PLANKTON FOOD WEB STRUCTURES AND INTERACTIONS IN THE EASTERN MEDITERRANEAN SEA

Dissertation

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Summary

Very little is known about the ecology and physiology of marine zooplankton under ultra-oligotrophic conditions. Providing new insight on this front is the main objective of the PhD thesis presented here. The marine environment around Crete (the Cretan Sea, the western and eastern Cretan Straits, and the Cretan Passage) is not only characterized by ultra-oligotrophic conditions, but most importantly, this region hosts (southeast of Crete) the Rhodes Gyre, a cyclone that is generally assumed to be the formation area of Levantine Intermediate Water (LIW). LIW is one of the most crucial water masses of the Mediterranean since it plays a key role in the deep convection both in the North Western Mediterranean and in South Adriatic, it contributes to the hydrodynamic exchanges in distant sea straits (Sicily and Gibraltar) and it fills nearly all the intermediate layers of the Eastern Basin. Most of the data on zooplankton around Crete stem from cruises studying the LIW (e.g. POEM, GOIN) during the mid-80s-mid-90s or the onset of the Eastern Mediterranean Transient, a very important climatically-induced shift in hydrography that occurred during 1989-1995. These data are mostly concentrated on the taxonomy, whereas few studies were conducted to study carbon budget and isotopes. Given the importance of this area for the ecology of the Eastern Mediterranean Sea, the literature on the mesozooplankton is very limited, indicating the need for further investigation.

The aim of the present thesis is to investigate the temporal and spatial distribution patterns of mesozooplankton as well as their metabolic rates and trophic interactions in relation to environmental factors in an ultra-oligotrophic environment around Crete (Cretan Sea, western and eastern Cretan Straits, Cretan Passage). Moreover we tried to underline the importance of the Rhodes Gyre.

Data collected from two different cruises (April and June 2016) were combined in order to answer our objectives. Four biochemical indices (ETS, spAARS, isotopes, fatty acids) were used to study the metabolic rates, trophic relations and feeding preferences of mesozooplankton as well as to verify the ultra-oligotrophic character of the EMS (first cruise). In synthesis **(Chapters 2 & 3)**, this study showed that mesozooplankton communities indicated a slight gradient in

total abundance and biomass, increasing from west to east in the Cretan Passage, probably due to the influence of Rhodes Gyre carrying high salinity and potentially nutrient-enriched Levantine Sea Water from the eastern Levantine Sea. The mesozooplankton community structure changed with depth, but not spatially (horizontally). Omnivory was the prevailing feeding mode. In combination with the results of the carbon flux budget (low respiration, growth and production rates), the oligotrophic character of the Cretan Passage was accentuated, indicating that the zooplankton is not well fed and that the organisms are living under oligotrophic stress.

The importance of the Rhodes Gyre was also highlighted in the results of the second cruise **(Chapter 4)**. A west-to-east increasing gradient (both in mesozooplankton and Chl *a*) was recorded in the Cretan Passage, whereas the lowest zooplankton abundances were recorded at stations along the Cretan Sea. The most significant aspect of copepod functional groups in the study area was the high dominance of small size species implying low metabolic rates and restricted energy demands. The dominance of small size species was also emphasized by the NB-SS (Normalized Biomass Size Spectrum) slope values. Small ambush feeding carnivores were found to be the most important component of the epipelagic zone at all stations. It is a well surviving model obtaining optimal resource allocation in this ultra-oligotrophic region since the species exhibiting ambush feeding mode have low energy demands, low predation risks, high longevity and low fecundity rates. Low copepod production results also highlighted the oligotrophic character of the studied area.

This thesis provides significant new insight on zooplankton ecology and ecophysiology under ultra-oligotrophic conditions. It also underlined the general, ultra-oligotrophic character of the Cretan Sea, the western and eastern Cretan Straits and the Cretan Passage by applying both classical and innovative methods, giving this study high importance as it advances the knowledge about the mesozooplankton communities, an important part of the pelagic food web of the studied area. The combination of the methods used illustrated an integrated image of the importance of hydrological features on mesozooplankton distribution and life strategies with regard to feeding preferences. It also proved that basic

exploratory research is still needed, while gaps in knowledge should be filled taking advantage of modern technologies and new approaches.

Zusammenfassung

Ueber die Ökologie und Physiologie des marinen Zooplanktons unter ultra-oligotrophen Bedingungen ist bisher sehr wenig bekannt. Ein wesentliches Ziel dieser Dissertation ist es, neue Kenntnisse in diesem Bereich zu gewinnen. Die marine Umwelt um Kreta (das Kretische Meeres, die westliche und östliche Straße von Kreta, und die Kreta-Passage) ist nicht nur durch ultra-oligotrophe Bedingungen charakterisiert, südöstlich von Kreta ist auch der Rhodoswirbel lokalisiert. Es wird angenommen, dass in diesem Wirbel Levantisches Zwischenwasser (LIW) gebildet wird. LIW ist eine der wichtigsten Wassermassen des Mittelmeeres, weil es eine Rolle in der tiefen Konvektion sowohl im nordwestlichen Mittelmeer als auch in der südlichen Adria spielt. Darüber hinaus trägt es zum hydrodynamischen Austausch in entfernteren Meeresstraßen (Messina und Gibraltar) bei und füllt fast alle Zwischenschichten des gesamten Mittelmeeres auf. Die meisten das Zooplankton betreffenden Daten um Kreta stammen von Ausfahrten von Mitte der 80er bis Mitte der 90er Jahre, deren Ziel die Untersuchung des LIW war (z.B. POEM, GOIN) sowie von Studien im Zusammenhang mit dem Eastern Mediterranean Transient, einer klimatisch-induzierten Verschiebung in der Hydrographie des östlichen Mittelmeeres zwischen 1989-1995. Diese Daten konzentrieren sich vor allem auf die Taxonomie, wogegen nur wenige Studien zum Kohlenstoffbudget und Nahrungsnetzen durchgeführt wurden. Angesichts der Wichtigkeit dieser Gegend für die Ökologie im östlichen Mittelmeer ist die Literatur über das Mesozooplankton sehr begrenzt, was die Notwendigkeit weiterer Forschungen aufzeigt.

Ziel der vorliegenden Arbeit war sowohl die Untersuchung der zeitlichen und räumlichen Verbreitungsmuster des Mesozooplanktons als auch seiner Stoffwechselraten und der trophischen Interaktionen in Relation zu den ökologischen und abiotischen Faktoren eines ultraoligotrophen Ökosystems (Kretisches Meer, westliche und östliche Straße von Kreta, Kreta-Passage). Die Arbeit basiert auf Freilandbeobachtungen, Laboruntersuchen und statistischen Analysen.

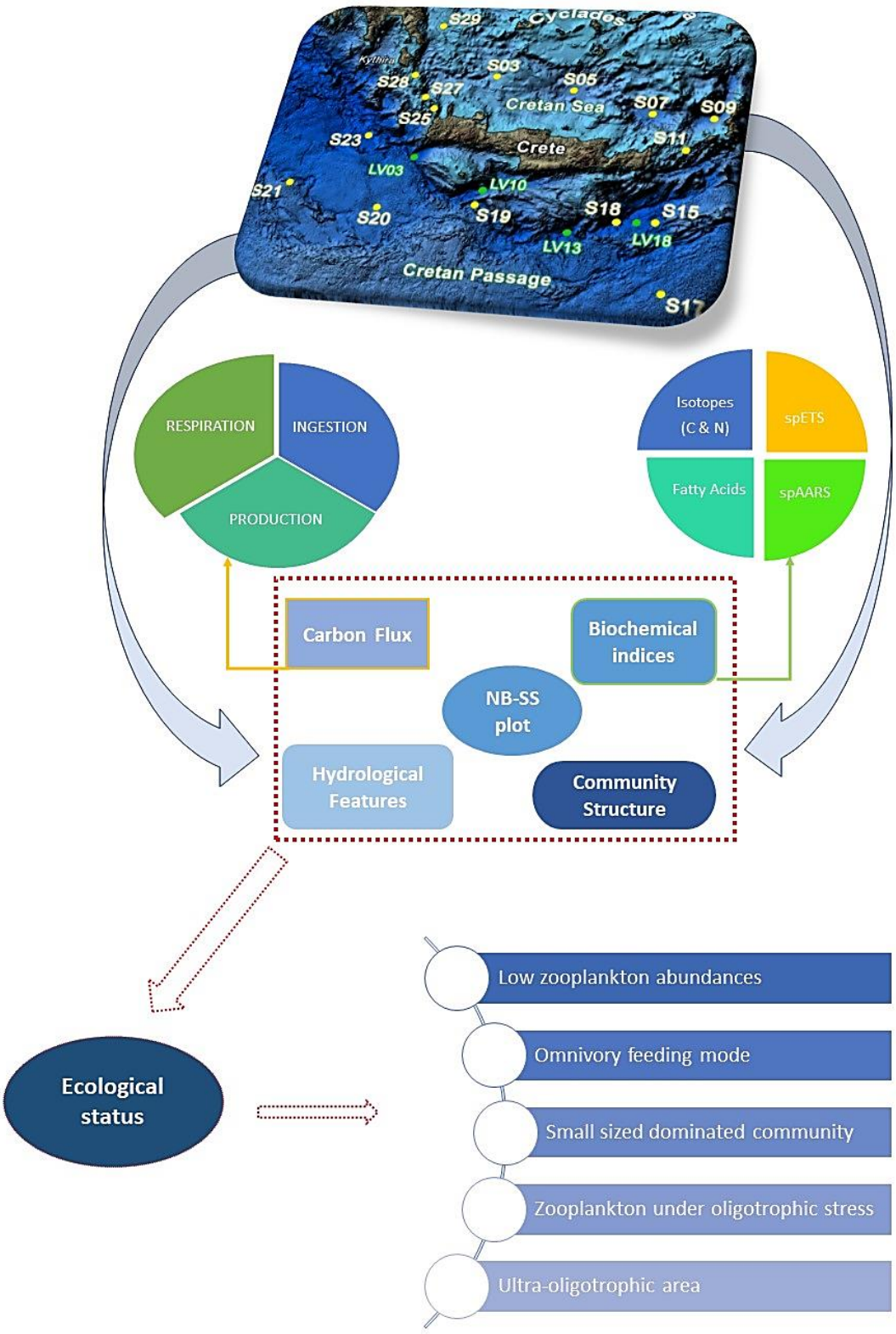
In Rahmen dieser Dissertation wurden vier biochemische Parameter benutzt um Stoffwechselraten, trophische Beziehungen und Futterpräferenzen des Mesozooplanktons zu untersuchen und, um den ultraoligotrophen Charakter des östlichen Mittelmeeres zu erfassen. Zusammenfassend **(Kapitel 2 & 3)** zeigte diese Studie, dass Mesozooplanktongemeinschaften vermutlich aufgrund des Einflusses des Rhodoswirbels im April 2016 einen leichten Gradienten von West nach Ost in der Kreta-Passage aufwiesen. Omnivorie war die vorherrschende Nahrungsstrategie. In Kombination mit den Daten des Kohlenstoffbudgets zeigen die Ergebnisse den oligotrophen Charakter der Kreta-Passage auf und das das Zooplankton unter oligotrophem Stress lebt und nicht gut ernährt wird.

Die Wichtigkeit des Rhodoswirbels wurde auch in den Untersuchungen der beiden Schiffs-Ausfahrten im April und Juni 2016 nachgewiesen **(Kapitel 2 & 4)**. Ein Gradient von West nach Ost (sowohl Mesozooplankton und Chl a) war in der Kreta-Passage zu beobachten, während die niedrigsten Zooplanktondichten an Stationen entlang des Kretischen Meeres gefunden wurden. Innerhalb der funktionellen Gruppen der Copepoden in der untersuchten Region war die starke Dominanz von Arten kleiner Größe auffällig, was niedrige Stoffwechselraten und damit niedrigen Energiebedarf impliziert. Die Dominanz von „kleinen“ Arten wurde auch von den Werten der Steigung des NB-SS (Normalized Biomass Size Spectrum) bestätigt. Kleine Räuber, die sich als aggressive Beutegreifer ernähren, waren die wichtigste Komponente der epipelagischen Zone an allen Stationen. Es handelt sich dabei um eine an diese ultra-oligotrophe Region gut angepasste Lebensweise, da Arten mit lauernd-angreifender Ernährungsweise einen niedrigen Energiebedarf verbunden mit einem niedrigen Risiko selbst gefressen zu werden haben. Langlebigkeit und geringe Reproduktionsraten gehören ebenfalls zu ihren Eigenschaften. Auch die Ergebnisse der Copepoden-Sekundärproduktion bestätigen den oligotrophen Charakter des untersuchten Gebietes.

Diese Dissertation konnte erhebliche neue Kenntnisse über die Ökologie und Ökophysiologie unter ultra-oligotrophen Bedingungen gewinnen. Der ultra-oligotrophen Charakter des Kretischen Meeres, der westlichen und der östlichen Kreta-Straße sowie der Kreta-Passage wurde durch Anwendung klassischer und innovativer Methoden bestätigt. Die Studie ist von hoher Bedeutung, da sie das Wissen über Mesozooplankton-Gemeinschaften im pelagischen Nahrungsnetz des

östlichen Mittelmeeres vermehrt. Die Kombination der angewendeten Methoden ermöglicht es, ein integriertes Bild über die Wichtigkeit abiotischer Einflussgrößen für die Verbreitung von Mesozooplankton-Gemeinschaften und ihre Lebensstrategien im Hinblick auf Nahrungspräferenzen zu zeichnen. Es konnte auch gezeigt werden, dass weitere Grundlagenforschungen zu diesem Thema in dem untersuchten Gebiet nötig sind, während Wissenslücken mit modernen Technologien und neuen Ansätzen geschlossen werden sollten.

“PLANKTON FOOD WEB STRUCTURES AND INTERACTIONS IN THE EASTERN MEDITERRANEAN SEA”



CHAPTER 1

1. General Introduction

1.1 The characteristics of Mediterranean Sea

The Mediterranean Sea (MS) is the largest (basin of 2.500.000 km^2) and deepest (average 1.500 m, max 5.267m) quasi-closed[1] sea on the Earth, with a surface similar to that of the largest semi-enclosed[2] (e.g. the Gulf of Mexico) and open (e.g., the Caribbean Sea) marginal seas of the extant ocean **(Fig. 1.1)**. However it comprises only 0.82% of the total surface area and 0.32% of the total volume of the world ocean (Defant, 1961; Bianchi & Morri, 2000; Meybeck *et al.*, 2007). The MS connects through the Strait of Gibraltar to the Atlantic Ocean in the west, and through the Strait of Bosphorus to the Sea of Marmara and the Black Sea in the northeast, while in the southeast the Suez Canal links the MS to the Red Sea and the Indian Ocean. The eastern basin (1.65 million km^2) and western basin (0.85 million km^2) of the Mediterranean are separated by the Strait of Sicily and the submerged Malta and Tunisian plateaus (Coll *et al.*, 2012). According to Siokou-Frangou *et al.* (2010), the size, location, morphology, and external forcing of the MS allow for a rich, dynamic and complex physical environment that includes: **i)** unique thermohaline features, **ii)** distinctive multilayer circulation, **iii)** topographic gyres, and **iv)** meso- and sub-mesoscale activity. Nutrients and chlorophyll a (chl a) levels rank the basin as oligotrophic to ultra-oligotrophic (Krom *et al.*, 1991; Antoine *et al.*, 1995). It has been known since the early 1980s that the very low concentration of inorganic phosphorus, which is assumed to limit primary production, is the main cause of oligotrophy (Berland *et al.*, 1980; Thingstad and

[1] A quasi-closed entity is defined by a relatively uninterrupted bank, encircling a mass of water of sufficient dimension so that the bank itself can be distinguished from the land behind it, and the connections are not immediate but sufficiently restricted so that it can be comfortably navigated over fairly short periods of time. (Sustainable Geography, R. Brunet, ISTE Ltd 2011)

[2] A gulf, basin or sea surrounded by two or more States and connected to another sea or the ocean by a narrow outlet or consisting entirely or primarily of the territorial seas and exclusive economic zones of two or more coastal States (United Nations Convention on the Law of the Sea. New York: United Nations, 1982)

Rassoulzadegan, 1995, 1999; Thingstad *et al.*, 2005). Other features of the MS are **i)** the decreasing west-east gradient in chl a concentration, as shown by color remote sensing (D'Ortenzio and Ribera d'Alcalá, 2009; Barale *et al.*, 2008) as well as by in-situ data (Turley *et al.*, 2000; Christaki *et al.*, 2001), **ii)** a high marine diversity compared to its surface and volume (Bianchi & Morri, 2000), and **iii)** a relatively high number of bioprovinces (*sensu* Longhurst, 2006), with boundary definitions mostly based on the distribution of the benthos and the nekton (Bianchi, 2007). All the aforementioned characteristics are expected to be reflected in the structure and dynamics of plankton communities.

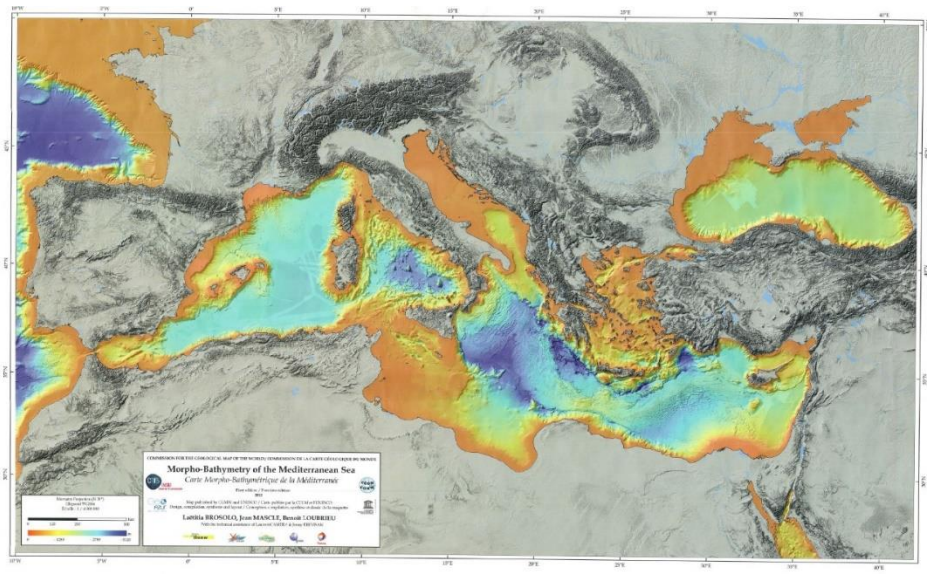


Figure 1.1 Morphobathymetric map of the Mediterranean Sea, publication CCGM/CGW, UNESCO, Paris (Brosolo *et al.,* 2012)

A first synthetic overview of the pelagic MS ecosystems was provided in the late 1980s by Margalef (1985), Moraitou-Apostolopoulou and Kiortsis (1985), and by Minas and Nival (1988). Primary productivity, chl a, mesozooplankton biomass and organism distributions were the first parameters to be explored, until the discovery of picoplankton (e.g., Waterbury *et al.*, 1979) and the consequent increased attention for the role of microheterotrophs within the pelagic food web, thus providing new perspectives for the understanding of oligotrophic seas such as the MS (Rassoulzadegan, 1977; Hagstrom *et al.*, 1988). Entering the nineties, many

research efforts were fervent to study carbon and nutrient fluxes in order to provide insight into the key players of the MS pelagic food web (e.g., Lipiatou *et al.*, 1999; Thingstad & Rassoulzadegan, 1999; Tselepides & Polychronaki, 2000; Monaco, 2002; Mazzocchi *et al.*, 2003; Krom *et al.*, 2005) and to the relevant biological processes and/or physiological rates (e.g., Calbet *et al.*, 1996; Estrada, 1996; Saiz *et al.*, 1999; Moutin & Raimbault, 2002), while the phosphorus limitation hypothesis has inspired studies on the effects of phosphorus enrichment on the pelagic food web (Thingstad *et al.*, 2005). Physical-biological coupling in general (Crise *et al.*, 1999; Pinardi *et al.*, 2004), as well as in relation to mesoscale dynamics, has also been addressed more frequently during the last decades (e.g., Champalbert, 1996; Alcaraz *et al.*, 2007). Clearly these studies have provided valuable insights on the components of the MS plankton in different areas of the basin.

1.2 The characteristics of Eastern Mediterranean Sea and West Levantine Sea

The Strait of Sicily (sill depth ~500 m) is the connection between the western and eastern basin of the MS. The Eastern Mediterranean Sea (EMS) is thus itself an isolated concentration basin where the evaporation is higher, causing the water level to decrease and salinity to increase from west to east. The resulting pressure gradient pushes relatively cool, low-salinity water from the Atlantic across the Mediterranean basin. This water warms up to the east, where it becomes saltier and then sinks in the Levantine Sea before circulating west and exiting through the Strait of Gibraltar. (Robinson *et al.,* 1992, Coll *et al.*, 2010).

According to Lascaratos *et al.* (1999), the circulation of the MS is usually described as an open thermohaline cell with two closed secondary cells, one for each sub-basin. The principal cell describes the transformation of the surface Atlantic Water (AW) to the Levantine Intermediate Water (LIW), which is the main contributor to the Mediterranean outflow into the Atlantic. The other two cells describe the transformation of surface and intermediate water to Western Mediterranean Deep Water (WMDW) and to Eastern Mediterranean Deep Water (EMDW). The existence of an intermediate depth cell is mainly controlled by the Gibraltar and Sicily Straits sills (Phillips, 1966). Additionally, the sill in the Strait

of Sicily prevents a direct communication between the EMDW and WMDW but coupling is achieved via the LIW layer.

The general circulation of the MS is also characterized by the presence of permanent or semi-permanent sub-basin gyres, which are mostly controlled by the topography (Robinson & Golnaraghi, 1994). Most important in the EMS is the cyclonic Rhodes Gyre (NW Levantine Sea), which is known to be the part where LIW is formed (Ovchinnikov, 1984; Malanotte-Rizzoli & Hecht, 1988; Lascaratos, Williams & Tragou, 1993; Lascaratos & Nittis, 1998). Some of the permanent mesoscale structures have been shown to heavily influence the local dynamics, affecting the distribution of nutrients and, as a consequence, the biologica1 activity. The cyclonic circulation (e.g. the Rhodes Gyre and the Cyprus Eddy) enriches the euphotic zone through the upwelling of nutrient-rich deep waters. On the other hand, downwelling processes occur in the anticyclonic areas (e.g. the anticyclonic eddies surrounding the Rhodes gyre), leading to an impoverishment of the surface waters (Mazzochi *et al.*, 1997).

1.3 The pelagic food web and the role of mesozooplankton

The physical and chemical features described above give the EMS the unique identity of one of the most oligotrophic areas of the world (Siokou-Frangou *et al.*, 2010; Zohary & Robarts, 1992). It is impoverished in terms of dissolved nutrients (Redfield *et al.*, 1963) and phytoplankton production (Dugdale & Wilkerson, 1988) with little fisheries yield and a limited influence on the global carbon cycle (Koppelmann *et al.*, 2004). Regionally, anthropogenic eutrophication such as in the Saronikos Gulf which had increased with rapid urbanization in the 20th century has turned to re-oligotrophication resembling the previous, non-impacted state (Tsiamis *et al.,* 2013). Organic carbon and nutrients are re-mineralized and recycled efficiently within a complex microbial food web with little energy transfer to the higher trophic levels (Turley, 2000; Van Wambeke, 1996). Hence, a west-to-east decrease of standing stock of zooplankton emerged from several studies (e.g. (Mazzochi *et al.*, 1997; Kovalev *et al.* 1999; Dolan *et al.*, 2002; Siokou-Frangou 2004, Nowaczyk *et al.*, 2011; Siokou-Frangou et al., 2019). For the above reasons, the EMS is interesting from both physical and biological perspectives.

The most widespread type of food web on Earth is the pelagic food web **(Fig. 1.2)**, and planktonic organisms involved in this type of food web are possibly the most abundant on Earth. Concerning only small planktonic marine copepods, they are the most abundant metazoans on Earth, including copepodites and adults of important calanoid genera such as *Paracalanus, Clausocalanus* and *Acartia*; cyclopoid genera such as *Oithona*, *Oncaea*, and *Corycaeus*; planktonic harpacticoids of the genus *Microsetella*; and nauplii of almost all copepod species (Turner, 2004). Therefore, it is not surprising that the dynamics of planktonic food webs have great impacts on significant issues such as world climate (e.g. Beaumont *et al.* 1998, Toole & Siegel 2004), global biogeochemical cycling (e.g. Dachs *et al.* 2002, Valdes *et al.* 2004) and the world food production (e.g. Meadows *et al.* 2004). To understand these impacts but also how the environmental changes, such as eutrophication and climate change, influence the function of food webs, we need to understand the processes determining food web structure.

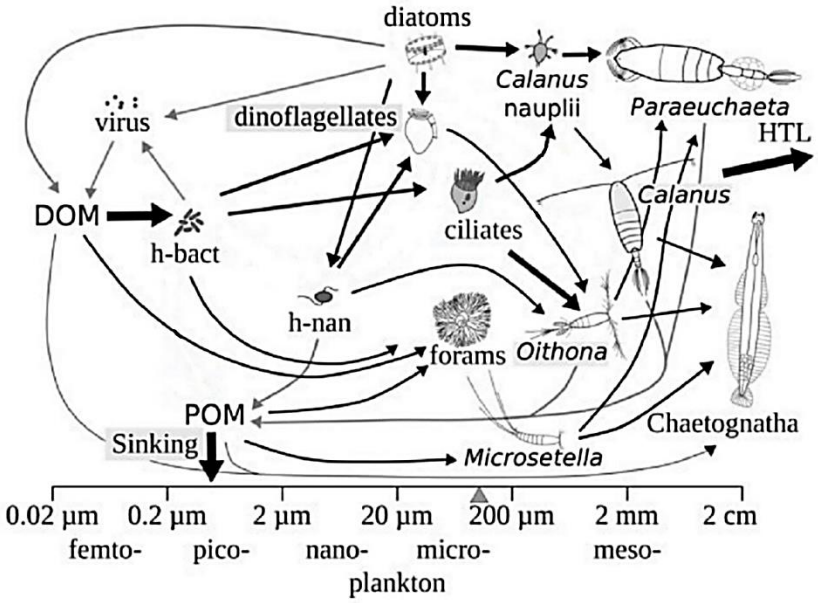


Figure 1.2 Basic food web with general key players in the marine pelagic and some of the observed species at our stations in the EMS. Several trophic pathways (black) and recycling pathways (grey) are shown, but by no means all (POM: Particulate Organic Matter, DOM: Dissolved Organic Matter, HTL: Higher Trophic Level). (Illustration from Basedow *et al.* 2016)

It is known that the concentrations of nutrients and the process of nutrient cycling greatly influence the dynamics and food web structure within pelagic systems. For example, in nutrient-rich waters the classical or herbivorous food web is predominant, while in nutrient-constrained environments, such as the one in the EMS, the microbial food web is of greater importance (Legendre & Rassoulzadegan, 1995). In the classical food web that consists of large phytoplankton, zooplankton and fish both resource and predation seem to be important structuring factors (Carpenter *et al.* 1985, 1987). Less clear is, however, the structure and regulation of the microbial food web.

According to Malone (1971) and Williams (1981), the recognition of the trophic importance of bacterioplankton and protozoans in marine waters has outdated the traditional model of a short marine food chain (phytoplankton - copepods - fish). Nowadays, it is accepted that a significant proportion of phytoplankton production is not consumed directly by zooplankton grazers, but is cycled by the microbial community ('microbial loop'), before it becomes available to consumers. Water-column bacteria, heterotrophic flagellates and ciliates are the primary organisms involved in the recycling activities of the microbial loop. This is particularly important in warm, low-nutrient waters, where microbes rapidly and efficiently recycle materials and thus limit the sinking of large amounts of organic matter to the bottom (Suthers & Rissik, 2009).

The scientific trend for monitoring ecological processes uses the measurement of carbon flux in the ocean through food webs. Carbon flux in the ocean depends mainly on the magnitude of primary production and the biochemical processes within the photic zone, as well as on the complexity of the pelagic food web, i.e. the relative abundance or biomass of its components and interactions between them. Therefore, whatever the productivity level, studies in the photic zone based on simultaneous estimates of the biomass and production of phytoplankton, bacteria, heterotrophic nano- and microplankton and mesozooplankton together are essential for the assessment of the carbon flux (Nielsen *et al.*, 1993; Nielsen & Hansen, 1995; Richardson *et al.*, 1998; Bradford-Grieve *et al.*, 1999). In oligotrophic areas, the food web is dominated by minute producers and consumers (Thingstad & Rassoulzadegan, 1995; Christaki *et al.*, 1996; Caron *et al.*, 1990; Turley *et al.*, 2000; Siokou-Frangou *et al.*, 2002) and most

of the carbon flow is through microbial communities (Azam *et al.*, 1983; Sherr & Sherr, 1988; Roman *et al.*, 1995).

Mesozooplankton plays a critical role in the pelagic carbon-flow processes (**Fig. 1.3**) through their interactions with higher and lower trophic levels within the water column or with the benthic community (Isari *et al.*, 2007). According to the ''traditional'' food web, copepods mediate between the primary producers (mainly diatoms) and the commercially important trophic levels—fish, without high loss in the energy transfer (Cushing 1989). This mainly applies to the large-sized copepods (i.e., large calanoids), whereas small-sized copepods (<1 mm in length) are capable of efficiently exploiting components of the microbial food web. They serve as major grazers of phytoplankton, as components of the microbial loop by preying upon bacterioplankton and heterotrophic protists, and as prey for ichthyoplankton and other larger pelagic carnivores (Turner, 2004).

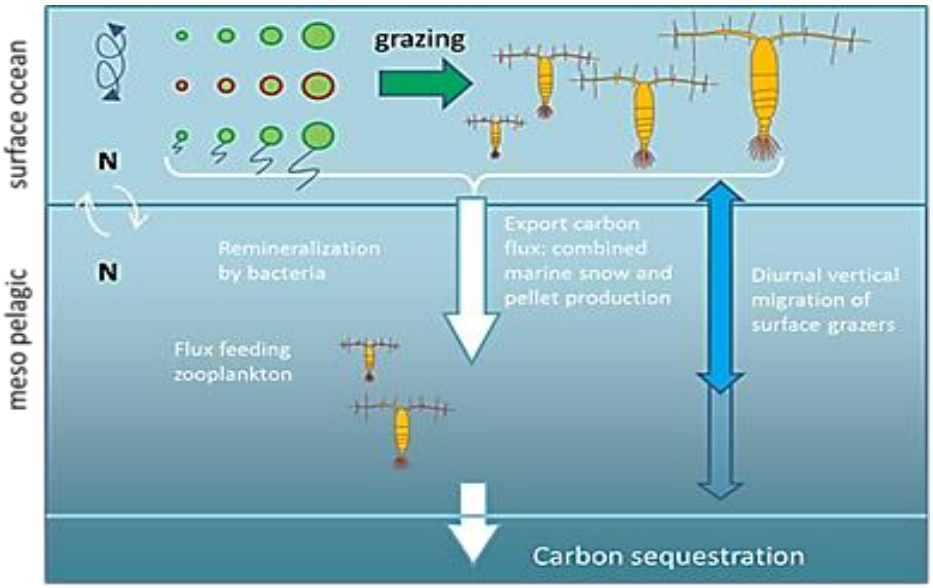


Figure 1.3 Carbon export flux and plankton traits. A full sized-based trophic model bridging from bacteria and phytoplankton to multicellular zooplankton by combining the unicellular and community models (http://www.mecano-plankton.dk/project/bio-pump)

According to Field *et al.* (1998) zooplankton consume a significant proportion of the primary production (~ 48.5 Pg (1 Pg=10^{15}g)) of carbon each year across the world's oceans, and through their metabolism play a key role in the recycling of carbon, nitrogen and other elements. The microzooplankton (<200 μm, the majority of which are heterotrophic protozoans) which are the principal grazers, consume on average 49-70% of the daily primary production across a range of habitats (Schmoker *et al.*, 2013), whereas mesozooplankton (>200 μm, including the abundant crustaceans) consume on average 10% to 40% of the daily primary production for the high- and low-productivity regions (Calbet, 2001, 2008).

By feeding in surface waters and producing sinking particles (e.g. fecal pellets), zooplankton contributes to the nutrient pool (Turner, 2015). Furthermore, by actively transporting dissolved and particulate matter to different depths via diel migration (e.g. Longhurst *et al.*, 1990; Steinberg *et al.*, 2000). According to Suthers and Rissik (2009) nutrient recycling is also assisted by the 'sloppy feeding' or partial ingestion of cells by herbivorous zooplankters (such as copepods), which results in the release of nutrient-rich cell sap following handling and rupture of captured cells. Zooplankton fecal pellets, molts, mucous products and carcasses help support the metabolism of deep-sea pelagic plankton and fish as well as benthic communities. Zooplankton grazing also largely determines the amount and composition of vertical flux. They affect the attenuation of sinking particle flux with increasing depth, through their feeding and metabolism of sinking Particulate Organic Carbon (POC) in the mesopelagic zone (Steinberg *et al.*, 2008), affecting the efficiency with which the Particulate Organic Material (POM) is exported and the sequestration of carbon in the deep ocean (Castellani and Edwards, 2017). This not only fuels the benthic community, but also contributes to the removal of surplus anthropogenic CO_2 from the atmosphere through sedimentation and burial of organic and inorganic compounds (Harris *et al.*, 2000).

Studies on food web dynamics may provide important information to understand baseline ecology of organisms, predict community-level consequences of abiotic and biotic changes and characterize trophic interactions. Traditional studies on food web dynamics have used gut content analysis and direct field observations to elucidate various aspects of population dynamics and community structure. While a great deal of information may be gleaned, these approaches are

labor intensive, logistically difficult and often ambiguous with regard to what was consumed and what was assimilated (Kelly & Scheibling, 2012). More recently, stable isotopes and lipid biomarkers (fatty acid analysis) have been used to identify specific food web relationships as they provide time-integrated information on an organism's assimilated diet (El Sabaawi *et al.*, 2009; Van den Meersche *et al.*, 2009; Allan *et al.*, 2010; Kelly & Scheibling, 2012)

1.4 Mesozooplankton abundance and composition

In the open MS, the bulk of epipelagic mesozooplankton is concentrated in the upper 100 m layer and sharply decreases beneath this depth (Scotto di Carlo *et al.*, 1984; Weikert & Trinkaus, 1990; Weikert & Koppelmann, 1993; Mazzocchi *et al.*, 1997). According to Longhurst and Harrison (1989), mesozooplankton plays a major role in biological processes in this layer, based on its linkage with phyto- and micro-zooplankton in the euphotic zone. During the night, the epipelagic layer is enriched by the diel migrants that ascend from the mesopelagic layer (Weikert & Trinkaus, 1990; Andersen *et al.*, 2001; Raybaud et al., 2008). However, the epipelagic mesozooplankton standing stocks do not differ significantly between day and night (Mazzocchi *et al.*, 1997; Ramfos *et al.*, 2006; Raybaud *et al.*, 2008). Zooplankton distribution patterns may show high local variability, with notable spatial changes even during the same season (Nival *et al.*, 1975).

