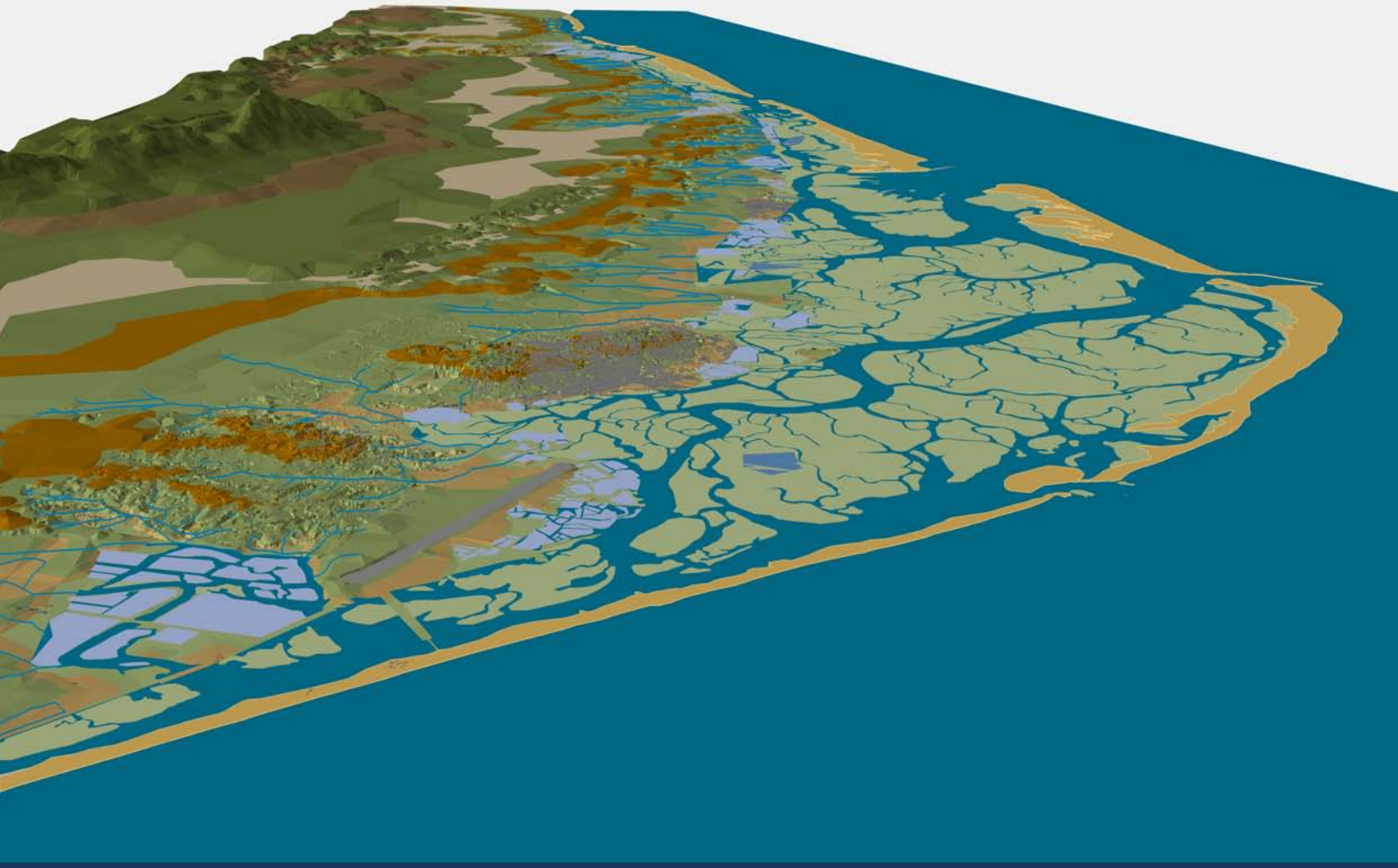

RIA FORMOSA

Challenges of a coastal lagoon in a changing environment

Edited by
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CENTRO DE INVESTIGAÇÃO MARINHA E AMBIENTAL

5. Role of microbes in the Ria Formosa lagoon

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5.1. What is role of microbes in C fluxes in the sea?

With the development of epifluorescence microscopy and sensitive radioisotope techniques, high abundance and activity of microorganisms was observed in marine waters since 1970s and 1980s. These observations resulted in a new concept of rapid turn-over and recycling of organic matter through a 'microbial loop' (Azam et al., 1983; Azam, 1998). Figure 5.1 illustrates fluxes of material through the marine microbial loop. Main processes are C fixation by photosynthetic microorganisms (prokaryotic and eukaryotic) with exudation losses of Dissolved Organic Matter (DOM), which is incorporated by heterotrophic bacteria. Phagotrophic protists in turn graze both autotrophs and bacteria producing 'sloppy feeding' loss of DOM, which returns to the loop. DOM is remineralized by all microorganisms into Dissolved Inorganic Nutrients (DIN), which are taken up by autotrophic and heterotrophic microorganisms. Thus, bacteria and the whole microbial loop function as a dynamic sink for C and monopolizes > 90% of C fixed by primary production in Ria Formosa waters (Ducklow et al., 1986; Pomeroy et al., 2007). The role of marine viruses still remains to be completely elucidated in the oceans, although it is known that viral lyses promotes biogeochemical fluxes by releasing both dissolved (DOM) and also particulate organic matter (POM) from lysed cells (Suttle, 2007). In Figure 5.1, viruses are depicted to produce mainly DOM, which is directly absorbed by bacteria, since the POM fraction is much smaller and has to be subjected to exoenzymatic hydrolysis into DOM by bacteria before incorporation.

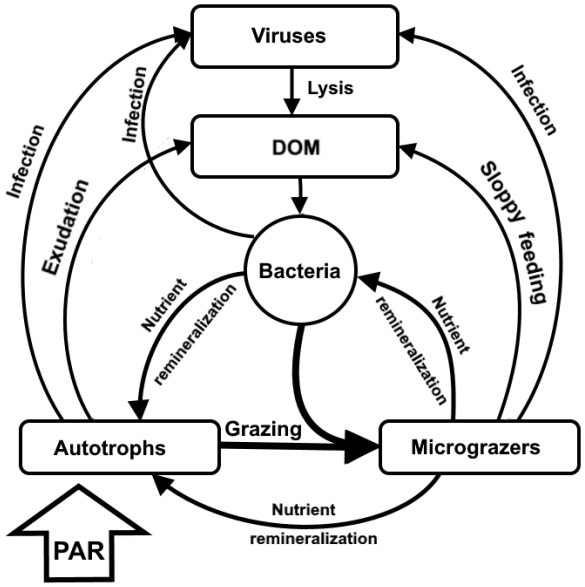

Figure 5.1.
Diagram of microbial loop illustrating concept of dynamic carbon sink. DOM: Dissolved Organic Matter; PAR: Photosynthetically Active Radiation.

5.2. Phytoplankton community in the Ria Formosa lagoon

Phytoplankton constitutes a community of photosynthetic microorganisms drifting in surface waters and span size ranges from 0.2 to 230 µm (see Box 5.1). They generate more than half the oxygen in the earth s atmosphere and constitute the base of all aquatic/marine foodwebs in surface waters.

