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Zooplankton spatial and temporal dynamics, community composition and bentho-pelagic coupling in Comau Fjord, northern Chilean Patagonia

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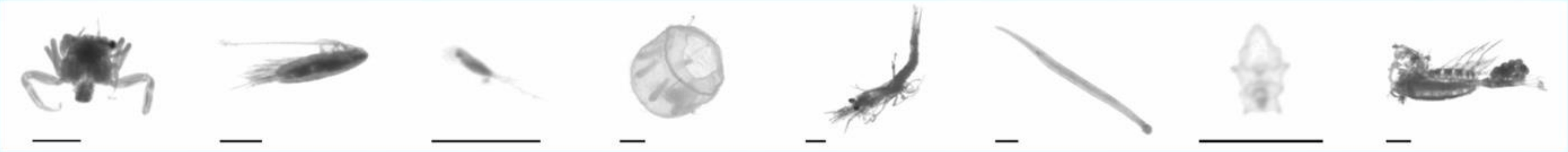

Zooplankton spatial and temporal dynamics, community composition and bentho-pelagic coupling in Comau Fjord, northern Chilean Patagonia

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“Un hombre que no tiene conocimiento del universo celestial ha perdido una porción de su herencia y es como una piedra que no sabe nada de la humanidad”

´Abdu´l-Bahá

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Summary

Zooplankton are key players in the functioning of the marine pelagic food web of high latitude ecosystems as they constitute an essential link between primary producers and higher consumers. In addition, zooplankton provide food for benthic invertebrates, such as cold-water corals. The steep walls of Comau Fjord, northern Chilean Patagonia, harbor a diverse and rich benthic community, including dense aggregations of cold-water corals that thrive even in adverse conditions such as aragonite-depleted waters. This paradox has been attributed to a rich food supply, zooplankton being the main diet of the corals. However, the information on zooplankton seasonal and vertical distribution, together with its community composition, is still very fragmentary in Patagonian waters. Furthermore, abiotic and biotic drivers influencing the zooplankton dynamics under seasonal environmental variations (e.g. changes in solar radiation and rainfall) and their potential linkage to the corals in Comau Fjord are so far unknown.

The aim of this thesis is to identify the drivers governing the zooplankton dynamics in Comau Fjord, and to understand the role of zooplankton in the bentho-pelagic coupling. To achieve this objective, the zooplankton distribution and composition were investigated both spatially and temporally. Stratified vertical hauls from surface waters to near the seabed were carried out both seasonally and during day and night by a closing Nansen net at different locations in the fjord. Subsequently, zooplankton samples were scanned with state-of-art digital imaging system (ZooScan), and zooplankton abundance, biovolume and biomass were determined for 41 zooplankton and 11 meroplankton taxa out of a total of 163,840 vignettes identified in EcoTaxa. The zooplankton availability was used to estimate spatial and temporal differences in food availability for benthic coral communities and were further compared to measured trophic markers in corals from different coral populations along the fjord.

The zooplankton distribution in Comau Fjord showed a pronounce seasonality (**chapter 2**). Its abundance peaked in austral spring, suggesting a close link between zooplankton reproduction and phytoplankton spring bloom. However, zooplankton biomass was the highest in summer and lowest in winter, indicating that during the warmer season, bigger individuals or larger taxa developed and during the cold season the low primary production was not enough to support a high zooplankton growth. Higher zooplankton biovolume were observed in the outer part of Comau Fjord compared to the central-inner fjord and could clearly be associated with a higher chlorophyll *a* concentration at the fjord mouth (**chapter 3**). Throughout the year and along the horizontal transect, zooplankton abundance was concentrated in surface waters, while highest biovolumes were found in intermediate waters during daytime, and in surface layers during nighttime, indicating the diel vertical migration of large zooplankton (**chapter 2 and 3**).

The community composition of zooplankton in Comau Fjord was mainly dominated by copepods, both in abundance and biovolume, followed by chaetognaths, mysids, cnidarians, and amphipods (in terms of biovolume) and meroplankton, appendicularians and ostracods (in terms of abundance) (**chapter 2 and 3**). Meroplankton, defined as planktonic life stages of benthic organisms, have an important role in the bentho-pelagic coupling of marine ecosystems. Meroplankton abundance concentrated in the upper 50 m of the water column (**chapter 4**), and was highest in spring, coinciding with higher chlorophyll *a* and oxygen concentrations, suggesting a close coupling between macrobenthos reproduction and meroplankton feeding with the phytoplankton spring bloom. The dominance of the different meroplankton taxa shifted seasonally, being echinoderms the most abundant in spring, gastropods in summer and autumn, and bryozoans in winter.

The fatty acid composition of corals from both shallow and deep water sites revealed strong differences in their main food source (**chapter 5**). In shallow, fatty acid markers indicated a phytoplankton-based (dominated by diatom) diet with contribution of small copepods, while corals from the deep sites showed a dominance of calanoid copepod-related markers that may explain the higher energy stores in deep corals. However, this is not reflected by the low zooplankton assessment (**chapter 2 and 3**). This discrepancy may derive from ecological differences at the two different depths as well as methodological issues, both discussed in this thesis.

Overall, this thesis provides novel insights into (a) the zooplankton community composition in Chilean Patagonian waters, mainly dominated by copepods, with an unprecedented level of spatial and temporal detail; (b) the main drivers governing the zooplankton dynamics, such as water column stratification, estuarine circulation or diel vertical migration; and (c) how the strong seasonal environmental variations of Comau Fjord affect not only the pelagic system (holo- and meroplankton), especially in the highly variable surface waters, but also the coral communities that populate the fjord. Furthermore, this thesis highlights the importance of zooplankton as an essential food supply to opportunistic benthic invertebrates that seasonally need to adapt to the high variations in zooplankton distribution and affect their trophic ecology. Additionally, the data clearly underscores the information gain through regular monitoring of pelagic communities in coastal ecosystems that allow to assess the natural extent of bentho-pelagic coupling as well as how anthropogenic perturbations may impact the functioning of the marine food web.

Zusammenfassung

Zooplankton spielt eine wichtige Schlüsselrolle für das pelagische Nahrungsnetz in marinen Ökosystemen der hohen Breitengrade, da es ein wichtiges Bindeglied zwischen Primärproduzenten und Konsumenten darstellt. Darüber hinaus dient Zooplankton als Nahrungsquelle für benthische wirbellose Tiere, wie beispielsweise Kaltwasserkorallen. Die Steilwände des Comau Fjords im Norden Chiles beherbergen eine vielfältige benthische Gemeinschaft, darunter dichte Ansammlungen von Kaltwasserkorallen, die selbst unter ungünstigen Bedingungen wie Aragonituntersättigung gedeihen. Dieses Paradoxon wird auf ein reiches Nahrungsangebot zurückgeführt, wobei Zooplankton die Hauptnahrungsquelle der Korallen darstellt. Es fehlen bisher jedoch noch wichtige Informationen über die saisonale und vertikale Verteilung und Artenzusammensetzung des Zooplanktons in den patagonischen Gewässern. Darüber hinaus sind die abiotischen und biotischen Faktoren, die die Zooplanktondynamik durch saisonale Umweltschwankungen beeinflussen (z. B. eine Veränderung der Sonneneinstrahlung und Niederschläge), und ihre mögliche Verbindung zu den Korallen im Comau Fjord, bisher unbekannt.

Ziel dieser Arbeit ist es daher, die treibenden Kräfte für die Zooplanktondynamik im Comau Fjord zu identifizieren und die Rolle des Zooplanktons in der bentho-pelagischen Kopplung zu verstehen. Um dieses Ziel zu erreichen, wurden sowohl die räumlichen als auch die zeitlichen Veränderungen der Verteilung und Zusammensetzung des Zooplanktons untersucht. Dafür wurde die gesamte Wassersäule vom Oberflächenwasser bis in die Nähe des Meeresbodens sowohl saisonal als auch tagsüber und nachts mit einem sich schließenden Nansen-Netz an verschiedenen Stellen im Fjord vertikal beprobt. Anschließend wurden die Zooplanktonproben mit einem hochmodernen digitalen Bildgebungssystem (ZooScan) gescannt. Danach wurden die Abundanz, das Biovolumen und die Biomasse des Zooplanktons für 41 Zooplankton- und 11 Meroplanktontaxa aus insgesamt 163.840 in EcoTaxa identifizierten Vignetten bestimmt. Anhand der Zooplanktonverfügbarkeit wurden die räumlichen und zeitlichen Unterschiede in der Nahrungsverfügbarkeit für benthische Korallengemeinschaften bestimmt und mit gemessenen trophischen Markern in Korallen von verschiedenen Populationen entlang des Fjords verglichen.

Die Verteilung des Zooplanktons im Comau Fjord zeigte eine ausgeprägte Saisonalität (**Kapitel 2**). Die Abundanz erreichte ihren Höhepunkt im Frühjahr, was auf einen engen Zusammenhang zwischen der Fortpflanzung des Zooplanktons und der Frühjahrsblüte des Phytoplanktons schließen lässt. Die Biomasse des Zooplanktons war jedoch im Sommer am höchsten und im Winter am niedrigsten. Dies deutet darauf hin, dass sich in der wärmeren Jahreszeit größere Individuen oder größere Taxa entwickelten und in der kalten Jahreszeit die geringe Primärproduktion nicht für einen hohen Zuwachs des Zooplanktons ausreichte. An der

Mündung des Comau Fjords war das Biovolumen des Zooplanktons höher als im zentralen und inneren Bereich des Fjords, was eindeutig mit einer höheren Konzentration von Chlorophyll-*a* an der Fjordmündung in Verbindung gebracht werden kann (**Kapitel 3**). Während des gesamten Jahres und entlang des horizontalen Transekts kam am meisten Zooplankton in der Oberflächenschicht vor. Die höchsten Biovolumina waren hingegen tagsüber in den mittleren Wasserschichten und nachts in der Oberflächenschicht zu finden, was auf die tägliche vertikale Migration von großen Zooplanktonorganismen hinweist (**Kapitel 2 und 3**).

Die Zusammensetzung der Zooplanktongemeinschaft im Comau Fjord wurde hauptsächlich von Copepoden dominiert, sowohl in Bezug auf die Abundanz als auch auf das Biovolumen, gefolgt von Chaetognathen, Mysiden, Nesseltieren und Amphipoden (in Bezug auf das Biovolumen) und Meroplankton, Appendicularia und Ostracoden (in Bezug auf die Abundanz) (**Kapitel 2 und 3**). Meroplankton, die planktischen Lebensstadien benthischer Organismen, spielt eine wichtige Rolle bei der bentho-pelagischen Kopplung mariner Ökosysteme. Das Meroplanktons war in den oberen 50 m der Wassersäule am häufigsten (**Kapitel 4**). Die Abundanz des Meroplanktons war im Frühjahr am höchsten, was mit höherem Chlorophyll *a*- und Sauerstoffkonzentrationen zusammenfiel und auf einen engen Zusammenhang zwischen der Fortpflanzung und Ernährung des Meroplanktons und der Phytoplanktonblüte im Frühjahr schließen lässt. Verschiedenen Meroplankton-Taxa waren saisonal dominierend, wobei Echinoderme im Frühjahr am häufigsten vorkamen, Gastropoden im Sommer und Herbst und Bryozoen im Winter.

Die Fettsäurezusammensetzung von Korallen aus flachen und tiefen Wasserschichten zeigte starke Unterschiede in der Hauptnahrungsquelle der Korallen (**Kapitel 5**). Im Flachwasser wiesen die Fettsäuremarker auf eine Ernährung auf Phytoplanktonbasis (vor allem Kieselalgen) und kleiner Copepoden hin. Die Korallen aus tieferen Wasserschichten wiesen hingegen überwiegend calanoide Copepodenmarker auf, was die große Menge an Speicherlipiden der Korallen in der Tiefe erklären kann. Dies spiegelt sich jedoch nicht in der geringen Bewertung des Zooplanktons wider (**Kapitel 2 und 3**). Diese Diskrepanz könnte auf ökologische Unterschiede in den beiden Wassertiefen, sowie auf methodische Probleme zurückzuführen sein, die in dieser Arbeit erörtert werden.

Insgesamt liefert diese Arbeit neue Erkenntnisse über (a) die Zusammensetzung der Zooplankton-Gemeinschaft in den chilenisch-patagonischen Gewässern, die hauptsächlich von Copepoden dominiert wird, mit einem noch nie dagewesenen Grad an räumlicher und zeitlicher Auflösung; (b) die wichtigsten Faktoren, die die Zooplanktondynamik bestimmen, wie z.B. die Schichtung der Wassersäule, die Ästuar-Zirkulation oder die tägliche vertikale Migration des Zooplanktons; und (c) die Auswirkungen der starken saisonalen Umweltschwankungen auf das pelagische System (Holo- und Meroplankton), insbesondere in dem stark schwankenden Oberflächenwasser und die Korallengemeinschaften in diesem Fjord. Darüber hinaus

unterstreicht diese Arbeit die Bedeutung des Zooplanktons als wichtige Nahrungsquelle für opportunistische benthische Wirbellose, die sich saisonal an die stark schwankende Zooplanktonverteilung anpassen müssen und deren trophische Ökologie beeinflussen. Darüber hinaus zeigen die Daten einen deutlichen Informationsgewinn durch die regelmäßige Beprobung pelagischer Gemeinschaften in Küstenökosystemen. Dies ermöglicht es das natürliche Ausmaß der benthisch-pelagischen Kopplung sowie die Auswirkungen anthropogener Störungen auf die Funktionsweise des marinen Nahrungsnetzes zu beurteilen.

Resumen

El zooplancton es un actor clave en el funcionamiento de la red trófica pelágica marina de los ecosistemas de altas latitudes, ya que constituye un enlace esencial entre los productores primarios y los consumidores superiores. Además, el zooplancton proporciona alimento a los invertebrados bentónicos, como por ejemplo los corales de aguas frías. Las escarpadas paredes del fiordo Comau, en el norte de la Patagonia Chilena, albergan una comunidad bentónica diversa y rica, incluyendo densas agregaciones de corales de aguas frías que prosperan incluso en condiciones adversas como, por ejemplo, aguas con déficit de aragonita. Esta paradoja ha sido atribuida a un alto abastecimiento de alimento, siendo el zooplancton la principal dieta de los corales. Sin embargo, la información sobre la distribución estacional y vertical del zooplancton, así como la composición de su comunidad, es aún muy fragmentaria en aguas patagónicas. Además, hasta el momento se desconocen los factores abióticos y bióticos que influyen en la dinámica del zooplancton bajo variaciones ambientales estacionales (por ejemplo, cambios en la radiación solar y las precipitaciones) y su posible relación con los corales del fiordo Comau.

El objetivo de esta tesis es identificar los factores que controlan la dinámica del zooplancton en el fiordo Comau y comprender el rol que juega el zooplancton en el acoplamiento bentopelágico. Para lograr este objetivo, se investigó la distribución y composición del zooplancton tanto espacial como temporalmente. Se llevaron a cabo lances verticales estratificados desde las aguas superficiales hasta cerca del fondo marino estacionalmente y durante día y noche, mediante una red Nansen de cierre en diferentes lugares del fiordo. Posteriormente, las muestras de zooplancton se escanearon con un sistema de imagen digital de última generación (ZooScan) y se determinó la abundancia, el biovolumen y la biomasa de 41 taxones de zooplancton y 11 de meroplancton de un total de 163.840 viñetas, identificadas en EcoTaxa. Los datos de distribución y abundancia de zooplancton se utilizaron para estimar las diferencias espaciales y temporales en la disponibilidad alimentaria para las comunidades coralinas bentónicas y estos resultados fueron comparados con los marcadores tróficos medidos en corales de diferentes poblaciones a lo largo del fiordo.

La distribución del zooplancton mostró una pronunciada estacionalidad a lo largo del año (**capítulo 2**). Su abundancia alcanzó el máximo en la primavera austral, sugiriendo una estrecha relación entre la reproducción del zooplancton y la floración primaveral del fitoplancton. Sin embargo, la biomasa de zooplancton más alta se encontró en verano y la más baja en invierno, indicando que durante la estación más cálida se desarrollaron individuos más grandes o taxones de mayor tamaño y durante la estación fría, la baja producción primaria no fue suficiente para sostener un crecimiento elevado del zooplancton. En la parte exterior del fiordo Comau se observó un mayor biovolumen de zooplancton en comparación con la parte central-interior del fiordo. Esto podría estar asociado a una mayor concentración de clorofila *a* en la desembocadura del fiordo (**capítulo 3**). Durante todo el año y a lo largo del transecto horizontal, la abundancia de zooplancton se concentró en aguas superficiales, mientras que los mayores biovolúmenes se encontraron en las aguas intermedias durante el día y en las capas superficiales durante la noche, indicando una migración vertical diurna del zooplancton de mayor tamaño (**capítulos 2 y 3**).

La composición de la comunidad de zooplancton en el fiordo Comau estaba principalmente dominada por copépodos, tanto en abundancia como en biovolumen, seguidos de quetognatos, mísidos, cnidarios y anfípodos (en términos de biovolumen) y meroplancton, apendicularios y ostrácodos (en términos de abundancia) (**capítulos 2 y 3**). El meroplancton, definido como las fases de vida planctónicas de los organismos bentónicos, desempeña un papel importante en el acoplamiento bentopelágico de los ecosistemas marinos. La abundancia de meroplancton se concentró en los 50 m superiores de la columna de agua (**capítulo 4**), y fue mayor en primavera, coincidiendo con concentraciones más altas de clorofila *a* y oxígeno, sugiriendo un estrecho acoplamiento entre la reproducción del macrobentos y la alimentación del meroplancton con la floración primaveral del fitoplancton. El predominio de los distintos taxones de meroplancton varió estacionalmente, siendo los equinodermos los más abundantes en primavera, los gasterópodos en verano y otoño, y los briozoos en invierno.

La composición en ácidos grasos de los corales de aguas someras y profundas reveló marcadas diferencias en su principal fuente de alimentación (**capítulo 5**). En aguas poco profundas, los marcadores de ácidos grasos indicaron una dieta basada en fitoplancton (dominada por diatomeas) con contribución de pequeños copépodos, mientras que los corales de aguas profundas mostraron un predominio de marcadores relacionados con copépodos calanoideos que podrían explicar las mayores reservas energéticas de los corales de aguas profundas. Sin embargo, esto no se reflejó en los datos cuantitativos del zooplancton (**capítulos 2 y 3**). Esta discrepancia puede deberse a diferencias ecológicas en las dos profundidades, así como a cuestiones metodológicas, ambas analizadas en esta tesis.

En general, esta tesis proporciona nuevos conocimientos sobre (a) la composición de la comunidad de zooplancton en las aguas patagónicas chilenas, dominada principalmente por copépodos, con un nivel de detalle espacial y temporal sin precedentes; (b) los principales factores que gobiernan la dinámica del zooplancton, tales como la estratificación de la columna de agua, la circulación estuarina o la migración vertical diurna; y (c) cómo las fuertes variaciones ambientales estacionales del fiordo Comau afectan no sólo al sistema pelágico (holo- y meroplancton), especialmente en las muy variables aguas superficiales, sino también a las comunidades coralinas que habitan en el fiordo. Además, esta tesis subraya la importancia del zooplancton como un suministro esencial de alimento para los invertebrados bentónicos oportunistas que necesitan adaptarse estacionalmente a las grandes variaciones en la distribución del zooplancton y que afectan a su ecología trófica. Asimismo, estos datos destacan la importancia de monitorizar regularmente las comunidades pelágicas en los ecosistemas costeros, permitiendo evaluar el impacto natural del acoplamiento bentónico-pelágico, así como la forma en que las perturbaciones antropogénicas pueden afectar al funcionamiento de la red trófica marina.

List of Abbreviations, Tables and Figures

Abbreviations

AWI	Alfred Wegener Institut für Polar und Meeresforschung
B	Biomass
BV	Biovolume
ca.	“circa” (about)
CD	Centroid Depth
Chl *a*	Chlorophyll *a*
CTD	Conductivity, Temperature, and Depth recorder
CWC	Cold-water corals
df	Degree of freedom
DM	Dry Mass
dpi	Dots per inch
DVM	Diel Vertical Migration
e.g.	“exempli gratia” (for example)
ESD	Equivalent Spherical Diameter
i.e.	“id est” (that is)
MSAAW	Modified Subantarctic Water
PCA	Principal Component Analysis
PERMANOVA	Permutational Multivariate Analysis of Variance
RDA	Redundancy Analysis
SD	Standard Deviation
V	Volume
V_N	Volume filtered by Nansen net

Tables

Figures

Chapter 1 – General Introduction and Methodology

1.1. Plankton

The origin of the term “plankton” comes from the Greek word *planktos* [πλαγκτός] that means “wandering or drifting” and was first introduced by Viktor Hensen in 1887. Plankton is defined as those aquatic organisms (plants and animals) that live suspended in water which do not have the ability to swim against currents (Lalli and Parsons, 1997). On the other hand, organisms that can actively swim against turbulent waters are typically called nekton and comprise a wide variety of organisms, from e.g. small fishes to large whales.

The plankton community englobes an extremely wide variety of organisms which can be classified depending on size or nutrition. Due to the enormous size range, plankton is grouped into the following seven categories (Table 1.1) (Larink and Westheide, 2011):

Table 1.1 Size fractions of the plankton community

Category	Size range	Examples
femtoplankton	0.02 – 0.2 µm	viruses
picoplankton	0.2 – 2.0 µm	bacteria, cyanophytes
nanoplankton	2.0 – 20 µm	small phototrophic flagellates
microplankton	20 – 200 µm	tintinnids, diatoms, dinoflagellates
mesoplankton	0.2 – 20 mm	copepods, many larvae, hydromedusae
macroplankton	2.0 – 20 cm	krill, arrow-worms
megaplankton	0.2 – 2.0 m	large jellyfish and tunicates

From the nutrition point of view, unicellular microscopic photoautotrophic algae are called “phytoplankton”. These algae carry out photosynthetic activity (the same way land plants do) and due to the presence of chlorophyll within their cells, phytoplankton takes up the nutrients present in the water and synthetize them into chemical energy. As phytoplankton needs solar energy to perform the photosynthesis, it inhabits the euphotic zone of the water column. Therefore, its growth primarily depends on photosynthetically active radiation and the availability of macronutrients, such as carbon dioxide, nitrogen, phosphorus and silicate (Berthold and Campbell, 2021). Phytoplankton abundance can expand exponentially under the correct circumstances, a phenomenon known as bloom, which duration differs depending on abiotic (e.g. light and nutrients availability or temperature) and biotic factors (e.g. grazing or phytoplankton physiology) (Miller, 2004; Hunter-Cevera et al., 2016). These blooms can be detected via satellite photos or by chlorophyll *a* concentration recorded during e.g. CTD profiles, and can span hundreds of square kilometers. Phytoplankton is considered the base of the ocean food web and sustain the rest of the trophic levels in the marine ecosystem (Palma and Kaiser, 1993).

Heterotrophic plankton organisms are called “zooplankton”. They are composed of non-photosynthetic protists (unicellular) and metazoans (pluricellular) which feed on phytoplankton (herbivores), on other zooplankton organisms (carnivores), or on dead organic material (detritivores) and have a huge heterogeneity in form, size, and locomotion systems (Fig. 1.1) (Lalli and Parsons, 1997).

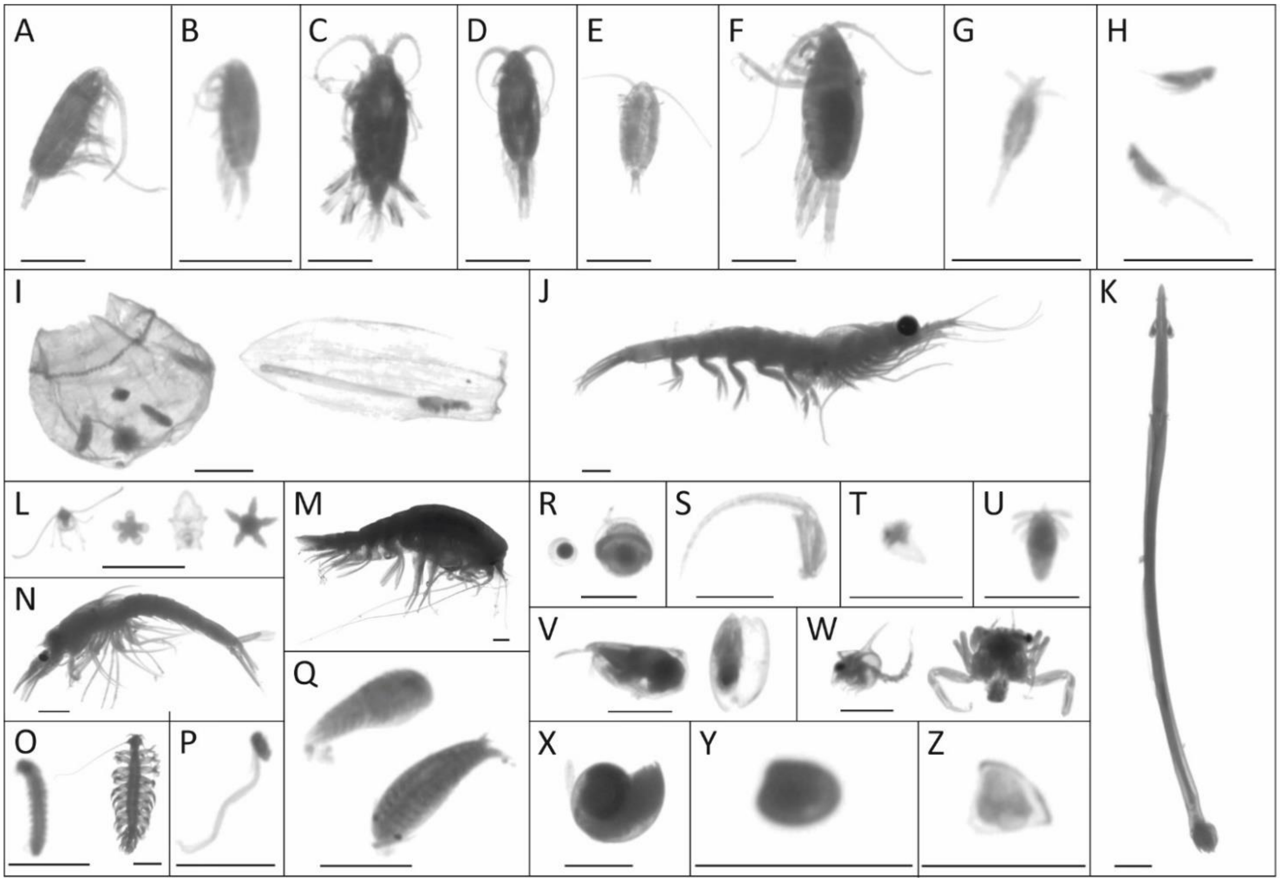

Fig. 1.1 Examples of vignettes of organisms from the ZooScan analysis of this thesis (scale bar 1 mm). A-H: Copepoda, (A) Calanidae, (B) Clausocalanidae, (C) Candacidae, (D) Metridinidae, (E) Aetideidae, (F) Euchaetidae, (G) Cyclopoida, (H) Harpacticoida, (I) Cnidaria, (J) Euphausiacea, (K) Chaetognatha, (L) Echinodermata, (M) Amphipoda, (N) Mysida, (O) Polychaeta, (P) Appendicularia, (Q) Isopoda, (R) eggs, (S) Ascidiacea, (T) Cladocera, (U) nauplii, (V) Ostracoda, (W) Decapoda, (X) Gastropoda, (Y) Bivalvia, (Z) Bryozoa.

Metazoan zooplankton may have certain swimming capabilities at small spatial scales, however, they are unable to swim against strong water currents, turbulence, and/or bulk density. According to their life cycle, zooplankton can be divided into two further categories: holoplankton and meroplankton. Holoplankton are composed of organisms that develop their whole biological life cycle in the water column (e.g. copepods, arrow-worms, amphipods or krill), whereas meroplankton comprise those organisms that spend part of their biological life cycle in the water column (typically their initial developmental stages) and later become part of the nekton or benthos (e.g. fish larvae, barnacles, mollusks or echinoderms) (Levinton 2001).

Within zooplankton populations, crustaceans usually dominate the community numerically. Crustacean zooplankton are generally composed of copepods, euphausiids, mysids, ostracods, cirripeds, amphipods and decapods, copepods being the most abundant type of zooplankton dominating nearly all marine ecosystems (Larink and Westheide, 2011; Levinton, 2017). They have an external skeleton of chitin and relatively impermeable material which is segmented with paired appendages. Other important taxa within the zooplankton community are 1) gelatinous zooplankton which are composed of jellyfish, siphonophores and appendicularians (commonly called larvaceans), and 2) chaetognaths (commonly called arrow-worms) (Levinton, 2017).

As a result of a) the high plankton heterogeneity (in size, morphology and dietary needs), and b) their dispersal, determined by oceanographic features, zooplankton taxa exhibit significant variations in their geographical, seasonal, vertical and daily distributions (Palma and Kaiser, 1993).

1.2. Zooplankton in coastal waters

In the marine environment, zooplankton are distributed throughout the global ocean. However, their terminology changes with the preferred habitat. Zooplankton on the continental shelf are called “neritic or coastal zooplankton”, while beyond the continental shelf seawards, the correct term would be “oceanic zooplankton” (Levinton, 2017). On a global scale, zooplankton taxonomic richness has been significantly associated to water temperature, being higher in tropical ecosystems and decreasing towards the poles (Rombouts et al., 2009; Yasuhara et al., 2012; Ibarbalz et al., 2019). However, when accounting for zooplankton biomass, temperate regions account for higher zooplankton biomasses and lower biomasses are found in tropical environments (Moriarty et al., 2013; Soviadan et al., 2022). Although in the global databases there are still many gaps of zooplankton biomass due to the time-consuming process of taxonomic identification (Everett et al., 2017), it can be perceptible that zooplankton biomass in coastal waters is higher than in oceanic waters (Fig. 1.2).

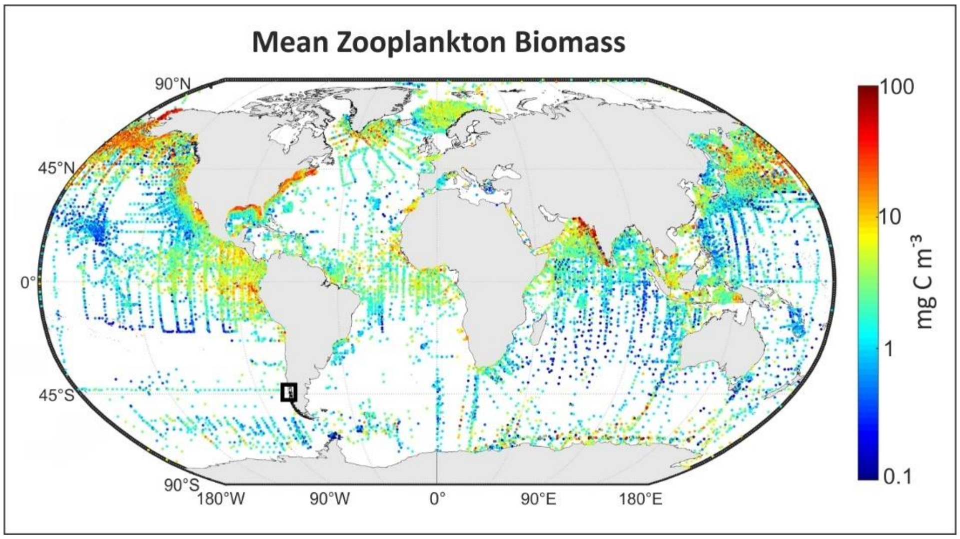

Fig. 1.2 Mean marine zooplankton biomass (mg C m^{-3}) for mesozooplankton (0 – 200 m). The study area of this thesis (Comau Fjord) is highlighted with a black square. Data from "COPEPOD: The Global Plankton Database". (Modified from Everett et al., 2017)

Coastal waters are among the highest productive ecosystems on earth (Mann and Lazier, 1991). They offer significant economic and ecological services, such as fisheries, biological diversity, coastal stability and erosion prevention, nutrient cycling and high genetic resources (Post and Lundin, 1996; Scavia et al., 2002; Escribano et al., 2003; Barbier, 2017). The value of ecological services provided by coastal and marine habitats is thought to be over half of the value of the total ecological services (Costanza et al., 1997). However, coastal ecosystems are being heavily exploited by human activities putting in risk the services these environments provide (Barbier, 2017). To name an example, anthropogenic activities can modulate the stoichiometry and increase nutrients concentration in the water of coastal environments leading to changes in the water quality and increase eutrophication, harmful algal blooms and negatively affect the structure of the marine food web (Anderson et al., 2002; Glibert, 2012). In ecosystems where an increment in nutrient loads has been observed, zooplankton variations in their distribution, abundance and biomass have been used as indicators of changes in the marine environment (Capriulo et al., 2002).

As an essential role in the marine food web, zooplankton are important components in the bentho-pelagic coupling of the oceans and in the carbon and nutrient cycles. They are considered as the principal link between primary producers (phytoplankton) and higher trophic levels (e.g. fish, cephalopods and marine mammals) (Fig. 1.3). For example, zooplankton are directly consumed by fish or marine mammals, transferring energy to the pelagic food web in coastal ecosystems (such as continental shelves, reefs and seagrass meadows) (Nemoto, 1970; Hamner et al., 1988; Kingsford and MacDiarmid, 1988; Edgar and Shaw, 1995; Marquis et al., 2011; Frisch et al., 2014; Champion et al., 2015). Zooplankton can also transfer energy to the deep layers of the ocean by the biological carbon pump: 1) passively, through the egestion and high sinking rates of their fecal pellets and the short food chains that produce those sinking particles, or 2) actively, by their diel vertical migration (Ducklow et al., 2001). Through their diel vertical migration, zooplankton transport the surface-ingested material (not yet digested) and metabolize it in deeper layers when they migrate downwards (Longhurst, 1976). The mortality of zooplankton in deep layers would also contribute to increment the concentration of assimilated organic material that can be consumed by other organisms (Fig. 1.3) (Longhurst, 1976).

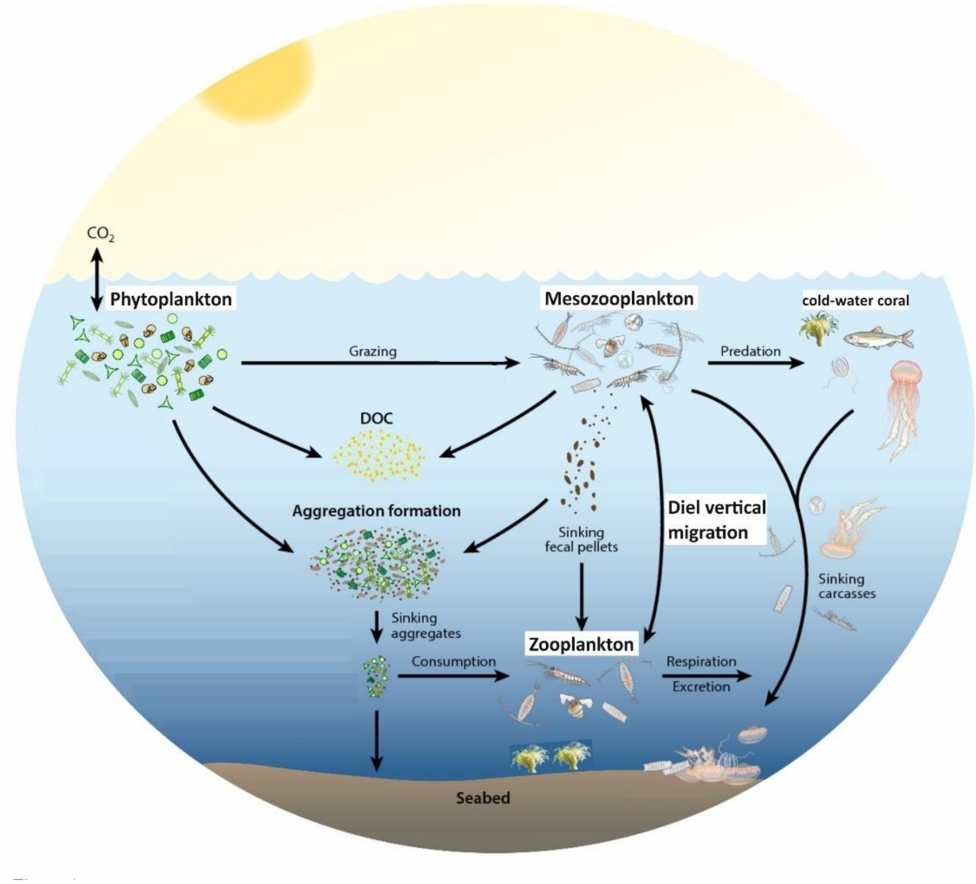

Fig. 1.3 Biological carbon pump and marine food web, showing that zooplankton are 1) the intermediate trophic level between producers and higher consumers, 2) the role of zooplankton in carbon and nutrient cycles. (Modified from Steinberg and Landry, 2017)

1.3. Zooplankton distribution

The mechanisms governing zooplankton distribution are multiple and complex. Regional temperature is likely one of the most important factors governing the distribution of zooplankton species in a medium regional scale (Scavia et al., 2002). Changes in the global temperature patterns, as ocean warming, are expected to cause poleward shifts in many zooplankton taxa, meaning that some tropical and lower-latitude species would shift their habitats to higher latitudes (Scavia et al., 2002). In warmer ecosystems (like the California current), an increment in water stratification led to a lower nutrient regeneration in the upper water layers resulting in a significant decline in zooplankton biomass (Roemmich and McGowan, 1995; McGowan et al., 1998). However, in high latitudes, an increment in surface temperature led to changes in productivity, significantly increasing the zooplankton biomass (Brodeur and Ware, 1992).

Coastal environments can be affected by a number of oceanographic and biological features and processes, such as upwelling, tidal mixing and turbulence, water stratification, estuarine circulation, freshwater and nutrient inputs, and complex trophic interactions, which all will determine the distribution of zooplankton in these ecosystems (Haury et al., 1978; Briggs, 1995; Palma and Silva, 2004; González et al., 2010; Meerhoff et al., 2014b). As zooplankton cannot overcome strong currents, horizontal transport of zooplankton has been associated to upwelling and semi-diurnal tides, which can increment the zooplankton abundance in small-scales (Rodríguez et al., 1999; Castro et al., 2011). Zooplankton distribution is also modulated by the presence of haloclines, both in controlled laboratory experiments and in the field, either by aggregating in or below this layer or by taxonomic depth differentiation (Lougee et al., 2002; Sánchez et al., 2011). Anoxic layers from upwelling systems can act as a barrier for many zooplankton taxa that cannot stand low oxygen concentrations, resulting in unusual high zooplankton concentrations in near-surface waters above the oxygen minimum zones (Judkins, 1980). Another strong driver of zooplankton distribution is the location of food patches, although there is no single pattern that can determine their distribution, as this is highly dependent on zooplankton taxa and oceanographic conditions (Folt and Burns, 1999). When predators are present, zooplankton aggregation in food-rich layers are often limited or even eliminated (Kleiven et al., 1996).

Within the zooplankton, there are some taxa that maintain a discrete depth throughout the water column, but there are also many taxa that undergo the so-called diel vertical migration (DVM) which determines the vertical distribution of zooplankton (Larink and Westheide, 2011). In terms of biomass, DVM is probably the largest migration in the world (Hays, 2003; Brierley, 2014), which due to its implications, has been considered a driver for carbon and nitrogen net removal from surface waters by international programs like the Joint Global Ocean Flux Study (JGOFS). The most common pattern of DVM involves plankton moving to deeper waters during day, and at night they migrate upwards to shallower depths. This event takes place in 24-hour cycles, so the most probable cue triggering DVM is changes in light intensity (Ringelberg, 1995). Zooplankton are so sensitive to light, that even in environments where modest light changes between day and night take place (such as polar nights in Arctic fjords, varying cloud cover, eclipses or lunar cycles), DVM can be activated (Tarling et al., 1999; Berge et al., 2009; Ochoa et al., 2013; Last et al., 2016; Omand et al., 2021). DVM occurs in many different marine and freshwater habitats and is performed by very different taxa, so it is unlikely that only one factor can explain the reason behind this process (Hays, 2003). One of the hypotheses is that DVM helps to protect the migrant organisms from ultraviolet radiation (Leech and Williamson, 2001), but that hypothesis would not explain why some species perform such extensive migrations beyond where light can penetrate (sometimes even for hundreds of meters in a few hours), which is astonishing for such small organisms (Hays, 2003; Brierley, 2014). Another hypothesis is the attraction for food, where herbivore zooplankton aggregate in the rich-phytoplankton layer in illuminated surface waters, where photosynthesis is possible, and carnivorous zooplankton follow their food patches (Hays et al., 1998; Brierley, 2014). However, solely this hypothesis would not explain either the true benefit of DVM, as it produces a high metabolic cost to zooplankton (Lampert, 1989; Aksnes and Giske, 1990). Therefore, the most plausible hypothesis is the “predation evasion hypothesis” (Zaret and Suffern, 1976). This hypothesis establishes that zooplankton inhabit the deep dark waters during the day, where the probability of being perceived by visually hunting predators (and hence eaten) is lower than if they remain in more illuminated waters, and at night, in the refugee of darkness, they migrate upwards to shallow waters where food is abundant. As a consequence, larger and more pigmented species tend to perform more pronounced DVM than smaller zooplankton (Wright et al., 1980; Hays et al., 1994). However, individual variability within a population also affects DVM, as it will depend on the body condition and their energy reserves. For instance, organisms with larger lipid reserves may remain at deeper layers during the night avoiding this way the risk of predation, until their energy reserves are depleted to a threshold when they must initiate again the upward migration to feed (Fiksen and Carlotti, 1998; Sekino and Yamamura, 1999; Hays et al., 2001). Fundamentally, the benefit of being protected from predators versus not being able to feed during the day when they remain in deeper and darker waters is higher for zooplankton, and likely the ultimate reason for DVM.

The extension of zooplankton DVM can be influenced by a number of factors, such as water temperature, dissolved oxygen or density changes (Southward and Barret, 1983; Pagès and Gili, 1992; Díaz-Astudillo et al., 2017; Pérez-Santos et al., 2018). Water column stratification and strong temperature gradients can act as a barrier for many zooplankton taxa preventing or slowing down their vertical migration (Southward and Barret, 1983; Pagès and Gili, 1992). For instance, the extension of the DVM in the mixed water column of the Ancud Gulf, Chile, was deeper than in a stratified fjord nearby (Díaz-Astudillo et al., 2017). The same applies for layers with low oxygen, where DVM is restricted and the compression of the habitat is significantly reduced when zooplankton do not enter deeper, hypoxic waters (Pérez-Santos et al., 2018).

1.4. Zooplankton in Chilean Patagonia

In coastal ecosystems, estuaries and channels comprise approximately 0.03% of global oceanic areas. However, due to their importance in oceanographic events and biological productivity (3.4% of total marine primary production occurs in estuaries), these regions are highly important for the carbon flow (Burrell, 1988). A fjord is a unique type of estuary where a long, narrow inlet with a deep basin and steep walls, created by a glacier, are connected to an adjacent ocean (Cameron and Pritchard, 1963; Freeland et al., 1980), transporting high amounts of organic matter from terrestrial to oceanic systems across continental slopes (Walsh, 1991). Fjords can be found around the globe, in regions such as Scotland, Norway, Greenland, Canada, New Zealand and the Chilean Patagonia (Silva et al., 2011). In this regard, the Chilean Patagonia is one of the most extensive fjord regions in the world (41.5°S - 55.9°S), with approximately 240,000 km^2, where very low populated areas can be found (Pantoja et al., 2011). However, an increment in anthropogenic activities over the last two decades is threatening the sustainable use of the fjords, particularly through an intensifying mariculture of mostly non-native species and a growing development of hydroelectric energy (Iriarte et al., 2010; Buschmann et al., 2021; Navedo and Vargas-Chacoff, 2021). Chilean fjords sustain rich pelagic and benthic fisheries (Buschmann et al., 2021), however, the state of alteration of these systems (depending on the area, e.g. very remote regions or quite accessible ones) greatly differ among each other. In affected systems, the comparison of biodiversity, productivity and dynamics could allow the development of conservation strategies for the protection of these remote and pristine ecosystems (Iriarte et al., 2010).

Chilean fjords, such as the Comau Fjord, are influenced by autochthonous (from planktonic productivity) and allochthonous (from terrestrial transport by river runoffs) organic matter (Vargas et al., 2011). Salinity, water temperature, nutrient concentration and phyto- and zooplankton biomass depend on the seasonal variation of solar radiation (high in summer), precipitation (>5,000 mm year^{-1}), tidal mixing, and freshwater runoffs (from rivers and glacial melt) (Pantoja et al., 2011). As a result, water stratification, light and nutrients govern the biological productivity of the fjords, where higher environmental variability occurs near the surface compared to deeper layers (Palma and Silva, 2004; Palma, 2008). Due to the seasonal variation and estuarine circulation occurring in the fjord, a strong vertical and horizontal gradient originate from opposing water masses. At the surface of the water column, there is a meteoric low salinity mass (<25) flowing seaward carrying oligotrophic but rich in silicic acid waters, which is fed by high precipitation, river runoff and meltwater from cordilleran glaciers. Underneath this water mass, there is a deep, saline (>31) oceanic Modified Subantarctic Water (MSAAW) flowing landward, loaded with macronutrients (phosphate and nitrate) from the adjacent ocean (Silva et al., 1997, 1998; González et al., 2011). These very different water masses create a strong pycnocline, which can vary depending on the strong seasonal variations or due to mixing by winds and tides and will directly affect the phytoplankton communities (Iriarte et al., 2007). As a result of the estuarine circulation and strong seasonal variations, phytoplankton and zooplankton typically have marked seasonal cycles (Fig. 1.4). Water column stratification together with the nutrient lateral advection promote that the phytoplankton algal cells remain in the photic zone leading to strong diatom blooms in spring providing food for zooplankton (Silva et al., 1997; Iriarte et al., 2007; González et al., 2010; Pérez-Santos et al., 2014). As a consequence, zooplankton biomass increase in summer (Sullivan, 1993; Antezana, 1999; González et al., 2010). In late autumn and winter, the low solar radiation leads to a minimum Chl *a* concentration (dominated by small phytoplankton) resulting in a low primary production, causing lower zooplankton abundances and biomasses during these cold seasons (González et al., 2010).

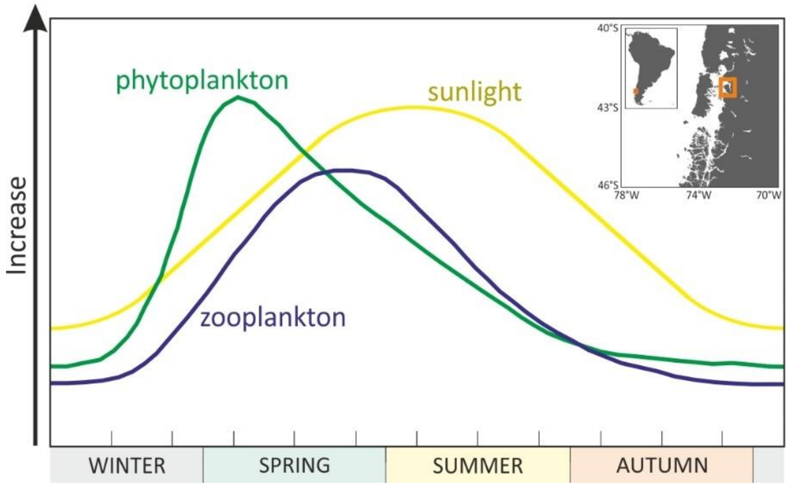

Fig. 1.4 Estimated annual cycle of phyto- and zooplankton in regards to sunlight availability at Comau Fjord.

The zooplankton biomass along Chilean channels and fjords is mainly composed of crustacean zooplankton (copepods and euphausiids) followed by the carnivorous taxa chaetognaths and cnidarians (medusae and siphonophores) (Palma, 2008). Along the entire Chilean Patagonia, higher zooplankton abundance, biomass and species diversity were observed in the northern zone (between Puerto Montt and Elefantes Fjord), gradually decreasing towards the southern-most area (down to Cape Horn) (Palma and Silva, 2004; Palma, 2008). The species richness in these areas is very low compared to those found for other coastal regions, but it is comparable to other fjords such as those from Scandinavia and Canada (Sands, 1980; Båmstedt, 1988; Richard and Haedrich, 1991; Palma, 2008). However, very few species have been able to adapt to the very difficult oceanographic conditions and the majority of species from interior waters come from oceanic waters (Palma and Silva, 2004).

In general, most Chilean Patagonia zooplankton research has been carried out in the central-southern region (from Penas Gulf to Cape Horn, 46.50°S-55.9°S), whereas research done in the northern region (from Puerto Montt to San Rafael Lagoon, 41.20°S-46.40°S) has mainly focused on specific microzooplankton taxa in the upper water column, on bulk zooplankton biomass measurements, or on the carbon cycle through the pelagic food web (e.g. Palma, 2008; Villenas et al., 2009; González et al., 2010, 2011; Palma et al., 2011; Sánchez et al., 2011). Other research focused on physical oceanographic processes and their effect on zooplankton distribution (Marín and Delgado, 2009; Castro et al., 2011), and their connection to zooplankton and ichthyoplankton growth and feeding (Landaeta et al., 2015a, 2015b). Acoustic backscatter and vertical velocity profiles have also been used in recent years to examine zooplankton migration patterns (Valle-

Levinson et al., 2014; Díaz-Astudillo et al., 2017; Pérez-Santos et al., 2018). However, besides all studies done in the area, the information on zooplankton seasonality, horizontal and vertical distribution and taxonomic composition is still very scarce.

1.5. Importance of zooplankton as a food source

As seen in previous chapters, zooplankton play a key role in the functioning of the marine food web of high latitude ecosystems. They constitute the principal link between primary producers and higher consumers, but they also provide food for invertebrate predators, such as cold-water corals (CWC) (Nemoto, 1970; Kingsford and MacDiarmid, 1988; Kiriakoulakis et al., 2005; Gili et al., 2006; Champion et al., 2015; Höfer et al., 2018). In this sense, CWC, in contrast to their tropical counterparts, do not have symbiotic zooxanthellae algae within their tissue, so they entirely depend on an external source of organic matter to maintain their physiological processes, such as respiration and growth (Carlier et al., 2009; Mayr et al., 2011; Naumann et al., 2011). Despite the widespread distribution of CWC in the marine environment, little is known about their natural diet and its availability, mostly due to their remoteness, deep depths and the difficulty of study them *in situ*. However, observations in laboratory and field experiments have provided evidence that CWC are opportunistic feeders and can feed on a wide range of food sources (from dissolve organic matter, bacteria and small phytoplankton to meso- and macrozooplankton) (Kiriakoulakis et al., 2005; Carlier et al., 2009; Dodds et al., 2009; Tsounis et al., 2010; Mueller et al., 2014; Gori et al., 2018; Höfer et al., 2018). Although these studies did not observed directly the uptake *in situ*, metabolic, stable isotope analyses and fatty acid trophic markers suggested that zooplankton seem to be the most important diet, which can sustain their metabolic needs (Carlier et al., 2009; Dodds et al., 2009; Mayr et al., 2011; Naumann et al., 2011, 2015; Maier et al., 2021; Rakka et al., 2021).

In Comau Fjord, Chilean Patagonia, CWC are widespread along the steep walls forming dense banks, and particularly abundant between 20-280 m water depth, even in areas where aragonite-undersaturated waters (low pH) are found (Häussermann and Försterra, 2007; Fillinger and Richter, 2013; Jantzen et al., 2013a). These unfavorable conditions may cause reduced growth and survival rates due to the dissolution of exposed skeleton parts (McCulloch et al., 2012; Maier et al., 2016). The calcification of the CWC skeleton is energetically costly, therefore under adverse environmental conditions, such as low pH, CWC food requirements are higher compared to normal conditions (Maier et al., 2016). In this case, an additional food source (such as krill) may provide the sufficient energy to counteract the unfavorable conditions and provide the corals enough energy to fulfill their metabolic needs, regardless of the water pH (Martínez-Dios et al., 2020; Maier et al., 2021). CWC densities in Comau Fjord decrease below 280 m water depth despite available substrate, suggesting that adverse conditions in the water (low pH and dissolved oxygen), together with low food availability, might be limiting coral growth (Fillinger and Richter, 2013).

Laboratory experiments have shown that CWC feeding and growth rates linearly increase with prey abundance (Mortensen, 2001; Höfer et al., 2018). However, zooplankton are highly variable, and the distribution of zooplankton communities is subjected to a number of oceanographic (e.g. water column mixing/stratification, horizontal transport, seasonality) and biological drivers (e.g. grazing, vertical migration), disrupting typical horizontal and vertical distribution patterns (Castro et al., 2011). While some CWC can adapt well to seasonal variations in food availability and can tolerate short periods of low food abundance, a prolonged period without enough food supply might jeopardize their physiological performance lowering their respiration, calcification and growth rates and their general fitness (Naumann et al., 2011; Larsson et al., 2013; Büscher et al., 2017; Maier et al., 2019; Martínez-Dios et al., 2020). The Comau Fjord, thus, provides the perfect natural laboratory to study the adverse effects of ocean acidification and future environmental changes that CWC might face, in combination with the CWC nutritional status and gain new insights on their natural food availability.

