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"Spatio-temporal variation of ecological indicators in the Albufera lagoon of Valencia"

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Abstract

The Albufera lagoon of Valencia is one of the most important wetlands in Europe and it is a paradigmatic example of the interaction between nature and human activity. Declared a natural park in 1986, it was also included in the RAMSAR agreement of the "International important wetlands". Around the lagoon there are approximately 14,000 hectares of rice fields, which are the park's largest environment and, at the same time, a key element for the conservation of biodiversity within the park. These fields, in fact, are periodically flooded in winter and summer period. The winter flooding is of fundamental importance for the conservation of numerous species of migratory birds that use this habitat for nesting and rearing. In summer period fields are flooded for rice production. In accordance with European legislation (Water Framework Directive 2000/60/EC) and for aquatic habitat conservation, it is important to determine the ecological status of water in both the flooding periods.

In this thesis, the assessment of ecological status of aquatic habitats has been carried out through the measurement of physical, physical-chemical and biological parameters around the lagoon. The benthic macroinvertebrate community has been used to evaluate the biological quality. Several indices (i.e., Shannon and Simpson Diversity Index, QAELS-Water Quality Index for Shallow Lentic Ecosystems, IMN-Nutrition Mode Index) have been calculated. A comparison between areas (mostly North and South Tancats) and years (2019 and 2018) was carried out to highlight possible response in terms of phyco-chemical and biological quality.

The analysis showed a worsening of the physico-chemical conditions in summer all around the lagoon (Dissolved Oxygen ANOVA*: F=21.78, d.f.=1;52, p-value=2.2E-5*; pH ANOVA: *F=68.87, d.f.=1;52, p-value=4.2E-11)*. This trend was also shown by the analysis of nutrients concentration in water (*Nitrates, ANOVA: F=4.19, d.f.=1;52, p-value=0.045; Phosphates, ANOVA: F=25.52, d.f.=1;52, p-value=5.74E-06*). The Northern areas, in particular way, were characterized by the worst valued for all the parameters, especially in summer season. The same trend was shown in 2018, even if some differences appeared (winter 2018-winter 2019, electrical conductivity, North Tancats, ANOVA*: F=91, d.f.=1;17, p-value=3.1E-8;* South Tancats, ANOVA*: F= 6.24, d.f.=1;19, p-value=0.02*; summer 2018-summer 2019*, dissolved oxygen, North Tancats, ANOVA: F=14 d.f.=1;7, p-value=0.006; South*

Tancats, ANOVA: F=10.47 d.f.=1;11, p-value=0.007; Jucar, ANOVA: F=6.48 d.f.= 1;11, p-value=0.02)

The analysis of the macroinvertebrates community and the biological indexes showed a heavily and stressed environment in almost all considered areas. It was not possible to underline significant differences between North and South Tancats area during the winter season. Diversity indexes in winter 2019 were significantly lower than in winter 2018 in South Tancats (*Simpson Index, ANOVA: F=6.78, d.f.= 1;12, p-value=0.017; Shannon index, ANOVA: F=4.37, d.f.=1;19, p-value=0.05).*

Interestingly, two classes of macroinvertebrates, Copepoda and Ostracoda, showed a significant correlation with phyco-chemical variables. Interesting correlations were found between these classes and some of the sampled parameters (i.e., *Copepoda: pH,* $\rho_{Spearman}$=0.55, *T* $\rho_{Spearman}$ = -0.65, *PO4* $\rho_{Spearman}$ = -0,3; *Ostracoda: PO4* $\rho_{Spearman}$ = -0.61, *pH* $\rho_{Spearman}$ =0.61, *T* $\rho_{Spearman}$ = -0,41). Random Forest classification models were applied in order to assess the most important physical-chemical variables in predicting the presence of these two macroinvertebrates classes. The models confirmed that these two classes may be used as good indicators for water quality in the Albufera because their high sensitivity to low concentration of dissolved oxygen, low values of pH and high concentrations of nutrients.

Contents

Chapter 1.

Introduction and Objectives

1.1 Human-made wetlands: an overview

Wetlands are complex ecosystems, defined by multiple aspects; water is mixed with soil, vegetation and biodiversity and all together give life to one of the most productive ecosystem on the Earth. Wetlands include also all human-made sites such as fishponds, rice paddies, reservoirs. In 1971, Ramsar Convention applied a more detailed definition of wetlands; in the Article 1.1 of the text of the Convention, wetlands are defined as *"area of marsh, fen, peatland or water, whether natural or artificial, permanent or temporary, with water that is static or flowing, fresh, brackish or salt, including areas of marine water, the depth of which at low tide does not exceed six meters*". In addition, the Convention (Article 2.1) provides that wetlands *"may incorporate riparian and coastal zones adjacent to the wetlands, and islands or bodies of marine water deeper than six meters at low tide lying within the wetlands*".

Figure 1. Different types of wetland: a mangrove wetland on the left and an inland wetland on the right (www.ramsar.org)

Wetlands have vital importance for the economy and the well-being of human population; they represent only the 6% of the surface of our planet but provide the 40 % of global annual ecosystem services: drinking water, resources and food supply, flood control, climate change mitigation, water purification and hydrological regulation. But, at the same time, they are very fragile ecosystems (Ting Xu, May 2019). A countless number of plants and animals depends on wetlands.

For these reasons it is necessary to protect these environments and ensure the maintenance of biodiversity; but, still today, despite the presence of international agreements and convention, these ecosystems are still in danger. Agriculture has been the main cause of wetland degradation all over the world. In fact, men have always exploited these areas, transforming them into agricultural land, not knowing or not appreciating their value. Moreover, agriculture has contributed indirectly to the degradation of wetland due to the input of polluting substances in its waters, such as nutrients.

This is a serious problem for wetlands all over the world, but especially for Mediterranean ones. The Natural Park of La Albufera of Valencia (Spain) is not unrelated to all these situations; this area has gone through a period of degradation which has started in the 1960s and which, still today, has not been completely restored. The whole area occupied by the Albufera Natural Park is symbol of a complex relationship between nature and human activity. Some of the natural aspects of this area are the results of human transformations (e.g. the presence of the rice crops which give to the area the wetland character). But, in the last 50 years, the transformations that this environment suffered have seriously damaged and affected it. Still today, the Albufera lake is considered a hypereutrophic system and the loss of biodiversity is one of the most alarming problem.

1.2 Objective of the work

This thesis was conducted with a research team of the Polytechnic University of Valencia, led by Professor Guillermo Salvador Palau; the object of the research was the study of the rice crops surrounding the Albufera lake in order to better understand the relationships between the ecological parameters of the wetland ecosystem and the human management of the area.

Following the EU Water Framework Directive200/60/EC, a study of water quality of the area was made, from a physical, chemical and biological point of view.

A physical-chemical analysis of water was carried out, through the measurement of physical-chemical parameters (dissolved oxygen, oxygen saturation in water, pH, temperature, electrical conductivity, nitrite, nitrate and phosphate concentrations). This analysis was necessary in order to better characterize the environment.

In order to evaluate the biological water quality, the macroinvertebrate community was studied. Benthic macroinvertebrates are one of the most widely used groups for this kind of analysis: they have a high taxonomic diversity, they are easy to collect and to analyse, they are widely distributed and are sensitive to contamination and alteration phenomena. IMN (*Índice de Modo de Nutriciòn-Nutrition Mode Index*) and QAELS (*Índex de Qualitat de l'Aigua d'Ecosistemes Lenítics Soms-Water Quality Index for Lentic Shallow Ecosystems*) Shannon-Wiener and Simpson diversity indexes were used for studying the macroinvertebrates community and for the assessment of the ecological status of the lagoon.

Comparisons between areas and over seasons were made, in order to evaluate differences related to water management. Datasets of 2019 were also compared with datasets from 2018, in order to evaluate differences or analogies and to better characterize the environment.

Moreover, the macroinvertebrates classes of Ostracoda and Copepoda were analysed. In fact, these classes are considered good indicators of water quality: they are sensitive to contamination and alteration phenomena and, particularly, they are useful bio indicators when studying eutrophication phenomena. This study can help to confirm the reliability of Ostracoda and Copepoda in studying eutrophication phenomena but also to find the variables which most influence the abundance of the aforementioned. In fact, in ecology, it is important to study the relationships between biological communities and the chemical-physical variables which describe an environment; this kind of study can help to understand the influences that the environmental variables have on the biological community and which are the limiting factors that contribute to the degradation of water quality.

Consequently, increasing knowledge about the relationships between environmental features and Ostracoda and Copepoda is therefore essential for the design of effective habitat conservation and wetland restoration actions.

1.3 Structure of the work

The present work is composed by 5 chapters.

In the first chapter, the general aspects and the objective of the work were briefly described, while in Chapter 2 further information about the Natural Park of the Albufera and the rice fields are provided; the ecological and economic importance of these environments are described, with a particular emphasis on the rice fields surrounding the lagoon. The guidelines and methodologies used in the development of the study in the different phases are set out in Chapter 3, in order to show and discuss the results obtained in Chapter 4. Finally, Chapter 5 sets out the conclusions and recommendations for future development.

Chapter 2.

Case study: the Albufera lagoon of Valencia

2.1. The Albufera Natural Park

2.1.1. Introduction

La Albufera of Valencia is one of the most iconic wetlands in Europe and represents a paradigmatic example of the difficult but essential interaction between nature and human activity; in fact, it is a symbol of the environmental importance of wetlands, but also of the socio-economic richness that revolves around these areas, of the environmental problems this ecosystem suffers and of the cultural heritage it represents.

La Albufera is a shallow coastal lagoon placed in the Mediterranean coast line, situated at 12 km south from the city of Valencia (Spain) (it is set between the parallels 39° 19' and 39° 22', and between the meridians 3°18' and 3°22').

Figure 2. Geographical identification of the Albufera Natural Park. On the left, Valencian Community is pointed out; on the right, the purple line borders the Natural Park of La Albufera

The Natural Park of Albufera has an extension of 21.120 ha and it takes place between the Turia (north) and Jucar (south) rivers.

In this environment, it is possible to differentiate three ecosystems (www.albufera.valencia.es, s.d.):

1. The Albufera lake: it occupies the central part of the Natural Park, with an extension of about 2.800 ha. The lagoon is semi-circular, with an 8 km diameter; the mean depth of water is about 1 m.

Figure 3. The Albufera lake

2. The "Marjal": it is the biggest environment in the park, with an extension of more than 14.000 ha (it takes up the 70% of the total surface of the Natural Park); it is composed by the lands which surround the lake and which are mainly used for agricultural purposes.

   It is possible to identify three sub-environments:

   - the rice fields: they are the object of the current study
   - the orchards, around the first ones
   - the irrigation system: an extensive network of ditches, canals, streams and drains where water circulates until it reaches the lake.

3. The Devesa: set between the Albufera lagoon and the Mediterranean Sea, it is a sandy stripe of 10 kilometres length. Devesa is a coastal forest of 850 ha where it is possible to find both a dune environment and a wild pine forest (Figure 4).

Figure 4. The different vegetation of the Devesa

The connection between the Albufera and the Mediterranean sea occurs through three channels, or *golas* (Pujol Nou, Perellò and Perellonet) and the water flow is regulated by floodgates that maintain the lake level at the most appropriate values for rice cultivation and inundation of the fields.

2.1.2. Historical Background

Originally, the Albufera was a large sea gulf. The sandy stripe that still separates the lagoon from the sea began its formation around 6000 years ago, thus giving rise to a large salty lake. At the end of the Middle Age, the Albufera was a mirror of salty water, connected with the sea through a wide gorge (of about 200 m length) (Sanchis-Ibor, 2016). The surface of the water was the triple of the current one (about 7.500 ha).

Figure 5. Evolution of the surface of the Albufera lagoon. Reproduction of the process performed by TYPSA (2005)

In the XVII century the process of desalination of the lagoon waters began; in fact, documents dating back to this century attest a progressive loss of salinity that was mainly connected to the construction of the irrigation systems in a large number around the lagoon, due to the preponderant development of the rice fields.

The result of this process of desalination was the disruption of the ecological and economic conditions of the lagoon. Aware of the impossibility of re-establishing the past conditions, the Spanish Crown decided to carry out a decisive change for the economy of the area: the control of the *golas* was taken away from the fishermen and was given to the *arroceros*; a new gorge was built, the Perellò gorge, whose construction was followed, several years later, by the one of Perellonet and Pujol Nou.

In the XVIII century, the enlargement of the Acequia Real on the lands situated to the west allowed a rapid expansion of the rice fields, and, consequently, the injection of large quantities of fresh water inside the lagoon, consolidating in definitive form the freshwater character of the Albufera water. Still today, the "*Comunidades de Regantes*" maintain a very important role for the management of irrigation water in the rice fields and their role is essential for the proper functioning of the whole lagoon.

At the end of the XIX century, those lands that were at a lower level than the sea (*Tancats*, in Valencian language) were transformed into rice paddies, for whose irrigation it was necessary to introduce a pump system.

However, as a result of the rapid urban and industrial development, the quality of the lagoon waters began to deteriorate as early as the 1940s, to take on a very abrupt collapse in the 60s and 70s. In fact, both industrial and urban development had been characterized by a very rapid growth rate, producing a chaotic and difficult to manage situation.

Between 1970 and 1980, because of the discharge of organic matter and inorganic nutrients coming from the discharge system, especially phosphorus and nitrogen compounds, and the growth of tourism within the park, the lagoon, which, until that moment, was characterized as an oligotrophic system, assumed a hypertrophic character (Soria, 2006). The accumulation of nutrients gave rise to the growth of phytoplankton and algae, which caused a high consumption of oxygen and prevented sunlight from penetrating into the water. In a few decades, the ecological condition of the lagoon waters has therefore suffered a sharp deterioration, which, even today, cannot be completely remediated.

2.1.3. The Natural Park as a protected environment

There are different authorities, both local and European, which, since the beginning of the XX century, started to protect and preserve the quality and the whole environment of La Albufera.

The whole system composed by the lagoon, the marshland and the sandy dunes was declared Natural Park by the Valencian Autonomous Government by decree

89/1986. Since the 1990 the Park has also been included in the RAMSAR agreement, so that the area was awarded the category "International Important Wetlands" (RAMSAR Site 454).

The area was also included in the European network Natura 2000 as a Special Protection Area (special area for bird protection) since 1991 and in 2001 the area was classified as "Site of Community Importance".

All these recognitions necessarily imply a conservation commitment, and make the Natural Park a preferred area for the European funding and for community, state and regional measures aimed at implementing "sustainable management of habitats of value for the Community and the world" (Seventh Environment Action Program for policy and action on the environment and sustainable development).

2.1.4. Economic importance of the Albufera Natural Park

The Park in its whole is not only important from an ecological and environmental point of view, but it is also essential for the economy of all the area surrounding the park. The contributes that this environment add to the local economy are different:

a. Urban: inside the park, we can find different urban centres, such as Pinedo, Saler, Perellonet and Palmar by the side of the city of Valencia; Perellà and Mareny Blau in Seuca; Mareny de Sant Llorens in Cullera.
b. Touristic: the beach and the area of the Devesa are the centre of an active touristic life which takes place at the Albufera. Other touristic or recreational activities are fishing, hunting and birdwatching.
c. Agricultural: one of the peculiar characteristics of the Natural Park is the agriculture-environment binomial. The environmental value of the park has often been threatened by human action, because of the economic

importance of agriculture. Moreover, this binomial is necessary for the ecological good functioning of the area, because, thanks to the cultivation of rice and the conditions that this type of agriculture requires, the Park can be considered a wetland. The crops we find in the Park can be divided into three zones: the coastal zone, where we can find cultivation in greenhouses; the central zone, or the *marjal*, where the rice crops are developed; the high zone, with the cultivation of vegetables and citrus.

In this regard, in addition to its ecological and environmental importance, a description of the main crop of the area will follow: the rice crops.

2.2 Rice cultivation in the Natural Park of the Albufera

The process of transformation of the M*arjal* into rice paddy begins around 1650 and culminates in the second decade of the twentieth century, as said before. This long process of agricultural transformation of marshes and salt marshes in rice paddies and the regulation of the hydrological functioning of the wetland to favor this crop, have been the main cause of modification of the original landscape of the Albufera, in which the influence of the sea was very important (Oficina de gestion tecnica del Parc Natural de l'Albufera, 2002). The importance of the rice sector in this territorial field constitutes the basic pillar on which the local economy is based, with a mean production of 115.000 t of rice every year.

Figure 6. View of the rice fields

This cultivation, despite the high degree of artificiality and human intervention required, implies the maintenance of specific environmental conditions so that this large land area can be considered as a wetland.

2.2.1. Environmental implications and benefits

The environmental implications of the rice cultivation in the Natural Park of the Albufera can be summarized into 4 different aspects:

1. The importance of the maintenance of the flooded area: the water needs of rice cultivation guarantee the semi-permanent flooding of a large area over a long period of time, allowing the maintenance of the characteristics of this space as a wetland. The existence of a large flooded area brings a series of beneficial effects:

Figure 7. Flooded rice fields during the winter season

- Allowing prolonged flooding of land is a key factor in favoring the development of a rich biological community, both of aquatic invertebrates and aquatic vegetation, which constitute the food base of most water birds present in the

Natural Park. In this way, there is a clear dependence between the agricultural ecosystem and the associated fauna.

