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Phytoplankton dynamics in Maltese coastal waters (Central Mediterranean) using in situ, remote sensing methods, and modelling techniques

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Phytoplankton dynamics in Maltese coastal waters (Central Mediterranean) using *in situ*, remote sensing methods, and modelling techniques

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Abstract

This study focuses on updating current knowledge concerning the quality of coastal water of the Maltese Islands (central Mediterranean) using *in-situ*, remote sensing and modelling techniques. *In-situ* data were collected from four sampling points around the Maltese Islands from April 2015 to January 2016. A weak, but statistically significant, relationship was observed between *in-situ* chlorophyll and chlorophyll derived from the MODIS satellite using a recently updated Mediterranean wide algorithm MedOC4 (Santoleri *et al.*, 2008). A ten year time series of satellite data shows that chlorophyll levels in this part of the Mediterranean are generally low (<0.5 mg m^{-3}) and show a regular maximum in December and January, with an occasional second maximum in the spring.

A two-layer bio-physical model has been constructed in order to test our understanding of this behaviour. The surface mixed layer in the model is forced by surface heating and wind-stirring. Phytoplankton growth in the surface is set to the minimum of light- and nutrient-limited growth. In the clear waters of the Mediterranean, with high levels of sunlight at all times of year, phytoplankton growth is almost always nutrient-limited. Maximum growth therefore occurs in the winter, when high wind speeds deepen the surface mixed layer and nutrients become available in the (still) sufficiently-lit surface waters. The *in situ* data are also used to construct a site-specific linear model for chlorophyll; this accounts for 52.6% of the variance of observed chlorophyll. The overall conclusions of this study are that phytoplankton blooms in Maltese waters occur mainly during the winter months and that these waters are nutrient limited at all times of the year. Further work needs to be done on the derivation of chlorophyll from ocean colour in these waters.

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I would like to thank both of my supervisors, Professor David Bowers and Professor David Thomas for their excellent supervision and guidance throughout my studies. Their advice and insight was second to none and it was a great experience and pleasure to work with them throughout the course of my studies. I would like to personally thank Professor David Bowers for the warmth and affection he showed me and everybody at the School of Ocean Sciences throughout his career and the comparatively short period that I was in Bangor.

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Chapter 1: Introduction and Literature Review

1.1 Phytoplankton Dynamics

Phytoplankton forms the base of the marine ecosystem by the process of photosynthesis and acts as the primary producer of the world's oceans. Phytoplankton growth is modulated by a number of chemical, physical and biological factors, such as the presence of predators and the availability of nutrients and light, which influence its population dynamics, community structure and biogeographical distribution.

Quantification of the photosynthetic pigment, chlorophyll, is widely used as an estimation of phytoplankton biomass (Cullen, 1982; Jeffrey & Vesk, 1997; Huot *et al.*, 2007; Novoa *et al.*, 2012). Chlorophyll is a photosynthetic pigment found in photoautotrophic 'algae'. By measuring the amount of chlorophyll found in the water in a particular area it is possible to estimate the concentration of phytoplankton present. It is unique in the fact that it is probably the only pigment that can directly transfer light energy to the photochemical reaction and therefore provide an index of production, or standing crop. The index generated by measuring chlorophyll can determine the location of fertile fishing grounds (Szekielda & Duvall, 1976) and primary productivity (Odum, 1971; Tester & Stumpf, 1989).

The Water Framework Directive (WFD) and the Marine Strategy Framework Directive (MSFD) (Borja *et al.*, 2011; Ferreira *et al.*, 2011) selected phytoplankton biomass to act as a parameter for water quality assessment; the main reason being that phytoplankton responds to nutrient enrichment when there is excess nutrient input, such as phosphate and nitrates, into the marine environment. Events such as increases in organic matter and turbidity and a reduction of dissolved oxygen (Bricker *et al.*, 2008) can disturb the balance of marine systems, thus reducing its ability to accommodate life. These factors could lead to water eutrophication (Halpern *et al.*, 2008; Nixon, 1995; Conley *et al.*, 2009).

Nutrient availability is dependent on biogeochemical and physical processes (Behrenfeld *et al.*, 2006; Rinaldi *et al.*, 2014) such as the solar cycle, atmospheric and local (riverine and anthropogenic sources) deposition, vertical/horizontal advection and ocean surface mixing. The latter two factors are in turn influenced by mesoscale/ sub-mesoscale processes, mixed layer dynamics, and also deep water formation (open ocean) or wind-driven upwelling. In order to implement good management techniques it is important that this ecosystem should be properly monitored and understood.

Different monitoring techniques can be utilised. *In situ* sampling has been used for many years with success, and was the only method available in the past. Today, new monitoring techniques have been developed that make monitoring more efficient and cost effective. These are remote sensing and modelling techniques. Remote sensing is a spectral tool whereby aerial images obtained from aircraft or satellites in orbit are used to obtain an estimation of the value or concentration of a physical parameter on land or in the marine environment. This means that temporal and spatial views of the surface are readily available, which gives remote sensing an edge over *in situ* measurements of water quality. That can be used very effectively when appropriately applied.

Modelling techniques are another method of obtaining estimations of the values of physiochemical parameters in the marine environment. This method requires a strong baseline foundation of the mechanisms controlling the structure of the ecosystem. Once these have been established, modelling techniques can prove to be a greater utility than the above two mentioned methodologies through their predictive and forecasting abilities. The latter two methodologies have been poorly explored in Maltese waters.

This study will be investigating phytoplankton dynamics in coastal waters. This is where most human activity is located. Consequently, the results and findings of this study will have a wider range of applications. The European Water Framework Directive (WFD) subdivides the definition of the coastal zone into two separate parts, open coastal waters and inshore coastal waters. Open coastal waters are defined as the one nautical mile limit from the baseline of territorial waters. These waters are not under the influence of terrestrial discharge. Physico-chemical and biological changes in these waters occur due to changes in natural conditions (Ferreira *et al.*, 2007). Inshore coastal waters consist of waters found along the coast that come under the influence of rivers, discharge from human activity, such as sewage or fish farms, and other terrestrial sources (Gohin, F. *et al*., 2008). This land-sea interaction creates a dynamic and complex system (Axiak *et al.*, 2002).

Humans have found coastal areas very attractive locations for habitation and development because of the many different provisions and cultural services that are provided in these areas. The global value of the coastal environment’s ecosystem service is 1.5 times higher than that of open oceans and has been estimated at a value of $8400 billion per year (Worm & Barbier, 2006). Shelf regions are key areas for biological activity and generate the biological production that supports 90% of the world's fish catches (Pauly, 2002). According to Constanza (1997) around 60% of the planet's population is found in these regions (Cloern, 2001). The ever increasing impact of human activities on coastal and shelf seas has generated tremendous pressure on these sensitive ecosystems. Studies that characterise and diagnose the present condition of coastal environments are urgently needed if we are to address problems caused by this pressure (Herrera, 2009).

1.2 The Mediterranean Sea

Malta is situated in the middle of the Mediterranean Sea. The Mediterranean is considered to be an oligotrophic sea which increases in oligotrophic nature from west to east and north to south as shown in Figure 1.2.2.

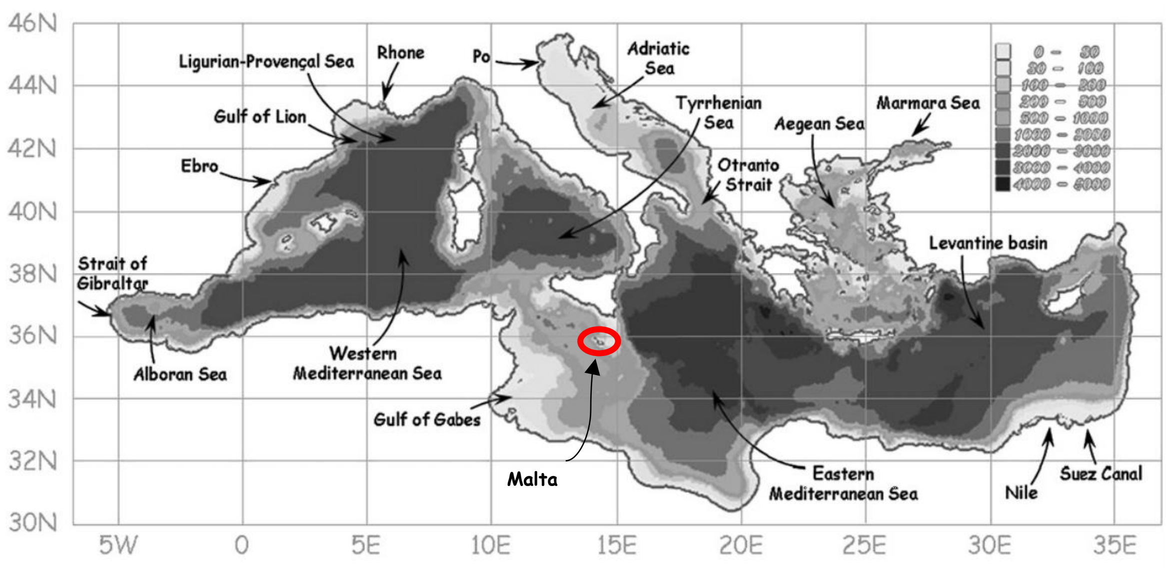


Figure 1.2.1: *showing a map of the Mediterranean Sea bathymetry and nomenclature, along with the location of the Maltese Islands (Adapted from Barale, Jaquet and Ndiaye (2008))*

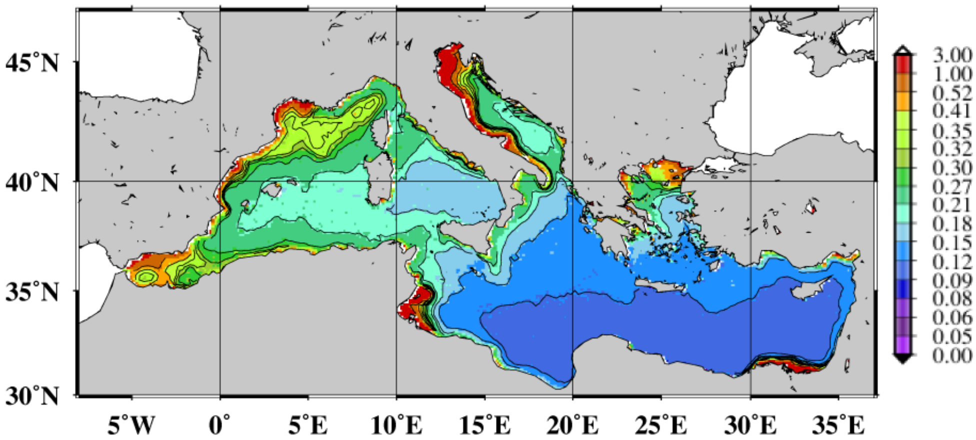


Figure 1.2.2: *Ten year climatological mean of chlorophyll concentration showing its spatial distribution throughout the Mediterranean Sea in mg m^{-3} (Siokou-Frangou et al., 2010)*

The reason for such a spatial distribution of chlorophyll throughout the Mediterranean Sea can be attributed to the transport of nutrient rich Atlantic Water entering the semi-enclosed Mediterranean Sea through the Gibraltar Straits in the western basin. This water flows through the basin and becomes what is known as Modified Atlantic Water (MAW). The Mediterranean is a concentration basin, meaning freshwater loss exceeds freshwater input. This is due to the imbalance between evaporation, transpiration and runoff. The further east that Atlantic water travels, the greater the change in its temperature and salinity. Thus, an open thermohaline cell is created between the two sub-basins that characterise the Mediterranean Sea (Siokou-Frangou *et al.*, 2010). This is characterised by a west to east surface (top 200m) transport of Modified Atlantic Water which overlays an east to west flow of saltier water known as the Levantine Intermediate Water (LIW).

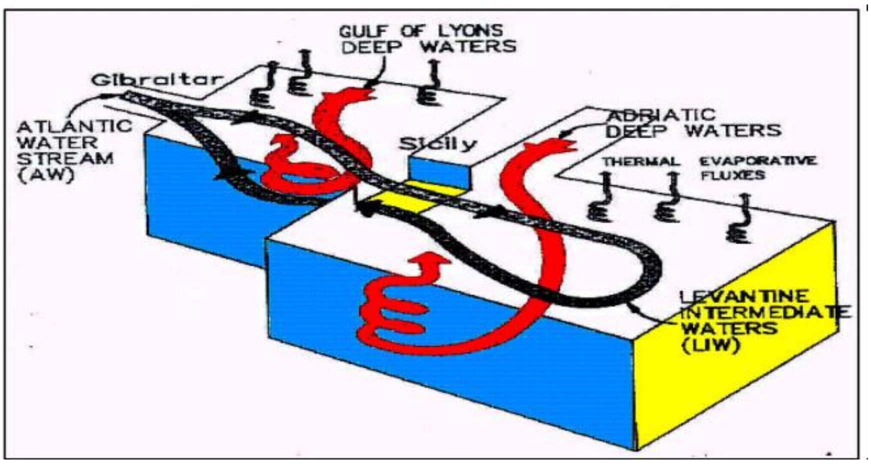


Figure 1.2.3: *Diagram showing the thermohaline circulation in deep-water formation in the Mediterranean as fresh MAW moves eastward as a surface flow (Drago et al., 2010)*

Malta is strongly influenced by the Atlantic Ionian Stream (AIS). This current transfers fresher MAW westward, across the Sicily Channel, into the warmer Ionian Sea. The contrast of temperatures produces the Maltese Front. The circulation and meandering of the AIS produces cyclonic and anticyclonic vortices off the southern coast of Malta and Sicily. When a cyclonic vortex is present, and is maintained, upwelling will occur at the centre of the vortex. This phenomenon takes place along much of the southern coast of Sicily and extends offshore, up to the Malta platform and beyond (Garcia Lafuente *et al*., 2002; Rinaldi *et al*., 2014). These upwelling events transport nutrients to surface waters, resulting in enhancing primary production. During the summer, a bloom event may sometimes take place south of the Maltese Islands, as a result of the westward moving LIW shifting vertically upwards and enriching surface waters. The LIW migrates north to the outer extent of the Maltese shelf. This produces significant upwelling which sometimes extends as far north as the southern coast of Gozo (Stelzenmüller *et al.*, 2008).

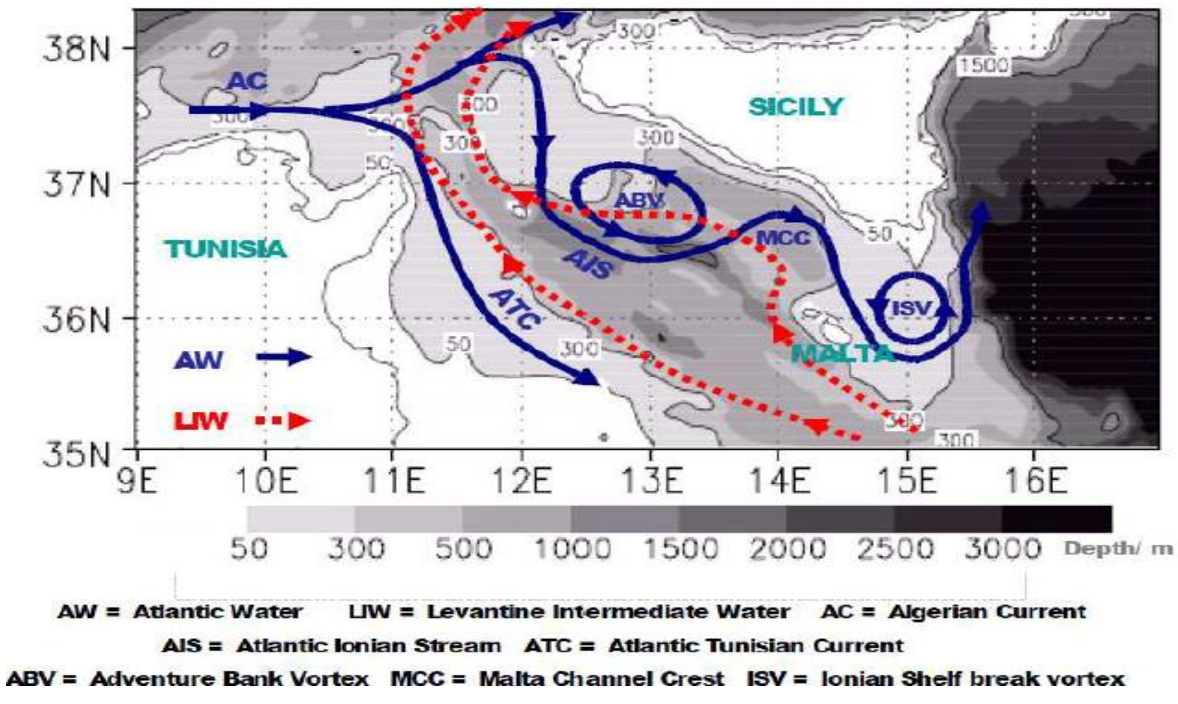


Figure 1.2.4: *Circulation in the Straits of Sicily (Astraldi et al., 2001)*

1.3 Mediterranean Seasonality

The yearly average value of chlorophyll concentration for the total Mediterranean Sea of 0.19 mg m^{-3} results from a combination of two extremes at either end of the basin: The oligotrophic Levantine Basin in the east and the seasonally productive regions in the northwest (~0.05 and ~0.26 mg m^{-3}, respectively) (Santoleri *et al.*, 2008).

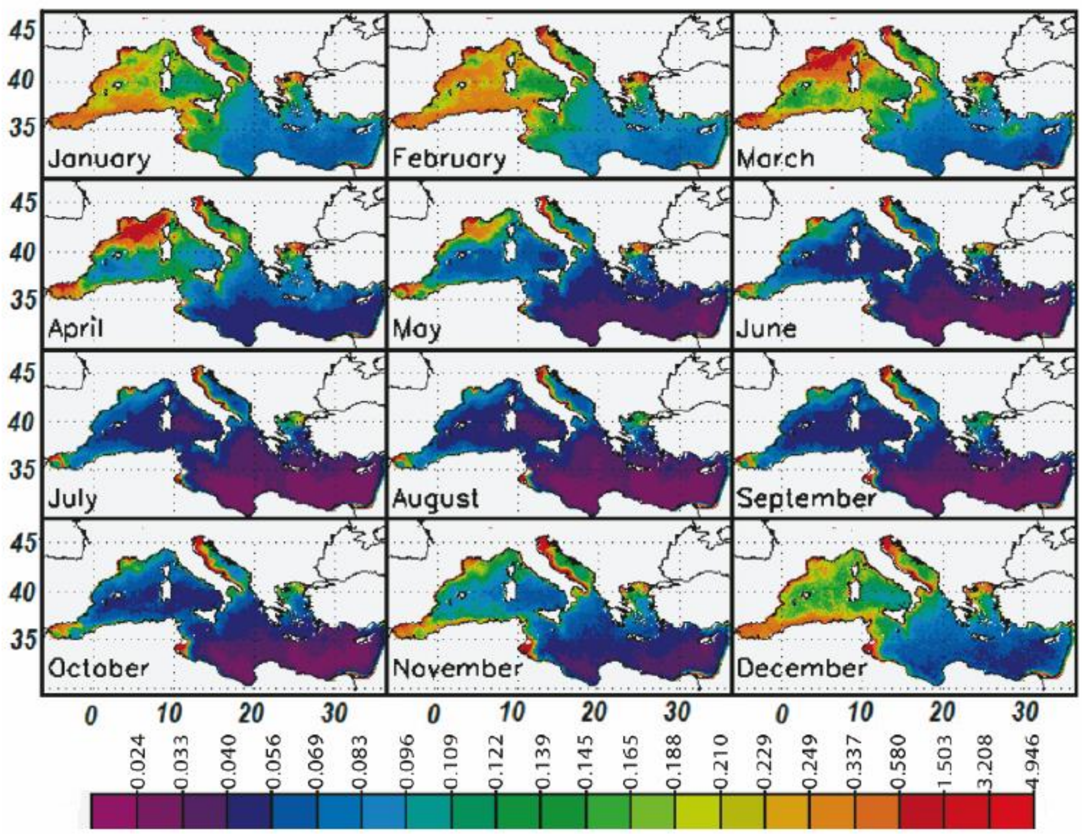


Figure 1.3.1: *SeaWiFS-derived (1998-2005) chlorophyll climatological monthly means in mg m^{-3} (MedOC4 algorithm) (Massi & Luca et al., 2011)*

Figure 1.3.1 shows that higher chlorophyll concentrations can be observed around the Ligurian-Provençal-Catalan region and Algerian-Alboran Basin from December to April. This is attributed to an increase in nutrient availability, which is introduced by wind induced meteorological forcing and the presence of quasi-permanent gyres found in that region (Buongiorno Nardelli *et al.* 2001). The eastern Mediterranean is characterised by low chlorophyll concentrations, with mean monthly values rarely exceeding 0.1 mg m^{-3} (Volpe *et al.*, 2012). Its seasonal cycle can be considered as being similar to that of sub-tropical gyres, as opposed to that of the western basin which can be considered similar to the North Atlantic's spring bloom (D'Ortenzio and Ribera d'Alcala, 2009).

Malta is situated in the Sicily channel. This channel divides the eastern Mediterranean sub-basin from the western Mediterranean sub-basin (Drago *et al.*, 2003). Here chlorophyll values range from 0.5 (winter) to 0.04 (summer) mg m^{-3}. Maximum values are measured during winter to spring, and have been observed to correlate with periods of low water column stratification and high levels of water mixing (Rinaldi, 2014).

Daily visualisations from the Sicily channel of SeaWiFS chlorophyll data by Rinaldi, 2014, showed that offshore sites have a stronger seasonal pattern than coastal sites. Rinaldi also observed that there are many different factors and oceanographic features that modulate the phytoplankton dynamics of an area, such as instabilities and frontal meanders and wind-induced coastal upwelling. Runoff from continental margins also contributes to increasing chlorophyll concentrations in coastal waters (Santoleri *et al.*, 2008).

This study will be analysing Maltese coastal waters in order to gain a deeper understanding of the coastal dynamics of the Maltese Island. The theories proposed by Santoleri (2008) and the observations made by Rinaldi (2014) regarding the improved accuracy of the new regional algorithms when applied to coastal waters, referred to later in this review, will be tested. Rinaldi's (2014) theories on coastal areas, suggesting that there is a less pronounced seasonal variation of chlorophyll throughout the year will also be investigated. Specifically, this study will address the following aspects of their hypotheses:

1. The correlation between surface chlorophyll *in situ* sampling values against remotely sensed chlorophyll values.
2. The seasonal variation in chlorophyll for both open coastal (1 nautical mile) and coastal sites for the central Mediterranean Island of Malta.

1.4 Malta

The Maltese Islands, located in the central Mediterranean, as seen in Figure 1.2.1, are characterised by well-mixed waters in the winter and strongly stratified waters in the summer. The European Union habitat classification has described the characteristics of the water column during summer as being “vertically stratified, with full salinity” and the winter water column as being “completely mixed with full salinity” (Malta Environment and Planning Authority, 2013). It has been reported that there is a stratification having a thermal step of 2.4^{o}C at 25 to

30 meters during the summer months (Malta Environment and Planning Authority, 2013). During this period, the near shore water column structure is dominated by a solar heated upper mixed layer, which averages 20 meters in depth. The salinity in the surface waters during summer months peaks at 38 psu. These waters are mainly composed of MAW. Towards the south of the Maltese Islands, salinity values go as high as 38.4 psu, indicating the presence of the LIW. Surface temperatures reach up to 27 °C during the summer. Bottom waters reach a temperature of around 15 °C. As winter approaches, thermal stratification is eroded and vertical mixing, along with surface cooling, takes place, producing a more homogenised water column, with mixing reaching down to 100 meters, where temperatures will be around 15 °C.

Figure 1.4.1: *Map showing the various localities throughout the Maltese Islands*

Few studies have been carried out in the central Mediterranean on coastal or offshore waters. Malta is strategically located in the centre of the Sicily Straits, which are right between the Western and Eastern basins of the Mediterranean Sea. This makes the Maltese archipelago ideal for monitoring waters flowing from one basin to another.

The first study to investigate chlorophyll concentrations in Maltese waters was carried out by Agius & Jaccarini in 1978 to determine the possibility of setting up oyster culture for *Crassostrea italies* and *Ostrea edulis* species. The study sites used in this study were Marsaxlokk Bay, Mistra Bay and Rinella. These sites are found towards the south and the east of the Maltese Islands. This is where sample sites C and D are located in the present study. Water quality parameters, such as salinity, temperature, dissolved oxygen, suspended particulate matter and chlorophyll concentrations were used to monitor water quality and its influences on trial growth of these two species.

Another study related to this area was also carried out by Agius & Jaccarini (1982). This study explored the effect of artificial nutrient (nitrates and phosphates) enrichment of phytoplankton in Marsaxlokk Bay, in the south of Malta, in order to identify the primary limiting nutrient on phytoplankton growth in this particular environment. It was concluded that phosphates were the primary limiting nutrient.

The studies that have explored marine productivity and water quality of the Maltese Islands using remote sensing techniques were the studies of Axiak & Adami (1997), Deidun, Borg, & Micallef, (2011), and most recently Azzopardi, Deidun, *et al.*, (2013).

The table below lists the studies that have been carried out indicating the location of the sites that were studied, the chlorophyll concentrations at the various sites and the methods used to determine the chlorophyll concentration.

References	Coastal water quality parameters monitored	Local sites	Concentrations of Chlorophyll	Method applied
Agius & Jaccarini ,1978	Chlorophyll (mg m^{-3})	Marsaxlokk, Mistra, and Rinella	0.22-1.23 0.19-0.92 0.15-10.4	Parsons & Strickland (1972)
Agius, & Jaccarini, 1982	Chlorophyll (mg m^{-3})	Marsaxlokk	0.29-0.57	Parsons & Strickland (1972)
Axiak & Adami, 1997	Chlorophyll (mg m^{-3})	Marsascala, Marsamxett Harbour, and Wied Ghammieq	0.55 (MEAN)	AQUATRACKA III (Chelsea Instrument)
Rizzo & Le Breton, 1997	Chlorophyll (mg m^{-3})	6 sites located near fish farms	0.06-0.97	Parsons & Strickland (1972)
Deidun *et al.*, 2011	Chlorophyll (mg m^{-3})	6 sites along the east coast of Malta	0.118-0.511 (April) 0.130-0.5 (September)	Parsons & Strickland (1972)

Table 1.4.1: *Past studies carried out in Maltese coastal waters and their corresponding chlorophyll concentrations.*

In 1997 Axiak & Adami carried out the first study which monitored water quality using satellite data. The study used LANDSAT-TM data to map water quality parameters along with *in situ* data collected at the time of satellite overpass. Parameters mapped included surface temperature, water transparency and ocean colour. These were collected on a one-day basis in a restricted spatial area. Algorithms were developed for satellite data in order to map spatial distribution of each water quality parameter. The data collected was used to establish a monitoring program and data bank of water quality. However the volume of data supporting its existence is limited (Balopoulos, E. T. 2003). Since this study was carried out, newer sensors have been deployed by the LANDSAT mission that have higher spectral sensitivities and could provide better quality data at a resolution as accurate as 15-30 meters.

In 2011, Deidun *et al.* explored the accuracy of Moderate Resolution Imaging Spectro-radiometer (MODIS) platform ocean colour data by simultaneously analysing *in situ* surface chlorophyll data from a number of field sites. Data was collected over a 12-month period. This resulted in the development of an ocean time-series dataset of chlorophyll. Results showed a peak in chlorophyll concentrations between January to February and also indicated that MODIS ocean data did not match accurately with *in situ* analysis.

In 2013, a study was published which analysed the seasonal and spatial variability of ocean colour chlorophyll values from 9 different coastal water sites (Azzopardi, Deidun, *et al., 2013*). Images were analysed on a monthly basis from 2003 to 2011 using values originating from MODIS, MEdium Resolution Imaging Spectrometer (MERIS) and Sea-viewing Wide Field-of-view Sensor (SeaWiFS) sensors available from the MyOcean Marine Core Service. The seasonal pattern of chlorophyll variability was seen to be fairly homogeneous, with the greatest variation during December and February. These trends could not be associated to any event or phenomenon.

1.5 Remote Sensing of Ocean Colour

Remote sensing is a spectral tool that can be used very effectively when applied appropriately. Temporal and spatial views of the surface are readily available, which gives remote sensing an edge over *in situ* measurements of water quality. Remote sensing techniques help minimise spatial and time issues by providing a synoptic view of the study area (Platt & Sathyendranath, 1988). This makes it a more economic operation compared to monitoring regimes based on *in* *situ* sampling. Scientists are becoming more reliant on this type of data. This coincides with great strides forward in the accuracy and functionality of this data source. It has become a crucial tool for marine systems dynamics and can be used to observe many different parameters ranging from sea surface temperature to wind speed.

The accumulation of phytoplankton cells brings about a change in the natural blue colour of the sea towards a greenish colour. Ultimately the colour of the sea depends on the Inherent Optical Properties (IOPs). The IOPs are made up of spectral absorption $a\ (\lambda)$, backscattering $b_b(\lambda)$ coefficients, and scattering $b(\lambda)$. These are significant in defining the marine optical field. These variables in turn affect the apparent optical properties (AOPs) that define the in-water radiation field. Irradiance reflectance $R(\lambda)$, diffuse attenuation coefficient (downwelling) $K_d(\lambda)$, and the remote sensing reflectance $R_{rs}(\lambda)$ are the three most used AOPs in remote sensing.

Remote sensing techniques are based on relationships between R(λ) and two inherent IOPs; total absorption (*a*) and backscattering (b_b) coefficients (Morel and Prieur, 1977; Gordon and Morel, 1983; Carder *et al.*, 1999; Stramska *et al.*, 2000);

$$R(\lambda) \propto \gamma \frac{b_b(\lambda)}{a(\lambda) + b_b(\lambda)} \tag{1.1}$$

The IOPs are determined by optically active water constituents in the marine environment which absorb and scatter light. Chlorophyll *a*, which is the main photosynthetic pigment, Coloured Dissolved Organic Matter (CDOM) (denotes the coloured material that passes through a 0.2 µm filter), produced during the decay of terrestrial vegetation or marine phytoplankton and Non-Algal Particles (NAP), of which the Suspended Particulate Matter (SPM), all influence the colour of the ocean, therefore determining the AOPs values.

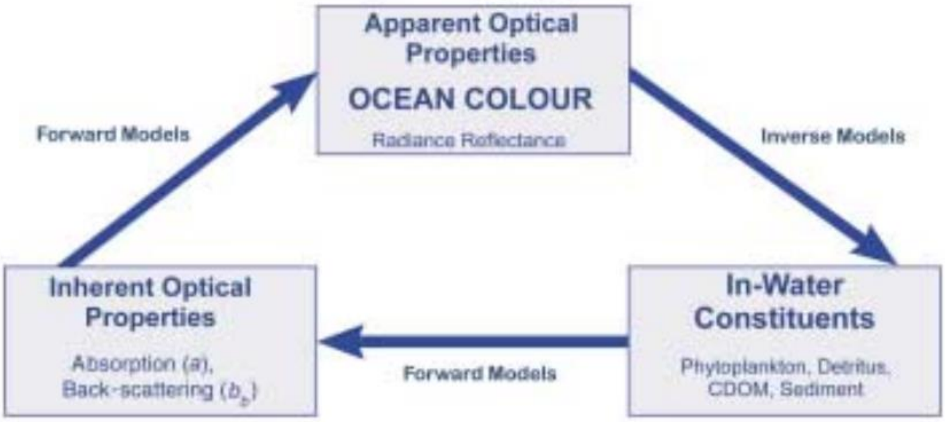


Figure 1.5.1: *Showing the relationship between optical properties (Sathyendranath, 2000)*

Water colour has been found to be dependent on water quality. In 1977 Jerlov devised a way to compare water samples to colour scale. Two broad categories of optical water types were identified (Morel *et al.*, 1977). "Case 1" waters was the label given to waters whose colour was a simple function of phytoplankton concentration. Colour dissolved organic matter (CDOM) and detritus are believed to co-vary with chlorophyll concentration. Prior to the mid 1990's, much of the focus in the field of remote sensing was centred on Case 1 waters and regions far from the coast.

Waters that are heavily influenced by resuspended sediment, highly concentrated phytoplankton blooms such as red tides or coccolithophores CDOM, resuspended sediment or terrigenous particulate and dissolved materials are known as "Case 2" waters. In Case 2 waters, the constituents mentioned above behave independently of one another and do not co-vary with chlorophyll. These factors change with geographic location and time due to inputs such as river discharge, or wind. Recent works have focused more on understanding the optics of coastal environments and other turbid Case 2 waters (Del Castillo, 2005).

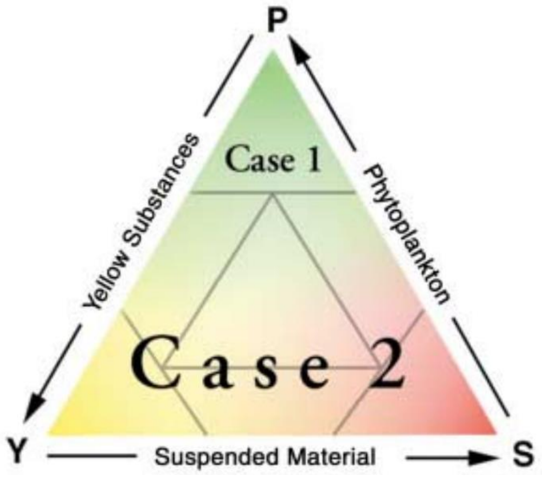


Figure 1.5.2: *Schematic representation of Case 1 and Case 2 waters, adapted from Prieur and Sathyendranath (1981)*

Chlorophyll concentrations of coastal and open waters have been obtained from remote sensing oceanic colour data using algorithms. This research began 30 years ago. Today there are many different types of algorithms and sensors which can be used. Different algorithms include spectral-band ratios, bio-optical models, reflection based classification algorithms and spectral band difference algorithms (Blondeau-Patissier *et al.*, 2014). Most chlorophyll algorithms are empirical algorithms that rely on switching band ratios (O'Reilly *et al*., 1998, 2000).

When phytoplankton is the main optically active substance in the water column, these algorithms perform well. However, they lose their accuracy when CDOM and total suspended matter (TSM) dictate the optical properties of water (Prieur and Sathyendranath, 1981; Bowers *et al*., 1996; IOCCG, 2006), as is the case in Case 2 and coastal waters. These errors have been observed in many near-coastal waters around the world and have been a major challenge for the operational satellite oceanography community (Sathyendranath, 2000).

Empirical algorithms are developed by correlating water leaving radiances to chlorophyll concentrations using large *in situ* datasets. Empirical blue-green spectral band ratios lie at wavelengths of around 440 to 550 nm. By using spectral bands which lie in the Near Infrared (NIR) (>700) and red (620-700 nm) wavelengths CDOM and TSM can be distinguished from chlorophyll (Gitelson *et al*., 2009).

MODIS band ratio algorithm OCM3 has been observed to overestimate the value of chlorophyll in various studies. Moore *et al*. (2009) have reported that, when looking at chlorophyll outside an ocean gyre using OCM3, the error can be greater than 50% and up to

100% when observing coastal waters. Another study by Komick *et al*. (2009) applied OCM3 to Western Canadian waters having a chlorophyll concentration lower than 0.13mg m^{-3} and observed similar results.

1.6 Mediterranean Regional Algorithms

The regional specificity of standard algorithms which calculate bio-optical characteristics from satellite ocean colour sensors has also not been given consideration, which gives rise to substantial errors. Data measured *in situ* from a given area is required as a base for regional algorithms. At a global scale OC2v4 and OC4v4 algorithms (O'Reilly *et al.* 1998) perform accurately. However, at a local/regional scale, such algorithms are inaccurate. In the Mediterranean Sea, NASA standard algorithms (OC2v4 and OC4v4) display a substantial overestimation of chlorophyll derived from the SeaWiFS sensor (above 70% for chlorophyll below 0.2 mg m^{-3}) (Bricaud *et al.*, 2002; Claustre *et al.*, 2002; D'Ortenzio *et al*., 2002; Volpe *et al*., 2007).

Many attempts have been made in the last decade, seeking to characterise bio-optical properties in the Mediterranean Sea, in order to apply them to satellite data. This was done either by plotting R_{rs} ratios against *in situ* chlorophyll values or by elaborating bio-optical models. With regards to the chlorophyll and R_{rs} ratios, a two-band R_{rs} ratio is used for all the Mediterranean adapted empirical algorithms. A blue to green ratio is used to get the best estimation of chlorophyll.

An algorithm's performance may be further enhanced by using multiple R_{rs} ratios, which decreases the signal-to-noise ratio (O'Reilly *et al.*, 1998). In a paper published by Volpe *et al.* (2007), it was demonstrated that two Mediterranean algorithms, DORMA (D'Ortenzio *et al.*, 2002) and BRIC (Bricaud *et al.*, 2002), were strongly linked to chlorophyll values. The inaccuracies of some global and regional algorithms in the Mediterranean area (DORMA, BRIC and OC4v4) were quantified by Volpe *et al.* (2007).

From these a new improved algorithm (MedOC4) was developed, which could provide high quality ocean colour datasets. This more advanced bio-optical algorithm is grounded on a fourth power polynomial regression between the log-transformed Maximum Band Ratio and the log-transformed chlorophyll. The accuracy of chlorophyll estimates in the Mediterranean was significantly improved by applying the MedOC4 algorithm. This was partly due to the fact that MedOC4 was calibrated using the most representative bio-optical dataset of its time.

Further explanation as to why global and regional algorithms exhibit such differences can be found in Santoleri *et al.* (2008). Hypothesis such as instrument calibration, the effect of different types of water stratification, or the impact of atmospheric correction algorithms are some of the reasons given. One reason that was provided to explain the different bio-optical composition of the Mediterranean Sea at a regional scale is the effect of ecological factors on the bio-optical characteristics.

Such claims are supported by other authors. Claustre *et al.* (2002) proposed that the observed bias could be a result of Saharan dust being present in the water column. D'Ortenzio *et al.* (2002), Gitelson *et al.* (1996) gave an alternate proposal and stated that "the relative abundance of coccolithophores could account, at least partially, for the observed discrepancy" (Volpe *et al*. 2007). These studies also believe that such an explanation is applicable to remote sensing platforms other than SeaWiFS such as MODIS or MERIS. In order to overcome problems such as the ones mentioned above, regional algorithms can provide a suitable solution (Garcia *et al*., 2005; Gitelson *et al*., 1996; Volpe *et al*. 2007).

Along with algorithm advancements, there have been improvements in the satellite sensors themselves. Satellites today are being equipped with broader spectral bands which utilise different portions of the light spectrum (Tilstone, 2013). The MODIS radiometer, which is on board NASA's Aqua satellite, is the main tool for taking chlorophyll measurements. This sensor has improved on the previous sensor SeaWiFS through its higher resolution and broader spectral bands.

MODIS can observe earth's surface at a high radiometric sensitivity (12 bit) at 36 spectral bands, ranging from 400 nm to1400 nm. The channels from the near infra-red and the visible ranges are used for ocean colour observations. These have a spatial resolution of 1km at nadir. MODIS data is supplied pre-processed. Data is often updated and reprocessed in order to prevent inaccuracies from sensor degradation and outdated atmospheric correction algorithms.

MODIS data for European waters is processed and provided by MyOcean Copernicus Mediterranean Ocean colour algorithms. Case 1 waters use MedOC4. Case 2 waters use the AD4 algorithm (D'Alimonte *et al*., 2003) which was calibrated as part of the CoASTS funded project (Berthon *et al*., 2002). These two algorithms are merged to distinguish between more optically complex Case 2 waters and the phytoplankton dominated Case 1 waters. The satellite spectrum is analysed at a pixel by pixel level and the appropriate algorithm is applied to its respective waters following the method described in D'Alimonte *et al.*, (2003).

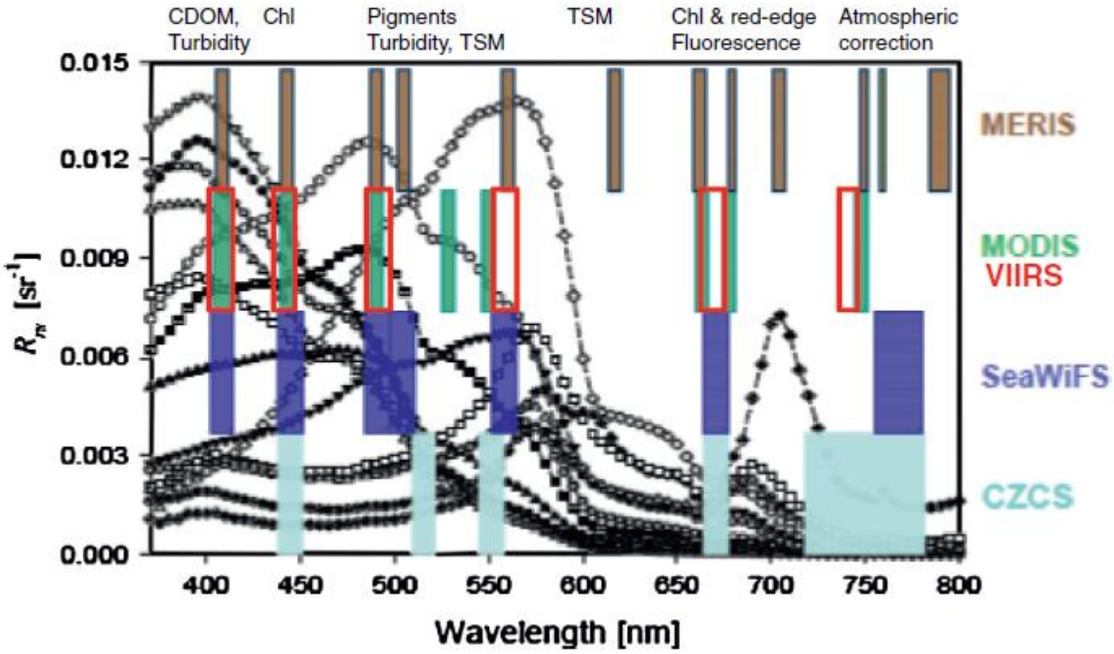


Figure 1.6.1: *Comparison of spectral band positions for 5 ocean colour sensors of the first (Coastal Zone Color Scanner (CZCS)), second (SeaWiFS), third (MERIS, MODIS) and Fourth (VIIRS) generation (Blondeau-Patissier et al., 2014)*

Today ocean products available for MODIS are still poorly explored in terms of accuracy assessment when applying them to coastal regions and Case 2 waters, where optical properties change more rapidly due to landward influences (Blondeau-Patissier *et al.*, 2014). Despite validation taking place for MODIS products, together with Indian Remote Sensing Satellite (ISR)-P4 Ocean Colour Monitor (OCM) and SeaWiFS products, few studies have investigated the accuracy of the newer generation ocean products on coastal waters.

1.7 Aims and Objectives

Clear oligotrophic waters of the central and eastern Mediterranean Sea have been shown to have phytoplankton blooms during winter as opposed to the spring blooms observed in the western Mediterranean. This study seeks to determine if this seasonal pattern is characteristic of Maltese waters and which parameters are driving this seasonal pattern. The general hypothesis of this study is that phytoplankton *growth is controlled by the flux of nutrients found in surface waters, which in turn will be influenced by water mixing and the rate at which effluents derived from human activities enter the water.* This hypothesis will be tested by *in situ* seasonal measurements of various physio-chemical parameters in the water column. The testing of this hypothesis is the main aim of this study. The process of testing this hypothesis will lead to this study achieving four objectives;

1. Identify drivers of change for chlorophyll seasonal patterns in Maltese waters.
2. Test the accuracy of the latest satellite platforms and algorithms in coastal waters through ground truthing exercises.
3. Build up a time series of approximately 10 years of satellite derived chlorophyll values in Maltese coastal waters and compare these with other regions of the Mediterranean.
4. Construct a mathematical model to test if observations from the multi-year time series can be reproduced.

Chapter 2: Field Study of phytoplankton dynamics and their influencing drivers in Maltese waters

2.1 Introduction

This chapter aims at identifying the parameters most significantly associated with the variation in chlorophyll, at a spatial and seasonal level. Statistical techniques based on Pearson's correlation and multiple linear regression are utilised for this analysis. The hypothesis that chlorophyll concentrations are affected primarily by nutrient availability in the surface layers, which in turn are controlled by coastal processes or meteorological forcing, will be investigated through *in situ* analysis.

2.2 Methodology

2.2.1 Description of sampling sites

Four sampling sites were chosen for this study. This number of sites is considered to be sufficient to obtain representative samples of the physiochemical and water quality conditions of Maltese coastal waters. This number of sites also allowed the collection and processing of the samples from all the sites to be carried out on the same day. A larger number of sampling sites could have compromised the quality of the water samples. Knapp *et al.* (1996) stipulates that, once sample collection has taken place, filtration of the samples must take place within an 8-hour time frame. Four sampling sites allows enough time for the island to be circumnavigated, the samples to be collected and returned to the lab, filtered and frozen within the 8 hour time frame.

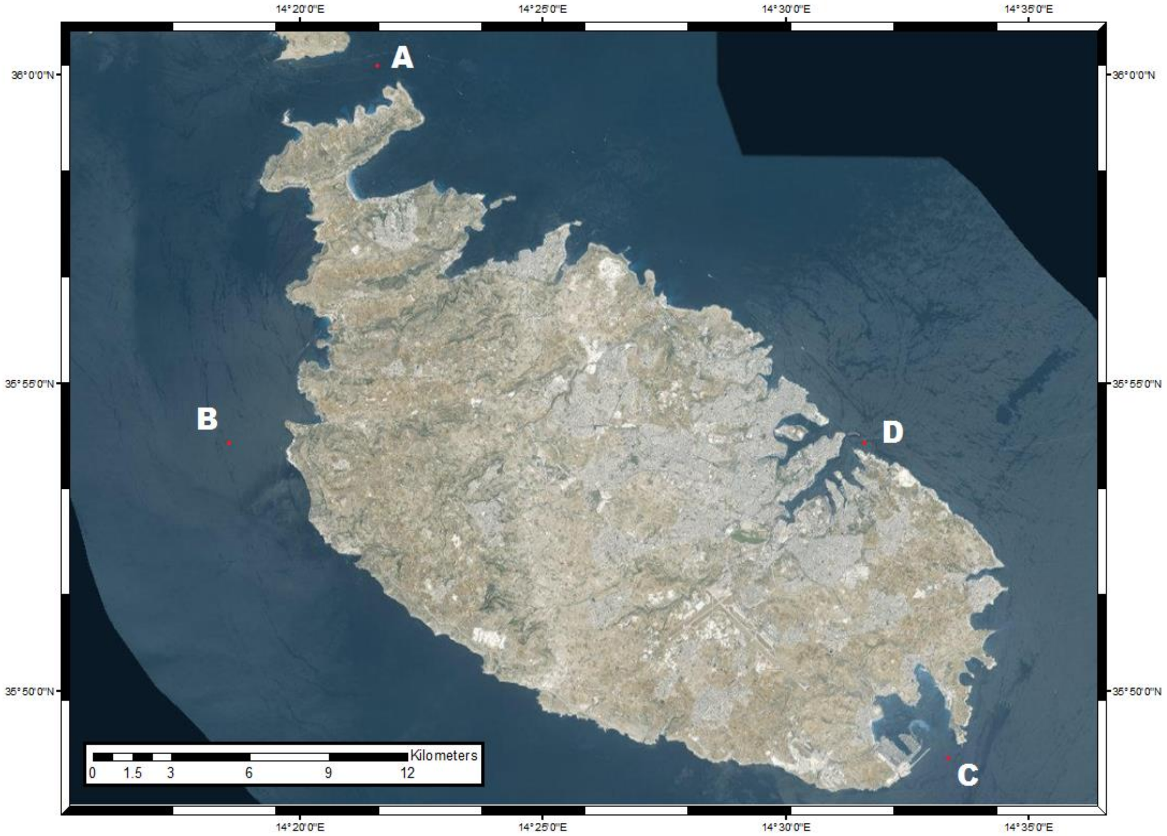


Figure 2.2.1.1: *Showing the location of the sampling sites in Maltese waters utilised in this study*

These stations were located on opposite sides of the island, so as to obtain a range of samples from either side of the island which are representative of Maltese waters. These were labelled as site A, B, C, and D. The geographical coordinates of each site are shown in Table 2.2.1.1.