Epipelagic mesozooplankton communities in the open MS are highly diversified in terms of taxonomic composition, but copepods represent the major group both in terms of abundance and biomass. The dominance of small copepods (mostly ≤1 mm in total length) in terms of both numbers and biomass represents the major feature of the structure of mesozooplankton communities at basin level. In samples collected with coarser mesh nets (333 μm), the 0.5–1 mm size fraction contributes 45–58% to the total mesozooplankton abundance in the open EMS (Koppelmann & Weikert, 2007). The importance of the small-sized copepods has also been highlighted in Mediterranean coastal and open sea waters (Calbet *et al.*, 2001; Zervoudaki *et al.*, 2007).

The zooplankton abundance in oligotrophic areas is typically low and it has been recorded not only in MS (Scotto di Carlo *et al.* (1984) for the Tyrrhenian Sea,

which is considered poorer in zooplankton biomass when compared to other parts of the Western Mediterranean (Scotto di Carlo & lanora, 1983), Siokou-Frangou *et al.*, 2002, 2010) but also for oligotrophic areas in the tropical and North Pacific Ocean (Zenkevitch, 1963) and in Sargasso Sea (Deevey & Brooks, 1977). As described previously, zooplankton distribution and abundance may be affected by local water mass circulation. The permanent or semi-permanent cyclonic gyres of the EMS (e.g., the Rhodos Gyre and the cyclonic gyre south-west of Crete) revealed higher mesozooplankton abundance than the neighboring anticyclonic gyres (Pancucci-Papadopoulou *et al.*, 1992; Mazzocchi *et al.*, 1997; Christou *et al.,* 1998; Siokou- Frangou *et al.,* 1999, 2004).

1.5 Tools to investigate the trophic interactions

A suitable approach to study mesozooplankton physiology and trophic interactions consists of using an array of biochemical indices such as electron transport system activity (ETS), amino acyl-tRNA synthetase activity (AARS), fatty acids (FA) and isotopes (^{13}C, ^{15}N) which have been applied to several zooplankton species (Schukat *et al.*, 2014; Teuber *et al.*, 2014)

The ETS technique was developed by Packard (1971a) and Packard *et al.* (1971b, 1974) and has subsequently been applied to estimate respiration in zooplankton (Bämsted, 1980; Hirch *et al.*, 2009; King & Packard, 1975; King *et al.*, 1978; Koppelmann & Weikert, 1999; Koppelmann *et al.*, 2004; Minutoli & Guglielmo, 2009; Owens & King, 1975; Packard *et al.*, 1974; Schalk, 1988), phytoplankton (Kenner & Ahmed, 1975; Packard, 1971) and bacteria (Arístegui & Montero, 1995; Packard *et al.*, 1983; Packard *et al.*, 1996). This technique is founded upon the notion that the ETS is at the biochemical basis of respiration and controls energy production via oxidative phosphorylation. This technique uses the reduction of an artificial electron acceptor, a tetrazolium-salt (INT), to stoichiometrically measure the capacity of mitochondria to consume O_2. This can be done because the reduction of 2 mol INT by the ETS is equivalent to the ETS-driven reduction of 2 atoms of oxygen (or 1 molecule of O_2; Packard, 1971). The respiratory enzymatic system is saturated with substrates (NADH, NADPH and succinate) to obtain the "potential" activity or maximum activity of the electron

transport system (Φ), as demonstrated in a recent study by Maldonado *et al.* (2012). ETS, as an ecological measure of respiration, is as reliable as any other proxy or index used in aquatic ecology or ocean geochemistry (Del Giorgio & Williams, 2005). ETS is ubiquitous in mitochondrial membranes and can be used as an indicator of organic matter remineralization, as it consists of a complex chain of cytochromes, flavoproteins and metabolic ions that transport electrons from catabolized food to oxygen. ETS activity is directly correlated to *in vivo* respiration (Owens & King, 1975) and can be used as a proxy of mesozooplankton respiration rates.

Aminoacyl-tRNA synthetases are the group of enzymes that catalyze amino acid activation and the aminoacylation of tRNA (Schimmel & Soll, 1979) which is the first step of protein synthesis. Chang *et al.* (1984) was the first to develop a very simple continuous assay for AARS activity, in which they measured the activity of different AARS based on the release of pyrophosphate (PPi) during aminoacylation of tRNA, assessed as the oxidation of NADH by the PPi (O' Brien, 1976). This method was later adapted by Yebra and Hernandez-Leon (2004), in order to be able to assay AARS activity without adding amino acids for measuring the capability to synthesize proteins that individuals have in the field, reflecting their previous food and development history. Positive relationships between AARS activity and growth have been observed in freshwater and marine crustaceans (Yebra & Hernández-Léon, 2004, Yebra *et al.*, 2005, 2006) making AARS activity a good candidate to be used as an index of growth in zooplankton (Herrera, 2014; McKinnon *et al.*, 2015).

Stable isotopes of the major constituents of organic molecules (H, C, N, O, S) may be indicators for the trophic level of an organism and its diet (Peterson & Fry, 1987). With this analysis, it is possible to monitor the state and the dynamics of food webs since heavier isotopes are enriched in organisms relative to their diet (Fry, 2006; Newton, 2010). For terrestrial as well as aquatic ecosystems, the usage of stable nitrogen and carbon isotopes for estimating the trophic position and the carbon flow inside a food web is very advantageous (Kling *et al.*, 1992; France, 1995; Post, 2002). To estimate the trophic position of an organism, the ratio of stable nitrogen isotopes ($\delta^{15}N$) can be used due to an ^{15}N enrichment of 3-4 ‰ per

trophic level (DeNiro & Epstein, 1981; Minagawa & Wada, 1984; Peterson & Fry, 1987) with a mean factor of 3.4 ‰ (Post, 2002). To obtain detailed information of the trophic position, a comparison with the trophic base is necessary. Depending on the examined food web, primary producers like algae (France, 1995) or detritus (Koppelmann & Weikert, 2003, Koppelmann *et al.*, 2009) or primary consumers (Vander Zanden & Rasumussen 1999, Post 2002) can be used as baselines. In contrast to the incremental increase of ^{15}N, the carbon isotope ratio ($\delta^{13}C$) changes only little (0-1 ‰) between trophic levels (Rounick & Winterbourn, 1986; Peterson and Fry, 1987). Therefore, $\delta^{13}C$ allows revealing the carbon sources of organisms inside food webs if they vary in their isotopic signature. Stable isotope analyses of plankton from the EMS were previously performed by Hannides *et al.* (2015), Koppelmann and Weikert (2003) and Koppelmann *et al.* (2009). Mixed zooplankton of different size classes showed relative low $\delta^{15}N$ values in the upper layers which could either be caused by the fixation of atmospheric nitrogen by diazotroph cyanophycea like Synechococcus (Li *et al.*, 1993, Detmer, 1995) or by a lack of significant denitrification in the basin and by particulate organic matter exported from surface waters during the $P_{limited}$ winter plankton bloom (Krom *et al.* 2004). Koppelmann and Weikert (2003) noted that the $\delta^{15}N$ signature increased in deeper layers. The authors determined the trophic level (TL) of zooplankton using $\delta^{15}N$ values of POM assuming that particulate organic matter (POM) is the main source of food in the deep sea (Angel, 1990).

Since stable isotopes are indicators for the origin of organic molecules and directly linked to the diet, the dimensions of the trophic niche may reflect the ecological niche of populations (Bearhop *et al.*, 2004). The isotopic niche can be defined as an area (δ space) with isotopic δ values as coordinates (Layman *et al.*, 2007, Newsome *et al.*, 2007). Jackson *et al.* (2011) developed a method, based on a Bayesian framework, to compare and to visualize the isotopic niches of individual communities (SIBER, Stable Isotope Bayesian Ellipses in R). Further to the definition of the trophic level, the expansion of the isotopic niche widths as well as the overlap can be calculated. In association with information of the trophic level, the niche widths of the copepod taxa show the trophic variability in δ space and how the trophic niches of the different species differ among themselves.

Fatty acids, some of the most important molecules transferred across the plant-animal interface in aquatic environments (Dalsgaard *et al.*, 2003; Allan *et al.*, 2010), can be used as trophic markers since they are transferred without change from primary producers to higher trophic levels within the food web (Alfaro *et al.*, 2006). FAs have a high biological specificity and in conjunction with stable isotope ratios can provide information on the assimilated diet of zooplankton (El-Sabaawi *et al.*, 2009; Van den Meersche *et al.*, 2009; Allan *et al.*, 2010; Kelly & Scheibling, 2012). More specifically, the use of these fatty acid trophic markers (FATMs) derives from the fact that predators retain the taxon-specific compounds that are produced by their prey (e.g bacteria, phytoplankton and microzooplankton) (Dalsgaard *et al.*, 2003). In copepods FATMs can give us information concerning the level of carnivory as well as the specific diet of a species. Carnivorous copepods and zooplankton in general have higher quantities of polar lipids which are rich in polyunsaturated fatty acids (PUFAs) and therefore their ratio to saturated fatty acids (PUFA/SFA) is used as a trophic marker of carnivory (Cripps & Atkinson, 2000; Stevens *et al.*, 2004). Highly unsaturated fatty acids (HUFA) such as the eicosapentaenoic acid EPA (20:5(n-3)) and docosahexaenoic acid (DHA; 22:6(n-3)) are considered to be essential compounds which are growth limiting for herbivorous zooplankton (Müller-Navarra 1995; Müller-Navarra *et al.* 2000; Ravet *et al.* 2003). DHA specifically is an important component of polar lipids and it is found to be highly preserved in the marine food-web (Scott *et al.* 2002; Veefkind, 2003). Since DHA is often dominant in dinoflagellates and EPA in diatoms (Viso & Marty, 1993; Kattner *et al.*, 2009), respectively, their ratio (DHA/EPA) can be used as a reflection of herbivorous and omnivorous copepods diets as well as an indication of their degree of carnivory (Dalsgaard *et al.*, 2003) and therefore higher ratios indicate higher trophic levels. Carnivory can also be inferred from the 18:1(n-9)/18:1(n-7) ratio (Auel *et al.*, 2002; Dalsgaard *et al.*, 2003) since it has been found that 18:1(n-9) is indicative of carnivorous feeding (Falk-Petersen *et al.*, 1990). This ratio has recently become more specific by the addition of other diatom and dinoflagellate FA markers and can therefore be used as 18:1(n-9)/Σ herb markers (Σ herb markers: 16:1(n-7) + 16:4(n-1) + 18:1(n-7) + 18:4(n-3); Schukat *et al.*, 2014). Another widely used trophic marker is the ratio of all diatom markers (D= 16PUFA + 16:1(n-7) + 20:5(n-3)) to all flagellate markers (F= 18PUFA + 18:2(n-

6) + 22:6(n-3)) which is used to distinguish between diatom/flagellate based diets (El-Sabaawi *et al.*, 2009). Terrestrial detritus and green algae in the diet of zooplankton can be inferred from the presence of high proportions of 18:2(n-6) (Dalsgaard *et al.*, 2003) whereas the presence of bacteria can be inferred from the sum of 15:0 and 17:0 fatty acids (Parkes, 1987; Vestal & White, 1989).

1.6 Goals and outline of the thesis

The present study aims to investigate the spatial and temporal distribution patterns of mesozooplankton as well as their metabolic rates and trophic relationships, in relation to the environmental factors and hydrological features of an ultra-oligotrophic environment, the Cretan Sea, the western and eastern Cretan Straits and the Cretan Passage.

In particular, this study will investigate the:

(i) vertical mesozooplankton distribution total abundance and species composition up to 1000 m at selected stations in the Cretan passage along with the plankton food web structure and estimation of carbon budget in the euphotic zone (**Chapter 2**);

(ii) dietary preferences, trophic interaction, potential respiration and somatic growth using biochemical indices and isotopes (**Chapter 2** & **Chapter 3**) and

(iii) mesozooplankton distribution, total abundance and species composition in the photic zone among the different transects, the contribution of important functional groups and the carbon requirements by coupling standing stocks estimations (abundance, biomass and size classes) and metabolic measurements (**Chapter 4**).

For a better understanding of processes and changes in the marine environment, and especially in an ultra-oligotrophic system, it is essential to have the knowledge of the hydrological features that locally affect the mesozooplankton communities, as well as the mesozooplankton trophic interactions and relationships. Though there have been previous studies regarding the trophic

relationships and vertical distribution of mesozooplankton in the present study area of the Eastern Mediterranean Sea, the knowledge on the plankton food web is still very limited. Thus, this study combining a large number of parameters that they have been collected by classical and innovative methods, will illustrate for the first time an integrated image of the importance of hydrological features on mesozooplankton distribution and life strategies with regard to feeding preferences.

1.6.1 Studied area

To investigate the community composition, trophic niche and carbon budget, field sampling in different transects, depths and seasons were combined with laboratory and statistical approaches. Sampling was performed on board R/V AEGAEO from 28 March 2016 until 17 April 2016 (LEVECO) at 4 stations in the Cretan Passage and from 3 until 9 June 2016 (Eurofleet 2) at 16 stations in the Cretan Sea, W and E Cretan Straits and Cretan Passage. During March-April 2016, mesozooplankton samples were collected at discrete layers from the surface until 1000 m by vertical hauls of a WP-2 net during daytime and with 0-100 & 0-500 m layers by vertical hauls of a WP-3 net. The samples from the WP3 net were used for biomarker analysis (spETS, spAARS, SIA, FA). During June 2016, mesozooplankton samples were collected at selected stations in the epipelagic zone (0-200 m) by vertical hauls of a WP-2. Environmental parameters (T, S, O_2, fluorescence and nutrients) as well the potential zooplankton prey (diatoms, dinoflagellates and ciliates) are also available from cruises.

1.6.2 Hydrological features of the studied area

The sampling area was the West Levantine Sea and more specific the Cretan Passage, Cretan Sea and W & E Cretan Straits **(Fig. 1.4)**. The Cretan Sea, where water masses from Ionian, Levantine and Black Seas interact, is a critical area for the formation and the transformation of the water masses of the EMS. It was shown by Georgopoulos *et al.* (1989) and Zodiatis (1991) that the northeastern shelf zone of Crete is a secondary region for LIW formation, thus making it an important area of the EMS. In comparison with other parts of the EMS (in particular, the Aegean Sea), this area is one of the least explored.

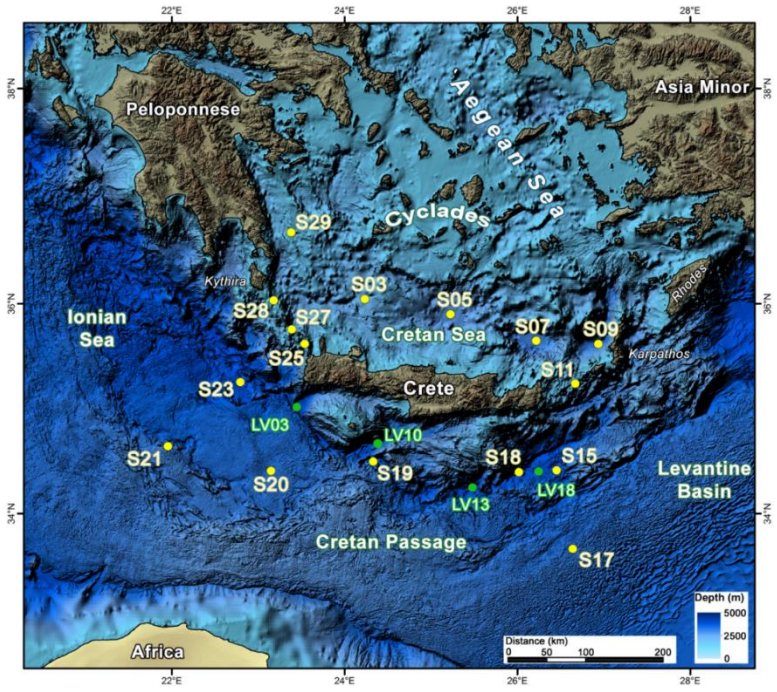


Figure 1.4 Mesozooplankton sampling stations in the Cretan Passage, Cretan Sea and W & E Cretan Straits during April (LV) and June (S) 2016.

The hydrographical status of the studied area according to CTD measurements is far different from the abrupt shift in the Mediterranean ''ocean climate'' that happened during 1989-1995, the Eastern Mediterranean Transient (EMT) period. According to Theocharis *et al.,* 2002, during this period large amounts of dense water flowed out of the Aegean and filled the eastern Mediterranean basins with a significant impact on the zooplankton (Weikert *et al.*

2001). Several hypotheses concerning possible causes of this unique thermohaline event have been proposed, such as: **(i)** changes in circulation patterns leading to blocking situations concerning the Modified Atlantic Water (MAW) and the Levantine Intermediate Water (LIW) (Malanotte-Rizzoli *et al.*, 1999) **(ii)** internal redistribution of salt (Klein *et al.,* 1999), **(iii)** changes in the local atmospheric forcing over the Aegean combined with long-term salinity change (Theocharis *et al.*, 1999; Lascaratos *et al.*, 1999) and **(iv)** variations in fresh water input coming from the Black Sea (Zervakis *et al.*, 2000). Whatever the percentage of contribution of each of the above proposed scenarios, there is still lack of a consistent and quantified theory of the EMT.

According to Velaoras *et al.* (2018), in the Cretan Sea, intermediate water masses of both Cretan and Levantine origins are detected. The stagnating bottom waters of this basin still hold high salinity, density, and dissolved oxygen values, remnant of the EMT deep water formation episodes. Characterized by low salinity and oxygen values, transitional waters of Mediterranean origin are present between intermediate and bottom layers throughout the Cretan Sea. Intermittent weak outflow of warm and saline water masses of Cretan origin towards the Eastern Mediterranean is observed at the bottom of both east and west Cretan Straits. In the Cretan Passage, there is no sign of the Ierapetra anticyclonic gyre, possibly related to the seasonality of the gyre or linked to larger scale Eastern Mediterranean circulation variability. The observed surface circulation in this area is comprised of a series of smaller gyres between the Cretan Cyclone and the Rhodes Gyre. The bottom waters of the Cretan Passage present a west-to-east gradient of increasing salinity and decreasing oxygen related to the propagation of new Adriatic Deep Water from the Ionian Sea towards the Levantine basin. **Figure 1.5** shows the geostrophic velocities over the absolute dynamic topography for April 15th and June 6th. The figure has been produced with the use of satellite-derived absolute dynamic topography generated by the SSALTO/DUACS delayed time altimeter data produced and distributed by the Copernicus Marine and Environment Monitoring Service (CMEMS) (http://www.marine.copernicus.eu). The station positions from the two cruises are also plotted in this figure. The velocity map provides

information about the distribution of the water masses in the euphotic zone generated by the appearing circulation structures (Velaoras *et al.,* 2018).

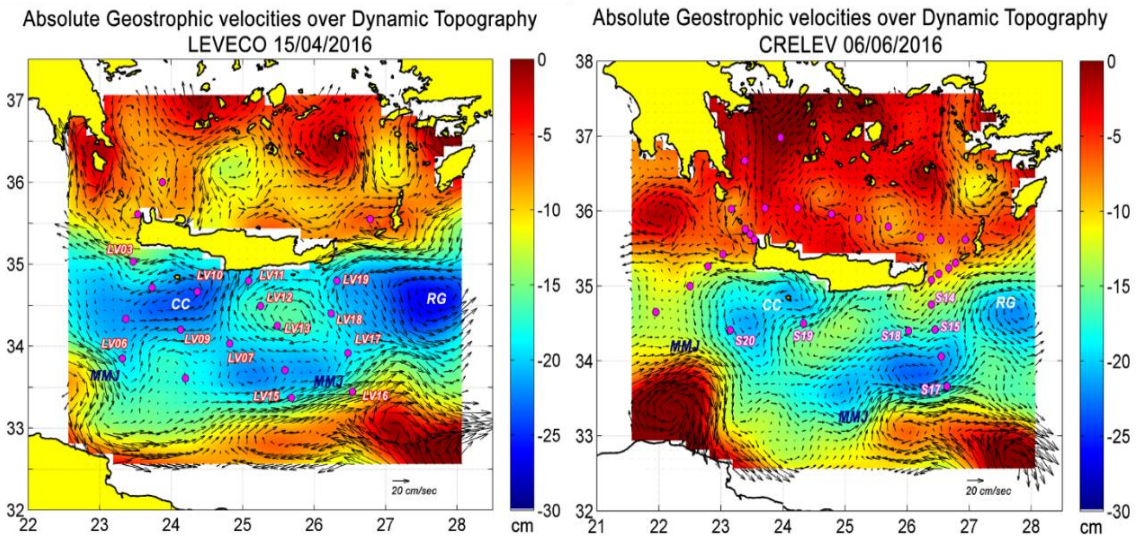


Figure 1.5 Absolute geostrophic velocities over dynamic topography on 15th April and 6th June during the two cruises. Station positions are plotted. Data originate from CMEMS (Velaoras *et al.*, 2018)

Oligotrophic areas, like the region investigated here, mainly depend on water mass circulation for nutrient supply. The hydrography of the studied area is influenced by a complex interaction of cyclonic and anticyclonic eddies. Cyclonic eddies move the isopycnals upward and anticyclonic eddies downward. The hydrographic survey has identified three circulation patterns in the upper ~1500 m that influence the sampling sites. Stations LV03, LV10, S19 and S20 are affected by the low salinity Atlantic water mass carried by a branch of surface water that circulates around the periphery of the Cretan Cyclone. Station LV13 is influenced by an anticyclone in the central part of the investigated area whereas stations LV18, S15 and S18 are influenced by a strong current flowing cyclonically around the periphery of the Rhodes Gyre carrying high salinity and potentially nutrient enriched LSW from the east Levantine Sea. The Rhodes Gyre is known as a feature of nutrient enrichment which may support higher biological activity (Salihoglu *et al.,* 1990).

1.7 Chapter outlines

So far, few studies have been conducted in this area. **Chapter 2** describes the vertical community structure of mesozooplankton in the Cretan Passage (Eastern Mediterranean Sea) from the cruise during April 2016 as well as the carbon requirements of mesozooplankton using enzymatic activity indices (AARS and ETS. It combined field sampling, lab work and statistical analysis, to examine the potential respiration, the somatic growth of mesozooplankton and the carbon budget in the photic zone. This study was undertaken to confirm the oligotrophic character of the studied area and furthermore to test the hypothesis that hydrographical features locally affect the mesozooplankton communities under ultra-oligotrophic conditions. The influence of the sampling area by the presence of the permanent Rhodes Cyclonic Gyre was highlighted.

We applied isotope and fatty acids analyses to further elucidate the dietary preferences and feeding strategies of mesozooplankton (**Chapter 3**). The isotopic composition of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) was used to determine the dietary preferences of copepod species/taxa as well as their trophic position. Furthermore, fatty acid trophic markers were used to characterize food preferences among copepod species/taxa since they provide a qualitative indicator of assimilated food. It is important to emphasize that only few papers about trophic interactions with the use of stable isotopes exist for the Cretan Passage and EMS (Koppelmann *et al.* 2003, 2009; Hannides *et al.*, 2015) in general. Significantly, the present study investigates lipid content and composition in copepods in the region; therefore it provides important insights and novel data about such relationships for copepod taxa which are prominent in the EMS.

The last chapter (**Chapter 4**) reports an investigation of the abundance and distribution of mesozooplankton in Cretan Sea, western and eastern Cretan Straits and Cretan Passage, from a cruise during June 2016. It combined field sampling, lab work and statistical analysis to examine the distribution in comparison with hydrological features. The aim of this study was to improve our knowledge on the mesozooplankton community structure as well as the carbon requirements in such an ultra-oligotrophic area by coupling standing stocks estimations (abundance, biomass and size classes) and metabolic measurements. The contribution of

important functional groups in the mesozooplankton community was also highlighted.

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CHAPTER 2

2. Zooplankton distribution, growth and respiration in the Cretan Passage, Eastern Mediterranean

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2.1 Abstract

The Eastern Mediterranean Sea (EMS) is an ultraoligotrophic semi-enclosed sea with low nutrient levels, low primary production rate, impoverished phytoplankton populations and low zooplankton standing stocks. The Cretan Passage in the western Levantine Sea is one of the least explored areas of the EMS. We measured the mesozooplankton biomass, abundance and taxon composition as well as biochemical parameters like Electron Transport System (ETS) activity and Aminoacyl-tRNA Synthetases (AARS) activities in late spring of 2016 (28 March - 17 April 2016). The sampling area is influenced by the presence of the permanent Rhodes Cyclonic Gyre east of Crete and a series of smaller permanent or recurrent gyres south of Crete which influence the pathway of the Atlantic Water from the Ionian Sea towards the Levantine Basin at surface and subsurface layers. Mesozooplankton abundance showed slightly increasing values from western to eastern stations of the Cretan Passage and although subsurface maxima were recorded in the 50-100 m layer, the abundance at all stations generally decreased with depth. The community was dominated by copepods at all stations. Juveniles of *Clausocalanus* followed by juveniles of *Oithona* and the *Oncaea media* group (*Oncaea waldemari, Oncaea scottodicarloi, Oncaea media*) were the most abundant taxa in the upper layer, whereas *Mormonilla minor*, *Haloptilus longicornis* and *Subeucalanus monachus* were mainly found below 100 m. ETS, AARS and biomass for carbon measurements were conducted in the same area for the 0-100 m and 0-500 m layers. ETS was highest at LV13 with 10.56±1.33 µl O_2 h^{-1} mg $protein^{-1}$ in 0-500 m and carbon biomass was highest at LV18 with 4.99 mg m^{-3} in 0-100 m. Lowest values of 4.52±1.46 µl O_2 h^{-1} mg $protein^{-1}$ at LV18 in 0-100 m were detected for ETS and 0.91 mg m^{-3} at LV10 in 0-500 m for carbon biomass. Specific AARS activities showed no significant differences among sites. On the basis of ETS and AARS, we calculated that the zooplankton carbon requirements follow the west to east trend of zooplankton biomass, however, the zooplankton is not well fed and the organisms are living under oligotrophic stress.

Keywords: Eastern Mediterranean Sea, Cretan Passage, Mesozooplankton, Distribution, ETS, AARS

2.2 Introduction

In order to understand the ecology and distribution of zooplankton in the Cretan Passage, the unique features of the Mediterranean Sea (MS), and in particular those of the Eastern Mediterranean Sea (EMS), have to be taken into account. Large variability of physical processes and interactions (Malanotte-Rizzoli and Robinson, 1988; Robinson *et al.*, 1992) in this semi-enclosed sea allow investigating the biological responses on changing abiotic parameters. The size, location and morphology of the MS and external forcing cause complex physical dynamics like: i) unique thermohaline features, ii) distinctive multilayer circulation, iii) topographic gyres, and iv) meso- and sub-mesoscale activity (Siokou-Frangou *et al.,* 2010). Overall, nutrients and chlorophyll *a* pools classify the basin as oligotrophic to ultraoligotrophic (Krom *et al.*, 1991) with little fishery yield and a limited influence on the global carbon cycle (Koppelmann *et al.*, 2004), but regional features enrich coastal areas through changing wind conditions, temporal thermoclines, currents, river outflows and municipal sewage discharges (Estrada, 1996). Additional features of the MS are i) strong longitudinal environmental gradients (Danovaro *et al.*, 1999) with increasing nutrient depletion from west to east (Christaki *et al.*, 2001), ii) a high diversity (7% of the world's marine biodiversity, Coll *et al.*, 2012) and a high rate of endemism (average of total endemics: 20.2%, Coll *et al.*, 2010) considering its relatively small surface area and volume (Bianchi and Morri, 2000), and (iii) strong productivity gradients decreasing from north to south and from west to east which are inversely correlated to the increase in temperature and salinity (Coll *et al.*, 2010). Hence, a west-to-east decrease of zooplankton standing stocks emerged from several studies (e.g. Mazzochi *et al.*, 1997; Kovalev *et al.* 1999; Dolan *et al.*, 2002; Siokou-Frangou 2004). All these contrasting characteristics should likely be reflected in the structure and dynamics of plankton communities (Siokou-Frangou *et al.*, 2010).

Plankton plays a pivotal role in oceanic carbon flux as the primary biological mechanism sequestering the carbon transferred from the atmosphere into surface waters to deeper layers and higher trophic levels. Organic carbon and nutrients are remineralized and recycled efficiently in this oligotrophic environment in a complex microbial food web with little energy transfer to the

higher trophic levels (Turley, 2000; Van Wambeke, 1996). Biochemical indices such as electron transport system activity (ETS) and amino acyl-tRNA synthetase activity (AARS) have been applied to several zooplankton species (Herrera *et al.*, 2012, 2014; Yebra *et al.*, 2005) to study their physiology and trophic interactions. ETS is ubiquitous in mitochondrial membranes and can be used as an indicator of organic matter remineralization since it consists of a complex chain of cytochromes flavo proteins and metabolic ions that transport electrons from catabolized food to oxygen. ETS activity can be correlated to *in vivo* respiration (Owens and King, 1975) and used as an estimation of mesozooplankton respiration rates. AARS are enzymes that catalyze amino acid activation and the aminoacylation of tRNA (Schimmel and Soll, 1979) which is the first step of protein synthesis. Positive relationships between AARS activity and growth have been observed in freshwater and marine crustaceans (Yebra and Hernández-Léon, 2004, Yebra *et al.*, 2005, 2006) making AARS activity a good candidate to be used as an index (proxy) of growth in zooplankton (McKinnon, 2015).

This study investigates the vertical community structure of mesozooplankton in the Cretan Passage (Eastern Mediterranean Sea) as well as the carbon requirements of mesozooplankton using enzymatic activity indices (AARS and ETS) to test the hypothesis that hydrographical features locally affect the mesozooplankton communities under oligotrophic conditions.

2.3 Materials and methods

2.3.1 Study area

A multidisciplinary oceanographic cruise (LEVECO cruise) was conducted south off Crete (Western Levantine Sea, Cretan Passage) on board the R/V AEGEAO (Hellenic Center for Marine Research) from 9 to 18 April 2016 in order to study the functioning of the pelagic ecosystem in the area **(Fig. 2.1)**. Sampling for mesozooplankton communities was conducted at 4 stations (LV03, LV10, LV13 and LV18) along a transect from western to eastern stations in the Cretan Passage **(Table 2.1)**.

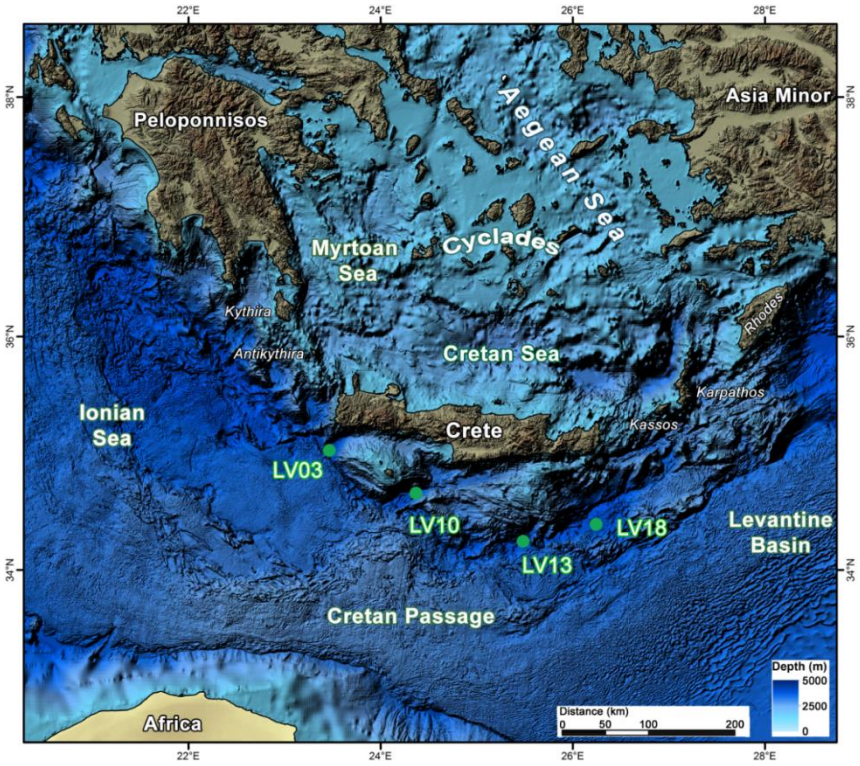

Figure 2.1 Mesozooplankton sampling stations in the Cretan Passage during April 2016.

Table 2.1 Station data for April 2016. All samples were collected during daylight hours and within a maximum of 4 hours.

Station	Region	Date	Haul	Sampled intervals (m)
LV03	35°03'N, 23°46'E	17/04/16	WP-2	1000-700-500-300-200-100-50 (7 samples)
LV10	34°66'N, 24°36'E	15/04/16	WP-2	1000-700-500-300-200-100-50 (7 samples)
LV13	34°25'N, 25°48'E	14/04/16	WP-2	1000-700-500-300-200-100-50 (7 samples)
LV18	34°43'N, 26°38'E	10/04/16	WP-2	1000-700-500-300-200-100-50 (7 samples)
LV03	35°03'N, 23°46'E	17/04/16	WP-3	0-100, 0-500 (2 samples)
LV10	34°66'N, 24°36'E	15/04/16	WP-3	0-100, 0-500 (2 samples)
LV13	34°25'N, 25°48'E	14/04/16	WP-3	0-100, 0-500 (2 samples)
LV18	34°43'N, 26°38'E	10/04/16	WP-3	0-100, 0-500 (2 samples)

2.3.2 Environmental data

Standard temperature and salinity (conductivity) measurements for the entire water column (max. sampling depth: 3950 m) were obtained with a Sea-Bird Electronics 11plus CTD deck unit, interfaced with a Sea-Bird Electronics 9plus underwater unit and a Sea-Bird Electronics 32 rosette sampler with 24 10L Niskin bottles.

Fluorescence was measured with a fluorometer (Chelsea Instruments AQUAtracka III; excitation 430 nm, emission 685 nm). Fluorometer readings were calibrated against Chl *α* estimates from discrete samples analyzed with high-performance liquid chromatography (HPLC) to obtain Chl *α* (µg L^{-1}). Chl *a* concentrations were converted to carbon biomass (mg m^{-3}) according to Malone *et al.* (1993) using the equation:

$$C = \text{Chl } a * e^{(3.89 - 0.01*z)}$$ (z = depth) for each station.

