A Global Mapping of Ocean Microbial Biomass

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Summary

Tons of ballast water housing thousands of species is carried around the world and introduced to new environments yearly. Organisms transported with ballast water risk disrupting ecosystems. The existing solution until vessels are forced to treat their ballast water is open oceans exchange. This is of several reasons not a sustainable solution. In this study a new method based on the cellular ATP content is applied for estimation of ocean microbial biomass, the method is described in details in this report. Water samples were collected, extracted and analyzed at sea during the Volvo Ocean Race 2008-2009. In addition, chlorophyll a has been measured via satellites and used to further evaluate the achieved sample data. The results in this study indicate a similar amount of biomass in open oceans and coastal areas, giving support for necessity of other solutions than open ocean exchange.. Interestingly, the relations found between ATP and chlorophyll-a is consistent with previously knowledge of open oceans and coastal areas. The ATP method has shown to be reliable. It is recommended that this method is also evaluated in comparison with existing methods which are more complicated and time-consuming to perform.

Introduction

Maritime traffic moves 90 per cent of the world's freight. An estimated three to five billion tons of ballast water is carried around the world and discharged per year in completely new environments. At any given time, the ballast water tanks of vessels are housing at least 7,000 species –mostly bacteria but also phytoplankton and zooplankton including eggs and planktonic stages of multicellular organisms from crustaceans to molluscs and fish.

In a new environment with no natural enemies, some of these alien species can become invasive, rapidly out-competing local fauna or flora. They can alter the local ecology, leading to the collapse of fisheries and threatening endangered species. Exotic algal species can also pose a risk to human health by contaminating seafood, and even human pathogenic bacteria have been documented in ballast water. Environmental experts claim that doing nothing about the ballast water is playing "ecological roulette".

In February 2004, the United Nations' Maritime Organization (IMO) established a convention that will, when it comes into force (earliest in 2012) require vessels to purify its ballast water before being discharged. For the convention to come into force it requires two things: "enough" ballast water treatment systems on the market (which by the IMO is defined as 3-5 systems that have gained their approval) and at least 30 countries, which account for 35 per cent of discharges of ballast water, signing the convention. At present (Feburary 2010) only 21 countries, which account for 23% of international merchant shipping tonnage, have ratified the convention. Without the convention, the majority of ship owners see no reason to treat ballast water, even though solutions do exist.

Treating ballast water is by no means a straightforward task. The market is stringent in its demands for treatment solutions that are both eco-friendly and financially justifiable. And it must be possible to use the systems on both old vessels and new buildings alike. The tough IMO requirements mean, for example, that the number of living organisms (>50 µm) must be reduced to fewer than 10 organisms/m^3. There are also separate requirements set up for organisms between 10µm and 50µm as well as for a few indicator bacteria.

Because the precise make-up of the water differs so widely from one sea to another when it comes to algae, bacteria, zooplankton, and potential contaminants, ballast water treatment systems are one of the most challenging areas of all in the field of water treatment.

The current solution to treat ballast waters is to conduct ballast water exchange. Briefly described, ballast water exchange is allowed 200 nautical miles out from shore with a minimum depth of 200 m. However, there are exceptions that allow ballast water exchange closer to land and at shallower water depths. Additionally, there are safety aspects that overrule the possibility to perform ballast water exchange; also ships might never cross a region where ballast water exchange is allowed.

There are approved methods of open-ocean exchange of ballast water. Either ballast water tanks are emptied and subsequently refilled, or the water is diluted until three times the volume has passed. The ballast water exchange can take up to two days depending on the volume of the ballast tanks. Regardless of the method employed, it should result in an exchange of at least 95 % of the ballast water.

An objective of this study is to raise the awareness to a wider public that the ballast water exchange is not a sustainable method as well as to inform them that there is abundant microbial life in the open oceans that will be transported via the ballast water tanks when waters have been exchanged.

The Volvo Ocean Race 2008-2009 started in October 2008 from Alicante (Spain) with stopovers in Cape Town (South Africa), Cochin (India), Singapore (Malaysia), Qingdao (China), Rio de Janeiro (Brazil), Boston (USA), Galway (Ireland), Gothenburg (Sweden) and Stockholm (Sweden) before concluding in St Petersburg (Russia) June 2009. Out of 8 boats starting at Alicante, 7 boats contributed data to the present study by sampling and analyzing biomass in water samples along the route using an automated assay system based on the measurement of adenosine-5'-triphosphate (ATP). ATP is present in all living organisms where it serves several vital functions. First and foremost, ATP is the energy currency of the cells linking metabolism (energy generation) to biosynthesis, growth and a range of additional energy demanding processes. ATP also serves as a biosynthetic precursor for nucleic acids. Based on laboratory studies of many marine organisms from bacteria to small zooplankton, ATP concentration is proportional to biomass with an average cell carbon-to-ATP ratio of 250:1 by weight. Hence, by measuring the ATP content it is also possible to estimate the biomass of living organisms in a sample of seawater.