Site	Coordinates	
Site A	N 36º 00' 05.2"	E 14º 21' 37.5"
Site B	N 35º 54' 01.7"	E 14º 19' 08.8"
Site C	N 35º 49' 04.9"	E 14º 33' 28.6"
Site D	N 35º 54' 00.4"	E 14º 31' 40.9"

Table 2.2.1.1: *Showing the coordinates of the sampling sites in Maltese waters utilised in this study*

Site A was located to the north of Malta, in a channel known as the South Comino Channel. The sampling site is located between Malta and Comino in the entrance to the channel. These waters are characterised by strong currents, due to the funnelling effect generated by this channel. The coastline is primarily composed of low rocky shores, having a karstic characteristic. The sea bed is composed of Posidonia meadows, with sporadic patches of coarse sand beds. Site A has a depth of 20 meters. A small beach, known as Armier Bay, is located to the north of the channel. When precipitation occurs this area experiences high levels of runoff. This could result in elevated sediment and nutrients entering the water. There are relatively small influences from human activity towards the north of the channel, which is where Site A is located. The south of the channel has a stronger anthropogenic influence with two hotels, a ferry terminus and fish farming pens located in the channel for a large part of the year.

Site B is found off the west coast of the Maltese Islands, one mile off Ras ir-Raheb. This site has a depth of 180 meters with predominant south-easterly currents. The coast around Ras ir-Raheb is composed of steep cliffs reaching a height of 80 meters. There is no development along this area of the western coastline. This site was utilised as an open coastal water reference site by this study.

Site C is located on the south coast of Malta, outside the industrial port of Marsaxlokk. This site is located directly in the middle of the entrance to the bay, 600 meters away from the coast. The site has a depth of 20 meters. The seabed is characterised by a coarse sandy bottom. The coastline can be described as having a low rocky characteristic. This area and the coastline inside the bay are seen to have a high degree of anthropogenic activity. There are two major fishing villages located here, and a Freeport with many cargo carrier vessels entering the port on a daily basis. A power station is also found here, along with extensive aquaculture activities, bunkering activities and a marine research centre. Located in open coastal waters, are two

Bluefin tuna (*Tunnus thynnus*) farms. This strong human activity has an effect on the quality of the water in this area.

Site D is also a site which comes under large human influence. It is located 300 meters offshore, on the east coast of Malta, directly in front of the entrance to the Grand Harbour. The Grand Harbour is one of the Mediterranean's largest natural ports. This harbour is a multi-purpose port equipped to offer a large spectrum of maritime services including cruise/ferry and cargo berths, petroleum installations and bunkering facilities. Site D is found close to the offshore sewage outflow at Rinella. Further down the coast there is a major sewage outflow, Ta' Barkat sewage outflow. This handles around 80% of all the sewage generated in the Maltese Islands. The coastline consists of low rocky coast. Site D has a depth of 20 meters and the seabed consists of coarse to fine sand grains.

2.2.2 Frequency of sampling

Sampling took place during the spring and summer seasons of 2015, May to September, and winter of 2016 (January). Sample collection took place twice to three times a month depending on weather conditions. If wind speeds were calm (below Beaufort wind force scale 4) sampling could take place. Sample collection was planned to coincide with satellite overpass when possible weather permitting.

2.2.3 Collection of water samples

Samples were collected from a boat at each of the sites. Samples were collected from two depths at each site using a Van Dorn water sampling device with a volume of 3.2 litres. The Van Dorn sampling device was lowered into the water and samples were collected from two different depths and stored in labelled dark blue polythene 5-litre capacity jerry cans. Opaque jerry cans were used in order to prevent light from contaminating the sample once it was collected.

One sample was taken from the surface and the other from the bottom depth. The bottom depth sample for Site A and C was taken at 20 meters. The bottom depth sample for Site D was taken at 15 meters and for Site B at 55 meters. For each depth at each site 10-litres of water were collected and used for all the analysis. On some occasions, especially during the winter season, the bottom depth sample was not obtained due to time and resource limitations. Originally the study was planning on analysing the differences between the surface depth and the bottom depth; however this became less of a priority as the project progressed since the main focus of this study was remote sensing which mainly consists of surface waters.

Gloves were used when handling the samples so as to avoid contamination. When the sample was on board the jerry cans were covered with a towel to prevent any exposure to the sun. The samples were transported back to the laboratory within seven hours of collection. Prior to every fieldwork session, the jerry cans were cleaned in the appropriate manner to avoid contamination. This was done by filling the jerry cans with Decon 90 (1% Decon) and left to stand overnight. The Decon 90 was removed and the jerry cans were then rinsed with deionised water (DI), filled with 10% HCl and left to stand for 24 to 48 hours. The acid was then removed and the jerry cans were rinsed from the acid using DI. The jerry cans were then placed in the sun to dry. Latex gloves were utilised when handling the jerry cans so as to avoid contamination. Jerry cans were stored with their caps on to avoid dust from entering the jerry can.

2.2.4 Parameters Measured

As stated in the aims and objectives section of this study, the drivers of seasonal change in chlorophyll dynamics were investigated. A number of physio-chemical parameters were chosen based upon the literature surrounding chlorophyll dynamics. The description of the methodologies used and their analysis are described below.

2.2.4.1 Suspended constituent analysis

2.2.4.1.1 Total Suspended Particulate Matter (SPM) concentration

The method described by Strickland and Parsons (1972) was used for determining Suspended Particulate Matter concentration. Whatman glass microfiber filters GF/F, of 47mm diameter were used to filter the samples. The filters were prepared by rinsing them with 500ml distilled water under vacuum in a Millipore filter housing. These were then placed on labelled aluminium trays and left to dry in an oven at 50^{o}C for 2 hours. The filters were then placed inside a muffle furnace for 3 hours at a temperature of 500^{o}C. These were then removed from the furnace, allowed to cool and stored in a desiccator until they were needed.

Prior to filtering the samples, the filters were weighed to the nearest 0.01mg. This weight was labelled as W_1.. The filter was placed inside a Millipore filter housing and the water sample was passed through the filter under vacuum. The sample was shaken vigorously before filtration in order to have a uniform distribution of material throughout the sample. Four litres of water were filtered through the filter. This was recorded as the volume, *V*. Strickland and Parson (1972) recommend that 2-5 litres of water are filtered if the sample is collected from the open ocean, and 0.5-1 litres if the sample is collected from coastal waters. However due to the oligotrophic nature of the water collected in this study, it was decided that 4 litres of water should be filtered in order to have a stronger signature in concentration.

After filtering the sample, the salt residue was removed from the filter by filtering 250 ml of DI water. The filters were then dried in an oven at 50°C for 3 hours. These were then weighed again. This weight was labelled as W_2. It represents the weight of the filter plus particulate matter. The dry weight of particulate material in the samples is calculated by the following equation;

$$\text{Dry weight (mg m}^{-3}\text{)} = (W_2 - W_1/V) \qquad (2.1)$$

2.2.4.1.2 Fluorometric determination of total chlorophyll and chlorophyll *a* concentration.

2.2.4.1.2.1 Sample preparation

A modified version of JGOFS 1942 Protocols was used for the determination of chlorophyll pigment. Whatman glass microfiber filters GF/F, 47mm diameter were used to filter the sample. Immediately after the sample was returned to the laboratory the filters were placed into a Millipore filter housing and 5 litres of the water sample was passed through the filter under a vacuum of less than 100 mm Hg. Due to the oligotrophic nature of Maltese waters it was decided that 5 litres of water would be a sufficient volume to filter. The filters were then folded in half, twice, in an inward direction, wrapped in aluminium foil, labelled and stored at -20°C.

2.2.4.1.2.2 Chlorophyll determination

The filter was removed from the freezer and allowed to defrost prior to analysis. When it reached room temperature the filter was unwrapped, rolled inwards into a cigar shape, and placed into a centrifuge tube. Care was taken when handling the filter. Forceps were used to handle the filters since the filters were fragile and susceptible to tearing. The filters were then submerged in 10 ml acetone in a 15 ml centrifuge tube. The centrifuge tube was then wrapped in foil to prevent light from entering the tube, labelled, and placed in the fridge where it was left to stand for 16 to 24 hours.

The fluorometer was allowed to stabilize for 30 minutes prior to use. This was zeroed using 90% acetone solution as the blank reference. This value was recorded and labelled as *B*. The sample was decanted from the centrifuge tubes into a 10 ml cuvette and placed inside the fluorometer for measurement. This value was recorded as *R* and used to calculate the total chlorophyll concentration in the sample. The sample was then acidified by adding 2 drops of 1.2 M HCl and a second value was obtained. This was labelled as R_{acid} and used to obtain a value of chlorophyll *a*.

These two values were applied to the following equation at separate instances to obtain a value for Total chlorophyll and chlorophyll *a*;

$$\text{Dividing Factor } (L) = \text{Volume of seawater filtered / Volume of acetone used} \quad (2.2)$$

$$\textit{Chlorophyll Concentration} = \text{Reading } (R) - \text{Blank } (B) \text{ / Dividing Factor } (L) \quad (2.3)$$

For some analysis acetonitrile was erroneously used instead of acetone. In order to obtain an accurate reading of chlorophyll concentrations for the sample analysed using acetonitrile that is comparable to the readings obtained using acetone for analysis, a correction procedure was applied.

2.2.4.1.2.3 Acetonitrile correction procedure

Due to a technical error in the supply of materials, acetonitrile was used instead of acetone, when analysing some of the samples for chlorophyll concentration. A correction method was devised in order to validate the chlorophyll readings of the samples analysed using acetonitrile to compensate for this error. A correlation coefficient was created to compare the chlorophyll concentrations obtained when using acetone and acetonitrile for analysis. Cultured alga *Tetraselmis chui*, grown by the School of Ocean Sciences at Bangor University, were identified as a source of chlorophyll. A series of samples was collected and analysed to determine the range of chlorophyll concentrations present in this culture. It was determined that the algae had a chlorophyll concentration of approximately 500 mg m^{-3}.

A 100 ml sample of *Tetraselmis chui* was utilised for this procedure. A 1 in 10 dilution was carried out on this sample, using filtered seawater (100ml sample and 900ml of filtered seawater). This was repeated once more, to produce a concentration of *Tetraselmis chui* of 5mg

m^{-3}. This was then serially diluted by a factor of 1 in 2 (500 ml of the 5mg m^{-3} alga solution and 500 ml filtered seawater). Nine 1 in 2 serial dilutions were carried out in order to prepare a range of standard chlorophyll concentrations.

250 ml of each of these dilutions were then filtered through two separate filter papers using the same method as described in section 2.2.4.1.2.2. After filtration, the filters were immediately placed into the different solvent solutions. One of the filters for each of the 10 dilutions was placed into an acetone solution and the other filter for each of the 10 dilutions was placed into acetonitrile solution. These two sets of samples were analysed using the method described in section 2.2.4.1.2.2., in order to obtain the total chlorophyll concentrations and the chlorophyll *a* concentrations. As a side note, whenever the term ‘chlorophyll’ is referred to in the remainder of this thesis it is referring to chlorophyll *a*.

When the chlorophyll concentrations were obtained, they were plotted against each other, to obtain a linear trend line. The results are presented in Figure 2.2.4.1.2.3.1. The value of the gradient of this trend line was utilised as well as the correlation coefficient.

In order to compare the values from the acetonitrile chlorophyll extraction method with the values from the acetone chlorophyll extraction method, a conversion factor was produced, based on the samples prepared above. The following equation was derived in order to calculate acetone chlorophyll values from chlorophyll values obtained from acetonitrile chlorophyll extraction;

$$Acetone\ extraction\ method\ chlorophyll\ values\ =\ 0.7208 * x - 0.0537 \quad (2.4)$$

x = Acetonitrile extraction method chlorophyll values

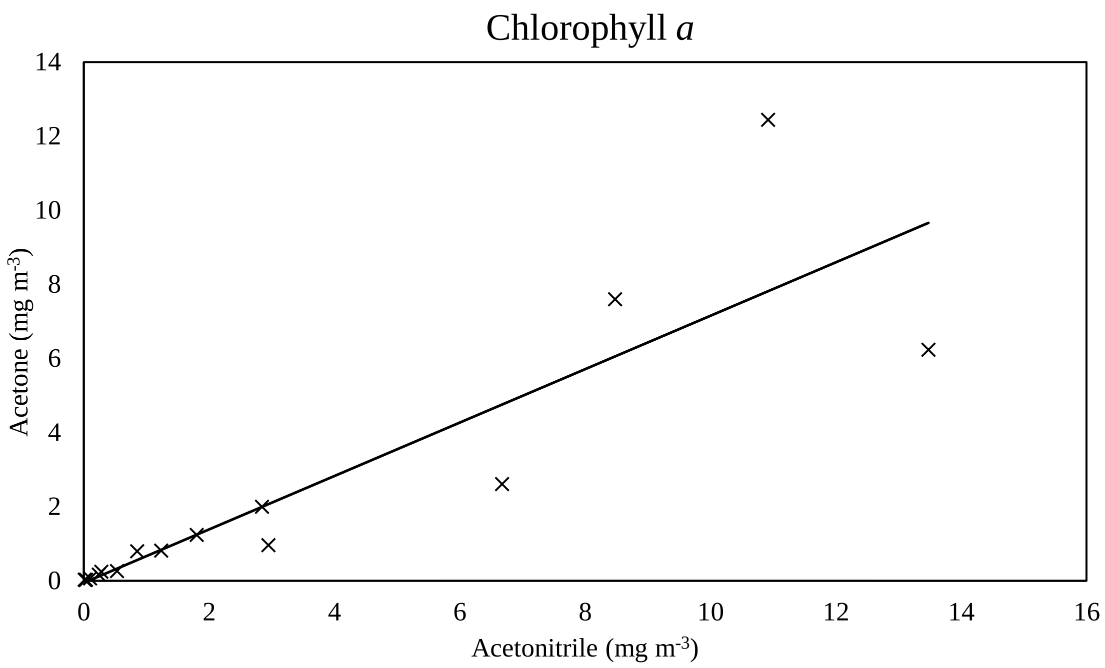


Figure 2.2.4.1.2.3.1: *Showing a scatter plot and the correlation between the two methods of determination of chlorophyll levels using the acetone and acetonitrile extraction.*

2.2.4.1.3 Identification of Phytoplankton species

The method described by Karlson *et al.* (2010) with some small modifications was used for the identification of phytoplankton. A plankton net of 50cm diameter and pore size 50 micrometres was lowered into the water to a depth of 15meters. This was then hauled to the surface in order to filter water through the net. Particulate matter bigger than 50 micrometres was retained by the net and deposited in the storage bottle attached at the base of the net. The water and particulate matter collected in the storage bottle was then transferred into a jerry can and transported to the laboratory for analysis. 0.1ml of Lugol's iodine solution was added to 50ml of each of the water sample collected through the net. These were then stored in a dark cool place for microscopic analysis.

Prior to analysis, the water samples were shaken thoroughly to homogenise the water and then transferred into Utermöhl chambers. The phytoplankton cells were allowed to settle on the bottom of the chamber under the influence of gravity. The settling time was dependent on the height of the chamber (Lund, Kipling & Cren, 1958). A chamber of 25 ml volume was utilised for this analysis with a settling time of 16 hours. Identification and enumeration of the plankton cells was then carried out using an inverted microscope with a magnification factor x400.

Organisms as small as nanoplankton could be identified using this technique. The plankton was identified down to a genus level.

When sedimentation was completed the chamber containing the sample was placed on the stage of the microscope. Counting was carried out at the lowest magnification in order to identify the larger species. Counts were carried out by traversing three transects from one end of the bottom of the chamber to the other. The number of cells counted is expressed in Cells/Litre. In order to calculate this value the following equation was applied;

$$Cells\ L^{-1} = N * \left(\frac{At}{Ac}\right) * \frac{1000}{V} \qquad (2.5)$$

where N is the number of cells counted for each genus, At, represents the total area of the counting chamber (mm^2), Ac represents the surface area of the counting chamber (mm^2) and V is the volume of the counting chamber (ml). The chambers were cleaned after use so they could be reused without the risk of cross contamination between the samples.

2.2.4.2 Analysis of dissolved constituent

2.2.4.2.1 Salinity

A HACH HQ40d Portable Multi-parameter meter with a HACH IntelliCAL™ CDC401 Standard Salinity / Conductivity probe was used to measure the salinity of the seawater according to the UNESCO 1981 practical salinity scale (PSU) (Lewis & Perkin 1981). The salinity meter was calibrated using a freshly prepared solution of 0.05 % sodium chloride solution, prior to every set of water measurements. The salinity of the sea water samples was measured and recorded as soon as the sample was brought to the surface. The probe had an accuracy of up to two decimal places and a range of 0 - 50 PSU.

2.2.4.2.2 Analysis of Dissolved Oxygen (DO) concentration

An HACH HQ40d Portable Multi-parameter meter, equipped with an IntelliCAL™ LDO101 Standard Luminescent / Optical Dissolved Oxygen (LDO) probe was used to determine the dissolved oxygen level in sea water according to Good Laboratory Practice Standards. The DO meter was factory pre-calibrated in accordance with Good Laboratory Practice Standards. The DO of the water samples from each sampling point was measured and recorded, to the second decimal of accuracy, as soon as the sample was brought to the surface.

2.2.4.2.3 Analysis of Chromophoric Dissolved Organic Matter (CDOM)

The CDOM absorption coefficient of the water sample was calculated using the method described by Bricaud, Morel & Prieur (1981) with a slight modification. A volume of 250 ml of sample water was filtered through a 0.2 µm membrane filter. A Whatman glass microfiber GF/F filter was used in the original method. This was modified so as to be used for measuring the CDOM concentration. This modification involved rinsing the filters with 250ml to 500ml distilled water under vacuum using a Millipore filter housing. These were then placed on labelled aluminium trays and left to dry in an oven at 50^{o}C for 2 hours. They were then placed inside a muffle furnace for 3 hours at temperature of 500^{o}C and then allowed to cool in a desiccator.

After the sample was collected and filtered, the filtrate was stored in a dark glass bottles at -20^{o}C for future analysis. The samples were analysed within a month of collection. These were analysed using a double beam spectrophotometer with monochromatic light to minimize the florescence of the sample. Quartz cuvettes with a 10cm path length were used for this analysis. A blank baseline reading was taken using DI water in both cuvettes. Spectrophotometric analysis of the water samples was carried out in the wavelength range 300 to 800nm at room temperature. This was carried out by placing the water sample in one of the cuvettes and measuring the absorbance against a cuvette containing DI water acting as a reference. The absorbance was converted to absorption by subtracting the absorbance value at 750 nm from the absorbance value at 440 nm for each sample and then applying the following equation;

$$a(\lambda) = ln(10) \times A(\lambda) / l \qquad (2.6)$$

where

λ = wavelength, normally 440nm.

A = absorbance measured by spectrophotometer

l = path length (0.1m for a 10cm cuvette)

2.2.4.2.4 Nutrients

2.2.4.2.4.1 Sample collection and storage

The water samples were collected as described in section 2.2.3. As soon as the samples were returned to the laboratory, 250ml were filtered through Whatman glass microfiber filters GF/F, 47mm diameter, using a Millipore filter housing under a vacuum of less than 100 mm Hg.

Three aliquots were taken from each sample and stored in 20ml opaque plastic vials that had been acid washed to remove all contaminants beforehand. The cleaning procedure is described in section 2.2.3. Every aliquot was clearly labelled and stored immediately at – 20^{o} C for analysis at a later date. These were stored upright to prevent contamination or leakage in the event of the samples defrosting.

2.2.4.2.4.2 Instrument calibration and sample analysis

Nutrients were analysed using a SEAL Analytical AA3 HR Nutrient Auto Analyser. The AA3 is a fully automated instrument designed for industrial and environmental nutrient analysis. It uses a continuous flow of solution that moves through the instrument, segmented by bubbles. The samples and the reagents are heated and mixed to produce a colour change. The intensity of colour produced is proportional to the nutrient concentration present in the sample. A fluorometer measures the absorbance at specific wavelengths. These are digitised and converted to a spectral signature representing the peaks of absorbance. The AA3 has five channels each with its own fluorometer and density manifold.

The day prior to running the analysis, samples were removed from the freezer and placed in a refrigerator to thaw. A standard was run in the Auto Analyser to ensure that the baseline stability, and peak shape were working correctly using the method described by Wurl (2009). Specific protocols for analysis using the AutoAnalyzer are provided by the manufacturer of the analyser; SEAL Analytical and can be obtained from Seal Analytical (2014). The following analysis was carried out on seawater;

2.2.4.2.4.3 Nitrate and Nitrite (NOx)

This automated procedure for the determination of nitrate and nitrite is based on the procedure whereby nitrate is reduced to nitrite at pH 7.5 in a copperized cadmium reduction coil. Nitrite is then reduced from nitrate with sulfanilamide to form a diazo compound that then couples with N-1-naphthylethylenediamine dihydrochloride (NEDD) to form a reddish-purple azo dye. This is then measured at wavelengths of 520 - 560 nm. This method of analysis is suitable for measurements of Nitrate and Nitrite in water in the range of 0 – 40.0 μmol/L with a detection limit of 0.04 μmol/L.

2.2.4.2.4.4 Analysis of Nitrite

This method of nitrite analysis is based on the Griess reaction, adapted to seawater by Bendschneider and Robinson (1952). Nitrite ions react with sulphanilamide to form a diazo compound, which then combines with N-naphthyl-ethylenediamine (NED) in acid conditions (at pH less than 2) to produce a final pink-coloured complex. This method of analysis is suitable for measuring nitrite levels in the range of 0.001 - 10 μmol/L of Nitrite in water with a detection limit is 1 nmol/L.

2.2.4.2.4.5 Analysis of Silicate

This automated procedure for the determination of soluble silicates is based on the reduction of silicomolybdate in acidic solution to molybdenum blue by ascorbic acid. This method was obtained from Grasshoff, Kremling and Ehrhardt (2009). The range of silicates that can be measured by this method is 0 - 100 μmol/L of silicate in water with a detection limit is 1 nmol/L.

2.2.4.2.4.6 Analysis of Phosphate

The analysis of phosphates is an automated procedure following the method of Murphy and Riley (1962), for the determination of ortho-phosphate based on the colorimetric method in which a blue colour is formed by the reaction of ortho-phosphate, molybdate ion and antimony ion followed by reduction with ascorbic acid at a pH of less than 1. The reduced blue phospho-molybdenum complex is measured at a wavelength of 880 nm. The [H+] : [Mo] ratio in the reaction mixture corresponds to the optimum determined by Drummond and Maher (1995). The range of phosphate concentrations that can be measured by this method is 0.01-1.7 mg P/L.

2.2.4.2.4.7 Analysis of Ammonium

The sample is reacted with o-phthalaldehyde (OPA) at 75°C in the presence of borate buffer and sodium sulphite to form a fluorescent species proportional to the ammonia concentration.

The fluorescence is measured at 460 nm following excitation at 370 nm. This method was obtained from Kérouel and Aminot (1997). The range of ammonium in water that can be measured using this method is 0.013- 5 μmol/L. The detection limit is 0.003 μmol/L.

2.2.4.3 Hydrographic measurements

2.2.4.3.1 Temperature-depth profiles

A Star-Oddi DST centi-TD temperature depth recorder was used to measure and create a temperature against depth profile of the water column. This probe was protected by an alumina housing with two holes at either end. This allowed the water to filter through. This was lowered from the boat at a rate of one meter every 30 seconds. Prior to launch it was placed in the water at a depth of 30 cm and allowed to stand for 2 minutes, in order for the temperature and depth gauge to reach a steady state of recording and provide accurate readings. The temperature response time of the probe is 20 second to adjust to 63% of the full value. The accuracy of the probe is +/- 0.1°C.

Mixed layer depth was calculated using the data collected by this probe. The criterion used for calculating mixed layer depth was a temperature change of 0.1ºC. This temperature change criterion was similar to the temperature change criterion used by previous studies. The study by D'Ortenzio *et al*. (2005) was found to be the most relevant study exploring mixed layer depth of the Mediterranean Sea. D'Ortenzio used a criterion applied by de Boyer Monte´gut *et al.* (2004) which utilised a $\Delta T = 0.2$ ºC. The decision of utilising a temperature change criterion of 0.1ºC was decided upon after applying a broad range of mixed layer depth temperature change criterion (0.025ºC, 0.05ºC, 0.1ºC, 0.15ºC, 0.2ºC) to each of the temperature profiles obtained from sampling. A visual depiction of this can be found in APPENDIX 2.

The temperature change criterion which had a stable variability from one field session to another was used for this analysis. This assumption was based on the high specific heat capacity of water which means that a lot of energy is required to change the temperature of a water body. Mixed layer depth is characterised by an overall gradual seasonal change, with the possibility of faster variation found in shallower waters (Oka, Talley & Suga, 2007). This can be seen in APPENDIX 2. Based on the graphs seen in APPENDIX 2 it was decided that the mixed layer depth temperature change criterion utilised by this study should be a temperature change of 0.1ºC.

2.2.4.4 *In situ* spectral radiometric measurements

2.2.4.4.1 Light intensity profiles diffuse attenuation coefficient (K_d)

A MDS Mk-L photo diode was used to measure the light intensity in the water column. The meter has a measuring range of 0 to 2000 µm/m^2.sec, a resolution of up to 1 µm/m^2.sec, and an accuracy of +/- 4 µm/m^2.sec. The diode is contained in a titanium housing. It is capable of measuring light intensities in the range of 390 to 690 nm. This is the approximate spectral bandwidth of light needed for photosynthesis. This was attached to a metal frame and lowered into the water. The temperature / depth probe was also attached to this metal frame, in order that light intensity measurements and temperature depth measurements are taken at the same depth.

This meter was used to calculate the diffuse attenuation coefficient of the water profile (K_d). K_d is a measure used to describe the light field of a water body in the marine environment. It is important to measure K_d when calculating the depth of the euphotic layer and determining how much light is available for photosynthesis by phytoplankton and other marine organisms (Mankovsky, 2014). The diffuse attenuation coefficient is the proportion of incident radiant flux that is attenuated by an infinitesimally thin horizontal plane parallel layer at a depth, z, divided by the thickness of the layer (Kirk, 1996). A method that is used for estimating the diffuse attenuation coefficient is by obtaining the slope value of the natural log of either a downwelling spectral irradiance, $E_d\,(z,\lambda)$ or upwelling spectral radiance, $L_u(z,\lambda)$ from the light profile obtained for each site. Light measurement intervals of a minimum of 4 to 10 meters is required to smooth over incident radiant flux fluctuations (Kirk, 1996).

2.2.4.4.2 Determination of Latitude and longitude

A handheld Garmin GPS 72H was used to identify the location coordinates of each site. This ensured that the same sampling locations were used throughout the entire survey. This is of utmost importance when conducting the ground truthing of remotely sensed data. The accuracy of the GPS is of approximately 15 meters. It can be as accurate as 3 meters if there is limited cloud cover.

2.2.4.4.3 Date and time

The date and time were recorded using the Garmin GPS 72H every time the boat arrived and left a sampling site. This ensured that all the data collection activities were logged in real time.

2.2.4.4.4 Collating Wind speed, wind direction, cloud cover, rainfall and swell direction data.

The wind speed and rainfall data were collected prior to sampling from coastal weather stations found close to the sampling sites. These provided an accurate and reliable source of meteorological data. The units for wind speed were measured in Beaufort wind force scale and rainfall was measured in mm. Wind direction data was also collected from the weather stations as well as being assessed by a wind vane located on the boat. Cloud cover and wind direction were based on experience of the skipper. The measurement of cloud cover was expressed as a percentage. Zero percent indicates clear skies and one hundred percent indicates very cloudy skies.

2.2.4.4.5 Measurement of pH

An HACH HQ40d portable multi-parameter meter, equipped with a HACH IntelliCAL™ PHC101 Standard Gel Filled pH Electrode probe was used to measure the pH of sea water according to Good Laboratory Practice Standards. The pH meter was calibrated using standard buffers of pH 4, 7 and 9. The pH of the water samples from each sampling point was measured and recorded within five hours of collection to the second decimal of accuracy.

2.2.4.4.6 Determining Secchi Depth

Secchi Depth is used as a measurement of transparency and is typical of many water quality studies. Poole and Atkins (1929) found that Secchi Depth has an inverse correlation with the diffuse attenuation coefficient (K_d) and can be used to empirically estimate the K_d. Kirk (1996) discussed the relationship between these two parameters. Secchi Depth is considered a simple and efficient way of assessing the strength of the light field in a water column.

A modified Secchi disk (20cm diameter) was used to estimate the turbidity at each of the four sites. The disk was lowered by hand on a rope that was labelled at 0.5m intervals. The disk had a black and white pattern on its surface. The Secchi Depth was determined as the depth at which the black and white pattern would disappear from view as seen from the surface. A pattern disappearance method was utilised as opposed to a disc disappearance method. This was done since most sites were shallow sites and the waters were mostly clear Case 1 waters. This meant that when conditions were right, the bottom was visible from the surface. The above method was used to obtain more accurate measurements.

In order to compare the standard disc disappearance method with the pattern disappearance method utilised in this study, a conversion factor was created based on the *in situ* sampling regime conducted in this study. The following equation was derived in order to estimate the disk disappearance depth from the pattern disappearance depth;

$$Disk\ Disappearance = 1.1297x + 1.5116 \qquad (2.7)$$

x = pattern disappearance depth

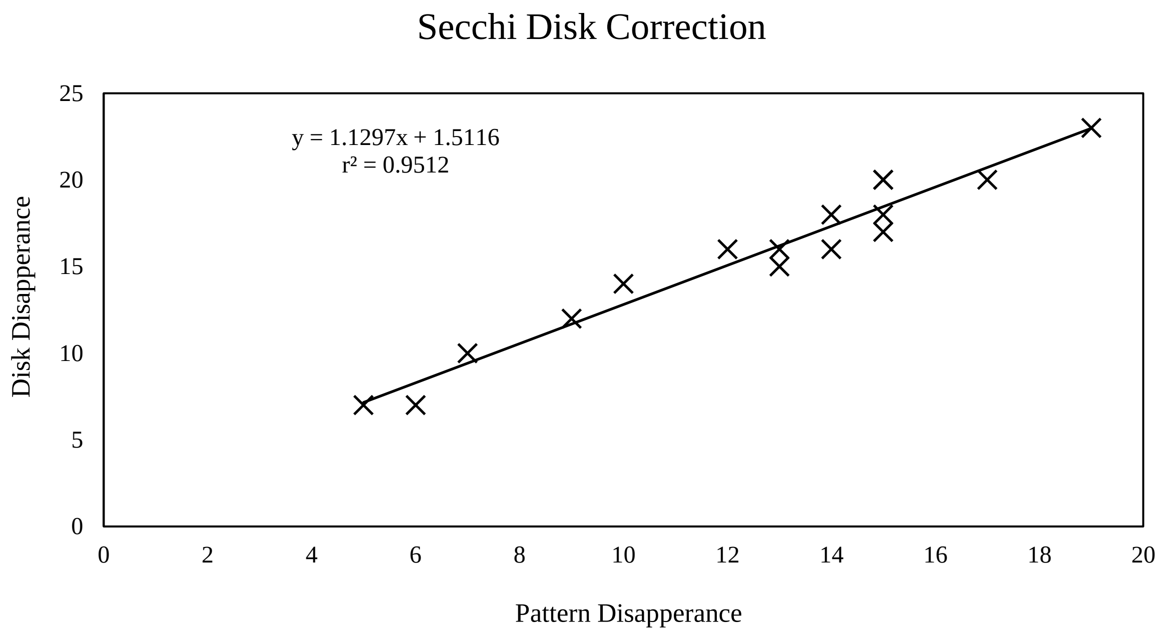


Figure 2.2.4.4.6.1: *Showing a scatter plot and the correlation between Secchi Depth pattern disappearance and disk disappearance in Maltese waters*

2.2.4.4.7 Correlation between the seasonal pattern of meteorological parameters and chlorophyll concentrations in Maltese waters

A Pearson product-moment correlation coefficient was used to assess the relationship between mean monthly meteorological data of air temperature, wind speeds and rainfall with mean monthly chlorophyll concentrations (obtained as described in chapter 3.2) of a seventeen year time series of Maltese waters. Historical meteorological data was obtained from Qrendi (Malta Weather Station, 2016) and Luqa (Weather Underground, 2016) weather stations. Both these stations are found in the south of Malta close to Site C. These were used to assess the importance of each of the meteorological parameters in driving chlorophyll dynamics in central Mediterranean shelf seas. This dataset represents one of the most comprehensive and lengthy time series of the Mediterranean Sea to have been analysed to date. This could yield new interesting data and patterns not observed by other studies. Chlorophyll data for this analysis was obtained from the same source described above (Section 3.2.1). Chlorophyll values of Maltese coastal waters was utilised for this analysis since the meteorological data was obtained from a site located on the coast.

2.3 Results and Discussion

The sampling data for all the parameters analysed in this study collected from the four different sites between spring of 2015 to winter of 2016 are shown in Appendix 1.

2.3.1 Tempo-spatial variation of sea surface temperature in Maltese waters

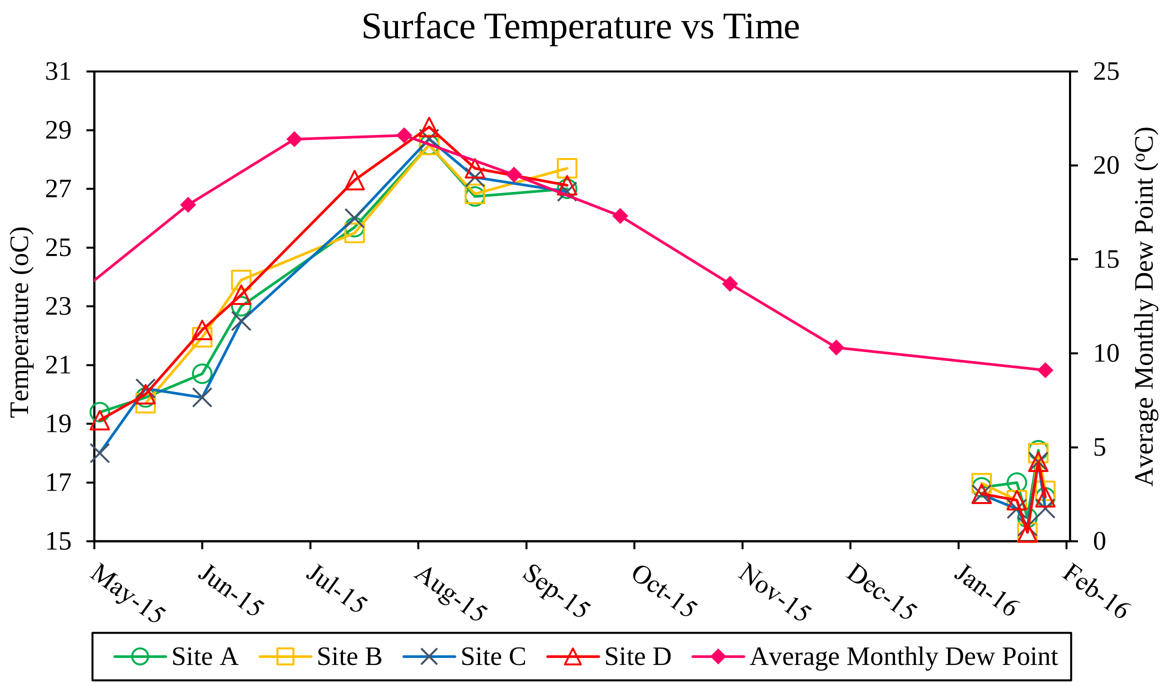


Figure 2.3.1.1: *Showing surface temperature and the average monthly dew point in Maltese waters over a series of time*

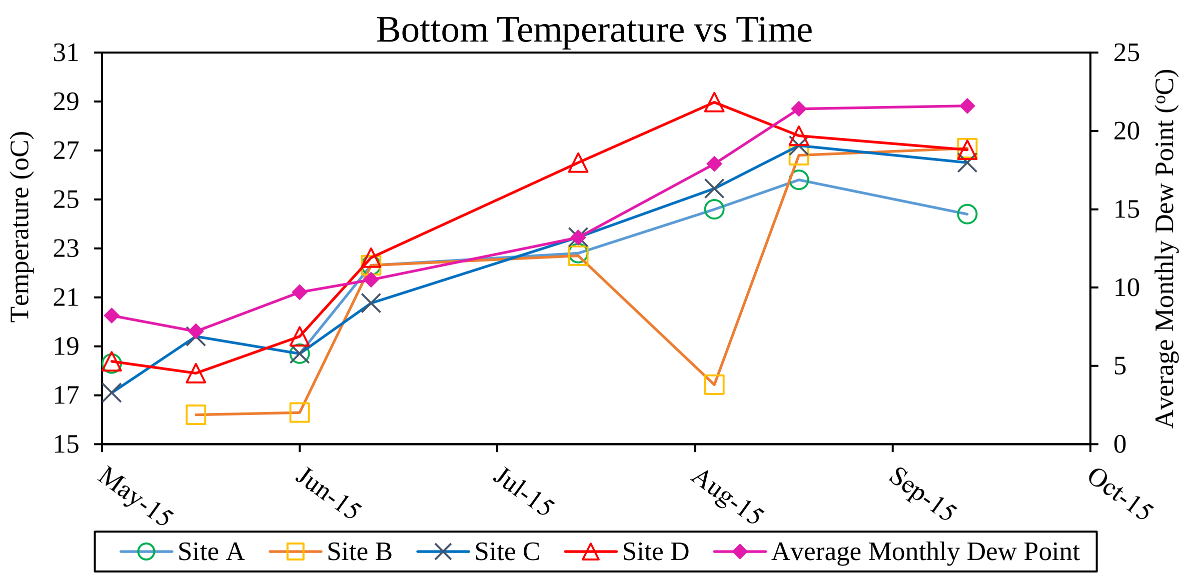


Figure 2.3.1.2: *Showing bottom temperature and the average monthly dew point in Maltese waters over a series of time*

Temperature has a strong seasonal variation. The surface temperature of the water column at each of the sampling sites was monitored in this study. As expected, surface water temperatures are highest during the summer when convective heating is at its highest, reaching a maximum temperature during August (29.1^{o}C). During the winter, the temperatures drop to their lowest point between January and March (15.3^{o}C) (Figure 2.3.1.1).

Site D, being the shallowest of the four sites, is seen to have the greatest temperature variation, recording the highest and the lowest temperatures in this study. This is related to the specific heat capacity of water whereby a considerable amount of energy is required to heat the water, resulting in deeper bodies of water needing more heat to bring about a change in temperature. This physical principle has been related to phytoplankton growth. Observations by Behrenfeld *et al.* (2006) resulted in the creation of a hypothesis which states that warm surface waters and strong water column stratification are related to lower levels of primary production.

This hypothesis is based on the observations that the greater the stratification in a body of water the less nutrients are transferred from nutrient rich, cold, dens deep waters to surface waters where light is available for photosynthesis. In this study temperature is seen to have an inverse correlation with chlorophyll concentrations (Appendix 3), thus agreeing with Behrenfeld's hypothesis.

The average monthly dew point is seen to follow the same seasonal cycle as temperature. The dew point is defined as the temperature to which air is cooled at a given pressure and water vapour content for the air parcel to reach saturation, resulting in condensation. The dew point temperature is always equal or lower to the air temperature. The highest dew point temperature recorded at Luqa airport (Weather Underground, 2016), which is close to Site C, was 21.6^{o}C in August and the lowest in January 9.1^{o}C. This is shown in Figure 2.3.1.1.

Maltese waters have a complex sea surface temperature field (Malta Planning and Environment Authority, 2013). The main factors dictating temperature patterns of the seawater column around the Maltese Islands include upwelling events taking place south of Sicily, heating and cooling of shallow continental shelf waters and the development of the Atlantic Ionian Stream (AIS).

During autumn, a number of fronts and thermal gradients are created due to the increased surface cooling characteristic at this time of year. The AIS, which is composed of warm, salty water, is stronger in summer and spans all Maltese waters at this time of year. In winter, the

AIS covers a smaller range and flows north of Malta. During this period, it flows down from Sicilian shores to a point around 45 kilometres north east of the Maltese Islands. This creates a front which is often broad and long, in the shape of a 'V' pointing northward. The Maltese Islands are often found situated at a frontal line whereby temperature variations between northern and southern shores are significant, with water bodies from the southern shores being warmer than those located in northern areas.

This front will affect the temperature and salinity gradients which will produce different stratification regimes. Mixed layer depth was used to represent the degree of stratification present in the water column at each sampling session. Its variability strongly influences upper ocean physics, along with important chemical and biological processes that have a large impact on the earth's climate (Falkowski *et al*., 1998).

2.3.2 Tempo-spatial variation of mixed layer depth in Maltese waters

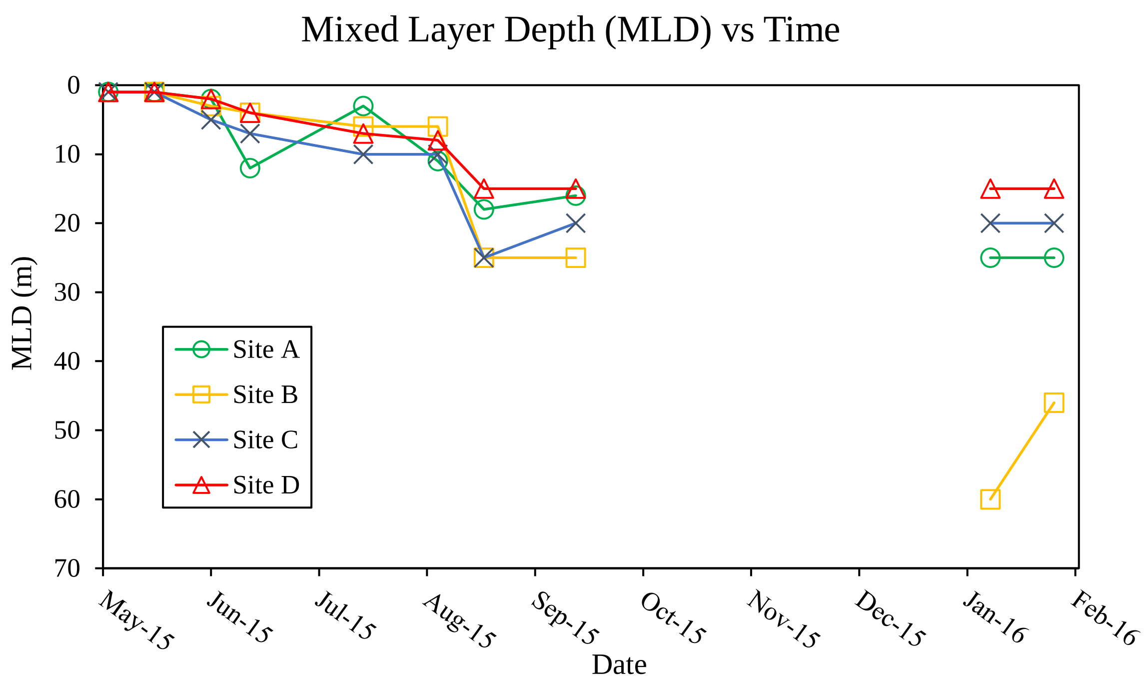


Figure 2.3.2.1: *Showing the Mixed Layer Depth in Maltese waters over a series of time*

The results from figure 2.3.2.1 show that Site B has the deepest mixed layer depth values and Site D the shallowest. The patterns for each of the site show a clear seasonal cycle with a deepening in the mixed layer depth from September to February and re stratification in what would most probably be March to April; however, since no sampling took place during these months this cannot be confirmed by this study. The coastal sites and open coastal water sites all seem to follow this diurnal variation despite the large differences in depth. These results are in line with the results from the first and most likely the only other study which utilised synoptic Conductivity (to compute salinity), Temperature and Depth (CTD) measurements in local waters (Drago *et al.,* 1997). This study showed that the upper 50 meters of the water column at an open coastal water site located north-west of the Maltese Islands had strong stratification during the hot season and a diurnal vertical oscillation of the thermocline throughout the year.

No overall correlation was observed between chlorophyll and mixed layer depth. In order to investigate this further, the data was divided by site and the correlation between chlorophyll and mixed layer depth was tested. It was found that chlorophyll concentrations at Site A and B had a significant correlation with mixed layer depth at the 99% confidence interval (Site A; r^2= 0.589, n=19) (Site B; r^2= 0.829, n= 17). Since these two sites are found in deeper waters, with a reduced influence from anthropogenic activities, and considered to be open coastal water sites (Site B); chlorophyll growth at these sites is influenced primarily by physical and meteorological drivers.

The significance of mixed layer depth affecting seasonal phytoplankton dynamics in open coastal water sites has been observed in other studies and has recently been shown to be a key driver of seasonal change in many Mediterranean waters. The study by D'Ortenzio *et al*. (2005) shows that the steep increase in biomass of the Mediterranean Sea during the winter months is seen to be in phase with the progressive, moderate deepening of the mixed layer (D'Ortenzio *et al*., 2005, Figure 1). During the summer period nutrients will be in low abundance and there will be insufficient concentrations for any increase in the levels of phytoplankton biomass. In November heat lost to the atmosphere at the sea's surface brings about a deepening of the mixed layer. This results in a greater concentration of nutrients entering the photic zone, which results in greater levels of phytoplankton growth during this time of the year.

2.3.3 Tempo-spatial variation of Secchi Depth in Maltese waters

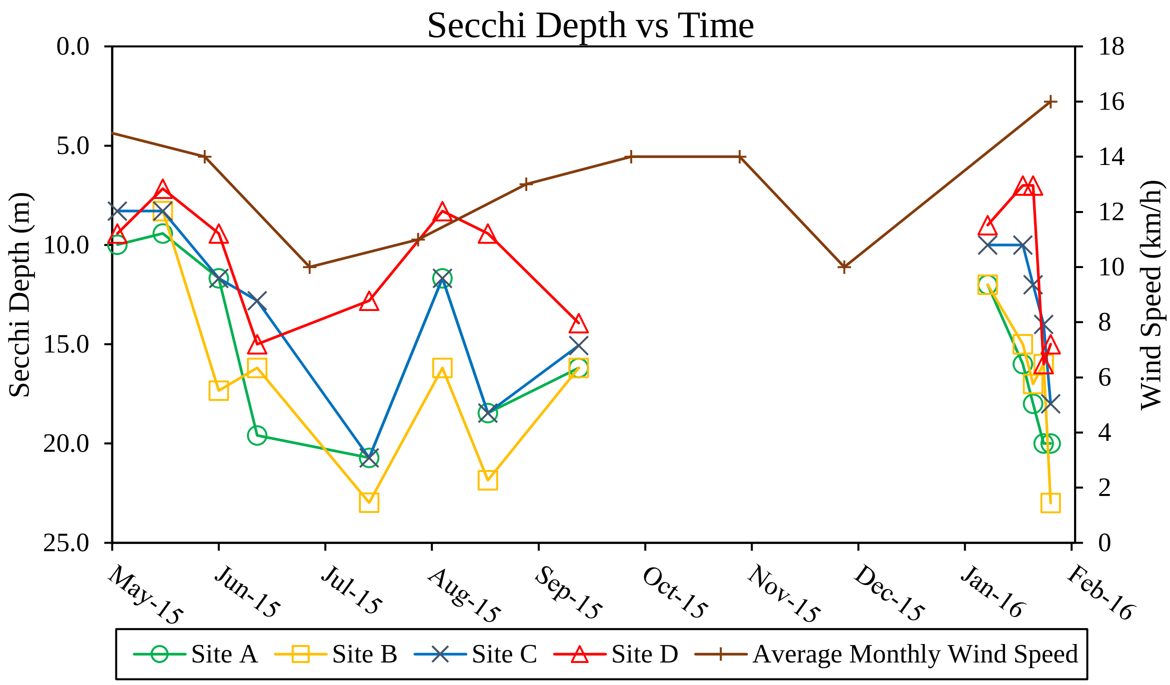


Figure 2.3.3.1: *Showing the Secchi Depth in Maltese waters and wind speed over a series of time*

Secchi Depth is seen to have a strong variation from one sampling session to another at each of the sites analysed in this study. Site D is seen to have the shallowest Secchi Depth. This is related to the fact that Site D is the site with the shallowest bottom depth. Site B is seen to have the overall deepest Secchi Depth whilst Site A and C have very similar Secchi Depths throughout the year. Site A and C both have similar bottom depth of 25 meters. Of the two, Site A is seen to have the deeper Secchi Depth. The reason for this is most likely related to the fact that a greater amount of suspended material, such as phytoplankton and suspended matter, is found in the water column at sites closer to the coast, even more so when there is a high degree of human activity at these sites.

Figure 2.3.3.1 shows that the average monthly wind speed has a similar timescale pattern to Secchi Depth. There is a statistically significant negative correlation, at the 95% confidence interval, between average monthly wind speed and Secchi Depth (r=-0.241, n= 75) in Maltese waters (Appendix 3). A similar observation was made by Sanden and Håkansson (1996) from their studies on Secchi Depth in the Baltic Sea. This correlation can be associated with greater wind speeds resulting in a greater disturbance of the water surface. This disturbance makes the surface ‘rougher’, thus reducing the contrast between the water colour and the Secchi disk, when looking down into the water directly from above the water’s surface (Preisendorfer, 1986). As wind speed increases, the visibility of the water column observed from the surface decreases.

