ESCUELA SUPERIOR POLITÉCNICA DEL LITORAL

College of maritime engineering, Biological, Oceanographic science and Natural Resources

"Plastic debris effect by Zooplanktonic Community in the Galápagos Islands – Santa Cruz and San Cristóbal cases"

By

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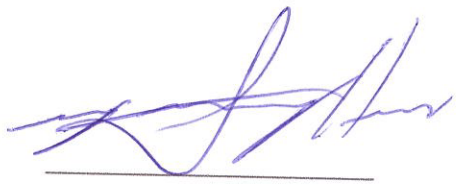
DEDICATION

To all readers interested in this investigation.

GRADUATION COMITEE



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PROJECT ADVISOR

DECLARATION

"The responsibility for the content of this Research Project, correspond exclusively to me; and the intellectual patrimony of the same to the ESCUALE SUPERIOR POLITÉCNICA DEL LITORAL"

(ESPOL Graduation Regulation).

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Plastic debris effect by Zooplanktonic Community in the Galápagos Islands - Santa Cruz and San Cristóbal cases

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Abstract
Plastic debris is known to undergo fragmentation at sea, which is accumulating in marine habitats, presence of plastic provides a potential pathway for the transfer of pollutants to organisms with uncertain consequences for their health.
Fifteen water samples were collected via vertical hauls during expedition in October, 2016 throughout Santa Cruz and San Cristobal Island of Galápagos Archipelago. The collection process was carried out using 150 µm mesh manta trawl, and the samples were analyzed for plastic debris and zooplankton organisms. Counting by Bogorov method was used to identify plastic debris types, categorized according to features and zooplankton species. Although in terms of abundance for zooplankton organisms and volume of plastic debris, correlation is insignificant; a considerable inverse relationship was found between zooplankton and plastic debris amount. Six zooplankton taxa were fewer in stations, Puerto Ayora Harbor and Puerto Baquerizo Moreno Harbor with highest amount of plastic debris. Findings imply that marine plastic debris may have a negative impact on zooplanktonic individuals, especially in areas with anthropogenic influence, such as harbors.

Keywords— plastic debris; zooplankton organisms; Galápagos Archipelago,

1. INTRODUCTION

Several studies from the environmental protection agency (EPA) about aquatic debris revealed a widespread distribution of plastic pellets in some harbors located on the Atlantic, Pacific, and the Gulf Coast. Miniature debris, so-called microplastic, have been the most common items found during previous research (US EPA & Division).

During the last years plastic materials have been used in a wide variety of products, and have displaced other materials, such as wood, metal, and glass (Gourmelon, 2015). There are a great variety of plastic materials, and the among the most commonly used can be mentioned: polyethylene (PE), polypropylene (PP), polystyrene (PS), polyethylene terephthalate (PET) and polyvinil Chloride (PVC). The mentioned types of plastics have high stability and durability (Bernes, 2009), and therefore; they do not degrade when released into the environment.

Plastic degradation in the environment can occur in four different ways: photodegradation, hydrolytic degradation, thermo-oxidative degradation and bio-degradation (Andrady, 2011). The degradation process commonly starts as photodegradation which conveys to thermos-oxidative degradation. Ultraviolet waves coming from the sun impact the plastic materials, and this energy produces an interaction between oxygen atoms and polymer (Gomes, 2014). As a result, the plastic turns into a crumbly and easily to broken material, facilitating its small particle production. This process is carried out until the plastic debris reach a low molecular weight due to its size. These small fragments, often invisible to the naked eye, can be confused by some marine animals, and consider the plastic detritus as food and/or other microorganisms.

