PERSEUS

POLICY-ORIENTED MARINE ENVIRONMENTAL RESEARCH IN THE SOUTHERN EUROPEAN SEAS

Implementation of the Continuous Plankton Recorder (CPR) in the Mediterranean Sea.

Deliverable Nr. 3.3

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EXECUTIVE SUMMARY / ABSTRACT

Here we describe the establishment and implementation of the Continuous Plankton Recorder (CPR) Facility and survey in the Mediterranean (MedCPR). The CPR is a robust sampling device used to collect plankton from horizontal transects, while towed by ships of opportunity (SOOP). PERSEUS has initiated the use of the CPR in the Eastern Mediterranean along the Cyprus-Haifa-Cyprus transect from the newly established MedCPR Facility at The Cyprus Institute (CyI). The CyI has kindly been provided with the use of the chemical tanker PETROLINA OCEAN as SOOP and well-trained crew members, for the deployment and towing of the CPR. After each deployment, the CPR device and all crucial equipment pass through strict inspection and maintenance procedures. Samples are analyzed in the aforementioned specially configured laboratory at the CyI. The CPR data include zooplankton and phytoplankton abundances and diversity, biological productivity measurements, as well as temperature and salinity data that are collected by a data logger attached to the CPR during each tow. The Levantine Sea is known as ultra-oligotrophic and is highly influenced by non-indigenous species (NIS) from the Red Sea that are introduced to the Levantine Sea through the Suez Canal. Data obtained from the Cyprus-Haifa-Cyprus transects will provide information on plankton populations from coastal and offshore waters, NIS, biological productivity, as well as sea surface temperatures and salinities on a high-resolution temporal and spatial scale. The realization of the Cyprus-Haifa-Cyprus route represents an important first step of a long term monitoring program and the first ever CPR survey in the Eastern Mediterranean.

SCOPE

This deliverable provides information to laboratories considering to establish a CPR survey facility, by sharing our experiences gained in the implementation of the MedCPR. Capacity building to enable the establishment of a CPR facility represents one of the goals of PERSEUS. The project provides the infrastructure and capability to operate a CPR facility in the Mediterranean region for the first time. These include initiating collaboration with commercial shipping companies, transferring CPR operation knowledge to the region and creating a data repository for the collection of CPR data. Implementing the CPR follows a steep curve, where the demands on effort and funding are significantly higher in the initial stage. The CPR has the potential of becoming a long-term monitoring tool covering the needs of the region for surface plankton data, tracking NIS, monitoring plankton population and abundance on monthly to annual and inter-annual basis. The abundance and species composition along a specific transect over an extended period of time will also provide an assessment of climate change impacts on marine ecosystems. The MedCPR facility will also pave the way for the implementation of CPR surveys in other Mediterranean and Black Sea routes. Extending the network of CPR sampling routes in the southern European Seas will contribute in filling the gap in ocean monitoring, an identified objective of the PERSEUS project (Crise et al, 2015), and will provide a better understanding of the dynamic, marine-biological regime in the Mediterranean Basin. The envisioned CPR network will also serve as an important tool for training and capacity building of young scholars.

ABBREVIATIONS

Chl-a: Chlorophyll-a pigment

CPR: Continuous Plankton Recorder

CSIRO: Commonwealth Scientific and Industrial Research Organisation, Australia

CTD: Conductivity, Temperature and Depth data logger.

CyI: The Cyprus Institute, Nicosia, Cyprus

EEZ: Exclusive Economic Zone

FOV: Field of view, here it is used to indicate the diameter of the observable surface when looking through a microscope.

GACS: Global Association of CPR Surveys

HCMR: Hellenic Centre for Marine Research

MedCPR: Mediterranean CPR Survey

NIS: Non-indigenous species

Nm: Nautical miles

MPD: miles per division, i.e. the number of Nm sampled by a 5in long silk roll division.

NOAA: National Oceanic and Atmospheric Administration, United States of America

NTL: Novel Technologies Laboratory building in the CyI campus

PCI: Phytoplankton Colour Index is a semi-quantitative method to study primary productivity.

SAHFOS: Sir Alister Hardy Foundation for Ocean Sciences, Plymouth, UK

SOOP: ship of opportunity, to denote a commercial vessel that is used opportunistically for the conduct of research

UK: United Kingdom

TITLE

Implementation of the Continuous Plankton Recorder (CPR) in the Mediterranean Sea.

Introduction

The Mediterranean Sea is an important ecologic and socio-economic resource. This mid-latitude sea covers 2,500,000 km^2 making it the largest semi enclosed basin. It communicates with the Atlantic Ocean, the Black Sea and the Red Sea through narrow passages, exchanging water masses and often species. Although it covers only 1% of Earth's oceans, it contains about 6% of marine species, including many endemics (Coll et al. 2010). Eighty two million people live in major cities built around the 46,000km long Mediterranean coastline running through 21 countries. Being subject to human intervention for millennia, the basin's ecosystems are under constant pressure.

Future projections estimate that global population will continue to grow while coastal urbanization will increase (UN 2013). Seasonal population pressures are also important. Over 100 million tourists visit Mediterranean beaches every year, a number expected to double by 2025. Catering for growing populations has increased the pressure on marine ecosystems. More than a quarter of global marine fish stocks are exploited at unsustainable levels (FAO 2014) and the alternative (i.e aquaculture) is one of the important ecological pressures of the region (UNEP 2007). As the Mediterranean is almost entirely landlocked, its waters have a very low renewal rate making them excessively sensitive to pollution (EEA 2006). Global temperature increase, untreated waste water, increasing industrial activities and commercial shipping contribute to the demise of indigenous ecosystems (UNEP 2007). The area has been dubbed as a climate change hotspot (Giorgi & Lionello 2008) and a natural laboratory, as biological responses to regime changes are expected to manifest earlier in the region than elsewhere.

Identifying, controlling, and preventing negative impacts on ecological and socio-economical aspects are of great concern all over the world. The goal is to design effective mitigation and adaptation measures based on sustainable solutions. In the Mediterranean, severe changes of the marine ecosystems are widely anticipated. At the Western and Central regions of the Mediterranean, it is well documented that climate conditions, impact of anthropogenic activities and natural disturbances are already affecting the health (Jimenez et al. 2014), composition and ecological functions of coastal (Bernhardt & Leslie 2013) and deep-sea habitats (Norse et al. 2012). The Levantine Sea, being characterized by low concentration of nutrients (oligotrophic) and specific thermal conditions, represents one of the most interesting research areas of the Mediterranean. The accelerated warming trend of sea surface temperatures in the Levantine basin is driving the already stressed marine ecosystems beyond their resilience thresholds (Jimenez et al. 2014). While there is scientific consensus that climate and pollution induced changes on Mediterranean regions are presently occurring and are projected to amplify in the future (Lejeusne et al. 2010), very little knowledge is available about the reliable quantification of these changes, which is hampered by a lack of suitable and cost effective monitoring systems.

Plankton, passively drifting plants (phytoplankton) and animals (zooplankton), are particularly good indicators of environmental change (Hays et al. 2005). Plankton includes many groups, including photosynthetic organisms and larval stages of commercially important species, providing a wide range of information including primary productivity estimates and predictions on future fish stock. Most species are short lived and not commercially exploited, thus plankton dynamics is directly coupled with environmental change and not largely affected by persistence of previous generations or fishing (Hays et al. 2005). Plankton populations expand and according to environmental conditions. Therefore, abundance and species composition of samples indicate subtle environmental disturbances stemming from the non-linear manifestation of environmental change in biological communities (Taylor et al. 2002). Plankton is often collected in long-term stations but only a few studies have a large spatial coverage (Durrieu de Madron et al. 2011).

The Continuous Plankton Recorder (CPR; Figure 1; described in detail in Appendix I) is a device used for marine biological sampling in long term monitoring surveys, designed for high throughput, quality results, used to indicate ecological responses to environmental change (McQuatters-Gollop et al. 2007; Hays et al. 2005; Mackas et al. 2012).

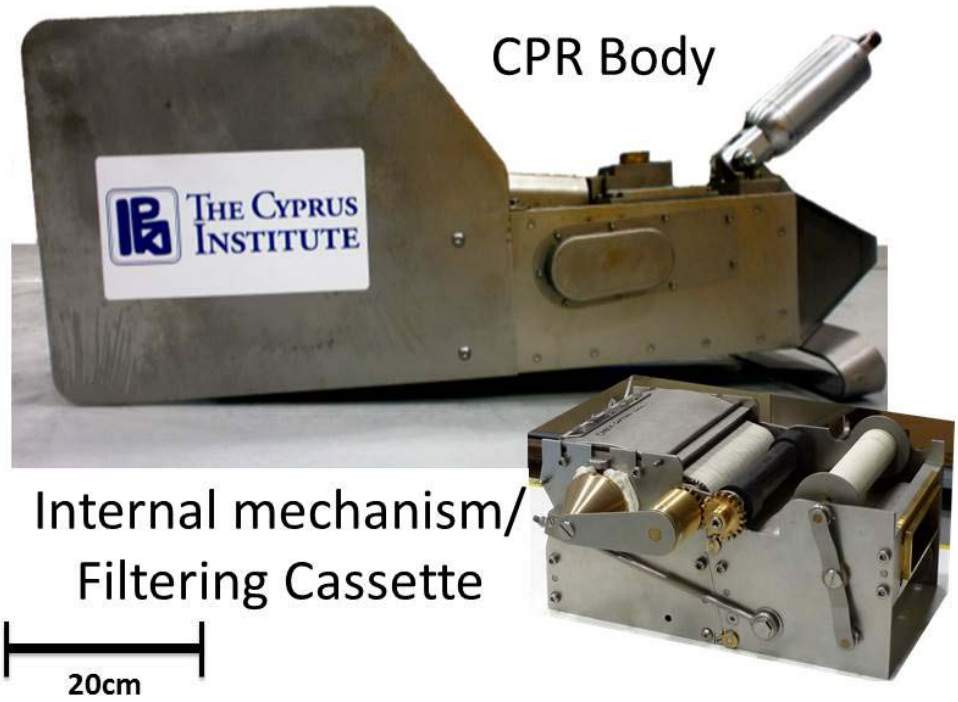

Figure 1: The Continuous Plankton Recorder device (above) and its internal filtering mechanism (bottom left).

Sampling plankton through the CPR has many advantages. The CPR is designed to be towed voluntarily by ships of opportunity (SOOPs) without interfering with the operation of the vessel. Thus, the survey integrates the need to monitor with the

increased commercial shipping activity in the Mediterranean. Oil tanker traffic through the Mediterranean alone, accounts for more than 20% of global traffic (Daffonchio et al. 2013). The CPR collects plankton from coastal and offshore surface waters continuously, maximizing the spatial coverage while minimizing costs. In addition, SOOPs perform a given route in high frequency, giving the opportunity to collect plankton in the required temporal resolution spanning from weekly to seasonal samplings. The CPR is also unique in that it can collect biological data from long transects in high spatial resolution. These attributes make it an attractive research solution to funding agencies and stakeholders, increasing the probability of sustaining the survey for many years.

Today, the recorder is established in the Atlantic, Indo- Pacific, Pacific and Southern Oceans, with new surveys benefiting from over 80 years of CPR monitoring experience. The Sir Alister Hardy Foundation for Ocean Sciences (SAHFOS), Plymouth, UK, is an institution dedicated to the CPR survey. SAHFOS, and nine other laboratories operating the CPR survey around the world, comprise the Global Alliance of CPR Surveys (GACS), dedicated to supporting and promoting plankton research.

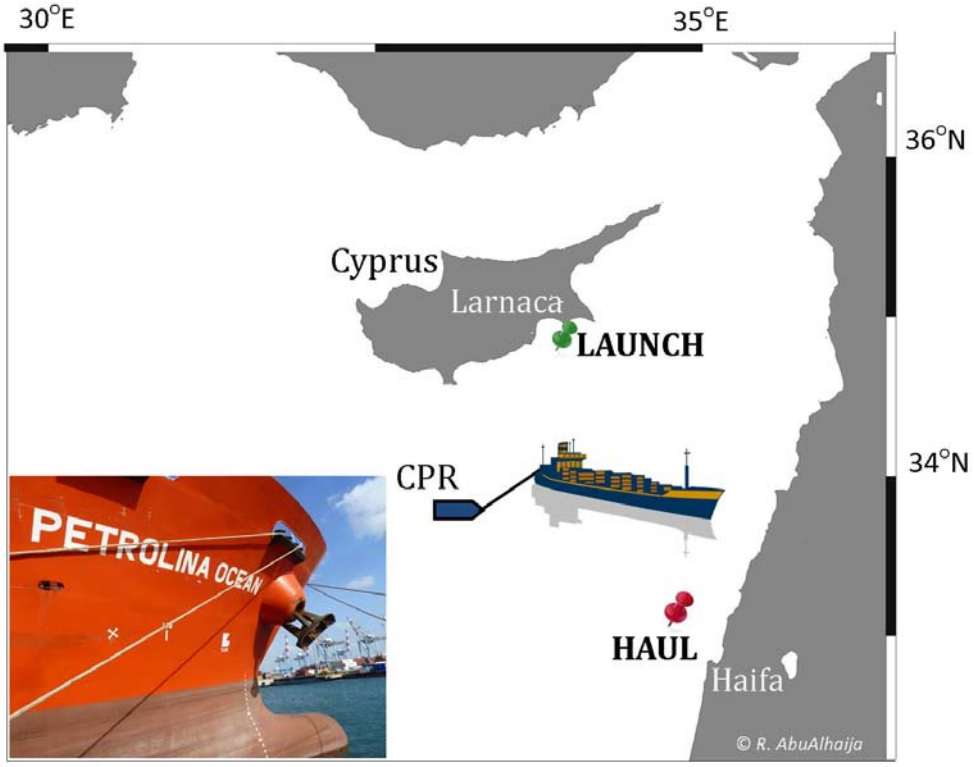

Figure 2: The first MedCPR route (Cyprus-Haifa), implemented through the PERSEUS project. The PETROLINA OCEAN tanker (insert) voluntarily tows the CPR.

Identifying the gap in knowledge (Crise et al. 2015) and the promising abilities of the CPR survey, PERSEUS has promoted its realisation in the Mediterranean, operating the newly founded survey from Cyprus. The island is strategically situated in the easternmost part of the basin and benefits from direct access to many coastal countries in the area, such as Israel. A transect from Cyprus to the Israeli port of Haifa, intersects the principal anticlockwise circulation of the Levantine (Menna et al.

2012). The currents disperse Red Sea water from the Suez Canal together with non-indigenous and Lessepsian migrants (Coll et al. 2010; Galil et al. 2014). The Mediterranean CPR survey, dubbed as MedCPR, is operated by The Cyprus Institute (CyI) through the MedCPR Facility. Data resulting from the MedCPR will later be made available through online databases and CyI data repository.

The CyI has succeeded in undertaking three pioneering tasks: (1) transferring the knowledge and experience of the CPR to the region, (2) implementing the survey in the Levantine basin along the Cyprus-Haifa route (Figure 2), and (3) initiating a network of marine laboratories interested in implementing CPR survey in the Mediterranean and Black Seas. The network will be strengthened and expanded through an upcoming CPR workshop to take place in September, 2015 at the CyI.