2.3.4 Tempo-spatial variation of surface nutrient concentrations in Maltese waters

2.3.4.1 Nitrate (NO_3) vs Time

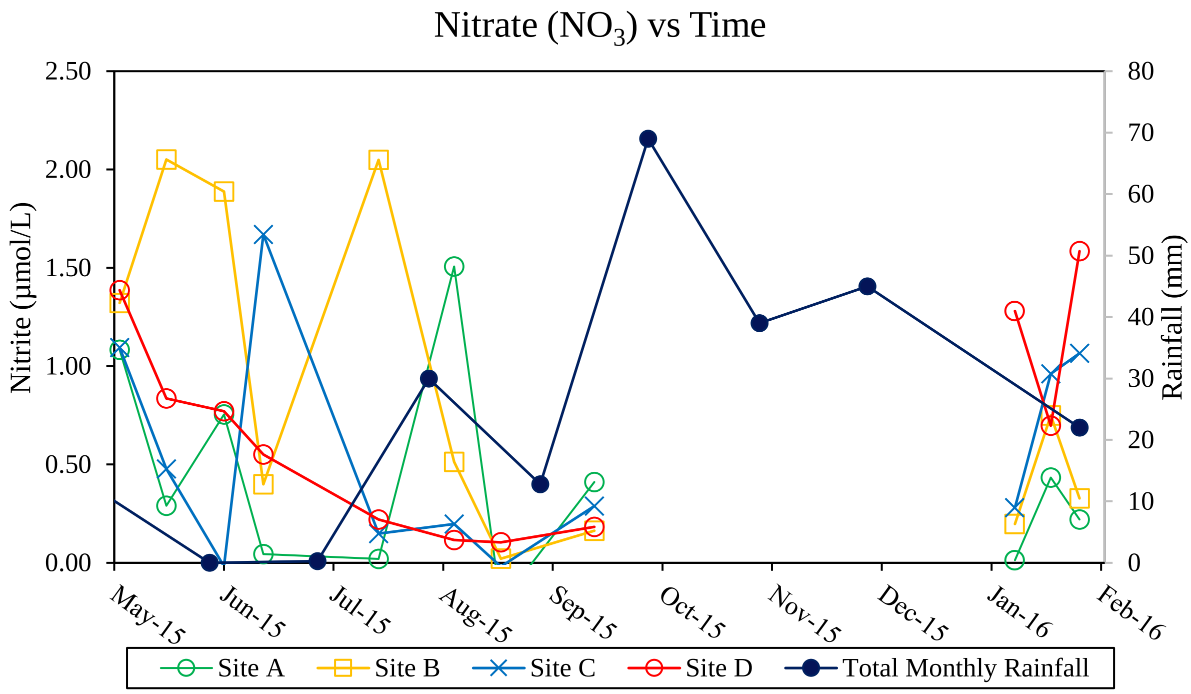


Figure 2.3.4.1.1: *Showing surface nitrate (NO_3) concentrations as well as total monthly rainfall in Maltese waters over a series of time*

Nitrate was plotted against time in order to investigate the seasonal variation and how it relate to other parameters measured in this study. Overall, the concentrations can be described as being oligotrophic. On some occasions, some of the sites experienced spikes in concentration. Site A and Site B are seen to have the greatest overall variation, with Site B experiencing the highest concentration observed during July (2.2µmol/L). Site C and Site D seem to have less variability than the other two sites, which is surprising given the high degree of anthropogenic activity taking place close to these sampling sites. These results do not follow the same seasonal pattern as the chlorophyll values obtained by this study, as was initially expected.

Nitrogen is an important element for marine life. The cycling of nitrogen is closely related to the cycles of other elements like phosphorus, carbon and sulphur. Nitrogen can be measured in the natural environment in its reduced forms of inorganic nitrogen (DIN) such as nitrate, nitrite, ammonium and organic molecules (DON). Nitrogen enters the marine environment through precipitation, river discharge and runoff. Once in the marine environment these are used for primary production by phytoplankton and macrophytes. Nitrogen can also enter the system through dinitrogen (N_2). Primary production is directly transported through the food web by grazing (zooplankton) and indirectly by mediation of the microbial loop as can be seen in Figure 2.3.4.1.2. Dead particulate biomass can sink to the sediment and is re-mineralized by bacteria. For example, dissolved dinitrogen (N2) can be produced and released into the water column by denitrification.

Human development is causing perturbations in the nitrogen cycle and is a major threat to the ocean and the coastal environment. Anthropogenically derived nitrogen mainly stems from agricultural practices and reaches the oceans via rivers and atmospheric deposition.

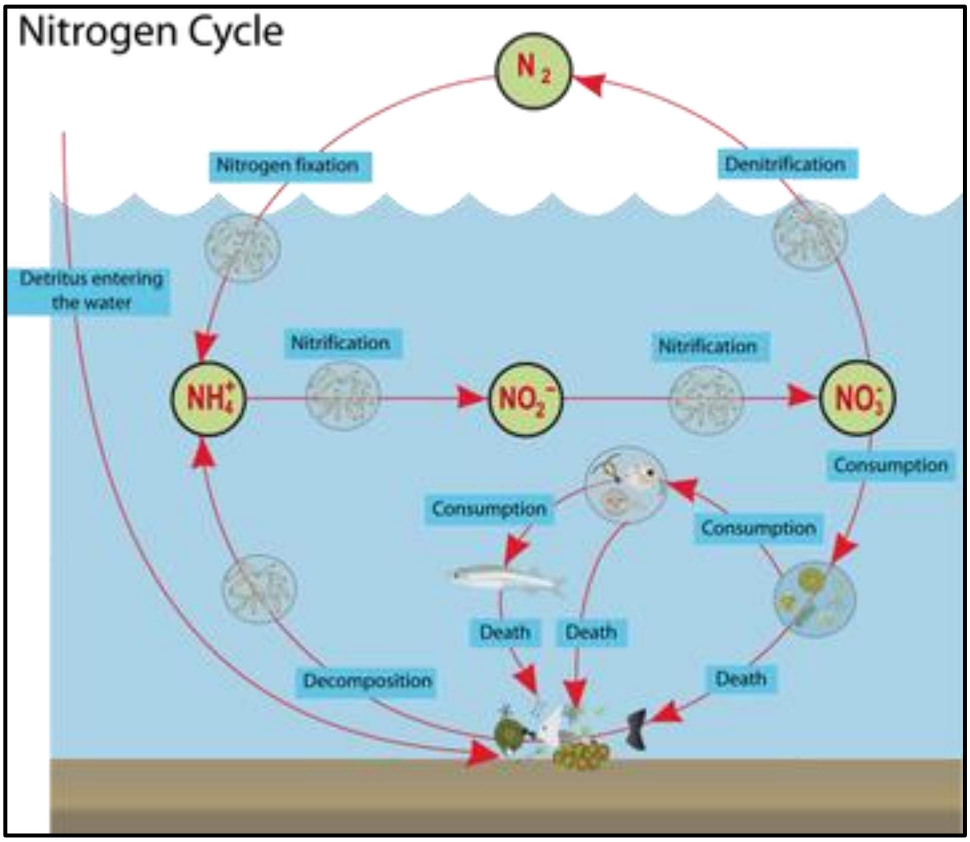


Figure 2.3.4.1.2: *The nitrogen cycle in the marine environment*

One possible reason for the elevated degree of variability at Site A and B could be associated with effluent being released from the waste water plants at Ras il-Ħobż, on the south coast of Gozo, and Iċ-Ċumnija, found on the northwest coast of Malta. The location of these sites is shown in Figure 2.3.2.1.3. These plants started to be used in November 2007 and November

2008, respectively. Only a small amount of wastewater in the Maltese Islands was treated prior to the development of these plants.

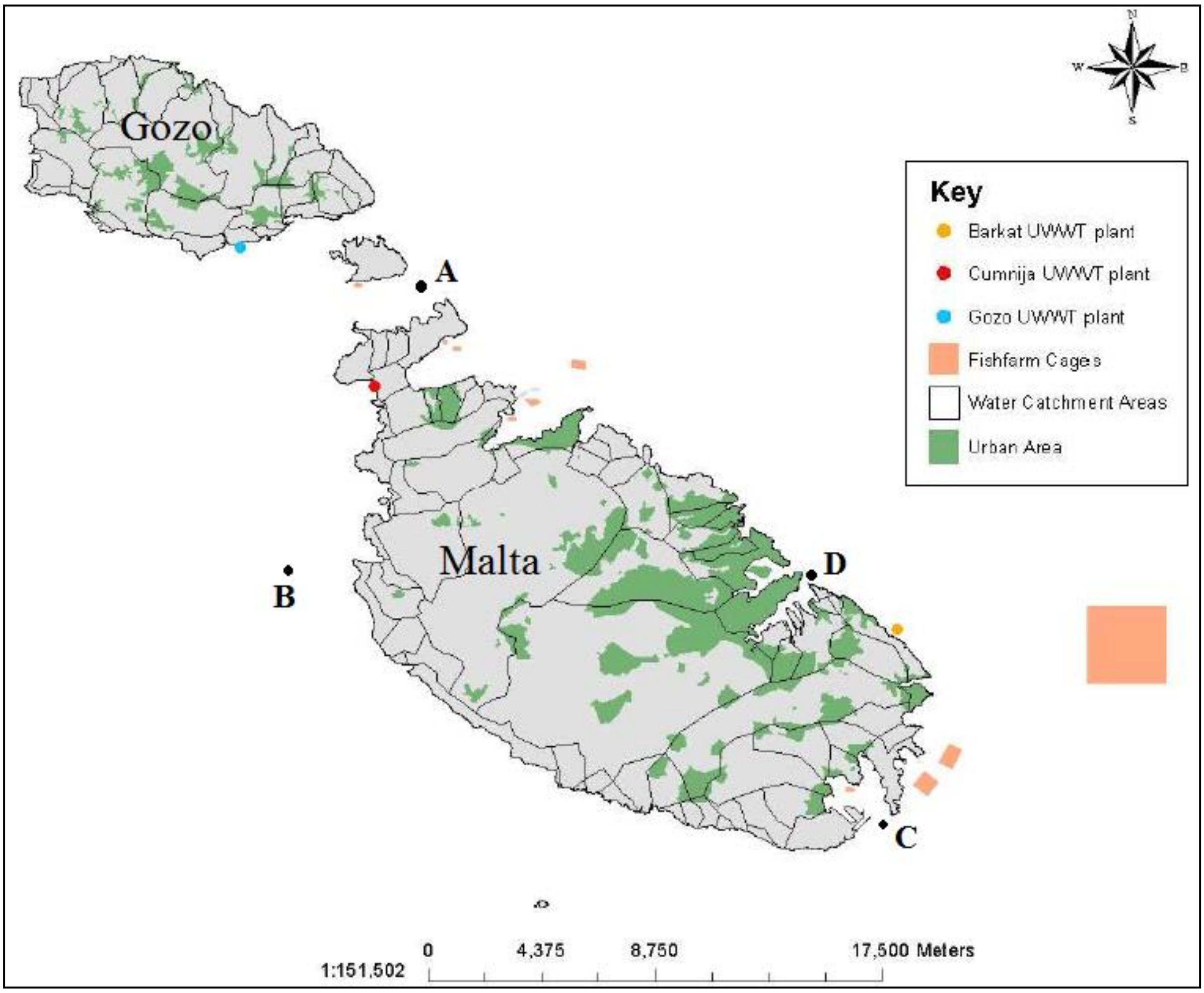


Figure 2.3.4.1.3: *Showing the location of various anthropogenic activity in the Maltese Islands*

Given that the Maltese Islands are characterised by a dominant north-westerly wind, it is possible that effluents from Iċ-Ċumnija and Ras il-Ħobż (Gozo) could be contributing to the elevated level of variability at Site A and B. There is a large distance between the sewage outfalls and the sampling sites A and B. Therefore, this might not be an accurate assessment of this observation, however it is still a possibility, especially when considering the variable pattern observed in figure 2.3.4.1.1.

A study carried out by Axiak *et al.* (2000) at Wied Ghammieq sewage outfall (located on the coast 1km south of Site D), prior to the construction of the Ta' Barkat wastewater treatment plant, showed that 85% of Malta's raw sewage was pumped into the marine environment via a 716m submarine pipe, with a thermal diffuser at a depth of 36 meters. The study showed that the pipes' impacts extended as far as 8km south east of the outfall. This is a very large stretch of coast, especially for an island as small as Malta. The impact of Ras il-Ħobż and Iċ-Ċumnija water treatment plants should be considerably less than that of raw sewage effluent; however it could possibly bring about an elevated nitrate concentrations at Site A and B.

Even if the modifying influence of the Ras il-Ħobż and Iċ-Ċumnija waste water treatment plants is able to alter nutrient concentrations at Site A and B, the exposure to effluents derived from anthropogenic sources such as Ta' Barkat and the fish farm cages found in the south of the island should produce a stronger modifying signature on nutrient concentrations at Site C and D.

Other possible reasons for such variability at Sites A and B could possibly be attributed to rainfall which would lead to runoff, resulting in elevated level of nutrients entering the water. However, the correlation matrix appendix 3 showed that average monthly rainfall had a significantly inverse correlation at the 95% confidence interval, with nitrate (NO_3) (r^2= -0.199, n= 75), thus ruling out this hypothesis. This is depicted in Figure 2.3.4.1.1.

The variable pattern exhibited by nitrate (NO_3) could not be attributed or explained by any particular phenomenon. Further studies having more samples collected at shorter intervals than the present study are required to shed light on the relationship of these two parameters.

2.3.4.2 Silicates

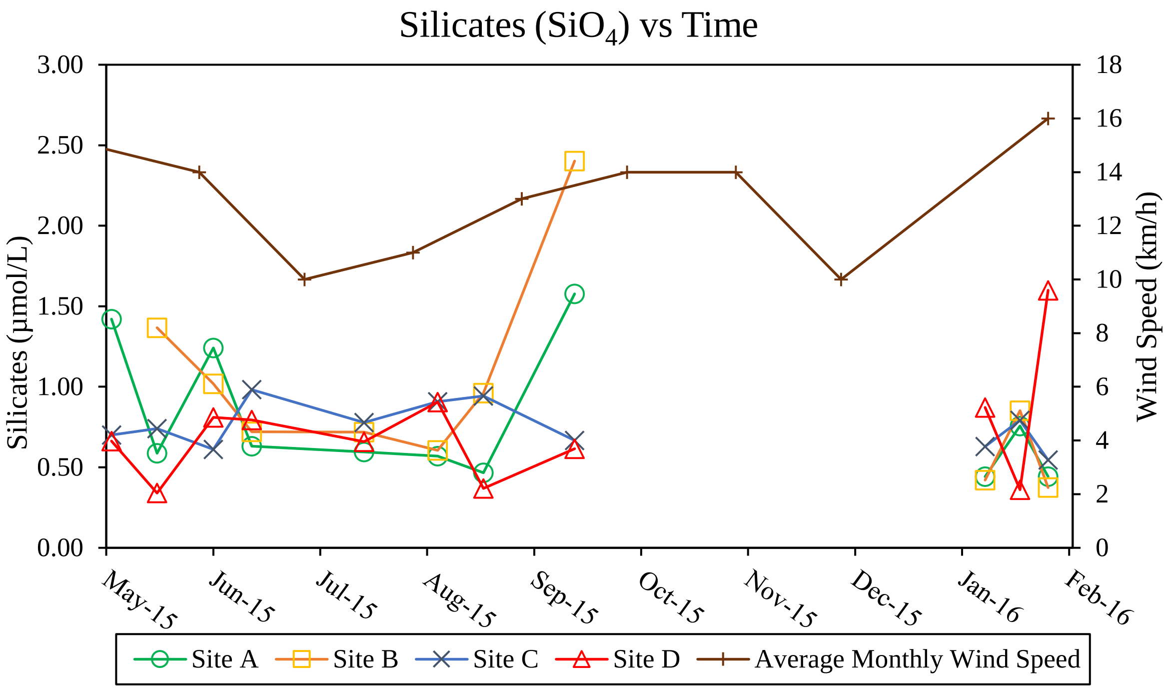


Figure 2.3.4.2.1: Showing the concentration of silicates (SiO_4) in Maltese waters and wind speed over a series of time

Figure 2.3.4.2.1 shows the seasonal variation of silicates in Maltese waters. The concentrations are in the range of 0.3 µmol/L and 1.5 µmol/L. There is a relatively small degree of variation from one sampling session to another, apart from Site A and B showing above average silicate concentrations in the samples collected on the 16th of September. The reason for this increase in concentration could be associated with increased wind speeds during September or an increased level of nutrients from coastal sources having entered the water. A correlation matrix was created in order to assess the correlations between all the parameters analysed in this study (Appendix 3). This showed that there was a relatively strong positive correlation between silicates and average monthly wind speed at the 95% confidence interval (r^2= 0.303, n= 75). Figure 2.3.4.2.1 also shows the seasonal variation of the average monthly wind speed in order to provide a visual representation of the correlation between the two variables. These monthly mean wind speed measurements indicated that wind speed is highest in spring with an average wind speed of 5.5 km/h during this season. These are followed by winter measurements which have a wind speed of 5.1 km/h and then summer which has a low of 4.5km/h.

Shanthi *et al.* (2014) stated that silicon may be present in sea water in suspension or found in particles of sand or clay. Falkowski, Barber & Smetacek (1998) reported that silicon availability is very dependent on freshwater runoff in coastal regions. The most likely explanation for the above observation is that the arrival of autumn brings about greater meteorological forcing and vorticity in the water column resulting in the stirring up of bottom sediments, thus releasing silicates that would have been contained on the seabed throughout spring and summer. These would then be transferred to surface waters, where there is enough light for phytoplankton to graze and grow resulting in an increase in the level of biomass.

2.3.4.3 Phosphates (PO_4) vs Time

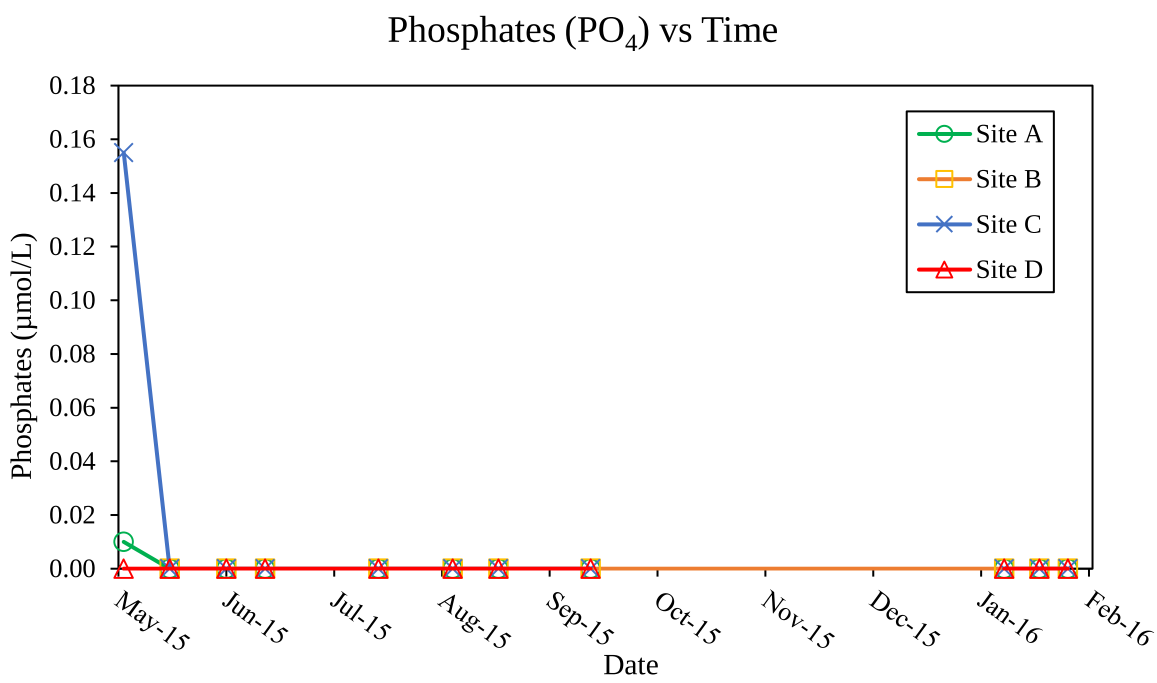


Figure 2.3.4.3.1: *Showing the phosphate (PO_4) concentration in Maltese waters over a series of time.*

Figure 2.3.4.3.1 shows the time series of phosphate concentrations in the samples collected for this study. These indicate that nearly all the samples were below the limit of detection for all the sites analysed. Such low concentrations were not expected. Agius & Jaccarini (1982) found that both nitrates and phosphates were very low in concentration in Maltese waters. Redfield (1958), and Frangou *et al.* (2010) stated that in many oceans around the world, nitrogen was the limiting factor to phytoplankton growth; however in the Mediterranean Sea phosphorus was the limiting factor. Agius & Jaccarini (1982) found this to be true for Maltese waters, specifically in Marsaxlokk Bay. The results of this study confirm that there are very low concentrations of phosphates in Maltese coastal waters.

2.3.4.4 Ammonium (NH3)

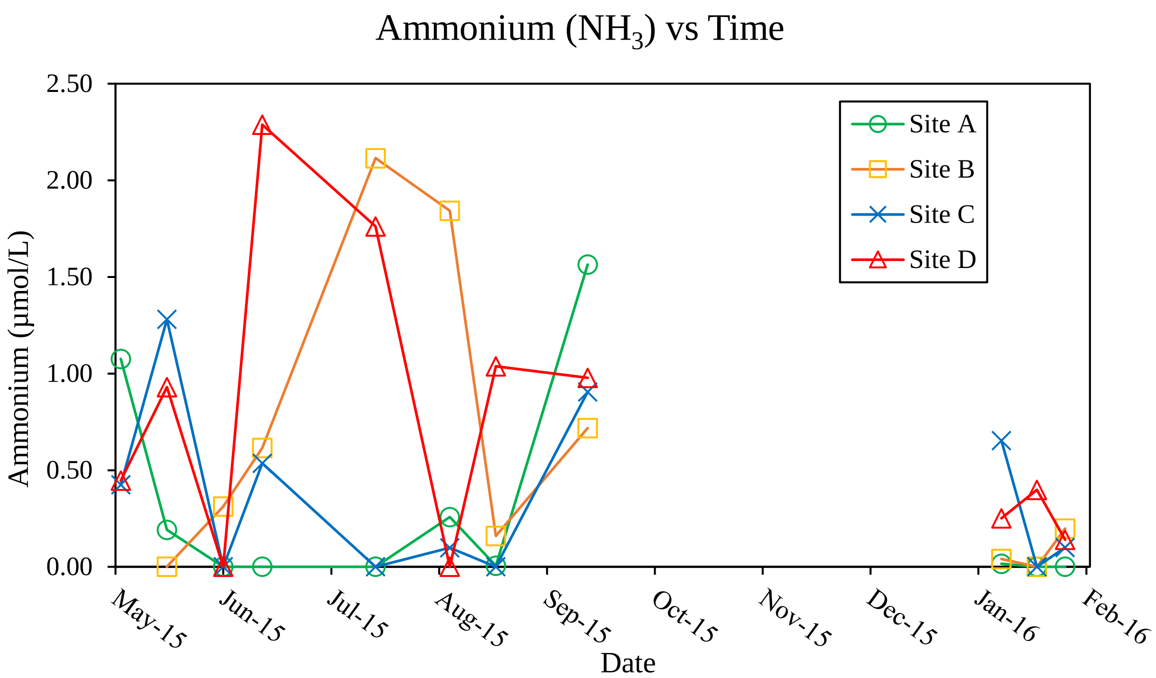


Figure 2.3.4.4.1: *Showing the concentration of ammonium (NH_3) in Maltese waters over a series of time*

The results of ammonium concentration over time are shown in Figure 2.3.4.4.1. The concentrations vary from one site to another and from one sampling date to another. There does not appear to be any clear pattern in the variation. During summer, the concentrations at Site B and D are higher than any other time of year, whilst Site A and C show opposite trends, with low concentrations for this time of year. Winter concentrations are lower than any other time of year. There are no significant correlations between ammonium and any of the other parameters measured that could provide an indication into the factors that might be driving the ammonium concentration in Maltese waters (Appendix 3).

2.3.4.5 Nitrite (NO_2)

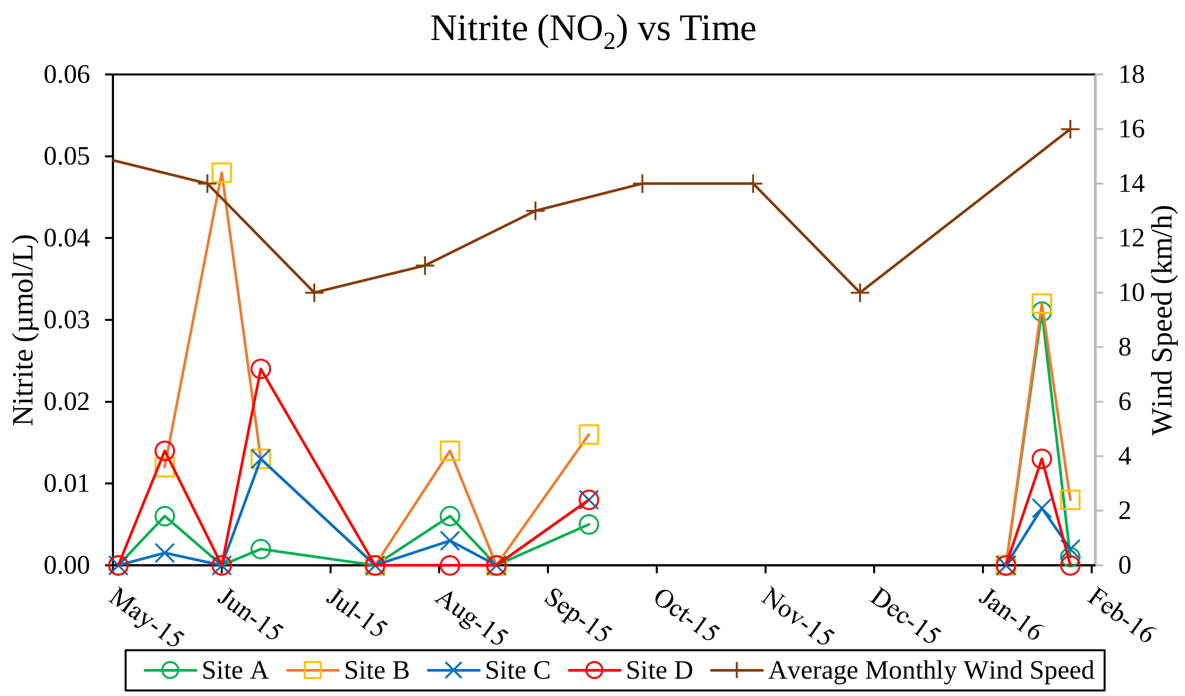


Figure 2.3.4.5.1: *Showing the nitrite (NO_2) concentration in Maltese waters and wind speed over a series of time*

The results obtained from measuring nitrite levels indicate that the concentrations in sea water are very low with some levels being under the limit of detection (Figure 2.3.4.5.1). The highest concentration was observed during May, at Site B (0.05 µmol/L). Nitrites at Site B were higher than the other sample sites throughout the entire study, followed by Site D. Site D and Site C were expected to have the highest level of nutrients in the water column due to the high level of anthropogenic activity occurring around these port areas. However, the results presented here do not show such a spatial pattern.

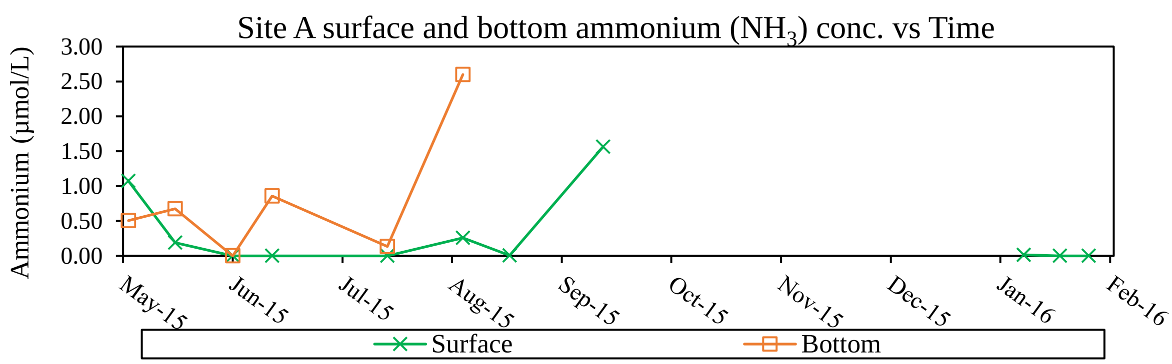
Site A follows a seasonal pattern similar to that of the chlorophyll results obtained by this study (Figure 2.3.6.1.2), with higher concentrations in the winter season; however this is the only site where nitrites can be described as having a diurnal oscillation in concentrations. The other three sites had a large variability in nitrite levels between one sampling session and another.

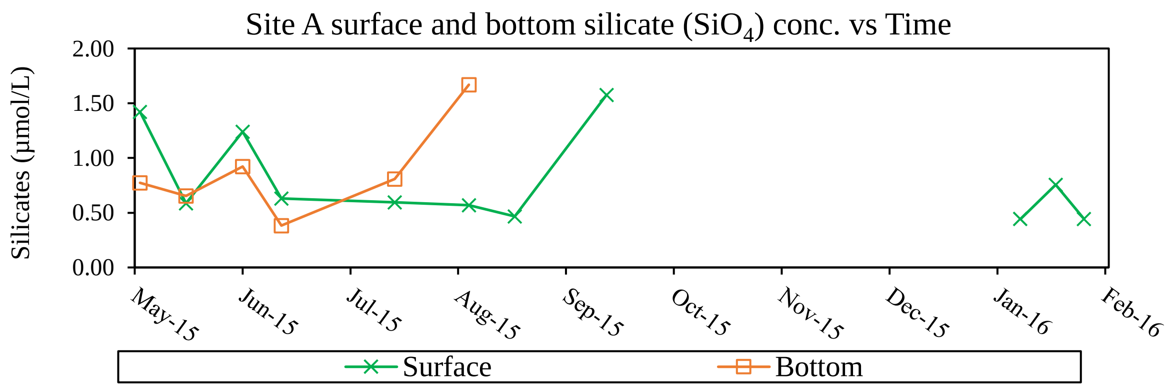
Nitrite levels were found to be significantly correlated with average monthly wind speed at the 95% confidence interval (r^2= 0.291, n=75) (Appendix 3). This indicates that wind could be an important parameter in controlling nitrite concentrations in Maltese coastal waters. Elevated wind speeds are most likely bringing about the stirring of the water column which will result in the mixing of surface waters with bottom waters, which contain higher levels of nutrients.

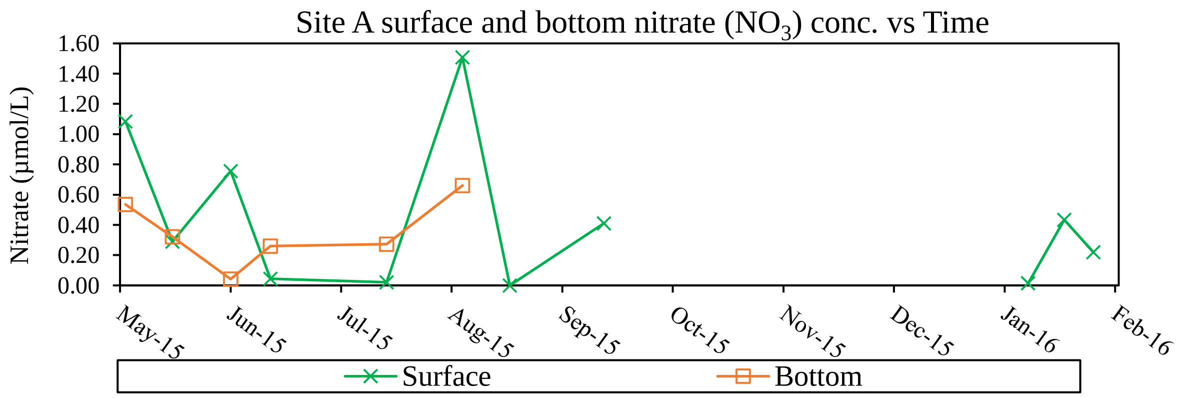
One can observe that elevated nitrite levels occurred at similar intervals across all the sampling sites, apart from 15th July at Site B. This provides further evidence to suggest that meteorological forcing, which due to Malta's small size is similar at each of the four sites, is controlling nitrite concentrations in Maltese waters.

2.3.5 Tempo-spatial variation of surface and bottom nutrient concentrations in Maltese waters

2.3.5.1 Site A nutrients







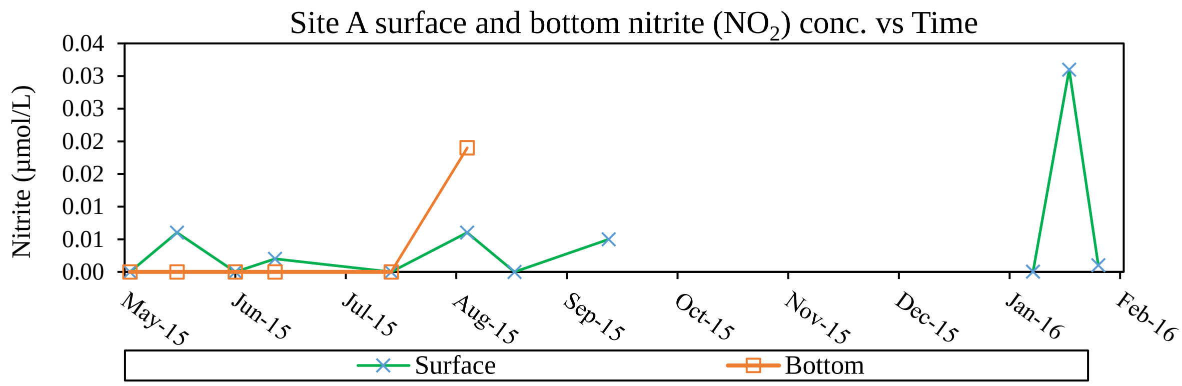
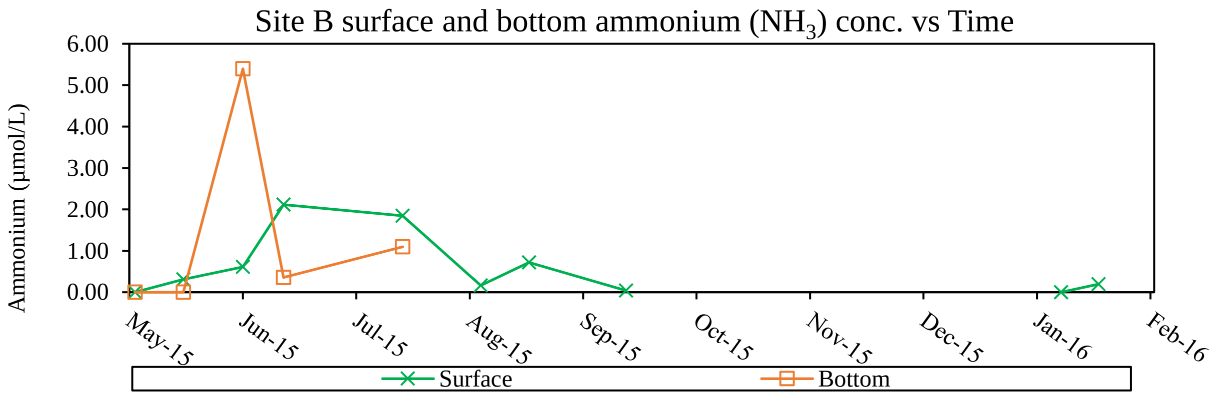
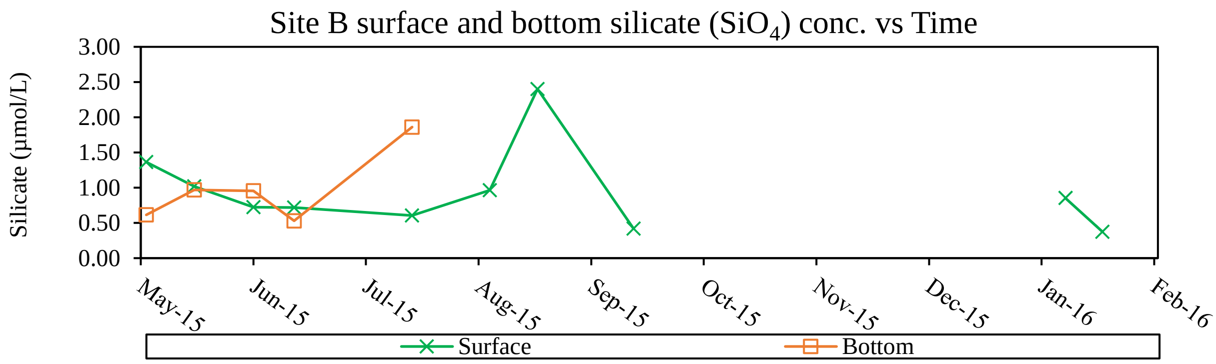
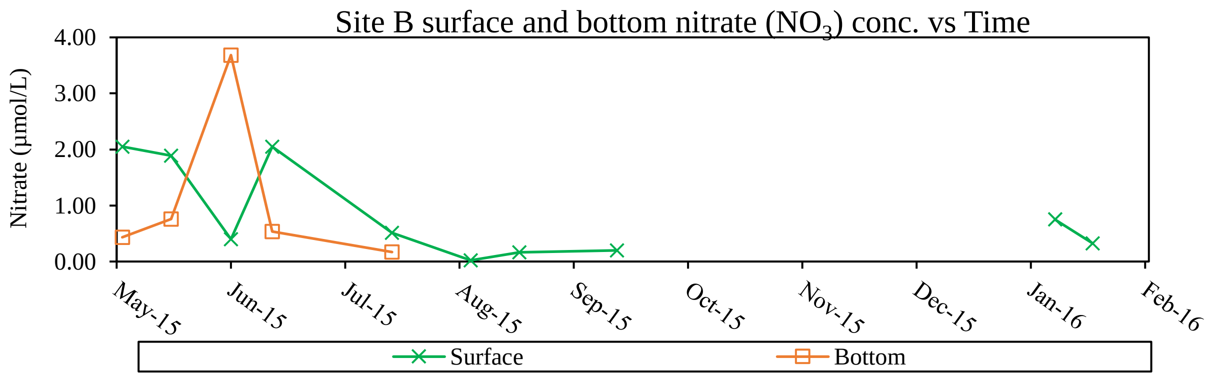


Figure 2.3.5.1.2: *showing the levels of ammonia, silicates, nitrates and nitrites over a time series in Maltese coastal water at Site A.*

2.3.5.2 Site B







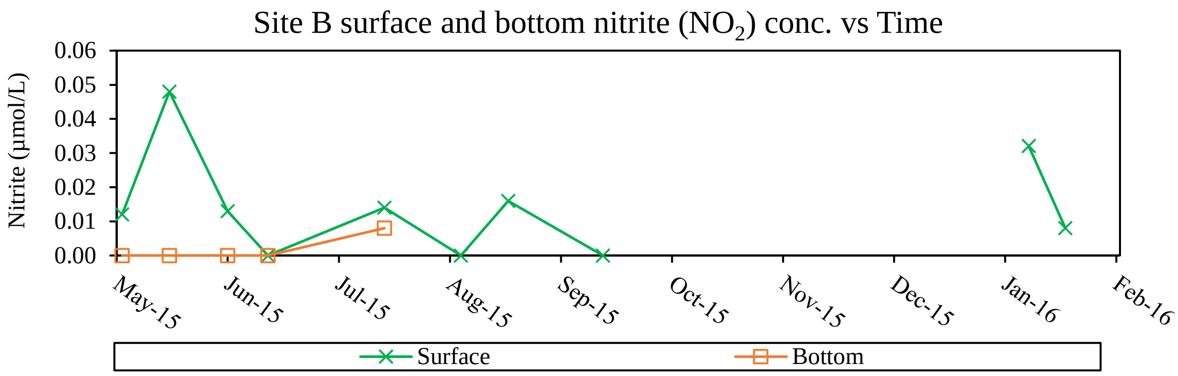
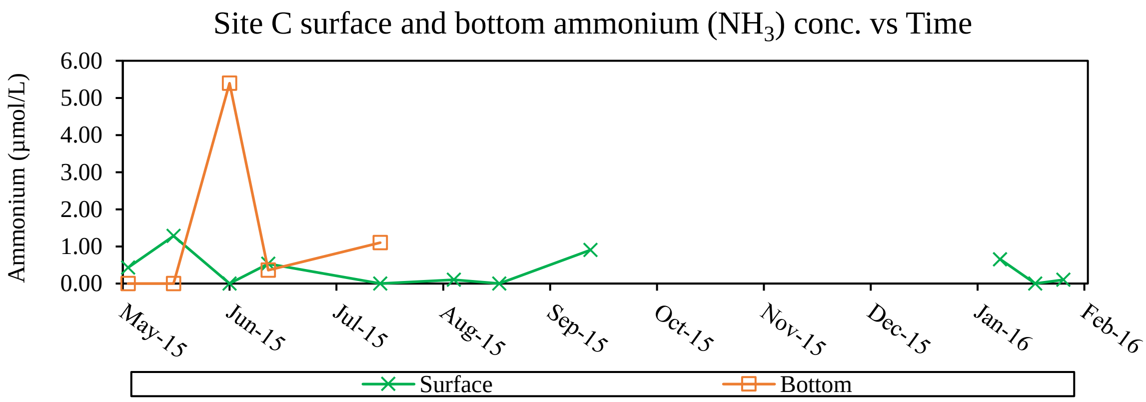
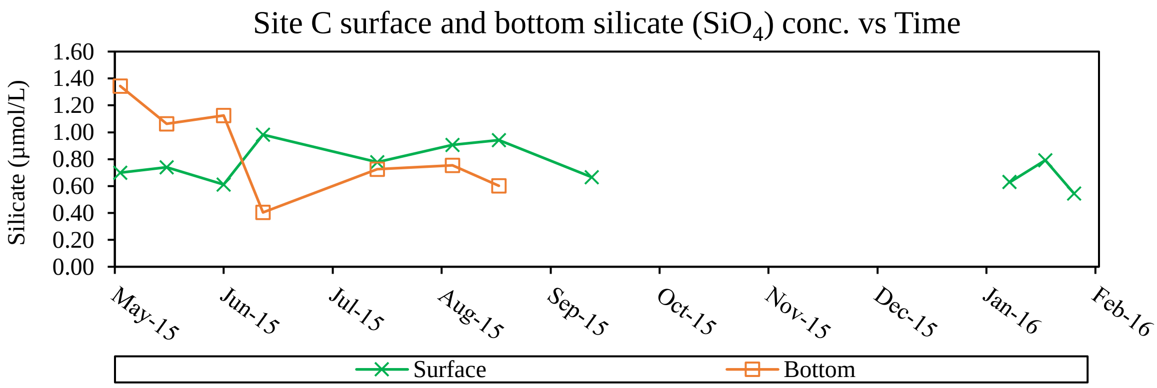
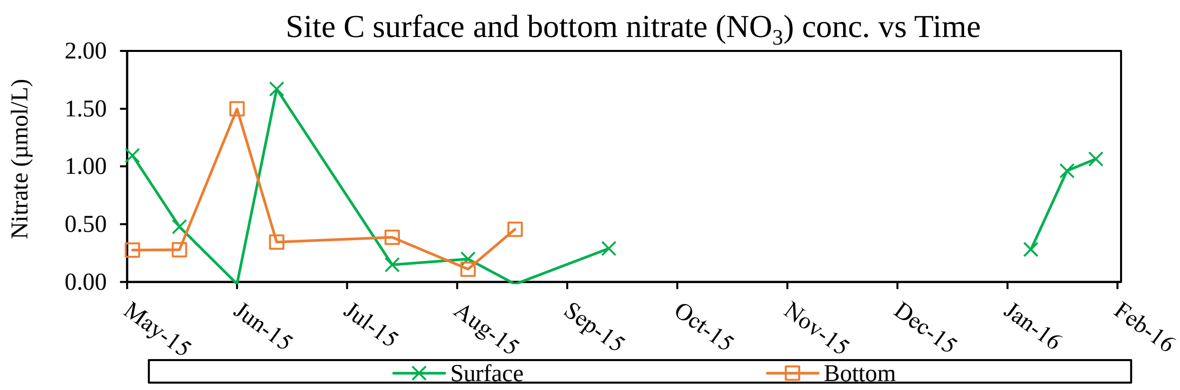


Figure 2.3.5.2.3: *showing the levels of ammonia, silicates, nitrates and nitrites over a time series in Maltese coastal water at Site B.*

2.3.5.3 Site C







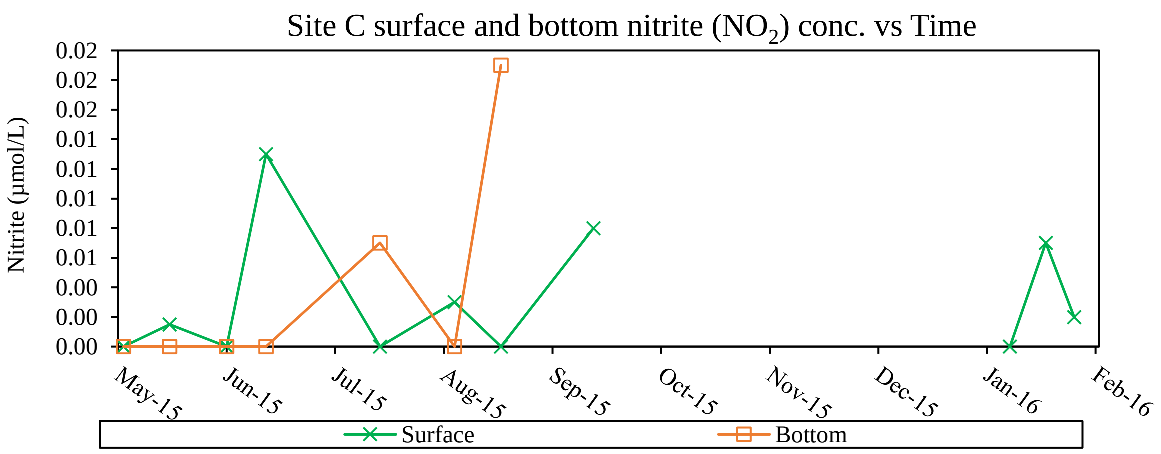
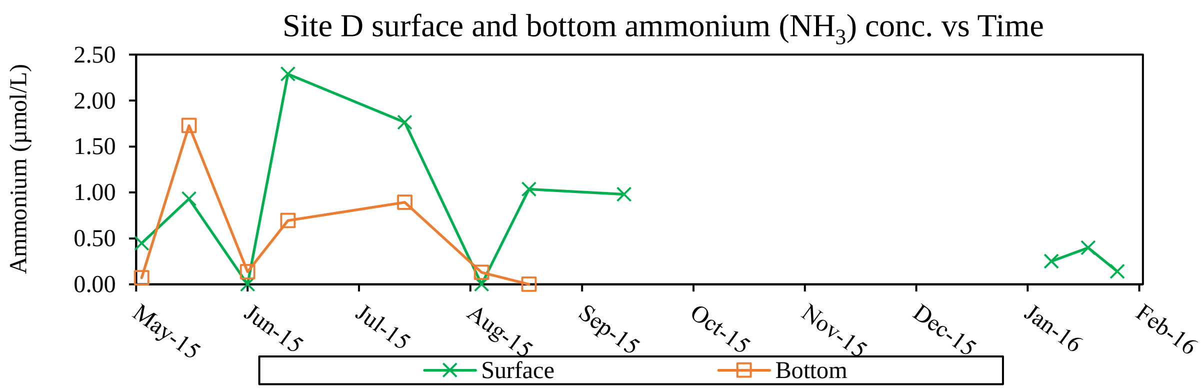


Figure 2.3.5.3.4: *showing the levels of ammonia, silicates, nitrates and nitrites over a time series in Maltese coastal water at Site C.*

2.3.5.4 Site D



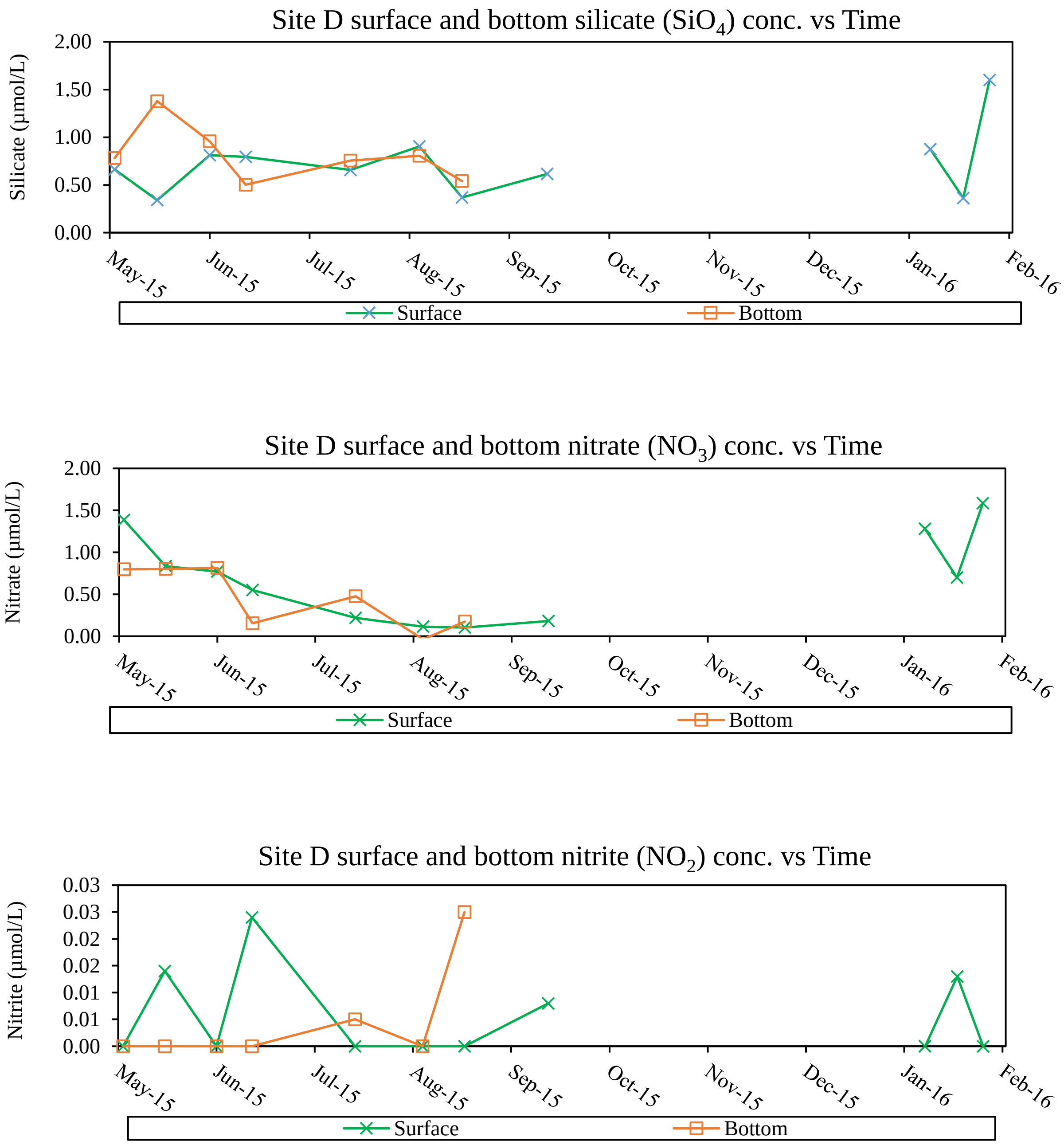


Figure 2.3.5.4.5: *showing the levels of ammonia, silicates, nitrates and nitrites over a time series in Maltese coastal water at Site D.*

2.3.5.5 Emerging patterns in surface and bottom nutrients of Maltese waters

The pattern of change in the nutrient levels (ammonium, silicates, nitrates and nitrites) in all the study sites is generally similar in surface water and bottom water. The level of nitrite is very low in all the sites over the entire study period. Site B, C and D show a similar pattern in the level of nutrients in the water column. There are higher levels of each of the nutrients measured between May and June than between June and September. There are some exceptions to this such as silicates at site B, nitrates and nitrites at site C and ammonium and nitrites at site D. At site A all the nutrient measured have higher levels between June and September than between May and June. These patterns could not be correlated to any events or phenomenon in Maltese coastal waters.