Thus, one of the most important question should be: How does plastic debris enter into the oceans? According to Browne, plastics into the oceans might be attributed to two possible ways: (a) direct introduction by runoff, it can be sewage outfalls, and (b) breakdown of plastics used in consumer products (Browne, 2006). Previous researchers have also demonstrated that the agglomeration growth of plastic debris is affected by: climatic forcing, geostrophic winds, caused by gradients of atmospheric pressure and solar radiation, stratospheric temperature and Coriolis effects (Kubota, 1993). These mentioned conditions in the coastal areas produce the movement of water, and the transport of macroscopic debris according to the size, shape and density. During this path, particles such as plastic, actives its degradation and together with mixed

external effects, plastic can be found in the water column (Edyvane, 2004). This situation increases the potential for ingestion and accumulation within the tissue of animals and microorganisms like zooplankton (Browne M. A., 2009).

Adverse effect on marine organisms due to plastic ingestion is not widely known. However, has been shown during laboratory analysis that organisms like worms, amphipods, mussels, sea cucumbers, decapods crustaceans, seabirds and fish have ingested tiny plastic particles (Graham, 2008). Evidence shows that plastic detritus of about 5 mm range of diameter produces a negative impact upon marine biota (Thomson, et al., 2004). The marine wildlife would be extremely in danger due to the ingestion of plastic debris, it might obstruct feeding appendages and block the alimentary canal, limit the food intake of an organism or even be translocated into the circulatory system. (Browne M. A., 2009) (Murray & Cowie, 2011).

In addition, plastic particle degradation shall import toxicant to the organisms: additives included in the plastic production to improve its properties. In general, phthalates are used for malleability, and polybrominated diphenyl ethers (PDE) increase the resistance to high temperatures. All these toxins can be extracted of weathered plastic detritus, another feature that may demonstrate the damage of plastic is its hydrophobic property which makes the plastic susceptible to the accumulation of other hydrophobic organic contaminants (HOCs) that can be dissociated post ingestion (Teuten et al., 2009). Considering the basic animals of the trophic chain, zooplankton has a vital ecological role in marine ecosystems because of they are primary consumer in the marine food web. Some laboratory experiments, in which plastic particles were used to model algal ingestion by some species of zooplankton, showed evidence of high ingestion in species of copepods, this examination also demonstrated some affinity for ingesting microplastics <100µm diameter (Cole, et al., 2013).

Galápagos Islands, also known as "Enchanted Islands" (recognized as a Natural Heritage on September 8 1986, Biosphere Reserve in 1985, Marine Reserve 1986 by UNESCO) has 25124 inhabitants, of which 61% living in Santa Cruz Island and 30% living in the San Cristóbal Island. Galápagos constitute one of the principal sights in Ecuador, a lot of foreign visitors arrive to Galápagos each year (225000 during 2015) according to Galápagos National Park foundation. The aim of this study is to investigate the effect of plastic debris on zooplanktonic community by means of the constitutive analysis of water samples from various stations. The stations are distributed around Santa Cruz and San Cristóbal Islands.