- Thanks to the storage capacity of a large volume of water, fields act like a temporary deposit and prevent and minimize the disastrous effects of floods.
- The flooding of the rice fields plays an important role in the prevention of the salinization of the water of the lagoon and especially in the reduction of the effect of the intrusion of seawater in the soil.

2. Conservation of the waterfowl community: the avifauna of the Albufera constitutes the main natural richness of this space, which is the reason why the park was awarded protected natural space and enjoy, still today, different degrees of protection. More than 330 species of birds have been cited in this space, of which about 90 nest in the park naturally. In this area there are also globally threatened species such as the *Marmaronetta angustirostris* and rare or threatened species in the European area, such as the *Aythya nyroca*, the *Ardeola ralloides* and the *Panurus biarmicus*.

Figure 8. Birds are the main beneficiaries of the presence of rice paddy

3. Purification and improvement of surface water quality: industrial and urban development throughout the environment of the Natural Park of the Albufera, together with the intensification in the use of agricultural chemicals (mainly fertilizers), have been, the most important cause in the deterioration of the quality of wetland waters . The Albufera constitutes a coastal wetland system that receives water from

both the drainage basin and the extensive and complicated irrigation network. These surface contributions include part of the industrial and urban wastewater that, without prior purification or with poor treatment, directly affects the system. In this sense, the surface of flooded rice fields that extend throughout the perimeter of the lagoon plays an important role as a biological filter and purification of these waters that arrive loaded with suspended materials and contaminants.

4. Maintenance of the traditional rural landscape: the practice of rice cultivation has been developed since ancient times in the area of the Albufera; for this reason, the different cultural practices and the continuous interventions carried out by farmers have configured a rural landscape of high value and great cultural and social singularity. An example is the innumerable agricultural constructions and buildings (mills, booths, warehouses) that are distributed throughout much of the rice paddy, bridges and old water supply facilities.

Figure 9. "Caseta" in the National Park of La Albufera

2.2.2. The Tancats

The "Tancats" are portions of lands which stand immediately close to the Albufera lake and are flooded directly by the lagoon. They are separated from the lagoon through "*motas*", land elevations built *ad hoc* to separate them from the lagoon in order to expand the cultivation fields.

The Tancats, according to their level of elevation, are divided into Lower and Upper.

The first ones are located at a lower level with respect of the level of the lake. Because of this difference, natural drainage cannot be applied; for this reason, this operation is performed through helical pumps. On the contrary, flooding occurs naturally from the lake to the fields.

The Upper Tancats, on the other hand, are at a higher level than that of the Albufera lagoon and further away from it; consequently, they need a pumping system for irrigation, but the drainage happens naturally.

2.2.3. The crop cycle

Generally, at the Albufera, the flooding of rice fields takes place in two periods of the year, between May and September, coinciding with the production of the cereal, and between October and February (Guillem, 2015). The flooding and drainage of the fields takes place through a complex network of channels and, consequently, the water management of the park is highly dependent on the rice crop cycle.

The rice cultivation cycle lasts in total one year, considering the time required for processing the land, flooding and draining the fields.

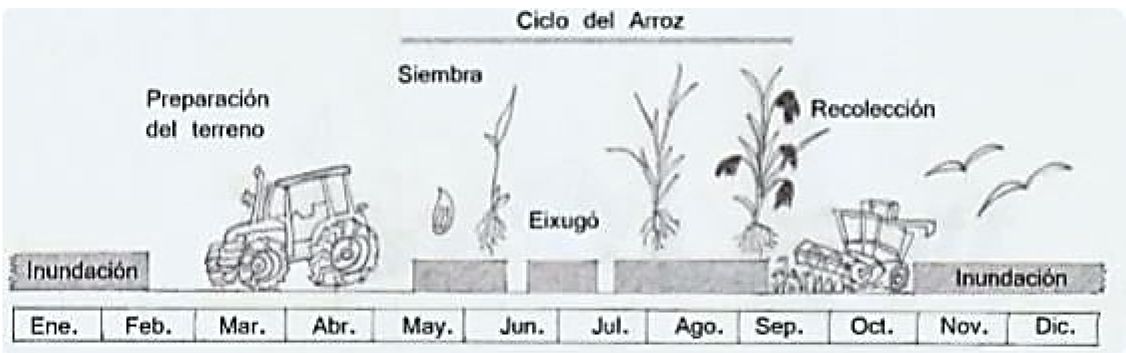

Figure 10. Crop Cycle

Between January and February, with a low level of water, the agricultures begin to "*fanguear*", to plow the land and add mud mixed with straw remaining from the previous year. Subsequently, in the months of March and April, the soil is dried and rest under the sun; the soil is turned and levelled, so that the largest grains are eliminated. In this period fertilizers are also distributed. On May, the land is prepared for "*la siembra*": fields are flooded and the seeds are submerged and scattered around the rice fields. During the summer months the completely submerged rice plants grow up to 30-40 cm; for brief periods, however, emptying operations are carried out (*eixugò*) in order to carry out a new dosage of fertilizers. In September fields are emptied and the harvest of the already mature rice begins, an operation that lasts until the middle of October; subsequently the floodgates that connect the Albufera with the sea are closed, the water level inside the lagoon rises and the fields are flooded again, throughout the winter.

2.3. Water regime of the Albufera

Due to its water regime, the Albufera differs from all other natural aquatic ecosystems, where seasonality and climatic conditions regulate the regime. In fact, the water supplies that reach the lagoon correspond to waters of different processes, with different chemical-physical properties.

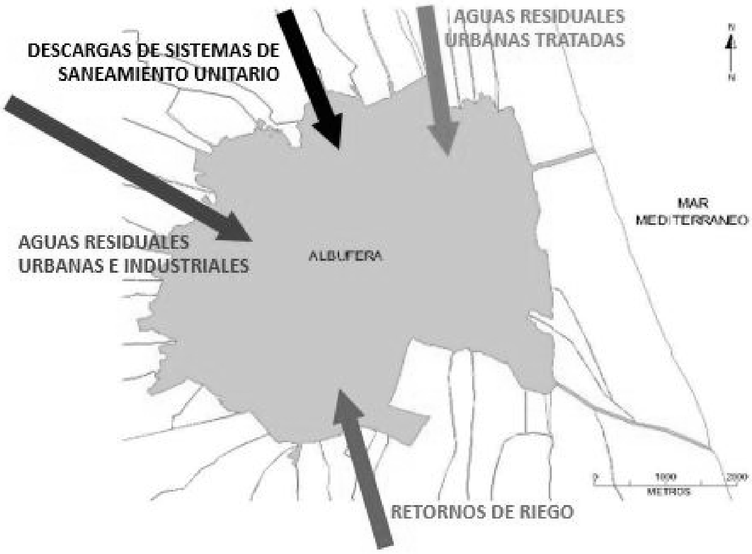

Figure 11. Anthropic pressure over the Albufera lake (Life Albufera, 2015)

In the northern part, in the past, before the urban and industrial development of the area, the Albufera received mainly the waters of the Turia and Acequia de Oro; subsequently the contributions in this area have assumed very different connotations and properties. In this area, in fact, the Albufera receives nowadays also the water contribute coming from the wastewater treatment plant of the City of Valencia (WWTP of Pinedo) and the untreated water of the collectors of the towns sprung up around the lagoon (Quart, Aldaia, Alaquas and others).

In the southern area, on the other hand, the contributions are essentially attributable to the Jucar river, to the discharges of the cities of Almussafes, Benifaiò, Alginet, Seuca, and to the various underground springs (*ullulas*) (Soria, Miracle, & Vicente, 1987).

Moreover, the lagoon receives the huge contribution from the water coming from the rice fields.

Summarizing, therefore, the Albufera Natural Park feeds mainly through 4 sources (Soria & Vicente, 2002):

1. Natural waters: essentially composed of the ravines which form the hydrographic basin that ends in the Albufera. The basin reaches the Albufera through various ravines, but the main ones are two: that of Beniparrel and that of Massanassa; both come directly into the waters of the Albufera, while the others flow into more or less large canals and only through these they pour their waters into the lagoon. In reality, this type of natural circulation contributes in an important way only in periods of intense rainfall, capable of producing runoff.
2. Groundwater: their contribution to the lagoon water balance is difficult to estimate; we have to remember also the contribution of *las ullals*, natural sources of fresh water
3. Wastewater: waters of urban and industrial origin; they come through their collectors or through natural channels or irrigation channels. In addition to the water coming from the wastewater treatment plant of the city of Valencia (EDAR), there are also untreated or pre-treated waters.
4. Irrigation waters: they reach the Albufera through 63 channels; their quantity is not constant but depends on the flooding of the rice fields.

Chapter 3.

Materials and Methods

3.1 Benthic macroinvertebrates as bio-indicator of water quality

Benthic macroinvertebrates are invertebrates visible to the naked eye; the term "benthic" means "bottom-living" (these organisms generally live in the lower layers of water bodies at least for a part of their life); the term "macro" indicates that they are retained by a 200-500 μm mesh (Rosenberg & Resh, 1993). In fact, these organisms have dimensions that usually exceed 3 mm, but in any case are not lower than 0,5 mm.

Benthic macroinvertebrates are found in all type of freshwater environments, such as rivers, lakes and lagoons. They mainly include arthropods (insects, arachnids and crustaceans), even in larval form; there are also oligochaetes and molluscs, although to a lesser extent.

Benthic invertebrates (and especially macroinvertebrates) are one of the most widely used groups as biological indicators of water quality. This is because they integrate many of the qualities expected from an indicator. Through their high taxonomic diversity, it is possible to explain different ecological, physic-chemical and biological aspects of an aquatic environment. According to the purpose of the EU Water Framework Directive 2000/60/CE, benthic invertebrates are considered useful for the detection and monitoring of the following types of pressures:

- physical-chemical pressures related to thermal pollution, changes in water mineralization, organic pollution, eutrophication, contamination by metals or other pollutants

- hydro morphological pressures related to change in flow rate, renewal rate, change in morphology of the fluvial bed

Among the advantages of the use of macroinvertebrates as biological indicators for water quality we can mention (Zúñiga, Rojas, & Caicedo, 1993):

- The sedentary nature of many species: this allows the spatial assessment of long-term adverse effects in the community
- They have relatively short life cycles compared to fishes and so, it is possible to explain more rapidly changes in the ecosystem structure through changes in their populations and communities
- They are widely distributed, abundant and easy to collect due to their size that makes them visible to the naked eye; sampling operations are generally not expensive
- They live and feed in or on sediments where toxins tend to accumulate
- They are sensitive to disturbance factors and respond to pollutants in both water and sediment

It is important to underline that all the different taxa have different tolerance to the different toxic compounds or to the alteration or degradation of the environment. In other words, there are very sensitive species that can disappear or reduce their abundance in presence of environmental contamination, while the more tolerant ones may, at the same time, increase their density. This property allowed the development of biotic indexes based on the tolerance of different macroinvertebrates (Gamboa, Reyes, & Arrivillaga, 2008).

Among the groups which are more sensitive to the alterations of the ecosystem there are the aquatic larvae of insects belonging to the orders Trichoptera, Ephemeroptera, Plecoptera (TPE) and larvae and adults of aquatic Coleopterans. These groups have shown a high sensitivity to pollution and degradation of Spanish aquatic ecosystems (Ribera, Aguilera, Hernando, & Millàn, 2002).

However, other groups show high resistance to disturbances and pollution, such as certain species of oligochaetes, dipterans and molluscs (e.g. Chironomidae) (Alonso & Camargo, 2005).

But this kind of analysis may also be difficult without the proper preparation and experience. The disadvantages of using macroinvertebrates as bio-indicators include:

- The macroinvertebrates' community is heterogeneous and the taxonomy of some groups is not well known; for this reason, it is crucial to have the proper knowledge and experience in the laboratory analysis for the identification of the different families of macroinvertebrates

- Seasonal or dynamic variations in the population may interfere with the interpretation or comparison of results

However, most researchers point out that within all groups considered as bio-indicators of environmental quality, aquatic macroinvertebrates are the best ones.

3.2. Sampling procedure

For the sampling procedures, the instructions indicated in the document "*Metolodogia para el establecimiento el Estado Ecologico segun la Directiva MARCO del Agua: Protocolos de muestreo y anàlisis para invertebratos bentonicos*" (2005) of the Ebro Hydrographic Confederation were followed.

3.2.1. Prior phase: identification of the sampling periods

Two sampling dates have been chosen during the year 2019, according to the flooding period; the first one, the 19th of January, was representative of the winter season, and the second one, the 15th and 16th of July, was chosen for the summer season.

It is important to make a distinction between these two periods because of the different roles the Albufera assumes during the year; in fact, in winter, the fields surrounding the lagoon are flooded because of their ecological role, which is preserving the habitat and the waterfowls that settle in the park during the winter months. During the summer season, on the contrary, the area surrounding the Albufera lagoon is devoted essentially to the rice cultivation.

There are, therefore, numerous differences between the two seasons: flooded area is different (in summer, it is bigger) and so are the water sources, as well as climatic conditions. Consequently, there are substantial differences between the chemical-physical-biological properties of water as well as in water management.

Now, a description of the two sampling periods will follow, with the aim of emphasizing the most relevant differences between the two.

- <u>Winter season</u>

In the study by Salvador Ibarra Galbis, " *Influencia de la inundaciòn invernal en la biodiversidad y calidad del agua de los arrozales del Parque Natural de la Albufera* " (2018), macroinvertebrate samplings were performed in winter 2017-2018. In order to investigate the variability of the biological parameters, samplings were carried out on 3 different dates, in December 2017, January 2018 and February 2018. On each date samplings were collected from 6 points, three located at north and 3 at south of the lagoon.

Figure 12. Sampling points in winter 2018

From his study it emerged that none of the biological water quality indexes (IMN, QAELS) showed significant differences between the three different dates.

Moreover, in January the diversity of macroinvertebrates should be at its maximum, because the fields are flooded and left to recover, as established in the Convention on Wetlands of International Importance.

In fact, through the use of satellite images, it is possible to observe the changes that the Albufera crosses during the winter season (Figure 13).

Figure 13. Satellite images (Sentinel 2B): variation of the flooded area during the winter season (from October 2017 to March 2018) (https://glovis.usgs.gov/)

In October the three *golas* are closed and the "*Perellonada*" begins, that is the flooding of the rice fields that surround the lagoon. Figure 13 shows the progress of the winter flooding in November and December. Until February the level of flooding remains unchanged: all the fields are flooded by water and there is no agricultural activity.

In the late February, the drainage process of the Marjal can be observed: the doors are opened, the natural drainage of the fields begins and, consequently, the lowering of the lagoon water level occurs. Between the end of February and the beginning of March the drainage process is concluded with the help of motors and pumps, so that fields can be prepared for sowings.

The study by Ibarra Galbis confirmed the hypothesis that in January the biodiversity is higer. In fact, the study of the diversity indexes of Shannon-Wiener and Simpson showed greater diversity in January than in December and February.

Consequently, for this campaign, it was decided to perform samplings on a single date, on 19/01/2019. However, the analysed sample was expanded; in this way it could be more representative of the study area and allow a more accurate and consistent study. Samples were in fact collected in 22 points; in this way it was possible to cover almost the entire study area.

- Summer season

In the study by Giulia Lucà "*Water resources management and ecological response in the Albufera lagoon of Valencia*" (2019), macroinvertebrates sampling was performed in the summer season of the year 2018. The samplings were made in 3 different months, June, July and September and 12 samples were collected from 12 different points from different areas.

From the study it emerged that in July there were the highest values of water quality indexes (IMN, QAELS). Also the values of the Shannon Diversity Index and of Simpson Index were higher than in the other two months.

For these reasons, for the summer campaign of 2019, it was decided to carry out the sampling operations only in the month of July. Also in this case, like in winter, the number of sampling points has been increased from 12 to 32, in order to better characterize the different areas.

Figure 14 shows summer flooding periods: in May, the flooding starts. In July fields are completely covered by water. In late July and August, rice plants grow and reach a height of 30-40 cm.

Figure 14. Satellite images (Sentinel 2B): variation of the flooded area in the summer season, from June to August (https://glovis.usgs.gov/)

3.2.2. Prior phase: identification of the sampling areas

As mentioned above, in the different seasons of the year, the Albufera lagoon and the surroundings assume a different role: in winter the ecological and environmental value dominates, while in summer the agricultural role is predominant. For these reasons, quantity and water sources are different.

In fact, in winter, when flooding is not carried out for agricultural purposes but only for the conservation and preservation of the habitat, fields are flooded mainly by the waters coming directly from the Albufera lake. Until 2017, in the northern part of the lagoon, water coming from the wastewater treatment plant of Pinedo was also used, but nowadays fields are flooded only by water coming from the lake. Consequently, the flooded area has decreased through the years, from about 120 km^2 to 80 km^2.