2.3.3 Zooplankton

Zooplankton samples were collected at discrete layers from the surface to 1000 m depth by vertical hauls (0.5 m sec^{-1}) of a WP-2 standard closing net from Hydrobios (Kiel, Germany) with 200 µm mesh size during daytime (Table 1) according to the zooplankton methodology manual (Sameoto *et al.*, 2000, Chapter 3). The filtered water volumes of the WP-2 net (V = A x L, m^3) were calculated by taking into account the area of the net mouth (A, m^2) and the length of the released wire (L, m). The final thickness of the sampled layer (ΔD, m) and the upper and lower depth limits of the layer (ΔL = Li-Lf, m) were computed considering the wire angle α (ΔD = ΔL cos α). The volume of filtered seawater was used to calculate the mesozooplankton abundance per m^3 for each haul. The nets were carefully rinsed and the samples were preserved in a seawater sodiumtetraborate buffered (Steedman, 1976) formaldehyde solution (4% final concentration) for later determination of zooplankton composition and abundance.

Rare species were searched in the total sample. Copepods were identified at species level whenever possible while other groups were mainly identified at higher taxonomic levels. Siphonophores were counted as part of colonies. Abundance was expressed as number of individuals in each layer per cubic meter (ind m^{-3}), whereas relative abundance (%) refers to the evenness of distribution of individuals among species in a community. Mean integrated abundance values (for the 0-100, 200-500 and 0-1000 m) in terms of depth were calculated according to the trapezoid rule. For statistical analysis mesozooplankton taxa (from WP-3 sampling, see below) were grouped in size fractions **(Table 2.2)**. Moreover, to test whether the degree of decrease in abundance with depth differs between the stations, a regression analysis was applied to the vertical profiles of $\log_{10}$ transformed abundance data of total mesozooplankton **(Table 2.3)**.

Table 2.2 Mesozooplankton taxa (WP-3 sampling) listed in size fraction (μm). Size fractionation was performed according to literature (Vives & Shmeleva, 2007).

0-100 m

300-500μm	**500-1000 μm**	**1000-1500 μm**	**>1500 μm**
Nauplii	Calanoids	Amphipoda	Calanoida
Oithonidae	Cirripedia	Appendicularia	Chaetognatha
Poecilostomatoida	Clausocalanidae	Calanoida	Decapoda lar
	Oithonidae	Clausocalanidae	Echinodermata lar.
	Lucicutia juv.	Oithonidae	*Eucalanus spp.*
	Mormonilla minor	*Haloptilus longicornis*	Euphausicea lar.
	Ostracoda	*Lucicutia spp.*	Fish larv.
	Poecilostomatoida	Poecilostomatoida	Medusae
			Poecilostomatoida
			Polychaeta larv.
			Pteropoda
			Salps
			Siphonophora

0-500 m

300-500 μm	500-1000 μm	1000-1500 μm	>1500 μm
Nauplii	Calanoida	Amphipoda	Calanoida
Poecilostomatoida	Clausocalanidae	Appendicularia	Chaetognatha
	Cirripedia	Calanoida	Decapoda lar.
	Lucicutia juv.	*Haloptilus longicornis*	Echinodermata lar.
	Mormonilla minor	*Lucicutia spp.*	*Eucalanus spp.*
	Oithonidae	Oithonidae	Euphausicea lar.
	Ostracoda	Poecilostomatoida	Fish larv
	Poecilostomatoida		*Lucifer sp.*
			Medusae
			Poecilostomatoida
			Polychaeta lar.
			Pteropoda
			Salps
			Siphonostomatoida
			Siphonophora

Table 2.3 Regression slopes of log transformed abundance (of total mesozooplankton) data versus depth, in the Cretan Passage during April 2016.

Site	Slope	r^2
LV03	0.0018	0.90
LV10	0.0019	0.92
LV13	0.0022	0.87
LV18	0.0023	0.81
Red Sea (Weikert, 1982)	0.0017	
S.Aegean (Siokou *et al.,* 2013)	0.0025	
N.Aegean (Siokou *et al.,* 2013)	0.0019	
Levantine (Weikert & Trinkhaus, 1990)	0.0011	

2.3.4 Electron Transport System activity (ETS) assay and respiration rates

Samples for biochemical analyses were collected with a Hydrobios (Kiel, Germany) WP-3 net (see zooplankton methodology manual by Sameoto *et al.*, 2000, Chapter 3) with 300 µm mesh size **(Table 2.1)** by vertical hauls (0.5 m s^{-1}) in 0-100 and 0-500 m layers. This larger net was only used to capture copepods for biochemical assays. The samples for enzyme assays (ETS, AARS) and biomass were initially frozen in liquid nitrogen and then stored at -80 ºC. Measurements were replicated three times for each sample in both analyses.

For the biomass estimation of the bulk population **(Table 2.5)**, the samples were split in halfs using a Folsom Splitter and drained by vacuum filtration on GF/C filters (25 or 47 mm diameter, pre-combusted at 400 °C for about 24 hours and weighted), after a quick final rinse with distilled water to eliminate the salts of seawater. Each filter was then placed in a small plastic Petri dish and dried in an oven at 60 °C for 24 hours or longer until completely dry, and weighed on an electronic microbalance. Dry weight was converted into carbon (mg C m^{-3}) using a factor of 0.45 (Larson, 1986).

Specific ETS (spETS) activity was measured according to the method of Kenner and Ahmed (1975) and Owens and King (1975) adapted to microplate reading by McKinnon *et al.* (2015). Samples were homogenized in ice-cold 0.05 M phosphate buffer pH 8.0, containing 0.2% v/v Triton X-100, 0.15% w/v polyvinylpyrrolidone and 75 µM $MgSO_4$ using a Potter-Elvehjem homogenizer (Heidolph Electro GmbH, Kelheim, Germany). Homogenates were centrifuged (Heraus Fresco 21 centrifuge, Thermo Scientific, Langenselbold, Germany) at 3,000 x g, 0°C for 15 minutes and ETS activities were assayed in the supernatant. 150 µl of substrate solution (0.25 mM β-nicotinamide adenine dinucleotide 2'phosphate, reduced form [NADPH], 0.835 mM NADH and 133 mM succinate in 0.05 M phosphate buffer pH 8.0, 0.2% v/v Triton X-100) and 50 µl INT solution (2 mg/ml 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyltetrazolium chloride) were added to the wells of a 96-well plate and incubated in a microplate reader (Synergie HT, BIOTEC) at 25°C for 10 minutes. Reaction was initiated by adding 50 µl of supernatant dilutions and absorbance was read at 490 nm for 35 min. spETS

measured as equivalent oxygen utilization (µl O_2 hr^{-1} mg $protein^{-1}$) was determined using the equation:

$$spETS\ (\mu l\ O_2\ h^{-1}\ mg\ protein^{-1}) = (slope \times V_{rm})/(1.42 \times F_c \times V_H \times P) \quad \textbf{(1)}$$

where slope is the rate of change (in absorbance units per hour) at 490 nm measured at least in two supernatant dilutions multiplied by the dilution factor, V_{rm} = 0.25 (reaction mixture volume (ml)), 1.42 is the molar equivalent conversion of INT to µl O_2, F_c = 0.69 (path length correction factor), V_H = 0.05 (homogenate volume (ml)) and P is the protein concentration of the homogenate (mg ml^{-1}) estimated by the method of Bradford (1976) performed on subsamples of the supernatant. Specific ETS (spETS) activitiy (µl O_2 hr^{-1} mg $protein^{-1}$) was corrected for *in-situ* temperature using the Arrhenius equation (Owens and King, 1975).

To convert spETS activity into carbon consumption rates, R (mg C m^{-3} h^{-1}), the following equation was used:

$$R = ([spETS] \times P{:}DW \times R{:}ETS \times R_q \times [12/22.4] \times DW)/1000 \quad \textbf{(2)}$$

where P:DW is the ratio of protein: dry weight (calculated from dry weight of zooplankton and protein concentration in the homogenate), R: ETS ratio = 0.5 (respiration rate, Ikeda *et al.*, 2000), R_q = 0.85 (respiratory quotient, Herrera *et al.* 2014), 12 is the weight (in µg) of 1 µmol C, 22.4 is the volume (µL) of 1 µmol O_2, DW is the dry weight of zooplankton (mg DW m^{-3}) and 1000 converts from µg C to mg C.

2.3.5 Aminoacyl-tRNA synthetases (AARS) activity and growth rates

Specific AARS (spAARS) activity was measured according to the method of Chang *et al.* (1984) and Yebra and Hernández-León (2004) adapted to microplate reading by McKinnon *et al.* (2015). Samples were homogenized in ice-cold Tris HCl pH 7.8. An aliquot was taken for dry weight determination and the remaining

homogenate was centrifuged at 3000 x g, 0°C for 15 min.120 µl water and 80 µl pyrophosphate reagent (Product No. P7275, Sigma) were added to the wells of a 96-well plate and incubated in a microplate reader at 25°C for 10 min. The reaction was initiated by adding 100 µl of supernatant dilutions and absorbance was read at 340 nm for 35 min. spAARS activity measured as the rate of pyrophosphate production (nmol PPi hr^{-1} mg $protein^{-1}$) was determined as follows:

$$\text{spAARS nmol PPi h}^{-1}\text{ mg protein}^{-1} = (\text{slope} \times V_{rm})/(A_{\beta\text{-nad}} \times F_c \times 2 \times V_H \times P) \quad \textbf{(3)}$$

where slope is the rate of change (in milli absorbance units per hour) at 340 nm measured at least in two supernatant dilutions multiplied by the dilution factor, V_{rm} = 0.3 (reaction mixture volume (ml)), $A_{\beta\text{-nad}}$ = 6.22 (millimolar absorptivity of β-nicotinamide adenine dinucleotide reduced form (NADH) at 340 nm), F_c = 0.83 (path length correction factor), 2 are the moles NADH oxidized per mole PPi consumed, V_H = 0.1 (homogenate volume (ml)) and P is the protein concentration of the homogenate (mg ml^{-1}) estimated by the method of Bradford (1976) performed on subsamples of the supernatant. Specific AARS activity was corrected for *in-situ* temperature using the Arrhenius equation (Owens and King, 1975). Total protein concentration was measured using bovine serum albumin (BSA) as a standard (Bradford 1976).

Our samples were dominated by small copepods of the calanoid family Clausocalanidae and the cyclopoid family Oithonidae. To convert spAARS to growth rate (G), we applied published relationships (Herrera *et al.*, 2012) between spAARS and directly measured G for a calanoid and for a cyclopoid copepod, both conducted at temperatures between 12 and 28°C. The calanoid relationship was established on the basis of experiments using nauplii of the copepod *Paracartia grani* **(Eq. 4).**

$$G' = 0.13 + 0.007 \times \text{spAARS} \quad \textbf{(4)}$$

For comparison, we also applied **equation (5)** published by Yebra *et al.* (2011) which was developed for nauplii and juveniles of the cyclopoid *Oithona davisae*:

$$G = \ln (spAARS/24.35) / 5.51 \quad \textbf{(5)}$$

For the spETS assays, we first calculated R (mg C m^{-3} d^{-1}) according to **Eq (2)**, and then G according to Ikeda and Motoda (1978):

$$G = 0.75 \times R \quad \textbf{(6)}$$

2.4 Statistical analysis

For statistical comparisons between groups, one-way analysis of variance (ANOVA) with the level of significance set at $p<0.05$, followed by Tukey-Kramer Multiple Comparison tests ($p \leq 0.05$) were used where appropriate. The Kruskal Wallis test and the Mann Whitney test were used when data did not comply to the assumptions of normality and homogeneity of variance checked by the Kolmogorov-Smirnoff test and Levene's test, respectively. The above tests were performed using SPSS statistics 20™.

Multivariate analyses were performed to examine changes both in the environmental conditions and in the structure of the zooplankton community, combining abundance and composition. Vertical changes in zooplankton community composition within each station were visualized by a non-metric multidimensional scaling (nMDS) on the Bray–Curtis dissimilarity matrix created from the datasets of each station based on square root transformed abundances. When the differences were significant, we applied the similarity percentages (SIMPER) analysis to assess the contribution of each taxon to the Bray–Curtis dissimilarities between assemblages.

A CorePlot in R was applied in order to evaluate the correlation, if any, between the biochemical indices (spETS , spAARS) and temperature (T), biomass (B, mg protein m^{-3}), important groups/species (Calanoida, Oithonidae, Poecilostomatoida, Clausocalanidae, *Mormonilla minor*, *Haloptilus longicornis*, Chaetognatha, Salps, Siphonophora, Appendicularia), total abundance (A) and

taxa abundance in size fraction (from WP-3 sampling) according to Fauna Iberica Vol. 29 & 33 (Vives & Shmeleva, 2007) (ZP1: 300-500, ZP2: 500-1000, ZP3: 1000-1500, ZP4: >1500μm). The above analyses were performed using Primer 6 package and R version 3.5.0 (packages ggplot2, corrplot and tidyr).

2.5 Results

2.5.1 Cretan passage environmental parameters

Hydrographic CTD measurements revealed five main water masses in the study area **(Fig. 2.2)**: (1) the Atlantic Water (AW), a surface/subsurface water mass (~0-50 m) of Atlantic origin moving eastwards towards the Levantine Basin with "low" salinity (S<39), (2) the Levantine Surface Water (LSW), a surface water mass created by strong evaporation mainly in the Levantine Basin with higher salinity (S>39), (3) the Levantine Intermediate Water (LIW), an intermediate (~100-400 m) water mass created by the winter convection of LSW mainly in the Rhodes Gyre area with salinities between 39.00 and 39.15, (4) the Eastern Mediterranean Deep Water (EMDW), a dense water mass occupying the bottom of the EMS mostly of Adriatic origin but with some Aegean contribution with σ_θ densities $\leq$29.2 kg m^{-3} and salinities around 38.74 and (5) transitional Mediterranean Water (TMW), water that occupies the layers between LIW and EMDW which is the oldest water mass found in the EMS.

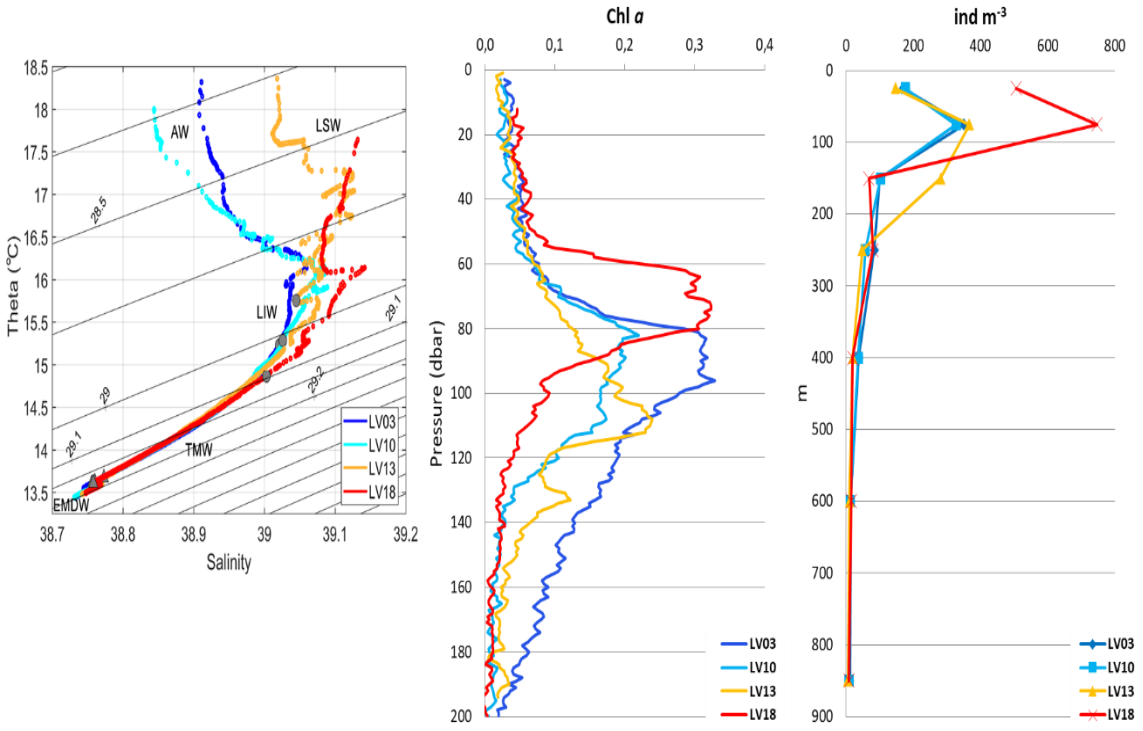

Figure 2.2 Potential temperature (Theta °C) and salinity (S) profiles (AW: Atlantic Water, LSW: Levantine Surface Water, LIW: Levantine Intermediate Water, EMDW: Eastern Mediterranean Deep Water). Bullets and triangles define 200 m and 1000 m depth, respectively. Chlorophyll α (Chl a) profile and vertical distribution of mesozooplankton abundance (ind m^{-3}) in the Cretan Passage during April 2016.

Figure 2.3 shows the geostrophic velocities over the absolute dynamic topography for April 10th. The figure has been produced with the use of satellite-derived absolute dynamic topography generated by the SSALTO / DUACS delayed time altimeter data produced and distributed by the Copernicus Marine and Environment Monitoring Service (CMEMS) (http://www.marine.copernicus.eu). LEVECO station positions are also plotted in this figure. The velocity map provides information about the distribution of the water masses in the euphotic zone generated by the appearing circulation structures. The westernmost part of the study area is affected by the presence of AW carried by the part of the surface current that splits at 34° N – 23.5° E and circulates around the periphery of the Cretan Cyclone. The northeastern part is dominated by the strong current flowing cyclonically around the periphery of the Rhodes Gyre carrying high salinity LSW from the eastern Levantine Sea. The central part of the study area around 34.5° N

– 25.5° E is influenced by an anticyclonic gyre. More analysis on water masses and circulation is presented by Velaoras *et al.* (2019).

Concentration of chlorophyll *α* (Chl *α*) ranged from 0.02 to 0.32 mg m^{-3}. Deep chlorophyll maximum (DCM) layers were detected between 75 and 100 m with values varied from 0.16 to 0.32 mg m^{-3}, whilst the surface values ranged from 0.02 to 0.05 mg m^{-3} **(Fig 2.2)**.

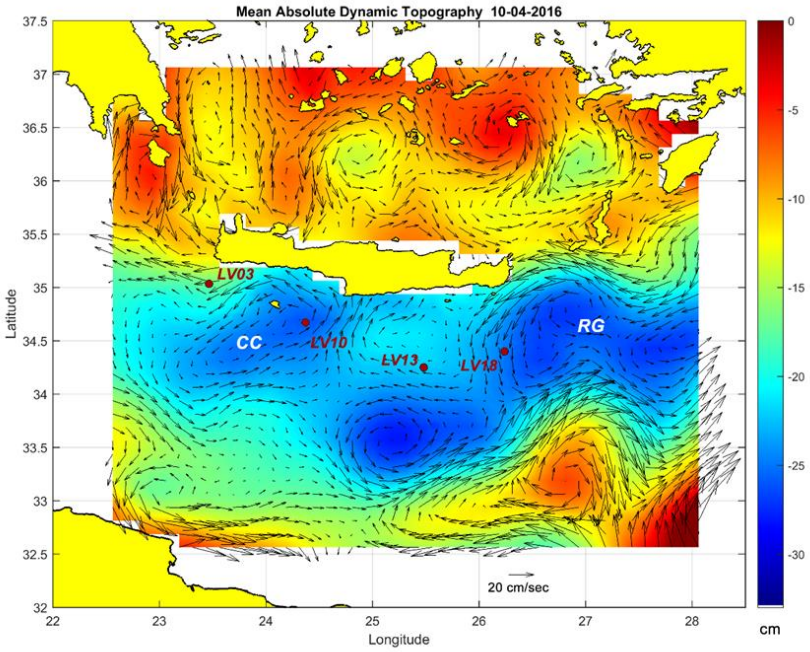

Figure 2.3 Geostrophic velocities over the absolute dynamic topography for April 10th (CC: Cretan Cyclone, RG: RhodosGyre)

2.5.2 Mesozooplankton abundance and composition

Abundance values decreased with depth at all stations below subsurface maxima in the upper 50-100 m to minima in the 700-1000 m layer **(Fig. 2.2)**. Highest mean integrated mesozooplankton abundance for 0-100 m layer was recorded at station LV18 (626 ind m^{-3}) with all other stations exhibiting much lower and almost the same values (LV03 (260 ind m^{-3}), LV10 (251 ind m^{-3}) and LV13 (257 ind m^{-3})). Regarding the 0-1000 m layer highest mean integrated mesozooplankton abundance was recorded once more at station LV18 (87 ind m^{-3}) followed by

stations LV13 (66 ind m^{-3}), LV10 (53 ind m^{-3}) and LV03 (58 ind m^{-3}). At all stations higher abundances were located in the upper layers. At stations LV10 and LV18 more than 70% (87% at LV18) of the abundance occurred in the upper 100 m, but only 60% at LV03 and LV13. The variability of the abundance decreasing pattern between stations was examined by comparing the regression slopes of log transformed abundance data with depth **(Table 2.3)**. The highest slopes in the abundance vertical decrease were found at stations LV18 and LV13 and the smallest slopes were found in LV03 and LV10. Copepods, ostracods, appendicularians, chaetognaths, salps and siphonophores accounted for >91% of total mesozooplankton abundance at each of the surveyed stations, followed by cnidarians, molluscs and polychaetes.

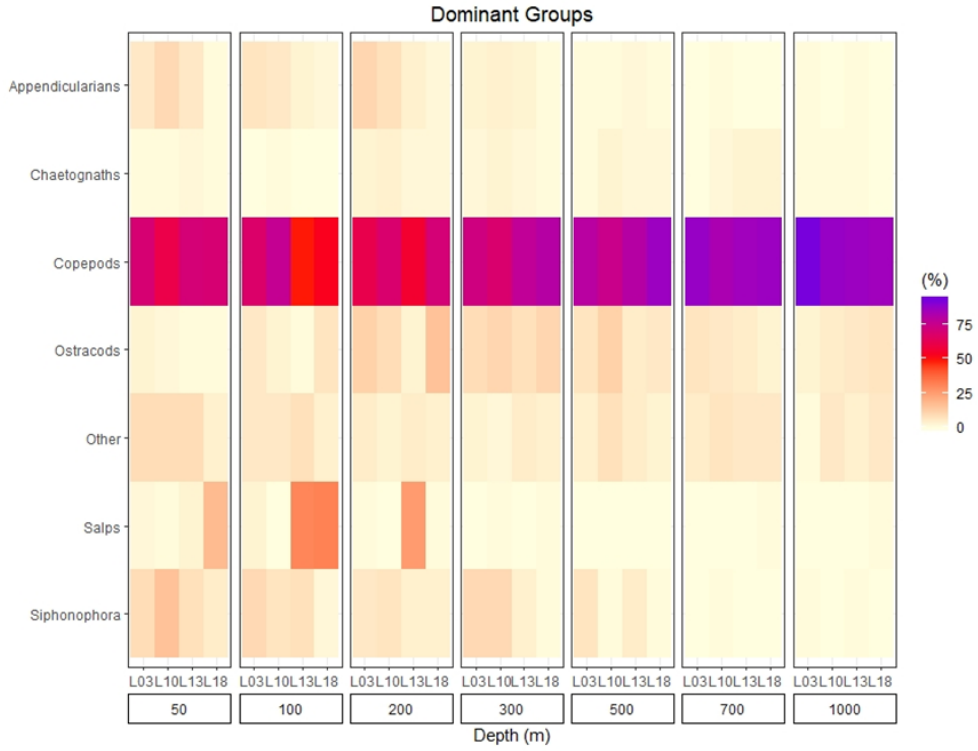

Figure 2.4 Heat map of the vertical distribution of the dominant mesozooplankton groups (relative abundances %) in the Cretan Passage during April 2016. Heat map is in a light yellow (low relative abundance), red (medium relative abundance) to blue (high relative abundance) gradient.

The communities in the entire water column were dominated by copepods at all stations and in all depth layers with a maximum relative abundance of 93% at station LV03 in 700-1000 m **(Fig. 2.4)**. Salps were of high importance in the euphotic zone at stations LV13 and LV18 with maximum relative abundances of 31% and 32% in 50-100 m, respectively. Ostracods had high contributions at all stations (except for LV13) with maximum values of 16% at LV18 (100-200 m) and 12 % at LV03 and LV10 (100-200 m and 200-300 m, respectively). Siphonophores were of high importance (16%) at station LV10 in 0-50 m, whereas they range between 1-10% at all other stations. Lastly, the contribution of appendicularians was found to be important, mostly at stations LV03 and LV10 with maximum relative abundances of 11% in 100-200 m and 10% in 0-50 m, respectively.

Among copepods, *Clausocalanus* and *Oithona* juveniles dominated the 0-300 m layer with relative abundances from 2 to 45 % and 2 to 18 %, respectively, whereas, *Mormonilla minor* dominated between 300 m and 1000 m **(Fig. 2.5)** with relative abundances from 2 to 28 %. Since there is little information on species composition in the study area, an effort has been made to identify rare and less abundant species (0.1-8.2 %) which are listed in **Table 2.4**.

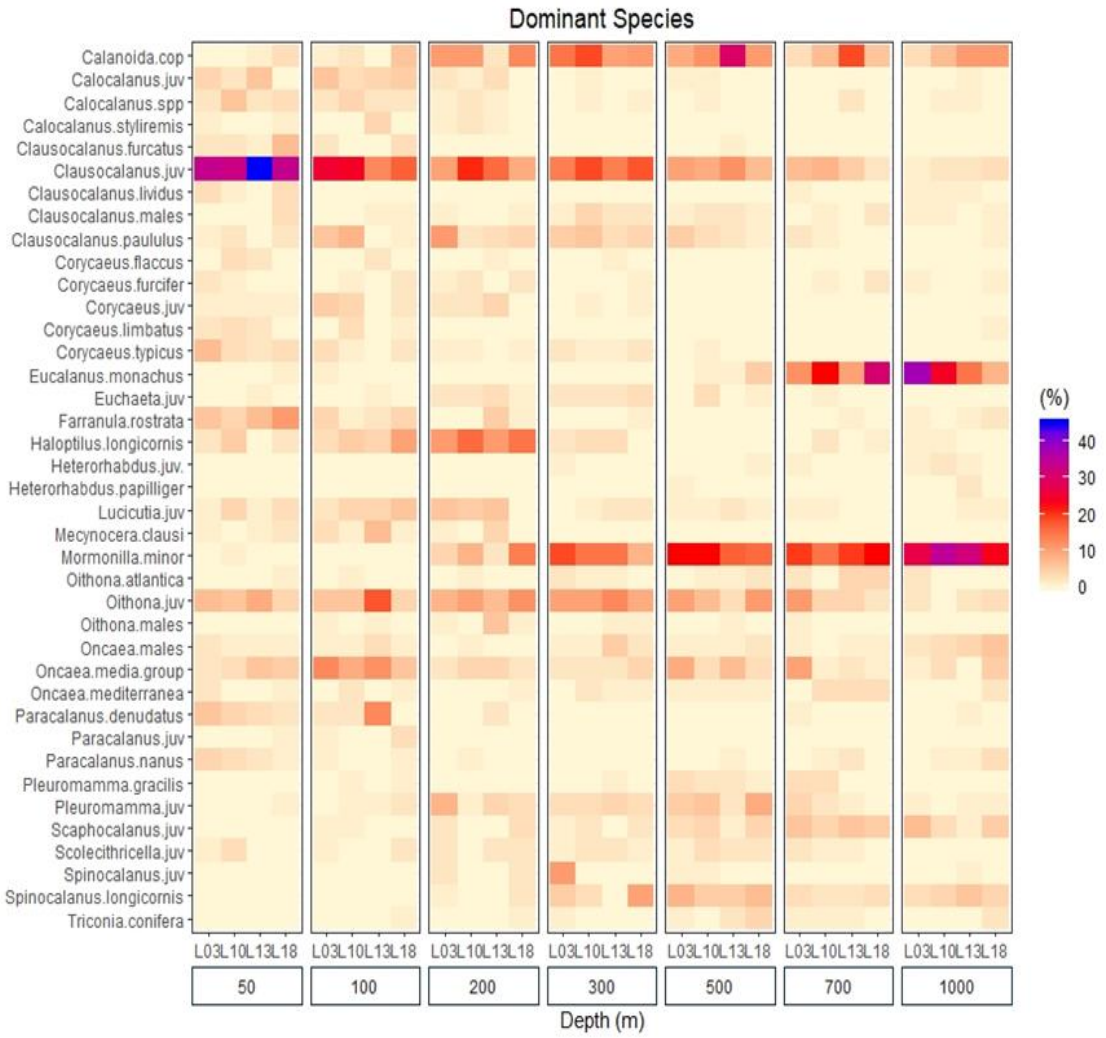

Figure 2.5 Heat map of the vertical distribution of the 10 most dominant species (relative abundances, %) per station and depth, among copepods, in the Cretan Passage during April 2016. Heat map is in a light yellow (low relative abundance), red (medium relative abundance) to blue (high relative abundance) gradient.

The differences in taxon composition, mainly correlated to depth (ANOSIM, R=0.82), drove the separation of samples into groups based on hierarchical clustering and nonmetric MDS **(Fig. 2.6)**. Three groups of samples were distinguished at a similarity level of 60 % (not shown here). The first group consisted of samples from the upper layers (0-50 m and 50-100 m, with the exception of LV13 100-200 m due to high abundance values, Fig. 2), the second group consisted of samples from 100-200 m and 200-300 m layers and samples from 300-500 m layer from stations LV03 and LV10, and the last group consisted of

samples from 300-500 m from stations LV13 and LV18 as well as samples from layers below 500 m. The separation of the 300-500 m layer is due to differences in abundance (LV03 and LV10 have 37 and 36 ind. m^{-3}, respectively, whereas LV13 and LV18 have 19 ind. m^{-3}).

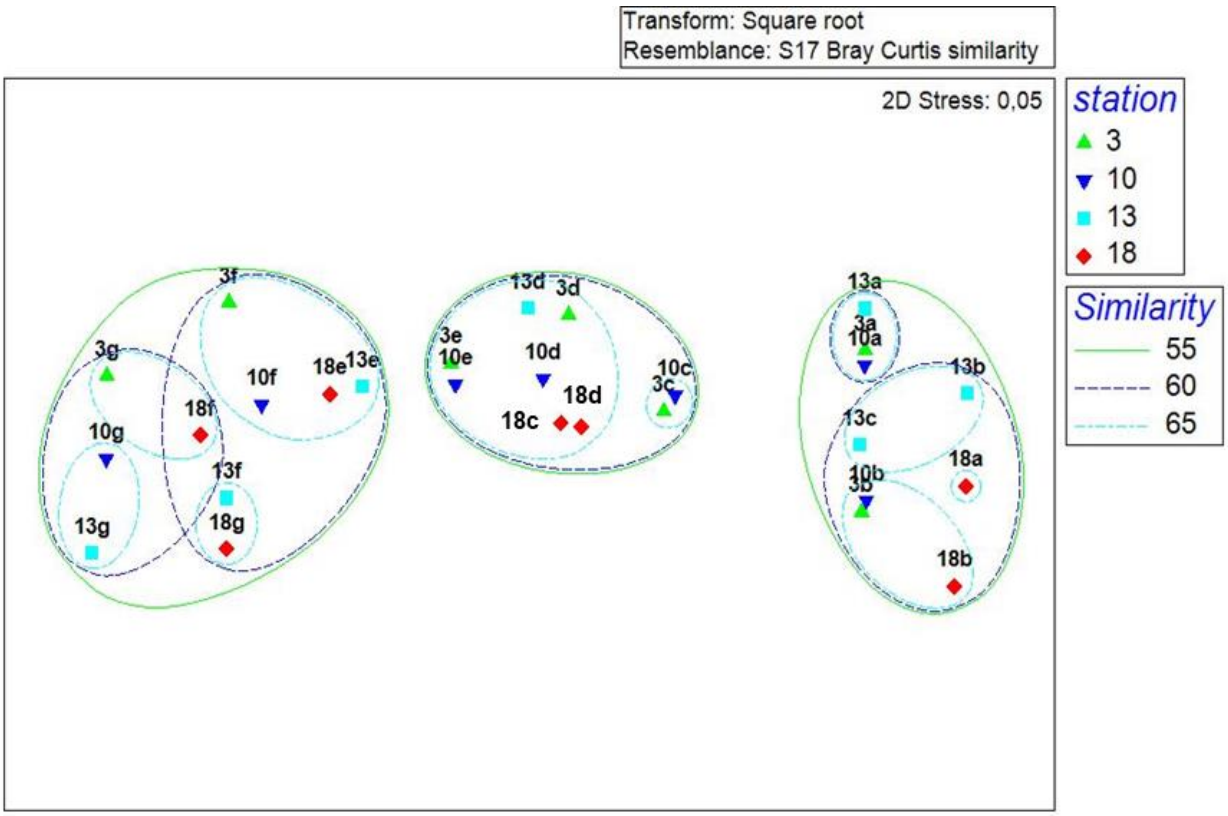

Figure 2.6 Non-metric multidimensional scaling (nMDS) on square root transformed abundances of the mesozooplankton in the Cretan Passage during April 2016 (a: 0-50 m, b: 50-100 m, c: 100-200 m, d: 200-300 m, e: 300-500 m, f: 500-700 m and g: 700-1000 m).

2.5.3 Zooplankton enzyme assays

Mesozooplankton biomass was estimated as mean integrated values of mg DW m^{-3} for the 0-100 m and 0-500 m layers of the studied area. It was calculated from WP-3 net samples with a maximum value of 4.99 mg m^{-3} for the 0-100 m layer at station LV18 and 1.67 mg m^{-3} for the 0-500 m layer at station LV13 **(Table 2.5).**

Specific ETS activities **(Table 2.5)** varied from 4.52 to 10.56 µl O_2 h^{-1} mg $protein^{-1}$ among stations showing lower values at LV18 (two way ANOVA, Tukey HSD test, $p<0.05$) while there were no significant differences in spETS activities between the 0-100 m and the 0-500 m layer (two way ANOVA, $p>0.05$).

Specific AARS activities were not significantly different between depth layers or stations **(Table 2.5)**. Both spETS and spAARS activity were highest at LV13 in the 0-500 layer.

Table 2.4 Rare and very low abundance species (relative abundance in the total copepods %) in the Cretan Passage during April 2016.