2.3.6 Tempo-spatial variation of chlorophyll concentrations in Maltese waters

The descriptive statistics regarding the surface chlorophyll concentrations for the samples collected throughout the seasons of spring and summer 2015 and winter 2016 are summarised in table 2.3.6.1 (spatial variation of chlorophyll in Maltese waters), and 2.3.6.2 (temporal variation of chlorophyll in Maltese waters). A visual representation of this is provided in Figure 2.3.6.2.

Report

Chlorophyll

Site	Mean	N	Std. Deviation	Minimum	Maximum	Std. Error of Mean
A	.062	19	.0344	.02	.15	.0078
B	.046	17	.0306	.01	.12	.0074
C	.098	19	.0351	.04	.17	.0080
D	.155	20	.0989	.03	.34	.0221

Table 2.3.6.1: *Showing the descriptive statistics of surface chlorophyll (mg m^{-3}) collected throughout spring 2015, summer 2015 and winter 2016 at Site A, B, C and D, found in Maltese waters*

Site D has the highest mean chlorophyll concentration (0.156 mg m^{-3}). Site D also has the highest maximum chlorophyll concentration (0.34 mg m^{-3}) and the greatest standard deviation in concentration (0.099 mg m^{-3}). Site B has the lowest chlorophyll concentration (0.0465 mg m^{-3}) along with the lowest minimum value (0.01 mg m^{-3}) and the lowest standard deviation (0.0306 mg m^{-3}). These values are within the same range as the remotely sensed values obtained and described in the next chapter, and similar to the ranges described by other studies carried out in Maltese waters (Table 1.4.1).

One study by Azzopardi *et al*. (2013) analysed the seasonal and spatial variability of ocean colour chlorophyll values from 9 different coastal water sites. Images were analysed monthly from 2003 to 2011 using values originating from MODIS, MERIS and SeaWiFS sensors. It was observed that two of the sites in this study (MTC 107 and 108) had elevated ocean colour satellite values during the years of 2010 and 2011, compared to the other 8 sites. These two sites were located in a similar position to Site C in the present study, located just outside Marsaxlokk Bay.

On occasions Marsaxlokk Bay was seen to display eutrophic conditions. On 9th December 1975 Marsaxlokk bay was seen to exhibit a chlorophyll concentration of 1.23 mg m^{-3} (Jaccarini, Agius & Leger, 1978). These values compare with some of the most productive waters throughout the world, such as the waters around Great Britain or those of the Peru coastal current (Guillen, de Mendiola, & de Rondan, 1973).

Another study by Jaccarini, Agius & Leger (1978) focused on setting up the culture of oyster *Crassostrea italies* and *Ostrea edulis* species in Maltese waters. The study sites used were Marsaxlokk Bay, Mistra Bay and Rinella, which are found towards the south and the east of the Islands, close to where sample sites C and D are located in the present study. Results from *in situ* analysis of chlorophyll indicated that Rinella creek, which is found inside the Grand Harbour, close to site D, exhibited concentrations which ranged between 0.18 - 1.47 mg m^{-3}. This site was said to be subject to a high degree of organic effluents and displayed values above the norm for most Maltese and Mediterranean waters. Jaccarini, Agius & Leger (1978) also observed that the mean chlorophyll value at Rinella Creek was six times higher than that of Marsaxlokk Bay and Mistra Bay.

Azzopardi *et al* (2013) attributed elevated chlorophyll values at Site C to the intensification of Bluefin tuna (*Tunnus thynnus*) farming in the year prior to the study taking place. The National Statistics Office (NSO) of Malta reported that gilt-head seabream (*Sparus aurata*) and European seabass (*Dicentrarchus labrax*) production decreased in 2010, whilst Atlantic Bluefin tuna obtained through capture-based aquaculture increased substantially. When Bluefin tuna penning began in Malta in 2000 the annual production was 300 tonnes. By 2003 two farms produced 3550 tonnes of tuna and by 2010 this increased to around 5000 tonnes.

Azzopardi also mentioned the possible influence of the sewage outflow at Ta' Barkat sewage outflow, which is found close to Site D. In 2010 the site was processing 80% all of the sewage generated in Malta. Despite most of this waste being treated to remove harmful materials, there are still sufficient nutrients left in the treated effluent to bring about elevated levels of chlorophyll, as observed in this study.

No past studies could be found where samples had been obtained close to Site A and B of the present study for comparison of past values with the ones obtained by this study. It can be observed that these sites have lower chlorophyll concentrations compared to Sites C and D. As described in section 2.2.1, these sites are further from the coast and have a smaller degree of anthropogenic activity than sites C and D, which are both located at the entrance of bays.

Report

Chlorophyll

Season	Mean	N	Std. Deviation	Minimum	Maximum	Std. Error of Mean
Spring	.073	26	.062	.01	.31	.012
Summer	.089	29	.070	.03	.32	.013
Winter	.123	20	.077	.05	.34	.017

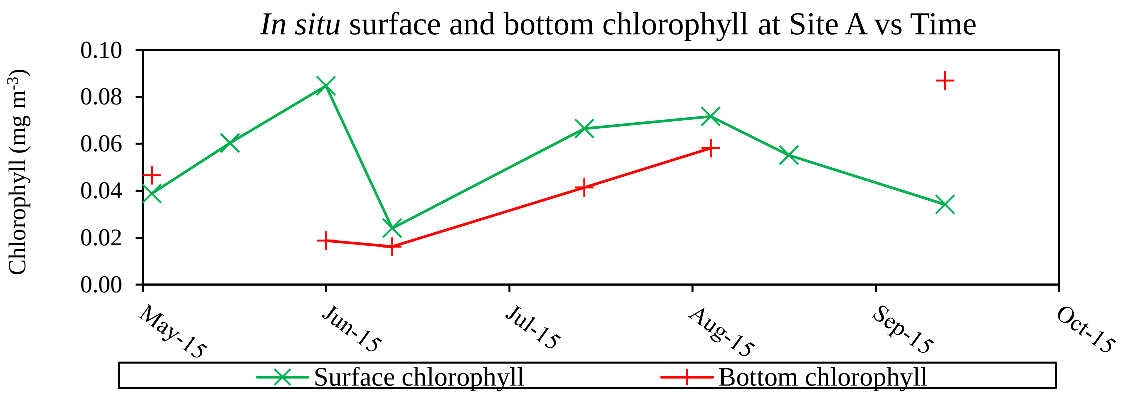
Table 2.3.6.2: *Showing the descriptive statistics of chlorophyll (mg m^{-3}) collected throughout Maltese waters for the seasons of spring 2015, summer 2015 and winter 2016*

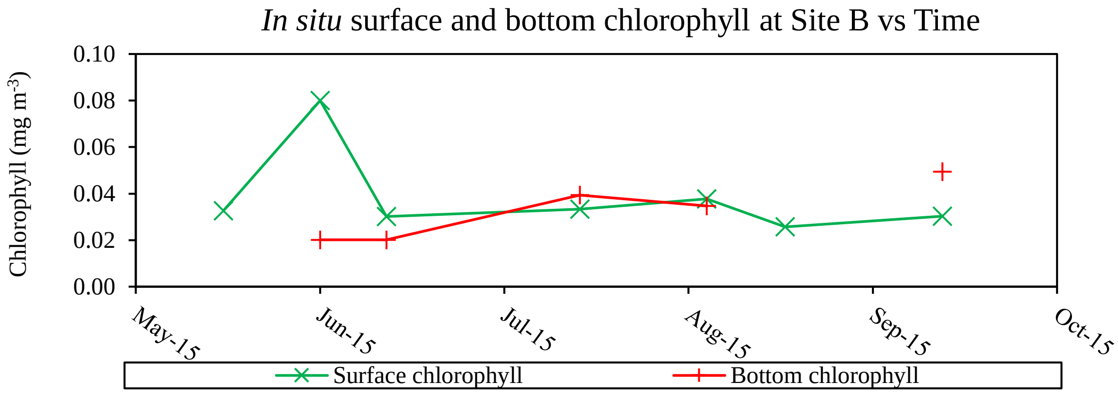
Table 2.3.6.2 shows that winter 2016 had the highest chlorophyll concentration (0.124 mg m^{-3}). It also had the highest maximum chlorophyll concentration (0.34 mg m^{-3}) and the highest standard deviation (0.077 mg m^{-3}). Spring had the lowest mean chlorophyll concentrations (0.073 mg m^{-3}), the lowest minimum value (0.01 mg m^{-3}) and the lowest standard deviation (0.063 mg m^{-3}) of all the three seasons.

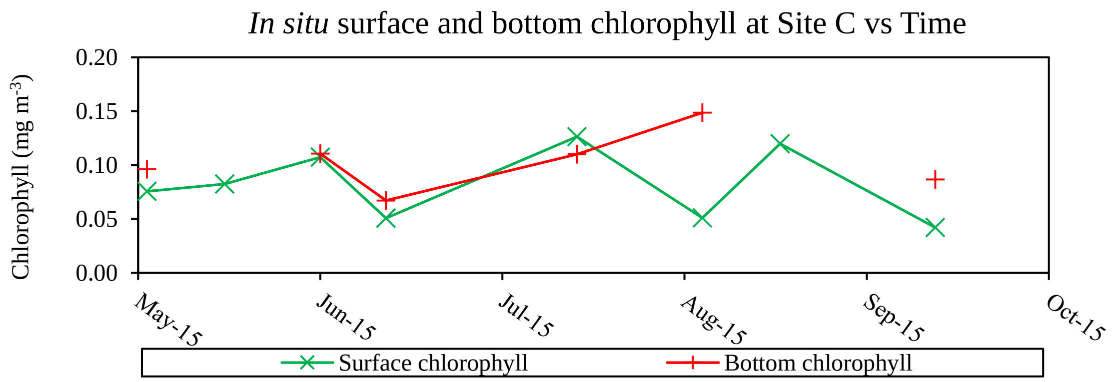
Previous studies demonstrate a similar seasonal pattern in chlorophyll concentration. Deidun *et al.* (2011) recorded the highest values on January 17th 2010 (1.36 mgm^{-3}). April and May were the months with the lowest chlorophyll concentrations (0.04 mgm^{-3}). Many areas throughout the Mediterranean Sea follow this type of seasonal pattern.

Azzopardi *et al.* (2013) analysed the seasonal and spatial variability of ocean colour chlorophyll values from 9 different coastal sites. Monthly images were analysed from 2003 to 2011 using values originating from MODIS, MERIS and SeaWiFS sensors. The seasonal pattern of chlorophyll variability was seen to be fairly homogeneous, with the greatest variation observed during December and February.

Agius, Jaccarini & Ritz (1978) found three chlorophyll peaks occurred in Rinella Bay, which is found close to Site D. One of these took place in January 1957 and another in February 1976. The other peak was observed in May 1976. Summer values were very low throughout the study. The results of the present study concur with the observations made by previous studies of Maltese waters.







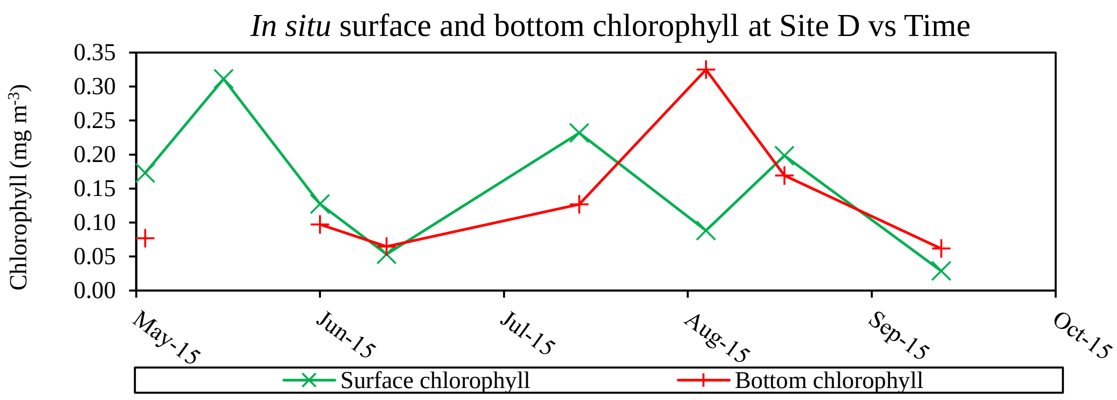


Figure 2.3.6.1: *Showing a time series of the variation of in situ surface and bottom chlorophyll in Maltese waters*

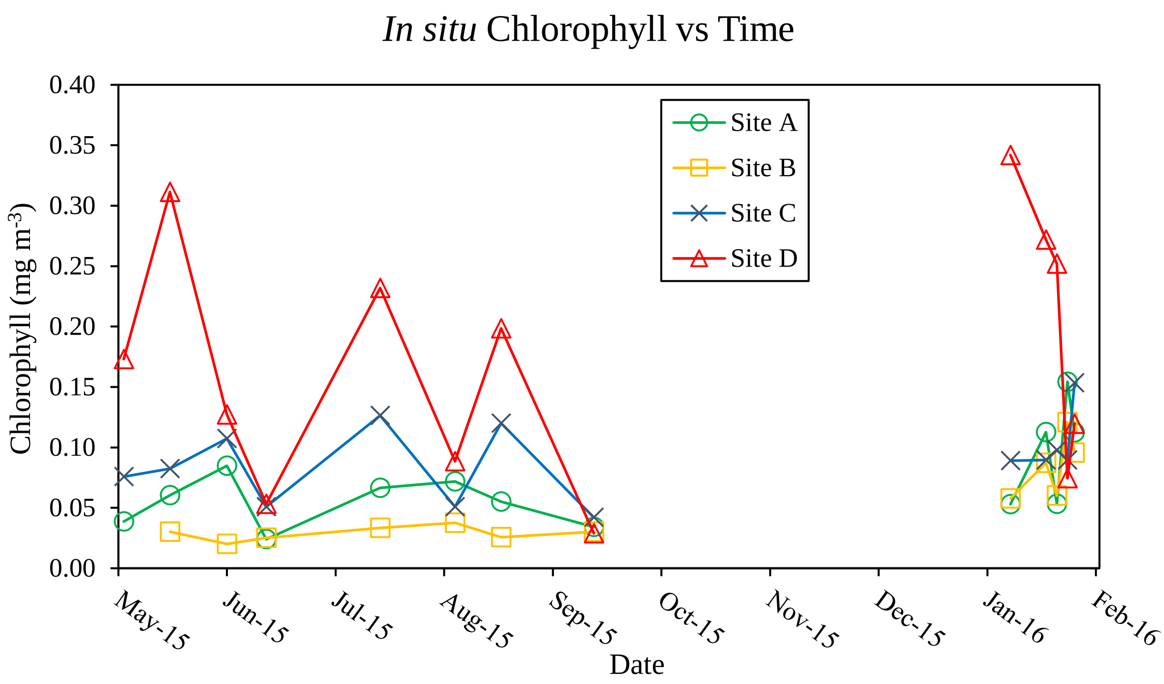


Figure 2.3.6.2: *Showing a time series of the variation of in situ surface chlorophyll found in Maltese waters over time*

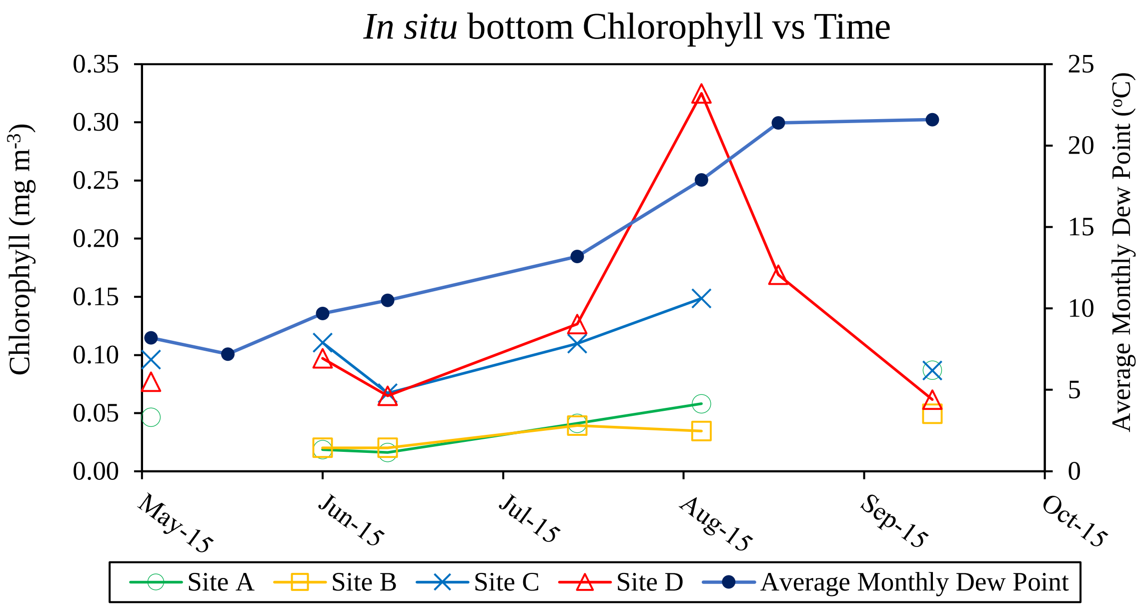


Figure 2.3.6.3: *Showing a time series of the variation of in situ bottom chlorophyll found in Maltese waters over time*

Figure 2.3.6.2 shows a seasonal pattern of chlorophyll concentration with time. There is a great deal of variation at Site D and much less variation at Site B, as is described in Table 2.3.6.1. The seasonal variation for each of the sites shows an overall increased level in concentrations during the winter period. This observation is confirmed by Table 2.3.6.2 where the mean winter chlorophyll concentration of all the sites combined is seen to be higher than that of the other two seasons analysed by this study.

This observation was also made by Azzopardi *et al.* (2013) who reported a significant variation in seasonal pattern in various sites around the Maltese coast. His results from an 8 year dataset obtained from the MODIS platform also indicated that there was little inter annual variability in the seasonal pattern. This is explored in more detail and described in the next chapter.

By utilising the chlorophyll concentrations obtained by this study, Rinaldi's 2014 theories regarding reduced seasonal variation in coastal regions can be tested (table 2.3.1.2 and figure 2.3.6.1). It can be seen that the coastal sites of this study, Site C and D, had a higher degree of variability from one sampling session to another and a smaller degree of seasonal variability compared to Site A and B. From figure 2.3.6.1, it can be seen that Site A and B exhibit more seasonal variability with higher concentrations in winter.

These observations complement Rinaldi's theory, in that seasonal variation is homogenised at coastal sites and there is a greater degree of variability from one sampling session to another and from one site to another, as opposed to the seasonal variation observed at the open coastal water site at Site B and channel site A. This study hypothesises that this day to day variability experienced by coastal sites is primarily controlled by coastal influences derived from effluents resulting primarily from human activities, along with meteorological forcing by the wind. This theory is further supported by the fact that there is a high degree of industrial activity in Marsaxlokk Bay and the Grand Harbour, which is where site C and D are found.

The doubling time of phytoplankton is around 2 to 4 days (Grahame, 1997). This suggests that a period of strong winds, followed by a period of calm conditions could result in a slow rate of water exchange in and out of the bays, thus providing stable conditions and increased phytoplankton growth. Phytoplankton growth in Maltese waters is not limited by the lack of light needed for photosynthesis during the winter period since the Maltese Islands have over 300 days of sunshine (NOAA, 2016). There is an abundance of light for photosynthesis to occur throughout the year. In shallow waters, such as Site C and D, the amount of energy needed to mix the water column is relatively low, when compared to deeper water bodies. This means that nutrients from the seafloor and bottom waters can frequently be brought to the photic zone, resulting in phytoplankton blooms throughout the year. In deep water sites, such as Site B, the energy required for mixing the entire water column and bringing bottom nutrients to the surface is much greater. This could be a possible reason for an overall lower concentration of phytoplankton at Site A and B and the reason for seasonal blooms in winter when water column mixing is greatest.

This was further analysed by separating the data according to site and then analysing the spatial and temporal patterns of all the physio-chemical parameters measured in this study in order to determine if there was any correlation between these parameters and chlorophyll concentration. Results indicated that Site A and B had a greater number of physio-chemical parameters having a significant correlation with chlorophyll than Site C and D. Site A and B had significant correlations at the 99% confidence interval with mixed layer depth, average monthly rainfall and average monthly dew point (not at site A). Secchi Depth was the only parameter that had a significantly correlation with chlorophyll at Site D (r^2= 0.653, n=20). There was no significant correlation between any of the parameters and chlorophyll at Site C.

This observation is most likely related to the fact that there are many different anthropogenic and natural influencing physio-chemical parameters effecting shallow coastal waters (Site C and D). These parameters have a variable pattern, due to human activities, as can be seen in the following sections of this chapter, thus producing a highly variable chlorophyll pattern in coastal waters. This provides further support for Rinaldi's theory on the homogenisation of seasonal variation at coastal sites and confirms part of the stated hypothesis in chapter 1.7 which indicates that the seasonal pattern of coastal sites is strongly influenced by human activities.

On the other hand, results indicate that chlorophyll concentrations at open coastal water sites have a similar seasonal pattern to physical parameters primarily influenced by meteorological forcing. This is reflected in mixed layer depth results found in section 2.3.4 below. Hence these sites will be dependent on weather systems, resulting in a diurnal oscillation in seasonal pattern, which is reflected in chlorophyll concentrations.

The variation of chlorophyll concentrations over time show a similar pattern in surface water and bottom water with site C and site D having higher levels of chlorophyll than site A and site B. A difference is observed between surface and bottom chlorophyll concentration in August at site D. In surface water the level of chlorophyll is lower in August than the level observed in July. In bottom water the level of chlorophyll is much higher in August than it is in July. This could be associated with the change in temperature and average monthly dew point in the bottom waters at site D (Figure 2.3.6.3).

The temperature in bottom water in July was 22^{o}C at all four study sites. The rate of temperature rise in bottom water at site D is much faster and reaches a higher temperature than the other sites, reaching a temperature of 27^{o}C by July and 29^{o}C by August. The other sites reached a temperature of 23^{o}C by July and an average of 27^{o}C by August. This is the only site and time period where there is such an observation. It does not correlate with all the other observations made in relation to temperature, dew point and chlorophyll concentration.

The results from phytoplankton identification experiments showed that a variety of phytoplankton species are present in Maltese coastal waters. The main species include diatoms, dinoflagellates, and coccolithophores. There was no overall predominant species present or any significant variation in the distribution of species between the different sites or the different seasons analysed in this study. In saying this, it must be noted that not all the samples collected were analysed. Therefore this part of the analysis is not based on a representative sample of the entire sampling period of this study. A conclusion could be drawn suggesting that the distribution of phytoplankton species in Maltese coastal waters is fairly constant in its community structure through the three seasons and four sites analysed by this study.

2.3.7 Analysis of the relationship between chlorophyll concentration and physiochemical parameters using multiple regression.

Chlorophyll concentration is a key ecological variable for setting the pace of life in high-latitude and temperate seas (Ferriera *et al*., 2015). Phytoplankton concentration is described to increase whenever growth rate exceeds loss rate (Sverdrup, 1953). When growth rate of phytoplankton is very high, phytoplankton blooms occur. As previously described there are many parameters that govern the growth of phytoplankton. There have been no studies prior to this that have tried to identify drivers of chlorophyll dynamics in Maltese waters. These results will provide new insights into which parameters drive the local ecosystem.

An analysis of the relationship between chlorophyll concentration, the dependant variable, and the physiochemical parameters measured, the independent variables, was carried out. Multiple regression was utilised for this analysis, in order to obtain a stronger statistical representation other than Pearson's correlation, and to determine which of the physiochemical parameters measured have the strongest relationship with chlorophyll concentration. This is based on correlation analysis, and allows for a more extensive analysis of the interrelationship among the sets of variables. This is a valuable method of analysis for complex real-life situations. Multiple regression analysis will determine how the independent variables, measured in this study, are correlated with chlorophyll concentrations. This can provide information on which variables influence chlorophyll concentration and can be used as predictors of the chlorophyll concentration.

A stepwise model was used for this analysis. The stepwise method is based on the p-value of F (probability of (ratio of two mean square values)). SPSS starts by entering the variable with the smallest p-value. The variables (from the list of variables not yet in the equation) with the smallest p-value for F are removed stepwise. Variables already in the equation are removed if their p-value becomes larger than the default limit due to the inclusion of another variable. The method terminates when no more variables are eligible for inclusion or removal. This method is based on both probability-to-enter (PIN) and probability-to-remove (POUT). The limits of the stepwise criteria for this study are based on a 0.05 level of significance.

A preliminary analysis was performed to ensure there was no violation of the assumption of normality, linearity, multicollinearity and homoscedasticity. The assumption of multicollinearity was found to have been violated. To rectify this, the variables temperature, salinity and average monthly rainfall were removed. Temperature was found to be significantly correlated with the variables dissolved oxygen, salinity and average monthly dewpoint above a bivariate correlation value of 0.7. Salinity was found to be correlated to temperature, and average monthly dew point above a bivariate correlation value of 0.7. Average monthly rainfall was found to be correlated to MLD above a bivariate correlation value of 0.7. This was taken into account when analysing the data.

Stepwise multiple regression was used to assess the ability of ten variables (pH, dissolved oxygen, Secchi Depth, suspended particulate matter, MLD, Average Monthly Wind Speed, Average Monthly Dew point, Nitrate and Nitrite (NO_x), Silicate (SiO_4) Phosphate (PO_4), Ammonium (NH_3), and Nitrite (NO_2)) to predict the chlorophyll concentration in Maltese waters based on the dataset ‘All Data’. This dataset contains all the data collected throughout all the field surveys.

Three steps were required to obtain a parsimonious model. This means that three variables were found to be significant predictors of chlorophyll concentrations. Step 3 of the analysis used Secchi Depth, average monthly wind speed and average monthly dewpoint in the regression equation. This was significantly correlated with chlorophyll concentration $F\ (3,71)= 12.64$, $p < 0.0001$. The multiple correlation coefficient was 0.59, suggesting that approximately 59% of the variance of the chlorophyll concentration could be accounted for by Secchi Depth, average monthly wind speed and average monthly dew point.

Thus, the regression equation for predicting chlorophyll concentrations in Maltese waters is:

$$\text{Chlorophyll conc.} = 0.519 - 0.07x_1 - 0.51x_2 - 0.004x_3 \qquad (2.8)$$

x_1= Secchi Depth; x_2=Average monthly wind speed; x_3= Average monthly dew point

The variable Secchi Depth, as indexed by its β value of -0.476, was shown to have the strongest relationship with chlorophyll concentrations. Average monthly wind speed came in a close second with a β value of -0.436 and average monthly dew point followed in third place, having a β value of -0.290.

A Pearson product-moment correlation coefficient was computed to assess the relationship between predicted chlorophyll concentrations *in situ* chlorophyll concentrations for Maltese waters. There was a significant positive correlation between the two variables, $r = 0.587$, $n = 75$, $p = 0.0001$. A scatterplot summarizes the results (Figure 2.3.7.1.1). There is a relatively strong, positive correlation between *in situ* and predicted chlorophyll concentrations in Maltese waters.

When looking at the results of this model one must keep in mind that *in situ* values from October to December were not collected. The lack of data during this period is most likely to have influenced results. If such data was collected, this model could have accounted for a greater extent of variance when predicting chlorophyll values in Maltese waters. Despite this the model accounts for a good 59% of the variance when predicting chlorophyll in Maltese waters.

Correlations

		Chlorophyll	Predicted Value
Predicted Value	Pearson Correlation	.587**	1
	Sig. (2-tailed)	.000	
	N	75	76

Table 3.2.7.6: *Showing the relationship between predicted chlorophyll va;ue and predicted chlorophyll values*

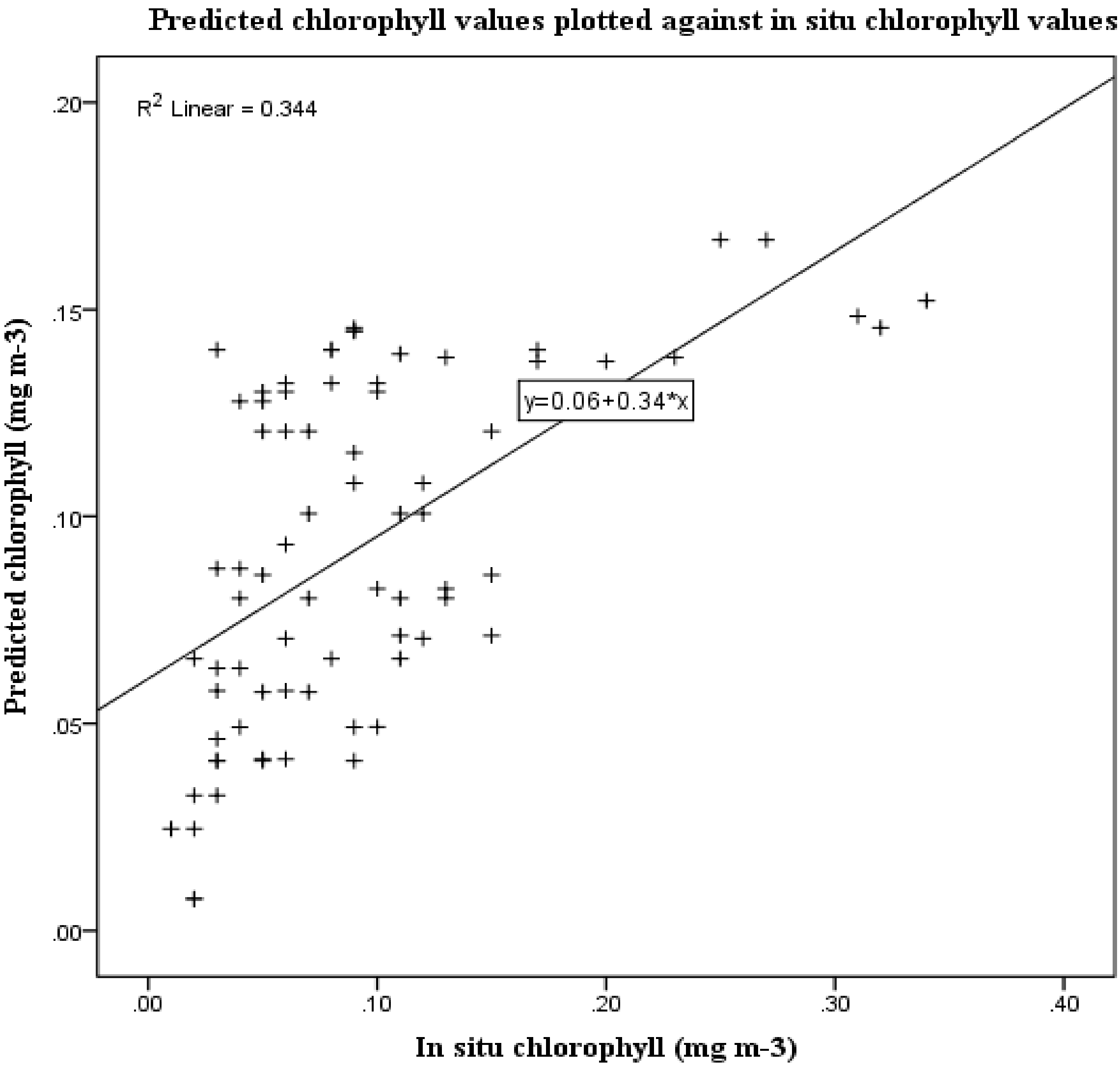


Figure 3.2.7.7: *Showing predicted chlorophyll values against in situ chlorophyll values*

From all the parameters inputted into this multiple linear regression analysis model, wind speed and temperature- two meteorological forcing parameters, along with Secchi Depth, had significant correlations with chlorophyll. This provides evidence on the importance of meteorological forcing in governing phytoplankton growth. This confirms the initial hypothesis of this study, despite the inverse correlation observed between wind speed and chlorophyll. This inverse correlation between the two parameters occurred due to spring 2015 having the highest mean wind speed (5.5 km/h) as opposed to what was predicted in the hypothesis of winter 2016 having an overall higher wind speed. 2016 registered an overall lower wind speed than that of spring (5.1 km/h).

Despite this result, the initial hypothesis of this study, which stated that Maltese waters are governed by metrological forcing parameters, can be confirmed. This hypothesis is reinforced by the results obtained from remotely sensed chlorophyll values' strong positive relationship with wind speed, which is explored later in this study (section 2.3.7).

A conclusion can be drawn, which states that meteorological forcing parameters such as wind speed and temperature are the primary drivers of the variation in chlorophyll concentration observed in this study. These parameters have all contributed to bringing about changes in the thermal stratification and nutrient exchange of the water column, which will in turn effect phytoplankton production.

Many parts of the Mediterranean are defined by a warm surface mixed layer with high light intensity, however depleted from nutrients. The sub-surface layer contains more nutrients, however is characterised by less light. A study by Lazzari *et al.* (2014) simulated the mixed layer depth throughout the Mediterranean Sea through the use of OGCM model. This showed the importance of the mixed layer depth in controlling primary producers both temporally and spatially in the Mediterranean Sea. This author goes on to quote D'Ortenzio *et al.* (2005)'s study as further evidence to this. Studies by Behrenfeld, 2010; d'Ortenzio and Ribera d'Alcalà, 2009; Casotti *et al.*, 2003 provide further evidence to this phenomenon. These studies along with the results of the present study indicate that that the steep increase in phytoplankton concentrations in the surface layer is in phase with the progressive deepening of the mixed layer. Despite the fact that the correlation between chlorophyll concentration and mixed layer depth observed in this study is not statistically significant, the overall seasonal pattern of the two is in phase (seen in Figure 2.3.6.2 and Figure 2.3.2.1 respectively).

In the current study nutrients did not have a significant correlation with chlorophyll. When taking into consideration the similarity of the seasonal pattern of all the parameters analysed in this study, it could be assumed that nutrients have a small influence on chlorophyll patterns and should not be considered as factors controlling phytoplankton growth in Maltese waters. However, based on previous literature, it is known that phytoplankton feed off nutrients found in the water column to sustain their growth. Mediterranean waters are known to have scarce nutrient stocks.

The results from the present study indicate that nutrient concentrations at all the sites analysed, varied drastically from one sampling session to another and their seasonal pattern did not match with that of chlorophyll. Studies have shown that nutrient concentrations can vary on timescales of hours to days, even more so at coastal sites (Romero *et al.*, 2013). Laboratory and field studies have shown that intermittent nutrient supplies can drastically change phytoplankton community structure and concentration (Roelke, Eldridge, & Cifuentes, 1999; Spatharis, Danielidis & Tsirtsis, 2007). The results of this study indicate that due to the high degree of variation of nutrients, there is no significant correlation between any of the nutrients analysed and chlorophyll concentrations.

Chlorophyll concentrations are also known to vary significantly over short timescales. Grahame (1997) described the growth rate of phytoplankton populations to be as rapid as having a doubling time of 2 to 4 days. The present study employed a series of bimonthly (approximately every three weeks) measurements for a period of a year, to monitor physiochemical variations throughout the Maltese Islands. This type of analysis is useful when analysing long term trends and extreme events, in order to assess how the community responds to seasonal change and episodic disruptions.

When it comes to measuring parameters that vary significantly over short time scales, such as phytoplankton concentrations, which also have the ability to migrate vertically through the water column, it would be desirable to increase the sampling frequency, along with adding vertical profiles of physiochemical parameters of the water column. This will enable the assessment of the variation of phytoplankton concentrations with depth and provide an estimate of the level of deep-water chlorophyll maximum, with short term changes in concentrations. This will increase the chance of observing and possibly obtaining a significant correlation between parameters such as chlorophyll and nutrient concentrations.

In order to obtain chlorophyll readings at a higher frequency, remote sensing could provide a good alterative. This would be a cost effective, readily available resource which scientists and planners could use with relatively ease. As mentioned in Chapter 1, remote sensing also provides a temporal view of surface chlorophyll which is of more use to most users. Unfortunately, the current technology has a few trade-offs and what it provides in efficiency and cost effectiveness it lacks in accuracy. This will be further explored in the next chapter.

2.3.8 Metrological parameters and their association with remotely sensed monthly mean chlorophyll levels and seasonal pattern in Maltese waters

In order to understand further drivers of change most strongly related to chlorophyll concentrations, the mean monthly remotely sensed chlorophyll time series (see chapter __ methodology) for Maltese waters was compared to meteorological parameters of wind speeds, temperature and rainfall. This was done using Pearson's correlation coefficient analysis. This would provide further evidence of the importance of metrological forcing parameters in controlling chlorophyll concentrations, based on long term datasets.

2.3.8.1 The association between wind speeds and chlorophyll levels in Maltese coastal waters.

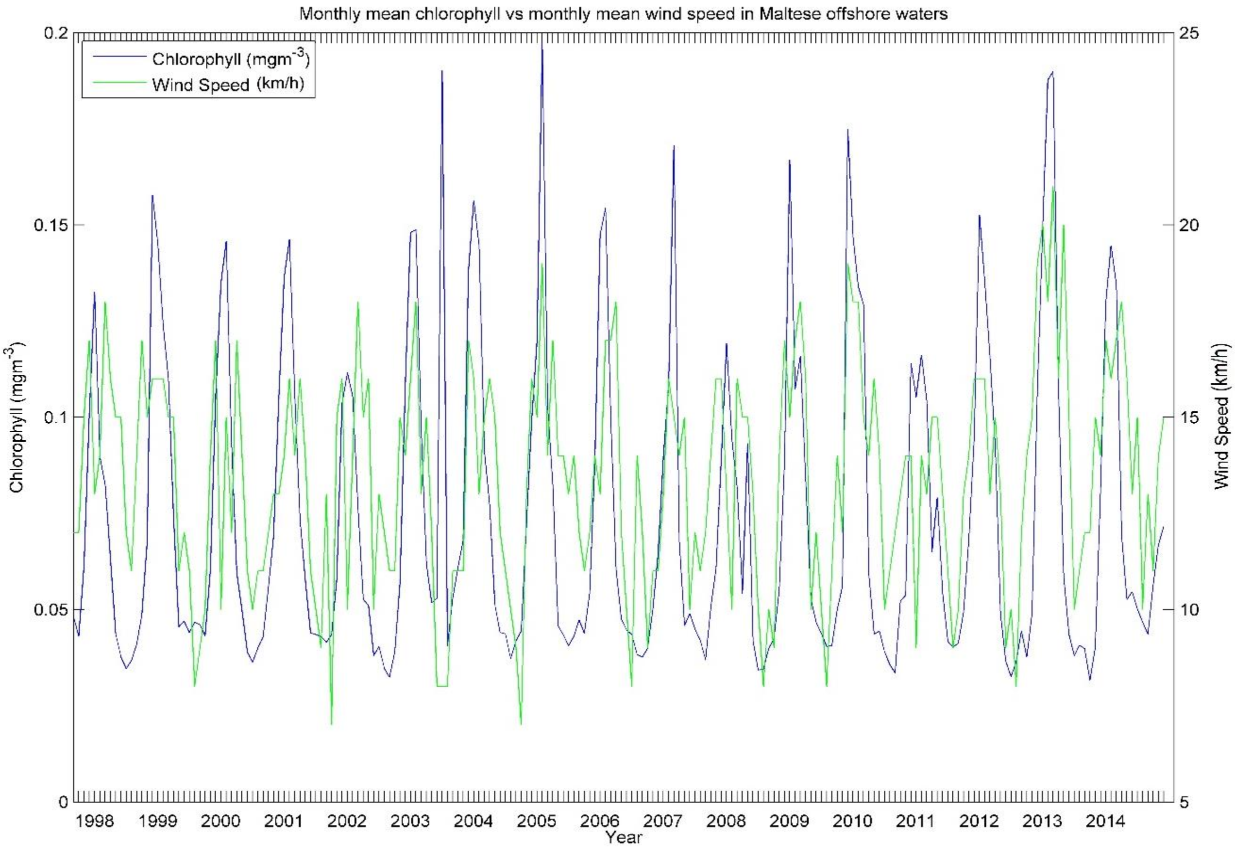


Figure 2.3.8.1.1: *Showing the variation of the average monthly chlorophyll concentration (mg m^{-3}) (MyOcean Copernicus Mission as indicated in chapter 3.2) and average monthly wind speeds (km/h) (Underground Weather) in Maltese coastal waters.*

Correlations

		Chlorophyll Coastal Waters	Wind
Chlorophyll Coastal Waters	Pearson Correlation	1	.529**
	Sig. (2-tailed)		.000
	N	204	203

**. Correlation is significant at the 0.01 level (2-tailed).

Table 2.3.8.1.1: *Showing the results of the correlation coefficient analysis between average monthly chlorophyll concentration and average wind speed in Maltese coastal waters.*

A Pearson product-moment correlation coefficient was computed to assess the relationship between average monthly chlorophyll concentrations and average monthly wind speeds in Maltese coastal waters, over a 17 year period. There was a significant positive correlation between the two variables, $r = 0.529$, $n = 203$, $p = 0.0001$. A scatterplot summarizes the results (Figure 2.3.7.1.2).

The quadratic correlation between log 10 chlorophyll concentration and log 10 wind speed suggest that there is a positive correlation between chlorophyll and wind speed up to a wind speed of 9 Km/hr. The graph shows that the optimal wind speed for phytoplankton growth is 9 Km/hr. Beyond this wind speed any further increase brings about a decrease in chlorophyll levels. The quadratic correlation coefficient ($r^2 = 0.359$).