1.6. New technological methods

Zooplankton abundance and biomass can be extremely variable within a wide range of temporal and spatial scales, where fluctuations in continental shelves might be particularly strong (Bode et al., 2013). Interannual time series would constitute an important tool to investigate the environmental and biological drivers that govern the zooplankton fluctuations in different temporal scales and ecological systems (Mackas et al., 2012). Yet, the knowledge of zooplankton dynamics and the influence of environmental variability on their populations is still very fragmentary, and therefore hard to interpret, especially in large scales. The reason behind this fact is that sampling methods, taxonomic identification and counting of organisms are usually very difficult to homogenize, labor-intensive and very time-consuming, limiting the number of samples that can be processed (Grosjean et al., 2004). For instance, plankton nets can be very useful tools to identify patches of zooplankton at specific times and depths for taxonomically identifying species, life stages and size-frequency distributions within a population, however, the spatial and temporal resolution is usually limited (Wiebe and Benfield, 2003; Gorsky et al., 2010). This sampling limitation reduces our understanding on different processes that might be affecting the zooplankton dynamics. On the other hand, high-resolution optic systems (acoustic and optical counters) can provide an almost *in situ* spatial and temporal distribution of zooplankton, but often at the cost of taxonomic identification. Some adult individuals of certain species or juvenile and larval stages can be very hard to identify even in very good laboratory conditions, let alone in a moving underwater optical device (Wiebe and Benfield, 2003).

The digitalization and computer processing of zooplankton samples constitute a good compromise between traditional and new techniques. Imaging systems can be very useful tools for producing data on zooplankton distribution at a fine vertical and horizontal scales (Davis et al., 1992, 2004). Image analysis can produce reliable data in a considerable shorter period of time than traditional sampling processes (Benfield et al., 2007). Many camera and imaging systems have been developed in recent years trying to extract useful information from zooplankton images with a more efficient method, for instance: FlowCam (Sieracki et al., 1998), Laser-Optical Plankton Counter (Herman et al., 2004), Video Plankton Recorder (Davis et al., 2005), Zooplankton Visualization system (Trevorrow et al., 2005), In Situ Ichthyoplankton Imaging System (Cowen and Guigand, 2008), Lightframe On-sight Keyspecies Investigation (Schulz et al., 2010), or ZooScan (Gorsky et al., 2010). A semi-automatic identification and classification of organisms, combined with better algorithms for machine learning already allowed to increase the number of net samples and to rapidly identify unbiased and quantitative data (Grosjean et al., 2004; Gorsky et al., 2010). In this sense, the combination of the high-resolution imaging system (ZooScan), with the image processor software (ZooProcess) and the web application for visual exploration and semi-automatic taxonomic identification of images (EcoTaxa) as an integrated analysis system for data acquisition allows a full and faster data processing of preserved samples, storing the information in a digitalized form and permitting the comparison of standardize images among different laboratories thanks to its built-in calibration method (Grosjean et al., 2004). Once the high-resolution image has been processed (Fig. 1.5), only zooplankton images can be selected excluding the non-living parts of the sample (e.g. detritus or sediments).

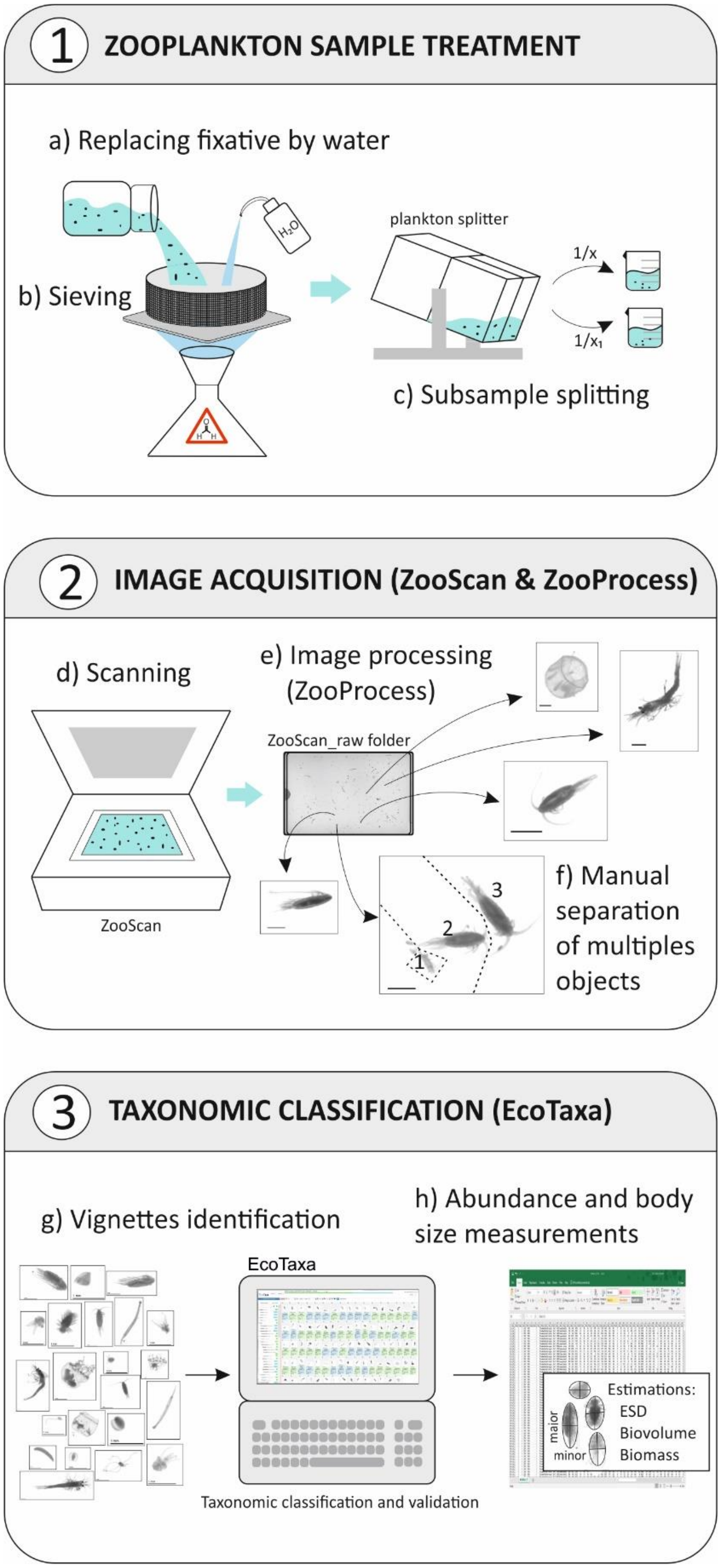

Fig. 1.5 Conceptual diagram of zooplankton sample pre-process, imaging analyses steps (ZooScan, Zooprocess and EcoTaxa) and data acquisition.

Overall, the ZooScan digital imaging system, together with the imaging processing software ZooProcess and EcoTaxa, a web-based machine learning application that uses training sets and random forest algorithms to semi-automatically classify the organisms as in integrated analysis system allow to 1) taxonomically identify and classify different individuals of a zooplankton population and 2) provide accurate measurements of body size and biomass estimations (Gorsky et al., 2010). The methodology approach used in this thesis thus provides a good compromise between the potential biases of the fully automatic classification method with the manual but accurate zooplankton identification.

1.7. Structure and main objectives

The aim of this thesis is to understand the zooplankton dynamics regarding its role in the trophic ecology of cold-water corals. Zooplankton distribution was assessed in order to identify spatial, seasonal, vertical and diel dynamics throughout the entire water column by taking advantage of novel methodological procedures that allowed to efficiently process a high number of zooplankton samples. I used the Comau Fjord as my focal area since little is known on seasonal and horizontal changes in zooplankton composition for the Patagonian Fjord systems. Additionally, Comau Fjord is known for its rich and diverse benthic community that essentially is dependent on planktonic food provision. Thus, the main objectives of this thesis are to identify 1) the mesozooplankton community and its size distribution, 2) the seasonality of meroplankton and its community composition, 3) how abiotic (e.g. changes in environmental variables) and biotic (e.g. diel vertical migration) drivers influence the zooplankton distribution and 4) potential natural food spectrum for key benthic taxa like cold-water corals. These data can be compared with nutritional status of benthic foundation species and identify trophic dynamics in the region. Based on these objectives, the following research questions are proposed:

- How zooplankton seasonality is influenced by environmental variations of fjords in temperate regions?

In temperate regions, ecological systems are influenced by strong seasonal variations in solar radiation, precipitation and terrestrial runoffs (Pantoja et al., 2011). As a result, physico-chemical characteristics of the water column changes seasonally and biological productivity, and therefore, phyto- and zooplankton abundance and biomass, are governed by these variations with marked seasonal cycles (Palma, 2008; González et al., 2010) with expectedly higher zooplankton abundance and biomass in spring and summer, coinciding with the phytoplankton spring bloom, compared to winter.

Approach: Zooplankton abundance, biovolume and biomass were seasonally assessed by the integrated analysis system (ZooScan, ZooProcess and EcoTaxa) in combination with the assessment of physico-chemical data recorded during CTD profiles (**chapter 2**).

- Do we find a clear vertical structure in zooplankton composition as well as temporal dynamics? Are temporal dynamics linked to known diurnal vertical migration patterns as for the well-known migrator *Metridia lucens*?

The major biotic driver governing the vertical distribution of zooplankton is the diel vertical migration (DVM). In order to protect themselves from predators, zooplankton migrate upwards to surface waters during night to feed, where they are less susceptible to be seen. It is therefore more typical to see bigger zooplankton performing DVM than small species or small individuals from certain populations (Wright et al., 1980; Hays et al., 1994) – a pattern that is expected to be observed for the Comau Fjord.

Approach: The vertical migration of big zooplankton taxa (copepods and other non-copepod zooplankton) was assessed by stratified vertical hauls throughout the entire water column during day and night, once in every season and along different locations through a central transect in Comau Fjord. The changes in the daily zooplankton biovolume and biomass among the different depth strata measured throughout the entire water column provide insights into migration patterns for specific zooplankton taxa e.g. large versus small taxa (**chapter 2 and 3**).

- Are oceanographic processes (such as water stratification, salinity gradients, low dissolve oxygen or high chlorophyll *a* concentrations) affecting the spatial and vertical distribution of zooplankton?

Temperature, salinity and dissolved oxygen play an important role on the vertical and horizontal distribution of zooplankton. Strong thermo-, halo- and oxyclines present in the water can modulate the dynamics of zooplankton populations (Lougee et al., 2002; Sánchez et al., 2011; Pérez-Santos et al., 2018), and strong chlorophyll *a* concentrations are usually associated to higher abundances and biomasses of zooplankton (Folt and Burns, 1999). Since higher chlorophyll *a* concentration is found in the surface waters of the fjord, it is expected that zooplankton accumulate in this layer.

Approach: The influence of the physico-chemical parameters on the vertical (**chapter 2**), and spatial (**chapter 3**) distribution of the mesozooplankton community, as well as on the meroplankton community (**chapter 4**) was evaluated using redundancy analyses between explanatory (environmental factors) and response (zooplankton abundance and biovolume) variables along the transect between Ancud Gulf and Comau Fjord.

- Are meroplankton abundance and composition clearly driven by seasonal changes? Are meroplankton also varying with depth?

Meroplankton constitute a key element in the bentho-pelagic coupling of ecological systems. Many benthic invertebrates reproduce by the spawning of pelagic larvae (meroplankton) into the water column. However, the spawning season and dispersal of meroplankton greatly differ among species and is usually associated to changes in environmental factors (temperature, phytoplankton abundance or salinity) (Giese and Kanatani, 1987). Most of the benthic invertebrates reproduce via planktotrophic larvae (which generally feed on phytoplankton), therefore it is expected that meroplankton abundance in Comau Fjord is linked, and therefore higher, in spring when the phytoplankton bloom occurs and in surface waters where phytoplankton is more abundant.

Approach: The meroplankton abundance and community composition are explored here for the first time for Comau Fjord by the integrated system analysis separating those images that belong to meroplankton taxa in order to obtain the total meroplankton abundance. These data provide new insights into spatial and vertical differences, as well as dominance from different taxonomic groups during different seasons. The data allow to further, describe vertical distribution patterns and evaluate the potential spawning periods for different benthic invertebrate taxa (**chapter 4**).

- What is the natural food spectrum of cold-water corals? What role zooplankton play on cold-water corals diet? Do they have to adapt to different food regimes?

Cold-water corals (CWC) depend on heterotrophic feeding to fulfill their basic metabolic needs (Carlier et al., 2009; Mayr et al., 2011; Naumann et al., 2011). They are able to feed on a wide range of food sources, however, zooplankton seem to be their most important diet (Dodds et al., 2009; Maier et al., 2021; Rakka et al., 2021). So far only nutritional information from CWC are available but not in combination with zooplankton data. In Comau Fjord, CWC are expected to deal with strong seasonal differences in food availability. Zooplankton should play a key role on the CWC's diet and the sites and depths where lower zooplankton availability is found, can be sites where corals are less well nourished. Thus, CWC should be adapted to different feeding regimes, however in areas where food is limited, their general performance can be negatively affected. It is expected that changes in zooplankton distribution both, horizontally and vertically, are reflected in the nutritional conditions of the corals with improved nutrition in shallow corals compared to deep ones.

Approach: To link zooplankton availability with CWC nutrition, the coral fatty acid composition was assessed. For that, fatty acid trophic markers from the CWC *Desmophyllum dianthus* were analyzed (**chapter 5**) relating that information to corals coming from sites with known differences in performance (Beck et al., 2022) to the naturally food availability and its distribution (i.e. zooplankton seasonal and vertical dynamics) (**chapter 2**). General CWC fitness, combined with changes in environmental conditions were also evaluated, and potential suggestions about CWC food requirements under a variable environment are made (**chapter 5**).

1.8. List of manuscripts and contributions of authors

Manuscript #1 - Chapter 2

Seasonal and diel variations in the vertical distribution, composition, abundance and biomass of zooplankton in a deep Chilean Patagonian Fjord

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Author contributions: NG-H, GF, HEG and CR conceived and designed the research; NG-H, AC, JL and JH performed field and laboratory work; NG-H, AC, BN and CR analyzed the data; NG-H prepared figures and tables; NG-H, AC, JL, BN, JH, GF, HEG and CR authored or reviewed drafts of the manuscript, and approved the final draft.

Manuscript #2 - Chapter 3

Spatial and temporal dynamics of zooplankton along a section through Comau Fjord to the Ancud Gulf, Chile

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Manuscript #3 - Chapter 4

Spatio-temporal dynamic of meroplankton in a deep temperate Patagonian fjord

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Manuscript in preparation

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Manuscript #4 - Chapter 5

Lipid biomarkers reveal trophic relationships and energetic trade-offs in contrasting phenotypes of the cold-water coral *Desmophyllum dianthus* in Comau Fjord, Chile

Manuscript under review at *Functional Ecology*

Marlene Wall, Kristina K. Beck, **Nur Garcia-Herrera**, Gertraud M. Schmidt-Grieb, Jurgen Laudien, Juan Hofer, Gunter Forsterra, Christoph Held, Gernot Nehrke, Matthias Woll, Martin Graeve, and Claudio Richter

Author contributions: GMSG, JL and CR designed the study; NG-H, GMSG, JL and GF contributed with background information and collected corals; MWall, KKB and MWoll conducted coral preparation and lipid measurements; KKB and GMSG contributed physiological background data; MWall and MWoll analyzed raw data; MWall and MG interpreted the data; MWall conducted the statistical analysis; MWall and KKB prepared the figures; MWall, KKB, NG-H, GMSG, JL, JH, GF, CH, GN, MWoll, MG and CR authored or reviewed drafts of the manuscript, and approved the final draft.

Chapter 2 – Zooplankton seasonal dynamics

Seasonal and diel variations in the vertical distribution, composition, abundance and biomass of zooplankton in a deep Chilean Patagonian fjord

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2.1. Abstract

Comau Fjord is a stratified Chilean Patagonian fjord characterized by a shallow brackish surface layer and a >400 m layer of aragonite-depleted subsurface waters. Despite the energetic burden of low aragonite saturation levels to calcification, Comau Fjord harbours dense populations of cold-water corals (CWC). While this paradox has been attributed to a rich supply of zooplankton, supporting abundance and biomass data are so far lacking. In this study, we investigated the seasonal and diel changes of the zooplankton community over the entire water column. We used a Nansen net (100 µm mesh) to take stratified vertical hauls between the surface and the bottom (0-50-100-200-300-400-450 m). Samples were scanned with a ZooScan, and abundance, biovolume and biomass were determined for 41 taxa identified on the web-based platform EcoTaxa 2.0. Zooplankton biomass was the highest in summer (13 g dry mass m^{-2}) and the lowest in winter (4 g dry mass m^{-2}). Abundance, however, peaked in spring, suggesting a close correspondence between reproduction and phytoplankton spring blooms (Chl *a* max. 50.86 mg m^{-3}, 3 m depth). Overall, copepods were the most important group of the total zooplankton community, both in abundance (64-81%) and biovolume (20-70%) followed by mysids and chaetognaths (in terms of biovolume and biomass), and nauplii and Appendicularia (in terms of abundance). Throughout the year, diel changes in the vertical distribution of biomass were found with a daytime maximum in the 100-200 m depth layer and a nighttime maximum in surface waters (0-50 m), associated with the diel vertical migration of the calanoid copepod family Metridinidae. Diel differences in integrated zooplankton abundance, biovolume and biomass were probably due to a high zooplankton patchiness driven by biological processes (e.g. diel vertical migration or predation avoidance), and oceanographic processes (estuarine circulation, tidal mixing or water column stratification). Those factors are considered to be the main drivers of the zooplankton vertical distribution in Comau Fjord.

Keywords:

Zooplankton | seasonality | diel vertical migration | cold-water corals (CWC) | ZooScan | EcoTaxa | Comau Fjord | Chilean Patagonia

2.2. Introduction

Coastal marine ecosystems are among the most productive on earth (Mann and Lazier, 1991). They provide substantial economic and ecological services, such as high biological production, nutrient cycling or shoreline stability and erosion control (Escribano et al., 2003; Liu et al., 2010; Pan et al., 2013; Barbier, 2017). The Chilean fjord region extends over large and complex hydrographic and geomorphologic conditions, particularly rich in productivity and biodiversity (Försterra et al., 2017; Häussermann et al., 2021b), but also endangered by human exploitation, which has been increasing significantly – e.g. salmon aquaculture – over the last two decades (Iriarte et al., 2010; Buschmann et al., 2021; Navedo and Vargas-Chacoff, 2021).

The hydrography of the fjords and channels of Patagonia can be considered a transitional estuarine-marine system where a surface layer of silicate-rich terrestrial freshwater meets nitrate- and phosphate-rich marine waters. These fjords receive freshwater from rivers, surface runoff and groundwater flow due to the high rainfall and glacier melting (Pantoja et al., 2011). The upper brackish layer within the top 10 m water depth is usually poor in nitrate and phosphate but rich in silicate and organic matter from terrestrial inputs (Sánchez et al., 2011). Below the halocline, a water mass with higher salinity (>31), named Modified Subantarctic Water (MSAAW), flowing landward from the adjacent oceanic area provides the fjords with macronutrients (nitrate and phosphate). Both water masses, surface-freshwater and MSAAW, generate a two-layer structure in the water column with sharp vertical and more gradual horizontal salinity gradients (Sievers and Silva, 2008; Pérez-Santos et al., 2014; Meerhoff et al., 2019). The summer stratification creates a barrier which may hinder the exchange of nutrients within the water column, altering the functioning of the pelagic food web and productivity patterns (Silva et al., 1997; González et al., 2011). During austral spring, the Comau Fjord receives an intense riverine input of fresh water, rich in silicic acid used by bloom-generating diatoms and, thus, leading to high primary production (González et al., 2010). In austral summer, the high concentration of phytoplankton promotes an increase in the abundance and biomass of zooplankton as secondary producers (Antezana, 1999; González et al., 2010). In fjord systems, seasonal patterns are modulated by other oceanographic processes, such as estuarine circulation (Palma and Silva, 2004), tidal regimes and lateral advection (Castro et al., 2011) or water column stratification (Sánchez et al., 2011) influencing the zooplankton biomass and community structure on shorter time scales.

Zooplankton plays an essential role in the functioning of marine ecosystems and in the oceanic carbon cycle. It includes a wide variety of organisms and displays extreme variability in terms of community composition and vertical, seasonal and geographical distribution (Palma and Kaiser, 1993). Many taxa are known to perform diel vertical migrations (DVM), most likely to evade predators (Stich and Lampert, 1981; Iwasa, 1982). According to the predator-evasion hypothesis, migrating zooplankton resides in deep waters during daytime hours where the probability of being perceived by visually orientated predators is lower than if they remained in better illuminated shallow waters, and at night, in the refuge of darkness, they migrate upwards to feed. However, DVM is not performed by all organisms in a zooplankton community or even not by all individuals of one species. For example, while late copepodites and adults of the copepod genus *Metridia* migrate, a large fraction of the young developmental stages remains in surface waters, saving the energy of performing the DVM, suggesting a lower probability of being perceived and consumed by visual predators (Hays, 1995). Both, migrant and non-migrant species are important elements of the biological carbon pump via the production of sinking fecal pellets that transport carbon from surface waters to the seafloor (Urrère and Knauer, 1981; Fowler and Knauer, 1986; Emerson and Roff, 1987). Zooplankton also provides a trophic link between primary production and higher consumers such as fish, birds and mammals, but also invertebrate predators, such as corals (Nemoto, 1970; Gili et al., 2006; Höfer et al., 2018).

Cold-water corals (CWC) rely on zooplankton as their principal food source to maintain their physiological processes, such as respiratory metabolism and growth (Carlier et al., 2009; Mayr et al., 2011; Naumann et al., 2011). Therefore, their diet depends on the zooplankton seasonal, diel and vertical distribution. In Comau Fjord, azooxanthellate scleractinian CWC are wide-spread even in deep aragonite-undersaturated waters (Häussermann and Försterra, 2007; Fillinger and Richter, 2013; Jantzen et al., 2013a). In the latter, the dissolution of exposed parts of the skeleton, enhanced bioerosion, and reduced CWC growth and survival have been observed (McCulloch et al., 2012; Maier et al., 2016). The calcification of the CWC skeleton is energetically costly, and thus food requirements in these adverse environments are higher compared to aragonite-saturated waters (Maier et al., 2016). According to Fillinger and Richter (2013), in Comau Fjord, the CWC *Desmophyllum dianthus* (Esper, 1794) thrives but coral densities decrease below 280 m despite available substrate, suggesting that lower oxygen and pH concentrations, combined with a shortage of zooplankton could be limiting coral growth. However, up to now little is known about the zooplankton of Comau Fjord in the northern Patagonian region and its role in sustaining the CWC communities living in the fjord. Most studies have been carried out in the central-southern part of Patagonia (from Penas Gulf to Cape Horn, S46.50°-S55.55°), while studies performed in the northern area (from Puerto Montt to San Rafael Lagoon, S41.20°-S46.40°) mainly focused on selected microzooplankton taxa in the upper water column, on bulk measurements of zooplankton biomass, or on the carbon flow through the pelagic food web (e.g. Palma, 2008; Villenas et al., 2009; González et al., 2010, 2011; Palma et al., 2011; Sánchez et al., 2011). Other studies addressed the physical oceanographic processes and their effects on zooplankton distribution (e.g. Marín and Delgado, 2009; Castro et al., 2011), and their relationship with zoo- and ichthyoplankton growth and feeding (Landaeta et al., 2015a, 2015b). Recent studies have investigated zooplankton migration patterns by acoustic backscatter and vertical velocity profiles (Valle-Levinson et al., 2014; Díaz-Astudillo et al., 2017; Pérez-Santos et al., 2018). The information on zooplankton diversity and migration patterns is, however, still very fragmentary and the linkage between the abundance of CWC and zooplankton supply in Comau Fjord remains unknown.

In this study, we aim to describe the diel, vertical, and seasonal distribution of mesozooplankton groups of Comau Fjord, with a focus on the dominating taxa, particularly those that migrate and are more likely to aggregate. Samples were collected at day and night hours with vertical net hauls through the whole water column in spring, summer, autumn and winter. They were processed with a high-resolution image analysis system (ZooScan, Gorsky et al. 2010). The zooplankton taxa were identified using EcoTaxa 2.0 (Picheral et al., 2017), allowing to assess the influence of seasonal environmental changes on zooplankton dynamics, and the food naturally available to CWC.

2.3. Materials & Methods

Field work was carried out in Comau Fjord, Northern Patagonia, Chile (Fig. 2.1). Zooplankton was sampled four times at a fixed station (42°14.95S, 72°28.83W) in central Comau Fjord: in austral spring (28th September 2016; three days before spring tide-new moon), summer (17th January 2017; two days after spring tide-full moon), autumn (11th May 2017; one day before spring tide-full moon), and winter (9th Aug 2017; spring tide-full moon), during both day (noon) and night (midnight). Samples were collected with a 70 cm-diameter Nansen closing net (mesh size: 100 µm) equipped with a non-filtering cod end. Vertical hauls were carried out at 0.45 m s^{-1} to sample the depth strata 0-50-100-200-300-400-450 m. Immediately after the collection, the samples were sieved through a 50 µm mesh and preserved in 4% borax-buffered formaldehyde for laboratory analyses. After every zooplankton haul, a CTD multi-probe (SBE 19plusV2 Profiler -

with RS 232 Interface, Sea-Bird Electronics Inc.) was deployed from the surface to the bottom, measuring conductivity, temperature, oxygen, pH and chlorophyll *a* (Chl *a*)-fluorescence.

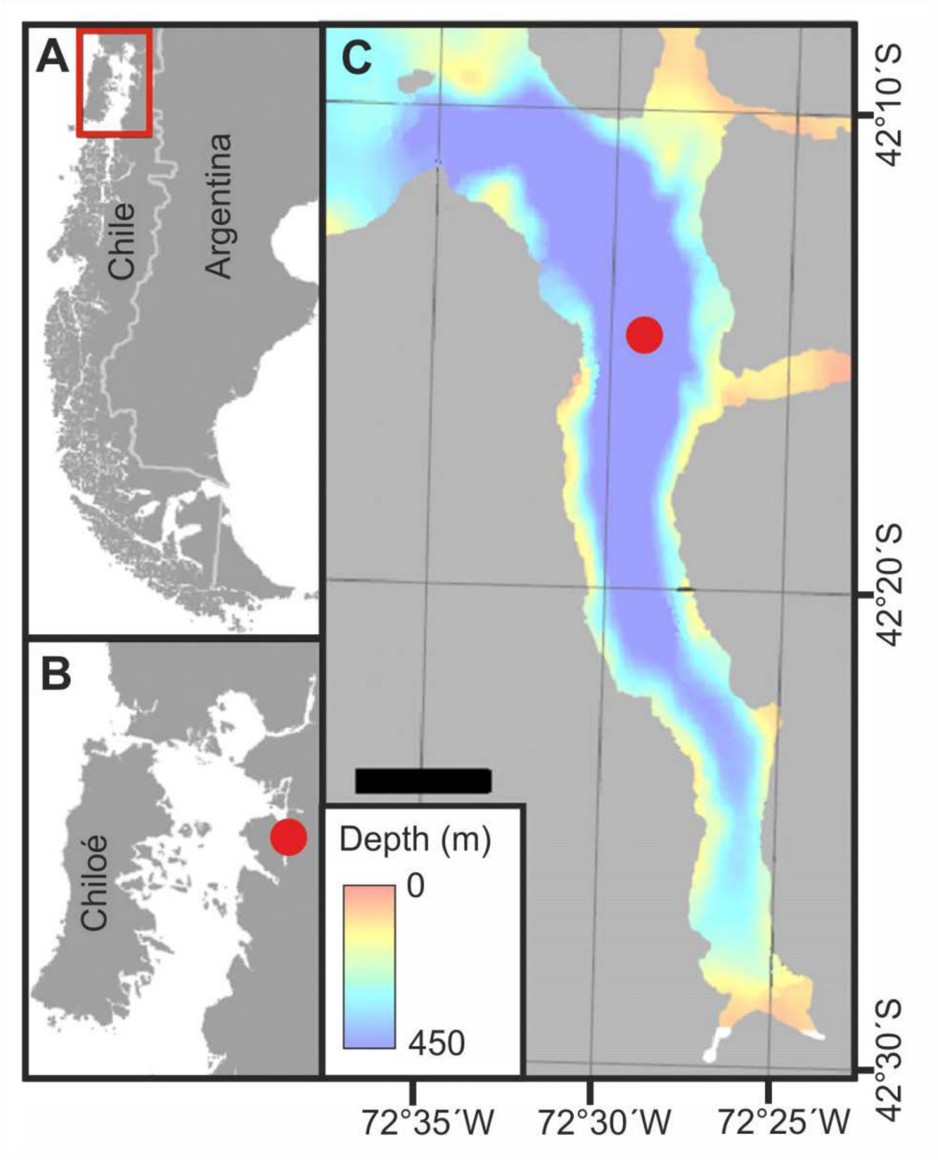

Fig. 2.1 Study site. (A) Overview of Chilean Patagonia. Red Square denotes area in B. (B) Inner Sea off Chiloé island, where Comau Fjord (red dot) is located. (C) Comau Fjord with the bathymetry and location of the stations where zooplankton samples were taken (red dot). Adapted from Fillinger and Richter (2013).
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In the laboratory, fixed zooplankton samples were washed with fresh water and prepared for analysis with a ZooScan digital imaging system (Grosjean et al., 2004; Gorsky et al., 2010). ZooScan (CNRS patent, www.hydroptic.com) provides a quick and reliable method for the analysis of preserved plankton samples, storing digitized images for later examination, reprocessing and dissemination. Concentrated samples were subsampled with a Folsom plankton splitter to avoid images being cluttered with more than approximately 1000-1500 individuals. Up to six binary splitting steps were carried out (corresponding to a minimum 1/64th fraction of the original sample). Routinely, the two final splits were scanned with ZooScan yielding images of 2400-dpi resolution (14200×22700 pixels). The hinged base of the ZooScan allowed the recovery of the complete undamaged subsample, which was later stored in 70% ethanol for archiving. Most overlapping individuals on the scanning surface were manually separated to ensure an even distribution before scanning. Image analysis was performed with the software ZooProcess (Gorsky et al., 2010), a plug-in for the image processing and analysis software ImageJ (Schneider et al., 2012). The processing involved (1) the automatic subtraction of background noise, (2) the automatic thresholding and detection of objects, and (3) the automated storage of detected objects in separate images ("vignettes"). Below 300 µm, organisms were often too blurred to be identified. Thus, the ZooScan detection limit was set at the standard of 300 µm so that detected zooplankton sizes ranged from 0.3 to 59 mm. The automatic processing of the scans was successful in 75-80% of the cases where vignettes with one individual were obtained. However, despite the manual separation, some individuals overlapped, resulting in vignettes with two or more objects. These objects on the pictures were manually separated using the "separation with mask" tool of the ZooProcess software. Separated vignettes were stored, while the original vignette, containing multiple objects was eliminated from the database to avoid duplicate counts. In some cases, the separation of individuals was not possible as cutting the vignette would mean losing information about the morphology of the organisms (i.e. cutting overlapping urosomes from two different copepods or small copepods embedded in cnidarians). Overall, the contribution of vignettes with multiple objects that could not be separated was always <10% of the total amount of vignettes.

Vignettes were subjected to the semi-automated taxonomic classification in EcoTaxa 2.0 (Picheral et al., 2017). This web-based machine learning application uses training sets of expert-identified taxa and random forest classification to automatically identify and sort the objects. Although EcoTaxa contains more than 160 million objects on its server, no ZooScan training set was available for Patagonian waters. Therefore, manual identification of individuals on a subset of the images was first necessary to train an initial model, which was later used by the system to classify the scanned organisms. The initial learning set with Patagonian organisms improved progressively its prediction by sorting more objects into the given categories. This produced the final learning set for the classification of the entire image data set. At the end, all classified objects were individually validated to assure a correct classification. The organisms were classified to the lowest possible taxonomic level; for most copepods this was the family level. However, small calanoid copepods (<1.5 mm) were not distinguishable on family level and were thus comprised as one category: “Calanoida (<1.5 mm)” including five groups (copepodites (all calanoid taxa, <1mm), Clausocalanidae, Microcalanidae, small Calanidae (*Neocalanus* spp.) and Paracalanidae). Developmental stages were included in the corresponding taxon as long as they were clearly identifiable. Only for calanoid copepods, the classification of some developmental stages was not clear and therefore, they were included into the category “copepodites”, which included developmental stages of different calanoid copepod taxa. The category Cnidaria was constituted by organisms from the class Hydrozoa (mostly medusa and Siphonophorae). Another category contained all images that were out of focus (“bad focus”) and likely comprised individuals from all copepod taxa, in total 6,766 vignettes. From the total of 83,516 vignettes, 23,227 could not be assigned to zooplankton taxa, but were labelled as “detritus”, “feces”, “fiber”, “leg” “bubble” and “other” and were not considered in our analyses. ZooProcess provides information about the length and width of each object, allowing the calculation of its volume as a proxy for its biomass (Gorsky et al., 2010). The program automatically fits an ellipse around the object, from which the major and minor axis and volume (V) is computed:

$$V\ (mm^3) = \frac{3}{4} \times \pi\ \times \frac{major\ axis\ (mm)}{2}\ \times \left(\frac{minor\ axis\ (mm)}{2}\right)^2$$

Biovolume (BV) was then calculated as the sum of the volumes of all objects (ΣV) divided by the fraction of the sample (e.g. F=1/64) and by the volume filtered by the Nansen net (V_N):

$$BV\ (mm^3/m^3) = \frac{\left[\frac{\sum V\ (mm^3)}{F}\right]}{V_N\ (m^3)}$$

V_N was calculated as:

$$V_N\ (m^3) = \left[\pi\ \times \left[\frac{net\ diameter\ (m)}{2}\right]^2\right] \times\ depth\ interval\ (m) \times\ filtration\ efficiency$$

where filtration efficiency was assumed as the theoretical 100% efficiency (value = 1). Flowmeter readings were not used because the speed of the net haul was below the measuring range of the mechanical flowmeter.

Table 2.1 Regression coefficients between individual dry mass and body area to estimate biomass [DM (µg) = (a A^b)] for different groups given by Lehette & Hernández-León (2009). Area provides the size range for each category observed in this study

Organism	*a*	*b*	*Area (mm²)*
Actinopterygii (eggs and larvae)	43.38	1.54	0.079 – 1.198
Appendicularia	43.38	1.54	0.056 – 6.071
Ascidiacea (larvae)	43.38	1.54	0.072 – 1.652
Amphipoda	43.38	1.54	0.103 – 59.854
Brachiopoda (larvae)	43.38	1.54	0.193 – 0.366
Bivalvia (larvae)	43.38	1.54	0.071 – 2.040
Bryozoa (larvae)	43.38	1.54	0.067 – 0.240
Chaetognatha	23.45	1.19	0.068 – 15.935
Cirripedia (larvae and cypris)	43.38	1.54	0.071 – 0.286
Cladocera	43.38	1.54	0.072 – 0.455
Cnidaria	4.03	1.24	0.051 – 95.743
Copepoda	43.97	1.52	0.068 – 9.177
Decapoda (zoea)	43.38	1.54	0.072 – 6.733
Echinodermata	43.38	1.54	0.070 – 0.757
Euphausiacea	43.38	1.54	0.145 – 461.813
Eggs	43.38	1.54	0.070 – 1.952
Gastropoda (larvae)	43.38	1.54	0.071 – 2.266
Isopoda	43.38	1.54	0.073 – 0.930
Mysidacea	43.38	1.54	0.126 – 43.504
Nemertea (pilidium)	43.38	1.54	0.082 – 0.777
Ostracoda	43.38	1.54	0.066 – 1.270
Platyhelminthes (larvae)	43.38	1.54	0.075 – 0.162
Polychaeta (larvae)	43.38	1.54	0.068 – 7.535

For the estimation of biomass, a regression between the dry mass (DM) of a specimen and its body area [DM (µg) = (a A^b)] was used (Hernández-León and Montero, 2006; Lehette and Hernández-León, 2009), where A is the area (mm^2) of each scanned individual. The regression required different conversion factors depending on the organism, as for instance, gelatinous zooplankton with high water content may not be compared to crustaceans or echinoderms (Table 2.1). Such coefficients have been successfully published in previous studies for mid-latitude shelf areas (Marcolin et al., 2015) or the Chilean upwelling region (Tutasi and Escribano, 2020).

The biomass (B) of each taxon was then calculated as the sum of the individual dry masses of the respective taxon (ΣDM) divided by the fraction of the sample (e.g. F=1/64) and by the volume filtered by the Nansen net (V_N):

$$B\ (mg\ dry\ mass/m^3) = \frac{\left[\frac{\sum DM(mg)}{F}\right]}{V_N(m^3)}$$

Biovolume and biomass were calculated to obtain the sum of the values of all individuals for a given taxon. In multiple vignettes, the automatic calculation of biovolume and biomass was not possible because of overlapping specimens from different taxa. Then, the organisms were counted manually and biovolume and biomass were estimated by multiplying the mean volume or DM of the given taxon from all automatic calculations by the extra number of multiple vignettes. Groups with large size variability (e.g. chaetognaths, cnidarians or Euchaetidae) were divided into two categories, small (0.003-4.242 mm^3) and large (4.243-90.083 mm^3) in order to get a better biovolume/biomass assessment.

The integrated values of abundance, biovolume and biomass, were calculated down to 400 m water depth for all seasons, taking out the last 50 m from summer and winter, to make it comparable to the spring and autumn seasons, where samples were collected down to 400 m.

The relationships among physico-chemical variables and each taxon's abundance were analyzed using a redundancy analysis (RDA). RDA is a constrained ordination procedure which allows the assessment of how much of the variation of one set of response variables (i.e. zooplankton abundances) is explained with another set of variables (i.e. physico-chemical variables). The RDA is a multiresponse analysis which summarizes the linear relationships among dependent and independent variables into a matrix followed by a principal component analysis (PCA). Mean values of temperature, salinity, oxygen, and Chl *a* (log transformed) for the entire water column were used as explanatory variables. RDA was performed in R (R Core Team, 2020) by using the *rda* function of the *vegan* package (Oksanen et al., 2019). The problems caused by non-normal distributions in testing the significance of RDA results were solved by a permutation test (10,000 iterations) (Borcard et al., 2011) using the *anova.cca* function from the *vegan* package. All abundance data were logarithmically transformed before analysis.

The centroid depths (CD) of the zooplankton groups for each sampling event were calculated as: CD = $\sum (p_k \times z_k) / \sum p_k$, where p_k is the number of organisms in the stratum k, and z_k is the mean depth of the stratum k. CD were calculated for abundance and biovolume – not biomass – to better represent the gelatinous zooplankton groups (e.g, Cnidaria). Due to the lack of replication, these values were compared using a contingency table by means of a Chi-square test, in order to test the significance of night-day changes in CD. The Chi-square test compares the critical values to assess their significance according to their degrees of freedom (df) = $(r - 1)(c - 1)$, where r is the number of rows and c the number of columns in the contingency table. After showing significant day-night differences in CD, a dissimilarity analysis (function *simper* from *vegan* package) performed pairwise comparisons of zooplankton taxa and estimated the average contribution of each taxon to the average overall Bray-Curtis dissimilarity. We listed the zooplankton taxa which cumulatively contributed at least to 70% of the night-day differences observed.

2.4. Results

The physico-chemical parameters measured throughout the water column of Comau Fjord showed a stronger seasonal variability in surface waters (0-50 m) than in deep waters (50-450 m) (Fig. 2.2). The temperature profile indicated summer stratification down to 17 m water depth, followed by surface cooling, breakdown of the thermocline in autumn, and reverse temperature gradients in winter and spring. Accordingly, the surface temperature values were the lowest in winter and spring (8.6-11 °C), and the highest in summer (16.7 °C), getting cooler again in autumn (12 °C). In deeper waters, temperatures were more stable with an average value of 11.4 ± 0.2 °C (mean ± SD; Fig. 2.2A). Salinity was between 10-30 in the upper 20 m and 32.9 ± 0.4 below 20 m (Fig. 2.2B). The pH ranged between 8.5 and 7.7 in the upper 50 m and was 7.9 ± 0.1 in deeper waters in all seasons, except for autumn, where we interpret the sudden drop of pH values as an instrument malfunction (Fig. 2.2C). Oxygen concentration showed the largest variations in the upper 50 m during the spring season (137.2-410.5 µmol kg^{-1}), while below 100 m depth it was on average 180 ± 9.3 µmol kg^{-1} (Fig. 2.2D). The chlorophyll *a* (Chl *a*) concentration peaked in early spring (50.86 mg Chl *a* m^{-3} at 3 m depth), followed by a decrease towards the end of the summer and low values through autumn and mid-winter (2.5-5 mg Chl *a* m^{-3} at 5-10 m depth) (Fig. 2.2E). Below 25 m, the concentration of Chl *a* was <1.8 ± 0.5 mg m^{-3} throughout the year (Fig. 2.2E).

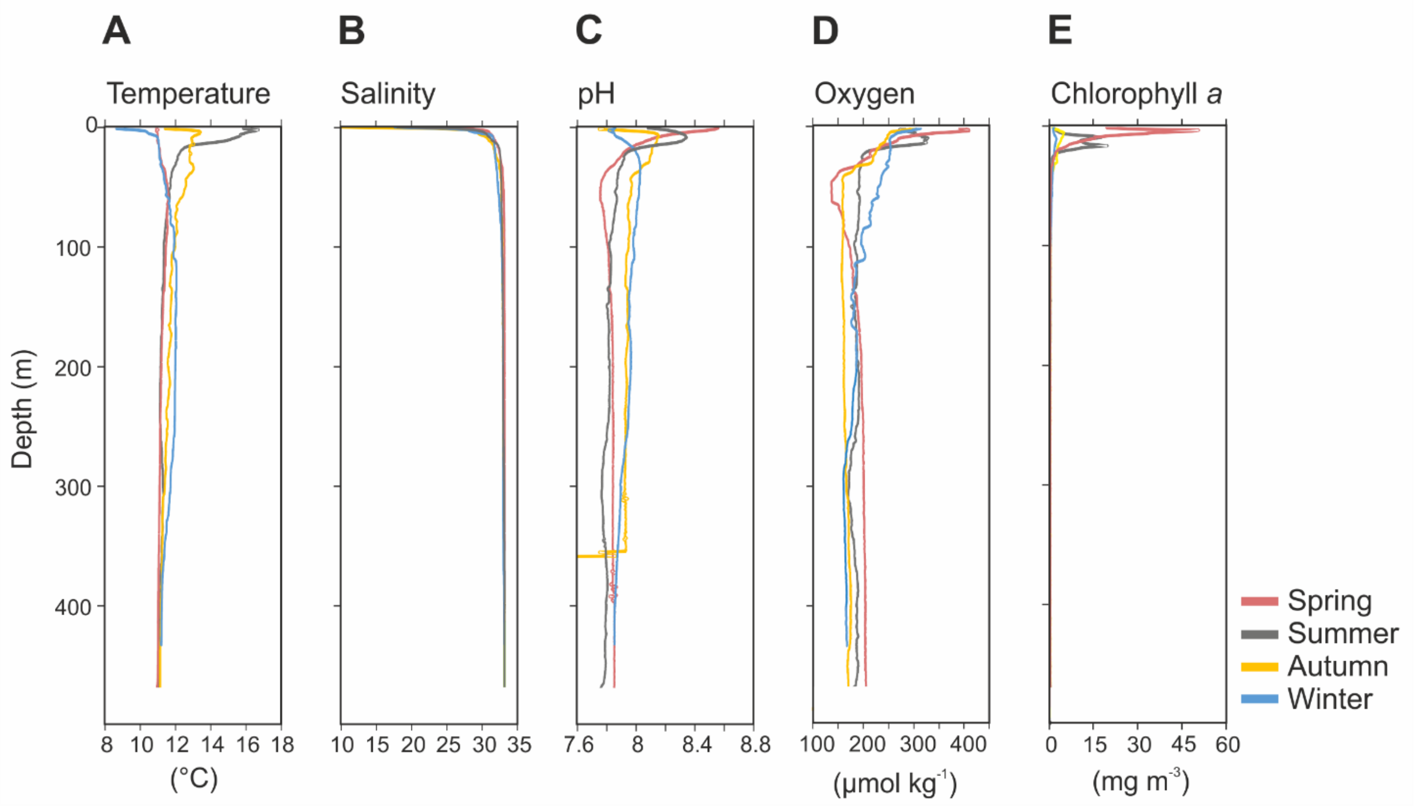

Fig. 2.2 Vertical profiles of physico-chemical parameters of Comau Fjord. (A) Temperature, (B) salinity, (C) pH, (D) oxygen and (E) Chlorophyll-*a*.

Full-size DOI: 10.7717/peerj.12823/fig-2

The zooplankton community exhibited large seasonal and diel differences. Abundance, integrated over the upper 400 m of the water column (individuals m^{-2}) showed the highest values in spring and the lowest during autumn for both day and night (Fig. 2.3A). The integrated biovolume (cm^{3} m^{-2}) and biomass (g dry mass m^{-2}) showed a different pattern with the highest values in summer and the lowest in autumn and winter (Figs. 2.3B, 2. 3C). Diel differences in integrated abundances generally showed higher values during the day than at night, except for autumn (Fig. 2.3A). Diel differences in integrated biovolume and biomass were surprisingly large, with generally higher values during the night, except for spring (Figs. 2.3B, 2.3C), indicating that during daytime, particularly in spring, zooplankton was more numerous but smaller in size.

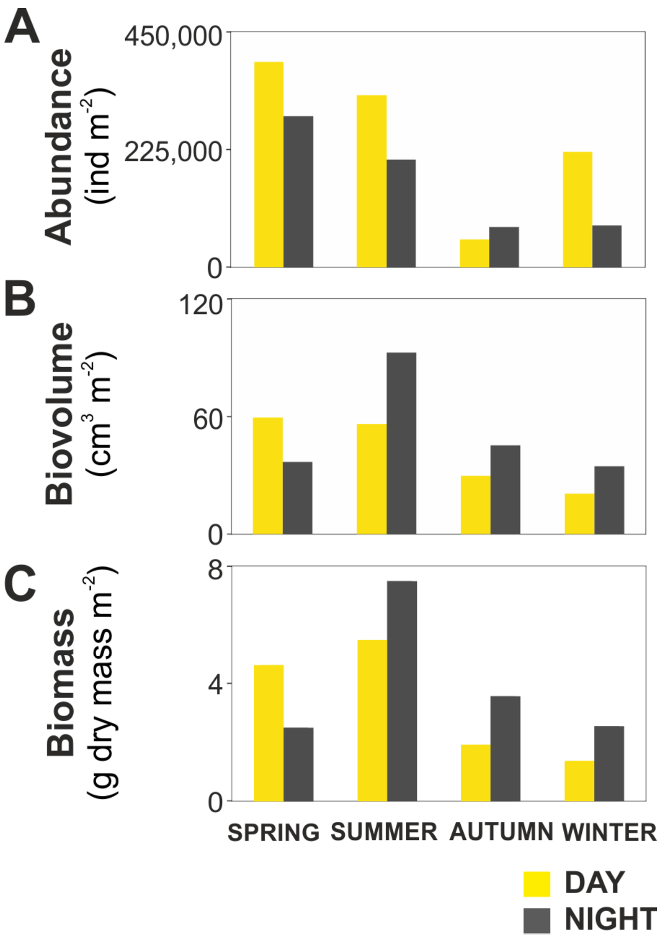

Fig. 2.3 Zooplankton seasonal and diel distribution. Seasonal distribution of integrated (A) abundance, (B) biovolume and (C) biomass of the zooplankton community during day and night.

Full-size DOI: 10.7717/peerj.12823/fig-3

Overall, the centroid depth dissimilarity analysis showed that in spring, fewer taxa contributed to the significant day-night differences (Chi-square Pr <2.2 e^{-16}, p <0.001) both in abundance and biovolume, while in summer and autumn the number of contributing zooplankton groups increased (Fig. 2.4). The zooplankton vertical distribution showed that abundance exhibited the highest values during day and night in the 0-50 m layer at all seasons, with the exception of autumn during day time, where abundances showed low values throughout the water column (Fig. 2.5A). The lowest abundances were found in >300 m depth in spring, summer and winter, and in 50-100 m depth in autumn during day and night time (Fig. 2.5A). The highest biovolume and biomass daytime values were observed in 0-50 m and 100-200 m water depth in spring and summer, and in 100-300 m in autumn. However, in winter, daytime biovolume and biomass were similarly low throughout the entire water column (Figs. 2.5B, 2.5C). At night, the highest biovolume and biomass values were found at the surface (0-50 m) in all seasons. The lowest biovolume and biomass values were found in >300 m depth in spring and summer, in 50-100 m and 200-300 m depth in autumn and in >50 m depth in winter (Figs. 2.5B, 2.5C).

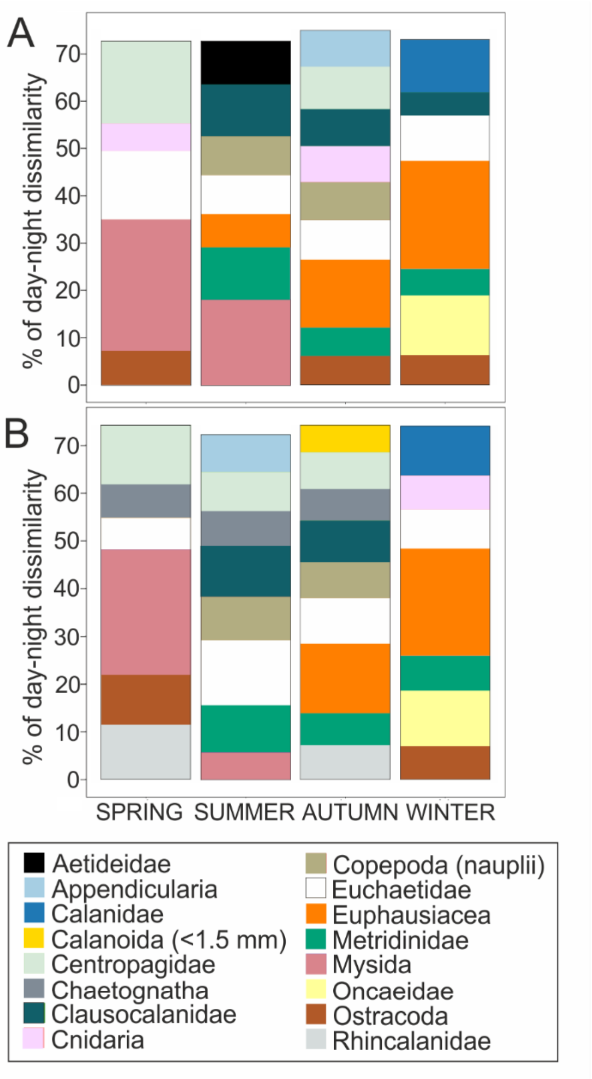

Fig. 2.4 Dissimilarity analysis of day-night differences in centroid depth (CD). Zooplankton taxa which contribute cumulatively to at least 70% of the day-night differences in (A) abundance CD and (B) biovolume CD.

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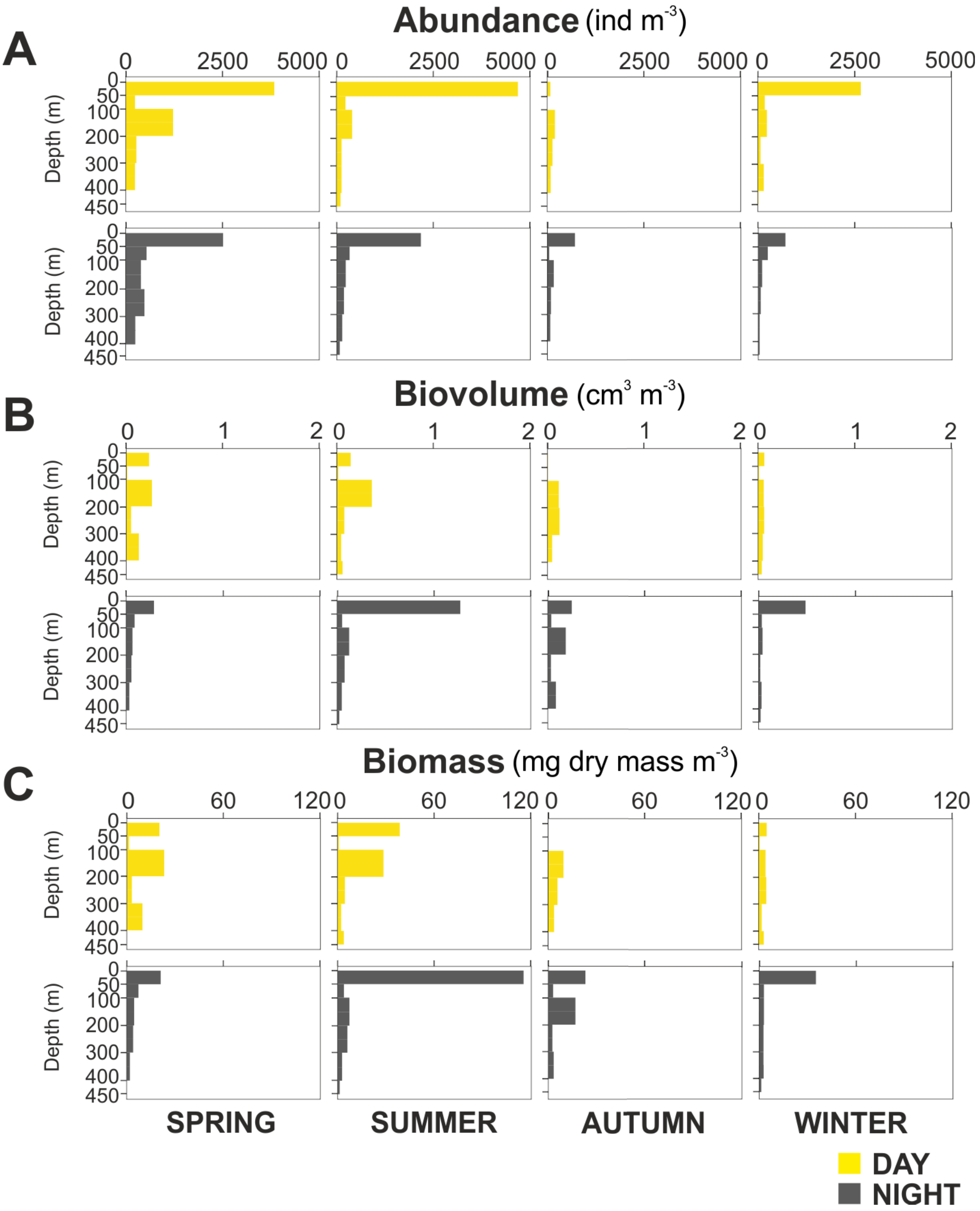

Fig. 2.5 Zooplankton vertical distribution. Seasonal, diel and vertical distribution of (A) abundance, (B) biovolume and (C) biomass of the zooplankton community during day and night.

