Phytoplankton Composition off the Coast of Point Revellata, Corsica, France

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Abstract

Phytoplankton are the base of the marine trophic food web, thus their distribution, composition and diversity are important in determining the condition of a given ecosystem. We looked at the composition of phytoplankton species in the Bay of Calvi in Corsica, France. Phytoplankton abundance is limited by both light (which attenuates with depth) and nutrient availability. While the Mediterranean is generally oligotrophic, previous studies have found the canopy created by the leaves of Posidonia oceanica trap and retain nutrients that may then diffuse into the water column above the canopy. We characterized the phytoplankton community composition across a depth gradient in the water column over P. oceanica meadows at 7.5m and 15m. Phytoplankton species composition was found to be affected by both depth and distance from meadow. However, proximity to the meadow had a larger effect on the species composition, likely due to increased nutrient availability.

Introduction

Phytoplankton are important primary producers that provide nutrients for zooplankton, euphausiids, and larger phytoplankton while also contributing to oceanic and atmospheric oxygen. As the base of the marine trophic structure, the abundance, distribution, and diversity of phytoplankton are useful in determining the condition of a given marine ecosystem. Abiotic factors such as light (a function of depth) and inorganic nutrient availability are crucial constraints of phytoplankton growth and reproduction (De Baar, 1994). Nutrients influence the type and succession of phytoplankton; small modifications in nutrient levels, when nutrients are very limiting, can have a profound impact on phytoplankton species composition (Barron et al, 2006). Nutrients are usually introduced into marine environments via upwelling events and coastal runoff. The nutrient profile changes with depth and light availability. In the shallow euphotic zone, nutrients are at low concentrations because they are quickly used up by phytoplankton during photosynthesis. However, as depth increases, light and consequently, the rate of photosynthesis decreases and the nutrients are regenerated via the microbial loop (Armbrust et al, 2010). As phytodetrital sedimentation and decay occur, upwelling brings those nutrients back into the upper water column once again and the nutrients are quickly consumed by phytoplankton (Smayda, 2002). As is the case with land plants, phytoplankton require nutrients like nitrate, phosphate, silicate and micronutrients like iron and copper to grow (Armbrust et al, 2010). Thus, factors such as light and nutrient availability will have an effect on the distribution of certain species in the water column.

The Mediterranean is a largely oligotrophic, nutrient limited region due to the lack of upwelling events and small amount of coastal/land runoff (Briand, 2010). It is thought to be the most severely phosphate limited marine area in the world (Briand, 2010). The productivity in the Northwest Mediterranean Sea occurs during the winter-spring transition period between the months of January and March. Components of this productive period include phytoplankton blooms, sardine spawning, and benthic invertebrate reproduction (Goffart et al, 2002). The Bay of Calvi experiences a seasonal upwelling event due the submarine canyon located nearby. As climate changes and human development continues to impact the Mediterranean Sea, the dynamics of this seasonal boost in productivity are changing and may also change the phytoplankton communities throughout the rest of the year (Goffart et al, 2002). Studying the composition of phytoplankton and zooplankton at different depths in the water column during a particularly oligotrophic time of year may give insight to distribution and diversity of phytoplankton in a changing environment (Goffart et al, 2002).

Few phytoplankton studies span long periods of time and include the species distribution in the water column. There are even fewer studies on specific phytoplankton species and community
composition (Briand, 2010). Spatial distribution of phytoplankton is a result of stratification of the water masses and relies heavily on movement of the ocean circulation and currents to bring together nutrients with light (Trainer et al., 2010). The Bay of Calvi in Corsica, where our studies took place, is part of the permanent Liguro-Provençal frontal zone which includes a complex network of divergences to bring water and nutrients to the surface (Goffart et al, 2002). Parameters that influence community composition include light and nutrient availability, trace elements and turbulence (Bates et al., 2006). These elements drive the composition of phytoplankton species at different depths in the water column.