The Ria Formosa lagoon (Fig. 5.2), located on the Atlantic ocean in the Algarve region of Portugal, is the most south-westerly of European lagoons. In contrast to microtidal conditions in most Southern European lagoons, the Ria has a mesotidal regime with a tidal range varying between 1.3 m at neap and 3.4 m at spring tides. This shallow network of saltmarsh, sediment flats and tidal channels covers an area of 58 km^2, and is an internationally recognized site of ecological importance (Newton et al., 2014), as well as supporting a fishery and aquaculture industry of national significance.

Box 5.1. What are size ranges of microbial plankton?

There are 4 size-classes of microscopic plankton:

1. Ultra- or virioplankton (< 0.2 µm): viruses and very small bacteria
2. Picoplankton (0.2 - 2 µm): heterotrophic and autotrophic (photosynthetic) bacteria
3. Nanoplankton (2 - 20 µm): heterotrophic and autotrophic nanoflagellates, small diatoms
4. Microplankton (20 - 200 µm): diatoms, dinoflagellates, ciliates

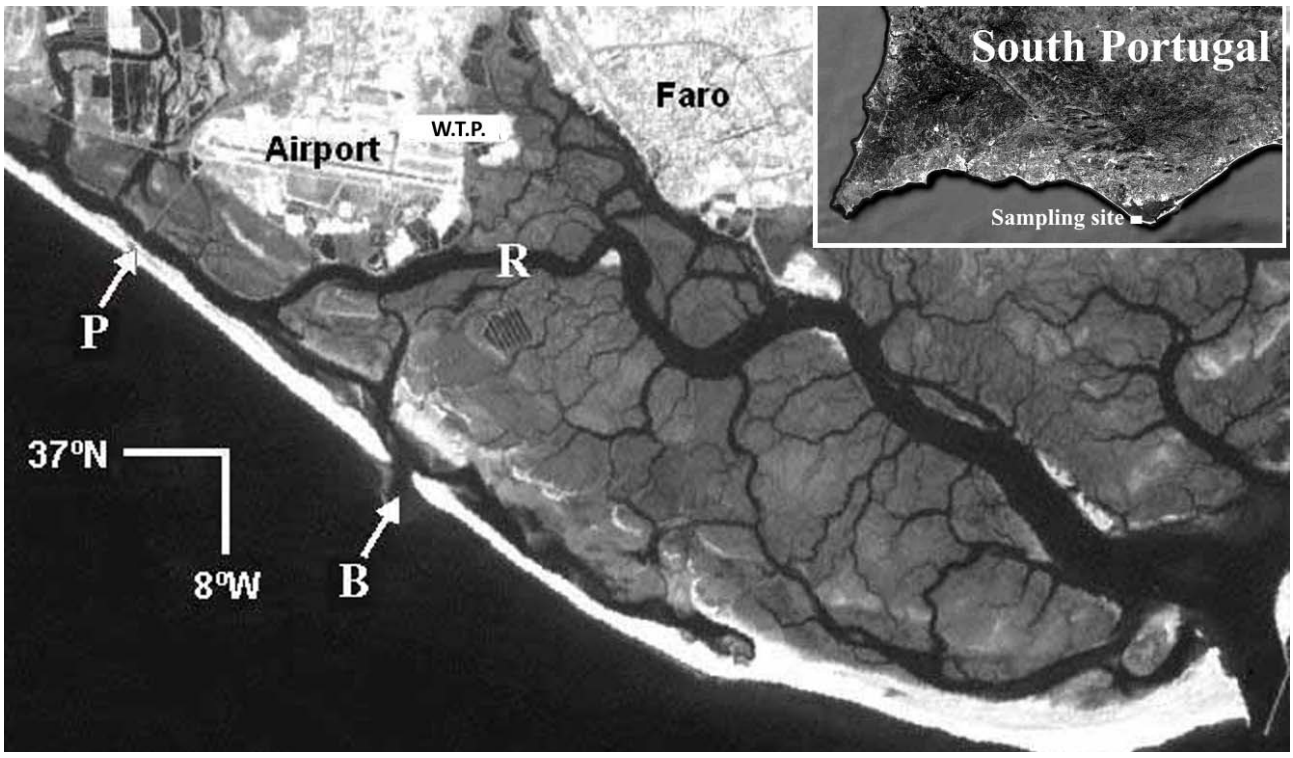

Figure 5.2.
Location of sampling stations (P: Ponte; B: Barra; R: Ramalhete) in the western region of the Ria Formosa. WTP: urban waste water treatment plant.

This study presents data on pelagic primary and bacterial production, as well as phytoplankton community structure at three stations representing contrasting situations within the lagoon, namely: an artificial inlet opened in 1997 prior to this study (B in Fig. 5.2), a channel draining salt marsh (P in Fig.

5.2), and a channel draining both salt marsh (R in Fig. 5.2) and the effluent from the main facility for urban Water Treatment Plant (WTP in Fig. 5.2) of Faro. The new outlet to the ocean (station B) changed hydrodynamics in Ria Formosa substantially (see Newton & Icely, 2002 for details). The sampling strategy was designed to assess microbial dynamics during extreme tidal conditions over the year. These conditions occur for neap tides close to Summer (June) and Winter (December) solstice and for spring tides during Autumn (September) and Spring (April) equinox.

Sampling campaigns were carried out on 13th June 2001, 18th September 2001, 8th December 2001 and 27th April 2002 (Table 5.1). A fifth campaign was added on 3rd July 2002 for phytoplankton microscopy enumerations. Water samples were collected during daylight at high water (HW), mid - ebb (EBB), low water (LW), and mid-flood (FLOOD) from Barra, Ponte and Ramalhete stations (B, P and R in Fig. 5.1) in the Ria Formosa. Water samples for bacterial and phytoplankton enumeration were preserved with particle-free 25% gluteraldehyde (2% final concentration) and kept refrigerated and processed within 24 h to minimize cell loss. Samples were filtered through 0.2 µm black polycarbonate filters mounted on 0.45 µm cellulose acetate backing filters and stained with 4 ,6-diamidino-2-phenylindole (DAPI) for 5 minutes (JGOFS 1994). Sample volumes of 2 ml were filtered for picoplankton and 20 ml for nanoplankton and small microplankton. Filters were mounted on a slide with Cargille type A non-fluorescent immersion oil and frozen until examination. DAPI was used as a fluorochrome dye and samples were observed with a Leica epifluorescence microscope using UV and blue filters for DAPI and for chlorophyll autofluorescence, respectively. The concentration of chlorophyll *a* (chl *a*) and pheopigments (pheo) in the water samples was determined fluorimetrically within days after sampling, following methods described in JGOFS (1994).

In microscope enumerations, phytoplankton cells were separated in two size fractions: 0.2-2 µm – picoplankton (cyanobacteria and picoflagellates) and 2-200 µm – nanoplankton and small microplankton (nanoflagellates, dinoflagellates, diatoms and some autotrophic ciliates). Cells were identified according to the following features: cyanobacteria – orange dots (< 2 µm diameter) under blue light; small flagellates – larger blue dots (nucleus ~1-2 µm diameter) surrounded by a paler blue halo (cytoplasm 3-10 µm diameter) under UV light and with orange/red aufluorescence under either UV or blue light; nanoflagellates and dinoflagellates (2-100 µm) – shapes, presence of chloroplasts and flagella (when visible); diatoms – shape, chloroplasts and frustules; ciliates – shape, presence of chloroplasts and cilia (when visible). At least 20 random fields of view were counted for each size fraction. Cell volumes for biomass determination were determined following formulas given in Box 5.2.