Full-size DOI: 10.7717/peerj.12823/fig-5

The taxonomic composition greatly differed when considering biovolume and abundance (Figs. 2.6, 2.7A; biovolume—not biomass—was used to better represent the gelatinous taxa). Copepoda were generally the dominant group, constituting 20-70% of the total biovolume, and 64-81% of the total abundance. Within the copepod community, individuals smaller than 1.5 mm included (a) copepodites and adults of small calanoid genera, such as Clausocalanidae, Microcalanidae, Neocalanidae and Paracalanidae; (b) cyclopoids of the genera *Oithona* and *Oncaea*; (c) harpacticoids; and (d) nauplii. These small copepods accounted for 58-86% of the total abundance within the copepod community. Overall, 14 out of 41 taxa contributed 45-98% of the total biovolume and 45-86% of the total zooplankton abundance (Fig. 2.6). The other 27 taxa were constituted by copepods (Acartiidae, Aetideidae, Calanoida (non-identifiable), Candaciidae, Centropagidae, Copepoda (non-identifiable), Eucalanidae, Heterorhabdidae, Lucicutiidae, Oncaeidae, Pontellidae, Rhincalanidae), Actinopterygii (eggs and larvae), Appendicularia, Ascidiacea (larvae), Brachiopoda (larvae), Bryozoa (larvae), Cirripedia (nauplii and cypris), Cladocera, Decapoda (larvae), Echinodermata (larvae), eggs, Isopoda, Nemertea (larvae), Platyhelminthes (larvae), Polychaeta (larvae) and non-identifiable organisms.

The contribution of groups other than copepods to the total zooplankton community differed regarding biovolume and abundance. For biovolume, Cnidaria (2-23%) and Mysida (1-21%) constituted a large part of the zooplankton community, followed by Chaetognatha (3-15%). Metanauplii and calyptopis stages of Euphausiacea were found mostly in spring and summer, accounting for 0-7% of the total biovolume. A single adult specimen found during winter in 0-50 m water depth at night raised the total biovolume of this taxon to 65%. Regarding abundance, copepod nauplii (2-9%, with their maximum in spring and minimum in autumn) and Appendicularia (0.4-6%) were the second and third most abundant groups after Copepoda, respectively. The fourth most abundant group differed among seasons: Echinodermata larvae (5% in spring), Mollusca larvae (2-5% in summer, 3-9% in autumn), Ostracoda (4-5% in autumn) and Bryozoa larvae (2-10% in winter). Across all samples and seasons, taxa that represented more than 5% of total biovolume were Cnidaria (13%), Calanidae (12.9%), Mysida (12.7%), Metridinidae (12.6%), Chaetognatha (9.6%) and Euchaetidae (8.7%). The most abundant groups with more than 5% of the total abundance were Harpacticoida (14.0%), Cyclopoida (9.1%) and Metridinidae (8.4%) (Figs. 2.6, 2.7A).

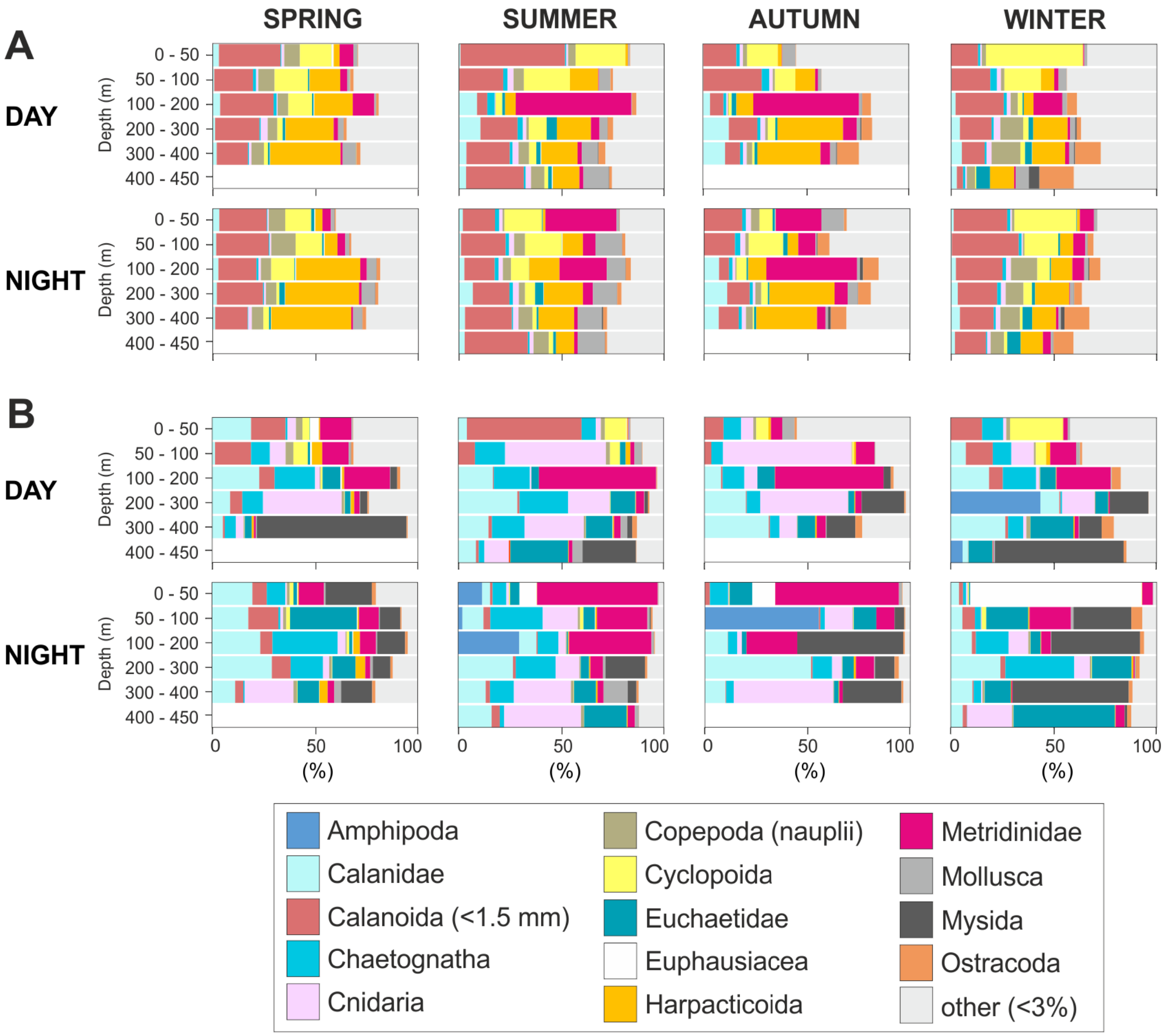

Fig. 2.6 Group dominance of the mesozooplankton taxa. Relative (A) abundance and (B) biovolume of major zooplankton groups. Taxa comprising less than 3% of the total zooplankton community (27 taxa) were pooled together as ‘‘other’’.

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The integrated abundance of small and large copepods and chaetognaths showed the highest abundance in spring and generally low values in autumn and winter (Table 2.2). Highest abundances of copepod nauplii and cnidarians were found in early spring with a minimum in autumn and raising up again in late-winter. Mysida presented a stable abundance throughout the year with a minimum in summer.

Table 2.2 Integrated abundances (ind. m^{-2}) of the most important zooplankton groups over the entire water column sampled on four dates throughout a year.

		SPRING		SUMMER		AUTUMN		WINTER	
		Day	Night	Day	Night	Day	Night	Day	Night
Cyclopoida		47,894	28,198	5,974	6,501	2,227	4,342	3,209	3,477
	Mean	38,046		6,237		3,284		3,343	
Harpacticoida		48,632	47,978	9,664	12,421	9,929	7,486	6,514	4,345
	Mean	48,305		11,042		8,707		5,429	
Copepoda (nauplii)		20,600	18,129	1,481	2,583	431	2,094	2,463	1,616
	Mean	19,365		2,032		1,263		2,039	
Metridinidae		27,772	9,287	25,691	8,105	12,696	18,262	4,438	1,684
	Mean	18,529		16,897		15,479		3,061	
Calanidae		11,288	7,447	5,898	2,827	3,622	3,087	1,977	889
	Mean	9,367		4,363		3,354		1,433	
Euchaetidae		1,840	4,259	1,731	1,110	790	873	1,060	733
	Mean	3,045		1,420		831		896	
Cnidaria		4,895	2,546	1,520	3,713	702	2,432	4,636	2,848
	Mean	3,721		2,616		1,567		3,742	
Chaetognatha		3,108	1,216	2,528	1,187	884	1,390	1,000	598
	Mean	2,162		1,858		1,117		799	
Mysida		239	171	62	164	151	411	239	166
	Mean	205		113		280		202	

These groups clearly presented different vertical distributions (Fig. 2.7). Metridinidae, a family of large copepods with high biovolume and abundance, resided generally above 200 m, exhibiting a peak between 100-200 m during the day. At night, 74% of their abundance and 77% of their biovolume were found in the upper 50 m (Fig. 2.7B). On average, larger individuals (0.62 ± 0.3 mm^3; average size ± SD) were found in 100-200 m water depth during day and night. In spring, a significant proportion of the Metridinidae population was found during the day in shallow waters where smaller individuals (0.14 ± 0.08 mm^3) were observed. At nighttime, a fraction of those large and small individuals residing at intermediate waters were found also in shallow waters (Fig. 2.8A). The Calanidae were found mainly in intermediate waters (100-300 m) during daytime, except in spring, where on average larger individuals (1.37 ± 0.5 mm^3) resided (Fig 2.8B).

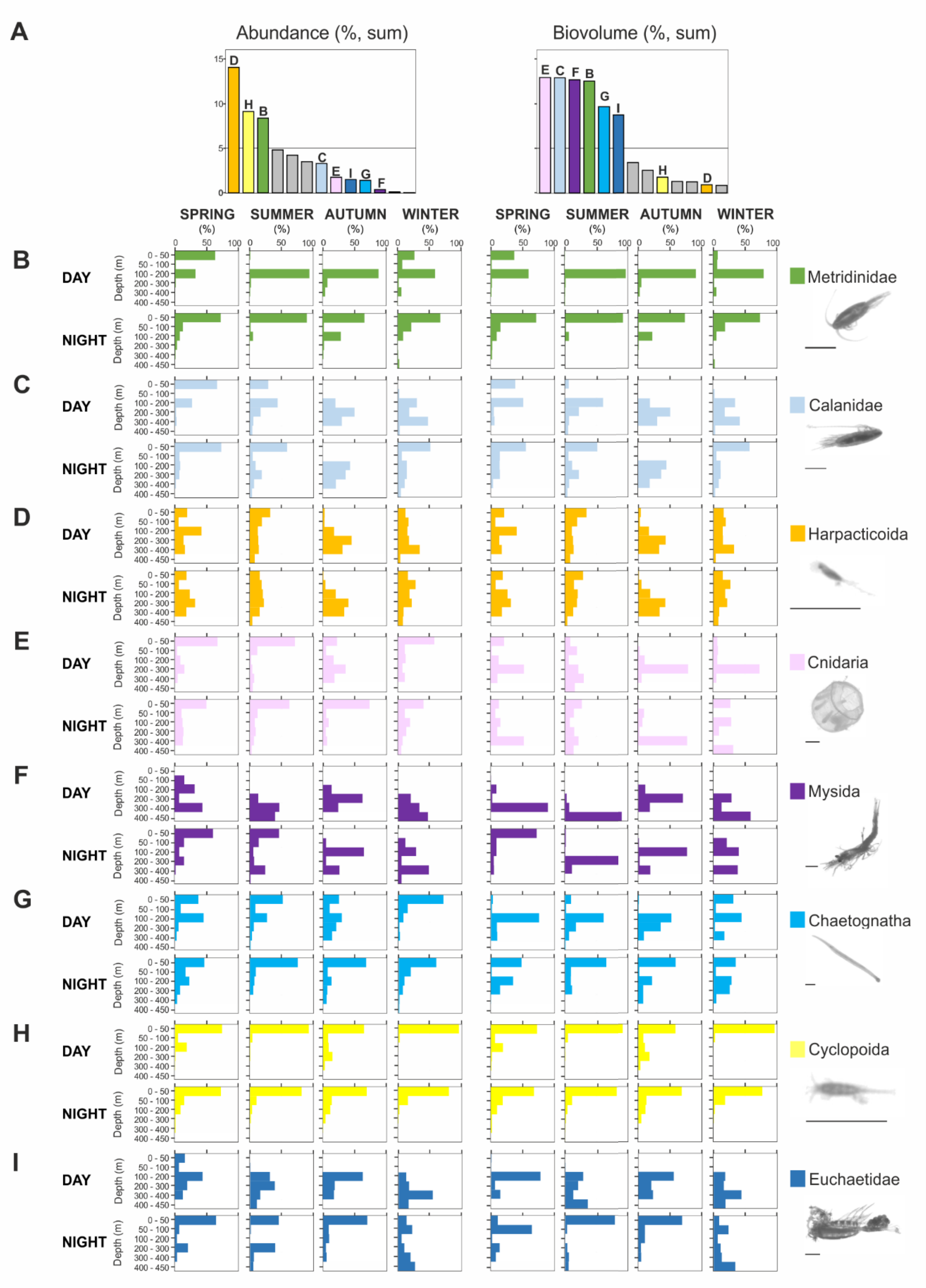

Fig. 2.7 Seasonal, diel and vertical distribution of the most important taxa. Vertical, diel and seasonal distribution of the most important taxa representing the percent of abundance and biovolume (%) in

relation to their total abundance and biovolume. (A) Total sum of biovolume and abundance in all samples. Vertical distributions of (B) Metridinidae, (C) Calanidae, (D) Harpacticoida, (E) Cnidaria, (F) Mysida, (G) Chaetognatha, (H) Cyclopoida and (I) Euchaetidae. The group ‘‘calanoids (<1.5 mm)’’, although more than 5% in both, biovolume and abundance, is not represented here as it is composed of a mix of taxa with different functions. Pictures scale bar = 1 mm.

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In spring, small specimens of Calanidae (0.30 ± 0.003 mm^3) were found in shallow waters also during daytime (Fig 2.8B). At nighttime, half of the population (61% and 54% of their abundance and biovolume, respectively) were present in shallow waters. In autumn, however, the whole population stayed between 100-400 m. Despite the low number of specimens of the Calanidae found at shallow waters, they contributed considerably to the biovolume in these layers (Fig. 2.6). Euchaetidae showed higher abundance and biovolume in the deep part of the water column during day time (100-450 m), ascending to shallower waters at night (Fig. 2.7I). Small copepods of the taxa Harpacticoida and Cyclopoida were very abundant, but, as expected, they only represented a small fraction of the total biovolume. Harpacticoida were distributed through the whole water column (Fig. 2.7D). Cyclopoida were mainly present in the upper 50 m during day and night in all seasons (Fig. 2.7H). Cnidarians were overall not very abundant, but constituted an important fraction of the biovolume, especially in 200-300 m depth during day in all seasons, and 50-100 m depth during day time in summer and autumn. Their highest abundances were generally found in 0-50 m during day and night, while higher biovolumes were detected in deeper layers 200-450 m (Fig. 2.7E). Throughout the year, Mysida were mainly present in >200 m depth during the day, accounting for 52-100% of its abundance and 89-100% of its biovolume. In spring, at night a high proportion of their abundance and biovolume were found in shallow waters while in summer only a fraction of their abundance was found in 0-50 m. In autumn and winter the shallowest depth mysids were found was up to 50 m both during day and night (Fig. 2.7F). Chaetognatha presented the highest abundances in the 0-50 m water depth during day and night in all seasons. Their biovolume, however, peaked at 100-300 m during day and at 0-50 m during night (Fig. 2.7G).

Overall, the four predictor variables (fluorescence (Chl *a*), oxygen, temperature, salinity) explained 44.3% and 33.69% of the total variation in abundance and biovolume, respectively (Fig. 2.S1). The first axis of the RDA explained 34.15% (abundance) and 25.4% (biovolume) of the total variation while the second axis only accounted for 10.15% (abundance) and 8.29% (biovolume).

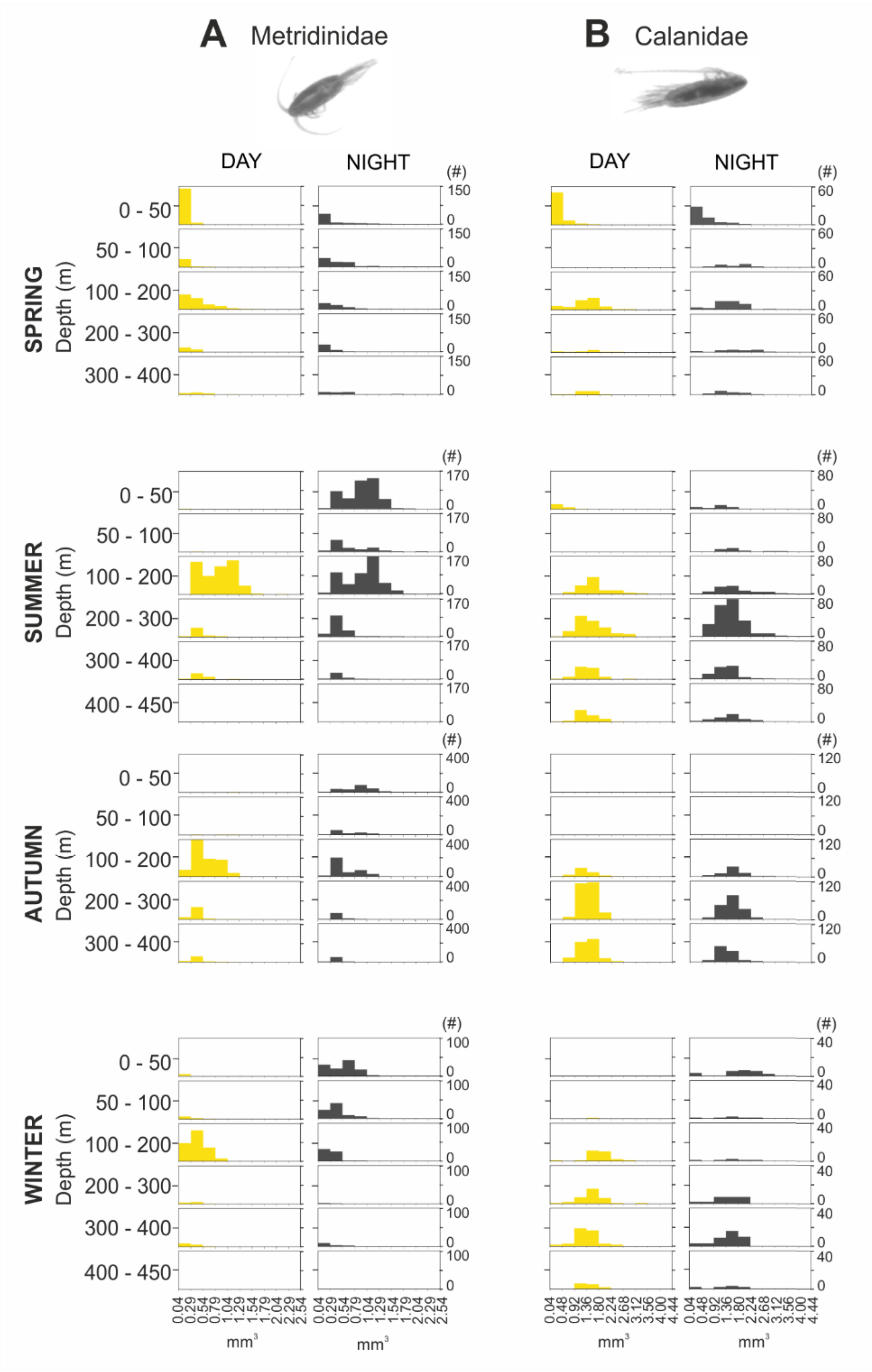

Fig. 2.8 Size frequency distribution of Metridinidae and Calanidae (Copepoda). Seasonal, diel and vertical size-frequency distribution of (A) Metridinidae and (B) Calanidae. (#) provides the number of individuals measured.

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Relationships between the parameters and the first RDA axis were highest for Chl *a*, while temperature was related to the second RDA axis. For both abundance and biovolume the main differences were observed between day and night samples in shallow waters (0-50 m), during spring and summer. The abundance and biovolume of the major zooplankton groups were positively influenced by Chl *a* concentration, except for mysids and ostracods, while temperature did not seem to have a strong influence on the majority of the groups, except for the large zooplankton (amphipods, Euphausiacea and euphausiids) (Fig. 2.S1).

2.5. Discussion

Physico-chemical properties and mesozooplankton seasonal dynamics

The physico-chemical observations in our study are consistent with earlier descriptions from Comau Fjord, a temperate fjord connected to the Pacific Ocean by the Chacao Channel and Ancud Gulf. Comau Fjord has an estuarine circulation and is characterized by a strong pycnocline, where surface waters (0-50 m) present higher variability than the deeper and quasi-homogeneous water layer (Fig. 2.2). Clear differences were observed in temperature, pH, oxygen and Chl *a* in relation to the season, likely caused by the strong seasonal variability in solar radiation (maximum between spring and summer), precipitation and river discharge (maximum in late autumn and winter) (González et al., 2010).

Pronounced seasonality of environmental variables often results in high biological production and are associated to seasonal changes in the holoplankton community (Mauchline, 1998; Balbontín and Bustos, 2005; Aracena et al., 2011), and meroplankton abundance (Ladah et al., 2005; Landaeta and Castro, 2006). In Comau Fjord, a thermal inversion of the surface water (0-50 m) in winter is visible (Fig. 2.2), probably due to heat loss in the surface layer caused by winds and the discharge of cold freshwater from rivers and glaciers (Silva et al., 1997). Later in the year, the thermal density stratification stabilizes the water column, triggering spring phytoplankton blooms (Iriarte et al., 2007), which usually follow rain events and thus the input of nutrients. This leads to a strong increase in Chl *a* (Fig. 2.2E). As shown by previous studies in the area (Palma and Silva, 2004; Vargas et al., 2008; González et al., 2010), this peak in Chl *a* was most likely due to blooming chain-forming diatoms. The high phytoplankton biomass is expected to be grazed predominantly by copepods, increasing their biomass and establishing the classical diatom-to-zooplankton food web (Palma and Silva, 2004; Vargas et al., 2008; González et al., 2010).

Accordingly, the population dynamics of copepods in this study followed the phytoplankton seasonal cycle, with the highest abundance in spring, associated with the maximum concentration in phytoplankton (Table 2.2). At this time, copepod nauplii and young stages of calanoid copepods, which are indicative of intense zooplankton reproduction, accounted for a large proportion of the zooplankton community. Considering that copepods are the main food source for cnidarians and chaetognaths (Palma and Kaiser, 1993), the higher abundance of carnivorous zooplankton occurring in spring (Table 2.2) can be attributed to the large copepod abundance at this time of the year. In summer, biovolume and biomass reached their maxima (Fig. 2.3), which together with a lower abundance, suggests the presence of larger individuals or different groups including species with larger individuals. Subsequently, in autumn and winter, zooplankton abundance, biovolume and biomass decreased. At this time, primary production should be low, as reflected by low Chl *a* values (Fig. 2.2E), and likely zooplankton growth was limited by food availability (Escribano et al., 2007). The overall decline in copepod abundance from spring to winter may be explained by the decrease in phytoplankton occurrence and the increasing predation pressure exerted by carnivorous zooplankton. In winter, the plankton in the fjord typically shifts towards a microbial loop based community grazed by heterotrophic nanoflagellates, which become the main mesozooplankton prey (Vargas et al., 2008; González et al., 2010) however, this does not support a high secondary production.

The northern part of the Chilean fjord region, the area between Puerto Montt and Guafo's Mouth, represents the most productive area of Patagonia in terms of primary production and zooplankton biomass (Palma, 2008). In contrast, the phytoplankton production in the southern area is low due to the stronger influence of glaciers, resulting in cold, fresh and turbid waters (Palma and Rosales, 1997; Palma and Silva, 2004; Iriarte et al., 2007; Palma, 2008), and consequently low zooplankton survival and growth (Giesecke et al., 2019). Previous studies described ranges for zooplankton biovolume, expressed as the plankton wet volume, of 65 to more than 1,386 ml zooplankton 1,000 m^{-3} outside Comau Fjord, in the Inner Sea off Chiloé island (Palma, 2008). This is in line with the present results (250-1,500 ml zooplankton 1,000 m^{-3}), showing an especially high biovolume during summer. Palma & Rosales (1997) also found the highest values of zooplankton biovolume in the northern part (interior of Reloncaví Fjord and Ancud Gulf) with values that ranged between 56 and 1,626 ml zooplankton 1,000 m^{-3}, but a low zooplankton biovolume in the inner of Comau Fjord. The observed variations may potentially be due to (a) interannual differences with a much lower Chl *a* concentration during the same season of their year of study (Ramírez, 1995); and/or (b) methodological and analytical differences, e.g.

different sampling gears and proxies for biomass estimation (i.e. measurement of zooplankton wet volume vs. image analysis in this study). Moreover, the vertical hauls used in this study may most likely underestimate the abundance of very motile organisms able to avoid nets, such as adult euphausiids, megalopae of *Munida gregaria* or large fish larvae, which are also important in terms of abundance and biomass across west Patagonia (Antezana, 1999).

In fjord systems, oceanographic processes such as estuarine circulation, tidal mixing or water column stratification may influence the composition and abundance of zooplankton communities (Palma and Silva, 2004; Sánchez et al., 2011). Overall, copepods were the main contributors to the total biomass and biovolume of the zooplankton community, especially during summer (69 – 78%). This is in agreement with previous studies showing that in Chilean fjords, planktonic crustaceans, such as copepods and euphausiids, have the highest abundances and biomasses, followed by chaetognaths and gelatinous plankton (Defren-Janson et al., 1999; Palma and Silva, 2004). Copepods are the most abundant and diverse component of marine zooplankton worldwide (Mauchline, 1998), and the abundance of small copepods (<1.5 mm) generally surpasses the abundance of larger ones (Fransz, 1988; Gallienne and Robins, 1998; Gallienne et al., 2001). Similarly, small copepods accounted for 58-86% of the total copepod community in the present study. Another important contributor to zooplankton communities in Chilean fjords is the euphausiid *Euphausia vallentini*. The present study revealed young stages of euphausiids during spring and summer but only one adult specimen in winter, indicating that euphausiids are present in Comau Fjord, but were not caught efficiently in our samples. This is likely related to the small volume filtered by our net, the patchy distribution of *E. vallentini*, and their ability of avoiding slow nets (Brinton, 1962). Like euphausiids, mysids can form dense swarms, making them a potential food resource for a wide range of organisms, from predatory fishes to benthic CWC. Despite their important contribution to the total zooplankton biovolume in deep waters (Fig. 2.6), poor attention has been given to their presence in Chilean Patagonia. To our knowledge, there are only two studies describing mysids in this area: Guglielmo & Ianora (1997) found that the most abundant species for the Strait of Magellan is the deep-dwelling *Boreomysis rostrata*; Díaz-Astudillo, Cáceres & Landaeta (2017) found higher abundances of mysids during night and inside the Reloncaví Fjord and Ancud Gulf. Thus, this study constitutes the first record of mysids in Comau Fjord.

Zooplankton diel vertical distribution and migration

Biological processes (e.g. diel vertical migration, predator avoidance, location of food patches and mating), together with oceanographic processes (e.g. estuarine circulation or water column stratification) are mechanisms by which the underlying zooplankton behavior presents high spatial heterogeneity (Folt and Burns, 1999). In the present study, zooplankton abundance, biovolume and biomass significantly differed between day and night. These differences were probably due to the high patchiness and the vertical distribution the zooplankton exhibited during day and night, especially by larger zooplankton individuals, those able to form swarms (i.e. mysids and euphausiids) and migrate (Fig. 2.7B-2.7I). For instance, Euchaetidae, Euphausiacea, Metridinidae and Mysida, four of the larger and most important zooplankton groups, changed their centroid depth between day and night contributing to 30-40% of the day-night difference in abundance and 30-38% in biovolume (Fig. 2.4). To a lesser extent, small organisms, e.g. Appendicularia, Clausocalanidae and Copepoda (nauplii) also contributed to these differences, especially in summer and autumn, however, their contribution was lower (0-29%) in comparison to larger zooplankton organisms.

Diel Vertical Migration (DVM) is usually associated with differences in light intensity within the photic zone, taking place periodically in 24 h cycles (Brierley, 2014). During daytime, zooplankton organisms migrate to deeper, darker waters to avoid visual predators, such as fishes, while they come to the surface for feeding at night (Hays et al., 1998). In Comau Fjord, DVM seemed to be related to the size of the zooplankton. Here, large zooplankton taxa, particularly large copepods, that inhabited the intermediate waters (100-300 m) during daytime ascended to shallow waters at night, while small organisms, mainly cyclopoids and harpacticoids, did not perform a clear DVM (Fig. 2.7B-2.7I). Throughout the year, Cyclopoida centroid depth fluctuated between 40-85 m water depth and Harpacticoida between 149-254 m water depth. These two copepod groups, however, differed in their vertical distribution. The highest abundances of cyclopoid copepods were mainly found in the upper 50 m, whereas harpacticoids were found throughout the water column (Fig. 2.7D).

Large individuals from several calanoid copepod taxa (Metridinidae, Calanidae, Euchaetidae), mysids, chaetognaths and cnidarians did perform DVM over 300 m. This agrees with the findings by Hays (1995) that DVM is pronounced in large and pigmented species due to their susceptibility of being perceived by visually orientated predators. Similarly, studies in northern Patagonia have shown that conspicuous zooplankton organisms tend to avoid well illuminated waters (Villenas et al., 2009). According to Hays, Kennedy and Frost (2001), large individuals of *Metridia* usually reside in deep waters, and only a fraction of these ascends to shallow waters at night, whereas smaller individuals stay at the surface. In Comau Fjord, Metridinidae showed the highest values of biovolume and abundance at intermediate depths (100-200 m) during daytime, but in the surface layer (0-50 m) at night, indicating that 77% of the entire population migrated towards the surface (Fig. 2.7B). In spring, a proportion of the Metridinidae population was found in shallow waters during the day. This was due to the smaller size of the individuals (Fig. 2.8A) and the higher amount of food available here. Following the same pattern as Metridinidae, larger individuals of Calanidae were found in deeper waters during daytime, whereas small specimens were found in shallow waters also during daytime in spring, where they may not be hunted by visual predators due to their small size. It is possible that those small organisms were individuals of earlier life stages (i.e. small) or species with a smaller size. Euchaetidae also performed DVM with most of the organisms living continuously in deep waters (100-450 m) during the day, but a large proportion of the population (60% and 53% of their abundance and biovolume, respectively) migrated to the surface during night, except in winter (Fig. 2.7I).

Mysids accounted for a big proportion (up to 70%) of the total zooplankton biovolume, especially in spring and winter (Fig. 2.6). During day, they were generally detected in deep waters (>200 m water depth) and at night they migrated upwards, although their DVM pattern was only clear in spring and summer. At night in spring, both abundance and biovolume were high in 0-50 m water depth, whereas in summer only a high abundance was detected at that water depth. The fact that biovolume peaked at 200-300 m in summer at night suggested that only small individuals migrated to shallow waters while larger ones stayed at deeper waters (Fig. 2.7F).

Chaetognaths were distributed throughout the water column with the highest biovolumes between 100-300 m, during both day and night (Fig. 2.7G). This is in accordance with the distribution found by Guglielmo and Ianora (1995) for the Strait of Magellan. A particularly high abundance of chaetognaths in the upper 0-50 m during both day and night and a high biovolume only at night indicated that larger individuals migrated to shallow waters at night likely to feed on other organisms. South of Comau Fjord, between Guafo's Mouth and the Pulluche Channel, the vertical distribution of cnidarians (i.e. Hydromedusae and Siphonophorae) showed highest abundances in the upper 100 m, specifically in the 20-50 m stratum (Palma et al., 2007). In this study, cnidarian highest abundances were found in 0-50 m during day and night. However, biovolume peaked in every season, except for summer, at 200-300 m, indicating that larger individuals of cnidarians stayed at deeper waters while small individuals resided in shallow waters.

In the 50-100 m water layer a minimum in zooplankton abundance and biomass was found in all seasons (Fig. 2.5). This “zooplankton gap” could be related to a high concentration of predators in this water depth. In northern Chilean fjords, the high biomasses and abundances of gelatinous organisms are correlated to a decrease in chitinous biomass of other organisms (Palma and Silva, 2004; Villenas et al., 2009). It is known that chaetognaths and cnidarians can grow at fast rates, forming dense aggregations that seasonally dominate the zooplankton biomass (Casanova, 1999; Brodeur et al., 2002) by feeding voraciously on copepods and larvae from other organisms (Lie et al., 1983; Palma and Rosales, 1997). In our study, biovolume of carnivorous organisms ranged between 18 and 83% of the total zooplankton in this water layer, reaching particularly high values in summer and autumn (50-83%) (Fig. 2.9). These previous evidences suggest that carnivorous organisms may have reduced zooplankton abundance in this depth stratum and predation may play a role in structuring the vertical zooplankton community in Comau Fjord.

Mesozooplankton plays an important role in the food web as a food source for many pelagic and benthic organisms (González et al., 2013), including CWC (Gili et al., 2006; Carlier et al., 2009; Mayr et al., 2011). Due to the difficulty of studying CWC *in situ*, little is known about their natural diet and its availability. In Comau Fjord, CWC thrive also in deeper, naturally acidified waters (Häussermann and Försterra, 2007; Försterra et al., 2017). Although the environment is unfavorable, this might be due to the high ingestion rate (e.g. of the CWC *Desmophyllum dianthus*) which showed a positive impact on their calcification rates, regardless of the seawater pH (Martínez-Dios et al., 2020). Zooplankton abundance and biomass are highly influenced by seasonality. Therefore, CWC must be adapted to differences in food availability, i.e. high zooplankton abundances during spring and summer and low concentrations during autumn and winter. Indeed high growth rates of *D. dianthus* were found in summer (Jantzen et al., 2013b) which may be associated with higher zooplankton availability. In winter, when zooplankton biomass is low, CWC may slow down their metabolism to cope with the lower food availability (Naumann et al., 2011). Only recently, a study confirmed that *D. dianthus* preyed on medium and large sized calanoid copepods and euphausiids (Höfer et al., 2018). Based on the present DVM data, shallow-dwelling CWC in Comau Fjord might feed on small copepods (e.g. cyclopoid and calanoid) during the day, and on larger organisms during night hours when zooplankton migrates upwards. Deeper-dwelling corals, by contrast, may mainly encounter larger prey, such as mysids or large calanoid copepods (Calanidae, Euchaetidae) and thus may gain enough energy to upregulate their internal pH in an acidified environment.

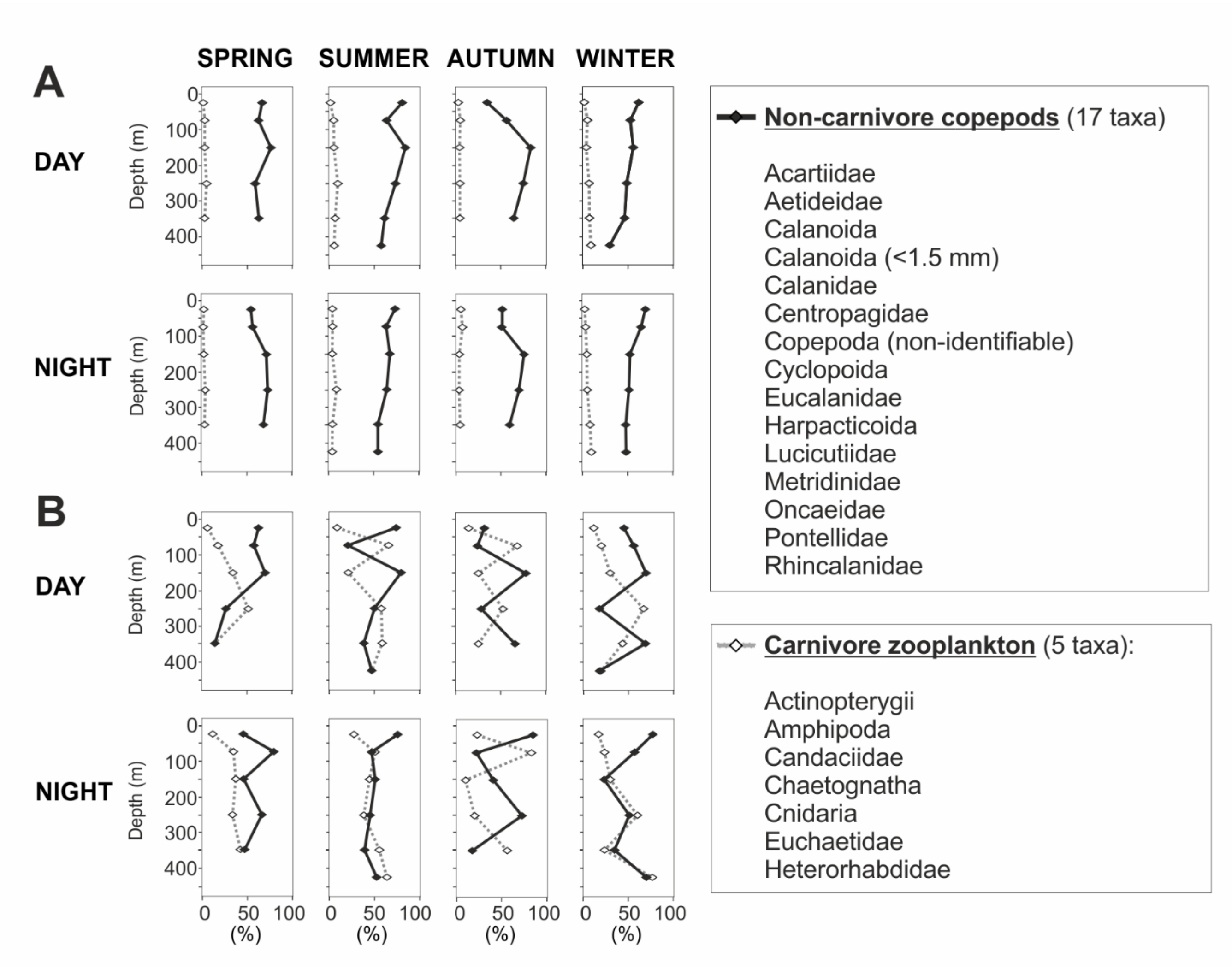

Fig. 2.9 Non-carnivore copepods *versus* carnivore zooplankton. Presented as percentages of (A) total abundance and (B) total biovolume.

Full-size DOI: 10.7717/peerj.12823/fig-9

2.6. Conclusions

The seasonal changes of zooplankton over the entire water column showed that abundance peaked in spring, likely due to spring phytoplankton blooms. In summer, biovolume and biomass were the highest and decreased thereafter over time, reaching the lowest values in late autumn and mid-winter. The low concentration of Chl *a* during the cold season suggests that primary production was insufficient to support high levels of secondary production. The vertical distribution of zooplankton biovolume and biomass differed between day and night, with a daytime maximum between 100 and 200 m water depth and a nighttime maximum in surface waters (0-50 m) associated with the diel vertical migration of calanoid copepods of the family Metridinidae. Overall, copepods were the dominant group of the total zooplankton community with an important contribution of small organisms (individuals <1.5 mm), followed by mysids, chaetognaths and cnidarians (biovolume and biomass), and nauplii and Appendicularia (abundance). The integrated abundance, biovolume and biomass also showed significant differences between daytime and nighttime values. These differences were probably due to the high zooplankton patchiness driven by both biological and oceanographic processes. Diel vertical migration, predation avoidance, location of food patches as well as estuarine circulation, tidal mixing or water column stratification are considered to be the main drivers of the zooplankton distribution in Comau Fjord.

2.7. Acknowledgements

We thank the team of PACOC which contributed to the success of the project. Support during sampling was provided by the scientific and logistic staff from the Fundación San Ignacio del Huinay. Henning Schröder and Manding Suwareh assisted with the sample collection. Thomas Heran, Simon Schöbinger and Jasmin Stimpfle helped with the treatment, analysis and scanning of the samples. Gertraud Schmidt-Grieb provided support in an early phase of the project.

2.8. Supplemental information

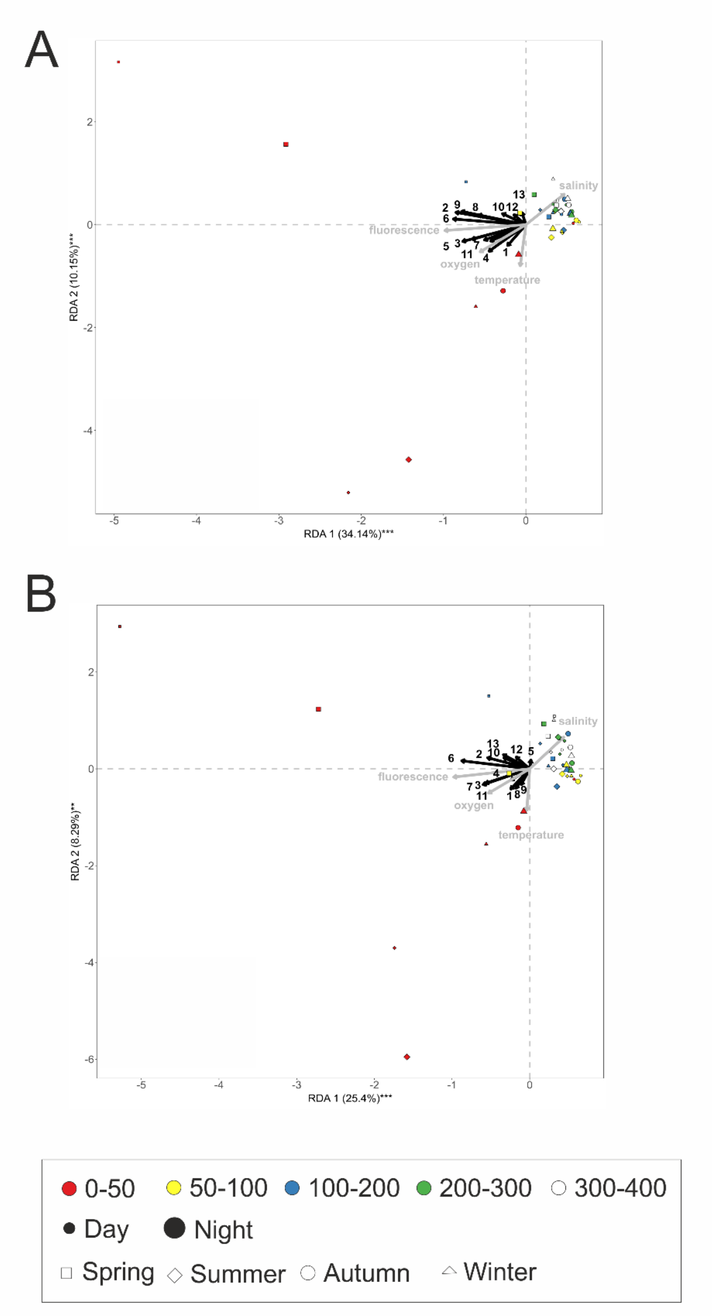

Fig. 2.S1 Effect of hydrographic and biological variables on zooplankton abundance and biovolume RDA ordination for (A) abundance and (B) biovolume of the most important zooplankton groups constrained by four variables. In the RDA correlation biplot, angles among explanatory (grey arrows) and/or response (black arrows) variables reflect their correlations. Depth strata are represented by color of data points, day/night sampling (size of data points) and seasons (symbols). Taxa code: 1 Amphipoda, 2 Calanidae, 3 Calanoida (<1.5 mm), 4 Chaetognatha, 5 Cnidaria, 6 Copepoda (nauplii), 7 Cyclopoida, 8 Euchaetidae, 9 Euphausiacea, 10 Harpacticoida, 11 Metridinidae, 12 Mysida, 13 Ostracoda.

Chapter 3 – Zooplankton spatial dynamics

Spatial and temporal dynamics of zooplankton along a section through Comau Fjord to the Ancud Gulf, Chile

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3.1. Abstract

Comau Fjord is a deep, stratified fjord located in the northern region of Chilean Patagonia, connecting to the shallow Ancud Gulf. Despite its importance as cold-water coral habitat and a growing salmon mariculture, the composition of the zooplankton community as basis of the food web as well as its spatio-temporal dynamics are poorly understood. Thus, we investigated the spatial (four fjord stations and one station in Ancud Gulf), seasonal (summer and winter) and diel (day and night) distribution of zooplankton over the entire water column. Stratified vertical hauls from the surface to near the seabed (max. sampling depth 450 m) were carried out with a Nansen net (100 µm mesh) and abundance and biovolume were determined for 40 taxonomic groups using ZooScan/EcoTaxa. Integrated zooplankton abundance and biovolume were on average two and three times higher in summer than in winter, respectively. In the outer fjord and Ancud Gulf, the integrated zooplankton biovolume was 6-fold higher than in the inner fjord, associated with a 4-fold higher Chl *a* concentration. During both, day and night, zooplankton abundance concentrated in the upper 0-50 m of the water column. However, zooplankton biovolume concentrated between 100-200 m during the day and between 0-50 m at night, indicating diel vertical migration of large zooplankton. In the deeper central stations, biodiversity increased compared to shallower stations and a higher number of larger zooplankton taxa was detected. Copepods dominated the zooplankton community (71.5 ± 10% and 52 ± 21% of total abundance and total biovolume, respectively), with Metridinidae and Calanidae constituting the most important groups. Chaetognaths, cnidarians, amphipods and mysids followed in rank of importance for biovolume, and meroplankton and ostracods for abundance. Salinity, temperature, oxygen and Chl *a* explained only 34% and 44% of the total variation of zooplankton abundance for summer and winter, respectively, suggesting that diel vertical migration (DVM) and other biological factors may play an important role in the horizontal and vertical distribution of zooplankton and the cycling and vertical flux of carbon in Comau Fjord.

Keywords:

Zooplankton distribution | ZooScan | EcoTaxa | Northern Chilean Patagonia | diel vertical migration (DVM)

3.2. Introduction

Fjords are semi-enclosed coastal water bodies with surface water diluted by freshwater and deep waters connecting to the adjacent ocean (Cameron and Pritchard, 1963; Freeland et al., 1980; Mardones et al., 2021), typically across a submarine sill (Matthews and Sands, 1973; Freeland et al., 1980). The regional oceanography as well as the geomorphology and vegetation of the watershed govern the nutrient input, autotrophic biomass and heterotrophic production (Iriarte et al., 2017). In this regard, the Chilean fjord region is one of the most biologically productive fjord regions worldwide (Escribano et al., 2003), sustaining rich pelagic and benthic fisheries (Buschmann et al., 2021). However, important interventions in the marine environment and watershed are threatening the sustainable use of the fjords, notably through an intensifying mariculture of mostly non-native species and a growing development of hydroelectric energy over the last two decades (Iriarte et al., 2010; Buschmann et al., 2021; Navedo and Vargas-Chacoff, 2021). As Chilean fjords are currently in different stages of alteration, the comparison of biodiversity, productivity and dynamics of differentially affected systems may serve as a baseline for developing conservation strategies for the more remote and pristine ecosystems (Iriarte et al., 2010).

The northern Chilean Patagonia provides an ideal natural laboratory to study the effect of changing abiotic environmental variables on the distribution of biotic communities. The Chilean Patagonian fjord system, covering an area of ca. 240,000 km^2, hosts a complex set of deep fjords and channels with deep basins and steep walls (Pantoja et al., 2011). It is characterized by an estuarine circulation resulting in strong vertical and horizontal gradients originating from opposing water masses. On the one hand is the meteoric freshwater mass at the surface, fed by river runoff, high precipitation and glacial melt, which carries oligotrophic but silicic acid rich water flowing seawards. On the other hand, there is deep saline oceanic Modified Subantarctic Water (MSAAW) flowing landwards, carrying high concentrations of macronutrients into the fjords (Silva et al., 1998; González et al., 2011). This two-layer water system is separated by a strong pycnocline, which may vary seasonally due to changes in solar radiation and freshwater input, but also on shorter time scales due to mixing by winds and tides. The variations in stratification along with the lateral advection of nutrients govern the primary production (Silva et al., 1997; González et al., 2010; Pérez-Santos et al., 2014). In austral spring, fjords typically receive high solar radiation and freshwater input, resulting in strong diatom blooms that provide food for zooplankton (González et al., 2010). This leads to an increase in zooplankton abundance and biomass in summer (Sullivan, 1993; Antezana, 1999; González et al., 2010). Lower primary production in fall and winter is reflected in the lowest values for abundance and biomass of zooplankton in the cold seasons (Garcia-Herrera et al., 2022).

Both, abiotic and biotic drivers affect the seasonal and vertical distribution, as well as the community composition of zooplankton. Among abiotic processes, presence of thermoclines and haloclines (Southward and Barret, 1983; Sánchez et al., 2011), layers with low oxygen concentration (Longhurst, 1976; Judkins, 1980; Pérez-Santos et al., 2018), tidal and turbulent mixing (Castro et al., 2011; Pérez-Santos et al., 2018), and estuarine circulation (Palma and Silva, 2004) influence the vertical distribution of zooplankton. For example, the intrusion of oceanic waters has led to successful colonization of oceanic zooplankton species that can form dense aggregations, affecting the species composition of endemic zooplankton and its distribution in the water column due to predation (Palma, 2008; Villenas et al., 2009).

Among the biological drivers that can influence the zooplankton dynamics, zooplankton behavior may lead to predator avoidance (Stich and Lampert, 1981; Ohman et al., 1983) and aggregation in phytoplankton-rich layers (Ortner et al., 1980; Castro et al., 1991). Both, food attraction and predator avoidance cause diel vertical migration (DVM) of some zooplankton species (Longhurst, 1976; Hays, 1995). DVM occurs in 24h cycles, likely triggered by light intensity, even in areas where the differences between day and night are very small (e.g. during polar nights in Arctic fjords), suggesting a high light sensitivity of zooplankton (Berge et al., 2009). Generally, migratory zooplankton descend to deeper, darker waters during the day to avoid being hunted by visual predators in well-illuminated surface areas. At night, herbivorous zooplankton migrate upwards to feed on phytoplankton, which is most abundant there, and carnivorous zooplankton pursue the migrating zooplankton (Hays et al., 1998; Brierley, 2014). However, increase and loss in zooplankton biomass can vary rapidly on relatively short time scales, due to DVM, rapid growth of some taxa, or mortality through high consumption (Hirst and Bunker, 2003; Frangoulis et al., 2010; Zhou et al., 2010). Therefore, a high zooplankton biomass can 1) attract nekton (such as fish, cephalopods or whales), transferring energy to higher trophic levels (Nemoto, 1970; Gili et al., 2006; Höfer et al., 2018) and 2) export carbon to deeper layers through the biological carbon pump by the egestion of fecal pellets and production of other sinking particles (Urrère and Knauer, 1981; Frangoulis et al., 2010; González et al., 2016).

Due to the importance of zooplankton in the marine environment, we investigated the abundance and biovolume of the mesozooplankton community of Comau Fjord in this study. We describe 1) the spatial, seasonal, vertical and diel distribution of mesozooplankton along the fjord and into the connecting Ancud Gulf, 2) mesozooplankton community composition with a focus on the dominant taxonomic groups, and 3) the diversity of the mesozooplankton in relation to water depth. In addition, we analyzed the spatial and temporal variation of the physico-chemical properties in the fjord to study how the changes of abiotic environmental variables and other oceanographic processes may influence the biological zooplankton distribution in Comau Fjord.