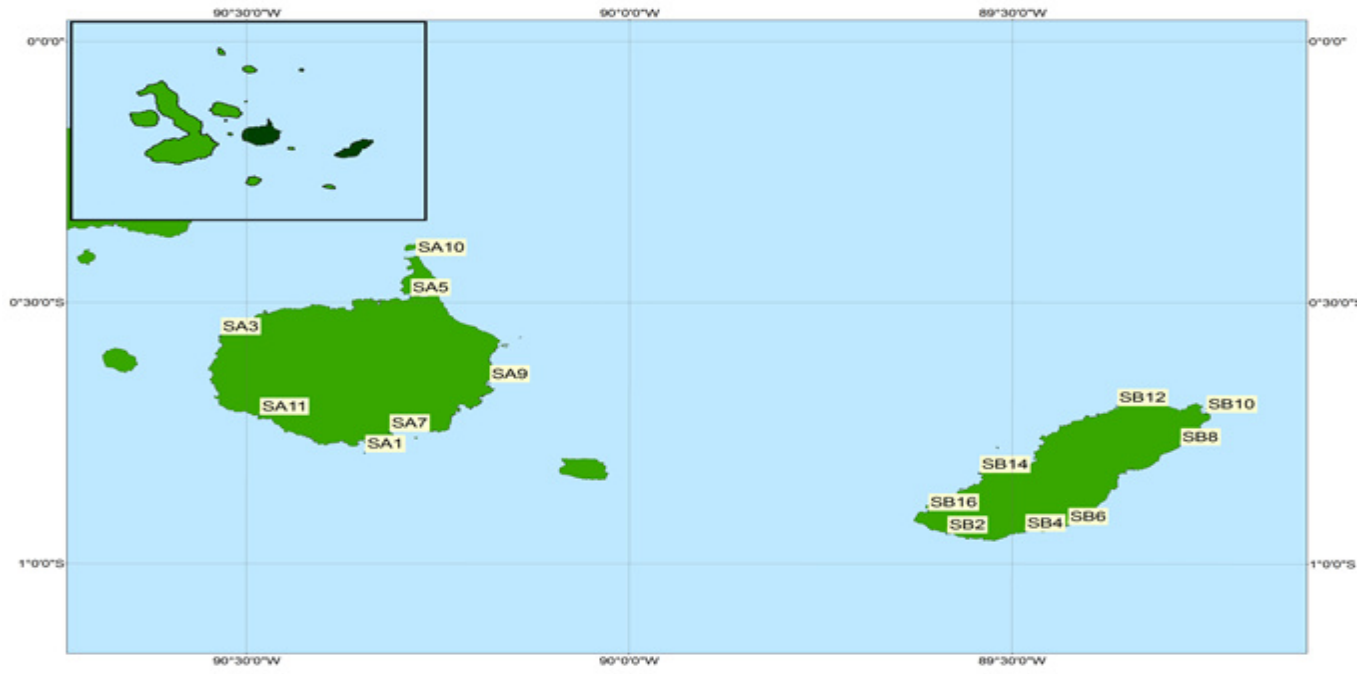

Figure 1.- Sampling locations in the Galálagos Archipelago. Alpha-numeric label corresponds to the locations presented in Table 1

2. MATERIALS AND METHODS

Fifteen remote locations throughout Santa Cruz and San Cristóbal Islands were considered, an expedition aboard a fishing boat collected the samples between October 21st and October 27th, 2016. Locations were randomly selected, considering places with and without anthropogenic impacts as shown in Figure 1. At each station, water samples were taken at near coast location, i.e., about 1.5 km – 2.0 km from the shoreline and 3.0 m – 5.0 m of depth.

A 150 µm mesh manta trawl plankton net with 0.60 m diameter ring mesh was used to collect water samples via vertical hauls. Collected samples were held in 150 ml bottles, and 2.0 ml of 5.0 % formaldehyde was added for fixation and preservation. All the samples were transported in a cool box to protect them of ultraviolet waves from the sun because they are photosensitive. Samples were taken to the facilities at Planktonic Laboratory (ESPOL, Ecuador). For all experimental and analysis procedures, water samples were maintained at ambient laboratory temperature (20 - 22°C).

2.1 TABULATION PROCESS

For each water sample, the presence of plastic debris and zooplankton were tabulated as follows:

- **ZOOPLANKTON**

Before counting, 14 ml of 5% formaldehyde was added for re-preserving the samples. Zooplankton sampling is divided into two equal portions using a Folsom plankton splitter to settle (Lab, 1994), then one sub-sample from the split is saved in the sample bottle; this procedure was repeated several times until having enough amount to put it in the Bogorov chamber. Organisms were counted for each sample at X57 magnification with an Olympus SZX9 stereomicroscope. Species identification was ascertained by hand selected and viewing specimens at X10 and X40 magnification with a Motic B1-220A microscope.

- **PLASTIC DEBRIS**

The method used in this investigation for counting plastic debris was closely similar to the zooplanktonic identification. Plastic was divided into four classes: fragment (bottles, cups, food containers, etc.), film (plastic bags, zip bags, etc.), line (plastic rope and fishing net detritus) and foamed (sponge, fomi, etc.). Bogorov chamber was used to manipulate water sample and the classification was done by Olympus SZX9 stereomicroscope. The magnification used depends on the plastic particle size (Sun & Liu, 2003), (Eriksen, et al., 2012)

2.2 STATISTICAL ANALYSIS

Zooplankton was examined in terms of species, the data were divided in two groups: (1) total individual counting per sample (150ml) and (2) zooplanktonic abundance (microorganism/m³). Species taxonomic was carried out to calculate the Shannon diversity index. For comparative purposes, data composition of plastic debris was divided in a similar way, but in three groups: (1) total plastic particles, (2) plastic deris class, and (3) total volume of plastic (mm³).

