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Analysis of zooplankton communities in Mediterranean coastal areas (El Kantaoui port – Tunisia)

Diogo da Silva Molinos Peixoto

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Faculdade de Ciências da Universidade do Porto, Università degli Studi di Firenze
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Departamento de Biologia

2016

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Todas as correções determinadas pelo júri, e só essas, foram efetuadas.

O Presidente do Júri,

Porto, _____/_____/_____

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Abstract

Mediterranean Sea is a land-locked relatively small marine system with high environmental variability. In last decades, it has become one of the most demanded destinations for organized touristic and commercial routes. The increase of maritime traffic of ships, boats and cargos and tourist frequentation of the Mediterranean coasts, particularly hosting ports, are at risk of irreversible environmental degradation that will in turn negatively affect the whole Mediterranean Basin. With this scenario, the MaPMed Project was developed under the first call for proposals launched by European Neighbourhood and Partnership Instrument Cross Border Cooperation in 2009, with the general aim of improve the environmental sustainability of tourist coastal areas in the Countries of the Mediterranean Sea Basin with the propose of monitoring and reduction of marine pollution.

The pollution induced by human activities may affect the coastal ecosystem and eventually cause rapid mortality of zooplanktonic organisms that cannot rapidly escape from negative conditions. The aim of this work was to analyse the zooplankton communities in four different seasons (July 2014 as summer, October 2014 as autumn, January 2015 as winter and March 2015 as spring) at El Kantaoui port – Tunisia, one of the study sites within the MaPMed Project. The zooplankton communities were sampled in different stations of the harbour, used for different tourist and maritime activities, and along a transect through the port. A total number of 54 samples were observed under the stereomicroscope, using a Bogorov counting chamber for zooplankton and the main taxa were identified at the lowest possible level (class, order, family and species), except for the larval stages. Multivariate analyses were performed trough the PRIMER software. As expected, the environmental variables and the mean densities of individuals (ind/m^3) had a seasonal variation from summer to spring. The highest mean densities of individuals were recorded in summer and autumn and the lowest ones in winter and spring. Furthermore, it was possible to observe a gradient of abundance and diversity of the communities in the different stations of the harbour from the inner stations to the outer ones.

Keywords

Zooplankton communities; zooplankton seasonality; Mediterranean Sea; MaPMed project.

Resumo

O Mar Mediterrâneo é considerado um oceano de pequena escala rodeado por terra com enorme variabilidade ambiental. Nas últimas décadas tem-se tornado um destino muito procurado para turismo organizado e rotas comerciais. O aumento do tráfego marinho de barcos e navios de carga neste mar, aumentam o risco de degradação ambiental irreversível que afetará negativamente o Bacia do Mediterrâneo. Com este cenário, o projeto MaPMed foi desenvolvido sobre uma primeira chamada de propostas lançada pela European Neighbourhood and Partnership Instrument Cross Border Cooperation, em 2009, com o objetivo principal melhorar a sustentabilidade ambiental das áreas costeiras turísticas em países do Mar Mediterrâneo com o propósito de monitorizar e reduzir a poluição marinha.

A poluição induzida pelas atividades humanas pode afetar o ecossistema costeiro e eventualmente provocar a rápida mortalidade dos organismos zooplantónicos que não conseguem escapar de condições adversas. O objetivo deste estudo é analisar a comunidade de zooplâncton em quatro estações do ano diferentes (Julho 2014 como verão, Outubro 2014 como outono, Janeiro 2015 como inverno e Março 2015 como primavera) no porto de El Kantaoui – Tunísia, um dos locais estudados no decorrer do projeto MapMed. Foram recolhidas amostras da comunidade de zooplâncton em diferentes estações do porto, usadas para diferentes atividades turísticas e marítimas, e ao longo de um transecto através do porto. Um total de 54 amostras foram observadas à lupa com a utilização de uma camara de contagem Bogorov para zooplâncton e os principais taxa foram identificados ao nível mais baixo possível (Classe, Ordem, Família e Espécie), exceto os estados larvares. As análises multivariadas foram realizadas através da utilização do programa PRIMER. Como era de esperar as variáveis ambientais e as densidades médias de indivíduos (ind/m3) apresentaram uma variação sazonal do verão para a primavera. As densidades médias de indivíduos mais altas foram registadas no verão e no outono e as mais baixas no inverno e primavera. Além disso foi possível observar um gradiente de abundância e diversidade da comunidade nas diferentes estações do porto, desde as estações internas para as estações mais próximas da saída do porto.

Palavras-chave

Comunidade de zooplâncton; Sazonalidade do zooplâncton; Mar Mediterrâneo; projeto MaPMed.

Contents

Figure list

Appendix list

Abbreviations

ENPI CBC Mediterranean Sea Basin Programme – European Neighbourhood and Partnership Instrument Cross Border Cooperation;

FST - Faculty of Science of Tunis, University of Tunis;

HCMR - Hellenic Center of Marine Research;

HMWB - Heavily Modified Water Bodies;

IGS - Institute for Graduate Studies and Research, University of Alexandria;

MapMed - European project Management of Port areas in the MEDiterranean Sea Basin;

MSFD – Marine Strategy Framework Directive;

RAS - HARDIS - Regione Autonoma della Sardegna - Head Office Regional Agency of the Sardinian River Basin Districto;

UNCED – United Nations Conference on Environment and Development;

UNICA - University of Cagliari;

UNIFI - University of Florence;

Environmental variables abbreviations

PO_4^{3-}(µM) – phosphate (µM);

Chl(mg/m^3) - chlorophyll-a (mg/m^3);

DIN (µg/L) – dissolved inorganic nitrogen (µg/L);

Dissolved O2 (mg/L) – dissolved oxygen (mg/L);

DOC (µg/L) – dissolved organic carbon (mg/L);

O2 saturation (%) – oxygen saturation;

Volume (m^{-3}) – volume of filtered water (m^3);

Analysis abbreviations

CLUSTER - Hierarchical Cluster analysis;

d - Margalef Index;

DistLM test - Distance-based Linear Models;

H'(loge) - Shannon Index;

J' - Pielou's evenness Index;

Lambda' - Simpson Index;

MDS - Non-metric Multi-Dimensional Scaling analysis;

PCA - Principal Component Analysis;

PERMANOVA test - Permutational MANOVA;

PRIMER 6 software - Plymouth Routines In Multivariate Ecological Research;

SIMPER analysis - SIMilarity PERcentage.

Introduction

Ecological state of the Mediterranean Sea

The Mediterranean Sea is a land-locked relatively small marine ecosystem that represents approximately 0,8% of the world's ocean surface area (Hassoun et al., 2015). It is connected to the Atlantic Ocean via the Strait of Gibraltar and with the Indic Ocean/Red Sea via the Suez Channel. It is considered a small-scale ocean with high environmental variability (Béthoux et al, 1999; Hassoun et al., 2015). Since the last century, Mediterranean Sea has become one of the most demanded destinations for organized touristic routes (MaPMed, W/D a). The Heavily Modified Water Bodies (HMWB) according to EEA (1999) are bodies of water which as a result of physical alterations by human activity are substantially changed in character and cannot, therefore, meet good ecological status. Ports areas (HMWB), as sea-land interface for humans activities are fast developing on the Mediterranean coasts to sustain the growing request for commerce and leisure activities. They have a decisive role in the economic development of coastal areas and the risk of impact of infrastructures and maritime traffic on the coastal zone is high (MaPMed, W/D a; Rossano & Scapini, 2014). The increasing traffic of ships, boats and cargos and tourist frequentation of the Mediterranean coasts, particularly those hosting ports, may be cause of irreversible environmental degradation that will in turn negatively affect the whole Mediterranean Basin (MaPMed, 2014; Rossano & Scapini, 2014). Ports are particularly critical environments because they can receive pollution coming from land, ships and the port facilities themselves (Senatore et al., 2012; MaPMed, W/D b). The major concerns in port areas is the presence of toxic pollutants (deriving from boat maintenance activities, e.g. antifueling) and their harmful effects on the marine ecosystems and human health. Furthermore, ports are not closed systems and their pollution may affect large parts of the adjacent coastal areas (MaPMep, W/D b). Tourist ports are subject to seasonal massive impact, however they are not considered natural area worth of protection, therefore are rarely studied from the ecological point of view (Rossano & Scapini, 2014).

The MaPMed project and its contribution to the ecological evaluation of Mediterranean coastal areas

Nowadays the European regulations are improving the sustainability of uses within the port areas through more strict rules and controls, but port areas are anyway subject to strong impacts. Therefore, actions of implementing good practices, monitoring and improving them are needed (Rossano & Scapini, 2014). A large and fast increasing number of environmental laws and regulations on sustainable management of coastal areas including ports exists. The Marine Strategy Framework Directive (MSFD) has been developed with the overall aim of promoting sustainable use of the seas and conserving marine ecosystems (Caroppo et al., 2013).

Port/Maritime Authorities and Licensed Port Company Operators have to find ways to implement them in practice, choosing among the many existing different solutions, which imply different costs and environmental effects and, as a consequence, influence port competition within and between different countries. Port Authorities and Operators frequently ask the scientific community for support to provide guidelines and tools, but rarely involve them in long term cooperation to guarantee their real application towards sustainable management (MaPMed, 2012).

Considering the effects of population increase along the Mediterranean coasts, the European Community, on the one hand, the environmental agencies and local authorities, on the other hand, are developing and applying strategies to preserve and restore ports and coastal environments from the many kinds of impacts due to anthropic activities (Bultrini et al., 2009; SuPorts, 2010; Rossano & Scapini, 2014). There is indeed a need to combine environmental protection with the growth of the ports in line with the logic of sustainable development. Strategies for this purpose have already been recognized in the United Nations Conference on Environment and Development (UNCED) which established that “States, acting individually, bilaterally, regionally or multilaterally […] should assess the need for additional measures to address degradation of the marine environment” from shipping and dumping (Agenda 21, 1992; Rossano & Scapini, 2014).

The MaPMed project (European project Management of Port areas in the MEDiterranean Sea Basin) was developed under the first call for proposals launched by ENPI CBC Mediterranean Sea Basin Programme (European Neighbourhood and Partnership Instrument Cross Border Cooperation) in 2009 (Rossano & Scapini, 2014). The overall aim of the project was to improve the environmental sustainability of tourist

coastal areas in the Countries of the Mediterranean Sea Basin (Fig. 1) through the promotion of a long term cooperation between Institutional Authorities, Port Authorities and the Scientific Community and, at a more specific level, to optimize, validate and transfer tools to guide Institutional Authorities in the sustainable management of tourist harbours with regard to monitoring and reduction of marine pollution (MaPMed, W/D a; Rossano et al., 2013; Rossano & Scapini, 2014).

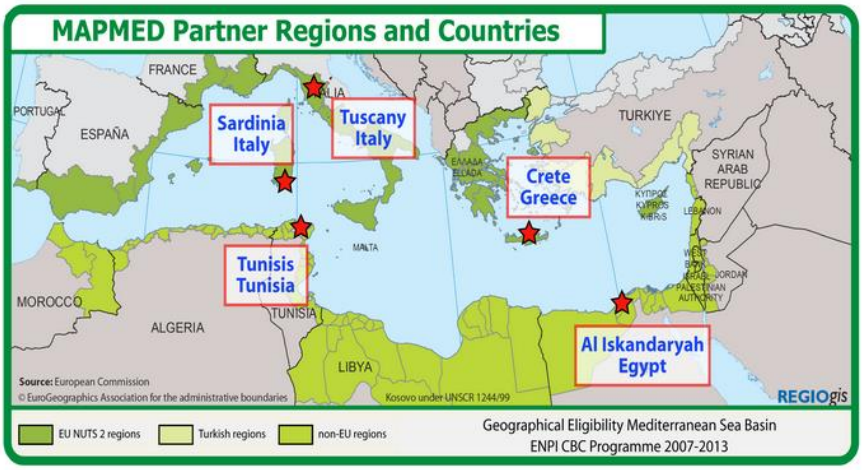


Fig. 1 - MaPMed Stations on Mediterranean Sea and partners participating on the project. In Italy the partners participating were, the University of Cagliari (UNICA, Department of Civil and Environmental Engineering and Architecture, Department of Biomedical Science and Department of Law), the Regione Autonoma della Sardegna (RAS-HARDIS, Head Office Regional Agency of the Sardinian River Basin Districto), the University of Florence (UNIFI, Department of Biology); Greece, participated with the Hellenic Center of Marine Research HCMR in Crete; Egypt, with the University of Alexandria (IGSR, Institute for Graduate Studies and Research) and Tunisia with the University of Tunis (FST, Faculty of Science of Tunis) (Rossano & Scapini, 2014; MaPMed, 2015).

This project pursued these objectives through an integrated multidisciplinary approach based on the skills and know-how of the scientists, technicians, socio-economic and legal experts involved in the implementation of the activities in different countries, to allow integration across the country's borders and add a comparative dimension (at the Mediterranean level) to the developed tools (MaPMed, W/D b; MaPMed, 2012).

The El Kantaoui port - Tunisia

This work was part of the monitoring campaign of the El Kantaoui port integrated in the MaPMed project. During the work the zooplankton communities were analysed for this port.

Zooplankton

"Plankton" is a term by the German founder of quantitative plankton and fishery research Victor Hensen (1887) and it is derived from the Greek word "planao". Meaning to wander and it has the same etymological root as "planet" (Harris et al., 2000).

Zooplankton are microscopic animal organisms at various life stages, from eggs to larvae and adults, they feed on phytoplankton and bacteria as primary consumers or on smaller zooplankton organisms as secondary consumers (MaPMed, 2014). They are drifting organisms with insufficient abilities of locomotion to withstand currents as the nekton, so they drift in water column of ocean, seas or fresh water bodies to move great distances (Harris et al., 2000; Ferdous & Muktadir, 2009). The zooplankton community succession is largely determined by the interactions and the seasonal cycles of physical-chemical factors and biological factors such as competition and predation, which varies in different periods of the year and also among aquatic ecosystems (Sommer et al., 1986; Leibold et al., 2004; Pinel-Alloul & Ghadouani, 2007 and Larson et al., 2009). They may represent early bioindicator of environmental changes, even if few studies exist on this subject (Yamada & Ikeda, 1999; Siokou-Frangou et al., 2010).

Ports may offer protected areas rich of nutrients, where zooplanktonic organisms perform their whole life cycle, or may represent nursery areas for early life stages (MaPMed, 2014). Nevertheless, in enclosed port sectors the human induced pollution may cause rapid mortality of zooplanktonic organisms that cannot rapidly escape from negative conditions (MaPMeD, 2014). For this reason, studies on the structure and dynamics of zooplankton communities in the open Mediterranean Sea have increased in the last decades (Siokou-Frangou et al., 2010; MaPMed, 2014). On the other hand, the Mediterranean port areas need to become target of scientific studies (Siokou-Frangou et al., 2010; MaPMed, 2014). In this sense, more data are needed to establish how human pressures can affect a planktonic component and how this component can affect other components of the ecosystem, and to establish if there are indicators which are able to meet the majority of criteria for good indicators in a holistic ecosystem-based assessment (Caroppo et al., 2013; Painting et al., 2013).

In the Marine Strategy Framework Directive (MSFD) it has been stated that zooplanktonic communities are relevant indicators for the definition of Good

Environmental Status (GES) (Caroppo et al., 2013). The response of zooplankton to environmental conditions is of relevant interest due to the central role that this group occupies as a trophic link between planktonic primary producers and larger consumers (Caroppo et al., 2013; Siokou-Frangou et al., 2010). Any variation in zooplanktonic biomass has implications on biogeochemical cycling, trophodynamics, fisheries and ecosystems services (Caroppo et al., 2013). Indeed, the presence or absence of zooplankton communities may represent the relative influence of different water types on ecosystem structures and they may serve as an early indication of a biological response to environmental and climatic variability and thus reflect changes in marine ecosystems (Hays et al., 2005; Ziadi et al., 2015).

Sizes categories and Life cycles of zooplankton

Zooplankton presents various size categories as microplankton (20–200 µm); mesoplankton (0.2–20 mm); macroplankton (2–20 cm) and megaplankton (Fig. 2) (Larink & Westheide, 2011). The microplankton category includes foraminiferans, ciliates, nauplii (early stages of crustaceans such as copepods) and others (Suthers & Rissik, 2009). The mesoplankton animals are very common and visible to the naked eye (Suthers & Rissik, 2009). They are diverse and include copepods, cladocerans, barnacles, many larvae and hydromedusae (Suthers & Rissik, 2009; Larink & Westheide, 2011). The macroplankton include large visible organisms such as krill, arrow worms (Chaetognata); lastly the megaplankton are large floating organisms that exceed 20 cm in length such as Appendicularia (Suthers & Rissik, 2009; Larink & Westheide, 2011).

Fig. 2 – A zooplankton collected at El Kantaoui port, with various taxonomic and size categories.

Most of mesozooplankton organisms have life cycles of a few weeks, while the macro- and megaplankton usually have life cycles spanning many months (Suthers & Rissik, 2009). Many zooplankton organisms spend their entire life cycle as part of the plankton (for example, copepods and some jellyfish) and they are called holoplankton. The meroplankton are planktonic only for part of their lives, usually at the larval stage,

and are seasonally abundant, especially in coastal waters (Suthers & Rissik, 2009; Larink & Westheide, 2011).

Zooplankton taxa important to this study

Myzozoa

The Phylum Myzozoa includes the Alveolata, which feed through myzocytosis. It is described as a phylum containing the subphylum Dinozoa and Apicomplexa (Suthers & Rissik, 2009). The Dinophyceae Class belongs to the subphylum Apicomplexa and includes *Noctiluca scintillans* (Fig. 3A). They are bioluminescent at night (Suthers & Rissik, 2009).

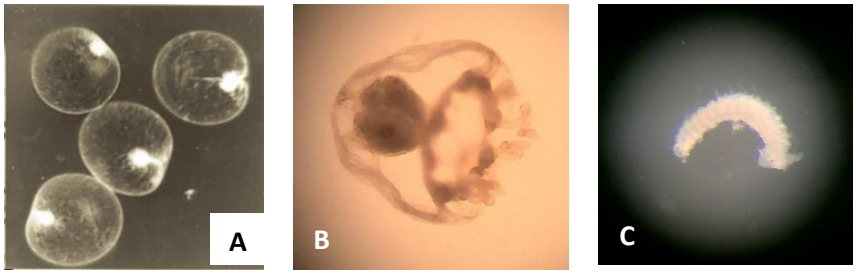


Fig. 3 – Zooplankton organisms in the samples collected at El Kantaoui port. **A**- *Noctiluca scintillans* (Suthers & Rissik, 2009); **B** – Hydromedusae and **C** – Spionidae larva.

Cnidaria

The phylum Cnidaria have two typically adult forms, polypoid (or hydroid), which are tubular and usually permanently attached to a substrate, and medusoid, which are in most cases free-swimming, flattened or bell-shaped (Conway, 2012 a). Some of them have only one of the two forms in their life cycle, others both. Most Cnidaria alternate between sexual and asexual stages and there are many variations in reproductive strategy (Conway, 2012 a). They have three representative classes: Anthozoa (including the sea anemones and corals), Scyphozoa (the large “jellyfish”) and Hydrozoa (the small “jellyfish”, as *Obelia sp*, and siphonophores) (Fig. 3B).

Nematoda

The Phylum Nematoda contains the most numerous species, currently around 20,000 occurring in almost every habitat, free-living, often in bottom sediments, or as parasites of a variety of plants and animals (Harris et al., 2000; Conway, 2015). They are occasionally found in plankton samples and may be free-living species, however, they may also be present because they are parasitic (Conway, 2015). They are elongated; worm-like shape is generally quite characteristic (Conway, 2015).

Platyhelminthes

The platyhelminths (observed in the zooplankton as Müller larvae and Fellodistomidae cercaria) are commonly known as “flatworms” and most marine classes of this phylum contain only parasitic species (Conway, 2012 a). They are usually found on or close to the sea bottom, but can be carried higher in the water column under turbulent conditions (Conway, 2012 a).

Nemertae

The Phylum Nemertae are a poorly known group of unsegmented, worm-like organisms with approximately 100 marine species found in European waters. The adult individuals are most abundant in coastal areas, generally found on the sea bottom. Some species live as commensals of other organisms and others are parasitic. They are mainly carnivorous and can be key predators, but some also scavenge on animal remains (Conway, 2012 a). The appearance and shape of the larvae is very variable, they are flattened, usually elongated and can have a single, or one or two pairs of ocelli on the anterior body (Conway, 2012 a).

Mollusca

This Phylum contains a diverse range of unsegmented soft-bodied organisms, partially or wholly covered by a mantle, a sheet of tissue exclusive to this Phylum. The body is often divided into a head, with eyes or tentacles, a muscular foot used for

locomotion, which is modified in some species for swimming, and a visceral mass housing the organs (Conway, 2012 a). Most molluscs have a protective shell, usually external, that is excreted by the mantle (as the Classes Bivalvia and Gastropoda) (Conway, 2012 a). The Bivalvia Class includes the oysters, mussels and clams, which are not planktonic at adult stages, but their larval stages can be very abundant in plankton samples (Harris et al., 2000; Conway, 2012 a). It is difficult to identify species in the early stages, but some later larvae can be identified using their shape and hinge structure (Brink, 2001). The Gastropoda Class is the largest marine molluscan Class (Conway, 2012 a). This Class includes the order Nudibranchia (with a right-coiled shell) and Thecosomata (with a left-coiled shell) (Conway, 2012 a). All thecosomes have shells, but they are very fragile and some of the species are described as having several sub-species or formae, which often show distinct morphological differences in separate parts of their geographic range (Conway, 2012 a).