3.3. Materials & Methods

Study site

This study was conducted in Comau Fjord, Northern Patagonia, Chile (Fig. 3.1). The fjord is 45 km long and 2-8.5 km wide, with a maximum depth of approx. 490 m in the center. The mouth connects to the shallower Inner Sea of Chiloé via the Ancud Gulf and to the Hornopirén Channel across the Cholgo Channel. The fjord has two main inflows of freshwater, one in the south by the Vodudahue and Leptepu rivers, and two in the eastern side by the river catchments of the Quintupeu and Cahuelmó Fjords and the Loncochaigua river. The freshwater inputs drive an estuarine circulation in which the seaward-flowing shallow brackish water mass (freshwater coming from precipitation, river runoffs due to glacier melt and rainfall, among others) is compensated by an opposing flow of deep high saline Modified Subantarctic Water (MSAAW) (Silva et al., 1997; Dávila et al., 2002; González et al., 2010). Changes in solar radiation (high in summer), precipitation (>5000 mm/year), river runoff, glacial melt, and tidal mixing (Pantoja et al., 2011) result in a much higher environmental variability in the surface layer than in deeper waters (Garcia-Herrera et al., 2022). As a result, stratification, light and nutrients govern the biological productivity near the surface (Palma, 2008).

Sample collection

Zooplankton samples were collected in austral summer and winter (January and August) of 2017, during day and night, at five stations along an oceanographic section through Comau Fjord and Ancud Gulf (Fig. 3.1, Table 3.1). Stratified vertical hauls were carried out with a 70 cm-diameter Nansen closing net (mesh size: 100 µm) equipped with a non-filtering cod-end, from the surface to 450 m depth, or to near the bottom at shallower sampling stations. The net was closed by a messenger at depth intervals of 0-50-100-200-300-400-450 m, or shallower, respectively. After collection, samples were sieved through a 50 µm plankton sieve and stored in Kautex vials filled with seawater and 4% buffered formalin until further laboratory analysis. In the open waters of Ancud Gulf, strong wind drift of the boat prevented the execution of vertical hauls to the seafloor on one occasion (summer night), causing an oblique haul down to 200 m (see below). At every zooplankton sampling occasion, a CTD multi-probe (SBE 19plusV2 Profiler - with RS 232 Interface, Sea-Bird Electronics Inc.) was deployed from the sea surface to the bottom, measuring density, salinity, temperature, oxygen, pH and chlorophyll *a* fluorescence (Fig. 3.2).

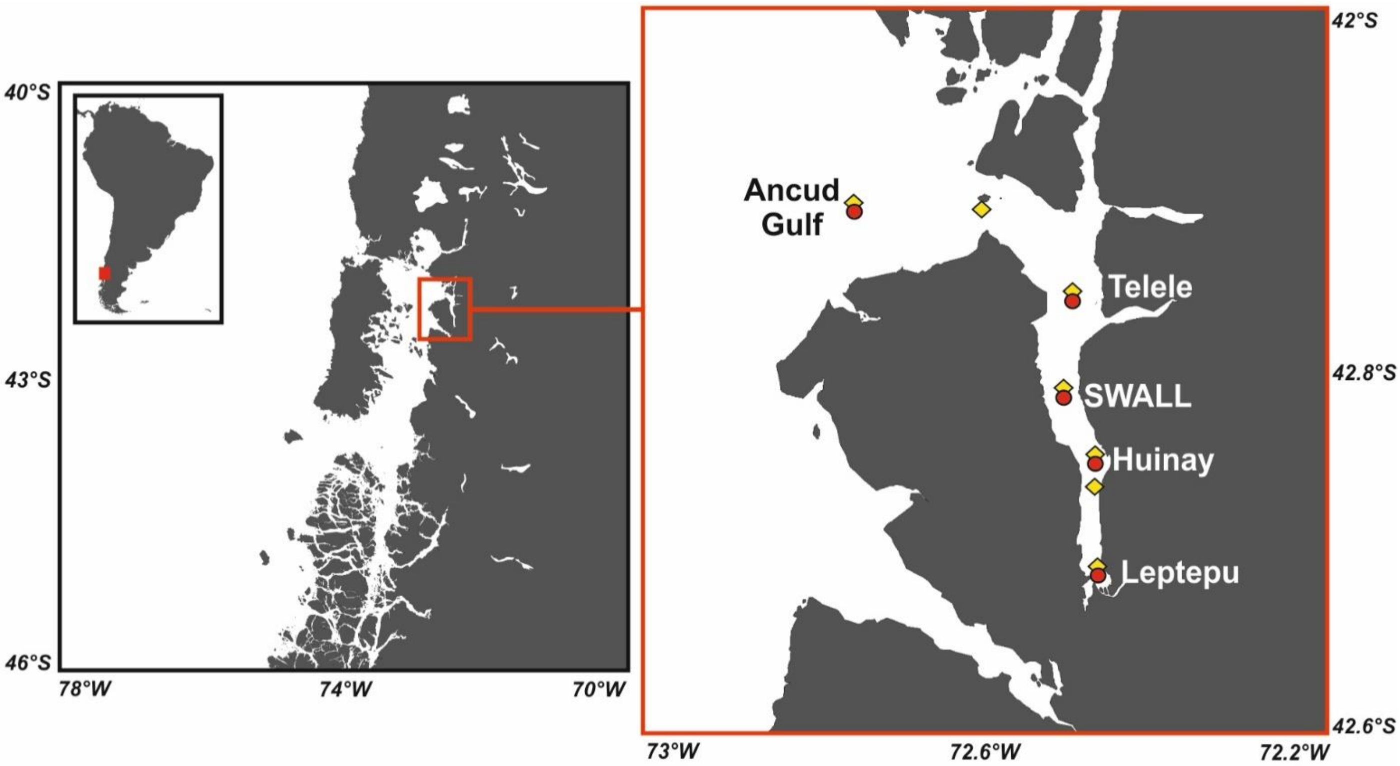

Fig. 3.1 Study site including the five zooplankton sampling stations (red circles) and seven hydrographic sampling stations (yellow diamonds) along Comau Fjord and the Ancud Gulf in Northern Chilean Patagonia.

Table 3.1 Metadata of sampling collection for all five zooplankton sampling stations along the fjord transect.

Station	Geographical Position (Latitude/Longitude)	Fjord position	Bottom depth	Haul depth	Season (date)	Diel (time)	Remarks
Ancud Gulf	-42.17297, -72.73635	Outside fjord	296 m	200 m	Summer (Jan 21st – 22nd 2017)	Day (12:40 – 13:44) Night (00:50 – 02:07)	Rope angle 45°
					Winter (Aug 4th – 5th 2017)	Day (14:49 – 16:15) Night (23:09 – 00:00)	
Telele	-42.24917, -72.48056	Outer fjord	485 m	450 m	Summer (Jan 17th – 18th 2017)	Day (12:00 – 15:10) Night (23:50 – 03:30)	
					Winter (Aug 9th – 11th 2017)	Day (12:06 – 15:31) Night (00:14 – 03:20)	
SWALL	-42.33102, -72.48082	Central fjord	465 m	450 m	Summer (14th – 15th 2017)	Day (12:19 – 15:18) Night (23:39 – 02:32)	
					Winter (Aug 6th – 7th 2017)	Day (11:31 – 15:46) Night (21:53 – 00:44)	
Huinay	-42.38745, -72.43662	Central fjord	417 m	400 m	Summer (Jan 12th – 13th 2017)	Day (12:07 – 15:05) Night (23:19 – 01:34)	
					Winter (Jul 28th – 29th 2017)	Day (11:02 – 13:15) Night (23:19 – 02:06)	
Leptepu	-42.48293, -72.43413	Inner fjord	108 m	100 m	Summer (Jan 11th – 12th 2017)	Day (12:21 – 12:43) Night (23:12 – 00:05)	
					Winter (Jul 24th – 25th 2017)	Day (11:54 – 12:32) Night (21:04 – 21:47)	

Mesozooplankton size measurements

In the laboratory, the samples were rinsed with freshwater and subdivided with a Folsom plankton splitter to reduce the number of organisms (minimum fraction 1/128th of the original sample) to a maximum of approximately 1500 individuals per fraction. The resulting subsamples were scanned with a ZooScan digital imaging system (CNRS patent, www.hydroptic.com), a waterproof scanner yielding images with a 2400-dpi resolution (14200x22700 pixels). Prior to scanning, individuals were manually separated to ensure an even distribution of the organisms and thus minimize the risk of overlapping. The scans were then processed with the software ZooProcess (Gorsky et al., 2010), a plugin of the image processing and analysis software ImageJ (Schneider et al., 2012). The image acquisition provides a high-quality raw image associated to the metadata in a full grey scale range, and from this, images (so called "vignettes") ideally showing only single objects were extracted. However, due to the small size of certain zooplankton organisms or occasional high amounts of detritus, in some cases overlapping individuals were detected in the same vignette and individual objects had to be manually separated using the Zooprocess "separation with mask" tool. Separated vignettes were saved, while vignettes with multiple organisms were deleted to avoid duplicate counts. Vignettes in which manual separation was not possible were grouped as "multiples" and then counted manually. Overall, the number of multiples did not exceed 10% of the total amount of vignettes per sample. The hinged base of the ZooScan allowed to recover and recollect the undamaged subsamples which, together with the rest of the sample, were later stored in 70% ethanol for archiving.

Vignettes were uploaded and identified by the semi-automated taxonomic classification in Ecotaxa 2.6 (Picheral et al., 2017). This web-based machine learning uses training sets and two-way random forest algorithms to automatically classify the objects on the vignettes. After the automatic classification, all objects were manually validated or reassigned to the correct category.

Zooplankton abundance was calculated based on the number of organisms per taxonomic category and the volume filtered through the net (V_N). V_N was calculated as:

$$V_N\ (m^3) = \left[\pi\ \times \left[\frac{net\ diameter\ (m)}{2}\right]^2\right] \times\ depth\ interval\ (m) \times\ filtration\ efficiency/drift$$

where filtration efficiency was assumed to be a theoretical 100% efficiency (value = 1). Current drift (drift) was assessed with a mechanical flowmeter, showing a linear response between rotor rotation and current velocity >0.3 m s^{-1}, and a non-linear response below the 0.3 m s^{-1} threshold.

Vertical hauls were conducted at <0.3 m s^{-1} winch speed, so the flow-meter readings fell within the non-linear range of the instrument and were thus not included in the calculation of the filtered volume under calm weather conditions when the towing rope angle was vertical. On one occasion (see above), we faced windy conditions (Ancud Gulf, summer, night) causing the boat to drift, resulting in a significant departure in the towing rope angle from vertical to 45°, i.e. an oblique haul with higher volume filtered. Winch speed and drift pushed current speeds above the 0.3 m s^{-1} threshold, resulting in a linear response of the flow-meter with accurate filtered volume readings. We used the filtered volume measured by the flowmeter to correct for the theoretical filtered volume due to drift.

The metadata of each vignette provide the length (major axis) and width (minor axis) of each individual. This allows to calculate body sizes (in equivalent spherical diameter, ESD) by fitting an ellipse around each object, computed as:

$$ESD\ (mm) = 2 * \sqrt{\frac{(major\ axis\ (mm)\ *\ minor\ axis\ (mm)}{\pi}}$$

Organisms with an ESD below the ZooScan standard detection limit of 300 µm were not considered in this study. Overall, the ESD of the objects found in the samples ranged from 0.3 to 30.66 mm.

The volume of the object can be considered as a proxy for zooplankton biomass (Gorsky et al., 2010). Therefore, the volume (V) of each object was computed as described in Garcia-Herrera et al. (2022):

$$V\ (mm^3) = \frac{4}{3} \times \pi \times \frac{major\ axis\ (mm)}{2} \times \left(\frac{minor\ axis\ (mm)}{2}\right)^2$$

Biovolume (BV) was then calculated as the sum of the volumes of all objects (ΣV) divided by the fraction of the sample (e.g. F=1/64) and by the volume filtered by the Nansen net (V_N):

$$BV\ (mm^3/m^3) = \frac{\left[\frac{\sum V\ (mm^3)}{F}\right]}{V_N\ (m^3)}$$

When two or more objects from different taxa overlapped, it was not possible to automatically calculate the biovolume of each one. Therefore, the objects were counted manually and their biovolume was estimated by multiplying the mean volume of the respective taxon by the number of multiple vignettes on which the taxon occurred. There were three groups with large size variability (chaetognaths, cnidarians and Euchaetidae among the calanoid copepods), which had to be divided into two categories: small (0.003-4.242 mm^3) and large (4.242-90.083 mm^3) in order to obtain a better representation of their biovolume.

Mesozooplankton community structure

The zooplankton community structure was determined using taxonomic composition, total abundance and total biovolume (ind. m^{-3} and mm^3 m^{-3}, respectively). The relative abundance and biovolume (%) and diversity of the zooplankton community were also calculated to assess different biological traits. To predict taxonomic categories in EcoTaxa, the training sets used in Garcia-Herrera et al. (2022) were applied. Of the total 114,141 vignettes, 39,761 (35%) were identified as non-zooplankton objects, labelled as “artefact”, “bubble”, “detritus”, “feces”, “fiber”, “leg” or “other”, and not considered in our analyses. Seven per cent of all images (i.e. 11,161 vignettes) were out of focus and did not allow to discriminate between zooplankton and non-zooplankton objects so that they were excluded from analyses. To account for the non-analyzed zooplankton, we applied a correction based on the vignettes that were in focus, assuming no systematic differences in zooplankton and particle composition between the vignettes that were in and out of focus, i.e. a proportion of zooplankton (69 ± 10%) and non-zooplankton objects (31 ± 10%) in both cases. In non-copepod zooplankton, the general level of identification was class/order, while in copepods it was the family level. Calanoid copepods that could not be distinguished at family level, were grouped as “Calanoida” (size >1 mm) and “copepodites” (size <1 mm), the developmental stages of calanoid copepods. The category “Cnidaria” included organisms from the class Hydrozoa (i.e. Medusae and Siphonophorae). “Meroplankton” included larval stages from the taxa Ascidiacea, Bivalvia, Bryozoa, Echinodermata, Gastropoda, Nemertea, Phoronida, Platyhelminthes, and Polychaeta, as well as unidentified larvae.

Taxonomic richness (R’) was assessed at all stations by the number of higher taxonomic groups within the zooplankton community and diversity by using Pielou’s eveness based on standardized density data (abundance, ind. m^{-3}) of the different zooplankton groups. Each diversity index reflects a different aspect (dominance and evenness). The Shannon’s diversity index (H’) considers the relative abundance of each taxonomic group in a sample and is calculated as $H' = -\sum p_i * \ln(p_i)$, where p_i is the proportion of species i in the entire community N, calculated as $P_i = n_i/N$, where n_i is the abundance of species i. It ranges between 1.5 (low diversity) and 3.5 (high diversity) and increases with species richness (Magurran, 2013). Based on Shannon’s diversity index, Pielou's evenness (*J'*) describes how abundances are distributed in the community. A community in which the individuals of the different species are equally distributed is considered even. Pielou's evenness was calculated as $J' = H'/\ln(S)$, where S is the total number of species. J' ranges from 0 (uneven) to 1 (even).

Statistical analysis

Prior to the statistical analysis, abundances were fourth-root transformed to reduce the effect of highly dominant taxonomic groups. A Permutational Multivariate Analysis of Variance (PERMANOVA) and post hoc corrected $p > 0.05$ using distance matrices and permutation tests was used to discriminate between factors, which may have been a source of variation in fitting linear models (i.e. season, station, depth, day/night). Collinearity among environmental parameters was tested to avoid potential problems of overfitting and increased residual variation (Dormann et al., 2013). Therefore, Pearson correlation coefficients were calculated for temperature, salinity, oxygen and fluorescence (<0.8), and included in the analysis. The relationship between environmental parameters and zooplankton abundances was analyzed using a redundancy analysis (RDA) (Fig. 3.8). RDA is a constrained ordination procedure that analyzes the impact of explanatory variables (i.e. environmental parameters) on response variables (i.e. zooplankton abundance). The RDA is a multi-response analysis in which the linear relationship among dependent and independent variables is summarized in a matrix, followed by a principal component analysis (PCA). In the RDA plot, only taxonomic groups that contributed >3% to total abundance or biovolume were displayed. In order to test the significance of the RDA results, a permutation test (ANOVA) for redundancy analysis was performed, which minimizes the problems caused by non-normal distributions (Borcard et al., 2011). Abundance and biovolume calculations were performed in R (R Core Team, 2021), statistical analyses were performed with the *vegan* package (Oksanen et al., 2019) and plots were displayed with the *ggplot2* package (Wickham, 2016).

3.4. Results & Discussion

Hydrographic conditions of Comau Fjord and Ancud Gulf

Seasonal variations in salinity, temperature, oxygen, and Chl *a* between summer and winter were the strongest in the shallow surface layer (0-50 m), while the remaining deeper water column was less variable (Figs. 3.2, 3.S1). Generally, the biological and physico-chemical parameters showed greater variability in summer than in winter. For instance, the temperature profile in summer ranged from a maximum temperature of 16.8 °C at the surface and an average of 11.2 ± 0.1 °C below the thermocline (25 m depth) (Figs. 3.2, 3.S1). In winter, however, temperature averaged 11.5 ± 0.4 °C for the entire water column with a thermal inversion in the surface layers, likely due to mixing caused by winds, atmospheric cooling and/or cold terrestrial runoff (Silva et al., 1997). Salinity was very variable in the upper first meter of the water column causing a strong horizontal gradient in summer (from 2 at Leptepu to 24 in the Ancud Gulf). The lowest salinity observed near Leptepu reflects the high freshwater input by the Vodudahue and Leptepu rivers (Sánchez et al., 2011) as a result of glacier melt during summer and this intensified run-off. In winter salinity was lower in the central fjord stations (Huinay and Telele, salinity = 17) than at Leptepu (salinity = 19) (Fig. 3.S1) likely due to an increment in the fluvial flow due to precipitation (Gonzalez et al., 2010) by the river catchments of the Quintupeu and Cahuelmó Fjords and the Loncochaigua river (near Telele and Huinay, respectively). A strong halocline was evident at 15 m water depth in summer and a weaker one at about 8 m in winter, except for Ancud Gulf in winter, where salinity was 32.6 ± 0.4 (mean ± sd) throughout the water column (Figs. 3.2, 3.S1), likely due to mixing in the water column. Below the halocline, salinity reached 30 at all stations, indicating the presence of Modified Subantarctic Water (MSAAW) (Silva et al., 1998; Palma and Silva, 2004; Pantoja et al., 2011), and below the halocline to the seabed, it varied little (average 33.0 ± 0.1 in summer and 32.6 ± 0.4 in winter) (Figs. 3.2, 3.S1). Oxygen concentration was higher at the Ancud Gulf and Telele and gradually decreased towards Leptepu, indicating the horizontal advection of adjacent higher oxygenated oceanic waters (Silva and Vargas, 2014). Maximum values (324.2-361.2 µmol kg^{-1}) between 10-20 m depth in summer and in the top 10 m in winter (264.4 ± 24 µmol kg^{-1}) (Figs. 3.2, 3.S1) were observed, while lowest values were found in the inner and deeper parts of the fjord in both summer and winter (Figs. 3.2, 3.S1). In the course of the estuarine MSAAW counterflow towards the fjord head (Leptepu), the respiration of allochthonous and autochthonous particulate organic matter takes up most of the oxygen dissolved in the water column reaching near-hypoxic levels (Silva and Vargas, 2014). In summer, Chlorophyll *a* (Chl *a*) concentration was the highest (12.5-19.6 mg m^{-3}) between 8-20 m depth (Fig. 3.S1), except for the station at Leptepu where the maximum Chl *a* concentration was only 4 mg m^{-3} at 12 m.

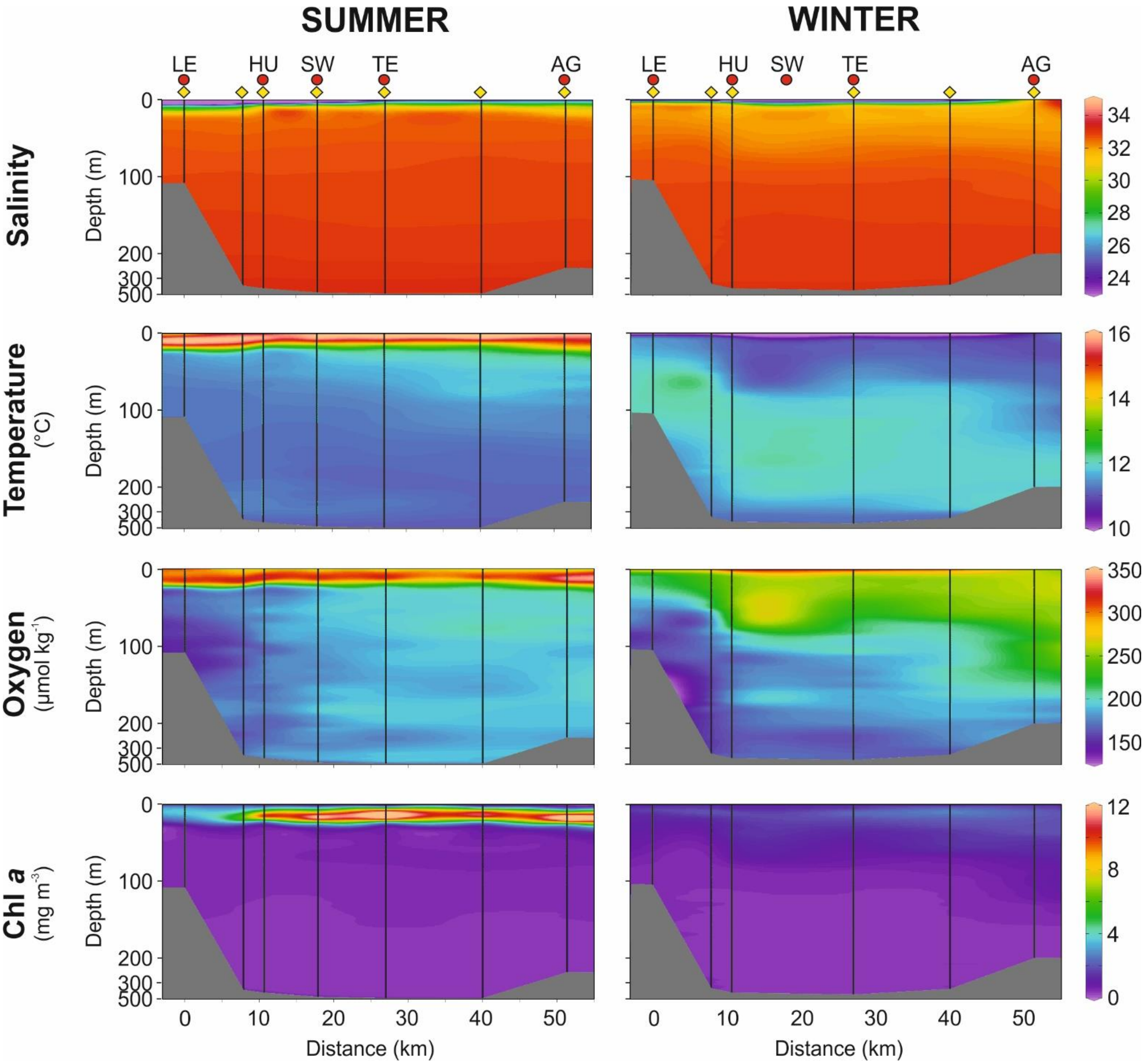

Fig. 3.2 Hydrographic sections for the entire water column along the south-north axis of Comau Fjord and Ancud Gulf. The distances on the x-axis refer to the southern station (0 corresponds to Leptepu station near the head of the fjord). Red dot and yellow rhombi define the locations of the stations where zooplankton and hydrography data were collected, respectively. Station names: (LE) Leptepu, (HU) Huinay, (SW) SWALL, (TE) Telele, (AG) Ancud Gulf. Figures created using Ocean Data View (https://odv.awi.de) with DIVA gridding.

Zooplankton seasonal and spatial dynamics

Throughout the water column, zooplankton abundance and biovolume showed significant seasonal differences ($p < 0.05$) (Fig. 3.3). Overall, integrated abundance and biovolume were about 2 and 3 times higher in summer than in winter, respectively. Higher water temperatures, stratification and nutrient input from river runoff and glacial melt provide perfect conditions for diatom-blooms (Pizarro et al., 2000; Iriarte et al., 2007; González et al., 2010), which are later grazed by zooplankton (González et al., 2010; Garcia-Herrera et al., 2022). The increase in solar radiation in summer re-established the thermal stratification in the upper 25 m, providing the basis for the vernal phytoplankton bloom (Chl *a* maximum, Fig. 3.2), leading to a subsequent peak in zooplankton abundance and biomass. In Comau Fjord, the decrease in zooplankton abundance and biovolume in winter was associated with low Chl *a* concentration, with highest values even <3.0 mg m^{-3} (6-12 m) (Fig. 3.2). It is likely that the reduction of light in winter resulted in lower phytoplankton abundance reflected by low Chl *a* concentrations (González et al., 2010). In turn, the latter does not necessarily support high zooplankton abundance and biomass (Escribano et al., 2007; Garcia-Herrera et al., 2022), as food for primary consumers may be limited.

Overall, we found higher zooplankton biovolumes seawards in summer, with significant differences among stations ($p < 0.05$). The abundance of zooplankton along the fjord did not vary a lot but the integrated biovolume in the Ancud Gulf and at Telele was higher than at Leptepu, indicating that larger zooplankton groups may inhabit the outer fjord and the Ancud Gulf (Fig. 3.3B). The high zooplankton abundance and biovolume at the outer station of the fjord (Telele) and in the Ancud Gulf in summer night was associated with higher Chl *a* concentrations, the maximum values of which were also found in these stations (Figs. 3.2, 3.S1), suggesting that zooplankton profited from the rich availability of food (Folt and Burns, 1999). Higher mean differences between day and night integrated biovolume values were generally observed in the Ancud Gulf and at Telele (Figs. 3.3B, 3.4), where bottom and sampling depths differed the most (delta-depths: Ancud Gulf = 96 m; Telele = 35 m; SWALL = 15 m; Huinay = 17 m; Leptepu = 8 m). Some of the daily migrating zooplankton that resided beneath the sampling depth during the daytime may have been missed, causing an underestimation of the daytime integrated values. Support for this possibility is given in Fig. 3.S2, where we plot the abundance and biovolume residuals as a function of the depth residuals, explaining up to 68% of the observed variability. The correlation was stronger for biovolume than for abundance, and stronger in summer than in winter (Fig. 3.S2). Our taxonomic analysis supported this observation, as we identified that diel vertical migration (DVM) was mostly performed by large taxa (see more details at DVM section

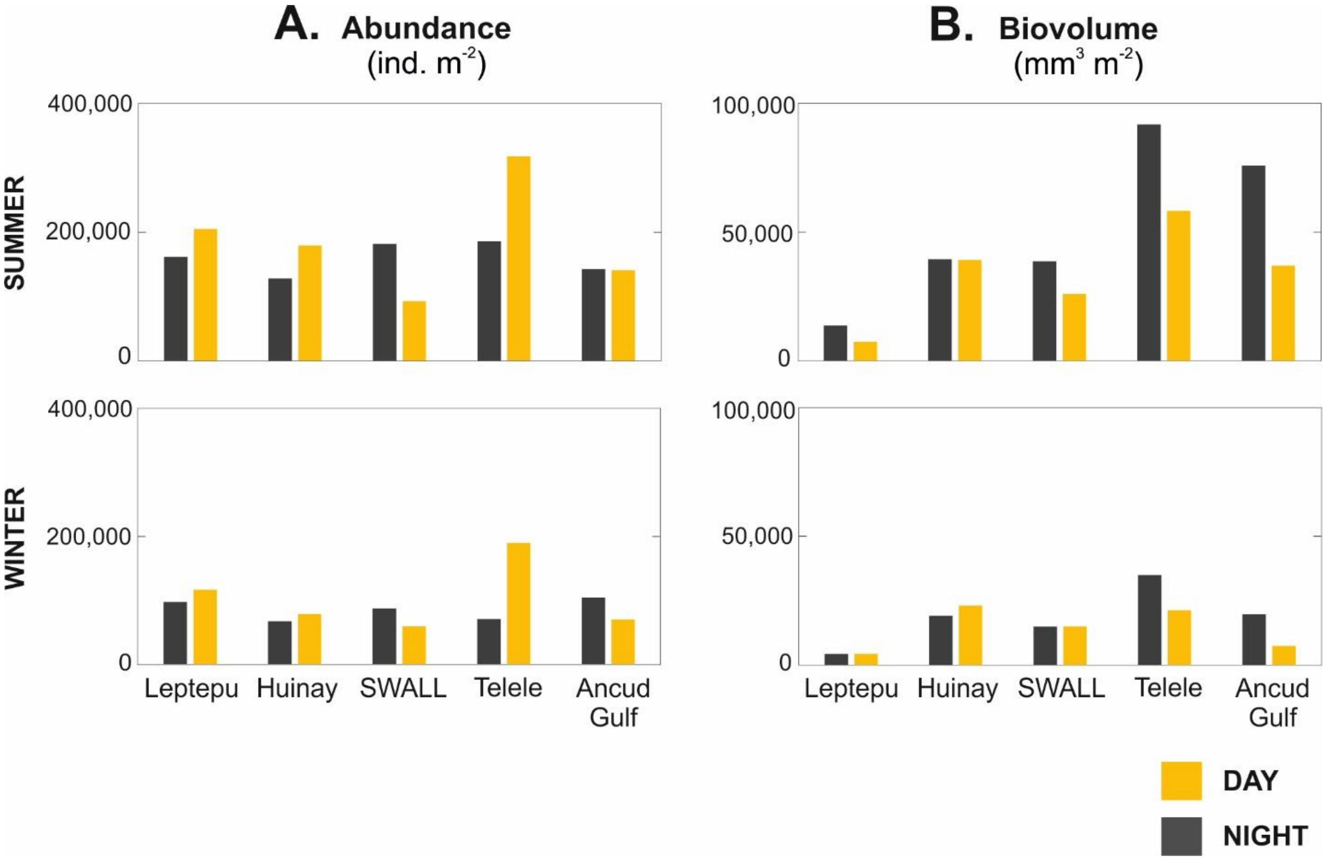

Fig. 3.3 Spatial and diel integrated abundance (ind. m^{-2}) (A) and biovolume (mm^3 m^{-2}) (B) of zooplankton throughout the whole water column (0-450 m) along a section through Comau Fjord to the Ancud Gulf in summer and winter. Note that each station has a different bottom depth (Leptepu - 100 m; Huinay - 400 m; SWALL and Telele - 450 m; Ancud Gulf - 200 m).

below). Larger zooplankton were therefore probably not caught during the day due to the residence of these organisms below the sampling depth, and at night, when they migrated upwards to surface waters, they were quantitatively caught and represented in the night biovolume.

The patterns of integrated abundance throughout the water column (Fig. 3.3A) and in the upper 100 m (comparable depth between all stations - Fig. 3.S3A) were similar, since the zooplankton distribution was depth related ($p < 0.05$). Most of the zooplankton concentrated in the top 0-50 m water depth, generally decreasing with depth (Fig. 3.4), often displaying the intermediate minimum at 50-100 m already noticed in Garcia-Herrera (2022). In Comau Fjord and Ancud Gulf, the highest abundances of zooplankton were found in surface waters during day

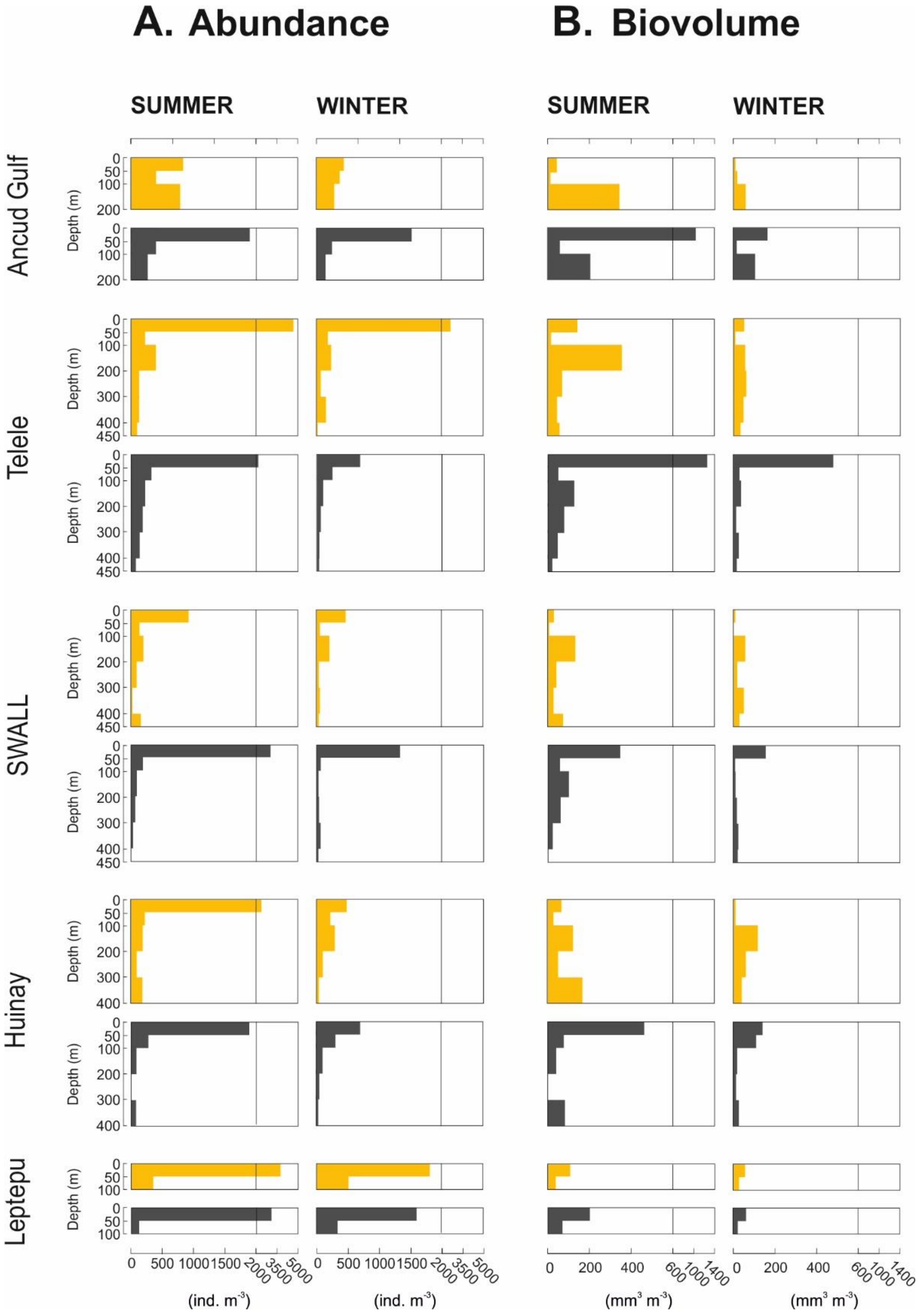

Fig. 3.4 Vertical distribution of the total zooplankton community abundance (ind. mm^{-3}) and biovolume (mm^3 m^{-3}) along a section through Comau Fjord to the Ancud Gulf. Note the break as a black line inside the charts. Yellow and black bars indicate day and night values, respectively.

and night, associated with the maximum phytoplankton biomass, most obvious in summer (Fig. 3.2). But the highest biovolumes were observed below 100 m depth during the day in summer, and mostly within 0-50 m at night in summer (Fig. 3.4B), indicating that vertical migration took place in part of the zooplankton community, mostly the larger zooplankton groups (Fig. 3.S4). No such pattern was observed at Leptepu, where abundance and biovolume were both concentrated in the upper 50 m of the water column. According to Hays et al. (2001), body condition can determine whether certain individuals perform diel vertical migration or not based on the energy reserves. If their energy reserves are filled, they might behave different from their own community and stay in deeper waters to avoid predation, especially in environments where planktivorous fish are abundant (Pearre, 1979; Hays et al., 2001). However, if their energy reserves are emptied, they would be forced to overcome the risk of migrating to surface waters (Sekino and Yamamura, 1999).

Community structure

Overall, the zooplankton community was primarily composed of copepods in terms of abundance and of amphipods, chaetognaths, mysids and gelatinous carnivores (i.e. medusae and siphonophores) in terms of biovolume. Similar dominant taxa have been found in other areas of northern Chilean Patagonia (Palma and Silva, 2004; Villenas et al., 2009; Díaz-Astudillo et al., 2017). However, the taxonomic composition along the fjord section was significantly different (p <0.005) between the shallow (Ancud Gulf and Leptepu) and the deep stations (Telele, SWALL, and Huinay). At the central fjord stations (SWALL and Huinay), a higher number of taxonomic groups, especially among larger copepods, was detected in summer during both day and night (Fig. 3.5), suggesting that biodiversity along the oceanographic section was related to depth. When accounting for the relative abundance of each taxonomic group, Pielou's evenness showed that the zooplankton community at Huinay had higher evenness in summer and at Leptepu in winter. During night, Huinay had the most even community along the fjord and SWALL during day.

In general, copepods (calanoids, cyclopoids and harpacticoids) dominated the zooplankton community, with seasonal and horizontal differences in the relative contribution of copepods vs. other zooplankton (Fig. 3.6). The relative abundance of copepods was higher in summer (77 ± 11%), especially in the shallow stations (Leptepu and Ancud Gulf, 87 ± 12%) than in winter (66 ± 9%), with a clear dominance of calanoid copepods (84%) over cyclopoids (12%) and harpacticoids (4%). In winter, calanoid abundances decreased (53 ± 10%) and cyclopoid (37 ± 8%) and harpacticoid (10 ± 5%) abundances increased, suggesting a second spawning season during late summer/autumn (Dvoretsky and Dvoretsky, 2009).

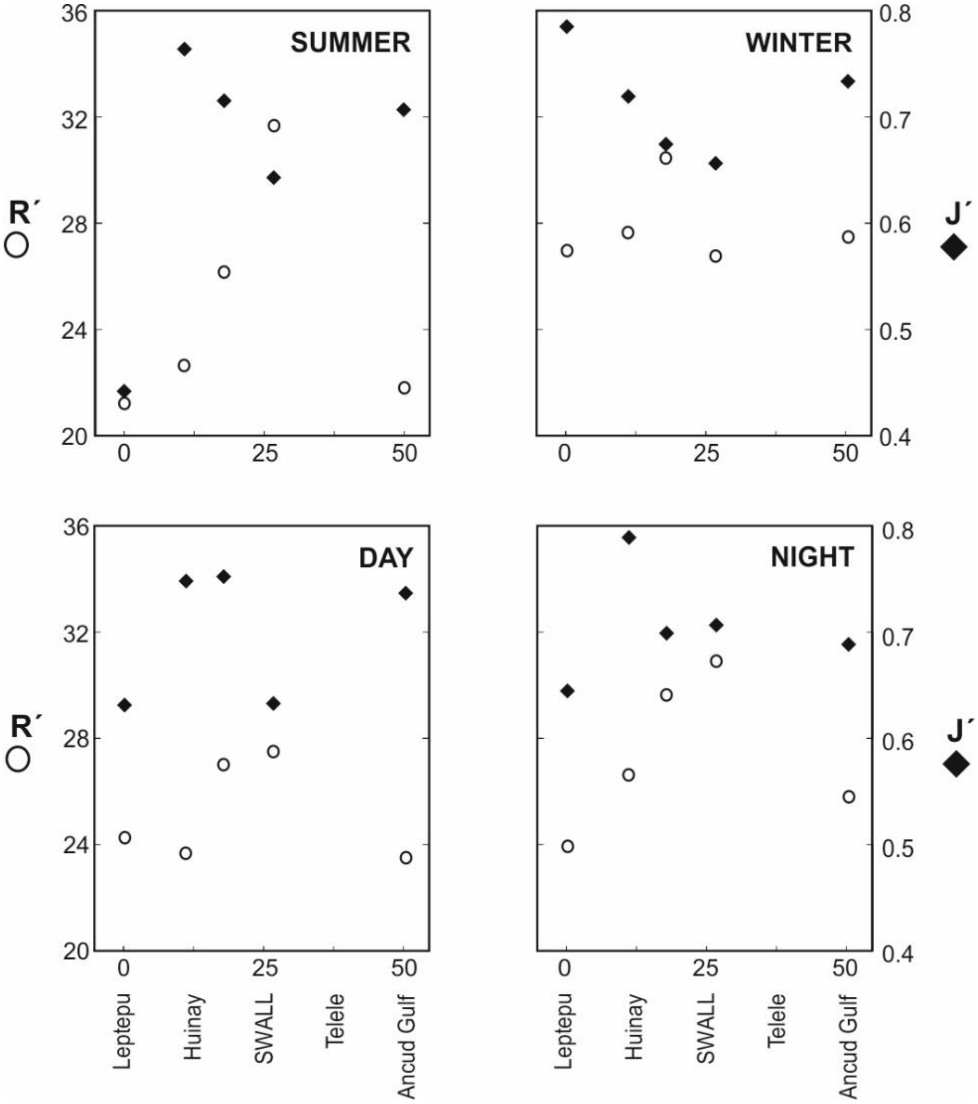

Fig. 3.5 Zooplankton diversity indices for the upper 200 m of the water column along a section through Comau Fjord to the Ancud Gulf. Taxonomic richness (R’) and Pielou’s evenness (J’) is represented for all stations along the fjord during summer and winter, day and night.

While in the deep fjord stations calanoids dominated the copepod community in both summer and winter (55 ± 13% and 60 ± 11%, respectively), harpacticoid copepods played a more important role (23 ± 10% in summer and 19 ± 8% in winter) than in the shallow stations (7 ± 4%). The relative biovolume of copepods was dominated by calanoid copepods, while the relative

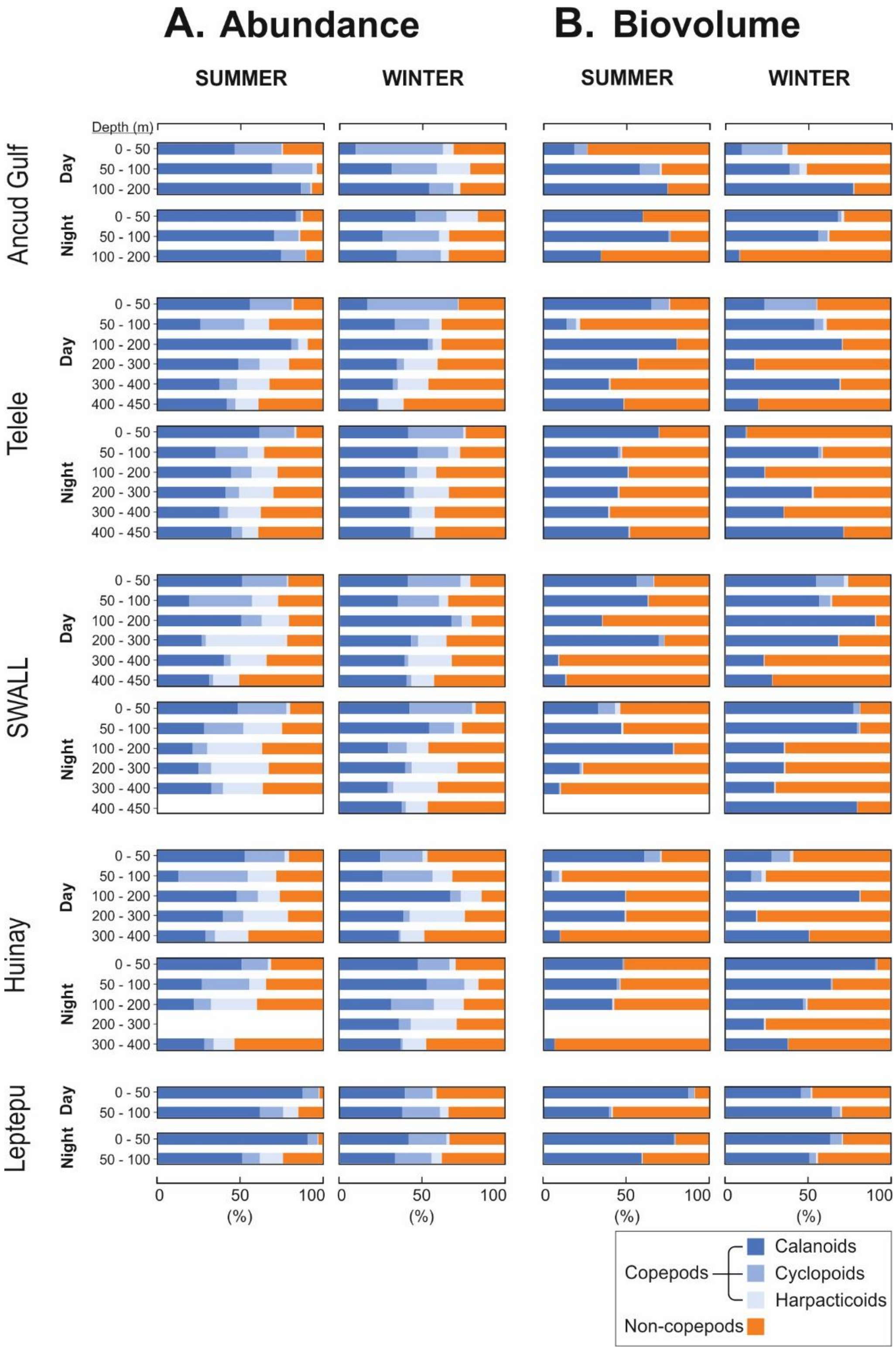

Fig. 3.6 Diel and vertical relative abundances and biovolumes of copepods (dark to light blue) and non-copepod (orange) zooplankton along a transect through Comau Fjord to the Ancud Gulf in summer and winter. Missing bars (SWALL: summer, night, 400-450 m; Huinay: summer, night, 200-300 m) indicate lacking samples

contribution of cyclopoids or harpacticoids was much lower (6% and 1% respectively) due to their small size. Calanoid copepods represented an important fraction of the zooplankton community, however, the relative contribution of biovolume from larger non-copepod zooplankton was higher compared to the abundances, reaching at Huinay maximum values of >90% in subsurface waters (50-100 m, day) and the deep layers (>300 m, day and night) in summer (Fig. 3.6). The relative contribution and distribution of both calanoid copepods and non-copepod zooplankton differed through the water column and along the fjord (Figs. 3.6, 3.7, 3.8). Generally, small zooplankton (copepodites, Clausocalanidae, Paracalanidae, small cnidarians, meroplankton and appendicularians) was relatively more abundant in surface waters. For instance, in shallower waters (0-100 m), the presence of small calanoids became very important, contributing 88 ± 9% of the total calanoid community during the day and 65 ± 18% at night. The calanoid families Acartiidae, Eucalanidae, Euchaetidae, Heterorhabdidae, Pontellidae and Rhincalanidae were only present in the deeper central fjord stations, especially in deep waters (>200 m).

The abundance of the non-copepod zooplankton community was mainly dominated by small zooplankton and ostracods (Fig. 3.8). Small zooplankton (i.e. crustacean larvae and meroplankton) accounted for 50 ± 12% of the total non-copepod zooplankton community in summer and 61 ± 13% in winter (Fig. 3.8). Ostracods were also an important contributor to the abundance in the deeper fjord's stations (Telele, SWALL and Huinay), usually associated with greater depths (100-400 m), except for the 50-100 m layer of Leptepu in summer (47 ± 18%). Similar findings were observed for sub-Antarctic waters, where the highest abundances of ostracods were found between 300-400 m water depth (Chavtur and Kruk, 2003).

Biovolume reflected larger individuals, and accordingly higher abundance and biovolume of larger calanoids (e.g. Calanidae, Euchaetidae or Rhincalanidae), amphipods, chaetognaths, cnidarians, euphausiids and mysids dominated here. Within the copepod community, Metridinidae and Calanidae played an important role, both in terms of abundance and biovolume (Fig. 3.7). For both taxa, abundance and biovolume were always higher in the Ancud Gulf and near the entrance of Comau Fjord (Telele) in summer than in winter, while the seasonal differences in the central and inner stations of Comau Fjord (i.e. SWALL, Huinay and Leptepu) were less obvious (Fig. 3.S5). Within the non-copepod zooplankton community, chaetognaths and cnidarians were present throughout the water column at each station, reaching relative biovolumes of 66 ± 7% in summer and 44 ± 14% in winter. Euphausiids contributed, albeit caught in low numbers, to a large proportion of the biovolume (59 ± 24%), both at the central deeper stations and in the Ancud Gulf.

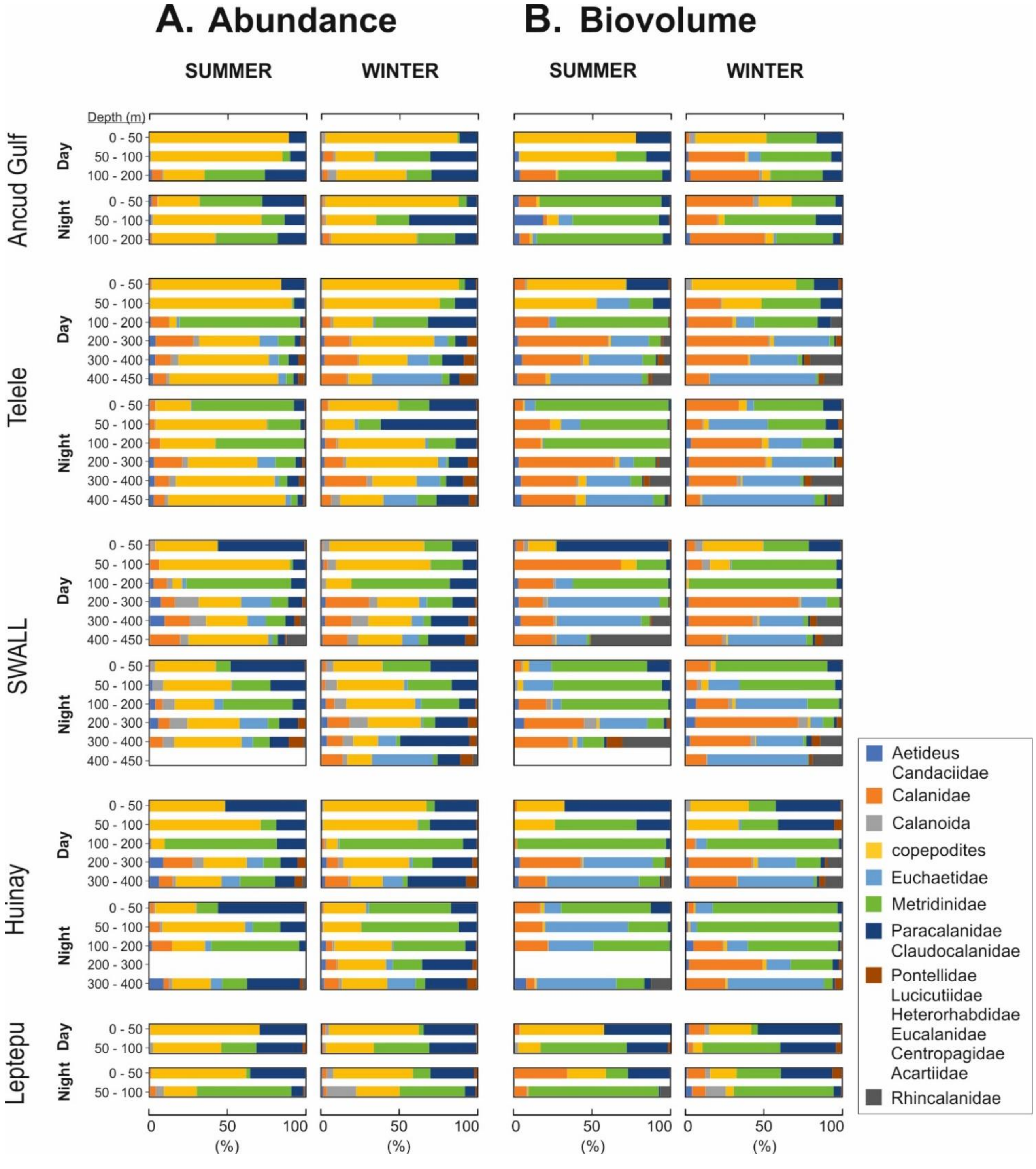

Fig. 3.7 Diel and vertical relative abundances and biovolumes for the calanoid copepod community along a section through Comau Fjord to the Ancud Gulf in summer and winter. Missing bars (SWALL: summer, night, 400-450 m; Huinay: summer, night, 200-300 m) indicate lacking samples

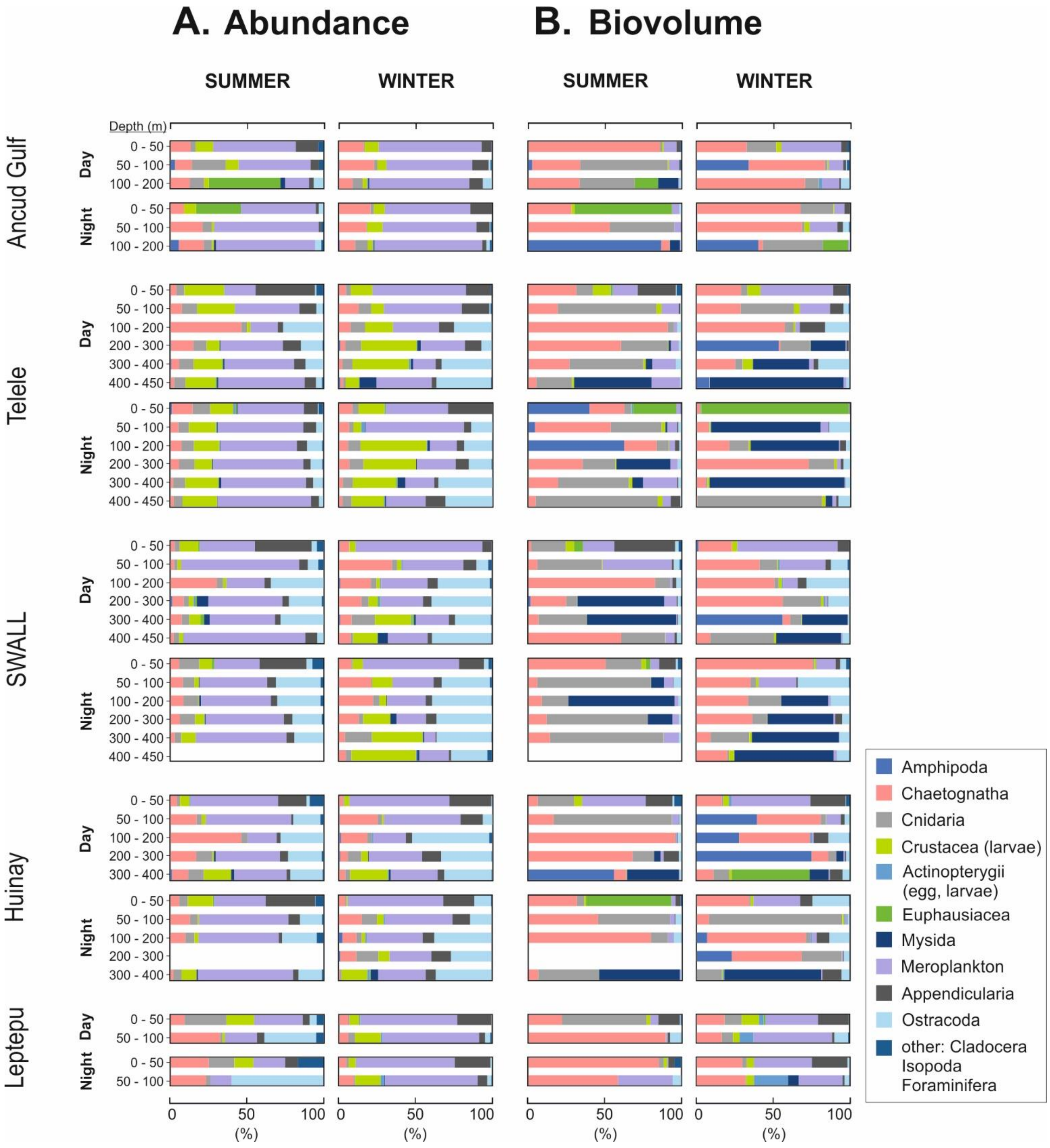

Fig. 3.8 Diel and vertical relative abundances and biovolumes for the non-copepod zooplankton community along a section through Comau Fjord to the Ancud Gulf in summer and winter. Missing bars (SWALL: summer, night, 400-450 m; Huinay: summer, night, 200-300 m) indicate lacking samples

In Patagonian waters, euphausiids are generally important contributors to the biomass of the zooplankton community (Palma and Silva, 2004; Valle-Levinson et al., 2014; Díaz-Astudillo et al., 2017). It is likely that euphausiids were not quantitatively caught by our 70 cm diameter net, owing to their distribution in swarms, and their ability to avoid slowly tawed nets (Brinton, 1962). Mysids were mainly found in the central fjord's stations >200 m water depth, both in summer and winter and during daytime and nighttime. Amphipods reached relative biovolumes of up to 87% (Ancud Gulf, 100-200 m, night) of the total non-copepod zooplankton community.