One of the many ATP-requiring cellular reactions that occur in some terrestrial and marine organisms is the production of light, a process termed bioluminescence. Because light can be measured very precisely and very accurately even at low photon fluxes, analytical biochemists have developed an ATP assay protocol that is based on the firefly bioluminescence system.

By setting up a specific chemical reaction that contains all necessary reactants except for ATP, the addition of a seawater sample containing extracted microbial

ATP will result in the production of light that is proportional in amount to the ATP in the sample. The light or glow produced in a laboratory sample can be measured in an instrument called a luminometer. By calibrating the intensity of light from a sample with the light produced from a sample with known amount of ATP, the biomass of the sample can be calculated.

In this study the ATP content of water samples collected, extracted and analyzed at sea during the Volvo Ocean Race route has been evaluated and is presented in relation to chlorophyll a which has been measured via satellites. Chlorophyll-a is a light-absorbing pigment found in plant matter such as phytoplankton. Ocean color satellite sensors measure light at different wavelengths and use an algorithm to estimate chlorophyll-a concentrations around the world. For this analysis, data were used from the MODIS sensor (Moderate Resolution Imaging Spectroradiometer) aboard the satellite AQUA. These data were provided by the National Aeronautics and Space Administration (NASA).

Unfortunately, clouds often block the view of the sensor. To reduce the effect of clouds, the MODIS data were averaged in both time and space to 8-day temporal resolution and 16 km spatial resolution, respectively. The chlorophyll-a values that corresponded to the locations and dates of the ATP data were then extracted.

Materials and methods

Assay of ATP

Water sampling for ATP analysis was conducted every third day, during a time interval from 10 PM to 2 AM local times. Most of the assays were performed in the open sea by the media person on the boat. These individuals had no previous experience in performing biochemical assays but had a short training in Alicante before the start of the race. Assays were performed in very cramped conditions in the boat far from a laboratory environment. There was very limited space on the boats to store the reagents and other disposables. Therefore these items were provided in the harbor at the start of each leg. All these items and the luminometer were stored in a specially made case to keep them dry.

Reagents: The 544-441 Ocean Water Biomass Kit was specially developed by BioThema AB for performing the assay on the sailing boats participating in the race. The kit consisted of 1) 15-051 ATP Reagent IS containing luciferase and luciferin, 2) 20-051 Diluent BS for reconstituting the ATP Reagent (5 mL), 3) 45-051 ATP Standard (10 µmol/L; 5 mL) and 4) 33-051 Extractant W (5 mL). One set of these four vials was intended for performing a triplicate assay of one water sample with a possibility to perform a fourth assay, if something went wrong.

Disposables: Each assay triplicate required: 1) Three 0.22 µm porosity filter (Millipore GSWP03700) mounted in a special filter holder (Millipore M000037P0) that were pre-assembled in a clean-room. 2) One 10 mL syringe (BD Plastipak 302188). 3) Six 1 mL syringes (BD Plastipak 300013. 4) Three 200 µL pipette tip (Eppendorf ep TIPS 0030 075.021) to be used with a 50 µL pipette.

Instrument: An FB12 Luminometer (Berthold Detection Systems), which is able to detect up to 9 attomol ATP, was used and powered by the 12 V power supply system on the boats.

The analytical procedure is as follows. First the ATP Reagent is mixed in the entire content of the Diluent BS vial. Before sampling the water is allowed to run for at least 2 min from the boat's seawater tap (inlet located 1-1.5 m below the sea surface). The sampling bucket is rinsed three times with the seawater and then filled. A 10 mL syringe is used to take a sample from the bucket. The water sample is thoroughly filtered through a holder containing the filter. Thereafter 1 mL of Extractant W and 1 mL of ATP Reagent IS are separately injected into the filter holder. After mixing of the liquids, the filter holder is placed in the luminometer where the light emission, I_{smp}, is measured. Finally, 50 µL ATP Standard is added to the filter holder and the light emission, $I_{smp+std}$, is measured again.

The ATP levels were calculated according to

$$ATP=I_{smp}/(I_{smp+std}-I_{smp})*50*10/0.01121 \text{ pmol/L}$$

ng ATP/L = pmolATP x 0.507

where I_{smp} is the light emission value from the sample and $I_{smp+std}$ is the signal after adding the ATP standard, 50 is the volume of ATP standard added (µL), 10 is the ATP standard concentration (µmol/L) and 0.01121 is the sample volume (L). The use of an internal ATP standard compensates for variations in temperature, instrument sensitivity, inhibition from sample and reagent sensitivity.