The obtained data were analyzed using RStudio 3.3.2 and Matlab. Correlations were used to indicate a predictive relationship between the selected variables. In addition, a Principal Component Analysis (PCA), to establish the relations between the different stations in terms of zooplankton and plastic, was applied. Finally, stations were represented by a dendrogram to visualize clusters.

3. RESULTS AND DICUSSIONS

Several particles were found in the collected samples. A total of 2201 plastic debris were counted. About zooplankton, a total of 6002 organisms were identifying as shown Table 1.

Stations	Locations	Plastic debris in 150ml	Zooplakton organisms in 150ml	Shannon Index
	Santan Cruz			
SA1	Tortuga Bay	93	396	4.1274
SA3	El Eden	159	276	3.5914
SA5	El Canal	111	1023	3.5386
SA7	Puerto Ayora Harbor	296	252	1.7582
SA9	Rocarfuerte	175	215	2.8953
SA10	Seymour	150	204	4.2250
SA11	Pelican Bay	60	560	3.7462
	San Cristóbal			
SB2	Las Negritas	84	402	4.1170
SB4	El Chorro	36	524	3.1722
SB6	Puerto Chino	249	240	3.8633
SB8	Puerto Colorado	108	327	2.9665
SB10	Punta Pitt	129	450	3.1628
SB12	Bahia Sardinas	120	474	3.6799
SB14	Manglarito	147	315	3.0849
SB16	Puerto Baquerizo Moreno Harbor	284	344	2.5917

Table 1 Locations of the analyzed stations in the current study. Plastic debris, zooplankton organisms and Shannon indexfor each station.