The Bay of Calvi near shore region is dominated by the sea grass *Posidonia oceanica*. Coastal sea grass communities such as *P. oceanica* are highly productive in oligotrophic waters due to their ability to obtain and conserve the relatively low amounts of nutrients present. The water flow is reduced under the canopy of leaves created by *P. oceanica*, enabling nutrients like nitrogen, phosphorous, and organic carbon to be trapped. The nutrients are relatively higher in the sea grass canopy than in the above waters (Barron et al, 2006) and can be used by phytoplankton. The objective of our study was to gain an understanding of how phytoplankton species composition varies as a function of water depth and proximity to sea grass *P. oceanica*. Our specific hypotheses were (1) phytoplankton composition varies across the three depths surveyed: 1.5m, 7.5m, 15m, (2) that phytoplankton composition varies with proximity to the bottom.

**Materials and Methods**

**Species Description:**

This study was conducted off the coast of Calvi, Corsica in the Mediterranean Sea. The Bay of Calvi is an oligotrophic or commonly nutrient limited ecosystem due to low input of nutrients and the quick turnover rate of phytoplankton (Goffart et al, 2002). The phytoplankton and radiolarian species which we observed and counted include: the diatom species *Chaetoceros* (species J), dinoflagellate species *Ceratium macroceros* (species A), *Peridinum* (species G), and *Pyrocystis* (species O), and protist species belonging to the class Acantharae (species B, E, I, Q, and W) known for its high variation in morphology and abundance in oligotrophic environments (Zettler, 1996). This family ranges in size from 50-800 micrometers and have very defined structures which have contributed to the amount of early studies and classifications done with microscopes starting in the 1880’s by Ernst Haeckel aboard the H.M.S Challenger Expedition (Zettler, 1996). However, these classifications were based on morphology which now seems to be a result of convergent evolution based on DNA and RNA analysis. Many studies have been done on Acantharae for a couple key reasons; their skeletons are made of strontium sulfate and play a key role in the strontium cycle in the world oceans, also they are often found in association with symbiotic algae (such as zooxanthellae) and play significant roles in primary production and microbial food-web dynamics (Zettler, 1996). The species we observed and labeled as “B” is most likely *Acanthometra fusiforme* which is slightly yet consistently different from “I” which we have matched to *Acanthometra fragilis*. *A. fusiforme* has slightly shorter and thicker spines when compared to *A. fragilis* which is also darker in color, larger, with a visible outer membrane stretching between the spines. Whereas species “E” is much like *A. fragilis* except the spines are slightly longer without a surrounding membrane. We were able to identify the genus of “E” as Acanthostauros although the species remains undefined. Species *Amphilonche heteracantha*, labeled “Q”, is a related and similar to the previous three discussed, yet it has elongated spines at each end with a thin membrane stretching between them, creating a pointed oblong shape. The final species we found in our samples belonging to this class is *Lithoptera muelleri* which had four longer silicate spines with a lattice or cross pattern on the tips. These were all identified using drawings by Dr. Gerhard Keuk (1999). For the remainder of this
paper species will be referred to by the letters noted above, for images and more species names see appendix 1.

Site Description:

The Mediterranean Sea is characterized by a low accumulation of dissolved nutrient salts, supporting but resulting in a sparse growth of algae and other organisms, and having high oxygen content due to low organic content (Millot, 1999). Corsica, where our study was performed, is located in the Northwestern region of the Mediterranean Sea in an area called the liguro-provencal basin. The liguro-provencal basin is semi-enclosed creating critical oceanic gateways for controlling circulation. The liguro-provencal current is driven by a number of interactive factors such as climate and bathymetry. The liguro-provencal current starts before the Ligurian Sea and continues south of the Channel of Ibiza (Millot, 1999). This study was conducted in the Bay of Calvi which is an oligotrophic or commonly nutrient limited ecosystem due to low input of nutrients and the quick turnover rate of phytoplankton.