Annelida

The Phylum Annelida is a large Phylum of segmented worms and is divided into two classes, Clitellata and Polychaeta (Conway, 2015). The Polychaeta (almost entirely marine) lack a clitellum and typically have paired, unjointed lateral outgrowths from their bodies called parapodia (Suthers & Rissik, 2009; Conway, 2015). Both classes usually have hair-like bristles known as chaetae (or setae) that are found along the body in various configurations and aid in locomotion, feeding and sometimes protection (Conway, 2015). A few Polychaeta are completely planktonic as adults but a large proportion of benthic species produce planktonic larvae, which can be very abundant in plankton samples, particularly in coastal areas (Dales & Peter, 1972). The Spionida Order includes some of the commonest larvae taken in inshore plankton samples (Fig. 3 C; Conway, 2015).

Crustacea

Crustaceans are represented in zooplankton by seven dominant Orders/Classes: Ostracoda, Cladocera, Copepoda, Cirripeda, Decapoda, Amphipoda, Isopoda (Harris et al., 2000).

Crustaceans have eyes and many limbs. The eyes are compound eyes, either stalked and obvious, or are sessile. Another distinction is the presence or absence of a carapace or shell that covers their thoracic limbs and gills. The cladocerans and ostracods are small crustaceans and are enclosed within their carapace. Crustaceans have two pairs of antennae on the head, which are usually composed of an inner and outer branch joined near the base (Harris et al., 2000; Suthers & Rissik, 2009).

The group of small crustaceans, the Ostracoda (Fig. 4F1, 4F2) are often benthic, with the head and eye completely contained within the carapace. They swim by twirling a powerful pair of antennae that they can retract safely within the two halves of the carapace (Suthers & Rissik, 2009). The Cladocera (e.g., *Evadne tergestina*; Fig. 4G2 and *Penilia avirostris*, Fig. 4G3) are small crustaceans, commonly called “water fleas” that can seasonally be very abundant (Suthers & Rissik, 2009; Conway, 2012 b). Marine Cladocera typically have an anterior, single, large compound eye and the head bears two pairs of appendages, the antennules that are usually tiny and unsegmented, bearing olfactory setae and the antennae that are used to swim. They are often found on the surface of samples, which may be due to trapped air inside the carapace (Conway, 2012 b).

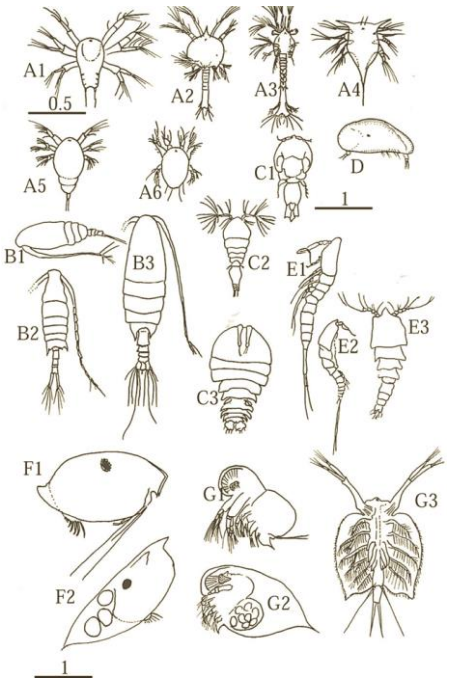


Fig. 4 - Smaller crustacean zooplankton line drawings showing. **A1 to A6** - various nauplius larval stages, **B1 to B3** - Calanoida copepods, **C1 to C3** - Cyclopoida copepods, **D** - Cirripeda cypris larva, **E1 to E3** - Harpacticoida copepods, **F1 to F2** - Ostracoda **G1 to G3** - Cladocera *Podon,Evadne, Penilia* (Suthers & Rissik, 2009).

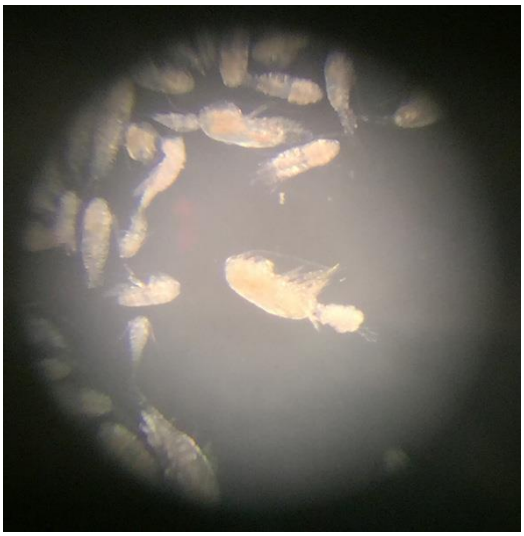


Fig. 5 – A sample of Copepoda collected at El Kantaoui port.

The Copepoda (Fig. 5) are the crustacean taxon with the largest number of species (Larink & Westheide, 2011). They account for most of the macroscopic zooplankton in the world's estuaries and oceans, with over 9,000 species (Suthers & Rissik, 2009).

The role of the subclass Copepoda in the pelagic ecosystem is crucial from a trophic point of view, as a link between the primary production and the larvae and juveniles of fishes and perhaps cephalopods, and characterize the secondary production of the sea (Razouls et al., 2016). They are the archetypal zooplanktonic organisms, growing from an egg, through six nauplius larval stages and a further six copepodite stages (juvenile stages) before becoming sexually reproducing adults (Suthers & Rissik, 2009). The nauplius larval stage is common to all Crustacea, it is around 0.5 mm in length, sometimes with a single compound eye; they have only two or three pairs of limbs (typically the antennae and the feeding limbs with long setae extending out) (Fig. 4) (Suthers & Rissik, 2009; Larink & Westheide, 2011). Juvenile and adult copepods are small (being 1 to 8 mm in length, with no carapace and having a sessile eye) (Fig. 4 and 5) (Suthers & Rissik, 2009). They have the toughest exoskeleton and the longest and strongest appendages that help them to swim faster than any other zooplanktonic organism (Ferdous & Muktadir, 2009).

Feeding habits differ in the main six orders of Copepoda, which are found in the zooplankton: Calanoida, Cyclopoida, Haparticoida, Monstrilloida, Siphonostomatoida and Misophrioida (Ferdous & Muktadir, 2009; Suthers & Rissik, 2009; Larink & Westheide, 2011; Conway, 2012; Razouls et al., 2016).

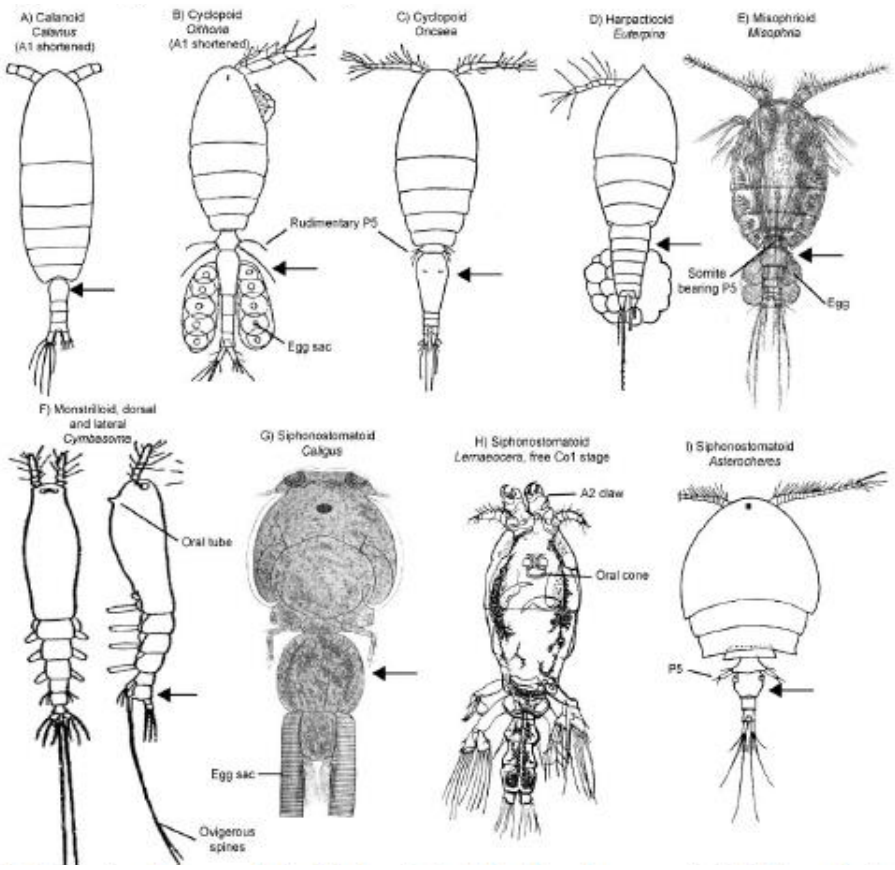


Fig. 6 – The six main different orders of Copepoda. A – Calanoida; B and C – Cyclopoida; D – Haparticoida; E – Misophrioida; F – Monstrilloida and G to I – Siphonostomatoida (Conway, 2012 b).

Calanoida

The Calanoida Order are the most abundant and the most important primary consumers in the pelagic marine ecosystems (e.g., *Acartia sp*., Pontellidae, *Isias sp*., *Centropages sp*. and *Parvocalanus sp*. (Fig. 6A). They are suspension-feeders using fast movements of head appendages to produce a continuous feeding current. Most of them are herbivorous but may consume small animals as readily as phytoplankton (Larink & Westheide, 2011).

The *Acartia sp*. are typical in the Indopacific and Atlantic seas and in all tropical and sudtropical seas (Razouls et al., 2016) but are spread everywhere and very common in the Mediterranean. They are usually larger and have long first antennae that almost reach the length of the animal and a thin abdomen (Fig. 7A). They scatter their eggs into the water, or retain them in a sac until these hatch (Fig. 5) (Suthers & Rissik, 2009).

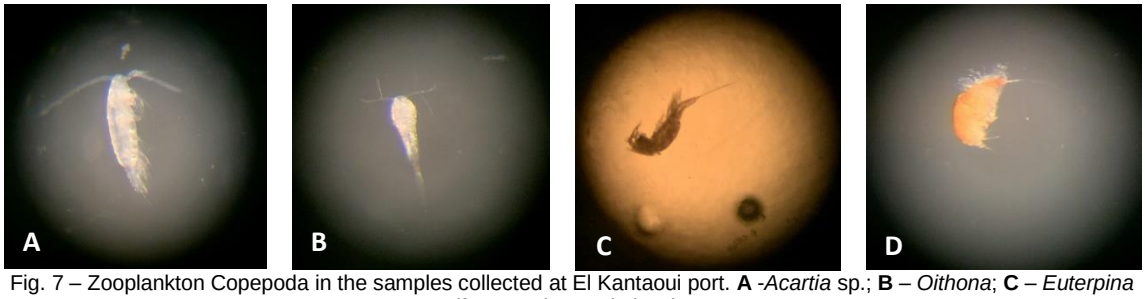


Fig. 7 – Zooplankton Copepoda in the samples collected at El Kantaoui port. **A** -*Acartia* sp.; **B** – *Oithona*; **C** – *Euterpina acutifrons* and **D** – *Diathrodes* sp.

Cyclopoida

In the Cyclopoida Order only 5% of the species are planktonic (Larink & Westheide, 2011). Some of them are carnivorous species, they feed on other zooplankton (such as *Oncaea* and *Oithona* (Fig. 6B, 6C and 7B)) and fish larvae. They also can feed on algae, bacteria and detritus (Ferdous & Muktadir, 2009; Suthers & Rissik, 2009). They are often small, with distinctively short antennae (Suthers & Rissik, 2009).

Harpacticoida

The animals who belong to the Harpacticoida Order are primarily benthic and mainly sediment dwellers, living in very great numbers in sand beaches and sea bottoms (Ferdous & Muktadir, 2009; Larink & Westheide, 2011). In the European marine waters, away from shallow coastal areas, they are usually one of the least commonly sampled of the copepod orders (Conway, 2012). They feed on detritus and protists (Larink & Westheide, 2011). Many Haparticoida species are smaller, elongate, have short antennae, egg sacs and have no difference in width between the thorax and abdomen. Some of them have distinctive very long tail setae (almost as long as the animal) (Fig. 6C and 6D) (Suthers & Rissik, 2009). Only a few Haparticoida species are holoplanktonic (e.g. *Euterpina acutifrons*, Fig. 6D and 7C), the other species temporarily go into the pelagic zone (Larink & Westheide, 2011).

Monstrilloida

Monstrilloida animals are mainly found close to inshore and the adults can be easily distinguished from other copepod orders by their elongate cylindrical shape (Conway, 2012 b). They are endoparasitic species of the Annelida Polychaeta in their nauplius stages and only the females are free planktonic life (Fig. 6F) (Larink & Westheide, 2011; Razouls et al., 2016). Since their digestive tract is vestigial, the

female lives on the reserves accumulated during its parasitic period. Their cephalathorax comprises almost half of the body and is filled with genital organs (Larink & Westheide, 2011).

Cirripeda

Cirripedia species (barnacles) also belong to the Subphylum Crustacea, are sessile and found attached to a wide range of inanimate surfaces in the sea, both fixed and free-floating. The adults are hermaphroditic and reproduce sexually by cross fertilisation (Suthers & Rissik, 2009). They can also attach externally to living organisms and are particularly common in the intertidal zone. Their nauplius (Fig. 8A) or cypris (Fig. 4D) stages often dominate the inshore plankton during their breeding season (Høeg et al., 2004; Conway, 2012 b). Cypris larvae are attracted to settle on hard substrates by the presence of other barnacles, ensuring settlement in areas suitable for barnacle survival and for obtaining future mates. After settling, the cypris releases a substance to permanently cement itself to the substrate. Calcareous plates then grow and surround the body. The appendages face upwards to form cirri which sweep food particles into the organism (Conway, 2012 b).

Malacostraca

Malacostraca is the largest of the six classes of Subphylum Crustacea and comprises 16 orders, characterised by a common body plan of head, thorax and abdomen. (Conway, 2012 b; Conlan & Bousfield, 2016). In the adult the head consists of five segments, the thorax of eight and the abdomen typically of six unfused segments (Conlan & Bousfield, 2016). They are abundant in the seas from the tropics to the poles and from the tidal zone to the abyss, in surface and subterranean fresh waters of all continents except Antarctica and terrestrially on all continental landmasses and all tropical and temperate islands (Conlan & Bousfield, 2016).

Cumacea

Cumaceans are almost entirely marine and brackish water crustaceans, abundant in shallow coastal areas, but also found at depth, where there is greater species diversity. They mainly feed on microorganisms and organic material. In a few species the mandibles are transformed into piercing appendages that may be used for predation on small organisms (Larink & Westheide, 2011; Conway, 2015).

Decapoda

Decapoda order is the largest order in Subphylum Crustacea with the most familiar crustaceans (Fig. 8B) (Suthers & Rissik, 2009; Larink & Westheide, 2011; Conway, 2015). There exist about 18,000 species with two extreme types: the elongate shrimp-like forms with swimming capability and the shortened crab-like animals with mainly crawling locomotion (Larink & Westheide, 2011). These animals have a division of their bodies into cephalothorax and pleon. A few shrimps are holoplanktonic in the epipelagial or mesopelagial zones of the seas, whereas most of the species are benthic (Larink & Westheide, 2011, Conway, 2015). However, the larval stages of decapods are part of the marine meroplankton (Larink & Westheide, 2011).

Isopoda

The Isopoda Order exhibit a great variety of body forms and while most are benthic grazers/detritivores or predators, some are wood-borers or parasites (mainly of decapod, ostracods and cirripedes as Epicaridium) (Fig. 8C) (Williams & Boyko, 2012; Conway, 2015). Epicarideans represent 8% of all described isopods and are unique in that they typically parasitize two different crustacean hosts during their life cycle, intermediate and definitive hosts, and include both endo- and ectoparasites (Conway, 2015). The intermediate host (pelagic copepod) is typically a Calanoida, but sometimes a Cyclopoida (Owens & Rothlisberg, 1995). Parasitisation may also affect the appearance, morphology and behaviour of hosts and may have an economic impact by reducing productivity of a variety of commercially important species (or of their prey) and negatively affecting saleability (Conway, 2015).

Amphipoda

The Subphylum Crustacea includes the Amphipoda Class who have several species living in the water column (Conway, 2015). They are generally detritivores or scavengers, but some are carnivorous, commensal or parasitic. They colonize all the aquatic environments and the most familiar are the terrestrial “sand hoppers” found under damp, decaying seaweed at the strand line on beaches (Suthers& Rissik, 2009; Conway, 2015).

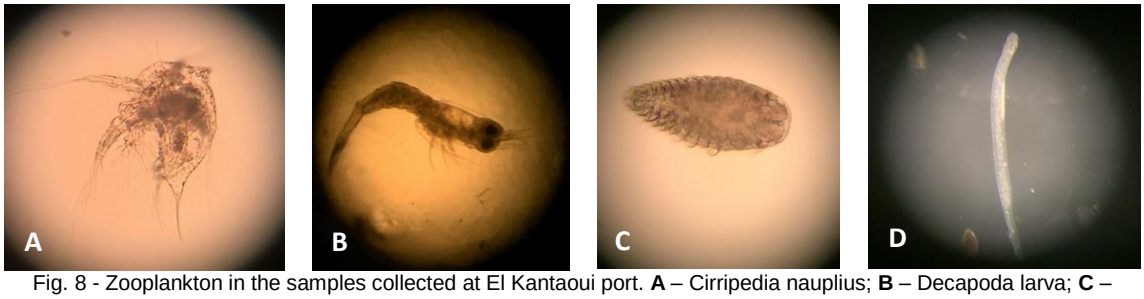


Fig. 8 - Zooplankton in the samples collected at El Kantaoui port. **A** – Cirripedia nauplius; **B** – Decapoda larva; **C** – Isopoda parasite of Copepoda (epicaridium larva) and **D** – Chaetognata.

Chaetognata

Chaetognata animals (or arrow worms) are holoplanktonic worm-like animals that are placed in their own phylum with about 100 species (Suthers & Rissik, 2009). They are 1–2 cm long and have fins (Fig. 8D) These animals are predator, with a row of bristles or spines at either side of the mouth (Suthers & Rissik, 2009). Most of them are pelagic, but around a quarter are benthic. Chaetognaths are generally quite transparent, making internal infestation by parasites easy to observe, typically by protozoans, nematodes; they are important vectors of these parasites (Conway, 2015).

Chordata: Ascidiacea, Appendicularia, Teleostei

The Phylum Chordata includes the Vertebrates, together with several invertebrates. They are united by having a notochord during some period in their life cycle, a hollow dorsal nerve cord, pharyngeal slits, an endostyle and a postanal tail.

The Ascidiacea Class are sac-like, solitary or colonial, sessile filter feeders, typically found on the seabed, or as fouling organisms on marine structures or ship bottoms (Fig. 9A and 9B). There are 57 species recorded, but the number in the European area has increased due to introduction of alien species (Conway, 2015). They were found in the zooplankton samples as Ascidiacea 1 composed by *Styla*-shaped species (with small dimensions and a body with an elongated shape) and Ascidiacea 2 composed by *Botryllus*-shaped species with large dimensions and a body with a rounded shape.

Appendicularia Class are planktonic fragile individuals, filter-feeding organisms, most only a few millimetres long, with a notochord that persists throughout their life (Conway, 2015). They can be very abundant in the zooplankton: *Oikopleura* are numerous during summer and *Fritillaria* during winter, sometimes found in coastal

waters (Arfi et al., 1982; Conway, 2015). Their numbers generally increase during elevated phytoplankton abundance.

The Actinopteri class includes the Teleostei larvae and fish eggs, which are usually perfectly spherical, each containing a ball of embryo delicately suspended inside (Fig. 9C; Suthers & Rissik, 2009).

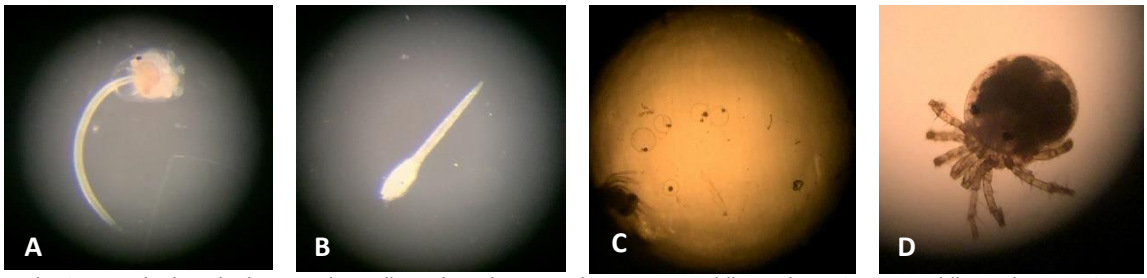


Fig. 9 - Zooplankton in the samples collected at El Kantaoui port. **A** – Ascidiacea larva 2; **B** – Ascidiacea larva 1; **C** – Fish eggs and **D** – Acarina.

Arachnida

The Order Acarina belongs to the Arachnida Class and is a group of primarily terrestrial arachnids. They are mainly found intertidally, but also below low tide level (sublittoral) to the very deep ocean (Conway, 2012). They present a short body, oval shape, outwardly showing little or no division into somites, bearing four pairs of legs, the anterior two pairs directed forwards and the posterior two pairs backwards (Fig. 9D) (Conway, 2012).

The influence of the environmental variables on zooplankton communities

Plankton has been used recently as an early bioindicator to monitor the aquatic ecosystems and integrity of water bodies (Hays et al., 2005; Ferdous & Muktadir, 2009; Ziadi et al., 2015). The potentiality of zooplankton as bioindicator is very high because its growth and distribution are dependent on some physical (as depth, water temperature, water salinity, pH), chemical and biological parameters (inorganic nutrients as dissolved oxygen, oxygen saturation, dissolved inorganic nitrogen, phosphate and organic nutrients as chlorophyll-a and dissolved organic carbon) (Ferdous & Muktadir, 2009; Bianchi et al., 2003). Williams (1998), and Wen et al. (2005) and D'Ambrosio et al. (2016), among others, have suggested that the structure

of a marine community is dictated by a combination of parameters including dissolved oxygen concentration, pH, hydrologic patterns and biotic interactions.