Within this report, we will discuss the intricate CPR methodology, possible pitfalls and alternative solutions in the implementation of the MedCPR survey. With this document, the CyI shares its experience in the implementation stages of the CPR survey providing solutions, advice and alerting laboratories to probable obstacles. Researches interested in establishing new survey routes, will also find here basic information regarding: the function of the CPR device and the maintenance procedure after each sampling; the CPR sample analysis protocol and the specialized equipment required for the process. Good record keeping, quality assessments and quality control complete the components of a successful CPR survey.

Implementation of the MedCPR

Study site/area

The Levantine Sea, and particularly Cyprus, is an excellent location to launch the MedCPR. First, the basin's open water has rarely been sampled in-situ for plankton communities. Over the years, research projects like the Physical Oceanography of the Eastern Mediterranean (POEM) project (Yacobi et al. 1995), have collected offshore plankton samples but to date there are no systematic surveys of offshore plankton in the area. Secondly, the Levantine has a set of almost unique characteristics. Its ultraoligotrophic warm waters have evolving patterns influencing not only local populations but also the region (Kress et al. 2013). The position of Cyprus is also a big asset to the survey. Cyprus is located amidst the basin, with close connections to the surrounding ports. For example, Haifa can be reached overnight, while the duration of a commercial voyage is only three days. Short routes and voyages are ideal for the initial stages of the survey, as to offer a better control of the on-board operation of the CPR, facilitate CPR researchers to board the SOOP and allow for immediate troubleshooting. Furthermore, being stable politically and with good diplomatic relationships to most Mediterranean countries, Cyprus is in an advantageous location within the Levantine basin. Lastly, the chosen route intercepts the non-native species' invasion pathway from the Red Sea.

A good percentage of the alien Indo-Pacific species in the Mediterranean comes from the Suez Canal (Koukouras et al. 2010; Katsanevakis et al. 2013; Galil et al. 2014). These species are referred to as Lessepsians and despite earlier indications of a halt in the rate of invasions (Por 1978), Lessepsian numbers are still increasing. This phenomenon is attributed partially to the increase of the water temperature in the

Levant, which follows the global climate change (Bianchi 2007), making the area suitable for warm-water marine species. Identifying future invaders is important for the design of mitigation and adaptation strategies.

A spatio-temporal identification of the Lessepsian introduction rate and pattern revealed that the latter follows the prevailing Mediterranean circulation (Coll et al. 2010; Koukouras et al. 2010; Tzomos et al. 2012; Galil et al. 2014). As Atlantic water travels Eastwards along the Southern coast of the Mediterranean, it passes in front of the Suez Canal, continues east and then northeast carrying with it Red Sea organisms. The PERSEUS initial proposal suggesting the engagement of a vessel with calls between Cyprus and Egypt ports, *was strategically redesigned to intercept the prevailing introduction pathway of Lessepsian species*. To that end, during the first year of the PERSEUS project, the marine science group of the CyI sought a vessel that has frequent calls between Cyprus and Haifa.

Securing the collaboration of a SOOP

The operation of the CPR is based on the participation of commercial vessels at the sampling stage. The communication with several vessels commenced in 2012. The PETROLINA OCEAN tanker, owned by Lefkaritis Marine Bros Ltd and managed by Columbia Shipmanagment, completes the Cyprus-Haifa-Cyprus route several times per month for commercial purposes. Having frequent calls to the port of destination secures a better temporal coverage of the area and reduces the risk of missing a tow. The CyI proposed the collaboration to the company which is now voluntarily participating in the survey. In 2013, we commenced a series of meetings to discuss the logistics of the tow of the CPR by the aforementioned vessel (Table 1). Apart from the logistics, building rapport and a steady two way relationship with SOOP stakeholders is also very important for the operation of the survey.

Table 1: Meetings with SOOP stakeholders.

No	Time	Objective
1	June, 2012	Visit to the office of Mr. Eduard Bucknall, former Technical Director of Columbia Shipmanagement Ltd. Agreement to work towards a bilateral collaboration.
2	July, 2012	First visit and tour to the PETROLINA OCEAN tanker. Meeting with Mr. Bucknall, Mr. Gurami Vasadze, former Technical Superintendent of the tanker. Familiarization with the on-board equipment available for the CPR tow.
3	September, 2013	Meeting with stakeholders from Lefkaritis Marine Bros ltd and Columbia Shipmanagment at Lefkaritis Marine Bros ltd headquarters. Discussion of suggested tow plan designs, probable benefits for the company (outreach programs etc.) and the company's requirements for the (e.g. permits from Cyprus and Israeli authorities).
4	October, 2013	Transfer of the CPR to the vessel as to familiarise the crew and stakeholders with the device. Meeting with the vessel's Captain

	and Mr. Timothy Hazeldine former Technical Superintendent onboard the PETROLINA OCEAN. Present the final tow design, discussion on crew training and first tow.

In order to get the approval for the tow, the CyI provided the ship owners with a series of documents including general CPR information, initial and alternative options for the tow plan and a waver letter to disengage the company from any damage sustained to CyI equipment, and staff as a result of the tow. The waver was "produced" after the exchange of information with the insurance provider of both parties (CyI and Lefkaritis Marine Bros ltd). The information included the nature of the CPR on board operation and the purpose of the tow.

Since the tow transect could cross three exclusive economic zones (EEZ), exchange of information and applications were necessary with embassies and consular offices of the respective countries. The process is time-consuming and often the not straight-forward communication contributes to sustaining the obstacle. The process was faster and less complicated when the local partners identified the exact office/institution/person responsible for granting research and transit permits. The permit was granted after the Israeli colleagues at the Ocean and Limnology Institute, Haifa, facilitated the paper work. To overcome pending issues with similar permits we have adjusted the route and operation of the CPR.

Once the administrative procedures were concluded and soon after receiving the official permission from the respective authorities, a test tow was performed in October 2014. The test was necessary in order to instruct the crew with the use and care of the CPR and resulting sample and to familiarize the captain and officers of the vessel with the acquisition of data during the cruise. Although all the plans for the on board tow design (i.e. architecture of the tow-system) were discussed and agreed upon prior to the first tow, the physical presence of a member of the laboratory on-board helps the interaction between stakeholders and contributes to the success of the research project. CyI personnel participated in three more tows and produced audio-visual material for the crew on the CPR use. As vessel crew changes frequently, CyI has scheduled continuing trainings.

MedCPR laboratory and capacity building

In the early stages of the project (2012-2013), the CyI prepared the infrastructure needed for the realization of the CPR survey which included specialized training and the procurement of equipment.

CyI was trained in CPR methodologies by the leading CPR laboratory, SAHFOS, Plymouth, UK. Two trainings took place at SAHFOS: the "CPR launch and maintenance" technical training (August, 2013) and the "CPR sample analysis and plankton identification" training (April-May, 2014). One more training took place at the Hellenic Centre for Marine Research (HCMR) in identification of Mediterranean mesozooplankton (June, 2013).

The conclusion of the technical training is imperative before the initiation of the tows as it provides important information on the care, deployment and maintenance of the CPR device. The training, as it is formulated by SAHFOS staff, includes specialised

advice for the communication with SOOPs, initiating collaboration, utilising the on-board equipment for the tow of the CPR and the launch and haul of the device. Several tow and CPR designs, operation templates and contact information of shipping companies, where kindly made available to CyI trainee, Dr. Carlos Jimenez. Troubleshooting several possible scenarios were covered, ranging from the loss of the CPR during a tow to the servicing a CPR after severe impacts to the side of the vessel. The following stage of the training involves the inspection of tow equipment, sample removal, and service of the CPR. The training also includes the transport protocol for CPR samples, CPR device and parts.

The second training at SAHFOS was focused on CPR sample analysis and plankton identification. The CPR survey, since its first years in 1930's, has a conserved protocol to ensure the comparability of results across different surveys and timeframes. CyI trainee, M. Sc. Rana Abu Alhaija, was trained in the function of the CPR, processing the plankton collected. Special attention was paid to the analysis of CPR samples for productivity measures, phytoplankton and zooplankton abundance and diversity measures and the application of specific factors to the analysis results. The training also included the practical sessions for the identification plankton collected by the CPR, as the plankton collected by the device is significantly damaged by the process. Therefore a plankton taxonomist analysing CPR samples must develop the ability of recognising specimens as they are collected by the device.

The majority of SAHFOS samples are of the Atlantic Ocean. Therefore a brief complementary training in the identification of Mediterranean mesozooplankton took place at HCMR, by Mediterranean plankton taxonomist Dr. Ioanna Siokkou (Greece).

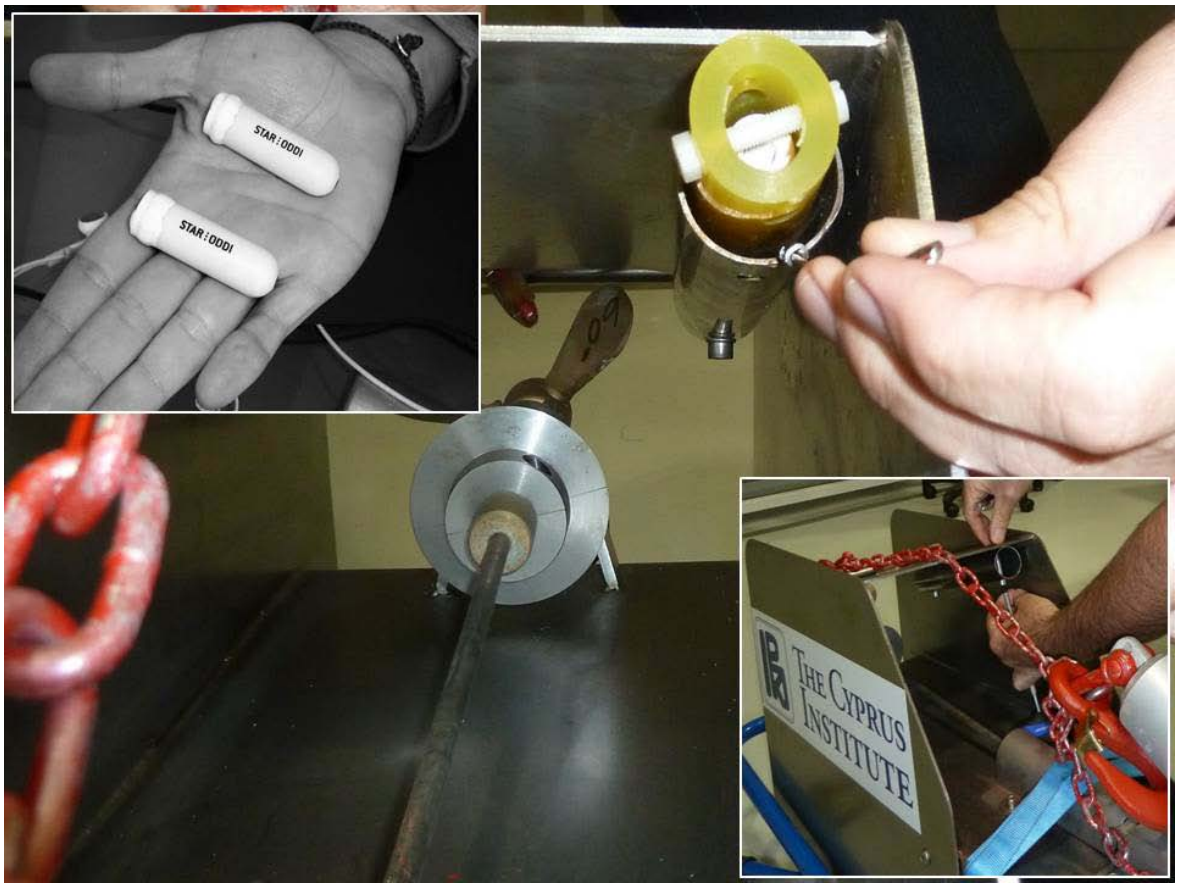

Figure 3: Data logger for temperature, salinity and depth. The logger is installed inside the box double tail fins of the CPR. During the tow, the logger measures once every minute and the data can be referenced according to the position and time of measurement.

Concurrently, a specialized marine laboratory was set up in the Novel Technologies Laboratory (NTL) building in the CyI campus. The laboratory has a complete set of instruments, consumables and resources for the realisation of the CPR tows and the analysis of CPR samples. These include a CPR body, CPR internal filtering cassette (Figure 1) and towing equipment, all procured through SAHFOS. The CPR body was also adjusted to receive a Conductivity Temperature and Salinity data logger (CTD, Star-Oddi, Island) to collect oceanographic data, along the CPR transect (Figure 3). A stereoscope modified for plankton viewing and a Nikon Ci-S microscope configured for the analysis of CPR samples complete the main instrumentation needed for the analysis of CPR samples (Figure 4).

CPR characteristics, accessories, and methodology are explained in more detail in Appendix I. The specifications and requirements of a CPR microscope are discussed in Appendix III.

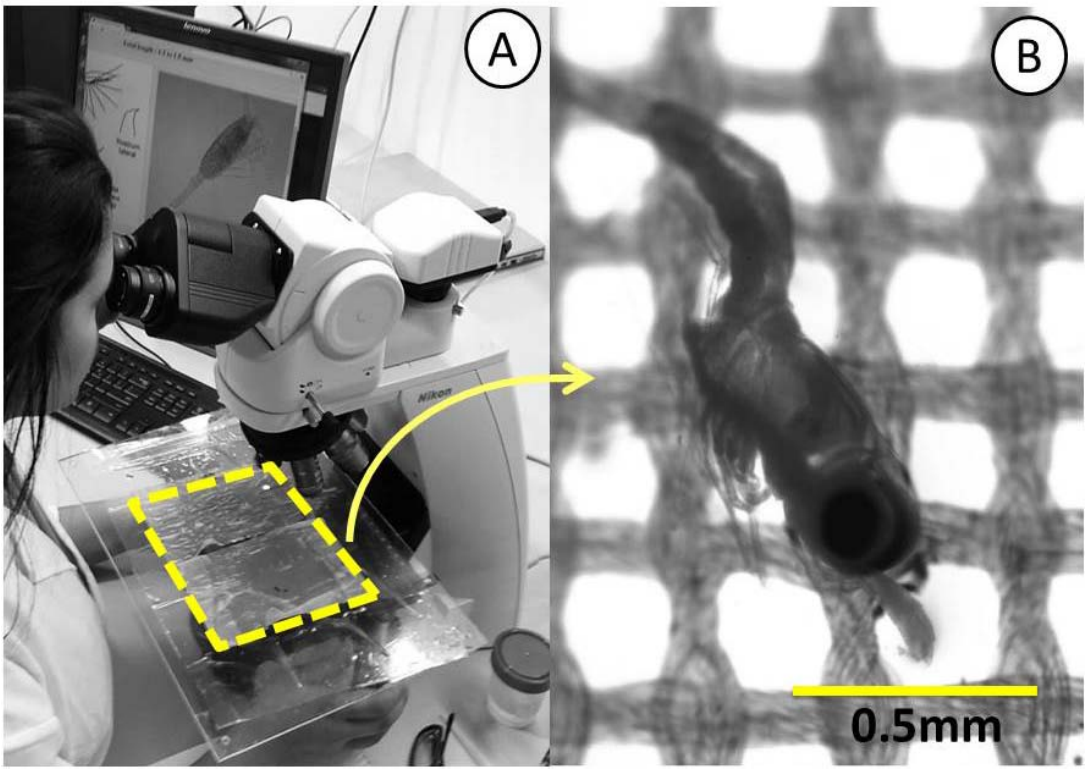

Figure 4: CPR on-silk analysis in the CyI MedCPR laboratory. (A) The sample is layed on a clear slate and analysed through the Nikon Ci-S microscope. (b) Specimen as it apears on the silk mesh.