Salinity levels are also high in the Mediterranean Sea. Evaporation is more relevant than the water from river out flow and precipitation. Thus the salinity in the Mediterranean is higher than in the Atlantic Ocean. The result is an outflow of warm saline Mediterranean water across the Strait of Gibraltar, and the inflow of a less saline surface current of cold Atlantic oceanic water (Millot, 1999). Nutrient depleted water from the Atlantic that flows into the Mediterranean exits after circulating the basin with nearly 10% salt content (Turley, 1999). High evaporation rates due to salinity levels, low land runoff, and seasonal rains that produce a deficit in hydrological balance make the Mediterranean very sensitive to climate changes (Turley, 1999). Nutrient levels have decrease with the damming of rivers such as the Nile. The basin wide circulation and variable seasonal climate combined with low land river runoff contribute to the low productivity of the Mediterranean (Millot, 1999).

Methods

To test our hypothesis that species composition varies with depth, we conducted an observational study about 150m offshore at a maximum depth of 15m over a bed of P. oceanica. To test our hypothesis that species composition must is function of proximity to the bottom, we took two different samples at a depth of approximately 7.5m. One sample was taken at a 7.5m depth near shore directly over a bed of P. oceanica on the sea floor; the other sample was taken at a 7.5m depth off shore. We also took two different surface samples at a depth of approximately 1.5m, and one deep water surface each collection day at a depth of approximately 15m. One surface sample was taken at a 1.5m depth near shore directly above the location that the near shore 7.5m sample was taken. The other surface sample was taken at a 1.5m depth directly above the location that the off shore 7.5m sample was taken. If our results show that the species composition of the sample taken off shore at a 1.5m depth is significantly different from the off shore sample taken at 15m deep, then they support our hypothesis that composition does vary with depth. If our results indicate that the species composition of the surface samples taken at 1.5m deep near shore and off shore are significantly similar, then there is no intrinsic difference in spatial origin. If our results show that the species composition of the sample taken at 15m off shore above a bed of P. oceanic a is significantly similar to the sample taken at 7.5m near shore above a bed of P. oceanic a, then species composition is a function of proximity to P.oceanica on the bottom.

Sample tows were taken using SCUBA and snorkel with an approximately 180 μm phytoplankton mesh net over a 120m transect. Data collection was on the north side of the boat harbor at the STARESO field station on Point Revellata October 8th-October 28th. The samples were taken at
approximate depths of 7.5m near shore, then 15m off shore, then 7.5m off shore, then at the surface off shore, and then at the surface near shore. For the sample at an approximate depth of 15m, at least two SCUBA divers laid out a 30m meter tape over the *P. oceanica* on the seafloor and sampled a total of 120m. Contamination was prevented by securing net between sample processing. Then a snorkeler dove down to retrieve the net and brought it to the surface. At the surface, two snorkelers untied the string around the net, while preventing the canister from making contact with the surface water. Excess water was then drained out of the side filters in the canister. The remaining fluid was poured into a sample container labeled with the depth of the sample and the date. The phytoplankton net was then tied off again and delivered back down to the SCUBA divers. All five samples followed the same procedure explained above, except that the plankton net was towed by a snorkeler for 120m during both surface samples.

To determine the relationship between light present and species composition, a light meter was used to measure the amount of light available for photosynthesis. At each sampled depth, the light meter was exposed and held flat for approximately one minute, accounting for about 6 readings of water temperature and light intensity measured in lum/ft².

Samples were processed within two days of collection and were observed on a depression slide with a volume of approximately .5 mL seawater under a Leitz DIALUX 20 EB microscope. Individuals were placed into categories A-Z based on similarities in structure and appearance. For every collection, four people analyzed two slides each from all five samples taken in the field. Thus a total of 4 mL of seawater was observed from each collection day. To account for grazing pressures on the phytoplankton we also counted the number of copepods in each slide. To make counting through the microscope easier and to prevent the copepods from consuming phytoplankton after samples were collected, a few drops of Acetone 90% was added to kill copepods. Initially we were adding acetone to each slide before counting. To maintain consistency and prevent any extraneous phytoplankton consumption we began adding acetone to the whole sample immediately after bringing them to the lab.