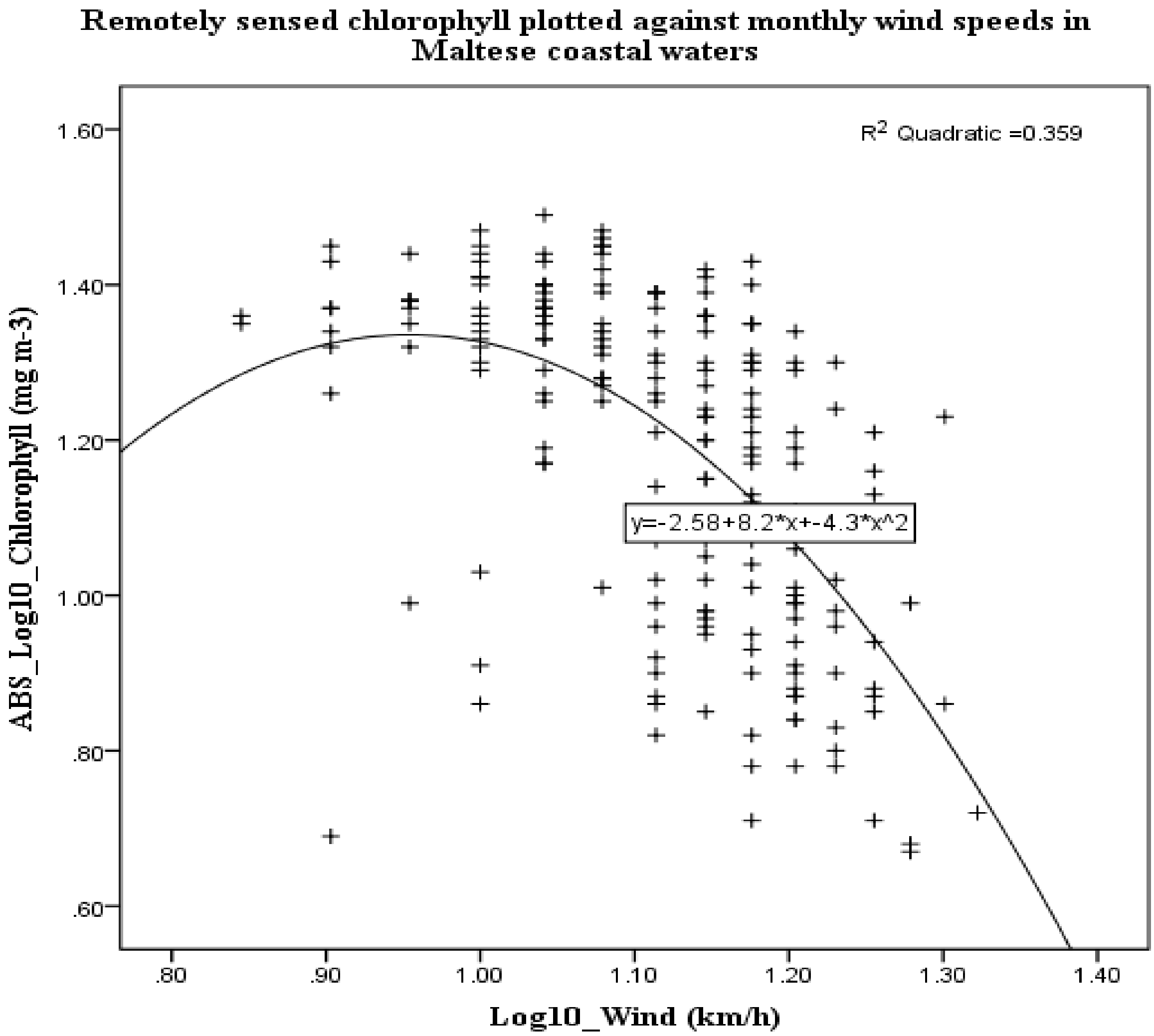


Figure 2.3.8.1.2: *showing a scatter plot and the correlation between average monthly chlorophyll concentrations and average wind speed in Maltese coastal waters.*

2.3.8.2 The association between air temperature and chlorophyll levels in Maltese coastal waters

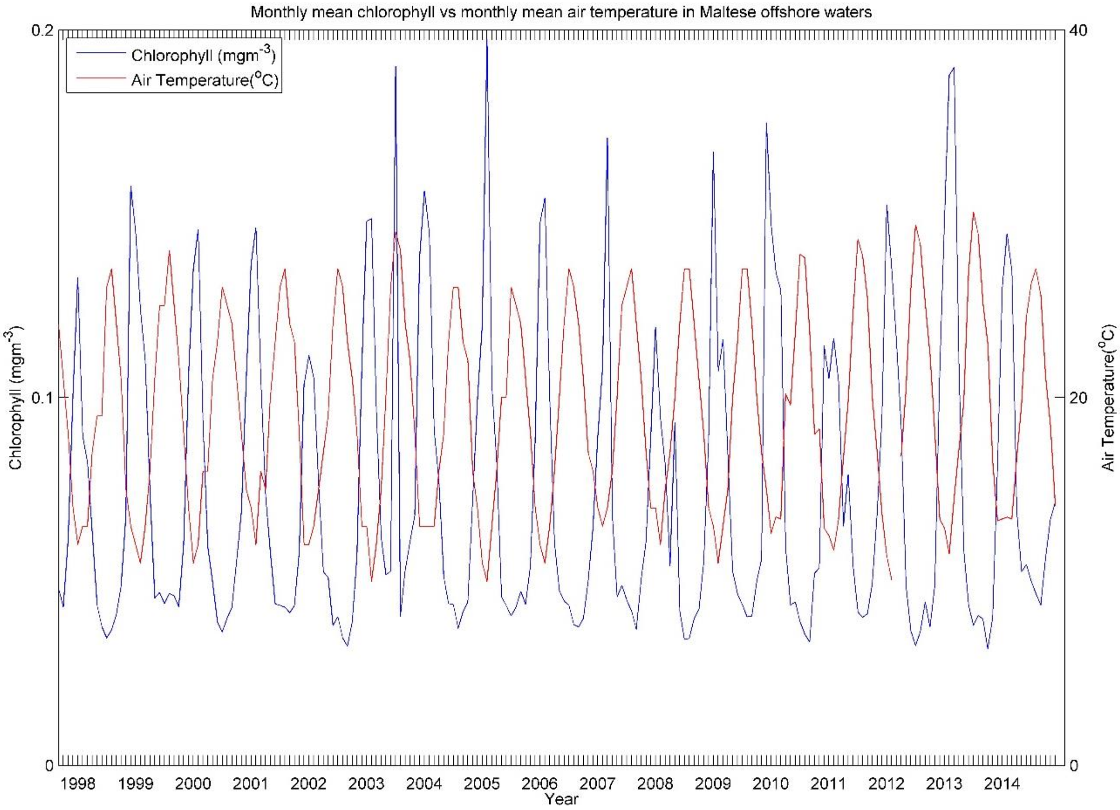


Figure 2.3.8.2.1: *Showing the correlation between average monthly chlorophyll concentration (mg m^{-3}) and mean monthly air temperature (°C) for Maltese coastal waters.*

Correlations

		Chlorophyll Coastal Waters	Air Temperature
Chlorophyll Coastal Waters	Pearson Correlation	1	-.734**
	Sig. (2-tailed)		.000
	N	204	203

**. Correlation is significant at the 0.01 level (2-tailed).

Table 2.3.8.2.1: *showing the correlation between mean monthly chlorophyll concentrations and average air temperature in Maltese coastal waters.*

A Pearson product-moment correlation coefficient was computed to assess the relationship between average monthly chlorophyll concentrations and mean monthly air temperature in Maltese coastal waters. There was a significant negative correlation between the two variables, r = -0.734, n = 203, p = 0.0001. The scatterplot (Figure 2.3.7.2.2) summarizes this result. There is a strong, negative correlation between monthly chlorophyll concentrations and air temperature. Increases in air temperature lead to a decrease in chlorophyll concentration.

The quadratic correlation between log 10 mean monthly chlorophyll concentration and log 10 mean monthly air temperature indicates that as the temperature increases the rate of growth of phytoplankton also increases. The optimal temperature for maximum phytoplankton growth is up to 12°C. Beyond this temperature the rate of phytoplankton growth decreases.

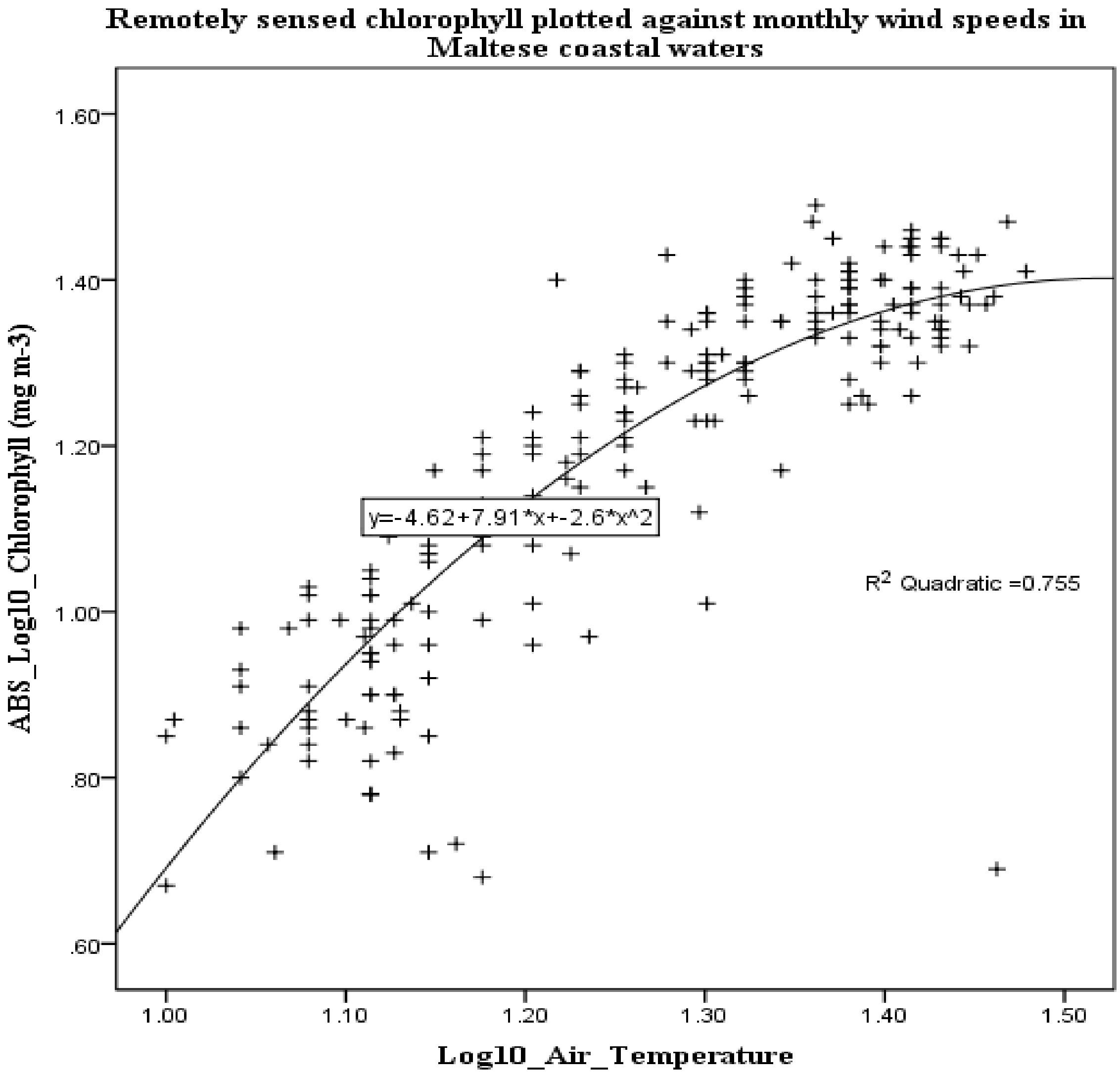


Figure 2.3.8.2.2: *Showing a scatter plot and the correlation between average monthly mean chlorophyll concentrations and average air temperature in Maltese coastal waters.*

2.3.8.3 The association between rainfall and chlorophyll levels in Maltese coastal waters

Correlations

		Chlorophyll Coastal Waters	Total monthly rainfall
Chlorophyll Coastal Waters	Pearson Correlation	1	.154
	Sig. (2-tailed)		.244
	N	204	59

Table 2.3.8.3.1: *Showing the correlation between mean monthly chlorophyll concentrations and total monthly rainfall in Maltese coastal waters.*

A Pearson product-moment correlation coefficient was computed to assess the relationship between average monthly chlorophyll concentrations and total monthly rainfall in Maltese coastal waters. . There was no correlation between the two variables, r = 0.154, n = 59, p = 0.244. A scatterplot (Figure 2.3.7.3.1) summarizes the results.

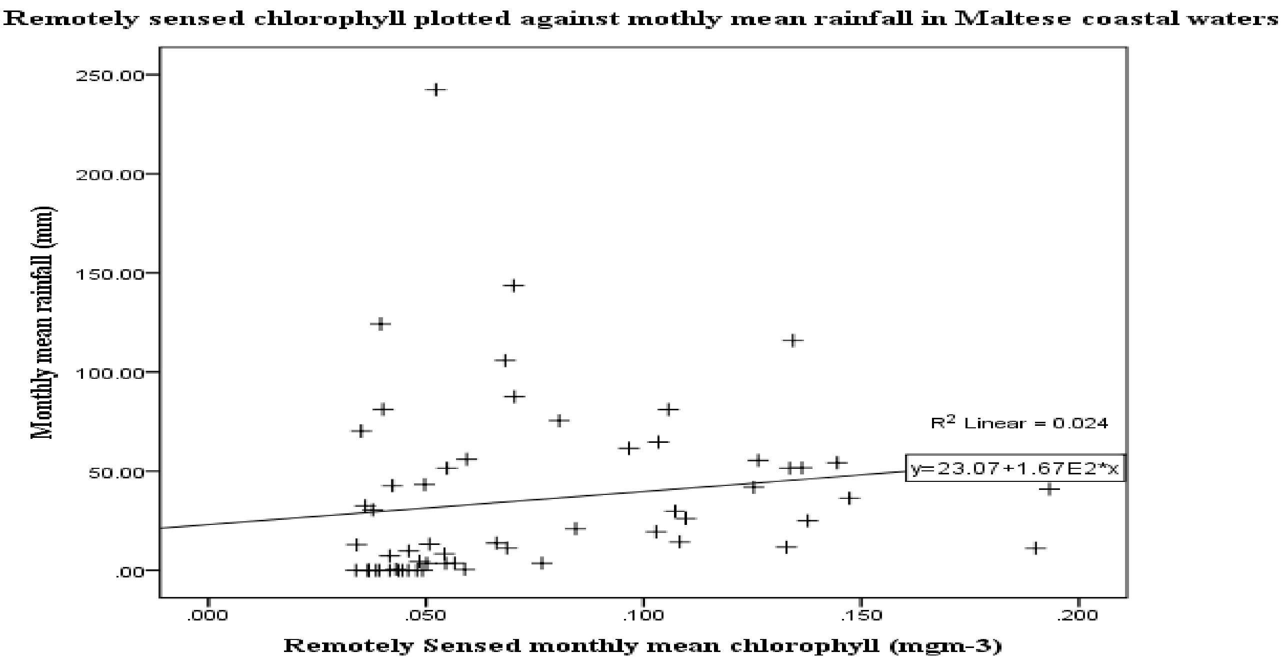


Figure 2.3.8.3.1: *showing a scatter plot and the correlation between average monthly chlorophyll concentrations and total monthly rainfall in Maltese coastal waters.*

2.3.8.4 Analysis of meteorological parameters and their association with remotely sensed monthly mean chlorophyll levels in Maltese waters

One of the hypotheses of this study is that the seasonal pattern of chlorophyll is controlled by meteorological conditions such as wind speeds, air temperature and rainfall. These parameters were chosen to be compared to remotely sensed chlorophyll concentrations over a 17 year period. The results indicate that that there is a significant correlation between air temperature and wind speeds and remotely sensed chlorophyll concentrations.

Air temperature had the strongest correlation with mean monthly chlorophyll concentration (Figure 2.3.7.2.1). This can be linked to Behrenfeld's hypothesis which states that warm surface waters and strong water column stratification are related to lower levels of primary production (Behrenfeld *et al.,* 2006). Air temperature will have a direct effect on heat transferred into the water column. Table 2.3.7.2.1, shows that there is an inverse correlation between air temperature and chlorophyll concentrations. This is in accord with observations made by D'Ortenzio (2005, Figure 1).

Wind speed brings about mixing of waters, and is characteristically higher during the winter months, which is when chlorophyll levels are highest in central and eastern Mediterranean waters (Figure 2.3.7.1.1). The results from the 17 year time series indicate a strong positive correlation with chlorophyll concentrations. This 17 year time series comparison between remotely sensed chlorophyll values and wind has not been analysed before in Maltese waters. This is a very robust result and important observation which enhances the knowledge about the effect of climatic factors on phytoplankton dynamics in the central Mediterranean area.

What seems to be happening in Maltese waters is that when temperatures are higher, stratification of the water column takes place. This increases temperature and salinity gradients in the water column, thus cutting off the surface and intermediate water from deeper nutrient rich waters resulting in low chlorophyll concentrations. The increased level of biomass in the Mediterranean Sea during the winter months, observed by remotely sensed and *in situ* results, is seen to be in phase with the progressive, moderate deepening of the mixed layer, as can be seen from the pattern observed in Maltese waters in Figure 2.3.2.1.

The mixed layer depth seasonal variability is characterised by a November to February/ early-March deepening, followed by a sudden re-stratification in April, which is sustained throughout summer and early autumn. At this time nutrients will not be abundant and there will be insufficient concentrations for any increase in the levels of phytoplankton biomass. As winter approaches water column vorticity becomes greater, thus bringing nutrients from deep waters to the surface, hence higher levels of phytoplankton growth occur. Wind speed seems to be a very important factor in controlling this process. Wind has also been observed to have a pivotal role in controlling the seasonal heat flux between the air and the sea, in other areas of the Mediterranean (Zecchetto & De Biaso, 2008; Burlando, 2009).

Another example of this is seen in the northwest basin of the Mediterranean Sea, where the cyclonic Gyre inside the Gulf of Lions exhibits a sequence of large blooms during spring. This pattern has been shown to be related to the seasonal pattern of the Mistral winds along with the convective processes found in this area of the Mediterranean. These produce deep water formation during the months of April and May (Morel & André, 1991; Barale, 2003). Strong northern Mistral winds come down to the sea over the continental landmass and mix these waters down to a depth of 1500-2000 meters, generating deep convective processes. The inefficiency of blooms in the winter in this part of the Mediterranean can be related to the conditions created by the overturning of much of the water column.

The prevailing wind, which generates turbulence and deep mixing precludes the stabilisation of algae in the well-lit upper layers of the water column. Upon the return of spring, the Mistrals decrease, and the water column returns to a more stable state, where stratification can set in. Nutrients would be abundant in surface layers, which are exposed to sufficient light, due to the prolonged winter mixing resulting in strong spring blooms in this area of the Mediterranean (Levy *et al*., 2000).

It is interesting and relevant to this study to compare chlorophyll dynamics from the Mediterranean Sea to oceanic waters elsewhere. The North Atlantic is known to be the one of the most productive ocean region, globally (Howarth *et al.,* 1996). The temporal and spatial distribution of chlorophyll throughout the North Atlantic is not uniform (Duklow & Harris, 1993). It was observed by Siegel *et al.* (2002), that blooms begin during winter, at the start of the year, for the regions between 45°N and 35°N, while regions further north would experience phytoplankton blooms during spring time (March-April). South of the 30°N latitude, the Atlantic Ocean is considered to have weak seasonal variation and to be oligotrophic. This is characteristic of a sub-tropical regime. The Mediterranean is located between these two limits of latitude having many regions characterised by oligotrophic, sub-tropical conditions, interwoven with areas where, given the correct hydrographic and atmospheric conditions, North Atlantic-like blooms are observed (D'Ortenzio & D'Alcalà, 2009).

2.4 Conclusion

The main conclusions that can be made from this study are:

- Maltese waters are oligotrophic with chlorophyll concentration, ranging from 0.01 mg m^{-3} to 0.34 mg m^{-3}.
- Chlorophyll is seen to have a strong seasonal variation, with the highest values in winter and lowest in summer.
- Site D had the highest mean chlorophyll concentration. This is probably being influenced by anthropogenic human activities that are densely concentrated at this coastal site.
- Site B had the lowest mean chlorophyll concentrations. This is most likely related to the fact that Site B is an open coastal water site, one mile away from the coast and its influences.
- Meteorological forcing parameters such as wind speed and temperature are the primary drivers of the variation in chlorophyll concentration observed in this study. This observation is based on the statistically significant results obtained in both *in situ* analysis (multiple linear regression) and remote sensing analysis (Pearson's correlation).

Based on these results, which have led to the above conclusions, the alternate hypothesis, which states that *phytoplankton growth is controlled by the flux of nutrients found in surface waters, which in turn will be influenced by water mixing and the rate at which effluents derived from human activities will enter the water,* is accepted.

Such analysis of phytoplankton dynamics has never been carried out in Maltese waters. This study has produced a statistical baseline for assessing variations of phytoplankton concentrations, along with investigating which physiochemical parameters are bringing about such a seasonal variation. The methods used for analysis provided a rigorous test, based on statistical significance, for the hypothesis that is being investigated.

In practical terms, this study has established which parameters govern phytoplankton growth in Maltese waters that will help to increase the growth of primary producers in the marine environment, upon which the marine system depends. These results have made progress in the development of a model which can be used to predict chlorophyll concentrations in Maltese waters. This model can be translated into a number of practical uses for industry and for future consecration efforts. This is discussed further in chapter 4.

Based on these results some limitations of this study have been observed. These limitations are associated with the study having a low frequency of sampling and measurements over a long period of time. The growth rate of phytoplankton populations is rapid with a doubling time of 2 to 4 days (Grahame, 1997). Given the highly variable nature of chlorophyll concentrations it would be desirable to increase sampling intervals along with physiochemical measurements of the vertical profile of the water column. This will enable the assessment of the variation of phytoplankton concentrations with depth and provide an estimate of the level of the deep-water chlorophyll maximum.

In order to further the understanding of the dynamics of phytoplankton populations in Maltese waters, further studies should be carried out using sampling stations that take repeated daily or weekly measurements at fixed points throughout the vertical water column. This will enhance the current knowledge about phytoplankton dynamics and provide important data needed to preserve as well as utilise this key natural resource found in Maltese coastal waters.

Chapter 3: Remote Sensing in the Mediterranean Sea and Maltese waters

3.1 Introduction

Remote sensing observation is a cost effective long term method used to assess water column dynamics which can take place at regional or basin levels. Such time series are of utmost importance in understanding the marine ecosystem, and help towards preventing damage of this valuable resource. Remote sensing is a spectral tool that can be used very effectively when applied appropriately. Temporal and spatial views of the surface are readily available. This gives it an edge over *in situ* measurements of water quality. Scientists are becoming more reliant on this type of data. This coincides with major improvement in the accuracy and functionality of this source of data.

Remote sensing of ocean colour has been used successfully in various areas around the globe to measure chlorophyll concentrations in the surface layer. This provides an efficient tool in managing the marine environment and monitoring ecosystem processes. Remote sensing is able to provide long-term, near real time, synoptic estimates of regional and global parameters which can be used to assimilate ecosystem models and validate high resolution models.

In the past three decades the Mediterranean Sea has been explored using remotely sensed products in order to evaluate chlorophyll dynamics of the area. This was primarily done using CZCS. Some examples of this include studies carried out by Morel and Andre (1991) and by Antoine *et al*. (1995). These laid the baseline research of spatial and seasonal dynamics of phytoplankton blooms in the Mediterranean Sea. These studies were followed by other studies. Barale (2003) analysed chlorophyll climatological CZCS data and compared it with satellite derived wind speed and sea surface temperature data collected by satellite throughout the 1980's and 1990's. These studies further enforced the western to eastern, north to south chlorophyll gradient described in Chapter 1, whilst ascribing driving forces of seasonal and annual change in chlorophyll levels with such gradients.

Despite the great advances that have taken place in satellite remote sensing over the last three decades, there still presents a significant degree of error in many remotely sensed products. Some of these errors can be assigned to atmospheric correction procedures whilst other are caused by inaccurate bio-optical algorithms. This study seeks to assess the degree of accuracy present in the MODIS Aqua satellite, which uses MedOC4 and AD4 algorithms, in Maltese waters. This study also seeks to build up on the past data generated by analysing the season variation of chlorophyll at four sites spread throughout the Mediterranean Sea. Statistical analysis is used to identify significant variations between seasonal and inter-annual variability for a 17 year dataset derived from a combination of historic ocean colour data.

3.2 Data and Methods

3.2.1 Tempo-spatial variation of chlorophyll at 4 different sites throughout the Mediterranean Sea over a 17 year period

Remotely sensed chlorophyll data was obtained from MyOcean Marine Core Service. This data is managed by the Satellite Oceanography (GOS-ISAC) of the Italian National Research Council (CNR) in Rome. This dataset consists of the Mediterranean Sea surface chlorophyll concentrations from multi satellite observations. The dataset spans seventeen years, with data being made available from September 1997 to December 2014. It has a 4km resolution and operates using the regional ocean colour algorithm MedOC4 (Volpe *et al*., 2007).

This data provides near real time, delayed time and re-analysis of merged remote sensing reflectance (*Rrs*) values from MODIS, MERIS and SeaWiFS sensors. This data is converted to chlorophyll concentrations by Plymouth Marine Laboratory. A hoc IDL script does this in a one-shot mode. MedOC4 algorithm utilises a blue-to-green maximum reflectance band ratio of *Rrs*443/*Rrs*555, *Rrs*490/*Rrs*555 and *Rrs*510/*Rrs*555. This product identifies the average chlorophyll content of the surface layer as defined by the first optical depth (roughly one fifth of the euphotic depth), which in the Mediterranean is about 15–35 meters on average. This is then mapped onto a grid of the Mediterranean Sea using a cylindrical equirectangular projection.

The mean monthly chlorophyll concentrations of four sites over a 17 year period was analysed in this study. These were Maltese coastal waters, Maltese offshore waters, the Ionian Sea and the Ligurian-Provencal Sea. This was done in order to identify trends that may be present in chlorophyll dynamics from different areas of the Mediterranean Sea. A square box was utilised for obtaining an average of the chlorophyll values at each of the chosen sites. Each box spanned a distance of 85km x 73 km (Figure 3.2.1.1). The box representative of Maltese coastal waters spanned a distance of 53km x 44km (Figure 3.2.1.1)

The analysis of spatial variation across the Mediterranean (Section 3.3.1) utilised all four boxes described above. Temporal variation analysis (Section 3.3.2) only utilised three of the above sites.

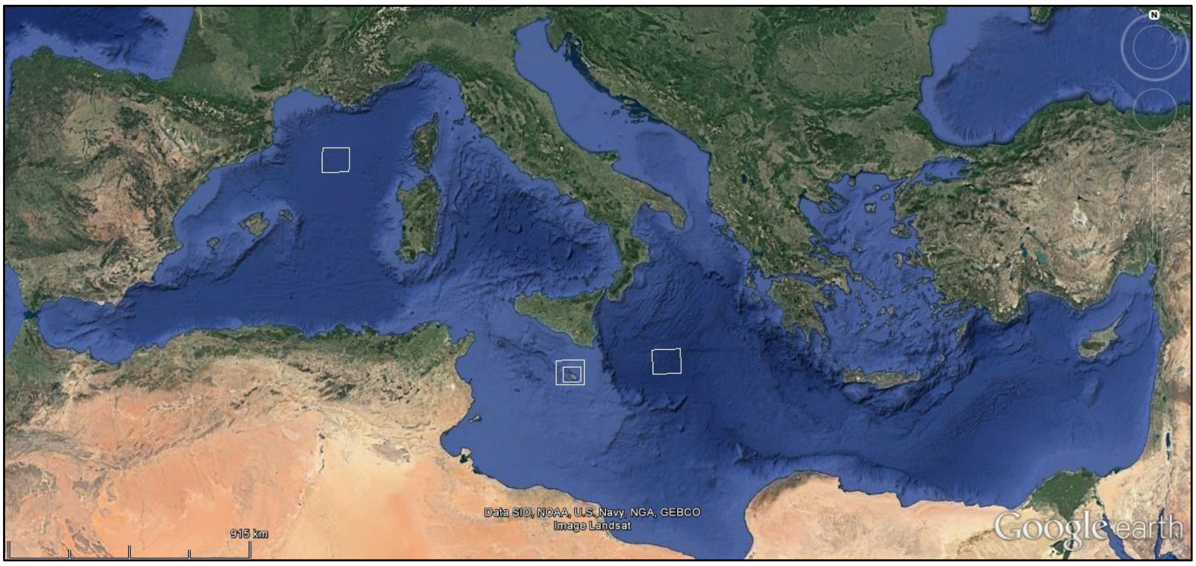


Figure 3.2.1.1: *Showing the size and location of each of the sites analysed by this study*

MATLAB was utilised to manipulate the dataset. The dataset containing daily chlorophyll values for each site was downloaded in segments, one year at a time. A script was designed whereby each dataset was loaded into MATLAB one year at a time. The script was designed to return the monthly mean, monthly minimum and monthly maximum chlorophyll concentrations, along with the monthly standard deviation and the number of days having data available as a percentage of each month, for each of the yearly segments loaded. The output was stored on an excel sheet and a plot of monthly mean chlorophyll values over a period of 17 years was created simultaneously. The results are shown in Figure 3.3.1.1 and 3.3.1.2.

Statistical analysis was carried out on the time series in order to test for any significant variations between sites, seasons, and yearly mean chlorophyll values. This was done using the statistical analysis package, SPSS. A One Way between groups ANOVA was chosen as the most efficient test for manipulating this particular dataset. When the data was found to be non-homogeneous and or had a non-normal distribution, the Kruskal-Wallis non-parametric test was used instead of a One Way ANOVA test. These were followed up with the *post hoc test;* Tukey's HSD (Honest Significant Difference) test (Hayter, 1986), in order to identify individual significant differences in chlorophyll concentrations between either sites, seasons or years.

This chapter presents the results obtained when testing for any significant spatial differences in chlorophyll concentration between the four sites analysed by this study (Section 3.3.1). This was followed by looking at the temporal variation of chlorophyll concentrations for these four sites (Section 3.3.2). Temporal analysis included Maltese offshore waters and Maltese coastal waters as one unit, due to their similarities in monthly mean chlorophyll concentration and seasonal pattern, as can be seen in Figure 3.3.1.2. Hence these were referred to as Maltese waters. Temporal analysis consisted of looking at the seasonal (spring, summer, autumn and winter) variation of chlorophyll concentrations in Maltese waters, Ionian waters and Ligurian-Provencal waters.

Analysis of the inter-annual variation of monthly mean chlorophyll concentrations of the three sites was then carried out on the 17 year dataset collected by this study (Section 3.3.3). The final set of analysis consisted of looking for significantly different winter (for all three sites) and spring (only for the Ligurian-Provencal Sea) monthly mean chlorophyll concentrations for the 17 year dataset, in order to identify significantly different trends between the years (Section 3.3.3). Winter and spring (only for the Ligurian-Provencal Sea) were chosen for this analysis since this was when phytoplankton blooms took place at the respective sites.

3.2.2 Accuracy of remotely sensed data when applied to Maltese waters

The accuracy of remotely sensed chlorophyll data when applied to Maltese waters was assessed by this study. This analysis involved downloading remotely sensed chlorophyll data from MyOcean Marine Core Service. The dataset utilised in this study consisted of Mediterranean Sea surface chlorophyll with a 1km resolution dataset. Data is relayed from the MODIS Aqua satellite platforms on a near real time and delayed time basis. MODIS was used because of the frequent satellite overpass, which takes place every one to two days (Ichoku, Remer, & Eck. 2005), whilst also having a relatively high spectral resolution of 1km. This makes MODIS Aqua a very useful tool for providing readily available data of temporal and spatial views of surface features (Platt & Sathyendranath, 1988).

The MODIS sensor is deployed upon the Aqua (EOS PM) and Terra (EOS AM) satellites. These satellites cross the equator at 13:30 and 10:30 Coordinated Universal Time (UTC), respectively. Every 16 days these satellites would overpass the exact same point on the earth’s surface. However, since each of the satellites has a swath width of 2330 km, observation of the same area is achievable every 1 to 2 days, depending on the latitude of the location from the satellite. The MODIS sensor has 36 spectral bands which range from 400nm to 14400nm, with a 12-bit radiometric sensitivity, which is considered to be high. When measuring ocean colour (chlorophyll) values, the visible and near infra-red channel ranges are most frequently used, since they have a higher spatial resolution. A number of different resolutions are achievable, with the best achievable spatial resolution being 1 km from nadir. This resolution, as provided by MyOcean Marine Core Service is georeferenced and interpolated onto maps, so as to cover coastal waters right up to the land sea boundary.

Mediterranean Ocean Colour algorithms are utilised in the processing of this data. A merged Case1 - Case2 dataset is utilised in this study. This works by using the AD4 algorithm for Case 2 waters type (D'Alimonte & Zibordi, 2003), and the empirical Mediterranean algorithm MedOC4 for Case 1 water types. Differentiation between the two water types takes place through satellite spectrum pixel-by-pixel comparisons of average spectral signature of each water type. The *in situ* dataset MedOC4 (Volpe *et al.*, 2007) is used to analyse Case 1 waters and the CoASTS dataset is used for case 2 waters (Berthon *et al.*, 2002). A method provided by D'Alimonte & Zibordi (2003) is then used to merge the information from the two datasets. A blue-to-green Maximum Reflectance ratio is utilised in the collection of this data.

This data is processed using the SeaWiFS Data Analysis System (SeaDAS) software, which is available from the NASA website (Volpe *et al*., 2012). This processes ocean colour sensor data from Level-1 to Level-3. Level-1 data is collected by the Group for Satellite Oceanography (GOS) from upstream providers. As soon as ancillary data is made available this data is processed. This usually takes place a few days after the satellite overpass has taken place. Standard masking criteria are applied in this process. These detect and correct for contamination factors such as clouds, land, atmospheric correction failure, sun glint, large spacecraft zenith angles greater than 56 degrees, large solar zenith angles greater than 70 degrees, high total radiance, negative water leaving radiance, and normalized water leaving radiance at 555 nm 0.15 Wm^{-2} sr^{-1}. Once the data has been merged and corrected it is mapped using a cylindrical equirectangular projection.

Data from Maltese waters for the years of 2015-2016 was utilised. The script was designed to select chlorophyll data from the coordinates of all four sampling points described in Chapter 1 (Site A, B, C and D). The specific dates at which *in situ* sampling took place were input into the script. The corresponding chlorophyll values were returned in mg m^{-3}. When no remotely sensed data was available for a particular day, the script was designed to provide remotely sensed chlorophyll values of the nearest day to the one requested. The output was designed to provide a chlorophyll value for the specific site that was requested, down to the nearest 0.5 kilometre along with the day the chlorophyll value was extracted. The chlorophyll values obtained from the remotely sensed dataset for each site were plotted against their corresponding *in situ* values in order to assess the accuracy of the remotely sensed data.

A Pearson product-moment correlation coefficient was used to assess the relationship between remotely sensed and *in situ* chlorophyll values. This was followed by performing an offline validation technique on the remotely sensed dataset (Volpe *et al.*, 2012). Offline validation refers to the estimation of basic statistical quantities between single sensor satellite observations and the corresponding *in situ* measurement. These include the correlation coefficient (r^2), the root mean square (RMS), the bias, and the relative (RPD) and absolute (APD) percentage differences.

The r^2 coefficient indicates the covariance between *in situ* (x) and remotely sensed chlorophyll concentration (y). The number of match up points between *in situ* (x) and remotely sensed chlorophyll is represented by N. The RMS indicates the spread of the data as compared to the best agreement and was defined as;

$$RMS = \sqrt{\frac{\sum_{i=1}^{N}(x-y)^2}{N}}$$

The mean bias is computed as;

$$BIAS = \frac{1}{N}\sum_{i=1}^{N}(y-x)$$

The RPD value is the mean percentage difference between *in situ* and remotely sensed chlorophyll concentrations. It provides an estimate of uncertainty as a function of the chlorophyll value and can be seen to be a relative BIAS. It is computed as;

$$RPD = \frac{1}{N}\sum_{i=1}^{N}\left(\frac{x-y}{y}\right) \times 100$$

The APD value is the difference between the remotely sensed estimate and the *in situ* measurement, weighted on the measured chlorophyll value, however as opposed to RPD, it does not provide any information regarding the direction of the discrepancy. This means that the difference can be either negative or positive and it represents a kind of relative RMS. This was computed as;

$$APD = \frac{1}{N}\sum_{i=1}^{N}\left|\frac{x-y}{y}\right| \times 100$$

3.3 Results and Discussion

3.3.1 Spatial variation of monthly mean chlorophyll concentrations across four sites in the Mediterranean Sea

The Mediterranean is a dynamic system which varies greatly in biotypes from one end to another, both north to south and west to east. This has been established by previous studies and lays the groundwork of information, upon which this study is based. The results below exhibit the different biotypes present in the Mediterranean Sea.

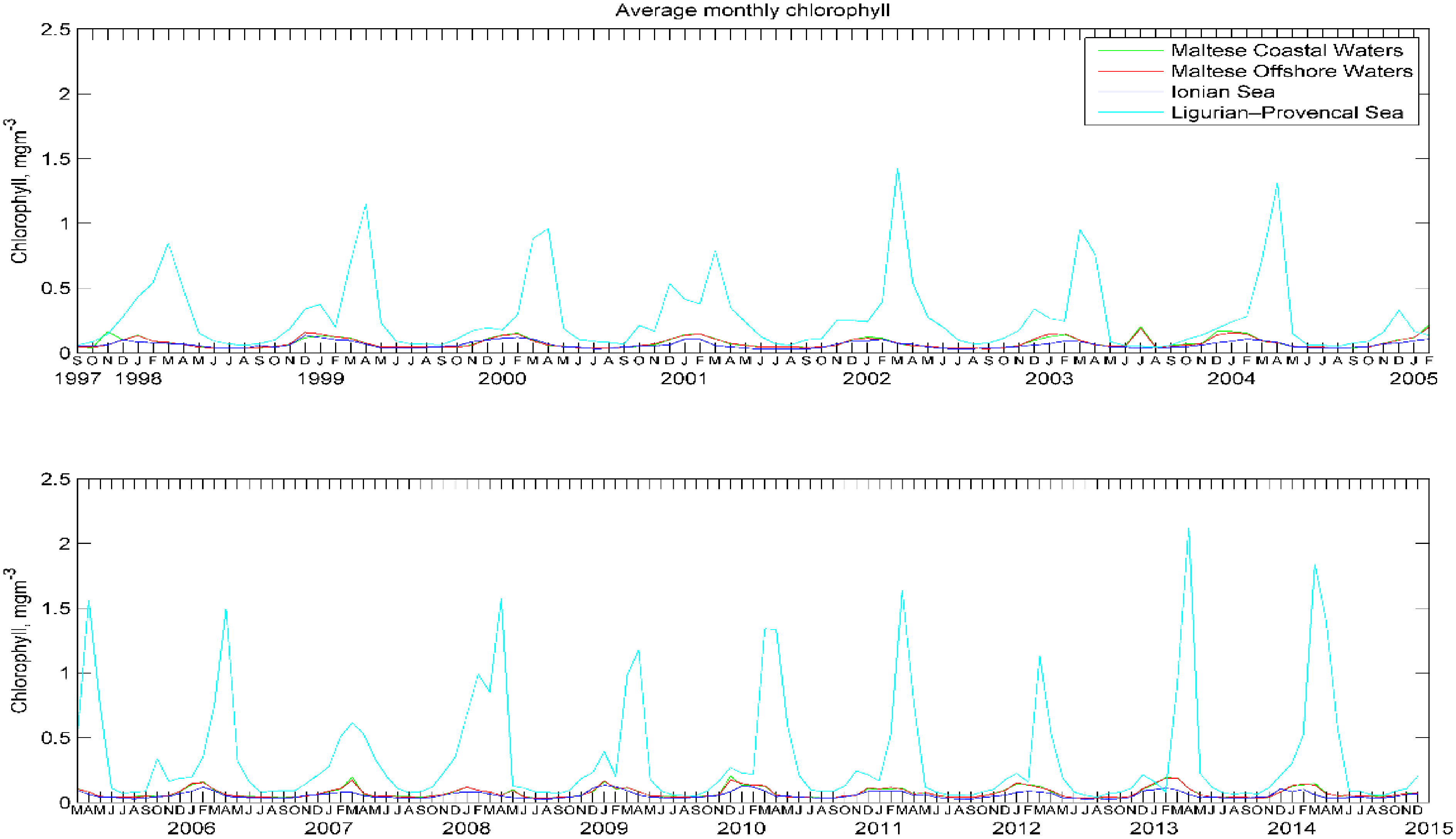


Figure 3.3.1.1: *Showing the variation of the average monthly chlorophyll concentrations in four areas at opposite ends of the Mediterranean Sea over a period of 17 years.*

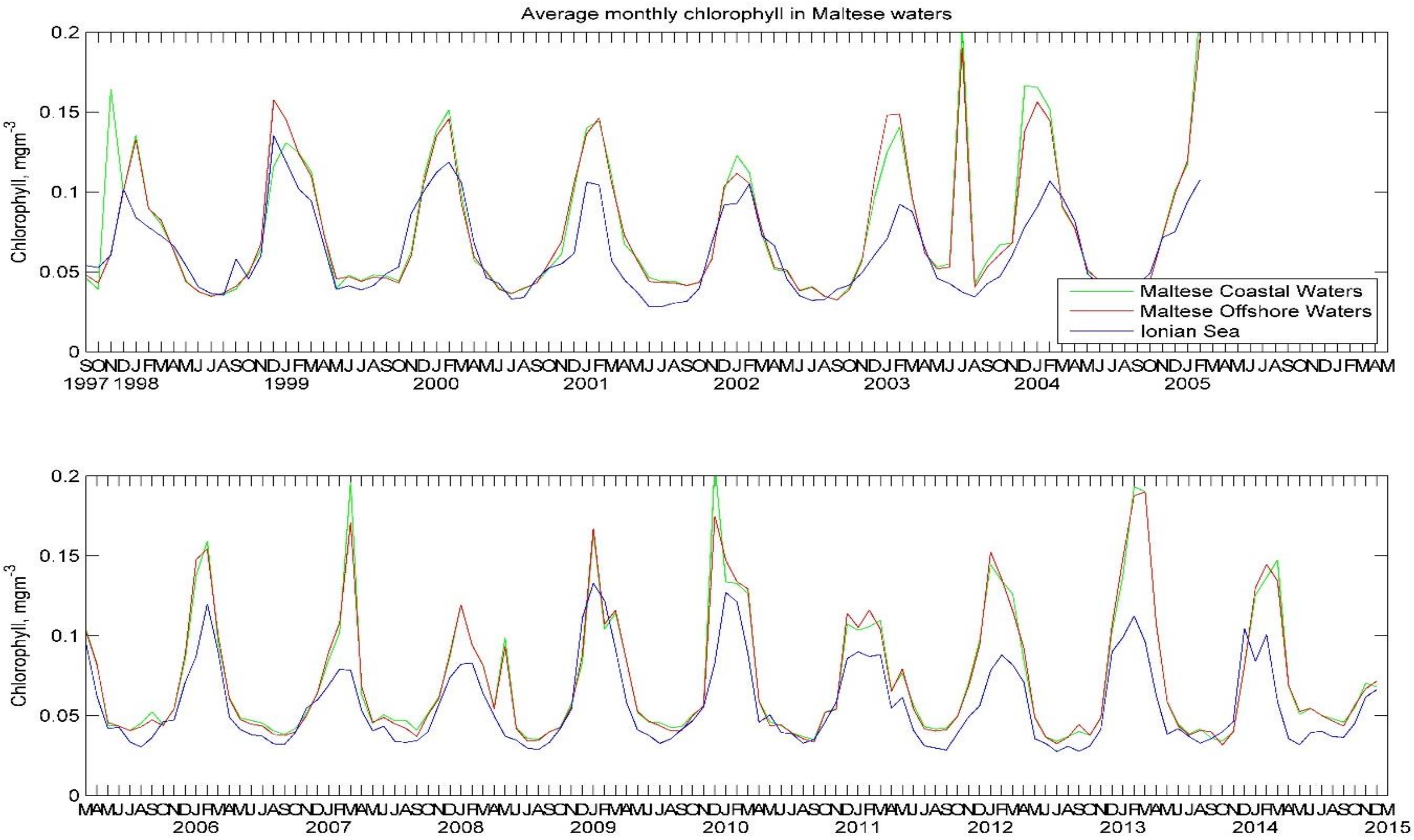


Figure 3.3.1.2: *Showing the variation of the average monthly chlorophyll concentration in Maltese waters and the Ionian Sea over a period of 17 years.*

Figure 3.3.1.1 and Figure 3.3.1.2 are showing the same results. Figure 3.3.1.2 presents results from 3 of the sites which have lower concentrations in order to make their representation more visible. Figure 3.3.1.1, shows that the Ligurian-Provencal Sea has much higher chlorophyll concentrations than the other three sites. Figure 3.3.1.2 shows that the Ionian Sea follows an almost identical seasonal pattern to Maltese coastal and offshore waters throughout the 17 year period, however slightly lower concentrations are observed. Maltese coastal and offshore waters are also seen to be very similar, however coastal waters seem to have slightly higher chlorophyll concentrations than offshore waters.

	N	Mean	Std. Deviation	Std. Error	Minimum	Maximum
Maltese Offshore Waters	204	.07433	.040386	.002828	.032	.197
Ionian Sea	204	.05926	.026560	.001860	.028	.135
Ligurian-Provencal Sea	204	.32092	.385617	.026999	.041	2.122
Maltese Coastal Waters	204	.07456	.041047	.002874	.032	.216
Total	816	.13227	.223508	.007824	.028	2.122

Table 3.3.1.1: *Showing the descriptive statistics of mean chlorophyll concentrations at four different sites throughout the Mediterranean Sea*

The descriptive statistic associated with mean chlorophyll levels across the 4 sites throughout the 17 year data series of Mediterranean waters is shown in Table 3.3.1.1. The lowest mean chlorophyll concentrations were found in the Ionian Sea (0.059 mg m^{-3}) and the highest in the Ligurian-Provencal Sea (0.321 mg m^{-3}). Maltese coastal waters (0.0746 mg m^{-3}) and Maltese offshore waters (0.0743mg m^{-3}) have very similar concentrations to each other. Maltese coastal and offshore waters have higher concentrations of chlorophyll than the Ionian Sea.

The maximum chlorophyll concentration in the Ionian Sea is 0.135 mg m^{-3} and the average yearly minimum level is 0.028 mg m^{-3}. The average yearly maximum in Maltese waters coastal waters is 0.216 mg m^{-3}, and Maltese offshore waters 0.197 mg m^{-3}. The average yearly maximum for the Ligurian-Provencal Sea for the years of 1997 to 2015 is 2.122 mg m^{-3}. This signifies a difference in mean chlorophyll concentrations by a factor of 10 between the eastern and western Mediterranean. The average yearly minimum value for the Ligurian-Provencal Sea is 0.041 mg m^{-3} and 0.032 mg m^{-3} for both Maltese offshore and coastal waters. These results suggest that there seems to be a clear spatial distinction in the trophic regions at opposite sides of the Mediterranean, with a clear west to east division. This is most evident during the bloom seasons.

In order to test if there is a significant difference in chlorophyll levels across the 4 different sites a One Way between groups ANOVA was performed. The current study seeks to build on the observations of previous studies, by searching for underlying trends through statistical analysis and by utilising the recently updated MedOC4 algorithm. Using a more finely tuned, sensitive algorithm, might make it possible to identify significant trends and patterns not observed by previous studies. Also by performing statistical analysis of chlorophyll values between different parts of the Mediterranean, a robust and scientifically sound analysis is provided.

Prior to conducting the ANOVA the assumption of normality was evaluated using a Kolmogorov-Smirnov and Shapiro-Wilk test.

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Chlorophyll	.320	816	.000	.420	816	.000

a. Lilliefors Significance Correction

Table 3.3.1.2: *showing the two tests of normality Kolmogorov-Smirnov and Shapiro-Wilk of mean chlorophyll concentrations at four different sites throughout the Mediterranean Sea*

The results suggest that the dataset analysed deviated significantly from a normal distribution (p=0.0001).

Furthermore, the assumption of homogeneity of variance was tested and satisfied based on Levene's F test, F (3,812) = 155.5, p=0.0001.

Test of Homogeneity of Variances

Chlorophyll

Levene Statistic	df1	df2	Sig.
155.504	3	812	.000

Table 3.3.1.3: *Showing Leven's test for homogeneity of variance for mean chlorophyll concentrations at four different sites throughout the Mediterranean Sea*

Since the assumption of normality was not fulfilled, the Kruskal-Wallis non-parametric test was used to determine if there is any significant difference in chlorophyll concentrations between the 4 sites.

Ranks

	Site	N	Mean Rank
Chlorophyll	Maltese Offshore Waters	204	360.55
	Ionian Sea	204	279.61
	Ligurian-Provencal Sea	204	630.51
	Maltese Coastal Waters	204	363.34
	Total	816	

Table 3.3.1.4: *showing the mean rank analyses of mean chlorophyll concentrations at four different sites throughout the Mediterranean Sea*

Test Statistics[a,b]

	Chlorophyll
Chi-Square	257.919
df	3
Asymp. Sig.	.000

a. Kruskal Wallis Test

b. Grouping Variable: Site

Table 3.3.1.5: *Showing the Kruskal-Wallis test for mean chlorophyll concentrations at four different sites throughout the Mediterranean Sea*

The results of the Kruskal-Wallis test showed a statistically significant difference x^2 (3, n = 81) = 257.92, p = 0.0001. Thus the alternate hypothesis, which states that there were significant differences between the means, was accepted. The Ligurian-Provencal Sea recorded a higher mean rank score than the other 3 sites, (Md = 630.51).

Tukey's HSD *post hoc* test revealed that four comparisons were found to be statistically significant ($p < 0.05$). The differences were observed when comparing the means of the Ligurian-Provencal Sea to each of the other four sites.

Figure 3.3.1.3 shows the mean and 95 % confidence interval for the chlorophyll concentrations at the 4 sites. It can be observed that the Ligurian-Provencal Sea has much higher chlorophyll concentrations and variability than the other three sites.