The initial sampling design was to collect 43 samples per boat or 301 in total during the worldwide race. For various reasons only 166 samples were collected giving 148 accepted results (89%). Considering the situation at sea this is considered to be a successful effort. There were triplicate measurements on each water sample. Because the media persons (with only limited training in sampling and biochemical analysis) were the main at-sea analysts, not to mention routinely rough sea conditions, this led to a situation where one of the triplicate measurement was sometimes an outlier. So rather than using the mean value of the triplicates it was decided to use the median value. We also calculated the mean of the two closest values. If this value deviated by more than 30% from the median value, we excluded the entire measurement from the triplicate data set. This was a way to ensure that at least two values of the triplicate data were close enough to make the median value reasonably reliable. In cases when there were only duplicates, the mean and the median values were the same. We then excluded data with CV >30%. When there was only one measurement that data set was excluded. Finally, the data set values from one boat, which provided only three measurements in total, was excluded.

Data Reduction and Global Analysis

The ATP level of each sample was recorded, together with position, temperature and weather conditions for each station along the global course (Figure 1).

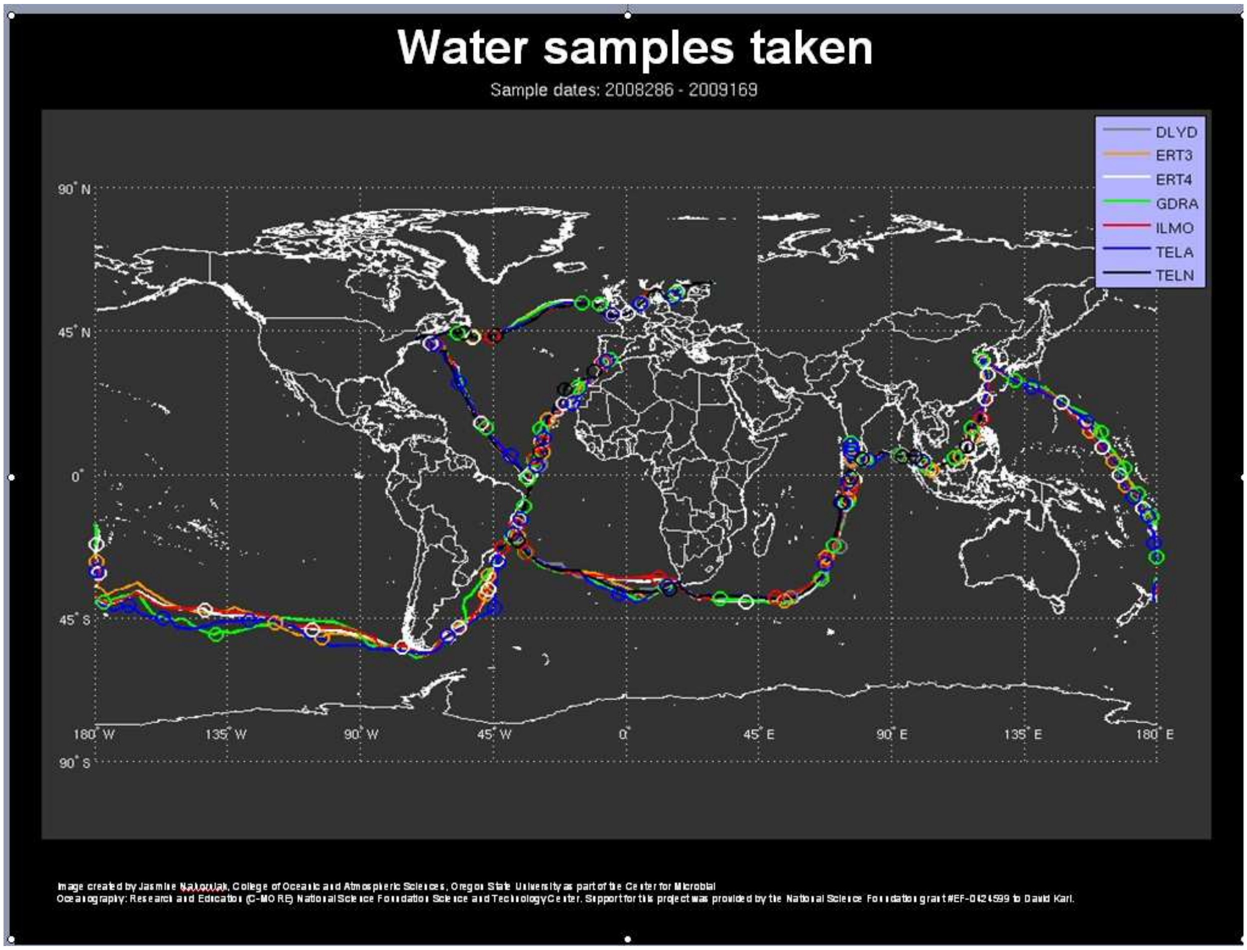


Figure 1. Worldwide race course and sampling locations of the participating boats.