Statistical analyses were done in JMP, PRIMER6, and Excel. Once the samples contents were recorded, we input all of our data into Excel. We used PRIMER6 for statistical analysis to assess the similarities and dissimilarities in species composition at different depths. We then used Excel and JMP to produce graphs of our results from PRIMER6. Permanova tests run in PRIMER6 were used to obtain the relatedness between phytoplankton species and depth. If there are species of phytoplankton at the greatest depth that are not present at another depth then we will conclude that that species will vary depending on depth and try to determine which mechanism might account for that by looking at the light meter readings or weather data collected. Meteorological data available online including significant wave height, wave period, cloud cover, and air temperature was recorded on every collection day to determine other possible factors contributing to phytoplankton growth.

**Results**

Phytoplankton Species Abundance Compared with Depth:

The 18 species shown in figure 1 were chosen according to average percent contribution at each depth. Any species which made up 1% or more at any depth (even if it was less than 1% in another depth) was included in the graph. The majority of phytoplankton composition at each depth is attributed to 5 main species. The first and most prominent in all depths measured is species A which makes up 25% ± 5 of each depth’s overall composition. Four of the other most prominent species were B, D, E, and L.
Species contributing 5% or more to the average overall composition at the offshore 1.5m depth location are: A 30%, B 22%, D 7%, E 15%, and L 5%. Species contributing 5% or more to the average overall composition at the offshore 7.5m depth location are: A 25%, B 22%, D 7%, E 16%, and Q 6%. Species contributing 5% or more to average overall composition at the offshore 15m depth location are: A 26%, B 16%, E 28%, L 6%. And Q 5%

Figure 1 shows that certain taxa dominate at specific depths. Each depth had a unique composition that was dominated by a few key species. At the surface level species A, B, D, and E contributed to approximately 79% of the total phytoplankton species composition. At 7.5m species A, B, D, E, J, and Q contributed to approximately 83% of the phytoplankton species composition. At 15m deep species A, B, D, E, L, and Q contributed to approximately 81% of the phytoplankton species composition. The rest of the species contributed less than 2% to the differences in composition.

The following species were observed at distinct depths of the water column. Species J and T were only found in abundance at the deep and mid-water samples. Species R was only found in abundance at the deep and surface samples. Species V was only found in abundance at the surface samples. Species X was only found in abundance at the mid-water and surface samples. Species Y was only found in abundance at the mid-water sample.

Figures 2-7 illustrate the key differences in phytoplankton species composition. This was done by comparing quantities between proximity to the shore and bottom over *P. oceanica* and depth, a proxy for light. The most notable differences were shown in figures 3, 5, and 6. Using Primer 6 we were able to see that the surface (1.5m) off shore sample and the deep water (15m) sample had the greatest differences. The off shore (7.5m/mid-water) sample and off shore surface (1.5m) sample were the most similar as displayed with Permanova in Table 1.
Figure 2. The species composition at the offshore location of surface water (1.5m) and the offshore location directly over the seagrass, *Posidonia oceanica* (15m).

Figure 3. The species composition at the offshore location comparing the seafloor sample and the mid water sample.
Figure 4. The species composition at the offshore location of surface water (1.5m) and the offshore location of mid-water (7.5m).

Figure 5. The species composition at the onshore location over the seagrass, *Posidonia oceanica* (7.5m) and the offshore location deep water over the seagrass, *Posidonia oceanica* (15m).
Figure 5. The species composition at the onshore location of surface water (1.5m) and the offshore location of surface water (1.5m).

Figure 6. The species composition at the onshore location over the seagrass, *Posidonia oceanica* (7.5m) and the offshore location of mid-water (7.5m).
Environmental Factors and Similarity by Permanova:

Other data gathered that helps describe the possible environmental impacts on phytoplankton composition includes: average light available at each depth and average copepods counted. The average light samples and copepod counts listed in Table 1 are partially reduced and only include the readings of sample days 8 through 13 where data was gathered for both onshore and offshore sites. This was done to prevent any discrepancies detected from previous sampling at the offshore site.