The environmental variables influence the structure and dynamics of zooplankton communities and determine the distribution and abundance of the species (Gyllström & Hansson, 2004). According to Ferdous *et al*. (2009), concentration of dissolved oxygen, temperature, total nitrogen, phosphate and pH can influence the growth of zooplankton and in some cases, the zooplankton population size is correlated with the biotic and abiotic parameters.

Aims

The aims of this work was to analyse and compare the zooplankton communities in four different seasons (July as summer, October as autumn, January as winter and March as spring) at El Kantaoui port – Tunisia. The zooplankton communities were sampled in different stations of the harbour not yet explored, used for different activities. This work was included in the monitoring campaign of this harbour and integrated in the European project Management of Port areas in the MEDiterranean Sea Basin (MapMed).

Materials and methods

The sampling campaigns were conducted at El Kantaoui Port, Soussa (Tunisia) during the months of July and October 2014 and January and March 2015. The laboratory work was carried out at Department of Biology, University of Florence - Italy, from October 2015 until April 2016.

Study sites

The selected touristic harbour for the development of this work was the El Kantaoui Port (Soussa - Tunisia, 35°53'38"N, 10°35'55"E, Fig. 10A). The harbour complex extends over an area of more than 300 hectares besides the marina with 550 berth for luxury yachts, has several golf courses and hosts sporting activities. The privileged localization of this harbour and its complex makes of it a desirable destination for many tourists (Magi and Fabbri, 2008; MaPMed, 2012).

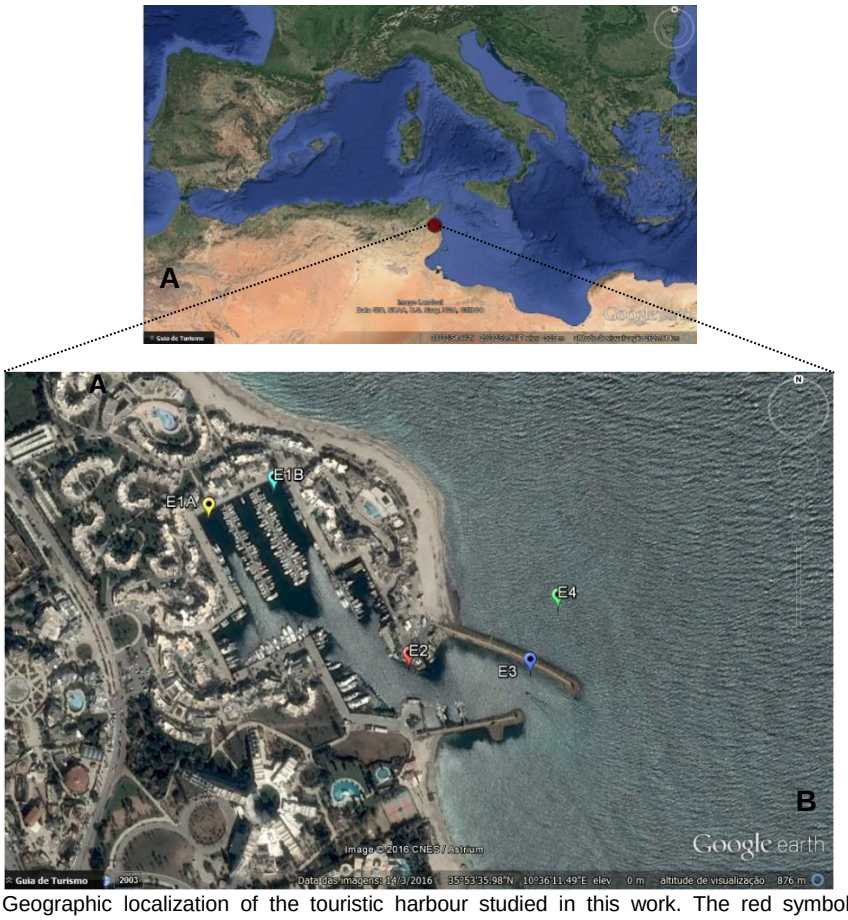


Fig. 10 – **A** - Geographic localization of the touristic harbour studied in this work. The red symbol indicates the localization of the El Kantaoui Port (Soussa - Tunisia, 35°53'38"N, 10°35'55"E, image from Google Earth, 2016); **B** - El Kantaoui Port with the four sampling stations. Station E1A - leisure boats sector (35°53'40.74"N,10°35'49.56"E); station E1B - leisure boats sector (35°53'41.82"N, 10°35'52.68"E); station E2 - fuel station sector (35°53'34.92"N, 10°35'59.22"E); station E3 - port entrance (35°53'34.68"N, 10°36'05.04"E); station E4 - outside port area (35°53'37.2"N, 10°36'06.4"E) (image from Google Earth, 2016).

Fieldwork

The sampling campaigns in El Kantaoui Port were conducted during four different months (July 2014, October 2014, January 2015 and March 2015). These sampling campaigns were included in the monitoring campaigns of this harbour and integrated in the European project Management of Port areas in the MEDiterranean Sea Basin (MapMed).

Five different stations were selected in different sectors of the harbour that were used for different activities. The selected stations were localized in the leisure boats sector (E1A - 35°53'40.74''N,10°35'49.56''E; and E1B - 35°53'41.82''N, 10°35'52.68''E), fuel station sector (E2 - 35°53'34.92''N, 10°35'59.22''E), port entrance (E3 - 35°53'34.68''N, 10°36'05.04''E) and outside the port area (E4 - 35°53'37.2''N, 10°36'06.4''E). This last station was sampled as control only during the last sampling campaigns (January and March) (Fig. 10B). The station E1A and E1B were symmetrical in the inner part of the port and within the same sector of leisure boats, so that one was control of the other.

Zooplankton sampling

An Apstein net for zooplankton was used during all the sampling campaigns. The net had a 200µm mesh (standard UNESCO mesh size for sampling zooplankton according with Harris *et al*., 2000), 40cm mouth diameter and was 1 meter long. The use of a smaller mesh size would have not allowed the sampling of all the zooplankton organisms, since larger and better swimming animals could have sensed the pressure wave in front of the net mouth and dodged it. Moreover in this case it was expected the risk of obstruction of a small mesh by the suspension in a muddy port with low depth and waste discharges. On the other hand if a larger mesh was used, the smaller zooplankton would have not been collected by the mesh (Suthers and Rissik, 2009).

The volume of water filtered by the Apstein net was calculated as

$$\text{Volume} = \text{mouth surface } (\pi r^2) \times \text{station depth}$$

and was used to estimate the densities of the individuals (ind/m^3). The calculated volume values are underestimated because the formula considered a vertical immersion, but natural factors like currents do not permit a completely vertical immersion of the net. With the increase of depth, the errors on the density values are lower.

During the sampling campaigns, five vertical tows had been taken (replicates a, b, c, d and e); three replicates (c, d and e) were analysed in this work.

After collection, zooplankton samples were fixed with 8% neutralized formalin solution neutralized with borax (pH=8). The neutralization of formalin with borax was necessary because pH value of formalin is 7 and to fix marine zooplankton the ideal value of pH should be 8 to prevent the decalcification of calcareous organisms before they are transferred to other preservatives (Motoda *et al.*, 1976).

Physical-chemical and biological factors

During the samplings campaigns at El Kantaoui Port the same physical parameters and environmental variables were measured at each station. Depth was recorded with the use of a depth meter, water temperature, water salinity, pH, dissolved oxygen and oxygen saturation through a multi-parametric probe.

The water samples for the analysis of chemical parameters and biological parameters were collected from the seawater surface for a total amount of 1L. These parameters were inorganic nutrients as dissolved inorganic nitrogen and phosphate and organic nutrients as chlorophyll-a and dissolved organic carbon. This amount was filtered using Whatman GF/F filters (47mm). Two filters (500ml of seawater were filtered through each filter) were stored in a freezer (-20°C) during the field campaign and later brought to the Department at the University of Cagliari (Italy) for the analyses. One filter was used for chlorophyll-a, and the other filter was for particulate organic carbon (POC) analysis. The analysis for inorganic nutrients in the seawater samples were performed according to the Strickland & Parsons (1972) method, while for the NH_4 analysis the Ivancic & Degobbis (1984) method was used. The chlorophyll-a was determined according to the method of Yentsch & Menzel (1963) and Arar & Collins (1992) (MaPMED, W/D).

Laboratory work

The analysis of the zooplankton community were performed at the Department of Biology, University of Florence - Italy, from October 2015 until April 2016. A total of 54 samples were analysed for the four months studied (July 2014, October 2014, January 2015 and March 2015). Three replicate samples (c, d and e) collected at each

station were sorted, the specimens were counted; then the analysis was performed for the number of individuals and taxa.

Before starting the identification of the samples, these were washed by rinsing with cold fresh water through a 100µm mesh to remove the formalin solution and possible fine sediment dirtiness (Fig. 11E). Each replicate was observed under a stereomicroscope type Wild M3 Heerbrugg (Fig. 12A) and a lighting type Olympus KL 1500LCD (Fig. 12B) using a Bogorov counting chamber for zooplankton (36mL) (Fig. 12D). The main taxa were identified at the lowest taxonomical level as possible, also including larval stages. In this study 52 different taxa were considered. All the species of the Class Copepoda were saved in Eppendorf tubes for a further identification that will be made by a specialist. The data were registered in a papery formulary (Protocol), appropriate for this study (Appendix 1) and an electronic database in Microsoft Office Excel was created with the results.

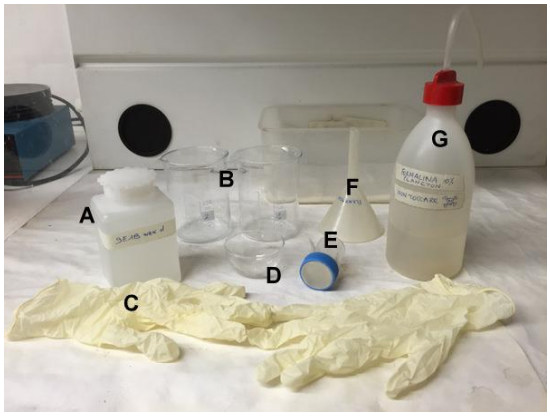


Fig. 11 - Material used to prepare the zooplankton samples. A – Sample bottle (250mL); B –Beaker (250 mL); C – Sterile gloves; D – Evaporating dish; E – 100µm sieve; F – Plastic funnel; G – Bottle with 8% neutralized formalin solution with borax.

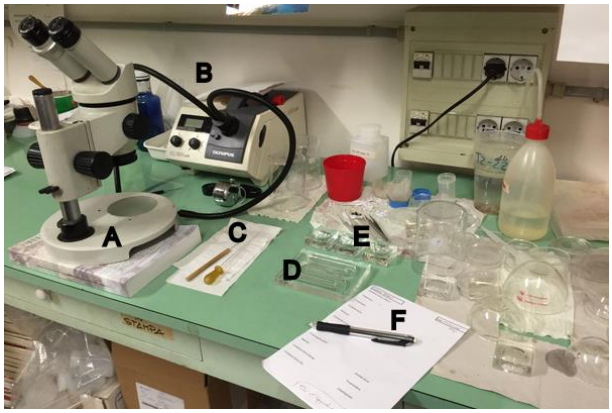


Fig. 12 - Material used to observe and count the zooplankton samples. A - Stereomicroscope type Wild M3 Heerbrugg; B - Stereomicroscope lighting type Olympus KL 1500LCD; C - Dropper pipet and needle; D – Bogorov counting chamber for zooplantkton (36mL); E – Glass dishes; F – Protocol.

The electronic database was created with the aim of organizing and analysing the results (Appendix .2). When the electronic format was completed, the density of individuals (ind/m^3) for each replicate was obtained dividing the number of individuals of each taxa for the volume of filtered water. On the same dataset the mean number of individuals among the three replicates was calculated and the mean densities over the three replicates for each taxa were calculated by dividing the mean number of individuals for the volume of filtered water (ind/m^3).

Statistical analysis

The excel database was primarily used to perform an inspection analysis through the elaboration of some of the histograms for this study. The statistical analysis of the biotic and abiotic data was performed using the PRIMER 6 software (Plymouth Routines In Multivariate Ecological Research). The PRIMER software is a statistical package that collects specialist univariate, multivariate and graphical routines for analysing species sampling data for community ecology with the aim of obtaining results and associations statistically relevant (Clarke & Gorley, 2015). For the statistical analysis, the biotic and abiotic data were imported to the PRIMER software as an Excel table. The first data analysed through this software were the biotic data. These were subjected to a pre-treatment: the Draftsman plots were used to inspect the influence of each diversity measure on the others; a fourth root overall transformation was used to transform the data to approximately normal distributions; a Draftsman plots was performed again after this transformation to check the accuracy of the pre-treatment. With the pre-treated data a resemblance matrix (similarity matrix) was created according to Bray-Curtis similarities index for the biotic densities. This resemblance matrix allowed to analyse the similarity among each station studied through the Hierarchical Cluster analysis (CLUSTER), which is represented by a dendrogram. After this analysis, the Non-metric Multi-Dimensional Scaling analysis (MDS) was performed, to show the relative distances among stations and the relative similarity/dissimilarity values. The data from the CLUSTER and MDS analyses were re-examined and the species contribution was determined using the SIMPER analysis (SIMilarity PERcentage). Species were separated in four groups (July, October, January and March). The SIMPER analysis decomposes the average Bray-Curtis similarities between all the pairs of groups into percentage contributions from each species, listing the species in decreasing order of such contributions (Clarke & Warwick, 2001). This analysis indicates which species were principally responsible for the groups.

After this analysis, the PERMANOVA test (Permutational MANOVA) was performed (PRIMER software). This test connects factors with the matrix of similarity of biological data. The selected factors were the month (July, October, January and March) and the distance of the stations from the port entrance (high distance at stations E1A and E1B, medium distance at station E2, low distance at station E3 and outside of the port at station E4).

The same pre-treatment steps of the biotic data were performed on the abiotic data also tabled in Excel. A normalization was performed on the dataset to better analyse the contribution of all the variables in the following analysis. As for the biotic data a resemblance matrix was created for the abiotic data where the method of the Euclidean distances was applied to analyse the similarity among the stations studied and to performed the Hierarchical Cluster analysis (CLUSTER). Starting from the normalized dataset a Principal Component Analysis (PCA) was performed through the Best routine, that reports the effect of each environmental variable recorded at each station.

Starting from the resemblance matrix of the biotic and abiotic data, the RELATE test was performed, to relate these two resemblance matrices superimposing their data and studying their variance. To perform this test the correlation method of Spearman (Rho coefficient) was used. The resemblance matrix of the biotic and abiotic data were also used to perform the DistLM test (distance-based linear models). This test relates the biotic and environmental variables with a number of permutations, with the purpose of predicting samples variation explained by the variation of specific variables. The DistLM test was applied using the AICc selection criterion and calculating R^2.

Through the PRIMER software, the biodiversity indexes of each station studied were also calculated. The biodiversity indexes calculated to describe the differences among the communities were Margalef Index (d), Pielou's evenness Index (J'), Shannon Index (H'(loge)) and Simpson Index (Lambda').

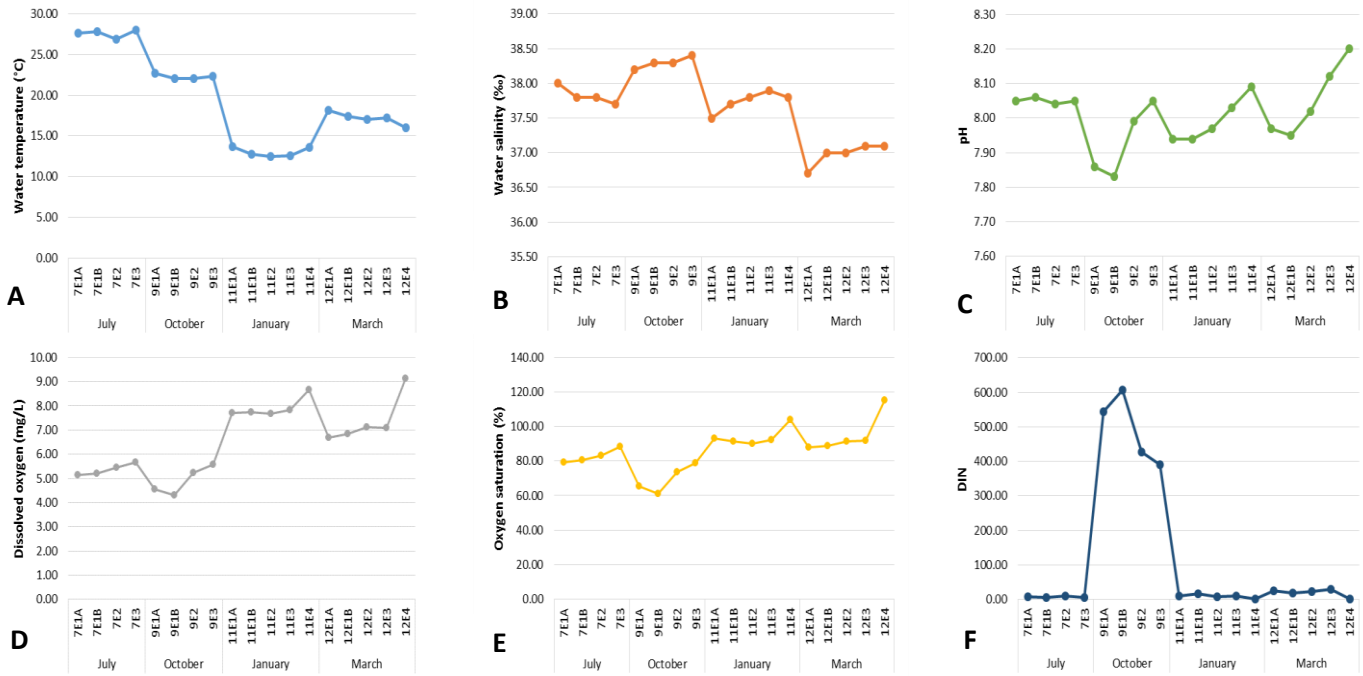
Results

Environmental variables

The environmental variables measured during this study in El Kantaoui Port were physical parameters (depth, water temperature, volume of filtered water), chemical parameters (water salinity, pH, dissolved oxygen, oxygen saturation, inorganic nutrients as dissolved inorganic nitrogen, phosphate and organic nutrients as chlorophyll-a and dissolved organic carbon). The abiotic variables are represented in Fig. 13.

The depth values recorded (m) were low and did not vary much, with the lower values at the inner stations than at outer stations. The highest value (3.90 m) was recorded in January at station E4 (outside port area) and the lowest value (2.17 m) was observed in March at station E1B (leisure boats sector).

As expected a monthly variation of water temperatures (°C) was observed (Fig. 13A). The maximum value recorded for water temperature was 28.00°C in July at station E3 (port entrance) and the minimum value was 12.50°C in January at the station E2 (fuel station sector).



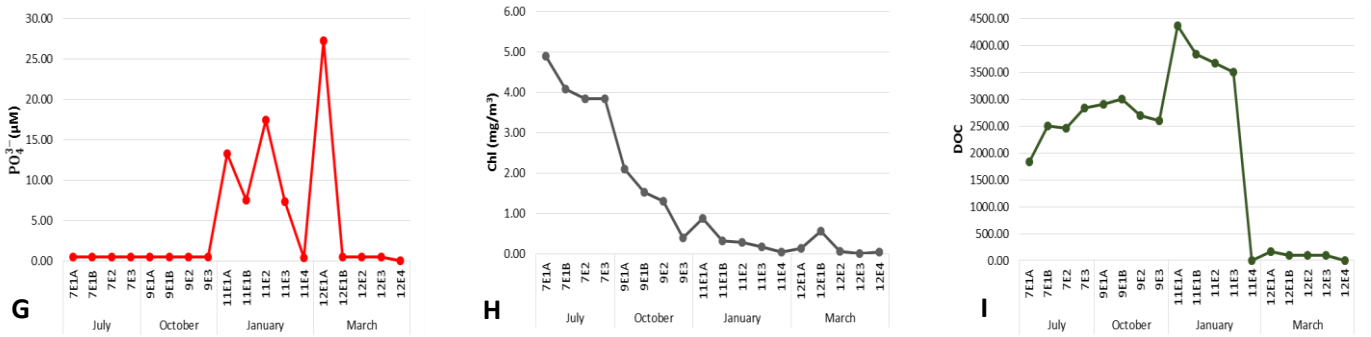


Fig. 13 - Spatial variation of the abiotic variables at each station: leisure boats sector (E1A and E1B), fuel station sector (E2), port entrance (E3) and outside port area (E4) for month (July 2014 - campaign 7, October 2014 - campaign 9, January 2015 - campaign 11 and March 2015 - campaign 12) recorded during the sampling campaigns in El Kantaoui Port. **Legend:A** – water temperature (°C); **B** – water salinity (‰); **C** – pH; **D** – dissolved oxygen (mg/L); **E** – oxygen saturation (%); **F** - dissolved inorganic nitrogen (µg/L); **G** - phosphate (µM); **H** - chlorophyll-a (mg/m^3); **I** –dissolved organic carbon (mg/L).

The water salinity recorded values peaked in October (38.40‰) at station E3 (port entrance) and the lowest value (36.70‰) was recorded in March at stations E1A (leisure boats sector) as expected from seasonal variation (Fig. 13B). A monthly variation of water salinity was indeed observed with the highest mean values in October (38.30‰) and in July (37.83‰), and the lowest in January and March (Fig. 13B).