So, the area examined in this study was flooded, during the winter period, by water coming directly from Lake of the Albufera. The 22 sampling points are located in the Tancats; we identified 2 zones, called "North Tancats" (906 ha) and "South Tancats" (4780 ha).

In these areas, quality and characteristics of the flooding water depend on quality and characteristics of the lake itself, which is considered a hypereutrophic system due to the intense concentrations of nutrients that are poured into it. But at the same time, the quality of the lake's water depends on the water supplies it receives, the quantity and the hydraulic regime.

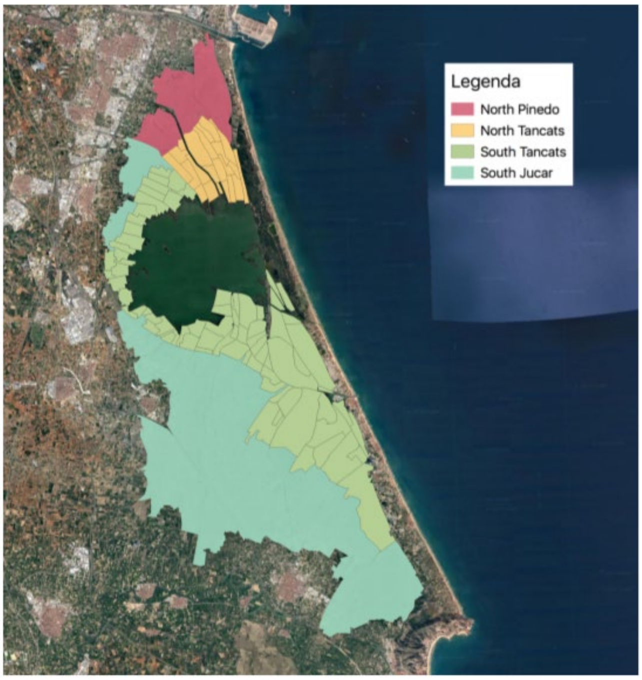

Figure 15. Sampling areas: in red the area North Pinedo is shown, in orange the area North Tancats, in green the area South Tancats and in blue the Jucar area (Lucà, 2019).

On the contrary, on summer, the surface of the flooded land is higher because summer flooding has essentially agricultural purposes: the rice fields need to be completely covered by water, in order to let the rice plants grow in a suitable environment. Consequently, water request is higher: the water coming from the lagoon is not enough to flood all fields, but additional water sources are needed.

In the study, four sampling areas have been identified; each area is supplied from a different water source. The four areas are called "Pinedo", "Jùcar", "North Tancats" and "South Tancats":

- Pinedo area is supplied mainly by the water of the wastewater treatment plant of Pinedo (1130 ha); it includes the rice fields of the "Acequia de Oro" and the "Acequia de Favara"

- Jùcar area is flooded by the water of the river Jùcar and includes the rice field of the "Acequia Real del Jucar" and the "Acequia of Seuca"; it incorporates irrigation sectors of Sueca, Silla, Sollana and Cullera. This area is much bigger than the northern areas (7400 ha) and it is also characterized by the presence of the so called "*ullals*" (groundwater springs).
- North and South Tancats areas are supplied directly by the lagoon; the distinction has been maintained in order to evaluate possible influences of the previous two zones on the Tancats areas.

3.2.3. Prior phase: identification of the sampling points

Once the temporal and spatial study of the area had been carried out, we proceeded with the collection, in field, of samples for the analysis of macroinvertebrates and for the study of physical-chemical parameters.

The choice of the parcels, in each studied area, is carried out randomly.

In winter, samplings were performed on 19/01/2019, for a total of 22 sampling points, each one belonging to a different parcel. A total of 10 samples were collected in the North Tancats area (N1-N11) and 12 in the South Tancats area (S1-S12).

In the South area, two points (S11 and S12) were sampled in the third type of Tancats, that is among those that need to use pumping both for flooding and draining the fields.

On July, a total of 32 samples were collected on two days, 15th and 16th. 6 points were collected from the North Tancats area and 6 from the Pinedo area (N1_A-N7_A). 10 samplings were collected from the South Tancats area (S1-S11) and 10 from the Jucar one (N8_A-N10_A; S1_A-S10_A).

Figure 16 and Figure 17 show the location of the sampled parcels.

Figure 16. Sampling point in the norther area of the lagoon. From N1 to N11: North Tancats area. From N1_A to N7_A: Pinedo area

Figure 17. Sampling points in the southern area of the lagoon. From S1 to S12: South Tancats area. From N8_A to S10_A: Jucar area.

3.3 Measuring of physico-chemical parameters

At each sampling points, measurements of physico-chemical parameters were done.

Measurements of physico-chemical parameters were made in situ, with different instruments that were calibrated before the campaign; the measures took into account:

- Dissolved oxygen (mg O_2/l)
- Percentage of oxygen saturation (%)
- Temperature (°C)
- pH (-)
- Electrical conductivity (mS/cm)

These parameters are fundamental for investigating water quality.

Dissolved oxygen is a clear indicator of the wellness of a water body; in fact, low dissolved oxygen concentrations can modify the structure of the ecosystem and cause problems of mortality of fish and living organisms, problems concerned with odour, aesthetic problems, etc. The minimum concentration of dissolved oxygen needed to assure the maintenance of all the living forms in water is 4 mg O_2/l.

The concentrations of dissolved oxygen in a water body may vary due to different processes which, more or less naturally, may occur within it. Photosynthesis and re-aeration by means of the atmosphere are processes considered as "source" of dissolved oxygen, because they increase oxygen concentration in water. The processes which, on the other hand, decrease oxygen concentration are the respiration of aquatic plants and the oxidation of organic matter and nitrogenous matter.

For oxygen concentration and saturation measurement, the instrument *DO 6 Economy Hand-held Dissolved Oxygen (Eutech instruments*) was used.

Figure 18. Intruments used for dissolved oxygen and pH measurement

Production and consumption of oxygen are also influenced by <u>temperature</u>, which modifies biological processes, accelerating them. In some critical summer periods, in fact, phases of anoxia can occur in some areas. All the instruments used were able to measure temperature, too.

<u>pH</u> is also an important parameter in defining water quality, which can be an indicator of acidification and eutrophication processes (*pH/mV & lon/pH Meter series, Eutech instruments*, was used). The optimal pH value is between 6 and 9; excessively low values could indicate an intense eutrophication phenomenon. The excessive growth of algae produces high concentrations of CO_2 in a water body due to the phenomena of respiration and this leads to a lowering of the pH value.

<u>Electrical conductivity</u> measures the content of salts dissolved in water in the form of free ions (*Hand-held conductivity/TDS Meter. Con 6/TDS 6, Eutech Instrument*).

Figure 19. Instrument used for electrical conductivity measurement

Salts dissolved in water allow the passage of the electric current: the higher the conductivity value, the greater the quantity of the mineral salts dissolved in water. Salinity is an ecological factor of considerable importance, which can influence the type of organisms that live in a waterbody and plant species that can grow in an aquatic environment. Values should not be higher than 2500 µS/cm. In this study, due to the high value measured in the water samplings, the electrical conductivity is expressed in mS/cm.

In addition to the analysis of the aforementioned parameters, an analysis of the chemical parameters of water was also carried out; the analysis focused on the concentrations of nutrients:

- Nitrites concentration (mg/l)
- Nitrates concentration (mg/l)
- Phosphates concentration (mg/l)

These analyses were performed in the laboratory of the Catholic University of Valencia and not in situ; the water to be analysed was sampled using 2-liter bottles and then taken to the laboratory as soon as possible. There the samples were stored at a temperature of 4 ° C until the analyses were performed (within 24 hours).

Nutrients analysis is essential to study a eutrophic environment like the Albufera.

The presence of nitrogen in form of nitrate is strongly conditioned by numerous factors, such as phytoplankton activity (which consumes nitrate as a nutrient), nitrification and denitrification processes, external inputs from agricultural activities. The maximum reference value is 1 mg N/l, maximum limit value in unaltered fresh water.

The presence of nitrites, on the other hand, is associated with phenomena of oxygen deficit in the system, since nitrites are formed by reactions of incomplete nitrification, due to the low concentration of oxygen. The target value for cyprinid waters is 0.03 mg / l.

There is no data on total phosphorus, but phosphate concentration was measured.

3.4 Macroinvertebrates sampling and identification

Sampling and identification of benthic macroinvertebrates as water quality elements must be carried out, in accordance with the Water Framework Directive, following a standardized procedure and with quality control systems. Even the identification of macroinvertebrates at the level of family, genus or species is an aspect of great importance, and which must be carried out according to the criteria and dictates of the European Directives.

3.4.1. In situ macroinvertebrates sampling

The European directive *EN27828: 1995: Water quality - Methods of biological sampling - Guidance on handnet sampling of aquatic benthic macro-invertebrates (ISO 7828: 19285)* was therefore applied for the sampling procedures.

The "dipping" technique was used for sampling. A manual sampling mash with a 250 µm light and an opening of 200 µm of diameter was used.

In dipping, the mesh is immersed in water and is pushed down to about 1 m from the bottom, then go up towards the surface, so that the benthic macroinvertebrates are captured inside the mesh.

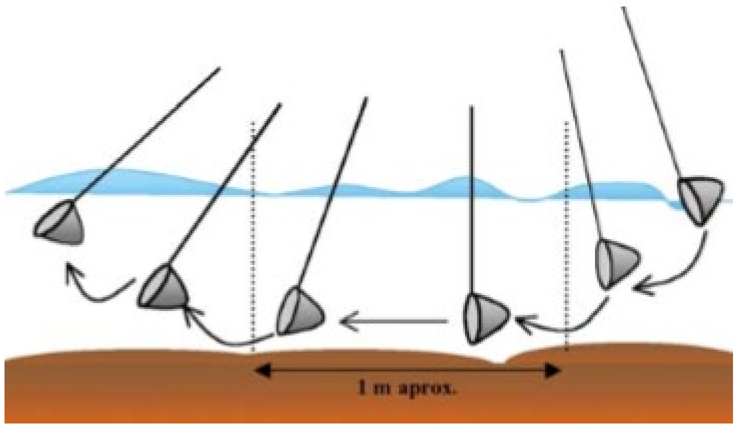

Figure 20. Dipping technique rappresentation (from: Metodología para el establecimiento del Estado Ecológico según la Directiva Marco del Agua).

A number of 3 repetitions were performed for each of the sampling points, so that the samples taken were found to be significant in the investigated area.

Figure 21. Sampling operation in January 2019 and July 2019

For each one of the investigated point, the content of the mesh has been properly emptied inside containers, previously cleaned and appropriately labeled (indication on the sampling point and date).

After each withdrawal, the mesh was cleaned, in order to prevent foreign elements to being found in the next sample.

3.4.2 Laboratory phase: macroinvertebrates identification

The identification and counting of the macroinvertebrates was carried out in the Ecology laboratory of the Department of Agroforestry Ecosystems E.T.S.I.A.M.N. of the Polytechnic University of Valencia.

Once in the laboratory, the collected samples were stored in refrigerators at 4°C in order to avoid deterioration and maintain macroinvertebrates alive.

Once analyzed, a sampling was poured onto white trays by adding water to separate macroinvertebrates from the slime and small macrophytes using tweezers, brushes and teaspoons.

Once isolated, they were put in Petri dishes; the larger, and clearly observable recognizable individuals were identified without the binocular. The rest was accounted for and identified under the binocular glass at the family level, except for some cases for which the classification stopped at the order or class level, because of the large number of the samples.

During the summer samplings, a total of 32 samplings were collected on two days (15/16 of July 2019). But, because of the high temperature and the huge number of collected samples, it was not possible to analyse and classify them in short times. For these reasons, great part of the macroinvertebrates died before the identification and it was not possible to proceed with the laboratory operation and with the evaluation of the quality and diversity indexes. It was possible to measure only the physical-chemical parameters.

3.5 Spatial analysis of water quality and biological data

In order to better understand and characterize the different areas and to compare among areas (North and South Tancats for winter, and North Tancats, Pinedo, South Tancats and Júcar for summer) and periods (winter and summer 2018 and 2019) it was necessary to apply some statistical analysis on the collected data.

On the new data collected through the year 2019, a normality test was applied, in order to verify the normality of the different dataset. The Shapiro-Wilk normality test was chosen, with a 95% significance level.

Comparison between areas and seasons were made. In winter, comparisons were made between the two areas North and South Tancats, while in summer among the areas North and South Tancats, Pinedo and Júcar. Dataset from winter 2019 (both physico-chemical and ecological) were compared with dataset from 2018, while for the two summer seasons (2018 and 2019) comparisons were possible only among the different physico-chemical parameters, because it was not possible to analyse and classify the macroinvertebrate community sampled in July 2019.

The ANOVA test was used in order to evaluate significant differences between two groups of data (comparisons over time or space); the ANOVA is so called because it compares the variance between different groups with the variability within each of the groups.

3.6. Assessment of the ecological status

Through the years, several indexes have been developed in order to evaluate the ecological status of water; many of them better adapt to rivers but this kind of study is not yet widespread for wetlands. In this thesis different indexes were used: Shannon-Wiener and Simpson indexes were used as a measure of the diversity of macroinvertebrates community, while IMN Index and QAELS Index were used as biological quality indexes of water.

- **Simpson index**

Simpson index (D), proposed in 1949 by the English Edward H. Simpson, measures the probability that two individuals randomly selected from a sample belong to the same species (or to the same category).

$$D = 1 - \sum_{i=1}^{S} p_i^2 \qquad p_i = \frac{N_i}{N}$$

where:

- S = number of species (richness)
- p_i= percentage of individuals of i-th specie with respect to total individuals (the relative abundance of species i)
- N_i = number of individuals of the i-th specie

- N = total number of all the species present in the sample

It is a measure of dominance: common species carry much weight as regards rare species. It ranges between 0 (when there is only one species) and $(1 - \frac{1}{S})$.

- **Shannon-Wiener diversity index**

Shannon or Shannon-Wiener index (1949) is one of the most used index in ecology to measure biodiversity. Shannon-Wiener index measures the average degree of uncertainty in predicting which species an individual chosen from a collection will belong to. It assumes that individuals are randomly selected and that all species are presented in the sample.

This index is expressed by a positive number, between zero, when there is only one species, and the logarithm of S (number of species), when all species are represented by the same number of individuals (Magurran, 1988). In most natural ecosystems it varies between 1 and 5. Exceptionally there may be ecosystems with higher values (tropical forests, coral reefs, ecosystems in which there is a huge biodiversity) or smaller ones (e.g. desert areas). The main limitation of this index is that it does not take into account the spatial distribution of species.

There is a relationship between Shannon-Weaver index and water quality (Margalef, 1993), and this becomes feasible by applying the base-2 logarithm instead of the natural logarithm, as the original formulation suggests. Therefore, this modified Shannon diversity index was chosen to express the results.

Shannon index is defined as:

$$H' = -\sum_{i=1}^{S} p_i log_2 p_i$$

As the number and distribution of taxa (biotic diversity) within the community increases, so does the value of H'.

Generally, a diversified community is index of good water quality; the more diversified is the macroinvertebrate community, the more unaltered is the studied system. In Table 3, the relationship between Shannon index and water quality is shown.

Table 1. Water quality according to Shannon-Wiener index

H′	Diversity level	Water Quality
<1	*Very low*	*Strongly polluted*
1-3	*Moderate*	*Moderately polluted*
>3	*High*	*Clear water*

- **IMN Index**

The IMN (*Índice de Modo de Nutrición- Nutrition Mode Index*) aims to evaluate the quality of the trophic structure of the macroinvertebrate communities according to the different food availability and according to the feeding mode of each species present in the water system (Rueda, Hernandez, Lòpez, & Martìnez, 2005). In Annex I the table with the different feeding modes and the consequent classification of macroinvertebrates is shown (Tachet, Bournaud, & Richoux, 1987).

It can be used for all type of aquatic systems, from rivers to lakes, from lagoons to rice fields.

IMN index is closely connected with the heterogeneity of the environment, so that we will notice a high diversity of invertebrates if the system is characterized by high heterogeneity, whereas if the environment tends towards homogeneity or it is altered, the diversity in the structure of macroinvertebrates will also be reduced, tending, in extreme cases, to a single nutrition mode.

The best condition in the ecosystem should show the presence of all trophic groups in proportions ranging between 5 and 15% for each of them, positively evaluating the presence of a certain group in the environment (greater than 0%) and negatively if it does not exist or its percentage is excessively high (over 40%). Finally, positive and negative values are added together; according to the final value of the IMN index it is possible to define the status of the macroinvertebrate community and that of water quality of the analyzed system.

Table 2. Definition of Food web diversity level and environmental status according to IMN values

IMN	**Class**	**Food Web Diversity Level**	**Environmental status**
>70	I	Very High	Unpolluted
55-69	II	High	Slightly impacted
40-54	III	Moderate	Moderately stressed
20-39	IV	Low	Stressed
0-19	V	Very Low	Heavily impacted

- **QAELS index**

QAELS index (*Qualitat de l'Aigua d'Ecosistemes Lenítics Soms-Water Quality Index for Shallow Lentic Ecosystems*) was created to study the Mediterranean shallow lentic ecosystems and was developed by the Agencia Catalana de l'Aigua in 2004 to adapt it to the guidelines of the EU Water Framework Directive (Agència Catalana de l'Aigua, 2004) . This index is based on the diversity and abundance of certain families of invertebrates and on quality scores these families get in terms of indicators.