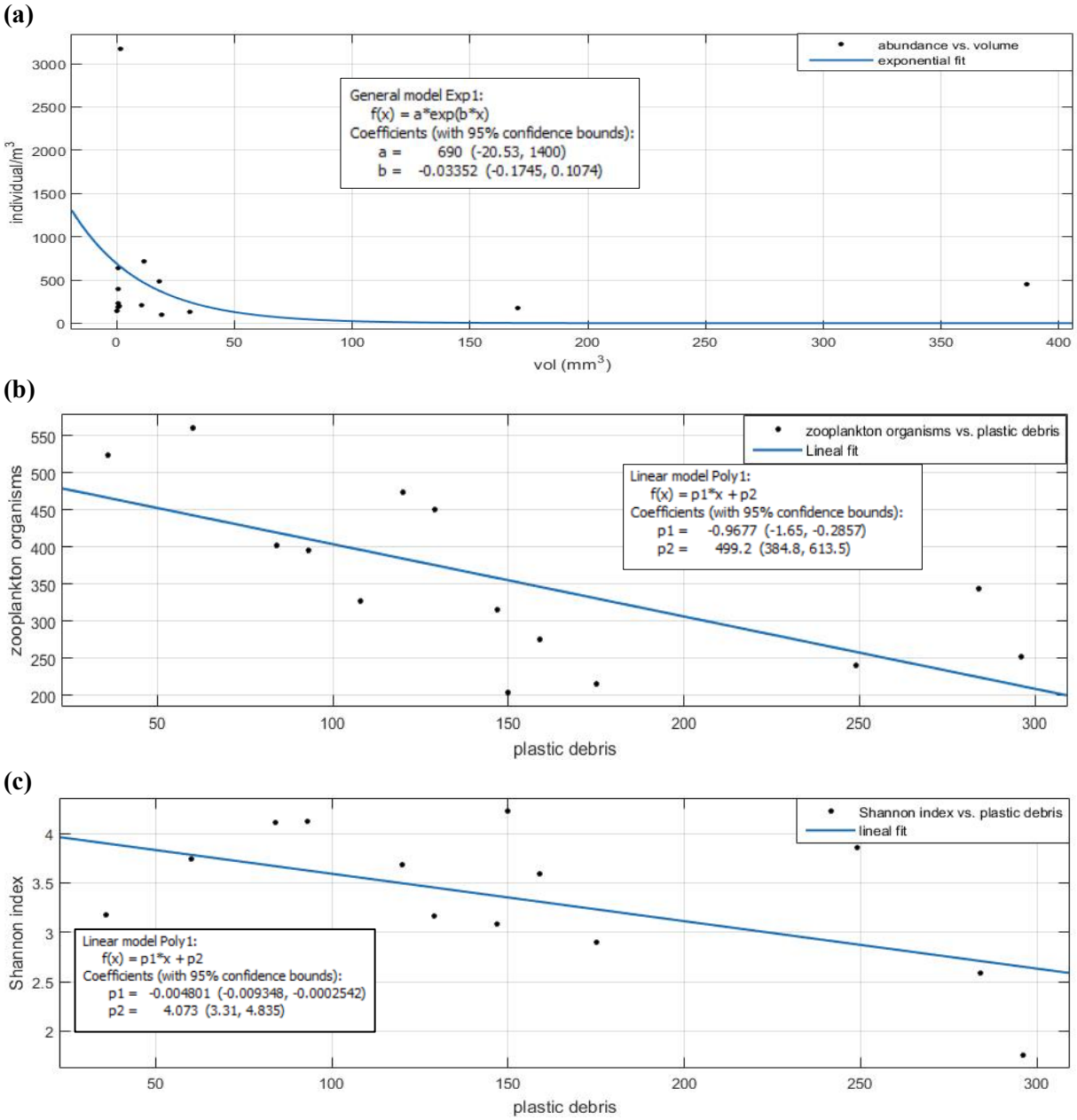

Figure 2 Normally distributed variables: (a) zooplankton abundance vs.plastic volume, variables with correlation coefficient $R^2 = 0.02967$, (b) zooplankton organisms vs. plastic debris, variable with correlation $R^2 = 0.4434$, (c) Shannon index vs. plastic debris, variablr with correlation $R^2 = 0.3061$. p-value< 0.5 in all correlations.

Initial visual analysis present the distribution both zooplankton abundance and microplastic abundance, data was better represented by and exponential function with $R^2 = 0.02967$ **(Figure 2a)**, unfortunately this value do not describe an important connection between data. With the aim of serch for any relation between zooplankton and plastic debris it was carried out a correlation among zooplankton organisms and the numbers of plastic debris counted per sample. The mentioned variables were better represented by a linear fit with $R^2 = 0.4434$ **(Figure 2b)**. For this correlation, the value of station SA5 was not consider, due to an abysmal difference of this point compared with all zooplankton organisms data set, this issue can be in **Table 1**. This is probably due to some error in the manipulation of the data or zooplankton counting.