The recorded values of pH in this study were characterized by a March peak (pH=8.20) at station E4 (outside port area) and by minimum value (pH=7.83) in October at station E1B (leisure boats sector) (Fig. 13C). In October, January and March the values increased at each station from the inner stations to the outer stations. On the other hand, in July, the variation of pH values from the inner stations to the outer stations was very low (Fig. 13C).

The highest value of dissolved oxygen (mg/L) was observed in March (9.14 mg/L) at station E4 (outside port area) and the lowest value in October (4.31 mg/L) at station E1B (leisure boats sector). In all the months, the values increased within each station from the inner stations to the outside stations, and stations E3 (port entrance) and E4 (outside port area) presented the highest values. (Fig. 13D).

The variation of the values of oxygen saturation (%) had the same trend of the dissolved oxygen variation (Fig. 13E). The highest value of oxygen saturation was in March (115.1%) at station E4 (outside port area) and the lowest value in October (61.00 %) at station E1B (leisure boats sector).

For the dissolved inorganic carbon (DIN) in October peak values were recorded at all the stations compared with the other three months, with a maximum value of 607.33 µg/L at station E1B (leisure boats sector) (Fig. 13F). The lowest value of DIN

was 0.07 µg/L in January at station E4 (outside port area). In March the values were higher than in July and January, but very low compared to October (Fig. 13F).

The phosphorus measured as phosphate (PO_4^{3-}) (µM) during the sampling campaigns at El Kantaoui port did not vary in July and October but was very variable in July and somehow in March. It presented a highest mean value in January (9.22 µM) and the highest at all was observed in March (27.33 µM) at station E1A (leisure boats sector) (Fig. 13G). In March the highest peak was recorded at station E1A and the lowest value (0.00 µM) at station E4 (outside port area) (Fig. 13G). In January and March the inner stations presented highest values than the outer stations (Fig. 13G).

The levels of chlorophyll-a (mg/m^3) decreased from July to March (Fig. 13H). The highest value of chlorophyll-a was in July (4.90 mg/m^3) at station E1A (leisure boats sector) and the lowest in March (0.00 mg/m^3) at station E3 (port entrance) (Fig. 13H). During all the samplings the inner stations (E1A and E1B, leisure boats sector) showed higher values than the outer stations (Fig. 13H).

The highest concentration of dissolved organic carbon (DOC) was measured in January (with a mean value of 3074.00 mg/L and peak of 4366.67 mg/L) and a collapse was observed in March (with a mean value of 93.97 mg/L and lowest value of 3.20 mg/L) when the lowest value were recorded. Station E4 in January was an exception because of its value comparable with the values in March (Fig. 13I). In October, January and March a spatial gradient with decreasing concentrations was observable from the inner stations to the outer stations whereas in July the opposite variation occurred (Fig. 13I).

To represent the effect of each environmental variable studied at each station in El Kantaoui Port the Hierarchical Cluster analysis, Non-metric Multi-Dimensional Scaling analysis (MDS) and the Principal Component Analysis (PCA) were performed through the PRIMER software. Hierarchical Cluster and MDS analysis were performed but the results are not presented here because they are resumed by PCA. Among these three analyses, the PCA was the one that best represented the data of the effect of each environmental variable studied at each station in El Kantaoui Port. The results of PCA are represented in Fig. 14.

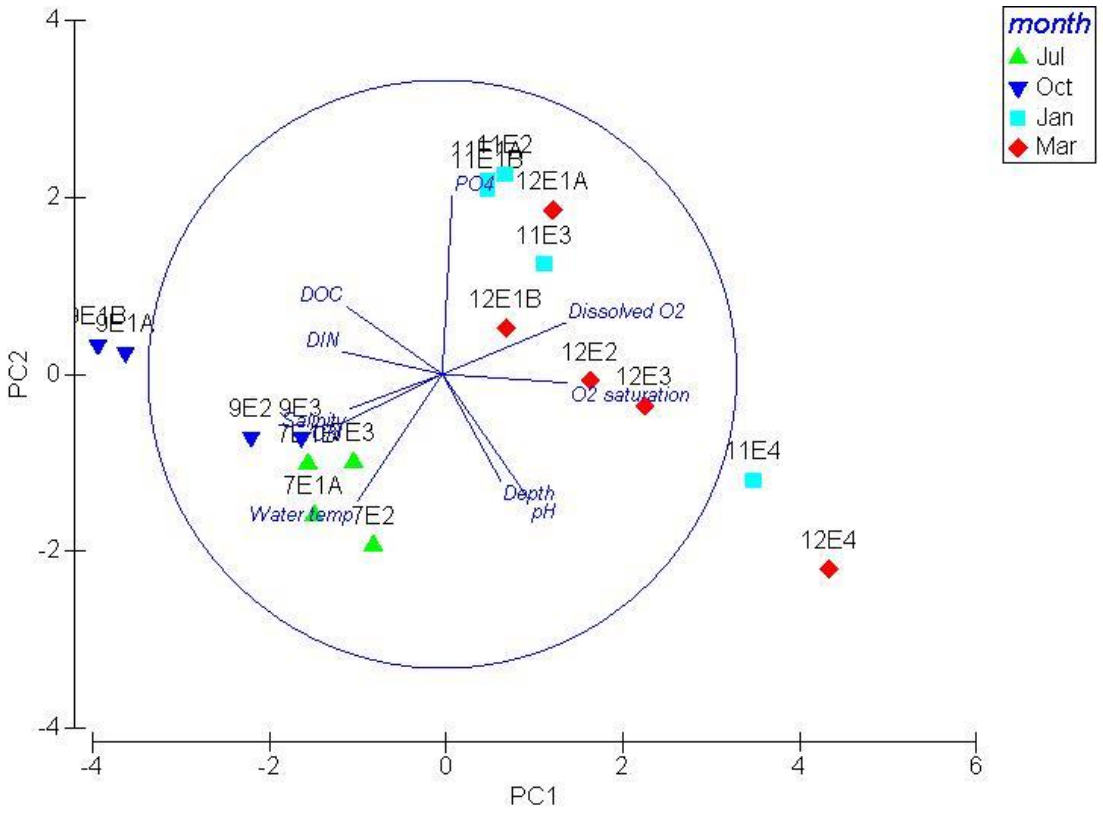


Fig. 14 – PCA, *Principal Component Analysis*. Coefficients in the linear combinations of environmental variables at each station (leisure boats sector (E1A and E1B), fuel station sector (E2), port entrance (E3) and outside port area (E4)) for month (July 2014-campaign 7, October 2014-campaign 9, January 2015-campaign 11 and March 2015-campaign 12) recorded during the sampling campaigns in El Kantaoui Port.

In Fig. 14, the most accurate representation of the true relationship between samples is summarised by the percentage of variation explained. The PC1 is mainly a combination of two variables (Appendix 3): dissolved oxygen and oxygen saturation that have the same trend and that separate July and October from January and March (Fig. 14). The PC2 is mainly a combination of three variables (Appendix 3): PO_4, water temperature and pH that approximately seem to separate the inner stations from the outer stations. By the point of view of the environmental variables, March was the month with the highest variability in the results, followed by January.

Zooplankton communities: abundance and composition

The total number of individuals sorted (N) in the 54 replicates of the four months studied (July 2014, October 2014, January 2015 and March 2015) during the sampling campaigns in El Kantaoui Port was of 41469 individuals and results are shown on

Appendix 4 and Fig. 15. The highest number of individuals (N) was recorded in October with 5727 individuals in the third replicate (e) at station E1A (leisure boats sector) and the lowest was recorded in January with 3 individuals in the third replicate (e) at station E1B (leisure boats sector) (Fig. 15). Concerning the abundances through the sampling campaigns, it can be observed that the individuals in July and in January were more abundant in the outer stations (E2 and E3) than in the inner stations (E1A and E1B) (Appendix 4 and Fig. 15). In July the mean number of individuals (± standard error) at stations E2 and E3 was several times the number of individuals collected at the inner stations (E1A and E1B) with the highest abundance at station E2 (1370±314.54 individuals) (Appendix 4 and Fig. 16). The opposite distribution occurred in October, where the highest frequencies at all were encountered and the distribution of the mean number of individuals was higher in the inner stations (E1A and E1B) than in the outer stations (E2 and E3) (Fig. 15). In March, the mean number of individuals was higher at the stations E1A, E2 and E4, and lowest at stations E1B and E3 (Fig. 15).

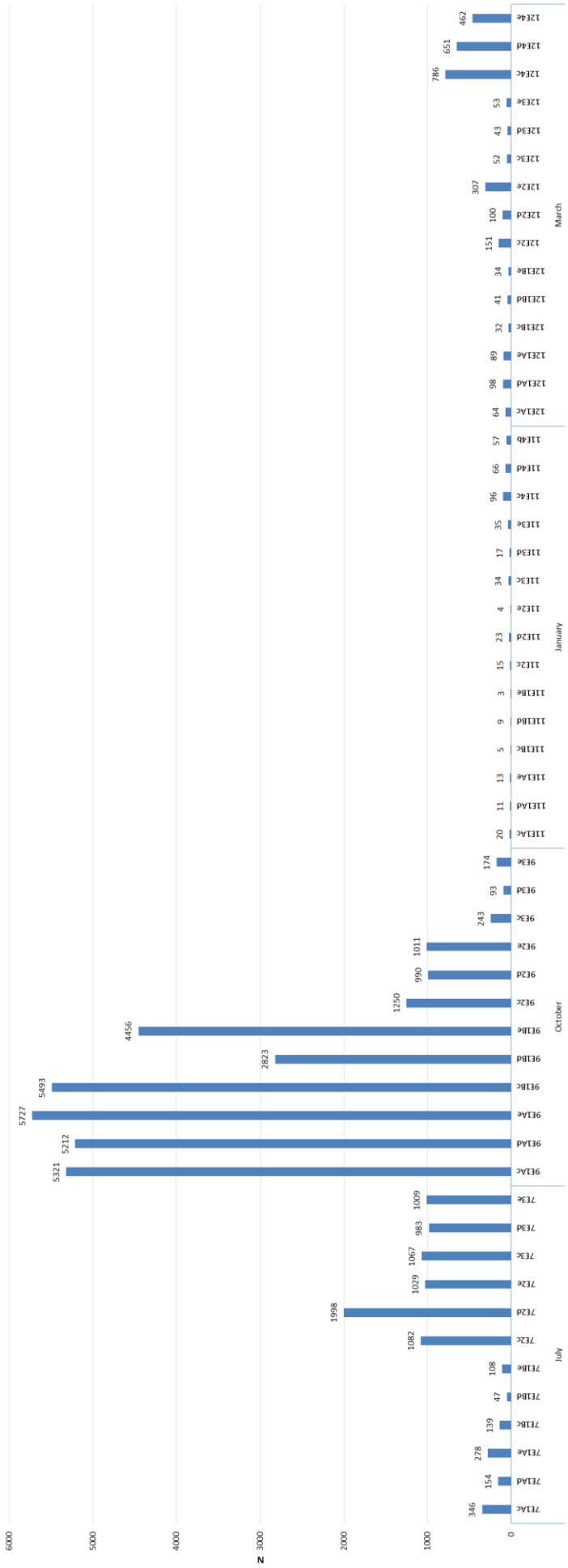


Fig. 15 - Total number of individuals (N) in 54 replicates at each station (leisure boats sector (E1A and E1B), fuel station sector (E2), port entrance (E3) and outside port area (E4)) of each month (July - campaign 7, October - campaign 9, January - campaign 11 and March - campaign 12) during the sampling campaigns at El Kantaoui Port.

In general, the replicates were quite homogeneous: only station E2 in July and station E1B in October showed big errors (Fig. 16 and Appendix 4). Therefore, since a consistent variability was observed in only 2 over 18 sampling stations, from this point on only the mean densities of individuals (ind/m^3) will be used for the following analyses. Mean densities were calculated as the ratio between the mean number of individuals (N) (Appendix 4) among replicates in each taxa and the volume of filtered water (m^3) at each station.

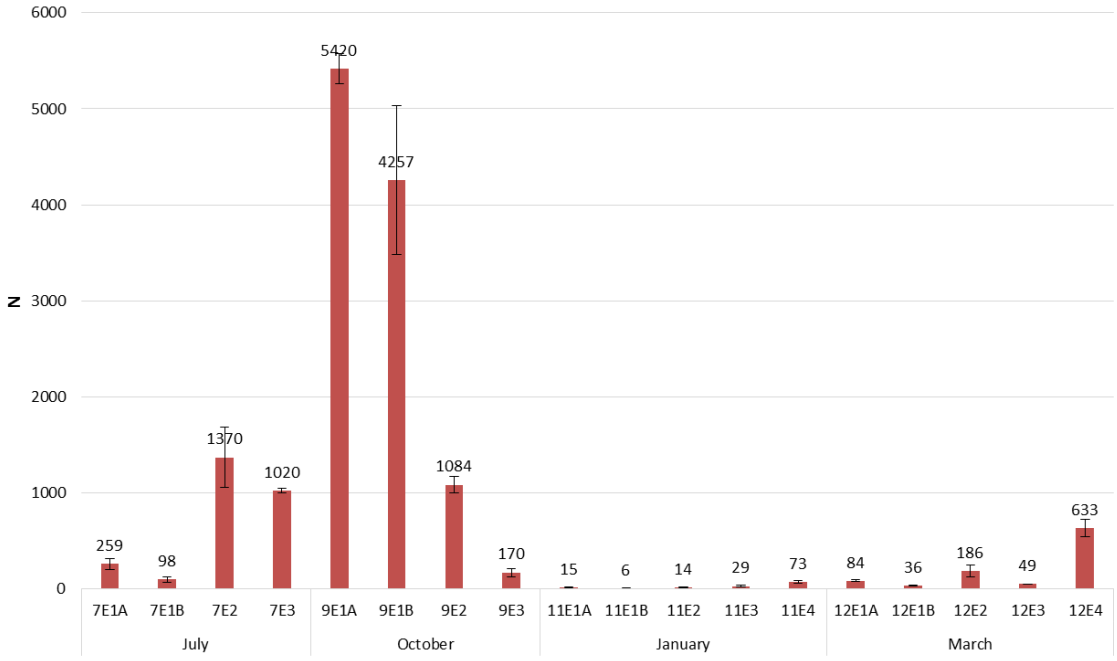


Fig. 16 - Mean of total number of individuals (N) (± standard error) of each replicate sample at each station (leisure boats sector (E1A and E1B), fuel station sector (E2), port entrance (E3) and outside port area (E4)) of each month (July - campaign 7, October - campaign 9, January - campaign 11 and March - campaign 12) during the sampling campaigns at El Kantaoui Port.

The mean densities of individuals (ind/m^3) (± standard error) at each station of the four months studied are shown in Appendix 4, Fig. 16 and Fig. 17. It is clear that the densities have the same pattern of the absolute numbers, being the depths at the different station in the port quite homogeneous. Fig. 17 also shows the proportion of Copepoda compared to the other taxa. The highest mean density of individuals recorded was in October with 14246±519.81 ind/m^3 at station E1A (leisure boats sector) and the lowest was recorded in January (19±5.77 ind/m^3) at station E1B (leisure boats sector) (Appendix 4).In July, the mean density of individuals was higher in the outer stations (E2 and E3) than in the inner stations (E1A and E1B) (Appendix 4). In this month the density of Copepoda was higher than the density of the other animals in the outside stations (E2 and E3) compared to the inner stations (E1A and E1B) (Fig.

17). In October the opposite pattern occurred, when at the inner stations the Copepoda percentage was higher than the density of the other animals (E1A and E1B) than at outside stations (E2 and E3) (Fig. 17). Since the mean number of individuals in January and March were lower than in July and October, the mean densities of individuals showed the same trend (Fig. 16 and Fig. 17). In January, the mean densities of Copepoda at stations E1B, E2, E3 and E4 were lower than the densities of the others animals. Only at station E1A the value of mean density of Copepoda were higher than the others animals (Fig. 17). The mean densities in this month were lower in the innerstations than at the outside stations (Appendix 4). In March, the Copepoda were less represented than the other animals at all the stations (Fig. 17). The stations with higher densities were station E2 (542 ind/m^3) and E4 (1400 ind/m^3) (Appendix 4 and Fig. 17).

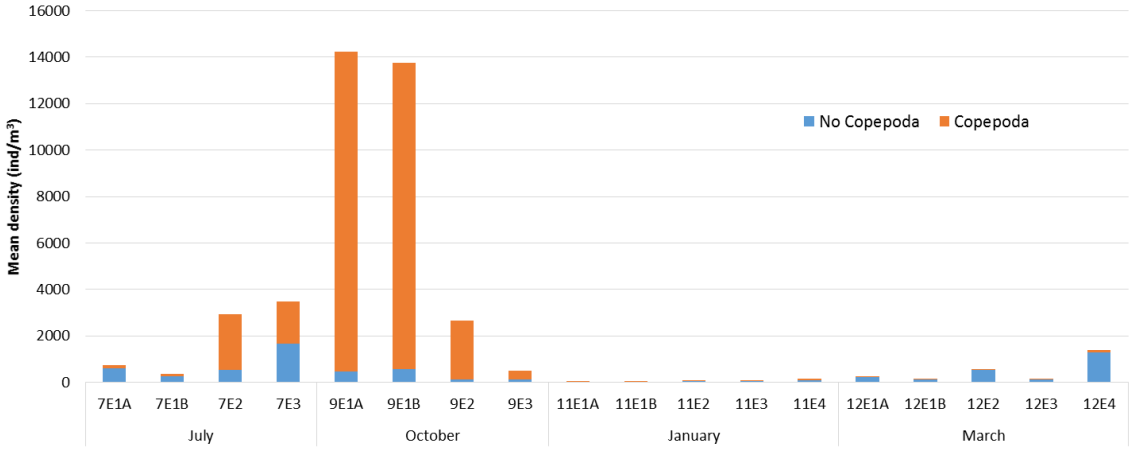


Fig. 17 - Mean densities of individuals (ind/m^3) present at each station (leisure boats sector (E1A and E1B), fuel station sector (E2), port entrance (E3) and outside port area (E4)) in the four months (July - campaign 7, October - campaign 9, January - campaign 11 and March - campaign 12) during the sampling campaigns at El Kantaoui Port. Each column of this figure were divided in two parts. The blue part of each column corresponds at the mean densities of zooplankton animals who are not included in Copepoda and the orange part of each column corresponds at the mean densities of zooplankton animals who are included in Copepoda.

In Fig. 18 the mean density values of each station (leisure boats sector (E1A and E1B), fuel station sector (E2), port entrance (E3) and outside port area (E4)) were summed and grouped by month and Copepoda were kept separated from the other taxa. As expected the mean density of Copepoda in July and October was higher than the mean density of individuals that are not included in the Copepoda. In January and Marchthe opposite distribution occurs (Fig. 18).

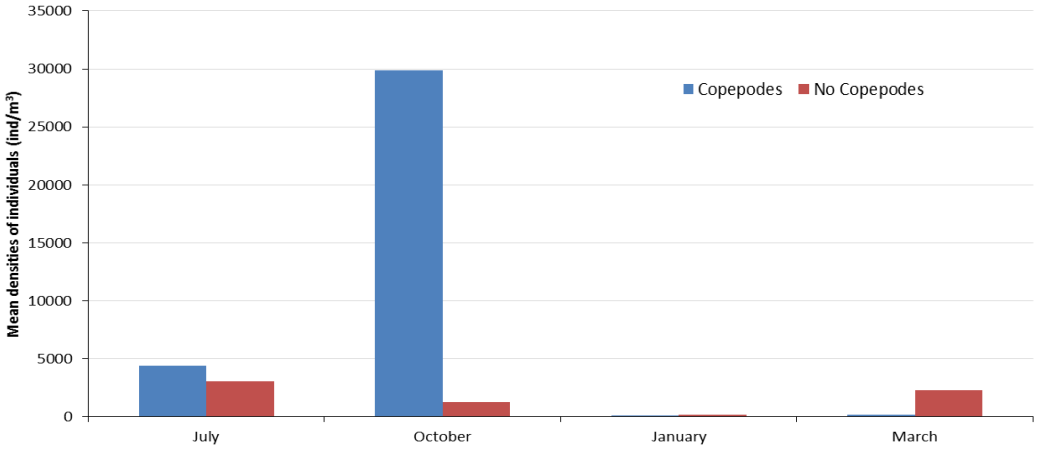


Fig. 18 – Mean density of Copepoda and not Copepoda (ind/m^3) in the four months (July - campaign 7, October - campaign 9, January - campaign 11 and March - campaign 12) during the sampling campaigns at El Kantaoui Port.

The taxa considered at the four/five stations of each month at El Kantaoui Port are presented in Appendix 5 and in Fig. 19. The highest mean number of taxa recorded was observed in July with 26 taxa at station E2 and the lowest was in January with a mean of 3 taxa at station E1B (Appendix 5 and Fig. 19). In July, the distribution of taxa was higher in the outer stations than in the inner stations (Fig. 19). In October, the mean number of taxa present at each station was almost the same (ranging from 18 to 19 taxa). In January and March, when the number of taxa was consistently reduced, the control station/outside station (E4) was the station where the highest mean number of taxa was observed (Fig. 19).