QAELS index combines two indexes in order to assess water quality: ACCO index (*Abundance of Copepoda, Cladocera and Ostracoda*) evaluates water quality through the abundance of species-indicators Cladocera, Copepoda and Ostracoda while RIC Index (*Richness of Insects and Crustaceans*) modulates this result through the taxonomic richness of the benthic community of insects and crustaceans.

It is evaluated by means of the following formula:

$$QAELS = (ACCO + 1) \times Log(RIC + 1)$$

According to QAELS value, it is possible to evaluate water quality:

Table 3. Water quality according to QAELS index

QAELS	**Water Quality**
<2	*Very Poor*
2-4	*Poor*
4-6	*Moderate*
6-8	*Good*
>8	*Very Good*

QAELS combines aspects of taxonomic richness with abundance ones. The abundance of macro-crustaceans and their quality values allow to obtain an index able to assess water quality. On the other hand, the richness of the species and the diversity of the benthic community are parameters commonly considered in the assessment of water quality of aquatic ecosystems.

ACCO index

Macro crustaceans such as Cladocera, Copepoda and Ostracoda have properties that make them suitable in the assessment of lentic water quality (Ruso, et al., 2017):

- They are present in all types of water bodies
- They respond to habitat disorders
- Their taxonomy is reasonably well defined

Through this index, each taxon is associated to an abundance value and to a quality value, ranging from 0 to 10. This value was firstly obtained from a canonical correspondence analysis between the presence of the different families of macro crustaceans and the variables that are index of good quality of water bodies (the Table is listed in the Annex I**);** a higher value was assigned when a certain family was strongly correlated with good quality variables.

Moreover, quality values vary depending on the kind of lentic ecosystems; in fact, three types of ecosystems have been identified: freshwater permanent wetland, freshwater temporary wetland and brackish water wetland.

ACCO index is so defined:

$$ACCO = \sum_{i=1}^{j} k_i \times n_i \qquad n_i = \frac{N_i}{N_{TOT}}$$

i: indicator taxa

j: number of indicator taxa

n_i: relative abundance of the i-th taxa

N_i: abundance of the i-th taxa

N_{TOT}: total abundance of the whole indicator taxa

k_i: quality value of the i-th taxa

In this study, the category “freshwater temporary wetland” was chosen, because fields do not remain flooded permanently, but they are periodically covered by water and drained.

Moreover, Ostracoda and Copepoda were analyzed and classified only until the “class” level; for this reason, an average value of the quality value was identified and assigned to this class of macroinvertebrates.

RIC index

It is calculated according to the following formula, based on the number of insects and crustaceans present in each sample:

$$RIC = N° \ of \ crustacean \ genera + N° \ of \ adult \ Coleoptera \ and \ Heteroptera + N° \ of \ insects' families \ and \ larvas$$

In fact, good quality waters are generally inhabited by a high number of different organisms, and for this reason RIC index evaluates the number of different species of insects and crustaceans.

3.7. Random Forest classification to predict macroinvertebrates presence

In this study, the interactions between the macroinvertebrate classes of Ostracoda and Copepoda and chemical-physical variables (Table 4) have been explored; in fact, these classes of macroinvertebrates are considered good indicators of water quality: they are sensitive to contamination and alteration phenomena and, particularly, are useful bio indicator when studying eutrophication phenomena. In fact, they are used for the evaluation of ACCO index and, consequently, for QAELS.

Table 4. Chemical-physical parameters used in the classification models

VARIABLE	***CODE***
Dissolved Oxygen [mg/l]	*D.O.*
pH	*pH*
Temperature [°C]	*T*
Electrical Conductivity [mS/cm]	*e.c.*
Nitrites [mg/l]	*NO_2*
Nitrates [mg/l]	*NO_3*
Phosphates [mg/l]	*PO_4*

The relationships between Ostracoda and Copepoda abundance and chemical-physical parameters (temperature, dissolved oxygen, pH, electrical conductivity, nitrites, nitrates and phosphates concentrations) were first explored, using the Spearman's rank correlation coefficient ρ as a measure of their correlation. Spearman's rank correlation coefficient is a non-parametric test, and it can vary between -1 (strong, negative correlation) and +1 (strong, positive correlation).

Datasets from 2018 and 2019 campaigns were used.

Random Forest (RF) was then used with the aims of identifying the most important variables in predicting presence of Ostracoda and Copepoda in the Albufera lagoon.

Random Forest (Breiman, 2001) is a machine learning technique, which is used both for classification and regression; it automatically combines a lot of decision trees and produce a predictive output. RF is considered a robust machine learning technique, especially for analysis of complex, nonlinear and highly dimensional data (Olaya-Marìn, Martinez-Capel, & Vezza, 2013). Classification is widely used to predict species distribution or habitat suitability. In this study, to produce Ostracoda and Copepoda distribution models, presence/absence was evaluated, and the importance of the collected chemical-physical variables over their presence was assessed, too. Temperature was not considered in the model because it is not a variable influenced by the agricultural practices. As the response variable was categorical (presence/absence) was categorical, classification mode was chosen.

Random forest was implemented in R (RStudio, 3.6.2.), by means of the randomForest package.

In Random Forest, each tree is trained by selecting a random bootstrap subset X_i from the original dataset X (i ranging from 1 to *ntree*), using two third of the total dataset and a random number of predictive variables (Vezza, Parasiewicz, Calles, Spairani, & Comoglio, 2014). Generally, the elements which are not included in the selection are called Out Of Bag (OOB); in this way, each element is an OOB in one third of the *ntree* iterations. For each bootstrap, a tree is grown. Within a tree, each node considers a randomized subset of the original predictive variables m; usually, in classification models m is chosen as:

$$m = \sqrt{n° \, variables}$$

The number of trees $ntree$ is generally chosen according to the OOB error: the number of trees which stabilizes the error could be the right one.

At the end, global RF accuracy and error rates are calculated, using the OOB predictions.

The importance of the predictive variables is assessed according to the Mean Decrease Accuracy (MDA) parameter. RF evaluates the importance of a variable by looking at how much prediction error increases when OOB data for that variables are permuted, while the other are left unchanged. The more the error increases when a variable is permuted, the more important is that variable.

Moreover, in Random Forest the model is represented from the forest itself, but it is not possible to see it as a result, through a graph or a formula. Despite this, it is possible to see, through the partial dependence plots, the marginal effects of a single predictive variable above the predicted one, averaging the effects of the other variables. They represent the average prediction between the individual chemical-physical variables and the predicted probability of macroinvertebrates presence.

The evaluation of the performance of the models was made through the analysis of the confusion matrix; in a classification model for presence/absence, the confusion matrix can be represented as follows (Figure 22).

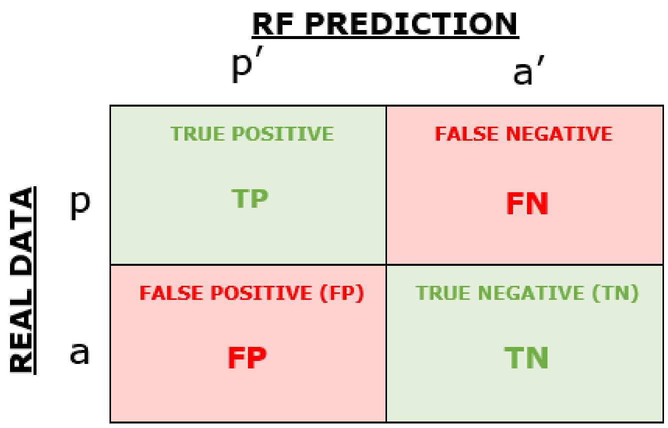

Figure 22. Confusion matrix for a classification model (p=presence, a=absence)

Real data were classified in two classes: present (p) and absent (a). Random Forest prediction classified the data in the same two classes: present (p′) and absent (a′). Consequently, the matrix shows:

- True Positive (TP): values that were classified as present in real data and that the model classified as present;
- False Negative (FN): values that were classified as present in real data, but as absent in the model;
- False Positive (FP): values classified as absent in real data, but as present in the model;
- True Negative (TN): values classified as absent both in real data and in the model.

Through the confusion matrix, it is possible to evaluate different model performance parameters; in this study, the following parameters were used:

- *Accuracy*: proportion of overall correctly classified elements

$$Accuracy\ (\%) = \frac{TP + TN}{TP + FN + FP + TN} \times 100$$

- *Sensitivity*: proportion of actual positives correctly identified

$$Sensitivity\ (\%) = \frac{TP}{TP + FN} \times 100$$

- *Specificity*: proportion of actual negatives correctly identified

$$Specificity\ (\%) = \frac{TN}{FP + TN} \times 100$$

- *TSS* (True Skill Statistics): defined as

$$TSS = Sensitivity + Specificity - 1$$

Chapter 4.

Results and Discussion

4.1. Physico-chemical parameters

The results of the analysis of the physical-chemical parameters are shown. A statistical analysis was conducted on the collected data; Shapiro-Wilk normality test was applied to study the normality of the series while the ANOVA test was used in order to identify significant differences between areas or periods. Moreover, 2019 datasets were compared with 2018 ones, in order to underline possible differences of similarities that could help in understanding the water management of the area.

In Table 5 median values of dissolved oxygen concentrations, oxygen saturation in water, pH and electrical conductivity (year 2019) are listed:

Table 5. Median values of the physical-chemical parameters (2019)

Season	Area	D.O. [mg/l]	Oxygen Saturation [%]	pH	Electrical Conductivity [mS/cm]
Winter	North Tancats	9.73	92.1	8.44	2.18
	South Tancats	10.48	103.5	8.17	1.72
Summer	North Tancats	4.11	50.44	7.48	2.38
	Pinedo	4.26	51.50	7.91	2.09
	South Tancats	5.46	69.10	7.63	1.82
	Jùcar	5.52	72.11	7.89	1.42

Significant differences appear between the two periods, with lower values of dissolved oxygen, oxygen saturation and pH in summer (D.O. ANOVA*: F=21.78, d.f.=1;52, p-value=2.2E-5*; pH ANOVA: *F=68.87, d.f.=1;52, p-value=4.2E-11*).

These results are quite understandable: due to the higher temperature of summer, the biological processes become faster in this season, and this provokes the decrease of the oxygen concentration in water, the increase of carbon dioxide concentration and, consequently, the decrease of pH values too. Oxygen concentrations are very low in summer (about 4 mg/l), and this shows the deep alteration of the environment.

Generally, a non-altered water body is characterized by electrical conductivity values of about 1000 μS/cm; in this case, median values are generally above the threshold value, and describe an altered ecosystem. However, electrical conductivity is not significantly influenced nor by seasons and areas.

North Tancats seem to be the more affected area around the Albufera lagoon; in fact, in this area it is possible to notice the worst values for all the parameters, both in winter and summer. This area is directly flooded by the lagoon; consequently, but North Tancats are also influenced by water coming from Pinedo wastewater treatment plant; this contribute does not seem to bring a positive influence over the water quality of this area.

Comparing the data of 2019 with the ones of 2018 (Table 6), in winter no peculiar differences appear between the two years, except for the electrical conductivity values, which result lower in 2019 than in 2018 (North Tancats, ANOVA*: F=91, d.f.=1;17, p-value=3.1E-8;* South Tancats, ANOVA*: F= 6.24, d.f.=1;19, p-value=0.02)*.

Table 6. In situ physical parameters: comparison between winter seasons of 2018 and 2019

Area	Year	Dissolved Oxygen [mg/l]	pH	Electrical conductivity [mS/cm]
North Tancats	Winter 2018	7.62	7.77	3.72
	Winter 2019	9.73	8.45	2.18
South Tancats	Winter 2018	12.72	8.21	2.19
	Winter 2019	10.78	8.18	1.70

This great difference is caused by an absence of rains during the months of October and November 2017. In the Albufera system, the lake level is traditionally measured on a fixed scale in the *Pujol gola,* whose "0" level is 18 cm above sea level. During the "*Perellonada*", if water level of the lake is lower than 0, there is the serious risk that seawater may enter inside the lake and increase its salinity.

Because of the absence of rains during October and November 2017, after the *Perellonada* the water level of the Albufera lake was under the 0 level: seawater entered inside the lake, even if for a short period. This increased the electrical conductivity of water and, consequently, higher values were measured in winter 2018 (Figure 23).

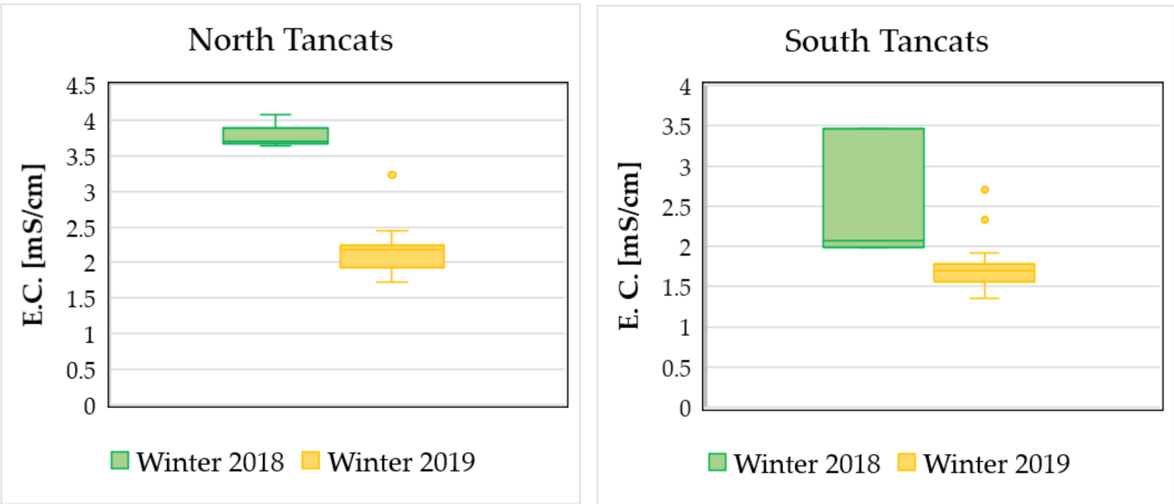

Figure 23. Electrical conductivity: comparison between winter 2018 and winter 2019

Even if no significant differences appeared from the ANOVA test, in both summer periods (2018 and 2019), the Northern areas (Tancats and Pinedo) showed worst values (lower concentration of dissolved oxygen, lower pH values and higher values of electrical conductivity). Table 7 shows median values of the two summer periods.

Table 7. Comparison between physical-chemical parameters between summer 2018 and summer 2019. Median values are shown

Area	*Year*	*Dissolved Oxygen [mg/l]*	*pH*	*Electrical conductivity [mS/cm]*
North Tancats	Summer 2018	8.10	7.75	2.64
	Summer 2019	4.11	7.49	2.38
Pinedo	Summer 2018	5.46	7.36	2.10
	Summer 2019	4.26	7.92	2.04
South Tancats	Summer 2018	8.71	7.82	1.59
	Summer 2019	5.46	7.61	1.83
Jùcar	Summer 2018	10.09	8.05	1.15
	Summer 2019	5.52	7.90	1.43

Significant differences appear between the oxygen concentration and saturation in the two years, except from the Pinedo area; in summer 2019, lower concentrations of dissolved oxygen are shown (Figure 24).