The boats did not cover the exact tracts or cover the course at the same speed. Therefore an average of all different routes sailed during the race has been chosen, herein called the transit line. This means, the distance is given in one dimension and the actual distance in between real sample positions might be larger than on the transit line, as illustrated in figure 2. Real sample positions (a, b, c, d) are represented by circles. Each sample position has been mirrored against the transit line in approximately 90 degrees angle as shown in figure 2. Hence, distances between the actual sample positions (a, b, c, and d) could be larger than the distances on the transit line (A, B, C, and D) used in the graphs. For example the dashed line between the sample positions b and c is larger than the distance between B and C, figure 2.

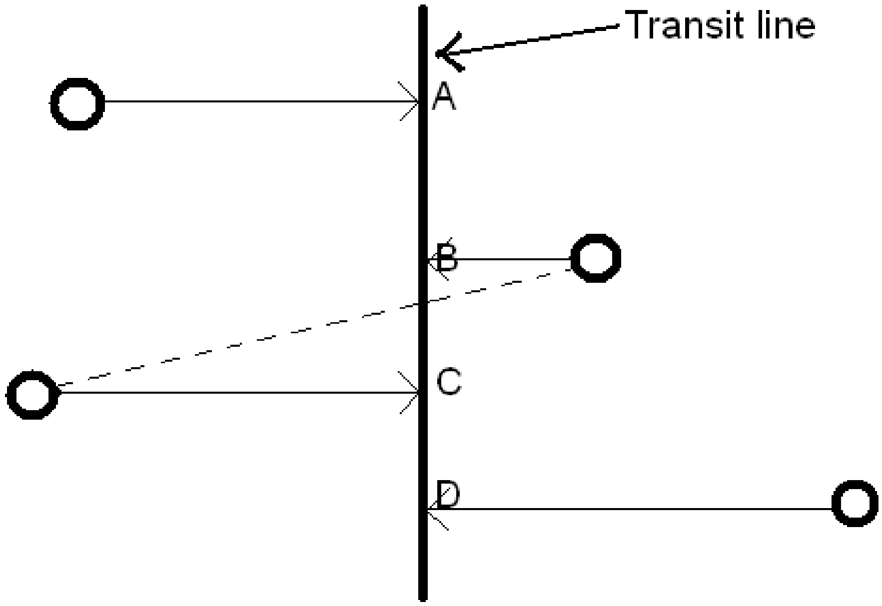


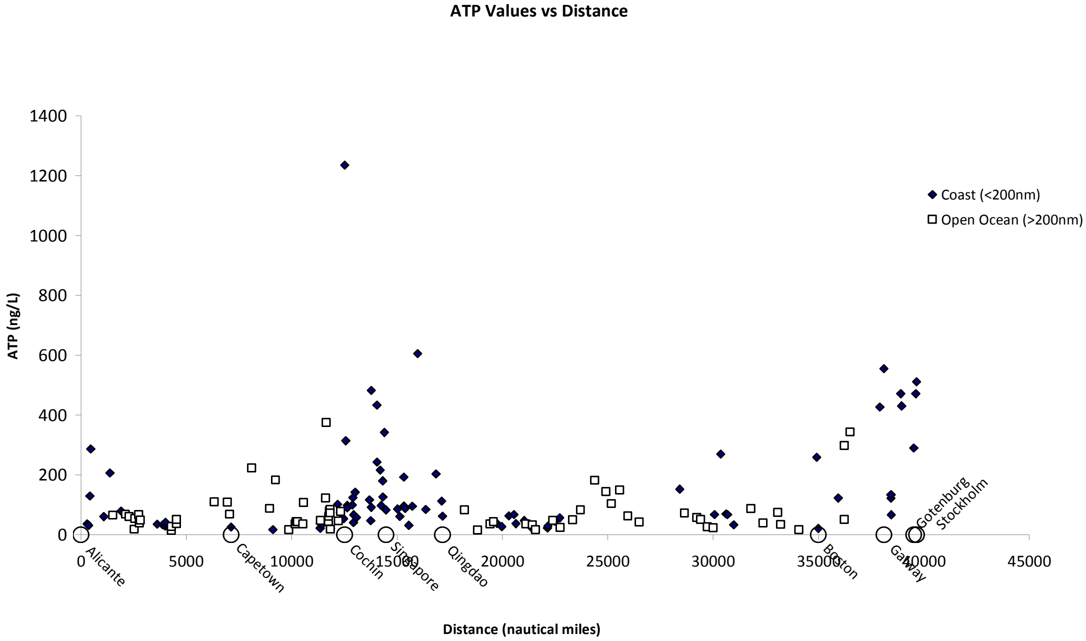
Figure 2. Principles of the average route estimation; each circle represents a sample position and the bold line (the transit-line) is the average route and the dashed line shows the actual distances between samples b and c.