Diel Vertical Migration (DVM)

Diel Vertical Migration (DVM), performed by a large number of taxa is among the most important biological drivers of zooplankton distribution (Folt and Burns, 1999). Migrant zooplankton typically reside in deep, dark waters during the day to minimize the risk of encountering visually orientated predators. At night, they migrate upwards to nutrient- and phytoplankton-rich areas, where they find safer feeding conditions than during daytime (Stich and Lampert, 1981; Iwasa, 1982; Hays et al., 2001). However, DVM is not performed by all individuals in the zooplankton community. It is more pronounced in large taxa due to their generally greater swimming capabilities, but also their higher risk of falling prey to visual predators such as fish (Hays, 1995). In our study, diel differences in biovolume indicated that DVM of larger zooplankton such as euphausiids, chaetognaths or large copepods (higher biovolume but lower abundance) occurred (Fig. 3.4B) when they migrate upwards in search for nutrient-rich areas. Large herbivore zooplankton probably followed the high Chl *a* concentrations in surface waters at night (Marín and Delgado, 2009), while carnivorous zooplankton, such as amphipods, chaetognaths, cnidarians and the large calanoid copepod Euchaetidae were attracted to the herbivorous, generally smaller and surface-resident zooplankton (Palma and Kaiser, 1993; Villenas et al., 2009). In our study, DVM was mostly performed by the large calanoid copepods (Metridinidae, Calanidae, and less evident for the rarer Euchaetidae) (Figs. 3.7, 3.S6). Similar circadian migration patterns were observed for Reloncaví Fjord, Ancud Gulf (Valle-Levinson et al., 2014; Díaz-Astudillo et al., 2017) and Puyuhuapi Fjord and Jacaf Channel (Pérez-Santos et al., 2018). Most large non-copepod groups (Amphipoda, Chaetognatha, Cnidaria, Euphausiacea, and Mysida) were not quantitatively samples as seen in the lack of symmetry in the diel changes in vertical distribution between the surface and deeper layers (Figs. 3.8, 3.S7).

In Comau Fjord, we observed that certain zooplankton taxa performed DVM at every station in both seasons while others were only found at certain stations, likely due to their low number found in our nets. Among the most dominant copepods performing DVM, Metridinidae and Calanidae were present at each station and mostly across the entire depth range with high abundances and biovolumes. Metridinidae population moved from mid-waters (50-200 m) during daytime to shallow waters (0-50 m) at nighttime (Fig. 3.7). It was less evident in winter. It appears that the largest proportion of the migrating population was composed by large individuals. This can be seen in the vertical distribution of the size frequencies. We found that smaller individuals of Metridinidae (mean biovolume ± sd of the Metridinidae population from 0-50 m: 0.21 ± 0.19 mm^3) stayed in surface waters during the day, while larger individuals resided in deeper waters (0.53 ± 0.13 mm^3), and that at night part of the population with larger individuals (0.60 ± 0.18 mm^3) migrated upwards. A similar pattern was found for the copepod *Metridia longa* in the North Atlantic and the North Sea, where the daily residence time in surface waters was shorter for larger copepods, as they were mainly present at night (copepodite stages C5-C6 *M. longa*) (Hays, 1995). Calanidae and Euchaetidae typically resided deeper than Metridinidae (200-450 m) during the day, but they were also observed migrating upwards to surface waters (0-50 m) at night, albeit in lower numbers (Figs. 3.S6, 3.S7).

In the deeper, central part of the fjord, the DVM of Calanidae, Chaetognatha, Cnidaria and Euchaetidae generally extended throughout the whole water column (0-450 m), while DVM for Mysida was more limited (migration range 100-450 m). The depth restriction of Mysida performing DVM could be due to its low tolerance to a lower salinity (Miyashita and Calliari, 2016). At the shallow stations (Ancud Gulf and Leptepu), only Calanidae, Metridinidae, Chaetognatha, Cnidaria and Euphausiacea were observed carrying out DVM. However, the DVM of Euphausiacea seemed to be restricted in winter and they were observed in the Ancud Gulf in deeper layers (50-200 m) only at night.

Amphipoda were detected at each station, except Leptepu. Generally, they constitute one of the three most important crustacean groups in the zooplankton community and have a very large depth range, inhabiting the Magellan Strait down to 700 m depth (Palma and Kaiser, 1993; Guglielmo and Ianora, 1997). Our low amphipod abundances make it very difficult to estimate DVM patterns. However, it was observed that amphipods in Comau Fjord and Ancud Gulf had a depth range of >50 m during day and migrating upwards to surface layers (0-50 m) at Telele in summer at night.

Influence of environmental parameters on zooplankton distribution

In Comau Fjord, the inner part was characterized by high freshwater inputs and low oxygen levels in the deeper water column compared with the rest of the fjord (Fig. 3.2). The wide fluvial valley of the Vodudahue river, which flows into this shallow area may lead to increase erosion in the watershed and higher nutrient input and sedimentation in these coastal waters (Labbé-Ibáñez et al., 2015; Soto et al., 2018). High concentrations of terrigenous organic matter accumulate on the seafloor, developing low dissolved oxygen waters, a product of respiration and rapid decomposition of autochthonous particulate organic matter, which is a recurrent condition for the eastern Patagonian fjord and channel system (Silva and Vargas, 2014). Plankton distribution, community composition and life cycles are influenced by the oxygen concentration of the waters they live in (Ekau et al., 2010). While migrating large calanoid copepods are able to avoid hypoxic waters, non-migrating large copepods such as *Rhincalanus* (Schnack-Schiel et al., 2008) and small copepods, euphausiids and cnidarians appear to have a higher tolerance to low oxygen conditions (Gonzalez and Quiñones, 2002; Ekau et al., 2010; Hauss et al., 2016; Pérez-Santos et al., 2018). Most of the zooplankton found at Leptepu belonged to the smaller size fractions of the zooplankton community: small copepods such as Clausocalanidae, Paracalanidae and *Neocalanus* (Calanidae), meroplankton, appendicularians, ostracods and chaetognaths. In the shallow inner fjord (only ca. 100 m depth), the paucity of macrozooplankton in the bottom waters of Leptepu could reflect a low tolerance to oxygen-poor water (but quantitative data to support this statement are so far lacking) (Figs. 3.2, 3.9). Habitat compression was likely a limitation for these vertical migrants that cannot tolerate low oxygen levels and could not migrate to deeper and more oxygenated waters (Roman et al., 2012).

Most of the major zooplankton groups were more abundant in surface waters (0-50 m) in summer and at stations with higher Chl *a*, oxygen concentrations and temperatures (Fig. 3.9). Only three groups (Amphipoda, Euchaetidae and Mysida) were highly related to salinity, and therefore, to deeper water layers. In winter, the abundances of many zooplankton groups were higher in surface/sub-surface waters (0-100 m) and were positively related to Chl *a* and oxygen concentrations, but negatively related to temperature and salinity. Large zooplankton (i.e. Mysida and Euchaetidae, and to a lesser extent Amphipoda, Calanidae and Euphausiacea) were positively related to salinity and temperature (Fig. 3.9), and therefore, found in the deeper part of the water column.

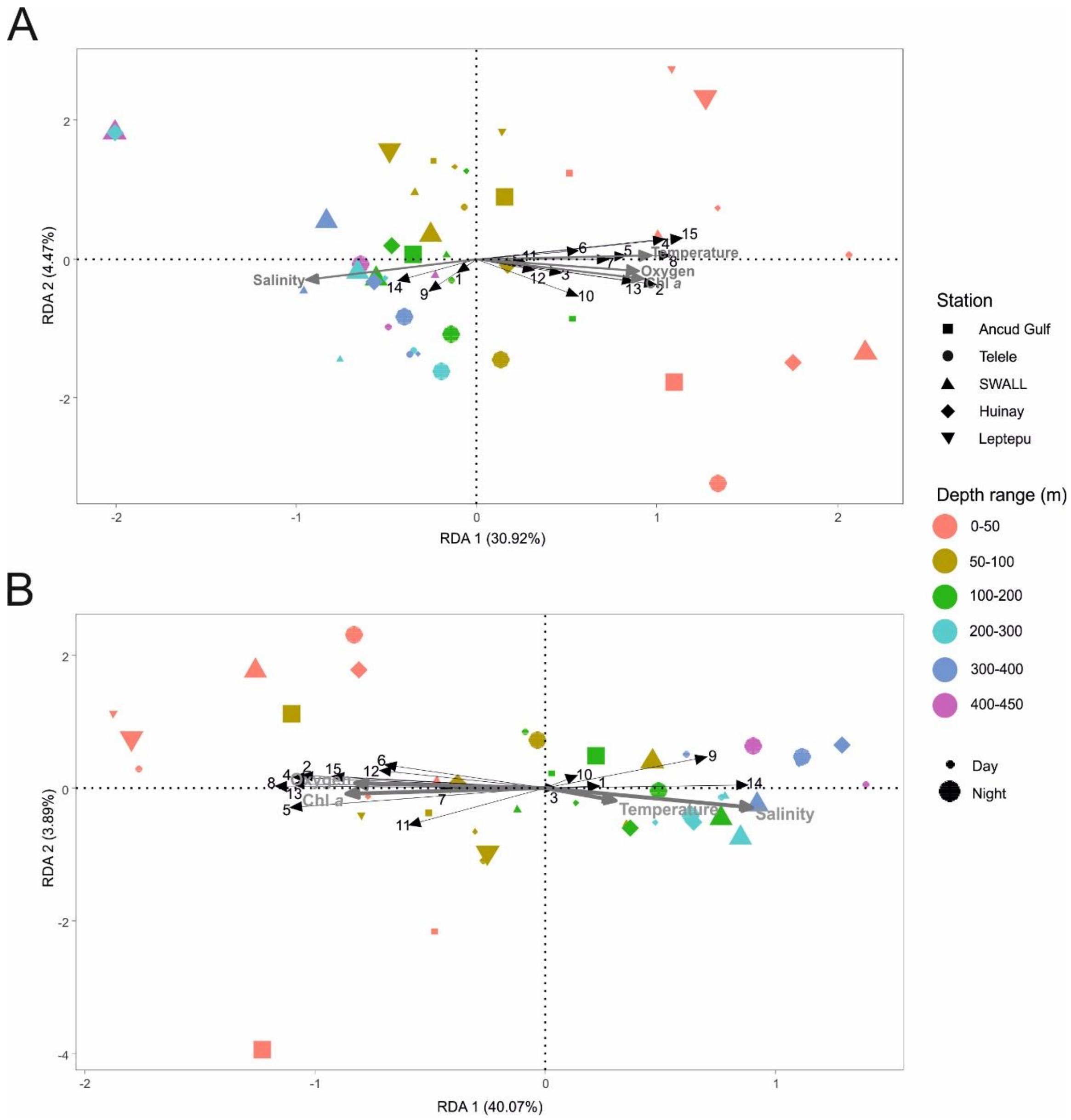

Fig. 3.9 Effect of hydrographic variables on zooplankton abundance (fourth root transformed) along a section through Comau Fjord to the Ancud Gulf. RDA ordination for (A) summer and (B) winter of the most important zooplankton groups constrained by four variables: temperature, salinity, oxygen and Chl *a*. In the RDA biplot, correlations between response variables (black arrows), the explanatory variables (grey arrows), and among themselves, are indicated by the angle between the arrows, which represents the intensity of the correlation (acute angle, right angle and obtuse/strait angle indicate positive correlation, no correlation and negative correlation, respectively). The length of the arrows indicates the strength of the correlation between the given variable and the ordination axis. The proportion of the total variance explained by each axis is given in brackets. Depth strata are represented by color, day/night by size and stations by symbols. Taxa code: 1 Amphipoda, 2 Appendicularia, 3 Calanidae, 4 Copepodites, 5 Chaetognatha, 6 Clausocalanidae, 7 Cnidaria, 8 Cyclopoida, 9 Euchaetidae, 10 Euphausiacea, 11 Harpacticoida, 12 Metridinidae, 13 Meroplankton, 14 Mysida, 15 Paracalanidae.

Overall, the environmental parameters (salinity, oxygen, temperature and Chl *a*) explained 34.24% and 43.93% of the total variation in zooplankton abundance in summer and winter, respectively (Fig. 3.9). However, not only oceanographic drivers such as estuarine circulation (Palma and Silva, 2004), the presence of thermoclines, haloclines and water with low oxygen concentrations (Longhurst, 1976; Southward and Barret, 1983; Sánchez et al., 2011; Pérez-Santos et al., 2018) and changes in the hydrography and nutrient exchange (Balbontín and Bustos, 2005; Aracena et al., 2011) influenced the horizontal and vertical distribution of the zooplankton community in Comau Fjord. The DVM performed by numerous taxa with high abundances and biovolumes may also play an important role in the exported production of the fjord, as DVM may increase the vertical carbon flux, contributing to the efficiency in the biological carbon pump in the ecosystem (González et al., 2016; Tutasi and Escribano, 2020).

In conclusion, the mesozooplankton community of Comau Fjord and Ancud Gulf presented strong seasonal, spatial and vertical dynamics linked to oceanographic and biological drivers. The variability of hydrographic conditions in Comau Fjord and Ancud Gulf was much higher in surface waters (0-20 m) than in the deeper water column due to seasonal variations in solar radiation, precipitation and river discharge. Summer conditions led to stratification of the first 25 m (Fig. 3.2) and higher surface temperatures, resulting in phytoplankton blooms followed by higher zooplankton abundances and biovolumes during this season. In the outer fjord and Ancud Gulf, zooplankton abundances and biovolumes were higher than in the inner part of the fjord, which was also linked to higher Chl *a* concentrations. Chl *a* was concentrated in the upper 20 m of the water column, reflected in the highest abundances of zooplankton in the surface layer (0-50 m). However, biovolume showed that larger zooplankton resided deeper in the water column during day (especially at 100-200 m; Fig. 3.4) in the refuge of darkness to avoid visual predators. At night they specimens migrated upwards to surface layers (0-50 m) in search of phytoplankton-rich waters. The central, deeper part of the fjord, where larger individuals from certain zooplankton groups performed extensive DVM, had higher diversity (in term of species richness) than the shallower areas (fjord head and Ancud Gulf) (Fig. 3.7). In the shallow innermost part of the fjord, oxygen levels were lower compared to the central and outer part, which influenced the zooplankton composition (zooplankton consisted mainly of groups with small individuals), and DVM seemed to be restricted to taxa with low oxygen tolerance that could not migrate to higher oxygenated waters. Overall, the results of this study suggest that zooplankton dynamics and community composition in Comau Fjord and Ancud Gulf are strongly dependent on hydrographic conditions and the seasonal phytoplankton occurrences. Climate warming (higher glacier melt and freshwater input; warmer and drier summers and autumns) and anthropogenic activities may significantly boost the terrestrial carbon pools into coastal waters and modulate the phytoplankton community dynamics (González et al., 2019; Torres et al., 2020), with serious consequences for the zooplankton community composition and dynamics. An increase in anthropogenic activities in the area (e.g. intensification of salmon farming, road construction) may destabilize the phytoplankton productivity and increase the occurrence of coastal and oceanic microbes (Iriarte, 2018). This indicates the importance of regular monitoring of pelagic communities to assess if natural and/or human-induced fluctuations are destabilizing the marine food web and its functions.

3.5. Acknowledgements

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3.6. Supplemental information

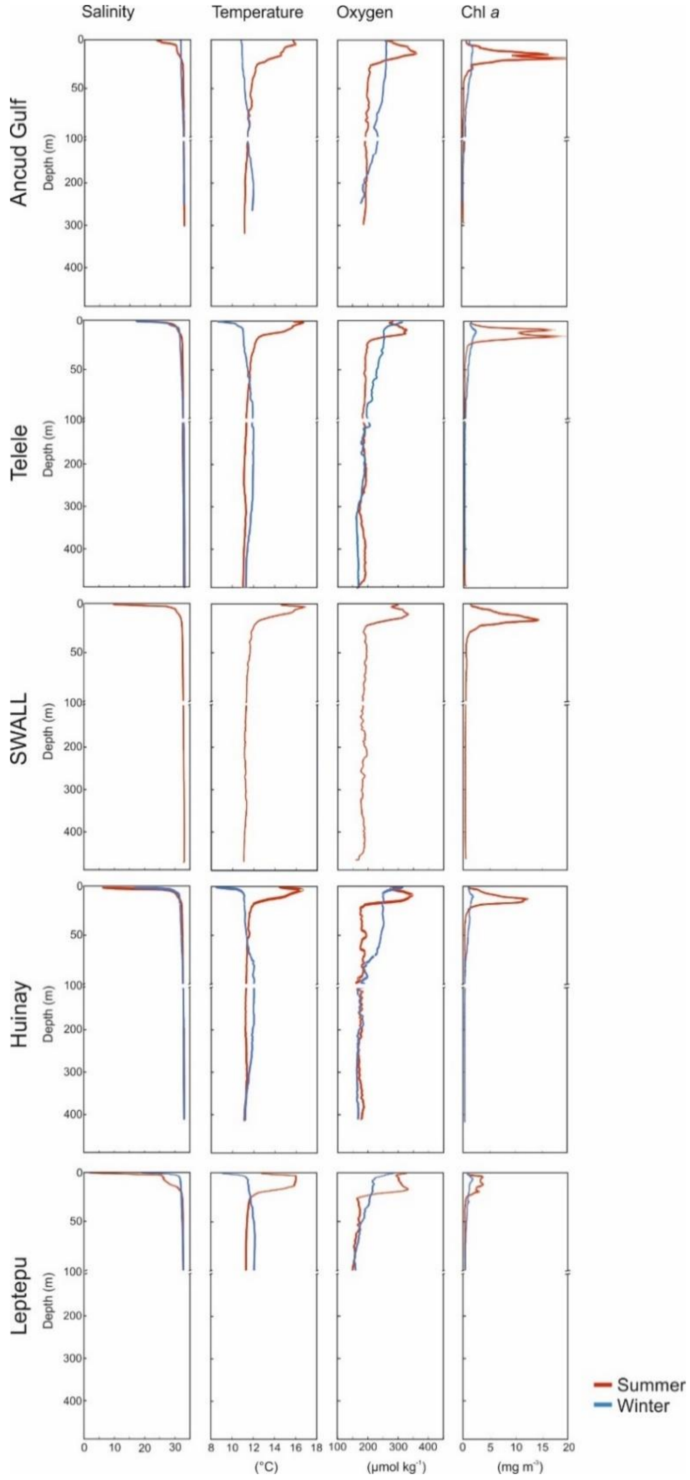

Fig. 3.S1 Hydrographic profiles for five stations sampled in summer and winter along an oceanographic section through Comau Fjord to the Ancud Gulf. Hydrographic data was not available for station SWALL in winter. Note the break at 100 m.

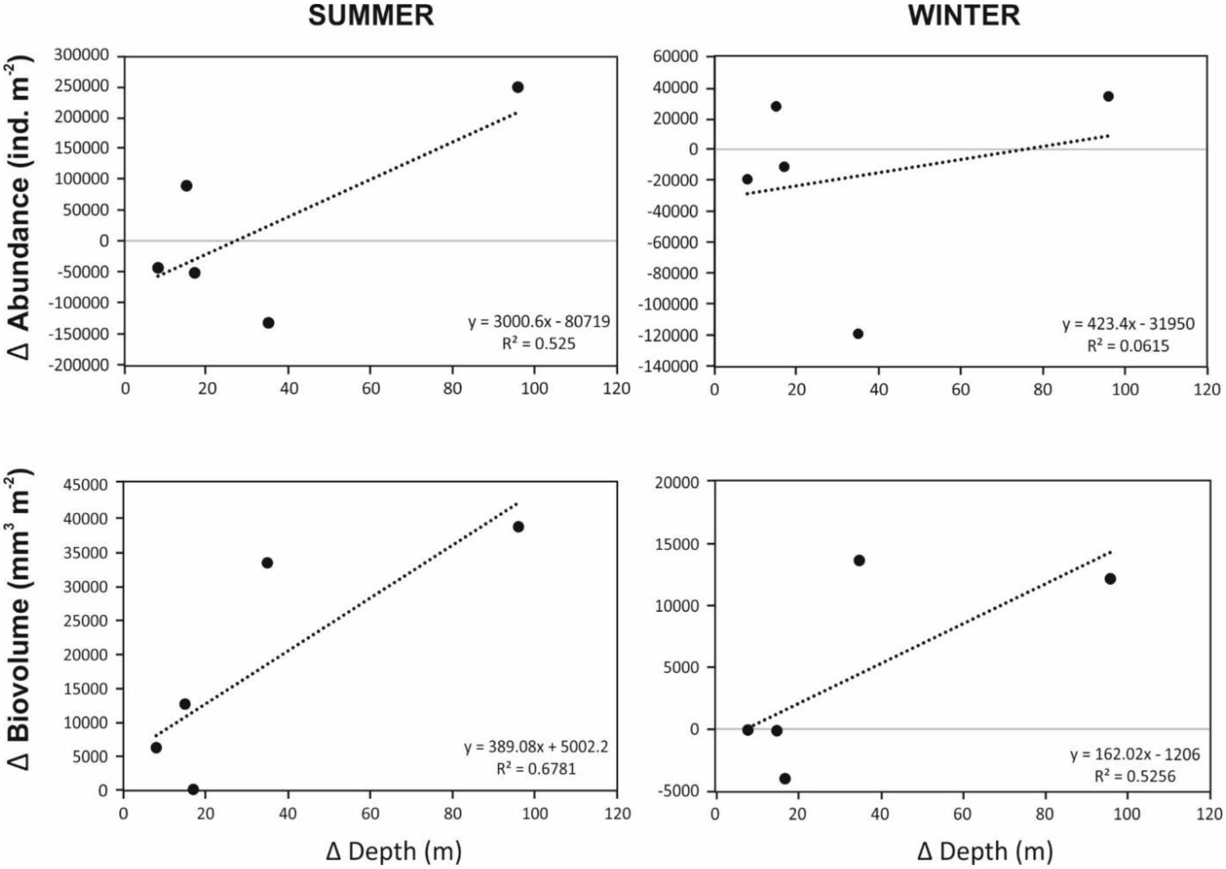

Fig. 3.S2 Integrated day/night differences in zooplankton abundance and biovolume below the sampling depth for summer and winter along a section through Comau Fjord to the Ancud Gulf. We considered the daily difference in abundance and biovolume (delta-abundance, delta-biovolume) as a function of the difference in depths (delta-depth) between the fjord bottom depth and our haul depth at the different stations (Table 1) (e.g. Huinay bottom depth = 417 m; haul maximum depth = 400 m; delta-depth = 17 m). R^2 indicates the variation of the dependent variable (e.g. delta-biovolume) that can be explained by the independent variable (delta-depth).

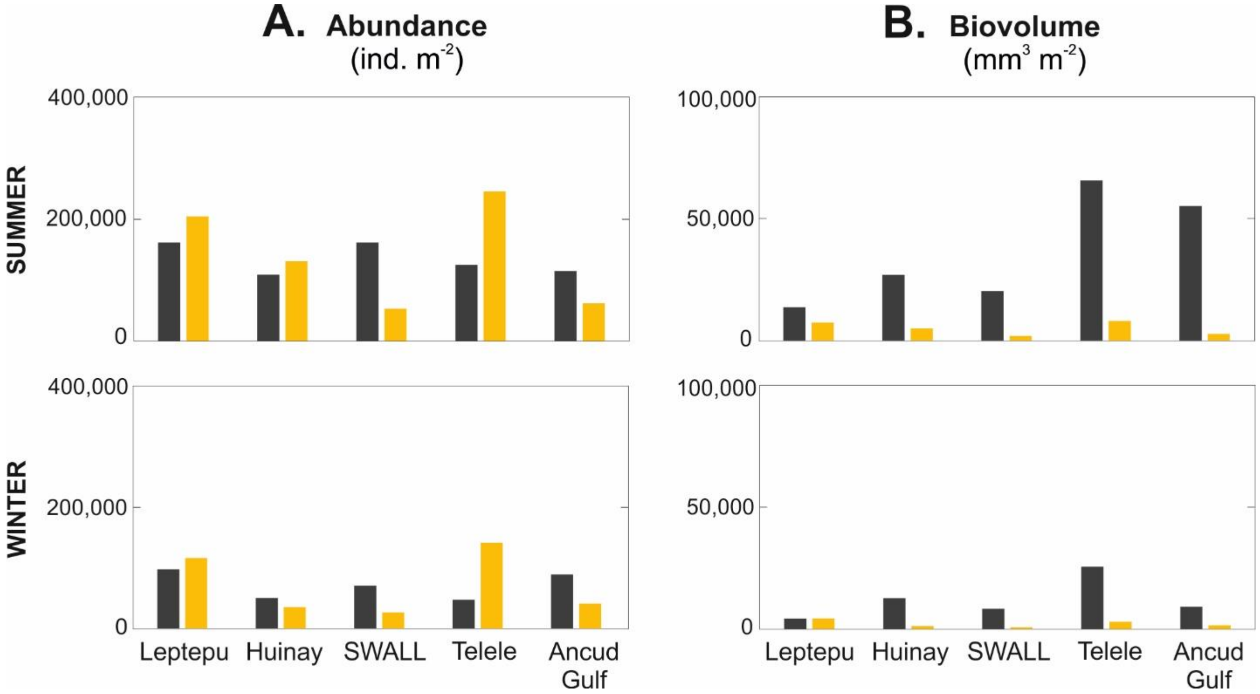

Fig. 3.S3 Spatial and diel integrated zooplankton abundance (ind. m^{-2}) and biovolume (mm^3 m^{-2}) of the upper 100 m water depth in summer and winter along the fjord transect of Comau Fjord and Ancud Gulf. Yellow and black bars represent day and night values, respectively.

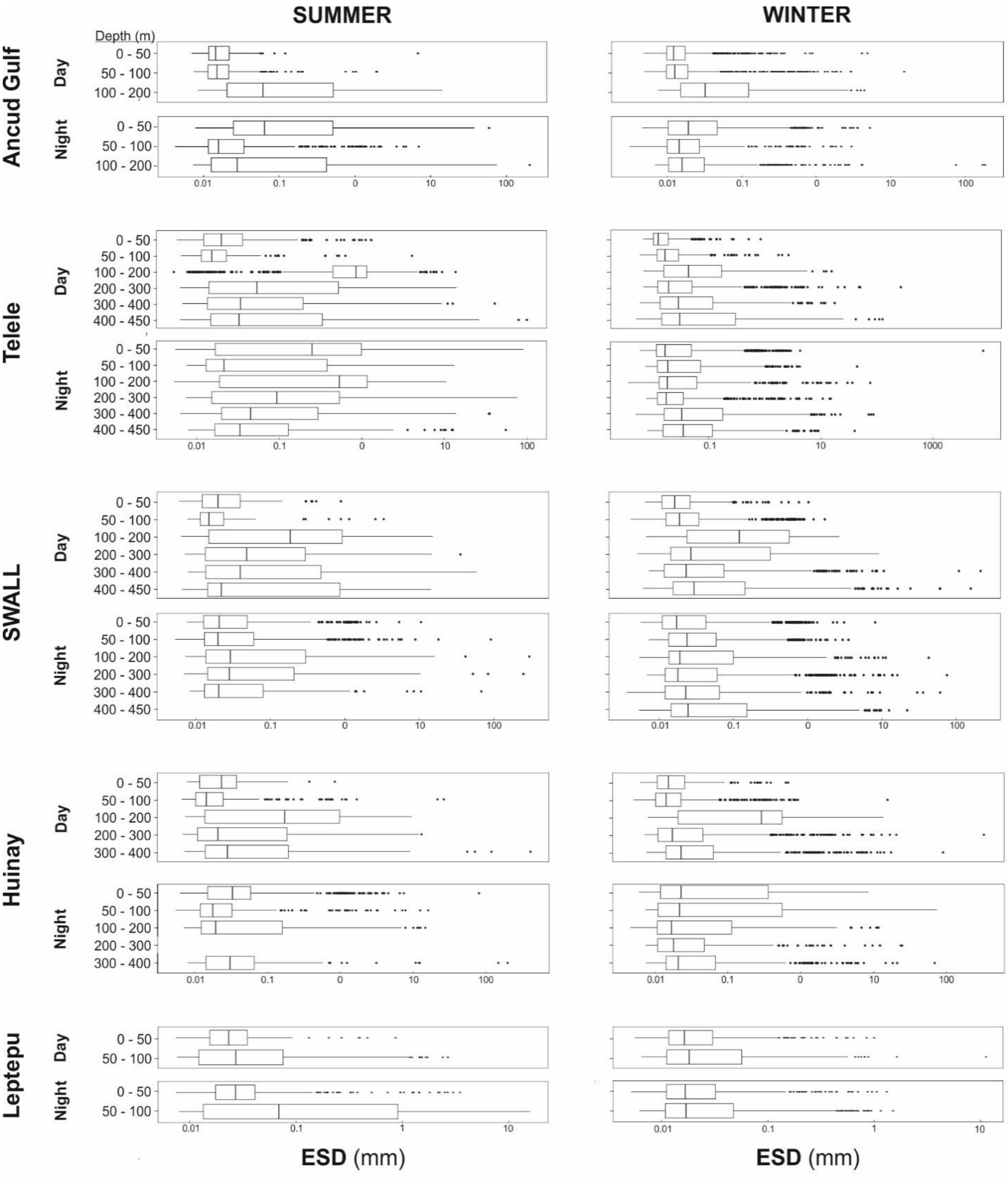

Fig. 3.S4 Equivalent Spherical Diameter (ESD) of individual zooplankton specimens within the mesozooplankton community at each sampling station along a transect section through Comau Fjord to the Ancud Gulf in summer and winter. In the boxplot graphs, the median is indicated by a vertical line, the box represents the 25 and 75 percentiles, and dots show the 1.5× outliers.

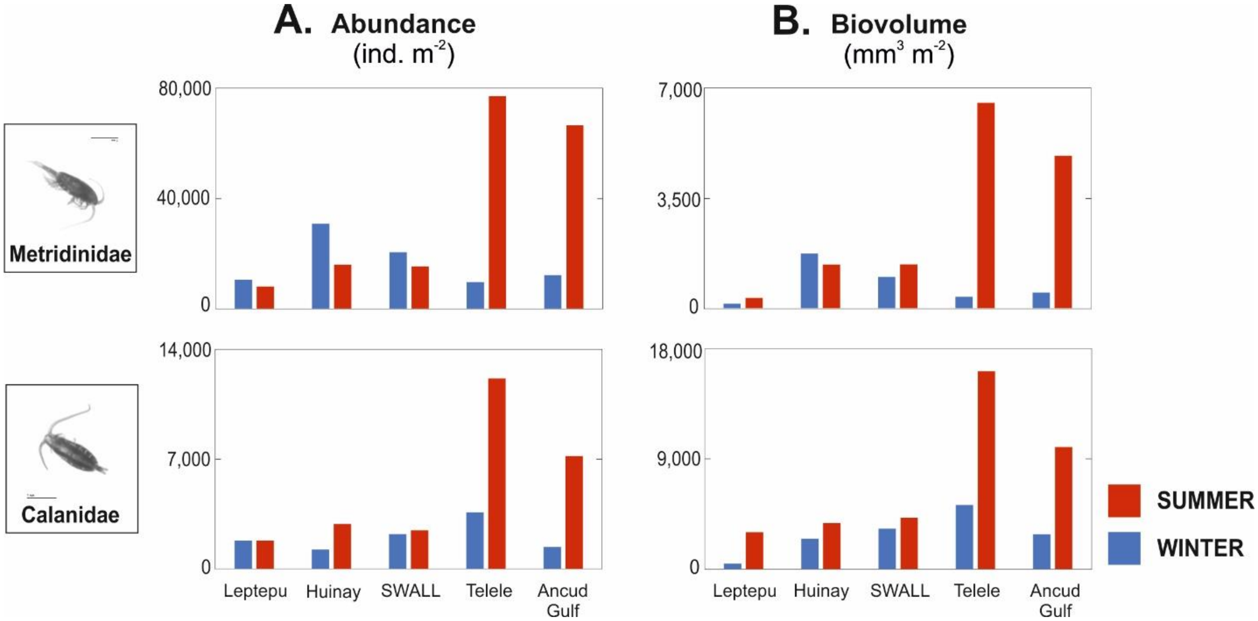

Fig. 3.S5 Summer and winter integrated abundances and biovolumes for the two numerically most dominant copepod taxa (Metridinidae and Calanidae) of the zooplankton community sampled throughout the entire water column along a transect section through Comau Fjord to the Ancud Gulf.

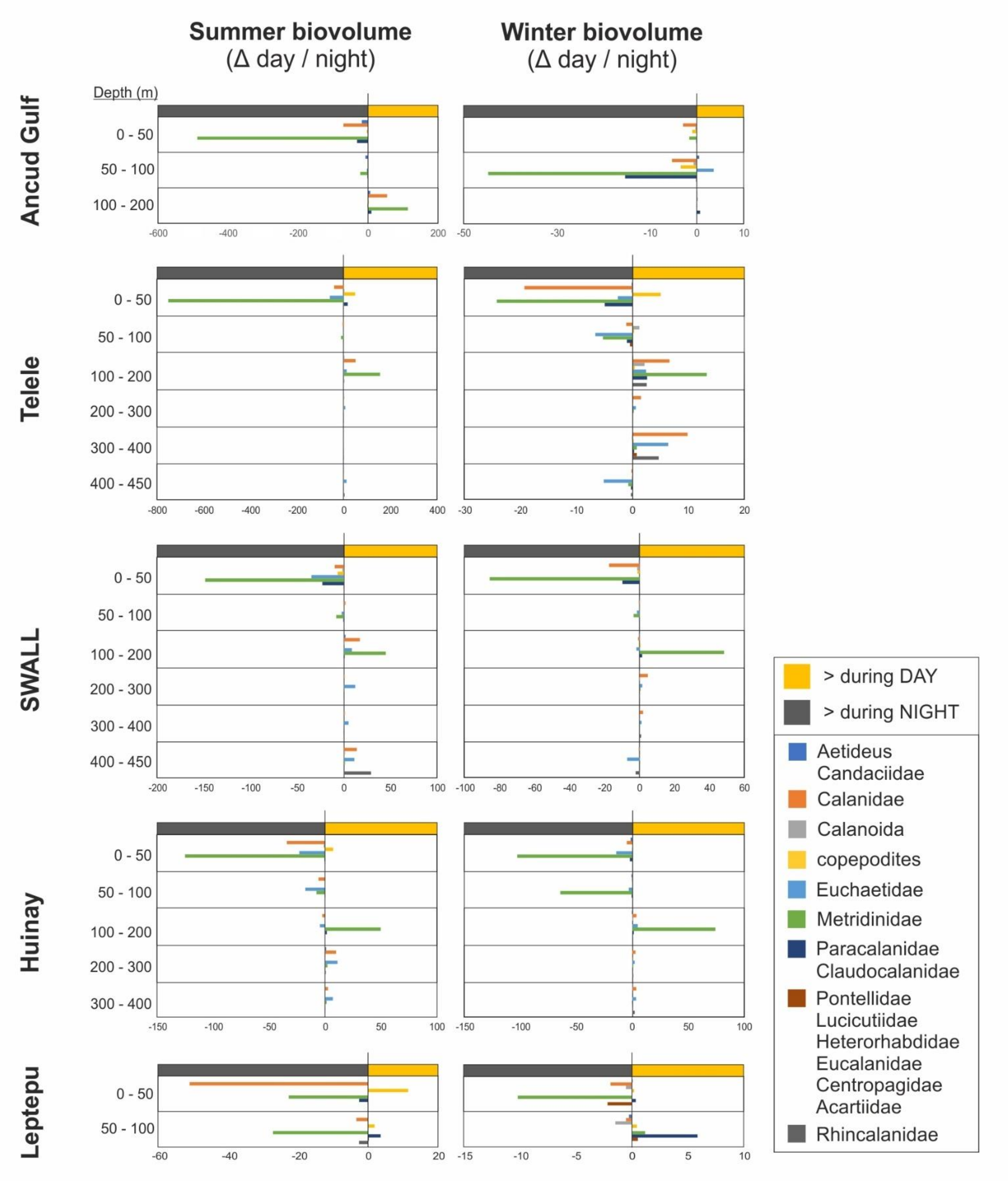

Fig. 3.S6 Differences between day and night absolute values for the calanoid copepod community. Positive values indicate a higher biovolume during day, negative values a higher biovolume during night in the respective sampled water stratum.

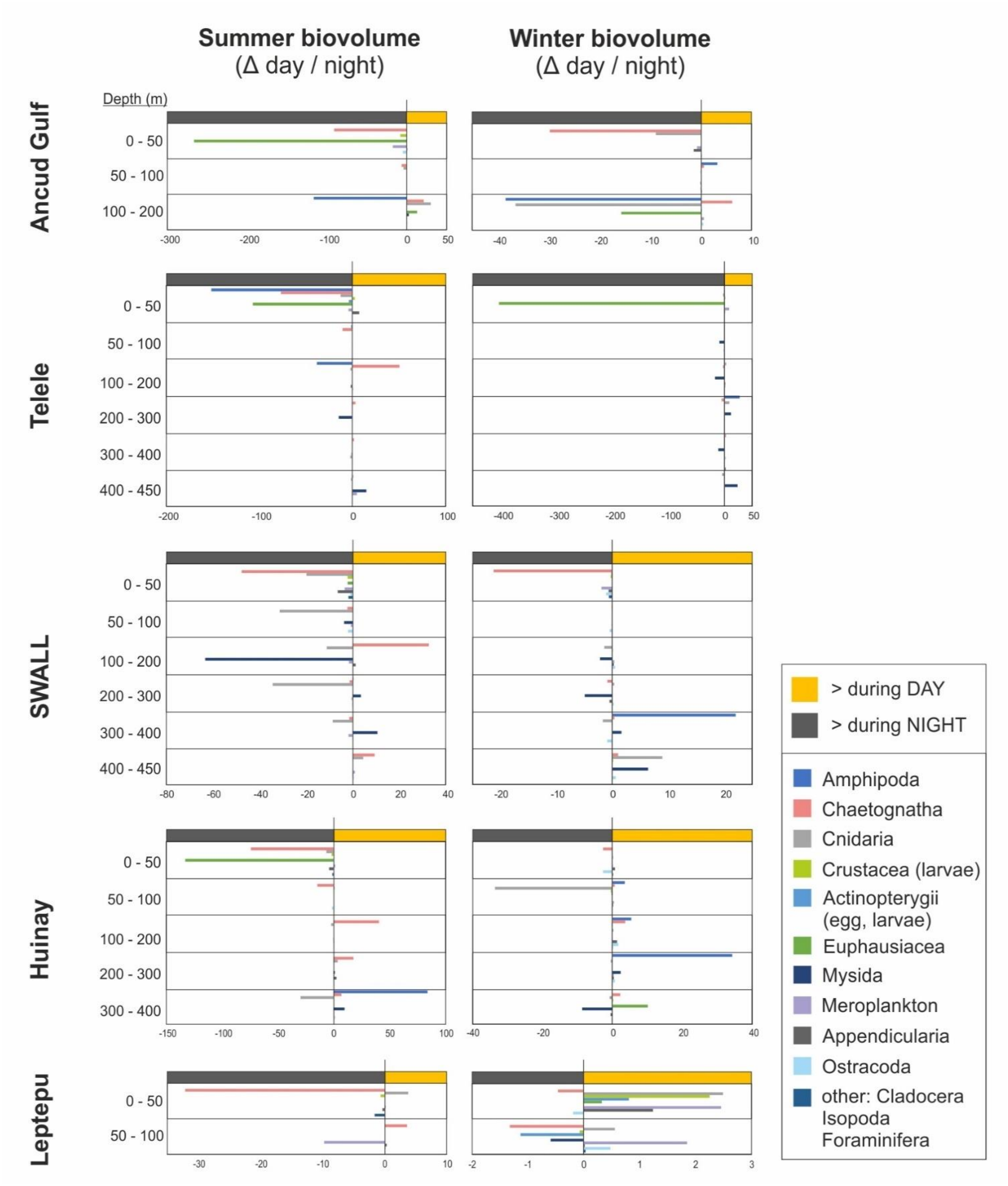

Fig. 3.S7 Differences between day and night absolute values for the non-copepod zooplankton community. Positive values indicate a higher biovolume during day, negative values a higher biovolume during night in the respective sampled water stratum.

Chapter 4 – Meroplankton seasonal and spatial dynamics

Spatio-temporal dynamic of meroplankton in a deep temperate Patagonian fjord

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4.1. Abstract

Meroplankton, pelagic larvae of benthic organisms, is a key element in the bentho-pelagic coupling of marine ecosystems. Rock faces of coastal waters of Chilean Patagonia harbor an exceptionally rich benthos, yet almost nothing is known about its meroplanktonic larvae, let alone their variability and environmental drivers. We fill the gap for Comau Fjord, a deep and productive stratified fjord harboring dense populations of cold-water corals. Meroplankton was collected with a Nansen closing net (70 cm diameter, 100 µm mesh) using stratified vertical hauls from the surface to the nearest interval above seabed (0-50-100-200-300-400-450 m depth). Day and night samples from four seasons were obtained at one station in the central fjord and at four additional stations along a fjord transect in summer and winter, respectively, scanned with a ZooScan and identified in EcoTaxa. Meroplankton abundance was highest in the upper 50 m in spring and coincided with highest chlorophyll *a* (Chl *a*) and oxygen concentrations, suggesting a close coupling of macrobenthos reproduction and subsequent meroplankton feeding with the phytoplankton bloom. Meroplankton composition was dominated by echinoderms in spring, by gastropods in summer and autumn, and by bryozoans in winter. These three taxa accounted for 66% of the total meroplankton on an annual average. Along the fjord transect, gastropods dominated the meroplankton community (38%), followed by bryozoans (20%) and polychaetes (13%). Abundance was higher during the day in spring and winter, but at night in summer and autumn. Overall, the meroplankton community was mainly influenced by Chl *a* and oxygen, while temperature had little effect. Salinity correlated negatively with the abundance of most meroplankton taxa, except for ascidians, which appeared to avoid the surface waters. Monitoring meroplankton dynamics provides useful information on the recruitment potential of ecologically and economically important benthic invertebrate populations of Comau Fjord.

Keywords:

Comau Fjord | benthic invertebrate larvae | ZooScan | seasonality | Chilean Patagonia | Chile

4.2. Introduction

Most marine benthic invertebrates are either sessile or slow moving, so their dispersal ability depends on a pelagic larval stage drifting with the currents. As a consequence of their floating habit, meroplanktonic larvae greatly differ from the adults in size, density, morphology and diet and remain in the pelagial over short (hours) to long (months up to one year) periods until they attach and undergo metamorphosis to the recruit juvenile stage (Riedl, 1966; Levin and Bridges, 1995; Pechenik, 1999; Young, 2002). Larval drift allows for genetic exchange with other populations and sustaining genetic diversity (Scheltema, 1986; Todd, 1998). Dispersal is influenced by currents, waves, turbulent mixing and resuspension (Pineda, 1991, 1994, 1999; Shanks, 1998, 2006; Isla et al., 2001), but also biological factors such as larval supply, growth, larval stage duration, physiological stresses in response to e.g. temperature and salinity fluctuations, as well as environmental and ecological factors such as food availability, settlement cues, availability of settlement substrate, or predation pressure (Pineda, 1994; Eckman, 1996; Johnson and Brink, 1998; Todd, 1998; Bhaud, 2000; Johnson and Shanks, 2003; Landaeta et al., 2013).

Spawning of benthic invertebrate can be triggered by a number of factors such as temperature, photoperiod, moon cycles, salinity or food supply (Giese and Kanatani, 1987). Therefore, meroplanktonic distribution patterns and seasonal dynamics significantly differ between species and higher taxonomic groups. Depending on habitat, nutrition and degree of parental care, larval development ranges between benthic and pelagic, feeding and lecithotrophic, free and protected, respectively (Poulin et al., 2001). However, from all benthic marine invertebrates, the majority of species produce planktotrophic pelagic larvae that depend to the seasonal plankton bloom and disperse over potentially large distances (Thorson, 1946; Palma and Aravena, 2001; Highfield et al., 2010). Only about 10% of benthic fauna produce lecithotrophic larvae, which derive their nutrition from a yolk sac and tend to show lower dispersal rates (Thorson, 1950).

High-latitude fjords are generally characterized by marked seasonal variability with high solar radiation in summer causing strong thermal stratification and high precipitation in winter. Such environmental variability results in intense phytoplankton blooms and high biological production in austral spring (González et al., 2010). Therefore, the synchronicity between spawning of benthic invertebrates and phytoplankton blooms, often results in meroplankton dominating the coastal zooplankton community during the reproductive season of benthic invertebrates (Thorson, 1946).

In Patagonian Fjords, the zooplankton community contains a diverse and abundant copepod community mixed with dense swarms of euphausiids, and a rich variety of cladocerans and meroplanktonic crustacean larvae (Marin and Antezana, 1985; Escribano et al., 2003; Palma, 2008; González et al., 2010; Garcia-Herrera et al., 2022). Salinity and temperature are key factors affecting the spatial and seasonal distribution of meroplankton, as well as its metabolism and swimming behavior (Benfield, 2013). The strong salinity and temperature gradients of the fjords that shape the hydrographic structure of the water column, affect the seasonal productivity patterns, community structure and larval dispersal of the benthic and pelagic community (González et al., 2010). The distribution and dynamics of these plankton assemblages are influenced by stratification and mixing (Sánchez et al., 2011; Meerhoff et al., 2014a), nutrient inputs (Castro et al., 1991; Hamame and Antezana, 2010), light and primary production (González et al., 2010) as well as degradation of organic matter and hypoxia (Castro et al., 2011; Pérez-Santos et al., 2018). For instance, in the Baker/Martinez fjord complex, seasonal shifts from Cirripedia larvae in early spring to Bivalvia larvae in late summer have been linked to hydrography and food availability (Meerhoff et al., 2014a). Meroplanktonic larvae are retained in fjords and bays by an estuarine circulation (North and Houde, 2001; Meerhoff et al., 2014b), in which freshwater inputs from precipitation, glacial melt and river runoff create low-salinity surface waters that flows seaward; and drive subsurface Modified Subantarctic Waters (MSAAW) carrying oceanic plankton and macronutrients landward (Silva et al., 1998).

A diverse benthic community with ca. 1,650 species has been reported in Patagonian fjord waters (Häussermann and Försterra, 2009). For Comau Fjord, specific papers are available on a number of taxa, such as bivalves, brachiopods, bryozoans, gastropods, cold-water corals, echinoderms, decapods, polychetes, and polyplacophorans (Schwabe et al., 2006; Häussermann et al., 2012, 2013, 2021a; Zapata-Hernández et al., 2014). But these are purely qualitative data on biodiversity and generally limited to diving depth. Presence/absence data have been reported for 114 taxa of sessile and mobile benthic invertebrates in the inner, middle and outer fjord and several depth strata down to 21 m (Villalobos et al., 2021). Semi-quantitative and anecdotal information is available on 28 species and 6 genera down to 40 m comparing temporal changes between 2003 and 2013 (Häussermann et al., 2013). Quantitative data covering the entire water column are available only for corals (*Desmophyllum dianthus*, Fillinger and Richter, 2013). Patterns of Deep-Water Emergence are reviewed on more than 1,000 invertebrate taxa along the entire Chilean coast (including the Comau Fjord) (Häussermann et al., 2021a). However, the lack of quantitative data for the whole benthic community, along with the scarcity of information on the reproductive cycles, is a bottleneck to assess the benthic sources for the meroplankton in our study. Notwithstanding, given the narrow topography of the fjord and its large benthic surface to pelagic volume, it is to be expected that an abundant meroplankton community exists here. However, studies on meroplankton in Patagonian waters, its distribution and relation to environmental and oceanography are scarce and limited to specific taxonomic groups such as Cirripedia and Decapoda (e.g. León et al., 2008; Meerhoff et al., 2014b, 2013; Mujica and Medina, 1997). Only a few studies investigated entire meroplanktonic assemblages (e.g. Antezana, 1999; Defren-Janson et al., 1999; Meerhoff et al., 2014a; Thatje et al., 2003). Most of these investigations have been conducted in southern Chilean Patagonia, and only Castro et al. (2011) provided some insights into meroplanktonic larval dispersal due to tidal mixing in northern Chilean Patagonia.

Boreal studies show that planktotrophic larvae occur in high densities in spring and summer, and are key elements in the pelagic food web as important phytoplankton grazers as well as potential food for larger zooplankton and fish in coastal environments (Thorson, 1950; Young and Chia, 1987). The supply of meroplankton is important for successful recruitment of adult populations, allowing fast colonization of bare substratum and new territories by benthic invertebrate communities (Thorson, 1950). Meroplankton are an essential component of the bentho-pelagic coupling of the ecosystem, determining the pathway for energy flow. In spite of the important role of meroplanktonic larvae in ecosystem processes, only few data on environmental variation and meroplankton dynamics are available from Chilean fjords (Thatje et al., 2003; Meerhoff et al., 2014a). Therefore, this study aimed to elucidate the distribution of meroplankton along a deep-temperate fjord in northern Chilean Patagonia. We describe the seasonal and spatial distribution of planktonic larval stages of benthic invertebrates in Comau Fjord and identify environmental drivers that may influence their abundance and community composition.

4.3. Materials & Methods

Study area

Comau Fjord is located in northern Chilean Patagonia. It is connected to the Inner Sea of Chiloé via the Ancud Gulf (Fig. 4.1). The fjord is approximately 45 km long, with a width between 2 and 8.5 km, and a depth of 480 m (outer - central area), and 100 m (fjord head) (Häussermann et al., 2012; Fillinger and Richter, 2013). The adjacent Ancud Gulf has a depth of ca. 300 m. Comau Fjord is characterized by high volcanic activity and pronounced environmental variations throughout the year (Soto et al., 2018). Particularly, high precipitation (>5000 mm/year) and a higher solar radiation in summer occur in this area (Pantoja et al., 2011). It has the typical estuarine circulation pattern, where the shallow water dynamics are driven by freshwater inputs, and Modified Subantarctic Water (MSAAW) enters the fjord in the deep. The freshwater inputs derive from rainfall (especially high in winter), glacial melt on the eastern side through Quintupeu and Cahuelmó Fjord, and from river runoffs on the southern side through the Vodudahue and Leptepu Rivers. The seasonal variation highly influences the hydrographic conditions, where a strong halocline, thermocline, oxycline and pycnocline forms in about 20 m water depth. Therefore, environmental parameters of shallow water (0-20m) are more variable than those of deeper water (Garcia-Herrera et al., 2022).