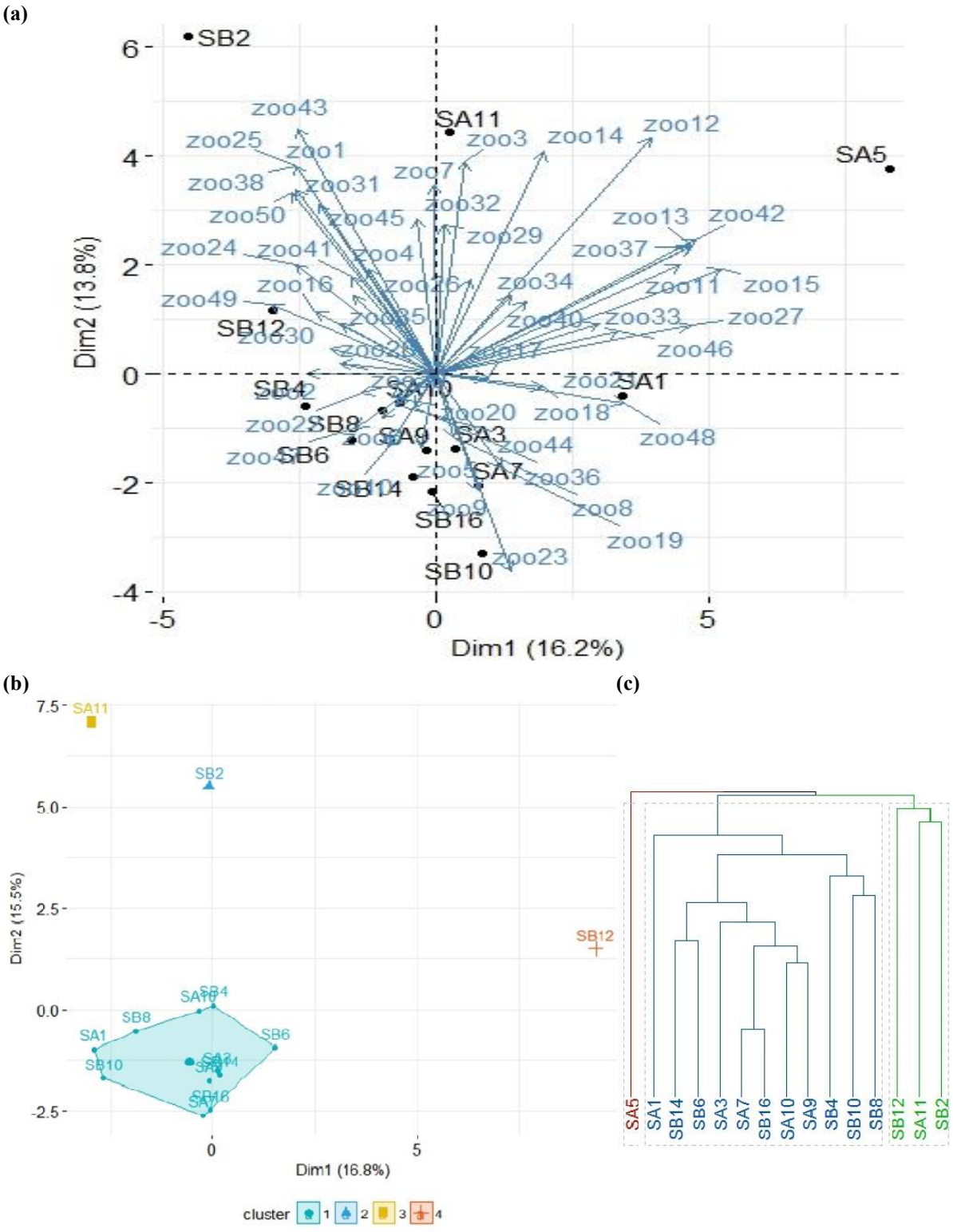

Figure 3 Exposure to including zooplankton species for each station: (a) PCA analysis, all fifty zooplankton species are represented in this bi-plot by a vector and statios by points. First principal component(Dim1) is represented by horizontal axis, while second principal component (Dim2) is represented by vertical axes. (b) Cluster analysis, represent the most related stations. (c) Dendrogram showing the similitaries and differences within station, shorter liner are stations very closely related.

During the species identification process, a total of 50 species were recognized. It was used to determine Shannon diversity index, with the objective to relate it to plastic debris amount. The correlation is given by a linear function with $R^2 = 0.3061$ **(Figure 2c)**. Probably this indicate that one class of plastic is affecting some selective species. The fitted curve is obtained with $p<0.5$.

A multivariate technique, principal component analysis (PCA) was used to analyze which stations are described by the quantified zooplankton species. Its goal is to extract important information from data set i.e., zooplankton species quantified by stations to represent as a set of new orthogonal variables called principal components. In this case these components have been denominated as dimension one (Dim1) and dimension two (Dim2).