CPR data availability

The data products derived from the analysis of CPR samples include the quantitative abundance and diversity of zooplankton and phytoplankton as well as a qualitative measure of ocean productivity referred to as the Phytoplankton Color Index (PCI). Due to the nature of the samples and the time needed for the taxonomic analysis of samples a time lag is created between the acquisition of the samples and the extraction of data. After the end of the PERSEUS project and an embargo period of 18 months, seasonal CPR data products will be made available (June, 2017) upon request through The Cyprus Institute.

A second set of data is produced through an oceanographic data logger (temperature, salinity and depth data). Time and position referenced salinity and temperature data derived from the logger will be made freely available to the public through the PERSEUS and CORIOLIS databases at the end of the project (December, 2015).

MedCPR Results

Since October 2014 and by July 2015, the MedCPR had collected ten samples (Table 2). The goal is to retrieve monthly CPR data for the MedCPR survey from the Levantine transect described above.

CPR tow schedule

PETROLINA OCEAN receives the CPR usually in the first week of each month, performs the tow, and returns the CPR to Cyprus. The SOOP has calls to two Cypriot ports, the Vasiliko and the Larnaca ports. Thus, to facilitate communication the route is dubbed as the Cyprus-Haifa route (route unique code: MCHL; additional information is given in Appendix I).

Table 2: CPR sampling in the Levantine Sea.

No	Tow Code	Date (dd/mm/yy)	Transect (From-To)
1	1MCHL	21/10/14	Larnaca-Haifa
2	2MCHL	05/11/14	Larnaca-Haifa
3	3MCHL	04/12/14	Larnaca-Haifa
4	4MCHL	22/01/15	Haifa-Larnaca
5	5MCHL	21/2/15	Vasiliko-Haifa
6	6MCHL	05/04/15	Vasiliko-Haifa
7	7MCHL	06/05/15	Larnaca-Haifa
8	8MCHL	24-25/06/2015	Vasiliko-Haifa
9	9MCHL	17-18/07/2015	Vasiliko-Haifa
10	10MCHL	19/07/2015	Haifa- Larnaca

Plankton is highly variable both in time and space, exacerbating the need for high resolution data. Concurrently, the continuation of the time survey is dependent on the proper function of the CPR instrument. The CyI realizing this need has already acquired an additional CPR internal. The internal (Figure 1) is the functional unit of the CPR. Having an alternative unit available, the CyI decreases the risk of interrupting the survey due to instrument malfunction. Secondly, by having two internals available both legs of the Cyprus-Haifa-Cyprus trip can be sampled, increasing both the spatial and temporal resolution. This becomes even more important when one of the legs is sampled during the day and the other during the night within the 1-6 day period of the round trip from Cyprus to Haifa. This new sampling mode has been quoted as twin-tows. The CyI has already successfully realized two twin tows in July and August, 2015. The first leg, Cyprus-Haifa, was sampled on the 17th -18th (overnight tow) while the second leg, Haifa-Cyprus, was sampled on the following day, the 19th. Sample analysis of twin tows is expected to provide information on diurnal variations and subtle changes in plankton abundance. Finally, with an additional internal we can extend the reach of a potential new CPR

route as a single internal is limited by the length of silk it can accommodate (approximately 500Nm can be sampled per internal per tow). For example, the currently available devices would be ideal for sampling transects between Cyprus and Athens (~800Nm) or Cyprus and Malta (~900Nm).

Data analysis and interpolation

The CPR produces three different data sets: two time-referenced tow position data sets, physical and biological data. The first tow position data set is provided by the crew of the SOOP (see Appendix II). The crew completes a tow log, where basic information includes date and coordinates where the CPR was cast and retrieved. The second vessel position data set is downloaded through an online Automatic Identification System (AIS) data provider (www.marinetraffic.com), and is reduced to correspond to the CPR tow. Time-referenced salinity and temperature data along the CPR track are collected through a CTD attached to the CPR body (Figure 3). Each tow results in a long roll of silk that has "recorded plankton continuously", as indicated by the name of the devise (see Appendix I, for a description of CPR methodology). This roll, which contains the biological sample, is then separated into equidistant samples that can be position and time referenced. Samples are kept in the MedCPR laboratory of the CyI where they are analysed (Figure 5). The CPR data set is then complete with the phytoplankton abundance index and phytoplankton and zooplankton abundance and diversity.

Figure 5: Samples are stored in the MedCPR laboratory in plastic boxes before the analysis [1-2]. The silk holding the sample can also be stored on the spool [3]. When samples are analysed the information is recorded on a notepad [4]. Later each sample is stored in indivual ziplock bags.

An important aspect of the CPR survey is the correct separation of the silk roll into individual samples and the assertion of the sampling location for each sample using tow-position information. This step is crucial for the correct connection of data to a specific section along the CPR transect. The ability to assert time and position for each unit of biological data is one of the important advantages of the CPR survey. Adjacent to this task is the visualisation of the CPR results. Once the samples are position referenced the data can be mapped and averaged both in time and space.

The development of an algorithmic tool that interpolates and visualises CPR data was part of Ms. Audrey Delpech's study, an undergraduate intern from ENSTA Paris Tech, Paris, France. The internship, completed at the CyI (May-July 2015), was arranged through the efforts of Dr. Laurent Mortier and co-financed by the Institut français de Chypre, Cyprus.

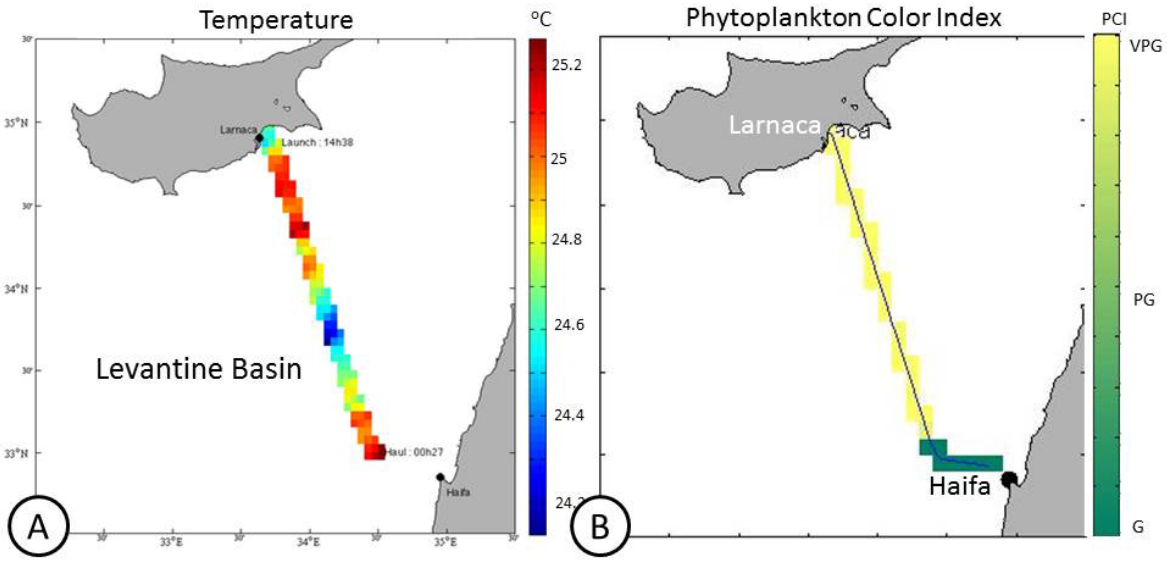

Figure 6: Temperature (A) and Phytoplankton color index (PCI; B) data interpolated through Matlab functions for the November 2014 transect. PCI (B) indicates the color of the CPR Silk (VPG: very pale green; PG: Pale green; G: Green).

During the internship algorithmic routines that integrate different CPR related data sets were produced in MatLab (Version 7.11.0584 (R2010b)); The MathWorks, Inc., United States). The initial part of the function resolves the position data for the performed tow and combines it with information on the length of the CPR silk roll to assert the length and position of each sample. The second part of the algorithm assigns location information to time-referenced oceanographic data. In the following step CPR datasets (plankton abundance, PCI and physical data) are position referenced and interpolated in time and space. Using the final part of the algorithm, the information can be visualised in customisable maps (Figure 6).

The code can be upgraded with the addition of a user interface that will facilitate the use of the functions and decrease the possibility of human error and an adjacent tool that will map and compare satellite data with CPR data.

Physical data

The oceanographic sensor adjusted to the CPR is pre-set to acquire measurements every minute for the duration of the SOOP's voyage. The data are then downloaded and processed using Microsoft Excel 2010 templates (Microsoft, USA) and Matlab algorithms developed for the MedCPR. Because of the time (diurnal, monthly) difference among observations it is perilous to extract any definite trends before the collection of a longer dataset. The temperature profile of the October and November tow show a warming of waters near the Israeli coast (Figure 7). This may be attributed to the time of the sampling since all three transects started from Cyprus in the morning and reached Haifa more than 10 hours later. In addition, there is an observed drop in offshore surface water between the coast of Larnaca and Haifa (e.g. Figure 6a, Figure 7). Localised drops in sea surface temperatures are often linked to water mixing by the Cyprus Eddy and other convection processes (Groom et al. 2005).

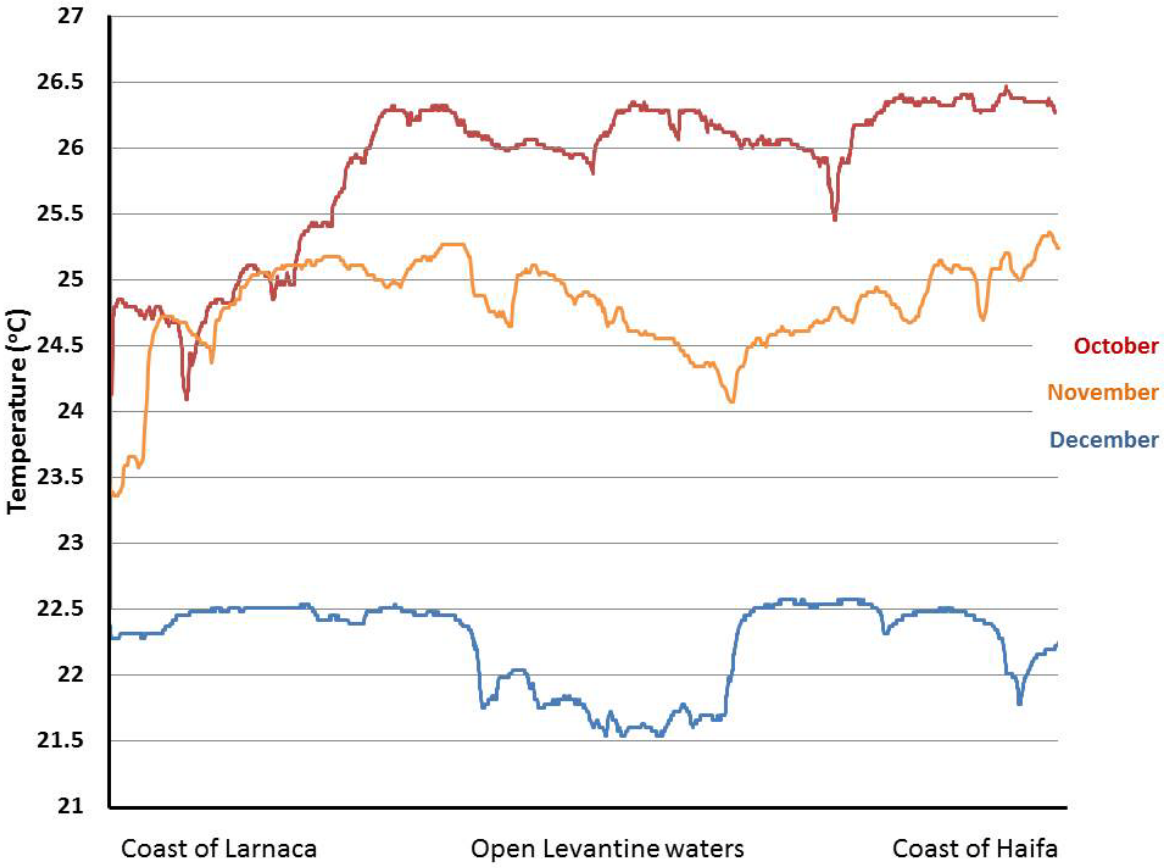

Figure 7: Surface Sea Temperature data collected by the CTD logger attached to the CPR.

As more samples are collected we expect to reduce noise and have a more coherent image of the basin and use physical data interpreting CPR biological information.

During the internship, Ms Delpech also worked with Prof. Lars Stemmann on the examination of oceanographic satellite and modelling data in order to link the increase of chlorophyll-a (chl-a) concentration to the Eastern Mediterranean Transient -like (EMT-like) event as this was described in Kress et al (2014). The

study, which will be made available online, describes the use of more than a decade's worth of satellite data in the recognition of long-term patterns in the circulation of the Eastern Levantine. Classical Eastern Mediterranean circulation is driven by the flow of cooler Atlantic water along the southeast coast. During an EMT-like event, water masses from the Adriatic and the Aegean Seas force the deflection of the Atlantic current to the west and the re-circulation of Levantine intermediate waters. Although subtle, it is hypothesised that these changes in circulation drive cascade changes in salinity, temperature and productivity of surface waters. The study has linked temperature and salinity changes to the increase in chl-a concentration but the lack of long term in-situ data prevents any certain conclusion on the adoption of the EMT-like event as the driver of these observations.

The CPR can play an important role in future studies of Levantine circulation as it provides physical and biological data that could be instrumental in the identification of change and its effect on plankton populations.

PCI and plankton information

Phytoplankton Colour Index (PCI) is a quantitative method of assessing primary production (details on the methodology are provided in Appendix I). Initial PCI observations depict the oligotrophic nature of the Eastern Mediterranean. Occasional increases in the PCI are observed near the coast of Haifa (Figure 6b), probably due to the flow of nutrients from coastal cities.

Preliminary results from analysed CPR samples (Figure 4) corroborate the initial hypothesis of oligotrophic warm waters. Based on the fall MedCPR samples analysed thus far, Tintinids (Figure 8d) are the most abundant group. Tintinids, tiny encased planktonic animals (ciliates), are characteristic of low productivity regimes since the latter favours smaller less complex predators. In addition, the southern part of the route, closer to Haifa, tends to have increased zooplankton abundance following the increase in productivity indicated by PCI results.