Results

Figures 3 and 4 give the measured ATP and chlorophyll a, respectively, for coastal (out to 200 nautical miles from shore) and open ocean areas along the race route. As can be seen, the values appear higher at certain positions especially in two coastal areas: Cochin-Singapore-Qingdao and Galway-Gothenburg-Stockholm, while other coastal areas can not be differentiated from the open ocean areas.

The ATP values range between 14-600 ng ATP/L if excluding the highest value at 1200 ng ATP/L which was found in the harbor of Cochin.

The distance of the actual sample position in 90 degrees angle to the transit line is in average 28 nautical miles and 330 nautical miles at most.



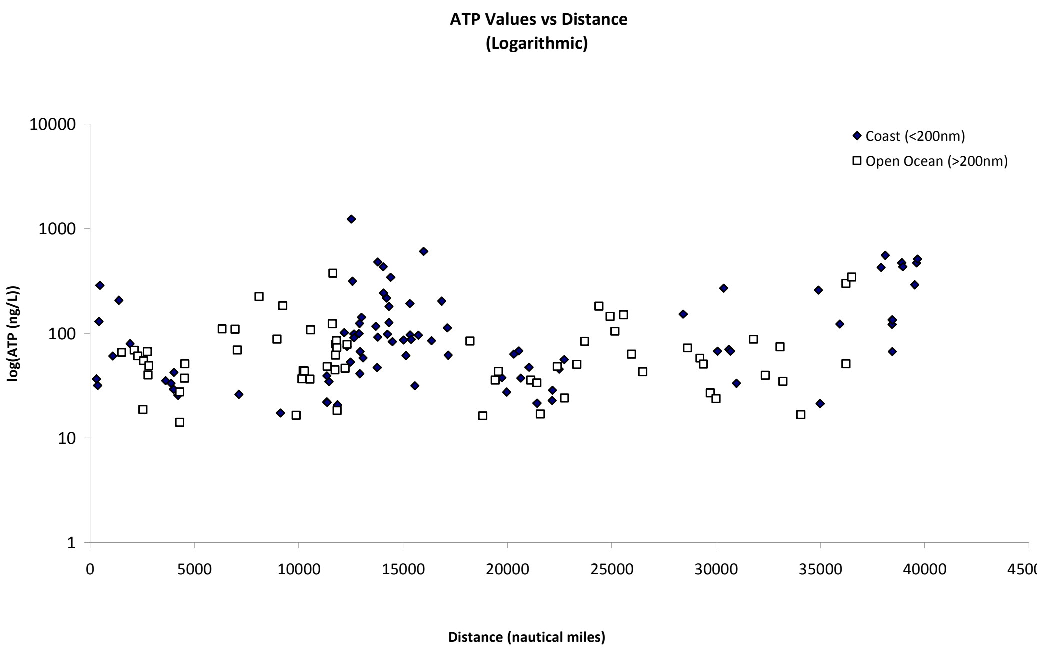
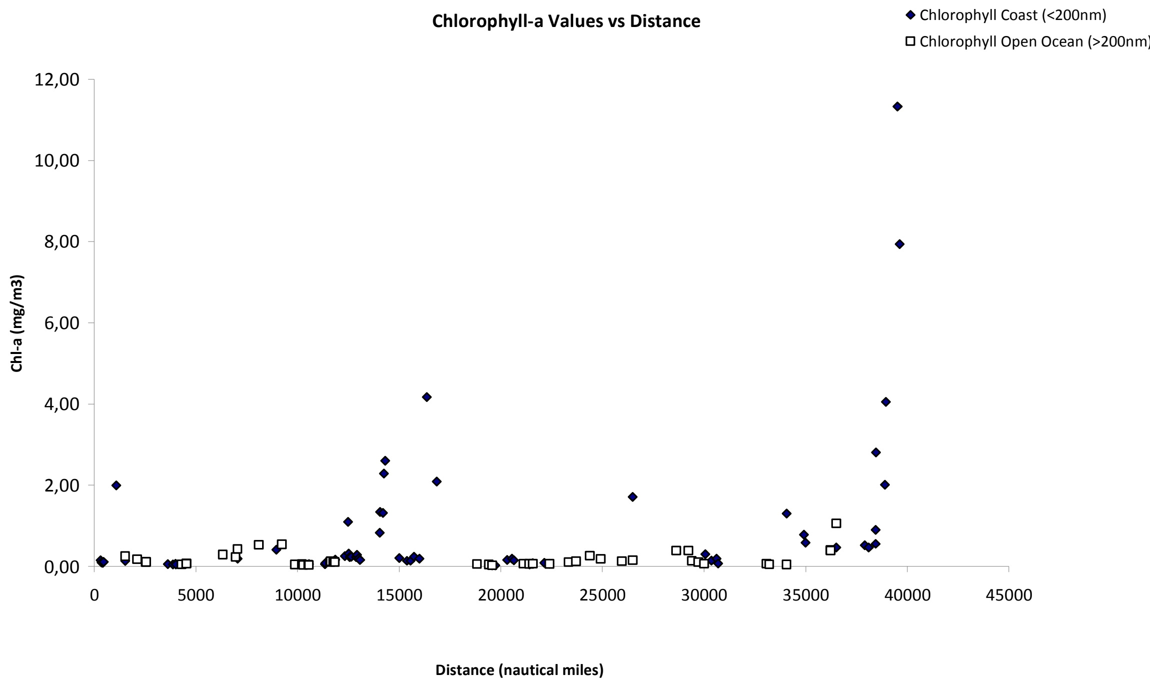