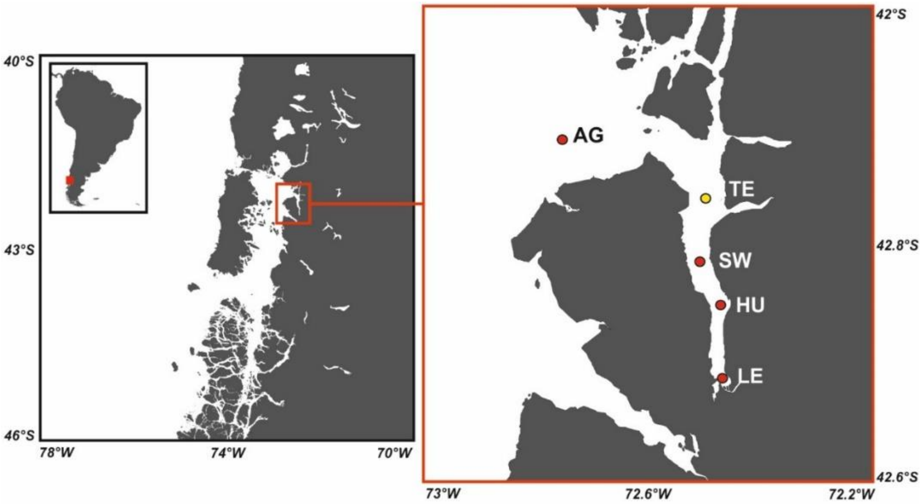

Fig. 4.1 Study site and sampling stations along Comau Fjord and Ancud Gulf, northern Chilean Patagonia. Samples from stations with red circles were visited in austral summer and winter, samples from Telele (yellow circle) were additionally visited in spring and autumn. Station names: (AG) Ancud Gulf, (TE) Telele, (SW) SWALL, (HU) Huinay, (LE) Leptepu.

Meroplankton and hydrography data collection

The distribution and variability of the meroplankton community was assessed by analyzing the daily, seasonal and spatial abundance at five stations along an almost north-south transect of the fjord (Table 4.1). Each station was sampled by stratified vertical hauls throughout the water column to the interval (0-50-100-200-300-400-450 m) nearest to the seabed, using a Nansen Closing Net with a 3 m long net bag, a net opening of 0.3848 m^2 and a mesh size of 100 µm.

Table 4.1 Station data.

Station	Geographical position	Fjord position	Bottom depth	Haul depth	Season (date)	Daytime (time)
Ancud Gulf	-42.17297, -72.73635	Outside fjord	296 m	200 m	Summer (Jan 21st – 22nd 2017)	Day (12:40 – 13:44) Night (00:50 – 02:07) *
					Winter (Aug 4th – 5th 2017)	Day (14:49 – 16:15) Night (23:09 – 00:00)
Telele	-42.24917, -72.48056	Outer fjord	485 m	450 m	Spring (Sep 28th 2016)	Day (11:30 – 15:00) Night (22:30 – 00:30)
					Summer (Jan 17th – 18th 2017)	Day (12:00 – 15:10) Night (23:50 – 03:30)
					Autumn (May 11th – 12th 2017)	Day (11:30 – 15:00) Night (22:11 – 00:30)
					Winter (Aug 9th – 11th 2017)	Day (12:06 – 15:31) Night (00:14 – 03:20)
SWALL	-42.33102, -72.48082	Central fjord	465 m	450 m	Summer (14th – 15th 2017)	Day (12:19 – 15:18) Night (23:39 – 02:32)
					Winter (Aug 6th – 7th 2017)	Day (11:31 – 15:46) Night (21:53 – 00:44)
Huinay	-42.38745, -72.43662	Central fjord	417 m	400 m	Summer (Jan 12th – 13th 2017)	Day (12:07 – 15:05) Night (23:19 – 01:34)
					Winter (Jul 28th – 29th 2017)	Day (11:02 – 13:15) Night (23:19 – 02:06)
Leptepu	-42.48293, -72.43413	Inner fjord	108 m	100 m	Summer (Jan 11th – 12th 2017)	Day (12:21 – 12:43) Night (23:12 – 00:05)
					Winter (Jul 24th – 25th 2017)	Day (11:54 – 12:32) Night (21:04 – 21:47)

* wind drift, rope angle 45°, resulting in oblique rather than vertical haul

All samples were preserved in buffered formaldehyde (4%) until further laboratory analysis. To assess if environmental parameters influence the seasonal and spatial distribution of meroplankton abundance, a CTD multi-probe (SBE 19plusV2 Profiler - with RS 232 Interface, Sea-Bird Electronics Inc.) was deployed. This allowed to obtain vertical salinity, temperature, oxygen and Chl *a* fluorescence profiles from the surface to the seabed.

In the laboratory, all plankton samples were scanned with a ZooScan digital imaging system (CNRS patent, www.hydroptic.com), which produces high-resolution images (2400-dpi resolution) and allows sharing and storage of information in digital form (Grosjean et al., 2004; Gorsky et al., 2010). Samples were fractioned with a Folsom plankton splitter to reduce the number of organisms in the scanner frame to ideally ca. 1250 organisms/scan. Samples and diluted subsamples (1-1/128 of the total preserved sample), respectively were scanned following Gorsky et al. (2010). In the scan frame, the objects were separated manually to assure an even distribution and to reduce the number of overlapping individuals. The resulting images were processed with the ZooProcess software (Gorsky et al., 2010), a plug-in of ImageJ (Schneider et al., 2012), which converts the high-quality image associated with the sample metadata into individual images (called ‘vignettes’), each usually showing only one object. When individuals overlapped within the same vignette, manual separation was performed using the ‘ZooProcess separation with mask’ digital tool, which draws lines to separate two or more individuals, and subsequently the vignettes were reprocessed. The original vignettes with multiple objects were deleted from the database to avoid double counting. After scanning, the sample was recovered and stored in 70% ethanol for archiving.

For semi-automated taxonomic classification, all vignettes were uploaded to the website Ecotaxa 2.6 (Picheral et al., 2017). This web-based machine learning application uses two-way random forest algorithms to predict and classify the objects on the vignettes using expert training sets. The accuracy of the semi-automatic classification highly depends on the number of vignettes included in each category and the required taxonomic resolution. To ensure the accuracy of the taxonomic identification, all classifications were manually validated or corrected. Larvae were determined by applying the training sets used in Garcia-Herrera et al. (2022). Vignettes containing meroplanktonic larvae were assigned to either phylum (e.g. Bryozoa, Echinodermata) or class (e.g. Ascidiacea, Bivalvia) and separated from the remaining images of the holoplankton. All organisms were counted based on the number of images per taxonomic category. Abundance was calculated as ind. m^{-3} based on the number of vignettes counted in a category and the volume filtered through the net, calculated as: $V = r^2 \times h$, where *r* is the radius of the net opening and *h* the range of each vertical haul. On one occasion (Ancud Gulf, summer, night) windy conditions caused the boat to drift, resulting in an oblique (rather than vertical) haul. Here, we used a mechanical flowmeter to measure the filtered volume. The flow meter was inaccurate at speeds <0.3 m s^{-1} and thus not used for the vertical hauls (winch speed ~0.3 m s^{-1}).

Data analysis

All abundance calculations and statistical analysis were performed in R (R Core Team, 2020) with the *vegan* package (Oksanen et al., 2019), and graphs were plotted using the *ggplot2* package (Wickham, 2016). Prior to statistical analysis, all abundance values were fourth-root transformed to reduce the influence of highly dominant taxonomic groups in the analysis. The influence of the environmental parameters on the meroplankton abundance along the fjord throughout the year was assessed by applying a redundancy analysis (RDA). RDA is a multiple linear regression between the mean of the explanatory variables (in our case, salinity, temperature, oxygen and Chl *a*) and the response variables (meroplankton abundance). RDA is a constrained ordination procedure which summarizes the multiple linear regressions, followed by the representation of a principal component analysis (PCA). To avoid possible overfitting and increased residual variation, collinearity between the explanatory variables was tested (Pearson correlation coefficient <0.8) and therefore included in the analysis (Dormann et al., 2013). In the final RDA analysis, a permutation test (ANOVA with 10,000 iteration) was performed to test for significant results. Additional comparisons between factors, which may have been source of variability in meroplankton abundance (such as stations, seasons or day/night) were used to discriminate significant differences using a Permutational Multivariate Analysis of Variance (PERMANOVA and pos hoc corrected $p > 0.05$) of distance matrices and permutation tests.

4.4. Results

Hydrographic conditions of Comau Fjord and Ancud Gulf

The hydrography of Comau Fjord showed strong variability in the upper 50 m of the water column, while the deeper layers showed only small vertical changes (Fig. 4.2). Although there were differences of the hydrographic patterns between summer and winter, they were similar in the respective season at all stations along the fjord, respectively (Fig. 4.2).

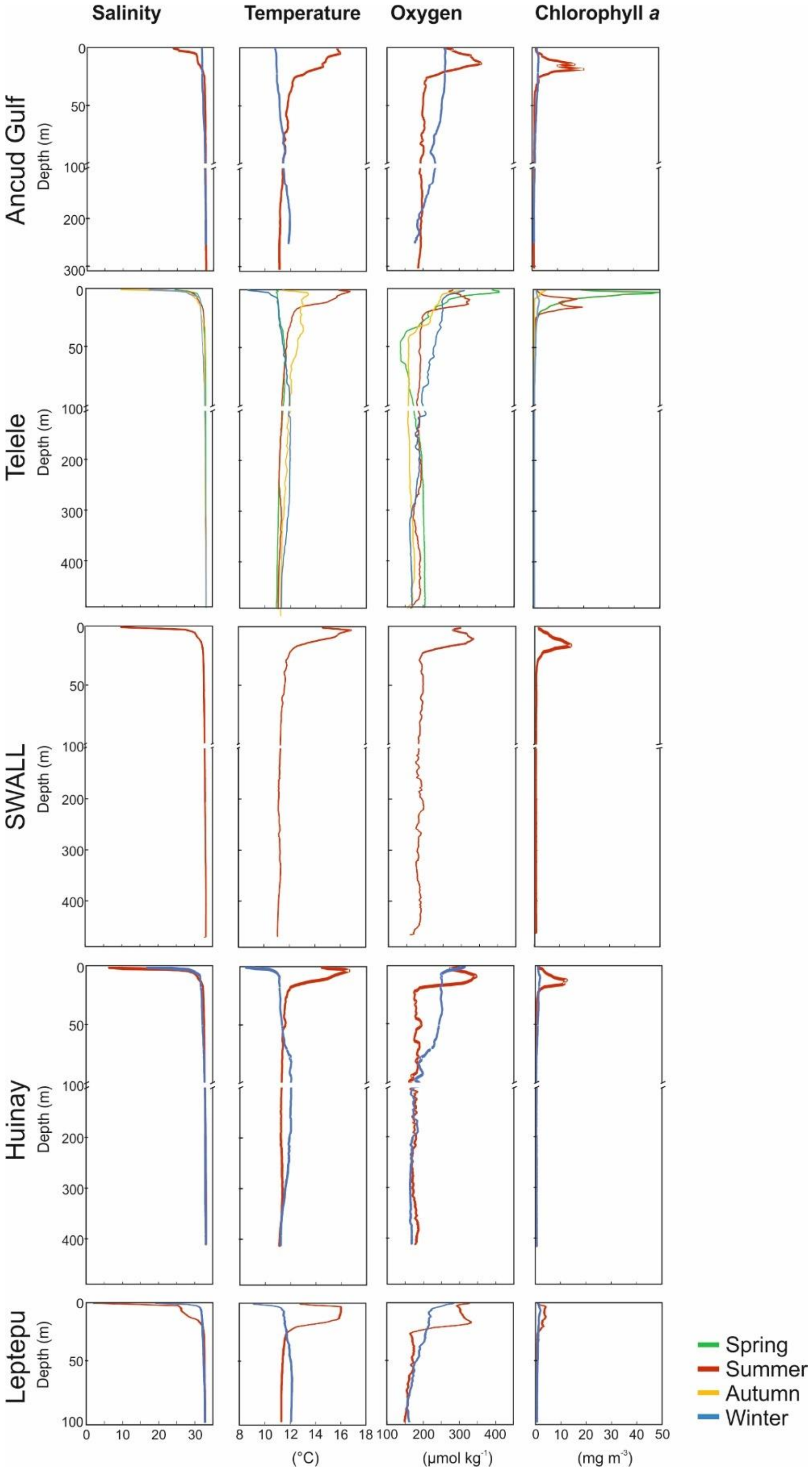

Fig. 4.2 Seasonal and spatial vertical profiles of environmental parameters of Comau Fjord and Ancud Gulf. Note the scale break at 100 m water depth. Hydrographic conditions for the station SWALL in winter are missing.

Salinity stratification was detected along the fjord, with the exception of the Ancud Gulf in winter, where salinity was nearly constant from the surface to the seafloor (32.6 ± 0.4). Surface waters also showed seasonal differences in salinity. In summer, the salinity in surface waters ranged between 2 and 24, while in winter it ranged between 17 and 20. The lowest values were measured at Leptepu in summer, gradually increasing towards the Ancud Gulf. In winter, slightly lower salinity values were observed in the central fjord (Telele, SWALL and Huinay) and slightly higher at Leptepu. Below the halocline, salinity was rather constant at all stations (32.8 ± 0.5). Temperature was similar throughout the water column at Telele in spring (note: this station was sampled quarterly), while in summer thermal stratification was observed (as at all stations), reaching maximum values of 16.7 °C at 2-4 m water depth, slowly decreasing until ca. 20 m, where values stabilized (11.3 ± 0.1 °C). Cooling caused a breakdown of the thermocline at Telele in autumn and a temperature inversion in the uppermost 20 m in winter (as at the other stations, except Ancud Gulf, where the brackish water layer is missing thus the surface temperature is higher), where values were lower (10.9 ± 0.5 °C) than deeper in the water column (11.7 ± 0.3 °C), indicating winter mixing. Oxygen concentration showed very high variability in the uppermost 50 m in spring (Telele) and in the uppermost 20 m in summer (all stations). Maximum oxygen values were observed in the uppermost 3 m in spring (410.5 µmol kg^{-1}) at Telele and were still high at all stations in summer with concentrations between 324 and 361 µmol kg^{-1}. In autumn, maximum values reached 302.7 µmol kg^{-1} (Telele) and in winter they ranged between 262 and 314 µmol kg^{-1} in the top 15 m. In winter, below 50 m water depth, the oxygen concentration at all stations averaged 183.4 ± 16.7 µmol kg^{-1}. The highest chlorophyll *a* (Chl *a*) concentration was observed in spring at Telele, reaching a maximum at 3 m water depth (50.86 mg Chl *a* m^{-3}), decreasing at this station over time. In summer, the Chl *a* peak was found at all stations between 4 and 16 m water depth. The lowest values were found at the stations in winter (2.5-1.8 mg m^{-3}).

Meroplankton spatial distribution

The spatial distribution of meroplankton showed significant differences among stations and depths (p <0.05). Among all stations, we observed that Telele had the highest abundances of meroplankton followed by Huinay and Leptepu (Fig. 4.3A). The high standard deviation of the meroplankton abundance in Leptepu was caused by the big difference between summer and winter abundances. At all stations, meroplankton showed clear differences in vertical distribution (p <0.05), with highest abundances in the upper 50 m (Fig. 4.3B). Below 50 m, the abundance generally decreased with depth reaching minimum values between 300-400 m water depth in SWALL (2.18 ind. m^{-3}).

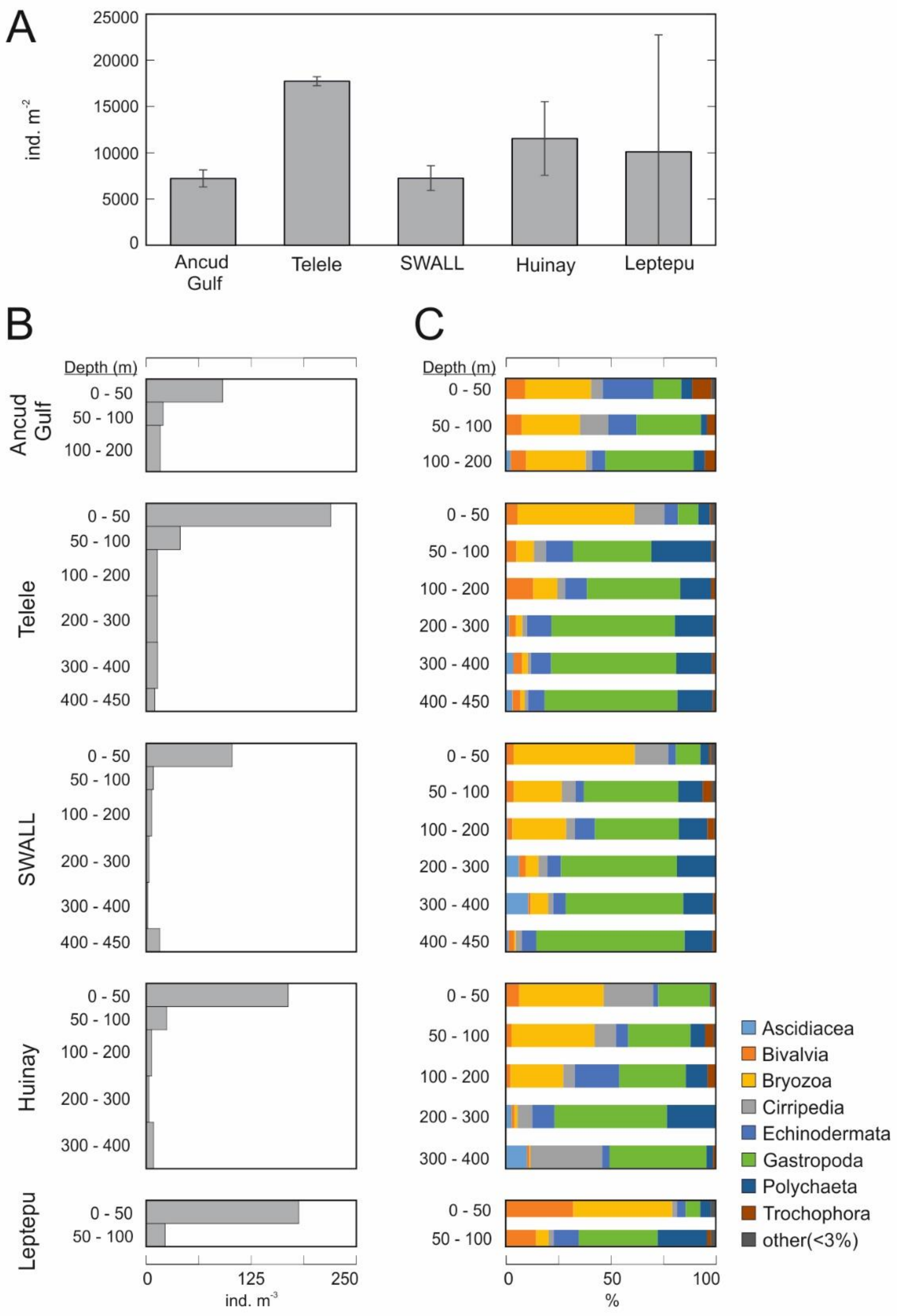

Fig. 4.3 (A) Mean spatial distribution (ind. m^{-2}), (B) vertical distribution (ind. m^{-3}) and (C) relative contribution (%) of the total meroplanktonic community abundance along the transect between the Ancud Gulf and Comau Fjord in summer and winter. Decapoda, Nemertea, Platyhelminthes and Phoronida larvae cumulatively represented less than 5% of the total abundance, and were thus pooled as “other (<3%)”.

The relative contribution of the different meroplankton groups also differed among stations and depths (Fig. 4.3C). Overall the community composition of meroplankton found in Comau Fjord and Ancud Gulf was composed of 11 different meroplankton taxa: Ascidiacea, Bivalvia, Bryozoa, Cirripedia, Decapoda, Echinodermata, Gastropoda, Nemertea, Phoronida, Platyhelminthes and Polychaeta (Fig. 4.4).

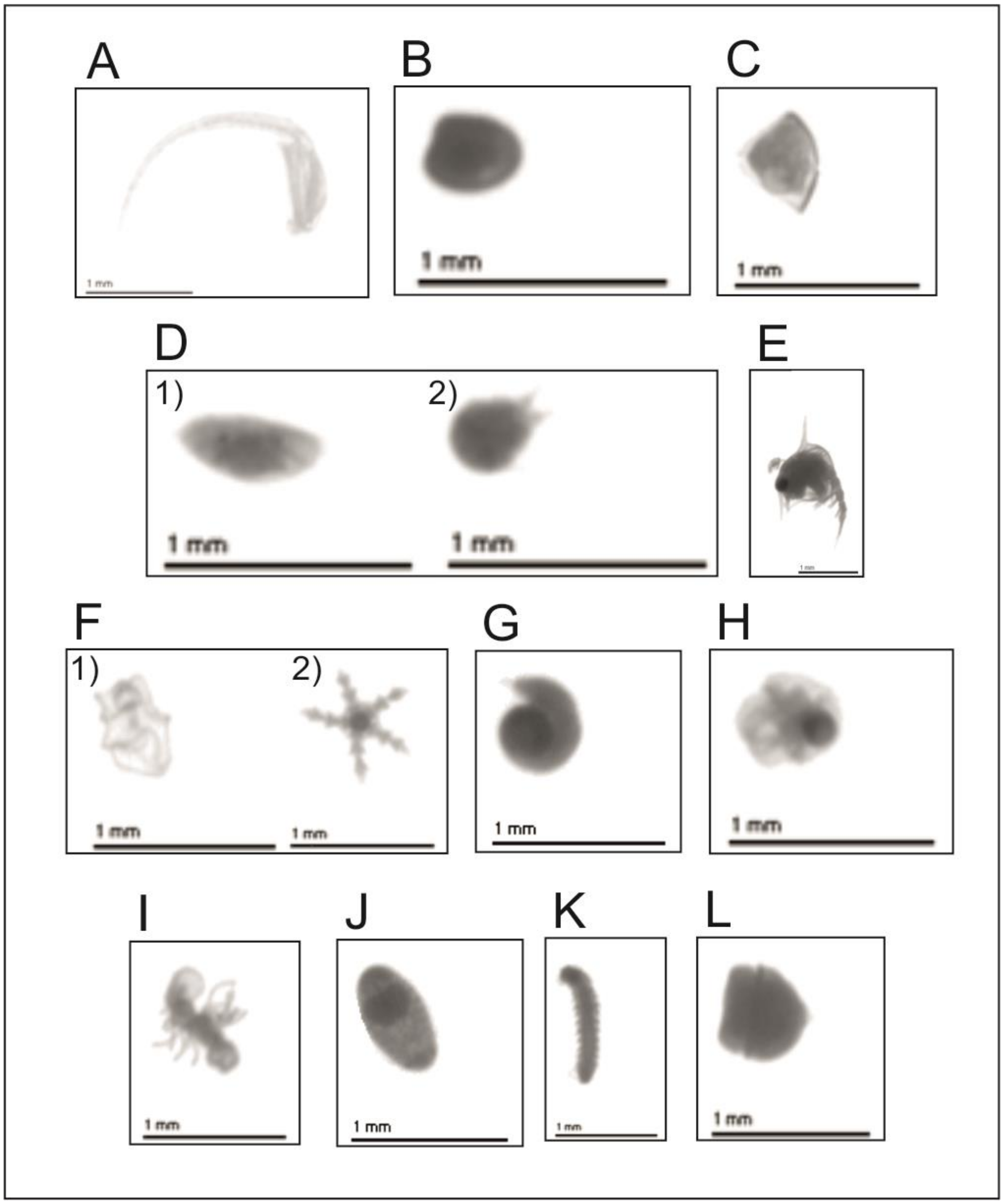

Fig. 4.4 ZooScan vignettes of the various meroplankton groups found in Comau Fjord and Ancud Gulf: (A) Ascidiacea, (B) Bivalvia (veliger), (C) Bryozoa (cyphonautes), (D) Cirripedia ((1) cypris, (2) nauplius), (E) Decapoda (zoea), (F) Echinodermata ((1) auricularia, (2) juvenile), (G) Gastropoda (veliger), (H) Nemertea (pilidium), (I) Phoronida (actinotrocha), (J) Platyhelminthes, (K) Polychaeta, (L) Polychaeta or Mollusca (trochophora)

In the Ancud Gulf, the meroplankton community was mainly composed of bryozoans, gastropods, echinoderms, and bivalves in order of abundance (29%, 28%, 14%, and 8%, respectively). Here, bivalves and bryozoans were similarly distributed throughout the water column but echinoderms were more abundant in the 0-50 m layer and gastropods below 50 m water depth. Trocophora larvae accounted for a relative abundance of 6%, and polychaetes were rare (4%).

In the rest of the stations, i.e. in Comau Fjord (Telele, SWALL, Huinay, Leptepu), gastropods dominated with a relative contribution of 38% of the total meroplankton community being more abundant below 50 m depth, followed by bryozoans (20%) which were more abundant in surface and intermediate waters (0-200 m) (Fig. 4.3C; Tables 4.2, 4.3). Polychaetes followed bryozoans with a relative contribution of 13% in Comau Fjord being more abundant below 50 m depth. Echinoderms, bivalves and cirripeds had a relative contribution of 9%, 8% and 7.3%, respectively. Echinoderms presented higher abundances below 50 m whereas the relative contribution of bivalves and cirripeds was slightly higher between 0-200 m, except in Huinay where a high abundance of cirripeds was found at 300-400 m. Ascidians were restricted to deep waters (100-450 m), which together with decapods, nemertean, platyhelminths and phoronids represented <1% of the total relative contribution.

Meroplankton seasonality

The meroplankton abundance differed significantly among seasons (p <0.05). Highest abundances were found in spring (day and night), while the lowest abundance was observed in autumn during the day (Fig. 4.5A). The meroplankton community was dominated by different taxa among seasons. Echinodermata were predominant in spring (12,722 ± 2,749 ind. m^{-2} (mean ± sd); 42% of the total meroplankton), Gastropoda prevailed in summer and autumn (4,003 ± 1,456 ind. m^{-2}; 35% of the total meroplankton in summer and 2,523 ± 2,620 ind. m^{-2}; 28% of the total meroplankton in autumn), and Bryozoa had the highest abundances in winter (6,550 ± 2,362 ind. m^{-2}; 56% of the total meroplankton; Table 4.2, Fig. 4.5B). Together, echinoderms, gastropods, and bryozoans accounted for 66% of the total meroplankton community on an annual average (spring 70%, summer 62%, autumn 60% and winter 72%). The larval abundance of Ascidiacea, Cirripedia, Phoronida (only present in spring), Nemertea and Platyhelminthes (absent in autumn) were low throughout the year.

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Table 4.2 Seasonal integrated abundances (ind. m^{-2}) of major meroplankton taxa over 450 m water depth. Spring and autumn values are exclusively from the station Telele, while summer and winter values are the average of the abundance from all stations (Ancud Gulf, Telele, SWALL, Huinay and Leptepu). Bold numbers indicate taxa with highest abundances within one season of the whole meroplanktonic community. *Note that Trochophora larvae represent both, polychaete and mollusc taxa.

Taxa			SPRING		SUMMER		AUTUMN		WINTER	
			Day	Night	Day	Night	Day	Night	Day	Night
Annelida	Polychaeta		3,378	4,028	757	878	348	3,352	711	758
Arthropoda	Crustacea	Cirripedia	613	806	1,593	2,033	96	133	671	423
		Decapoda	166	21	8	148	0	26	146	55
Bryozoa			3,087	2,022	1,469	1,996	572	1,333	**8,220**	**4,880**
Echinodermata			**14,666**	**10,778**	1,147	1,322	1,000	1,078	734	467
Mollusca	Bivalvia		4,386	1,710	595	907	702	915	2,218	1,118
	Gastropoda		3,368	7,733	**2,974**	**5,034**	670	**4,376**	1,539	776
Nemertea			1,029	275	38	59	10	21	66	33
Phoronida			104	0	0	0	0	0	12	34
Platyhelminthes			0	99	19	21	0	0	11	17
Tunicata	Ascidiacea		114	130	95	45	91	104	97	34
		*Trochophora	873	676	164	1,190	78	429	100	51
	TOTAL		31,784	28,276	8,793	13,405	3,568	11,766	14,291	8,507

Table 4.3 Mean vertical distribution of meroplankton taxa (ind. m^{-3}) along a transect in Comau Fjord. *Note that Trochophora larvae represent both, polychaete and mollusc taxa.

			SUMMER																					
			Ancud Gulf			Telele						SWALL						Huinay					Leptepu	
			0-50	50-100	100-200	0-50	50-100	100-200	200-300	300-400	400-450	0-50	50-100	100-200	200-300	300-400	400-450	0-50	50-100	100-200	200-300	300-400	0-50	50-100
Annelida	Polychaeta		6	0	0	13	6	3	4	4	2	6	2	1	1	0	2	2	2	1	1	0	5	2
Arthropoda	Crustacea	Cirripedia	4	0	0	33	4	0	0	0	0	32	1	0	0	0	0	76	4	1	0	6	5	1
		Decapoda	2	0	0	2	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	2	0
Bryozoa			17	1	1	63	2	1	0	0	0	32	2	0	0	0	0	31	2	0	0	0	3	0
Echinodermata			40	4	1	13	7	2	2	2	1	5	0	1	0	0	1	3	2	2	0	1	1	1
Mollusca	Bivalvia		13	3	1	5	1	1	1	1	1	2	1	0	0	0	0	19	1	0	0	0	0	0
	Gastropoda		15	7	7	33	27	11	15	15	12	22	7	5	3	2	11	75	12	3	1	8	2	1
Nemertea			0	0	0	2	1	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0
Phoronida			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Platyhelminthes			0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tunicata	Ascidiacea		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
		*Trochophora	17	1	1	3	0	0	0	0	0	2	0	0	0	0	0	4	1	0	0	0	0	0

Continued Table 4.3 Mean vertical distribution of meroplankton taxa (ind. m^{-3}) along a transect in Comau Fjord. *Note that Trochophora larvae represent both, polychaete and mollusc taxa.

			WINTER																					
			Ancud Gulf			Telele						SWALL						Huinay					Leptepu	
			0-50	50-100	100-200	0-50	50-100	100-200	200-300	300-400	400-450	0-50	50-100	100-200	200-300	300-400	400-450	0-50	50-100	100-200	200-300	300-400	0-50	50-100
Annelida	Polychaeta		4	1	1	11	17	1	1	1	1	3	0	0	0	0	1	1	1	0	0	0	12	9
Arthropoda	Crustacea	Cirripedia	6	5	1	30	1	1	0	0	0	1	0	0	0	0	0	3	1	0	0	0	3	0
		Decapoda	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	7	1
Bryozoa			40	10	9	182	4	2	1	1	0	87	2	3	0	0	0	105	17	3	0	0	165	3
Echinodermata			4	1	1	15	3	1	1	0	0	3	0	0	0	0	0	5	1	1	1	0	14	4
Mollusca	Bivalvia		4	0	1	20	3	3	0	1	0	5	0	0	0	0	0	3	0	0	0	0	116	6
	Gastropoda		9	5	7	10	3	1	2	2	1	2	0	1	1	0	1	9	3	2	2	1	23	15
Nemertea			0	0	0	2	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0
Phoronida			1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Platyhelminthes			0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tunicata	Ascidiacea		0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		*Trochophora	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0	1

Abundance also differed between day and night, in which the abundance of daytime was higher in spring and winter, but higher in nighttime in summer and autumn. (Fig. 4.5A). Higher abundances of gastropods were observed in spring and autumn at night whereas in summer and winter similar daily abundances were found. On the other hand, bivalves had higher abundances during day in spring and autumn and similar abundances in summer and winter. In winter, bryozoans were much more abundant during day than at night.

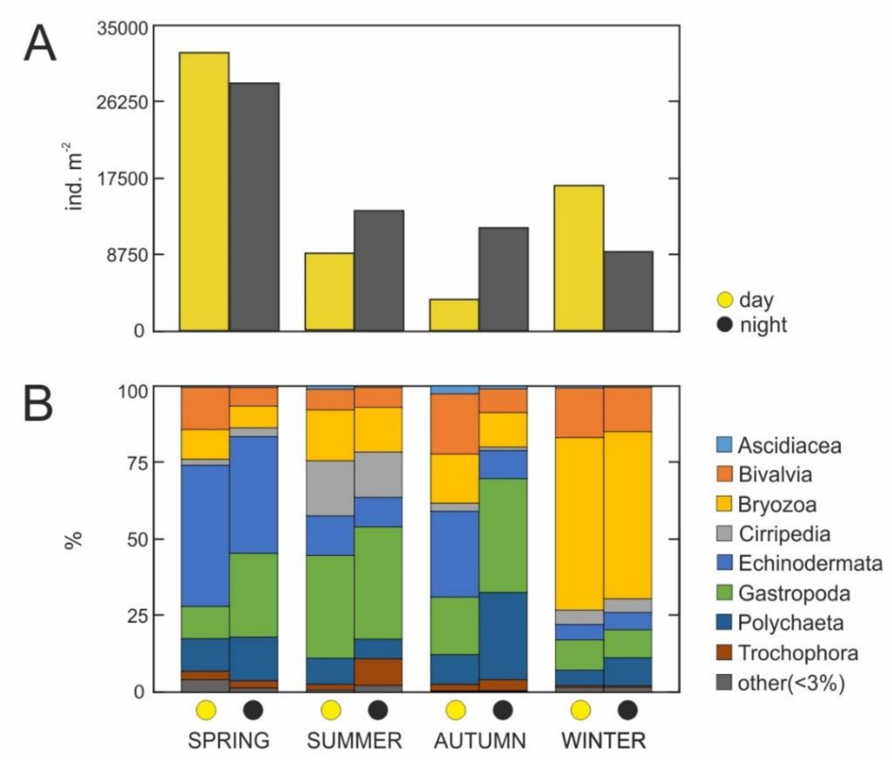

Fig. 4.5 Seasonal distribution of (A) total integrated meroplanktonic abundance and (B) meroplankton community composition. Note that spring and autumn are only from the central station Telele while summer and winter include also the other Comau Fjord stations and Ancud Gulf.

The multiple linear regression analysis (RDA) showed only weak correlations between environmental parameters (temperature, salinity, oxygen and Chl *a* concentration) and meroplankton abundance (p <0.05), explaining 28.52% of the total variability (Fig. 4.6). The first RDA axis explained 24.86% of the meroplankton abundance variability, which was primarily related to oxygen and Chl *a* and oxygen concentration as well as salinity. The second axis only explained 3.66% of the abundance variability and was mostly related to temperature. Therefore, RDA results indicated that the meroplankton was weakly related to changes in oxygen and Chl *a* as well as (inversely) to salinity. This was in association with the depth of the water column throughout the fjord, with shallow waters showing a strong correlation with oxygen and Chl *a* concentration as well as temperature, whereas salinity had a stronger influence in deeper waters (Fig. 4.6A). The abundance of most taxa was influenced by oxygen and Chl *a* concentration (Fig. 4.6B), with higher abundances in surface waters where higher concentrations of these parameters were detected. Ascidians, by contrast, were influenced by salinity, reflecting their higher occurrence below the halocline. Oxygen was inversely correlated with salinity, the abundances of Bryozoa, Cirripedia and Decapoda were positively related to oxygen concentration and thus to shallow water layers. Temperature explained less than 4% of the observed variability (Fig. 4.6B), suggesting a minor role on meroplankton distribution.

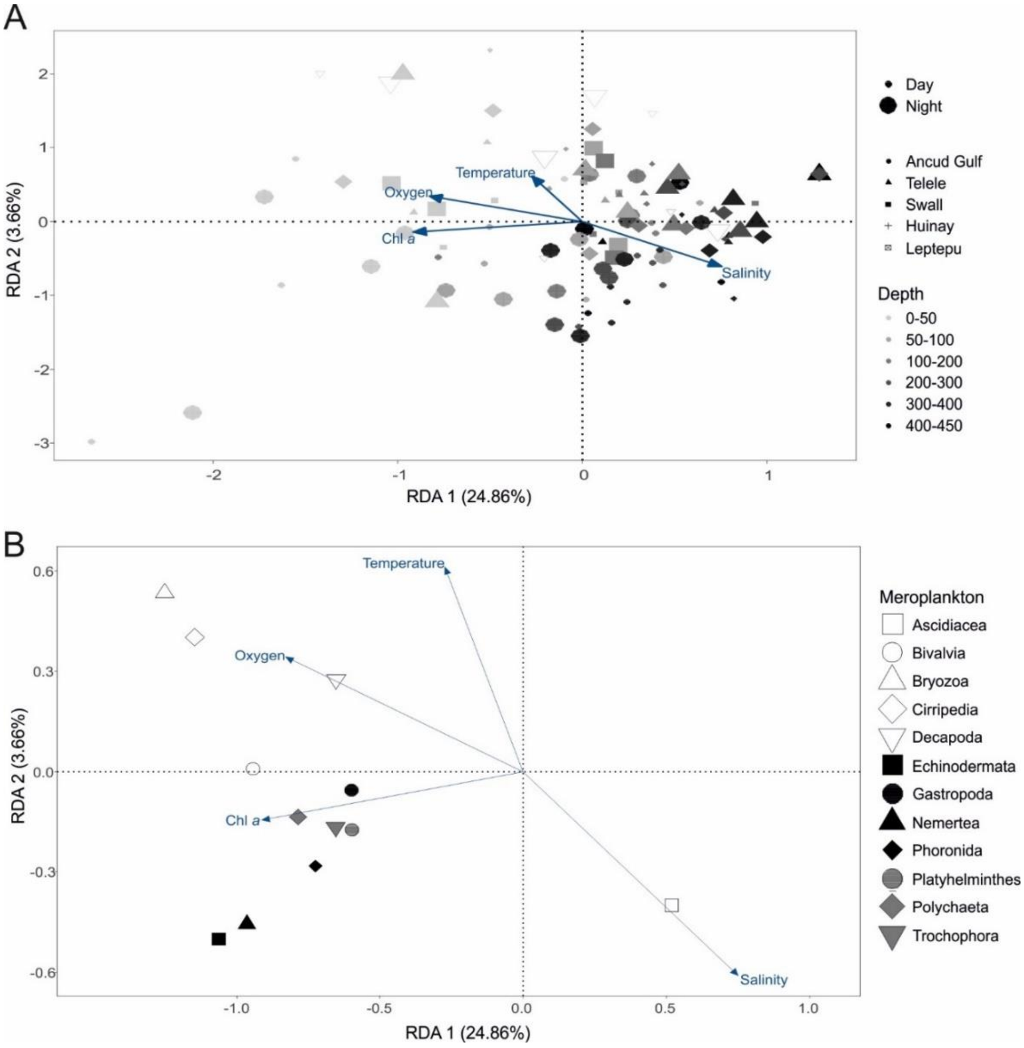

Fig. 4.6 Redundancy analysis (RDA) ordination biplot for meroplankton composition (all samples) constrained by four variables: temperature, salinity, oxygen and Chl *a*. (A) represents the effects of environmental factors on seasonality and (B) on meroplankton taxa.

4.5. Discussion

Data from this study revealed that meroplankton assemblages in Comau Fjord have a marked vertical and seasonal distribution. The most abundant taxonomic groups observed within the meroplanktonic community were bivalve, bryozoan, cirriped, echinoderm, gastropod and polychaete larvae. All of these benthic invertebrates reproduce by pelagic planktotrophic larvae (Strathmann, 1971; Scheltema, 1986; Ó Foighil, 1989; Newman and Ross, 2001; Young, 2002), which main source of food comes from phytoplankton feeding. Typically, benthic invertebrates that produce planktotrophic larvae release an extremely high number of pelagic larvae to counterbalance the high mortality rates (Jablonski and Lutz, 1983). When food is abundant, usually in spring, these groups have been observed to dominate the meroplankton abundance in in other fjords and channels from southern Patagonia (i.e. Gallegos Sound, Magellan Strait and Beagle Channel) (Thatje et al., 2003; Aguirre et al., 2012; Pineda-Metz and Montiel, 2021).

In Comau Fjord, the thermal density stratification combined with a silicate-rich surface waters from freshwater input triggered an increase in phytoplankton biomass mirrored in a high chlorophyll *a* fluorescence in spring and summer. Meroplankton abundances were higher in spring than in the rest of the year (except winter in Leptepu), suggesting that most of these larvae depend on phytoplankton production as a food source. Higher abundances in spring were also observed for meroplankton in the Magellan region (Antezana, 1999; Palma, 2008), in the Southern Ocean (Schnack-Schiel and Isla, 2005), off central Peru (Criales-Hernández et al., 2008), in Abrolhos Bank, eastern Brazil (Koettker and Lopes, 2013), and in northern Norway (Falk-Petersen, 1982). Chl *a* fluorescence peaked in the uppermost 5 m in spring in Telele, indicating optimal conditions for a rapid spring phytoplankton bloom and high primary production (Iriarte et al., 2007; González et al., 2010). Higher abundances of meroplankton were observed in surface layers (0-50 m) (Fig. 4.3). Vertical distribution patterns were similar at each station (i.e. highest abundances were always found in surface waters) (Fig. 4.6), suggesting that the estuarine circulation along the fjord acts as a retention area for larvae of benthic invertebrates, and that larval dispersal may occur within the range of this area restructuring the benthic community (Acha et al., 2004).

Spawning of benthic invertebrates has often been associated with cues provided by phytoplankton (Starr et al., 1990, 1991), and thus spring is the start of the period when meroplankton appears (Michelsen et al., 2017). However, not all benthic species have the same spawning cues nor times, resulting in very different meroplankton communities throughout the year, which also holds true for Comau Fjord. For instance, the spawning of cirripeds is triggered by higher phytoplankton concentrations, and therefore higher abundances of cirripeds are usually found in spring when phytoplankton blooms develop (Falk-Petersen, 1982; Starr et al., 1991; Meerhoff et al., 2014a; Stübner et al., 2016; Michelsen et al., 2017). Our results, however, showed that cirriped larvae were most abundant in summer when Chl *a* concentration was high. As drifting time of cirriped larvae can take between one week to almost two months (Riedl, 1966), it is possible that cirripeds spawned during the spring bloom and we sampled the drifting larva few weeks later in summer. The predominant meroplanktonic group in spring was echinoid larvae (Fig. 4.5), which is in accordance with previous findings from the Magellan region under stratified conditions (Defren-Janson et al., 1999). Accordingly, juvenile ophiuroids were found in the plankton from summer onwards and in higher abundances in autumn, suggesting that settlement of these taxa occurred within a few months. Gastropod and bivalve larvae were also common in spring (Table 4.2). Similarly, these groups were also found dominating in Peconic Bay, in the Long Island estuary during spring and early summer (Turner, 1982). Over time, the meroplankton in Comau Fjord shifted towards a gastropod dominated community in summer and a bryozoan dominant community in winter. The dominance of bryozoans in winter has also been found in the Gallegos Sound, Magellan region (Pineda-Metz and Montiel, 2021), and in sub-Arctic environments such as Porsangerfjord and Adventfjorden in northern Norway (Kuklinski et al., 2013; Stübner et al., 2016; Michelsen et al., 2017), and in Godthåbsfjord in southwest Greenland (Smidt, 1979). The fact that high abundance of certain meroplankton groups may be found in winter indicates that some benthic invertebrates are spawning during this time so that their larvae are able to feed upon the winter phytoplanktonic community, which is different from that of spring (González et al., 2010), if they are planktotrophic. Otherwise, lecithotrophic larvae will use up their yolk. One of the advantages of spawning during seasons with lower phytoplankton concentration (i.e. autumn or winter) is that larvae are less exposed to predation than taxa that spawn in spring or summer, when abundances of predatory zooplankton are highest. If the planktotrophic larvae have a long residence time, they are present when the phytoplankton blooms occur, so they can benefit from an abundant food source without delay.

One important driver in structuring the meroplankton dynamics and distribution is the variability of hydrographic conditions (Schlüter and Rachor, 2001; Fetzer and Arntz, 2008). A strong vertical salinity and temperature gradient was observed along the hydrographic section through Comau Fjord to the Ancud Gulf, similar to other Patagonian fjords and channels, such as Reloncaví fjord, the Puyuhuapi, Martinez and Baker channels (Silva et al., 1997). According to our hydrographic profiles, surface temperature was always higher in summer and autumn (Fig. 4.2A), which is related to the warming of the surface water due to the increase in solar radiation (Silva et al., 1997). Rising water temperatures often act as a trigger for benthic invertebrate spawning and must be above a minimum value for benthic organisms to spawn (Thorson, 1936). However, in Comau Fjord, temperature seemed to have little impact on the meroplankton abundance suggesting that other parameters may have a higher impact. Chl *a* and oxygen concentrations, on the other hand, showed a significant relationship with the abundance of most taxonomic groups, suggesting that meroplankton abundance matched with the spring phytoplankton bloom when there was a greater food supply. We also observed that the abundances of most meroplankton larvae were inversely related to salinity (except for ascidians), suggesting that most of the groups were found in surface waters where lower salinity was detected. However, it is possible that the influence of Chl *a* concentration (higher in surface waters) had a stronger effect on the abundance of meroplankton and that their physiological response to salinity was more resilient in environments with high food availability. Only the abundance of ascidian larvae was highly influenced by salinity, suggesting that these larvae preferred to reside in deeper, more saline water close to their parental habitats (Vázquez and Young, 1996).

Inter-annual variability in hydrographic conditions can cause large differences in meroplankton abundance and dynamics from one year to another. Inter-annual variation in mollusc, echinoderm and decapod larvae is common due to changes in food availability and temperature conditions (Kirby et al., 2008). For instance, in 2009-2010, lower Chl *a* concentrations and sea surface temperatures lead to a disruption in larval supply in the Inner Sea of Chiloé (Lara et al., 2016). In 2019, Wiesebron et al. (2022) found much higher Chl *a* concentrations in the Ancud Gulf in autumn than those we observed during the present study in 2017. At that time, the meroplankton community was dominated by bryozoan and echinoid pluteus larvae, whereas in our study neither of these groups was particularly dominant during the autumn season. Therefore, this inter-annual variability in the hydrography has profound consequences for meroplankton dynamics and composition, as well as influencing the spawning cues for benthic invertebrates.

Understanding seasonal and inter-annual variations in meroplankton dynamics will help to develop management strategies in environments where anthropogenic perturbations may have consequences for bentho-pelagic communities. Recent investigations suggest that rapid changes in environmental parameters due to human actions have higher implications for meroplanktonic communities than for holoplankton (Richardson, 2008). For instance, alterations in aragonite and calcite saturation and sea-surface temperature have negative consequences for the spawning cues and survival of larval stages of benthic shelled molluscs, affecting their growth rates, as their shell formation requires $CaCO_3$ available in seawater (Iriarte, 2018). In Chilean fjords, human activities (e.g. nutrient inputs from extensive aquaculture or terrestrial changes due to forestry) have been increasing over the past decades (Iriarte et al., 2010; Buschmann et al., 2021; Navedo and Vargas-Chacoff, 2021). Therefore, as meroplanktonic communities are the foundation for the survival of benthic invertebrate populations, it would be important to monitor the abundance, composition and developmental stages of meroplankton in the fjords, to understand the roles of natural and anthropogenic factors on bentho-pelagic coupling. Only this way we can predict the composition and abundance of future meroplankton communities and thus benthic communities.

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Chapter 5 – Trophic relationships

Lipid biomarkers reveal trophic relationships and energetic tradeoffs in contrasting phenotypes of the cold-water coral *Desmophyllum dianthus* in Comau Fjord, Chile

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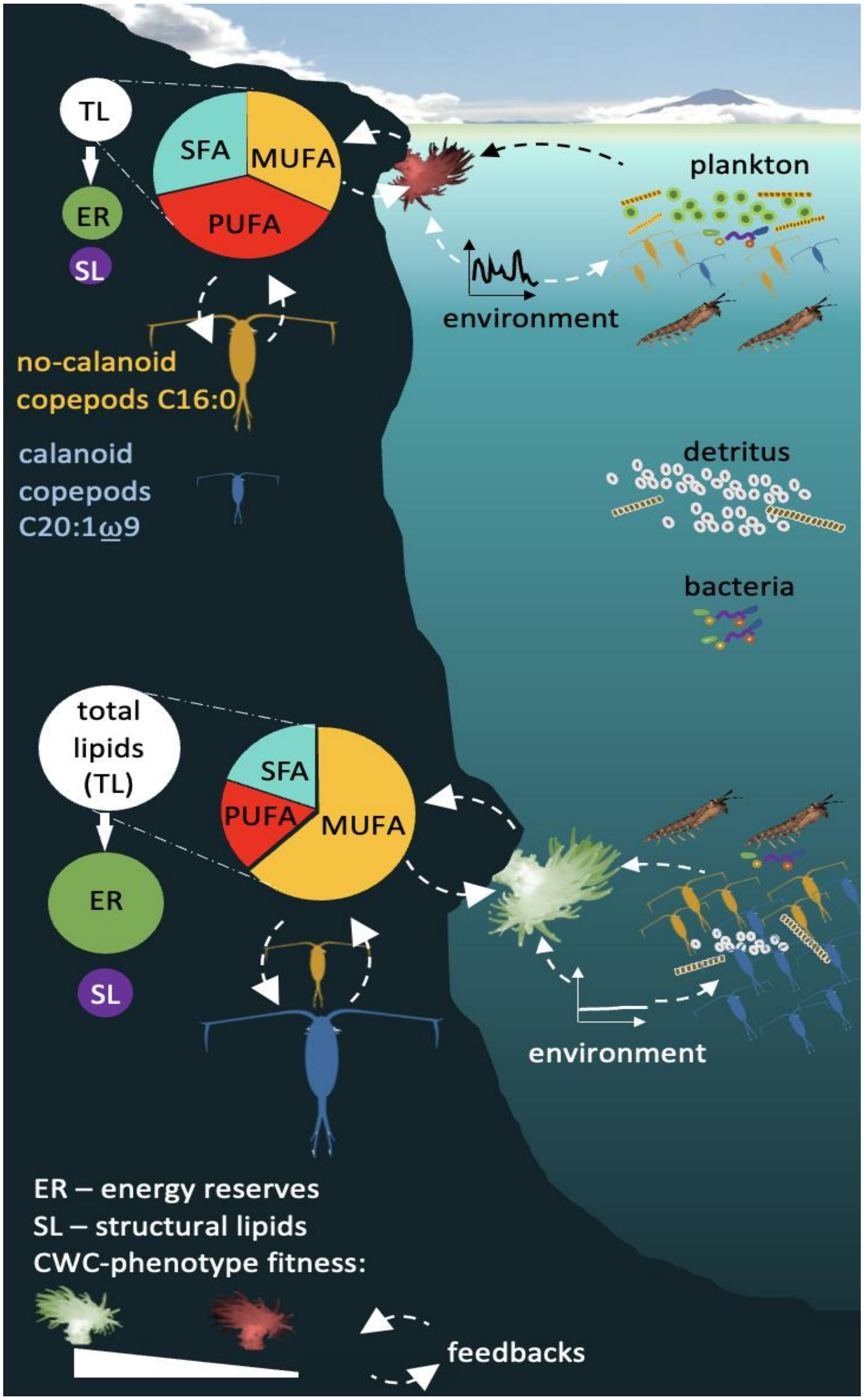
5.1. Abstract

Cold-water corals (CWCs) are considered wide-ranging opportunistic filter feeders, but little is known on their actual food *in situ*. To tackle this limitation, fatty acid trophic markers (FATM) have been employed to gain dietary insights. Yet, these *in situ* studies have not been combined with physiological investigations to understand how physiological limitations modulate the coral biochemistry. Here, we studied *Desmophyllum dianthus* in its natural habitat in Comau Fjord (Northern Patagonia, Chile) to assess the trophic ecology of this pan-globally distributed CWC in response to differing physico-chemical (variable vs. stable) and ecological drivers (food availability). Contrasting coral phenotypes were collected from shallow (20 m) and deep (300 m) sites with known fitness differences and improved performance (growth, biomass, energy reserves) in 300 m water depth. We analyzed the corals' fatty acid composition to evaluate the utility of FATM profiles to gain dietary insights and assess how fitness trade-offs potentially modulate the corals' FATM composition. We find that 20:1(n-9) zooplankton markers dominate the deep site, while 20:5(n-3) and 22:6(n-3) diatom and flagellate markers, respectively, are more prominent in shallow waters. The zooplankton diet supports higher energy stores in deep corals, in spite of measured lower zooplankton availability. Interestingly, essential FA concentrations are conserved across sites and likely reflect required levels for coral functioning and survival. While deep corals can easily meet these requirements, shallow-water corals appear to need more energy to maintain vital levels in their variable environment, potentially causing intrinsic re-allocations of energy and enrichment in certain essential markers (20:5(n-3), 22:6(n-3)). Our analysis highlights the biological and ecological insights that can be gained from FATM profiles in CWCs, but also cautions the reliability of FATM as diet tracers under limiting environmental conditions.