The average light readings followed the predicted pattern that more light is available at the surface and decreases with depth. Average copepod counts were recorded to act as a proxy for predation of phytoplankton at depth. The amounts of copepods were very similar at the *P. oceanica* sites; 100 copepods at 15m offshore and 85 copepods at 7.5m onshore. Also close in number were the mid water offshore sample (256 copepods) and onshore surface sample (221 copepods). The offshore surface sample had the greatest average amount of copepods (706). We can compare these factors to the similarities found through Permanova analysis to determine which impact (light, nutrients, or predation) may be the greatest limiting factor of phytoplankton composition. Table 1 gives P-values that were calculated using all comparative data collected on composition including date, location, and depth.

Table 1. Light readings (in Lumens/ft^2) and Copepod counts are averaged from the final six days of sampling when onshore collections began. Comparison of all data between depths show the similarity.

<table>
<thead>
<tr>
<th>Location</th>
<th>Depth (m)</th>
<th>Avg. Light (lumens/ft^2)</th>
<th>Avg. Copepods</th>
<th>vs. 15m Offshore</th>
<th>vs. 7.5m Offshore</th>
<th>vs. 1.5m Onshore</th>
</tr>
</thead>
<tbody>
<tr>
<td>Offshore</td>
<td>15</td>
<td>230.63</td>
<td>100</td>
<td>.</td>
<td>.</td>
<td>.</td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>301.3</td>
<td>256</td>
<td>0.109</td>
<td>.</td>
<td>.</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>1453.63</td>
<td>706</td>
<td>0.016</td>
<td>0.097</td>
<td>0.286</td>
</tr>
<tr>
<td>Onshore</td>
<td>7.5</td>
<td>267.83</td>
<td>85</td>
<td>0.939</td>
<td>0.429</td>
<td>0.225</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>1244.57</td>
<td>221</td>
<td>.</td>
<td>.</td>
<td>.</td>
</tr>
</tbody>
</table>

Other key Permanova analyses (which are not depth specific) show that the greatest amount of dissimilarity throughout all data was between day to day data (p-value of 0.059). The high variance in data between days contributes to noise in the data, especially because the duration of the experiment was quite short. When we narrowed the Permutation sample to comparing all data across depths and days within the limits of only the offshore samples the p-values for days and depth were 0.001 and 0.012 respectively. However between locations the difference was not significant (p-value=0.326).

Discussion

Our data provide support for the specific hypotheses that (1) phytoplankton composition vary with depth, and (2) that phytoplankton composition vary with proximity to the bottom. Differences in phytoplankton composition are likely due to variation in nutrient and light availability. Light and nutrient levels are the primary constraints on phytoplankton communities (Hecq Pers comm). Nutrient levels in this region of the Mediterranean have been shown to be highest in the benthic zone and lowest
near the surface. This distribution is due to nutrient retention in *Posidonia* meadows (Hecq, Pers comm). Light availability, which was found to decrease as a function of depth (Table 1), and nutrient abundance, which increases with proximity to the bottom, are likely the primary driving forces for the observed compositional differences in phytoplankton communities.

Our comparisons of the off shore communities (Fig.2-4) provide support for the first part of our hypothesis. The greatest statistical difference (p-value=0.016) occurred between the surface and the benthos (Table 1). Samples from these two depths existed in environments most dissimilar in terms of light and nutrient availability.

Extreme similarity (p-value=0.939) was noted between benthic communities at 7.5m on shore and 15m off shore (Table 1). This resemblance was attributed to their proximity to *Posidonia* and its associated nutrient abundance. When compared, on and off shore communities at 7.5m (Table 1) exhibited similarities as well, but to a lesser degree (p-value=.429). We ascribe their similarity to their common depth and similar photic exposure. These data suggest that while both light and nutrients play a role in phytoplankton community composition, nutrient availability is more influential.

Several species were observed at distinct depths in the water column (Fig.1). Species J and T were found in abundance in the deep and mid-water samples, while species V was primarily found at the surface. This is likely due to species-specific sensitivities to light and nutrient availability. Species R was found in abundance in the deep and surface samples. This pattern may be driven by selective herbivory by planktivorous fishes. *Chromis chromis*, one such species, was seen in abundance in the mid-water. Species such as R may be particularly susceptible to this pressure, resulting in their relative mid-water absence.