To determine Total Bacteria Number (TBN) and Bacterial Biomass (BB) compiled in Table 5.2, a minimum of 300 heterotrophic bacteria (without autofluorescence) and 25 fields of view

Box 5.2. How is phytoplankton cell volume and carbon determined?

Biovolumes were calculated on the basis of pre-defined 3-dimensional shapes and their respective stereometric formulas recommended by Hillebrand et al. (1999). Measurements of linear dimensions were made with a calibrated ocular micrometer scale in the microscope eyepiece.

Carbon content (CC) was estimated from mean cell volume (MCV) using following non-linear equations:

- Cyanobacteria and Picoflagellates:
  $CC\ (pgC.Cell^{-1}) = 0.436 \times MCV^{0.863}$
- Nanoflagellates (2-20 µm):
  $CC\ (pgC.Cell^{-1}) = 0.216 \times MCV^{0.939}$
- Diatoms:
  $CC\ (pgC.Cell^{-1}) = 0.288 \times MCV^{0.811}$
- Dinoflagellates:
  $CC\ (pgC.Cell^{-1}) = 0.760 \times MCV^{0.819}$

Total biomass was then calculated by multiplying CC with abundance.

were counted. Cell dimensions of 50 randomly selected bacteria were measured in each sample with a New-Porton calibrated graticule (Graticules Ltd.). Cell carbon content and biomass were calculated individually with the carbon to volume allometric relationship derived by Norland (1993).
Total phytoplankton enumerated microscopically averaged 23 000 x 10^3cells L^{-1} at HW and 12 000 x 10^3cells L^{-1} at LW at all stations. Phytoplankton abundance showed significant differences between HW and LW. Lowest abundance occurred in December 2001 with 2700 x 10^3cells L^{-1} at Ponte during HW and with 2400 x 10^3cells L^{-1} at Barra during LW. Highest abundance was observed in July 2002 with 78 000 x 10^3cells L^{-1} at Barra during HW and 29 000 x 10^3cells L^{-1} at Ponte during LW. Figure 5.3 shows that seasonal fluctuations in phytoplankton abundance were not as marked as in chlorophyll *a* and phaeopigments, when pigment concentrations in winter were less than half of that in summer.

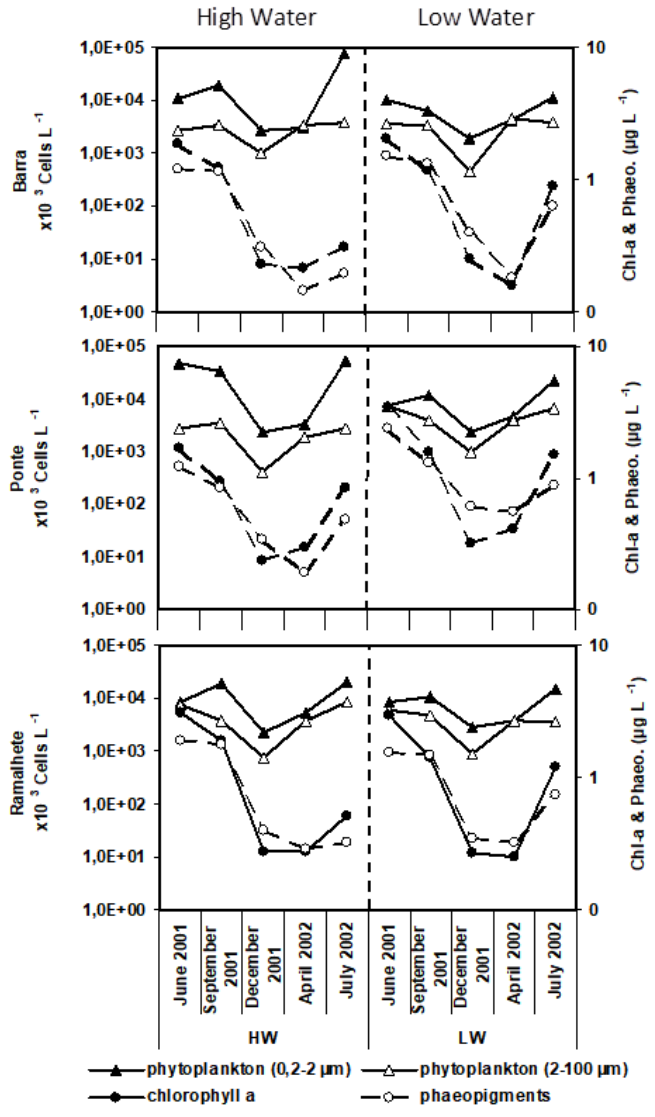

Figure 5.3.
Seasonal variations in photopigment concentrations and abundance of two main phytoplankton size- fractions at the three stations. Note Log scale in y-axes.

Variations in abundance of different phytoplankton groups are depicted in Figure 5.4. Cyanobacteria were the most numerous organisms at all sites ranging from a mean of 22 300 x10^3cells.L^{-1} (HW-Ponte) to 5 300 x10^3cells.L^{-1} (LW-Barra). Pico- and nanoflagellate abundance ranged from 5 100 x10^3cells.L^{-1} (HW-Ponte) to 1 200 x10^3cells.L^{-1} (LW-Ramalhete) for picoflagellates, and from 27 00 x10^3cells.L^{-1} (LW-Ponte) to 1 300 x10^3cells.L^{-1} (HW-Ponte) for nanoflagellates. At both HW and LW, picoflagellates were more numerous

than nanoflagellates at Ponte, but were less numerous at Barra and Ramalhete. Diatoms varied between 2 400 x10^{3}cells.L^{-1} (HW-Ramalhete) and 480 x10^{3}cells L^{-1} (HW-Barra). Dinoflagellates and ciliates were the least abundant taxa, ranging between 260 x10^{3}cells.L^{-1} (HW-Barra) and 110 x10^{3}cells.L^{-1} (LW-Ramalhete) and between 120 x10^{3}cells.L^{-1} (HW-Ramalhete) and 50 x10^{3}cells.L^{-1} (HW-Barra or Ponte), respectively. Overall variability was higher at HW than LW for cyanobacteria, picoflagellates, dinoflagellates and ciliates, but not for nanoflagellates and diatoms, which varied more at LW. Typical seasonal fluctuations in phytoplankton groups can also be seen in Figure 5.4 with decreasing numbers in winter and increasing during spring-summer, particularly for cyanobacteria and picoflagellates. Both dinoflagellates and ciliates occurred generally in low numbers without any evident seasonal or spatial pattern. Comparison of seasonal abundance of phytoplankton taxa and photopigments (chl) revealed generally good correlations at all 3 stations ($n = 10$; $r \geq 0.76$; $p<0.05$), whereas diatoms appeared better correlated with phaeopigments (eg. Ponte: $n = 10$; $r = 0.82$; $p<0.01$).