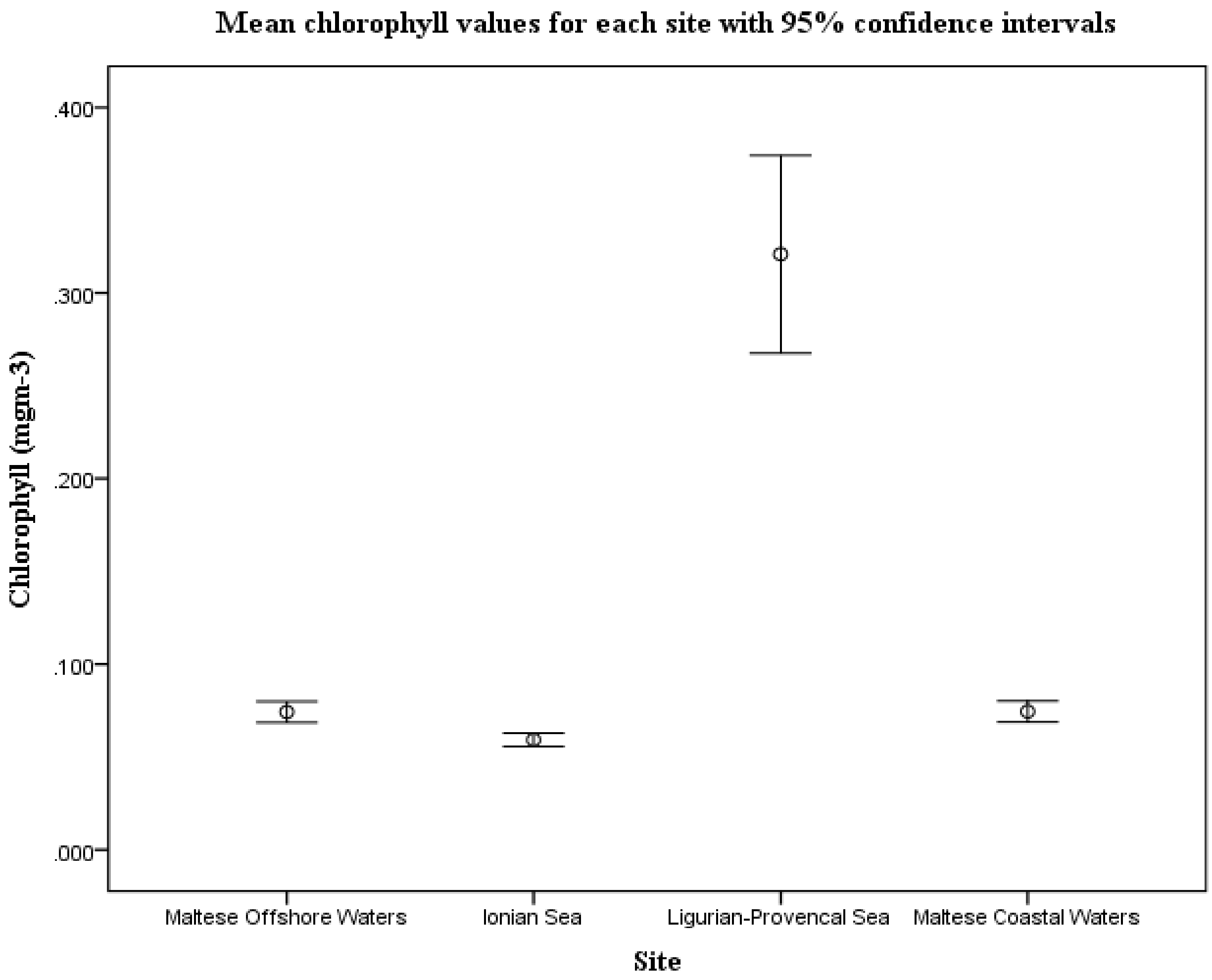


Figure 3.3.1.3: *Showing variation of the mean chlorophyll concentrations in four areas of the Mediterranean Sea with error bars at the 95% confidence interval.*

An important observation can be made by looking at mean monthly chlorophyll (Figure 1.2.1 and Figure 3.3.1.3) concentrations throughout the Mediterranean. This shows that there is an overall difference in chlorophyll concentrations from one side of the Mediterranean to the other. The western Mediterranean seems to be characterised by a higher chlorophyll concentration than the eastern Mediterranean. The eastern Mediterranean has very low oligotrophic conditions. This is visually depicted in Figure 3.3.1.2. These graphs show that chlorophyll concentrations in the Ligurian-Provencal Sea is characterised by high background productivity, a different seasonal pattern and greater inter-annual variability, when compared to Maltese waters (central Mediterranean) and the Ionian Sea. The statistical analysis carried out in section 3.3.1.1 further confirms this observation at a statistically significant level. The results of the Kruskal-Wallis test and Tukey's HSD *post hoc* test showed a statistically significant difference between the means of the four sites analysed.

This was also observed by Santoleri *et al.*, (2008). The oligotrophic Levantine Basin was seen to have concentrations of around 0.05 mg m^{-3}. The seasonally productive regions in the northwest of the Mediterranean Sea were seen to have concentrations of around 0.26 mg m^{-3}. The reason for such a spatial distribution of chlorophyll throughout the Mediterranean Sea can be attributed to the transport of nutrient rich Atlantic Water entering the semi-enclosed Mediterranean Sea through the Gibraltar Straits in the western basin. This water flows through the basin and becomes what is known as Modified Atlantic Water (MAW). The Mediterranean is a concentration basin, meaning freshwater loss exceeds freshwater input. This is due to the imbalance between evaporation, transpiration and runoff. The further east that Atlantic water travels, the greater the change in its temperature and salinity. Thus an open thermohaline cell is created between the two sub-basins that characterise the Mediterranean Sea (Siokou-Frangou *et al*., 2010). This is characterised by a west to east surface (top 200m) transport of Modified Atlantic Water which overlays an east to west flow of saltier water known as the Levantine Intermediate Water (LIW).

3.3.2 Temporal variation of remotely sensed monthly mean chlorophyll concentration over the 17 year time series at each site

Figure 3.3.1.1 and Figure 3.3.1.2 show temporal variation over time and periods of high and low chlorophyll concentrations occurring at each site. The Ligurian-Provencal Sea experiences an overall increase in chlorophyll concentrations during the month of September. This phytoplankton bloom is seen to increase continually until it peaks during March and April. After this, concentrations decrease until a minimum value of 0.1 mg m^{-3} is reached in June. In many years this bloom is seen to peak at two separate instances. A small bloom begins in September, and peaks in December at values of around 0.3 mg m^{-3} and then it decreases until it reaches a minimum chlorophyll concentration of 0.1 mg m^{-3} during January. Immediately a second larger bloom is initiated, rising to a concentration that usually exceeds 1 mg m^{-3} during March and April and takes until June to return to a minimum chlorophyll concentration. Maltese waters and the Ionian Sea have a different pattern to that of the Ligurian-Provencal Sea. A bloom event in these waters begins in November and lasts until April (Figure 3.3.1.1). This bloom event is seen to peak between the months of January and February at concentrations of around 0.15mg m^{-3}. Following this an oligotrophic state prevails throughout the summer months of June to September. The Ligurian-Provencal Sea retains a slightly higher chlorophyll concentration during this period, but summer is still the season with lowest chlorophyll concentrations for each of the study sites.

Temporal variation of remotely sensed chlorophyll concentration over a period of 17 years		
Location	Result (Statistical Test)	Tukey's HSD Test (Stat. Sig/ Non-stat. Sig)
Variation of seasonal mean chlorophyll concentration		
Maltese waters	x^2 (3, n=204)=132.5, p = 0.0001 (Kruskal-Wallis test- Significant)	All seasons significantly different values to each other
Ionian Sea	x^2 (3, n=204)= 144.2, p = 0.0001 (Kruskal-Wallis test- Significant)	All seasons significantly different values to each other
Ligurian-Provencal Sea	x^2 (3, n=204)=119.2, p = 0.0001 (Kruskal-Wallis test- Significant)	All seasons significantly different values to each other
Variation of annual mean chlorophyll concentration		
Maltese waters	x^2 (16, n=204)=7.91, p = 0.951 (Kruskal- Wallis- Non Significant)	All years not significantly different to one another
Ionian Sea	x^2 (16, n = 204) = 9.72, p = 0.881 (Kruskal- Wallis- Non Significant)	All years not significantly different to one another
Ligurian-Provencal Sea	x^2 (16, n=204)=8.73, p = 0.924 (Kruskal- Wallis- Non Significant)	All years not significantly different to one another
Variation of mean annual winter and spring chlorophyll concentrations		
Winter in Maltese waters	F(16,34)=1.41, p=0.192, η^2= 0.4 (ANOVA- Non significant)	All years not significantly different from one another
Winter in the Ionian Sea	F(16,34)=2.57, p=0.01, η^2= 0.547 (ANOVA- Significant)	All years not significantly different to one another
Winter in the Ligurian-Provencal Sea	x^2 (16, n=51)= 10.29, p = 0.851 (Kruskal- Wallis- Non Significant)	All years not significantly different to one another
Spring in the Ligurian-Provencal Sea	x^2 (16, n=51) = 7.11, p = 0.971 (Kruskal- Wallis- Non Significant)	All years not significantly different to one another

Table 3.3.2.1: *Statistical analysis of temporal variation of remotely sensed chlorophyll concentration over the 17 year time series.*

The results from statistical analysis indicate that there is a strong mean seasonal variation of chlorophyll at each of the sites analysed in this study (Table 3.3.2.1 and APPENDIX 4). The Kruskal-Wallis non-parametric test indicated that the mean chlorophyll value across the four seasons, at each of the sites analysed in this study, were significantly different at the 99% confidence level (Table 3.3.2.1). Tukey's HSD *post hoc* test indicated that each season is significantly different to each of the other seasons for each of the sites (APPENDIX 4). In Maltese waters and the Ionian Sea, winter is seen to have the highest mean chlorophyll concentrations. The Ligurian-Provencal Sea is seen to have winter and spring as the peak seasons for chlorophyll concentrations, with spring having greater variation at the 95% confidence level.

A strong seasonal variation has been observed by many studies which analysed chlorophyll dynamics in the Mediterranean Sea (Lacombe *et al*., 1972; Morel and Andre', 1991; Antoine *et al*., 1995; Bosc *et al*., 2004; D'Ortenzio and d'Alcala, 2009; Siokou-Frangou *et al*., 2010; Volpe *et al*., 2012; Rinaldi, 2014). This study emphasises this observation by validating it with statistically significant results. D'Ortenzio and d'Alcala (2009) compared the seasonal cycle of the eastern basin to that of a sub-tropical gyre and the seasonal cycle of the western basin to that of the North Atlantic. From the results obtained in this study the seasonal cycle of the central Mediterranean (Maltese waters) seems to be similar to that of the eastern basin, with slightly higher chlorophyll concentrations.

3.3.3 Inter-annual variation of mean monthly chlorophyll concentrations throughout the Mediterranean Sea

The inter-annual variability of the mean chlorophyll levels for the 17 year time series were investigated in this study using ANOVA. The results (Table 3.3.2.1) suggest that none of the three sites explored had any statistically significant differences in annual mean chlorophyll concentration between one year and another.

Figure 3.3.1.1 indicates that the inter-annual variability of chlorophyll levels in the western Mediterranean basin is seen to be greater than that of the eastern basin. It can also be seen that the variation of chlorophyll from one year to another, at each of the sites analysed in this study, can vary by a factor of two (Maltese waters and Ionia Sea) or three (Ligurian-Provencal Sea), mainly during the winter and spring months. This observation is similar to what was reported by Barale *et al* (2008), who observed that at a pixel level, the inter-annual variations of mean monthly chlorophyll concentration in the western Mediterranean Basin exceed a factor of four. On the other hand the summer and autumn inter-annual variability in the Ligurian-Provencal Sea were observed to be very small (Figure 3.3.1.1).

This analysis was taken a step further by looking at the inter-annual mean chlorophyll variation across the 17 year period for the bloom seasons, winter in Maltese waters and Ionia Sea and winter and spring in the Ligurian-Provencal Sea at each of the three sites. The results in table 3.3.2.1 indicate that the Ionian Sea was the only site to have statistically different variations in the mean winter chlorophyll concentration for the 17 years analysed in this study (Appendix 5). This indicates that there seems to be a greater level of variability in the eastern basin during the bloom period. Similar observations were made by Bosc *et al.,* 2004. To date these are the only studies that have made this observation. All other studies have spoken of the low concentrations and stable levels of chlorophyll concentrations in the eastern basin of the Mediterranean Sea. This is an interesting anomaly and should be investigated further.

In order to identify similar trends between the inter-annual variability monthly mean chlorophyll concentrations between sites, further analysis was undertaken. The highest and lowest means for each of the years, at each of the sites, was analysed. A trend emerged, whereby the year of 2010 was the year with the second highest annual mean chlorophyll level throughout the entire 17 year dataset for both the Ionian Sea (0.065 mg m^{-3}) and Maltese waters (0.082 mg m^{-3}). The second highest peaks in wind speed in Maltese waters throughout the 17 year dataset were also seen in 2010 (Figure 2.3.7.1.1). This is further evidence of the importance of meteorological forcing, specifically by the wind as a primary driver of phytoplankton growth in the Mediterranean Sea.

In order to identify similar trends between the inter-annual variability of winter and spring in the Ligurian-Provencal Sea, monthly mean chlorophyll concentrations between sites were analysed further. The results indicate that winter of 2008 had the second lowest mean chlorophyll content in Maltese waters and the Ionian Sea throughout the 17 years analysed in this study (Figure 3.3.1.2). This also corresponded with relatively low wind speed during the winter of 2008 compared to the climatological mean wind speed of Maltese waters (Figure 2.3.7.1.1). The similarity in pattern between wind speed and chlorophyll provides further evidence regarding the importance of wind speeds controlling chlorophyll concentrations.

A similar observation was also made in the winter of 2005. During this year Maltese waters experienced the second highest mean chlorophyll levels throughout the 17 years analysed by this study. This was found to correspond with the Ligurian-Provencal Sea having the lowest winter chlorophyll content, followed by the second highest spring chlorophyll concentration throughout the 17 years analysed by this study. Font *et al.* (2007) observed that a lower level of winter convection was present in the Ligurian-Provencal Sea during the year 2005 as compared to the climatological mean. Salat *et al.* (2007) had reported that for the same area the increased level of winter convection extended over a larger area than it would normally in other years. Smith *et al.,* (2008) reported that during 2005 Argo float measurements discovered a new location for the formation of deep water in the Ligurian-Provencal basin.

His study suggests that this new location for deep water formation could be the reason for such low levels of chlorophyll during winter 2005, which then resulted in the second highest mean spring chlorophyll content in the Ligurian-Provencal Sea throughout the 17 years remotely sensed dataset analysed by this study (Appendix 6). The remotely sensed results of this study are picking up on such trends in chlorophyll concentrations, which despite not being significant, can still add insight and give a synoptic view of results when comparing local chlorophyll values with those of other sites found throughout the Mediterranean Sea.

3.3.4 Overall increasing or decreasing mean monthly chlorophyll concentration at each of the sites analysed in this study

The overall trend of increasing or decreasing mean monthly chlorophyll levels from 1997 to 2014 at each of the four sites could be determined. This was done by obtaining the gradient of the mean chlorophyll values for the 17 year time series for each site. The results (Figure 3.3.1.1) indicate that the level of chlorophyll in the Ligurian-Provencal Sea is increasing at a rate of 0.0004 mg m^{-3} every month over the 17 year period analysed in this study. On the other hand the chlorophyll level in the Ionian Sea is seen to be decreasing at a rate of 0.0001 mg m^{-3} every month over the 17 year period analysed in this study. Maltese coastal and offshore waters do not seem to experiencing the same level of change as the other two sites. The chlorophyll level in Maltese offshore waters are seen to be increasing at a rate of 0.6×10^{-7} mg m^{-3} every month and in Maltese coastal waters it is decreasing at a rate of 0.6×10^{-6} mg m^{-3}. Barale *et al*. (2008) reported an overall decreasing trend in the chlorophyll level of 20% based on the climatological average value for the entire Mediterranean Sea between the periods 1997-2003.

The results of this study do not provide evidence as to why there are overall increases and decreases in chlorophyll concentrations in either of the two basins; however further analysis of extended time series, similar but longer than the one analysed in this study, might help identify inter-annual patterns. The analysis of analogues water quality parameters such as the ones analysed in Chapter 2 over a decadal time period will help identify drivers of long term change and provide a valuable insight into the basic workings of ecosystem change over extended time periods. Accurate remote sensing datasets are pivotal in achieving this aim.

3.3.5 Assessing the accuracy of remotely sensed data when applied to Maltese waters

3.3.5.1 Assessing the correlation between remotely sensed and *in situ* analysis of chlorophyll using the entire data set

Correlations

		Remotely Sensed Chlorophyll	*In situ* Chlorophyll
Remotely Sensed Chlorophyll	Pearson Correlation	1	.410**
	Sig. (2-tailed)		.003
	N	52	51

**. Correlation is significant at the 0.01 level (2-tailed).

Table 3.3.5.1.1: *Showing the correlation between in situ and remotely sensed chlorophyll concentrations for the entire data set*

A Pearson product-moment correlation coefficient was computed to assess the relationship between *in situ* and remotely sensed chlorophyll concentrations for the entire data set of chlorophyll samples obtained by this study (Table 3.3.5.1.1). There was a significant positive correlation at the 99 % confidence level between the two variables, $r = 0.410$, $n = 51$, $p = 0.003$. A scatterplot (Figure 3.3.5.1.1) summarizes the results. A relatively strong, positive correlation between the two methods of measurement of chlorophyll concentrations can be observed.

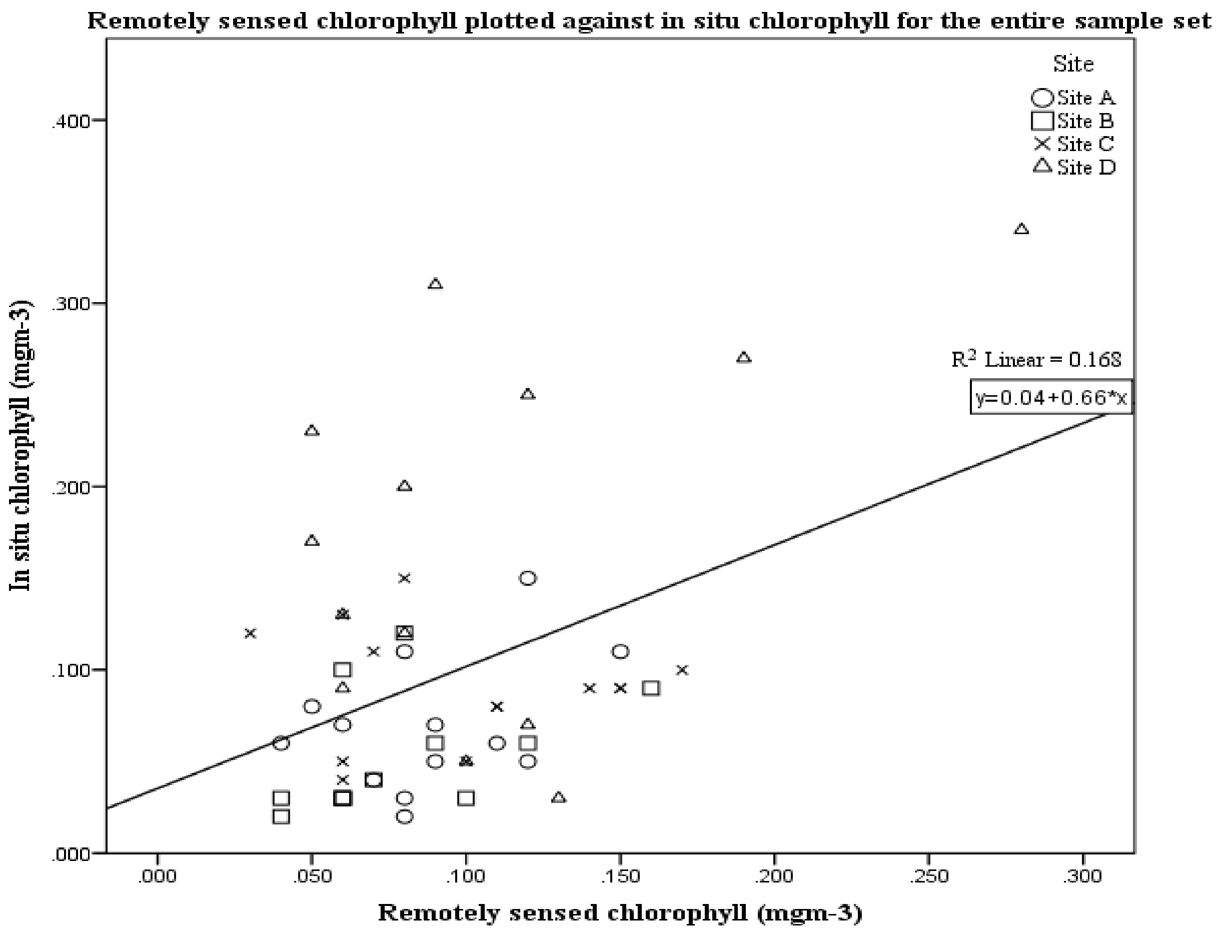


Figure 3.3.5.1.1: *Showing the scatterplot and correlation between in situ and remotely sensed measurement of chlorophyll using the entire data set*

3.3.5.2 Assessing the correlation between remotely sensed and *in situ* analysis of chlorophyll concentration when removing outliers.

The outliers were removed in order to obtain a more representative dataset for measuring the accuracy of the remotely sensed data. The criteria used for removing outliers was based on omitting paired data where either chlorophyll values were measured using the acetonitrile method (refer to chapter 2.2.4.1.2.3) and/or data points where *in situ* samples did not have a same day match with a satellite overpass.

Correlations

		Removing Outliers Remotely Sensed Chlorophyll	Removing Outliers *In situ* Chlorophyll
Removing Outliers Remotely Sensed Chlorophyll	Pearson Correlation	1	.720**
	Sig. (2-tailed)		.002
	N	16	16

**. Correlation is significant at the 0.01 level (2-tailed).

Table 3.3.5.2.1: *showing the correlation between in situ and remotely sensed chlorophyll concentrations for the data set which excludes outliers.*

A Pearson product-moment correlation coefficient was computed to assess the relationship between *in situ* and remotely sensed chlorophyll concentrations for the data set which excludes outliers (Table 3.3.5.2.1). There was a positive correlation at the 99% confidence level between the two variables, r = 0.720, n = 16, $p = 0.002$. A scatterplot summarizes the results (Figure 3.3.5.2.1). There is a strong, positive correlation between *in situ* and remotely sensed chlorophyll concentrations. An increase in chlorophyll concentrations in the *in situ* data correlates well with an increase in chlorophyll concentrations derived from the remotely sensed data. The removal of the outliers brings about a much stronger correlation between the two methods of chlorophyll determination.

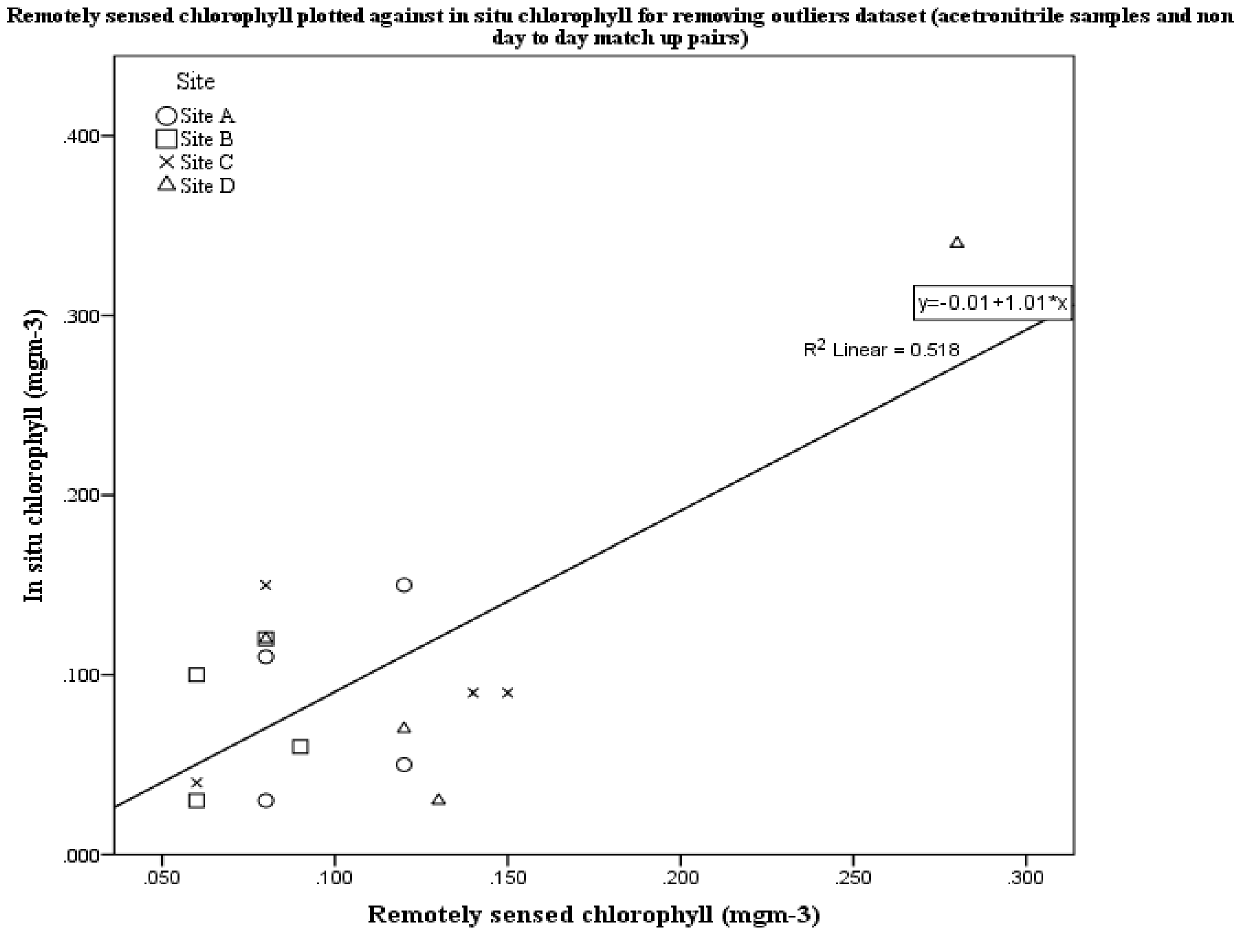


Figure 3.3.5.2.1: *Showing the correlation between in situ and remotely sensed chlorophyll concentrations for the data set which excludes outliers.*

3.3.5.3 Offline validation statistical technique of remote sensing datasets

Dataset	**r^2**	**RMS**	**Bias**	**RPD**	**APD**
Entire Dataset	0.168	0.067	-0.004	39.7	75.70
Site A	0.149	0.040	0.018	54.3	73.4
Site B	0.199	0.043	0.026	79.2	91.1
Site C	0.007	0.056	0.010	24.3	56.1
Site D	0.201	0.111	-0.066	1.1	77.7
Removing Outliers	0.518	0.052	0.009	50	77.1
MedOC4	0.620	0.730	-0.110	40	3

Table 3.3.5.3.1: *Showing the Correlation coefficient (r^2), Root Mean Square (RMS), Mean Bias Error (BIAS), Relative Percentage Difference (RPD) and Absolute Percentage Different (APD) between in situ chlorophyll measurement and Remotely Sensed chlorophyll values for each of the ground truthing datasets analysed in this study*

The statistical parameters utilised to validate the remote sensing results are shown in Table 3.3.5.3.1. These include all the datasets along with the dataset MedOC4 from Volpe *et al* (2007). The MedOC4 algorithm dataset was included for comparison with the datasets obtained from this study. This dataset was used since it was composed of a large number of validated *in situ* and remotely sensed match up points. The dataset Removing Outliers (r^2= 0.518) has the best r^2 value form all the datasets analysed. The dataset Removing Outliers is an improved version of the dataset Entire Dataset. The r^2 value of the dataset Entire Dataset was 0.168. This indicates a significantly weaker correlation than the dataset Removing Outliers. Site A (r^2= 0.149) and Site C (r^2= 0.007) datasets have a significantly weaker correlation between remotely sensed and *in situ* determined chlorophyll levels, than the dataset Site D (r^2= 0.201) and Site B (r^2=0.199).

Site D has the highest spread of data with an RMS value of 0.11 mg m^{-3}. All the other sites, along with the datasets Entire Dataset and Removing Outlier dataset have similar RMS values, ranging from 0.04 mg m^{-3} (Site A) to 0.07mg m^{-3} (Entire Dataset).

The dataset Removing Outliers had the lowest BIAS (0.0094), followed by the dataset Entire Dataset (-0.0305). By removing outliers from the dataset Entire Dataset, a positive BIAS is produced. The datasets representing each of the sites had larger BIAS's values. The reason for this is related to the fact that magnitude of BIAS is associated with the size of the various data sets. The datasets for each of the individual sites had a smaller sample size (*n*= 13) compared with the datasets Entire Dataset and Removing Outliers dataset with a sample size of *n*= 51.

3.3.6 Interpretation of ground truthing results

The results obtained by analysing the dataset Removing Outliers show that there is a relatively strong significant correlation between remotely sensed chlorophyll levels and *in situ* determined chlorophyll levels. However when comparing this with the validation dataset utilised by Volpe in the production of the algorithm MedOC4, some discrepancies were observed. The MedOC4 statistical parameters are shown in Table 3.3.5.3.1, in order to compare the values obtained by this study with those obtained by Volpe *et al* (2007). Volpe's study was chosen for its large validation dataset MedOC4, which is made up of a broad range of Mediterranean offshore waters. The correlation coefficient between remotely sensed and *in situ* determined chlorophyll values in Maltese waters for the dataset Removing Outliers have a similar correlation coefficient (r^2= 0.518) to that of the MedOC4 dataset (r^2 = 0.62).

Despite this, the values for bias and RMS of the Removed Outlier datasets are much lower than that of the MedOC4 dataset. This is attributed to the lower concentrations present in the current study, along with the dataset Removing Outliers having a much smaller number of match up sample points, compared to the dataset utilised in validating the MedOC4 algorithm (Removing Outliers *n*= 16; MedOC4 *n* = 440). This is also reflected in the APD values which are 77.1% and 3% respectively. One would argue that the reason for this large difference is due to the much larger sample size of the MedOC4 dataset.

Despite taking this into account, the difference in APD between the Removing Outliers dataset and the MedOC4 dataset is still very large and can be of concern when using MyOcean MODIS level-2 products to monitoring Maltese waters. The APD value for Maltese waters (77.1 - Removing outliers dataset) is much larger than that of the MedOC4 dataset (3), meaning that a greater difference is present between *in situ* and remotely sensed chlorophyll values. Maltese waters, like much of the central and eastern Mediterranean are considered to be Case 1 waters, due to their clarity and oligotrophic nature. Despite this it is possible that at times these waters might be considered Case 2 waters due to a change in the bio-optical characteristics of the water column brought about by land based influences or stirring up of bottom sediments in shallower seas. A possible explanation for the variation in ADP between the two datasets might be related to a change in the bio-optical characteristics of the water column in waters within 1 km vicinity of the coast.

Operational satellite chlorophyll algorithms tend to be empirical switch band ratios (O'Reilly *et al.*, 1998, 2000). As discussed in Chapter 1 these estimate chlorophyll accurately when the majority of optically active material in the water column is made up of phytoplankton (Sathyendranath *et al*., 1999), but give rise to inaccuracies when colour dissolved organic matter and suspended particulate matter are also found in the water column. Bottom reflectance along with the above contribute to the water leaving signal (Prieur & Sathyendranath, 1981; Darecki & Stramski, 2004), since these bring about spectral signatures of a complex nature.

In order to correct for errors such as the ones mentioned above, semi-analytical ocean colour algorithms have been created. The basis of these algorithms lies in the radiative transfer solutions derived from prior knowledge of IOP's. This is derived from large *in situ* databases for the particular region of study. (Sathyendranath *et al*., 2001; Maritorena *et al*., 2002; Tilstone *et al.*, 2012).

In this study the remotely sensed chlorophyll algorithms utilised by MyOcean Marine Core Service takes this possible variation between Case 1 and Case 2 waters into account. A merged Case 1 – Case 2 dataset based on the MedOC4 algorithm is used for case 1 waters, and the AD4 algorithm is used for Case 2 waters. This method requires the utilisation of the entire light spectrum ranging from the near infra-red to the blue band for both Case 1 and Case 2 waters (D'Alimonte & Zibordi, 2003).

When merging the products retrieved from different algorithms which originated from different water types, a challenge presents itself in trying to identify the differences between Case 1 and Case 2 waters (Volpe *et al.*, 2012). The flag for MODIS and SeaWiFS identifying turbid Case 2 waters is set to the point at which "Rrs (670) exceeds by 25% the expected value for pure water at this particular band" (Hooker *et al*., 2003).

This method has been utilised for this database. The underlying principal in this approach is that the influence of the water leaving radiance in Case 1 waters is close to nill, whilst in Case 2 waters the influence of the water leaving radiance is significant. The reasoning behind performing such a merger is that by producing a mean water type spectral signature from *in situ* measurements of both Case 1 and Case 2 waters, a greater assurance is provided when selecting for the two water types. This will also remove any false gradients that might form due to the application of different algorithms.

Despite the pairing of MedOC4 and the AD4 algorithm, there still seems to be the error mentioned above. Based on the current literature for these two algorithms, such an error is probably related to the AD4 algorithm. Volpe *et al*. (2007) indicated that one of the main reasons for inaccurate chlorophyll retrievals when applying global algorithms to regional seas, was the presence of differences in bio-optical characteristics of an ecological nature. Metsamaa *et al*. (2006) found that different plankton community structures could alter the spectral signature of the water column.

The AD4 algorithm was designed and calibrated based on the *in situ* dataset CoASTS (D'Alimonte & Zibordi, 2003). Most of the *in situ* samples from the dataset were collected from the North Adriatic Sea, at sites found approximately eight nautical miles southeast off the Venice Lagoon. The likelihood that the plankton community structure outside the Venice Lagoon and Maltese coastal waters have a similar plankton community structure and similarities between IOP’s is very slim. D'Alimonte & Zibordi (2003) also stated that the AD4 algorithm tends to overestimate the chlorophyll concentrations in oligotrophic conditions, as in the case in Maltese coastal waters. The reason for this could be associated with the observation described. This is just a hypothesis and has no background upon which to provide a definite conclusion. In order to test this hypothesis the plankton community structure of the two areas would need to be assessed and a remote sensing validation procedure similar to the one described above would need to be carried out for Adriatic waters.

The current study did not have the time or resources available to further verify this. It could be very interesting and important to achieve accurate remotely sensed chlorophyll retrievals in coastal waters by the MyOcean paired MedOC4 - AD4 algorithm. To date few studies have tested the accuracy of the AD4 algorithm, and even fewer have tested it when used in conjunction with the MedOC4 algorithm.

Other influencing factors that could be causing errors in the measurement of chlorophyll concentrations in coastal waters for both empirical algorithms and semi-analytic algorithms, especially when waters are clear Case 1 waters, is bottom reflectance (Carder *et al*., 2005; Cannizzaro & Carder, 2006; Hu, 2008). Ocean colour observations of satellite sensors are limited to the first optical depth, which given the clear nature of many Mediterranean coastal and offshore waters can range, on average, between 15 and 35 meters.

This depth will most of the time exceed the bottom depth in many coastal waters, which will result in the interference of light rays reflecting off the bottom and back out of the water column, thus creating a strong enough water leaving radiance to interfere with chlorophyll measurements. Level 2 products shallower than 30m are flagged as having possible errors. It is important to keep this in mind when analysing the results of this study. Results indicate that the offshore site, Site B, had a relatively good r^2 value and ADP, however in saying this, so did Site D, which has a depth of 15 meters.

The overall results of the study present good chlorophyll satellite retrievals. Further studies are needed to obtain more accurate and reliable information through remotely sensed images using MODIS Aqua data. A similar conclusion was made by (Antoine *et al.*, 2008) and extended to MERIS and SeaWiFS. One of the first steps in such an effort would be to incorporate new algorithms for dealing with bottom reflectance, such as the one utilised by Carder & Cannizzaro (2006), and applying them to MODIS, MERIS and SeaWIFS.

In this study, a method was developed for quantifying chlorophyll concentrations more accurately in oceanic regions containing unknown bottom reflectance contributions. The method is computationally efficient and requires $^{Rrs}\lambda$ data at only four wavebands (412, 490, 555, and 670 nm). These or similar wavebands are currently available for several operational satellite ocean colour sensors (SeaWiFS, MODIS, and MERIS). In this study, shipboard hyperspectral $^{Rrs}\lambda$ data collected from the west Florida shelf and Bahamian waters (1998–2001; n =451) are partitioned into water column and bottom reflectance spectra using the Lee & Carder (2004) optimization technique. The percentage contribution that the bottom reflectance makes to $^{Rrs}(555)$ could then be calculated.

Using this technique, the data are categorised as optically shallow, optically deep, or transitional based on criteria developed for the relationship between the band-ratio $^{Rrs}(412)/^{Rrs}(670)$ and the spectral curvature about $^{Rrs}(555)$, $[^{Rrs}(412) *^{Rrs}(670)]/^{Rrs}(555)$. Chlorophyll concentrations for data classified as optically deep are calculated from $^{Rrs}(490)/^{Rrs}(555)$ using a cubic polynomial function developed in this study for data with bottom reflectance contributions at 555 nm less than 25%. An alternative empirical algorithm for data classified as optically shallow based on the band-ratio $^{Rrs}(412)/^{Rrs}(670)$ is developed from the bottom reflectance at 555nm less than 25% data. The logic behind using $^{Rrs}(412)/^{Rrs}(670)$ instead of $^{Rrs}(490)/^{Rrs}(555)$ for optically shallow waters is that $^{Rrs}(412)$ and $^{Rrs}(670)$ are typically located outside of the spectral transparency window and influenced less by bottom reflectance. Algorithm switching artefacts are avoided by using a weighted blend of chlorophyll concentrations derived by both band-ratio algorithms for data classified as transitional.

The results of Carder & Cannizzaro (2006) showed that the root-mean-square error (RMSElog10) calculated for the entire data set using the new technique was 26% lower than the error derived using the standard blue-to-green, band-ratio algorithm. This study demonstrates that the potential of quantifying chlorophyll concentrations more accurately from multi-spectral satellite ocean colour data in oceanic regions containing optically shallow waters and has been described by the author as applicable to different water regimes, however the empirical algorithms and classification criteria may have to be adjusted regionally taking into account local CDOMλ to SPMλ ratios.

3.4 Conclusion

The main conclusions that can be made from this study are:

- A statistically significant variation in chlorophyll concentration between the four sites found in the Mediterranean Sea analysed in this study. This was dominated by a west to east gradient increase in the oligotrophic nature of sea water. This corroborates with observations made by previous studies.

- There was a significant seasonal variation at each site with each season having a significantly different mean concentration to the other. This indicates that seasonality is a significant driver of chlorophyll dynamics. The physiochemical parameters utilised in chapter 2, such as sea surface temperature, mixed layer depth, wind speed and rainfall, are used as a measure of seasonality by this study. The variation in of chlorophyll from one season to another as measured by remote sensing values provides evidence that these physiochemical parameters, which vary seasonally, have a strong influence of change in chlorophyll concentrations.

- The ANOVA and Kruskal Wallis analysis indicated that Maltese waters and Ionian Seas had no significant variation in annual mean chlorophyll levels in the years of 1997 to 2014. The Ligurian-Provencal Sea was seen to have significant differences over the entire dataset, but the *post hoc* analysis could not identify any individual year to be significantly different to any other year during the 17 year period analysed.
- The Ionian Sea was the only site of the three sites analysed which had significant variations in the annual winter mean chlorophyll concentrations over the 17 years analysed by this study.

- Mean chlorophyll concentration of the 17 year dataset in the Ligurian-Provencal Sea seems to be increasing and that of Ionian Sea seems to be decreasing. Maltese waters appear to have a very slow rate of change. This study could not come to any concrete conclusions as to why these trends might be occurring.

- Remote sensing is a very useful tool for assessing long term changes in chlorophyll. For this reason it should be a priority for the scientific community investigating chlorophyll dynamics to obtain accurate remote sensing retrievals. The results indicated that the dataset' Entire Dataset' did not perform well (r^2= 0.168). There was a major improvement in the performance when the outliers were removed (r^2= 0.518). Despite the strong r^2 value, the offline validation techniques based on the dataset 'Removing Outliers' dataset had a low RMS value (0.052), and a high RPD (50) and APD (77). The reasons for this were most likely attributed to errors in estimating chlorophyll concentrations by the semi analytical algorithm AD4, due to its calibration using North Adriatic waters, resulting in a specific set of IOPs, along with the effect of bottom reflectance interference.

The methods used to test the accuracy of the latest Mediterranean Ocean Colour algorithms provided a rigorous test of the applicability of such algorithms to central Mediterranean coastal and offshore waters. As yet, such analysis using the combination of a merged Case1 - Case2 dataset (MedOC4 and AD4 algorithms) has not been tested in the central Mediterranean region. This study provides a robust statistical baseline upon which to evaluate the performance and accuracy of the combination of this dataset.

Future studies are recommended to focus on creating larger more robust algorithms based on extended time series data having a basin wide dataset upon which to make measurements, in order to get as wide a range of Mediterranean Sea values and water types as possible. It is recommended that algorithms such as the one developed by Carder & Cannizzaro (2006) should be utilised for waters shallower than 30 meters.

Chapter 4: A bio-physical model of chlorophyll dynamics in Maltese waters (Central Mediterranean)

4.1 Introduction

Monitoring programs have recently been introduced to Maltese waters. Remote sensing observations are an efficient way of extracting a synoptic view of a number of parameters in the marine environment. However, as described in the previous chapter, these can perform poorly in coastal areas. *In situ* sampling is a more accurate method of obtaining information about the marine environment than remote sensing techniques. The drawback of these techniques is that they are considered to be expensive, labour intensive, and only provide a single point view of a zone in which parameter variation often follows steep gradients over a relatively small area. Biogeochemical gliders have started to be used in other coastal zones in various parts of the world. Due to the high cost associated with the use of such tools and limited funding it is unlikely that these resources will be utilised in the Mediterranean region, in the near future.

Modelling techniques provide a good alternative to the methods described above. When a sound understanding of the physical and biological property dynamics has been established, a reliable and efficient simulation of the marine environment can be created. This is attained by gathering the theoretical knowledge of shelf seas, such as remotely sensed and *in situ* observations, along with equations quantifying these physio-chemical properties; and then coding them into a model. This provides a good insight as to how the system functions when the experimental observations are limited.

The agglomeration of knowledge of the physics of shelf seas has shown that seasonal cycles and regional distribution of phytoplankton is dependent on the relationship between the requirements of phytoplankton, such as light and nutrients, and vertical mixing (Pingree *et al.*, 1978). By running numerical simulations of this relationship, it is possible to predict the outcome of phytoplankton concentration in shelf seas.

Sharples & Tett (1994) used such a setup in constructing a bio-physical model. A level 2 turbulence closure scheme was utilised to calculate the temporal development of depth-varying vertical diffusivities. This was coupled with a cell-quota limitation threshold biology to simulate phytoplankton growth in a seasonally stratified shelf sea. This was then used to predict a realistic midwater maximum of chlorophyll concentrations.

An adapted version of Sharples & Tett's (1994) model is utilised by this study. A simulated two-layer biophysical setup was used that could simulate surface chlorophyll concentrations. The seasonal variation of depth-varying diffusivities was also applied to this model. The surface mixed layer in the model is forced by surface heating and wind-stirring. Phytoplankton growth in the surface is set to the minimum of light and nutrient-limited growth. The generated vertical distribution of mixing and photosynthetically active radiation is controlled meteorologically. This was designed to produce a concentration of surface chlorophyll concentrations in Maltese shelf seas.

The results of such a climatological model will provide better understanding of past, present and future physical and bio-geochemical properties of the Central Mediterranean shelf seas. This information could be utilised as evidence in supporting regulation and legislation for the protection of the marine environment. An example of such an application could be in the updating of the European Marine Strategy Framework Directive. It could also provide a point of comparison for cross-site analyses and future studies.

4.2 Methodology

4.2.1 Model overview

A two-layer bio-physical model has been constructed in order to test our understanding of this behaviour. The model is an adaptation of the microbiological model created by Tett (1990) for shelf seas (Sharples and Tett, 1994). This is adapted and applied to shelf seas in the Central Mediterranean of depths of around 200 meters. In the model the surface mixed layer is forced by surface heating and wind-stirring. Phytoplankton growth in the surface is set to the minimum of light- and nutrient-limited growth. The output of the model will be the surface temperature, mixed layer depth, dissolved nutrient concentration and biomass concentration. MATLAB was utilised to create and present the results obtained by this model.

4.2.2 Physical Model

The water column is split into two layers; An upper layer of depth *h* and uniform temperature T_s, and a lower layer depth of *d*-*h* and temperature T_b. *d*. This is the total depth of water column from surface to bottom.

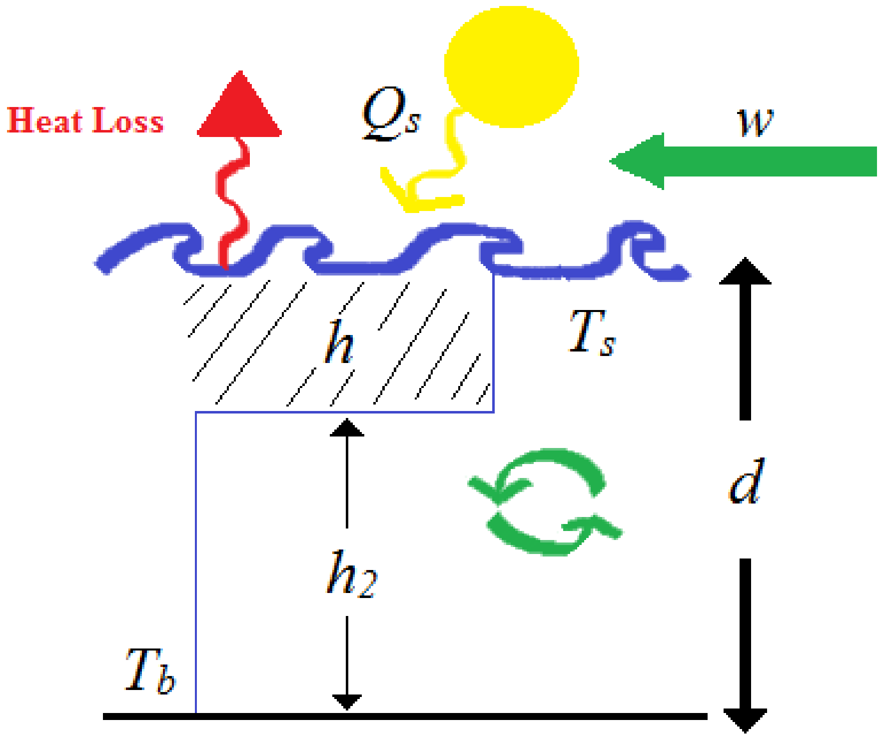


Figure 4.2.2.1: *Showing a visual representation of the two-layer bio-physical model designed to calculate the daily change in surface chlorophyll concentrations*

The physical model works by matching the heat content and potential energy of the two-layer water column to values predicted from the heat flux through the sea surface and the stirring of the water column by the wind. The heat content is predicted by integrating, with respect to time, the heat flux through the sea surface. The potential energy is predicted by allowing fixed fractions of the turbulent kinetic energy, put into the sea by wind, to increase the potential energy of the water column. The temperatures and layer thickness are predicted by this local balance of heating and stirring.

The heat content of the water column is given by

$$Q = (c_p \rho)[d \bullet T_b + h \, \Delta T] \qquad (4.1)$$

Where c_p is the specific heat of seawater and ρ is the density of seawater (see table 4.2.2.1.1). ΔT (=T_s-T_b) is the temperature difference between layers. The rate of change of heat content is equal to the net flux of heat through the sea surface, Q_{dot}. Starting from initial conditions on day 1, the change in heat content in a day is given by Q_{dot}. In a permanently stratified water column, c_p, ρ, d and T_b are all constant, so the value of Q_{dot} allows for the calculation of the change in the product $h\Delta T$.

The potential energy of a two layer water column, relative to the mixed state is

$$E = 0.5\, \rho \alpha g\, [hdT]\, (\mathrm{d} - \mathrm{h}) \tag{4.2}$$

Where α is the thermal expansion coefficient of seawater (see table 4.2.2.1.1). The change in potential energy, brought about by heating and stirring, in one day is

$$\frac{dE}{dt} = \frac{\alpha g\, Q_{dot}\, d}{2c_p} - \delta\, k_s\, \rho_a w^3 \tag{4.3}$$

Where g is the acceleration due to gravity, δ is an efficiency of mixing, k_s a surface drag coefficient, ρ_a is air density and w, wind speed. The first term on the right hand side of this equation represents the increase in potential energy (relative to the mixed state) caused by thermal expansion and the second term the reduction in potential energy produced by mixing.

The model works by calculating the change in energy in one day from equation 4.3. The new potential energy and the value of $h\Delta T$ from equation 4.1 are used to calculate the new mixed layer depth using equation 4.2. Once the new mixed layer depth is known the temperature difference ΔT can be calculated from the known value of $h\Delta T$ and the new surface temperature can be calculated from T_s=T_b+ΔT.

Occasionally in shallow water, the wind may mix the interface between surface and bottom layers right down to the sea bed. When this happens, the bottom temperature T_b is set equal to the surface temperature T_s and the potential energy relative to the mixed layer is set equal to zero.