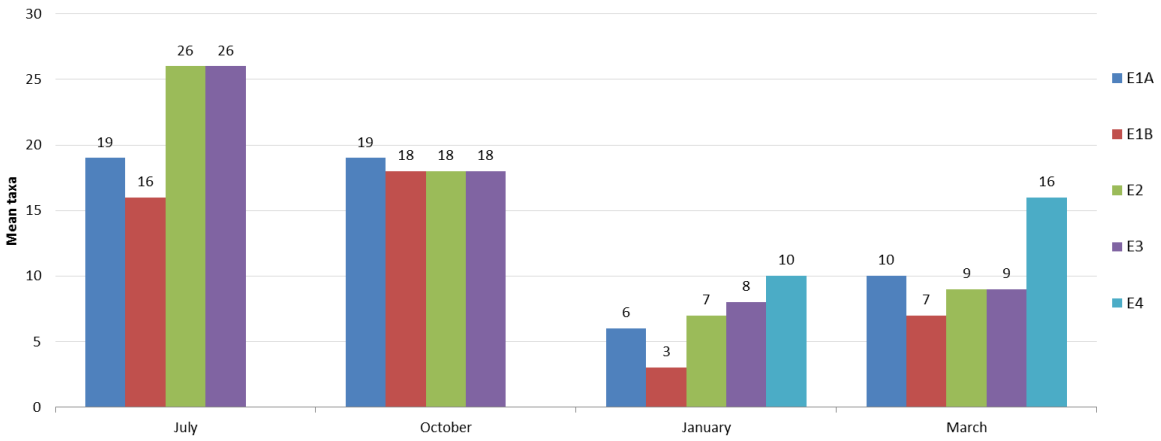


Fig. 19 - Mean number of taxa who were present at each station (leisure boats sector (E1A and E1B), fuel station sector (E2), port entrance (E3) and outside port area (E4)) in the four months (July - campaign 7, October - campaign 9, January - campaign 11 and March - campaign 12) during the sampling campaigns at El Kantaoui Port.

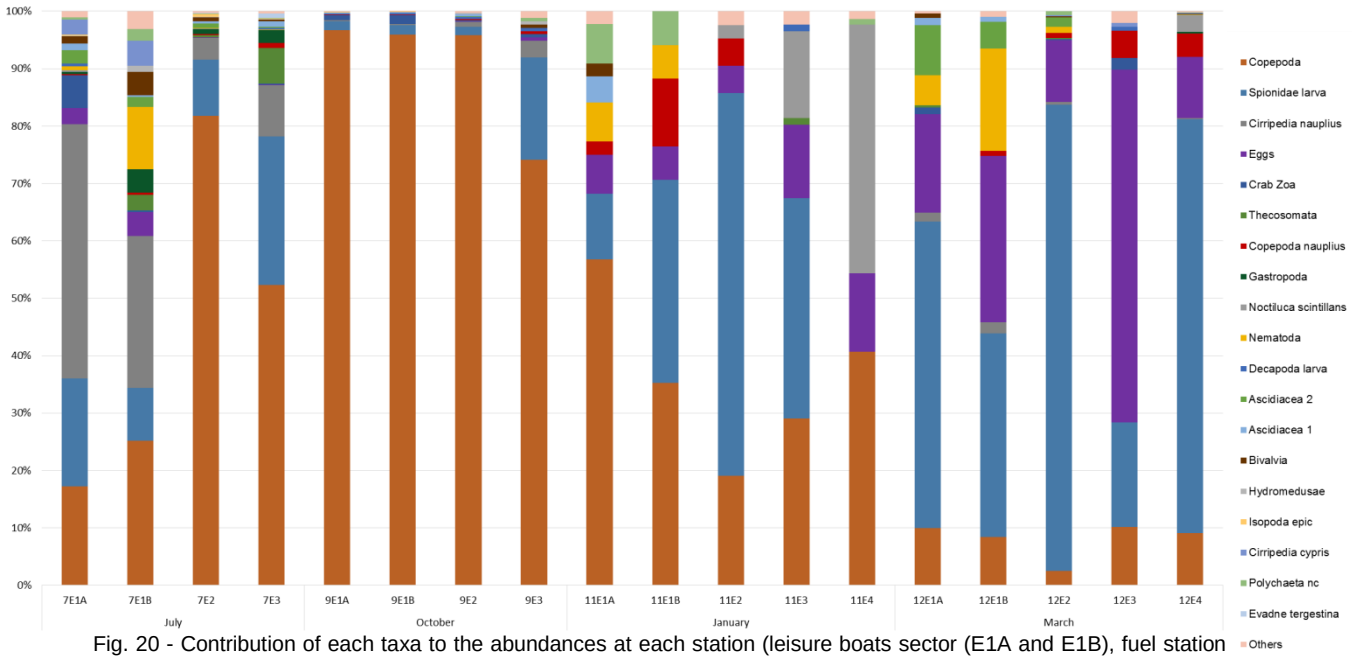


Fig. 20 - Contribution of each taxa to the abundances at each station (leisure boats sector (E1A and E1B), fuel station sector (E2), port entrance (E3) and outside port area (E4)) in the four months (July - campaign 7, October - campaign 9, January - campaign 11 and March - campaign 12) during the sampling campaigns at El Kantaoui Port. In this figure only was considered the taxa with a mean density of individuals superior than 20 ind/m^3 (approximately 5% of total mean densities). **Legend:** Isopoda epic – Isopod epicaridium larva ; Polychaeta nc – Polychaeta non identify.

In Fig. 20 the contribution of each taxa to the abundances at the four/five stations studied during the sampling campaigns are presented. To elaborate this figure (Fig. 20) only the taxa with a mean density of individuals superior than 20 ind/m^3 (approximately 5% of total mean densities) were considered to individuals series. The total contribution of the remaining taxa were grouped in a unique series (Others). The Ascidiacea were subdivided in two morphological groups: Ascidiacea 1 composed by *Styla* shape species (with small dimensions and a body with an elongated shape); Ascidiacea2 composed by *Botryllus* shape species with large dimensions and a body with a rounded shape.

In July, the high abundance of Cirripedia nauplii compared to Copepoda, Nematoda and Spionidae larvae was evident at the inner stations (E1A and E1B),nonetheless at outer stations (E2 and E3) the abundance of Copepoda was higher than Spionidae larvae and Cirripedia nauplii (Fig. 20).

At stations E1A, E1B and E2 in October the abundance of Copepoda was more than 90% and at station E3 was more than 70%. Crab zoea provided some relevant contribution to the abundances at all the stations (Fig. 20).

In January, Copepoda abundance was higher than Spionidae larva and Decapoda larva at station E1A, nonetheless at stations E1B and E2 the abundance of Spionidae larva was higher than Copepoda and *Noctiluca scintillans.* Outside the port (station E4) the highest abundance was of *Noctiluca scintillans* compared to Copepoda and Spionidae larva (Fig. 20).

In March, at stations E1A, E1B, E2 and E4 Spionidae larva dominated the community followed by the Ichthyoplankton and Copepoda. At station E3 the highest abundance was given by Ichthyoplankton compared to Spionidae larva and Copepoda (Fig. 20).

In Fig. 21 the contribution of each identified taxon of Copepoda to the abundances at the four/five stations studied during the sampling campaigns is presented.

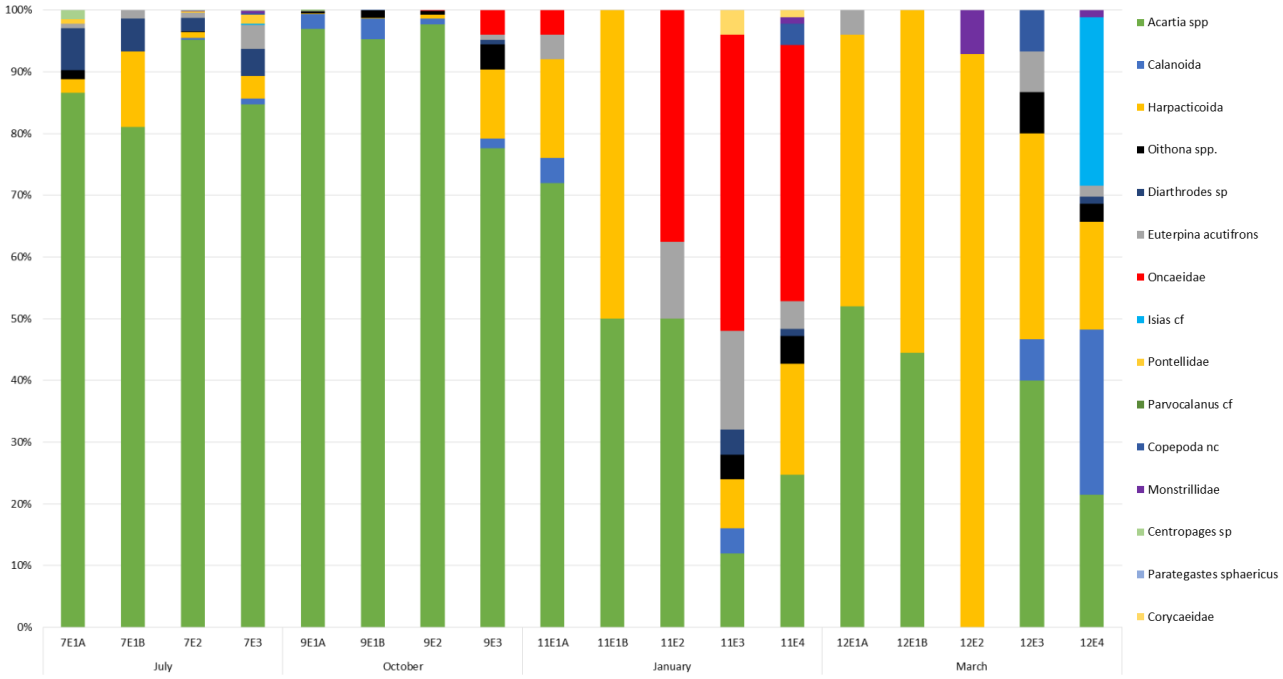


Fig. 21 - Contribution of each taxon of Copepoda to the abundances at each station (leisure boats sector (E1A and E1B), fuel station sector (E2), port entrance (E3) and outside port area (E4)) in the four months (July - campaign 7, October - campaign 9, January - campaign 11 and March - campaign 12) during the sampling campaigns at El Kantaoui Port. **Legend:** nc -not identified; cf – not certain identification.

In July and October, *Acartia spp*. dominated the community at all the stations. Moreover in July the contribution to the community abundance of the unidentified Haparticoida, and identified *Diarthrodes sp.* and *Euterpina acutifrons* was recorded at all the stations. On the other hand in October the community was a little changed and

the contribution of unidentified Calanoida, Haparticoida and *Oithona spp*. was recorded at all the stations (Fig. 21).

In January the high abundance of Acartia spp. compared to Haparticoida, Oncaeidae and *Euterpina acutifrons* was evident at the inner stations (E1A and E2), except at station E1B where the abundance of *Acartia spp.* and Haparticoida was comparable. At the outer stations (E3 and E4) the abundance of Oncaeidae was higher than *Acartia spp*. (Fig. 21).

In March, the abundance of *Acartia spp*. was higher at stations E1A and E3 compared to Haparticoida and at stations E1B and E2 the opposite distribution of abundances occurred since Haparticoida were the most abundant taxon. At station E4 *Isias cf.* was the most abundant taxon followed by Calanoida, *Acartia spp*. and Haparticoida.

In Fig. 22, 23 and 24 the results by the point of view of the feeding ecology are presented and the taxa were grouped in Carnivorous, Omnivorous and Suspension feeders.

In Fig. 22 the contribution of the Carnivorous taxa is presented at each station. In July Pteropoda were the main contributors to the total abundancies compared to Hydromedusae and Chaetognatha at stations E1B, E2 and E3, nevertheless at station E1A the abundance of Chaetognatha and Hydromedusae was of 50% for each (Fig. 22).

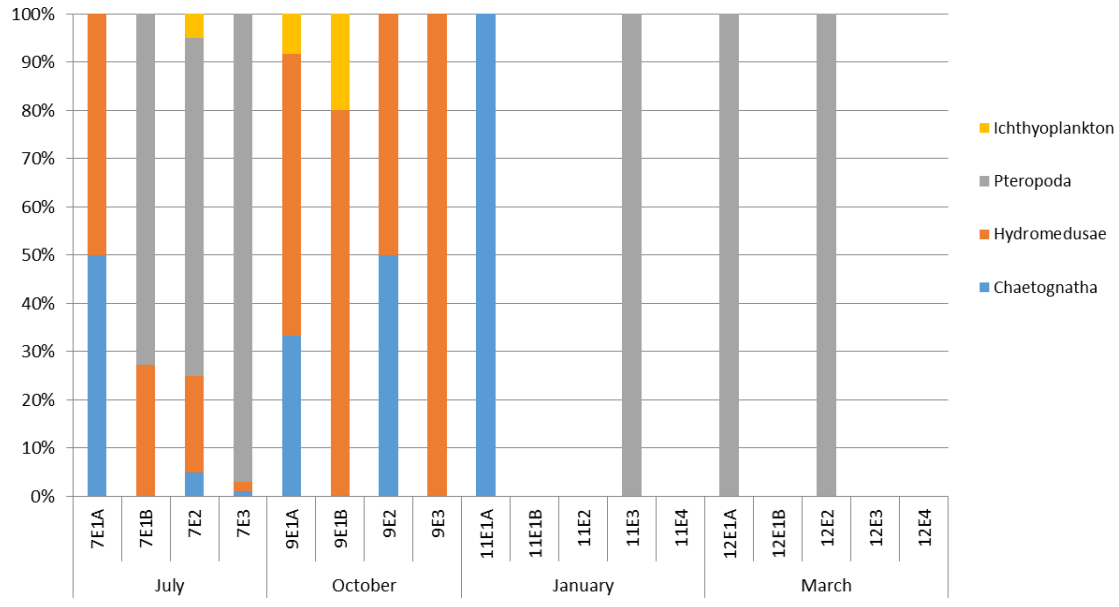


Fig. 22 - Contribution of the Carnivorous taxa to the abundances at each station (leisure boats sector (E1A and E1B), fuel station sector (E2), port entrance (E3) and outside port area (E4)) in the four months (July - campaign 7, October - campaign 9, January - campaign 11 and March - campaign 12) during the sampling campaigns at El Kantaoui Port. **Legend:** Ichthyoplankton – only considered the taxa Teleostei.

In October, Hydromedusae were the most abundant at all the stations, except at station E2 where the abundance was the same of Chaetognatha (50%). At station E1A in January only the abundance of Chaetognatha as carnivorous taxa was recorded and at station E3 only was recorded the abundance of Pteropoda. In March, the Pteropoda was recorded at stations E1A and E2 (Fig. 22) and it was the only carnivorous taxon recorded.

In Fig. 23 the contribution of omnivorous taxa to the abundances at each station is presented.

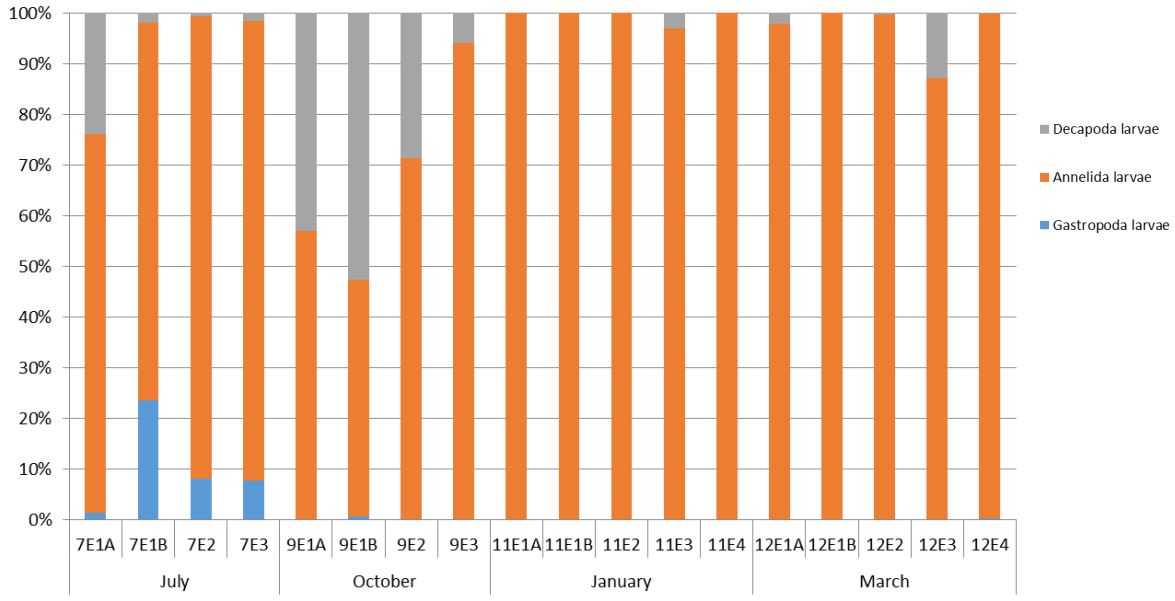


Fig. 23 - Contribution of the Omnivorous taxa to the abundances at each station (leisure boats sector (E1A and E1B), fuel station sector (E2), port entrance (E3) and outside port area (E4)) in the four months (July - campaign 7, October - campaign 9, January - campaign 11 and March - campaign 12) during the sampling campaigns at El Kantaoui Port.**Legend:** Gastropoda larvae , Annellida larvae - considering the taxon Spionidae larva, Sabellida and Polychaeta nc and Decapoda larvae - considering the taxon Decapoda larva, crab zoea and *Porcellana* sp.

The contribution of Annelida to the abundances of the omnivorous taxa in all the months was the highest compared to the others, except at the station E1B in October, where the highest contribution was of Decapoda larvae (Fig. 23). It can be observed that the Gastropoda larvae were present in a very low percentage in July compared to the other taxa in this group and the Decapoda larvae presented a higher contribution at October (Fig. 23).

In Fig. 24 it is presented the contribution of Suspension feeders taxa to the abundances in each station. In July at the inner stations, the highest contribution to the abundances of the suspension feeders taxa was of Cirripedia nauplii and cypris compared to Copepoda and at the outer stations the opposite trend occurred (Fig. 24). In October, the Copepoda dominated the contribution to the abundances in all the

stations followed by a few Cirripedia nauplii at the outer stations (Fig. 24). The same trend occurs in January, where the Copepoda dominated the contribution to the abundances at all the stations followed by Ostracoda only at station E3 (Fig. 24).

In March, the highest contribution to the abundances of the suspension feeders taxa was from Copepoda compared to Cirripedia nauplii and cypris, followed by Ostracoda at stations E1B and E3 (Fig. 24).

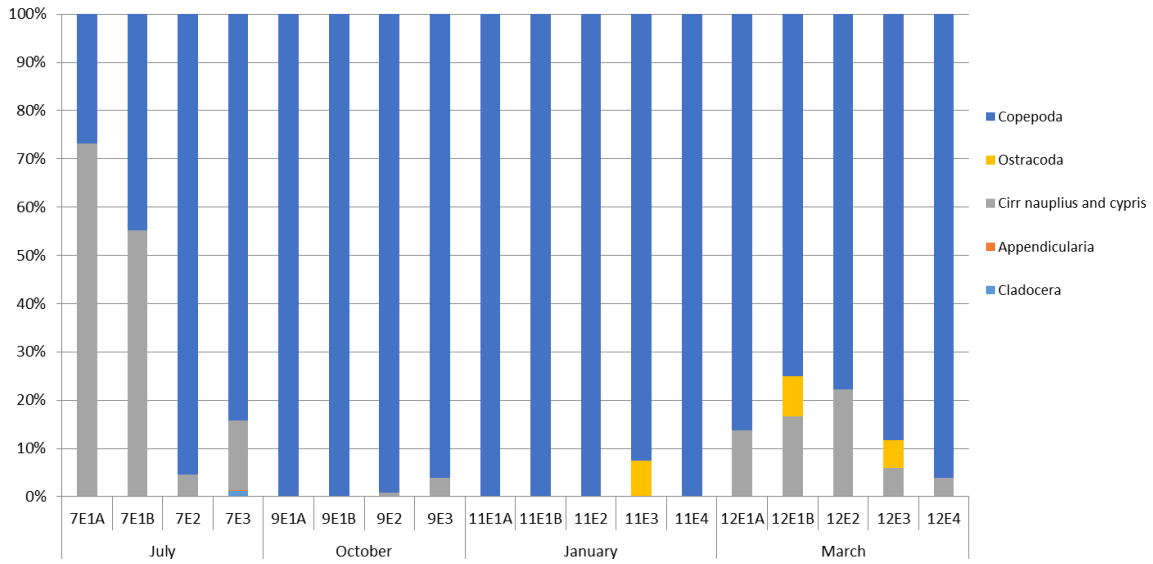


Fig. 24 - Contribution of the Suspension feeders taxa to the abundances at each station (leisure boats sector (E1A and E1B), fuel station sector (E2), port entrance (E3) and outside port area (E4)) in the four months (July - campaign 7, October - campaign 9, January - campaign 11 and March - campaign 12) during the sampling campaigns at El Kantaoui port. **Legend:**Cirr – Cirripedia; Cladocera – *Penilia avirostris* and *Evadne tergestina*.

In Fig. 25 the biodiversity indexes are represented as calculated from the mean densities (ind/m^3) at each station. As expected from the high abundances compared to the relatively low number of taxa, the Margalef Index (d) was lower in October at stations E1A and E1B and higher in July at stations E1A, E2 and E3. In January, the Pielou's evenness Index (J') was higher at station E1B. The Shannon (H') and Simpson Indexes (Lambda') were higher in July at station E1B (Fig. 25).