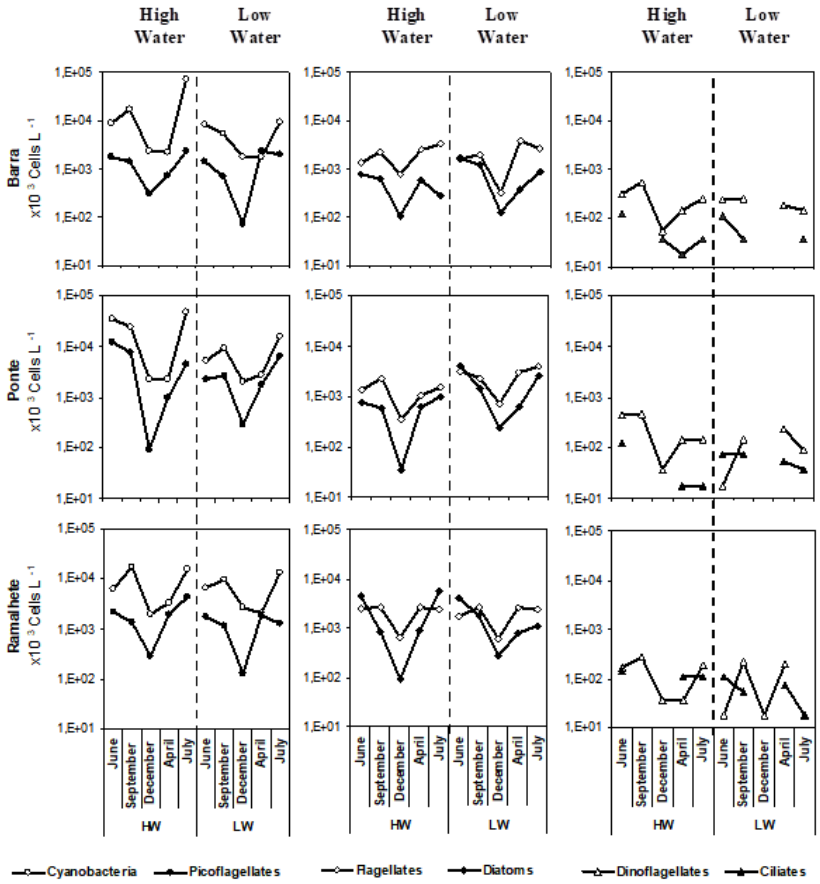

Figure 5.4.
Seasonal variations in different phytoplankton groups at the three stations. Note Log scale in y-axes.

Mean biomass of different phytoplankton groups are presented in Table 5.1. Total phytoplankton biomass throughout the sampling period averaged 158 µgC. L^{-1} during HW and 318 µgC.L^{-1} during LW. Minimum phytoplankton biomass was detected in December 2001 with 12 µgC. L^{-1} at Ponte during HW and with 26,19 µgC. L^{-1} at Ponte in April 2002 during LW. Diatoms exhibited highest mean biomass at all stations ranging fom 571 µg C. L^{-1} (LW-Ponte) to 36.5 µgC. L^{-1} (HW-Ponte). Dinoflagellate and nanoflagellate mean biomass values were comparable, ranging from 62.8 µgC. L^{-1} (HW-Barra) to 18.3 µgC. L^{-1} (HW-Ramalhete) for dinoflagellates, and from 52.7 µgC. L^{-1} (LW-Ponte) to 26.4 µgC. L^{-1} (HW-Ponte) for flagellates. Cyanobacteria were always the most abundant group, but due to very small cell sizes, had low mean biomass ranging between 23.2 µgC L^{-1} (HW-Ponte) and 6.4 µgC. L^{-1} (LW- Barra). Picoflagellates exhibited lowest mean biomass with ranges between 5.6 µgC. L^{-1} (HW-Ponte) and 1.2 µgC. L^{-1} (LW-Ramalhete).

Table 5.1.
Mean biomass of different phytoplankton groups (µg L^{-1}) at the three stations for the complete sampling Period (June 2001 to July 2002) during High Water and Low Water tidal stages. Note standard deviations in italics and coefficients of variation in %

	HIGH WATER			LOW WATER		
	BARRA	**PONTE**	**RAMALH.**	**BARRA**	**PONTE**	**RAMALH.**
Cyanobacteria	**22.27** *32.99* 148%	**23.22** *19.72* 85%	**9.80** *8.70* 89%	**6.44** *4.63* 72%	**8.24** *7.58* 92%	**8.11** *5.90* 73%
Picoflagellates	**1.37** *0.77* 56%	**5.56** *5.35* 96%	**1.90** *1.79* 94%	**1.21** *0,89* 74%	**2.65** *3.05* 115%	**1.22** *0.61* 50%
Nanoflagellates	**39.31** *29.78* 76%	**26.42** *17.58* 67%	**39.88** *21.83* 55 %	**41.67** *25.85* 62%	**52.67** *45.94* 87%	**28.53** *7.72* 27%
Dinoflagellates	**62.78** 54.60 87%	**30.18** *32.29* 107%	**18.35** *25.80* 141%	**40.45** *14.56* 36%	**40.53** *32.68* 81%	**26.52** 11.11 42%
Diatoms	**79.56** *87.41* 110%	**36.56** *46.53* 127%	**78.31** *79.62* 102%	**84.35** *60.05* 71%	**571.31** *1 076.22* 188%	**62.92** *37.63* 60%
TOTAL (g C. L^{-1})	**205.29**	**121.94**	**148.23**	**174.13**	**675.41**	**127.29**

Several trends could be ascertained from microscopical analyses of phytoplankton community, namely:

a) Although overall mean abundance of phytoplankton was much higher during HW (23.0x10^6 cells. L^{-1}) than during LW (12.0 x10^6 cells. L^{-1}), mean total biomass was half (158 µgC. L^{-1}) during HW than during LW (318 µgC.L^{-1}) due to abundant and ubiquitous picophytoplankton. This fraction demonstrated high variability which explained the lack of correlation between chlorophyll-derived biomass and microscopy-derived biomass, which resulted in carbon content overestimation of smallest size fraction (0.2-2 µm). In fact, observed biomass was 5.5-fold higher than calculated biomass in this study, resulting in a C:Chl ratio of 275 rather than the classic ratio of 50.

b) Seasonal patterns in phytoplankton community structure included summer blooms of oceanic cyanobacteria