Depth (m)	Station	*Aetideus acutus*	*Clausocalanus lividus*	*Euaugaptilus hecticus*	*Euchirella messinensis*	*Euterpina acutifrons*	*Haloptilus ornatus*	*Scaphocalanus curtus*	*Scaphocalanus similis*	*Scaphocalanus invalidus*	*Spinocalanus longicornis*	*Spinocalanus magnus*	*Temeropia mayubensis*	*Temeropia minor*	*Vettoria longifurca*
0-50	LV03	-	1.8	-	-	-	-	-	-	-	-	-	-	-	-
	LV10	-	0.5	-	-	-	-	-	-	-	-	-	-	-	-
	LV13	-	0.1	-	-	-	-	-	-	-	-	-	-	-	-
	LV18	-	1.8	-	-	-	-	-	-	-	-	-	-	-	0.2
50-100	LV03	-	-	-	-	-	-	-	-	-	0.2	-	-	-	-
	LV10	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	LV13	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	LV18	-	-	-	-	-	-	-	-	-	-	-	-	-	-
100-200	LV03	-	-	-	-	-	-	-	-	-	0.8	-	-	-	-
	LV10	-	-	0.2	-	-	-	-	-	-	-	-	-	-	-
	LV13	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-
	LV18	-	-	-	-	0.2	0.2	0.3	-	-	1.2	-	-	-	0.5
200-300	LV03	-	-	-	-	-	-	-	0.8	-	3.9	-	-	-	-

	LV10	-	-	-	-	-	-	0.4	-	-	2.2	-	-	-	-
	LV13	0.2	-	-	-	-	-	-	-	-	0.2	-	-	-	-
	LV18	-	-	-	-	-	-	0.3	-	-	8.2	-	-	-	-
300-500	LV03	-	0.3	-	-	-	-	-	-	-	6.6	-	-	-	-
	LV10	-	-	-	-	-	-	0.2	-	-	3.7	-	-	-	-
	LV13	-	-	-	-	-	-	-	-	-	4.3	-	-	-	-
	LV18	-	-	-	-	-	-	0.5	-	-	5.8	-	-	-	-
500-700	LV03	-	0.6	-	-	-	-	-	-	-	3.0	-	-	-	-
	LV10	-	-	-	-	-	-	-	-	-	1.5	-	-	-	-
	LV13	-	-	-	-	-	-	-	0.2	-	1.7	-	-	-	-
	LV18	-	-	-	-	-	-	0.3	-	-	3.0	-	-	-	-
700-1000	LV03	-	0.5	-	-	-	-	-	-	1.0	3.0	-	-	-	-
	LV10	-	1.2	-	0.4	-	-	0.8	-	-	3.2	-	-	-	-
	LV13	-	0.5	-	0.3	-	-	0.3	-	-	5.7	0.5	0.3	1.2	-
	LV18	-	-	-	-	-	-	0.4	-	-	3.1	-	-	-	-

Table 2.5 Biomass (mg m^{-3}), specific ETS activity (spETS) (µl O_2 h^{-1} mg $protein^{-1}$), respiration rate (R) (µg C m^{-3} h^{-1}), specific Aminoacyl-tRNA Synthetases (spAARS) activities (nmol PPi h^{-1} mg $protein^{-1}$) and Gelatinous:Copepods ratio (G:C) of zooplankton samples collected from four sites in the Cretan passage during April 2016 (mean ± SE) (n=3) at two depth layers (m).

Site	Layer	Biomass	spETS	R	spAARS	G:C ratio
LV03	0-100	1.33	9.26 (±0.27)	0.85 (±0.07)	32.72 (±7.76)	0.09
	0-500	1.03	8.96 (±0.19)	0.48 (±0.09)	32.55 (±3.64)	0.11
LV10	0-100	1.70	8.10 (±0.77)	1.01 (±0.10)	44.84 (±11.09)	0.22
	0-500	0.91	8.29 (±1.23)	0.63 (±0.08)	38.95 (±4.05)	0.08
LV13	0-100	2.85	6.94 (±1.69)	1.47 (±0.45)	41.72 (±8.15)	0.54
	0-500	1.67	10.56 (±1.33)	1.34 (±0.68)	48.98 (±11.17)	0.50
LV18	0-100	4.99	4.52 (±1.46)	2.18 (±0.78)	35.48 (±4.34)	0.71
	0-500	1.23	4.68 (±1.18)	0.26 (±0.09)	33.92 (±7.54)	0.16

To interpret the spETS and spAARS data obtained from the samples collected, the ratio between the gelatinous and crustacean taxa (from WP-3) was calculated (**Table 2.5)**. In terms of total zooplankton abundance in the studied area, the gelatinous taxa represented 7% at station LV03 and up to 37% at station LV18 in the 0-100 m layer. Crustaceans represented 56% at station LV18 and up to 84% at station LV03. The ratio between the gelatinous and crustacean taxa ranged from 0.09 at station LV03 to 0.66 at station LV18. Gelatinous taxa represented 6% at station LV10 and up to 31% at station LV13 in the 0-500 m layer. Crustaceans represented 62% at station LV13 and up to 82% at station LV18. The ratio between gelatinous and crustacean taxa ranged from 0.08 at station LV10 to 0.50 at station LV13. The ratios were slightly higher in 0-100 m (except for LV03) and followed the same trend as spAARS (except for LV13), but were not similar to spETS.

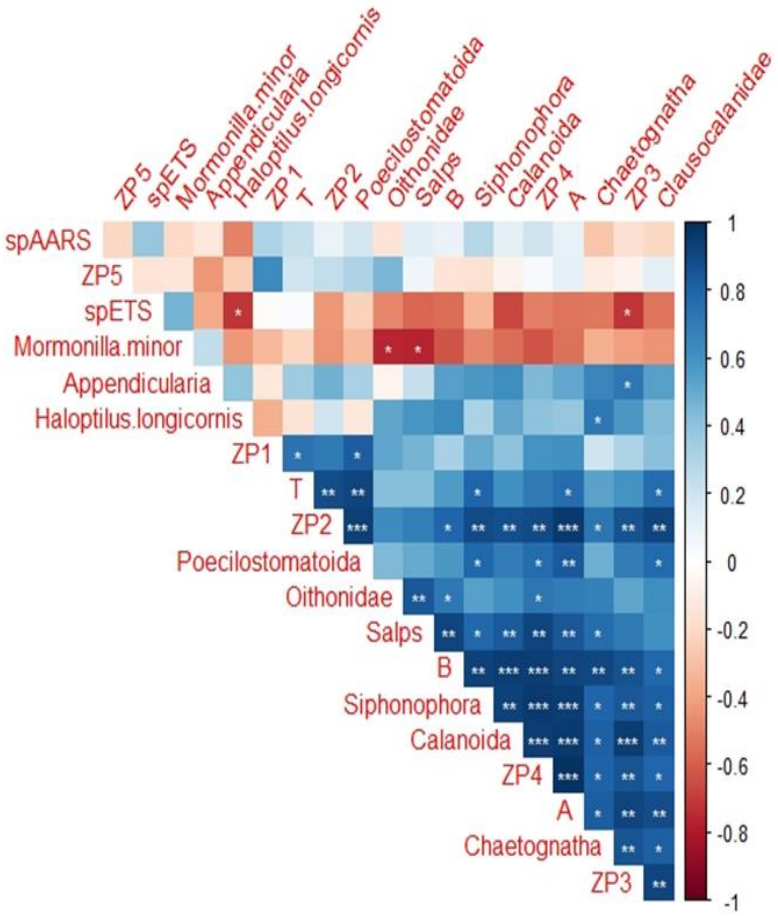

Figure 2.7 A correlation matrix between spETS, SPAARS and environmental and biological parameters represented as a tiled heat map (upper triangle) with asterisk correlation coefficients (sign. level .001, .01, .05) in the Cretan Passage during April 2016. (T: temperature, B: biomass (mg protein m^{-3}), A: total abundance and abundance in size fraction ZP1: 300-500, ZP2: 500-1000, ZP3: 1000-1500, ZP4: >1500μm and).

CorePlot analysis showed no correlation between spAARS and the aforementioned parameters, whereas spETS was found to be negatively correlated with the size fraction 1000-1500 μm and *Haloptilus longicornis* **(Fig. 2.7)**.

2.5.4 Zooplankton respiration, growth and production

Respiration rate estimates from ETS measurements in the 0-100 m layer were highest at LV18 (2.18±0.78 μg C m^{-3} h^{-1}) and lowest at LV18, while in the 0-500 m they were highest at LV13 **(Table 2.5)**. However, there were no significant differences between stations (Kruskal Wallis test, $p>0.05$) and they were higher in the 0-100 m layer among all stations (Mann Whitney test, $p<0.05$).

Zooplankton production (ZP) was calculated as the product of C-specific biomass and growth (G) derived from respiration as well as from the product of C-specific biomass and G calculated from spAARS **(Table 2.6)**. On average, G calculated on the basis of **Eq (4)** was 5.3 times higher than that calculated on the basis of **Eq (5)**. The estimation of ZP using G from **Eq (4)** was on average 16.25 times higher than the value estimated from R, whereas ZP estimated using G from **Eq (5)** was on average 3.14 times higher than that estimated from R **(Table 2.6)**.

There was remarkably little variation in R, G or ZP between stations and layers with higher values in the 0-100 m layer **(Table 2.6)**.

Table 2.6 Summary of biomass (B, mg C m^{-3}), respiration rate (R, mg C m^{-3} d^{-1}), growth (G d^{-1}), zooplankton production (ZP, mg C m^{-3} d^{-1}) and ingestion (I, mg C m^{-3} d^{-1}) estimates for each station and depth layer. Growth has been calculated according to the empirically derived relationships between spAARS activities and independently estimated G for *Paracartia grani* **(Eq 4)** and for *Oithona davisae* **(Eq 5)**. The subscripts Para and Oith refer to calculations made on each of these bases, respectively. ZP has been calculated 3 ways comparison; on the basis of R **(Eq 6)**, and on the basis of each estimate of G. The values are means and standard deviations of each set of n estimates for each station and sample layer.

Site	Layer	B	R	G_{Para}	G_{Oith}	ZP_R	ZP_{Para}	ZP_{Oith}	ZP_{aver}	I (R)
LV3	**0-100**	0.60	0.02 (±0.003)	0.36 (±0.05)	0.04 (±0.05)	0.009 (±0.001)	0.22 (±0.06)	0.02 (±0.05)	0.08 (±0.04)	0.05 (±0.01)
	0-500	0.46	0.01 (±0.004)	0.36 (±0.03)	0.05 (±0.02)	0.004 (±0.001)	0.17 (±0.02)	0.02 (±0.02)	0.06 (±0.01)	0.03 (±0.01)
LV10	**0-100**	0.77	0.02 (±0.004)	0.44 (±0.08)	0.10 (±0.04)	0.014 (±0.003)	0.34 (±0.10)	0.08 (±0.05)	0.14 (±0.05)	0.06 (±0.01)
	0-500	0.41	0.02 (±0.003)	0.4 (±0.03)	0.08 (±0.02)	0.005 (±0.001)	0.16 (±0.02)	0.03 (±0.01)	0.07 (±0.01)	0.04 (±0.01)
LV13	**0-100**	1.28	0.04 (±0.019)	0.42 (±0.06)	0.09 (±0.04)	0.034 (±0.018)	0.54 (±0.13)	0.12 (±0.09)	0.23 (±0.08)	0.09 (±0.05)
	0-500	0.75	0.03 (±0.028)	0.47 (±0.08)	0.12 (±0.04)	0.018 (±0.016)	0.35 (±0.10)	0.09 (±0.05)	0.15 (±0.06)	0.08 (±0.07)
LV18	**0-100**	2.25	0.05 (±0.033)	0.38 (±0.03)	0.07 (±0.02)	0.088 (±0.054)	0.85 (±0.12)	0.16 (±0.08)	0.37 (±0.09)	0.13 (±0.08)
	0-500	0.55	0.01 (±0.004)	0.37 (±0.05)	0.05 (±0.04)	0.002 (±0.001)	0.20 (±0.05)	0.03 (±0.04)	0.08 (±0.03)	0.02 (±0.01)

2.6 Discussion

2.6.1 Zooplankton distribution and species composition.

Oligotrophic areas, like the region investigated here, mainly depend on water mass circulation for nutrient supply. The hydrography of the studied area is influenced by a complex interaction of cyclonic and anticyclonic eddies. Cyclonic eddies move the isopycnals upward and anticyclonic eddies downward. The hydrographic survey has identified three circulation patterns in the upper ~1500 m that influence the sampling sites. Stations LV03 and LV10 are affected by the low salinity Atlantic water mass carried by a branch of surface water that circulates around the periphery of the Cretan Cyclone. Station LV13 is influenced by an anticyclone in the central part of the investigated area. Station LV18 is influenced by a strong current flowing cyclonically around the periphery of the Rhodes Gyre carrying high salinity and potentially nutrient enriched LSW from the east Levantine Sea. These waters derive from the Rhodes Gyre and were enriched with nutrients and dissolved and particulate matter derived from a pronounced phytoplankton bloom that occurred prior to this survey (as retrieved from remote sensing chlorophyll *a* imagery). The Rhodes gyre is known as a feature of nutrient enrichment which may support higher biological activity (Salihoglu *et al.,* 1990). According to this pattern, the observed deep chlorophyll maximum (DCM) was located between 75-100 m at stations LV03, LV10 and LV18, whereas it was deeper (114 m) at station LV13, apparently related to the anticyclonic flow impacting this station. Ediger and Yilmaz (1996) also reported that the DCM is usually formed at shallower depths (28-75 m) in cyclonic eddy fields than in anticyclonic eddies (77-100 m). The Chl *a* values, however, were opposite to the zooplankton abundance distribution (with the exception of LV18), maybe denoting the grazing pressure upon phytoplankton.

Phytoplankton vertical distribution in terms of Chl *a* concentrations showed a pronounced deep maximum (60 - 100 m) which indicates an early onset of the water column stratification. This was rather unexpected for the time of the experiment (early spring) and was indicative of a 'post-bloom' situation **(Fig. 2.2)**.

The zooplankton data collected during the LEVECO cruise support the current notion that the EMS is one of the most oligotrophic marine basins in the world. In the entire area, depth-integrated abundances (0-1000 m) were fairly low, averaging from 53 to 87 ind m^{-3}. The values recorded are in similar ranges as reported for other oligotrophic areas (Zenkevitch, 1963: total zooplankton in the tropical and North Pacific Ocean; Deevey and Brooks, 1977: copepods in the Sargasso Sea). They were also similar to those reported by Scotto di Carlo *et al.* (1984) for the Tyrrhenian Sea, which is considered poorer in zooplankton biomass when compared to other parts of the Western Mediterranean (Scotto di Carlo and lanora, 1983). Increasing zooplankton abundances at local sites caused by the Rhodes Gyre were also reported for spring 1986 by Pancucci-Papadopoulou *et al.* (1992) and Mazzocchi *et al.* (1997). Stations LV13 and LV18 showed maximum zooplankton abundance in the upper 100 m in compliance with Chl_{max} and Phy_{max}. The observed smooth decrease of abundance ($r^2 \geq 0.9$) at stations LV03 and LV10 could be attributed to the positioning of the stations near the Cretan Cyclone; this gyre probably entraps zooplankters towards deeper layers, as indicated by the increase of integrated abundance values in 200-500 m layer (LV03:53, LV10:43 ind m^{-3}). On the other hand, a less smooth decrease of abundance ($r^2 > 0.8$) was evident at stations LV13 and LV18 located over an anticyclone eddy and Rhodos gyre respectively (LV13: 28, LV18: 39 ind m^{-3}). Although LV18 exhibits high integrated abundance value in 200-500 m layer, the slope is less smooth due to the reflection of Rhodos gyre in the primary production in the 0-100 m layer and subsequently in zooplankton abundance. Similar slope values have been exhibited by Siokou *et al.* (2013) for the Aegean Sea and by Weikert (1982) for the Red Sea, whereas much lower values were reported by Weikert & Trinkhaus (1990) for the Levantine Sea **(Table 2.3)**.

Copepoda was the dominant taxon group at all stations, consistent with previous reports by Kimor and Berdugo (1967), Moraitou-Apostolopoulou (1985), Pancucci-Papadopoulou (1992), Mazzocchi *et al.* (1997), Koppelmann *et al.* (2009), Siokou-Frangou *et al.* (2010) and Christiansen and Weikert (2017) for the Eastern Mediterranean. Total mesozooplankton abundance was mainly concentrated in the upper 100 m layer, with *Clausocalanus* juveniles dominating the communities at all stations. *Clausocalanus* is a mainly surface-living genus, although some taxa can

extend their vertical range to about 500 m depth (Raymont, 1983). Noteworthy is the occurrence of *Clausocalanus lividus* at all stations. It is a typical species in the North Atlantic Ocean (European waters), but it has been rarely recorded in the Cretan Sea and the Straits of the Cretan Arc (Gotsis-Skretas *et al.*, 1999) and it is for the first time recorded in the Cretan Passage in such high abundances. The abundance of *Haloptilus longicornis* in the 100-200 m layer was also important and has been mentioned by Weikert and Trinkaus (1990), Siokou-Frangou *et al.* (1996, 1997 and 2010) and Koppelman *et al.* (2009). *Mormonilla minor* showed high dominance values at depth below 200 m at all stations.

According to Basescu (1985), the Eastern Mediterranean zooplankton community is distinguished by a high percentage of thermophile tropical and subtropical elements. In our study, the contribution of cyclopoids (33%) and poecilostomatoids (5%) to the copepod community was quite important. Though this could be underestimated since the mesh size of the net was 200 μm and these species are often smaller. The abundance and diversity of the cyclopoid *Oithona* and the poecilostomatoids *Oncaea, Corycaeus* and *Farranula* support the notion of the subtropical character of Eastern Mediterranean zooplankton because these genera are abundant in warm seas (Raymont, 1983). Species composition was primarily differentiated according to depth; copepod assemblages occupied discrete layers, each of them being dominated by different genus/species, such as *Clausocalanus* juveniles, *Oithona* juveniles or *Mormonilla minor*. Within the mixed layer (0-100 m), the epipelagic community composition was quite homogeneous, however, the abundance distribution was higher in the subsurface layer (50-100 m).

Regarding the rare and less abundant species detected in this study, it was of high importance to study taxonomy at species level as there is little zooplankton biodiversity information available for the Cretan Passage. For example, there are studies by Weikert and Koppelmann (1993) and Koppelmann *et al.* (2007) that have taxonomic information but mostly down to genus level and by Christiansen & Weikert (2017), that have information (not always at species level) for the surrounding area e.g. the Levantine Basin etc. We know that the rare and less abundant species (Table 4) are species of the EMS. Apart from *Aetideus acutus* and *T. minor*, the others have been recently listed as species of the Italian Seas by

Mazzocchi & Di Capua (2010). Also according to the list of Razouls *et al.* (2005-2018), all species, except *Scaphocalanus similis, Vettoria longifurca, A. acutus* and *Temeropia mino*r, are present in the Levantine Basin and except *Scaphocalanus invalidus, S. similis, Spinocalanus longicornis, S. magnus A. acutus* and *T. mino*r, all are present in the Aegean Sea. This means that the species most likely are widespread in the study area but were not recorded so far.

2.6.2 Zooplankton respiration, growth and production

There were no consistent trends in enzyme data visible between stations when both examined layers (0-100 m and 0-500 m) are considered together. However, the values for spAARS were higher in the 0-100 m layer indicating higher specific growth rates in the euphotic zone, where primary production occurs (see also Yebra *et al.*, 2009).

Herrera *et al.* (2012) detected high specific AARS activities at low growth rates under limiting food concentration and low individual biomass. This was not the case in our study where high spAARS values were observed when biomass values were high and vice versa (with exception of LV13). On the contrary, spETS negatively followed biomass values (with exception of LV03). Nevertheless, it should be underlined that in this study spAARS and spETS represent bulk zooplankton communities.

Growth and respiration rates could be modified by changes in zooplankton communities or trophic conditions. For example, when we tried to correlate spAARS and spETS values with gelatinous-crustacean ratios, it seems that the two indices respond differently. It is known from literature (Schalk, 1988), that crustaceans (only copepods in this study) show higher respiratory activity than gelatinous species, therefore, we expect higher values in spETS when the ratio is low. This, in fact, was underlined by our results. On the other hand, regarding spAARS, we expect that the activity follows the gelatinous abundance values because growth rates are higher when gelatinous blooms occur (Alldredge, 1984). This, however, does not seem to be the case in this study which could be caused by two reasons. First of all, Alldredge (1984) refers to all categories of gelatinous species, whereas we mainly detected salps in our study. Although it is known that

spring is the bloom period for salps (Menard *et al.*, 1994, Boero *et al.*, 2013, Pascual & Fuentes, 2015), we cannot provide evidence that our sampling occurred during a salp bloom.

To evaluate the role of zooplankton in the carbon budget in the pelagic ecosystem, ETS values were converted to respiration rates. Converting our oxygen-specific (spETS activity) units to carbon-specific units, we detected almost 10 times smaller values in the 0-100 m layer than in the 0-500 m layer at station LV18. This difference could be due to differences in the zooplankton abundance or it could be derived from a combination of higher temperature and turbulence and changing food availability. The aforementioned values are close to the ones reported by Herrera *et al.* (2014) for the Western Mediterranean, but very low compared to the values reported by Minutoli and Gugliemo (2009) for the Western Mediterranean and Balearic Islands and also compared to the values reported by King *et al.* (1978) for the Eastern Tropical North Pacific. An increasing gradient of respiration from LV03 towards LV18 was evident, but with no significant statistical differences.

To estimate ZP, we used the grand mean of estimates from spETS and the two published spAARS-G relationships. The estimation of ZP using G from **Eq (4)** was higher than the one estimated using G from **Eq (5)**. This was expected since **Eq (4)** was established on a basis of experiments using nauplii of the copepod *Paracartia grani*, a much larger copepod than the nauplii and juveniles of *Oithona davisae* that were used to establish **Eq (5)**. The resulting overall mean of ZP in the 0-100 m layer was 0.46 mg C m^{-3} d^{-1} whereas it was 0.20 mg C m^{-3} d^{-1} in the 0-500 m layer. Our enzyme-based methods have generated very low zooplankton production rates in comparison to other measurements from different regions, for example 1.21 and 6.78 mg C m^{-3} d^{-1} in North West Australia (McKinnon *et al.* 2015).

In area specific terms, the zooplankton production in the study area ranged from 0.64 to 5.24 mg C m^{-2} d^{-1} for the upper 100 m and 3.18 to 26.20 mg C m^{-2} d^{-1} for the upper 500 m. These values are very low compared to eutrophic regions. McKinnon *et al.* (2015) recorded values of 42 and 278 mg C m^{-2} d^{-1} in water depths of 35 and 41 m, respectively, in North West Australia. Newbury *et al.* (1976) measured 151 mg C m^{-2} d^{-1} in Kaneohe Bay, Hawaii, and Peterson (1995) estimated

values as high as 400 mg C m^{-2} d^{-1} in the Eastern Agulhas Bank of the Benguela Upwelling System. On the other hand, for a more oligotrophic area such as the EMS, previous studies of the copepod production in the Northern Aegean Sea by Zervoudaki *et al.* (2007) presented values of 15 mg C m^{-2} d^{-1} in late summer and 36 mg C m^{-2} d^{-1} in spring for the upper 100 m depth layer, which are values closer to the ones reported in this study. It has to be noted that the previous estimates of zooplankton production are based on artificial cohort experiments focused solely on copepods dominant in these systems, whilst our enzyme methods were conducted with mixed plankton populations.

Finally, applying Dagg's (1982) equation for ingestion rates, we calculated very low values, ranging from 0.02 (LV18 0-500 m) to 0.13 (LV18 0-100 m) mg C m^{-3} d^{-1}.

The primary production (PP) estimated during our cruise by Livanou *et al.* (this issue) for the 0-100 m layer showed highest values at station LV10 (221.3 mg C m^2 d^{-1}), similar values at LV18 (183.0 mg C m^2 d^{-1}) and LV03 (172.9 mg C m^2 d^{-1}) and lowest values at LV13 (109.6 mg C m^2 d^{-1}). The PP values are similar to those reported by Siokou-Frangou *et al.* (2002) and they follow the mean integrated phytoplankton abundance values, but not the mean integrated values of zooplankton, which show a trend of increasing biomass from western to eastern stations of the Cretan Passage.

The very few measurements of carbon flux in the southern Aegean Sea (Siokou-Frangou *et al.*, 2002) limit our understanding of the fate of the pelagic production in this ecosystem. During our study, as an attempt to illustrate the pelagic food web in the upper 100 m with special emphasis on zooplankton, we have established carbon flux budgets for the studied sites **(Fig. 2.8)**. Given that copepods comprise ~80% of mesozooplankton abundance, we estimated the carbon demand of the zooplankton community from production rates by assuming a one-third gross growth efficiency (Kiorboe *et al.*, 1985; Peterson, 1988). In order to examine whether available food is sufficient for the zooplankton community, we calculated their carbon demand and consumption and related it to phytoplankton biomass and production. The available phytoplankton production could cover the zooplankton carbon demand at all stations, however, only 5 to 6% of the primary

production was consumed by zooplankton at the western stations while 14 to 16% of this rate was consumed at the eastern stations. Therefore, high grazing impacts of zooplankton on phytoplankton biomass were detected (21-71%). This can be explained, at least for the eastern station LV13, by the increased relative contribution of the >2 μm size fraction (nano + microphytoplankton) to total PP (Livanou *et al.,* this issue) which involves cells in the size range that can be effectively grazed by zooplankton. Nevertheless, in the study area, most of the PP was performed by picophytoplankton size fraction (Livanou *et al.,* this issue) which

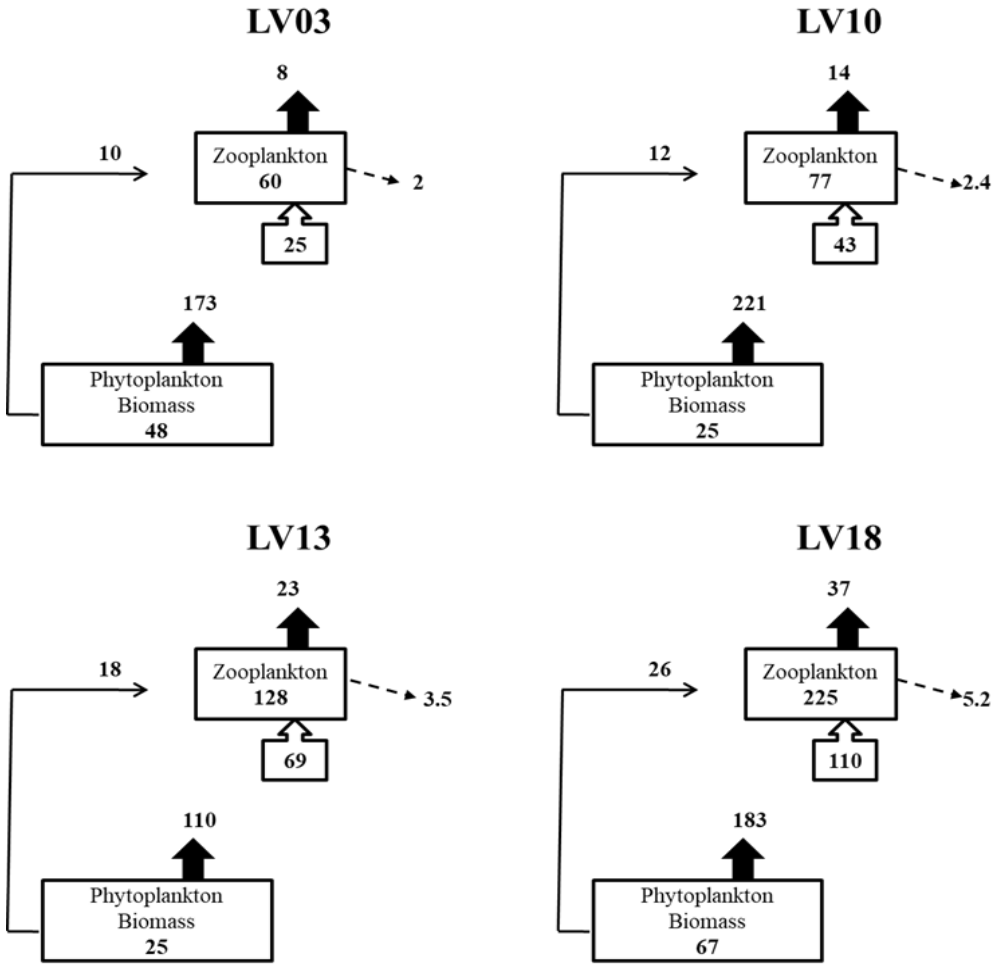

Figure 2.8 Carbon flow diagrams of the planktonic food web established for the Cretan Passage during April 2016 for the 0-100 m layer. Numbers in boxes show biomass (mg C m-2), black thick arrows show the carbon production and the white boxes with arrow show carbon demand (mg C m-2 day-1). Narrow arrows show the consumption (mg C m-2 day-1) of the zooplankton and the dashed arrow show the respiration.

is not efficiently grazed by zooplankton (Zervoudaki et al., 2007). Although the available food satisfies the zooplankton carbon demands, we can assume that only a part of the available phytoplankton production is consumed because not all autotrophs provide adequate food quality for zooplankton. Thus, it seems that there is a strong need for alternative food sources for zooplankton such as protozooplankton like in other picoplankton-dominated marine systems (Siokou-Frangou *et al.*, 2002, Zervoudaki *et al.*, 2007). The applications of enzymatic indices of *in-situ* growth or respiration results are in agreement with other studies, confirming that mesozooplankton consume ~12% of the primary production (Calbet *et al.* 2001). Zooplankton (carbon) losses through respiration were low compared to the respiration measurements from the global ocean (Hernández-León and Ikeda, 2005). This means that the carbon available for zooplankton is mainly used for its production in the study area.

In synthesis, this study showed that mesozooplankton communities manifested a slight gradient from western to eastern stations of the Cretan Passage in total abundance and biomass, probably due to the influence of Rhodos gyre in station LV18. The results from the carbon flux budget underline the oligotrophic character of the Cretan Passage and indicate that the zooplankton is not well fed and that the organisms are living under oligotrophic stress.

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CHAPTER 3

3. Trophic positioning of prominent copepods in the epi- and mesopelagic zone of the ultra-oligotrophic Eastern Mediterranean Sea

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3.1 Abstract

Combined analyses of Fatty Acid Trophic Markers (FATM) and Stable Isotopes (SI) were used to characterize food preferences among copepod species/taxa and to trace their food sources in the ultra-oligotrophic Cretan Passage of the Eastern Mediterranean Sea (EMS). FATMs are based on the conservation and transfer of specific source Fatty Acids (FAs) through the food web, providing a qualitative indicator of assimilated food types. SI provide information about trophic levels and food sources of specific organisms. Most species/taxa exhibited low $\delta^{15}N$ ratios caused by low $\delta^{15}N$-PON base values at the study site. A cluster analysis based on the mean Fatty Acid (FA) compositions of nine copepods from all stations identified three distinct groups (group 1: *Clausocalanus lividus* and *Euchaeta* spp.*;* group 2: *Pleuromamma* spp.*, Haloptilus longicornis*, *Lucicutia* spp., *Clausocalanus* spp. and *Corycaeus* spp.; group 3: *Pareucalanus attenuatus* and *Oncaea* spp.), coinciding with similarities in feeding behavior. *H. longicornis,* however, revealed similar trophic positions but different food resources by FATM and SIA analyses. Overall, we conclude that omnivory was the prevailing feeding mode, demonstrating a high degree of opportunistic feeding of copepods in these ultra-oligotrophic waters. This study shed first light on the zooplankton taxa/species life strategies with regard to feeding preferences and lipid storage mechanisms in the oligotrophic EMS, where lack of relevant information exists. Although further investigation is required, the good agreement between FA and SI in some species of copepods emphasize the applicability of lipid trophic markers. Results benefit from the coupling of these indices even in oligotrophic regions of the world ocean.

KEYWORDS: zooplankton, fatty acids, stable isotopes, trophic markers, oligotrophy, Mediterranean Sea

3.2 Introduction

The Mediterranean Sea is characterized by a strong eastward gradient in nutrients, phytoplankton biomass and primary production (Siokou-Frangou et al., 2010) with ultra-oligotrophic conditions being found in the Levantine Basin (Krom et al., 1991; Moutin and Raimbault, 2002; Ignatiades, 2005). Similar patterns have also been reported on the basin scale for mesozooplankton abundance (Mazzocchi et al., 1997; Dolan et al., 2002; Siokou-Frangou, 2004; Minutoli and Guglielmo, 2009; Nowaczyk et al., 2011).

Diatoms and dinoflagellates, typical primary producers in higher latitudes and eutrophic systems with characteristic marker FAs, are less abundant in oligotrophic tropical environments (Calbet and Landry, 1999; Gaudy et al*.,* 2003). Nevertheless, phytoplankton alone usually does not cover carbon demand in oligotrophic regions (Siokou et al., 2010). The potentially limited availability of phytoplankton is therefore often compensated by feeding on microzooplankton (Kleppel, 1993; Calbet and Landry, 1999; Calbet and Saiz, 2005, Zervoudaki et al., 2007), which results in a rather mixed diet and a more opportunistic and omnivorous feeding mode. Copepods are the dominant group of mesozooplankton and play a key role in the food web as they form a link between primary producers and secondary consumers (Richmond et al., 2007; Guschina and Harwood, 2009). Studies on food web relationships may provide important information to understand organisms' baseline ecology, predict community-level consequences of abiotic and biotic changes and characterize trophic interactions. Traditional studies on food web dynamics have used gut content analyses and food removal methods. While a great deal of information may be gleaned, these approaches are labor intensive, logistically difficult and often ambiguous with regard to what was consumed and what was assimilated (Kelly and Scheibling, 2012). For many years now, stable isotopes and lipid biomarkers (fatty acid analysis) are being used to identify specific food web relationships as they provide time integrated information on an organism's assimilated diet (Graeve et al., 1994a; Auel et al., 2002; Dalsgaard et al., 2003; El-Sabaawi et al., 2009a, b; Van den Meersche et al., 2009; Allan et al., 2010; Kelly and Scheibling, 2012; Schukat et al., 2014). Trophic

biomarkers integrate dietary signals over longer time periods of days to several weeks depending on the species (Graeve et al., 1994a; Gentsch et al., 2009).