Figure 3. The amount of ATP in coastal and open ocean areas at elapsed distances of the race. Each symbol is a median determination from each sample analyzed by one of the 7 vessels competing in the worldwide race.



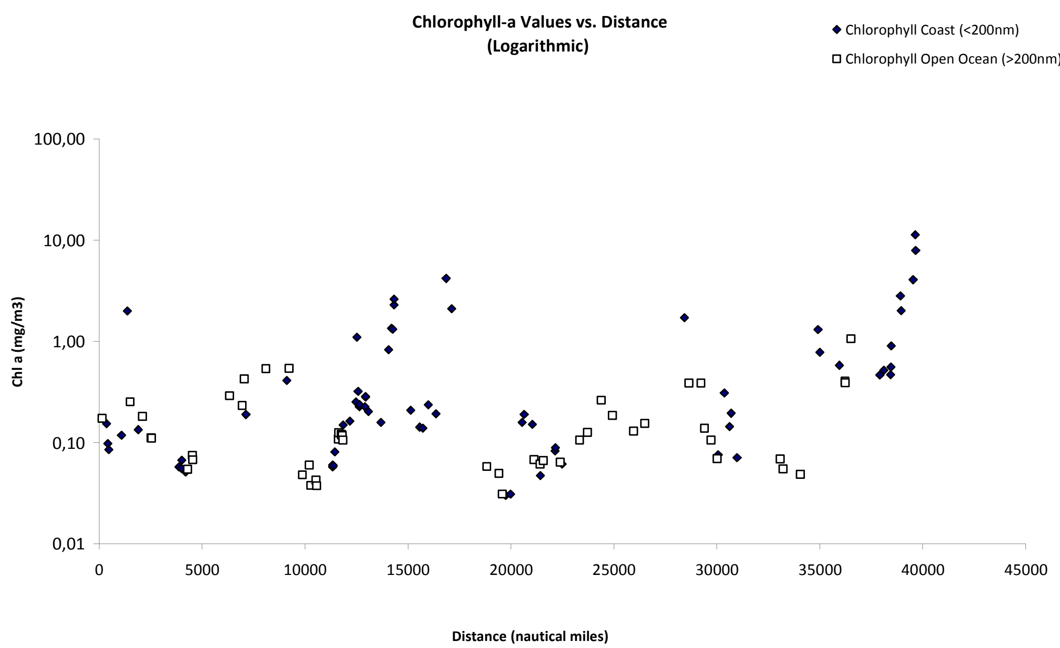


Figure 4. The amount of chlorophyll a in coastal and open ocean areas at elapsed distances of the race. Approximate locations of the ports is shown.

The figure (5) below shows all ATP values for all boats participating versus satellite determined chlorophyll a.