4.2.2.1 Calculating the net surface heat exchange

The heat flux through the sea surface Q_{dot} is calculated from the following equations

$$Q_{dot} = Q_s - KK(T_s - T_d) \qquad (4.4)$$

Where Q_s is solar radiation falling on the sea surface and KK is a heat exchange coefficient. T_d is the dew point temperature in the atmosphere above the sea. It is calculated from;

$$KK = 4.5 + 0.05T_s + (B + 0.47)f_w$$

Where;

$$B = 0.35 + 0.015T_m + 0.0012{T_m}^2$$

And

$$T_m = 0.5(T_s + T_d)$$

$$f_w = 9.2 + 0.46w^2$$

Values of solar radiation, Q_s, wind speed, w and dew point temperature's, T_d, were obtained by fitting a sinusoidal curves to the data in order to represent the day to day variation over the year. The following equations were used to represent these variables;

$$Q_s = 224 + 6.36sin\left(\frac{2\pi}{365}\right) - 118cos\left(\frac{2\pi}{365}\right);$$

$$w = winda(year) + windb(year)sin\left(\frac{2\pi}{365}\right) + windc(year)cos\left(\frac{2\pi}{365}\right)$$

$$T_d = dewa(year) + dewb(year)sin\left(\frac{2\pi}{365}\right) + dewc(year)cos\left(\frac{2\pi}{365}\right)$$

Constant Value	***Constant Name***
$\rho=1025$	Density of water (m^{-3})
$c_p=3900$	Specific heat of sea water ($Jkg^{-1o}C^{-1}$)
$g=9.81$	Gravity (ms^{-2})
$\alpha=0.00021$	Thermal expansion coefficient for sea water ($^oC^{-1}$)
$\rho_a=1$	Density of air (kgm^{-3})
$K_s=0.002$	Surface Drag coefficient (dimensionless)
$\delta=0.0007$	Efficiency of wind mixing (dimensionless)

Table 4.2.2.1.1: *Showing the constants used in the physical model*

4.2.3 Biological Model

Phytoplankton growth in the marine environment depends mainly on two factors; the availability of light and the availability of nutrients. In the model, the rate of phytoplankton growth is assumed to increase in a linear manner relative to the level of light as shown in figure 4.2.3.1. The line has a gradient α, which corresponds to the photosynthetic efficiency and intercepts the x-axis at an irradiance value Ic, which corresponds to the compensation irradiance.

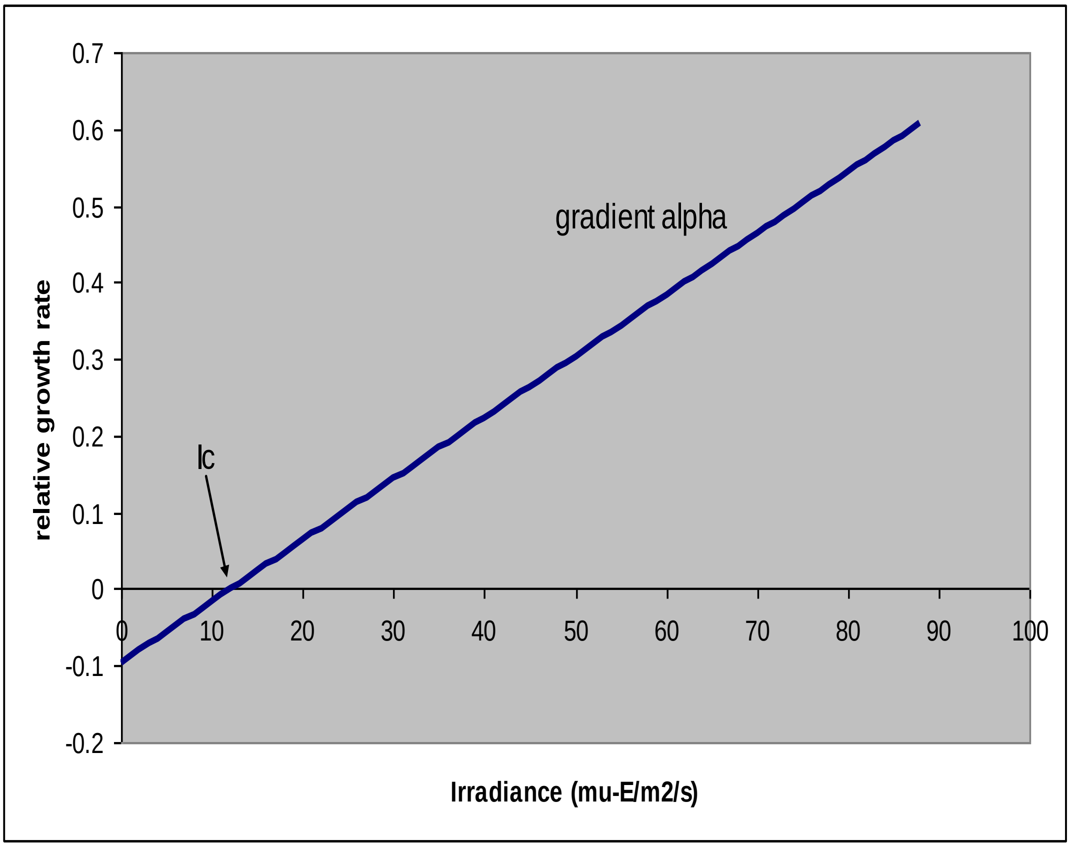


Figure 4.2.3.1: *Showing the growth rate of phytoplankton as a function of Irradiance*

Below the compensation irradiance, the light levels are so low that respiration exceeds photosynthesis by the algae and the biomass decreases. The line shown in the figure 4.2.3.1 can be represented by the equation;

$$dB/dt = \alpha(I - Ic)B \qquad (4.5)$$

dB/dt is the rate of change in biomass (for light limited growth), α is the photosynthetic efficiency, *Ic* is the compensation irradiance and *B* is the biomass at the start of the day. The timestep in solving this equation is 1 day.

The average solar energy arriving at the sea surface every day is known as the direct solar heating flux and is measured in Wm^{-2}. This seasonal variation was calculated for the Maltese Islands as described in the previous section. Data estimating the solar irradiance reaching Maltese waters was obtained from Farrugia *et al.* (2005). Around 40% of this energy comprises of visible light, and is the proportion of light that will be used for photosynthesis. This is calculated by the following equation;

$Io = 2 * Q_s$ (4.6)

where *Io* is the irradiance at the sea surface in $\mu E/m^2/s$.

Light decays exponentially with depth in the sea. The irradiance at depth *z* is given by a rule called the *Beer Lambert- Law*;

$$I = Io\, exp(-kz)$$

The factor *k* (m^{-1}) is called a *diffuse attenuation coefficient*. The higher the value of *k* the more quickly the light diminishes as depth increases. Central Mediterranean waters are considered clear Case 1 waters meaning that they have a very low k value. It can be shown that as depth increases, irradiance will decay at a rate that is proportional to the clarity of the water, *k*. The mean value of the irradiance from the surface down to the depth, *h*, of the thermocline is given approximately by

$I(mean) = Io / (kh)$ (4.7)

The diffuse attenuation coefficient depends on the turbidity of the water. This in turn depends on the suspended sediment load and the chlorophyll concentration, since phytoplankton absorb light and contribute to light attenuation. We can estimate the diffuse attenuation coefficient from the following expression;

$\mathrm{k} = k_0 + k_1\mathrm{B}$ (4.8)

Where k_0 is the background level of attenuation in Maltese waters and k_1 represents the increase in attenuation per unit increase in Biomass (see table 4.2.3.2).

The equations above will simulate phytoplankton growth in a light dependant environment; however they do not take into account nutrient limiting effects on biomass. Small amounts of inorganic nutrients are needed to sustain primary productivity. The main nutrients are nitrate, phosphate, silicate and iron. This model focuses on the effect of nitrate on phytoplankton. In reality the nutrient that is in least supply will be the one controlling phytoplankton growth in light abundant conditions.

The mean content of phytoplankton in seas and oceans is described by the Redfield ratio. This describes the ratio of carbon, nitrogen and phosphorus found in phytoplankton. The Redfield empirically developed stoichiometric ratio is found to be C:N:P = 106:16:1. One gram of chlorophyll is taken in our model to be equivalent to 50 grams of carbon which is 4.17 Moles of carbon. Based on the ratio of *C(6):N(1),* 4.17 Moles of carbon is equal to 0.7 moles of nitrogen. Therefore for every micro-gram of chlorophyll that is produced by microalgal growth, 0.7 micro-Moles of nitrogen will be required and hence taken out of the water. Therefore the rate of change of nitrogen in the surface mixed layer is calculated by;

$$\frac{\mathrm{d}N}{dt} = -0.7 * \frac{\mathrm{d}B}{dt} + \left(\frac{dh}{dt}\right)\left(\frac{1}{h}\right) Nd$$

The first term on the right hand side of this equation represents the removal of nitrate by phytoplankton growth. The second term represents the replenishment of surface nitrogen as the mixed layer deepens and nitrate is transferred from deep water (where the concentration is *Nd*). The second term only applies when *dh/dt*>0. When the mixed layer is shallow, the second term is set to zero.

The daily increase in biomass when growth is nutrient limited is then calculated from

$$dB/dt = \left(\frac{N}{0.7}\right)$$

where *N* is the concentration of nitrate in the surface layer at the start of the day. For example, if N= 0.7 micro-Moles, then dB=1 mg.chl/m^3 per day. If this is less than the change in biomass predicted by the light-limited growth equation, then this is the rate of change of biomass on that day. Finally, grazing is allowed for by removing a constant fraction (equal to 1/10) of the biomass each day.

The equations from the two sections of the model were used to work out how temperature, mixed layer depth, dissolved nitrate concentration and chlorophyll biomass concentrations are expected to vary over a defined period of time. This was done by taking each of these parameters and carrying out a forward time stepping procedure. Initial values of each parameter on day 1 were defined. Then, the concentration on day two was calculated by adding the value of change of this parameter on day two to the value of day one. This procedure was repeated for 19 years and compared to MODIS MedOC4 remotely sensed chlorophyll values in Maltese offshore waters for the period between 1997 and 2014. The results are shown in the next section.

Constant Value	***Constant Name***
$k_0 = 0.01$	Background level of light attenuation m^{-1}
$k_1 = 0.012$	Self-shading constant $m^{-1}/(mg\ chl/m^3)$
$\alpha = 0.008$	Photosynthetic efficiency growth/unit light $(0.008/day/(uE/m^2/s))$
$I_c = 10$	Compensation irradiance $(muE/m^2/sec)$
$Nd = 2$	Nutrient concentration in the deep layer (mM/m^3)

Table 4.2.3.1: *Showing the constants used in the biological model*

Biomass and nutrient concentrations on the first day of the model (corresponding to January 1^{st} 1997) were set as follows;

$b = 0.1$; biomass at day 1 $(mgchl/m^3)$

$N = 0$; initial surface nitrogen concentration (mM/m^3)

4.3 Results and Discussion

4.3.1 Accuracy of model simulations

Numerical simulations give a virtual depiction of real life events, which can be manipulated and applied to different scenarios and provide high frequency results that help quantify and predict outcomes that are likely to occur.

The results from the model simulated by this study were overlaid by remotely sensed monthly mean chlorophyll values in order to produce a comparative schematic, thus making it possible to visually analyse the similarity between the two. Remotely sensed data was obtained from the same source utilised in Section 3.2 of this study. Since the model is designed for shelf seas in the central Mediterranean, remotely sensed data from Maltese offshore waters was utilised for this comparison. Remotely sensed chlorophyll data was obtained for the period 1997 up to 2014. Model simulations were run for the same period. The results from the model produced daily chlorophyll concentration as opposed to mean monthly chlorophyll levels provided by the remotely sensed data.

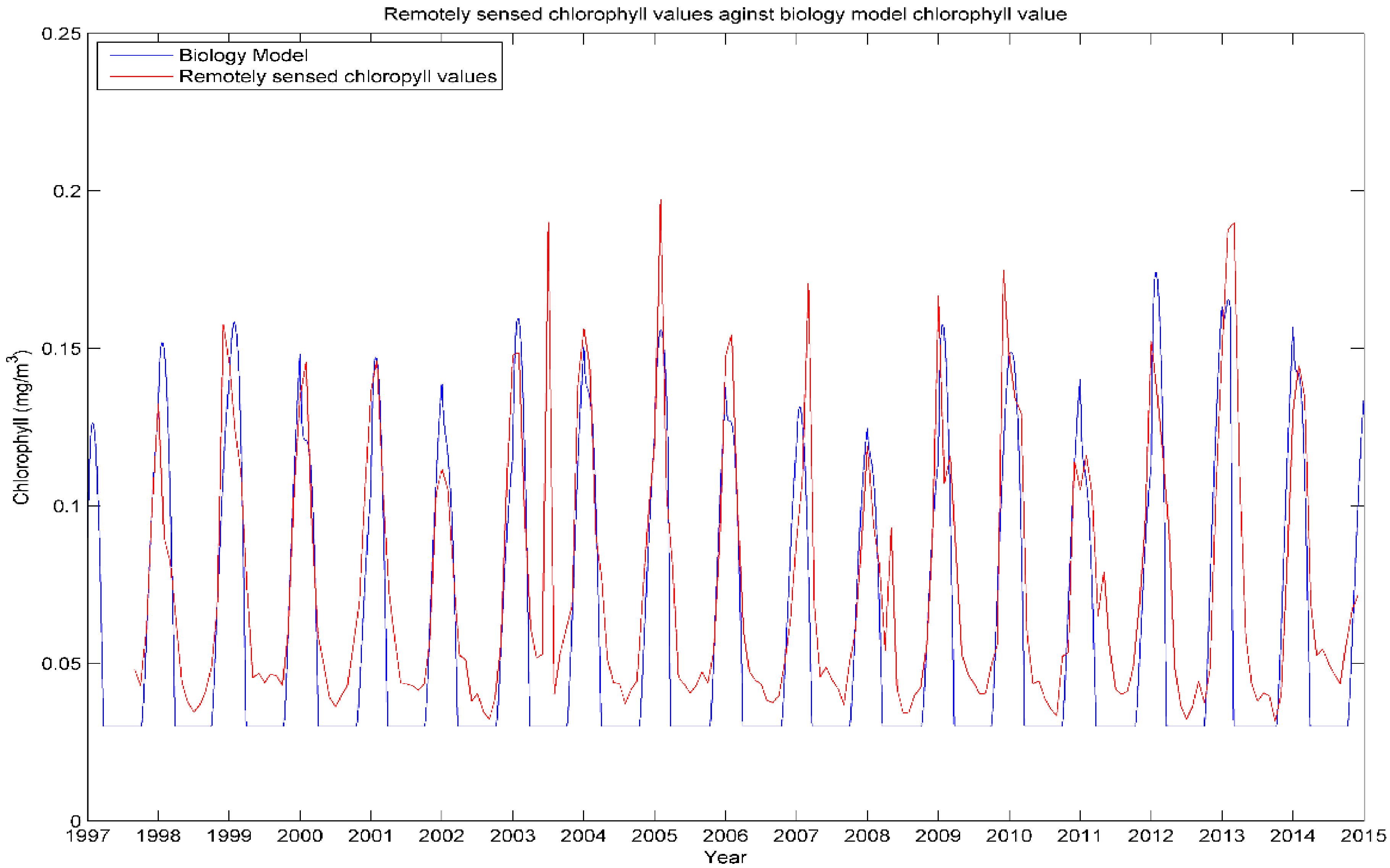


Figure 4.3.1.1: *The results of the biology model overlain by the remotely sensed monthly mean chlorophyll data from 1997-2014*

The results from the model (Figure 4.3.1.1) present a trend which is very similar to the remote sensing measurements of chlorophyll. There is an almost identical pattern between remotely sensed chlorophyll values and modelled chlorophyll values on an annual basis. Chlorophyll values range from 0.03mg m^{-3} to 0.15mg m^{-3}. Strong peaks in annual chlorophyll concentrations are observed in 2005 and 2013, where chlorophyll values of around 0.2 mg m^{-3} are registered by the MODIS satellite. The biological model matches these peaks closely, however just falls short and reaches maximum peak levels of around 0.15mg m^{-3}, with the highest chlorophyll value obtained by the model being observed in 2012 (0.169mg m^{-3}).

The annual seasonal variation of chlorophyll also appears to be in sync with the remotely sensed data. There does not appear to be any lag between the model and the remotely sensed chlorophyll values. A misalliance that was observed is that the model did not detect the small spring blooms that occurred in 2003, 2008 and 2011.

In the late spring of 2003 the remotely sensed data exhibits a bloom that rose to a very high concentration. The concentration was particularly high for central Mediterranean waters, for that time of year. The remote sensing dataset was compiled by determining the average monthly chlorophyll values for Maltese surface waters within a radius of 52 miles from a point one mile offshore from Malta. The chances that such an extensive bloom occurred throughout these waters during a period when surface waters are nutrient depleted is highly unlikely. The most logical explanation for this observation is that there could have been an error in the algorithm data itself, which might have caused such a high late spring bloom in 2003.

In the years where the remote sensing observations register lower annual mean chlorophyll values, the model produces an identical response, exhibiting values which are lower in concentration, than the previous years. Examples of this can be seen in the years 2002, 2008 and 2011, where unusually low chlorophyll concentrations were observed by the remotely sensed data. The model reproduced trends where both the concentration and the period of the winter bloom are reproduced in each of those years. This indicates that the model is working well and producing accurate determinations of chlorophyll concentrations.

A Pearson product-moment correlation coefficient was computed to assess the relationship between the biology model predicted mean monthly chlorophyll concentrations and remote sensing mean monthly chlorophyll concentrations for Maltese waters. There was a significant positive correlation between the two variables, r = 0.781, n = 208, $p = 0.0001$. There is a strong, positive correlation between remote sensing and predicted chlorophyll concentrations in Maltese waters.

Correlations

		Biology_model_monthly_CHL_mg_m3	Sat_monthly_CHL_mg_m3
Biology_model_monthly_CHL_mg_m3	Pearson Correlation	1	.781**
	Sig. (2-tailed)		.000
	N	208	208

**. Correlation is significant at the 0.01 level (2-tailed).

Table 4.3.1.1: *Showing the correlation between the biology model and satellite chlorophyll values*

Overall the model appears to be more accurate in calculating chlorophyll concentrations in years that register low chlorophyll concentrations during the bloom season, rather than when high chlorophyll concentrations are being measured. The reasons for this may be related to radiation and wind speed dynamics, which are both inputs of the model. This will be further discussed in section 4.3.3 below.

4.3.2 Seasonal pattern

In order to further validate the model's accuracy in determining chlorophyll concentrations in Maltese shelf seas, seasonal values were analysed. Figure 4.3.2.1 was compiled by taking the average and standard deviation values of remotely sensed chlorophyll concentration from 1997 to 2014. These were overlaid onto the chlorophyll concentration values determined by the model for 1998. The model and the remotely sensed values are in phase both in the period of bloom and concentration. A clear seasonal cycle is observed with a maximum during the winter months January-February and a minimum during the summer months in both the model and remotely sensed chlorophyll levels. This pattern is typical of mid-latitudes seasonal cycle, as described in the previous chapters and by other authors; (Longhurst, 2001; Bosc *et al.*, 2004).

Therefore, this model can be applied for calculating chlorophyll concentrations to most central and eastern Mediterranean shelf seas, with depths of around 200 meters,

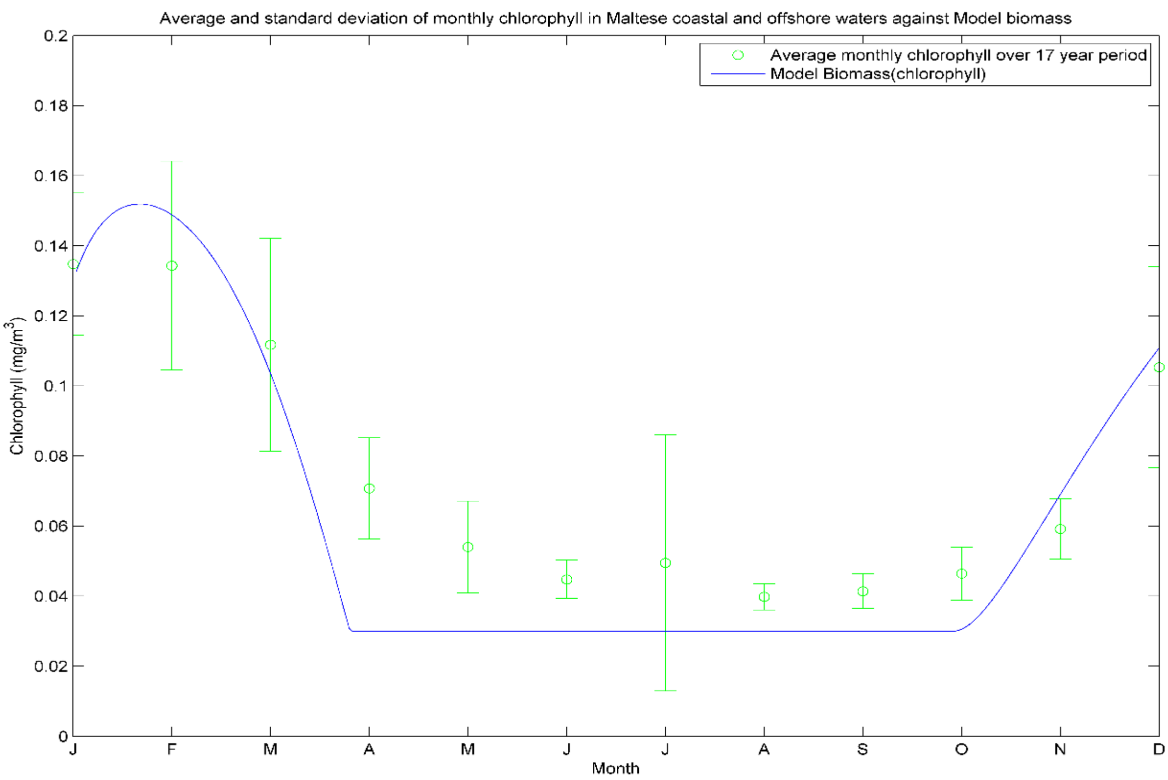


Figure 4.3.2.1: *Showing the results of the biomass levels obtained for the year 1998 determined by the model against the average monthly chlorophyll level and standard deviation extracted from the MyOcean chlorophyll multi-satellite reprocessed dataset in the period 1997-2014 in and around Maltese waters*

The chlorophyll values determined by the model during the summer months are slightly out of phase when compared with mean chlorophyll values obtained by the remotely sensed data. Between March and April the chlorophyll concentrations determined by the model decrease drastically to a level of 0.03mg m^{-3}. This is maintained throughout the spring and summer months. This then starts to increase gradually in October. The reason this occurs is due to the design of the model which has been designed to output a chlorophyll concentration of 0.03mg m^{-3} whenever the daily calculated chlorophyll value falls beyond 0.01mg m^{-3}. This was done in order to prevent the chlorophyll value from becoming negative in the nutrient depleted summer period. This results in the model showing constant ultra-oligotrophic conditions by the beginning of April.

The remotely sensed data shows a gradual decrease in chlorophyll concentration throughout spring and summer, until minimum concentrations are observed in August. There is a small increase in chlorophyll concentrations during the following month of September and further small increases, as autumn and winter set in, reaching peak concentrations in January-February of the following year.

The biological model follows a less harmonic pattern whereby chlorophyll concentrations are seen to settle at a constant minimum value from April up until October. As autumn and winter set in, the chlorophyll concentrations start to resemble the chlorophyll concentrations recorded by the MODIS satellite more accurately. The drivers of change of the chlorophyll concentrations are controlled by various physio-chemical properties of the water column. These are further explored in section 4.3.3 below.

4.3.3 Drivers of change in Maltese shelf seas

The previous chapters and other studies have identified thermal stratification, net surface heat input and surface and bottom nutrient concentrations to be the primary influencing physiochemical factors affecting phytoplankton growth. The model simulated values of these parameters for Maltese shelf waters. These are shown in Figure 4.3.3.1. Biomass is included in this figure and represents chlorophyll concentration.

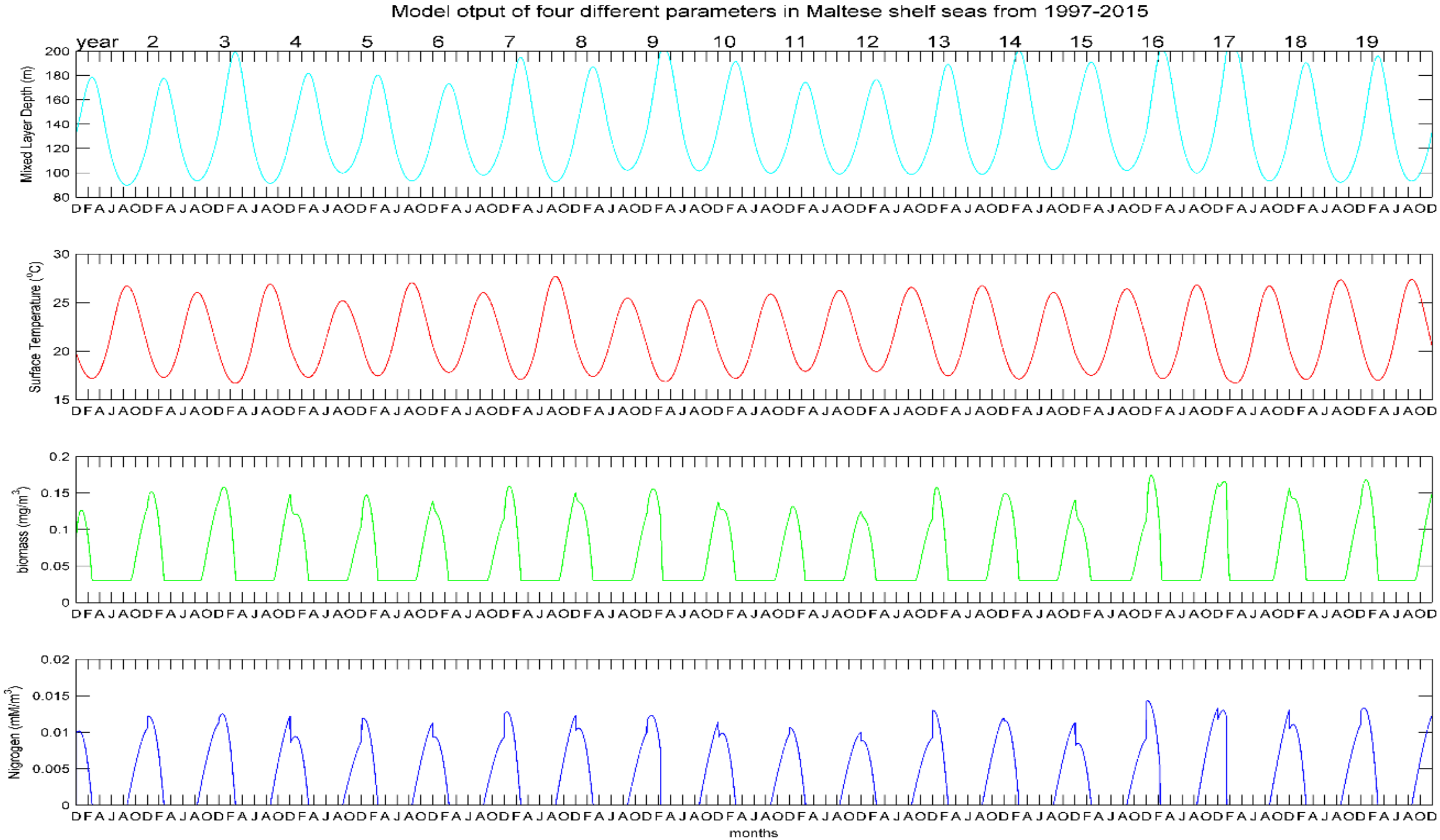


Figure 4.3.3.1: *The results of the model for four different parameters; mixed layer depth, sea surface temperature, biomass (chlorophyll concentration) and nitrogen (representing nutrients) over a 19 year period from 1997 to 2015.*

Mixed layer depth plays an important role in regulating the production of phytoplankton both temporally and spatially. The mixed layer depth simulated by this model shows depth values rising to a depth of 80 meters during the summer months, reaching a peak during August and September. As winter sets in, the mixed layer depth is pushed deeper, as the upper layers of the water column become more homogeneous. The maximum depth, of the mixed layer depth, of around 180 to 200 meters is reached during the month of March. The inter-annual variability of the mixed layer depth has a relatively high degree of consistency, with little variation from one year to another in both period and magnitude, for both the winter and summer months.

In spring the waters become stratified again, as calmer conditions are seen in surface waters. This pattern corroborates with other studies for the central and eastern Mediterranean Sea, most notably with the study of D'Ortenzio *et al.* (2005), who identified a significant spatial and temporal correlation between phytoplankton and mixed layer depth dynamics.

The pattern exhibited by mixed layer depth corresponds to that of surface temperature. As can be seen from the description of the model in section 4.2.2.1, these values are influenced and determined by the direct solar heating flux, *Qs*, the dew point temperature *Td*, along with the heat exchange coefficient, *KK*, which is dependent on wind speed, *w*. Thus, the highest temperatures were observed during the summer months, with max surface water values peaking at around 27.5^{o} C during late August and September.

The lowest temperatures were recorded during March with values reaching a minimum of 16^{o}C. There was little inter-annual variability in the peaks and seasonal period of sea surface temperatures. This dynamic confirms observations made by other studies carried out on sea surface temperature in the central and eastern Mediterranean Sea, most notably that of Behrenfeld *et al.* (2006). Behrenfeld stated that warmer surface waters and stronger water column stratification are related to lower levels of primary production.

Nutrients have a pivotal role in regulating phytoplankton growth throughout marine waters. In Mediterranean waters, where there is a high level of irradiance throughout the year, nutrients have an even more vital role, as the primary limiting factor that regulate phytoplankton growth. Whether nitrates or phosphates are most limiting to phytoplankton growth was not analysed by this study. This model focused on determining the influence of nitrates on phytoplankton growth. As can be seen from figure 4.3.3.1 the changes in nitrogen levels found in surface waters have an almost identical pattern to chlorophyll levels. Nitrate concentrations are seen to be very low, ranging between 0.0001 to 0.0125 mM/m^{-3}, thus reflecting the oligotrophic nature of the central and eastern Mediterranean Sea.

The highest levels of phytoplankton growth occur in the winter, when high wind speeds deepen the surface mixed layer. By December the convective mixing results in a vertically mixed water column. Thus, nutrients found in deeper waters are made available for phytoplankton in the photic zone, resulting in the highest nutrient concentrations being observed by December. As calmer weather conditions set in and mixing of the water column is reduced, the surface nutrient concentrations decrease drastically due to phytoplankton grazing utilising the surface water column stock of nutrients provided in the winter months.

The re-stratification of the water column stops the supply of nutrients from bottom depths to surface layers. By March the nutrient levels are almost negligible. These low nutrient concentrations remain at a low level throughout spring and summer due to the thermocline preventing bottom nutrients from reaching the surface, until the winds start to pick up again between August and September. This pattern of change in nutrient concentrations is close to being identical to that of chlorophyll levels. This indicates that nutrients play a vital role in phytoplankton dynamics of central Mediterranean waters.

4.4 Conclusion

A model describing the seasonal variation of surface chlorophyll concentrations in Maltese shelf waters for the period 1997-2014 is presented in this study. Model simulations show that the simulated chlorophyll values have a high degree of accuracy when compared to remotely sensed chlorophyll values of Maltese shelf seas. The seasonal variations of the chlorophyll values determined by the model are in coherence with the Longhurst biological domain for the Mediterranean Sea; which is a "subtropical nutrient-limited winter, to winter-spring production period".

The physical and biological components of the model have effectively been combined together, creating a model with an accurate representation of how the physical dynamics of the local area has a direct influence on phytoplankton growth. This biological and physical interaction occurs primarily by vertical turbulent diffusivities modelled as a function of local stability and applying them to time and depth-dependant equations which process the biological factors.

The results simulated by the model in this study indicate that surface wind stress is a predominant factor in controlling phytoplankton dynamics in the photic zone. When forcing by the wind is strong, the thermocline barrier is eroded, which allow nutrient rich deep water to enter the surface layers of the water column. A flux in nutrients into the thermocline can be seen to bring about elevated chlorophyll concentrations. This is caused by a peak in the strength of the mixing processes.

The performance of the temporal variability of chlorophyll concentration suggests that a two layer model such as the one used in this study constitutes a good and useful tool for exploring physio-biological interactions of seasonal variability in shelf seas. This model can be a very useful tool when used for establishing conservation policies, and assessing the impact of developments and activities on phytoplankton along the Maltese coast. It can also be used to predict the possible effect and outcome in terms of phytoplankton growth when changes in the biotope occur due to predetermined or unforeseen events.

Chapter 5: Overall conclusion

Results from this study indicate that mixed layer depth in Maltese waters deepens to 100 meters during the winter season (Figure 2.3.2.1). This depth varies from year to year. Convective mixing during this season can involve the entire column of water with temperatures at the surface being very similar to those found in bottom waters. This was also observed in other regions of the Mediterranean (Gascard *et al.*, 1978).

The peak of winter mixing in Maltese waters occurs around December and January. This introduces nutrients to the surface layers where greater light concentrations are available for photosynthesis. This results in elevated levels of plankton growth during the winter months. As the summer months set in, stabilisation of the surface layer occurs. This stabilisation increases in depth from April to September. During this period nutrients are depleted from surface layers.

These observations are based on the results obtained though both in situ measurements and remotely sensed results. Such observations are similar to measurements made by other studies such as Lazzari *et al.* (2014)'s mixed layer depth study of Mediterranean waters. This study utilised an OGCM model to show how mixed layer depth controls primary production both temporally and spatially in Mediterranean waters. This study quotes D'Ortenzio e*t al.* (2005)'s study as further evidence.

Based on these results, which have led to the above conclusions, the alternate hypothesis, which states that *phytoplankton growth is controlled by the flux of nutrients found in surface waters, which in turn will be influenced by water mixing and the rate at which effluents derived from human activities will enter the water,* is accepted.

Data collection for this study was carried out throughout the year, thus providing a broad spectrum of data for different parameters from different seasons. Such a broad study, containing such a quantity of data on phytoplankton dynamics has never been carried out in Maltese waters.

This study has produced a statistical baseline for assessing variations of phytoplankton concentrations, along with investigating which physiochemical parameters are bringing about such a seasonal variation. The methods used for analysis provides a rigorous test, based on statistical significance, for the hypothesis that is being investigated.

In practical terms this study has established which parameters govern phytoplankton growth in Maltese waters. This will help towards increasing the overall understanding of the processes controlling the growth of primary producers in the marine environment, upon which the marine ecosystem depends. These results have given contributed towards the development of a model that can be used to predict chlorophyll concentrations in Maltese waters. This model can be applied to a number of practical uses for industry and future conservation efforts. This model produced accurate readings that had a strong correlation with remote sensing results ($r = 0.781$, $n = 208$, $p = 0.0001$).

The analysis of the Med OC4 algorithm and OC4 algorithm and its accuracy in coastal and offshore sites in the central Mediterranean was also tested by this study. This combination of algorithm had not been tested in central Mediterranean waters prior to this study. The results obtained here provide a robust statistical baseline upon which to evaluate the performance and accuracy of this algorithm. The ground truthing exercise carried out found that such a combination of algorithms produced relatively accurate chlorophyll retrievals in Maltese waters, however considerable work still needs to be done in order to reduce errors such as high RPD (50) and APD (77) values.

Some limitations that could be worked on in future studies is applying improved algorithms which will address bottom reflectance in coastal waters, where the depth is less than 30 meters. This study gives the example of Carder & Cannizzaro (2006) which reduces the error attributed in bottom depth reflectance by 26%. This is of even greater importance in clear case 1 waters. The next step in such a study would be to apply these algorithms to a higher resolution sensor. This would make satellites more adapted to measuring values in coastal waters.

Another issue highlighted by this study was that in order to further improve remote sensing retrievals from the marine environment, the calibration of algorithms must be carried out using datasets which have IOP's similar to the area being investigated. The phytoplankton species community structure in a body of water most likely has a stronger influence on retrievals than expected. Future studies should treat this issue as a priority when validating remote sensing images from marine habitats.

The main limitation associated with the study is the low frequency of sampling. The growth rate of phytoplankton populations is rapid with a doubling time of 2 to 4 days (Grahame, 1997). Given the highly variable nature of chlorophyll concentrations it would be desirable to increase sampling intervals along with frequent physiochemical measurements of the vertical profile of the water column. This will enable a more realistic assessment of the variation of phytoplankton concentrations over time, and with depth, and provide an estimate of the level of the deep-water chlorophyll maximum over shorter timescales.

In order to further the understanding of the dynamics of phytoplankton populations in Maltese waters, further studies should be carried out using sampling stations that take repeated daily or weekly measurements at fixed points throughout the vertical water column. This will enhance the current knowledge about phytoplankton dynamics and provide important data needed to preserve as well as utilise this key natural resource which forms the basis of the marine food chain.

Appendix 1: Survey sheet showing all the data collected for the parameters analysed by this study

Date: 07/05/15		**Site:** A		**Coordinates;** N 36º 00' 5.22" E 014º 21' 37.5"										
Depth (m)	Wind Speed (mph)	Wind Direction	Temp (ºC)	pH	Dissolved Oxygen (mg/L)	Secchi Depth (m)	Conduct (mS/cm)	Cloud Cover (%)	Rainfall (mm)	Chlor *a* (mg m^{-3})	SPM (mg m^{-3})	MSS (mg m^{-3})	CDOM	Salinity (PSU / PPT)
0.3	13	NW	19.4	7.83	8.82	7.5	53.9	0	0	0.04	5.84	/	0.2048	40.5
20	13	NW	18.29	7.9	8.89	7.5	52.8	0	0	0.05	5.50	/	0.1451	39.6
		Site: D		**Coordinates;** N 35º 54' 00.36" E 014º 31' 40.98"										
0.3	13	NW	19.12	8.1	8.87	7	52.5	0	0	0.17	4.37	/	0.1945	39.6
15	13	NW	18.38	7.95	8.85	7	52.7	0	0	0.08	6.19	/	0.1978	38.5
		Site: C		**Coordinates;** N35º 49' 04.98" E 014º 33' 28.62"										
0.3	13	NW	18	7.31	8.5	6	53	0	0	0.08	5.94	/	0.0178	41.2
20	13	NW	17.1	7.35	8.3	6	52.4	0	0	0.10	5.70	/	0.1149	41.5
Date: 20/05/2015		**Site:** A		**Coordinates:** N 36º 00' 5.22" E 014º 21' 37.5"										
0.3	9	SE	19.9	8	8.3	7	55.6	0	0(Rained 2 days earlier)	0.06	0.98	/	0.1315	41.5
20	/	/	/	/	/	/	/	/	/	/	/	/	/	/
		Site: B		**Coordinates;** N35º 54' 01.68" E 014º 19' 08.82"										
0.3	9	SE	19.7	8.1	8.5	6	53.1	0	0	0.03	1.34	/	0.1190	39.6
55	/	/	16.2	/	/	/	/	/	/	/	/	/	/	/
		Site: C		**Coordinates:** N 35º 49' 04.98" E 014º 33' 28.62"										

0.3	9	SE	20.2	7.58	8.61	6	54.3	0	0	0.08	0.99	/	0.1237	40.1057
20	/	/	19.4	/	/	/	/	/	/	/	/	/	/	/
		Site: D		**Coordinates:** N 35º 54' 00.36" E 014º 31' 40.98"										
0.3	9	SE	20	7.81	8.27	5	53.8	0	0	0.31	1.08	/	0.1320	39.9
15	/	/	17.9	/	/	/	/	/	/	/	/	/	/	/
Date: 05/06/2015		**Site:** A		**Coordinates:** N 36º 00' 5.22" E 014º 21' 37.5"										
0.3	7	N	20.7	7.75	8.61	9	55.2	0	0	0.08	-1.39	/	0.1641	40.4
20	7	N	18.7	7.53	8.76	9	57	0	0	0.02	1.07	/	0.0780	41.3
		Site: B		**Coordinates:** N35º54' 01.68" E 14o 19' 08.82"										
0.3	7	N	21.95	7.49	8.85	14	57.8	0	0	0.02	1.28	/	0.0536	41.3
55	7	N	16.29	7.54	/	14	58.5	0	0	0.01	1.59	/	0.0613	41.6
		Site: C		**Coordinates:** N35º 49' 04.98" E 014º 33' 28.62"										
0.3	7	N	19.9	7.57	8.87	9	58.7	0	0	0.11	0.69	/	0.1948	44.12
20	7	N	18.7	7.62	8.77	9	58.7	0	0	0.11	0.66	/	0.1301	45.42
		Site: D		**Coordinates:** N35º 54' 00.36" E 014º 31' 40.98"										
0.3	7	N	22.2	7.59	8.47	7	57.3	0	0	0.13	1.11	/	0.254	40.6
15	7	N	19.4	7.78	8.75	7	57.9	0	0	0.10	1.03	/	0.135	43.45

Date: 16/06/2015		**Site:** A		**Coordinates:** N 36º 00' 5.22" E 014º 21' 37.5"										
0.3	10	N-NE	24.2	7.14	8.2	16	59.1	0	0	0.02	0.88	/	0.195	40.2
20	10	N-NE	22.8	7.31	8.4	16	59.2	0	0	0.02	0.84	/	0.1921	41.6
		Site: B		**Coordinates:** N35º54' 01.68" E 14º 19' 08.82"										
0.3	10	N-NE	24.7	7.17	8.13	13	59.5	0	0	0.03	0.9	/	0.1823	40.1
55	10	N-NE	21	7.39	8.78	13	59.3	0	0	0.02	0.96	/	0.0846	43.48
		Site: C		**Coordinates:** N35º 49' 04.98" E 014º 33' 28.62"										
0.3	10	N-NE	24.1	7.24	8.32	10	59.5	0	0	0.05	0.69	/	0.0946	40.6
20	10	N-NE	22.5	7.52	8.45	10	59.6	0	0	0.07	1.07	/	0.1173	42.1
		Site: D		**Coordinates:** N35º 54' 00.36" E 014º 31' 40.98"										
0.3	10	N-NE	24.9	7.2	8.16	19	59.4	0	0	0.05	0.68	/	0.0843	39.8
15	10	N-NE	24.1	7.61	8.42	19	59.5	0	0	0.06	0.77	/	0.1029	40.6
Date: 18/07/2015		**Site:** A		**Coordinates:** N 36º 00' 5.22" E 014º 21' 37.5"										
0.3	8	N	26.4	7.4	7.82	17	58.9	0	0	0.07	1.34	/	0.1125	38.2
20	8	N	21.9	7.44	8.86	17	58.7			0.04	2.17	/	0.0972	42

		Site: B		**Coordinates:** N 35º54' 01.68" E 14º 19' 08.82"										
0.3	8	N	25.7	7.75	8.06	19	59	0	0	0.03	1.31	/	0.0352	38.8
55	8	N	21.1	7.57	8.5	19	58.7	0	0	0.04	0.87	/	0.0996	42.8
		Site: C		**Coordinates:** N 35º 49' 04.98" E 014º 33' 28.62"										
0.3	8	N	26.3	7.75	7.97	17	59.3	0	0	0.13	0.87	/	0.1931	38.6
20	8	N	24.9	7.7	8.17	17	59.1	0	0	0.11	1.01	/	0.2665	39.6
		Site: D		**Coordinates:** N 35º 54' 00.36" E 014º 31' 40.98"										
0.3	8	N	28	7.75	7.75	10	59	0	0	0.23	0.75	/	0.2129	37
15	8	N	27.5	7.78	7.7	10	59	0	0	0.13	0.78	/	0.2136	37.4
Date: 08/08/2015		**Site:** A		**Coordinates:** N 36º 00' 5.22" E 014º 21' 37.5"										
0.3	12	N	29.4	7.39	7.38	9	59.7	0	0	0.07	0.33	0.11	0.0538	36.3
20	12	N	28.3	7.49	7.72	9	59.6	0	0	0.06	1.08	0.525	-0.265	37.1
		Site: B		**Coordinates:** N 35º54' 01.68" E 14º 19' 08.82"										
0.3	12	N	28.7	7.64	7.53	13	59.6	0	0	0.04	0.45	0.1925	0.0628	36.8
55	12	N	25.2	7.68	8.46	13	59.6	0	0	0.03	0.45	0.19	0.1382	39.7

		Site: C		**Coordinates:** N 35º 49' 04.98" E 014º 33' 28.62"										
0.3	12	N	28.7	7.71	7.38	9	59.7	0	0	0.05	0.58	0.2475	0.1450	36.9
20	12	N	26	7.83	8.19	9	59.1	0	0	0.15	0.73	0.315	0.2082	38.6
		Site: D		**Coordinates:** N 35º 54' 00.36" E 014º 31' 40.98"										
0.3	12	N	28.9	7.84	7.45	6	59.7	0	0	0.09	0.50	0.205	0.1581	36.7
15	12	N	29	7.85	7.37	6	59.6	0	0	0.32	2.05	0.8725	0.2626	36.6
Date: 21/08/2015		**Site:** A		**Coordinates:** N 36º 00' 5.22" E 014º 21' 37.5"										
0.3	13	NW	27.4	7.61	7.71	15	60.2	0	0	0.06	0.23	0.0125	0.0359	38.3
20	/	/	/	/	/	/	/	/	/	/	/	/	/	/
		Site: B		**Coordinates:** N 35º54' 01.68" E 14º 19' 08.82"										
0.3	13	NW	26.7	7.81	7.81	18	59.8	0	0	0.03	0.32	0.035	0.0906	38.5
55	/	/	/	/	/	/	/	/	/	/	/	/	/	/
		Site: C		**Coordinates:** N35º 49' 04.98" E 014º 33' 28.62"										
0.3	13	NW	27.7	7.93	7.52	15	59.8	0	0	0.12	0.12	0.09	0.0333	37.7
20	13	NW	26.9	7.99	7.52	15	60.2	0	0	/	0.36	0.055	0.0714	38.6

		Site: D		**Coordinates:** N 35º 54' 00.36" E 014º 31' 40.98"										
0.3	13	NW	27.4	8.05	7.87	7	59.9	0	0	0.20	0.60	0.17	0.1454	38.1
15	13	NW	27.2	8.1	7.7	7	59.9	0	0	0.17	0.84	0.1575	0.0821	38.2
Date: 16/09/2015		**Site**: A		**Coordinates:** N 36º 00' 5.22" E 014º 21' 37.5"										
0.3	11	S	27	7.72	7.08	13	58	0	0	0.03	0.74	0.058427	0.0405	37
20	11	S	24.4	7.99	8.93	13	57.5	0	0	0.09	1.59	0.05	0.1060	38.8
		Site: B		**Coordinates:** N 35º54' 01.68" E 14º 19' 08.82"										
0.3	11	S	27.7	8.05	7.23	13	57	0	0	0.03	0.85	-0.03765	0.0716	35.8
55	11	S	27.5	8.07	7.4	13	57.2	0	0	0.05	1.14	0.177273	0.0472	36.1
		Site: C		**Coordinates:** N35º 49' 04.98" E 014º 33' 28.62"										
0.3	11	S	27.1	8.15	7.56	12	58.3	0	0	0.04	1.53	0.179167	0.0410	37.3
20	11	S	26.8	8.16	7.56	12	58.2	0	0	0.09	1.11	0.1075	-0.0020	37.34
		Site: D		**Coordinates:** N35º 54' 00.36" E 014º 31' 40.98"										
0.3	11	S	27.2	8.19	7.53	11	57.7	0	0	0.03	1.63	0.2325	0.0244	36.7
15	11	S	26.9	8.22	7.5	11	58.2	0	0	0.06	0.63	-0.1275	0.0166	37.3