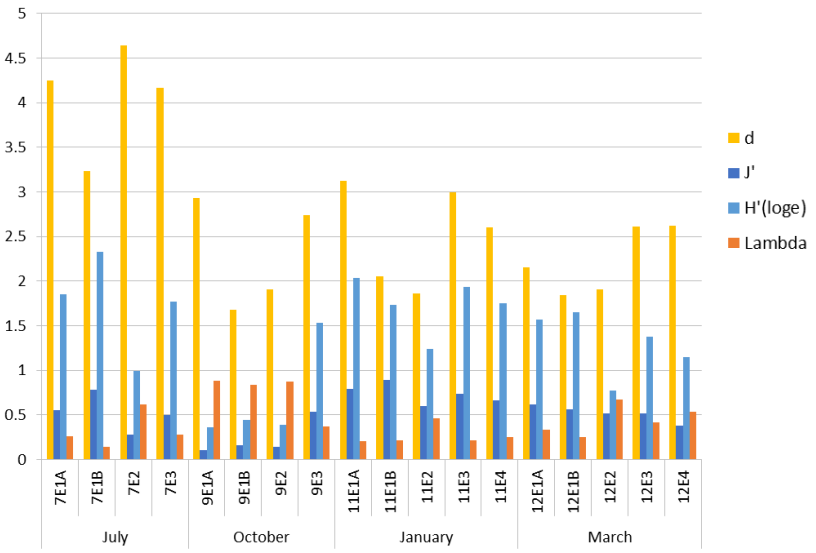


Fig. 25 - Biodiversity indexes calculated from the mean densities of individuals (ind/m^3) at each station (leisure boats sector (E1A and E1B), fuel station sector (E2), port entrance (E3) and outside port area (E4)) in the four months (July - campaign 7, October - campaign 9, January - campaign 11 and March - campaign 12) during the sampling campaigns at El Kantaoui Port. **Legend**: d - Margalef Index; J' - Pielou's evenness Index; H'(loge) - Shannon Index and Lambda' - Simpson Index.

To analyse the similarity between each station studied the Hierarchical Cluster analysis (CLUSTER, PRIMER 6) was performed starting from the resemblance matrix. The results are represented in Fig. 26.

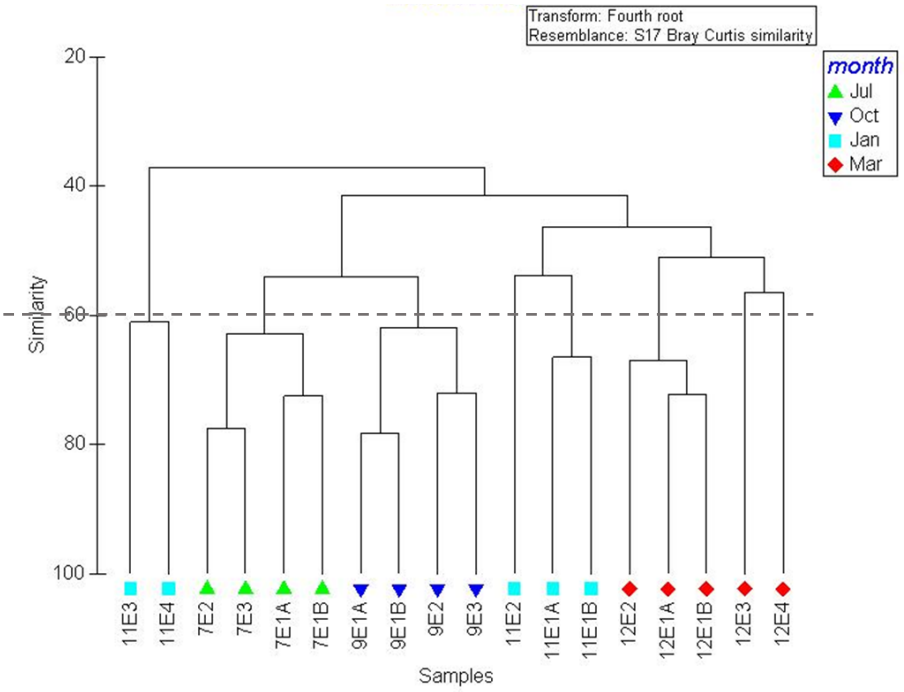


Fig. 26 –CLUSTER, *Hierarchical Cluster analysis*. Dendrogram representation of the dataset at each station (leisure boats sector (E1A and E1B), fuel station sector (E2), port entrance (E3) and outside port area (E4)) for month (July - campaign 7, October - campaign 9, January - campaign 11 and March - campaign 12) recorded during the sampling campaigns in El Kantaoui Port.

The Hierarchical Cluster analysis shows that the samples were grouped by month and that the communities in each month were similar up to the 60%. As expected the inner stations (E1A and E1B) were more similar between them than the outer stations (E2 and E3) and the outside station (E4) and vice versa (Fig. 26). Nevertheless, in January and March the outside station (E4), was more similar to station E3 and stations E1A, E1B and E2 were grouped together. July and October had a similarity of about 50%, and January and March up to 45% (Fig. 26).

The MDS analysis (PRIMER software) was performed to show the relative distances among the stations and the relative similarity/dissimilarity among them. The results are based on the similarity matrix as for the Cluster analysis and represent the same data in a different way (Fig. 27). The stress <0.15 (stress = 0.11) indicates an acceptable representation of the distribution of the data.

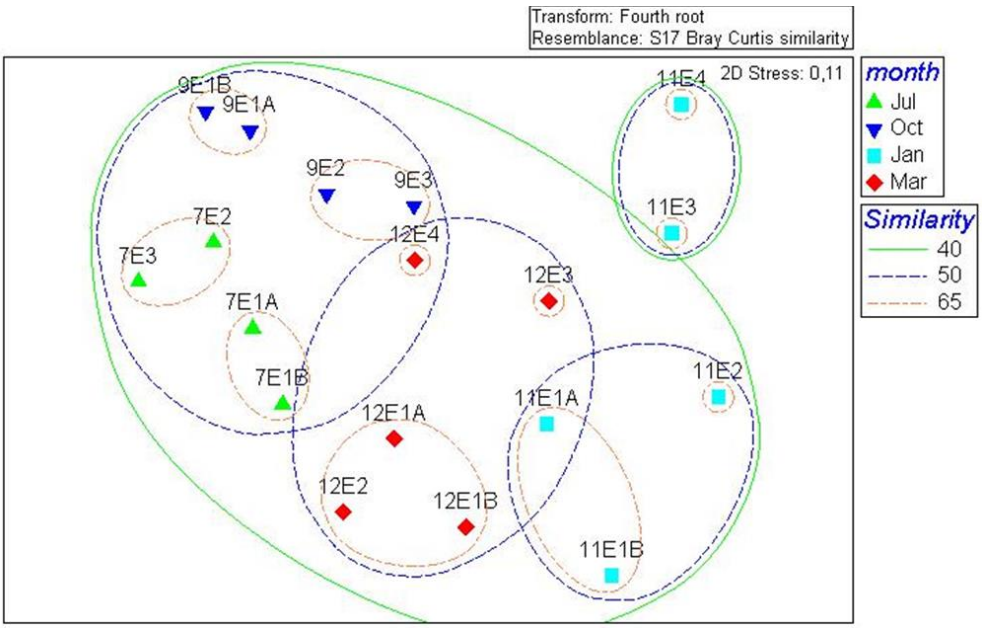


Fig. 27 – MDS, *Non-metric Multi-Dimensional Scaling*. Graphic representation of the relative distances among stations and the relative similarity/dissimilarity. Stress = 0.11.

The huge majority of stations was similar at 40% of similarity, except stations E3 and E4 in January (Fig. 27). These two stations in January show a dissimilarity at 65% between them. At 50% of similarity, occurs a monthly separation and a separation by inner and outer stations (Fig. 27). As expected, at 65% the inner stations (E1A and E1B) were more similar between them than the intermediate-outer stations (E2 and E3) and the outside station (E4) and vice versa, except in March where the stations E1A, E1B and E2 were similar to each other (Fig. 27).

The SIMPER test (PRIMER software) was performed to analyse the contribution of each taxa to the similarity/dissimilarity for month (Appendix 6). July was the month with the highest similarity among stations (mean similarity of 66.93%) and January was the month with the lowest similarity (mean similarity of 48.96%), so it was more diverse than the other months (Appendix 6). In July, October and January the taxa that gave the highest contribution to the similarity were *Acartia spp.* (in July with a contribution of 9.75%, October with a contribution of 21.75% and January with a contribution of 19.76%); in March the highest contribution was given by the Spionidae larvae with the 21.42% (Appendix 6). In July the next taxa with highest contribution to the similarity were Cirripedia nauplii and Spionidae larvae, in October Spionidae larvae and Calanoida (Appendix 6), In January Ichthyoplankton and Spionidae larvae and in March Ichthyoplankton and Haparticoida (Appendix 6).

The highest mean dissimilarity was between July and January (67.49% of dissimilarity), where the Cirripedia nauplius was the taxon with more contribution to this dissimilarity with 8.23%, followed by *Acartia spp*. (6.48%) and by Spionidae larvae (4.59%) (Appendix 7). The lowest mean dissimilarity was between July and October (45.96% of dissimilarity), where the *Acartia spp*. was the taxon that gave the biggest contribution to this dissimilarity with 8.97%, followed by Calanoida (5.35%) and *Oithona spp.* (4.46%) (Appendix 7).

In Fig. 28 the RELATE test (PRIMER software) is presented, with the goal of relating two superimposed resemblance matrices – Biotic and Abiotic matrices. The correlation of the similarity matrix of the biotic and abiotic data was evaluated and the result of the RELATE test was Rho = 0.493. The correlation between these two matrices presented some similarities, but not high enough to be statistically significant, since the Rho was lower than 0.6.

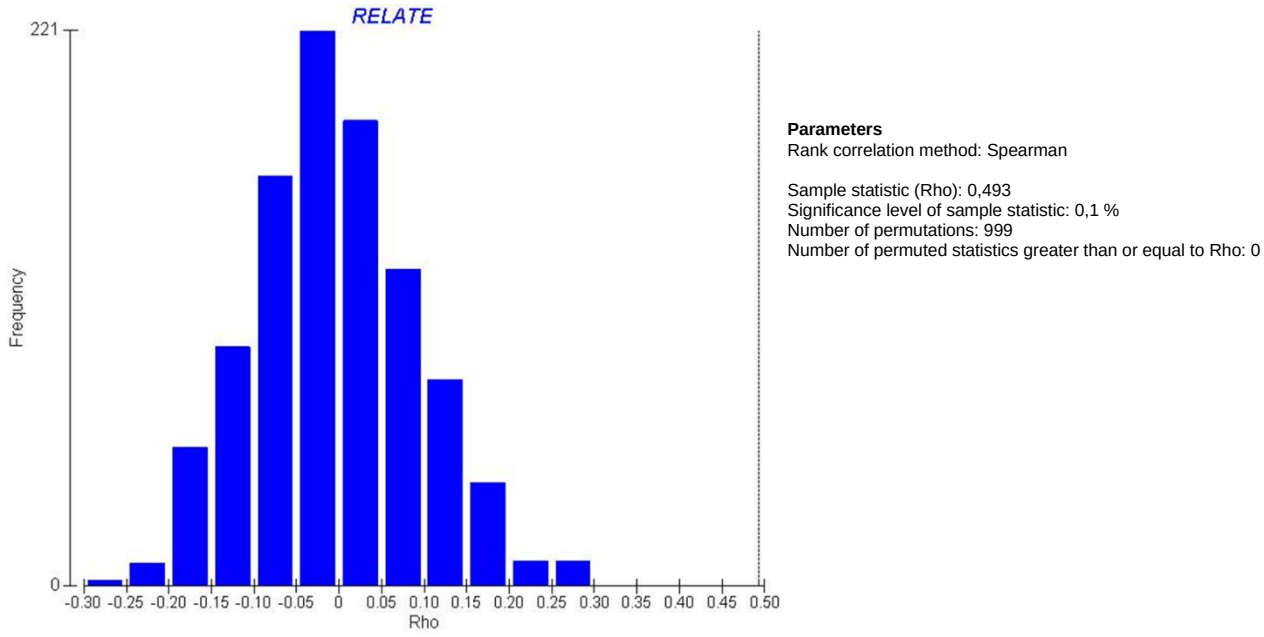


Fig. 28 – RELATE test, *Testing matched resemblance matrices*. Distribution of the Rho values calculate through the PRIMER software.

The DistLM test was performed through the PRIMER software to produce the most synthetic model resuming the most effective variables in shaping the biotic community. The results are presented in the Appendix 8 and Fig. 29. This test relates the biotic and environmental variables with a number of permutations, with the aim of predicting samples variation and explaining the selected variables. The DistLM test in this work was run selecting the AICc selection criterion and calculating R^2. The selected model with minor AICc (128.42) and significant R^2 (0.53642), shows that the water temperature, the pH and salinity were significant parameters in defining the community structure of the samples and confirmed the BEST analysis restricting the effect to three variables (Appendix 8).

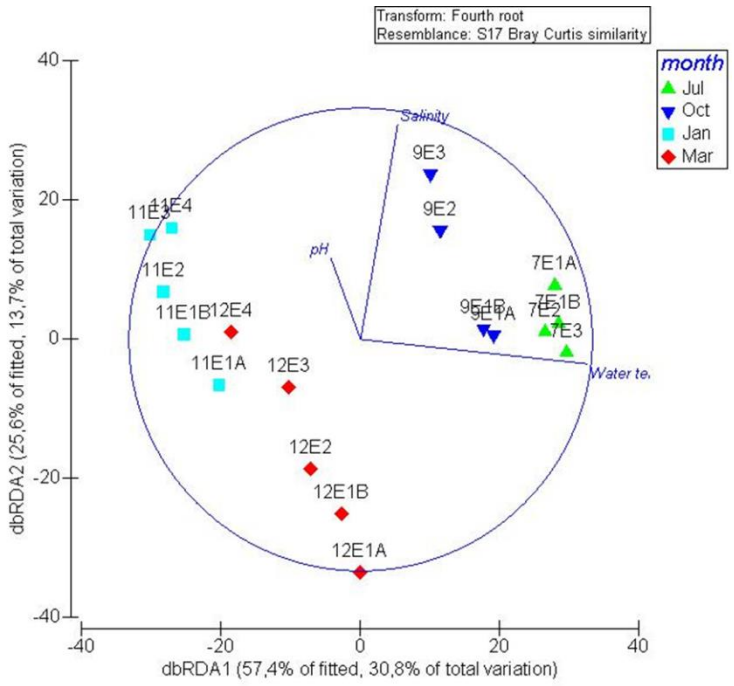


Fig. 29 – DistLM test, *Distance based linear models*. Graphic results of dbRDA performed through the PRIMER software.

The dbRDA provides a good representation of the DistLM data, since the first two axes graph is representing 82.93% of the variation of the model itself and explains the value that represents about 44.49% of the total variation in the similarity matrix (Appendix. 8). July was a month not much diverse, while October, January and March were more diverse (Fig. 29). The water temperature was the important environmental variable to the communities separating July and October from January and March and pH and salinity were important to the communities in separating the stations in each month (Fig. 29).

BEST analysis (Biota and/or Environment matching) in PRIMER was performed to inspect which was the 'best' match between the multivariate among-sample patterns of an assemblage and from environmental variables associated with those samples. The extent to which these two patterns match, reflects the degree to which the chosen abiotic data 'explains' the biotic pattern (Clarke, 1993). These results confirm the results of dbRDA. The environmental variables who better explains the biotic pattern were: water temperature (°C), pH, water salinity (‰), dissolved oxygen (mg/L) and oxygen saturation (%) (Appendix 9).

Through the PRIMER software also the PERMANOVA test was performed and the results are presented at Appendix 10. This test connects factors with the matrix of similarity of biotic data. The factors selected were the month (July, October, January

and March) and the distance of the stations from the port entrance (high distance at stations E1A and E1B, medium distance at station E2, low distance at station E3 and open sea station E4). The results show that the month was the factor statically most significant (Pseudo-F = 10.76, *p* = 0.001) compared to distance (Pseudo-F = 4.2426, *p* = 0.015) that was also significant. The interaction between the two factors resulted significant also (month x distance, Pseudo-F = 2.2778, *p* = 0,006) (Appendix 10).

The Copepoda swarms observed in October and the presence of few individuals in January and March can be explained by the levels of chlorophyll-a. Acartia spp. was the copepod with highest abundances (Fig. 21). The curves of monthly variation of Acartia spp. (ind/m^3) and chlorophyll-a (mg/m^3) at each station are represented in Fig. 30.

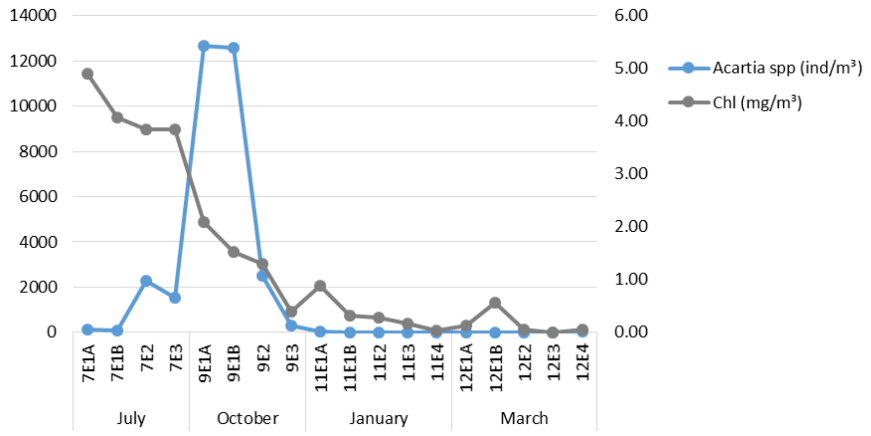


Fig. 30 - Curve of month variation of Acartia spp. (ind/m^3) **(blue line)** and chlorophyll-a (mg/m^3) **(grey line)** at each station (leisure boats sector (E1A and E1B), fuel station sector (E2), port entrance (E3) and outside port area (E4)) of El Kantaoui Port during the sampling campaigns (July-campaign 7, October-campaign 9, January-campaign 11 and March-campaign 12).

At the inner stations in July the abundance of *Acartia spp*. was lowest than in the intermediate and outer stations and the opposite trend was observed in the levels of chlorophyll-a, which were higher at the inner stations (E1A and E1B) than at the outer ones (E2 and E3) (Fig. 30).

In October, there was an *Acartia spp.* peak at stations E1A and E1B and this matched with a decrease of the levels of chlorophyll-a (Fig. 30). At the outer stations (E2 and E3) the levels of chlorophyll-a and the abundance of *Acartia spp*. were comparable. In this month the levels of chlorophyll-a and the abundance of *Acartia spp*. decreased from the inner stations to the outer stations (Fig. 30).

In January and March some fluctuations were observed: the levels of chlorophyll-a and the abundance of *Acartia spp.* were very low, except at stations E1A

in January and E1B in March, where the levels of chlorophyll-a were higher than in other stations (Fig. 30).

Discussion

In order to investigate the environmental effects on the water ecosystems through different stations of the Port area it was needed to analyse both the physico-chemical and biological factors of the water samples. A total amount of 54 samples (collected in July and October 2014 and January and March 2015) were analysed, and they included the zooplankton communities and relative water physico-chemical factors in the four seasons (summer and autumn 2014 and winter and spring 2015). The analysis of the zooplankton communities in the four seasons allowed us to observe a variation of the communities at each station and a seasonal pattern, thus contributing to the aim of this thesis.

The environmental variables measured during this study, such as water temperature and water salinity presented a seasonal variation, with higher values in the inner stations in summer and in the outer station in autumn (Fig. 13A and Fig. 13B), and as expected (such in the study of Guermazi *et al*., 2012) appeared to affect the zooplankton communities in the port (Fig. 14). The lower water salinity in winter and spring can be due to the flow of freshwater (rain) from the inland, while the higher levels of water salinity in summer and autumn can be due to the effect of high temperatures in summer, inducing water evaporation and to the low inflow of freshwater during these seasons (Borghini et al., 2014).

The pH recorded in the four seasons was around 7-8 (Fig. 13C) and according to Brett (1989) these values did not affect zooplankton communities.

The dissolved oxygen and the oxygen saturation had the same trend (Fig. 13D, 13E). These two variables appeared to affect in the same way the zooplankton communities at the outer stations in winter and the inner ones in spring (Fig. 14). At the outer station E4 in winter and spring, the values of oxygen saturation were higher than 100%; this means that in those stations there was oxygen production likely due to algal production and wave action (Fig. 13E). These two parameters had the opposite trends of the water temperature and salinity (Fig. 13A, 13B, 13D, 13E). These results confirm literature findings: when water temperature and salinity are lower, the dissolved and saturated oxygen in the water are higher (Borghini et al., 2014) whereas the

consumption by higher abundances of zooplankton can further reduce the values of both parameters in summer and autumn (Fig. 16). Moreover, phosphorus and DIN contribute to enhance algal growth and subsequent decomposition reduces oxygen availability to sea creatures (NASA, 2016). In Fig.13D, 13E, 13F it is possible to observe the reduction of oxygen availability in comparison with highest values of DIN in autumn. However, the PO_4^{3-}recorded values had higher expression at all the stations in winter (except at the outer station – E4) and at station E1A in spring (Fig. 13G and Fig. 14). According to Oram (2014) these higher values can be due to runoff from agricultural sites and application of some lawn fertilizers that in the study area can mainly derive from the maintenance of the extended golf club nearby the port. Phosphate stimulate the growth of plankton and chlorophyll-a that are PO_4^{3-} consumers (Oram, 2014), so this fact can explain the lower values observed in summer and autumn, when the abundance of zooplankton community was higher (Fig. 16). The decrease of chlorophyll-a from summer to autumn and winter matches with the natural cycles of phytoplankton in coastal waters and with the presence of swarms of copepods (*Acartia spp*, grazers) in summer and autumn (Fig. 13H and Fig. 17). According to Ambler (2002) high concentrations of phytoplankton increase the swarms densities of copepods.

According to Johannes & Webb (1970) zooplankton communities may release significant amounts of DOC and Webb & Johannes (1967) estimated that marine zooplankton could release the equivalent of the dissolved free amino acids present in the water during one month. In fact, the highest values of DOC were found in winter, a season that follows two seasons with high abundances of zooplankton (summer and spring) (Fig. 13I and Fig. 15).

