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Environmental Characterization of the Stagno Longu of Posada, Sardinia: Assessing the Benthic Community and Ecological Quality Status.

UNIVERSIDADE DO ALGARVE
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Mestrado em Biologia Marinha

Trabalho efetuado sob a orientação de:
Doutore David Cabana
Professore Karim Erzini

UNIVERSIDADE DO ALGARVE
Faculdade de Ciências e Tecnologia
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Declaração de autoria de trabalho

Environmental Characterization of the Stagno Longu of Posada, Sardinia: Assessing the Benthic Community and Ecological Quality Status.

Declaro ser o autor deste trabalho, que é original e inédito. Autores e trabalhos consultados estão devidamente citados no texto e constam da listagem de referências incluída.

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(Cristina Vanda Orro)

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Resumo

Os invertebrados macrobentónicos foram investigados em seis locais na Stagno Longu, uma lagoa costeira do nordeste da Sardenha (Itália), a fim de realizar a primeira caracterização ambiental de sua fauna bentónica. Apesar da lagoa pertencer à Rede Natura 2000, à lista da Convenção de Ramsar das zonas húmidas de importância internacional e de ter sido recentemente adicionada ao Programa Homem e Biosfera (MAB) da UNESCO, ainda falta um plano de gestão especifico, bem como programas de avaliação e monitorização ambiental. Em 2000, a Diretiva-Quadro da Água da União Europeia (DQA) estabeleceu uma estrutura jurídica para a proteção e melhoria de todas as massas de água europeias, através de uma abordagem holística baseada no ecossistema. A DQA exigiu que todos os Estados-Membros (EM) protegessem e melhorassem a qualidade em todas as suas massas de água e definiu como objetivo final alcançar pelo menos um "bom estado" até 2015 ou, o mais tardar, até 2027. A diretiva requer, de todos os EM, a avaliação do Estado da Qualidade Ecológica (EQS) para todas as massas de água.

A composição e a abundância de macroinvertebrados bentónicos, além do seu papel central no funcionamento do ecossistema aquático, é um Elemento de Qualidade Biológica (BQE) amplamente utilizado na avaliação do estado da qualidade ecológica em águas costeiras e de transição. Muitos estudos descreveram como as biocenoses macrobentónicas respondem de forma relativamente rápida a perturbações de origem antropogénica. Deste modo, está bem documentado que atividades humanas que resultem na emissão de esgotos urbanos ou industriais, as atividades agrícolas e a destruição de habitats aumentam as concentrações de compostos orgânicos e de nutrientes, provocam alterações em todo o ecossistema e em particular nas comunidades de macroinvertebrados bentónicos.

Para cumprir a implementação da DQA e determinar a integridade biológica, houve necessidade de aplicar metodologias que incorporassem respostas bióticas através da avaliação de todos os processos, desde os indivíduos aos ecossistemas. Em particular, numerosos índices baseados nos macroinvertebrados foram desenvolvidos para avaliar o EQS em lagoas costeiras europeias.

Através do recente exercício europeu de intercalibração, diferenças significativas na classificação do EQS entre os EM foram harmonizadas, comparando e ajustando os limites de “Bom estado ecológico” em grandes unidades geográficas com tipos semelhantes de massas de água.

Para as águas de transição italianas no Mediterrâneo, os índices adotados foram derivados de AMBI multivariado (M-AMBI) (Muxika et al., 2007) e usados em conjunto com o Índice Biótico Marinho (AMBI). Estes índices dependem das características das comunidades de macroinvertebrados bentónicos, como riqueza de espécies, diversidade de espécies, abundância e sensibilidade das espécies a perturbações antropogénicas.

A estrutura taxonómica da comunidade bentónica de Stagno Longu foi caracterizada como típica de lagoas de água salobra com fauna de meios mesohalinos, comparável à de outras lagoas do Mediterrâneo. Apesar do pequeno tamanho e baixa profundidade média da lagoa, a sua comunidade bentónica era numericamente dominada por crustáceos, poliquetas e bivalves. Os taxa predominantes incluíram o poliqueta depositívoro *Neanthes nubila*, e organismos que se alimentam de detritos e restos de matéria orgânica à superfície do sedimento, de pequeno tamanho, como o poliqueta *Streblospio sp*. e os anfípodes *Corophium ascherosicum*, *Gammarus sensibilis* e *Corophium sextonae*. Os filtradores foram quase exclusivamente representados pelo bivalve *Scrobicularia plana*.