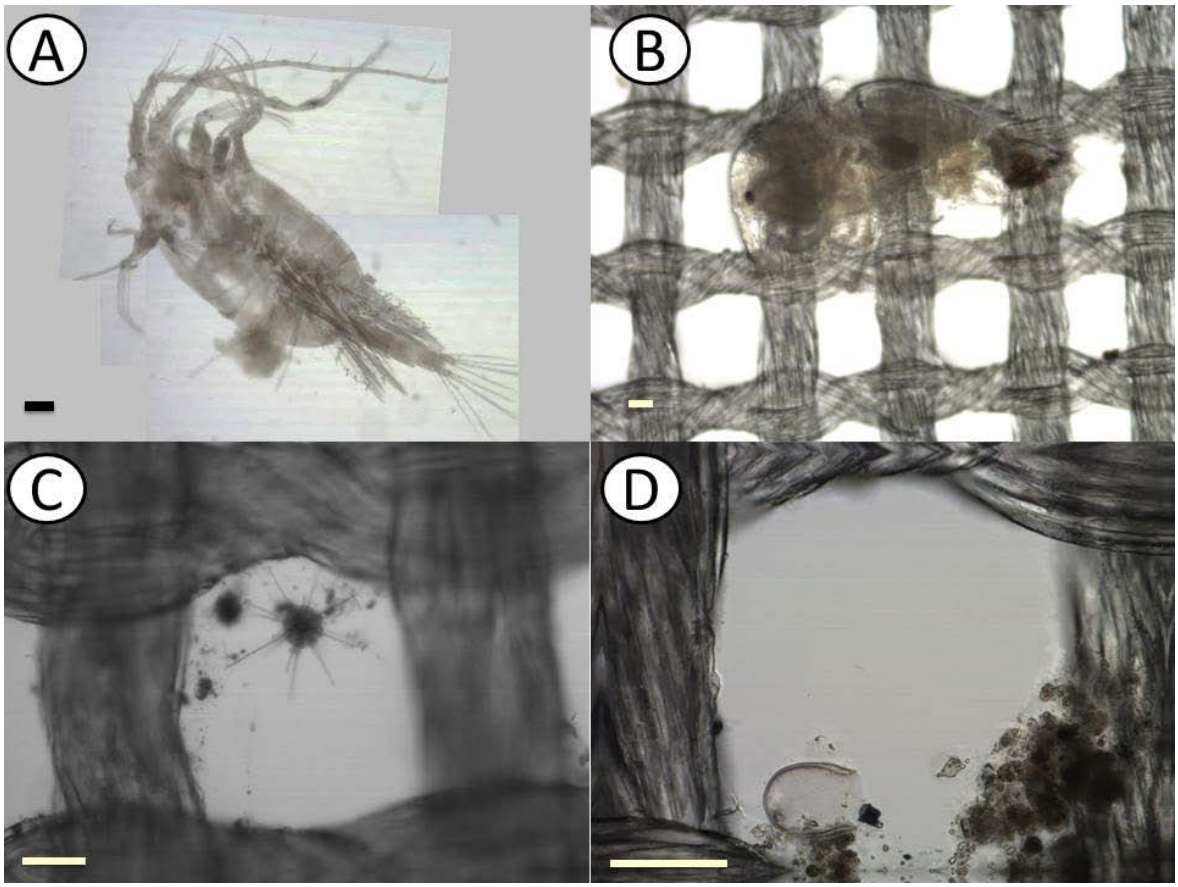

Figure 8: Copepods (A, B) and micro-zooplankton (C, D) collected by the CPR in the Eastern Mediterranean in November 2014 (pictures where edited). The method calls for the on silk analysis of samples (silk visible in B, C and D). Scale bar: 0.1mm

The MedCPR is intended to be a long term monitoring survey where with the increased number of analysed samples trends as well as isolated events will be revealed.

Conclusion

The Cyprus Institute has completed the successful implementation of the CPR survey in the Mediterranean Sea. Although this tool has been used in the study of plankton for more than 80 years, this is the first time it samples the Eastern Mediterranean basin and the first time operated by a Mediterranean country in the region.

The MedCPR survey, which is expected to complete fifteen months of successful sampling by the end of the PERSEUS project (December 2015), has marked the beginning of a baseline database for the Levantine. The expanding of the Suez canal (Galil et al. 2014), temperature increases (Samuel-Rhoads et al. 2010) and intense hydrocarbon exploration are all expected to change the Levantine environment. The availability of a monitoring system able to deliver physical and biological data, during and after these events will be an asset to scientists and stakeholders of the region.

The CPR survey has a steep implementation curve. A substantial amount of effort and time is put into the initial set up of a new survey. This includes the communication

with shipping companies, setting up the laboratory and troubleshooting region specific challenges. This was not experienced only by the CyI team working on the implementation of the MedCPR, but also by other CPR sister surveys (AusCPR, SO-CPR etc.). The eminence of this survey is that once the initial effort is put in, and funding is provided, the CPR has the potential of being a high-throughput monitoring system.

Through this experience, the CyI has gained valuable insights which can facilitate the expansion of the survey in the region. The infrastructure, capacity building and network acquired by the CyI are an advantage to the region and gives the potential for the continuance of the CPR Survey in the Levantine area and the Southern European Seas in general. The untapped commercial shipping activity in the area has the dynamic of becoming an asset to marine plankton science (Figure 9). With the engagement of regional partners and the support of the community, the MedCPR survey could grow, giving information of the current state and the changing ecosystems of the Mediterranean.

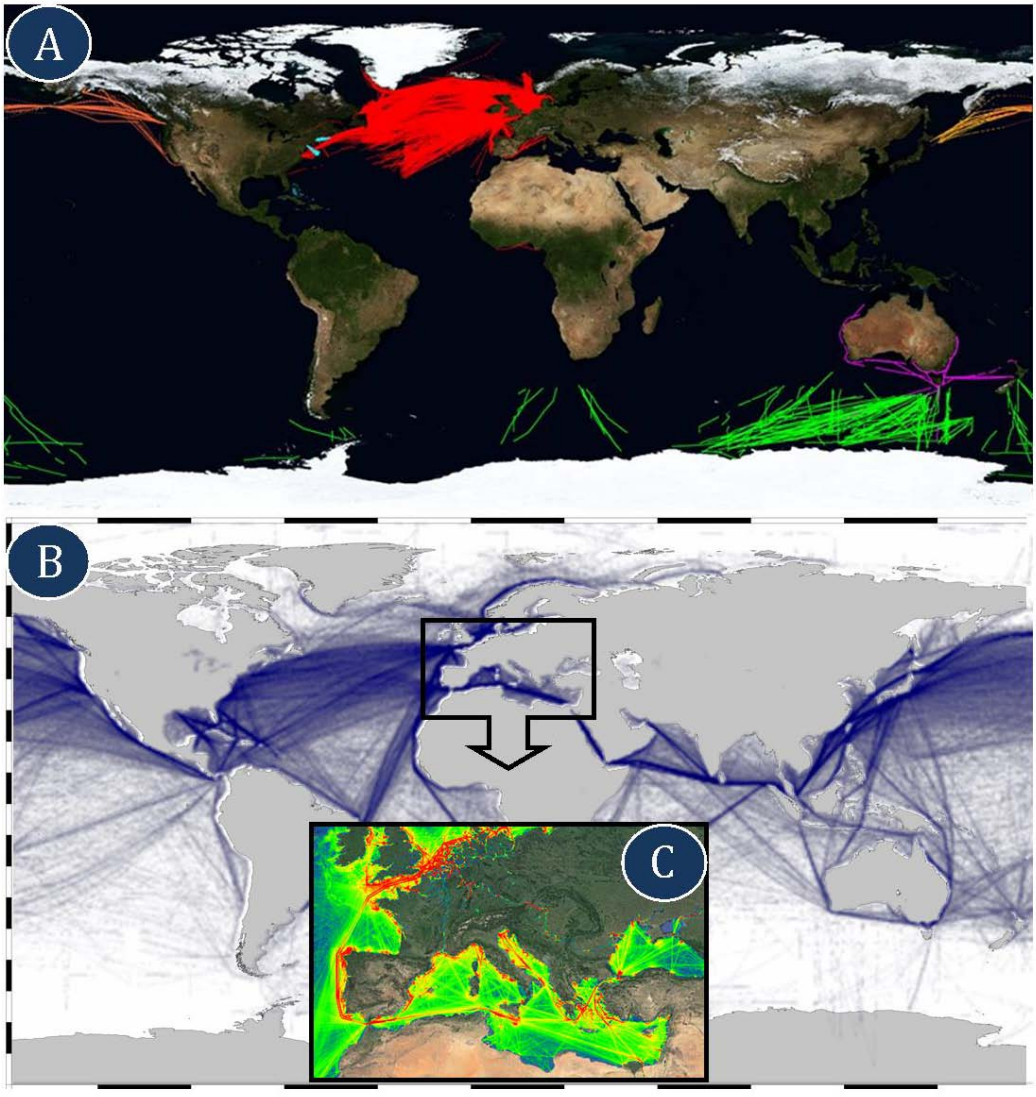

Figure 9: CPR sampling routes (A). Colours represent different CPR surveys (SAHFOS; SO-CPR; AusCPR; NOAA; SAHFOS 2013). Worldwide vessel density map (®Grolltech) (B) The increased

commercial shipping activity in the region (www.marinetraffic.com)(C). Each track is a potential CPR sampling route.

Colleagues working on Mediterranean and Black Sea plankton communities have already shown interest in exploring such options. In addition, the CPR workshop to be held in Cyprus (Milestone 42, WP8, PERSEUS Project), has the potential to consolidate a network of collaborators interested in expanding the survey.

The PERSUS objective was to set up the MedCPR Facility and initiate the survey within the project timeframe. Its aspiration is to continue and expand the survey, utilizing the current infrastructure. This translates into continuing the current route, establishing new routes operated by the CyI or other regional laboratories and expanding the marine research collaborations in the area. Within this, Cyprus will act as a point of reference and a centre for the transfer of CPR knowledge to the region as is the vision of the PERSEUS project.

Bibliography

Bernhardt, J.R. & Leslie, H.M., 2013. Resilience to Climate Change in Coastal Marine Ecosystems. *Annual Review of Marine Science*, 5(1), pp.371–92.

Bianchi, C.N., 2007. Biodiversity issues for the forthcoming tropical Mediterranean Sea. *Hydrobiologia*, 580(1), pp.7–21. Available at: http://www.springerlink.com/index/10.1007/s10750-006-0469-5 [Accessed November 14, 2012].

Coll, M. et al., 2010. The biodiversity of the Mediterranean Sea: Estimates, patterns, and threats. *PLoS ONE*, 5(8), p.e11842.

Crise, A. et al., 2015. A MSFD complementary approach for the assessment of pressures, knowledge and data gaps in Southern European Seas: The PERSEUS experience. *Marine Pollution Bulletin*. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0025326X15001691.

Durrieu de Madron, X. et al., 2011. Progress in Oceanography Marine ecosystems ' responses to climatic and anthropogenic forcings in the Mediterranean. *Progress in Oceanography*, 91(2), pp.97–166. Available at: http://dx.doi.org/10.1016/j.pocean.2011.02.003.

EEA, 2006. *Priority issues in the Mediterranean environment*, Copenhagen, Denmark. Available at: http://www.eea.europa.eu/publications/eea_report_2006_4.

Galil, B.S. et al., 2014. " Double trouble ": the expansion of the Suez Canal and marine bioinvasions in the Mediterranean Sea. *Biological Invasions*, pp.1–4.

Giorgi, F. & Lionello, P., 2008. Climate change projections for the Mediterranean region. *Global and Planetary Change*, 63, pp.90–104.

Groom, S. et al., 2005. Satellite-derived spatial and temporal biological variability in the Cyprus Eddy. *Deep Sea Research Part II: Topical Studies in Oceanography*, 52(22-23), pp.2990–3010. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0967064505002079 [Accessed November 8, 2012].

Hays, G.C., Richardson, A.J. & Robinson, C., 2005. Climate change and marine plankton. *Trends in Ecology and Evolution*, 20(6 SPEC. ISS.), pp.337–344.

Jimenez, C. et al., 2014. Mortality of the scleractinian coral Cladocora caespitosa during a warming event in the Levantine Sea (Cyprus). *Regional Environmental Change*, 14(6).

Katsanevakis, S. et al., 2013. Invading European Seas: Assessing pathways of introduction of marine aliens. *Ocean & Coastal Management*, 76, pp.64–74. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0964569113000562 [Accessed June 4, 2014].

Koukouras, A. et al., 2010. Evolution of the entrance rate and of the spatio-temporal distribution of Lessepsian Crustacea Decapoda in the Mediterranean Sea. *Crustaceana*, 83(12), pp.1409–1430. Available at: http://booksandjournals.brillonline.com/content/10.1163/001121610x539498 [Accessed May 13, 2013].

Kress, N., Gertman, I. & Herut, B., 2013. Temporal evolution of physical and chemical characteristics of the water column in the Easternmost Levantine Basin (Eastern Mediterranean Sea) from 2002 to 2010. *Journal of Marine Systems*, 135, pp.6–13. Available at: http://dx.doi.org/10.1016/j.jmarsys.2013.11.016.

Lejeusne, C. et al., 2010. Climate change effects on a miniature ocean: the highly diverse, highly impacted Mediterranean Sea. *Trends in Ecology and Evolution*, 25(4), pp.250–260.

Mackas, D.L. et al., 2012. Changing zooplankton seasonality in a changing ocean: Comparing time series of zooplankton phenology. *Progress in Oceanography*, 97-100, pp.31–62. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0079661111001236 [Accessed March 5, 2013].

McQuatters-Gollop, A. et al., 2007. A long-term chlorophyll dataset reveals regime shift in North Sea phytoplankton biomass unconnected to nutrient levels. *Limnology and Oceanography*, 52(2), pp.635–648.

Menna, M. et al., 2012. On the surface circulation of the Levantine sub-basin derived from Lagrangian drifters and satellite altimetry data. *Deep-Sea Research Part I: Oceanographic Research Papers*, 65, pp.46–58.

Norse, E. a. et al., 2012. Sustainability of deep-sea fisheries. *Marine Policy*, 36(2), pp.307–320. Available at: http://dx.doi.org/10.1016/j.marpol.2011.06.008.

Samuel-Rhoads, Y. et al., 2010. Sea surface temperature and salinity rise in the Levantine basin. *Rapid Communications int. Mer Méditerranea*, 39, p.177.

Tzomos, T. et al., 2012. Evolution of the entrance rate and of the spatio-temporal distribution of Lessepsian Mollusca in the Mediterranean Sea. *Journal of Biological Reasearch*, 17, pp.81–96.

UNEP, 2007. *Approaches to the assessment of eutrophication in Mediterranean coastal waters*, Athens, Greece. Available at: http://195.97.36.231/acrobatfiles/07WG321_Inf6_eng.pdf.