Comparing the zooplankton with environmental factors, we observed that the zooplankton communities sorted varied significantly together with physico-chemical parameters among the different seasons (Fig. 13A-I and Fig. 15). On seasonal scale and unlike other Mediterranean areas, two zooplankton peaks were recorded (Kamburska & Fonda-Umani, 2009; Drira et al., 2014). The higher mean density of individuals was observed during summer and autumn, and was mainly due to the presence of swarms of copepods at all the stations (Fig. 17). These swarms were mostly constituted by *Acartia spp*. (including all copepodid stages with adults being the predominant stage) (Fig. 21). As observed by Emery (1968) and confirmed by other authors (Ueda et al., 1983; Aleya, 2015) this can be explained by the fact that these copepods form swarms only during the day and disperse at night and are enhanced by the environmental factors. According to Ambler (2002), the proposed zooplankton

swarming is usually hypothesized by the high local availability of food. In summer and autumn, the mean density of Copepoda was higher than the mean densities of the other animals and explained the higher mean densities of individuals in the community analysed (Fig. 17 and Fig. 18). The opposite trend occurred in winter and spring (Fig. 17 and Fig. 18.

In all the seasons studied, the intermediate and outer stations (E2, E3 and E4) had higher mean numbers of taxa observed than the inner stations (E1A and E1B), except in autumn where mean number of taxa in all the stations was almost equal (Fig. 19). Nonetheless, observing the distributions of Fig. 20 it is possible to note that in all the seasons at inner stations only few taxa gave a high contribution to the abundances than compared to outer stations, that means lower evenness. The diversity indexes (Fig. 25), showed a higher species richness (Margalef Index) in summer and a higher evenness (Pielou's evenness Index) in winter. Amphipods, mysids, ostracods, spionid larvae, *Noctiluca scintillans* and ichthyoplankton exhibited an increase in abundance, reaching a maximum in winter and spring, most likely due to exploitation of the phytoplankton (Fig. 20) (Dhib et al., 2015).

Observing the distributions in Fig. 21, it was possible to note that among Copepoda, *Acartia* gave in general a big contribution to their abundance in the four seasons, and was the principal responsible of the swarms. According to Dhira et al. (2009), *Acartia* exhibits a high spectrum of distribution in the Mediterranean Sea and it was found in high numbers in other Mediterranean ecosystems (Blanc et al., 1975; Benon et al., 1976; Calbet et al., 2001) and coastal waters. Other studies indicated that *Oithona* dominated in summer in the Bay of Blanes (coastal north-western Mediterranean Sea) (Calbet et al., 2001) and in the Tunis North Lagoon (Annabi-Trabelsi et al., 2005). Haparticoida, Calanoida, *Oithona*, *Diarthrodes* and *Euterpina acutifrons*, Oncaeidae and *Isias* gave a relevant contribution to the abundances of Copepoda in the studied seasons (Fig. 21). All these taxa found in our samples are typical, with different frequencies, of Mediterranean coastal waters. If we consider the El Kantaoui port a HMWB, the expectation was to find no rich communities in the samples, nevertheless we found zooplankton communities that may be comparable to coastal zooplankton communities for abundances and diversity (Larink & Westheide, 2011).

The zooplankton community was also characterized by the point of view of the feeding ecology (Fig. 22, 23 and 24; carnivorous taxa, omnivorous taxa and suspension feeders taxa respectively). The contribution of carnivorous taxa to the

abundances at stations E1B, E2 and E3 in summer, E3 in winter and E2 in spring was characterized by the abundance of Pteropoda (Fig. 22). The higher abundance of Pteropoda in these stations can be due to their reproductive cycle. According to Dadon & Cidre (1992), the abundance in summer and spring can be associated with the reproductive season and in winter with the development season (Fig. 22). Hydromedusae were observed in summer and at all the stations in autumn. At station E1A in summer, they had a similar contribution than Chaetognatha (Fig. 22). Hydromedusae are warm-season species with a hot temperature affinity, so this fact can explain these contributions to the abundances in summer and autumn (Fig. 13A and Fig. 22) (Goy, 1991). Chaetognata are predators of copepods (Brusca & Brusca, 2003; Margulis & Chapman, 2010; Ramel, 2012; Shapiro, 2012) and were recorded in summer and autumn, when the presence of copepods was higher (Fig. 20, Fig. 22 and Appendix 5). The omnivorous taxa were mostly represented by Annelida larvae (Fig. 23). The observed taxa are able to tolerate great variations of temperature, salinity and survive drastic conditions of hypoxia (Scaps, 2002). The Decapoda larvae gave high contribution to the abundances in autumn (Fig. 23). According to Colloca (2009), this season is the spawning season of Decapoda. The contribution of the suspension feeders taxa to the abundances at each station in general was represented by Copepoda, as reported above (Fig. 24). The low abundance of Cirripedia nauplii and cypris may be explained by different factors as high salinity, depth, stratification and limited connection with the open sea, which may all be considered stress factors that act directly upon the development and the survival of nauplii (Berger, 2004; Berger et al., 2006).

The analyses performed trough the PRIMER software on zooplankton communities, and the results obtained by Hierarchical Cluster analysis (Fig. 26) and MDS analysis (Fig. 27) show that samples are grouped by season and in each season stations have a gradient through the port except in winter. In all the seasons, the inner stations E1A and E1B were very similar, as it was expected because E1B was chosen as control of E1A. Furthermore, as expected, the intermediate and outer stations (E2, E3 and E4) were more similar among them than with the inner stations (including the station E2 in winter and spring). This can be explained by the different characteristics of the stations studied (such as the proximity to the entrance of the harbour) and by the composition of the zooplankton community at each station (Fig. 26 and Fig. 27). Summer, autumn and the station E4 in spring had 50% of similarity, as all the stations in spring and the station E1A in winter (Fig. 27). According to the results of the SIMPER test, all the stations in summer had the highest mean similarity among them,

followed by autumn (Appendix 6). In summer, autumn and winter the highest contribution was given by *Acartia* and in spring by spionid larvae (Appendix 6). In winter, the inner and intermediate stations (E1A, E1B and E2) and the stations closer to the port entrance or outside the port (E3 and E4) had less than 40% of similarity. This similarity can be due to the high abundances of Cirripedia nauplii and ichthyoplankton at stations E3 and E4 in comparison with the inner stations in spring, where they had high abundances of spionid larvae (Fig. 20, Fig. 27 and Appendix 6). The results of MDS analysis (Fig. 27) are therefore explained by the lower mean similarity among stations obtained with the SIMPER test (Appendix 6). In other words, winter and summer had the highest dissimilarity and summer and autumn had the lowest (Appendix 7). Nonetheless, spring had a dissimilarity superior than 50% with the other seasons.

Through the results of DistLM test and BEST analysis it was possible to resume which were the most effective variables in shaping the biotic communities (Fig. 28, Fig. 29, Appendix 8 and Appendix 9). DistLM data presented at dbRDA (Fig. 29) show that in the seasons with highest densities of individuals (summer and autumn) (Fig. 17) water temperature was the environmental variable mostly affecting the communities and separating summer and autumn from winter and spring; pH and salinity affected the communities and separated the stations in a gradient in each month. In winter and spring the stations were more diverse than in summer and autumn (Fig. 29). Comparable results for the seasonality were found by Dai et al. (2014), where they noted that the zooplankton communities were correlated with water temperature.

The Copepoda swarms observed in autumn and the presence of few individuals in winter and spring can be explained by the levels of chlorophyll-a and therefore by the seasonality and temperature variation (Fig. 30). *Acartia* that is a grazer, was indeed the copepod mostly contributing to the high abundances (Fig. 21). In other studies it was noted that the food availability may have influenced zooplankton distribution, in species such as the copepod *Acartia clausi* (Boucher et al., 1987; Drira et al., 2010; Estrada et al., 2012) and Neila et al. (2012) had recorded that *Acartia clausi* was significantly correlated with chlorophyll-a. These can confirm the seasonal parallel trend of abundances of phytoplankton and suspension feeders or grazers.

On the other hand no clear difference among the stations was highlighted. The gradient from the inner to the outer stations can be explained by the low hydrodinamicity of the port (that can be observed also through the gradient of oxygen

concentration) and by the progressive similarity of the stations from inside to outside the port, with the open sea due to some nutrients accumulation in specific seasons.

Conclusions

The seasonal diverse compositions of the zooplankton communities and their densities from July 2014 to March 2015 in El Kantaoui port can be due to many factors. Within this study it was possible to observe a seasonality of the zooplankton communities. The zooplankton communities found on the samples were comparable to coastal zooplankton communities. Furthermore, it was possible to note a gradient of abundance and diversity of the communities on the different stations of the harbour from the isolated inner to the outer stations (near of the open sea), possible due to the low water circulation and by the presence of nutrients that concentrate in a specific season.

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Appendix

Appendix 1 – Protocol used to register the data from each replicate of this study at El Kantaoui Port.

Campione		Data	
Appendicolaria (Oikop.)			
Cladocera ( )			
Polychaeta			
Cirripeda larva			
Mollusca	Ostracoda		
Copepoda (Calan+Cyclop)			
Copepoda (Harpactic)			
Nauplii			
Echinoderma larva			
Medusa	Ascidia larva		
Crostacea larva Decap.	Foraminifera		
Crostacea larva Zoa	Chaetognata		

Appendix 2 – CD-Rom with the electronic database created with the results of this study performed through the Microsoft Office Excel software.

Appendix .3 – Results of the PCA analysis (*Principal Component Analysis*) performed through the PRIMER software

Eigenvectors

Variable	PC1	PC2	PC3	PC4	PC5
Water temp	-0,290	**-0,434**	0,210	-0,308	-0,111
pH	0,294	**-0,425**	0,182	0,052	0,418
Salinity	-0,314	-0,117	-0,028	0,628	0,307
Dissolved O_2	**0,418**	0,175	0,050	0,172	0,154
O_2 saturation	**0,426**	-0,029	0,235	0,053	0,167
DIN	-0,339	0,077	-0,584	0,039	0,110
PO_4	0,034	**0,607**	0,272	0,100	-0,334
Chl	-0,335	-0,164	0,542	-0,016	-0,240
DOC	-0,328	0,232	0,374	0,346	0,312
Depth	0,198	-0,363	-0,125	0,587	-0,624

Appendix 4 – Table with data of the total number of individuals (N) in each replicate sample in the four/five (leisure boats sector (E1A and E1B), fuel station sector (E2), port entrance (E3) and outside port area (E4)) of each month (July, October, January and March) during the sampling campaigns at El Kantaoui Port.

Season	Station / Replicate	Total number of individuals (N)	Mean of total number of individuals (N) / Station	Standard error
July	7E1Ac	346		
	7E1Ad	154	259	56.21
	7E1Ae	278		
	7E1Bc	139		
	7E1Bd	47	98	27.02
	7E1Be	108		
	7E2c	1082		
	7E2d	1998	1370	314.54
	7E2e	1029		
	7E3c	1067		
	7E3d	983	1020	24.83
	7E3e	1009		
Ocotober	9E1Ac	5321		
	9E1Ad	5212	5420	148.87
	9E1Ae	5727		
	9E1Bc	5493		
	9E1Bd	2823	4257	925.55
	9E1Be	4456		
	9E2c	1250		
	9E2d	990	1084	252.57
	9E2e	1011		
	9E3c	243		
	9E3d	93	170	33.07
	9E3e	174		

Season	Station / Replicate	Total number of individuals (N)	Mean of total number of individuals (N) / Station	Standard error
January	11E1Ac	20		
	11E1Ad	11	15	2.73
	11E1Ae	13		
	11E1Bc	5		
	11E1Bd	9	6	1.76
	11E1Be	3		
	11E2c	15		
	11E2d	23	14	5.51
	11E2e	4		
	11E3c	34		
	11E3d	17	29	5.84
	11E3e	35		
	11E4c	96		
	11E4d	66	73	11.79
	11E4b	57		
March	12E1Ac	64		
	12E1Ad	98	84	10.17
	12E1Ae	89		
	12E1Bc	32		
	12E1Bd	41	36	2.73
	12E1Be	34		
	12E2c	151		
	12E2d	100	186	62.27
	12E2e	307		
	12E3c	52		
	12E3d	43	49	3.18
	12E3e	53		
	12E4c	786		
	12E4d	651	633	93.96
	12E4e	462		

Appendix 5 – Table with data of the taxa considered and mean density (ind/m^3) at each stations (leisure boats sector (E1A and E1B), fuel station sector (E2), port entrance (E3) and outside port area (E4)) of each month (July, October, January and March) during the sampling campaigns at El Kantaoui Port.

		July				October				January					March				
Phylum/Class	**Taxa**	**7E1A**	**7E1B**	**7E2**	**7E3**	**9E1A**	**9E1B**	**9E2**	**9E3**	**11E1A**	**11E1B**	**11E2**	**11E3**	**11E4**	**12E1A**	**12E1B**	**12E2**	**12E3**	**12E4**
Dinophyceae	*Noctiluca scintillans*	2	0	1	0	0	0	0	0	0	0	1	12	65	0	0	0	0	41
Hyrozoa	Hydromedusae	1	4	3	5	14	13	5	3	0	0	0	0	0	0	0	0	0	0
Hyrozoa	*Obelia sp*	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hyrozoa	Cnidaria nc	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Nematoda	Nematoda	5	39	4	5	1	3	0	0	3	1	0	0	0	14	23	6	0	1
Platyhelminthes	Müller larva	2	5	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Plagiorchiida	*Fellodistomidae cercaria*	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Palaeonemertea	Palaeonemertea	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bivalvia	Bivalvia	9	14	21	8	0	0	0	3	1	0	0	0	0	2	0	1	0	1
Gastropoda	Gastropoda	3	14	26	77	0	3	0	0	0	0	0	0	0	0	0	0	0	3
Gastropoda	Thecosomata	0	10	10	217	0	0	0	0	0	0	0	1	0	1	0	1	0	0
Gastropoda	Mollusca nc	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Polychaeta	Spionidae larva	137	33	286	901	224	223	40	90	5	7	29	30	0	140	47	440	27	1010
Polychaeta	*Sabellida*	1	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Polychaeta	Polychaeta nc	3	7	2	3	1	0	0	3	3	1	0	0	1	0	0	4	0	1
Ostracoda	Ostracoda	0	0	0	0	1	0	0	0	0	0	0	2	0	0	1	0	1	0
Cladocera	*Evadne tergestina*	0	0	4	26	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cladocera	*Penilia avirostris*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Copepoda	*Oithona*	2	0	1	0	42	160	16	15	0	0	0	1	3	0	0	0	1	4
Copepoda	Calanoida	0	0	7	18	340	437	24	6	1	0	0	1	0	0	0	0	1	34
Copepoda	*Parvocalanus cf*	0	0	0	0	17	0	0	0	0	0	0	0	0	0	0	0	0	0
Copepoda	*Centropages sp*	2	0	4	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Copepoda	*Acartia spp*	109	72	2273	1540	13337	12587	2489	283	19	3	4	3	15	14	5	0	6	27
Copepoda	Oncaeidae	0	0	1	0	3	0	3	15	1	0	3	11	25	0	0	0	0	0
Copepoda	Harpacticoida	3	11	23	66	15	19	16	41	4	3	0	2	11	12	6	13	5	22
Copepoda	*Parategastes sphaericus*	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0
Copepoda	*Euterpina acutifrons*	1	1	18	72	11	0	0	3	1	0	1	4	3	1	0	0	1	2
Copepoda	*Diarthrodes sp*	8	5	54	80	0	0	0	3	0	0	0	1	1	0	0	0	0	1
Copepoda	Monstrillidae	0	0	1	7	1	0	0	0	0	0	0	0	1	0	0	1	0	1
Copepoda	*Isias cf*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	35
Copepoda	*Isias clavipes*	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Copepoda	Corycaeidae	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0
Copepoda	Pontellidae	1	0	3	27	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Copepoda	Copepoda nc	0	0	1	5	4	0	0	0	0	0	0	0	2	0	0	0	1	0
Copepoda	Copepoda nauplius	2	1	8	28	7	9	5	3	1	2	2	0	0	0	1	5	7	58
Cirripeda	Rhizocephala	0	0	1	3	0	0	0	3	0	0	0	0	1	0	0	0	0	0
Cirripeda	Cirripedia nauplius	323	94	110	313	23	31	21	15	0	0	0	0	0	4	2	3	0	3
Cirripeda	Cirripedia cypris	20	16	1	1	1	0	0	0	0	0	0	0	0	0	0	1	1	2
Decapoda	Decapoda larva	4	0	1	10	35	38	11	3	0	0	0	1	0	0	0	0	1	1
Decapoda	*Porcellana sp*	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Decapoda	Crab Zoa	41	1	1	5	135	214	5	3	0	0	0	0	0	3	0	1	3	0
Malacostraca	Cumacea	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Isopoda	Gnathiidae	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Isopoda	Isopoda epic	1	0	14	3	15	13	0	0	0	0	0	0	0	0	0	0	0	0
Amphipoda	Gammaridae	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amphipoda	Corophiidae cf	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chaetognatha	Chaetognatha	1	0	1	2	8	0	5	0	1	0	0	0	0	0	0	0	0	0
Ascidiacea	Ascidiacea 1	8	1	10	35	5	9	11	0	2	0	0	0	0	3	1	0	0	0
Ascidiacea	Ascidiacea 2	17	6	22	6	1	0	3	0	0	0	0	0	0	23	6	9	0	0
Appendicularia	*Oikopleura sp*	1	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Arachnida	Acarina	1	0	1	1	1	0	3	3	0	0	1	0	0	1	0	0	2	2
Actinopteri	Teleostei	0	0	1	0	2	3	0	0	0	0	0	0	0	0	0	0	0	0
Eggs	Eggs	21	14	2	3	1	3	3	3	3	1	2	10	20	45	38	58	89	149
	Mean densitities (ind/m³)	729	355	2921	3479	14246	13769	2659	495	47	19	43	77	149	263	131	542	145	1400
	Standard error	157.94	97.80	670.79	84.72	519.81	2442.39	221.30	126.26	8.69	5.77	14.33	15.67	24.07	31.96	10.03	181.37	9.38	207.81
	Mean taxa	19	16	26	26	19	18	18	18	6	3	4	8	10	10	7	9	9	16
	Standard error	1.45	2.60	0.33	1.20	2.03	0.88	0.33	2.08	0.67	0.33	1.20	0.67	0.58	0.33	0.67	0.58	0.58	0.58

Appendix 6 – SIMPER test (*Similarity Percentages*) performed through the PRIMER software. Table with the taxa contributions for the similarity at each station (leisure boats sector (E1A and E1B), fuel station sector (E2), port entrance (E3) and outside port area (E4)) for month (July, October, January and March) during the sampling campaigns at El Kantaoui Port.

July

Mean similarity: 66,93%

Species	Mean Abund.	Mean Sim.	Sim/SD	Contrib%	Cum.%
Acartia spp	4,83	6,53	4,36	9,75	9,75
Cirripedia n	3,70	6,26	6,34	9,36	19,11
Spionidae l	3,85	5,55	7,61	8,29	27,40
Bivalvia	1,88	3,32	4,93	4,96	32,36
Diarthrodes	2,22	3,24	6,63	4,84	37,19
Gastropoda	2,12	3,11	4,43	4,65	41,84
Ascidiacea	1,83	3,08	5,09	4,61	46,45
Harpacticoi	2,04	3,01	5,05	4,50	50,94
Nematoda	1,72	2,74	5,62	4,10	55,04
Eggs	1,66	2,67	2,55	4,00	59,04
Ascidiacea	1,74	2,55	5,75	3,81	62,84
Polychaeta	1,38	2,39	5,27	3,57	66,42
Cirripedia c	1,51	2,24	1,73	3,34	69,76
Copepoda r	1,55	2,21	8,64	3,30	73,06
Euterpina a	1,75	2,17	4,21	3,24	76,30
Hydromedu	1,28	2,17	5,16	3,24	79,54
Crab Zoa	1,53	2,12	6,14	3,17	82,71
Müller larva	1,15	1,91	3,80	2,85	85,56
Thecosoma	1,84	1,58	0,90	2,37	87,93
Centropage	0,94	0,99	0,91	1,48	89,41
Isopoda epi	1,07	0,91	0,91	1,37	90,77

January

Mean similarity: 48,96%

Species	Mean Abund.	Mean Sim.	Sim/SD	Contrib%	Cum.%
Acartia spp	1,62	9,67	4,63	19,76	19,76
Eggs	1,49	8,23	7,49	16,82	36,58
Spionidae larva	1,55	7,67	1,10	15,67	52,24
Harpacticoida	1,15	4,89	1,11	9,98	62,23
Oncaeidae	1,28	4,58	1,11	9,36	71,59
Euterpina acutifrons	0,94	3,88	1,15	7,93	79,52
Copepoda nauplius	0,69	2,83	0,60	5,78	85,30
Noctiluca scintillans	1,14	2,29	0,61	4,67	89,97
Polychaeta nc	0,69	2,11	0,61	4,30	94,27

October

Mean similarity: 66,33%

Species	Mean Abund.	Mean Sim.	Sim/SD	Contrib%	Cum.%
Acartia spp	8,13	14,23	3,36	21,45	21,45
Spionidae l	3,33	6,93	7,53	10,44	31,89
Calanoida	3,16	5,12	3,12	7,71	39,61
Cirripedia n	2,17	4,93	6,89	7,43	47,04
Oithona	2,51	4,92	7,73	7,42	54,46
Harpacticoi	2,14	4,80	5,35	7,24	61,70
Crab Zoa	2,51	3,97	3,12	5,99	67,69
Decapoda l	2,00	3,89	5,85	5,86	73,55
Hydromedu	1,66	3,49	8,50	5,26	78,81
Copepoda r	1,55	3,40	7,58	5,13	83,94
Eggs	1,25	2,87	3,87	4,33	88,27
Ascidiacea	1,27	1,76	0,88	2,65	90,93

March

Mean similarity: 56,96%

Species	Mean Abund.	Mean Sim.	Sim/SD	Contrib%	Cum.%
Spionidae larva	3,71	12,20	4,92	21,42	21,42
Eggs	2,88	11,53	5,95	20,24	41,66
Harpacticoida	1,79	7,10	6,03	12,47	54,13
Acartia spp	1,45	4,08	1,13	7,17	61,29
Nematoda	1,32	3,48	0,93	6,12	67,41
Copepoda nauplius	1,38	3,32	1,12	5,82	73,23
Cirripedia nauplius	1,06	3,24	1,12	5,69	78,92
Ascidiacea 2	1,10	2,43	0,62	4,26	83,18
Crab Zoa	0,73	1,56	0,61	2,74	85,93
Acarina	0,68	1,24	0,61	2,17	88,10
Cirripedia cypris	0,64	1,16	0,61	2,04	90,14

Appendix 7 – SIMPER test (*Similarity Percentages*) performed through the PRIMER software. Table with the taxa contributions for the dissimilarity among months (July, October, January and March) during the sampling campaigns at El Kantaoui Port.