Keywords:

Cold-water coral | *Desmophyllum dianthus* | lipid biomarker | trophic ecology | essential fatty acids | physiological requirements

Graphical abstract



5.2. Introduction

Cold-water corals (CWCs) are important foundation species in all deep and dark ocean waters (Freiwald and Roberts, 2005; Roberts et al., 2006), where they create complex habitats that sustain a high biodiversity of up to 900 species (Rogers, 1999), including many commercially important fish species (Fosså et al., 2002; Buhl-Mortensen and Mortensen, 2004; Roberts et al., 2006). Their remoteness and depth have hampered early discovery and exploration, but advances in underwater technology providing submersible and remotely operated vehicles (ROV) has allowed much progress over the last decades (Freiwald et al., 2009). The combination of CWC distribution data, physico-chemical characteristics (temperature, salinity, oxygen, turbulence) and modelling now allow to predict suitable CWC habitats (Davies et al., 2008; Davies and Guinotte, 2011; Morato et al., 2020). In addition, both *ex situ* (e.g. Georgian et al., 2016; Maier et al., 2016; Büscher et al., 2017) as well as *in situ* (e.g. Jantzen et al., 2013a; Lartaud et al., 2017; Büscher et al., 2019; Chapron et al., 2020; Rossbach et al., 2021) manipulative experiments enable us to identify the drivers governing their health and vulnerability to human pressure as well as climate change and thus, their occurrence as well as distribution today and in the future. Among the biological drivers, productivity and the supply of particulate organic carbon are considered important (e.g. Davies et al., 2008; Davies and Guinotte, 2011; Morato et al., 2020), but not consistently linked to habitat suitability (see discussion in Davies and Guinotte, 2011). Field observations underpin such variable modelling results and CWC reefs can be found in areas of variable (both high and low) surface productivity and thus, food availability (e.g. Dodds et al., 2009; Cau et al., 2017; Gori et al., 2018; Hebbeln et al., 2020). This indicates the corals' broad tolerance towards a range of nutritional conditions as well as their ability to take up various food sources (Naumann et al., 2011; Mueller et al., 2014; Höfer et al., 2018; Maier et al., 2019).

The availability of food, however, affects the corals' physiological performance, like their metabolic rate and growth (Naumann et al., 2011; Larsson et al., 2013; Maier et al., 2021) and it was shown that higher food availability can compensate for otherwise limiting conditions (e.g. Georgian et al., 2016; Maier et al., 2016; Büscher et al., 2017; Martínez-Alarcón et al., 2019). While CWCs may be quite tolerant and resilient to projected future changes in temperature and aragonite saturation state alone (e.g. Hennige et al., 2015; Büscher et al., 2017; Dorey et al., 2020; but also see e.g. Maier et al., 2009; Georgian et al., 2016; Gori et al., 2016), combined changes in physico-chemical conditions and food availability have been shown to compromise the calcification rates of CWCs (Hennige et al., 2015; Büscher et al., 2019). Corals are suspension-feeding omnivores gaining their metabolic energy from a variety of food particles ranging from microbes to phyto- and zooplankton (Duineveld et al., 2004; Mueller et al., 2014; Höfer et al., 2018; Maier et al., 2019; Rakka et al., 2021), where the zooplankton and krill appear to provide a key energy source to sustain their metabolic needs (Naumann et al., 2011; Maier et al., 2019, 2021). Thus, the relative availability of food along the plankton size spectrum *in situ* may be crucial to CWCs' response to future changes in their environment. However, still little is known about CWCs' feeding ecology in the field and the role of food on coral *in situ* fitness as well as performance.

Lipids, which constitute up to 40% of the corals' biomass (Harland et al., 1993) and their fatty acids, are promising biochemical markers providing insights into the corals' nutritional status, ecology and health (e.g. Dalsgaard et al., 2003; Imbs and Yakovleva, 2012; Rocker et al., 2019; Kim et al., 2021). Lipids provide more metabolic energy per gram than any other tissue compound like proteins or carbohydrates (Bureau et al., 2002) and represent a diverse group of large biological molecules (Joseph, 1979; Imbs et al., 2019). Dietary lipid is required by animals for the provision of metabolic energy, generated as ATP through the oxidative metabolism of fatty acids, and for the production of polar lipids, i.e. phospholipids and sphingolipids, necessary for the formation of cell membranes. This dual role is reflected in the compartmentalization of body lipid into adipose tissue, composed mainly of wax esters and/or triacylglycerols, and cell membrane lipid composed mainly of polar lipids and cholesterol (Sargent et al., 1993). Their balance is critical for the corals' stress resistance (Yamashiro et al., 2005; Imbs and Yakovleva, 2012). Lipids and their fatty acids (FAs) are involved in the majority of biochemical and physiological processes in organisms (Tocher, 2003; Russo, 2009).

The analysis of FA and fatty alcohols (FAlc) composition - so called fatty acid trophic markers (FATM) - has been widely used to trace the main food sources in marine organisms (e.g. Graeve et al., 1997; Dalsgaard et al., 2003; Budge et al., 2006). The lipid content as well as lipid class composition, together with the FATM profiles of marine organisms provide insight into the biochemical and ecological conditions of their surroundings (Bergé and Barnathan, 2005). Similarly, specific fatty acid ratios (e.g. DHA/EPA, n-3 PUFA/n-6 PUFA) serve as useful tracers of nutritional conditions (Graeve et al., 1997; Dalsgaard et al., 2003; Budge et al., 2006). At the same time, many PUFAs are essential for coral functioning and health and their biosynthetic pathways together with the organism's ability to modify FATM marker signal should be considered as this can modify the pure dietary FATM signal (Rocker et al., 2019; Galloway and Budge, 2020; Kim et al., 2021). Biosynthetic pathways are clearly described for phyto- and zooplankton and these pathways underscore their FATM profiles as well as their ability of trophic upgrading (i.e. the modification of essential dietary precursor PUFAs like 18:3n-3 (ALA - alpha linolenic acid) to essential LC PUFAs like EPA; a schematic biosynthesis pathway are depicted in Fig. 5.S1). *De novo* synthesis of long-chain PUFAs, however, is linked to the unique possession of specific desaturase enzymes (Δ12, Δ15; Kabeya et al., 2020). These are key enzymes in primary producers that synthesize LC PUFA precursors, but these enzymes are lacking in higher trophic levels, e.g. fishes. Recently, evidence is accumulating that also intermediate consumers and invertebrate groups, including tropical corals, do possess such enzymes (Kabeya et al., 2018; Monroig and Kabeya, 2018). A matter of fact that needs to be considered in future FATM studies.

In CWCs, FATM analyses, together with the study of the corals' isotopic composition, could identify the corals *in situ* main food sources, distinguish between phytoplankton- (Duineveld et al., 2004, 2012; Kiriakoulakis et al., 2005) and zooplankton-dominated diets (Kiriakoulakis et al., 2005; Carlier et al., 2009; Dodds et al., 2009) at low and high supply levels (Dodds et al., 2009; Gori et al., 2018). Thus, biomarkers can provide a powerful tool to identify potential differences in prey composition. Since corals are omnivorous (Duineveld et al., 2004; Carlier et al., 2009; Höfer et al., 2018) and not actively select their prey (Höfer et al., 2018) but consume what is provided, CWCs represent an ideal system to infer nutritional condition and trophic ecology from FATM signal (Dodds et al., 2009; Duineveld et al., 2012; Naumann et al., 2015; Gori et al., 2018). It has to be borne in mind, however, that physiological responses to unfavorable conditions (e.g. like eutrophication in tropical corals; Rocker et al., 2019; Kim et al., 2021) may affect the trophic transfer or modification of biomarkers and potentially distort the diet signal (Galloway and Budge, 2020). While in tropical corals first studies addressed the potential changes in biosynthetic pathways under optimal and suboptimal conditions and identified putative FA indicators for coral health (Rocker et al., 2019; Kim et al., 2021), similar investigations for CWCs are still missing.

Here we investigated the suitability of biomarkers to assess dietary composition and health in CWC from contrasting habitats, using *Desmophyllum dianthus* in its natural Comau Fjord environment as our model species. We analyzed the FATM signal in *D. dianthus* individuals sampled along strong environmental gradients (both horizontal and vertical), and analyzed the results against the background of concomitant data on coral physiology (Beck et al., 2022) and zooplankton composition (Garcia-Herrera et al., 2022). This allowed us to assess the FATM signal links to the available diet and possible shifts due to coral physiology influencing the FATM composition. Ultimately, how contrasting CWC phenotypes modulate their lipid composition provides a first baseline on potential physiological constraints on FA biosynthesis pathways in

CWCs, and an important step forward to advance this line of research in these deep-sea foundation species.

5.3. Materials & Methods

Site and collection

The study was conducted in the Comau Fjord, Northern Patagonia, Chile (Fig. 5.1), one of the few places worldwide where CWC emerged from the deep and inhabited shallow waters (Häussermann et al., 2021b). Thus, local populations of *D. dianthus* are found within the same region in very contrasting environments, between deep and shallow, but also between fjord head and mouth (Beck et al., 2022). To represent the horizontal and vertical environmental gradients, corals were collected at 20 m at three stations (A, E, F) along the horizontal in the fjord, and at two depth (20 m and 280-290 m) at station (E), midway along the horizontal transect. At each sampling location, six *D. dianthus* individuals were collected in September 2016 (06.09.-22.09.) by SCUBA divers (shallow sites) and ROV (deep site, Commander 2, Mariscope Ingeniería, Puerto Montt, Chile; modified with manipulator arms and high-resolution camera). The collection of deep-water specimens required the ROV to be equipped with a wire frame and a water-tight bag attached to scrape the corals from the wall. The detached corals were secured in the bag, sealed and brought to the surface avoiding sample contact with the brackish surface layer.

Environmental and physiological background data

The physico-chemical environmental conditions during the sampling period were characterized along the entire fjord by CTD vertical profiles ranging from the surface to the bottom of the fjord. These CTD casts were conducted between 07.09-12.09.2016 at a total of seven stations in the centre of the fjord (Fig. 5.1, yellow triangles). The CTD (SBE 19plus V2 SeaCAT profiler CTD, Sea-Bird Scientific, USA) was equipped with oxygen (SBE 43 dissolved oxygen, Sea-Bird Scientific, USA) and pH sensors (SBE 18 pH, Sea-Bird Scientific, USA).

Coral calcification, respiration and biomass were assessed subsequently to the coral sampling in a separate year-long *in situ* experiment. Corals were collected at the four sites and prepared for re-installation and re-collected seasonally after 4, 8 and 11 months to assess their performance. In brief, growth was measured through the buoyant weight technique (Jokiel et al., 1978) and respiration through closed-cell incubations. Further, separate corals were prepared and collected at the same seasonal time scales, snap frozen and the coral tissue biomass determined.

The procedure and seasonally resolved responses are presented in Beck et al. (2022). Here, we averaged all seasonal data to obtain a site-specific representation of coral performance. In addition, scaled pictures of the lateral side of the corals were taken in September 2016 after collection to show visual differences of the corals between sites.

Coral sample processing and FATM analysis

After collection, corals were transported to the Huinay field station and maintained in the flow-through aquaria system (for approx. 3-14 days). Corals were then snap-frozen in liquid nitrogen and maintained at -80°C until further processing. In the laboratory, corals were crushed and ground to a relatively fine and homogeneous powder in a liquid nitrogen cooled stainless steel mortar. Subsequently, the total ground material was weighed (ranging from 970-8250 mg per coral individual) and subsampled for downstream analyses (i.e. ash free dry weight (AFDW) determination and fatty acid analysis) as follows: approximately 300 mg per sample were used to determine ash free dry weight (approx. 10% of the whole sample) and a minimum of 700 mg per sample (700-6600 mg, approx. 80% of the whole animal) for lipid concentration and fatty acid composition analyses. The remaining material (approx. 10%) was snap frozen and returned to -80°C. Ash free dry weight was determined by transferring the subsample to a pre-combusted aluminium weighing pan and dried to constant dry weight for approx. 24 hours in an oven at 60°C. Subsequently, the sample was weighed and combusted at 450°C for 6 hours. Ash free dry weight was used to normalise the quantitative lipid and fatty acid composition analyses by extrapolating measurement to the total crushed coral sample and standardised to the organic fraction of the samples.

The lipid sub-sample was freeze-dried for approx. 24 hours and total lipid extracted in dichloromethan:methanol (DCM:MeOH, 2:1 per volume), modified after Folch et al. (1957). Aqueous KCl solution was added prior to centrifugation for phase separation. The extracted total lipid mass was determined gravimetrically and expressed as normalised to AFDW. As an internal standard, tricosanoic acid methyl ester (23:0) was added to each sample (1 µg μl^{-1} in n-Hexan). Transesterification of the lipid extracts was performed with 3% sulfuric acid in methanol for 4 hours at 80°C under nitrogen atmosphere. A gas chromatograph (HP 6890 N, Agilent Technologies, Inc) was used to determine the fatty acid methyl esters and fatty alcohols (Kattner and Fricke, 1986; Graeve et al., 1997). Individual fatty acids (FA) and fatty alcohol compounds (FAlc) were identified by their retention time and compared to known standards. Unknown peaks with less than one percentage contribution were excluded and the remaining chromatograms were evaluated using the Clarity Chromatography Software from DataApex. Total lipid mass per individual was derived by GC-FA/FAlc content from the measurements. Individual FA and FAlc compounds were calculated as relative percentage based on the total FA or FAlc concentration and also normalised to AFDW.

Data analyses and statistics

Analyses were performed using RStudio 1.3.1073 with R version 4.0.2 (R Core Team, 2020; RStudioTeam, 2020). Site-specific differences in both FA and FAlc percentage composition were visualized using heatmaps with hierarchical clustering of both samples and FA/FAlc (package *ComplexHeatmap*, v2.6.2, Gu et al., 2016) and characterized by ordination analyses based on weighted log-ratio analysis (LRA) using the package *easyCODA* (Greenacre, 2018). Log-ratio analyses were performed on the total FA as well as total FAlc marker sets. Within LRA biplots loading vectors were limited to the major contributing markers following the procedure described in *easyCODA* or Greenacre (2018) and Graeve and Greenacre (2020). Here we also summed markers for bacteria-specific origin: i15:0, ai15:0, i17:0 and 18:1(n-7) (Meziane and Tsuchiya, 2000; Boschker and Middelburg, 2002; Brett et al., 2006) to identify their diet contribution. In addition, the sum of FA and FAlc groups (SFA - saturated fatty acids/alcohols, MUFA - mono-unsaturated fatty acids/alcohols and PUFA - poly-unsaturated fatty acids) were derived as both relative and absolute concentration and in addition, ratios of putative FA trophic marker (e.g. 18:1n9 / 18:1n7, DHA / EPA) as well as putative health indicators (e.g. EPA / ARA, PUFAn3 / PUFAn6; Rocker et al., 2019; Kim et al., 2021) were calculated from the FATM signal. One-factorial ANOVA (normal and equal variance), Welch One-Way test (violation of equal variance) or Kruskal-Wallis test (violation of both equal variance and normality) were performed on total lipid composition, FA or FAlc classes, important dietary compounds/ratios (i.e. 20:1(n-9), trophic marker ratio: 18:1(n-9) / 18:1(n-7)) as well as essential FAs or health markers (e.g. EPA, DPA) to identify site-specific differences. Pairwise tests were used (with Tukey or Bonferroni correction) to reveal post hoc differences between individual sites. Fatty acid class composition differences between sites were tested with PERMANOVA in the R package *vegan*.

Plots of the CTD data were performed with Ocean Data View (Schlitzer, 2021). In the along-fjord profiles (Fig. 5.1B), the color scales were adapted to highlight the structure of the entire water column. Site-specific profiles were derived from the CTD profiles closest to each station and depth related average values obtained from these profiles per depth (shallow: 20-30 m and deep: 280-300 m to cover the maximum diurnal range for the region).

5.4. Results

Environmental background

The sampling period marks the end of the austral winter, characterized by a strong temperature inversion near the surface due to atmospheric cooling (Fig. 5.1B, 5.1C). Winter mixing of the upper water column was evident in all parameters measured, showing deep mixing (down to 120 m) of oxygen- and pH-rich surface water in the outer two stations, eroding the suboxic and low-pH subsurface (30-80 m) layer of below the persisting pycnocline in the inner parts of the fjord, where mixing remained shallower than 30 m. Below 200 m, we found uniform conditions with only small vertical or lateral gradients. Shallow corals thrive at the lower edge of the halocline under slightly lower salinity (32.49 ± 0.104 95%-CI) compared to deep corals (33.15 ± 0.0002 95%-CI, Fig. 5.S2).

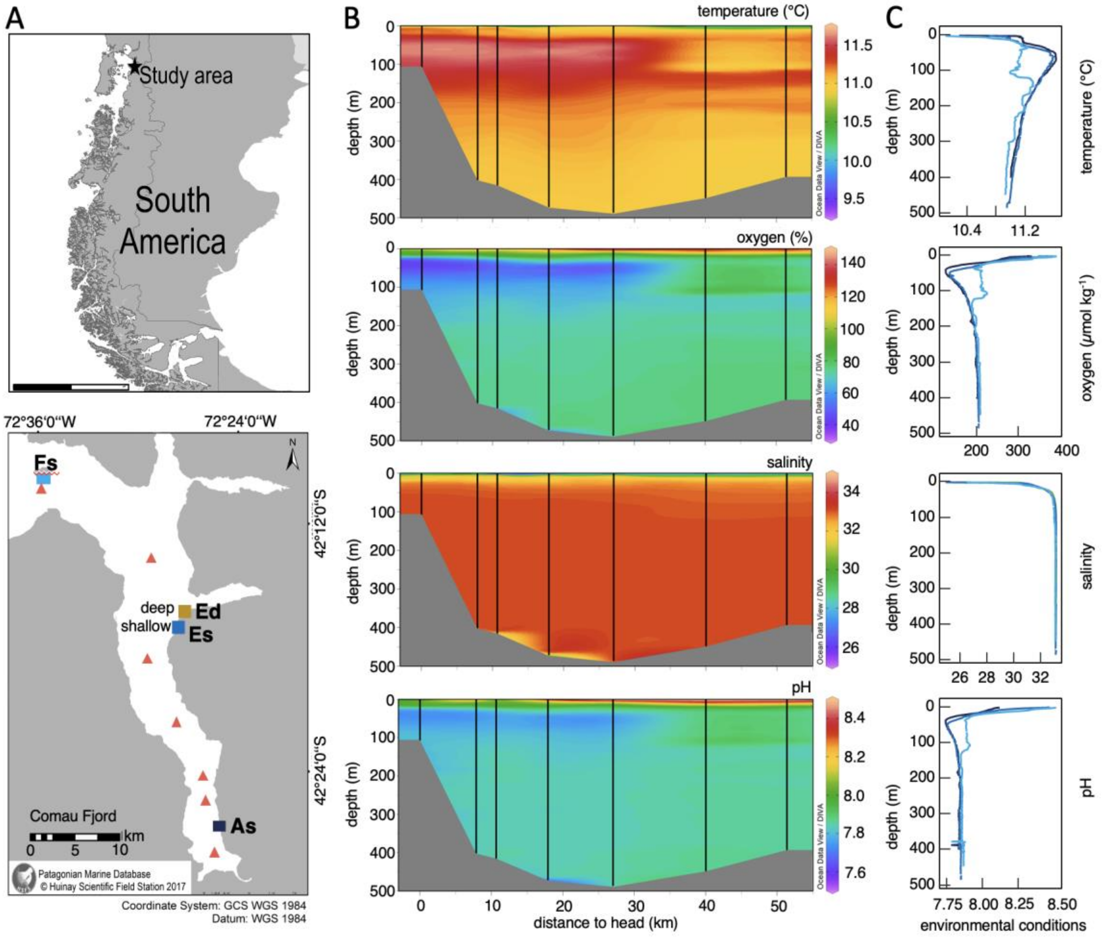


Fig. 5.1 Sample sites and conditions. A) Study area is located in South America (upper panel) in the Fjord Comau in Northern Patagonia, Chile (lower panel). Shallow (20 m) coral sampling sites are denoted with

light to dark blue squares (stations A, E, F), the deep site (280-290 m) in E is denoted in dark yellow. Oceanographic stations along the fjord are marked with red triangles. B) Cross sections of temperature (°C), oxygen saturation (%), salinity and pH from fjord head to mouth and deep to shallow are provided. Sampling stations (triangles in A) are shown by vertical black lines. C) Vertical profiles of temperature, oxygen saturation, salinity and pH at the sampling stations (A, E, F) are shown. Line colors correspond to the blue color hues in (A).

Coral phenotypes and its relative biochemical composition

Desmophyllum dianthus expressed different coral phenotypes between shallow and deep sites in Comau Fjord that differed visually, metabolically as well as biochemically (Fig. 5.2A-5.2D). Corals with highest calcification rates can be found at the deep site and within the shallow sites we found a clear gradient of coral growth from the head towards the fjord mouth (Fig. 5.2B). Biochemically, shallow water corals from all sites were very similar and have almost equal proportion of SFA, MUFA and PUFA (30.04 ± 1.22%, 29.50 ± 1.43%, 40.33 ± 1.54%, respectively), compared to the significantly different deep corals with a specific fatty acid group composition (PERMANOVA df = 3, F = 112.81, p-value <0.001, Fig. 5.2C). Deep coral fatty acids were dominated by MUFA (60.75 ± 1.62%) with lower but equal parts of PUFA and SFA (18.81 ± 0.99%, 20.44 ± 0.71%, respectively). Deep corals stood out with a significantly higher total lipids content (4-fold increase, Welch-test, df = 3, F = 4.09, p-value = 0.038) and content of fatty alcohols (One-Way ANOVA, df = 3, F = 103.95, p-value <0.001, 33.8 ± 4.0% compared to 10 ± 2.8% across all shallow sites, Fig. 5.2C, 5.2D).

Fatty acid trophic marker composition

A total of 44 FA and 8 FAlc markers were determined with very characteristic and different compositions at the two depths. The main FAs differentiating among depth were a long LRA dim 1, which explained 79.1% of the observed variance and in particular involved the FAs 20:1(n-9) and 22:1(n-11), as well as essential FAs like 20:5(n-3) and 22:5(n-3) (Fig. 5.3A). Thus, the clear distinction between deep and shallow phenotypes is mirrored also in the FA and FAlc composition (Fig. 5.S3, Table 5.S1), indicating pronounced dietary differences between deep and shallow corals.

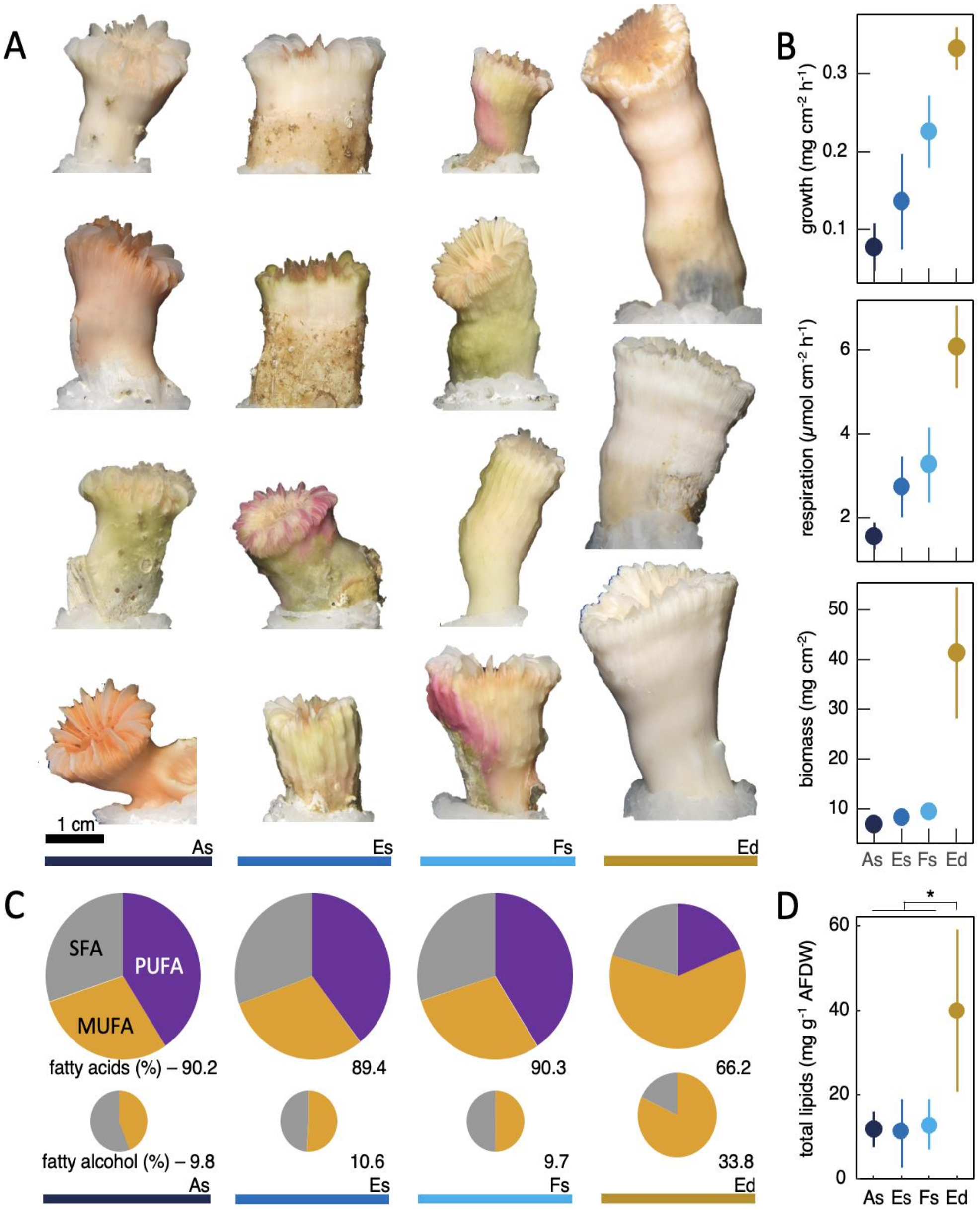


Fig. 5.2 Coral phenotype and lipid composition across sites. A) Coral phenotypes at the shallow sites (first three columns; sites are depicted by dark blue, blue and light blue color bars) and the deep site (dark yellow horizontal bar). B) Coral traits across sites in terms of growth, respiration and biomass are provided and assessed during September 2016 to August 2017 (Beck et al., 2022). C) Coral fatty acid trophic marker (FATM) composition is depicted in terms of fatty acids and fatty alcohols (upper and lower panel, respectively; circle diameters reflect percentage of fatty acids and fatty alcohols of total lipids at the given sites) and pie charts further indicate their specific composition (SFA: saturated fatty acid/alcohols, grey; MUFA: monounsaturated fatty acids/alcohols, orange; PUFA: polyunsaturated fatty acids, violet). D) The corals' total lipid content across sites. Shallow sites are indicated by As, Es, Fs (colored in dark blue, blue and light blue, respectively) and the deep site by Ed (colored in dark yellow). Data are mean ± 95%-confidence intervals.

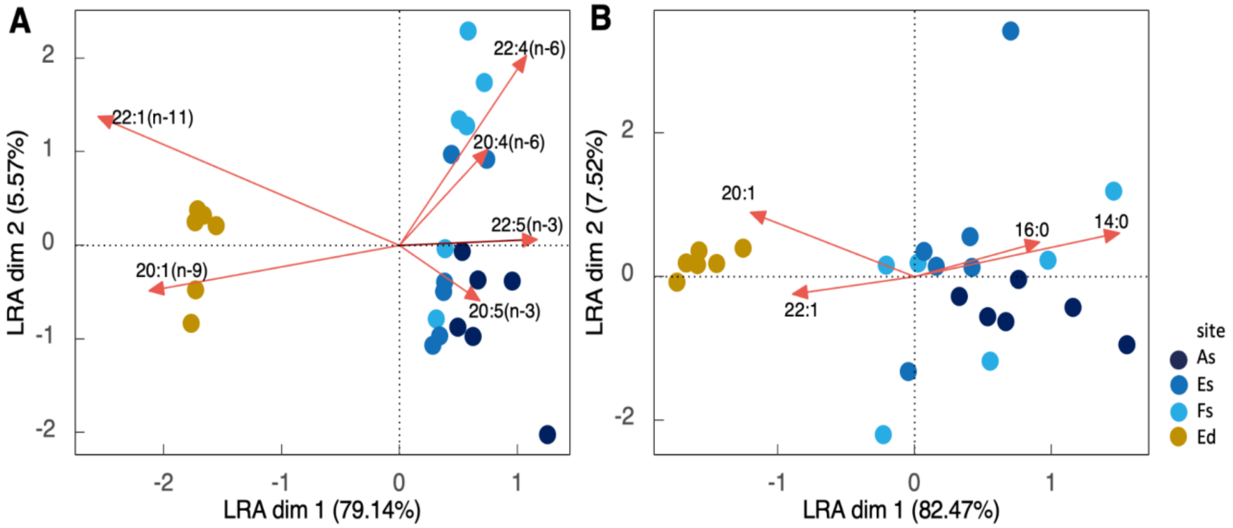


Fig. 5.3 Log-ratio analysis (LRA) of the relative fatty acid trophic marker composition of *Desmophyllum dianthus*. Biplot of LRA of 44 fatty acids (FA, in A) and 8 fatty alcohol (Falc, in B) in specimens (circles) from four different sites. The LRA dimension 1 and 2 are differentiated by depth and by within shallow variance, respectively. Only markers (FA, Falc) that contribute highly to the separation of the samples are shown in red.

The trophic marker ratio 18:1(n-9)) : 18:1(n-7) >3, (Fig. 5.4A, Graeve and Greenacre, 2020) is slightly higher at the deep site, indicating a higher trophic level, i.e. more carnivorous diet. Phytoplankton markers, by contrast, are higher in shallow water corals (e.g. 20:5(n-3) and 22:5(n-3) with 14-12% and 4.5-3.8% in shallow water compared to 7 and 2.5% in deep, respectively; Figs. 5.3A, 5.S3), indicating a more herbivorous diet. The shallow corals' FATM profiles (Fig. 5.3A) showed a stronger contribution of diatom markers (20:5(n-3), EPA) compared to dinoflagellates markers (22:5(n-3), DHA) across sites (Figs. 5.3A, 5.4B). However, EPA and DHA are also major compounds of the membranes and other algal markers are less distinct (e.g. 16:1(n-7) or 18:4(n-3) with 4.12 ± 0.84 and 0.87 ± 0.25 in shallow compared to 6.53 ± 0.61 and 0.57 ± 0.09 in deep, respectively). In addition, the contribution of bacterial markers to the individuals' biochemical composition is higher in shallow corals (marginally significant, $F = 2.798$, $p = 0.067$), but in general its overall contribution to the trophic structure was negligible.

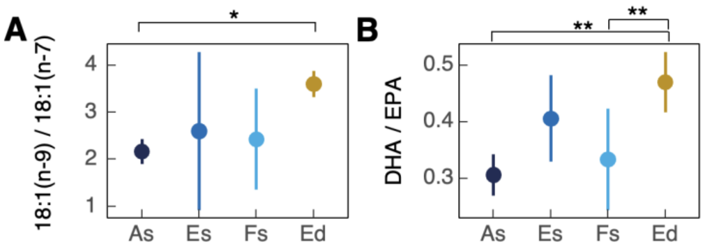


Fig. 5.4 Fatty acid trophic marker ratios. Ratio of specific markers across sites provide insights into trophic position A) 18:1(n-9) / 18:1(n-7) or trophic network composition B) DHA / EPA (DHA: docosahexaenoic acid 22:6(n-3), EPA: eicosapentaenoic acid 20:5(n-3)). Data are mean ± 95%-confidence intervals. Shallow sites are indicated by As, Es, Fs and colored in dark blue, blue and light blue, respectively, and the deep site by Ed and colored in dark yellow. Significant differences are denoted by * ($p \leq 0.05$), ** ($p \leq 0.01$).

Absolute fatty acid composition, essential fatty acids and potential mobilization and modification of fatty acids

The higher lipid content but lower proportion of PUFA and SFA in deep corals result in very similar total PUFA (One-Way ANOVA df = 3, F = 1.621, p-value = 0.216, 4.38-7.60 mg g^{-1} AFDW) and SFA concentrations (Welch-test, df = 3, F = 2.468, p-value = 0.119, 3.56-8.24 mg g^{-1} AFDW) across all sites. By contrast, the MUFA content differed significantly with an almost 7-fold change between deep and shallow sites (Kruskal- Wallis test, df = 3, X^2 = 12.96, p-value = 0.005, 3.57 ± 0.95 vs. 24.16 ± 11.43 mg g^{-1} AFDW; Fig. 5.5A-5.5C), dominated by increases in 20:1 and 22:1 fatty acids in deep corals (Fig. 5.3A). Similar clear differences were found in the wax ester content (Kruskal-Wallis test, df = 3, X^2 = 12.98, p-value = 0.005) as well as the storage-to-structural-compound ratio (SFA+MUFA vs. PUFA) that is 2.9-fold enriched in deep corals (One-Way ANOVA, F = 221.6, p-value <0.001, Fig. 5.5D, 5.5E).

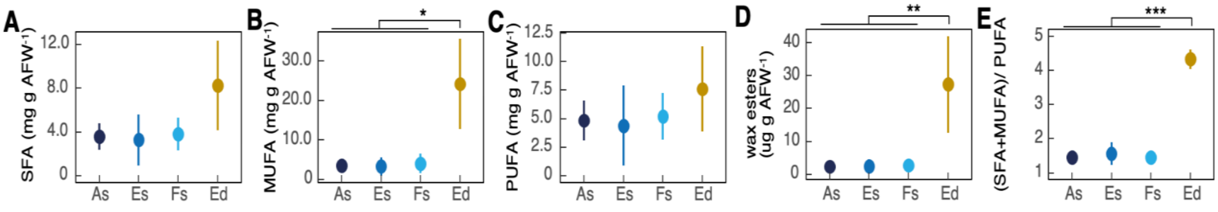


Fig. 5.5 Coral fatty acid group concentrations and energy reserves. A-D) Absolute fatty acid group concentrations (SFA: saturated fatty acid; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids) and wax ester content across sites. E) Fatty acid class ratios reflect the storage capacity ((SFA+MUFA)/PUFA) and are depicted across sites. Shallow sites are indicated by As, Es, Fs and colored in dark blue, blue and light blue, respectively and the deep site by Ed and colored in dark yellow. Data are mean ± 95%-confidence intervals.

Fatty acid analysis indicated that SFA markers like palmitic acid (PA, 16:0) and stearic acid (SA, 18:0) together with oleic acid (OleA 18:1(n-9)) were enriched in absolute concentration in deep corals, though not all significantly differ between shallow and deep sites (Fig. 5.6; PA: Kruskal-Wallis test p-value = 0.057, X^2 = 7.533, SA: One-Way ANOVA, p-value = 0.090, F = 2.488, OleA: p-value = 0.001, F = 7.867). Oleic acid forms one important substrate for n-3 and n-6 PUFA synthesis pathways and their intermediates linoleic acid (LA, 18:2(n-6)) as well as alpha linoleic acid (ALA, 18:3(n-3)) were reduced in shallow corals (p-value = 0.002, F = 6.932 and p-value = 0.066, X^2 = 7.193, respectively). While most PUFAs were elevated in deep corals, some essential PUFAs were remarkably similar and non-significantly different concentrations across sites (Fig. 5.6; arachidonic acid ARA, 20:4(n-6): p-value = 0.29, F = 1.338, Adrenic acid AdA 22:4(n-6): p = 0.115, X^2 = 5.927, EPA 20:5(n-3): p-value = 0.453, F = 0.912, docosapentaenoic acid DPA 22:5(n-3): p = 0.879, F = 0.224). Putative health indicators (e.g. EPA / ARA, PUFA(n-3)/PUFA(n-6)), were also very similar across sites (Fig. 5.S4).

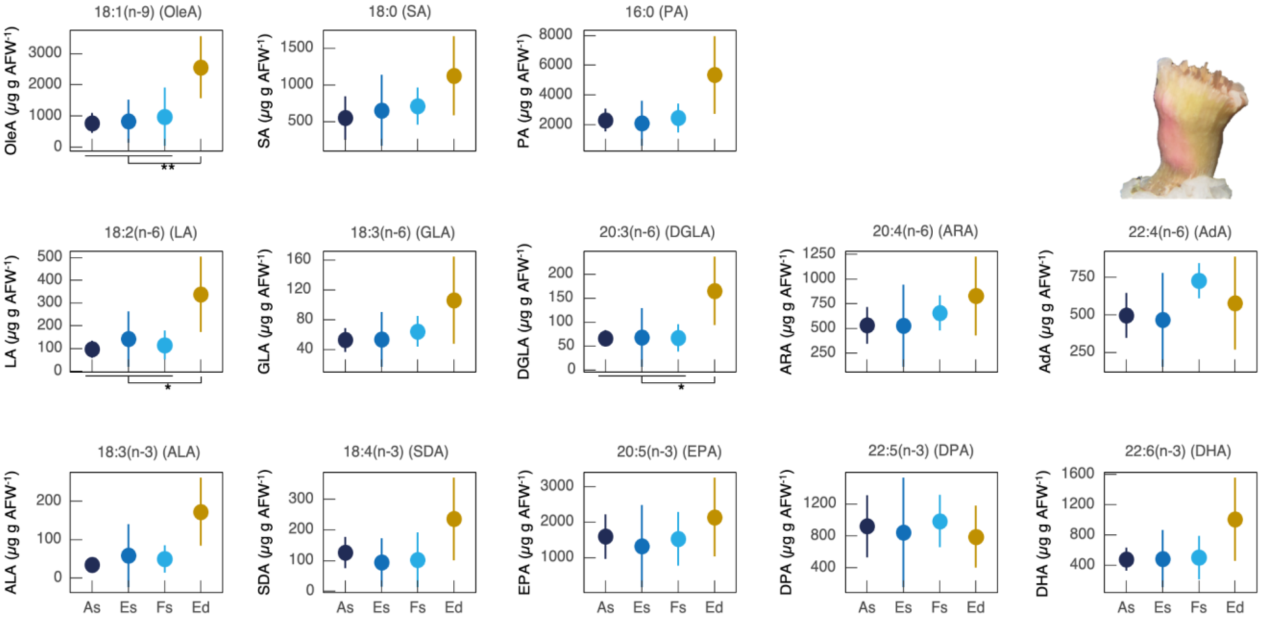


Fig. 5.6 Fatty acid concentration through potential biosynthetic pathways. Schematic fatty acid synthesis pathways are provided in the supplementary material (Fig. 5.S1) and some saturated, monounsaturated and in particular polyunsaturated fatty acids are provided for the different sites (As, Es, Fs and Ed). Shallow sites are indicated by As, Es, Fs and colored in dark blue, blue and light blue, respectively, and the deep site by Ed and colored in dark yellow. For the fatty acids acronyms are used: OleA: oleic acid, SA: stearic acid, PA: palmitic acid, LA: linoleic acid, GLA: gamma-linoleic acid, DGLA: dihomo-gamma-linoleic acid, ARA: arachidonic acid, AdA: adrenic acid, ALA: alpha linoleic acid, SDA: stearidonic acid, EPA: eicosapentaenoic acid, DPA: docosapentaenoic acid, DHA: docosahexaenoic acid. Significant differences are denoted by * (p ≤ 0.05), ** (p ≤ 0.01). Data are mean ± 95%-confidence intervals.

5.5. Discussion

Fatty acid trophic markers in deep and shallow *D. dianthus* clearly distinguish between a deep lipid-rich phenotype and a leaner phenotype in shallow, despite expectedly more challenging conditions like year-round aragonite undersaturation and lower temperature (Fillinger and Richter, 2013; Jantzen et al., 2013b). Our findings suggest that food is sufficient for the buildup of energy reserves (e.g. wax esters) in deep corals, in line with their higher calcification rates (Beck et al., 2022) and this may be linked to a more carnivorous diet. Shallow water corals do not possess the ability to accumulate much energy reserves, underpinning their lower calcification rates (Beck et al., 2022) and potentially subsisting on a more vegetarian diet. This is in conflict, however, with measured higher zooplankton quantity in shallow waters (Garcia-Herrera et al., 2022). To resolve this paradox, we link coral physiological performance with FATM data and provide hypotheses explaining the contradictory observations in the context of physico-chemical parameters and resource availability.

Trophic network inferences

The Comau Fjord and other Chilean fjords are productive environments sustaining high zooplankton stocks (Palma and Silva, 2004; González et al., 2010; Garcia-Herrera et al., 2022). Zooplankton composition and biomass vary over wide temporal (seasonal, tidal, diel) and spatial (horizontal and vertical) scales (Palma and Silva, 2004; Hamame and Antezana, 2010; Garcia-Herrera et al., 2022), resulting in contrasting nutritional environments to their coral predators. We thus expect moderate horizontal differences in trophic networks between head and mouth, and large differences between shallow and deep waters that should be reflected in the corals' FATM signal. Overall, the *D. dianthus'* rich biochemical composition supports their omnivore feeding behavior (Gori et al., 2018; Höfer et al., 2018), similar to other deep sea corals (Orejas et al., 2001; Rakka et al., 2021). It also clearly indicates differences in prey community between deep and shallow sites, but not along the head-mouth axis of the fjord (Fig. 5.S3). Along the same isobath, the FATM signals cluster together but we do see a stronger spread as well as variation within this cluster even within the same site (Figs. 5.3A-5.3C, 5.S3). This rather points towards stronger spatial gradients within sites in shallow waters (Fig. 5.S3) and it may reflect local microenvironments of similar trophic niche across sites with less clear differentiation of the plankton community along the fjord.

As observed for other CWCs (Naumann et al., 2015; Gori et al., 2018; van Oevelen et al., 2018), bacterial markers appear in *D. dianthus'* fatty acids profile, however, their contribution to the corals' diet is negligible with a slightly larger relative contribution in shallow waters (Fig. 5.S3). Phytoplankton and phytodetritus may serve as potential food sources for deep sea corals (Orejas et al., 2001, 2016; Maier et al., 2019) and could thus, directly account for these specific markers. Inputs are visible through a higher proportion of algal related markers (EPA, DHA and slightly for 18:4(n-3)), especially in shallow corals (Figs. 5.3A, 5.S3; Harwood and Russell, 1984; Dalsgaard et al., 2003) and the ratio DHA/EPA indicates a diatom signal prevailed over dinoflagellate (Falk-Petersen et al., 1987; Auel et al., 2002; Scott et al., 2002) at both depths (Fig. 5.3A). This also coincides with a predominant pattern of diatom-zooplankton succession in the plankton communities of Chilean fjords (González et al., 2010; Montero et al., 2017), which is reflected in the corals' FATM signal. However, we also need to consider the importance of EPA and DHA as major membrane constituents that will be discussed in more detail below.

From an energetic perspective, bacteria, phytoplankton and phytodetritus are insufficient to sustain the corals' metabolic needs compared to a zooplankton-based diet (Naumann et al., 2011; Maier et al., 2019; Rakka et al., 2021). Phytoplankton markers are ambiguous, indicating either direct uptake of phytoplankton, or the uptake of herbivorous zooplankton containing accumulated phytoplankton markers. Other FAs, like 14:0, 16:0, 18:0, are also major structural elements in copepods (Boissonnot et al., 2019) and together with EPA also indicate herbivore copepods. Between depths, however, they differ in relative abundance (Figs. 5.3A, 5.3B, 5.S3). The 16:0 fatty alcohol in particular is dominant in shallow corals (Fig. 5.3B) and such short-chained fatty alcohols (similar to FAlc 14:0) are found to be more prevalent in small or non-calanoid species (e.g. cyclopoid copepods; Sargent and Falk-Petersen, 1988; Kattner et al., 2003; Lischka and Hagen, 2007). As non-calanoid copepods (e.g. Cyclopoida) and small calanoid copepods (e.g. Paracalanus, Clausocalanus) are abundant in the shallow water layers of Comau Fjord (Garcia-Herrera et al., 2022), they may have contributed to the observed shallow water phytoplankton FATM signal in the corals.

The biomarkers 20:1 and 22:1 (both the FA and FAlc) clearly differ between deep and shallow corals. They are enriched in coral tissue of deep waters and only found in a reduced proportion in shallow corals (Fig. 5.3A, 5.3B). These markers are mainly synthesized *de novo* by calanoid copepods (Sargent and Whittle, 1981; Dalsgaard et al., 2003), in particular by the family Calanidae (Hagen and Auel, 2001). While calanoid copepods occur in almost similar biomass across all depths in winter and spring (around the time of sampling), compositional differences exist. For instance, Calanidae are more prevalent in deeper sites, while Metrinidae occur at shallower water layers. Yet, they have shown diurnal migration patterns and may still contribute to the diet in both depths

(Garcia-Herrera et al., 2022). The low concentration of LC MUFA in shallow corals, however, may result from these two different dominating families. It has been shown that they have distinct FATM signals in other regions with *Calanus* spp. accumulating 20:1(n-9) and 22:1(n-11) FA and FAlc as major end products compared to *Metridia* spp. with 14:0 and 16:0 FAlc as well as 16:1(n-7) and 18:1(n-9) FA as major end products (Hagen and Auel, 2001). This suggests that even though both groups of zooplankton migrate across several water layers (though at different extent), the family Calanidae dominates the deep corals' diet while contributing little to the shallow corals' nutrition.

The trophic marker ratio 18:1n-9/18:1n-7, indicating a higher trophic level in the zooplankton community (Graeve et al., 1997; Legeżyńska et al., 2012), suggests a higher contribution of carnivorous zooplankton to the deep corals' diet (Fig. 5.4). This may indicate that the algal markers found in shallow corals arose from a consumption of herbivorous zooplankton, not by direct phytoplankton uptake. Similarly, the deep corals' rich calanoid signal may not necessarily derive from direct consumption alone. Biomarkers also accumulate at the higher trophic level (Dalsgaard et al., 2003) and it is, thus, possible that they actually prey on larger zooplankton species that feed on calanoid copepods (Kobari et al., 2021). For instance, mysids and Euchaetidae are abundant in the deep fjord region and can represent an important food source for deep corals (Garcia-Herrera et al., 2022). Euchaetidae are large carnivorous copepods consuming smaller calanoid copepods (Yen, 1985), which could have accounted in part to the calanoid signal. While mysids are in general omnivores (Siegfried and Kopache, 1980; Grossnickle, 1982), adults may become more carnivorous (Fulton, 1982) and potentially also prey on calanoid copepods (Crescenti, 1997; Díaz-Astudillo et al., 2017).

Overall, we do see clear differences across depths in the corals' FATM signals that can be linked to available zooplankton data and support the dominance of certain zooplankton groups (e.g. Calanidae vs. Metridae; Garcia-Herrera et al., 2022). Based on markers for trophic position we can speculate whether some markers derive from direct consumption or through accumulation along the food chain but this requires more detailed food web analyses. So far, we compared the relative FATM signal without considering absolute values, the clear differences in the corals' energetic status and physiological performance observed across sites. It is also possible that FATM profiles are differently modulated by the organisms in concert with the ecological settings and this may further distort the trophic network signal. Subsequently, we will discuss what we can learn from the lipid data in terms of zooplankton biomass availability across depths and potential consequences that may arise for the corals' FATM signal.

Energy availability in the fjord

In Comau Fjord, the zooplankton biomass and abundance vary across depths with highest abundances and biomasses in the upper 50 m of the water column (Garcia-Herrera et al., 2022). Thus, shallow water corals should thrive in a land of plenty compared to deep water corals. Yet, this is neither reflected in the directly measured coral traits (Beck et al., 2022; data summarized in Fig. 5.2), nor in the biochemical signature of the corals. Deep corals have first and foremost approx. 4-fold higher lipid content in addition to an elevated ratio of energy storage (SFA+MUFA) to structural (PUFA) FAs as well as 3.4-fold higher wax ester content. Both highlight a thicker coral tissue with high energy storage capacity reflected by the wax ester content in deep corals. There are a number of potential explanations for this food paradox that are not mutually exclusive. It may be possible that a) shallow corals grow in denser banks along with other suspension and filter feeders compared to deep corals, hence experiencing greater competition due to interdependence of competition for space and food (Buss, 1979), b) shallow corals are exposed to other environmental drivers, not considered in this study, that inflict an energetic burden, c) major differences in plankton biomass and composition exist between the central water column and the plankton close to the steep walls harbouring the corals (e.g. wall effects) and/or d) plankton communities represent different food qualities for corals.

In the Chilean fjords, CWCs emerged from the deep and conquered the shallows, inaccessible for CWCs in most other regions (8 m; Försterra et al., 2005; Häussermann et al., 2021a). Emergence may be associated with *D. dianthus*' strong phenotypic plasticity and ability to acclimate to very contrasting conditions (Häussermann et al., 2021a; Beck et al., 2022), but may come at a cost and may only be possible in rather plankton-rich environments (reviewed in Häussermann et al., 2021a), such as the Comau Fjord (Garcia-Herrera et al., 2022). Recent studies suggest that *D. dianthus* in the shallow environments experiences stronger environmental variability and conditions potentially more stressful for corals (Beck et al., 2022). Together with pressures from infestations of their skeleton by microscopic endolithic photo-autotrophs (like *Ostreobium queckettii* or the cyanobacterium *Plectonema terebrans;* Försterra et al., 2005; Försterra and Häussermann, 2008; Hassenrück et al., 2013) and other epiand endolithic organisms (Försterra et al., 2005), the emerged corals may be at their physiological limits. Ecological factors may add to this, such as competition. Coral banks in the regions are dominated by *D. dianthus* and they can be associated with other suspension or filter feeders across depths, e.g. brachiopod banks occur from shallow to deep, mussel and barnacle banks are restricted to the intertidal (Häussermann and Försterra, 2009; Betti et al., 2017) and *Acesta patagonica* co-occurs with *D.*

dianthus below 60 m (Fillinger and Richter, 2013; Häussermann et al., 2013). The shallow regions, however, can be particularly diverse (Försterra et al., 2017) and CWCs co-occur with a myriad of both sessile and mobile species that decrease with depth (Försterra et al., 2005). This probably leads to competition for food and depletion of the rich plankton community in shallow waters, which may likely be reflected in the overall lower calcification rates of these corals.

Additionally, the strong environmental variability in shallow waters, in particular registered salinity fluctuations (Beck et al., 2022), may affect the plankton composition. For instance, some zooplankton groups are known for their higher salinity tolerance (e.g. Cyclopoida, with known FATM characteristics such as high 16:0 and 14:0 FAlc, 16:0 FA visible in shallow corals; Lischka and Hagen, 2007), whereas others are more sensitive (Magouz et al., 2021). This may also affect the abundance of the zooplankton (Laprise and Dodson, 1994; Wells et al., 2022) and potentially restrict certain zooplankton groups to deeper waters. However, as the available plankton data derive from integrated plankton tows across the upper 50 m of the water column (Garcia-Herrera et al., 2022), they are too coarse to elucidate small scale patterns. They can only serve as first approximations to evaluate the response of the zooplankton community to environmental variability to better understand its cascading effects on the physiological performance of corals and their FATM signal.

Zooplankton abundance and biomass diminish with increasing depth, yet the biomass per prey item increases with depth (Garcia-Herrera et al., 2022). Thus, we calculate that shallow corals may have a 10-fold higher individual capture rate compared to deeper ones, however, this difference shrinks to a 2-fold difference when the biomass per prey is considered (Garcia-Herrera et al., 2022). This does not consider prey handling, which may be energetically unfavourable, when dealing with many small items versus few large ones (Sebens, 1982). Nor does it include differences in food quality (Dessier et al., 2018; Schaafsma et al., 2018). Certain calanoid copepods are known for their high wax ester and thus, energy content (Hagen and Auel, 2001; Dessier et al., 2018; Schaafsma et al., 2018). Additionally, even within the group of calanoid copepods energy density may differ, with up to one third lower energy content in some genera compared to others (e.g. *Calanus hegolandicus* vs. *Metridia* sp. or *Temora longicornis*; Dessier et al., 2018). The same accounts also for other taxa and strong variability in energy density within zooplankton groups (reviewed in Schaafsma et al., 2018). Thus, it may be possible that different calanoid copepod species dominating the deep and shallow corals diet (e.g. Metrinidae vs. Calanidae; Garcia-Herrera et al., 2022) have potentially very different energy content. While the FATM signal may not be affected, it has consequences for the corals and can contribute to the distinct performance observed at different depths.

Sampling bias may also account for some inconsistencies and limit a more precise FATM signal interpretation. For instance, plankton tows were performed in the center of the fjord, but the zooplankton community may be different closer to the steep fjord walls (Greene et al., 1988; Hirche et al., 2016). Additionally, larger zooplankton and micronekton is mobile and capable of avoiding the nets (Brinton, 1962). *Euphausia vallentini* (krill) is common in Comau Fjord (Sánchez et al., 2011) however, it has not been caught efficiently by vertical plankton tows (few young stages & one adult individual; Garcia-Herrera et al., 2022). Similarly, euphausiids employ a number of unique adaptations to entrapped life in fjords and for instance, take advantage of demersal habitats where they exploit a rich and alternative food source compared to their open ocean counterparts (Hamame and Antezana, 2010). Potentially, euphausiids play a critical role in the Northern Chilean fjords (Maier et al., 2021), representing an energy-rich diet for deep corals in Comau Fjord (Maier et al., 2021) together with Euchaetidae and mysids (Garcia-Herrera et al., 2022). Certain Euphausiids can also accumulate wax esters as well as a calanoid signal (like 20:1(n-9) FAlc; Falk-Petersen et al., 2000; Hagen and Auel, 2001). Thus, a revised interpretation of the deep corals' Calanidae FATM signal (20:1(n-9) & 22:1(n-11) FA & FAlc) is possible and potentially krill can additionally account for such a signal in deeper sites.

All these mentioned aspects can contribute to the apparent resource limitation and stronger differences in energy availability than originally expected between deep and shallow waters of the Comau Fjord. While sampling biases, with underrepresented zooplankton groups, can have a direct effect on the interpretation of the FATM signals, resource limitation can also result in a modification of fatty acids and a distortion of the FATM signal. In the latter case, the FATM signal may rather be driven by the corals' metabolic needs than their diet. Subsequently, we discuss what we can learn from the physiological background data in terms of energy allocation and finally discuss insights gained from the FATM signal regarding the corals' potential active fatty acid modifications.