Yacobi, Y.Z. et al., 1995. Chlorophyll distribution throughout the southeastern Mediterranean in relation to the physical structure of the water mass. *Journal of Marine Systems*, 6(3), pp.179–190. Available at: http://linkinghub.elsevier.com/retrieve/pii/092479639400028A.

Appendix I: CPR Methodology

Within this section the CPR methodology is briefly illustrated. This document does not only include parts of the basic CPR training received by CyI staff from SAHFOS, UK, but also lessons learned through experience in the implementation of the survey in the Levantine Sea.

General description of the CPR device

The CPR is a metal (stainless steel) instrument of about 100kg, 1m length, 40cm high and 40cm wide, with a pyramid-shaped front nozzle, and rectangular middle and wide vertical flanks on the sides (Figure I- 1). A propeller is located on the rear end of the CPR. The CPR is streamed using a wire shacked to a shock absorber pin in the front of the CPR.

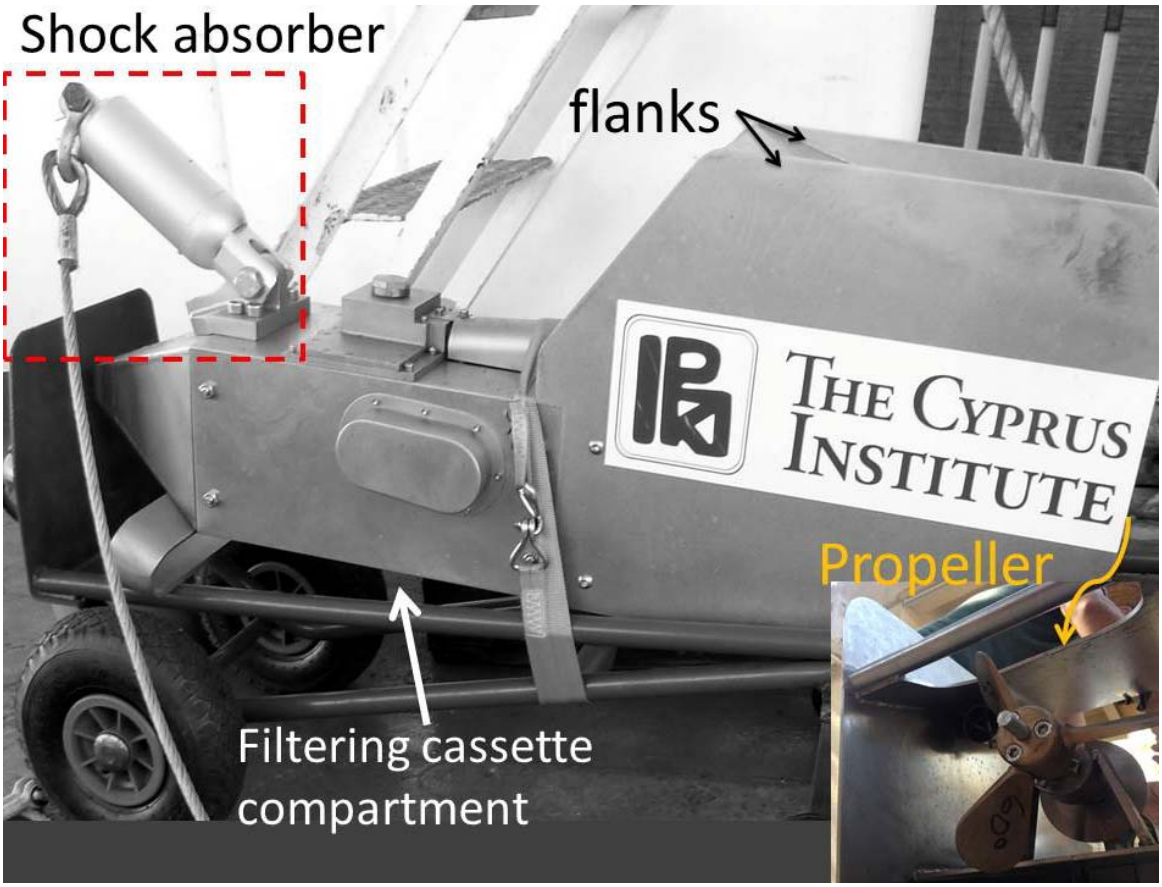

Figure I- 1: The CPR device and some of its integral parts.

The CPR body has a removable cover that closes an internal compartment holding the functional unit of the CPR (Figure I- 1), henceforward referred to as the "*internal*" or "*cassette*" (Figure I- 2). The internal is the part which performs the sampling and ultimately preserves the sample. Each internal has a serial number from the manufacturer; since some components were hand-made, it is often the case that the parts of one internal will not fit exactly into another.

The internal, when prepared for sampling, holds two long strips of finely weaved silk, rolled on separate spools (Figure I- 2). Each strip has a unique function and characteristics. The first, called the *filtering silk*, is single, unfolded, divided in numbered 5cm sections and passes from the middle of the internal. The second, called the *covering silk*, runs across the top part of the internal and is folded so that about 2cm at the side is double. The folded part is sniped diagonally every 10cm and glued, securing it to the strip. Both strips have the same width (about 15cm). Within the internal, the ends of the two silk strips are brought together, with the folded part of the covering silk in the middle.

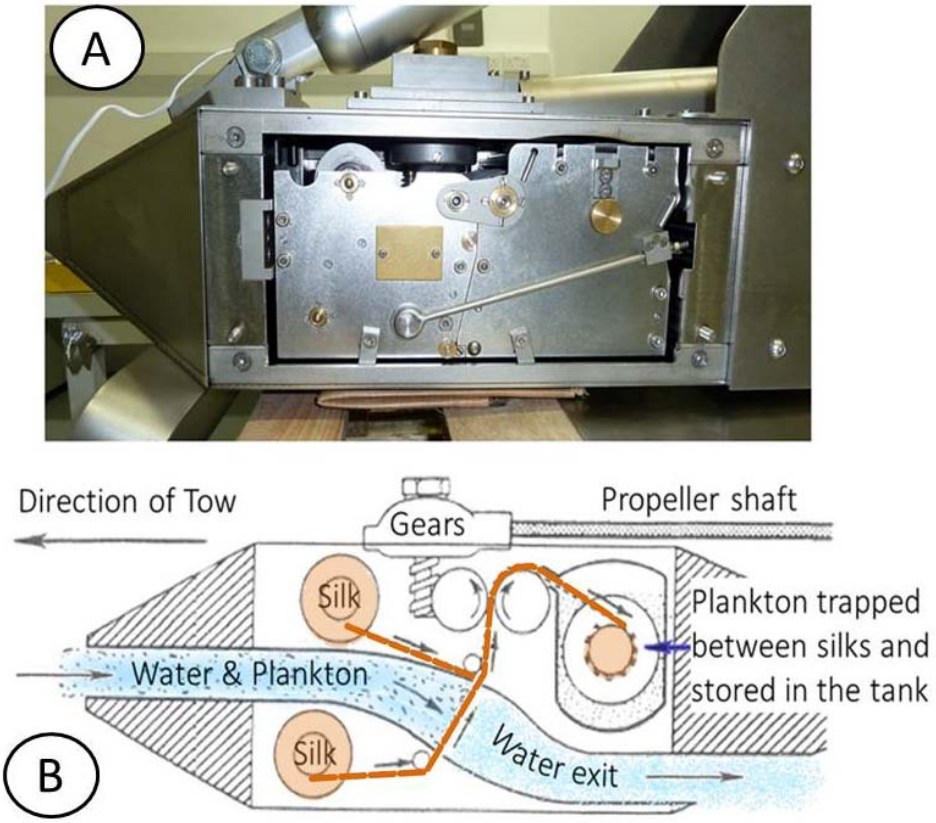

Figure I- 2: Plankton filtering mechanism. Water and plankton enter through the orifice in the nozzle of the CPR. Plankton is then filtered and trapped between two bands of silk (orange). The rolling of the bands is controlled by the gears and propeller shaft. Diagram modified from an original provided by SAHFOS Lab., Plymouth.

The preservative used in the MedCPR is alcohol. Alcohol fixates plankton allowing for stereoscope and genetic analysis of samples. Using alcohol as a preservative facilitates the analysis process since fewer precautions need to be taken as opposed to other alternatives (e.g. formalin solutions) (Table I- 1).

As the CPR is hauled at speeds between 6-15knots, water goes through the ~1.27cm^2 aperture in the front of the CPR and into the pyramidal compartment, causing it to slowdown and enter the internal (Figure I- 2). The rushing water is filtered by a piece of filtering silk that is exposed within the internal. The silk (mesh size 270um) allows for the sampling of mesozooplankton and smaller zoo- and phyto-plankton groups because of it's intertwine weave pattern. Simultaneously, as the CPR moves forward,

the propeller drives a continuous pull, slowly bringing a fresh part of the silk roll in front of the CPR aperture, while the cover silk seals the filtering silk, forming a plankton sandwich. It is estimated that 1.17m^3 of surface sea water are filtered per 5Nm travelled by the CPR, represented by a 5cm division of the silk. The two overlapping strips of silk are rolled on a spool in the preservation compartment. This tank communicates with an enclosed part of the internal that is filed with cotton, soaked in undissolved preservation liquid. Sea water entering the internal helps dissolve this liquid within the preservation compartment and thus preserve the sample.

Table I- 1: Fixating and preserving CPR samples across different laboratories. MedCPR: Mediterranean CPR operated by The Cyprus Institute; SAHFOS: Sir Alister Hardy Foundation for Ocean Sciences, operating many CPR routes mainly in the Atlantic; AusCPR: Australian CPR, operated by Commonwealth Scientific and Industrial Research Organisation (CSIRO), Australia and the Australian Antarctic Division. PGP: Propylene Phenoxetol and Propylene glycol based solution

Preservation liquid for:	MedCPR	SAHFOS	AusCPR
Fixation	90% alcohol	40% buffered formalin	40% buffered formalin
Analysis	70% alcohol, water	PGP solution	PGP solution, water
Long-term storage	4% buffered Formalin	4% buffered Formalin	PGP solution

The internal mechanism or filtering cassette is a robust self-contained cartridge. It is loaded with the filtering silks (Figure I- 2) and is put inside the CPR right before the tow. The cassette needs to be aligned with the rails and the vertical worm drive gear of the CPR. The cassette is not forced into the casing but rather slided in being careful not to disturb the fusee and fusee wire on the port side of the internal (Figure I- 2). When the internal is in, the gears mesh up and the item slides in place.

CPR tow

The laboratory delivers to the SOOP all the equipment needed to tow the CPR. This will include the CPR body, a loaded CPR internal, 100m long steel wire, tow blocks, quick-release hook and shackles. All items are carefully inspected and the condition recorded in logbooks for each component. Deteriorated or time-expired items are discarded and replaced with new ensuring that the CPR and accessories are in proper condition for the safety of the crew and the CPR.

For the transfer of the CPR body a modified hand trolley is used, while the internal is kept in a dedicated hard-case. *The CPR and all equipment are always clean, well maintained and shipshape.* The characteristics and equipment of the SOOP dictate how the CPR will be towed. An available tow davit on-deck is usually the straight-forward method, but if one is not available, other solutions can be found. For example,

the CPR laboratory may fund the built of a dedicated tow-davit on the SOOP. On board the PETROLINA OCEAN, the CPR is towed using the panama bow.

The CPR is connected to the tow wire by a shackle, and tows between 6 to 10m depth below the sea surface and about 70-80m from the ship (Figure I- 6). The maximum pull on the wire is 0.4 tonne at 20 knots (speed of other SOOPS not in Cyprus). The recorder CPR requires no special attention from the ship's crew and requirements are limited to launch and recovery.

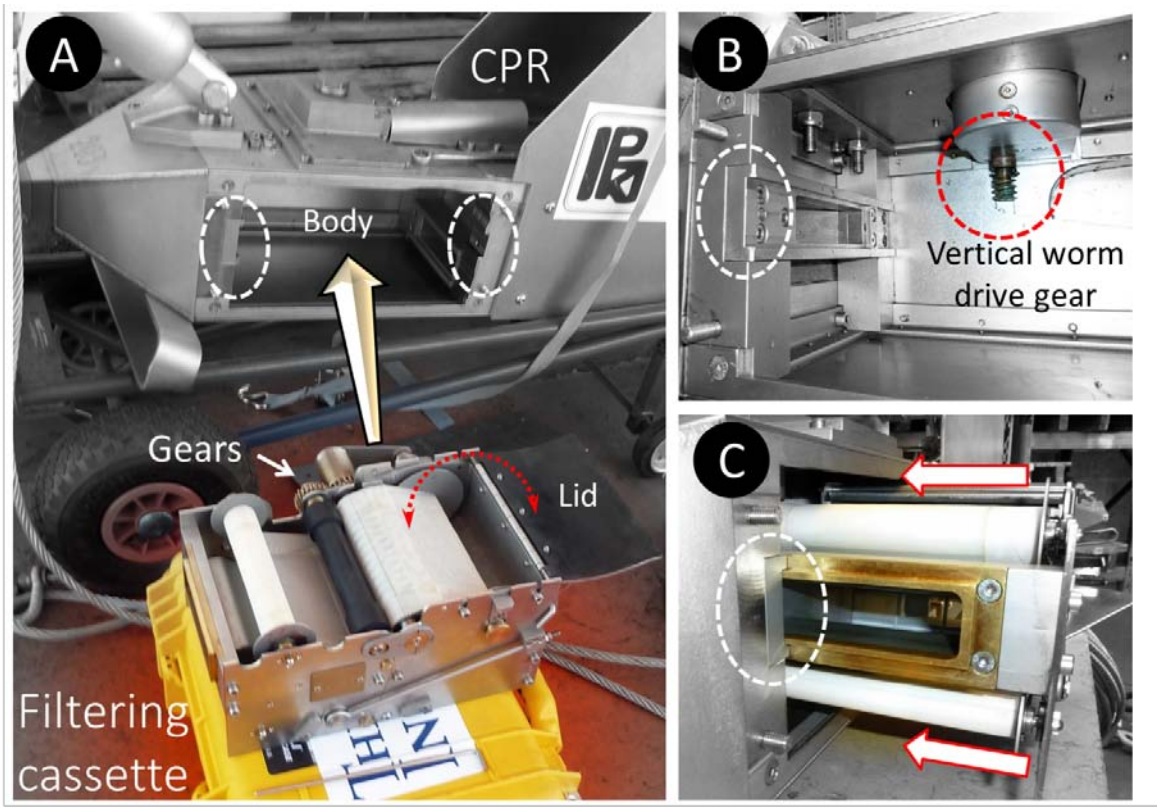

Figure I- 3: Internal mechanism or filtering cassette. It is shown here with an open lid (red arrows) and the CPR; both port side view. (B) Inside of the body where the filtering cassette is inserted. Gears of the cassette need to mesh with the vertical worm drive gear of the CPR. (C) The internal cassette is placed into the body by aligning its frame and sliding it along the notches.