July and October
Mean dissimilarity = 45,96%

Species	July Mean Abund.	October Mean Sim.	Av.Diss	Diss/SD	Contrib%	Cum.%
Acartia spp	4,83	8,13	4,12	1,45	8,97	8,97
Calanoida	0,92	3,16	2,46	1,47	5,35	14,32
Oithona	0,57	2,51	2,05	2,12	4,46	18,78
Diarthrodes sp	2,22	0,33	1,89	2,59	4,11	22,88
Gastropoda	2,12	0,33	1,87	2,17	4,07	26,95
Thecosomata	1,84	0,00	1,85	1,43	4,02	30,97
Bivalvia	1,88	0,33	1,63	2,38	3,54	34,51
Cirripedia nauplius	3,70	2,17	1,62	2,56	3,53	38,04
Cirripedia cypris	1,51	0,23	1,49	1,51	3,25	41,29
Ascidiacea 2	1,83	0,54	1,37	1,82	2,98	44,27
Nematoda	1,72	0,60	1,32	1,17	2,88	47,15
Crab Zoa	1,53	2,51	1,30	1,33	2,82	49,97
Müller larva	1,15	0,00	1,27	2,68	2,75	52,73
Euterpina acutifrons	1,75	0,78	1,26	1,67	2,75	55,47
Decapoda larva	1,02	2,00	1,15	1,24	2,51	57,98
Oncaeidae	0,27	1,14	1,13	1,25	2,46	60,44
Spionidae larva	3,85	3,33	1,12	1,43	2,45	62,89
Pontellidae	1,14	0,00	1,10	1,45	2,39	65,27
Isopoda epic	1,07	0,96	1,03	1,27	2,23	67,50
Polychaeta nc	1,38	0,59	0,88	1,16	1,91	69,41
Chaetognatha	0,78	0,80	0,85	1,42	1,84	71,25
Centropages sp	0,94	0,27	0,84	1,34	1,82	73,07
Evadne tergestina	0,91	0,00	0,83	0,92	1,80	74,87
Ascidiacea 1	1,74	1,27	0,81	1,03	1,76	76,64
Sabellida	0,64	0,00	0,79	0,88	1,72	78,36
Copepoda nc	0,64	0,35	0,65	1,01	1,42	79,78
Monstrillidae	0,68	0,23	0,64	1,04	1,40	81,18
Rhizocephala	0,57	0,33	0,63	0,95	1,38	82,56
Teleostea	0,23	0,63	0,62	1,04	1,36	83,91
Acarina	0,73	0,87	0,61	1,01	1,33	85,24
Harpacticoida	2,04	2,14	0,57	1,40	1,23	86,48
Noctiluca scintillans	0,52	0,00	0,55	0,92	1,21	87,68
Oikopleura sp	0,55	0,00	0,55	0,96	1,20	88,88
Eggs	1,66	1,25	0,52	1,17	1,14	90,02

July and March
Mean dissimilarity = 52,93%

Species	July Mean Abund.	March Mean Sim.	Av.Diss	Diss/SD	Contrib%	Cum.%
Acartia spp	4,83	1,45	4,17	1,99	7,89	7,89
Cirripedia nauplius	3,70	1,06	3,51	2,96	6,64	14,53
Diarthrodes sp	2,22	0,22	2,58	3,07	4,88	19,41
Gastropoda	2,12	0,26	2,45	2,39	4,63	24,04
Thecosomata	1,84	0,40	2,03	1,52	3,84	27,88
Spionidae larva	3,85	3,71	1,76	1,38	3,32	31,20
Hydromedusae	1,28	0,00	1,71	4,01	3,23	34,44
Bivalvia	1,88	0,66	1,69	1,70	3,19	37,63
Ascidiacea 1	1,74	0,48	1,60	1,99	3,03	40,66
Müller larva	1,15	0,00	1,60	2,50	3,02	43,68
Eggs	1,66	2,88	1,52	3,05	2,88	46,55
Euterpina acutifrons	1,75	0,65	1,41	1,34	2,66	49,21
Cirripedia cypris	1,51	0,64	1,39	1,16	2,64	51,85
Pontellidae	1,14	0,00	1,34	1,49	2,53	54,37
Polychaeta nc	1,38	0,50	1,30	1,30	2,45	56,82
Isopoda epic	1,07	0,00	1,28	1,51	2,42	59,24
Calanoida	0,92	0,68	1,26	1,16	2,38	61,62
Crab Zoa	1,53	0,73	1,22	1,13	2,30	63,92
Ascidiacea 2	1,83	1,10	1,17	1,12	2,20	66,12
Centropages sp	0,94	0,00	1,15	1,63	2,17	68,29
Nematoda	1,72	1,32	1,10	1,07	2,09	70,38
Decapoda larva	1,02	0,42	1,07	1,33	2,03	72,41
Copepoda nauplius	1,55	1,38	1,04	1,38	1,97	74,38
Sabellida	0,64	0,00	1,03	0,88	1,94	76,32
Noctiluca scintillans	0,52	0,51	1,03	1,03	1,94	78,26
Evadne tergestina	0,91	0,00	1,00	0,93	1,88	80,14
Chaetognatha	0,78	0,00	0,95	1,62	1,79	81,93
Monstrillidae	0,68	0,42	0,83	1,10	1,57	83,50
Oithona	0,57	0,48	0,81	1,05	1,53	85,03
Copepoda nc	0,64	0,20	0,75	1,03	1,43	86,46
Acarina	0,73	0,68	0,74	1,04	1,40	87,86
Oikopleura sp	0,55	0,00	0,68	0,97	1,28	89,14
Harpacticoida	2,04	1,79	0,66	1,59	1,26	90,39

July and January
Mean dissimilarity = 67,49%

Species	July Mean Abund.	January Mean Sim.	Av.Diss	Diss/SD	Contrib%	Cum.%
Cirripedia nauplius	3,70	0,00	5,55	4,63	8,23	8,23
Acartia spp	4,83	1,62	4,38	2,19	6,48	14,71
Spionidae larva	3,85	1,55	3,10	1,94	4,59	19,31
Gastropoda	2,12	0,00	3,09	4,31	4,58	23,88
Ascidiacea 2	1,83	0,00	2,76	4,26	4,09	27,97
Diarthrodes sp	2,22	0,38	2,65	2,74	3,92	31,89
Bivalvia	1,88	0,20	2,56	2,65	3,80	35,69
Cirripedia cypris	1,51	0,00	2,45	1,85	3,63	39,32
Thecosomata	1,84	0,19	2,43	1,48	3,61	42,93
Crab Zoa	1,53	0,00	2,34	2,08	3,46	46,39
Ascidiacea 1	1,74	0,24	2,17	2,71	3,22	49,61
Nematoda	1,72	0,47	1,99	1,33	2,96	52,57
Hydromedusae	1,28	0,00	1,93	3,77	2,86	55,43
Müller larva	1,15	0,00	1,81	2,38	2,69	58,12
Oncaeidae	0,27	1,28	1,68	1,37	2,49	60,61
Noctiluca scintillans	0,52	1,14	1,54	1,14	2,28	62,90
Pontellidae	1,14	0,00	1,48	1,52	2,20	65,09
Isopoda epic	1,07	0,00	1,42	1,52	2,11	67,21
Harpacticoida	2,04	1,15	1,37	1,24	2,03	69,23
Decapoda larva	1,02	0,19	1,29	1,41	1,91	71,15
Centropages sp	0,94	0,00	1,28	1,63	1,89	73,04
Euterpina acutifrons	1,75	0,94	1,28	1,23	1,89	74,93
Calanoida	0,92	0,40	1,23	1,28	1,83	76,76
Copepoda nauplius	1,55	0,69	1,20	1,41	1,77	78,53
Sabellida	0,64	0,00	1,18	0,88	1,76	80,29
Polychaeta nc	1,38	0,69	1,12	1,09	1,65	81,94
Evadne tergestina	0,91	0,00	1,10	0,94	1,63	83,57
Chaetognatha	0,78	0,20	0,97	1,35	1,43	85,00
Acarina	0,73	0,20	0,91	1,28	1,34	86,34
Oithona	0,57	0,45	0,89	1,02	1,32	87,67
Monstrillidae	0,68	0,18	0,87	1,05	1,28	88,95
Copepoda nc	0,64	0,24	0,85	1,00	1,25	90,20

October and March
Mean dissimilarity = 55,35%

Species	October Mean Abund.	March Mean Sim.	Av.Diss	Diss/SD	Contrib%	Cum.%
Acartia spp	8,13	1,45	9,92	2,73	17,93	17,93
Calanoida	3,16	0,68	3,87	1,96	7,00	24,92
Oithona	2,51	0,48	3,17	2,29	5,73	30,65
Hydromedusae	1,66	0,00	2,55	7,96	4,61	35,26
Crab Zoa	2,51	0,73	2,52	1,48	4,56	39,82
Eggs	1,25	2,88	2,51	4,94	4,53	44,36
Decapoda larva	2,00	0,42	2,43	2,25	4,40	48,75
Oncaeidae	1,14	0,00	1,89	1,39	3,42	52,17
Spionidae larva	3,33	3,71	1,77	1,49	3,20	55,37
Cirripedia nauplius	2,17	1,06	1,74	1,75	3,15	58,52
Nematoda	0,60	1,32	1,71	1,18	3,09	61,61
Ascidiacea 2	0,54	1,10	1,58	1,27	2,86	64,47
Ascidiacea 1	1,27	0,48	1,58	1,43	2,85	67,33
Isopoda epic	0,96	0,00	1,27	0,96	2,30	69,62
Chaetognatha	0,80	0,00	1,20	0,95	2,17	71,79
Euterpina acutifrons	0,78	0,65	1,19	1,29	2,15	73,94
Copepoda nauplius	1,55	1,38	1,06	1,18	1,91	75,85
Bivalvia	0,33	0,66	1,01	1,09	1,83	77,68
Polychaeta nc	0,59	0,50	1,01	0,98	1,83	79,51
Acarina	0,87	0,68	0,98	1,02	1,77	81,28
Cirripedia cypris	0,23	0,64	0,91	1,11	1,65	82,93
Teleostea	0,63	0,00	0,84	0,95	1,52	84,45
Ostracoda	0,27	0,41	0,76	0,86	1,38	85,83
Diarthrodes sp	0,33	0,22	0,72	0,69	1,31	87,14
Harpacticoida	2,14	1,79	0,68	1,16	1,23	88,36
Monstrillidae	0,23	0,42	0,67	0,89	1,21	89,57
Gastropoda	0,33	0,26	0,65	0,71	1,18	90,75

October and January
Mean dissimilarity = 64,69%

Species	October Mean Abund.	January Mean Sim.	Av.Diss	Diss/SD	Contrib%	Cum.%
Acartia spp	8,13	1,62	11,00	2,94	17,00	17,00
Calanoida	3,16	0,40	4,65	2,34	7,18	24,18
Crab Zoa	2,51	0,00	4,22	3,02	6,53	30,71
Cirripedia nauplius	2,17	0,00	3,91	5,54	6,04	36,75
Oithona	2,51	0,45	3,68	2,36	5,69	42,44
Decapoda larva	2,00	0,19	3,17	3,21	4,90	47,33
Spionidae larva	3,33	1,55	3,00	1,94	4,64	51,97
Hydromedusae	1,66	0,00	2,94	8,44	4,54	56,51
Ascidiacea 1	1,27	0,24	2,01	1,41	3,11	59,62
Noctiluca scintillans	0,00	1,14	1,95	1,02	3,01	62,63
Harpacticoida	2,14	1,15	1,90	1,26	2,94	65,57
Oncaeidae	1,14	1,28	1,50	1,15	2,31	67,88
Acarina	0,87	0,20	1,49	1,30	2,30	70,18
Copepoda nauplius	1,55	0,69	1,44	1,50	2,23	72,42
Isopoda epic	0,96	0,00	1,43	0,97	2,21	74,62
Euterpina acutifrons	0,78	0,94	1,39	1,36	2,15	76,78
Chaetognatha	0,80	0,20	1,39	1,04	2,15	78,93
Polychaeta nc	0,59	0,69	1,15	1,05	1,78	80,71
Nematoda	0,60	0,47	1,11	1,00	1,71	82,42
Ascidiacea 2	0,54	0,00	0,98	0,85	1,52	83,94
Diarthrodes sp	0,33	0,38	0,97	0,87	1,50	85,44
Teleostea	0,63	0,00	0,94	0,95	1,46	86,90
Rhizocephala	0,33	0,22	0,86	0,70	1,32	88,22
Bivalvia	0,33	0,20	0,86	0,71	1,32	89,54
Copepoda nc	0,35	0,24	0,72	0,73	1,12	90,66

January and March
Mean dissimilarity = 56,19%

Species	January Mean Abund.	March Mean Sim.	Av.Diss	Diss/SD	Contrib%	Cum.%
Spionidae larva	1,55	3,71	5,29	1,68	9,41	9,41
Eggs	1,49	2,88	3,80	2,24	6,76	16,16
Oncaeidae	1,28	0,00	3,26	1,66	5,81	21,97
Ascidiacea 2	0,00	1,10	3,19	1,16	5,67	27,64
Noctiluca scintillans	1,14	0,51	3,03	1,14	5,39	33,03
Nematoda	0,47	1,32	3,02	1,33	5,37	38,40
Cirripedia nauplius	0,00	1,06	2,83	1,76	5,04	43,44
Copepoda nauplius	0,69	1,38	2,39	1,53	4,26	47,70
Crab Zoa	0,00	0,73	2,06	1,15	3,66	51,36
Harpacticoida	1,15	1,79	1,89	1,01	3,36	54,72
Polychaeta nc	0,69	0,50	1,82	1,10	3,23	57,96
Calanoida	0,40	0,68	1,80	1,02	3,20	61,16
Acartia spp	1,62	1,45	1,73	1,13	3,08	64,23
Acarina	0,20	0,68	1,66	1,11	2,95	67,18
Euterpina acutifrons	0,94	0,65	1,62	1,05	2,89	70,07
Cirripedia cypris	0,00	0,64	1,61	1,16	2,86	72,93
Bivalvia	0,20	0,66	1,59	1,09	2,82	75,76
Ascidiacea 1	0,24	0,48	1,53	0,87	2,73	78,49
Oithona	0,45	0,48	1,47	1,01	2,62	81,11
Ostracoda	0,23	0,41	1,39	0,87	2,48	83,59
Thecosomata	0,19	0,40	1,20	0,86	2,13	85,72
Decapoda larva	0,19	0,42	1,14	0,86	2,04	87,76
Monstrillidae	0,18	0,42	1,10	0,88	1,96	89,71
Diarthrodes sp	0,38	0,22	1,06	0,89	1,88	91,59

Appendix 8 – Results of DistLM (*Distance based linear models*) performed through the PRIMER software.

DistLM2
Distance based linear models

Resemblance worksheet
Name: ResemBio(2)
Data type: Similarity
Selection: All
Transform: Fourth root
Resemblance: S17 Bray Curtis similarity

Predictor variables worksheet
Name: Data6
Data type: Environmental
Sample selection: All
Variable selection: All
Transform: Fourth root
Normalise

Selection criterion: AICc
Selection procedure: Best

VARIABLES

1	Water temp	Trial
2	pH	Trial
3	Salinity	Trial
4	Dissolved O2	Trial
5	O2 saturation	Trial
6	DIN	Trial
7	PO4	Trial
8	Chl	Trial
9	DOC	Trial
10	Depth	Trial

Total SS(trace): 26316

MARGINAL TESTS

Variable	SS(trace)	Pseudo-F	P	Prop.
Water temp	7977	6,9595	0,001	0,30312
pH	2558	1,7227	0,1	9,7203E-2
Salinity	3911,9	2,7937	0,015	0,14865
Dissolved O2	6840,4	5,6196	0,001	0,25993
O2 saturation	4875,2	3,638	0,005	0,18525
DIN	3894,9	2,7795	0,014	0,148
PO4	3697,2	2,6153	0,021	0,14049
Chl	5238,9	3,9769	0,005	0,19907
DOC	2205	1,4632	0,192	8,3788E-2
Depth	2719,2	1,8437	0,086	0,10333

BEST SOLUTIONS
BEST RESULT FOR EACH NUMBER OF VARIABLES

AICc	R^2	RSS	No.Vars	Selections
129,48	0,30312	18339	1	1
128,82	0,4284	15042	2	1;3
128,42	0,53642	12200	3	1-3
128,71	0,62113	9970,5	4	1;3;6;8
130,78	0,67145	8646,3	5	1;3;6;8;10
134,06	0,71053	7617,9	6	1-3;6;7;10
138,67	0,74377	6743	7	1-4;6;7;10
145,09	0,77164	6009,7	8	1-7;10
153,38	0,80281	5189,2	9	1-5;7-10
164,93	0,83327	4387,8	10	All

OVERALL BEST SOLUTIONS

AICc	R^2	RSS	No.Vars	Selections
128,42	0,53642	12200	3	1-3
128,43	0,53614	12207	3	1;3;6
128,43	0,53593	12213	3	1;3;4
128,44	0,53571	12218	3	1;3;5
128,58	0,53228	12309	3	3-5
128,71	0,62113	9970,5	4	1;3;6;8
128,78	0,6195	10013	4	1;3;6;10
128,79	0,52665	12457	3	1;6;10
128,8	0,52637	12464	3	1;3;10
128,82	0,4284	15042	2	1;3

Percentage of variation explained by individual axes

	% explained variation out of fitted model		% explained variation out of total variation	
Axis	**Individual**	**Cumulative**	**Individual**	**Cumulative**
1	57,38	57,38	30,78	30,78
2	25,55	82,93	13,71	44,49
3	17,07	100	9,16	53,64

dbRDA coordinate scores

Sample	dbRDA1	dbRDA2	dbRDA3
7E1A	27,896	7,783	-6,4379
7E1B	28,436	2,2658	-8,7881
7E2	26,479	1,0878	-6,1032
7E3	29,579	-1,8669	-8,208
9E1A	19,103	0,61148	19,259
9E1B	17,651	1,5696	23,721
9E2	11,425	15,667	4,3737
9E3	10,017	23,783	-2,3821
11E1A	-20,37	-6,5557	9,8534
11E1B	-25,39	0,71095	11,465
11E2	-28,334	6,817	8,5775
11E3	-30,269	15,081	1,8706
11E4	-27,148	16,076	-6,405
12E1A	-0,1203	-33,517	-0,39439
12E1B	-2,774	-25,073	4,0057
12E2	-7,1934	-18,596	-4,2114
12E3	-10,33	-6,909	-15,662
12E4	-18,656	1,0649	-24,535

Appendix 9 – Results of the Best analysis (*Environment matching*) performed through the PRIMER software.

BEST

Biota and/or Environment matching

Data worksheet
Name: Data6
Data type: Environmental
Sample selection: All
Variable selection: All

Resemblance worksheet
Name: ResemBio(2)
Data type: Similarity
Selection: All

Parameters
Rank correlation method: Spearman
Method: BIOENV
Maximum number of variables: 5
Resemblance:
Analyse between: Samples
Resemblance measure: D1 Euclidean distance

Variables

1 Water temp
2 pH
3 Salinity
4 Dissolved O2
5 O2 saturation
6 DIN
7 PO4
8 Chl
9 DOC
10 Depth

Best results

No.Vars	Corr.	Selections
2	0,621	1;4
3	0,617	1;4;9
1	0,598	1
4	0,586	1;4;5;9
4	0,584	1;4;9;10
5	0,582	1;4;5;9;10
3	0,576	1;4;7
3	0,576	1;5;9
3	0,575	1;4;10
5	0,575	1;4;6;9;10

Appendix 10 – Results of PERMANOVA (*Permutational MANOVA*) performed through the PRIMER software.

PERMANOVA
Permutational MANOVA

Resemblance worksheet
Name: ResemBio(2)
Data type: Similarity
Selection: All
Transform: Fourth root
Resemblance: S17 Bray Curtis similarity

Sums of squares type: Type III (partial)
Fixed effects sum to zero for mixed terms
Permutation method: Permutation of residuals under a reduced
Number of permutations: 999

Factors

Name	Abbrev.	Type	Levels
month	mo	Fixed	4
dist	di	Fixed	4

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
mo	3	12553	4184,3	10,76	0,001	998
di	3	4949,4	1649,8	4,2426	0,015	999
moxdi**	7	6200,2	885,74	2,2778	0,006	999
Res	4	1555,5	388,87			
Total	17	26316				

** Term has one or more empty cells

Details of the expected mean squares (EMS) for the model

Source	EMS
mo	1*V(Res) + 3,9238*S(mo)
di	1*V(Res) + 4,0586*S(di)
moxdi	1*V(Res) + 1,2286*S(moxdi)
Res	1*V(Res)

Construction of Pseudo-F ratio(s) from mean squares

Source	Numerator	Denominator	Num.df	Den.df
mo	1*mo	1*Res	3	4
di	1*di	1*Res	3	4
moxdi	1*moxdi	1*Res	7	4

Estimates of components of variation

Source	Estimate	Sq.root
S(mo)	967,28	31,101
S(di)	310,68	17,626
S(moxdi)	404,44	20,111
V(Res)	388,87	19,72