Energy turn-over and trade-offs

Physiological background data support higher net energy availability for deep corals, yet they also reveal energetic trade-offs. Energy is channeled into 2.3-fold higher calcification, 5.0-fold higher tissue biomass and 3.4-fold higher energy reserves (e.g. wax esters) in deep water corals, compared to shallow corals. Energy reserves prevailed through winter in spite 2- to 3– fold lower zooplankton availability during the cold season (Garcia-Herrera et al., 2022), typical also of other CWC regions (Wiborg, 1954; Gaard, 1999; González et al., 2010). The prevalence is even more remarkable considering the reproduction of *D. dianthus* which, in spite of being energetically costly (Calow, 1979), peaks in austral winter (Feehan et al., 2019). Population genetic analyses indicated a mixed population from shallow to the deep sites within Comau Fjord and speculated about a source/sink separation and that potentially the deep corals serve as an important source of coral recruits in general (Addamo et al., 2021). This represents a likely scenario based on our data. Yet, it is also plausible that the reduced energy reserves derive from a similar or even higher investment into reproduction that deplete these reserves in shallow water. Physiological trade-offs and shifts in energy allocation are prevalent in an organism's life history and are strategic processes underpinning the success and physiological performance of organisms, especially under resource limitations (Leuzinger et al., 2012; English and Bonsall, 2019). Under severe limitations there are two possible strategies: a) invest in reproduction at the risk of death or b) invest into somatic growth to endure periods of scarcity (Stearns, 1989; Fischer et al., 2009). In a tropical coral, resource limitations lead to a substantial reduction in somatic growth, while calcification and reproduction was maintained and energy transfer increased. However, under severe resource scarcity reproduction was halted, but growth to some extent maintained in the same study (Leuzinger et al., 2012). Applied to our study, this could mean that somatic growth (in terms of reduced biomass in Fig. 5.2B or seen in some corals with lower tissue cover in Beck et al., 2022) is sacrificed to maintain calcification and reproduction. While we do see such higher energy transfer into growth, investment into reproduction requires further investigation. However, such physiological trade-offs likely require the mobilization of energy reserves and contribute to the distortion of the FATM signal, which must be taken into account.

Fatty acids as indicators of coral health and functionality

Despite clear differences in total lipids as well as energy storage capacity (Fig. 5.2, 5.5E), the similar total PUFA concentration across sites and depths is striking (Fig. 5.5C). It may represent the corals' dependencies on specific concentrations of essential fatty acids for their general metabolic regulation and point towards their need to maintain them across sites. Polyunsaturated FA are key molecules for metabolic regulation and support structural integrity, membrane functioning and immune system competency (Tocher, 2003; Russo, 2009; Kim et al., 2021). Under stressful conditions, invertebrates were found to metabolise SFA and MUFA, while preserving PUFA as long as possible (Schlechtriem et al., 2008; Mezek et al., 2010). Here we also find very low MUFA content in shallow corals but similar PUFA content, in line with the lower calcification rates in shallow water, the limiting conditions and need to mobilize fatty acids like MUFAs. Marked differences in absolute lipid concentration can, thus, result in an over-representation of these essential lipids in the relative FATM signal (e.g. algal markers like EPA, ARA in shallow corals; Fig. 5.3C). In turn, it potentially also results in a lack of certain markers (e.g. LC MUFAs like Calanidae markers 20:1n-9) that were readily metabolised to fuel the organism's metabolic demands. This clearly shows the duality of some FA markers, i.e. the derivation from their diet or the modification through their catabolism (fatty acid integration "black box"; Galloway and Budge, 2020; Helenius et al., 2020; Kabeya et al., 2020).

Underlying drivers for such modified levels may be selective retention of essential FAs (Yasuda et al., 2021), trophic upgrading (Helenius et al., 2020) or *de novo* synthesis by the coral (Kabeya et al., 2018). The significantly lower linoleic acid (LA, 18:2(n-6)) and reduced ALA (18:3(n-3)) concentration may indicate their trophic upgrading into LC PUFAs (Fig. 5.5), but it may also be possible that *D. dianthus* is capable of *de novo* synthesis following all PUFA biosynthesis steps (Fig. 5.6) through the activity of methyl-end desaturation (Δ12, Δ15) enzymes (Kabeya et al., 2018). For example, *Lophelia pertusa* - a sibling species - is able to synthesize essential fatty acids *de novo* including PUFA(n-3) (EPA and DHA; Mueller et al., 2014). Similar observations exist for tropical corals, where the two essential PUFAs (LA and ALA) were identified (Kabeya et al., 2018). Yet, the energetic costs and the potential rate of modification (that may still be limited in animals) of this *de novo* synthesis need to be evaluated. Besides, a capability does not necessarily indicate its activity. Chronic stress, e.g. eutrophication, has been shown to actually limit the ability for lipid synthesis in tropical corals (Kim et al., 2021).

Notably, fatty acid modifications can be linked to organism physiology, and in tropical corals PUFA ratios (e.g. DHA:EPA, EPA:ARA) were employed as indicators for water quality as well as putative coral health (Rocker et al., 2019; Kim et al., 2021). In this study, we expect that the FATM signal of the deep coral phenotype more closely resembles their diet, while the limiting conditions in shallow waters warrant further investigation of the corals' fatty acid metabolism and thus potentially stronger modification of their FATM signal, especially following winter. This also adds caution to the interpretation of the FATM profiles from other CWC species, in particular from areas with distinct and in particular low background productivity (first insights and comparisons are provided in the supplementary material). Analogue to studies in tropical corals (Rocker et al., 2019; Kim et al., 2021), specific PUFA ratios may rather be used to differentiate between optimal versus suboptimal environmental conditions, but this will require more detailed experimental studies to support such application in *D. dianthus* as well as other CWC species.

5.6. Conclusion

In the past, FA profiles in CWCs helped to gain insight into their trophic ecology and the ecological setting they flourish in. Yet, none have combined *in situ* sampling with physiological investigations to understand how physiological limitations modulate the coral biochemistry in CWCs. Here FATM provides insights into the corals' potential *in situ* food sources, but we also emphasize the need for a more holistic view and better integration of physiological (metabolic rates, fitness) and ecological information (e.g. food availability and sources, physico-chemical conditions) with FATM analysis. While FATM and lipid content analyses gained a whole new perspective on corals in Comau Fjord, it also revealed numerous open questions that warrant further investigations. In this respect, the Comau Fjord represents an ideal natural laboratory that allows the design of experiments able to trace lipid metabolism and energy allocation pathways. This will provide a more precise understanding of the CWCs' ability to cope with future changing conditions in particular under contrasting productivity regimes.

5.7. Acknowledgements

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5.8. Supplemental information

5.8.1. Supplemental text

Comparison of the biochemical composition with other *Desmophyllum* and other cold-water coral species

Deep coral lipid composition markedly differs from other CWCs analyzed so far. CWCs show a pan-global distribution inhabiting oligotrophic waters like the Mediterranean Sea (Freiwald and Roberts, 2005; Lo Iacono et al., 2018) and high productivity areas as described for the North and South Atlantic (Dodds et al., 2009; Hebbeln et al., 2020). In the latter, they can even endure anoxic conditions as observed in subtropical oxygen minimum zones (Colman et al., 2005; Le Guilloux et al., 2009; Hebbeln et al., 2020). In terms of lipid composition derived from various CWC reefs, the contrasting content of PUFA sticks out particularly. PUFAs are the major FA group in *Dendrophyllia cornigera* in both Menorca and Cantabria (Gori et al., 2018, 67%) as well as *D. dianthus* or *Madrepora oculata* in the Mediterranean and NE Atlantic (Kiriakoulakis et al., 2005, 42-45%; Naumann et al., 2015, ~50%) and they share this characteristic with *D. dianthus* obtained in shallow stations in the Comau Fjord (40%). *Desmophyllum pertusum* samples from the NE Atlantic represent an exception with similarly low or even lower values like the deep *D. dianthus* in the Comau Fjord (Kiriakoulakis et al., 2005, 7-25%). Previously, PUFA dominance was explained as an expected characteristic of marine lipids (Yamashiro et al., 1999; Meyer et al., 2019) yet not supported in the fittest CWCs phenotype studied here. This stark difference is accompanied by a 2.9-fold higher level of storage compounds compared to structural lipids. However, even the storage to structural ratio of shallow *D. dianthus* corals is significantly higher compared to *D. cornigera* (Gori et al., 2018), but similar to *D. dianthus* from the Mediterranean (Naumann et al., 2015). This might reflect species-specific differences but may also reflect limited food supply. Often a higher proportion of PUFA coincides with low productivity areas as reflected in *D. cornigera* in the Menorca channel (Gori et al., 2018). Similarly, *D. pertusum* corals collected in the NE Atlantic are characterized by enrichment in storage lipids likewise as the deep coral phenotype in this study (or even higher up to 8-fold enrichment in storage compounds, Kiriakoulakis et al., 2005; Dodds et al., 2009) and therefore, indicate high productivity regions and thus healthy and fit CWC populations. It can be expected that these populations employ similarly elevated growth rates, respiration rates and coral biomass as observed for the deep coral population in the Comau Fjord and may indicate flourishing 3-dimensional reef structures in these regions.

5.8.2. Supplemental tables

Table 5.S1 List of fatty acids (FA) as well fatty alcohols. Individual marker concentrations are given as relative abundance as well as absolute concentration per site. All values are stated as mean ± standard deviation (SD) and fatty acids as well as fatty alcohols with a relative abundance <0.5% were excluded from the data set.

	relative concentration (%)				**absolute concentration (μg g^{-1} AFDW)**			
FA	As	Es	Fs	Ed	As	Es	Fs	Ed
14:0	2.21 (0.3)	2.11 (0.37)	1.23 (0.26)	2.82 (0.4)	264.5 (101.4)	215.1 (154.8)	168.3 (109.8)	1161.4 (647.2)
16:0	19.51 (1.86)	19.5 (2.22)	19.22 (1.24)	13.3 (0.69)	2317.6 (743.8)	2108.3 (1440.5)	2453 (927.8)	5349.8 (2491.9)
16:1(n-5)	1.06 (0.1)	0.96 (0.1)	0.87 (0.19)	1.28 (0.13)	126.3 (45.6)	105.7 (74.4)	119.7 (75.1)	514 (251.6)
16:1(n-7)	4.55 (0.49)	4.22 (0.57)	3.6 (1.15)	6.53 (0.61)	552.5 (238.6)	450.1 (335.3)	509.6 (374.6)	2634.5 (1254.9)
16:2(n-4)	0.79 (0.16)	0.55 (0.14)	0.52 (0.18)	0.86 (0.19)	96 (46.9)	64.3 (49.7)	72.8 (51.9)	359.9 (201.6)
16:3(n-4)	0.61 (0.13)	0.41 (0.24)	0.35 (0.11)	0.21 (0.09)	74.4 (37.9)	46.5 (32.3)	48.8 (35)	90.2 (67.5)
16:4(n-1)	0.71 (0.15)	0.4 (0.24)	0.37 (0.19)	0.17 (0.05)	87 (43.4)	53.7 (43.9)	46.6 (26.5)	70.3 (39.2)
17:0	0.57 (0.09)	0.3 (0.48)	0.64 (0.06)	0 (0)	68.2 (22.9)	16.1 (28.4)	80.9 (24.8)	0 (0)
18:0	4.71 (1.8)	5.94 (0.48)	5.63 (0.42)	2.83 (0.2)	548.8 (284.1)	653.3 (458.9)	712.3 (241.1)	1124.8 (515.5)
18:1(n-5)	2.94 (0.22)	2.93 (0.63)	2.58 (0.44)	2.44 (0.3)	350.3 (123.1)	351 (284.5)	337.2 (171.2)	973.1 (444.3)
18:1(n-7)	2.98 (0.24)	3.02 (0.45)	2.67 (0.27)	1.84 (0.08)	356.8 (130.3)	324.2 (247.7)	357.5 (197.8)	724.1 (312.6)
18:1(n-9)	6.43 (0.58)	7.5 (3.75)	6.56 (3.05)	6.62 (0.6)	774.6 (306.3)	830.1 (661.3)	978 (885.7)	2560.5 (954)
18:2(n-6)	0.81 (0.11)	1.33 (0.3)	0.9 (0.35)	0.84 (0.05)	96.5 (35.2)	142.3 (115.3)	115 (60.2)	337.6 (157.5)
18:3(n-6)	0.45 (0.04)	0.49 (0.3)	0.52 (0.07)	0.26 (0.05)	52.7 (15.3)	53.7 (34.8)	64.4 (19.5)	105.9 (55.7)
18:4(n-3)	1.07 (0.22)	0.82 (0.17)	0.73 (0.26)	0.57 (0.1)	126.8 (48.7)	94.2 (75.3)	102.7 (85.4)	235.8 (128.5)
20:0	1.43 (0.06)	1.47 (0.25)	1.38 (0.22)	0.59 (0.07)	168.4 (49.1)	172.9 (137.6)	174.1 (60.3)	236.9 (114.7)
20:1(n-11)	0.24 (0.03)	0.21 (0.18)	0.26 (0.05)	0.85 (0.03)	29.3 (11.3)	18.2 (23.9)	34.8 (20.6)	340.3 (155.1)
20:1(n-5)	1.12 (0.13)	0.61 (0.71)	1.22 (0.26)	0 (0)	132.2 (40.1)	29.6 (48.3)	149.7 (40)	0 (0)
20:1(n-7)	2.09 (0.33)	1.99 (0.13)	2.24 (0.23)	0.79 (0.07)	250.5 (87.7)	214.6 (145.9)	281.8 (89.4)	315.5 (145.7)
20:1(n-9)	1.48 (0.24)	1.9 (0.27)	1.66 (0.24)	12.85 (0.51)	177.8 (69.8)	208.4 (148.6)	225 (129)	5133.8 (2419.5)
20:2(n-6)	0.73 (0.25)	0.59 (0.35)	0.91 (0.46)	0.52 (0.1)	83.3 (29.2)	77 (59.5)	109.6 (47.7)	211.3 (109.8)
20:3(n-6)	0.57 (0.05)	0.53 (0.27)	0.53 (0.07)	0.42 (0.05)	66.4 (16.7)	68.5 (58.4)	67.2 (27.1)	164.9 (68.1)

Table 5.S1 (continued)

20:4(n-3)	0.62 (0.09)	0.42 (0.35)	0.62 (0.23)	0.77 (0.11)	73.7 (29.2)	67.7 (66.6)	88.6 (77.8)	313.5 (155.3)
20:4(n-6)	4.49 (0.51)	5.3 (1.36)	5.33 (0.92)	2.1 (0.49)	531.7 (176)	528.5 (394.7)	657.2 (170.6)	828.9 (378.4)
20:5(n-3)	13.33 (0.89)	11.59 (2.94)	11.64 (1.58)	5.26 (0.49)	1596.2 (596.9)	1324.6 (1101.6)	1528.9 (724.2)	2142.5 (1056.9)
22:0	0.48 (0.27)	0.23 (0.36)	0.63 (0.18)	0 (0)	60.1 (36.2)	14.8 (28.5)	79 (32.7)	0 (0)
22:1(n-11)	1.59 (0.72)	2.67 (0.7)	2.7 (0.27)	22.26 (0.81)	201.7 (120)	318.9 (250.7)	351.2 (159)	8869.2 (4095.2)
22:1(n-7)	0.89 (0.36)	0.76 (0.37)	0.9 (0.44)	0.51 (0.11)	100.1 (40.6)	79.8 (63.6)	111.8 (57.7)	199.5 (93.2)
22:1(n-9)	1.77 (0.94)	1.76 (0.78)	1.6 (0.18)	2.43 (0.31)	194.5 (64.4)	161.3 (98.6)	206.5 (88.6)	955.9 (422.5)
22:4(n-6)	4.24 (0.62)	4.6 (1.31)	6.11 (1.66)	1.48 (0.41)	495.5 (142.8)	468 (296.8)	727.4 (112.3)	577.3 (294.7)
22:5(n-3)	7.66 (0.8)	7.41 (1.8)	7.82 (0.75)	1.99 (0.34)	921.4 (368.9)	842.7 (652.9)	985.8 (313.3)	789.9 (369.1)
22:6(n-3)	4.09 (0.61)	4.56 (0.79)	3.78 (0.47)	2.46 (0.2)	479.8 (146.1)	486.4 (360.2)	501.2 (272.2)	1007.9 (523.5)
24:1(n-11)	0.64 (0.12)	0.33 (0.39)	0.89 (0.22)	1.54 (0.42)	74.9 (22.4)	29.8 (42.9)	106.4 (18.1)	616 (366.2)
24:1(n-9)	1.17 (0.13)	1.08 (0.22)	1.1 (0.19)	0.81 (0.03)	137.7 (46)	123.9 (95.5)	150.1 (91.5)	322.4 (146.9)
FAlc	**relative concentration (%)**				**absolute concentration (µg g^{-1} AFDW)**			
14:0	13.9 (2.72)	11.67 (5.7)	9.41 (4.48)	2.68 (0.6)	1711.7 (843.4)	958.2 (490)	1297.8 (905.9)	1029 (389.2)
16:0	35.64 (4.52)	32.12 (6.15)	35.24 (10.2)	13.42 (1.34)	4288.1 (1657.4)	3457.5 (2542.7)	4966.8 (3477.2)	5308.9 (2221.1)
16:1(n-7)	3.73 (0.73)	2.35 (2.38)	4.54 (2.52)	2 (0.13)	432.7 (131)	332.3 (291.2)	523.2 (175.3)	801.4 (375.8)
18:0	6.42 (0.96)	5.27 (1.45)	5.29 (1.63)	1.9 (0.12)	763.4 (267)	610.3 (479.4)	676.7 (301.9)	755.3 (323.1)
18:1(n-7)	0.68 (1.09)	0 (0)	0.77 (0.89)	0.73 (0.05)	82.3 (132.6)	0 (0)	128.1 (158.4)	293.6 (144.2)
18:1(n-7)	5.8 (0.65)	4.44 (2.52)	4.6 (1.92)	2.18 (0.16)	687 (242.7)	585.8 (468.5)	564.2 (240.1)	872 (407.3)
20:1	9.11 (3.45)	14.42 (3.94)	11.88 (4.98)	26.42 (0.52)	1024.1 (290.8)	1754.1 (1429.2)	1403 (468.2)	10618.8 (5038.8)
22:1	24.71 (5.61)	29.73 (6.38)	28.27 (8.27)	50.68 (1.76)	2908.3 (1040)	3209.6 (2205.8)	3433.4 (1107.9)	20318.2 (9643.1)

5.8.2. Supplemental figures

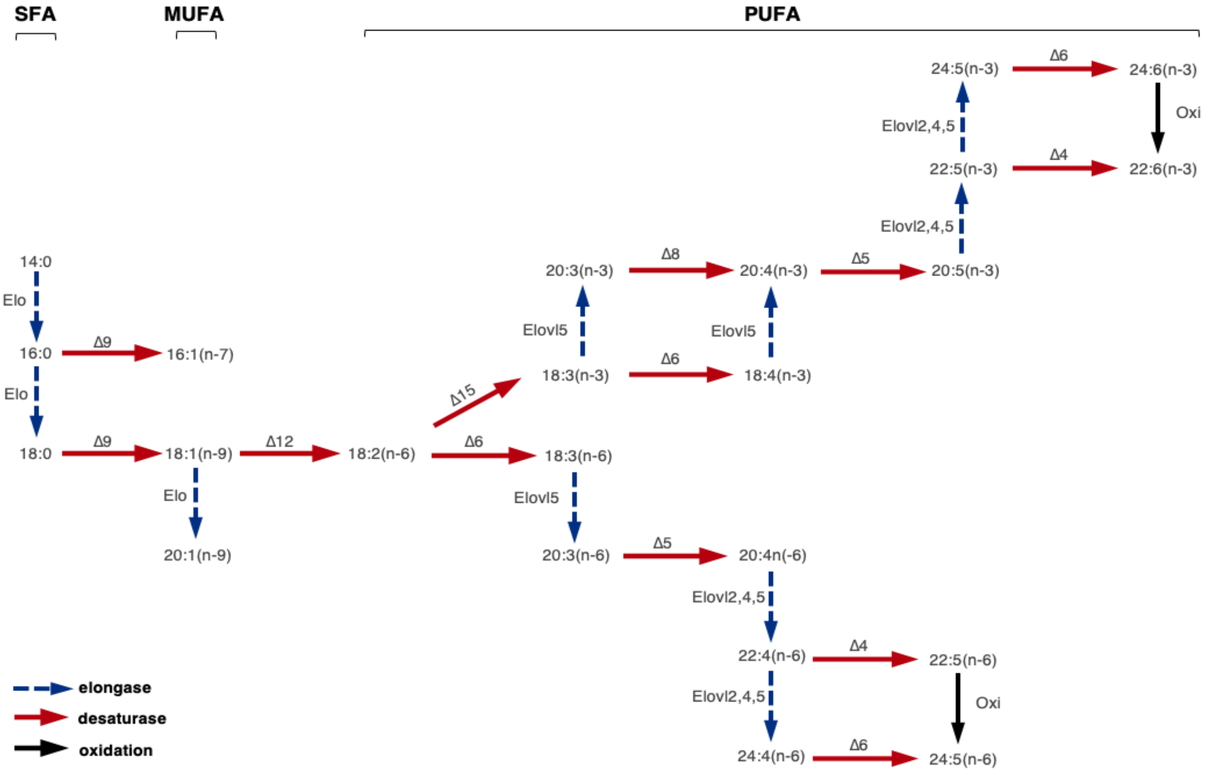

Fig. 5.S1 Fatty acid biosynthesis pathways. Schematic fatty acid synthesis pathways provided with solid red line arrows indicate desaturase enzymes (with type specified as ΔX), blue dashed line arrows indicate elongase enzymes and black arrow represent β-oxidation involved in a potential coral biosynthesis pathway. Fatty acid groups are indicated above showing saturated (SFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA). Pathways modified and adapted from Dalsgaard et al. (2003), Monroig et al. (2013), Rocker et al. (2019), Kim et al. (2021).

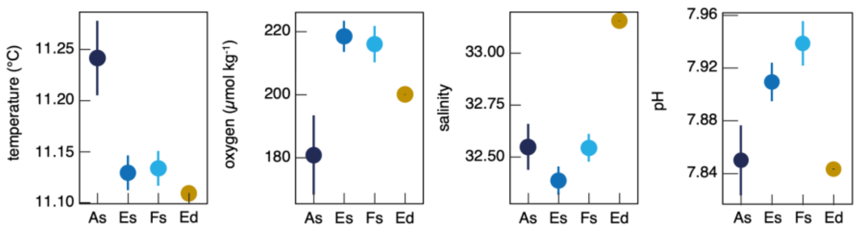

Fig. 5.S2 Average environmental conditions during sampling. Site-specific average conditions (temperature, oxygen, salinity and pH) are provided per station (A, E, F) at the shallow sites (20-30 m depth range for As, Es, Fs) and the deep site (280-300 m depth range, Ed). Shallow sites are indicated by As, Es, Fs and colored in dark blue, blue and light blue, respectively, and the deep site by Ed and colored in dark yellow. Data are mean ± 95%- confidence intervals.

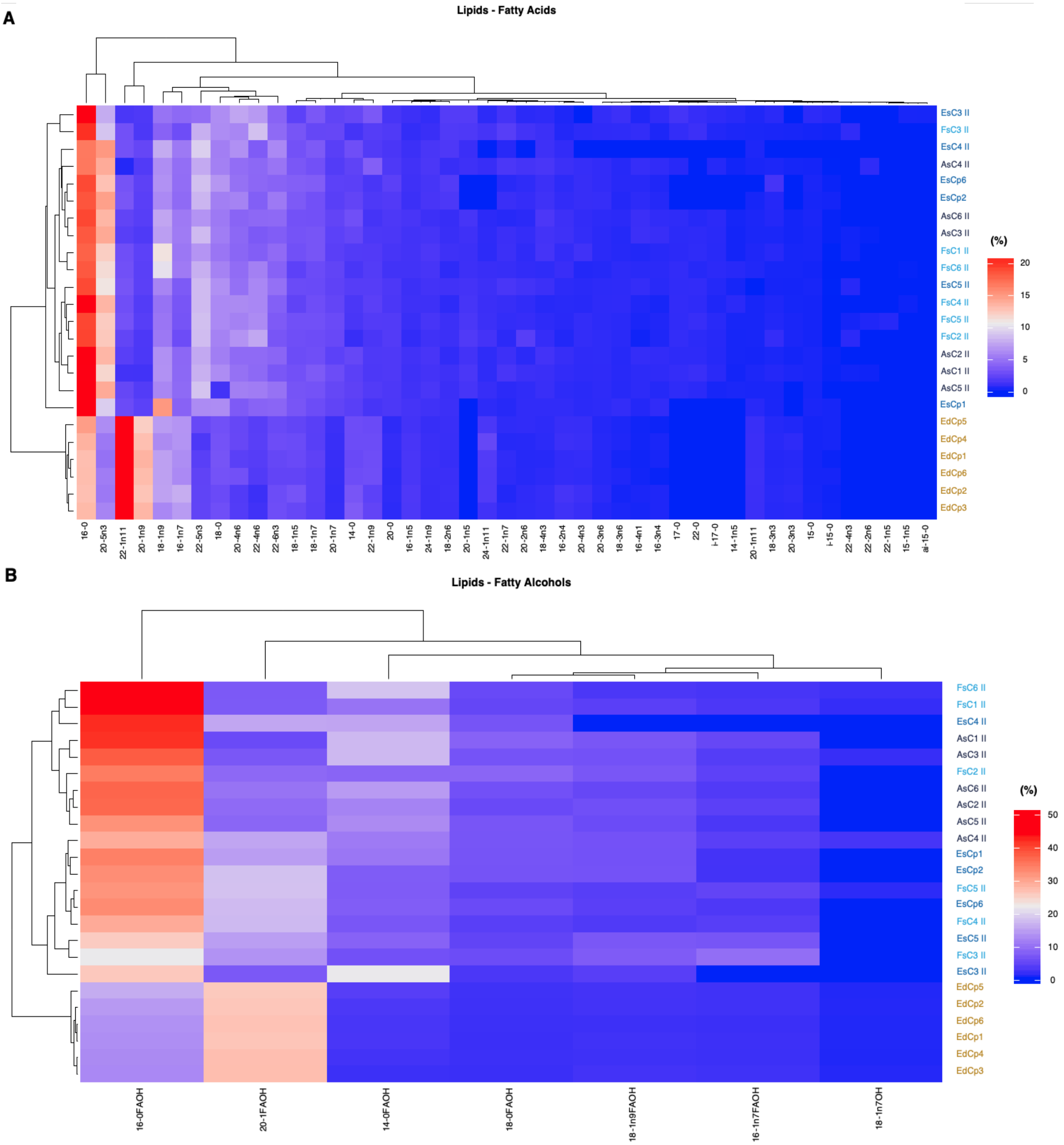

Fig. 5.S3 Heatmaps of fatty acid and fatty alcohol composition of individual corals. Heatmaps depict the relative percentage of individual fatty acid (a) and alcohol (b) markers per sample. Trees are hierarchical clustering of samples based on a complete linkage method of the relative abundance across sample or marker. Coral samples were collected at shallow waters (20 m depth) of Comau Fjord at sites As, Es, Fs (dark blue, blue and light blue colors, respectively) and at one deep site (300 m depth) at site Ed (dark yellow).

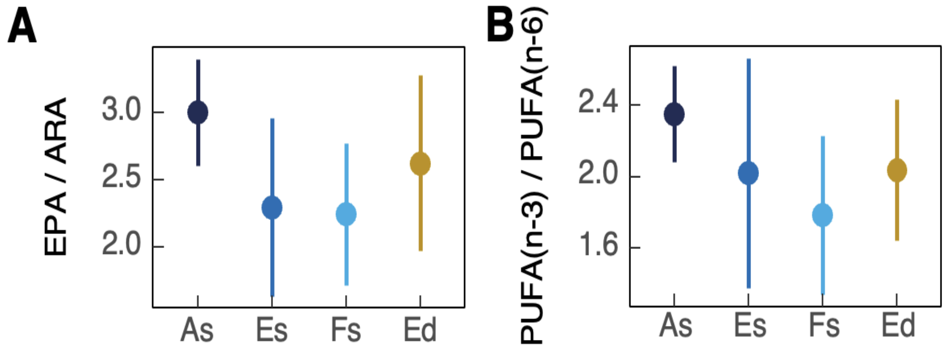

Fig. 5.S4 Fatty acid ratio as putative health indicators. Ratio of specific markers across sites that have been used as putative health indicators in tropical corals (Rocker et al., 2019; Kim et al., 2021). A) represents the ratio EPA / ARA (EPA: eicosapentaenoic acid 20:5(n-3) and ARA: arachidonic acid, 20:4(n-6)) and B) PUFA(n-3) / PUFA(n-6) (PUFA: polyunsaturated fatty acids and n-3 and n-6 represent the sum of all omega-3 and omega-6 PUFA, respectively). Shallow sites are indicated by As, Es, Fs and colored in dark blue, blue and light blue, respectively, and the deep site by Ed and colored in dark yellow. Data are mean ± 95%-confidence intervals.

Chapter 6 – Concluding discussion and key findings

6.1. Concluding discussion

The overarching aim of this thesis was to investigate the seasonal and diurnal zooplankton dynamics along and throughout the entire water column of Comau Fjord, Chilean Patagonia. The underlying idea was to provide novel and in-depth characterization of the zooplankton to better understand their key role in the diet of the numerically abundant cold-water corals living in the deep aragonite undersaturated waters of the fjord. In order to accomplish this, a novel state-of-art methodological procedure was applied – an integrated image analysis system (ZooScan, ZooProcess and EcoTaxa) – that allowed to efficiently process a high number of zooplankton samples. Using this approach, spatial and temporal zooplankton population dynamics, as well as the ecological drivers influencing their distribution, the community composition, size frequency distributions and individual variability within the same community could be provided (**chapters 2, 3 and 4**). Further, an experimental study assessed the natural food spectrum of cold-water corals along the fjord and with depth using the corals fatty acids composition and the findings were discussed in the light of the assessed depth-specific zooplankton composition as well as the corals general health status (e.g. growth and energy reserves) (**chapter 5**). Altogether, the results of this work provide the following answers to the previously proposed research questions:

- *How is zooplankton seasonality influenced by environmental variations of fjords in temperate regions?*

Seasonal environmental fluctuations clearly influenced the general dynamics of zooplankton. The results showed that integrated zooplankton abundance peaked in spring, while the highest integrated biovolume and biomass were found in summer decreasing thereafter over time, to find minima of abundance, biovolume and biomass in late autumn and mid-winter (**chapter 2**). Large seasonal environmental variations often derive from high biological productivity associated to changes in the zooplankton population dynamics (Mauchline, 1998; Balbontín and Bustos, 2005; Ladah et al., 2005; Landaeta and Castro, 2006; Aracena et al., 2011). Between spring and summer, a thermal density stratification was observed in Comau Fjord, probably caused by the increase in solar radiation, triggering a spring phytoplankton bloom reflected in a high chlorophyll *a* concentration. The high phytoplankton biomass (mainly composed by chain-forming diatoms) was as expected grazed by the gradual increment in zooplankton abundance (especially copepods), establishing the classical diatom-to-zooplankton food web (Palma and Silva, 2004; Vargas et al., 2008; González et al., 2010). Accordingly, zooplankton abundance peaked in spring when copepod nauplii and young stages of calanoid copepods accounted for a large proportion of the zooplankton community, indicating an intense zooplankton reproduction. During spring, also carnivorous zooplankton (chaetognaths and cnidarians) occurred in higher abundances, attributed to the high copepod abundance.

In summer, zooplankton (in particular copepods) biovolume and biomass was the highest of the year while abundance decreased, suggesting the presence of larger individuals or taxa (**chapter 2**). In autumn and winter, zooplankton abundance, biovolume and biomass found their minimum, likely affected by low primary production where zooplankton growth was limited by food availability and the phytoplankton community composition (i.e. microbial loop based community) (Escribano et al., 2007; Vargas et al., 2008; González et al., 2010). The low chlorophyll *a* concentration recorded by our CTD profiles in autumn and winter was probably affected by a low solar radiation causing the thermal inversion observed in surface waters, likely due to heat loss caused by winds, precipitation and cold freshwater discharge from rivers and glaciers (Palma and Silva, 2004; González et al., 2010). The overall decline in copepod abundance and biomass from spring and summer to winter can be explained by the decrease in phytoplankton availability and the increasing predation pressure built by carnivorous organisms.

For the first time, the combined seasonal dynamics of environmental conditions and the spatial and vertical zooplankton distribution throughout the entire water column in Comau Fjord have been assessed. Climate warming and increasing anthropogenic activities (e.g. development of hydroelectric energy and mariculture of non-native species), however, may have the ability to modulate the plankton succession patterns and food web structure and it is thus, important to implement a more regular monitoring to be able to better project future changes.

- *Do we find a clear vertical structure in zooplankton composition as well as temporal dynamics? Are temporal dynamics linked to known diurnal vertical migration patterns as for the well-known migrator Metridia?*

Zooplankton abundance and biovolume differed in their vertical structure (**chapters 2 and 3**). Zooplankton abundance concentrated in surface waters during both day and night, whereas zooplankton biovolume was higher in intermediate waters during daytime and in surface waters during nighttime. This indicated that smaller zooplankton remained in surface waters during day and night, and that vertical migration (reflected in the diel differences in biovolume) was mainly performed by larger zooplankton.

Diel vertical migration (DVM) is usually more pronounced in large taxa, due to their susceptibility of being perceived by visual hunting predators and their generally greater swimming capabilities (Hays, 1995). For instance, in Comau Fjord, small copepods such as cyclopoid and harpacticoids, did not performed a clear DVM. In this thesis we detected that DVM (over 300 m) was mainly performed by large calanoid copepods (Metridinidae, Calanidae and to a lesser extent Euchaetidae) and by large non-copepod taxa (chaetognaths, cnidarians, mysids, amphipods and euphausiids) (**chapters 2 and 3**). Similar circadian migration patterns were found for other Patagonian fjords and channels, for example Reloncaví Fjord, Ancud Gulf (Valle-Levinson et al., 2014; Díaz-Astudillo et al., 2017) and Puyuhuapi Fjord and Jacaf Channel (Pérez-Santos et al., 2018). Probably, large herbivore zooplankton migrated upwards where chlorophyll *a* concentration was higher (and hence, phytoplankton abundance), while carnivorous zooplankton followed the herbivorous, generally smaller and surface-resident zooplankton (Palma and Kaiser, 1993; Villenas et al., 2009). However, individual variability within the calanoid copepod populations was found in Comau Fjord. From the Metridinidae and Calanidae populations, a portion of the entire population carried out the DVM, which was mostly performed by the largest individuals, while smaller individuals remained in surface waters during daytime, likely due to the small size of the organisms and the high food availability. The same pattern was also found for the copepod *Metridia pacifica*, in Dabob Bay, a fjord in Washington state, linking the body condition of zooplankton (in terms of energy reserves) with the DVM (Hays et al., 2001).

In Comau Fjord, the presence of high concentration of predators in certain water layers also influenced the vertical distribution of zooplankton (**chapter 2**). Generally, the 50-100 m water layer presented a very low zooplankton abundance and biovolume. This was attributed to the high contribution in the relative biovolume of gelatinous carnivores (chaetognaths and cnidarians) within the zooplankton community. These taxa (common in the area) are known to be voracious predators of copepods and larvae from other organisms and can grow at fast rates forming dense aggregations that can seasonally dominate the community composition (Lie et al., 1983; Palma and Rosales, 1997; Casanova, 1999; Brodeur et al., 2002; Villenas et al., 2009). When predators are present, zooplankton aggregation in nutrient-rich layers can be limited or even eliminated (Kleiven et al., 1996). Thus, even though these carnivorous zooplankton had low abundances they can contribute to the reduction of other zooplankton taxa and play a role in structuring the vertical zooplankton community.

- *Are oceanographic processes (such as water stratification, salinity gradients, low dissolved oxygen or high chlorophyll a concentrations) affecting the spatial and vertical distribution of zooplankton?*

Here, the environmental parameters explained in average one third of the total variation in zooplankton abundance in Comau Fjord. The most influential factors on zooplankton distribution were in general chlorophyll *a* and oxygen concentrations. Spatially, higher zooplankton abundances and biovolumes were found in the outer fjord and Ancud Gulf compared to the central-inner parts, linked to higher chlorophyll *a* concentration in these areas (**chapter 3**). Vertically, chlorophyll *a* and oxygen concentrations were the highest in the top 20 m of the water column showing a significant relationship with most of the major zooplankton groups (holo- and meroplankton) (**chapters 2, 3 and 4**). Overall, higher zooplankton abundances were observed in surface waters, suggesting that zooplankton was linked with phytoplankton abundances where food was highly available. Few taxa (Amphipoda, Ascidiacea, Euchaetidae and Mysida) were positively related to salinity and to a lesser extent, temperature, indicating the presence of these groups in deeper and more saline waters.

The strong salinity gradient of the fjord and the low oxygen concentration in certain parts of the water column seemed to restrict the DVM of some zooplankton groups. For example, in the deeper, central part of Comau Fjord, the DVM range of mysids was more limited than for other migrating taxa (**chapter 3**). Mysids have generally a low tolerance to lower salinities (Miyashita and Calliari, 2016), thus the low salinity found in surface waters could be limiting the mysid upward migration into shallow waters at night. The shallow innermost part of the fjord was characterized by high freshwater inputs and low oxygen concentration in the deeper water column compared to the central and outer part, mainly due to the high river runoffs and rapid decomposition of autochthonous particulate organic matter coming from nutrient inputs and sedimentation (Silva and Vargas, 2014; Labbé-Ibáñez et al., 2015; Soto et al., 2018). Most of the zooplankton found in this area mainly belonged to the smaller size fraction of the zooplankton community. The DVM of larger taxa seemed to be restricted by their low tolerance to hypoxic waters (although quantitative data are so far lacking) that could not migrate to deeper and more oxygenated waters (Roman et al., 2012).

Overall, environmental variations in salinity, temperature, oxygen and chlorophyll *a* (as a proxy of phytoplankton biomass) significantly influenced the zooplankton dynamics and community composition in Comau Fjord and Ancud Gulf. Altogether, the results of this thesis suggest that these abiotic factors may play an important role in the spatial and temporal dynamics of zooplankton and the biological processes that occur in Comau Fjord.

- *Are meroplankton abundance and composition clearly driven by seasonal changes? Are meroplankton also varying with depth?*

Meroplankton dynamics revealed a marked vertical and seasonal distribution in the study area of this thesis (**chapter 4**), probably governed by the variability of hydrographic conditions (Schlüter and Rachor, 2001; Fetzer and Arntz, 2008). The highest abundances of meroplankton in Comau Fjord were detected in surface waters gradually decreasing with depth coinciding with the highest chlorophyll *a* and oxygen concentrations. This suggests a close coupling between most of the meroplankton groups and phytoplankton abundance. Despite decreasing abundance with depth, the majority of meroplankton taxa were distributed throughout the entire water column. The only exceptions represent bryozoan larvae, which mainly inhabited surface and intermediate waters, and ascidian larvae, which resided only in deep waters.

Spawning of benthic invertebrates has often been associated with cues provided by phytoplankton (Starr et al., 1990, 1991), so the spring phytoplankton bloom is usually linked with a high meroplankton dominance in zooplankton populations (Michelsen et al., 2017). Accordingly, in this thesis the highest meroplankton abundances in Comau Fjord were found in spring, suggesting that most of these larvae depend on phytoplankton production as a food source. However, the spawning cues or residence times of the very diverse and distinct benthic invertebrates can greatly vary among each other, contributing to the shifting of the meroplankton composition throughout the year. For instance, in Comau Fjord, the meroplankton community composition shifted from an echinoderm dominated community in spring, towards a gastropod dominant community in summer and autumn, and a bryozoan dominant community in winter (**chapter 4**).

Meroplankton are key elements on the bentho-pelagic coupling of ecological systems, where abundant and healthy communities will constitute the foundation for the survival of the benthic invertebrate populations. Monitoring meroplankton dynamics can provide important insights into the fitness of benthic communities. This thesis therefore, underscores the importance of assessing the meroplankton community and that it may serve as useful tool to identify biological shifts in the ecosystem under anthropogenic perturbations. Such pressures are increasing in Patagonian fjords so the assessment of meroplankton dynamics is key to evaluate its potential consequences for the fjord communities.

- *What is the natural food spectrum of cold-water corals? What role zooplankton play on cold-water corals diet? Do they have to adapt to different food regimes?*

The biochemical composition of the cold-water coral *Desmophyllum dianthus* presented in this thesis support that corals are able to feed on a wide range of plankton organisms (**chapter 5**) (Gori et al., 2018; Höfer et al., 2018). A rich food supply has been attributed to the success of cold-water corals in Comau Fjord, even in adverse conditions. Yet, trophic markers showed that corals from the deep sites have different dominating markers compared to shallow corals. Characteristic fatty acid trophic marker ratios in deep corals suggested a higher contribution of carnivorous zooplankton to their diet, whereas diatom and flagellate markers were more dominant in the shallow coral's diet. Phytoplankton markers, however, are often ambiguous, indicating either the direct phytoplankton uptake or the consumption of herbivorous grazers (e.g. cyclopoid copepods) containing accumulated phytoplankton markers (Sargent and Falk-Petersen, 1988; Kattner et al., 2003; Lischka and Hagen, 2007). In Comau Fjord, the high abundances of cyclopoid copepods and small calanoid copepods in shallow waters (**chapters 2 and 3**) might have contributed to the phytoplankton markers observed in shallow corals. Similarly, the rich calanoid signal observed from deep corals might not necessarily derived from direct uptake but from larger carnivorous zooplankton that fed on calanoid copepods. The higher abundances of mysids and the carnivorous calanoid copepod Euchaetidae observed in deeper waters might have contributed as an important food source for the deep corals in Comau Fjord.

It has been shown in this thesis that zooplankton distribution and dynamics can be highly variable in temporal and spatial scales. For instance, zooplankton abundance and biomass are highly influenced by strong environmental variations (**chapter 2**), therefore it is expected that cold-water corals are adapted to seasonal differences in food availability, i.e. high zooplankton abundances in spring and low concentrations in autumn and winter. Physiological data supported the prevalence of energy reserves throughout the year (**chapter 5**), even in winter despite the low zooplankton availability. Spatially, the highest abundances of zooplankton were found in shallow waters (**chapters 2, 3 and 4**), therefore shallow corals should thrive in an environment where food is abundant compared to deep corals where zooplankton availability was lower. Yet, deep corals had a higher lipid content and a thicker coral tissue with high energy storage capacity (**chapter 5**) and have been found to grow faster (Beck et al., 2022). This difference in zooplankton abundance and coral energetics may be due to a) the high competition for space and food (Buss, 1979) of shallow corals which grow on denser banks along with a diverse benthic invertebrate community compared to deep corals, b) the high environmental variability observed in shallow waters inflicting an energetic burden, c) differences in the zooplankton composition from the central water column and the plankton close to the corals, as well as the different quality as a food source of the zooplankton composition from the shallow and deep zooplankton populations, or d) the bias towards larger mobile zooplankton taxa (see 6.2. Methodological discussion).

In addition, the assessment of the fatty acid trophic markers provides new insights into the suitability of these biomarkers to assess the natural diet in *Desmophyllum dianthus* populations that differ in their health (metabolic rates and energy reserves). These corals derive from contrasting habitats where distinct conditions, such as physico-chemical conditions (Beck et al., 2022) and food availability (**chapter 2 and 3**), have been observed and together contribute to these differences in health.

6.2. Methodology discussion

This chapter discusses the potential advantages and constraints of the integrated analysis system in the data acquisition and image processing used in this thesis. As seen in previous chapters, ZooScan provides the digitalization of every preserved zooplankton sample, resulting in a high-resolution image associated to specific metadata (e.g. name of location, date and time of sampling collection, bottom and sampling depth, net type, fraction splitting ratio, etc) (Gorsky et al., 2010). One of the advantages of the scan digitalization of the zooplankton samples is the permanent record of the sample content, which can be shared with other laboratories and compared with other studies. The scanned image is segmented based on a scale of different grey levels and the objects are detected and processed with the ZooProcess into separated individual images (“vignettes”). These vignettes provide estimates of body length and width, which can be converted to size spectra calculating the biovolume of each organism in a fairly fast process. The plankton identifier software EcoTaxa combines a) the semi-automatic classification with computer speed of plankton vignettes and counting of organisms from each sample and b) human skills to discriminate organisms with a very high level of detail. Thus, the integrated analysis system, consisting of ZooScan, ZooProcess and Ecotaxa, allows to efficiently process a high number of fixed zooplankton samples in a shorter period of time compared to traditional manual techniques (e.g. taxonomic identification and counting of organisms by microscopy).

On the other hand, there are three biases that might alter the size spectra results at different steps of the image processing that need to be considered (Vandromme et al., 2012). The first bias comes from objects touching each other during image acquisition. When one vignette contains multiple touching objects, it is considered as a single, large object modifying the abundances and biovolumes of the different categories found in the zooplankton population. In this case, ZooProcess provides a solution by using the “separation with mask” tool in the generated digital image, where lines have to be manually drawn to separate the touching objects. However, this is a highly time-consuming process and sometimes separation cannot be done when taxonomic resolution might be reduced (e.g. cutting overlapping urosomes from two different copepods). Generally, the number of touching objects decrease by reducing the concentration of the zooplankton sample in the scanning frame or it may increase if a lot of sticking material (e.g. mucus or detritus) is found within the sample, so it is always a trade-off between the sample concentration and the quality of the resulting image. The second bias comes from errors in the automatic classification of objects into different zooplankton categories. The accuracy of the automatic classification highly depends on the level of taxonomic identification, the number of vignettes contained in each category and the shape of the organisms. At the end of the automatic classification, individual manual validation of all objects needs to be done by an expert to assure a correct classification. The third bias is the choice of a single geometrical model (usually Equivalent Spherical Diameter (ESD), as used in this thesis) to calculate biovolume for all zooplankton categories. Normally, copepods, the dominant group of zooplankton populations, are well represented by an ellipsoid (Herman et al., 2004), however, this tends to over- or underestimate the biovolume estimations of zooplankton groups that are not typically spherical and have very different morphologies. For example, for large not-rounded individuals (e.g. appendicularians, chaetognaths, or polychaetes) overestimation in the biovolume calculations have been found compared to microscopy or C/N relationships (Gorsky et al., 2010; Naito et al., 2019).

The use of digital images of zooplankton organisms might also have some taxonomic limitations. The use of grey silhouettes can sometimes suffer from reduced resolution in comparison with other manual visualization techniques limiting the taxonomic level that can be reached (order or family level) (García-Comas et al., 2016). For instance, ZooScan might not be the best option for meroplankton identification (**chapter 4**), as larvae are usually quite small and

ZooScan detection limit might not quantitatively identify the morphological differences between some larvae. The most optimal plankton size range for ZooScan is 10^2-10^4 µm in ESD (Lombard et al., 2019), therefore it might be better for future meroplankton research the use of other optical devices, such as FlowCam or FlowCytoBot (best plankton size range 10^1-10^2 µm, Lombard et al., 2019), which are more suitable for smaller organisms. Also, as a future perspective, to better identify and reduce the number of touching objects in the resulting scan (which manual separation is time-consuming), net samples could be first size fractioned using the ZooScan for objects >500 µm and image the small organisms with another and more suitable device.

One methodological concern in this thesis was the use of a small mesh size net (100 µm) for collecting the zooplankton samples. This type of net may not efficiently sample large and more mobile zooplankton taxa. For instance, euphausiids are important contributors to zooplankton communities in Chilean fjords (Antezana, 1976; Guglielmo and Ianora, 1997; Palma and Silva, 2004). However, they were severely underestimated in our net catches and not quantitatively caught. This was likely related to the small filtered volume, the patchy distribution of euphausiids and their ability to escape slow nets (Brinton, 1962). Similarly, other large and motile zooplankton (e.g. amphipods, cnidarians, chaetognaths, mysids or carnivorous copepods) might have also been quantitatively undersampled by our nets. The ability of avoiding towed nets by large zooplankton is one of the major biases for underestimation of the zooplankton abundance measurements (Wiebe and Holland, 1968). No single net can quantitatively sample the entire zooplankton size spectra (Clutter and Anraku, 1979). Therefore, ideally the best method would have been the use of two nets, one small net (e.g. 100 µm) for sampling small zooplankton, which was very well represented in our results, and another bigger net (e.g. 300 µm) for sampling larger zooplankton taxa.

Another methodological concern came regarding the depths of the stratified vertical hauls, especially in the surface layer, where the highest environmental variations were found (**chapters 2, 3 and 4**). The biggest changes in salinity, temperature and oxygen were mostly observed in the upper 20 m of the water column, therefore, our 0-50 m depth stratum was too coarse to detect the influence of the halo-, thermo-, and oxyclines present in the water column on the zooplankton distribution in a finer scale. For example, vertical migration might have been limited to a depth of 20-30 m, and zooplankton with low salinity tolerance might have not been able to migrate upwards to the water surface into brackish waters. The here used spatial resolution was not able to discriminate between zooplankton groups with different salinity tolerance levels and up to which depth vertical migration took place. A finer vertical resolution, e.g. sampling zooplankton communities every 10 m, would have been clearly preferable. Despite some limitations, the here presented studies provide important new insights into the zooplankton community of Comau Fjord, previously barely known but critical for its rich benthic communities. Further, this thesis provides important steps forward in the ecological assessment of Comau Fjord and underscores key aspects essential to be integrated in its long-term monitoring.

6.3. Key findings

The take home messages of this thesis are summarized by the following concluding remarks:

- Seasonal distribution:
    - The zooplankton distribution showed a pronounced seasonality in Comau Fjord, associated to the phytoplankton annual cycles.
    - Zooplankton abundance peaked in spring, while highest biovolumes and biomasses were observed in summer. Lowest zooplankton abundance, biovolume and biomass were found during the cold seasons, in late autumn and winter.

- Spatial, vertical and diel distribution:
    - Higher zooplankton biovolume was found in the outer part of Comau Fjord, indicating the presence of larger taxa or individuals, associated to higher chlorophyll *a* concentration.
    - Highest abundances were observed in surface waters during day and night, whereas biovolume was higher in intermediate waters during daytime and in surface waters at nighttime due to the vertical migration of large zooplankton.

- Community composition:
    - Zooplankton was mainly dominated by copepods, followed by chaetognaths, mysids, cnidarians and amphipods (in biovolume) and meroplankton, appendicularians and ostracods (in abundance).

- Meroplankton dynamics:
  - Meroplankton showed a strong seasonality and vertical distribution, where highest abundances were detected in spring and in surface waters, suggesting a close coupling between macrobenthos reproduction and phytoplankton feeding by meroplankton.
  - Different meroplankton taxa dominated seasonally, with echinoderms dominating in spring, gastropods in summer and autumn, and bryozoans in winter.

- Trophic interactions:
  - Corals from the deep sites showed a higher zooplankton dominance in their fatty acid trophic markers, while diatom and flagellate markers were more prominent in corals from shallow sites.
  - While deep corals can easily meet the necessary levels to maintain their general fitness, shallow corals appear to need more energy under the prevalent environmental conditions with observed strong fluctuations.

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Contribution to multi-author article/manuscript

Declaration on the contribution of the candidate to a multi-author article/ manuscript which is included as a chapter in the submitted doctoral thesis

Manuscript 1: Seasonal and diel variations in the vertical distribution, composition, abundance and biomass of zooplankton in a deep Chilean Patagonian Fjord

Contribution of the candidate in % of the total work load (up to 100% for each of the following categories):

Experimental concept and design: ca. 85 %

Experimental work and/or acquisition of (experimental) data: ca. 85 %

Data analysis and interpretation: ca. 90 %

Preparation of figures and tables: ca. 100 %

Drafting of the manuscript: ca. 90 %

Manuscript 2: Spatial and temporal dynamics of zooplankton along a section through Comau Fjord to the Ancud Gulf, Chile

Contribution of the candidate in % of the total work load (up to 100% for each of the following categories):

Experimental concept and design: ca. 80 %

Experimental work and/or acquisition of (experimental) data: ca. 85 %

Data analysis and interpretation: ca. 90 %

Preparation of figures and tables: ca. 95 %

Drafting of the manuscript: ca. 90 %

Manuscript 3: Spatio-temporal dynamic of meroplankton in a deep temperate Patagonian fjord

Contribution of the candidate in % of the total work load (up to 100% for each of the following categories):

Experimental concept and design:	ca. 85 %
Experimental work and/or acquisition of (experimental) data:	ca. 90 %
Data analysis and interpretation:	ca. 90 %
Preparation of figures and tables:	ca. 100 %
Drafting of the manuscript:	ca. 90 %

Manuscript 4: Lipid biomarkers reveal trophic relationships and energetic trade-offs in contrasting phenotypes of the cold-water coral *Desmophyllum dianthus* in Comau Fjord, Chile

Contribution of the candidate in % of the total work load (up to 100% for each of the following categories):

Experimental concept and design:	ca. 5 %
Experimental work and/or acquisition of (experimental) data:	ca. 5 %
Data analysis and interpretation:	ca. 15 %
Preparation of figures and tables:	ca. 5 %
Drafting of the manuscript:	ca. 15 %

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