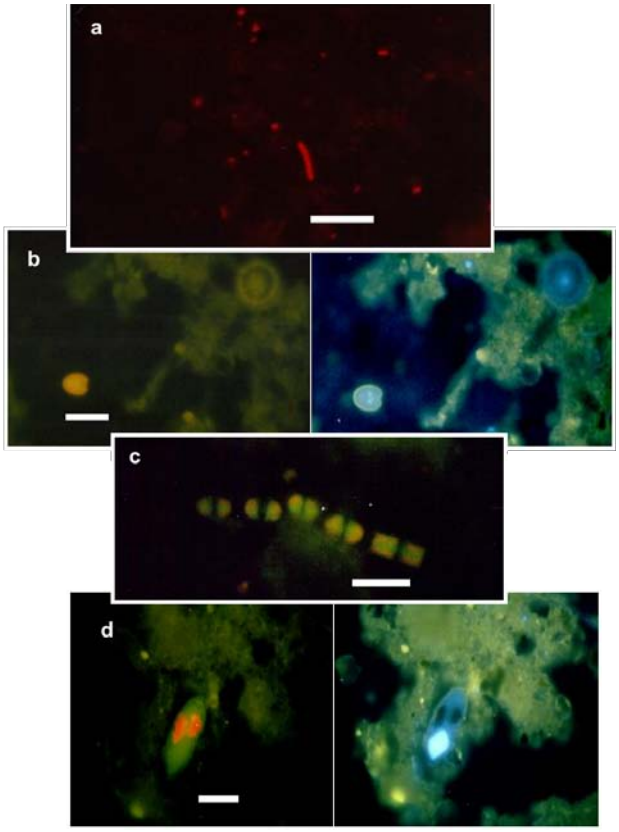

Figure 5.5.
Photomicrographs of pico- and nanophytoplankton taken with epifluorescence microscopy. 5.5.a: orange-red autofluorescence of chroococcoid and short chain-forming cyanobacteria in unstained sample under green light; 5.5.b: autofluorescent nanoflagellate under blue light (left) and stained with DAPI under UV light (right); 5.5.c: autofluorescent Chaetoceros spp. chain under blue light; 5.5.d: autofluorescent dinoflagellate under blue light (left) and stained with DAPI under UV light (right). Bars: 10 µm. Courtesy of Sandra M. Caetano.

(*Synechococcus* spp.) at Barra and predominance of benthic diatoms during summer. Species of dinoflagellates and ciliates (mainly *Myrionecta rubra*) occurring during summer blooms at Barra were typically oceanic and seemed to have been transported inshore.

c) Diatom abundance and biomass were significantly correlated with chl *a*. There was a significant correlation between the seasonal patterns of chlorophyll and diatom abundance and biomass. Indeed, diatoms contributed an overall average of 53% of total phytoplankton biomass during LW and 35% of total biomass during LW with several conspicuous species (*Chaetoceros*, *Nitzchia* and *Pseudonitzchia* spp). This diatom predominance was confirmed by High Performance Liquid Chromatography (HPLC) analyses of photopigments during same time period (Pereira et al., 2007).

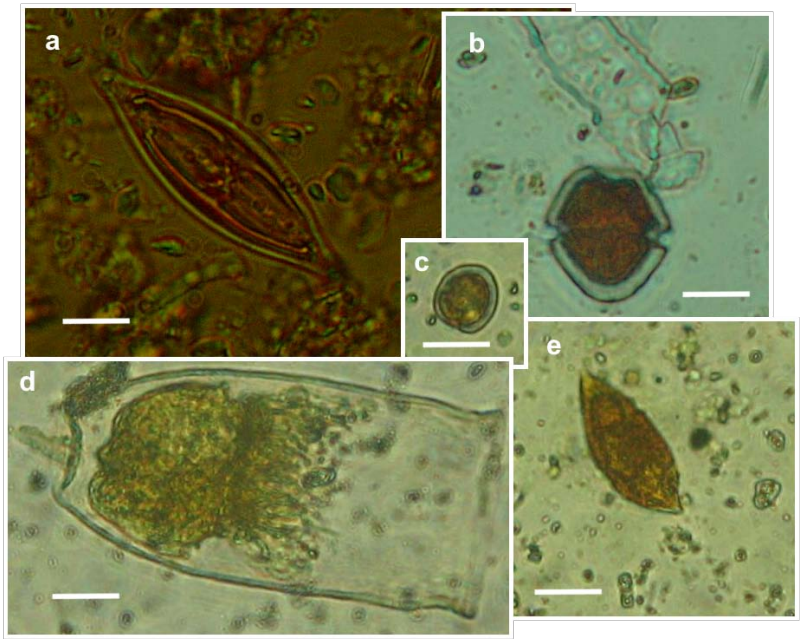

Figure 5.6.
Photomicrographs of larger microplankton species taken with lugol stained samples observed under phase-contrast inversion microscopy with 400X magnification. 5.6.a: pennate diatom *Navicula* sp.; 5.6.b : unidentified thecate dinoflagellate; 5.6.c: bloom-forming dinoflagellate *Prorocentrum minimum*; 5.6.d: large herbivorous tintinnid ciliate; 5.6.e: thecate dinoflagellate *Gyrodinium* sp. Courtesy of Rita B. Domingues. Bars: 20 µm

d) Large dinoflagellates were usually more conspicuous during HW at all 3 stations contributing an overall mean of 35% to total biomass during HW and only 16% during LW (mainly *Ceratium* and *Prorocentrum* spp.). Nanoflagellates (2-20 µm) were abundant and ubiquitous (mostly Cryptophytes) contributing an overall mean of 25% to total phytoplankton biomass regardless of tidal stage.

These trends were explained by the hydrodynamic regime of the Ria Formosa lagoon. Earlier observations confirmed that 80% of water exchange in the western lagoon occurred through the Faro-Olhão and Armona inlets (Silva et al., 2002). Newton and Icely (2002) stressed the important influence of the artificial inlet opened in June 1997 at Barra location (B in Fig. 5.2). In effect, floodwater from Barra inlet reduced water inflow of water from Ramalhete channel into the western Ancão basin, allowing rapid and substantial exchange of water between Barra and Ponte stations over a tidal cycle, but effectively reduced water exchange between Ramalhete and other stations. This circulation pattern in the Ancão Basin explained why patterns in phytoplankton community composition at Ramalhete in inner lagoon were strikingly different from those observed at Ponte and Barra in outer lagoon.
Photomicrographs of typical phytoplankton species are illustrated in Figure 5.5 and Figure 5.6. Figure 5.5 depicts typical pico- and nanophytoplankton taxa taken with epifluorescence microscopy stained with DAPI under UV or blue light, while Figure 5.6 photomicrographs of larger conspicuous species were taken using phase contrast inversion microscopy with Lugol staining (JGOFS Protocols 1994).

5.3. Quantification of main carbon fluxes in Ria Formosa

Phytoplankton primary production (PP) and bacterial production (BP) were determined following sampling strategy described in previous section using standard C14 incorporation methods. BP using ^{14}C-leucine incorporation followed method described in Chin-Leo and Kirchman (1988), whereas PP was determined by ^{14}C-bicarbonate fixation according to JGOFS standard protocols (1994). Figure 5.7 illustrates incubation set-up (5.7.a) for PP, filtration ramp for C14 incorporations (5.7.b) and bubbling method to purge unfixed CO_2 (5.7.c) in PP determinations.