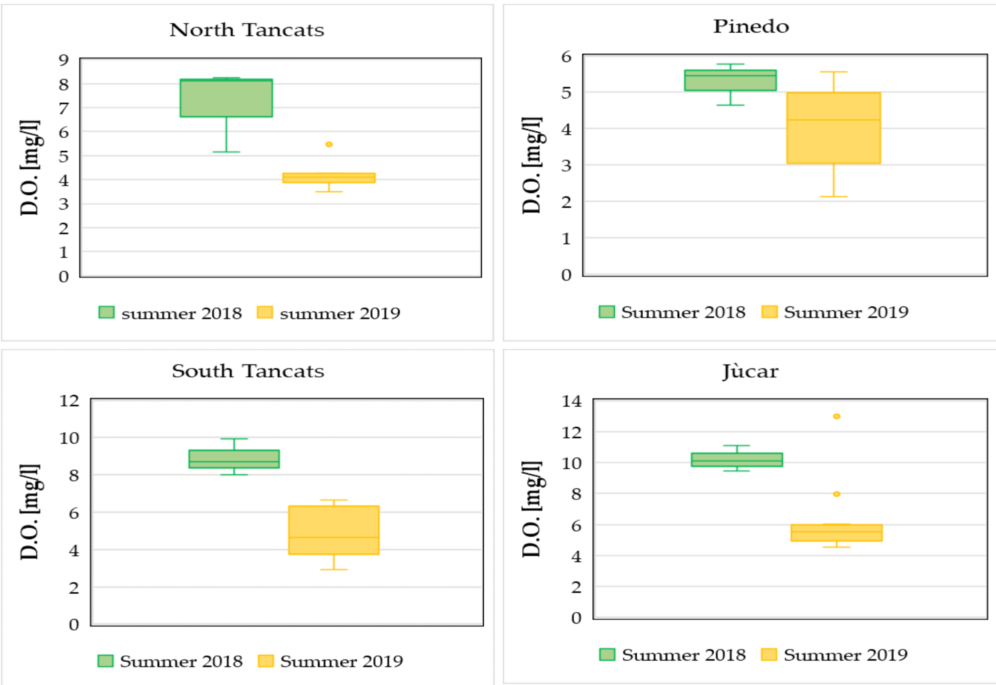

Figure 24. Dissolved oxygen concentration in summer 2018 vs summer 2019

Moreover, concentrations of nitrites, nitrates and phosphates were measured [mg/l]; these substances are important and fundamental while studying the trend of phenomena such as eutrophication. In Table 8 results for the aforementioned analysis are listed (median values of the concentrations):

Table 8. Median values of nitrites, nitrates and phosphates concentration (2019)

Season	Area	NO_2 [mg/l]	NO_3 [mg/l]	PO_4 [mg/l]
Winter	North Tancat	0.03	0.9	0.2
	South Tancat	0.03	0.9	0.2
Summer	North Tancat	0.01	4.22	0.9
	Pinedo	0.07	2.17	0.8
	South Tancat	0.15	1.68	0.95
	Jucar	0.14	2.72	1.2

Clear differences appear between the two seasons, winter and summer (NO_3, ANOVA: *F=4.19, d.f.=1;52, p-value=0.045*; PO_4, ANOVA: *F=25.52, d.f.=1;52, p-value=5.74E-06*).

In winter, both nitrates and phosphates mean concentrations are under the detection limit of the instruments (0.9 mg/l for nitrates and 0.2 mg/l for phosphates). No differences occur between North and South Tancats. Moreover, median concentrations are lower than the threshold concentrations for good quality water; in fact, in this period, fields are flooded only for the maintenance of the biodiversity of the lagoon, and no agricultural practices occur.

On the contrary, in summer, higher concentrations of all the parameters have been measured. These values are surely the results of the agriculture activities, which include the use of fertilizers containing nitrates and phosphates (Figure 25).

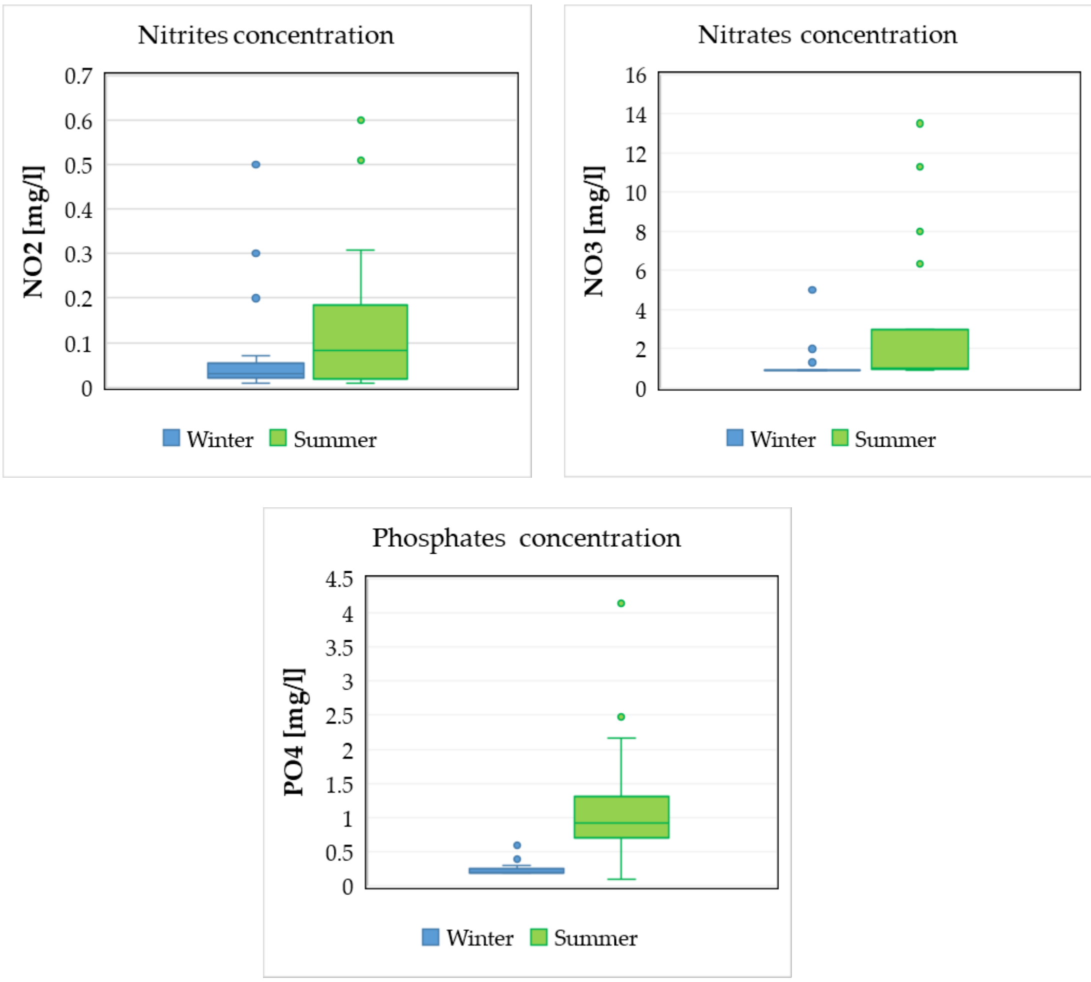

Figure 25. Nutrients concentration: comparison between winter and summer 2019

In summer, concentrations of nitrates exceed the threshold value of 1 mg/l and concentrations of nitrites exceed 0.03 mg/l, showing the critical situation of the Albufera lagoon. In particular, nitrates concentration is almost the double of the threshold limit. Phosphates concentrations exceed the limit imposed by the legislation of 0.4 mg/l.

Moreover, in summer, nutrients values oscillate among the different areas, but no significant differences appear (Figure 26 and Figure 27). Consequently, it is not possible to underline differences among the different areas due to the different sources of water used for rice field flooding operations. The influence of the wastewater treatment plant of Pinedo and of the Jucar river does not contribute to improve the lagoon water quality.

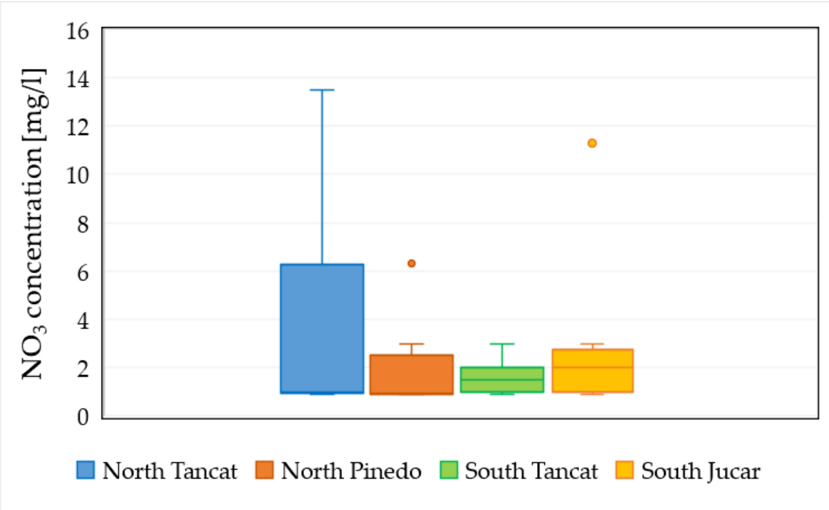

Figure 26.Nitrate concentration values in summer 2019: comparison among the 4 sampling areas

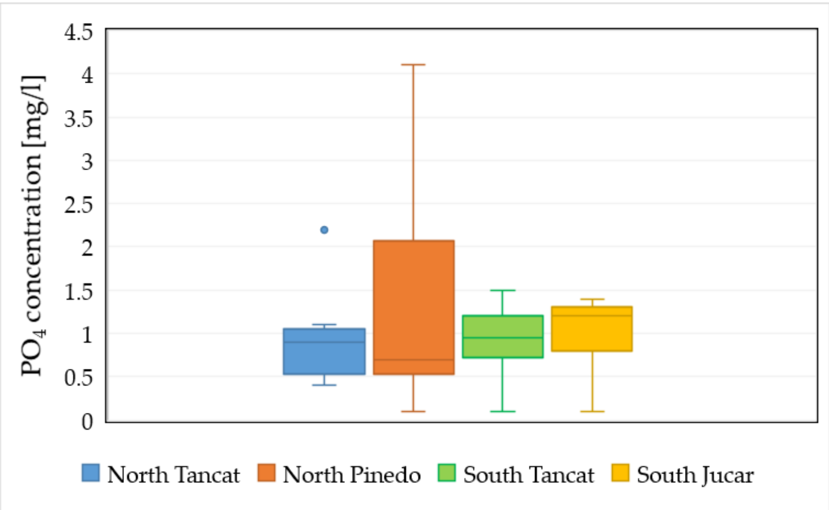

Figure 27. Phosphates concentration values in summer 2019: comparison among the 4 sampling areas

As shown in Table 9 and Table 10, no differences occur between 2018 and 2019, both winter and summer. Median values slightly vary between the zone, but with no significant deviations.

Table 9. Nutrient concentrations: comparisons between winter seasons 2018 and 2019.

Area	Year	NO_2 [mg/l]	NO_3 [mg/l]	PO_4 [mg/l]
North Tancats	Winter 2018	0.01	0.9	0.73
	Winter 2019	0.03	0.9	0.20
South Tancats	Winter 2018	0.02	0.9	0.93
	Winter 2019	0.03	0.9	0.20

Table 10. Nutrients concentrations: comparison between summer 2018 and 2019

Area	Year	NO_2 [mg/l]	NO_3 [mg/l]	PO_4 [mg/l]
North Tancats	Summer 2018	0.05	1.82	0.70
	Summer 2019	0.01	4.22	0.90
Pinedo	Summer 2018	0.26	2.00	0.80
	Summer 2019	0.07	2.17	0.80
South Tancats	Summer 2018	0.05	1.17	0.58
	Summer 2019	0.15	1.68	0.95
Jùcar	Summer 2018	0.09	1.8	0.35
	Summer 2019	0.14	2.72	1.20

4.2 Macroinvertebrates sampling

During the winter samplings, a total of 22 samples were collected on 19/01/2019. 10 samples were collected in North Tancats area and 12 in South Tancats area. Samples were analysed during the following week in the laboratory of the Universidad Politecnica de Valencia: they were separated and classified.

Macroinvertebrates collected in winter season were used in order to evaluate Shannon-Wiener and Simpson diversity indexes and IMN and QAELS quality indexes for each sampled parcel.

In order to compare the two zones (North and South Tancats), the values have been unified, according to the belonging area, and median values have been used. In fact, it is supposed that parcels belonging to the same area are flooded by water of the same origin.

The indexes were than compared with the ones evaluated in winter 2018, in order to underline possible differences.

4.2.1. Benthic macroinvertebrates identification

During the winter samplings, a total of 80.966 macroinvertebrate individuals were counted and identified in the 22 analyzed parcels.

Table 11. Macroinvertebrates families sampled in winter 2019 (N=North; S=South)

Phylum	***Class***	***Order***	***Family***	***N***	***S***
Annelida	Clitellata	Oligochaeta		X	
Arthropoda	Branchiopoda	Cladocera	Daphniidae	X	X
Arthropoda	Insecta	Diptera	Chironomidae	X	X
Arthropoda	Insecta	Diptera		X	X
Arthropoda	Insecta	Ephemeroptera	Baetidae	X	X
Arthropoda	Insecta	Rhynchota	Corixidae	X	X
Arthropoda	Insecta	Diptera	Limoniidae		X
Arthropoda	Insecta	Odonata	Zygoptera		X
Arthropoda	Insecta	Diptera	Ceratopogonidae		X
Arthropoda	Insecta	Odonata	Aeshnidae		X
Arthropoda	Insecta	Coleoptera	Dytiscidae		X
Arthropoda	Insecta	Coleoptera	Hydrophilidae		X
Arthropoda	Insecta	Odonata	Cordulegastridae		X
Arthropoda	Malacostraca	Decapoda	Cambaridae	X	X
Arthropoda	Copepoda			X	X
Arthropoda	Ostracoda			X	X
Chordata	Actinopterygii	Cyprinodontiformes	Poeciliidae	X	
Mollusca	Gastropoda	Planorboidea	Physidae	X	X
Mollusca	Gastropoda	Planorboidea	Planorbidae	X	X

They were classified in 4 phylum, 8 classes, 18 orders and 16 families. Some species of macroinvertebrates are classified only until class (Copepoda, Ostracoda) because they were too abundant and the classification until the family level would have required a huge amount of time and a deeper level of knowledge.

Even though different families have been recognized, only few of them were particularly numerous. The most numerous were: Daphnidae (51,89 %), Copepoda (32,10 %) and Ostracoda (14,28 %). All the other families are under the 1% (Physidae 0,95 %; Chironomidae 0,33 %; Ceratopogonidae 0,19 %; Planorbidae 0,11%).

These species are all important and significant from an ecological point of view.

We can notice that Daphnidae (Cladocera), Copepoda and Ostracoda are the most abundant species in all the parcels we analysed. They all belong to the crustaceans' subphylum and they are fundamental for QAELS index calculation.

In general, in ecology it is known that crustaceans, among all the different taxa, are one of the least tolerant groups to different types of pollution (Ruiz, et al., 2013). The high sensitivity of this group to low concentrations of dissolved oxygen generates, in case of eutrophication, an impoverishment of its populations, due to the hypoxia that is generated in the aquatic environment. Obviously, the degree of tolerance depends on the different families.

Some of the main characteristics of Copepoda, Ostracoda and Daphnidae are reported below:

- *Daphnidae*: Cladocera order. Cladocera are important biological indicators for a wide range of environmental variables (Zawiska, Zawiska, & Correa-Metrio, 2016). They are resistant to high values of electrical conductivity but can live in freshwater too; they are generally resistant and versatile to different types of environments.

- *Copepoda* is considered a good bioindicator of water quality by some authors, especially for problems connected to eutrophication. In general, this class is very sensitive to low concentration of dissolved oxygen in water, which can explain Copepoda sensitivity to eutrophication (Ruso, et al., 2017). Copepoda live as plankton or at the bottom of the water and their feeding habits are very variable depending on the species: filters, predators or parasites of fish (sometimes mollusks).
- *Ostracoda* has been considered a good indicator against the processes related to eutrophication. Its wide distribution in aquatic environments, the ease of its collection, its high density and its response to pollution, make them a useful bioindicator (Ruiz, et al., 2013). The processes of eutrophication affect Ostracoda populations and so does organic polluted water.

Then, some information about the other abundant species found in the samplings are listed.

Physidae: this family has been reported in several studies as a good indicator of contamination, due to some of its characteristics (longevity, sedentary lifestyle, resistance to changes in the characteristics of water bodies) (García, 2003). They live essentially in freshwater, and can withstand a very wide temperature range. Some authors claim that this family, among the mollusk reign, is the one that most resists contamination phenomena. Physidae macroinvertebrates are used, in ecology, as a biological indicator for contamination phenomena due to the increase in nutrients.

Chironomidae: they are a family of dipterans of worldwide distribution. They are strongly correlated with organic pollution phenomena. The increase of organic matter in the water produces a proliferation of the microorganisms responsible for its decomposition, which a reduction in the concentration of dissolved oxygen in water and an increase in the concentration of inorganic nutrients, such as ammonium and phosphate (Paggi, 1999). Most invertebrates are sensitive to this reduction of dissolved oxygen, so that they reduce their abundance, or even disappear. On the contrary, other groups, such as Chironomidae, tolerate well the low concentrations of dissolved oxygen; in this way a high abundance of these groups with respect to natural conditions or reference is an indicator of this type of contamination (Alonso & Camargo, 2005).

Ceratopogonidae: the insects of the order of the Diptera, they have small size (1-4 mm) and are distributed worldwide, with exception of the polar zones. Their presence is often connected with Chironomidae's one. They are indicated as organisms capable of resisting at organic pollution. They are commonly used as bioindicators in the evaluation of water quality (Cazorla-Perfetti, 2014)

Planorbidae: this is a family of air-breathing, freshwater snails, belonging to the class of Gastropoda molluscs. They usually inhabit clear freshwater either stagnant or slow running and feed on algae and fine organic deposits (Waikagul & Thaenkham, 2014).

Between the two studied zones, no significant differences in the composition of the macroinvertebrates community were identified.

In North Tancats, the 46.566 macroinvertebrates collected are classified into 4 phylum, 8 classes, 10 orders and 11 families, while in South Tancats, 33.400 macroinvertebrates have been identified and classified in 2 phylum, 6 classes, 11 orders and 15 families.