The MedCPR tows may be performed either from Cyprus to Haifa or on the way back. Which leg of the round trip, and if the CPR will be towed at all, is left to the discretion of the ship's master. In general, there are a series of suggestions on when it is not advisable to tow the CPR. These include fog or conditions of reduced visibility, heavy weather conditions and in the presence of fishing vessels. Once the vessel is underway, and the captain gives the order, and the wire is shackled to the shock absorber on the nose of the CPR. The other end of the wire is fed through the head block, down through the panama bow, bitts and rollers (Figure I- 4) and onto the winch drum with a minimum of five turns around it.

The mooring winch raises the CPR through the tow-block and lowers it into the water steadily (Figure I- 4B), until it sinks at 6-10m; then, the block is secured to the side of the ship. The sinking depth of the CPR is regulated by the wire length paid, which in

turn will depend on the ship's speed. A yellow tow mark, about 22m upstream from the CPR (Figure I- 5), indicates the wire that must be paid. At around 12knots vessel speed, the yellow mark should be at sea surface, so that the CPR is at the required depth (6-10m). If speed is 10knots, the crew must shorten tow wire, bringing yellow tow mark in 10m out of the sea. If ship reduces to 5knots, the tow mark is about 20m out of the sea. The depth at which the CPR sinks is crosschecked with information from the CTD and the speed of the vessel given in the tow-log form or online vessel tracking webtools (e.g. www.marinetraffic.com).

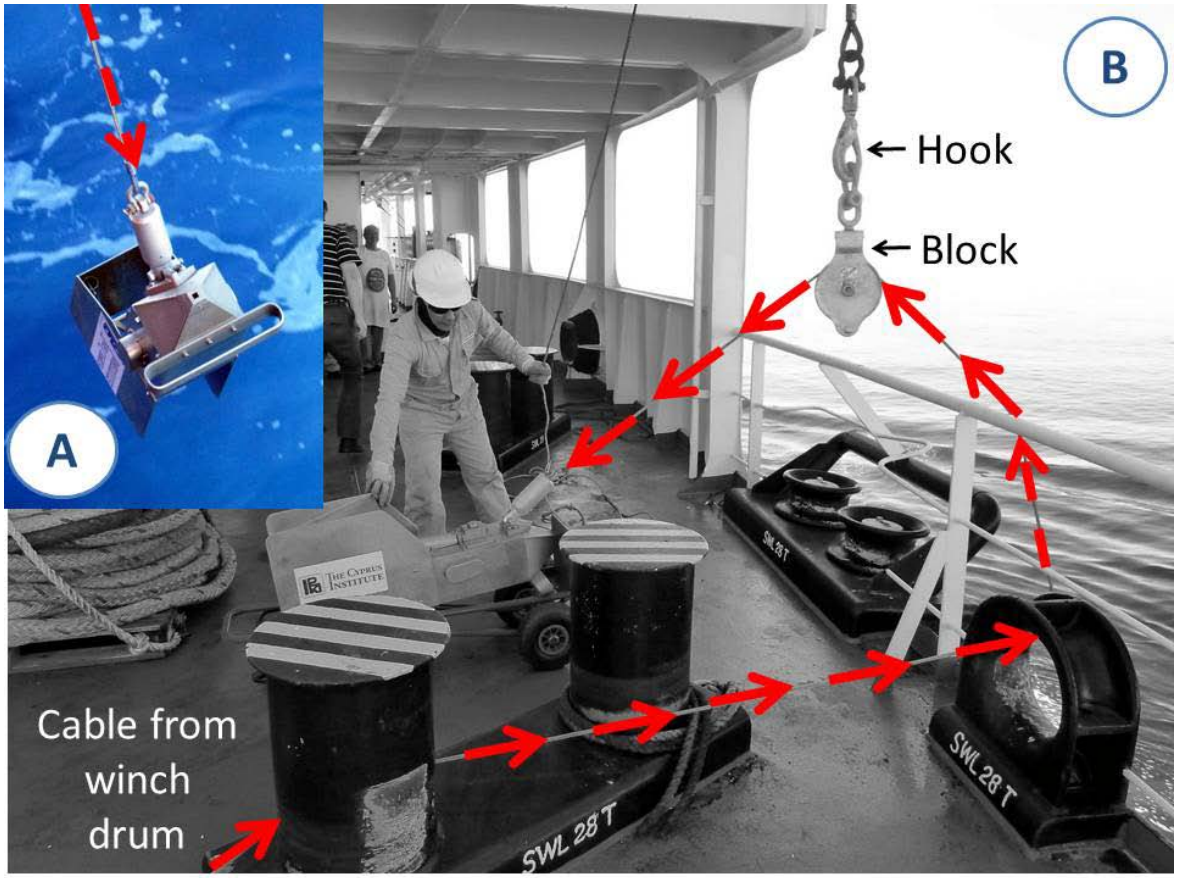

Figure I- 4: (A) Arrangement onboard of the vessel for the CPR tow. (B) The CPR before it is streamed into the Levantive Sea for the first time (Oct. 21st, 2014).

During the first MedCPR tows, the CPR did not sink to the required depth possibly because the speed of the vessel was more than 12knots. When the speed is above 12knots more wire must be paid. Therefore, we increased the wire paid and made a new, red mark on the inboard part of the wire.

Once the CPR is sunk (Figure I- 5), it ultimately takes its normal towing position, which is usually dead astern or slightly angled to either side depending on the ship's propeller wake eddies. The CPR is designed as to request minimum attention and work from the vessel crew and almost no effort while underway. At this time, a crew member assumes the responsibility of keeping the tow record by filling the provided form. This will include time, date, position, course and speed of the vessel at CPR launch, haul and every two hours while the CPR is in the water. Brief notes on the

weather conditions and cloud cover during the tow are also logged. An example of the tow-log is given in Appendix II.

The CPR is not hauled until the end of the route, only when speed is below 3knots or approaching the harbour or mooring station. For the haul, the CPR is brought to the surface and onto the deck while the vessel is underway. This process must be performed accurately and with care. Two basic points of caution are taken into consideration. First, the CPR is hauled away from the side of the vessel to avoid impacts. Secondly, the winch operator must stop wiring the cable before the CPR is too close to the head block.

When the CPR is on board, the wire is released, the side of the CPR body is opened and the filtering cassette is removed and put into the protective case. All the equipment is washed with fresh water then inspected for damage or deterioration and stored at the designated CPR area on-board the PETROLINA OCEAN. As the CPR collects plankton continuously, the last samples remain outside the preservation tank when the recorder is hauled. Therefore, the last action of the vessel crew, regarding the survey, is to pour preservative liquid over the silk once or twice a day, until the CPR body and internal are unloaded from the vessel.

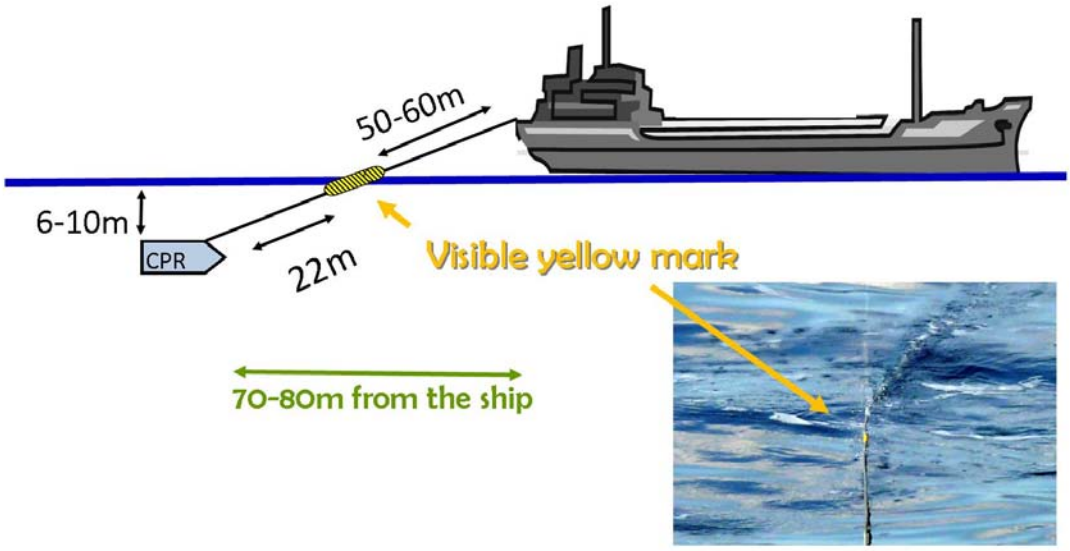

Figure I- 5: Towing the CPR. At ship speed of about 12knots the CPR sinks at the required depth (6-10m) when the yellow mark is visible at sea surface and about 70-80m from the ship.

The CPR is loaded/unloaded from the vessel either through a launch (if weather conditions allow it) or directly from the marina if the vessel is alongside. In the case of the Cyprus-Haifa route, the wire, blocks and other CPR equipment remain on-board where a CPR technician together with trained crew members check and certify that all the equipment is up to standards. The technical workshop at the CyI will then receive the CPR body, the internal, the remaining preservative liquid and the filled tow-log form. When the tow-log form is received, the CPR laboratory offers the crew of the SOOP a small compensation for their help. The amount of the *crew*

compensation (€75) has been relatively unchanged through the years and around the globe, a tradition continued by the CyI.

CPR route names

Each route has a unique code as to distinguish it from past and future CPR tows. The code given to the route is MCHL: M: Mediterranean (to be given in the future to all Mediterranean CPR routes), C: CyI-Cyprus (to denote Mediterranean routes operated from Cyprus/CyI), H: Haifa, L: Larnaca. The code also facilitates the incorporation of MedCPR data into CONSOLE, an algorithmic tool developed by SAHFOS for the integration of CPR data. CONSOLE needs a unique identifier for each route. Each tow also has a serial number. For example, the first tow, taking place in October, 2014 is 1MCHL. All samples from that tow are marked with the same code followed by the serial number of each sample. This way we can trace time and location of each sample within the survey.

CPR maintenance

When back in the lab the sample is removed from the internal and both the internal and the CPR body go through a strict maintenance protocol. All parts are checked, repaired or rectified (if needed), cleaned and greased.

Maintenance of CPR body

The CPR may be damaged at any part of the CPR tow especially while it is being hauled. The CPR is hauled while the vessel is underway so there is an increased possibility that the CPR will hit the side of the vessel as it is coming up, especially with high wind or swell conditions. While all precautions against CPR and vessel damage are made, the CPR workshop should be prepared for the worst. Furthermore, even if there is no readily visible damage, it is of paramount importance that the CPR, the internal, and accessories are checked and well maintained after each tow.

The internal is removed from the CPR body as soon as the CPR is hauled, so when the CPR body reaches the technical workshop it is void. The CPR technician starts by inspecting the pin at the front part of the CPR. The *pin* holds the shock absorber (Figure I- 1). If it seems loose or not in its correct position, the pin is disassembled, the inside is repositioned, greased and put back together. The *flanks* are exposed and could be bent after a tow. In the workshop they are checked and fitted. The *propeller* (Figure I- 1) *and gears* (Figure I- 3) are responsible for the movement of the CPR silk within the internal. The three fans of the propeller are adjusted in the angle that matches the speed of the SOOP and results in the sampling of 5Nm on a 2.5in long silk division. The propeller is turned to ensure that the gears are correctly moving in-sync with the propeller and the later are cleaned and greased. The *nozzle*, *internal slot* and all other parts of the body are also checked for damage and repaired if needed. The CPR body is then cleaned and sprayed with a lubricant for further protection.

Maintenance of CPR internal

The internal is brought to the workshop holding the plankton sample. The end of the silk with the last samples is still exposed as the CPR is continuously sampling until the moment it is hauled. The silk is marked and the technician calculates the true end of

the CPR tow on the silk. The spool holding the sample is removed; additional silk is rolled on the spool and then cut. At this point the spool is either stored or the silk is removed to start the sample analysis promptly. If the spool is to be stored, a piece of lint is rolled on top of the sample, which is then placed vertically in a plastic container with preservation liquid (see Figure 5). The route code and date are marked on the container.

After the silk is removed, the internal is cleaned and checked for damage. As a first step, the cotton in the preservative compartment is removed. The internal is disassembled and all parts are scrubbed clean using a brush and a specialised cleaning soap. All parts are put in a container marked with the serial number of the internal. After all the parts are dry, any remaining impurities are sanded off and all gears and corners are checked for damage. Each part is individually lubricated and then the internal is put back together.

Before the next voyage, the internal is loaded with fresh rolls of silk and cotton (Figure I- 3). The fusee is loaded with a thin wire is to connect and drive the movement of the spools to the propeller. The fusee and wire are at fragile positions on the side of the internal, so that they communicate with the cog wheels that transmit the movement of the propeller, so care is taken when handling the loaded internal. Finally, preservative liquid is used to soak the cotton through the small holes (~1mm Ø) present between the cotton and preservation compartments.

During the first ten MedCPR tows, the CPR has not suffered any severe damage.

Maintenance and other CPR forms

As outlined above, the unloading maintenance and loading of the CPR internal is a process that requires many steps and is often completed over the period of several days. In order to keep track of the intricate parts of the protocol, a form that includes all the necessary information, dates and person responsible is filled. One more form is compiled to keep track of the major steps in the process, such as CPR delivery to the vessel and tow realisation. Good record keeping facilitates quality control processes and locating possible mistakes or omissions. Examples of the forms used in the MedCPR are found in Appendix II.

CPR calculations: CONSOLE and its alternatives

SAHFOS has developed an algorithmic tool that has the ability to store, analyse and report CPR information. CONSOLE accepts tow-log data (date, time and coordinates) from which it calculates ship speed, the miles per division factor (MPD) and the sample cutting points. In addition, the application includes a built-in mapping tool, where the route is mapped and a tool that can be used to visualise CPR data. MedCPR, as an active member of GACS, has access to CONSOLE through remote desktop connection to the SAHFOS system. Although CONSOLE is a powerful and relatively easy tool to use, remote connections are not always stable and it was preferred the data were not stored only on the remote server but also locally. Therefore algorithmic tools for all the functions readily needed, were developed by the MedCPR laboratory using available software solutions. The coordinates are inserted as points on the Google Earth mapping application (Google Inc., California), where the distance between each one is calculated using the measuring tool. Using Microsoft Excel

(Microsoft Corp., Washington) the sum of the distance of the sections is divided by the duration of the tow to calculate speed and the number of silk divisions to find he MPD factor. A template worksheet was created which outputs the cutting points (Appendix II). For quality control, the results are often checked against CONSOLE cutting points. CPR sample analysis data are also entered in an excel worksheet.

CPR sample analysis

The samples recovered from the CPR internal are analysed for primary productivity, phytoplankton and zooplankton abundance and diversity. After the tow, the CPR silk is cut into individual samples which represent a specific part of the transect. The calculations for cutting of the silk are calculated by an algorithm. Each sample goes through three stages of analysis: the Phytoplankton Colour Index analysis (PCI), the phytoplankton and the zooplankton enumeration. Once all samples from a given route are analysed, differences in abundance, species composition and phytoplankton concentration can be seen along the analysed transect.

