.0845	64	-1.225	0.8232
C-Dd	0.064	0.0776	64	0.825	0.9619
C-E	0.0829	0.0713	64	1.164	0.8522
Ds-Dd	0.1675	0.0845	64	1.981	0.3642
Ds-E	0.1865	0.0788	64	2.367	0.1836
Dd-E	0.0189	0.0713	64	0.266	0.9998

Supplementary Table 11: Post hoc test for generalised linear model of relative carbohydrate content of *Desmophyllum dianthus* in Comau Fjord. Statistically significant results are highlighted in bold.

contrast	estimate	SE	df	t.ratio	p.value
A-B	0.00415	0.0861	64	0.048	1
A-C	-0.01913	0.0909	64	-0.21	0.9999
A-Ds	-0.17319	0.0986	64	-1.756	0.5012
A-Dd	0.27057	0.0909	64	2.978	**0.0452**
A-E	0.33358	0.0838	64	3.979	**0.0024**
B-C	-0.02328	0.0838	64	-0.278	0.9998
B-Ds	-0.17734	0.0922	64	-1.924	0.3974
B-Dd	0.26641	0.0838	64	3.179	**0.0264**
B-E	0.32942	0.0761	64	4.328	**0.0007**
C-Ds	-0.15406	0.0966	64	-1.594	0.6053
C-Dd	0.28969	0.0887	64	3.267	**0.0207**
C-E	0.3527	0.0815	64	4.33	**0.0007**
Ds-Dd	0.44375	0.0966	64	4.592	**0.0003**
Ds-E	0.50676	0.0901	64	5.627	**<.0001**
Dd-E	0.06301	0.0815	64	0.774	0.971

Supplementary Table 12: Post hoc test for generalised linear model of approximated total energy reserves per surface area of *Desmophyllum dianthus* in Comau Fjord. Statistically significant results are highlighted in bold.

contrast	estimate	SE	df	t.ratio	p.value
A-C	-0.401	0.134	76	-3.001	**0.0101**
A-Dd	-0.613	0.147	76	-4.178	**0.0002**
C-Dd	-0.212	0.145	76	-1.467	0.3122

Supplementary Table 13: Post hoc test for generalised linear model of approximated total energy reserves per polyp of *Desmophyllum dianthus* in Comau Fjord. Statistically significant results are highlighted in bold.

contrast	estimate	SE	df	t.ratio	p.value
A-C	0.452	0.136	76	3.316	**0.004**
A-Dd	-0.47	0.15	76	-3.141	**0.0067**
C-Dd	-0.922	0.148	76	-6.249	**<.0001**

Universität Bremen

Offizielle Erklärungen von

Nachname: ___Krämer_____ Vorname: _____Carina_____
Matrikelnr.: 4588863

A) Eigenständigkeitserklärung
Ich versichere, dass ich die vorliegende Arbeit selbstständig verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel verwendet habe.
Alle Teile meiner Arbeit, die wortwörtlich oder dem Sinn nach anderen Werken entnommen sind, wurden unter Angabe der Quelle kenntlich gemacht. Gleiches gilt auch für Zeichnungen, Skizzen, bildliche Darstellungen sowie für Quellen aus dem Internet.
Die Arbeit wurde in gleicher oder ähnlicher Form noch nicht als Prüfungsleistung eingereicht.
Die elektronische Fassung der Arbeit stimmt mit der gedruckten Version überein.
Mir ist bewusst, dass wahrheitswidrige Angaben als Täuschung behandelt werden.

B) Erklärung zur Veröffentlichung von Bachelor- oder Masterarbeiten

Die Abschlussarbeit wird zwei Jahre nach Studienabschluss dem Archiv der Universität Bremen zur dauerhaften Archivierung angeboten. Archiviert werden:
1) Masterarbeiten mit lokalem oder regionalem Bezug sowie pro Studienfach und Studienjahr 10 % aller Abschlussarbeiten
2) Bachelorarbeiten des jeweils ersten und letzten Bachelorabschlusses pro Studienfach u. Jahr.

☐ Ich bin damit einverstanden, dass meine Abschlussarbeit im Universitätsarchiv für wissenschaftliche Zwecke von Dritten eingesehen werden darf.

☐ Ich bin damit einverstanden, dass meine Abschlussarbeit nach 30 Jahren (gem. §7 Abs. 2 BremArchivG) im Universitätsarchiv für wissenschaftliche Zwecke von Dritten eingesehen werden darf.

☒ Ich bin nicht damit einverstanden, dass meine Abschlussarbeit im Universitätsarchiv für wissenschaftliche Zwecke von Dritten eingesehen werden darf.

C) Einverständniserklärung über die Bereitstellung und Nutzung der Bachelorarbeit / Masterarbeit / Hausarbeit in elektronischer Form zur Überprüfung durch Plagiatssoftware

Eingereichte Arbeiten können mit der Software *Plagscan* auf einen hauseigenen Server auf Übereinstimmung mit externen Quellen und der institutionseigenen Datenbank untersucht werden.
Zum Zweck des Abgleichs mit zukünftig zu überprüfenden Studien- und Prüfungsarbeiten kann die Arbeit dauerhaft in der institutionseigenen Datenbank der Universität Bremen gespeichert werden.

☐ Ich bin damit einverstanden, dass die von mir vorgelegte und verfasste Arbeit zum Zweck der Überprüfung auf Plagiate auf den *Plagscan*-Server der Universität Bremen hochgeladen wird.

☐ Ich bin ebenfalls damit einverstanden, dass die von mir vorgelegte und verfasste Arbeit zum o.g. Zweck auf dem *Plagscan*-Server der Universität Bremen hochgeladen u. dauerhaft auf dem *Plagscan*-Server gespeichert wird.

☒ Ich bin nicht damit einverstanden, dass die von mir vorgelegte u. verfasste Arbeit zum o.g. Zweck auf dem *Plagscan*-Server der Universität Bremen hochgeladen u. dauerhaft gespeichert wird.

Mit meiner Unterschrift versichere ich, dass ich die oben stehenden Erklärungen gelesen und verstanden habe. Mit meiner Unterschrift bestätige ich die Richtigkeit der oben gemachten Angaben.

17.02.2023, Bremen
Datum, Ort

Unterschrift