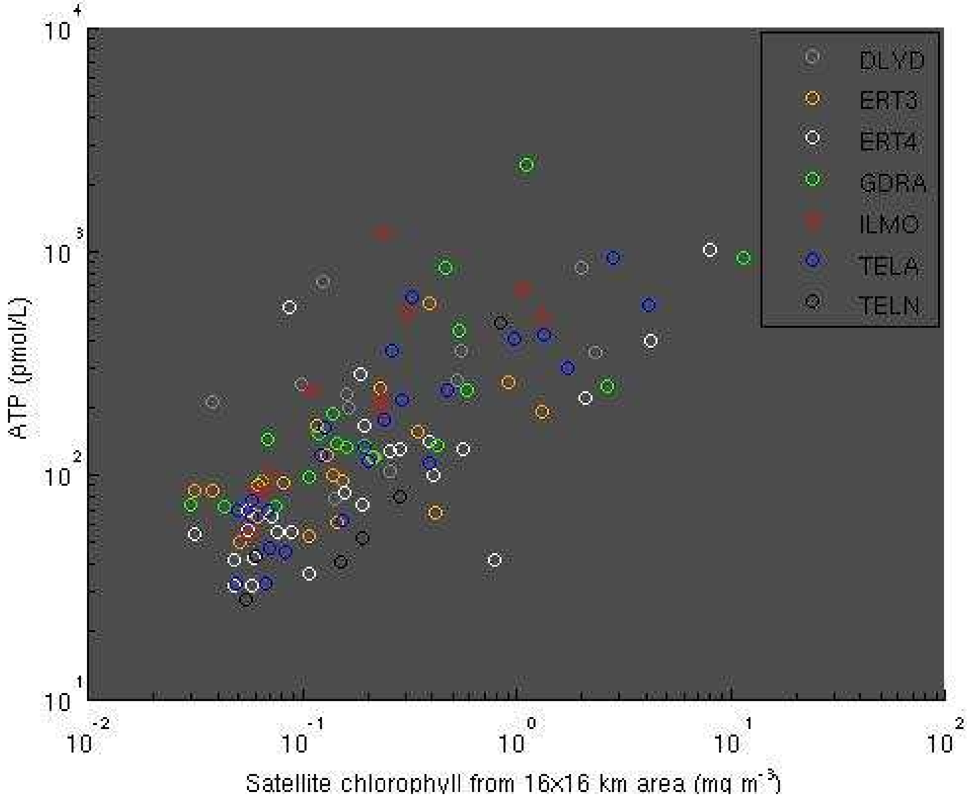


Figure 5 Chlorophyll-a and ATP values with each vessel represented by a color.

A correlation between measured ATP and chlorophyll a is presented for coastal and open ocean areas in figure 6 and 7, respectively To give a more equal weight to the data points when the range covers several orders of magnitude log scales are used. Otherwise a single high value will get a too strong influence on the linear regression analysis. Furthermore it gives an indication of the type of relationship between the two parameters. In the graphs of log (ATP) versus log(Chl) the slopes are 0.5 and 0.64 for coastal water and open sea, respectively.

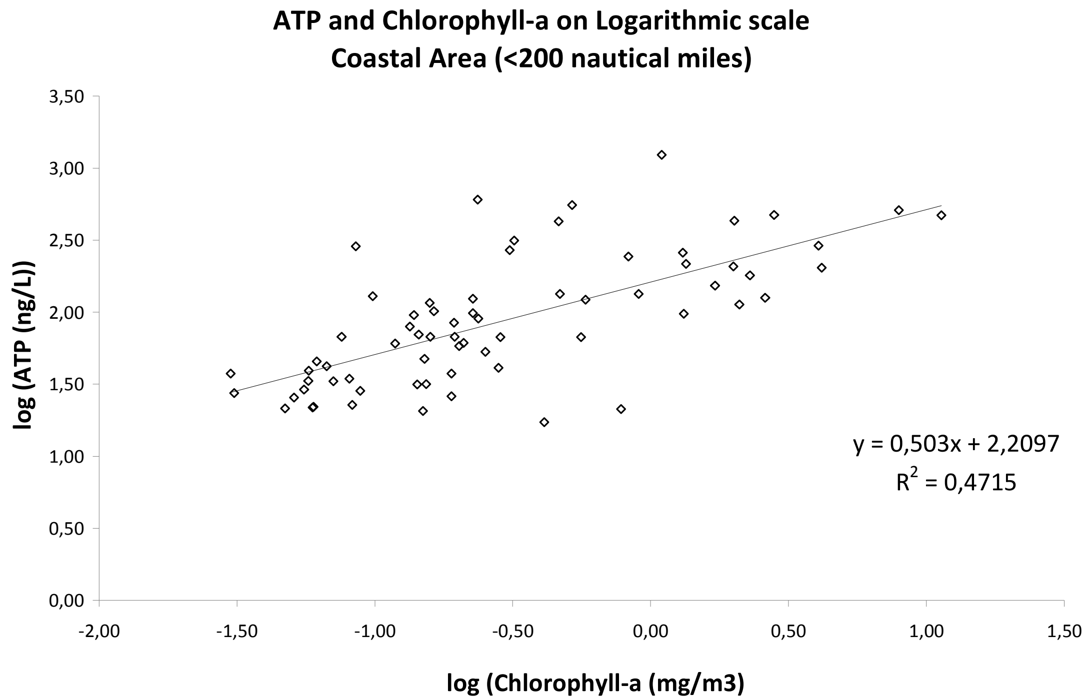


Figure 6 ATP and chlorophyll-a values on a logarithmic scale for coastal area.