Stable isotopes of the major constituents of organic molecules (H, C, N, O, S) may be indicators for an organism's trophic level and its diet (Petersen and Fry, 1987). With this analysis, it is possible to monitor the state and the dynamics of food webs since heavier isotopes are enriched in organisms relative to their diet (Fry, 2006; Newton, 2010). For terrestrial as well as aquatic ecosystems, the use of stable nitrogen and carbon isotopes for estimating the trophic position and the carbon flow inside a food web is very advantageous (Kling et al., 1992; France, 1995; Post, 2002). To obtain detailed information of the trophic position, a comparison with the trophic base is necessary. Depending on the examined food web, primary producers like algae (France, 1995), or detritus (Koppelmann et al., 2003a, Koppelmann et al., 2009) and primary consumers (Vander Zanden and Rasmussen 1999, Post 2002) can be used as baselines. In contrast to the incremental increase of $\delta^{15}N$ (3-4 ‰, DeNiro and Epstein, 1981; Post, 2002), the $\delta^{13}C$ changes only little (0-1 ‰) between trophic levels (Rounick and Winterbourn, 1986; Peterson and Fry, 1987). Therefore, $\delta^{13}C$ allows revealing the carbon sources of organisms inside food webs if they vary in their isotopic signature. Koppelmann et al. (2003a, b), Koppelmann et al. (2009) and Hannides et al. (2015) already performed stable isotope analyses on plankton of the eastern Mediterranean Sea in 1999, 2001 and 2010 respectively. Mixed zooplankton of different size classes showed relatively low $\delta^{15}N$ values in the upper layers which could either be caused by the fixation of atmospheric nitrogen by diazotrophic cyanobacteria like *Synechococcus* (Li et al., 1993, Detmer, 1995) or by a lack of significant denitrification in the basin and by particulate organic matter exported from surface waters during the $P_{limited}$ winter plankton bloom (Krom et al. 2004). Koppelmann et al. (2003a) noted that the $\delta^{15}N$ signature increased in deeper layers. The authors determined the trophic position (TP) of zooplankton using $\delta^{15}N$ values of POM assuming that particulate organic matter (POM) is the main source of food in the deep sea (Angel, 1990).

Since stable isotopes are indicators for the origin of organic matter and directly linked to the organisms' diet, the dimensions of the trophic niche may reflect the ecological niche of populations (Bearhop et al., 2004). Furthermore, the

expansion of the isotopic niche widths as well as the overlap can be calculated to define the trophic level. In association with information of the trophic level, the niche widths of the copepod taxa show the trophic variability in δ space and how the trophic niches of the different species differ among themselves.

Fatty acids, some of the most important molecules transferred across the plant-animal interface in aquatic environments (Dalsgaard et al., 2003; Allan et al., 2010), can be used as trophic markers since they are transferred without change from primary producers to higher trophic levels within the food web (Alfaro *et al.*, 2006). Fatty acids have a high biological specificity and, in conjunction with stable isotope ratios, can provide information on the diet assimilated by zooplankton (El-Sabaawi et al., 2009a, 2010; Van den Meersche et al., 2009; Allan et al., 2010; Kelly and Scheibling, 2012). More specifically, the use of FATMs derives from the fact that predators retain the taxon-specific compounds that are produced by their prey (e.g. bacteria, phytoplankton and microzooplankton) (Dalsgaard et al., 2003). In copepods, FATMs provide information about the level of carnivory as well as the specific diet of a species.

Only few studies on trophic interactions with the use of stable isotopes exist for the eastern Mediterranean Sea and the Cretan Passage (Hannides et al., 2015; Koppelmann et al. 2003a,b; 2009), whereas lipid content and composition in copepods in the same area have not been investigated so far. Thus, this is the first study to elucidate the dietary preferences and feeding strategies of major copepod species based on the lipid composition combined with stable isotope analyses of prominent copepod taxa/species from an ultra-oligotrophic environment of the Eastern Mediterranean Sea, the Cretan Passage.

3.3 Material and Methods

3.3.1 Sampling site

The eastern Mediterranean Sea consists of two major basins: The Ionian Basin east of the Strait of Sicily and between the coasts of Italy, Greece and Libya and the Levantine Basin south of Asia Minor. The Cretan Passage, a submarine ridge between Crete and Barqah (Libya), separates the above two basins. A

multidisciplinary oceanographic cruise was conducted south of Crete Island (western Levantine Sea) on board the *R/V* AEGEAO (Hellenic Centre for Marine Research, Greece) from the 9th to the 18th of April 2016 and was dedicated to the study of the functioning of the pelagic ecosystem in the area. The sampled stations (LV03, LV10, LV13 and LV18) along a transect from west to east south of Crete are shown in **Figure 3.1**.

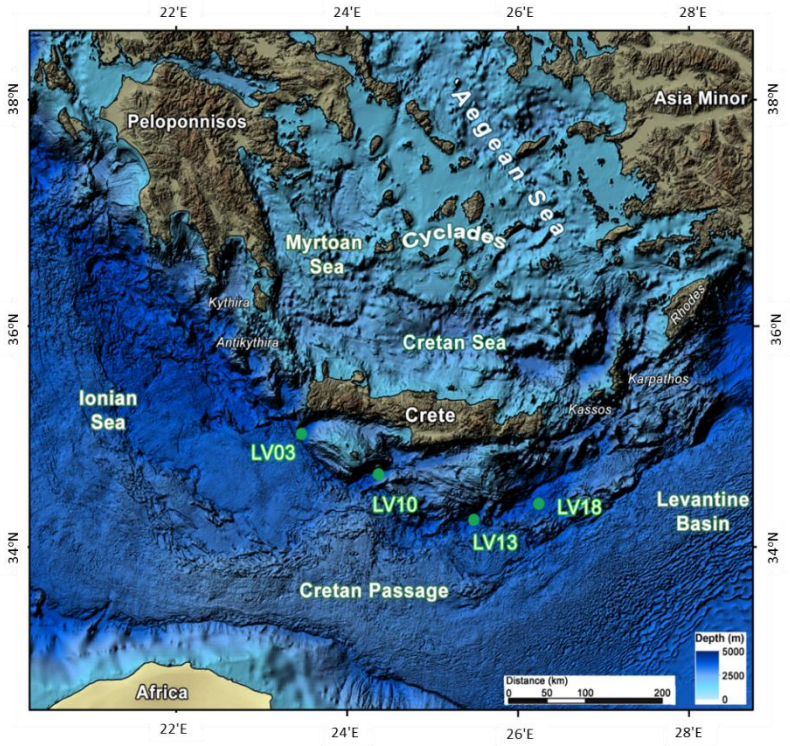

Figure 3.1 Sampling grid in the Cretan Passage during April 2016.

3.3.2 Hydrography

Standard temperature and salinity (conductivity) measurements for the entire water column (max. sampling depth: 3950 m) were obtained with a Sea–Bird Electronics 11plus™ CTD deck unit, interfaced with a Sea–Bird Electronics™ 9plus underwater unit and a Sea–Bird Electronics 32 rosette sampler with twenty-four 10–L Niskin bottles.

Fluorescence was measured with a fluorometer (Chelsea Instruments AQUAtracka™ III; excitation 430 nm, emission 685 nm). Fluorometer readings were calibrated against Chl-α estimates from discrete samples analyzed with high-

performance liquid chromatography (HPLC) to obtain Chl-α (μg L^{-1}). The environmental parameters are described in detail by Protopapa et al. (2019) and Velaoras et al. (2018).

3.3.3 Sampling

Samples for isotope analyses, FAs and biomass were collected by vertical hauls with a Hydrobios (Kiel, Germany) non-closing WP-3 net with a mesh size of 300 μm at all four sites **(Table 3.1)**. The hauls were obtained in depth ranges from 0 to 100 m and 0 to 500 m. The WP-3 net was used to collect large adult copepods in high concentrations. For zooplankton taxonomy and isotope analyses of formaldehyde samples, the samples were collected in discrete layers from the surface to 1000 m depth by vertical hauls of a WP-2 standard closing net from Hydrobios (Kiel, Germany) with 200 μm mesh size during daytime according to the "Zooplankton methodology manual" (Sameoto et al., 2000, Chapter 3).

Table 3.1 Station data for April 2016. The local time (UTC +2h) denotes the sampling period

Station	Region	Date	Local time	Sampled intervals (m)
LV03	35°03'N, 23°46'E	17/04/16	07:50-08:10	0-100
			10:10-10:50	0-500
LV10	34°66'N, 24°36'E	15/04/16	09:25-09:45	0-100
			12:00-12:45	0-500
LV13	34°25'N, 25°48'E	14/04/16	09:00-09:20	0-100
			11:00-12:00	0-500
LV18	34°43'N, 26°38'E	10/04/16	09:40-10:00	0-100
			11:53-12:43	0-500

3.3.4 Taxonomy and abundance

Copepods were identified at species level whenever possible while other groups were mainly identified at higher taxonomic levels. Their abundance was expressed as relative abundance (%) referring to the evenness of distribution of individuals among species in a community.

3.3.5 Stable isotopes

In order to prepare the WP-3 samples for isotope analysis, the zooplankton were washed on board with seawater on a circle of gauze with a diameter of 5 cm and a mesh size of 300 µm. Subsequently the gauze was stored in a freezer at -20°C until further analysis. The samples were defrosted in the lab in a cooling box filled with ice to identify species for isotope analysis. A subsample was placed under a stereo-microscope in a petry dish filled with sea water with a salinity of 38 and placed on a plate on ice. Single copepods were picked and sorted at species and genus level. The individuals were washed in a bowl of tap water for several seconds to get rid of the salt. After washing, the copepods were placed on glass fiber filters (GF/C 25 mm). Only males and females without eggs or CIV and CV copepodite stages were used for isotope analyses. The number picked for each species and genus depended on size and estimated dry weight **(Table 3.3)**. The filters were folded and put into small Eppendorf tubes for drying at 60°C for 24 hours. After drying, the tubes were closed and stored into a bag filled with silica gel. The WP-2 samples used for isotope analyses were rinsed with Milli-Q water for 60 seconds to get rid of the formaldehyde and transferred into a petri dish. Single copepods of *Haloptilus longicornis* and *Subeucalanus monachus* were treated like above.

Filters with the dried copepods were teared into pieces and stuffed into 5x9 mm zinc capsules. For gaining the precise dry weight of the organic material, the weight of the assembled filters was subtracted from the blank weight, which was determined before. The closed capsules were stored in a 96x well plate before filling the auto-sampler of the mass spectrometer. Stable isotope studies were performed at the University of Hamburg in the Institute of Plant Science and Microbiology. A CNHO isotope mass spectrometer (Nu Horizon Stable Isotope Mass Spectrometer, Nu Instruments Ltd.) with an associated CHNSO element analyzer

(EURO- EA 3000, Euro Vector, Italy) with standard configuration was used for the analyses. N_2 was used as nitrogen standard and PDB as standard for carbon. Calibrations were rendered with a certificated IAEA - 600 caffeine standard ($\delta^{13}C$ = - 27.77 ‰, VPDB, SD = 0.043, $\delta^{15}N$ = +1.0 ‰ air N_2, SD 0.2 ‰) and IAEA-NO- 3 potassium nitrate ($\delta^{15}N$ = +4.7 ‰ airN2, SD 0.2 ‰). The analytical error of this method is ≤0.1‰. Stable isotope values are expressed in δ notations as parts per thousand (‰), where R is the ratio of $^{15}N/^{14}N$ and the standard is atmospheric nitrogen:

$$\delta^{15}N\ [‰] = ((R_{sample}/R_{standard})-1)*1000$$

For the stable isotope analysis of suspended and sinking particulate matter (POM), water was collected by sampling bottles with closing mechanism at the same stations except for LV03 where no samples were obtained. The amount of water collected in every haul and depth ranged between 15 and 20 l each **(Table 3.2)**. The collected water was filtered on a glass fiber filter (GF/C, 90 mm) and frozen at -20°C. For isotope analyses, filters were defrosted and dried for 48h at 60°C. The amount of material from 200 and 500 m depth was too low for the $\delta^{15}N$-PON analysis and these samples were not treated further. Deep chlorophyll maximum (DCM) layers were detected between 75 and 100 m.

3.3.6 Fatty acids

Copepods for fatty acid analysis were collected on board immediately after recovering of the nets. Sorting was done on ice to avoid any extraction of FAs from the copepod tissues. For each selected taxa/species, intact individuals were randomly picked under a stereo-microscope and washed with cold pre-filtered seawater (pore size 0.2 μm) to remove potential particulate matter stuck to their exoskeleton. Overall, two replicates were prepared whenever possible (due to low abundances). Each sample consisted of 10-70 individuals, depending on the taxa/species size and on the availability of animals, in the sample, to ensure sufficient material and to minimize individual variability. The samples were stored

in cryogenic vials in liquid nitrogen during the sampling period and in the lab at -80 °C until further analysis. The copepod taxa/species collected were *Clausocalanus lividus, Clausocalanus* spp., *Corycaeus* spp., *Pareucalanus attenuatus, Euchaeta* spp., *Haloptilus longicornis, Lucicutia* spp., *Oncaea* spp. and *Pleuromamma* spp.

All samples were lyophilized for 24 h. Afterwards dry masses were determined using a microbalance (Sartorius ISO 9001, ±2 µg), while the samples were kept in a vacuum desiccator to prevent hydration during measurements. Lipid extraction was performed following a modified protocol of Folch et al. (1957). In detail, copepods were transferred into glass vials with 4 mL of dichloromethane:methanol (DcM:MeOH) (2:1/*v:v*) for at least one week and stored at -30 °C. Tricosanoic acid (S23:0) was added as internal standard and samples were filled up to 5 mL with DcM:MeOH. The extract was washed using 1 mL of an aqueous KCl solution (0.88 %), centrifuged for 15 min with 1200 rpm at 0 °C and the unipolar phase containing the lipids was separated and vaporized with N_2. An aliquot of the extracts was mixed with 1 mL of methanolic H_2SO_4 (3 %) and heated up to 80 °C for 4 h to esterify the FAs into their methyl ester derivates (FAMEs) (Kattner and Fricke, 1986). For the FAME extraction, 2 mL of aqua bidest and 1 mL of hexane were added. The extraction with hexane, centrifugation for 10 min with 1200 rpm at 0 °C and vaporization with elemental nitrogen (N_2) was repeated three times for each sample. FAMEs were identified by gas chromatography using retention times, derived from known composition of herring oil (Marinol) using the software Agilent OpenLab Data analysis, as well as analyzed manually afterwards. Dirt and blurred peaks were excluded.

3.3.7 Data treatment and Statistical Analysis

The trophic position (TP) was modelled with the $\delta^{15}N$ values of the copepod samples, using a trophic enrichment factor (TEF) of 3.4 ‰ following the suggestions of Post (2002). The stable isotope signatures of filtered water samples were used as a baseline (Table 3). The following formula was used to calculate the trophic position:

$$TP_{onsumer} = TL_{base} + (\delta^{15}N_{consumer} - \delta^{15}N_{base}) / \delta^{15}N$$

where $TP_{consumer}$ are the sampled copepods and TL_{base} the trophic level of POM. The baseline was set to an intermediate value of 1.5 since POM consists mostly of phytoplankton (TL = 1) and micro- and mesozooplankton (TL = 2) (Costalago et al., 2012; Albo-Puigserver et al., 2016). $\delta^{15}N$ represents the applied TEF of 3.4 ‰ per trophic level.

The isotopic niche width ($‰^2$) was estimated using the standard ellipse function SEA of the R (R Core Team, 2016)-based Stable Isotope Analysis package (SIAR, v4.2.2) according to Jackson et al. (2011). The ellipses were calculated in SIAR default mode including 40 % of the data, using a correction mode (SEAc) for small sample sizes. The individual isotopic niche width (SEAc) was calculated for every taxon. In addition to individual niche width, the overlap of competing niches was analyzed to see if there are groups that are located in the same niche. Therefore, a Bayesian estimate of standard ellipses was calculated with the Stable Isotope Bayesian Ellipse sub-function SIBER, to compare the niche width areas SEAb. Moreover, the overlapping areas of the isotopic niches were analyzed and their proportion was designed by using the niche widths of the standard ellipses.

In order to reveal dietary preferences of the species/taxa investigated, basic trophic marker ratios (FATMs) were estimated (DHA/EPA, PUFA/SFA, 16PUFA/18PUFA, D/F, 18:2(n-6), 18:1(n-9)/18:1(n-7), 18:1(n-9)/Σherb.markers and 15:0 + 17:0). A cluster analysis based on a similarity matrix was performed applying the group average linkage technique (Primer v6 software, Clarke and Warwick, 1994) to identify similarities in FA compositions between different copepod species/taxa, Similarities in FAs among species were calculated by the Bray–Curtis similarity index.

3.4 Results

3.4.1 Mesozooplankton abundance and composition

The communities in the entire water column were dominated by copepods at all stations and in all depth layers with a relative abundance from 49 to 93 %. The relative abundance of the species/taxa collected for FA and SI ranged from 0.1 to 1.9 % for *C. lividus*, from 2.6 to 34.1 % for *Clausocalanus* spp., from 0.2 to 9.0

% for *Corycaeus* spp., from 0.2 to 0.5 % for *P. attenuatus*, from 0.1 to 2.4 % for *Euchaeta* spp., from 0.1 to 11.0 % for *H. longicornis*, from 0.4 to 4.8 % for *Lucicutia* spp., from 1.5 to 16.7 % for *Oncaea* spp., and from 0.2 to 9.2 % for *Pleuromamma* spp. Analytical results on the vertical distribution of zooplankton from the same studied area are reported in Protopapa et al. (2019).

3.4.2 Isotopic signature of filtered POM

$\delta^{15}N$ values of POM in 5 m and 75 m depth range from 0.4 to 1.7 ‰ with lowest values at LV13 and highest values at LV03 **(Table 3.2)**. $\delta^{13}C$ ranged between -23.9 and -27.0 ‰.

Table 3.2 Isotopic signature of analysed POM at the sampling sites and corresponding depths.

Station	Depth (m)	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)
LV03	5	1.5	-27.0
	75	1.7	-24.6
LV13	5	0.4	-23.9
	120	0.3	-26.0
LV18	5	1.1	-26.5
	75	0.7	-25.6

3.4.3 Stable isotopic composition of prominent copepods

The WP-3 samples provided sufficient material for the stable isotope analysis of five different taxa. The spatial distributions of $\delta^{15}N$ and $\delta^{13}C$ of each taxon illustrated a clustered arrangement **(Table 3.3)**, where *H. longicornis*, *Corycaeus* spp. and *Pleuromamma* spp. were arranged around values of -22.0 ‰ in $\delta^{13}C$. There was a distinct difference in $\delta^{15}N$ between *H. longicornis* (3.6 ± 0.4 ‰) and *Corycaeus* spp. (1.7 ± 0.2 ‰). *Pleuromamma* spp. filled the gap between the two taxa with 2.2 ± 0.7 ‰ in $\delta^{15}N$. The $\delta^{15}N$ signatures of *C. lividus* and *Lucicutia* spp. (both 1.8 ± 0.5 ‰) were located close to the $\delta^{15}N$ level of *Corycaeus* spp., however, with lower $\delta^{13}C$ values between -23.4 and -23.5 ‰. This structure was also visible

for the individual trophic levels of the copepod taxa. *H. longicornis* had the highest TP of 2.29 and *Lucicutia* spp. the lowest with 1.22 **(Table 3.3)**.

Table 3.3 Mean $\delta^{15}N$ and $\delta^{13}C$ (‰) values of specific copepod taxa from frozen samples sorted by sampling sites and corresponding depth levels. n (ind) = number of replicates and individuals/replicate, SD = standard deviation between the stations. Trophic position (TP) determined with $\delta^{15}N$ values of analyzed POM used as baseline (TL = 1.5) listed depth levels. Niche width calculated with correction for small sample size (SEAc). Values are given as mean ± standard deviation for n ≥ 3, if n = 2; values are arranged according to the scheme: sample 1 data/sample 2 data

Taxa	n (ind)	Station	Depth (m)	$\delta^{15}N$	$\delta^{13}C$	C/N	TP (TEF = 3.4 ‰)	Niche width (‰², SEAc)
Haloptilus longicornis							**2.3**	**0.3**
	3 (50)	LV03	0-100	3.9±0.1	-22.0±0.1	4.1±0.1	2.2	
	3 (50)		0-500	3.9±0.5	-22.1±0.2	4.0±0.3	2.2	
	3 (50)	LV10	0-100	3.9±0.2	-22.2±0.2	4.1±0.2	2.4	
	3 (50)		0-500	3.5±0.4	-22.0±0.1	4.0±0.1	2.3	
	3 (50)	LV13	0-100	3.5±0.2	-22.0±0.2	4.2±0.1	2.4	
	3 (50)		0-500	3.8±0.5	-22.0±0.4	4.5±0.2	2.5	
	3 (50)	LV18	0-100	3.2±0.4	-22.2±0.4	4.4±0.3	2.2	
	3 (50)		0-500	3.3±0.3	-22.2±0.3	4.5±0.4	2.2	
Pleuromamma spp.							**1.9**	**0.6**
	2 (10)	LV03	0-500	2.9/2.1	-22.1/-22.5	3.4/3.7	1.8	
	2 (15)	LV10	0-500	1.5/2.6	-22.1/-22.2	3.5/3.6	1.8	
	2 (20)	LV13	0-500	2.5/2.8	-21.8/-21.7	3.6/3.7	2.2	
	3 (15)	LV18	0-500	1.8±0.9	-21.9±0.3	3.8±0.3	1.8	
Clausocalanus lividus							**1.6**	**0.2**
	1(20)	LV03	0-100	2.2	-23.3	4.5	1.7	
	1 (11)		0-500	1.9	-23.6	4.8	1.6	
	1(20)	LV10	0-100	1.2	-23.1	4.3	1.6	
Lucicutia spp.							**1.2**	**0.3**
	2 (15)	LV03	0-500	1.4/1.9	-24.0/-23.6	5.0/4.8	1.5	
	2 (15)	LV10	0-500	2.5/1.7	-23.5/-23.5	5.1/5.5	1.8	
	2 (15)	LV18	0-500	0.5/1.6	-23.9/-21.9	4.8/3.8	1.5	
Corycaeus spp.							**1.7**	**0.6**
	3 (40)	LV03	0-500	2.0±0.3	-22.2±0.1	3.8±0.2	1.6	
	3 (40)	LV10	0-500	1.6±0.1	-22.3±0.1	3.9±0.2	1.67	
	3 (40)	LV13	0-500	1.7±0.0	-21.8±0.1	3.9±0.1	1.9	
	2 (40)	LV18	0-500	1.6/1.7	-22.0/-22.5	3.6/4.0	1.5	

While WP-3 samples cover a wider species range, only *H. longicornis* and *S. monachus* were collected from WP-2 formaldehyde samples. These samples also covered a larger depth range (1000 m) instead of the WP-3 samples (500 m). The average difference in $\delta^{13}C$ between the two species was minor with -22.3 ‰ for *H. longicornis* and -21.8 ‰ for *S. monachus* **(Fig. 3.2)**, but with the addition that the values of *S. monachus* were more spread (±1.1 ‰), while *H. longicornis* showed a more cohered character (±0.3 ‰). The $\delta^{15}N$ values, corrected by 0.51 ‰ for formaldehyde samples according to Koppelmann et al. (2009), of *H. longicornis* were higher than described before with a mean value of 5.6 ‰ ± 0.7 ‰. *S. monachus* showed the highest mean values in $\delta^{15}N$ of all copepod sampled analyzed so far with 6.2 ‰ ± 1.3 ‰ **(Table 3.4)**. Although the material was treated with formaldehyde, $\delta^{13}C$ values did not vary from those of frozen samples and will be used for further interpretations. In total, the variance of values was higher at these samples, which, however, had a larger depth range. At this point, it is advantageous to have a look on the particular depth zones and their isotopic signature. Clear discernible is the already pointed depth zonation of both species, where *H. longicornis* occurred mostly in the epipelagic zone, while *S. monachus* was mostly found in the meso- and bathypelagic zone. The $\delta^{15}N$ values increased slightly with depth which lead to the higher mean $\delta^{15}N$ signature of *S. monachus*.

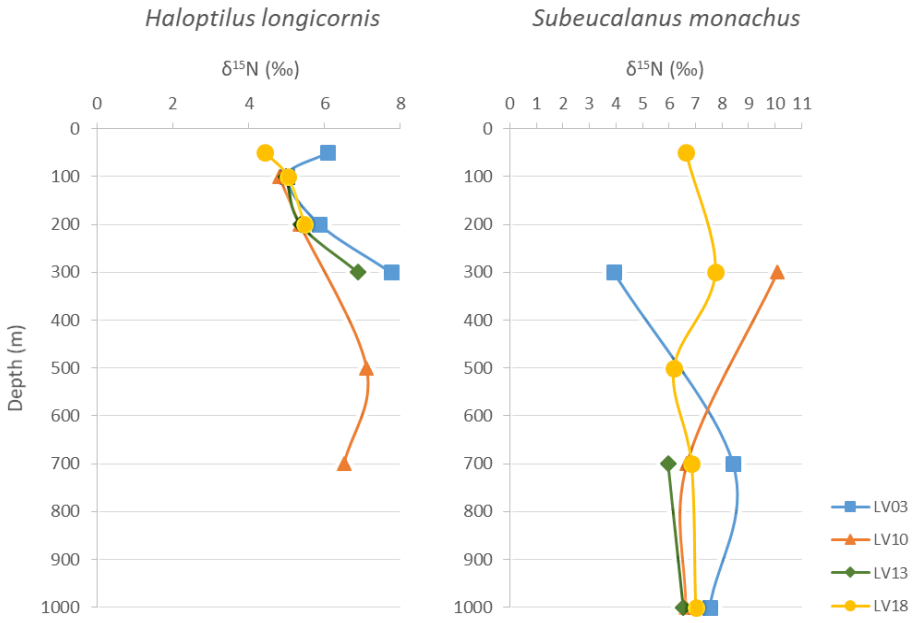

Figure 3.2 Vertical distribution of δ15N mean values (‰) of Haloptilus longicornis and Subeucalanus monachus at the four sampling sites in the eastern Mediterranean.

Table 3.4 Mean $\delta^{15}N$ and $\delta^{13}C$ (‰) values from all stations of formaldehyde samples sorted by corresponding depth levels. ($\delta^{15}N$ values corrected according to Koppelmann et al. 2009).Trophic Position (TP) determined with $\delta^{15}N$ values of analyzed POM used as baseline (TL = 1.5) following Koppelmann et al. (2003a). Niche width calculated with correction for small sample size (SEAc). n = number of replicates, SD = standard deviation between the stations. Values are given as mean ± standard deviation for n ≥ 3, if n = 2; values are arranged according to the scheme: sample 1 data/sample 2 data

Taxa	n	Depth (m)	$\delta^{15}N$	$\delta^{13}C$	C/N	TP (TEF = 3.4 ‰)	Niche width (‰^{2}, SEAc)
Haloptilus longicornis			**6.1±0.7**	**-22.4±0.2**	**5.6±0.3**	**2. 5**	**0.8**
	3	0-50	4.5±1.0	-22.2±0.3	5.2±0.4	2.4	
	8	50-100	4.4±0.2	-22.2±0.2	5.1±0.2	2.4	
	8	100-200	5.0±0.9	-22.2±0.3	5.4±0.4	2.5	
	2	200-300	7.2/6.4	-22.4/-22.4	6.1/6.1	2.0	
	1	300-500	6.6	-22.7	6.4	3.0	
	1	500-700	6.0	-22.4	5.6	2.8	
Subeucalanus monachus.			**6.8±1.3**	**-21.9±0.9**	9.2**±1.1**	**2.9**	**4.0**
	1	0-50	6.1	-21.5	8.9	2.9	
	3	200-300	6.7±3.1	-22.2±1.4	8.3±1.8	3.0	
	2	300-500	5.4/6.0	-22.2/-22.3	9.9/9.9	2.7	
	11	500-700	6.2±0.7	-21.4±1.1	9.0±1.4	2.9	
	13	700-1000	6.5±0.7	-21.9±1.2	10.1±1.2	3.0	

3.4.4 Differences in the trophic level of species among stations

H. longicornis isotopic data are available for all stations in the 0-100 m samples, whereas data for *C. lividus* are only available at LV03 and LV10. *H. longicornis* had the highest mean TP at station LV13 and the lowest at station LV18 (2.44 and 2.17, respectively). On the other hand, *C. lividus* showed almost similar values at the two stations (1.7 and 1.6, respectively) **(Table 3.3)**. For the 0-500 m layer, *H. longicornis, Pleuromamma* spp. and *Corycaeus* spp. had more or less similar values among stations, with the exception of station LV13 where all three taxa had highest values of 2.5, 2.2 and 1.9, respectively. For *Lucicutia* spp., data are available only for stations LV03, LV10 and LV18 with highest value at LV10 (1.8) **(Table 3.3)**.

Regarding the formaldehyde samples, the calculated trophic position of *S. monachus* (TP = 2.9) was slightly higher than the trophic position of *H. longicornis* (TP = 2.5) exhibiting almost the same values in all depths whereas *H. longicornis* TP values seem to increase with depth **(Table 3.4)**.

3.4.5 Isotopic niche analysis

The isotopic niche widths for the copepod taxa of the WP-3 samples were visualized as the most likely standard ellipses (SEAc) in **Figure 3.4a**. In addition to that, the convex hull, embracing the total area (TA) is presented in Figure 3 to get an indication of the complete potential niche space occupied by the organisms with regard to their maximal extent in δ space (Layman et al. 2007). The comparison of individual niche positions showed that there are overlaps between *Pleuromamma* and *Corycaeus* spp., as well as between *Lucicutia* spp. and *C. lividus*, while *H. longicornis*, with the highest mean $\delta^{15}N$ values, is isolated. The niche widths, calculated with the Bayesian approach, ranged from 0.2 ‰^{2} (*Corycaeus* spp.) to 0.6 ‰^{2} (*Pleuromamma* spp.). The overlapping couple *Pleuromamma* spp. and *Corycaeus* spp. showed a match of 0.2 ‰^{2}, which is 85% of the niche width of *Corycaeus* spp., while the overlap of *Lucicutia* spp. and *C. lividus* was 0.1 ‰^{2}, where *C. lividus* occupied 25.5 % of the *Lucicutia* spp. niche. Although the standard ellipses varied in their size, there were no significant differences between the estimated

standard ellipse areas **(Fig. 3.3a)**. Standard ellipses of *H. longicornis* and *S. monachus*, taken from formaldehyde samples varied strong in their proportions (*H. longicornis* = 0.8 ‰^{2}, *S. monachus* = 4 ‰^{2}, **Fig. 3.3b**). The comparison of estimated standard ellipses showed a significant difference in size ($p < 0.0001$, **Fig. 3.3b**). The overlapping area in δ space between the taxa was at 0.3, where *S. monachus* matched with *H. longicornis* with 33.6 %. Standard ellipses including the whole copepods sampled by WP-2 and WP-3 revealed, that there were two different trophic niches, which were occupied by the copepod community regarding to depth **(Fig. 3.4b)**. The proportions of niche areas were 2.3 ‰^{2} for the upper 500 m and 2.9 ‰^{2} for the depth zones from 500 m to 1000 m. There were no significant differences in size comparing the estimated standard ellipses and no significant overlap of the standard ellipses.

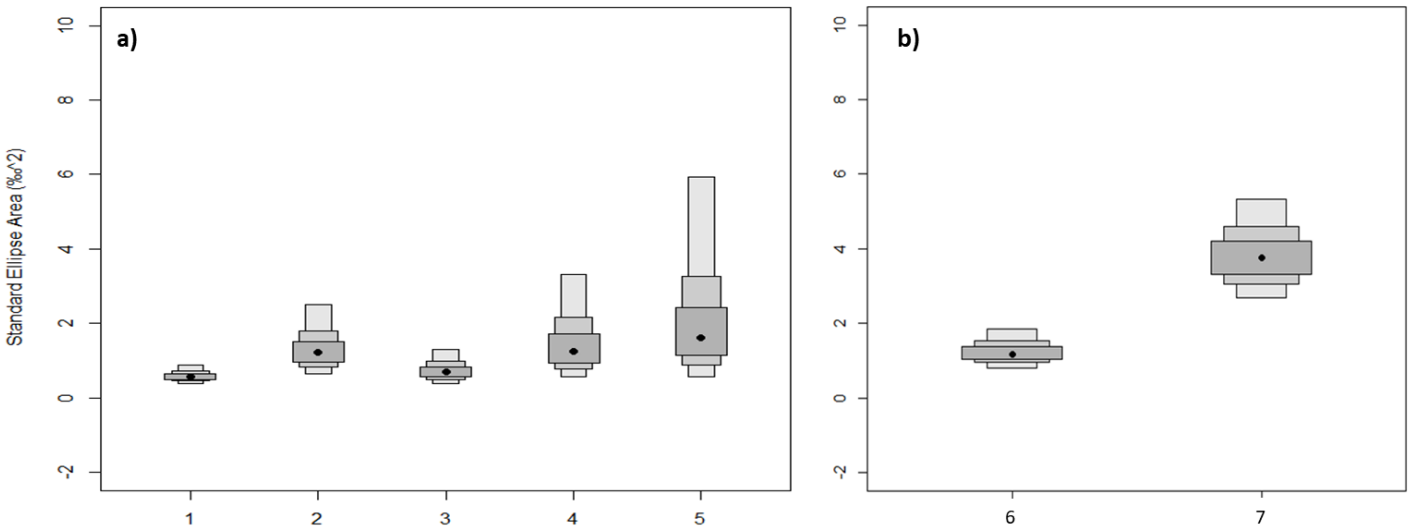

Figure 3.31 Density plots of the modeled estimated standard ellipses of the analyzed copepods sampled with WP-3 **(a)** and WP-2 **(b)**. Black dots belong to the mean standard ellipses, while boxes represent the 50 %, 75 % and 90 % credible intervals for mean estimation. **a)** *1: H. longicornis, 2: Pleuromamma spp., 3: Corycaeus spp., 4: Lucicutia spp., 5: C. lividus* **b)** *6: H. longicornis, 7: S. monachus*

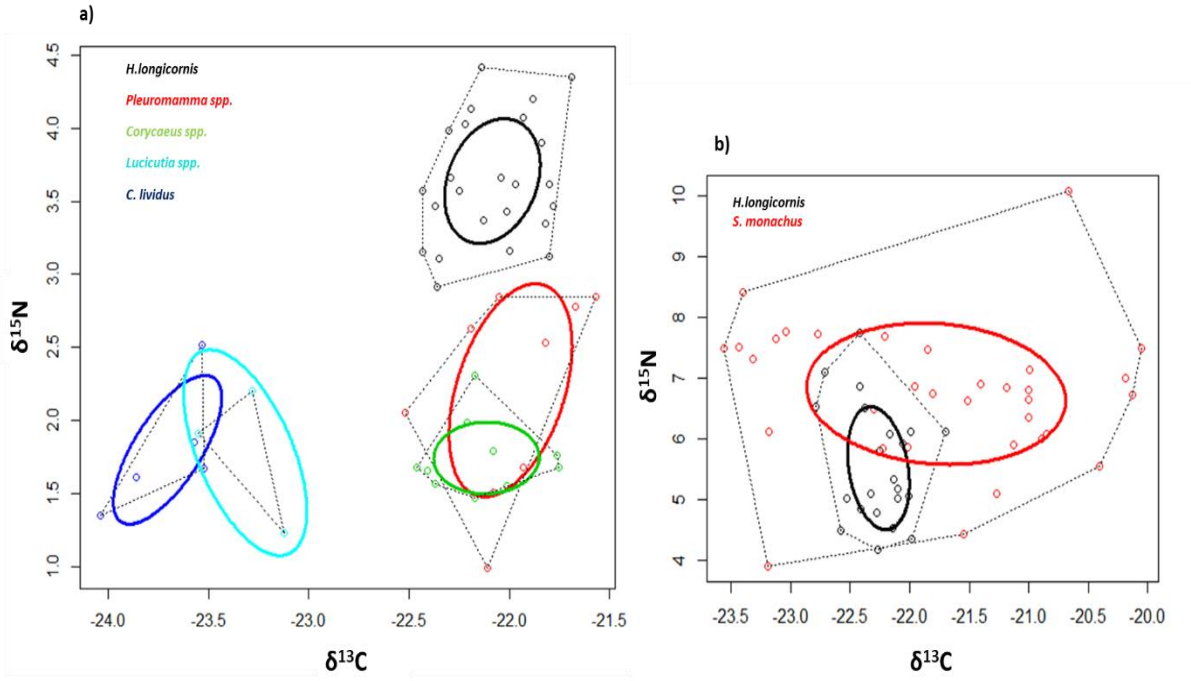

Figure 3.4 Stable isotope biplot with standard ellipses representing the trophic niche width of 40 % corrected for small sample size (SEAc) of copepods sampled with WP-3 **(a)** and WP-2 **(b)**. Dashed lines show the associated convex hulls.