Silk Cutting points

CONSOLE (or its alternative) is used to insert tow-log data and output sample cutting points. The CPR propeller and gears are configured in such a way, so that *each division (5cm of silk roll) represents 5Nm along the transect*. Unforeseen conditions, such as alterations of ship's speed and course, have an effect on the length of silk needed to sample 5Nm. The total number of miles travelled/total number of divisions rolled is an estimate of the true length of sea transect sampled by each division, i.e the miles per division factor (MPD). If MPD is lower than four and or higher than six then the CPR propeller is reconfigured by the CPR technician. As MedCPR sample analysis is based on the examination of 5Nm samples the next step is to calculate how many divisions are in a 5Nm transect. This information will give rise to the points where the silk roll needs to be cut in-order to acquire 5Nm samples. Since all silk rolls used in CPR surveys are pre-marked with divisions and numbers, it is easy to use this information to cut the samples. The cutting points indicate the division point where each sample starts and finishes. For example, if for a given route, 1.5 divisions represent 5Nm, then the start and finish points for the first division would be at 0 and 1.5 division respectively, for the second at 1.5 and 3.0 and so forth. The points are put in the cutting report.

To separate the CPR silk in 5Nm samples, the silk is unrolled, marked (Figure I- 6A) and then cut. The start of the route is usually at the beginning of the first division but one should always check the CPR forms and the cutting report which include this information. The silk may be distorted with the middle section pulled. To find the cutting points, a ruler is put in the middle of the silk and using the marked division lines as guidance the silk is marked according to the cutting points in the cutting report. Unless there was no preservative used to fill the tank of the CPR internal cassette the process should be concluded in a fume-hood or a well aerated space according to the best practices and governing laws of the country hosting the CPR laboratory and the chosen preservative liquid. The analysis of the MedCPR survey samples takes place in a well aerated laboratory since alcohol solutions are used for the fixation and preservation of plankton.

Thick colour pencils are used to mark the silk (Figure I- 6A). Red, blue and purple make for good marking colours as they are easily distinguished on the silk. The pencils need to be tested beforehand to make sure that they do not smear or are erased on the moist silk. The route code and the sample number are also marked on each sample. As a precaution the information is also written on a piece of tracing paper that will accompany the sample through the analysis and storage.

In laboratories with more than one analyst, it is advisable that the latter are assigned non-consecutive samples. This way it is easier to trace mistakes, possible misidentifications or omissions.

Figure I- 6: CPR sampling results in a long silk-plankton-silk strip. The silk is unrolled, marked (A) and then analysed. For the Phytoplankton Colour Index analysis the colour of the silk is compared to a range of green colors (B-C) and given one of four values: Nil, Very Pale Green (VPG), Pale Green (PG) or Green (G).

Phytoplankton Colour Index-PCI

The Phytoplankton Colour Index (PCI) is a semi-qualitative measure of phytoplankton production deducted from the observed colour of the CPR silk. This is the first step of the sample analysis and it precedes the cutting of the silk roll.

After the removal of the silk from the CPR internal, the long strip is unrolled on a bench and carefully marked so that each sample represents 5Nm from the sampled transect, as explained above. Holding a white sheet behind the silk we assess the greenness of each sample under sunlight conditions, using colour palettes that contain light to dark green shades (Figure I- 6). The palettes cover a range of greens

to include almond, chartreuse, kelly, hunter and olive green, as to represent the possible pigmentation. The analyst must decide whether the sample is "Very Pale Green-VPG", "Pale Green-PG", "Green-G", "Very Green-VG" or is void of phytoplankton ("Nil") (Figure I- 6B). As it is difficult to decide that there is absolutely no phytoplankton in the sample, an empty piece of silk, or the beginning of the silk roll, is used for comparison. One of the four values above is assigned to each sample and then the information is entered in the results database. Any other general observation, such as presence of large ichthyoplankton, is also noted down.

After PCI is assigned, samples are cut and stored individually. Plastic sheets can be used to stack cut samples and avoid contamination.

PCI importance and bias

The PCI has been used widely in climate studies (Kirby et al., 2007), productivity assessments (McQuatters-Gollop et al., 2011) or to evaluate the ecological status of an area (Edwards et al., 2002). It is very attractive to climate modellers since the protocol has been relatively unchanged and results are available for a wide temporal and spatial coverage. The PCI was mentioned in more than 200 publications (Google Scholar search, on March 12, 2015). PCI has the most complete record within the CPR database because the analysis is straightforward and requires minimum resources.

Nevertheless, PCI is partially a semi-qualitative method. The palettes (Figure I- 6) offer guidance and a reference point as to decide where on the range of green shades better resembles the colour of the sample. During the necessary stage of training, the trainee assigns a colour to the sample and is then corrected as needed by an experienced analyst. Laboratories with more than one analyst have continuing trainings as to correct any potential errors. Inter-calibrations between laboratories that operate the CPR also take place with every opportunity. One such opportunity is the GACS annual meeting, taking place in Plymouth, UK, in September of each year. Members of CPR laboratories around the globe are asked to assign PCI values on samples and compare results. Another point of caution is sunlight. The traditional SAHFOS protocol calls for the use of natural and not electric lighting for the completion of the PCI analysis. This may be difficult as the sun is not equally bright or available throughout the year in all countries. In the UK for example, the analysis may be postponed because of overcast conditions and cloudy days. The location of the laboratory is also important. For example, in Cyprus, the CPR laboratory is located in the basement of the main lab building, with no access to ambient light. CAGS has suggested the use of white light bulbs that will be consistent among different laboratories and provide a constant practice between samples of the same survey. To date, the suggestions haven't been widely implemented. Samples of the MedCPR are analysed for PCI under white electrical light.

The PCI is also influenced by the chosen preservation technique. Phytoplankton pigments are sensitive to light exposure and preservatives. Although formaldehyde generally maintains green pigmentation the same does not apply for alcohol, where the loss of colour is proportional to the time the sample remains in alcohol. Ergo, the preservation liquid that is put in the tank of the CPR internal should be chosen carefully, while the time between sample arrival and PCI analysis should be minimized. The MedCPR has also scheduled a test with a non-fixed sample where no

preservation liquid will be put in the in the tank. The sample will be stored in a cooler and transferred immediately to the lab. This will allow for a comparison with an alcohol fixed sample in regards to PCI colour and plankton analysis. In any case, PCI from samples preserved in alcohol and samples preserved in formaldehyde should be compared with caution.

Plankton Identification

Plankton is trapped between two silk pieces, the filtering and the cover silk which are now cut in smaller sections; each sample is about 6x15cm. The following steps require a microscope and a stereoscope to analyse plankton on and off silk. A microscope is used for the on-silk analysis. The microscope has to have some minimum requirements: (1) large movable stage to accommodate the CPR sample, so that by moving the stage the analyst can view the complete sample, and (2) have the possibility to adjust the field of view (FOV) to match the requirements of the phytoplankton and zooplankton analysis (see Appendix I for information in microscope requirements). The FOV is used as a set frame within which all phyto- and zooplankton are identified and enumerated.

On –Silk: Phytoplankton analysis

First the sample is analysed for phytoplankton and then for zooplankton abundance and diversity. The silk is laid open on a transparent plate such that the plankton filled side is facing up and put under the microscope (see Figure 4). To prevent contamination between samples, a plastic transparent sheet is laid on the plate under the silk. The on-silk phytoplankton analysis calls for the use of a microscope system that provides a field of view (FOV) of 295um. Starting from the bottom right side of the filtering silk the analyst sets the view so that a silk mesh opening is in the middle of the FOV (Figure I- 7). All non-repetitive taxa of phytoplankton are counted within that circle. Phytoplankton is identified to the lowest taxonomic group according to the knowledge of the analyst and the availability of information, as often the specimen will be broken or destroyed. In the case that part of the organism is within the FOV then the analyst will enumerate them only if specific parts of their anatomy are visible. For example, dinoflagellates are enumerated only if the girdle, a belt-like groove that runs through the middle of the organism, is visible. If the analyst can see and identify a specimen but the countable part of it is not in the FOV then the organism will be recorded as present. If the same species is counted in the next FOVs then the presence record is erased.

When all the non-repetitive phytoplankton organisms within the FOV are noted down, the analyst moves the stage diagonally as to bring into view a second silk opening. The process is continued so that ten FOVs are analysed on the bottom-right-to-upper-left diagonal of the cover silk. The bottom-left-to-upper-right diagonal is analysed in the same manner as to ultimately create an X. The filtering silk is not analysed for phytoplankton. In total, 20 phytoplankton 295um Ø FOVs are analysed per sample.

Since only non-repetitive organisms are logged, the maximum number of counts per organism for each sample is 20. Historically, different groups have been identified from analysed samples. A survey may start by grouping all dinoflagellates together

during analysis. If lower taxonomic groups as *Ceratium*, *Gonyaulax* and other genera are distinguished, it is advised that the dinoflagellate record is also kept, for continuity. The same should apply if different *Ceratium* species are identified when a *Ceratium* field is also logged. Counts cannot be added and only one of each group is enumerated per FOV. For example, consider the occasion where FOV1 has one *Gonyaulax* sp., FOV2 has two *Ceratium* species a *C. tripos* and a *C. furca* and FOV3 has five *C. tripos* and one *Gonyaulax* sp. The results (totals) for the three FOVs would be as follows: Dinoflagellates – 3; *Ceratium*- 2; *Gonyaulax*- 2; *C. tripos*- 2; *C. furca*- 1

Phytoplankton is analysed on-silk by all CPR laboratories.

On–Silk: Zooplankton analysis

While the CPR sample is still laid open under the microscope it is analysed for zooplankton, in a similar manner. The magnification used is between 40- 100x and the FOV is 2.06mm (Figure I- 7). This will constrain a 2.06mm height traverse along the silk to be observed for the presence of zooplankton species. Starting again from the bottom right of the filtering silk, and taking care as to avoid the area covered by the flaps of the covering silk, a rectangle that covers a fifth of the width of the silk is analysed continuously by moving the stage to the right. Then the stage is moved downwards (towards the analyst) and slightly to the right to start the second box. The process is continued until five transects, set in a uniform stepwise arrangement, are analysed on the surface of the filtering silk. Since the two sheets of silk trap plankton between them, it is possible that some specimens remain on the covering silk. Therefore, the covering silk is also analysed with the same stepwise manner as to mirror the analysis of the cover silk.

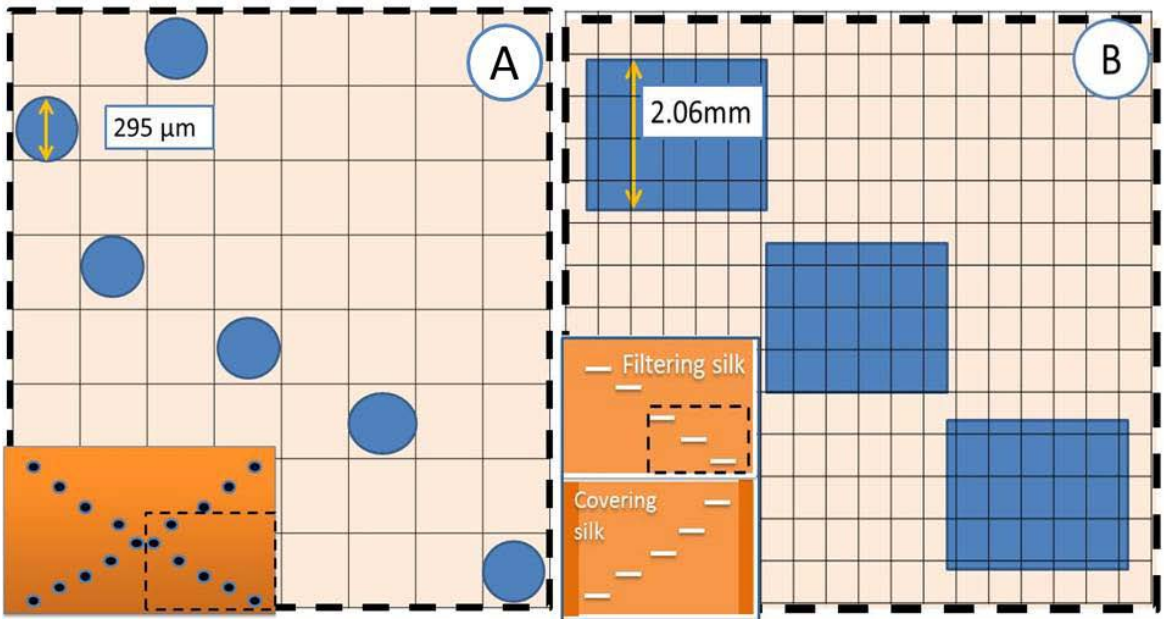

Figure I- 7: Graphical representation of the on-silk phytoplankton (A) and zooplankton (B) analysis. Inserts represent a miniature of the sample as it is laid under the microscope; grids

represent the mesh; blue circles and rectangles represent the area analysed during each process

During the on-silk zooplankton traverse, the analyst records zooplankton species smaller than 2mm to the lowest taxonomic group possible, following the same principle as the phytoplankton analysis. Information on the frequency they appear in the sample (abundance measurements) is also recorded. Groups that are often encountered are copepods, foraminiferans, molluscs, barnacle larvae, early life-stage forms of crustacean, fish and copepod eggs. Abundance measures are recorded for all these groups during the zooplankton traverse. Nematocysts, which indicate the presence of jellyfish are also noted but only as presence/absence data. If part of an animal is in the examined FOV, the analyst will identify it only if a specific part of its anatomy is visible. Copepods, for example, will be counted only if the base of the antenna on the top of the head of the animal is within the FOV. Species that are visible but will not be counted are recorded as present. If the same species is counted further down then the presence record is erased.

During recent years, microplastics are also logged in CPR surveys. A survey may choose to include this information as notes to a sample or enumerate and describe different kinds of plastic. In the MedCPR survey, plastics are distinguished as threats or fragments while any other specific characteristics (e.g. colour) are put down as notes.

As the analysis may take a long time (20-120min), it is wise to squirt the sample with some preservation liquid from time to time as to avoid the drying of the specimens.

Off-silk: Zooplankton eye-count

The final step in the CPR sample analysis is the off-silk enumeration of zooplankton under a stereoscope. During the analysis it is possible that some specimens where not included in the FOV or where very large to be identified under the microscope. For the final step, plankton from the cover and filtering silk (including plankton trapped under the flaps) is removed using a fine painters brush and put into a counting tray. Brush strokes across the silk must be gentle as not to damage specimens and collect as much of the plankton as possible. Specimens may also be washed out using a squirt bottle. According to availability, a Bogorov counting tray (also known as S-tray or Zooplankton Chamber), a grid chamber or simply a marked petri-dish can be used to hold the plankton. Water can be used as the medium for this process. This helps to avoid using the stereoscope inside a fume-hood.

During the off-silk zooplankton eye-count, all animals larger than 2mm are identified and enumerated. This will include, among others, ichthyoplankton, amphipods, isopods, chaetognaths and larger groups of copepods. Other small plankton species that were missed during the traverse are also noted down as present.