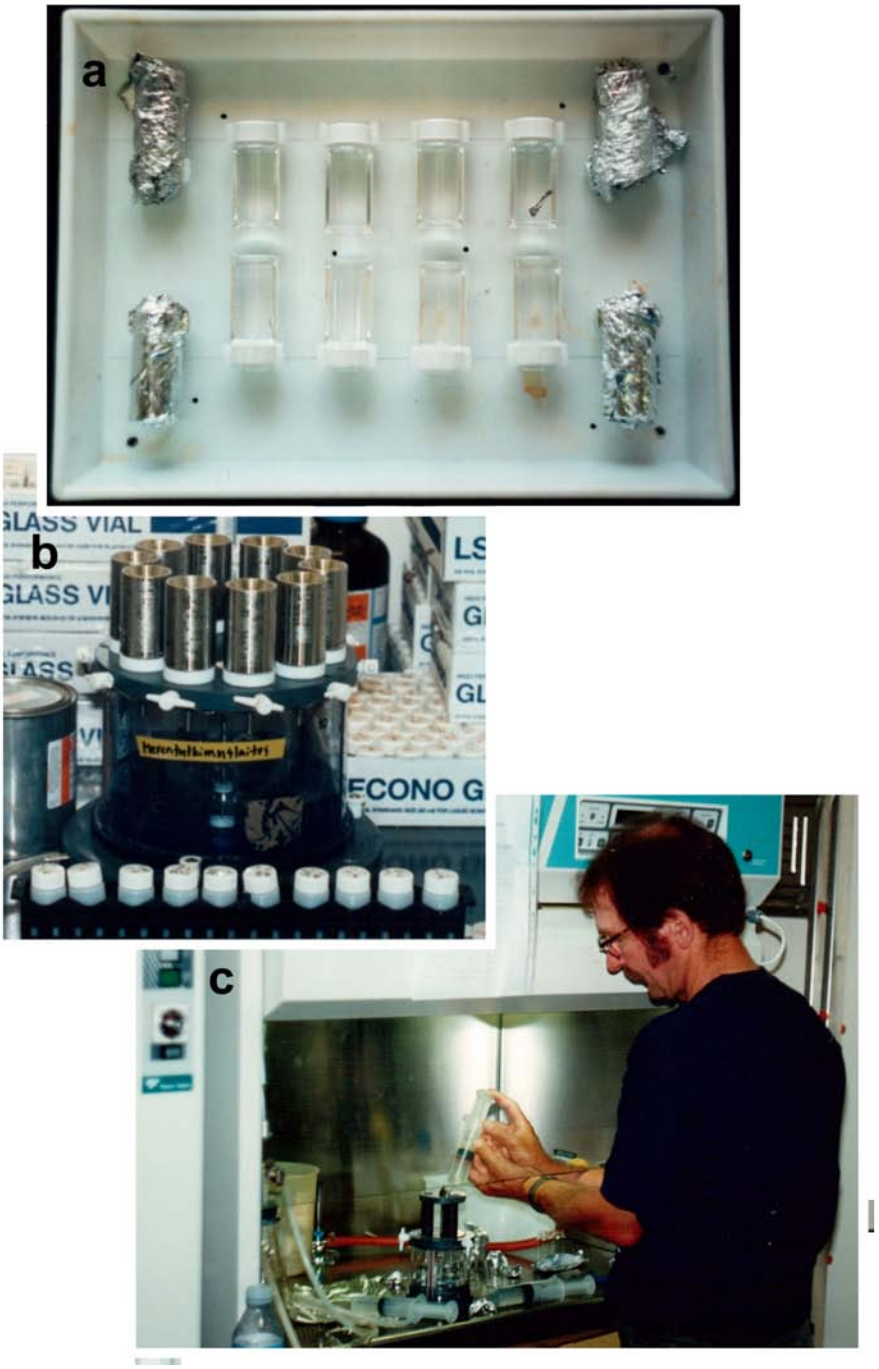

Figure 5.7.
Equipment used in C14 methodology. 5.7.a: Primary Production (PP) incubation set-up; 5.7.b: filtration ramp used in C14 incorporations; 5.7.c: bubbling method used in PP. Courtesy of Pedro. A. Mendes.

Table 5.2 compiles means of bacterial abundance or Total Bacteria Number (TBN), Bacterial Biomass (BB), Phytoplankton Biomass (PB), Bacterial Production (PB) and phytoplankton Primary Production (PP). Bacteria growth is calculated by BP:BB and phytoplankton growth by PP:PB. Bacterial Carbon Demand was calculated by

BCD = BP / BGE whereby Bacterial Growth Efficiency (BGE)

BGE = (0,037+0,65 BP) / (1,8+BP) according to del Giorgio and Cole (1998).

Table 5.2.

Microbial variable means at three stations for four sampling campaigns. TBN: Total Bacteria Number; BB: Bacteria Biomass; BP: Bacteria Production; BCD: Bacterial Carbon Demand; PB: chlorophyll-derived Phytoplankton Biomass; PP: Primary Production. Note ratios BP: BB = bacterial growth rates; PP:PB = phytoplankton growth rates. BCD:PP > 1: heterotrophy; BCD:PP < 1 autotrophy.

		June 2001			September 2001			December 2001			April 2002		
	Tide	**B**	**P**	**R**	**B**	**P**	**R**	**B**	**P**	**R**	**B**	**P**	**R**
TBN	HW	2.2	8.6	8.1	2.8	2.8	6.3	1.8	1.2	2.3	0.8	1.1	2.9
(10^9cells. L^{-1})	LW	6.9	1.2	7.7	4.4	5.8	9.4	2.1	3.8	2.9	3.5	5.4	4.2
BB	HW	24.3	98.2	86.1	29.2	31.9	86.6	22.9	12.7	24.2	8.3	11.5	35.5
(mgC. m^{-3})	LW	79.4	147.5	87.7	47.2	72.0	109.9	31.5	47.3	37.9	28.9	74.1	47.5
BP	HW	13.7	13.5	3.6	2.1	3.2	7.6	0.5	1.5	4.1	1.4	2.5	4.9
(µgC. L^{-1}. h^{-1})	LW	16.5	17.0	16.4	6.8	9.6	5.0	2.4	4.5	1.9	9.3	19.3	8.7
BCD	HW	23.8	23.4	8.1	5.9	7.5	14.4	3.1	4.9	9.0	4.8	6.4	10.3
(mgC. m^{-3}. h^{-1})	LW	28.0	28.9	28.0	13.1	17.5	10.3	6.3	9.5	5.4	17.0	32.3	16.1
BP:BB	HW	***0.56***	***0.14***	***0.04***	***0.07***	***0.10***	***0.09***	***0.02***	***0.12***	***0.17***	***0.17***	***0.22***	***0.14***
(h^{-1})	LW	***0.21***	***0.11***	***0.19***	***0.14***	***0.13***	***0.04***	***0.08***	***0.09***	***0.05***	***0.24***	***0.26***	***0.18***
PB	HW	92.9	83.6	154.2	61.6	46.7	96.3	11.4	11.7	13.8	10.8	14.8	13.7
(mgC. m^{-3})	LW	102.6	173.4	147.9	58.6	78.1	71.1	12.7	16.0	13.3	7.9	20.7	12.5
PP	HW	30.3	32.3	35.4	10.5	14.3	32.1	1.8	2.1	2.5	4.2	7.6	8.7
(µgC. L^{-1}. h^{-1})	LW	19.8	73.8	46.5	9.3	27.7	23.3	2.0	3.1	2.2	4.9	15.8	8.3
PP:PB	HW	***0.33***	***0.39***	***0.23***	***0.17***	***0.31***	***0.33***	***0.16***	***0.18***	***0.18***	***0.39***	***0.51***	***0.63***
(h^{-1})	LW	***0.19***	***0.43***	***0.31***	***0.16***	***0.35***	***0.33***	***0.16***	***0.19***	***0.16***	***0.62***	***0.76***	***0.66***
BCD:PP	HW	***0.79***	***0.72***	***0.10***	***0.23***	***0.56***	***0.53***	***0.45***	***1.72***	***2.33***	***3.60***	***1.14***	***0.84***
	LW	***1.41***	***0.39***	***0.35***	***0.60***	***1.41***	***0.63***	***0.44***	***3.15***	***3.06***	***2.45***	***3.47***	***2.04***

Total bacterial numbers (TBN), bacterial biomass (BB), bacterial production (BP), bacterial carbon demand (BCD) in Table 5.2 were consistently higher at LW throughout the year at Barra. A similar pattern occurred at Ponte except for a higher value of TBN at HW in June. However, in the case of Ramalhete, values were higher at HW, for TBN in June, for BP in September and December and for BCD in September and December. On the other hand, phytoplankton biomass (PB) and primary production (PP) were higher during HW at Barra in September and April, and at Ramalhete for all four seasons. PP had higher values for HW in June and September at Barra, and in September, December and April at Ramalhete. PB and PP values were consistently lower at HW compared to LW.