O poliqueta depositívoro *Neanthes nubila* foi a espécie mais abundante na bacia norte (estações 1 e 2) durante as amostragens de inverno e verão. A comunidade desta bacia apresentou a menor abundancia e riqueza de espécies. A bacia oeste mostrou uma estrutura diferente da comunidade: durante o inverno, a parte interna (estacão 3) foi dominada por *Scrobicularia plana*, enquanto a parte externa (estacão 4) por *Streblospio sp*.. Durante o verão, *Scrobicularia plana* continuou a dominar toda a bacia, juntamente com *Corophium*. A bacia leste, a mais próxima do canal do mar, apresentou a maior abundancia e riqueza de espécies (estações 5 e 6) e caracterizou-se pela dominância do poliqueta *Streblospio sp*. e dos crustáceos *Corophium ascherosicum*, *C. sextonae*, *Gammarus insensibilis* e *Cyathura carinata*. Essas espécies são geralmente inteiramente marítimas, encontradas em estuários e águas costeiras (Levin, 2001), mas provavelmente colonizam também áreas lagunares que estão em contato imediato com o mar.

Entre as variáveis espaciais e temporais medidas, a localização da bacia e a composição dos sedimentos foram os fatores que mais contribuíram para a estrutura e distribuição das comunidades de macroinvertebrados bentónicos.

Estas comunidades foram submetidas à variabilidade espacial das variáveis físicas da lagoa, sobretudo no nível de confinamento relacionado com a distância à entrada do mar. Os resultados da análise multivariada n-MDS, com base nas semelhanças da fauna betónica, confirmaram que a lagoa Stagno Longu é caracterizada por três comunidades distintas, altamente correlacionadas com sua localização na bacia. A composição dos sedimentos (das camadas superficiais e sub-superficiais) da entrada em direção à ligação marítima de cada bacia influenciou a composição e a estrutura da comunidade de macrofauna betónica ao longo da lagoa. A bacia norte, mais confinada, apresenta sedimentos com elevado conteúdo de finos (areia fina, silte e argila) e matéria orgânica, sendo a comunidade bentónica dominada por um elevado número de depositívoros de superfície (*Neanthes nubila*) e de espécies oportunistas como *Streblospio sp*. e *Polydora hoplura*. Indo em direção ao centro, a bacia oeste – composta por carbonato de cálcio (principalmente conchas de bivalves), cascalho e argila – mostrou-se dominada por filtradores; e a bacia leste, por sua vez, apresentou maior diversidade de depositivores de superfície e de sub-superfície e de filtradores, sendo composta principalmente por areia.

Um facto interessante é a alteração sazonal da dominância dos poliquetas sobre os crustáceos, durante o inverno, para a relação inversa durante o verão. Além disso, as espécies de crustáceos (*Corophium ascherosicum*, *Corophium sextonae* e *Melita palmata*), mesmo que em pequeno número, estão entre as que mais contribuíram para distinções entre bacias, nas duas estações do ano. Quanto ao padrão espacial, foram obtidos valores mais altos do Índice de Diversidade de Shannon-Winner em estações próximas ao canal do mar (bacia leste e oeste) e valores mais baixos nas estações mais internas (bacia norte).

Com base nos resultados de AMBI, todos as estações, tanto no inverno quanto no verão, foram classificadas como levemente perturbadas (BI-2), enquanto através da aplicação do M-AMBI se concluiu que o estado ecológico da maioria dos estações – tanto no inverno como no verão – era moderado exceto no estação 5, que apresentou um bom estado no inverno, mas moderado no verão; e na estação 6, que foi sempre classificada como tendo um bom estado ecológico.