In the northern area, a major abundance of macroinvertebrates was found, although the number of sampled parcels was lower (10 instead of 12). On the other hand, in South Tancats, despite the lower number of individuals sampled, the macroinvertebrates were more diversified, with individuals belonging to a greater number of families.

The most abundant species, both north and south of the lagoon, turned out to be the same. In order they were so distributed:

- North Tancats: Daphnidae (46.6%), Copepoda (38.5%) and Podocopida (13.9%). Chironomidae (0.39%) and Physidae (0.54%) are found in minor quantity but their presence is important from an ecological point of view.

- South Tancats: Daphnidae (59.5%), Copepoda (23%); Podocopida (15%); Physidae (1.5%); Ceratopongidae (0.46%), Planorbidae (0.26%), Chironomidae (0.25%).

In North Tancats a major abundance of Chironomidae was found (184 units vs 83 units in South Tancats). This could show a higher contamination in the northern part of the lagoon. The influence of water coming from the Pinedo wastewater treatment plant can be the cause of this type of contamination.

In South Tancats, 19 units belonging to the Baetidae family (Ephemeroptera order) were present; these families are generally indicators of good quality water.

4.2.2. Evaluation of the ecological status

Shannon and Simpson diversity indexes were evaluated, both for North and South Tancats. The results are shown in the following table:

Table 12. Simpson D and Shannon-Wiener S Index: winter 2019 (median values)

Index	*Area*	*Median Value*
Simpson	North Tancats	0.48
	South Tancats	0.45
Shannon	North Tancats	1.18
	South Tancats	1.20

Both the indexes were very low, both in North and South Tancats. This shows a low biodiversity in the macroinvertebrates community of the studied area and, consequently, a significantly stressed environment.

It is not possible to underline any spatial difference between North and South Tancats.

In 2018, the same trend was observed in North Tancats, and no significant difference appeared between the two years (Table 13).

Table 13. Simpson and Shannon-Wiener indexes in winter 2018 and 2019

Area	Year	Simpson Index	Shannon Index
North Tancats	Winter 2018	0.50	1.19
	Winter 2019	0.48	1.18
South Tancats	Winter 2018	0.59	1.51
	Winter 2019	0.45	1.20

On the contrary, in South Tancats higher values of the two diversity indexes were observed in 2018 (Figure 28), showing a higher biodiversity than in 2019 (Simpson Index, *ANOVA: F=6.78, g.d.l.=1;12, p-value=0.017; Shannon Index, ANOVA: F=4.37, g.d.l.=1;19, p-value=0.05*).

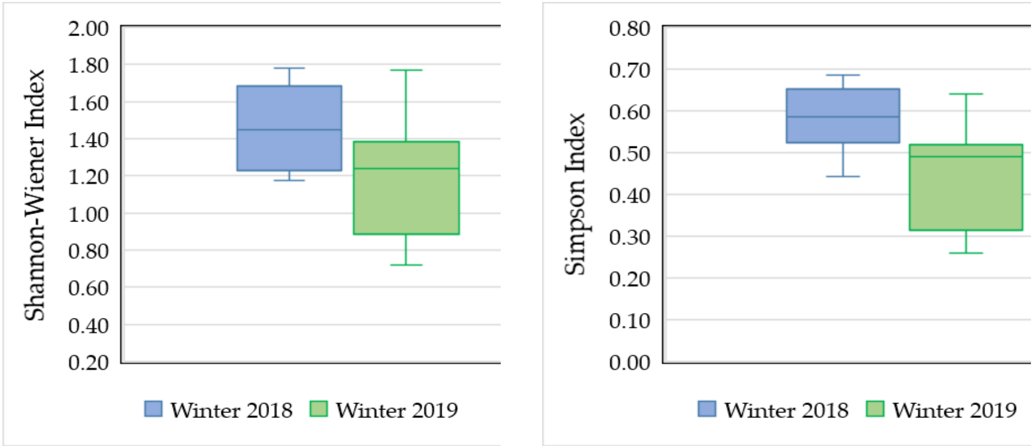

Figure 28. Shannon-Wiener and Simpson Indexes: comparison between South Tancats values in winter 2018 and 2019

So, in 2018, a clear difference was shown between North and South Tancats (Shannon-Wiener, *ANOVA: F=10, d.f.=1,16, p-value=0,0506;* Simpson Index, *ANOVA: F=8,127, d.f.=1,16, p-value=0,0116)*, resulting in a major biodiversity and water quality in the southern area of the lagoon. But the same can't be said about winter 2019, where this difference disappeared and the measured values resulted more homogenised in all the studied area. This can be caused by the higher number of sampling points.

For water quality assessment in winter period, QAELS index and IMN index were evaluated both for North and South Tancats (Table 14).

Table 14. QAELS and IMN indexes: comparison between winter 2018 and winter 2019

Area	Year	QAELS	IMN
North Tancats	Winter 2018	2.6	24
	Winter 2019	2.35	26
South Tancats	Winter 2018	2.15	27
	Winter 2019	2.54	27

Both for QAELS and IMN indexes, in 2019 no statistical differences appeared between the two sampled areas (QAELS, ANOVA: *F=1.6177, F crit= 4.4138, p value= 0.7711*; IMN, ANOVA: *F=0.4084, Fcrit=4.4138, p-value=0.5308*).

Both in North and South areas, according to the definition of QAELS, water quality is classified as "poor". These values showed no relation with the proximity to the lagoon.

Because of the great abundance of Ostracoda, Copepoda and Cladocera, ACCO Index resulted higher than QAELS in the majority of the sampling points; in fact, these three species of macroinvertebrates are generally the most abundant in the Albufera environment. ACCO values were modulated by RIC, which depend on the number of species of insects and crustaceans. RIC measures the taxonomic richness. Consequently, the low values of QAELS depend on the poor taxonomic richness: in fact, good quality waters are generally characterized by a large number of different organisms. This shows not only the poor level of water quality of the Albufera, but also the poor richness of biodiversity.

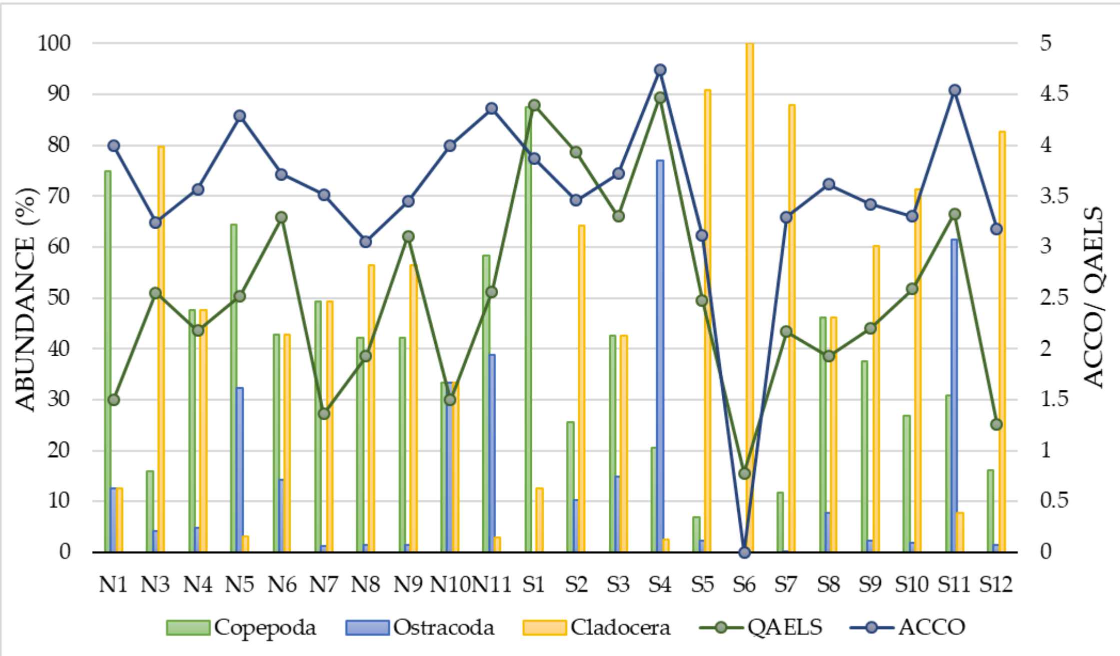

Figure 29. ACCO and QAELS variation according to the abundance of Ostracoda, Copepoda and Cladocera

The values of the IMN indicate that there is a low food web diversity level: according to the definition of this index, the higher is the homogeneity of the macroinvertebrates community, the lower is the quality of the environment. In this case, the low values of IMN index show that the Albufera is a stressed and impacted ecosystem.

With respect to the values of QAELS and IMN measured during the winter season 2018, there is no significant difference (Figure 30 and Figure 31).

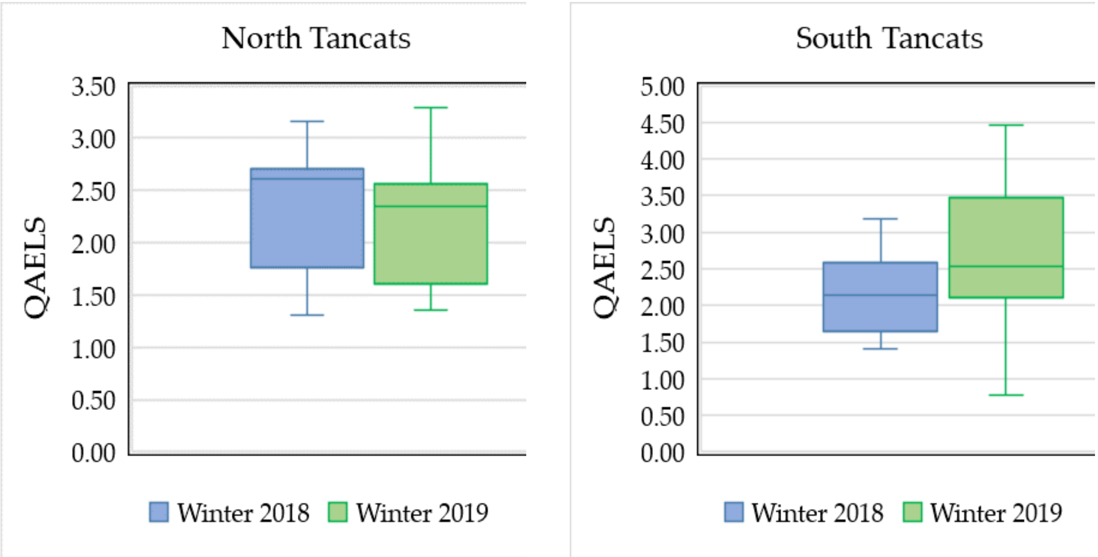

Figure 30. QAELS Index: comparison between winter 2018 and winter 2019

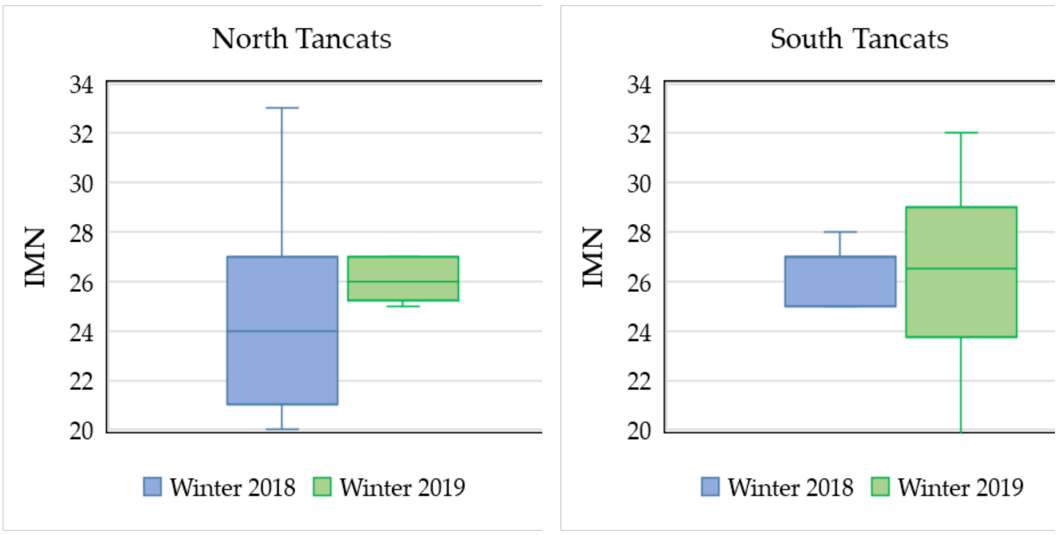

Figure 31. IMN Index: comparison between winter 2018 and winter 2019

4.3. Correlation between physical-chemical variables and abundance of Ostracoda and Copepoda

Because of their ecological importance (Paragraph 4.2.1.), Copepoda and Ostracoda classes were studied in relationship with the chemical-physical parameters (Table 4). Their abundances (in logarithmic scale, in order to obtain the normality of the series) were correlated to the variables through the Spearman's rank correlation.

The abundance of Ostracoda showed a positive correlation with pH values ($\rho_{Spearman}$= 0.61) and a negative one with temperature ($\rho_{Spearman}$= -0.41), showing that with pH values lower than 8, the abundance of the macroinvertebrates decreases.

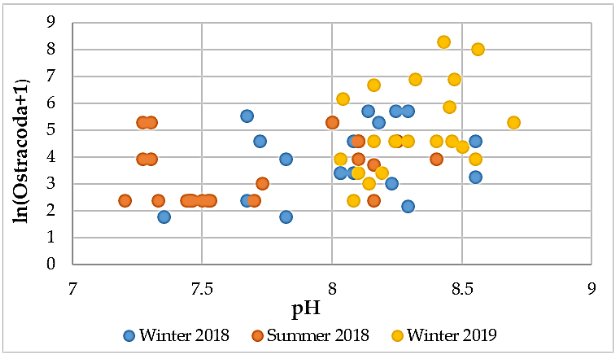

Figure 32. Scatter plot of the abundance of Ostracoda and pH values

Dissolved oxygen concentration seemed to have a moderate positive effect on the abundance of Ostracoda ($\rho_{Spearman}$= 0.30).

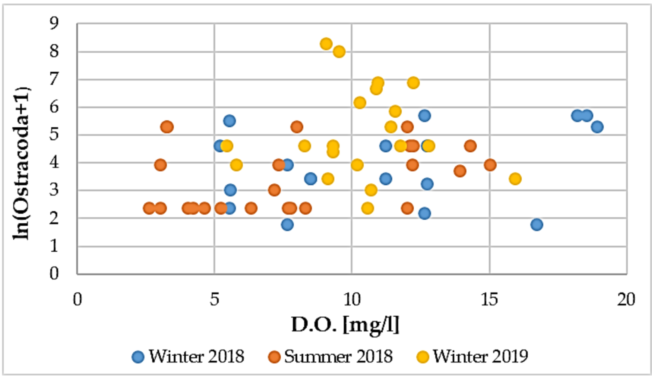

Figure 33. Scatter plot of Ostracoda abundance and dissolved oxygen concentration

Electrical conductivity does not show correlation ($\rho_{Spearman}$= -0.18).

Phosphates concentration strongly affects the abundance of the aforementioned macroinvertebrates ($\rho_{Spearman}$=-0,61); on the contrary, nitrites and nitrates one does not seem to have any particular effect (nitrates concentration, $\rho_{Spearman}$= -0.17; nitrites concentration $\rho_{Spearman}$= -0.16).

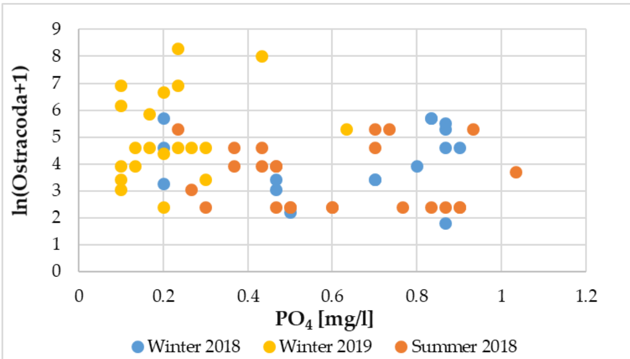

Figure 34. Phosphates concentration vs Ostracoda abundance

<u>Copepoda</u> class showed some correlation with the variables, too. The highest correlations were shown, as in the previous case, with pH ($\rho_{Spearman}$= 0.55), temperature ($\rho_{Spearman}$-= *-0.65)* and phosphates concentration ($\rho_{Spearman}$=-0,31).