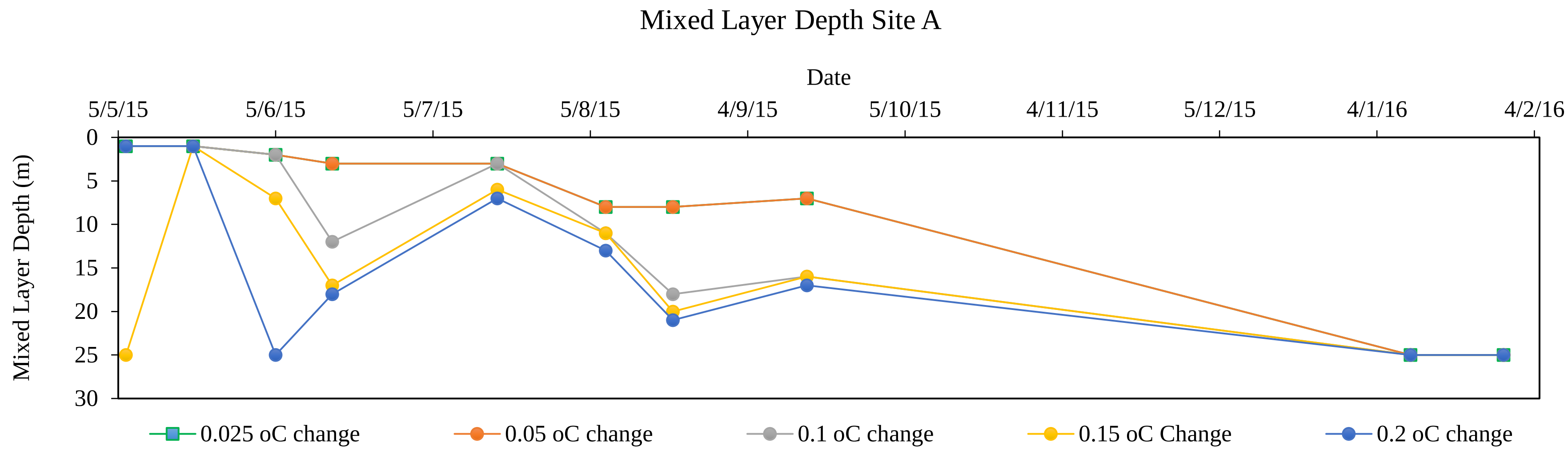
Date: 11/01/2016		**Site:** A		**Coordinates:** N 36º 00' 5.22" E 014o 21' 37.5"										
0.3	13	N	16.83	7.49	9.01	12	53.6	0	0	0.05	0.78	0.3775	-0.4178	42.4
20	/	/	/	/	/	/	/	/	/	/	/	/	/	/
		Site: B		**Coordinates:** N 35º54' 01.68" E 14º 19' 08.82"										
0.3	13	N	16.98	7.62	9	12	55	0	0	0.06	0.68	0.35	0.1043	44
55	/	/	/	/	/	/	/	/	/	/	/	/	/	/
		Site: C		**Coordinates:** N 35º 49' 04.98" E 014º 33' 28.62"										
0.3	13	N	16.59	7.75	9.65	10	54.4	0	0	0.09	0.41	0.1875	0.0848	43
20	/	/	/	/	/	/	/	/	/	/	/	/	/	/
		Site: D		**Coordinates:** N 35º 54' 00.36" E 014º 31' 40.98"										
0.3	13	N	16.62	7.76	9.22	9	54.5	0	0	0.34	1.00	0.515	0.0878	43
15	/	/	/	/	/	/	/	/	/	/	/	/	/	/
Date: 21/01/2016		**Site:** A		**Coordinates:** N 36º 00' 5.22" E 014º 21' 37.5"										
0.3	6	E-NE	17	8.24	8.98	16	56.8	30	0	0.11	8.41	6.465	0.0396	45
20	/	/	/	/	/	/	/	/	/	/	/	/	/	/

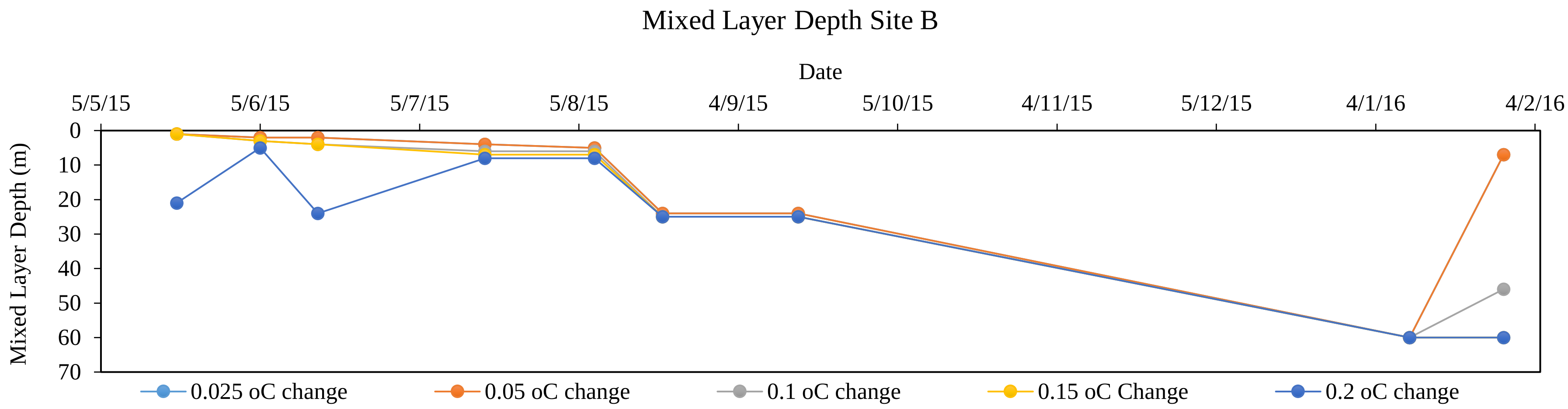
		Site: B		**Coordinates:** N 35º54' 01.68" E 14º 19' 08.82"										
0.3	6	E-NE	16.4	8.29	9.15	15	56.9	30	0	0.09	5.67	4.2575	0.0968	46
55	/	/	/	/	/	/	/	/	/	/	/	/	/	/
		Site: C		**Coordinates:** N 35º 49' 04.98" E 014o 33' 28.62"										
0.3	6	E-NE	16.1	8.34	9.18	10	57	30	0	0.09	10.1	8.016667	0.0982	46.8
20	/	/	/	/	/	/	/	/	/	/	/	/	/	/
		Site: D		**Coordinates:** N35º 54' 00.36" E 014º 31' 40.98"										
0.3	6	E-N E	16.4	8.34	9.14	7	56	30	0	0.27	5.90	4.3375	0.1180	45.6
15	/	/	/	/	/	/	/	/	/	/	/	/	/	/
Date: 24/01/2016		**Site:** A		**Coordinates:** N 36º 00' 5.22" E 014º 21' 37.5"										
0.3	8	NW	15.8	7.99	9.05	18	56.7	18	0	0.05	7.62	5.95	0.2350	46.9
20	/	/	/	/	/	/	/	/	/	/	/	/	/	/
		Site: B		**Coordinates:** N 35º54' 01.68" E 14º 19' 08.82"										
0.3	8	NW	15.3	8.22	9.11	17	56.9	18	0	0.06	6.66	5.0475	0.7996	47.7
55	/	/	/	/	/	/	/	/	/	/	/	/	/	/

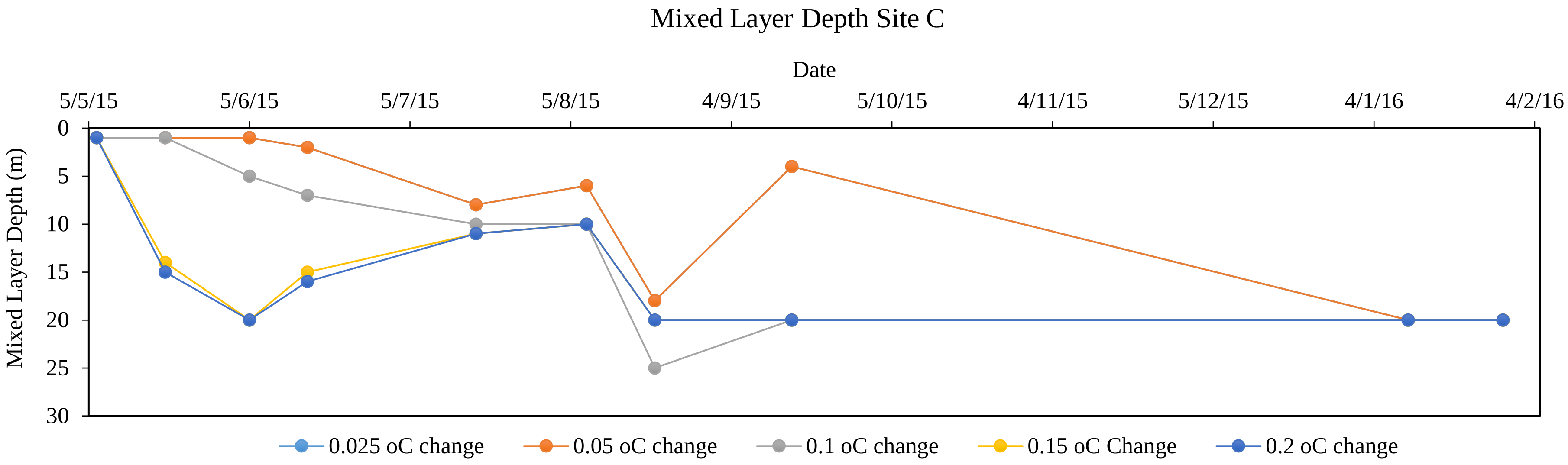
		Site: C		**Coordinates:** N 35º 49' 04.98" E 014º 33' 28.62"										
0.3	8	NW	15.5	8.36	9.1	12	56.8	18	0	0.10	8.89	7.05	0.1263	47.4
20	/	/	/	/	/	/	/	/	/	/	/	/	/	/
		Site: D		**Coordinates:** N 35º 54' 00.36" E 014º 31' 40.98"										
0.3	8	NW	15.3	8.38	9.26	7	56.2	18	0	0.25	8.22	6.1875	0.1730	47
15	/	/	/	/	/	/	/	/	/	/	/	/	/	/
Date: 27/01/2016		**Site:** A		**Coordinates:** N 36º 00' 5.22" E 014º 21' 37.5"										
0.3	3	W-NW	18.1	8.25	9.07	20(B)	56.6	10	0	0.15	/	/	0.2254	44.3
20	/	/	/	/	/	/	/	/	/	/	/	/	/	/
		Site: B		**Coordinates:** N 35º54' 01.68" E 14º 19' 08.82"										
0.3	3	W-NW	18	8.56	9.02	16	56.9	10	0	0.12	0.58	0.325	0.0891	44.6
55	/	/	/	/	/	/	/	/	/	/	/	/	/	/
		Site: C		**Coordinates:** N 35º 49' 04.98" E 014º 33' 28.62"										
0.3	3	W-NW	17.7	8.56	9.15	14	56.9	10	0	0.09	0.94	0.485	0.1134	44.9
20	/	/	/	/	/	/	/	/	/	/	/	/	/	/

		Site: D		**Coordinates:** N 35º 54' 00.36" E 014º 31' 40.98"										
0.3	3	W-NW	17.7	8.56	9.03	16	56.7	10	0	0.07	8.4	6.115	0.0912	44.7
15	/	/	/	/	/	/	/	/	/	/	/	/	/	/
Date: 29/01/2015		**Site:** A		**Coordinates:** N 36º 00' 5.22" E 014º 21' 37.5"										
0.3	5	N	18.4	8.36	9.02	20(B)	57	0	0	0.11	0.71	0.4225	0.0352	44.2
20	/	/	/	/	/	/	/	/	/	/	/	/	/	/
		Site: B		**Coordinates:** N 35º54' 01.68" E 14º 19' 08.82"										
0.3	5	N	18.2	8.3	8.98	23	56.8	0	0	0.10	1.57		0.0386	44.3
55	/	/	/	/	/	/	/	/	/	/	/	/	/	/
		Site: C		**Coordinates:** N 35º 49' 04.98" E 014º 33' 28.62"										
0.3	5	N	/	8.31	9.05	18	56.5	0	0	0.15	0.45	/	0.0404	44.1
20	/	/	/	/	/	/	/	/	/	/	/	/	/	/
		Site: D		**Coordinates:** N 35º 54' 00.36" E 014º 31' 40.98"										
0.3	5	N	/	8.23	9.07	20(B)	56	0	0	0.12	0.48	/	0.0298	43
15	/	/	/	/	/	/	/	/	/	/	/	/	/	/

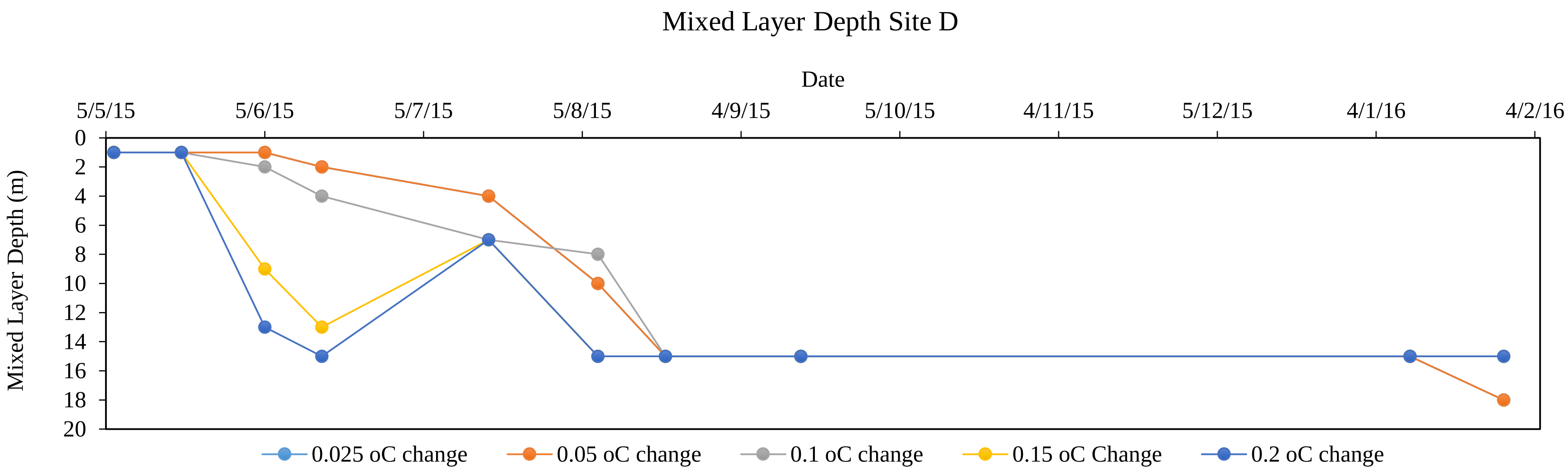
Appendix 2: Assesing Mixed Layer Depth criterion for each of the sites







Mixed Layer Depth Site C
Date
5/5/15
5/6/15
5/7/15
5/8/15
4/9/15
5/10/15
4/11/15
5/12/15
4/1/16
4/2/16
Mixed Layer Depth (m)
0
5
10
15
20
25
30
0.025 oC change
0.05 oC change
0.1 oC change
0.15 oC Change
0.2 oC change



Mixed Layer Depth Site D
Date
5/5/15
5/6/15
5/7/15
5/8/15
4/9/15
5/10/15
4/11/15
5/12/15
4/1/16
4/2/16
Mixed Layer Depth (m)
0
2
4
6
8
10
12
14
16
18
20
0.025 oC change
0.05 oC change
0.1 oC change
0.15 oC Change
0.2 oC change

Appendix 3: Correlation matrix showing the correlation between all the parameters analysed in this study

Correlations

		Chlorophyll	Depth	Wind Speed	Wind Direction	Temperature	pH	Dissolved Oxygen	Secchi Depth	SPM	CDOM	Salinity	MLD	Kd	Average Monthly Rainfall	Average Monthly Wind Speed	Average Monthly Dewpoint	Nitrate (NO_3)	SiO_4	PO_4	NH_3	Nitrite (NO_2)
Chlorophyll	Pearson Correlation	1	-.100	-.067	.025	-.125	.278**	.105	-.421**	.112	.117	.118	.015	.181	.266*	-.200*	-.207*	.043	-.169	.034	-.040	-.082
	Sig. (1-tailed)		.198	.285	.415	.143	.008	.186	.000	.168	.159	.157	.451	.135	.010	.042	.037	.358	.074	.387	.366	.241
	N	75	75	75	75	75	75	75	75	75	75	75	75	39	75	75	75	75	75	75	75	75
Depth	Pearson Correlation	-.100	1	.207*	-.091	.189	-.208*	-.126	-.022	-.142	.020	-.199*	-.267**	-.079	-.335**	-.039	.405**	-.016	.090	.041	.199*	-.158
	Sig. (1-tailed)	.198		.037	.218	.051	.036	.138	.425	.110	.432	.043	.010	.316	.002	.369	.000	.446	.220	.362	.043	.087
	N	75	76	76	76	76	76	76	76	76	76	76	76	39	76	76	76	76	76	76	76	76
Wind Speed	Pearson Correlation	-.067	.207*	1	.182	.475**	-.414**	-.421**	-.240*	-.187	-.049	-.602**	-.197*	.155	-.157	-.179	.430**	-.144	.081	.057	.021	-.191*
	Sig. (1-tailed)	.285	.037		.058	.000	.000	.000	.018	.053	.338	.000	.044	.174	.088	.060	.000	.108	.244	.312	.428	.049
	N	75	76	76	76	76	76	76	76	76	76	76	76	39	76	76	76	76	76	76	76	76
Wind Direction	Pearson Correlation	.025	-.091	.182	1	-.110	.421**	.045	-.072	.410**	.109	.048	.197*	-.197	.182	.041	-.217*	-.055	.223*	.112	.001	-.074
	Sig. (1-tailed)	.415	.218	.058		.172	.000	.350	.269	.000	.174	.341	.044	.115	.058	.361	.030	.319	.026	.168	.497	.262
	N	75	76	76	76	76	76	76	76	76	76	76	76	39	76	76	76	76	76	76	76	76

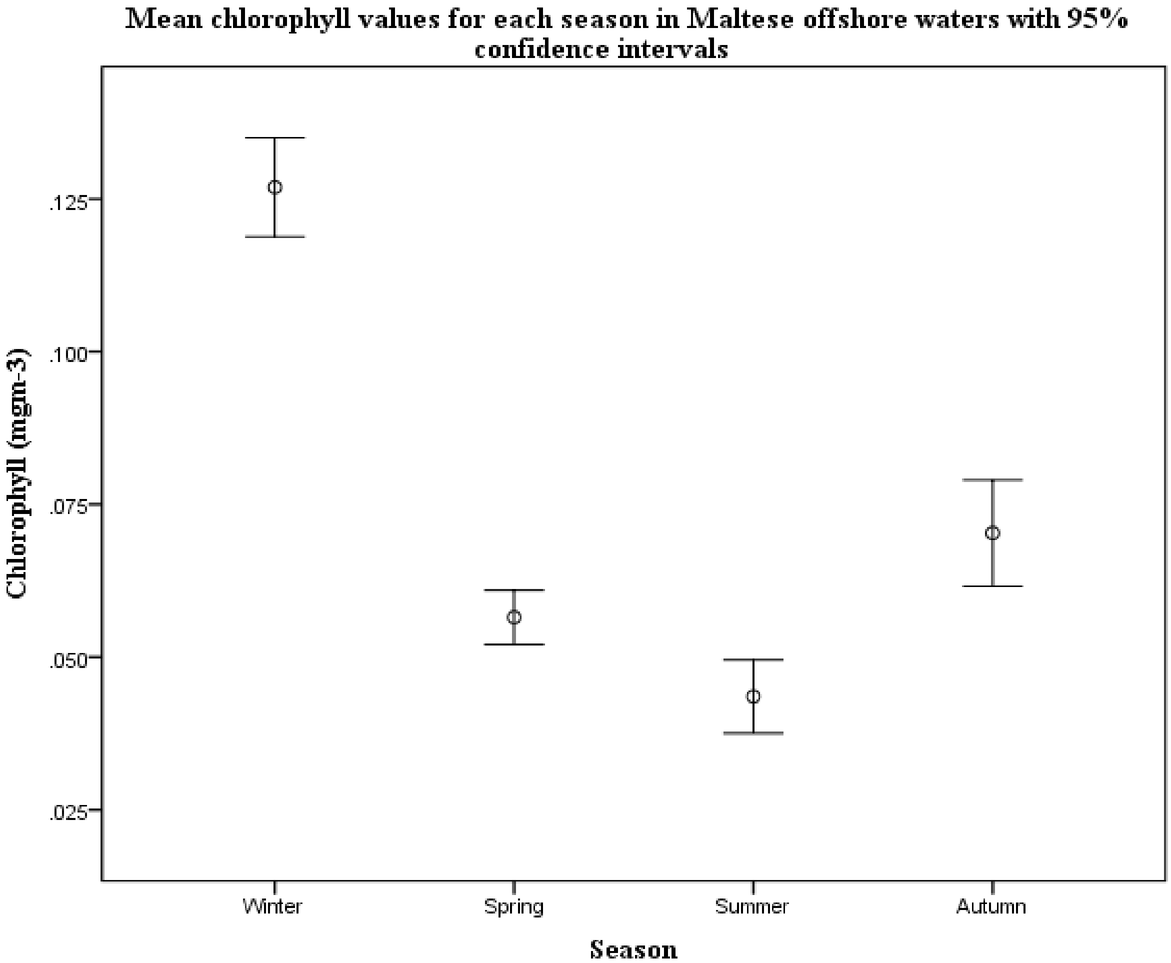
Temperature	Pearson Correlation	-.125	.189	.475**	-.110	1	-.310**	-.881**	.183	-.551**	-.121	-.884**	-.237*	-.118	-.259*	-.425**	.896**	-.270**	.066	-.128	.216*	.011
	Sig. (1-tailed)	.143	.051	.000	.172		.003	.000	.057	.000	.148	.000	.020	.237	.012	.000	.000	.009	.286	.135	.030	.462
	N	75	76	76	76	76	76	76	76	76	76	76	76	39	76	76	76	76	76	76	76	76
pH	Pearson Correlation	.278**	-.208*	-.414**	.421**	-.310**	1	.206*	-.019	.383**	.003	.320**	.499**	.177	.635**	-.019	-.493**	-.039	.048	-.171	-.026	.162
	Sig. (1-tailed)	.008	.036	.000	.000	.003		.037	.434	.000	.489	.002	.000	.140	.000	.435	.000	.369	.340	.070	.411	.081
	N	75	76	76	76	76	76	76	76	76	76	76	76	39	76	76	76	76	76	76	76	76
Dissolved Oxygen	Pearson Correlation	.105	-.126	-.421**	.045	-.881**	.206*	1	-.166	.493**	.198*	.850**	.167	.102	.200*	.350**	-.762**	.316**	-.085	.060	-.152	.016
	Sig. (1-tailed)	.186	.138	.000	.350	.000	.037		.076	.000	.044	.000	.075	.268	.042	.001	.000	.003	.233	.302	.094	.444
	N	75	76	76	76	76	76	76	76	76	76	76	76	39	76	76	76	76	76	76	76	76
Secchi Depth	Pearson Correlation	-.421**	-.022	-.240*	-.072	.183	-.019	-.166	1	-.210*	-.081	-.040	.293**	-.372**	.065	-.252*	.191*	-.050	-.097	.036	.243*	.077
	Sig. (1-tailed)	.000	.425	.018	.269	.057	.434	.076		.035	.243	.365	.005	.010	.288	.014	.049	.335	.202	.380	.017	.255
	N	75	76	76	76	76	76	76	76	76	76	76	76	39	76	76	76	76	76	76	76	76
SPM	Pearson Correlation	.112	-.142	-.187	.410**	-.551**	.383**	.493**	-.210*	1	.233*	.533**	.122	-.132	.247*	.123	-.557**	.171	.104	.136	-.138	.118
	Sig. (1-tailed)	.168	.110	.053	.000	.000	.000	.000	.035		.021	.000	.147	.212	.016	.145	.000	.069	.186	.121	.117	.155
	N	75	76	76	76	76	76	76	76	76	76	76	76	39	76	76	76	76	76	76	76	76
CDOM	Pearson Correlation	.117	.020	-.049	.109	-.121	.003	.198*	-.081	.233*	1	.203*	.144	.053	-.029	-.057	-.086	-.014	-.022	-.093	-.015	-.171
	Sig. (1-tailed)	.159	.432	.338	.174	.148	.489	.044	.243	.021		.040	.107	.374	.403	.311	.229	.453	.427	.212	.448	.070

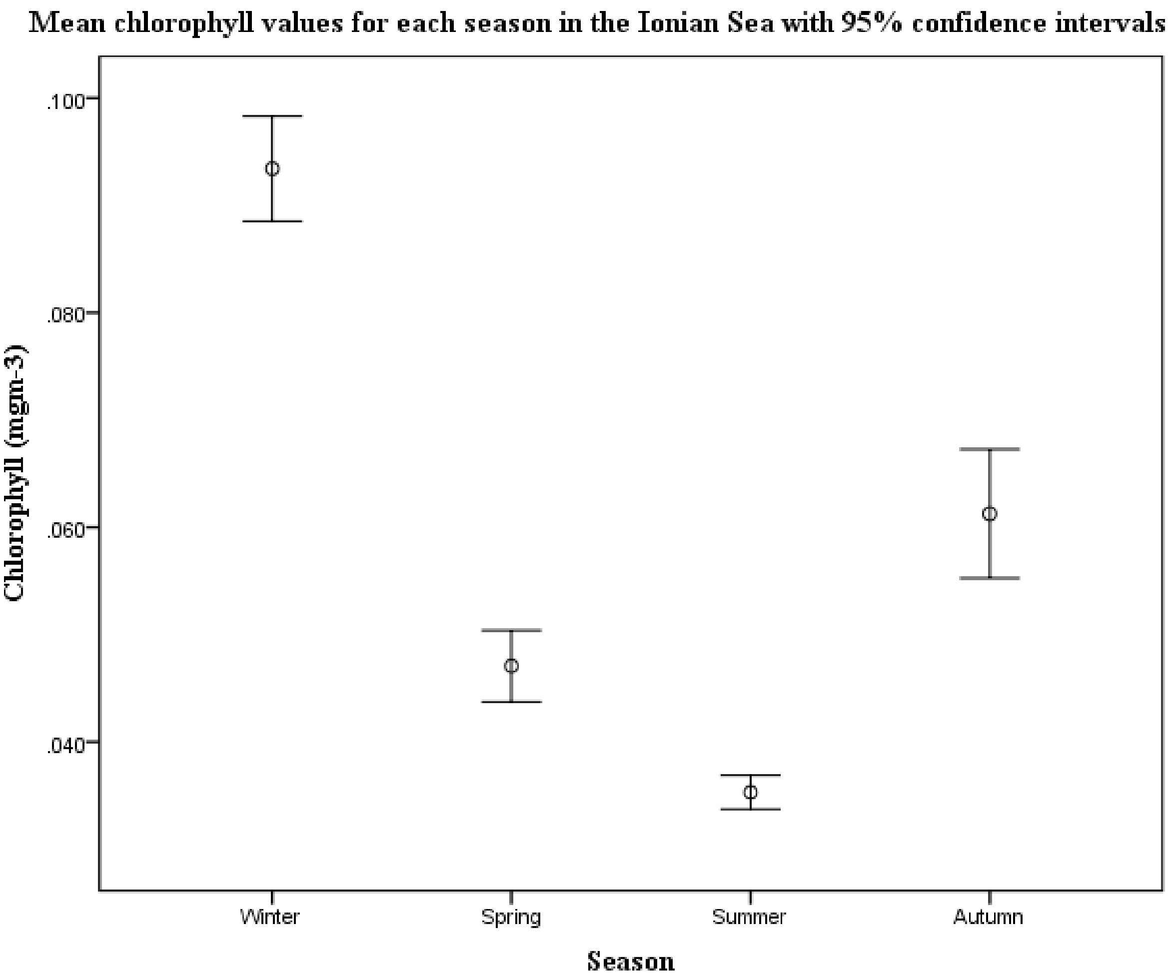
	N	75	76	76	76	76	76	76	76	76	76	76	76	39	76	76	76	76	76	76	76	76
Salinity	Pearson Correlation	.118	-.199*	-.602**	.048	-.884**	.320**	.850**	-.040	.533**	.203*	1	.368**	.139	.379**	.343**	-.788**	.236*	-.094	.086	-.179	.032
	Sig. (1-tailed)	.157	.043	.000	.341	.000	.002	.000	.365	.000	.040		.001	.200	.000	.001	.000	.020	.209	.230	.060	.391
	N	75	76	76	76	76	76	76	76	76	76	76	76	39	76	76	76	76	76	76	76	76
MLD	Pearson Correlation	.015	-.267**	-.197*	.197*	-.237*	.499**	.167	.293**	.122	.144	.368**	1	.248	.704**	.009	-.423**	-.219*	-.043	-.116	-.095	.172
	Sig. (1-tailed)	.451	.010	.044	.044	.020	.000	.075	.005	.147	.107	.001		.064	.000	.470	.000	.029	.357	.160	.208	.069
	N	75	76	76	76	76	76	76	76	76	76	76	76	39	76	76	76	76	76	76	76	76
Kd	Pearson Correlation	.181	-.079	.155	-.197	-.118	.177	.102	-.372**	-.132	.053	.139	.248	1	.529**	.080	-.159	-.142	-.010	-.044	-.005	-.029
	Sig. (1-tailed)	.135	.316	.174	.115	.237	.140	.268	.010	.212	.374	.200	.064		.000	.313	.167	.195	.477	.396	.488	.432
	N	39	39	39	39	39	39	39	39	39	39	39	39	39	39	39	39	39	39	39	39	39
Average Monthly Rainfall	Pearson Correlation	.266*	-.335**	-.157	.182	-.259*	.635**	.200*	.065	.247*	-.029	.379**	.704**	.529**	1	-.125	-.499**	-.203*	-.127	-.154	-.164	.088
	Sig. (1-tailed)	.010	.002	.088	.058	.012	.000	.042	.288	.016	.403	.000	.000	.000		.141	.000	.040	.137	.093	.079	.224
	N	75	76	76	76	76	76	76	76	76	76	76	76	39	76	76	76	76	76	76	76	76
Average Monthly Wind Speed	Pearson Correlation	-.200*	-.039	-.179	.041	-.425**	-.019	.350**	-.252*	.123	-.057	.343**	.009	.080	-.125	1	-.399**	-.003	.303**	-.123	-.225*	.297**
	Sig. (1-tailed)	.042	.369	.060	.361	.000	.435	.001	.014	.145	.311	.001	.470	.313	.141		.000	.491	.004	.145	.025	.005
	N	75	76	76	76	76	76	76	76	76	76	76	76	39	76	76	76	76	76	76	76	76
Average Monthly Dewpoint	Pearson Correlation	-.207*	.405**	.430**	-.217*	.896**	-.493**	-.762**	.191*	-.557**	-.086	-.788**	-.423**	-.159	-.499**	-.399**	1	-.129	.061	-.011	.269**	-.105

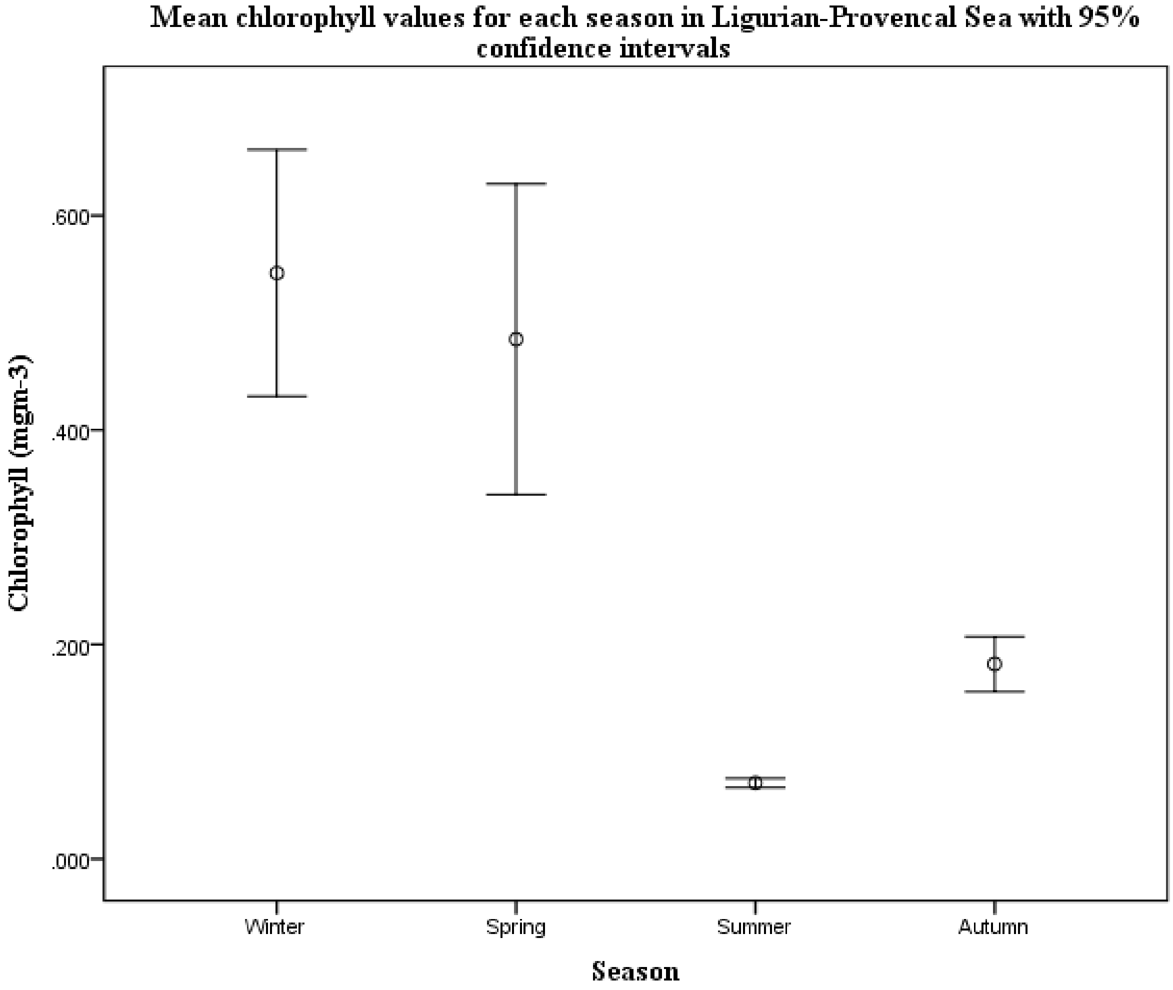
	Sig. (1-tailed)	.037	.000	.000	.030	.000	.000	.000	.049	.000	.229	.000	.000	.167	.000	.000		.134	.300	.463	.009	.182
	N	75	76	76	76	76	76	76	76	76	76	76	76	39	76	76	76	76	76	76	76	76
Nitrate (NO_3)	Pearson Correlation	.043	-.016	-.144	-.055	-.270**	-.039	.316**	-.050	.171	-.014	.236*	-.219*	-.142	-.203*	-.003	-.129	1	.194*	.474**	.419**	.073
	Sig. (1-tailed)	.358	.446	.108	.319	.009	.369	.003	.335	.069	.453	.020	.029	.195	.040	.491	.134		.046	.000	.000	.266
	N	75	76	76	76	76	76	76	76	76	76	76	76	39	76	76	76	76	76	76	76	76
SiO4	Pearson Correlation	-.169	.090	.081	.223*	.066	.048	-.085	-.097	.104	-.022	-.094	-.043	-.010	-.127	.303**	.061	.194*	1	.017	.111	.124
	Sig. (1-tailed)	.074	.220	.244	.026	.286	.340	.233	.202	.186	.427	.209	.357	.477	.137	.004	.300	.046		.443	.169	.143
	N	75	76	76	76	76	76	76	76	76	76	76	76	39	76	76	76	76	76	76	76	76
PO4	Pearson Correlation	.034	.041	.057	.112	-.128	-.171	.060	.036	.136	-.093	.086	-.116	-.044	-.154	-.123	-.011	.474**	.017	1	.397**	-.111
	Sig. (1-tailed)	.387	.362	.312	.168	.135	.070	.302	.380	.121	.212	.230	.160	.396	.093	.145	.463	.000	.443		.000	.169
	N	75	76	76	76	76	76	76	76	76	76	76	76	39	76	76	76	76	76	76	76	76
NH3	Pearson Correlation	-.040	.199*	.021	.001	.216*	-.026	-.152	.243*	-.138	-.015	-.179	-.095	-.005	-.164	-.225*	.269**	.419**	.111	.397**	1	.103
	Sig. (1-tailed)	.366	.043	.428	.497	.030	.411	.094	.017	.117	.448	.060	.208	.488	.079	.025	.009	.000	.169	.000		.187
	N	75	76	76	76	76	76	76	76	76	76	76	76	39	76	76	76	76	76	76	76	76
Nitrite (NO_2)	Pearson Correlation	-.082	-.158	-.191*	-.074	.011	.162	.016	.077	.118	-.171	.032	.172	-.029	.088	.297**	-.105	.073	.124	-.111	.103	1
	Sig. (1-tailed)	.241	.087	.049	.262	.462	.081	.444	.255	.155	.070	.391	.069	.432	.224	.005	.182	.266	.143	.169	.187	
	N	75	76	76	76	76	76	76	76	76	76	76	76	39	76	76	76	76	76	76	76	76

**. Correlation is significant at the 0.01 level (1-tailed). *. Correlation is significant at the 0.05 level (1-tailed).

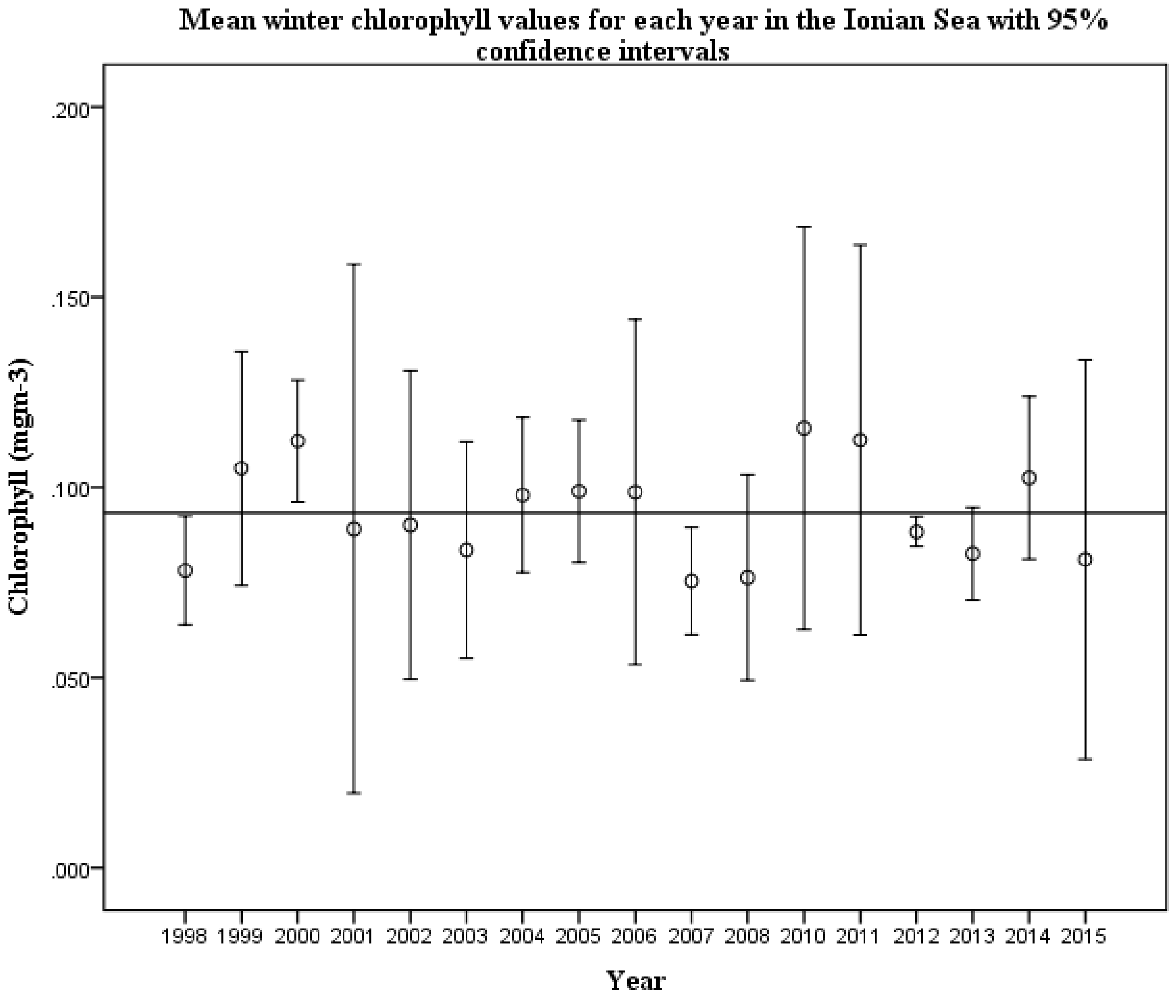
Appendix 4: Showing the mean seasonal variation of chlorophyll at each of the sites analysed in this study



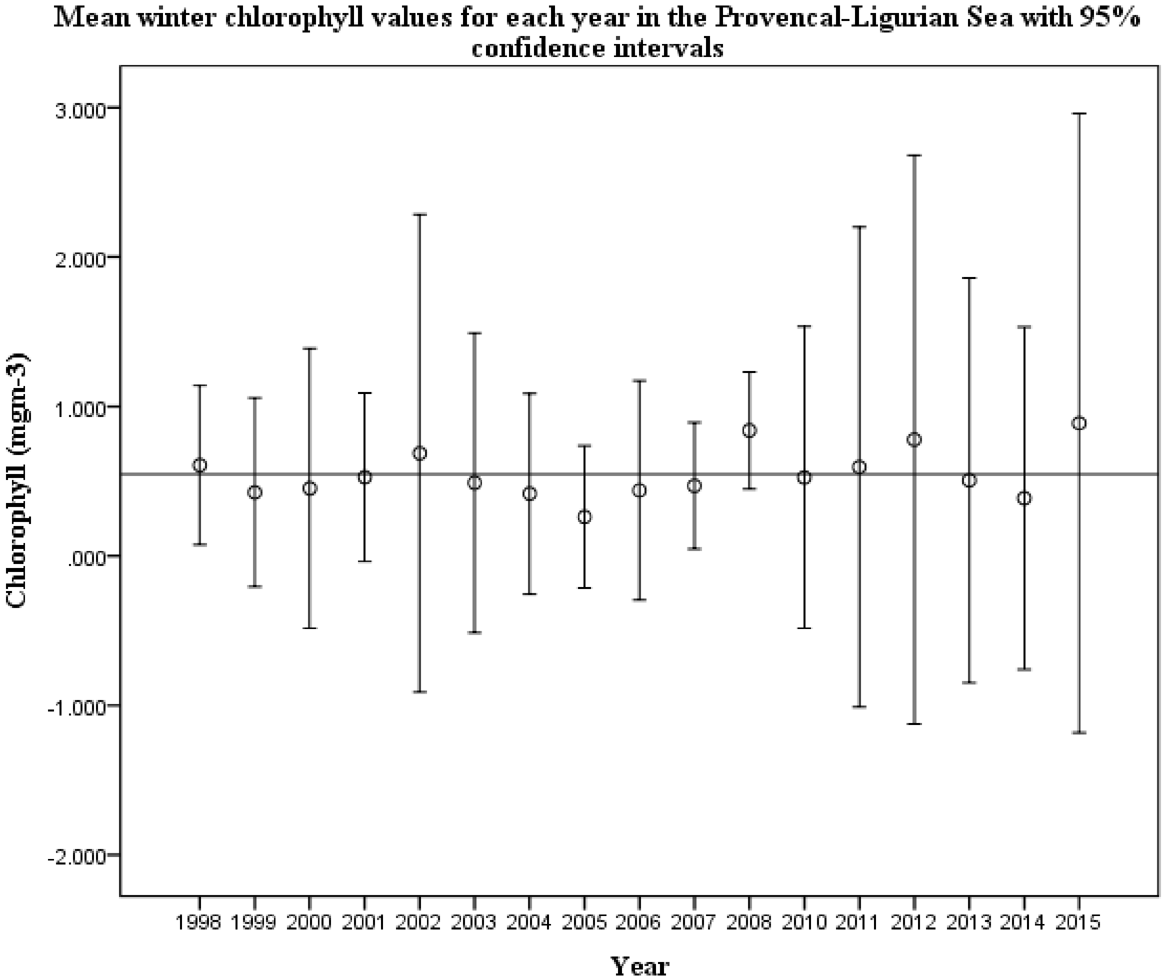

Mean chlorophyll values for each season in the Ionian Sea with 95% confidence intervals
.100
.080
.060
.040
Chlorophyll (mgm-3)
Winter
Spring
Summer
Autumn
Season

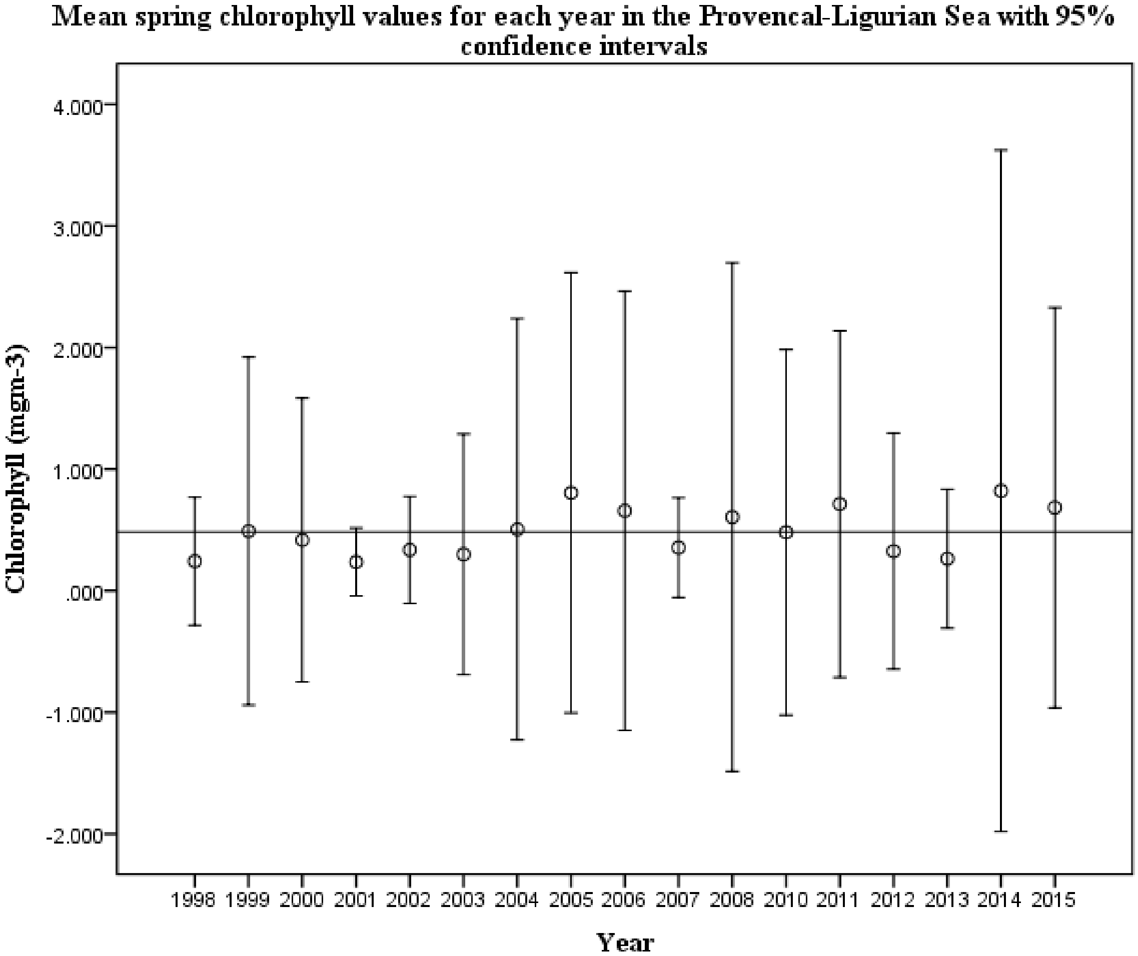

Mean chlorophyll values for each season in Ligurian-Provencal Sea with 95% confidence intervals
Chlorophyll (mgm-3)
.600
.400
.200
.000
Winter
Spring
Summer
Autumn
Season

Appendix 5: Showing variation of the chlorophyll levels in the winter season over a period of seventeen years in the Ionian Sea with error bars at the 95% confidence interval



Appendix 6: Variation in yearly mean winter and spring chlorophyll levels in the Ligurian-Provencal Sea




Mean spring chlorophyll values for each year in the Provencal-Ligurian Sea with 95% confidence intervals
4.000
3.000
2.000
1.000
.000
-1.000
-2.000
Chlorophyll (mgm-3)
1998 1999 2000 2001 2002 2003 2004 2005 2006 2007 2008 2010 2011 2012 2013 2014 2015
Year

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