De particular interesse é o facto das estações na parte interna da lagoa apresentarem valores mais altos do valor do AMBI em comparação às externas, o que aponta para ambientes ligeiramente perturbados. Há que realçar que faltam os valores das condições de referência da lagoa, o que faz com que os resultados do M-AMBI devam ser avaliados com precaução, sendo necessário mais estudos para confirmar o estado ecológico da lagoa.
Este estudo representa uma primeira caracterização ambiental e análise quantitativa de comunidades macrobentónicas e as relações com a composição de sedimentos no Stagno Longu da Posada. Foi demonstrado que as comunidades de macroinvertebrados bentónicos foram influenciadas por variações temporais e espaciais.
Este trabalho fornece conhecimentos fundamentais sobre as características básicas da área acima mencionada, sobre a sua fauna betónica e o estado ecológico. Essas informações representam referências básicas para futuros programas de monitorização, que serão essenciais para garantir a gestão dos recursos hídricos e a implementação de outras ações politicas. No caso da lagoa costeira de Stagno Longu, este estudo oferece as informações necessárias para realizar com êxito pesquisas futuras, sobretudo com foco em programas de monitorização das condições ambientais, da hidrologia da área assim como das suas comunidades, o que permitirá uma gestão baseada nos ecossistemas, no uso sustentável de recursos e, por fim, promover a conservação e a proteção de modo eficaz, necessárias para melhorar a coerência entre os objetivos económicos, sociais e ambientais.

Palavras-chave

Macroinvertebrados bentônicos; Lagunas Costeiras; Mar Mediterrâneo; Índices Bióticos; Status de Qualidade Ecológica; Composição de Sedimentos.

Abstract

Macrobenthic invertebrate assemblages were investigated at six sites in Stagno Longu, north-eastern Sardinian costal lagoon (Italy), in order to perform the first environmental characterization of the benthic fauna and to assess the ecological quality status. Despite the fact that this coastal lagoon belongs to Nature2000 sites, Ramsar list of wetlands of international importance and recently was added to the Man and Biosphere program (MAB), it still lacks specific management plan as well as environmental assessment and monitoring programs. Benthic assemblages comprised 32 taxa, of which *Streblospio sp., Neanthes nubila*, *Corophium ascherusicum* and *Scrobicularia plana* contributed the most to the abundance. Multivariate analysis showed the role of seasonal variations (higher species richness during summer season and more abundance during winter), basin's location and its relative location into the lagoon in influencing benthic assemblages composition. This study also explored the relationship between macrobenthos community and sediment composition which was found to influence abundance, diversity and richness of benthic assemblages. Sediments from the North basin of the lagoon was mostly muddy (rich in OC (organic matter content), H_2O content and fine sands) presenting high numbers of sub-surface deposit feeders (*Neanthes nubila*); the West basin comprised calcium carbonate (CC) (mostly death hard-shell of Bivalvia), gravel and clay and, dominated by filter feeders, while the East basin was mainly composed by sand and showed higher diversity of surface, sub-surface deposit feeders and filter feeders. Recently, several indices have been proposed to be used as ecological indicators in estuarine and coastal waters. The ecological quality of the Stagno Longu was assessed using biotic indices AMBI and M-AMBI which revealed the lagoon to be in a moderate and moderate to good status, respectively. In conclusion, our results provide fundamental knowledge on the basic characteristics of the above-mentioned area, its benthic fauna and the current EQS. Altogether, those information represents basic references for further monitoring programs, which will be essential to ensure management of water resources and implementation of further policy action in order to improve the coherence between economic, societal and environmental goals.

Keywords

Benthic Macroinvertebrates; Coastal Lagoons; Mediterranean Sea; Biotic Indices; Ecological Quality Status; Sediment Composition.