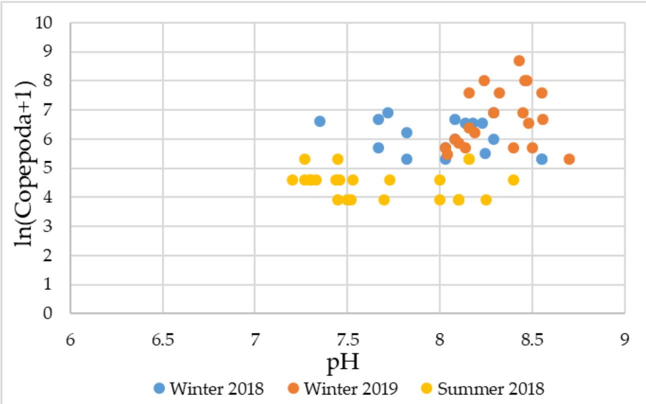

Figure 35. Scatter plot of Copepoda abundance and pH

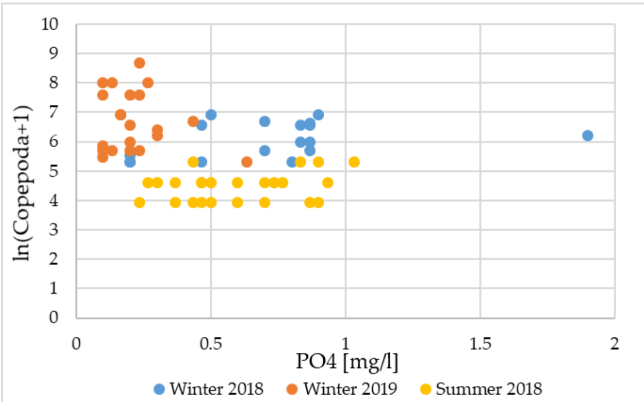

Figure 36. Scatter plot of Copepoda abundance and phosphates concentration

The other variables showed moderate-low correlation with the abundance of Copepoda (i.e., dissolved oxygen $\rho_{Spearman}$= 0.28, electrical conductivity $\rho_{Spearman}$= 0.26, nitrates concentration $\rho_{Spearman}$= -0.25) and no correlation was found with nitrites concentration ($\rho_{Spearman}$= -0.07).

All these characteristics show that Ostracoda and Copepoda may be good indicators of water quality in the lagoon of La Albufera, especially in the study of the eutrophication phenomena. In fact, with the increase of temperature and nutrients concentration (especially phosphates) during the summer season, and the intense decrease of oxygen content and pH, the abundance of these classes tend to decrease, showing the worsening of the ecological status of the lagoon

4.4. Classification models results

RF classification models were built in order to predict presence of these classes in relationships with the chemical-physical variables in Table 4 (an example of the script in R is attached in Annex II).

4.4.1. Copepoda classification model

Considered the wide range of abundance of Copepoda, the cut-off value between the two classes (presence/absence) was set equal to 100; so, a total of 38 elements were identified as present and 20 as absent (Figure 37).

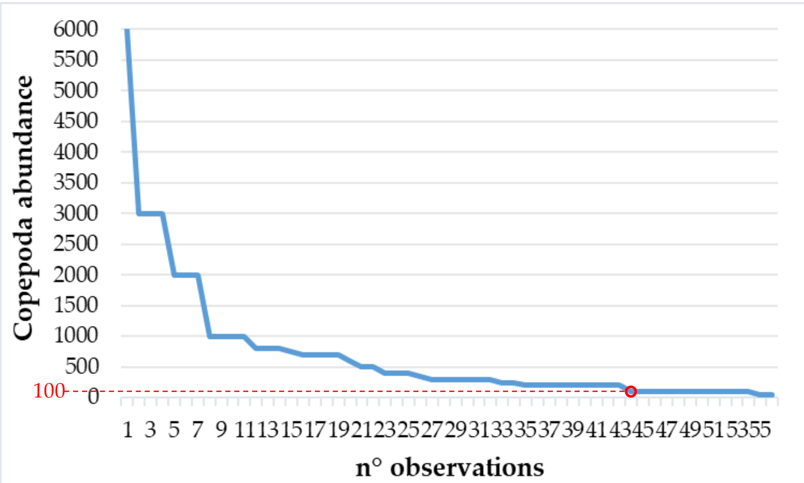

Figure 37. Copepoda abundance

The number of trees was decided according to the OOB error; in fact, it was possible to observe a stabilization of the OOB error (Figure 38). The parameter *m* (number of variables to split at each node) was set equal to 2.

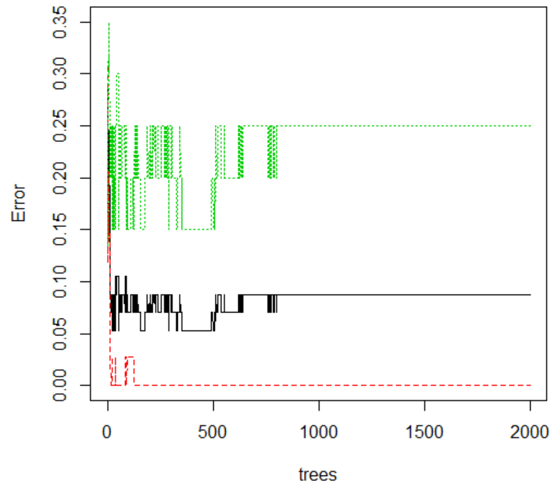

Figure 38. OOB error vs n° of trees. Black trend represents the absolute error, red one represents the error on the class absence, while green one on the class presence.

With regards to the model performances, the results showed a model which was pretty accurate (Overall Accuracy=92%; sensitivity= 100%; specificity= 75%; TSS=0.75).

The confusion matrix in Figure 39 shows the results of the classification.

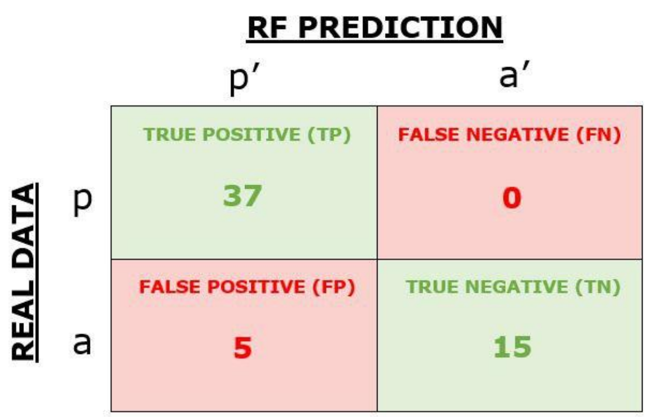

Figure 39. Confusion matrix of Copepoda presence/absence classification model

The importance of the 6 variables was evaluated according to the MDA parameter (Figure 40).

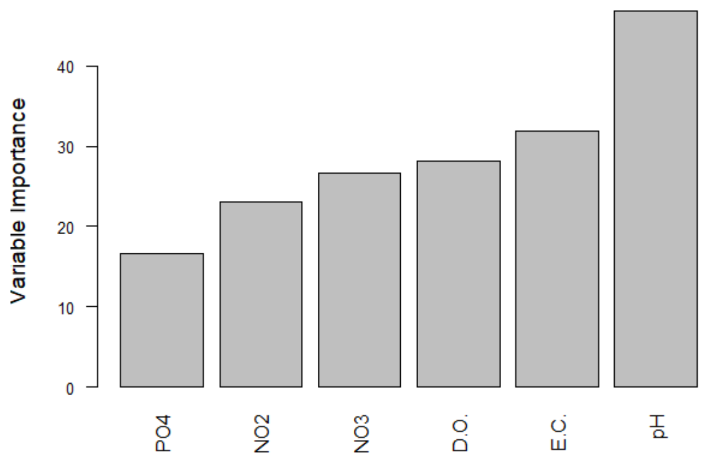

Figure 40. Variable relative importance over the prediction of Copepoda presence

pH, electrical conductivity and dissolved oxygen were the most important variables in the model, while nitrates, nitrites and phosphates concentrations seemed to play a less important role in the prediction of Copepoda presence.

Through the partial dependence plots (Figure 41), it is possible to see the marginal relationships between the probability of presence of Copepoda and each of the predictive variables, averaging out the effect of the other variables.

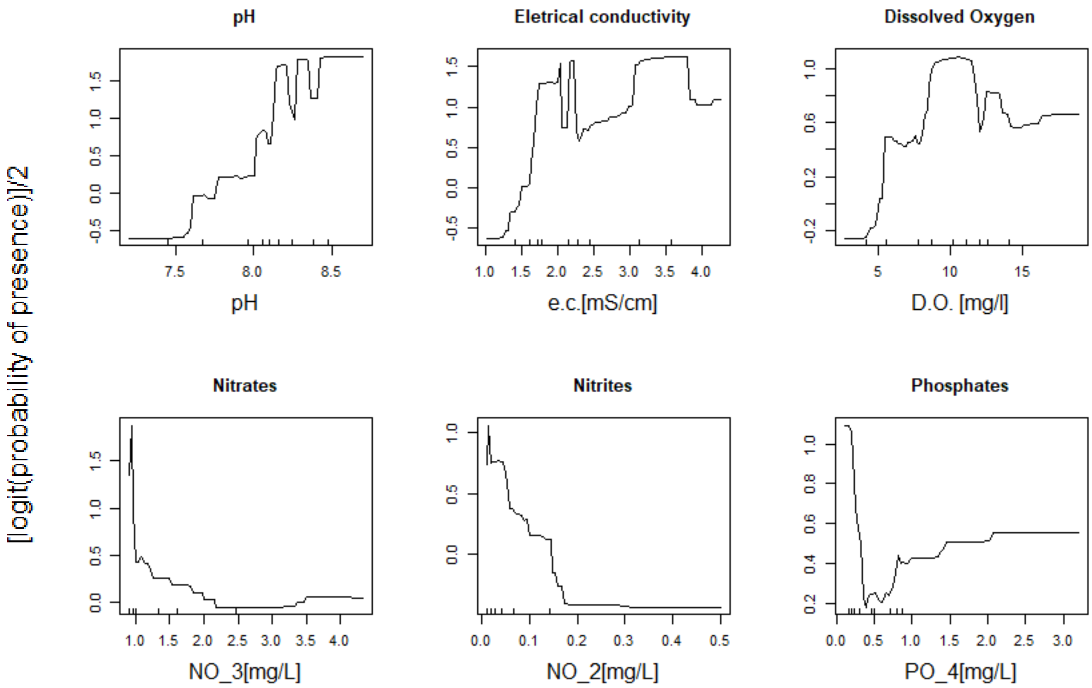

Figure 41. Partial dependence plots show the marginal effect of the selected variable on the predicted probability of presence of Copepoda

Considering Copepoda preference for the most important variables (pH, dissolved oxygen), a general agreement among existing studies is found; in fact, Copepoda is generally very sensitive to oxygen depletion; when dissolved oxygen concentration are lower than 5 mg/l, the probability of founding this type of macroinvertebrates is really reduced. Moreover, pH values really influence Copepoda presence. They seem to prefer slightly basic condition in water; this could be connected to the sensitivity of this class to eutrophication problems. In fact, when the respiration of the aquatic plants is faster, the production and the concentration of carbon dioxide in water grows; this led to a decrease of pH values.

No information in literature was found about the sensitivity of this class to electrical conductivity but, from this study, it seems that Copepoda prefer saltier water (higher values of electrical conductivity).

The effect of nitrites and nitrates concentration on the presence of Copepoda is quite clear and explicative: in both cases, it is possible to observe a decrease when nutrients concentration become higher; this reduction is sharper when NO3>1 mg/l, while it is slower with the increase of NO2 concentration.

In summer, because of the intensifying of the agricultural practices, fertilizers are used in copious quantity, causing the worsening of chemical-physical conditions of the whole lagoon. In fact, even if temperature is not a variable depending on eutrophication itself, it is during the summer season that the eutrophication phenomena occurs. With the increase of temperature and nutrients concentration in water, there is an intense decrease of oxygen content and pH. This negative trend of the ecological status is reflected also in the quantity of Copepoda, confirming that this class may be a good indicator of water quality in the lagoon of the Albufera.

4.4.2. Ostracoda classification model

In Ostracoda classification model, the cut-off value between the two classes presence/absence was set equal to 50. In this case, 28 elements were identified as present (47% of the total dataset), and 31 as absent (54% of the total dataset).

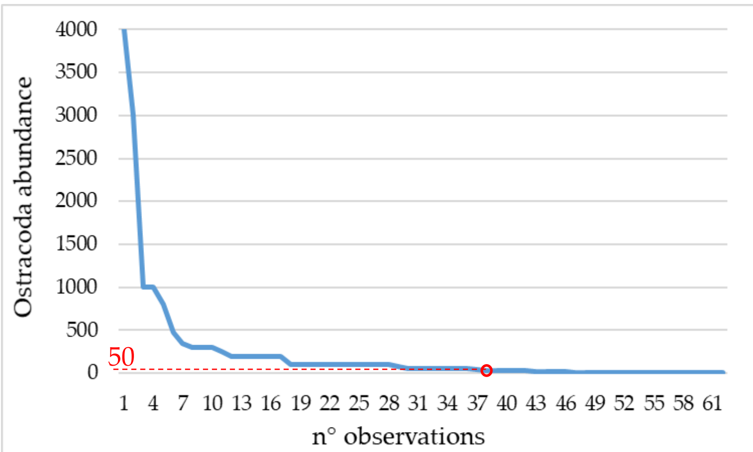

Figure 42. Ostracoda abundance vs n° of observations

The number of trees *ntree* was set equal to 2000 (Figure 43) and the m parameter equal to 2, as explained in the previous model.

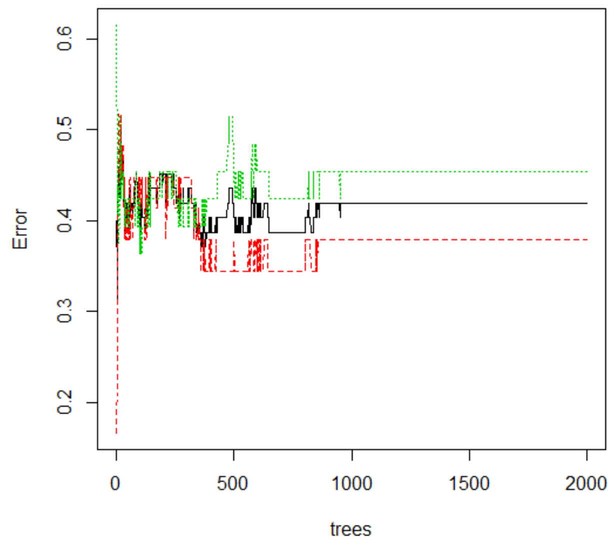

Figure 43. OOB error vs number of trees- Ostracoda

The performance of the model was lower than the Copepoda's one (Total Accuracy=0.63, Sensitivity=0.65, specificity=0.61, TSS=0,26). In Figure 44 the confusion matrix shows the results of the classification.

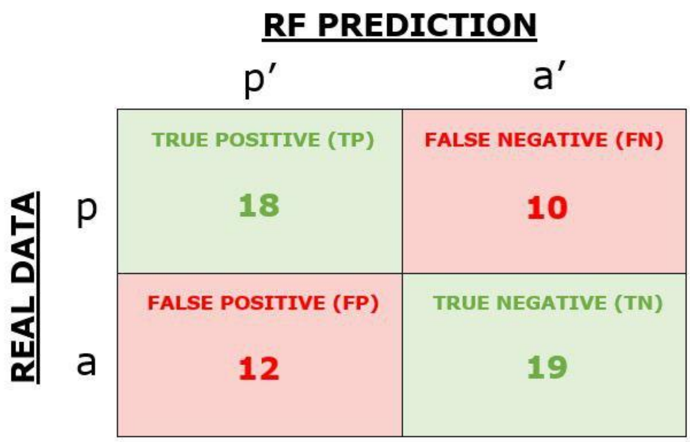

Figure 44. Confusion matrix of the classification model for Ostracoda presence

Mean decrease accuracy measured the importance of the six variable over the predictions of presence of Ostracoda. pH, NO2 and D.O. are the variables that have the greatest influence on the presence of Ostracoda in the Albufera lagoon, as it possible to see in Figure 45.

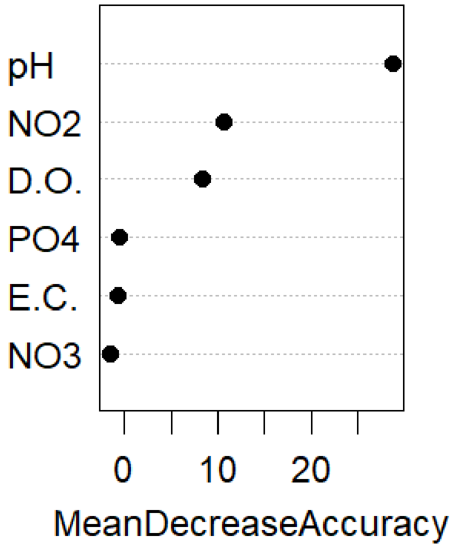

Figure 45. Variable importance for Ostracoda presence

PO4, e.c. and NO3 are pretty irrelevant on the prediction, contrary to what we expected based on what we found in scientific literature.

Partial dependence plots show the trend of the probability of presence in function of the 6 selected variables; explicative results are shown only in case of pH, NO2 and D.O. concentration. Also Ostracoda seem to prefer slightly basic water and are very sensitive to NO2 concentration. Their probability of presence is really reduced when dissolved oxygen concentration is lower than 7 mg/l.