When the process is finished, plankton is picked up slowly using the same brush and put back on the silk. Collect the least amount of medium possible and spread out the organisms.

Sample long term-storage

To store the sample, the cover silk is put on top of the filter silk and squirted with preservation liquid. The sample is then folded as to minimise the possibility of losing specimens. The information of the sample (i.e. route code, sample number and analyst that analysed the sample) is written on a piece of tracing paper with pencil. Pencil written labels on tracing paper can withstand many preservatives including formalin and alcohol. The plastic sheet put beneath the silk can be used to wrap and store the sample. Alternatively, the sample can be put in a plastic zip lock bag (see Figure 5. It is advisable that samples cut from the same route are put in order in a plastic box with easy visual access to the sample label. A piece of lint soaked in preservative material is put in the bottom of the box. If a survey sustains more than one route, each route could have a separate box. The route code and range of samples are marked on each box. Full boxes can be then stored in a cool place, where one can go back and re-analyse samples if needed.

Post processing the results and record keeping

The results of the analysis are recorded on a piece of paper, preferably a notebook. As this is a long-term survey, a good practice is to keep all paper records in a safe place, to come back to if need be. The information is then logged in a software where data may be stored and analysed. CONSOLE has a specialised function for inserting, storing and retrieving CPR data. All taxa currently and historically recorded by SAHFOS are listed by the software and the information is inserted and checked twice. The same is done for the MedCPR survey slowly accumulating the taxa found on CPR samples. Information like the name of the analyst, microscope and stereoscope used and date are also inserted. The analysis, as described above, is designed such as a representative surface of the silk is examined. To estimate the number of individuals present on the sample, we need to multiply by a factor corresponding to the surface of the sample. Where CONSOLE calculates and applies this factor automatically to all analysed samples from a given tow, through the CPR Microsoft Excel workbook the process is semiautomatic. In Excel, the user creates a data workbook from a template created in the lab, inserts the factor in the appropriate column and calculates the result. All the results are pulled together in a common Excel sheet. Looking ahead, the MedCPR lab will work on creating an algorithmic solution to map CPR data.

Alterations of CPR protocol

As mentioned above, the PCI and Phytoplankton on-silk analysis are unchanged and performed using the above method across CPR surveys. The Zooplankton analysis differs slightly in the Australian (AusCPR) and Southern Ocean (SO-CPR) CPR surveys. In these laboratories, all zooplankton are washed off and enumerated, with no size distinction, after the on-silk phytoplankton analysis. In addition, the final step in the CPR sample analysis is the dry weight determination. This method is constant and serves the needs of the two surveys.

At SAHFOS and other CPR laboratories, 10Nm are considered as one (1) sample while only every other sample is examined. MedCPR, considers 5Nm as one sample and examines all samples. AusCPR and SO-CPR also consider a 5Nm section as a sample but only examine every other sample. Since the examined sample is also unusable after the dry-weight determination this also serves as a way to store samples for use

in the long-term. In the case of the Mediterranean, similar to the Australian coast, specimens tend to be smaller and usually less abundant. Hence, by examining a bigger subsample we decrease the possibility of losing important information. In both cases, results are multiplied so that there are no gaps of information.

Appendix II: MedCPR forms and reports

In this section we provide copies of the forms used to keep record of:

1. CPR components before the tow (CPR checklist)
2. Tow-log form
3. CPR internal loading /CPR maintenance form, and
4. Silk cutting points

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CPR OPERATION Checklist

Date/Route ID					
Communication with vessel					
Expected date at port/ departure					
Destination					
Port					
Forms					
Leave requests					
Petty cash/compensation					
Technical assistance					
Vehicle availability					
Lab to vessel					
Loaded internal					
Preservative tank					
CTD program					
CTD load					
Cyl logo					
CPR delivery to port					
CPR body/hand troley/chain					
Internal/peli case/packaging foam					
tow-log forms					
Squirt bottle with alchohol					
Tow equipment inspection					
Notification of CPR delivery					
CPR pick up from port					
75€ -crew compensation					
Invoice					

Form 1: Check-list of the components and preparations concerning the tow of the CPR.

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CPR tow record sheet. Route ID:

Return to Cyl _____ Tow date _____ Sensor ID 7909

Route _____ Ship PetrolinaOcean CPR Mech. No. _____

Internal **Loading**		Internal **Unloading**		Internal Maintanance		CPR body maintanance	
DATE		DATE		DATE		Dispatch DATE	
Name(s)		Name(s)		Name(s)		Name	
Silk loading (flaps and orientation check)		Silk reading at Gasket		Cogwheels		Return Date	
Silk start		Actual Stop (Gasket+2.4)		Maintanance		Pin Check	
Wire (tension/glue)		Silk distortion		Grease		Propeller	
Cotton/ Preservative		Sample unload		Brush, Sanding		Flanks	
Comments		Comments		Gears/parts check		Gear box/ Cogwheels	
Sensor Loading/ Unloading				Clean up		Grease	
Sensor preparation		Sensor unload				Clean up/repaint	
Sensor loading		Data extraction		Comments		Comments	
Comments							

Form 2 CPR tow record sheet. This form includes information from the loading of the CPR (before the tow), unloading the sample and maintenance.

THE CYPRUS INSTITUTE

Continuous Plankton Recorder Survey in the Levantine Sea (MedCPR)

Please return this log form to the following contacts in the **Marine Science Group of the Cyprus Institute**: Dr. Carlos Jimenez (c.jimenez@cyi.ac.cy), MSc. Rana Abu-Alhaija (r.abualhalja@cyl.ac.cy), MSc. Marina Evriviadou (m.evriviadou@cyl.ac.cy). **Phone: +357-2220-8600 Fax: +357-2220-8625 20 Konstantinou Kavafi St., 2121 Aglanzia, P.O. Box 27456, 1645 Nicosia, Cyprus**

Name of Ship: Petroliva Ocean Master: Capt Sergiy Avdyeyenko

Route from: Larnaca to: Haifa

Writer(s) of Log: A. Rustamelnyk / A. Baleta / V. Tsikonia Rank(s): 2/off / 3rd/off / Cdet

Date UTC	Time UTC	CPR SHOT	Alterat. Course	CPR HAUL	Latitude	Longitude	Course	Distance	Wind/ Sea state/ Air °C/ Sea °C/ Cloud cover (1/8 etc.)
05.11.14	1140	✓			34°32.33N	033°49.34E	162°		light airs / Smooth / 22° / 21° / clear sky.
05.11.14	1200				34°47.70N	033°43.18E	162°	4.9	light airs / Smooth / 22° / 21° / clear sky.
05.11.14	1400				34°21.60N	033°53.51E	162°	27.8	Light airs / Smooth / 22° / 21° / Clear sky
05.11.14	1600				33°54.83N	034°03.73E	162°	27.9	Light airs / calm / 20° / 21° / partly Cloudy
05.11.14	1800				33°28.32N	034°13.91E	162°	27.9	Light airs / calm / 20° / 21° / partly cloudy.
05.11.14	2000				32°00.31N	034°25.60E	162°	28.0	Light airs / calm / 20° / 21° / partly cloudy
05.11.14	2120		✓	✓	32°56.20N	034°46.20E	099°	18.6	Light airs / calm / 20° / 21° / partly cloudy
							Total n.m.	135.1	

Form 3: Example of a filled tow-log form. Basic information includes vessel positions and times while the CPR is in the water and at the launch and haul.

When commercial microscopes do not offer the specifications required for the on-silk CPR sample analysis the following solutions have

The Cyprus Institute
MedCPR

Cutting report **2MCHL** **Nov-15**

CUTTING POINTS Calculation

Cutting factor	Sample	Cutting point
1.110288675		1.0
1.110288675	1	2.1
1.110288675	2	3.2
1.110288675	3	4.3
1.110288675	4	5.4
1.110288675	5	6.6
1.110288675	6	7.7
1.110288675	7	8.8
1.110288675	8	9.9
1.110288675	9	11.0
1.110288675	10	12.1
1.110288675	11	13.2
1.110288675	12	14.3
1.110288675	13	15.4
1.110288675	14	16.5
1.110288675	15	17.7
1.110288675	16	18.8
1.110288675	17	19.9
1.110288675	18	21.0
1.110288675	19	22.1
1.110288675	20	23.2
1.110288675	21	24.3
1.110288675	22	25.4
1.110288675	23	26.5
1.110288675	24	27.6
1.110288675	25	28.8
1.110288675	26	29.9
1.110288675	27	31.0

Average Speed (Knots)	14.37234
Miles/division	4.503333
Divisions/5Miles	27.02
Cutting factor (5Nm)	1.110289

Form 4: Cutting report of 2MCHL route. The numbers of the third column indicate the point where the silk is marked and cut so that each sample represents 5Nm.

Appendix III: Troubleshooting microscope requirements

The CPR on-silk analysis has some specific demands that do not fall into habitual plankton sample analysis. Therefore, the microscope used has to have some specific characteristics and in particular a defined field of view (FOV) and large movable stage. The available options range from ordering a custom built instrument to configuring an available device. The choice of microscope or the modifications to be applied is not trivial. A thorough research must be made into all the available options while having an a priori knowledge of the CPR analysis method. Even then, the system might need additional tweaks and adjustments to best serve the needs of the laboratory.

The on-silk plankton analysis calls for the use of a microscope system that provides a FOV of 295um. The FOV of a microscope system is the diameter of the circle one can observe through the eye-piece at any given time. The FOV is determined by the abilities of the microscope system and specifically the eye piece and the objective lens. Microscopes usually have more than one objective each with a different magnification power (MP). FOV decreases by increasing the MP. For the phytoplankton on-silk analysis the MP used is 400-600x (e.g. 10x ocular and 50x objective lens=500MP), while for the zooplankton on-silk analysis 40-100x.

When commercial microscopes do not offer the specifications required for the on-silk CPR sample analysis the following solutions have been used by different laboratories.

Requirement 1: Set FOV

As microscopes evolve, they provide a wider FOV even for high MPs. For the purposes of CPR sample analysis the following solutions can be used:

- Find the correct combination between ocular and objective. Different companies might offer combinations of lenses that will give the required FOV, usually using a high MP objective. Local microscope providers are often ready to provide possible suggestions.
- Create a ring. Usually the FOV is larger than needed. By creating a ring on the objective intended to be used for each of the zoo- and phytoplankton, the required FOV is created. Information outside that is dismissed. The ring would be created by the factory where the objective is purchased, thus making this solution expensive. An objective with a custom drawn ring has been fitted to the CPR Nikon ECLIPSE L200ND microscope at SAHFOS.
- Use a digital frame. At the MedCPR laboratory the microscope is connected to a camera and accompanying software. With the silk placed on the stage, the part of the silk to be analysed is brought in focus on the screen. Then a measured object or line is dawn on the captured snippet equal to the dimensions needed for the analysis, using the software application. This object serves as a frame to guide the analysis.

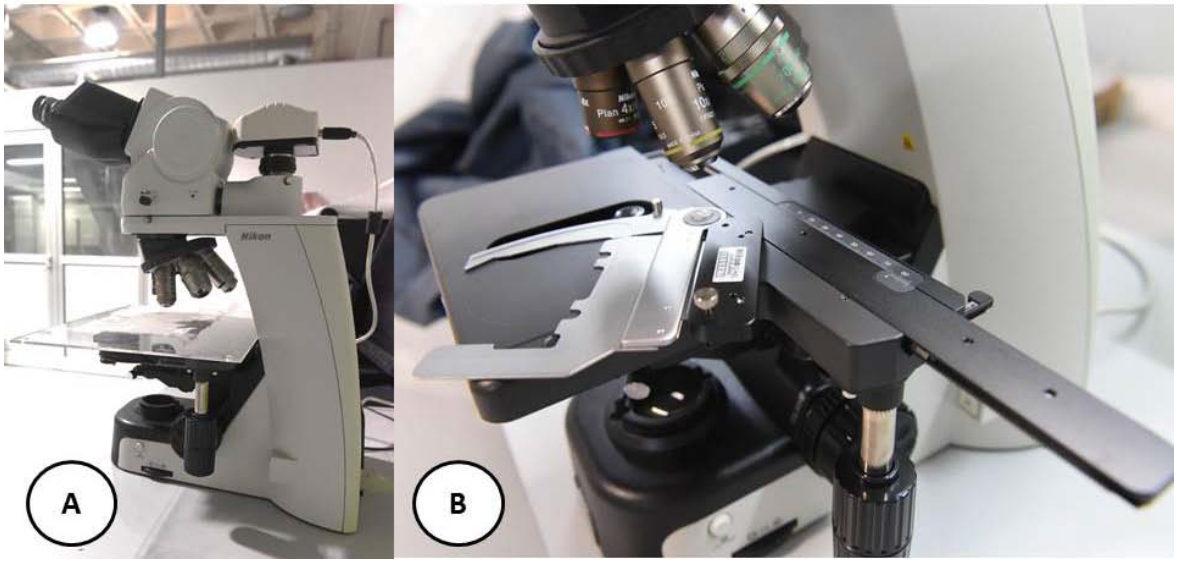

Figure III- 1: (A) The Nikon Ci-S microscope used for the on-silk analysis of plankton at the CyI. (B) The slide holder is removed and a fiberglass slate is fitted onto the stage.

Requirement 2: Large movable stage

The CPR sample is opened on the microscope stage. The best case scenario is having a microscope stage that brings the complete surface of the laid out silk in view by scrolling in all directions (↑, ↓, ←, →). This can be achieved by:

- Purchase of a *custom made microscope*. One of the challenges faced when configuring the microscope for this requirement is that the microscope body must be at least 30cm deep as to allow for the forward/backward movement of the stage and sample. A custom made microscope would have a steady head and condenser held by a deep body. The stage could be independent with a movable piece of glass on a track. As custom-made microscope makers are becoming scarce this solution is not widely available.
- Purchase of a microscope with a large stage. Although not habitually used for scientific research, *industrial microscopes* with deep bodies can provide an alternative solution. For example the Nikon ECLIPSE L200ND, industrial microscope, has a deep body and can be configured for CPR analysis by fitting a custom made glass stage to it. Märzhäuser Wetzlar GmbH & Co, Wetzlar, Germany, is a company specialised in fitting a range of stages on mass-produced microscopes and is able to provide one such stage. One such CPR microscope is used by SAHFOS. Industrial microscopes are expensive and when adding to that the cost of the custom made stage, the budget for such a microscope is further increased.
- Configuring an available microscope. *Commonly used microscopes* usually have configurable stages where one can interchange the slide holder to a petri-dish holder or some other accessory. Once the slide-holder is removed a custom made stage can be attached either directly or by removing the original stage. At the MedCPR laboratory an inexpensive transparent slate is fastened to the original stage. The disadvantage of this solution is that the analyst needs to turn the sample around in order to bring the complete surface into the FOV of the microscope. On the other hand, the microscope can be reconfigured to

support multiple common applications within the laboratory. *The microscope used at the MedCPR survey is a Nikon Ci-S fitted with four objectives (4x, 10x, 20, 50x).*

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