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List of Abbreviations

AMBI – ATZI' Marine Biotic Index

BC – Biotic Coefficient

BQE – Biological Quality Element

CC – Calcium Carbonate Content

d – species richness

EQR – Ecological Quality Ratio

EQS – Ecological Quality Status

H' – Shannon-Wiener diversity Index

MAB – Man and Biosphere Program

M-AMBI – Multivariate ATZI Marine Biotic Index

MS – Member States

N – Number of individuals

nMDS – non-metric Multidimentional technique

OC – Organic Matter Content

PCA – Principal Component Analysis

S – Number of species

TW – Transitional Waters

UNESCO – United Nations Educational, Scientific and Cultural Organization

WFD – Water Framework Directive

CHAPTER 1: Introduction

1.1 Transitional Water Ecosystems

Transitional water (TW) ecosystems are intricately open systems, connected and in-between upstream terrestrial/freshwater and downstream marine ecosystem. They serve many important biological functions in coastal waters: they have extremely high primary and secondary productivity and support a great abundance and diversity of fish, invertebrates and sub-merged vegetation which, altogether provide filtering and detoxification services (Lotze *et al.* 2006). TW such as estuaries and coastal lagoons in particular, are known to face severe exposure to humankind activities and consequently are some of the most heavily used and globally threatened naturally systems (Halpern *et al*. 2008). Those ecosystems have been under pressure from many different uses and from a variety of sectors such as agriculture, tourism, transport and recreational activities. In the past decades, they have suffered rapid transformation which had caused loss of biodiversity, coastal vegetation and ecosystem functions that may have contributed to biological invasion, decrease in coastal protection from flooding to storm events and declining water quality (Brodhead *et al.* 2007, Koch *et al.* 2009, Cochard *et al.* 2008).

For a considerable time now, there has been an increasing awareness and concern on the importance of water quality and high demand by citizens and environmental organisations for cleaner groundwaters, rivers and coastal systems (Wang *et al.*, 2018). As it has been understood water is not a commercial product, but it represents a common good and a limited resource that needs to be protected and used in a sustainable way, both in terms of quality and of quantity. Therefore, a common policy that ensure sufficient quantity of good-quality water for both people's need and for the environment is essential in order to maintain and improve the essential functions of all water-related ecosystems, including coastal and marine areas, by providing good management and monitoring programs (EEA, 2016c).

1.1.1 Mediterranean Coastal Lagoons

Coastal lagoons represent important transitional zones between the land and the sea. They are enriched by continental and marine inputs offering high biological productivity and they are among the most productive aquatic ecosystems (Nixon, 1988) as they play an important economic role. Worldwide, they occupy up to 13% of the coastline (Carrada, 1990) and only the Mediterranean region hosts around 400 coastal lagoons, covering a surface area of over 641000 hectares (Cataudella *et al.*, 2015).

Since ancient times, Mediterranean coastal lagoons represent a source of livelihood for human settlements because they provide important goods and services (Pérez-Ruzafa *et al.*, 2011). They support important fisheries (Perez-Ruzafa *et al.*, 2012): for their high biological productivity they provide incomes based on the quality and price of their natural products, so they can be used for intensive and extensive aquaculture exploitation. In addition, they are also suitable places for nautical sports, swimming and welfare providing key tourist and recreational services .

Those ecosystems are naturally stressed, and similarly to other coastal marine ecosystems, are highly exposed to a severe degree of anthropogenic stresses. It is well documented that human activities such as urban sewage, industrial activities, aquaculture and habitat destruction (Frontalini *et al.* 2009, Bouchet and Sauriau, 2008, Simboura and Zenetos, 2002) increase nutrients and organic matter loading which lead to eutrophication. Variations in the organic input in such areas, whether from biotic or abiotic stresses, result in changes of the whole ecosystem. Due to their particular character, coastal lagoons are considered a very sensitive aquatic ecosystem where processes and benthic inhabitants play an important regulatory function for the entire ecosystem (Viaroli *et al.*, 2004).

Their high biological productivity (Allongi, 1998) provides a collection of habitat types for resident species and also functions as nursery and feeding ground for endangered and migratory species (Levin, 2001). Furthermore, they contribute to climate and atmospheric regulation, disturbance prevention and water quality improvement, by decreasing the pollution loads brought by the rivers in coastal environments (Levin, 2001).

Each coastal lagoon has unique properties arising from its geomorphology and configuration (Pérez-Ruzafa *et al.*, 2007a). They are resilient and productive systems within the coastal landscape. They are ecotones (Basset *et al.*, 2006) where the ecological gradient, due to the transition from land to the sea, creates peculiar and often unpredictable conditions. Those ecosystems are characterized by the presence of physical boundaries between the land and waters, between the water column and the sediment layers and the atmosphere, between the lagoon and the sea and, often, between the waters in the lagoon and freshwater inputs (Perez-Ruzafa *et al.*, 2012). Each boundary allows the formation of strong physical and ecological gradients, which make them highly dynamic systems (UNESCO, 1981). They have relatively shallow depths and quiet waters in which seawater renewal depends on the morphology (eg. degree of enclosure/confinement) and hydrology (eg. tidal amplitude) of the lagoon (Perez-Ruzafa *et al.*, 2012).