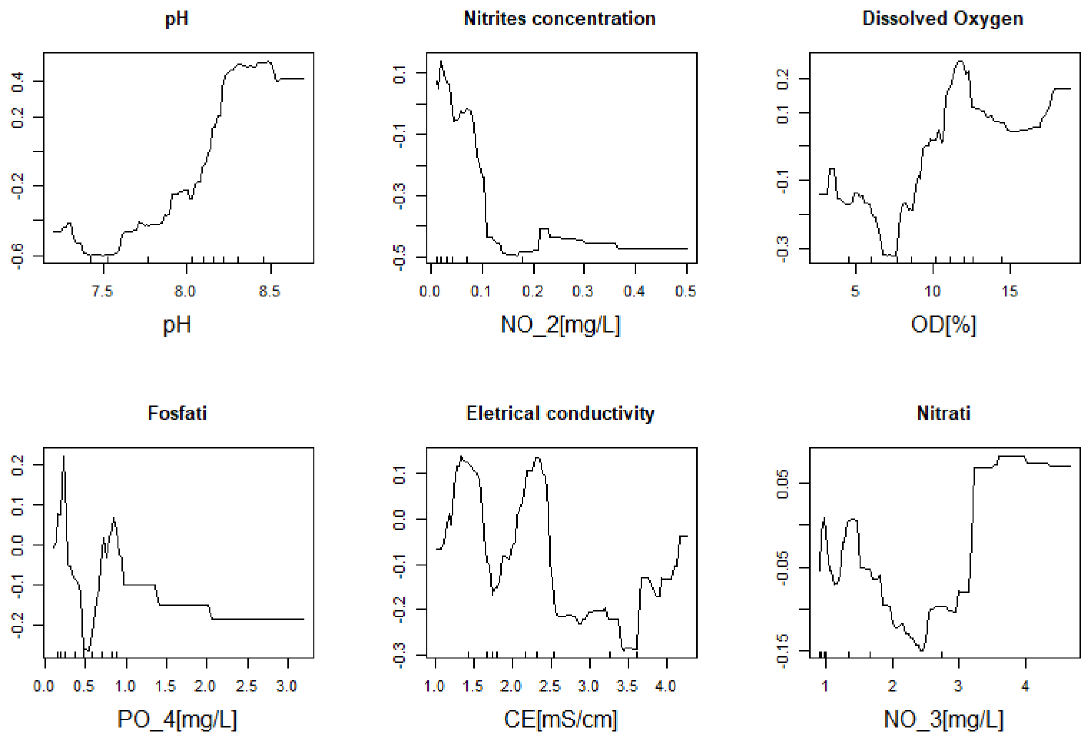

Figure 46. Partial dependence plots- Ostracoda presence classification model

In partial dependence plots of PO4, e.c. and NO3, it is not possible to see any significant trend.

In this case, the model was not as robust as in the previous case (Copepoda model); in fact, especially in PO4 and NO3 models, results did not correspond to what we expected. Presence of Ostracoda should be reduced with the increase of nutrients in water. Anyway, the response of Ostracoda to pH, NO2 and D.O. is similar to Copepoda's one. This shows a partial response of this class to the phenomena which occurs in the Albufera lagoon in summer season.

4.4.3. Comparison between the two models

Both the models produced good and quite robust results. In Table 15, the two models performance parameters are shown.

Table 15. Performance parameters of the two models

Performance parameters	***Copepoda***	***Ostracoda***
Accuracy	0.92	0.63
Sensitivity	1	0.65
Specificity	0.75	0.61
TSS	0.75	0.26

The most importance variables in the two models was pH (Table 16), while, for the other variables, there are not great similarities.

Table 16. Variable importance (MDA) for the two models

Predictive Variables	***Copepoda***	***Ostracoda***
D.O.	28.15	8.30
E.C.	31.93	-0.68
NO2	23.13	10.58
NO3	26.68	-1.51
pH	46.82	28.74
PO4	16.66	-0.61

Chapter 5.

Discussion and Conclusions

The objective of this thesis was the spatio-temporal analysis of the ecological indicators in the Albufera lagoon of Valencia; for this reasons, physico-chemical parameters and macroinvertebrates communities were sampled both in winter and summer season of 2019. They were then compared with the same parameters and variables sampled in winter and summer 2018.

Chemical-physical parameters were sampled and analysed both in winter and in summer 2019. Remarkable differences were observed between the two seasons; in summer, a critical scenario was observed, all around the lagoon, with oxygen concentration between 4 and 5 mg/l, and nutrients concentrations higher than the quality threshold values (D.O. ANOVA*: F=21.78, d.f.=1;52, p-value=2.2E-5*; pH ANOVA: *F=68.87, d.f.=1;52, p-value=4.2E-11)*. This trend was also shown by the analysis of nutrients concentration in water (*NO3, ANOVA: F=4.19, d.f.=1;52, p-value=0.045; PO4, ANOVA: F=25.52, d.f.=1;52, p-value=5.74E-06*). Electrical conductivity did not show significant differences nor between seasons (winter-summer) nor between areas, but the values overcame the threshold value of 1000 μS/cm all around the lagoon. The Northern areas, in particular way, were characterized by the worst valued for all the parameters, especially in summer season. The influence of Pinedo wastewater treatment plant over the Northern areas is not contributing positively to water quality. The presence of a major number of macroinvertebrates belonging to the Chironomidae family was found in North Tancats; further study could be done in order to verify the presence of organic pollution phenomena in the Norther areas (North Tancats and Pinedo).

The same trend was shown in 2018, even if some differences appeared, mainly caused by different meteorological conditions (winter 2018-winter 2019, electrical conductivity, North Tancats, ANOVA*: F=91, d.f.=1;17, p-value=3.1E-8;* South Tancats, ANOVA*: F= 6.24, d.f.=1;19, p-value=0.02*; *summer 2018-summer 2019, dissolved oxygen, North Tancats, ANOVA: F=14 d.f.=1;7, p-value=0.006; South Tancats, ANOVA: F=10.47 d.f.=1;11, p-value=0.007; Jucar, ANOVA: F=6.48 d.f.= 1;11, p-value=0.02)*

The analysis of the macroinvertebrates community and the biological indexes showed a heavily and stressed environment in almost all considered areas. It was not possible to underline significant differences between North and South Tancats area during the winter season. In South Tancats diversity indexes in winter 2019 were significantly lower than in winter 2018 (*Simpson Index, ANOVA: F=6.78, d.f.= 1;12, p-value=0.017; Shannon index, ANOVA: F=4.37, d.f.=1;19, p-*

value=0.05). This obviously led to a higher homogeneity of the results and the differences between the two areas were flattened.

Furthermore, Copepoda and Ostracoda classes were studied and presence/absence prediction models were developed with Random Forest. Copepoda showed a high sensitivity to pH and dissolved oxygen concentration, but also to nutrients, confirming that they can be used as bio indicators of eutrophication in the Albufera environment. The same results were found for Ostracoda as regards pH, dissolved oxygen and nitrites concentration, but not for the other nutrients (nitrates and phosphates). Both the models showed high performance parameters in predicting presence of these two classes (i.e., *overall accuracy*: Copepoda=0.92; Ostracoda=0.63).

Future activities, already planned in the near future, will focus on collecting more data during the summer period.

Classification at the family level for the different species are needed in order to better define the quality of water and the biodiversity indexes; special attention should be given to Copepoda, Ostracoda and Cladocera class in order to assess QAELS index with major accuracy. Moreover, a deeper attention could be given on the accounting in laboratory of these classes that seem to respond to water quality.

Acknowledgments

This thesis was developed in a multidisciplinary research group led by Professor Guillermo Palau, professor at the Polytechnic University of Valencia.

A sincere thanks to my supervisor, the professor Paolo Vezza, for his continuous availability and encouragement given to me in these months.

A special thank also to prof. Guillermo Salvador Palau for welcoming me into the team in the months spent in Valencia.

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Annex I

Tabla 1. Recopilación de los macroinvertebrados con sus afinidades tróficas (Tachet et al, 1987). MN = Modo de nutrición; Ad = Adultos; La = Larvas; H = Herbívoros; O = Omnívoros; D = Detritívoros; P = Predadores; Rm = Ramoneadores; Rs = Raspadores; F = Filtradores; L = Limnívoros; ChH = Chupadores herbívoros; ChP = Chupadores predadores.

Taxón	MN
PORIFERA, BRYOZOA	F
CNIDARIA, NEMERTINA: **Tetrastemmatidae, Hirudidae;** *Haemopis* sp. **Erpobdellidae**	P
PLATHYHELMINTHA, NEMATHELMINTHA: Nematodo, Gordiacea	ChP
Aelosomatidae, Naididae	O
Branchiobdellidae, Glossiphoniidae, Piscicolidae, Hirudidae; *Hirudo* sp.	ChP
Otros anélidos	L
Acroloxidae, Ancylidae, Bythinellidae, Ferrissiidae, Melanopsidae, Neritidae, Valvatidae	Rs
Bithyniidae, Hydrobiidae, Lymnaeidae, Planorbidae, Physidae, Viviparidae	Rm
Bivalvia	F
Acari La.	ChP
Acari Ad.	P
Ostracoda, **Triopsidae, Gammaridae,** Decapoda	O
Chirocephalidae, Limnadiidae	F
Argulidae	ChP
Asellidae	D
Caenidae, Ephemerellidae: *Ephemerella* sp., **Ephemeridae, Leptophlebiidae, Oligoneuriidae, Polymitarcidae, Potamanthidae, Siphlonuridae, Capniidae, Leuctridae, Nemouridae**	D
Prosopistomatidae, Heptageniidae, Taeniopterygidae: *Brachyptera* sp., *Rhabdiopteryx* sp.	Rs
Baetidae (excepto *Raptobaetopus*)	Rm
Raptobaetopus sp.	P
Ephemerellidae: *Torleya* sp.	H
Taeniopterygidae: *Taeniopteryx* sp.	D
Chloroperlidae, Perlidae, Perlodidae, Odonata	P
Heteroptera (excepto Corixidae)	ChP
Corixidae	Rs
Agriotypidae, Sisyridae	ChP
Osmylidae, Sialidae	P
Pyralidae	H
Dytiscidae: Ad., **Hygrobiidae, Hydrophilidae:** Larvas, **Gyrinidae**	P
Dytiscidae: La.	ChP
Elmidae,Eubriidae, Haliplidae, Helodidae, Hydraenidae, Hydrophilidae: Ad.; **Limnebiidae**	Rm
Chrysomelidae, Helophoridae: Ad., **Hydrochidae**	H
Helophoridae: La.	O
Dryopidae	D
Ecnomidae, Molannidae, Polycentropodidae, Rhyacophilidae	P
Brachycentridae, Glossosomatidae, Goeridae, Helichopsychidae, Leptoceridae, Drusinae, Stenophilacini, Chaetopterygini, **Odontoceridae, Thremmatidae,**	Rs
Hydroptilidae	ChH
Hydropsychidae, Philopotamidae, Psychomyiidae	F
Phryganeidae	O
Beraeidae, Limnephilidae: Apataniinae,	H
Dicosmoecinae, Limnephilini, **Lepidostomatidae, Sericostomatidae, Calamoceratidae,**	D
Blephariceridae, Orthocladiinae, Diamesinae, Corynoneurinae; **Dixidae, Psychodidae**	Rs
Tipulidae, Ephydridae	D
Limoniidae, Chaoboridae, Chironomidae: Tanypodinae	P
Cylindrotomidae	H
Ptychopteridae, Chironomidae: Chironominae	L
Culicidae, Simuliidae, Stratiomyidae, Syrphidae	F
Ceratopogonidae	O
Anthomyidae, Athericidae, Dolichopodidae, Empididae, Muscidae, Rhagionidae, Scatophagidae, Sciomyzidae, Tabanidae,	ChP

Figure 47. Classification of macroinverebrates according to the nutrition mode; used in IMN index assessment

TALASSOHALINES	k_i	N_i	n_i	k_i*n_i
CLADÒCERS				
Chydorus	5			
Daphnia	1			
Pleuroxus	3			
Simocephalus	4			
COPÈPODES				
Acanthocyclops	4			
Calanipeda	6			
Canuella	4			
Cletocamptus	4			
Cyclops	7			
Diacyclops	7			
Eucyclops	3			
Eurytemora	7			
Halicyclops	5			
Harpacticus	7			
Mesochra	10			
Nitokra	7			
Pseudonychocamptus	5			
Tisbe	3			
Tropocyclops	9			
OSTRACODES				
Cyprideis	5			
Cypridopsis	7			
Eucypris	6			
Heterocypris	4			
Loxoconcha	5			
Sarscypridopsis	1			
Xestoleberis	6			

DOLCES-OLIGOHAL. PERMANENTS	k_i	N_i	n_i	k_i*n_i
CLADÒCERS				
Alona	8			
Bosmina	5			
Ceriodaphnia	4			
Chydorus	3			
Daphnia	2			
Moina	1			
Oxyurella	8			
Pleuroxus	5			
Scapholeberis	8			
Simocephalus	7			
COPÈPODES				
Acanthocyclops	4			
Calanipeda	6			
Cyclops	8			
Ectocyclops	7			
Eucyclops	4			
Macrocyclops	8			
Megacyclops	10			
Tropocyclops	6			
OSTRACODES				
Cypria	3			
Cypridopsis	8			
Eucypris	8			
Herpetocypris	4			
Heterocypris	1			
Paracyclops	1			

DOLCES-OLIGOHAL. TEMPORÀNIES	k_i	N_i	n_i	k_i*n_i
CLADÒCERS				
Alona rectangula	3			
Ceriodaphnia quadrangula	5			
Ceriodaphnia reticulata	3			
Chydorus sphaericus	6			
Daphnia curvirostris	10			
Daphnia magna	3			
Daphnia obtusa	1			
Daphnia pulicaria	7			
Moina braquiata	5			
Simocephalus expinosus	6			
Simocephalus vetulus	7			
COPÈPODES				
Acanthocyclops gr. *robustus*	5			
Canthocamptus staphylinus	9			
Cyclops sp.	5			
Diacyclops bicuspidatus	8			
Diacyclops bisetosus	4			
Diaptomus cyaneus	10			
Megacyclops viridis	5			
Metacyclops minutus	7			
Mixodiaptomus incrassatus	7			
Mixodiaptomus kupelwieseri	6			
Neolovenula alluaudi	4			
OSTRACODES				
Cyclocypris ovum	4			
Cypridopsis vidua	8			
Eucypris virens	5			
Herpetocypris chevreuxi	7			
Heterocypris barbara	4			
Heterocypris incongruens	5			
Plesiocypridopsis newtoni	4			

Figure 48. Quality values assigned to Cladocera, Copepoda and Ostracoda class, in order to evaluate ACCO index

Annex II

```
copepoda<-subset(DATA, select=c(1))
Copepoda<-copepoda[,1]

p_a<-c()

for(ii in 1:length(Copepoda)){
if(Ostracoda[ii]>100){p_a[ii]='I'}
else{p_a[ii]='O'}
}

dip.data<-data.frame(p_a)

Presence<- length(which('I'==p_a))
Absence<- length(which('O'==p_a))

pred.data<-subset(DATA, select=c(4:9))

num_var<-ncol(pred.data)
ncol
num_var
m<-sqrt(num_var)
if(m<2)
  m<-2
m

sampsize=c(Presence,Absence)
set.seed(100)
RF <- randomForest(pred.data, dip.data[,1], data=pred.data, ...
...importance=T, ntree=2000, mtry=m, sampsize=sampsize, proximity=TRUE,outscale=TRUE)
RF
importance(RF)
plot(RF)
varImpPlot(RF,pch=19,cex=1.5,col="black",lwd=2)


#partial dependence plot
dev.new(width=9, height=6)
par(xpd=T, mar=par()$mar+c(1,0,0,0))
par(mfrow=c(2,3),oma = c(0, 2, 0, 0))
partialPlot(RF, pred.data=pred.data, x.var=pH,n.pt=100, main="pH", ...
...ylab="[logit(probability of presence)]/2", xlab="pH", cex.lab=1.5)
partialPlot(RF, pred.data=pred.data , x.var=c.e., n.pt=100,...
...main="Eletrical conductivity", xlab="e.c.[mS/cm]", cex.lab=1.5)
partialPlot(RF, pred.data=pred.data , x.var=O2,n.pt=100,...
... main="Dissolved Oxygen",xlab="D.O. [mg/l]", cex.lab=1.5)
partialPlot(RF, pred.data=pred.data , x.var=Nitrati,n.pt=100, main="Nitrates",...
... xlab="NO_3[mg/L]",ylab="[logit(probability of presence)]/2", cex.lab=1.5)
partialPlot(RF, pred.data=pred.data , x.var=Nitriti,n.pt=100, main="Nitrites",...
... xlab="NO_2[mg/L]", cex.lab=1.5)
partialPlot(RF, pred.data=pred.data, x.var=Fosfati, n.pt=100,main="Phosphates",...
... xlab="PO_4[mg/L]", cex.lab=1.5)
```

Figure 49. Random Forest script (R 3.6.2)

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