The obtained results, the PCA graph of the parameters zooplankton species (zoo#) and stations are shown in **Figure 3a.** Zooplankton species and stations were compared the first principal component (Dim1) accounted for 16.2% of the total range, while the second principal component (Dim2) accounted for 13.8%. Therefore, Dim1 and Dim2 together accounted for 30% of the total range. **Figure 3a** also shows that on axis Dim1, the most important parameters are *Scolecithrix danae* (zoo49) with a negative coefficient and *Coenophthalmus tridentatus* (zoo15) with a positive coefficient. For Dim2, the most important pararemeters are *Oncaea venusta* (zoo 43) and *Clausocalanus furcatus* (zoo12), both with a positive coefficient. These species were not founded repeatedly in all stations. The direction and length of the vector indicate the station on which the number of organisms was concentrated.

As result, between quadrant III and IV station measuring similar attributes were located in close proximity, and therefore, shewed that the trend with respect to the zooplankton species are very similar, as shown in **Figure 3a and 3b.** Interesting finding corresponds to SA3, SA7, SA9, SA10, SB6, SB8, SB14, SB16, these stations have lowest amount of zooplankton organisms as shown in **Table 1**, but in the other hand, most of these stations present highest plastic debris amount **(Figura 4)**.

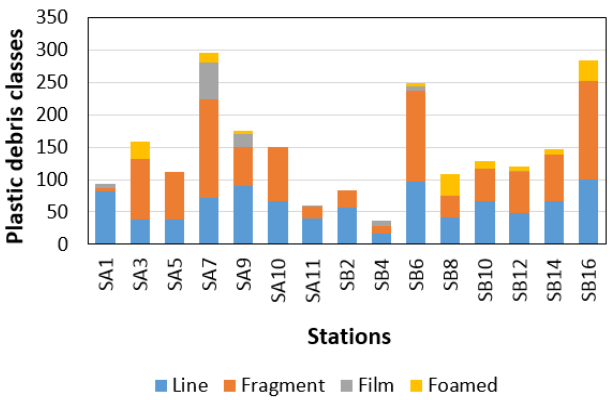

Figure 4: Identity and class of plastic debris collected in each station.

Longer lines in our dendrogram indicate a greater distance between clusters **(Figure 3c),** represented by stations. This allows a more detailed analysis and visualize of the completely closest stations, having as a result SA7 and SB16 with shortest lines which means high connection whereas that station SA5 it is completely separated.

Comparing this result with **Figure 4**, it is observed that the same stations present the highest values plastic debris quantity, in turn **Table 1** indicates that Shannon diversity index is the lowest for both stations H= 1.758 for SA7and H= 2.591 for SB16. This is due to low quantity in *Euterpina acutifrons* (zoo27), *Clausocalanus acuicornis* (zoo11) *Clausocalanus furcatus* (zoo12), *Acrocalanus gibber* (zoo2), *Fish larvae* (zoo34), *Microsetella Norvegica* (zoo36) species. Same species are abundant in stations with low plastic debris quantity. It is suitable note that "fragment" plastic debris class prevail in SA7 and SB16. It is posibe that this type of plastic cause an adverse effect into species previously mentioned.

4. CONCLUSIONS

Plastic usage and changing demographics go hand in hand. It will result in an increase of plastic debris in the ocean environment. As zooplankton species constitute the very foundation of the marine food web its coexistence with plastic can lead to decay of some species. Large plastic particles are just the beginning because they do not represent a big effect into zooplankton organisms. However, small fragments affect adversely some species, especially in sites with high anthropogenic influence as harbors.

There is an urgent need to identify and quantify the magnitude of these potential outcomes and asses the future impacts of increasing plastic debris levels on the oceans.

NOMENCLATURE

EPA	Environmental protection agency
Dim1	Dimension 1
Dim2	Dimension 2
HOC	Hydrophobic Organic Contaminants
PCA	Principal Components Anlysis
PDE	Polybrominated Diphenyl Ethers
PE	Polypropylene
PP	Polypropylene
PS	Polystyrene
PVC	Polyvinil Chloride
SA#	Santa Cruz station
SB#	San Cristóbal station
zoo#	Zooplankton species

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