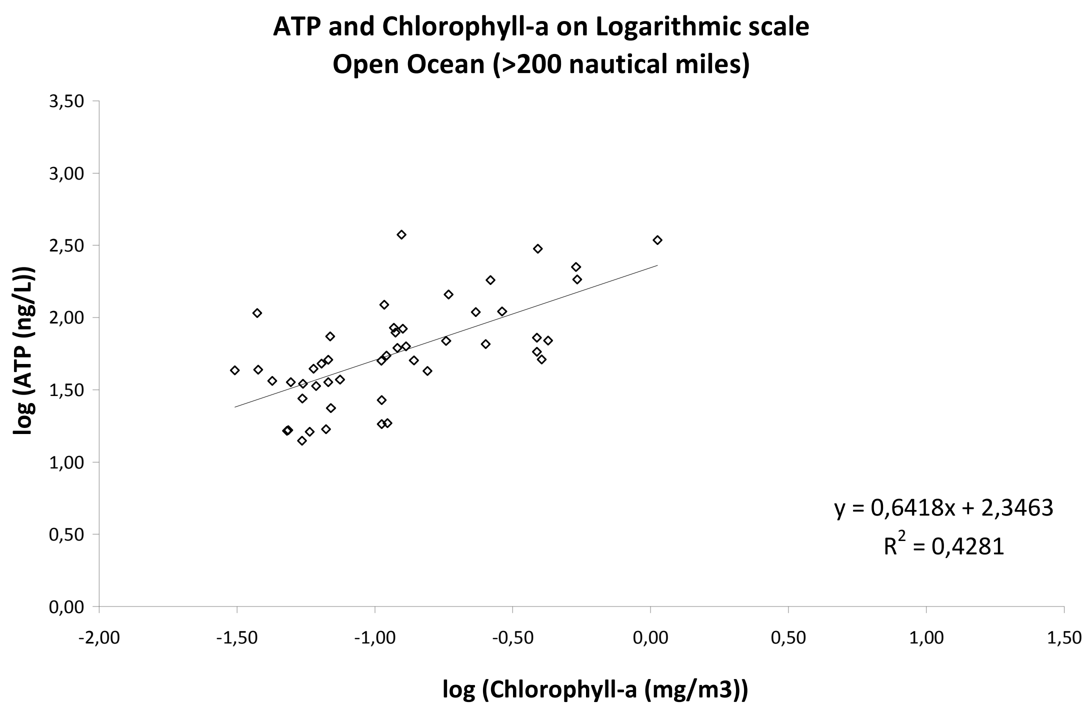


Figure 7. ATP and chlorophyll-a values on a logarithmic scale for open oceans.

Discussion and conclusions

The range of ATP (14-600 ng/L) is consistent with previously published data of ATP in the oceans. The ATP method used in this project is sensitive and handy compared to other approved methods. However, an extensive evaluation is needed and recommended to compare this method with existing methods.

The highest value, which appear extreme because it is twice as high compared to any other sample, was sampled in the harbor of Cochin, India. From the few in harbor samples that were taken there is no obvious trend that those are significantly higher than other coastal samples, this does not include the temperature differences, time of year or geographical position. However, more in-harbor samples in this geographical region are necessary to understand what a normal value should be this close to shore.

The ATP vs. chlorophyll-a graphs (fig 6 and 7) indicates a square root relation rather than a linear relation. When the chlorophyll level goes up the ATP level follows but not to the same extent. At infinitely low chlorophyll-a there is still ATP (according to the regression) which would make sense since all microbes contain ATP but only a subset contains chlorophyll. In the surface ocean, worldwide, both autotrophs which use nutrient in water for growth and heterotrophs which feed on other organisms should be present. It looks like the coastal regions are more autotroph rich (more chl per unit ATP, e.g phytoplankton) and the open ocean is more heterotroph rich (less chlorophyll, e.g. zooplankton), which is consistent with a priori predictions about metabolism in the sea.

None of the boats/participants appeared to have systematic errors as none seem to have higher or lower values than another boat (fig 5). The conclusion is that they all appear to have significantly achieved the same range of values, also when comparing data to chlorophyll-a which also appear consistent.

An important result from this study is that we could see similar level of biomass content in coastal and open ocean areas, and this supports that the current solution with ballast water exchange, as a solution until the convention has been ratified, is not sustainable and difficult to guarantee as a solution.

Reflections and recommendations for future biomass mapping

These samplings have been run during circumstances far from conditions normally found in laboratory environment, sometimes forcing the analysis to be interrupted in the middle of an assay. From a methodological point of view there was no possibility to test this method in these particular conditions in advance. Also, when analyzing data we realized that it would have been beneficial to note the air temperature as well as water temperature as this parameter probably had more influence of the assays. Furthermore the storage of reagents were far from storage conditions found in an ordinary laboratory, both temperature and storage time differed widely from stopovers and the duration of the legs.

It is also possible that a larger water volume would have increased the accuracy of the measurements. Also pre-filtration to achieve a specific range of microorganisms could have been interesting to evaluate the method at more specified size ranges of organisms. Qualification and quantification of the ATP measurements could have been achieved by examining the samples under a microscope; however this was not possible to do in this study.

It might not be as simple as looking at distance from shore (200 nautical miles) to distinguish coastal from open ocean areas, some regions have open ocean conditions lapping up on their shores and in other areas the coast extends out over a very broad continental shelf. Maybe for future projects the vessels can be equipped with nitrate sensors so they can use the nutrient loading criterion for coastal vs. open oceans.

Acknowledgements

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