3.4.6 Fatty acid composition of prominent copepods

Fatty acids of nine copepod species from all four stations (LV03, LV10, LV13, LV18) were measured **(Table 3.5)**. The fatty acid data showed relatively similar results with high abundances of polyunsaturated fatty acids (PUFA) that ranged between 27 % and 58 % of the total fatty acid content (% tFA) in each species (Table 5). PUFAs were dominated by the highly unsaturated docosahexaenoic acid 22:6(n-3) (DHA) (between 9 % and 40 %) and to a lesser extent by the eicosapentaenoic acid 20:5(n-3) (EPA) (between 5 % and 14 %). Although DHA concentrations were high throughout all species, *P. attenuatus* had much lower values at 9 % and 15 % at the stations LV13 and LV18, respectively. EPA on the other hand showed no considerable differences between species and stations. Arachidonic acid 20:4(n-6) (ARA) was found in minor concentrations except for *P. attenuatus* and *H. longicornis* (mean values 1 % and 2 %, respectively). Monounsaturated fatty acids (MUFA) were mainly represented by the 18:1(n-9) fatty acid, which was present in similar concentrations (on average in 12% of tFA)

throughout all species and stations. Exceptions were found for *C. lividus* (stations LV03 and LV10) and *Euchaeta* spp. (stations LV03, LV10 and LV13), which showed slightly higher values of 23 % - 28 % and 22 % - 25 %, respectively. 16:1(n-7) was present in minimal concentrations throughout all species (average of 3.1% tFA in all species), but showed a noticeable exception in *P. attenuatus* at LV13 and LV18 with concentrations of 22 % and 20 %, respectively. Saturated fatty acids (SFA), which ranged between 15 % - 44 % tFA, were mainly composed of 16:0 (palmitic acid) (between 10 % and 26 %) and 18:0 (stearic acid) (between 2 % and 7 %). *Euchaeta* spp. at stations LV03, LV10 and LV13 had considerably lower concentrations of SFAs, which ranged between 16 % - 19 %.

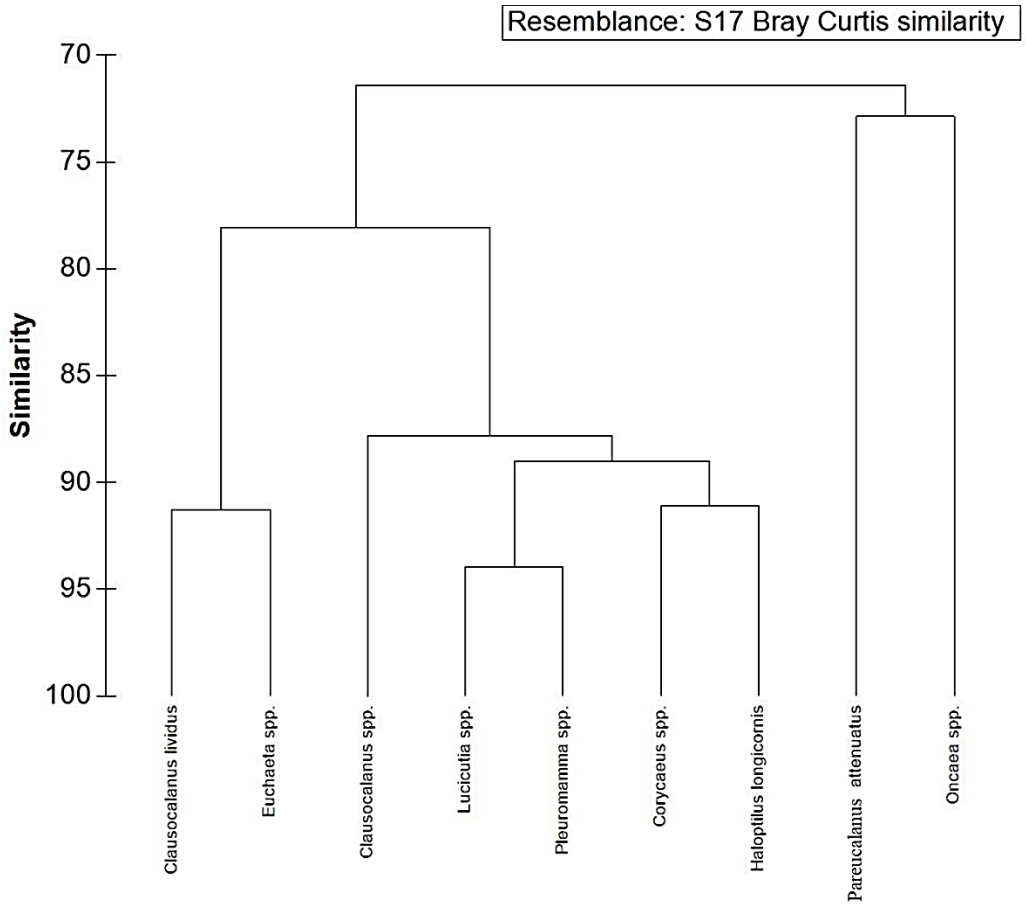

Figure 3.5 Clusters of copepods from the Cretan Passage with similar patterns in fatty acid illustrated as a dendrogram

Cluster analysis of mean FA abundances from all stations separated the copepods into three groups **(Fig. 3.5)**. Cluster 1 contained *C. lividus* and *Euchaeta* spp. and is characterized by high proportions of DHA, followed by 18:1(n-9) and secondarily EPA and 16:0 in similar relative abundances. Cluster 2 contained *Pleuromamma* spp., *H. longicornis*, *Lucicutia* spp., *Clausocalanus* spp. and *Corycaeus*

spp. and is characterized by high proportions of DHA, followed by 16:0 and secondarily EPA and 18:1(n-9). Cluster 3 contained *P. attenuatus* and *Oncaea* spp., which are, in contrast to all other taxa, characterized by the lowest relative proportions of DHA, and mixed (higher or lower) relative proportions for 16:0, EPA and 18:1(n-9) **(Table 3.5)**, respectively.

3.4.7 Trophic markers FA ratios

The trophic marker FA ratios (carnivory/herbivory ratios), in particular 18:1(n-9)/S herb. markers (Σ herb markers: 16:1(n-7) + 16:4(n-1) + 18:1(n-7) + 18:4(n-3)) (Schukat et al., 2014), serve as indices of the degree of carnivory in marine invertebrates. The copepod *P. attenuatus,* sampled between 0-500 m*,* exhibited the lowest degree of carnivory with a ratio of 0.7. The majority of copepods displayed intermediate ratios between 1 and 3. A higher degree of carnivory (ratio 5) was determined in the deeper-living species *Euchaeta* spp., sampled in 0-500 m, and in *C. lividus,* sampled in 0-100 m **(Tables 3.5 and 3.6)**.

Table 3.5 Fatty acid trophic markers considered in this study ([a] PUFA represents the sum of all polyunsaturated fatty acids, SFA represents the sum of all saturated fatty acids, [b] 16PUFA includes all PUFA with 16 carbon atoms, 18PUFA includes all PUFA with 18 carbon atoms, [c] D= 16PUFA+16:1(n-7) + 20:5(n-3) (all diatom fatty acids), F=18PUFA+18:2(n-6) + 22:6(n-3) (all flagellates fatty acids), [d] Σ herb. markers is the sum of 16:1(n-7) + 16:4(n-1) + 18:1(n-7) + 18:4(n-3)).

Trophic marker	Source/ Characteristic	Reference
DHA/EPA	Dinoflagellates/Diatoms, Carnivory	Budge and Parrish, 1998
PUFA/SFA[a]	Carnivory	Stevens et al., 2004
16PUFA/18PUFA[b]	Diatoms/Flagellates	Budge and Parrish 1998, Alfaro et al., 2006
D/F[c]	Diatoms/Flagellates	Dalsgaard et al., 2003
18:2(n-6)	Green algae, Terrestrial detritus	Dalsgaard et al., 2003
18:1(n-9)/18:1(n-7)	Carnivory or omnivory	Auel et al., 2002, Dalsgaard et al., 2003
18:1(n-9)/Σ herb. markers[d]	Carnivory or omnivory	Schukat et al., 2014
15:0 + 17:0	Bacteria	Kaneda, 1991

Table 3.6 Fatty acid averages (% of tFA content) and trophic markers ratio values of copepod taxa. (N.D: Not detected, PUFA: polyunsaturated fatty acids, DHA: docosahexaenoic acid 22:6(n-3), EPA: eicosapentaenoic acid 20:5(n-3), SFA: saturated fatty acids, D/F: Diatoms/Flagellates, Σ herb markers: 16:1(n-7) + 16:4(n-1) + 18:1(n-7) + 18:4(n-3))

Fatty acid	***Clausocalanus lividus***	***Clausocalanus spp.***	***Corycaeus spp.***	***Pareucalanus attenuatus***	***Euchaeta spp.***	***Haloptilus longicornis***	***Lucicutia spp.***	***Oncaea spp.***	***Pleuromamma spp.***
14:0	2.8	3.4	6.8	5.4	2.0	3.4	2.7	8.3	4.2
15:0	0.6	0.9	1.3	0.6	0.5	1.2	1.0	1.5	1.3
16:0	15.0	18.4	22.0	16.6	11.4	19.9	20.8	24.8	19.4
17:0	0.9	1.0	1.5	1.0	0.6	1.7	1.9	0.9	1.7
18:0	3.7	4.5	5.6	2.5	3.0	5.6	4.9	6.9	4.3
14:1(n-5)	N.D	N.D	N.D	N.D	N.D	0.5	0.2	1.2	0.1
16:1(n-7)	2.5	3.5	2.8	15.3	3.3	2.1	1.4	4.4	2.8
18:1(n-7)	1.3	1.6	1.3	4.3	1.0	2.4	1.2	1.9	1.4
18:1(n-9)	19.4	16.7	8.1	14.4	23.9	11.5	6.6	19.7	6.5
16:2(n-4)	0.5	0.4	1.7	1.2	1.1	1.4	0.8	0.9	0.6
16:3(n-3)	0.5	0.3	0.1	0.7	0.6	0.1	0.3	0.2	0.3
18:2(n-6)	2.9	2.4	2.1	1.7	1.9	2.0	2.0	2.7	2.1
18:3(n-6)	0.5	N.D	0.1	N.D	0.3	0.4	0.6	0.9	0.4
18:4(n-3)	N.D	1.3	0.1	N.D	0.3	0.4	0.6	N.D	0.4

18:5(n-3)	2.4	2.5	1.8	0.7	2.2	0.6	1.3	1.2	2.2
20:4(n-3)	2.3	1.7	0.4	N.D	0.4	1.7	1.0	1.1	0.9
20:4(n-6)	0.5	0.5	0.6	2.0	0.5	1.4	0.7	N.D	0.8
20:5(n-3)	11.4	10.7	9.6	12.8	9.6	8.7	10.7	4.8	12.2
22:6(n-3)	27.5	28.0	30.8	17.0	31.4	30.1	37.4	13.1	35.4
Trophic marker									
DHA/EPA	2.4	2.6	3.2	1.3	3.3	3.5	3.5	2.7	2.9
PUFA/SFA	2.2	1.7	1.3	1.4	2.8	1.5	1.9	0.6	1.9
16PUFA/18PUFA	0.2	0.1	0.4	0.8	0.4	0.4	0.2	0.3	0.2
D/F	0.4	0.4	0.4	1.5	0.4	0.4	0.3	0.6	0.4
18:2(n-6)	2.9	2.4	2.1	1.7	1.9	2.0	2.0	2.7	2.1
18:1(n-9)/ 18:1(n-7)	14.6	10.7	6.0	3.3	23.4	4.7	5.3	10.6	4.6
18:1(n-9)/Σ herb. markers	5.1	2.6	1.9	0.7	5.2	2.3	2.1	3.1	1.4
15:0 + 17:0	1.5	1.9	2.8	1.6	1.1	2.9	2.9	2.4	3

Table 3.7 Trophic level (TL) based on stable isotopes, main fatty acid (FA) marker and resulting diet characterization of analyzed taxa according to this study and literature results. (N.D: no data available)

	This study		Literature results		
Species/taxa	**TL**	**FA main marker**	**Diet**	**TL based on SI**	**Other Analyses[3]**
Oncaea spp.	N.D	Dinoflagellate	Omnivory	N.D	Detritivory (6)
Corycaeus spp.	1.73	Dinoflagellate	Omnivory	2.16 (8)	Carnivory (1,2,3,4,5,6)
Clausocalanus lividus	1.62		Carnivory	N.D	Herbivory-omnivory (6) Non-carnivory (5)
Clausocalanus spp.	N.D	Dinoflagellate	Omnivory	N.D	Herbivory-Detritivory (6)
Pareucalanus attenuatus	N.D	Diatom	Herbivory	2.97 *E. monachus* (8)	Herbivory (6)
Pleuromamma spp.	1.88	Dinoflagellate	Herbivory	1.82 (8)	Omnivory (7) Herbivory-Omnivory (6) Non-carnivory (5)
Haloptilus longicornis	2.29	Dinoflagellate	Omnivory	2.77 (8)	Omnivory (6) Carnivory (5)
Lucicutia spp.	1.22	Dinoflagellate	Omnivory	1.72 *L. flavicornis* (8)	Herbivory-omnivory (6) Non-carnivory (5)
Euchaeta spp.	N.D		Carnivory	2.39 (8)	Carnivory (6)

[3] 1) Wickstead (1962) based on literature, 2) Timonin (1969) for the Indian Ocean, 3) Turner et al. (1984) feeding experiments, 4) Landry et al. (1985) feeding experiments, 5) Benedetti et al. (2015) for the Mediterranean, 6) Benedetti et al. (2018) for the Mediterranean, 7) Teuber et al. (2014) for the SE Atlantic, 8) re-calculated after Koppelmann et al. (2009) for the Levantine Basin

Table 3.8 Mean $\delta^{15}N$ values of specific copepod taxa sorted by sampling sites and corresponding depth levels. n = number of replicates, SD = standard deviation between the stations. Koppelmann et al. (2003b, 2009).

	This study				**Koppelmann et al. 2009**		**Koppelmann et al.2003b**	
	WP-3 samples		**WP-2 samples**					
Species	**Depth**	**$\delta^{15}N$**	**Depth**	**$\delta^{15}N$**	**Depth**	**$\delta^{15}N$**	**Depth**	**$\delta^{15}N$**
Haloptilus longicornis	0-100	3.7±0.2	0-50	4.5±1.0	200-300	5.4±0.2	0-400	3.4±0.5
	0-500	3.6±0.4	50-100	4.4±0.2				
			100-200	5.0±0.9				
			200-300	6.4±0.6				
			300-500	6.6				
			500-700	6.0				
Pleuromamma spp.	0-500	2.3±0.6			350-600	2.2±0.1		
Clausocalanus lividus	0-100	1.7±0.5						
	0-500	1.9						
Lucicutia spp.	0-500	1.6±0.6			50-300	2.4		
Corycaeus spp.	0-500	1.7±0.1			50-100	3.3		
Subeucalanus monachus			50	6.1	600-1050	6.1±0.2	0-2750	6.9
			200-300	6.7±3.1				
			300-500	5.7±0.5				
			500-700	6.2±0.7				
			700-1000	6.5±0.7				

3.5 Discussion

This study presents a set of lipid and stable isotope data, reflecting potential dietary preferences as well as potential trophic interactions of copepods in the eastern Mediterranean Sea. The $\delta^{15}N$ signatures of *S. monachus* are in agreement with values reported by Koppelmann et al. (2003b, 2009) for the same area. *H. longicornis* ranged between 3.6 and 6.6 ‰ $\delta^{15}N$ in our study **(Table 3.8)**, which is in the same range as values of 3.4-5.4 ‰ published by Koppelmann et al. (2003b, 2009). The variability could be due to different feeding depths and/or feeding preferences. The $\delta^{15}N$ signatures of *S. monachus* and *H. longicornis* from greater depths were 2–3 ‰ higher than $\delta^{15}N$ values of the sampled taxa from the surface layers (<500 m) which lead consequently to a higher TP. Based on this, the hypothesis, that the trophic levels show differences in depth is vindicated but a precise determination of the TP with contemporary analysed depth related POM data was not possible. Another explanation for a higher TP can be exemplified by *H. longicornis*. This species shows a higher TP in the upper layers than the other examined taxa. Assuming that the feeding ecology of *H. longicornis* (Augaptilidae) is mostly carnivorous (Mullin, 1966; Timonin, 1971; Beckmann, 1995), the ascertained TP ranged between 2 and 3, indicating a first level predatory based trophic position. The TPs of the other taxa can be explained by herbi- or omnivorous feeding strategy since all mean values ranged between a TP of 1 and 2.

Based on the definition of Hutchinson (1957, 1978), the position of the trophic niche can be expressed by a n-dimensional hypervolume. Partitioned axes represent the environmental (scenopoetic) and ecological components, primary linked to the trophic parameters of the niche space. These axes could be defined using stable isotopes since there is a direct relationship of stable isotopes to the diet of organisms (Bolnick et al., 2003; Bearhop et al., 2004) and can be described as the isotopic niche. Focusing on stable nitrogen isotopes, the incremental enrichment of $\delta^{15}N$ with each trophic level (Minagawa and Wada, 1984; Fry, 1988) may have an increasing impact on the position of the isotopic niche and is partially correlated to the trophic niche parameters. As a result, it is possible to define the

individual variation in diet between members within a community (Bearhop et al., 2004). The positions of the isotopic niches vary in their distribution.

The isotopic niche of *H. longicornis* shows the highest position on the $\delta^{15}N$ axis whereas the other taxa are beneath. The position and also the compactness of the isotopic niche of *H. longicornis* could be an indicator for a more selective, predatory feeding mode while herbi- or omnivorous feeders probably tend to show a more opportunistic feeding strategy, which may lead to a broader niche width. The extensive isotopic niche of *S. monachus* can be explained with this assumption. This species seems to be a very opportunistic feeder in comparison with the other analysed taxa resulting in a broad range of $\delta^{13}C$ signatures as a result of a wide range of carbon sources. Nevertheless, a significant difference between the isotopic niche widths could only be validated for *H. longicornis* and *S. monachus.* On the other hand, the case of *H. longicornis* has to be considered with caution since the isotope results exhibited a carnivory preference whereas the FA ratios and the low relative proportions of diatom and flagellate markers indicate omnivory **(Tables 3.5, 3.6 and 3.7)**. However, $\delta^{15}N_{POM}$ does not represent a pure signature of copepod diets because it is composed of a heterogeneous mixture of particles, not all of which are consumed by copepods. At present, there is no realistically feasible way of acquiring a $\delta^{15}N$ signature of pure marine phytoplankton -which is also due to the large phylogenetic diversity of phytoplankton-, except for measuring the signature of pure chlorophyll (Sachs *et al.* 1999), which is a costly procedure that is also fraught with technical issues. Another problem associated with instantaneous measurements of $\delta^{15}N_{POM}$ as a representative of copepod diet is that its turnover rates are expected to be much faster than those of copepods. In order to accurately represent the $\delta^{15}N_{POM}$ ingested by copepods, an average estimate of $\delta^{15}N_{POM}$ is needed, preferably over a time scale that represents the period in which copepod tissues turn over. However, this approach can be subjective (see El-Sabaawi et al., 2009b and O'Reilly et al., 2002 for examples). Very few studies have attempted to address the isotopic turnover rates of copepods, and little is known about how isotopic turnover rates of copepods vary across species or across environmental conditions (e.g., Checkley and Entzeroth 1985; Klein-Breteler et al., 2002; Sommer et al. 2005; Tiselius and Fransson, 2016).

Pleuromamma spp. are known to occupy various trophic positions (Morris and Hopkins, 1983; Longhurst, 1985; Morales et al., 1993, Benedetti et al., 2015, 2018), thus according to our results and in agreement with Teuber et al. (2014), it seems to exhibit an opportunistic feeding behavior, while their FA composition point towards high levels of herbivorous feeding **(Tables 3.5 and 3.7)**. These species are active diel vertical migrators (Morris and Hopkins, 1983; Madhupratap and Haridas, 1990; Auel and Verheye, 2007) and follow a different life strategy in terms of energy metabolism (Teuber et al., 2013). They migrate to the surface at night to benefit from a richer food supply in epipelagic layers. The low C/N ratio implies high protein content, which is necessary to support a strong musculature in Diel Vertical Migration species (Morris and Hopkins, 1983).

The isotopic niches were calculated with a correction for small sample sizes as it was the case for *C. lividus* and *Lucicutia* spp. and this might have an impact on the niche width. *Corycaeus* spp. showed also a tight isotopic niche albeit its TP was on a similar level like *Pleuromamma* spp*., C. lividus* and *Lucicutia* spp. Several authors described members of the *Corycaeidae* to be carnivorous (Wickstead, 1962, Timonin, 1969; Turner et al., 1984; Landry et al., 1985) which could, like in the case of *H. longicornis*, lead to a tighter niche width. However, according to DHA/EPA, they exhibited a dinoflagellate diet, reflecting probably an opportunistic behavior. The isotopic niches vary also in their location on the $\delta^{13}C$ axis. *H. longicornis, Pleuromamma* spp. and *Corycaeus* spp. form a group near the -22‰ and *Lucicutia* spp. and *C. lividus* are combined between -23‰ and -24‰ in the upper 500 m. This may lead to the assumption that the origin of assimilated carbon has different origins. One can assume that the carbon source of *Lucicutia* spp. and *C. lividus* is different from the source of the other taxa. The values of the DHA/EPA ratio for *Lucicutia* spp*., H. longicornis* and *Corycaeus* spp. suggest that these species prefer a dinoflagellate diet, whereas, according to PUFA/SFA and 18:1 (n-9)/18:1(n-7) ratios, *C. lividus* exhibits high levels of carnivory. The different origin of assimilated carbon for the *Lucicutia* spp. and *C. lividus* samples, based on 1.5-2 ‰ difference from *H. longicornis*, *Pleuromamma* spp. and *Corycaeus* spp., could be attributed to feeding on micro- or nanophytoplankton. Livanou et al. (this issue) reported from data of the same cruise that most of the PP in the studied area was produced by picophytoplankton, a size fraction that is not efficiently grazed by

zooplankton (Zervoudaki et al., 2007). Protopapa et al. (2019) reported also from data of the same cruise that the available phytoplankton production could cover the zooplankton carbon demand at all stations, however, zooplankton consumed only 5 to 16% of the primary production. Although the available food satisfies the zooplankton carbon demands of the study area, it seems that only a part of the available phytoplankton production is consumed because not all autotrophs provide adequate food quality for zooplankton. Thus, it seems that there is a strong need for alternative food sources for zooplankton such as protozooplankton like in other picoplankton-dominated marine systems (Siokou-Frangou et al., 2002; Zervoudaki et al., 2007). Since the assumed enrichment in $\delta^{13}C$ is low (0.4 ‰, Post, 2002), the grouped position of *H. longicornis, Pleuromamma* spp. and *Corycaeus* spp. could point to a directly linked predator-prey relationship between *H. longicornis* as predator and *Pleuromamma* spp. and *Corycaeus* spp. as prey. However, this again cannot be verified by the FA results since *H. longicornis* seems to follow a dinoflagellate diet. The grouping of isotopic niches indicates an overlap, which suggests that there are similarities in the origin of the carbon sources. It also shows that the isotopic niches of overlapping taxa are located close together. The potential carnivorous *H. longicornis* is isolated due to its higher trophic level whereas the lower level taxa show an overlap. However, there is no overlap between all taxa due to the differences in $\delta^{13}C$. The degree of the overlapping area can be used as a scale for the trophic resemblance of two or more taxa. Linnebjerg et al. (2016) also mentioned that their analyzed species show a wide range of isotopic niches and are feeding on several trophic levels. This study covered a broad spectrum of different taxa, including invertebrates as well as marine mammals. Albo-Puigserver et al. (2016) estimated the isotopic niches for several fish species and revealed different trophic food levels in a predator community.

The indices of carnivory derived from FA compositions agreed quite well with trophic positions determined by nitrogen stable isotope analyses, supporting the validity of the lipid biomarker concept. Furthermore, cluster analyses **(Fig. 3.5)** grouped copepod species with similar FA compositions in broad clusters that were often characterized by similarities in feeding behavior. This is obvious for *C. lividus* and *Eucheata* spp., which exhibited the highest level of carnivory, and for *P. attenuatus* with *Oncaea* spp. with high levels of herbivory. Although *de-novo* synthesis

of the FA 16:1(n-7) is possible in many marine animals, it has been confirmed that 16:1(n-7) in herbivorous calanoid copepods predominantly derives from dietary sources, i.e. diatoms (Graeve et al., 1994a; Dalsgaard et al., 2003). A herbivorous diet in *P. attenuatus* is also supported by its lowest FA carnivory/ herbivory ratio of all copepods (0.7).

3.6 Conclusion

In conclusion, the present study revealed a diversity of taxa/species life strategies with regard to feeding preferences and lipid storage mechanisms. However, omnivory was the prevailing feeding mode, demonstrating a high degree of opportunistic feeding in oligotrophic copepods. The two complementary trophic biomarker approaches lead to similar results and emphasize the applicability of lipid trophic markers even in ultra-oligotrophic regions. Comparing the trophic levels received from both sampling methods reveals that the TP increases with greater depths, achieving the highest TP with *S. monachus*.

Despite the apparent strength of combining stable isotopes and fatty acids as trophic markers, many gaps in knowledge remain about the physiological relationships between these markers. For example, although few studies addressed the quick response of copepod fatty acids on dietary changes (e.g., Graeve et al., 1994a; Stevens et al., 2004; Bell et al., 2007), no studies addressed how turnover rates of stable isotopes and fatty acids vary relative to each other. It is clear that more experiments are needed to address how these tracers relate to copepod physiology across a wide range of taxa and environmental and dietary conditions.

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CHAPTER 4

4. Mesozooplankton community structure in the Eastern Mediterranean Sea

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4.1 Abstract

Mesozooplankton group composition was examined in the Cretan Sea, the Cretan Passage and the eastern and western Cretan Straits including one station in the Mirtoan Sea over a grid of 16 stations sampled during June 2016. Samples were taken in the epipelagic zone (0-200 m) and were analyzed at species level. The sampling area is influenced by the presence of the permanent Rhodes Cyclonic Gyre east of Crete and a series of smaller permanent or recurrent gyres south of Crete that influence the anti-estuarine pathway of the Atlantic Water from the Ionian Sea towards the Levantine Basin at surface and subsurface layers. Mesozooplankton abundance exhibited almost similar values (mean value 312 ind m^{-3}) between transects with a higher value at the western Straits (S25: 562 ind m^{-3}). Copepods were the dominant group at all stations with almost the same relative contributions among stations (76 %) but slightly higher values at stations S17 (84 %) and S19 (83 %). Among the different areas, copepods exhibited higher mean values in the Cretan Passage (80 %). The contribution of copepod functional groups in the mesozooplankton community is also highlighted. The importance of small sized copepods was underlined by the small ambush feeding carnivores, prevailing at all stations and demonstrating a high degree of opportunistic feeding of copepods in these ultra-oligotrophic waters. Copepod production was generally low whereas and only in the Cretan Sea was estimated to be higher than the other areas.

KEYWORDS: mesozooplankton, functional groups, normalized biomass size spectrum, copepod production, oligotrophy, eastern Mediterranean Sea

4.2 Introduction

The Mediterranean Sea is a basin with a surface circulation mainly driven by the inflow of low salinity/density Atlantic Water (AW) through the Straits of Gibraltar in order to compensate the water deficit of the basin. AW is converted into a more saline and dense intermediate water mass known as Levantine Intermediate Water (LIW) by open-sea convection processes during winter in the easternmost part (Levantine Basin) (POEM Group, 1992; Robinson *et al.*, 2001). LIW along with Levantine Surface Water (LSW) are water masses that occupy the intermediate and surface layers of the Cretan Sea (CS), Cretan Passage (CP) and Cretan Straits (CS) (Velaoras *et al.*, 2018)

The oligotrophic character of the Eastern Meditteranean Sea, in terms of both primary productivity and chlorophyll α (Chl α), has been widespread since the early 80s (Berman *et al.,* 1984a; Berman *et al.,* 1984b; Azov, 1986; Yacobi *et al.,* 1995), with phosphorus to be a limiting factor (Krom et al., 1991, 1992; Ignatiades, 1992). According to Berman *et al.* (1984b), a west-east gradient in nutrient deficiency creates an ultra-oligotrophic environment in the easternmost part of the EMS, the Levantine Sea.

Mesozooplankton distribution also follows the aforementioned gradient (Nowaczyk *et al.*, 2011; Siokou *et al.*, 2019), with higher abundances concentrated in the upper 100 m layer, sharply decreasing with depth, and characterized by the dominance of copepods, especially small sized animals, (≤1mm) (Siokou-Frangou *et al.*, 2010, 2019). Functional zooplankton traits are classified according to ecological functions (e.g. feeding, growth, reproduction, survival) and types (e.g. morphological, physiological, behavioral and life history) (Litchman *et al.*, 2013). Functional traits can be used to gather species with similar traits into functional groups (Gitay & Noble, 1997) or to describe functional diversity of zooplankton communities (Barnet *et al.*, 2007; Pomerleau *et al.*, 2015). It is important to understand and describe functional groups as they will increase our understanding of zooplankton ecological roles in marine ecosystems (Benedetti *et al.*, 2016).

The main goal of this study is to improve our knowledge on the mesozooplankton community structure as well as the carbon requirements in the ultra-oligotrophic area of the Southern Aegean Sea by coupling copepod standing

stock estimations (abundance, biomass and size classes) and metabolic measurements (according to size). The contribution of important functional copepod groups in the mesozooplankton community is also highlighted.

4.3 Materials and methods

4.3.1 Cruise tracks and environmental parameters

4.3.1.1 Cruise transect

A multidisciplinary oceanographic cruise was conducted in the Cretan Sea (S03, S05, S07, S09), the Cretan Passage (CP: S15, S17, S18, S19, S20, S21, S23), the Cretan Straits (WS: S25, S27, S28 and ES: S11) and the Myrtoan Sea (MS: S29) on board the R/V AEGEAO (Hellenic Center for Marine Research) from 2 to 9 June 2016 in order to study the functioning of the pelagic ecosystem in the area **(Fig. 4.1) (Table 4.1)**.

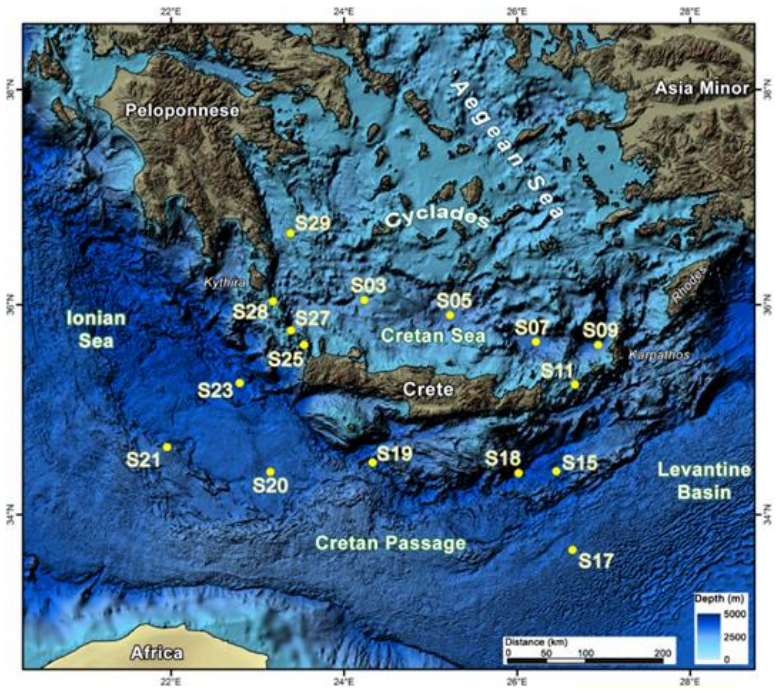

Figure 4.1 Mesozooplankton sampling stations in the Cretan Passage, Cretan Sea and Cretan Straits during June 2016.

Table 4.1. Station data for June 2016. The local time (UTC +2h) denotes the sampling period. All samples were collected during daylight hours in the 0-200 m layer.