June and September 2001 values for bacterial production (BP) in the Ria were higher than most values reported for estuaries and salt marshes (Billen et al., 1990), whereas December and April values were consistent with other published values. All were within the range reported by Billen et al. (1990) for

estuarine environments, and consistent with values reported for Ria de Aveiro (Almeida et al., 2002). In June, BP and PP appeared to have inverse trends. However, statistical analyses showed no correlation between BP and either PP, pigments, TBN or BB. Furthermore, plotting Log BB vs. Log BP showed no C limitation of the bacterial community. According to the Billen et al., 1990 model, this indicated that bacteria were not controlled by bottom-up mechanisms, such as DOM availability.

In April 2002, an increase in bacteria and phytoplankton production was observed, although standing stocks remained similar to December 2001, resulting in a marked increase in specific production. BP was generally higher than PP, but there was no significant correlation between BP and PP. (Fig. 5.3). These observations suggested there was no coupling between PP and BP during spring. However, plotting Log BB vs. Log BP Billen et al. (1990), model application indicated that bacterial community was strongly limited by availability of dissolved organic carbon. Studies that show that bottom-up and top-down processes could change quickly within days and that the strength of bottom-up control could be influenced by changes in temperature (Vaqué et al., 2014). This is not surprising, since marine microorganisms in temperate regions survive near optimal temperature during summer and near null growth temperature during winter despite acclimatization and seasonal succession (Pomeroy and Wiebe, 2001).

Grazing (top-down control), substrate supply rates (bottom-up control) and temperature have been considered as main regulation factors of bacterial production (BP) in marine environments, whereas primary production (PP) is regulated by the combined effect of light, nutrients and temperature, as well as grazing (Vaqué et al., 2014). During summer, nutrients and organic matter input increased in the Ria Formosa due to a rise in tourist population. In fact, nutrient concentrations (data not shown) showed maxima in DIN (Dissolved Inorganic Nitrogen) and phosphates (P) in June 2001 with generally higher values observed at LW. Silicate (DSi) maximum values occurred in September 2001 (data not shown) with higher values at LW. This pattern of P and DSi variability indicated that sediments were main source of nutrients in the Ria Formosa. According to Justi et al. (1995) criteria for stoichiometric nutrient balance in coastal waters, there was a potential limitation in DIN, but not in P or DSi.

Phytoplankton exudation provides the main source of DOM for heterotrophic bacteria (see section 1). To assess to what extent Bacterial Carbon Demand (BCD) was satisfied by phytoplankton, an average of 20% of Primary Production was assumed to be lost to exudation. Then, Primary Production fulfilled at most 87.4 % of BCD in June 2001, 45.5 % in September 2001, 11.6 % in December 2001 and 17.5 % in April 2002. Therefore, the remainder dissolved carbon had to be supplied internally by protist "sloppy-feeding" (see section 5.1) or by external sources (land derived). Since grazing rates by phagotrophic protists were not available for this study, this additional source of carbon could not be assessed. However, recent studies in productive coastal waters, determined that 49% to 65% of Bacterial Production was grazed by protists (Vaqué et al., 2013), and a large fraction (>50%) of prey biomass is lost by "sloppy-feeding", thus this mechanism could largely supply remainder dissolved carbon necessary for BCD. So, the role of bacteria as land-derived DOM consumers could only be significant during cold seasons.

Furthermore, annual variations in BCD: PP (Table 5.2) indicated that the Ria underwent a marked shift from strongly autotrophic in June and September 2001 to heterotrophic in December 2001 and April 2002. A similar shift has also been reported for Ria de Aveiro (Almeida et al., 2002). This is explained, by the typical light limitation of phytoplankton in RF during winter, whereas during summer both light and temperature increase. Differences in phytoplankton community composition (see Fig. 5.3 and 5.4) could explain PP variations at HW and LW (Table 5.2) between Ramalhete and other stations. Furthermore, Newton and Mudge (2003) reported much lower water exchange rate at Ramalhete than at Barra and Ponte. Since Ramalhete is located at the main sewage treatment plant outlet for Faro (Fig. 5.2), this station is considerably more vulnerable to anthropogenic impact. When lagoon shifts from an autotrophic to heterotrophic regime during the cold season, this could enhance role of bacteria as DOM consumers and remineralizers, which is an important self-purification process in natural waters.

5.4. Assessment of trophic or ecological status of Ria Formosa

Previous Ria Formosa eutrophication reports concluded poor or pristine status depending on classification criteria, either nutrients only, or, in combination with chlorophyll and oxygen saturation (Newton et al., 2003). In contrast, criteria selected for eutrophication by the US National Estuarine Eutrophic Assessment based on symptoms, such as high chlorophyll *a* concentrations and low oxygen saturations, suggested that the lagoon was near pristine (Newton et al., 2003). To reconcile differences in assessing trophic status in Regions of Restricted Exchange (RRE), Tett et al. (2003) proposed measuring relative contribution of autotrophic and heterotrophic components of pico-nano and microplankton (size range: 0.2 to 200 µm) to pelagic production which can be estimated by comparing primary and bacterial production.

Annual Phytoplankton Primary Production (APPP) in RF (Table 5.3) was estimated to reach an overall mean of 533 g C. $m^{-2.}$ yr^{-1} at Barra, 317 g C. $m^{-2.}$ yr^{-1} at Ponte and 203 g C m^{-2} yr^{-1} at Ramalhete. APPP was calculated assuming a well mixed water column and that depth of euphotic layer was approximately 3 x Secchi depth (A. Barbosa, pers. comm.), so that the whole water column is euphotic in RF. APPP values were higher than overall mean of 252 g C .m^{-2}.yr^{-1} reported by Cloern et al. (2014) when reviewing data from 131 estuaries, which exhibited a large range from −105 (net pelagic production, Scheldt Estuary, Belgium) to 1890 g C. m^{-2}.yr^{-1} (Tamagawa Estuary, Japan). Following Scott Nixon's classification (Nixon, 1995), oligotrophic ecosystems possess APPP < 100 g C. m^{-2}. yr^{-1}, mesotrophic 100– 300 g C. m^{-2}.yr^{-1}, eutrophic 300–500 g C. m^{-2}. yr^{-1}, and hypertrophic APPP > 500 g C. m^{-2}. yr^{-1}. Thus, RF can be considered as eutrophic. However, since PP was determined only at the surface without any vertical profiles, APPP is only a rough estimate presuming mixed layer depth as euphotic layer (B: 10 m; P: 3 m; R: 2m).