In the rapidly changing Mediterranean, phytoplankton studies are more valuable than ever. Modifications in agricultural practices and altered river flow influence the concentration of nutrients flowing into the sea (Millot, 1999). It is predicted that human population and tourism in the Mediterranean will continue to increase, especially along southern shores. Without adequate management, this will likely result in increased pollution and eutrophication (Millot, 1999). Phytoplankton studies are the key to understanding the effects of changing nutrient levels because nutrients are a limiting factor for phytoplankton. Even small modifications in nutrient levels can have a profound impact on phytoplankton species composition (Barron et al, 2006). The location we studied was ideal for investigation of these concerns. Point Revellata remains fairly pristine, yet is a region similar to those currently in danger. This study and future similar surveys will become increasingly important as potentially damaging practices continue.

Phytoplankton studies commonly use satellite information collected on the presence of chlorophyll *a* (Eppley et al. 1985, Longhurst et al. 1995) Satellite studies, while providing data on abundance, offer no information on species composition. Information available by our method of sample analysis was complementary. A combination of the approaches would provide a more complete survey.

We have taken the first steps toward characterizing the composition of phytoplankton communities present in the region immediately north of the STARESO research facility in the Mediterranean Sea. If this survey is continued, and perhaps expanded to provide information on abundance, it will provide a vital source of information on the status of phytoplankton communities—the foundation of the Mediterranean food web.
Works Cited


Appendix 1
Species observed:

Species A: *Ceratium macroceros*
Species B: *Acanthostaurus fusiforme*
Species C: Unidentified
Species D: Unidentified Diatom
Species E: *Acanthostaurus sp.*
Species F: *Striatella unipunctata*
Species G: *Peridunum sp.*
Species H: Unidentified
Species I: *Acanthometra fragilis*
Species J: *Chaetoceros sp.*
Species K: Unknown Foraminifera
Species L: Unknown
Species M: Unknown Foraminifera
Species N: *Scaphodinium sp.*
Species O: *Pyrocystis*
Species P: Unknown
Species Q: *Amphilonche heteracantha*
Species R: Unknown diatom
Species S: Snail shell, not a Phytoplankton
Species T: *Cladopaxis sp.*
Species V: *Rhizosolemia*

Species W: *Lithoptera*

Species W: *Lithoptera sp.*

Species X: Unknown

Species Y: *Dorataspis costata*
Appendix II
COMMENTS ON EXPERIMENTAL DESIGN

Although the net was rinsed cursorily at each new location and depth, contamination likely occurred. The 50 foot sample, when compared to other depths, was relatively lacking in species overlap, which may be because it was consistently the first sample taken, and therefore remained uncontaminated.

The practice of adding acetone or formaldehyde to kill copepods was introduced only after herbivory upon phytoplankton was notice in our samples. It is possible that data taken before this practice was introduced are skewed by selective copepod herbivory.

The presence of jellies and radiolarians in the sample area was sporadic but extreme. When present, they filled the net, turning our samples into a gelatinous mass. This created difficulties for sample examination.

One surprising aspect of survey was the extreme temporal variability in species present. Certain species which were seen in abundance directly prior to the investigation were entirely absent from our samples. This point in particular illustrates the need for a longer sampling period. On a shorter time scale, day to day variation of composition was significant, particularly at the surface for reasons which were unclear. The surface samples taken near and off shore varied more than expected (p-value=0.286) (Table 1).

ADVICE FOR FUTURE RESEARCH

A subject for future study is the in depth comparison of light and nutrient levels with the actual species which inhabit these depths. A quantification of the abundance of copepods and other herbivores would be a useful addition. By establishing the conditions in which specific species thrive, it may be possible to predict changes in phytoplankton compositions in response to environmental changes.

The approach we took to data collection was low budget and fairly labor intensive. A minimum of five researchers (two SCUBA divers and three snorkelers) was present for each. The use of a boat could have reduced the number of researchers required for sample collection. The most important modification needed for eliminating artifact phytoplankton is the addition of nets for each sample depth/location.