Coastal lagoons constitute heterogeneous environments (Gomez *et al.*, 1998) highly variable both in the water column (Mistri *et al.*, 2002) and under the influence of high sedimentation rate (Gianmarco *et al.*, 1997). Those characteristics consequently affect most physical and chemical environmental variables such as salinity, temperature and dissolved oxygen (Elliott and Quintino, 2007) on both spatial and temporal scales.

1.2 European Water Framework Directive

In 2000, the European Water Framework Directive (WFD) (European Commission, 2000) established a main basis legal framework for the protection and improvement of all European waters in an eco-system-based, holistic approach. The WFD demanded to all Member States (MS) to protect and improve water quality in all waters, and defined their final objective as to achieve at least 'good water status' by 2015 or, at the latest, by 2027. It requires all MS to assess the Ecological Quality Status (EQS) for all water bodies. "Surface water" were classified into five categories, those included: coastal waters, rivers, lakes, artificial and heavily modified bodies of water and transitional waters (TWs) (European Commission, 2000). The term TWs was coined and firstly used during the implementations of the directive and includes salt marshes, fjords, rias, river estuaries and coastal lagoons.

According to the WFD directive, the EQS is assigned through the assessment of biological, hydromorphological and physio-chemical quality elements, and determined by comparing data obtained from monitoring networks with reference (possibly undisturbed/pristine) conditions, thereby deriving an Ecological Quality Ratio (EQR) (European Commission, 2000). The EQR is expressed as a numerical value between 0 and 1, with ‘High status’ represented by value close to 1 and ‘Bad status’ represented by value close to 0. The range is divided into five EQS categories: ‘High’, ‘Good’, ‘Moderate’, ‘Poor’ and ‘Bad’. The Biological Quality Elements (BQE) proposed by the WFD that must be included in the EQS assessment as quality elements for the classification of TWs are the composition, abundance and biomass of phytoplankton, aquatic flora (i.e. macroalgae and angiosperms), benthic invertebrate fauna and fish (European Commission, 2000).

The WFD has already led to a significant shift in MS’s water management: it has increased the availability of information to the public and it is providing a much better understanding of the status and pressures on aquatic ecosystems, as well as of measures to reduce pressures and achieve quality status improvements (EEA, 2016c).

Over the past few decades, clear progress has been made in reducing emission into surface waters, leading into improvements in waste water treatments across European continent (EEA, 2016c, 2017b), but results also showed that European waters remain under pressure from multiple sources which affect the functioning of water-related ecosystems, contributing to biodiversity loss and threatening long-term delivery of ecosystem services and benefits to society and to the economy (EEA, 2017b).

1.2.1 Benthic Macroinvertebrates

Benthic macroinvertebrates composition and abundance, beside their central role in marine ecosystem functioning, are a well-established target and are the most widely used and reliable BQE in the evaluation of EQS in coastal waters (Teixeira, 2010).

Many studies have used benthic macroinvertebrate communities and described how macrobenthic biocoenosis evolution respond relatively rapidly under the influence of perturbations (such as anthropogenic and natural influences) (Simboura and Zenetos, 2002;

Bouchet *et al.*, 2018; Basset *et al.*, 2006; Ponti and Abbiati. 2004; Reizopoulou and Nicolaidou, 2007), basing their work on the publication of Pearson & Rosenberg (1978) and in the Mediterranean by Pérès & Bellan (1970).

The community of organisms that live on, or in, the bottom of a water body is known as "benthos". It is a complex community that includes a wide range of organisms from bacteria to plants (phytobenthos) and animals (zoobenthos). The benthic macroinvertebrates are those >1.0 mm in size (generally defined as organisms retained by a 1 mm sieve) that inhabit soft bottoms environments and include groups of benthos organisms such as worms (e.g.: polychaetes and oligochaetes), molluscs (e.g.: bivalves and gastropods) and crustaceans (e.g.: amphipods and decapods) (Tagliapietra & Sigovini, 2010). They are potentially considered good indicators for the following characteristics: (i) they include several infaunal species and are relatively sedentary as they cannot avoid deteriorating water/sediment quality condition and therefore are most likely to respond to local environmental impacts; (ii) they have relatively long life-spans thus, they indicate and integrate water/sediment quality condition over time, (iii) they include a broad range of taxonomic, functional and trophic groups that exhibit different tolerances to different source of disturbance, (iv) most species are sensitive to disturbances of the habitat such that the communities respond fairly quickly with changes in species composition and abundance. In addition, (v) most species that compose benthic assemblages are important components of the food chain, having a role in cycling nutrients and materials between the underlying sediments and the overlying water column, and often act to transport not only nutrients, but also toxicants to the rest of the system.