Table 5.3.

Annual Phytoplankton Primary Production (APPP) and Annual Bacterial Production (ABP) at the three stations assuming mixed layer depth approx. equal to euphotic layer (Barra: 10 m; Ponte: 3 m; Ramalhete: 2 m)

	Barra	Ponte	Ramalhete
APPP (g C. m^{-2}. yr^{-1})	533	317	203
ABP (g C. m^{-2}. yr^{-1})	653	279	166

On the other hand, applying Carlson s (1977) Trophic State Index (TSI) developed for North American lakes, TSI based on chlorophyll was calculated to be 47.1 and based on Secchi depth 40.7. So, on a TSI scale of 0-100, RF could be classified as mesotrophic. However, considering Carlson s TSI general scheme, the range of chlorophyll values determined in RF (0.22 - 3.90 µg. L^{-1}) and overall mean of 1.18 µg. L^{-1}, RF would be classified closer to oligotrophic (0-2.6 µg. L^{-1}), rather than mesotrophic (2.6-20 µg. L^{-1}). Moreover, a trophic state index (TRIX) developed by Vollenweider (1998) and recommended by the European Environmental Agency (see Box 5.3) to monitor eutrophication in coastal waters (EEA report 7/2001), was calculated using overall mean chlorophyll (1.18), DIN (54.73), phosphates (42.11) in mg m^{-3} and 90th percentile oxygen saturation (130%) reported by Newton et al. (2003). This yielded a Trophic Score of 4.91 and a Trophic Index (TRIX) of 5.32, which falls within the lower range of TRIX values

Box 5.3. How is Trophic State Index (TRIX) recommended by European Environmental Agency calculated?

According to Vollenweider (1998), and as recommended in EEA report 7/2001
TROPHIC INDEX = (LOG [Ch*aD%O*N*P] -[-1.5])/1.2
Ch: chlorophyll *a* in mg. m-3
aD%O: absolute value of (% Oxygen - saturation)
N: Dissolved Inorganic Nitrogen concentration ($NH_4^+ + NO_3^- + NO_2^-$) in mg. m^{-3}
P: Dissolved Inorganic Phosphate (PO_4^{3-}) in mg. m^{-3}
Example:
Given following concentrations,
chl *a*: 22.3 mg. m^{-3}; % Oxygen saturation: 184%; N: 343 mg. m^{-3}; P: 5.0 mg. m^{-3}
Trophic Score = log(Ch*D%0*N*P) = **6.51**
Trophic Index = (6.51 + 1.51)/1.2 = **6.88**
Although Vollenweider (1998) developed this Index for Mediterranean waters, the EEA recommends its application for European NE Atlantic coastal waters using seasonal and regional means for limits. Thus, TRIX seasonal values vary from 4.9 and 7.1 in Danish waters (Kattegat) and from 7.5 to 9.0 in the North Sea (EEA 7/2001).

determined in Northern European coastal waters (EEA report7/2001) during 1998. Unfortunately, since there are no measurements of oxygen saturation in this study, no useful information could be derived from spatial and temporal variations.
Towards the implementation of European Water Framework Directive (WFD 2000/60/EC) references and boundary conditions were determined in Portuguese Coastal and Transitional Waters as well as in Coastal Lagoons at selected reference sites. Boundary and reference values are generally expressed for the metric chl *a* 90th percentile (see Box 5.4). The High/Good boundary corresponds to a 50% deviation from the reference condition and the Good/Moderate boundary corresponds to a 50% from the High/Good boundary (see box 5.4). Brito et al. (2012) proposed an increase in chl *a* reference conditions of High/Good ecological status from 5.3 mg m^{-3} for Portuguese coastal lagoons (Coutinho et al., 2012) to 8 mg m^{-3} due to longer water residence times in inner

Box 5.4. What are existing Reference and Boundary Conditions in coastal and lagoon waters in Portugal

Following WFD recommendations, Reference and Boundary conditions should be established using all data available or at least during 6 months. Reference conditions depending on water typology should be established using 90% percentile as Reference condition and 50% deviation from this as High/Good Boundary and again 50% deviation from the latter as Good/Moderate boundary. The most commonly used Biological Quality Element is chlorophyll *a*, although other elements can be used such as total phytoplankton abundance and frequency of blooms above a certain threshold, also to be defined according to local phytoplankton community.
In Portugal, several coastal water typologies have been defined such as adjacent Coastal Waters (CWs) with strong or moderate upwelling and Coastal Lagoons (CW-Ls) . According to Brito et al. (2012) and Coutinho et al. (2012) studying several coastal lagoons in southern (Ria Formosa, Ria de Alvor) and northern Portugal (Óbidos, Albufeira, St. André) reference and boundary conditions for chlorophyll *a* (mg. m-3) are as follows:

	Reference	High/Good	Good/Moderate
Coastal Waters	4.0	6.0	9.0
Southern Lagoons	5.3	8.0	12.0
Northern Lagoons*	6.7	10.0	15.0

(*open to ocean regime)

lagoon. Regardless of different proposed boundaries, the Ecological Status of RF as defined by the WFD could be considered as High .

In conclusion, the evaluation of trophic or ecological status in the RF lagoon using different indices and classification systems yielded ambiguous, if not contradictory results. Ideally, trophic status should be ascertained from microbial processes rather than standing stocks. Unfortunately, only "state" environmental variables such as nutrient and chlorophyll concentrations are routinely monitored. Rather than rendering judgement on a particular location or time period, it would be advisable to analyse longer time series of environmental variables. For the Ria Formosa lagoon, water quality indicators such as dissolved inorganic nutrients, transparency (Secchi depth) and chlorophyll could now be compiled for at least a 50-year period, albeit from different sources. Brito et al. (2012), as well as Barbosa (2010) reported decreasing long-term trends in chl. This decreasing trend in chlorophyll was attributed to increase bivalve filtering by Brito et al. (2012), while Barbosa (2010) analysing the 1967-2008 decadal period suggested that a global warming trend could be responsible.

Regrettably, scant information on oxygen saturation is available for the Ria. It is postulated here that a global warming trend could further deteriorate oxygen conditions with increasing water temperature and salinity. This could result in the development of hypoxia, particularly at night, which could drive denitrification processes and further decrease N budget as well as phytoplankton production in the lagoon. This potential oligotrophication trend should be addressed in future time series analyses.

Finally, there is increasing interest in using earth observations (EO) to monitor ecosystems by remote sensing. EO is a cost-effective tool to assess environmental systems at a synoptic scale with high spatial and temporal capacity on a global scale with ranges from years to decades. However, the use of EO for microbial populations and processes has been limited to relatively few studies. For example, Larsen et al. (2015) used remotely sensed environmental parameters to create a system-scale model of marine microbial metabolism for the Western English Channel. In Chapter 10, S. Cristina et al. discuss how the Sentinel satellites developed by the European Space Agency could be used for EO of the RF. These observations would enable future studies of the microbial population and processes of RF lagoon with increasing temporal resolution.

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