Region	Station	Latitude (ºN)	Longitude (ºE)	Date
Cretan Sea (CS)	S03	34º02'	24º14'	03/06/2016
	S05	35º54'	25º13'	03/06/2016
	S07	35º39'	26º13'	04/06/2016
	S09	35º37'	26º56'	04/06/2016
Eastern Straits (ES)	S11	35º39'	26º13'	04/06/2016
Cretan Passage (CP)	S15	34º25'	26º27'	05/06/2016
	S17	34º39'	26º39'	06/06/2016
	S18	34º23'	26º01'	06/06/2016
	S19	34º30'	24º19'	06/06/2016
	S20	34º24'	23º08'	07/06/2016
	S21	34º38'	21º57'	07/06/2016
	S23	35º15'	22º47'	08/06/2016
Western Straits (WS)	S25	35º37'	23º32'	08/06/2016
	S27	35º45'	23º23'	09/06/2016
	S28	36º01'	23º10'	09/06/2016
Myrtoan Sea (MS)	S29	36º40'	23º22'	09/06/2016

4.3.1.2 Environmental data

Standard temperature and salinity (conductivity) measurements for the entire water column (max. sampling depth: 3950 m) were obtained with a Sea-Bird Electronics 11plus CTD deck unit, interfaced with a Sea-Bird Electronics 9plus underwater unit and a Sea-Bird Electronics 32 rosette sampler with 24 10L Niskin bottles.

4.3.2 Zooplankton

4.3.2.1 Sampling strategy

Zooplankton samples from 200 m were collected by vertical hauls (0.5 m s^{-1}) of a WP-2 standard closing net from Hydrobios (Kiel, Germany) with 200 μm mesh size during daytime **(Table 4.1)** according to the zooplankton methodology manual (Sameoto *et al.*, 2000, Chapter 3). The filtered water volumes of the WP-2 net (V = A x L, m^3) were calculated by taking into account the area of the net mouth (A, m^2) and the length of the released wire (L, m). The final thickness of the sampled layer (ΔD, m) and the upper and lower depth limits of the layer (ΔL = Li-Lf, m) were computed considering the wire angle α (ΔD = ΔL cos α). The volume of filtered seawater was used to calculate the mesozooplankton abundance per m^3 for each haul. The nets were carefully rinsed and the samples were halved, on board with a Folsom splitter. The first half was used for biomass determination and the other half was preserved in a seawater sodium tetraborate buffered formaldehyde solution (4% final concentration) (Steedman, 1976) for later determination of zooplankton composition and abundance.

4.3.2.2 Biomass and Chl *α* sampling

The subsample for bulk biomass measurement was filtered onto pre–weighed and pre-combusted, glass–fiber filters (Whatman GF/C) and dried at 60°C for 24 h onboard. Dry weight (mg) of samples was calculated from difference between the final weight and the weight of the filter and biomass (mg DW m^{-3}) was extrapolated from the total volume sampled by the net.

Water samples (2 l) were filtered onto 0.2-μm polycarbonate Millipore filters. The filters were extracted in 90% acetone for 24 h, and Chl *α* was determined using a TURNER 00-AU-10 fluorometer (Holm-Hansen et al. 1965). Chl *α* concentrations were converted to carbon biomass (mg m^{-3}) according to Malone *et al.* (1993).

4.3.2.3 Microscopic analyses

Taxonomic identification and counts of zooplankton were performed in the laboratory using an Olympus SZX-TR30 SZx12 dissecting microscope. Identification of the copepods community was made at species level and rare species were searched in the total sample. Holoplankton organisms other than copepods as well as meroplankton were identified at coarser taxonomic levels. Siphonophores were counted as part of colonies.

Abundance was expressed as number of individuals per cubic meter (ind m^{-3}), whereas relative abundance (%) refers to the evenness of distribution of individuals among species in a community.

4.3.2.4 Digital imaging approach using Imagepro-Plus software

A known fraction (1/5 to 1/10) of the subsample was scanned on an Epson Perfection 4990 photo color flatbed scanner similarly to Bell and Hopcroft (2008), at 2400 dpi (for samples collected by 200-μm and 500-μm nets) or 4800 dpi (for 45-μm net samples). Image analysis was completed using the software Imagepro plus 6.0 and processing similarly to Frangoulis *et al.* (2010). In each fraction (all net types) 500–1000 organisms were identified and measured (automatically by the software then checked and corrected – if necessary- by the user). The zooplankters were classified into the following groups: copepods (adult and copepodite stages), nauplii of all crustaceans (including copepod nauplii), appendicularians, decapod and euphausiid larvae, cladocerans, gelatinous carnivorous (chaetognaths, siphonophores, medusae), other meroplankton and other holoplankton. The sizes of zooplankters were converted into carbon based on literature size-carbon relationships (Uye, 1982; Alcaraz *et al.*, 2003; Lombard *et al.*, 2009).

The size spectrum of each sample (only for copepods) was then measured using the NB-SS (Normalized Biomass Size Spectrum) calculation (Yurista *et al.*, 2005; Herman & Harvey, 2006). The slope of NB-SS linear regression for each sample provides information on the community size-structure. Any size class with zero biomass was not included in the analysis, leaving an average of 4 size classes for each sampling station. High negative slopes are linked to higher percentages of small organisms while low negative slopes, close to zero, reveal high percentages of large organisms (Sourisseau & Carlotti, 2006).

4.3.2.5 Copepods growth rates, production and ingestion rates

Only copepod data were used for these analyses. Following the equation by Zhou *et al.* (2010), the growth rate (G, d^{-1}) of copepods was estimated for every station. Copepod production (P, mg C m^{-3} d^{-1}) was then calculated as the product of each estimate of G and the Imagepro plus estimated carbon biomass of copepods (mg C m^{-3}). Respiration (R, mg C m^{-3} d^{-1}) was estimated according to the equation R= 0.75*P (Ikeda & Motoda, 1978). We assessed the copepods potential ingestion (I; mg C m^{-3} d^{-1}) from production rates assuming a gross growth efficiency of 30% (Ikeda & Motoda, 1978)

4.3.3 Data analysis

Multivariate analyses were performed to examine spatial differentiation of the zooplankton community, if any, combining abundance and composition. Spatial differentiation of the zooplankton community among stations were visualized by a non-metric multidimensional scaling (nMDS) on the Bray–Curtis dissimilarity matrix created from the datasets of each station based on square root transformed abundances. Diversity indices such as Shannon diversity index (H′) and Species richness (S) were also calculated. All above parameters were calculated with Primer 6.0 software (Clarke & Warwick, 1995).

Relationship was tested between zooplankton parameters (abundance, biomass). A multiple regression analysis in Statgraphics Plus software ® was applied in order to evaluate the correlation, if any, between the environmental

parameters (temperature, salinity, oxygen) and Chl α, biomass and mesozooplankton abundance.

The copepods species identified were categorized in seven functional groups according to Benedetti *et al.* (2018). G1: large carnivores, G2: large filter feeding omnivores-herbivores, G3: Small ambush feeding carnivores, G4: Small filter feeding herbivores sac-spawners G5: Small sac-spawning detritivores G6: small filter feeding herbivores-omnivores (mostly broadcasters) and G7: small ambush feeding omnivores (sac spawners).

4.4. Results

4.4.1 Hydrography

Hydrographic CTD measurements revealed four main water masses in the study area **(Fig. 4.2): (1)** the Atlantic Water (AW), a surface/subsurface water mass (~0-50 m) of Atlantic origin moving eastwards towards the Levantine Basin with "low" salinity (S<39), **(2)** the Levantine Surface Water (LSW), a surface water mass created by strong evaporation mainly in the Levantine Basin with higher salinity (S>39), **(3)** the Black Sea Water (BSW), a surface water mass coming from the central Aegean with salinities between 38.87 and 39 and the **(4)** Levantine Intermediate water (LIW), an intermediate water

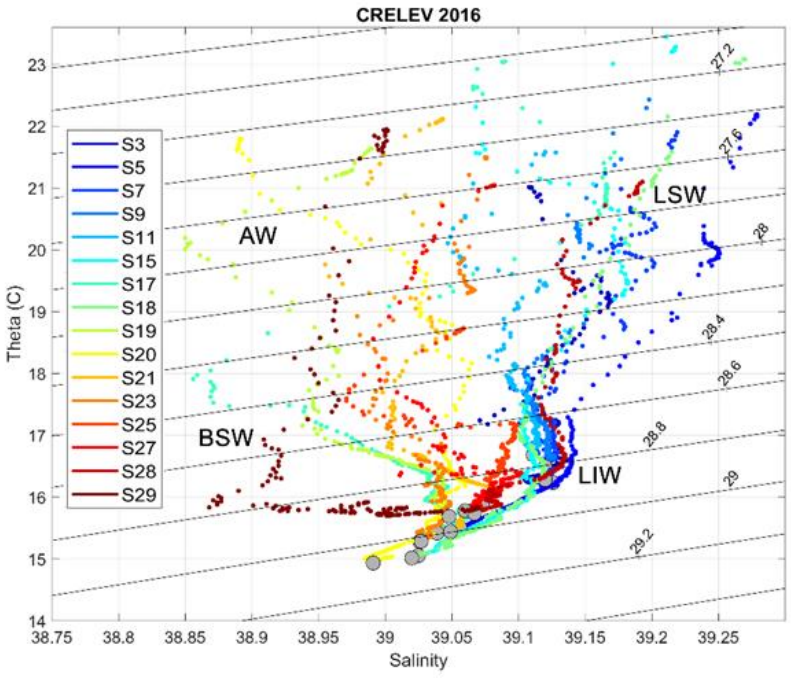

Figure 4.22 Potential temperature (Theta °C) and salinity (S) profiles (AW: Atlantic Water, LSW: Levantine Surface Water, LIW: Levantine Intermediate Water, BSW: Black Sea Water). Bullets define 200 m

mass below ~100 m with salinity ranging between 39.05 and 39.15, formed mainly in the Levantine basin (Rhodes Gyre) but also inside the Cretan Sea.

Surface values at station S29, which represents the western boundary of the MS, indicate the presence of BSW with a salinity of less than 39 in the 0 – 100 m layer whereas LSW with salinities ranging between 39.1 and 39.3 in the upper 100 m occupied all other stations. Saline LIW was observed below 100 m in the surveyed area expanding well below the maximum sampling depth of 200 m.

Low salinity AW (core salinity ≤38.9) was prominent at stations S17, S19, and S20 in the CP, while LSW with a salinity of ≥39 occupied stations S15 and S18. The Mid-Mediterranean Jet (MMJ) is directly affecting station S17 in the east and S20 in the west of the station grid **(Fig. 4.3)**, thus reducing local surface layer salinity. The Cretan Cyclon (CC) centered around 34.5° N – 24° E was influenced at its eastern periphery by the MMJ branch splitting off at around 34° N – 23.5° E. Station S19 situated between the CC and the anticyclone centered around 34.5° N – 25° E is affected by the MMJ branch. Stations S15 and S18 with surface salinities > 39.2 were directly influenced by LSW carried by the flow at the periphery of the Rhodes Gyre. The CS controls the water exchange between the Aegean Sea and the EMS. The upper 300 m are occupied with saline (S>39.05) and oxygen rich (dis. oxygen>5 mL/L in the west and>4.8 mL/L in the east)

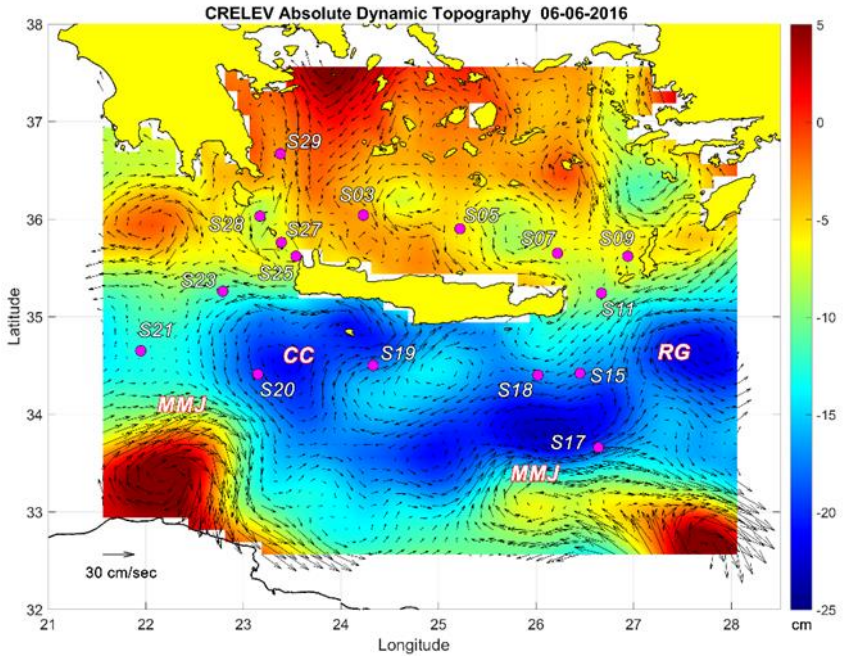

Figure 4.3 Geostrophic velocities over the absolute dynamic topography for June 6th

waters. More analysis on water masses and circulation is presented by Velaoras *et al.* (2018).

4.4.2 Zooplankton abundance and biomass distribution

Zooplankton abundance in the upper 200 m layer **(Fig. 4.4)** varied across the five geographic areas (CS, CP, WS, ES and MS), with values (mean ± SD) of 247 ± 17.1, 308 ± 63.9, 351 ± 151, 507 and 286 ind m^{-3}, respectively. Abundance was higher at the stations located in the CP, but station S25 in the WS exhibited the highest value among all stations (562 ind m^{-3}). The lowest abundance was found in the CP at station S23 with 198 ind m^{-3}. In addition no significant spatial differences were found between the five geographic areas (ANOVA, $p > 0.05$).

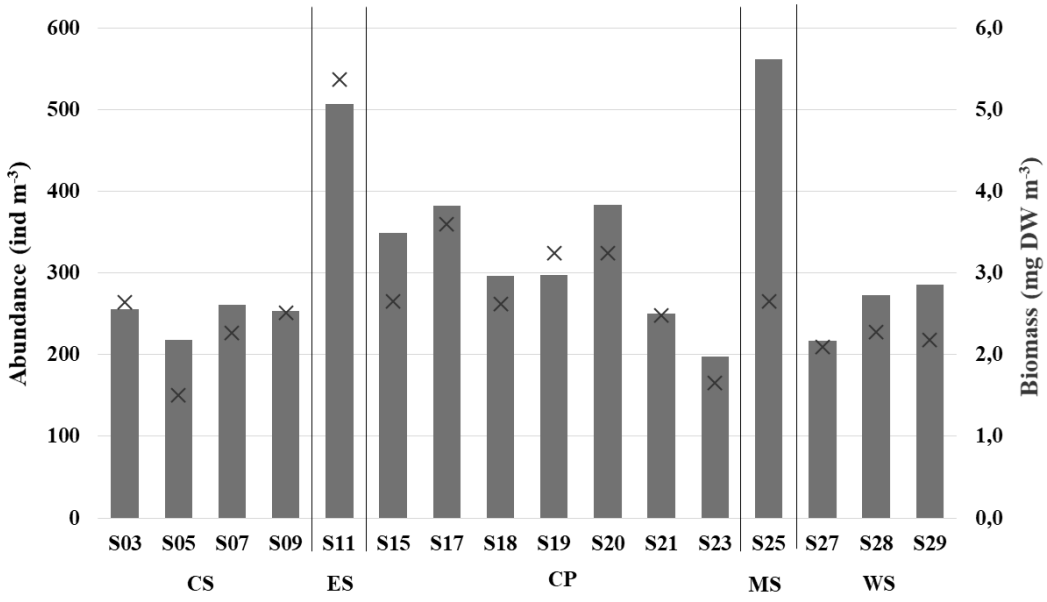

Figure 4.4 Spatial distribution of zooplankton abundance (bars, ind m^{-3}) and biomass (cross, mg DW m^{-3})

Zooplankton biomass (mg DW m^{-3}) was not correlated with abundance (ind m^{-3}) (R^2=0.01, n=16, $p > 0.05$). Biomass values ranged from 1.5 mg DW m^{-3} (S5) to 5.4 mg DW m^{-3} (S11) **(Fig. 4.4)**. Significant spatial differences were observed between the five geographic areas (ANOVA, $p < 0.05$). Multiple range tests showed no correlation only among CS-CP, CS-WS and CP-WS.

Chl *α* concentrations ranged from 236 (S20) to 678 (S11) mg C m^{-2} with no significant spatial differences among the five geographic areas (ANOVA, $p>0.05$).

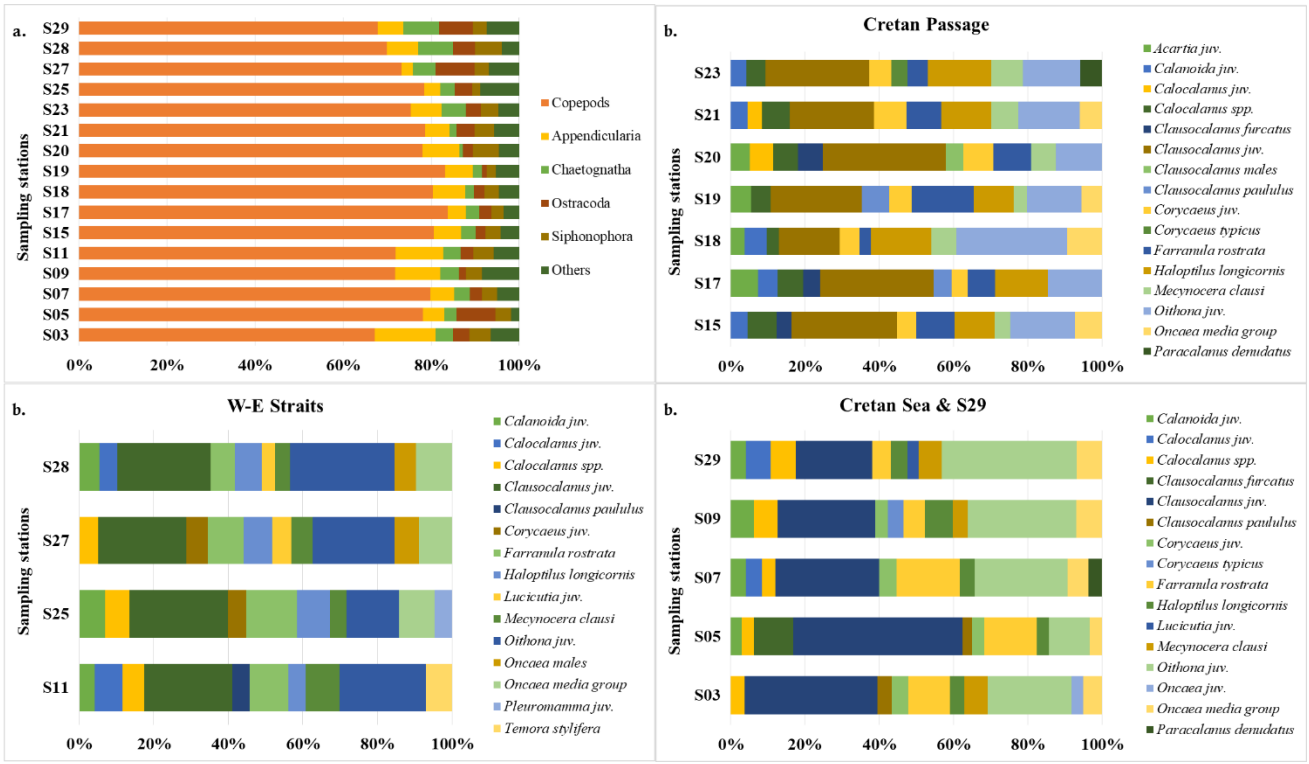

Figure 4.5 Relative abundance of (**a)** dominant mesozooplankton groups and (**b)** dominant copepods among mesozooplankton at the examined stations in the Cretan Passage, Cretan Sea, West & East Cretan Straits and Mirtoan Sea (S29) during June 2016.

4.4.3 Mesozooplankton community composition and distribution

92 taxa were identified in total during this study, with 74 genera/species of copepods, 11 taxa of holoplankton and 7 taxa of meroplankton. Nauplii were also present in the samples. Copepods represented 76 ± 5% of total mesozooplankton abundance and were dominated by 4 taxa: *Clausocalanus spp., Oithona spp.*, *Farranula rostrata* and *Haloptilus longicornis* which represented ~ 55% of the copepod community **(Fig. 4.5a)**. The first two taxa consisted mainly of juveniles whereas the other two were dominated by adults. The Shannon diversity index was similar among stations varied from 4.3 (S05) to 5.1 (S25) bits ind^{-1}, whereas the species richness varied from 52 (S09) to 67 (S25). The differences in taxon composition

and abundance caused the separation of samples into groups based on hierarchical clustering and nonmetric MDS **(Fig. 4.6)**. Three groups of samples were distinguished at a similarity level of 70% (not shown here). The first group consisted of samples from station S25, the second of samples from stations S03, S05, S07, S09, S21, S23, S27, S28 and S29 and the third of samples from stations S11, S15, S17, S18, S19 and S20.

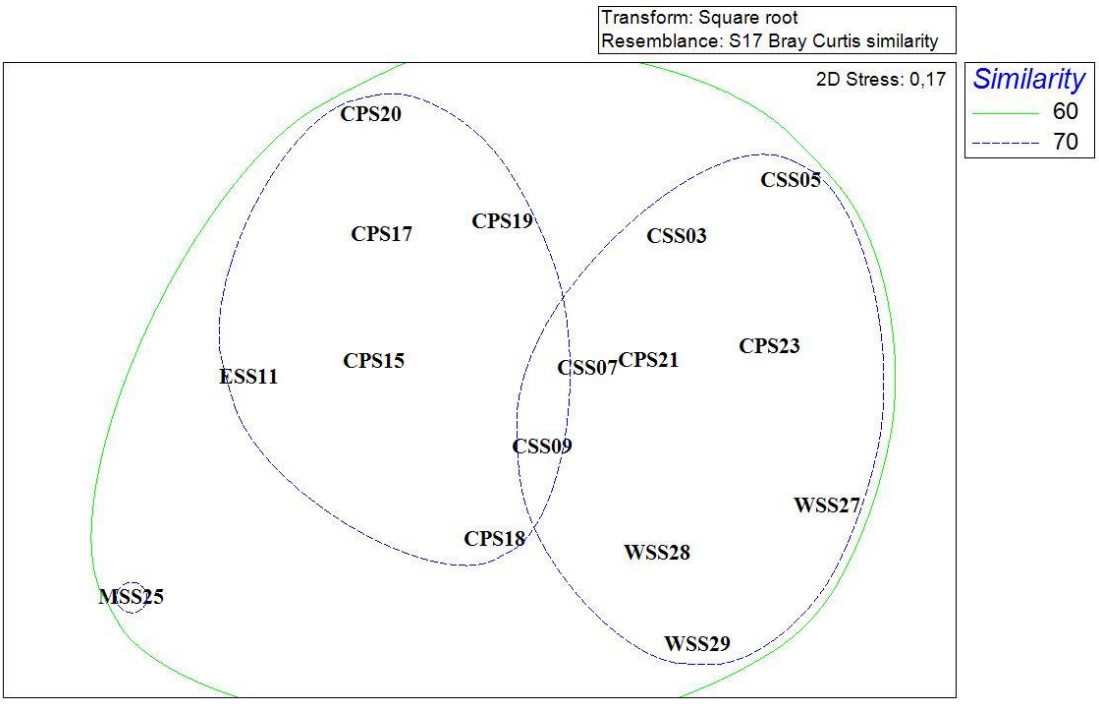

Figure 4.6 Non-metric multidimensional scaling (nMDS) on square root transformed abundances of the mesozooplankton in the Cretan Passage (CP), Cretan Sea (CS), Myrtona Sea (MS) and Cretan Straits (WS, ES) during June 2016. The first two letters on the graph indicate the region.

Comparing the mesozooplankton composition and distribution between the different regions, CP had the highest mean relative abundance of copepods (80 ±3 %). *Clausocalanus* and *Oithona* juveniles dominated all stations, followed by *H. longicornis*. The cluster analysis exhibited 70 % resemblance among stations with higher similarities between stations S21 and S23 (78%). The CS stations exhibited a mean relative abundance of copepods of 74 ±5 % with *Clausocalanus* and *Oithona* juveniles dominating the stations followed by *F. rostrata.* The cluster analysis indicated 73 % resemblance among stations, with higher similarities between S07

and S09 (76%). The WS also exhibited a mean relative abundance of copepods of 74 ±3 %, with *Clausocalanus* and *Oithona* juveniles dominating the stations, followed by *F. rostrata.* Cluster analysis showed 76% resemblance among stations, with higher similarities among S27 and S28 (73%). ES and MS are both dominated by *Clausocalanus* and *Oithona* juveniles with relative abundance of 72% and 68%, respectively.

Non-copepod holoplanktonic species mainly appendicularians, chaetognaths, ostracods and siphonophors contributed with 22 ± 5% to the mesozooplankton abundance, while meroplanktonic species were scarce (2 ± 1 %) **(Fig. 4.5b)**.

4.4.4 Functional copepod groups

Small ambush feeding carnivores (G3) was the most important group, with the higher abundance values at all stations. The contribution of large carnivores (G1) was important at stations S17 and S15 (mostly *Mesocalanus tenuicornis*), and large filter feeding omnivores-herbivores (G2) were most important at station S15. Also, small filter feeding herbivores sac-spawners (G4) were most important at stations S5 and S23 in the CS and CP, respectively. Small sac-spawning detritivores (G5) were most abundant at station S27, small filter feeding herbivores-omnivores (mostly broadcasters, G6) at stations S3, S7, S15 and S25 and lastly the small ambush feeding omnivores (sac spawners, G7) were most abundant at stations S3, S15 and S18 **(Fig. 4.7)**. Moreover, sac spawners represented 42% of the community encountered in the entire water column down to 200 m, whereas broadcasters contributed with 18%.

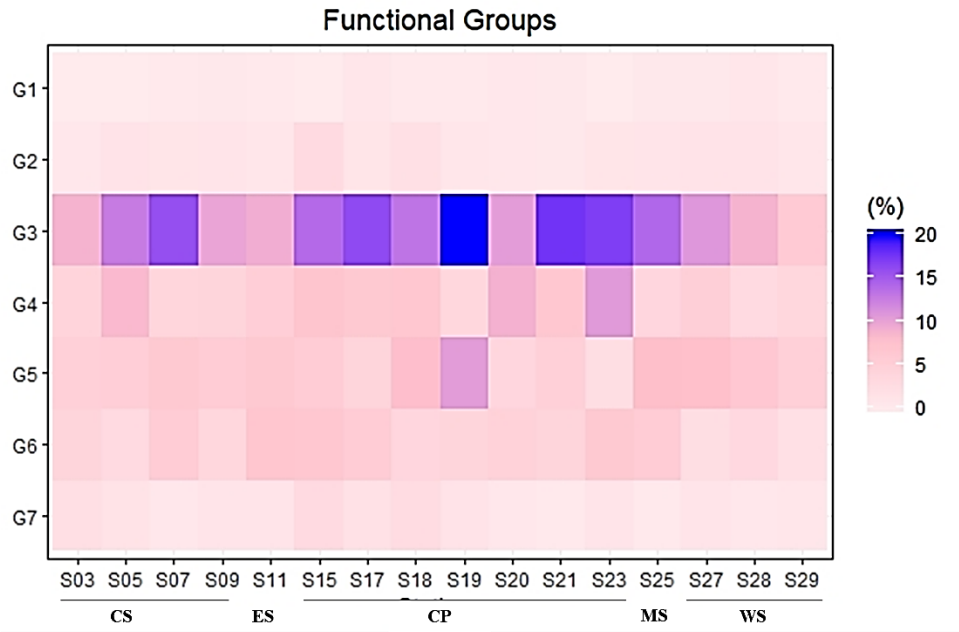

Figure 4.7 Heat map of the functional groups per station during June 2016. Heat map is in a light pink (low relative abundance), purple (medium relative abundance) to blue (high relative abundance) gradient. G1: large carnivores, G2: large filter feeding omnivores-herbivores, G3: Small ambush feeding carnivores, G4: Small filter feeding herbivores sac-spawners G5: Small sac-spawning detritivores G6: small filter feeding herbivores-omnivores (mostly broadcasters) and G7: small ambush feeding omnivores (sac spawners).

4.4.5 Copepod size structure

Contrary to NB-SS slopes (ANOVA, Df: 4, F: 3.6, $p < 0.05$), biovolume (Imagepro Plus determinations, data not shown) exhibited no significant relationship between the five investigated geographic areas. Moreover, the NB-SS slopes exhibited higher negative slopes in the WS indicating a higher relative abundance of small organisms (-0.81) whereas the CS had mean average of -0.65 and the CP -0.44. Total copepods abundance was not correlated with NB-SS slopes ($p > 0.05$) **(Fig. 4.8)**.

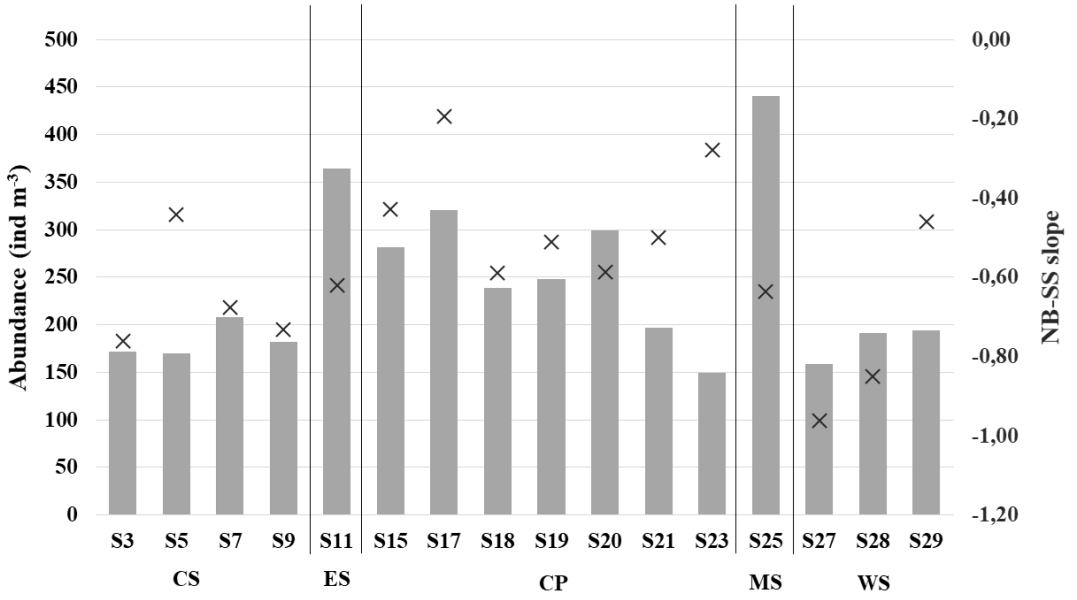

Figure 4.8 Spatial distribution of total copepods abundance (vertical bar) and values of normalized biomass size spectra (NB-SS) slopes (cross) along the transects

4.4.6 Relationships between mesozooplankton and environmental parameters

No significant correlations between the different environmental variables of temperature, salinity, oxygen, Chl *α* and mesozooplankton abundance were found, while multiple linear regression showed positive correlation between biomass and temperature (Df: 4, F: 1.8, $p < 0.05$).

4.4.7 Copepods growth rates, production and ingestion rates

According to the results **(Table 4.2)** temperature and biovolume was almost similar at all stations. Total Chl *α* was higher at station S11 and lower at station S20. The rest of the estimated values exhibited high values at stations S11, S15, S17, S25 and S29, with S11 exhibiting the highest value whereas S20 the lowest. In terms of region, Chl *α* was higher in ES and MS, whereas CS, CP and WS exhibited almost the same values.

Table 4.2 Total Chl *α* , temperature, copepod biovolume , copepod abundance, copepod biomass and copepod growth rate, production and ingestion on phytoplankton biomass.

Region	Station	Chl a (mg C m^{-2})	Temperature (°C)	Biovolume (mg C)	Growth rate (d^{-1})	Abundance (ind m^{-3})	Biomass (mg C m^{-2})	Production (mg C m^{-2} d^{-1})	Respiration (mg C m^{-2} d^{-1})	Ingestion (mg C m^{-2} d^{-1})
Cretan Sea (CS)	**S3**	494	17.30	0.002	0.03	172	66	1.84	2.45	5.52
	S5	665	17.21	0.002	0.03	170	65	1.78	2.38	5.35
	S7	541	17.84	0.002	0.03	208	84	2.20	2.93	6.59
	S9	670	17.67	0.002	0.03	182	77	2.32	3.10	6.97
Eastern Straits (ES)	**S11**	678	17.82	0.002	0.03	365	155	4.84	6.46	14.53
	S15	621	17.10	0.003	0.03	281	166	4.46	5.94	13.37
	S17	461	17.21	0.003	0.03	321	177	4.50	6.00	13.50
Cretan Passage (CP)	**S18**	628	16.88	0.002	0.02	239	112	2.72	3.63	8.17
	S19	582	17.10	0.002	0.03	248	115	3.53	4.71	10.59
	S20	236	16.82	0.002	0.01	299	122	1.20	1.60	3.59
	S21	439	17.10	0.002	0.03	197	84	2.38	3.17	7.14
	S23	378	16.85	0.003	0.01	149	92	1.37	1.83	4.12

Western Straits (WS)	**S25**	501	16.84	0.003	0.02	441	221	4.33	5.77	12.99
	S27	599	16.84	0.002	0.02	159	54	1.28	1.70	3.84
	S28	523	17.08	0.002	0.02	191	71	1.54	2.06	4.63
Myrtoan Sea (MS)	**S29**	579	16.64	0.006	0.02	194	218	4.46	5.95	13.38

4.5 Discussion

4.5.1 Mesozooplankton abundance, composition and biomass distribution

Zooplankton abundance values, recorded during this cruise in June 2016, are similar to those reported for the area in previous studies (Protopapa *et al.,* 2019 for April 2016; Mazzocchi *et al.*, 1997). The lowest zooplankton abundances were recorded at stations along the CS, whereas a west-to-east gradient was recorded in the CP. Chl *α* values are in agreement with previous studies (Mazzochi *et al.* 1997, Yacobi *et al.* 1995, for the Levantine Sea, and Rabitti *et al.* 1994) and similarly exhibited an increasing west to east gradient along the CP with high values at stations S15 and S18. This was probably due to the influence of the Rhodes Gyre, coming in agreement with previous studies for the area (Protopapa *et al.*, 2019 for April 2016). Stations S15 and S18, occupied by high salinity LSW carried by the flow at the periphery of the Rhodes Gyre, exhibited high abundance values **(Table 4.2)**. Station S25 exhibited the highest abundance value (562 ind m^{-3}) probably due to the influence of the less saline BSW, which as know from literature is enriched in dissolved organic carbon (Sempere *et al.*, 2002) and nitrogen rather than inorganic nutrients (Polat and Tugrul, 1996). Likewise, station S11 exhibited high abundance value (507 ind m^{-3}) but also the highest Chl *α* value, indicating probably a nutrient inflow, perhaps from Rhodos Gyre. For the purposes of this paper the stations were grouped according to their geographical position. This "grouping" however was not evident from the MDS results, possibly due to the hydrological features affecting each station.

On the other hand the biomass distribution did not show any large scale trend with average values (2.4 mg DW m^{-3}) similar between regions (see also Protopapa *et al.*, 2019; Siokou-Frangou *et al.*, 2010; Mazzochi *et al.*, 1997). Station S25 displayed high abundance but rather low biomass (2.5 mg DW m^{-3}) while station S11 exhibited a biomass 2 times higher than almost all other stations (5.4 mg DW m^{-3}). This could be due to the very high abundance of appendicularians at S11 (55 ind m^{-3}). The apparent paradox of the CP between the trend in abundance and no trend in biomass might be explained by differences in size-spectra between

the eastern and western part. The eastern stations were more abundant in larger species, such as *Haloptilus longicornis*, *Mesocalanus tenuicornis* and *Pareucalanus attenuatus.* Therefore, large species contributed to the low NB-SS slopes observed at the eastern stations.

The zooplankton composition recorded in the regions was in general agreement with the published data on the EMS community (Protopapa *et al.*, 2019, Siokou-Frangou *et al.*, 1997, 2010, 2019) whereas Shannon diversity index proved the high species diversity of the studied area, with values lower than the ones reported for the S. Aegean Sea by Moraitou-Apostolopoulou *et al.,* (2000). Though some stations were more abundant in larger species, overall the mesozooplankton community showed no clear distinction in taxonomic composition between regions and was dominated by copepods, particularly by small size species (< 1mm). *Clausocalanus* spp. along with *Oithona* spp. were the dominant genera, as described for the study area by Protopapa *et al.* (2019), which is typical for the EMS (Siokou-Frangou *et al.*,1997,2010; Zervoudaki *et al.*, 2007; Mazzocchi *et al.*, 1997).

The significance of small size species has been underlined not only from the NB-SS slopes but also by the functional group analysis, coming in line with Siokou *et al.* (2019). Small species implies low metabolic rates, thus restricted energy demands (Kiørboe & Hirst, 2014), an important advantage for species surviving in such an oligotrophic environment. In addition, small ambush feeding carnivores (*Corycaeidae, Augaptilidae, Candaciidae*) was the most important group at all stations (31-51% of the total functional groups). Species that exhibit ambush feeding behavior have low energy demands, low predation risks, high longevity and low fecundity rates (Kiørboe, 2011; Kiørboe & Sabatini, 1994; Kiørboe *et al.*, 2015); thus they obtain optimal resource allocation in the oligotrophic Mediterranean Sea. Based on the findings of Benedetti *et al.* (2018), carnivorous functional groups should dominate community composition in tropical conditions. Our results are also in agreement with Woodd-Walker *et al.*, (2002) who found a higher proportion of carnivorous copepods at lower latitudes, relative to herbivorous and omnivorous copepods. The influence of the Rhodos Gyre might favor the large filter feeding herbivores at station S15 as well as the small sac spawning detritivores at S18. The Cretan Cyclone and the anticyclone, that influence S19, might affect the abundance

of the small filter feeding herbivores-sac spawners, which is the lowest among all stations (9%), whereas in S20, influenced by the MMJ, exhibits the highest abundance (30%). Investigating the relationship between environmental niches and copepod functional groups Benedetti *et al.* (2018) concluded that large and small feeding herbivores are related with colder, more seasonally varying and productive conditions. The reproduction mode of sac spawner species represented 42% of the copepods community whereas broadcasters only 18%, giving them the advantage of avoiding their eggs consumption by predators thus higher survival rates.

4.5.2 Copepod growth, respiration and production

To evaluate the role of copepods in the carbon budget in the pelagic ecosystem, copepod growth, production, respiration and ingestion on phytoplankton biomass were estimated.

The resulting overall mean of copepod production was 2.8 mg C m^{-2} d^{-1}, whereas in area specific terms it ranged from 1.20 (S20) to 4.84 (S11) mg C m^{-2} d^{-1} for the upper 200 m. Some stations exhibited very high values, such as S11 and S29, these differences detected could be due to differences in the copepod abundance or it could be derived from a combination of higher temperature and turbulence and changing food availability. These values are similar to the ones reported for the same region by Protopapa *et al.* (2019) in spring 2016 for the total zooplankton production of the upper 100 m (0.64 to 5.24 mg C m^{-2} d^{-1}), by Siokou-Frangou *et al.* (2002) for the copepod production in the South (March 1997: 5 mg C m^{-2} d^{-1}, September 1997: 6 mg C m^{-2} d^{-1}) and North Aegean Seas (March 1997: 5 mg C m^{-2} d^{-1}) and by Fonda Umani (1996) for the mesozooplankton production in the Adriatic Sea (0.6-3 mg C m^{-2} d^{-1}). The values are very low compared to a previous study of Zervoudaki *et al.* (2007) who presented values of 15 mg C m^{-2} d^{-1} in late summer and 36 mg C m^{-2} d^{-1} in spring for the upper 100 m depth layer in the Northern Aegean Sea. Also very low compared to the tropical area of the Kimberley coast (North West Australia) with a primary production ranging from 1.5 to 3.5 g C m^{-2} d^{-1} and zooplankton production values of 42 and 278 mg C m^{-2} d^{-1} in water depths of 35 and 41 m, respectively (McKinnon *et al.,* 2015).

Given the oligotrophic status of the MS, prey availability can affect mesozooplankton. Applying Dagg's (1982) equation for ingestion rates, we calculated very low values, ranging from 0.02 to 0.07mg C m^{-3} d^{-1}, similar to the ones reported by Protopapa *et al.* (2019) for April 2016 (0.02 to 0.13 mg C m^{-3} d^{-1}).

In synthesis, this study showed that mesozooplankton communities did not differ a lot among stations, whereas a slight gradient from west to east in total mesozooplankton abundance and Chl α was recorded in the Cretan Passage, probably due to the influence of Rhodos gyre. The results of the carbon flux budget were in agreement with previous studies highlighting the oligotrophic character of the studied area. It is of high importance to underline that the studied area and especially the region around Rhodes Gyre although has a critical impact on the whole Mediterranean functioning, with the formation of the Levantine Intermediate Water, is still tremendously under sampled and many problems and questions remain and require further investigation and confirmation.

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4.7 Rerences

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CHAPTER 5

The present thesis has contributed to the understanding of the effects of the hydrological features as well as the ultra-oligotrophic conditions of the studied area to the distribution, composition and metabolic rates of the mesozooplankton population. For this reason a large number of parameters were collected by classical and innovative methods, illustrating for the first time an integrated image of the structure as well as the trophic interactions among the mesozooplankton components in the Eastern Mediterranean Sea.

5.1 Mesozooplankton community under ultra-oligotrophic conditions in the Eastern Mediterranean Sea: Cretan Passage, Cretan Sea, east & west Cretan Straits.

5.1.1 General plankton distribution in relation to the hydrological patters

Ultra-oligotrophic areas, like the region investigated here, mainly depend on water mass circulation for nutrient supply. The hydrography of the studied area is influenced by a complex interaction of cyclonic and anticyclonic eddies. Cyclonic eddies move the isopycnals upward and anticyclonic eddies downward. The physical characteristics of the water masses revealed that intermediate water masses of both Cretan and Levantine origins are present in the Cretan Sea (**Chapter 2 & 4**). The surface circulation in this area is influenced by a series of smaller gyres between the Cretan Cyclone and the Rhodes Gyre (Velaoras *et al.*, 2018). Indeed, a strong influence on mesozooplankton abundance was observed in this study.

The influence of **Rhodes Gyre** (Cretan Passage), was observed in both cruises. During the first cruise (April), mesozooplankton abundance exhibited a west-to-east gradient. In contrast, Chl *α* values were opposite to the general pattern, maybe denoting the grazing pressure upon phytoplankton. Opposite to the general

pattern was also the diatoms abundance, whereas in terms of biomass they displayed markedly increasing values towards the east and deep layers, with important implications for the food web interactions and trophic relationships. Almost the same pattern was observed during the second cruise in June. Chl α values exhibited almost same values among stations with no clear patterns, whereas diatoms and mesozooplankton abundance exhibited a west-to-east gradient. Station S17 along with S20, both affected by the **Mid-Mediterranean Jet**, exhibited the higher values of mesozooplankton abundance, whereas S20 exhibited the lower diatom abundance value. This could be due to high abundance of appendicularians (8.4 %) whereas only 4.1 % in S17. The food of an appendicularian consists of micro-organisms, especially small and smooth unicellular algae and protozoans.

The zooplankton data collected during the two cruises did not exhibit differences in terms of abundance or community structure, though the samplings were conducted in different seasons.

For the **first cruise,** in the entire area, depth-integrated abundances (0-1000 m) were fairly low, averaging from 53 to 87 ind m^{-3} whereas for the 0-200 m layer averaged from 177 to 347 ind m^{-3}. During the **second cruise** the abundances ranged from 198 to 562 ind m^{-3} with the Cretan Passage exhibiting the higher mean abundance values (308 ind m^{-3}). This can be explained due to the higher abundances recorded at stations S17 and S20 which are influenced by the less saline Atlantic water as well as due to the high abundance value recorded at S15 which is influenced by the Rhodes Gyre.

The values recorded in the studied area in both cruises support the current notion that the EMS is one of the most oligotrophic marine basins in the world and are in line with similar ranges reported for other oligotrophic areas (Zenkevitch, 1963: total zooplankton in the tropical and North Pacific Ocean; Deevey and Brooks, 1977: copepods in the Sargasso Sea). They are also similar to those reported by Scotto di Carlo *et al.* (1984) for the Tyrrhenian Sea, which is considered poorer in zooplankton biomass when compared to other parts of the Western Mediterranean (Scotto di Carlo and lanora, 1983). Increasing zooplankton abundances at local sites caused by the Rhodes Gyre were also reported for spring 1986 by Pancucci-Papadopoulou *et al.* (1992) and Mazzocchi *et al.* (1997).

Copepoda were the dominant taxon group for both cruises at all stations, consistent with previous reports by Kimor and Berdugo (1967), Moraitou-Apostolopoulou (1985), Pancucci-Papadopoulou (1992), Mazzocchi *et al.* (1997), Koppelmann *et al.* (2009), Siokou-Frangou *et al.* (2010) and Christiansen and Weikert (2017) for the EMS. Total mesozooplankton abundance was mainly concentrated in the euphotic zone, with *Clausocalanus* and *Oithona* juveniles dominating the communities. Noteworthy is the occurrence of *Clausocalanus lividus* at almost all stations in both cruises. It is a typical species in the North Atlantic Ocean (European waters), but it has been rarely recorded in the Cretan Sea and the Straits of the Cretan Arc (Gotsis-Skretas *et al.*, 1999) and it is for the first time recorded in the Cretan Passage in such high abundances. The abundance of *Haloptilus longicornis* in the 100-200 m layer was also important in both cruises and has been mentioned by Weikert and Trinkaus (1990), Siokou-Frangou *et al.* (1996, 1997 and 2010) and Koppelman *et al.* (2009). *Mormonilla minor* showed high dominance values at depth below 200 m at all stations (Aprils' cruise).

According to Basescu (1985), the Eastern Mediterranean zooplankton community is distinguished by a high percentage of thermophile tropical and subtropical elements. In our study, the contribution of cyclopoids (33% April and 23% June) and poecilostomatoids (5% April and 7% June) to the copepod community was quite important. Though this could be underestimated since the mesh size of the net was 200 μm and these species are often smaller. The abundance and diversity of the cyclopoid *Oithona* and the poecilostomatoids *Oncaea, Corycaeus* and *Farranula* support the notion of the subtropical character of Eastern Mediterranean zooplankton because these genera are abundant in warm seas (Raymont, 1983).

Regarding the rare and less abundant species detected in this thesis, it was of high importance to study taxonomy at species level as there is little zooplankton biodiversity information available for the Cretan Passage, Cretan Sea, as well as the western and eastern Cretan Straits. For example, there are studies by Weikert and Koppelmann (1993) and Koppelmann *et al.* (2007) that have taxonomic information but mostly down to genus level, while the study by Christiansen &

Weikert (2017), provides information (again, not always at species level) for the surrounding area e.g. the Levantine Basin etc.

The most significant aspect of copepod functional groups in the study area **(Chapter 4)**, during the second cruise, was the high dominance of small size species (especially the small ambush feeding carnivores) implying low metabolic rates (Kiørboe and Hirst, 2014), thus restricted energy demands. The dominance of small size species was also underlined by the NB-SS slope values (mean average among stations - 0.58), where high negative slopes are linked to higher percentages of small organisms (Sourisseau and Carlotti, 2006). Moreover, sac spawner species represented 42% of the community encountered in the entire water column down to 200 m. whereas broadcasters 18%. Station S15, influenced by the Rhodos Gyre, though it exhibited the highest abundance among all stations of large carnivores (mostly *Mesocalanus tenuicornis*) it was characterized by the important contribution of small filter feeding herbivorous-mixed omnivorous broadcast spawners and small ambush feeding omnivores sac spawners, which was also in line with the NB-SS slope value (- 0.43).

Small ambush feeding carnivores were found to be the most important component of the epipelagic zone at all stations. It is a well surviving model obtaining optimal resource allocation in this ultra-oligotrophic region since the species exhibiting ambush feeding mode have low energy demands, low predation risks, high longevity and low fecundity rates (Kiørboe, 2011; Kiørboe and Sabatini, 1994; Kiørboe et al., 2015).

5.2 Challenges using "new tools"

It is important to underline here that all of the biochemical indices used in this study are not actually new, but in terms of oceanographic studies some of them are very novel (such as spAARS) and the literature concerning most of them is very limited especially regarding the area studied here. Consequently, it is more complicated to have a proper understanding of the data and a comparison with other studied areas.

This study is the first attempt to elucidate the metabolic rates based on ETS and AARS as well as the dietary preferences and feeding strategies of major

copepod species/taxa based on the Fatty Acid composition combined with Stable Isotope Analyses of prominent copepod taxa/species from an ultra-oligotrophic environment of the Eastern Mediterranean Sea, the Cretan Passage (**Chapters 2 and 3**).

5.2.1 Biochemical Indices (ETS, AARS, SIA & FA)

Within the framework of this thesis, four biochemical indices were used in order to study the metabolic rates, trophic relations and feeding preferences of mesozooplankton as well as to verify the oligotrophic character of the EMS in general and the Cretan Passage in particular. Biochemical indices ETS and AARS are described in **Chapter 2** whereas Stable Isotopes Analysis (SIA) and Fatty Acids (FA) are covered in **Chapter 3**.

The literature for the Mediterranean Sea (MS) and especially for the EMS and the Cretan Passage, regarding the aforementioned biochemical indices, is very limited, particularly for AARS and FA. AARS is newly introduced in zooplankton studies and mostly directed in a species-related study (e.g. *Calanus helgolandicus* by Yebra *et al.* 2005, *Calanus finmarchicus* by Yebra *et al.* 2006, *Paracartia grani* by Herrerra *et al.* 2012), whereas for bulk communities there are very few studies and no for the MS (e.g. Yebra *et al.* 2009: Antarctic Peninsula and McKinnon *et al.* 2015: Tropical Indian and Pacific Ocean). On the other hand, the large majority of studies employing FA as trophic markers have been conducted in marine planktonic systems to examine the flow of lipids across the phytoplankton–zooplankton interface (Graeve *et al.* 1994, Dalsgaard *et al.* 2003).

In **Chapter 2** spETS and spAARS biochemical indices of the EMS and Cretan Passage were determined. It should be underlined that these data represent bulk zooplankton communities. According to Minutoli and Guglielmo (2009), the patterns of carbon demand from zooplankton estimated from measurements of ETS activity indicate spatial and day/night variations in the MS. Demand is significantly lower in the western (mean 290 μg C g wet wt^{-1} d^{-1}) than in the eastern (mean 387 μg C g wet wt^{-1} d^{-1}) sector. The increasing west-east gradient observed for both day and night is not due to structural properties of zooplankton

communities but likely related to zooplankton ETS activity and seawater temperature.

Our enzyme indices results exhibited no consistent trends among stations. SpAARS values, come in agreement with Yebra *et al.* (2009) where higher values are displayed in the euphotic zone, indicating higher specific growth rates where primary production occurs. The correlation of spAARS with biomass in our study showed opposite results of that presented by Herrera *et al.* (2012). After correlating food limitation and individual biomass in *P. grani* nauplii, she detected high specific AARS activities at low growth rates under limiting food concentration and low individual biomass. This was not the case in our study, in most stations, where high spAARS values were observed when biomass values were high and vice versa. On the contrary, spETS, again in most stations, negatively followed biomass values.

Another correlation was made among the enzymatic indices and gelatinous-crustacean ratios. For the spETS it has been underlined by our results what is known from literature (Schalk, 1988), that crustaceans (only copepods in this study) show higher respiratory activity than gelatinous species, therefore, higher values in spETS when the ratio is low. Conversely, regarding spAARS, we aspect that the activity follows the gelatinous abundance values because growth rates are higher when gelatinous blooms occur (Alldredge, 1984: referring to all gelatinous species). This, however, does not seem to be the case in this study, since we mainly detected salps and they didn't exhibit high abundance values in order to prove any bloom period either.

In **Chapter 3** stable isotope analysis along with fatty acids of specific species/taxa was reported. SIA has proven to be a useful tool in reconstructing diets, characterizing trophic relationships, elucidating patterns of resource allocation, and constructing food webs (Fry, 1991; Schukat *et al.*, 2014). On the other hand, the large majority of studies employing FA as trophic markers have been conducted in marine planktonic systems to examine the flow of lipids across the phytoplankton–zooplankton interface (Graeve *et al.,* 1994; Dalsgaard *et al.,* 2003). Trophic biochemical indices integrate dietary signals over longer time periods of days to several weeks depending on the species (Graeve *et al.*, 1994; Gentsch *et al.*, 2009). To realize the full potential of the FA biomarker approach, it

is critical to know the extent to which consumer FA composition is influenced by and is different from their known diets; however, for many organisms this is difficult to ascertain (Ravet *et al.*, 2010).

The present study revealed a diversity of taxon-/species-specific life strategies with regard to feeding preferences and lipid storage mechanisms. Our results are in agreement with literature studies on functional traits of copepods (e.g. Benedetti *et al.*, 2015 & 2018) and general literature studies based on the morphological characteristics of the copepods or experiments such as the case of Corycaeidae (e.g Wickstead, 1962, Timonin, 1969; Turner *et al.*, 1984; Landry *et al.*, 1985) (**Chapter 3, Table 3.6**). Omnivory was the prevailing feeding mode, demonstrating a high degree of opportunistic feeding in oligotrophic copepods. The two complementary trophic biomarker approaches led to similar results, though there can be some exceptions such as the case of *H. longicornis,* which emphasize the applicability of lipid trophic markers even in ultra-oligotrophic regions.

5.3 Metabolic rates and carbon budget

The metabolic rates of bulk mesozooplankton communities of the epipelagic MS have been examined only in a very limited number of studies conducted in the Western MS (Alcaraz, 1988; Calbet *et al.*, 1996; Gaudy and Youssara, 2003; Gaudy *et al.*, 2003), except for one trans-Mediterranean cruise in the spring of 2007 (Minutoli and Guglielmo, 2009). Furthermore for the EMS Cretan Passage, Koppelmann *et al.* (2004) studied the zooplankton carbon consumption rates but for the deep water.

To evaluate the role of zooplankton in the carbon budget in the pelagic ecosystem during spring (first cruise), ETS values were converted to respiration rates converting the oxygen-specific units to carbon-specific units **(Chapter 2)**. During summer (second cruise) carbon budget, only from copepods, was estimated by coupling standing stocks estimations (abundance, biomass and size classes) and metabolic measurements. Cretan Passage exhibited higher respiration than Cretan Sea. Also in the Cretan Passage, during both cruises, an increasing gradient of respiration from west to east was evident, whereas an almost 10-fold difference between layers (first cruise) was obvious, with higher values in the 0-500 m layer.

This difference could be due to differences in the zooplankton abundance or it could be derived from a combination of higher temperature and turbulence and changing food availability. The aforementioned values are close to the ones reported by Herrera *et al.* (2014) for the Western Mediterranean, but very low compared to the values reported by Minutoli and Gugliemo (2009) for the Western Mediterranean and Balearic Islands and also compared to the values reported by King *et al.* (1978) for the Eastern Tropical North Pacific.

The zooplankton production and copepod production of the Cretan Passage is in agreement with previous studies of the copepod production in the Northern Aegean Sea by Zervoudaki *et al.* (2007) but exhibits very low values when compared with eutrophic regions (North West Australia: McKinnon *et al.*, 2015; Kaneohe Bay, Hawaii: Newbury *et al.,* 1976; Eastern Agulhas Bank of the Benguela Upwelling System: Peterson, 1995). It has to be noted that our enzyme methods were conducted with mixed plankton populations whereas the previous estimates of zooplankton production are based on artificial cohort experiments focused solely on copepods dominant in these systems.

Finally, ingestion rates indicated very low values, in both cruises, whereas the primary production were similar to those reported by Siokou-Frangou *et al.* (2002) and they follow the mean integrated phytoplankton abundance values, but not the mean integrated values of zooplankton, which show a trend of increasing biomass from west to east.

The very few measurements of carbon flux in the southern Aegean Sea (Siokou-Frangou *et al.*, 2002) limit our understanding of the fate of the pelagic production in this ecosystem. During our study, as an attempt to illustrate the pelagic food web in the euphotic zone (0-100 m for the first cruise and 0-200 m for the second cruise) with special emphasis on zooplankton and copepods respectively, we have established carbon flux budgets for the studied sites. It seems that, during the first cruise, the available phytoplankton production covers the zooplankton carbon demand at all stations, however, only 5 to 16% of the primary production was consumed by the zooplankton. Therefore, high grazing impacts of zooplankton on phytoplankton biomass were detected (21-71%). It has been underlined from our study that although the available food can satisfy the

zooplankton carbon demands, only a part of the available phytoplankton production is consumed because not all autotrophs provide food of adequate quality for zooplankton. This was also obvious from the SIA and FA results (**Chapter 3**). The different origin of assimilated carbon for the *Lucicutia* spp. and *C. lividus* samples, based on 1.5-2 ‰ difference from *H. longicornis*, *Pleuromamma* spp. and *Corycaeus* spp., could be attributed to feeding on micro- or nanophytoplankton. Livanou *et al.* (2019) reported from data of the same cruise that most of the PP in the studied area was produced by picophytoplankton, a size fraction that is not efficiently grazed by zooplankton (Zervoudaki *et al.*, 2007). Thus, it seems that there is a strong need for alternative food sources for zooplankton such as protozooplankton like in other picoplankton-dominated marine systems (Siokou-Frangou *et al.*, 2002; Zervoudaki *et al.*, 2007).

The results from the carbon flux budget, from both cruises, underline the oligotrophic character of the studied area indicating that the zooplankton is not well fed and that the organisms are living under oligotrophic stress.

5.4 Biochemical indices: an applicable tool

Biochemical indices can provide us with really important information when used separated and/or in combination, such as SIA-FA or spETS-spAARS. Depending on the objectives of a given study they can be used to study food web structures, or carbon budget or both as in this study, and with the modern analytical techniques e.g. thin layer chromatography, gas liquid chromatography, automated plate readers etc., the analysis of biochemical indices are revolutionized, making them easily applicable by the marine community.

Regarding the metabolic rates (ETS, AARS) there are some pros and cons when working with experimental samples. An obvious problem is the lack of a clear index to judge whether or not the animals which are obtained from the field are in good condition or not. A second challenge is the difficulty to have environmental conditions in a laboratory experiment, which affect the metabolism of zooplankton. The third problem is the lack of adequate techniques to control swimming activity of test zooplankton during experiments, which affect metabolic rates, not so much for the small zooplankton but for bigger and more active such

as euphausiids. The fourth challenge is the establishment of standard methods for measuring metabolic rates in highly diverse group of animals, the zooplankton (differences in body-size range, locomotory activity etc.). Though the problems and challenges are many, getting this type of information from lab experiments is required for the better estimation of metabolic rates of zooplankton in the field.

On the other hand, when working with field samples the most important challenge is that it is impossible to work at species level. Since we are talking about metabolic rates, the samples have to be frozen (-80ºC) very fast, which is another difficulty when working on sampling cruises for several weeks.

Detailed analysis of lipid composition may help reveal dietary preferences of the species investigated. The concept of lipids as trophic markers makes use of the fact that specific fatty acids are characteristic of specific groups of phytoplankton such as 16:1 (n-7) for diatoms or 18:4 (n-3) for dinoflagellates (Harrington *et al.*, 1970; Lee *et al.*, 1971; Falk-Petersen *et al.*, 1990). According to Graeve *et al.* (1994a, b), these fatty acids are incorporated largely unaltered by phytophagous species revealing their dominant diet. Moreover, their major advantage over the gut content analyses is their integration of trophic information over a period of weeks and months yielding long-term mean feeding preferences. Another important information they can provide us with is the adaptive differences in the lipid economy of species with a different evolutionary or biogeographical background (Kattner *et al.,* 1994; Kattner and Hagen, 1995; Albers *et al.*, 1996)

While the technical aspects of stable isotope analysis have become easier and more affordable in recent years because of instrumental developments and increased number of commercial SIA laboratories stable isotope analysis is now routinely used in studies of food webs and ecosystem structure. Stable carbon isotopes in particular are commonly used to quantify food sources and energy flow in aquatic ecosystems, since carbon stable isotopes are known to fractionate little between each trophic transfer (DeNiro and Epstein, 1978; Peterson and Fry, 1987). Stable nitrogen isotopes fractionate more and are typically used to infer trophic positions of consumers in food webs (Minagawa and Wada, 1984; Peterson and Fry, 1987; Begon *et al.*, 2006; Syvaranta & Rautio, 2010)

Fatty acids have a high biological specificity and in conjunction with stable isotope ratios can provide information on the zooplankton's assimilated diet (El-Sabaawi *et al.*, 2009; Van den Meersche *et al.*, 2009; Allan *et al.*, 2010; Kelly and Scheibling, 2012).

5.5 Future scenarios for Cretan Passage, Cretan Sea and West, East Straits, Eastern Mediterranean Sea.

There is no doubt that climate change (rising temperatures, ocean acidification) and other human-driven environmental perturbations are influencing marine ecosystems causing reductions in biodiversity and impacting the functioning of ecosystems on a global scale (Küpper & Kamenos, 2018). With these changes happening, it is important to understand how organisms itself react and what impact this has at ecosystem level. Changes at the base of the food web, such as altered community structure in phytoplankton (Dutkiewicz *et al.,* 2015) or size structure and biomass of copepod communities (Taucher *et al.*, 2017) under increasing ocean acidification will consequently affect ecosystem structures. But still we don't know how the ecosystem structures will change, especially in ultra-oligotrophic systems such as the Cretan Passage, Cretan Sea, and the W and E Cretan Straits.

A study reporting a zooplankton time-series from the Balearic Islands indicated a correlation between copepod abundance and large-scale climatic factors (e.g., North Atlantic Oscillation, NAO) suggested that they act as main driver of the zooplankton variability. (Fernández de Puelles *et al.,* 2007).

Another study, on *Centropages typicus,* in the MS indicated that the regional differences observed in the long-term patterns of *C. typicus* populations suggest that the temporal dynamics of this species are significantly more affected by local conditions making them more sensitive to climate forcing (Mazzocchi *et al.*, 2007).

Mesocosm experiments can also provide us with important information. In 2014, such an experiment examined effects of sea surface warming on marine plankton indicating that warming treatments had positive direct effects on phytoplankton biomass, but were overcompensated by the negative effects of

decreased nutrient flux. Zooplankton switched from phytoplankton to grazing on ciliates. These results contrasted with previous experiments under nutrient-replete conditions, where warming indirectly reduced phytoplankton biomass via increased zooplankton grazing. Thus the effect of ocean warming on marine plankton depends on the nutrient regime, which provides a mechanistic basis for understanding global change in marine ecosystems (Lewandowska *et al.*, 2014).

Global warming is an "environmental reality" that highly concerns the scientific communities. Zooplankton are poikilothermic, indicating their physiological processes, such as ingestion, respiration, and reproduction, are highly sensitive to warming, with rates doubling or even tripling with temperature rise (Mauchline, 1998). Furthermore, according to the species shift hypothesis, global warming will likely cause decreasing body-size and an overall increase in the proportion of smaller size species at the community scale (Hays *et al.,* 2005) with consequences for community structure and biotic interactions.

All the above lead the scientific communities to realize the importance of monitoring the marine environment, and for this reason monitoring programs such as the Marine Strategy Framework Directive (MSFD: 2008/56/EC) and the Water Framework Directive (2000/60/EC) have been established. The scope of these programs is to measure biotic and abiotic parameters of the marine environment, with predefined spatial and temporal schedule, in order to produce datasets that can be used for application of assessment methods and derive credible conclusions (with defined confidence) on whether Good Environmental Status (as defined by the Marine Directive[4]) is achieved or not for the marine area concerned.

The aforementioned studies and concerns are just few of many that prove the importance of having good ecological knowledge of the environment, having time-series and conducting mesocosm experiments. Biochemical indices, such the ones used in this thesis, can provide us with valuable information on animal physiology-related parameters (spETS, spAARS), how this might be affected by

[4] The main goal of the Marine Directive is to achieve Good Environmental Status of EU marine waters by 2020. The Directive defines Good Environmental Status (GES) as: "The environmental status of marine waters where these provide ecologically diverse and dynamic oceans and seas which are clean, healthy and productive" Article 3

global warming or ocean acidification and thus how the food web structures (SIA, FA) could possibly be altered.

5.6 Outlook

The changes in the marine environments due to anthropogenic threats like overfishing, environmental pollution and climate change provide good reasons to gain further and deeper knowledge about the functioning of ecosystems. We have a certain understanding about the processes involved in carbon cycling and energy transfer in the food web. But the conditions during periods of low primary production remain poorly studied, making it difficult to resolve trophic interactions. Particularly in an ultra-oligotrophic area such as the EMS which is characterized by a permanent low primary production. Though MS is characterized by low primary production fisheries are richer than expected (Fiorentini *et al.*, 1997) which is the origin of the Mediterranean Paradox[5] (Sournia, 1973; Estrada, 1996). The MSs' very flexible food web contributes in minimizing carbon loss to deeper layers and predators can optimally profit from carbon produced and transformed within the system, thus being the ultimate controllers of plankton abundance in the MS (Siokou-Frangou *et al.*, 2010).

This thesis shed some light on zooplankton ecology and ecophysiology in a less well studied area. Taking this further will inevitably require more experimentation. Drawing solid conclusions about trophic links is certainly difficult as scientific field cruises generally just provide a snapshot of the current situation. In this sense, the combined use of measurements of biochemical indices (spETS, spAARS), SIA and FA helped to identify some potential relationships between mesozooplankton communities. For example, it seems that in ultra-oligotrophic areas, mesozooplankton tends to be more opportunistic than selective feeders.

For future studies it seems that the combination of direct methods, such as gut content analysis, and indirect biochemical and molecular techniques (SIA, FA,

[5] In aquatic biology, the paradox of the plankton (originally described by G. Evelyn Hutchinson in 1961) describes the situation in which a limited range of resources supports an unexpectedly wide range of plankton species, apparently flouting the competitive exclusion principle which holds that when two species compete for the same resource, one will be driven to extinction.

ETS, AARS and RNA/DNA) will be a more fruitful path to investigate mesozooplankton prey preferences as well as long- and short-term diets under controlled laboratory and field studies. Implementing field data on mesozooplankton into statistical and individual-based models are a valuable addition to the techniques described above.

It has to be noted the importance of this thesis for this, not well studied, ultra-oligotrophic area. A combination of innovative and more classical methods was used, proving that basic exploratory research is still needed, while gaps in knowledge should be filled taking advantage of modern technologies and new approaches. This kind of research has to be continued in order to a better understanding of how carbon is transferred through the food web and recycled in the marine system. Plankton is a valuable indicator of ecosystem status, thus it should become a pre-requisite part of routine surveys for environmental management.

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Outline of publications

The chapters of this thesis are written as manuscripts and are either published or prepared for submission in a peer-reviewed scientific journal. In the following the scientific contributions of each author are described in detail.

Manuscript 1

Zooplankton distribution, growth and respiration in the Cretan Passage, Eastern Mediterranean

MariaProtopapa, Soultana Zervoudaki,Cathrin Tsangaris, Dimitris Velaoras, Rolf Koppelmann, Stella Psarra & Christian Möllmann

The study was designed by Maria Protopapa (MP), Soultana Zervoudaki (SZ) and Cathrin Tsangaris (CT). Sampling was conducted by MP and SZ. Biomarker indices lab analysis was performed by CT, analysis of the hydrological data by Dimitris Velaoras (DV) and sample and data analysis was performed by MP under close cooperation with SZ and Rolf Koppelmann (RK). The manuscript was written by MP under supervision of SZ, RK and Christian Möllmann (CM). All authors contributed to reviewing and editing the manuscript.

The manuscript has been publish in Deep-Sea Research Part II (in press) https://doi.org/10.1016/j.dsr2.2019.03.001

Manuscript 2

Trophic positioning of prominent copepods in the epi- and mesopelagic zone of the ultra-oligotrophic Eastern Mediterranean Sea

Maria Protopapa, Rolf Koppelmann, Soultana Zervoudaki, Carsten Wunsch, Jana Peters, Constantine Parinos, Francesca Paraschos, Alexandra Gogou, Christian Möllmann

The study was designed by MP, SZ and RK. Sampling was conducted by MP and SZ. Isotope lab analysis was performed by CW, analysis of the fatty acids by Jana Peters, Constantine Parinos, Francesca Paraschos and Alexandra Gogou. Sample and data analysis was performed by MP under close cooperation with SZ and RK. The manuscript was written by MP under supervision of SZ, RK and CM. All authors contributed to reviewing and editing the manuscript.

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Manuscript 3

Mesozooplankton community structure in the South Aegean Sea

Protopapa Maria, Soultana Zervoudaki, Georgia Assimakopoulou, Dimitris Velaoras, Rolf Koppelmann

The study was designed by MP and SZ. Mesozooplankton sampling was conducted by MP and SZ. Sampling and data analysis for Chl a was performed by Georgia Assimakopoulou, analysis of the hydrological data was conducted by DV, and mesozooplankton sample and data analysis was performed by MP under close cooperation with SZ and RK. The manuscript was written by MP under supervision of SZ and RK. All authors contributed to reviewing and editing the manuscript.

The manuscript has been submitted to Journal of Marine Systems

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Eidesstattliche Versicherung

Declaration on oath

Hiermit erkläre ich an Eides statt, dass ich die vorliegende Dissertationsschrift selbst verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt habe.

I hereby declare, on oath, that I have written the present dissertation by my own and have not used other than the acknowledged resources and aids.

Hamburg, den Unterschrift