From a practical point of view, benthic communities are easy to sample and preserve; they provide an "*in situ*" measure of relative biotic integrity and habitat quality and also allow comparison on a geographical scale as some species are widely distributed (Dauer, 1993). For their several characteristics, macrobenthos are commonly used as indicators for the assessment of the biological condition of coastal waterbodies and routinely used in environmental bio-monitoring studies (e.g. Grall & Glemarec 1997; Borja *et al.*, 2003; Bouchet & Sauriau 2008).

1.2.2. Biotic Indices

In order to fulfil the requirements, to comply with the implementation of the WFD and accurately determine the biological integrity, there was the need of a method that incorporate biotic responses through the evaluation of all processes, from individuals to ecosystems (Allan *et al.*, 2006). Recently, a variety of biological assessment tools have been developed (Salas *et al.*, 2006), allowing a better assessment of environmental perturbations on biological systems, which facilitates possible interpretation to improve understanding of ecosystem functioning. Nowadays, numerous indices based on macrobenthos fauna are available for such purpose and in order to assess the EQS based on benthic community conditions in coastal aquatic ecosystems: AMBI (Borja *et al.*, 2000, 2007), BENTIX (Simboura and Zenetos, 2002), BQI (Rosemberg *et al.*, 2004), BOPA (Dauvin and Ruellet, 2007), M-AMBI (Borja *et al.*, 2004; Muxika *et al.,* 2007) and BO2A (Dauvin and Ruellet, 2009). Among these, ATZI's Marine Biotic Index (AMBI) and multivariate-AMBI (M-AMBI) are the most used indices around the world, they summarize the ecological quality status of the water body and allow the results to be easily interpreted. Those taxonomic biotic indices rely on some properties of the benthic assemblages like species richness, species diversity, abundance of the indicator species and species sensitivity to anthropogenic disturbance (Diaz *et al.*, 2004). They use benthic macroinvertebrates as a measure of ecosystem health and constitute the most straightforward and easy to present to potential users. The core feature of these indices is to reduce or summarize environmental quality or conditions to a number, which will become the basis for making management decisions regarding environmental condition and trend (Szyszczak, 2006). An essential tool for making sustainable decisions for coastal ecosystems and resources and also economic growth and social welfare (Szyszczak, 2006).

Most of the diversity indices are based on the Person-Roseberg paradigm (Pearson and Rosenberg, 1978) which describes a generalized pattern of benthic community response along a gradient of organic enrichment. It states that (i) species richness tends to increase, (ii) dominance tends to decrease, and (iii) the proportion of sensitive species tends to increase during secondary succession following disturbance (Pearson and Rosenberg, 1978).

The marine biotic index AMBI is based on the distribution of individual abundances of the soft-bottom communities into five ecological groups (EG) (Grall and Glémarec, 1997), classified accordingly on their sensitivity to the toxicant:

- Group 1: species belongs to this group are very sensitive to organic enrichment and are in abundance at unpolluted conditions;
- Group 2: these species are present in low densities and are relatively constant in abundance over time, unaffected by pollution;
- Group 3: species of this group are tolerant to high organic matter enrichment and are stimulated by an increase in organic pollution.
- Group 4: Species belong to this group are second-order opportunistic species which are favoured by unbalanced conditions.
- Group 5: This group species are first-order opportunistic species which are even more favoured by unbalanced conditions.

The index value is calculated using the following equation including the percentage of each group and the sensitivity coefficient for each group (Borja *et al.*, 2000):

$$AMBI = [(0x\%EGI)+(1.5x\%EGII)+(3x\%EGIII)+(4.5x\%EGIV)+(6x\%EGV)]/100$$

AMBI is based on the percentage of abundance of each ecological group of one site (biotic coefficient (BC)). The species were distributed in those groups according to their sensitivity to an increasing stress gradient (enrichment of organic matter) (Hily, 1984; Glémarec, 1986) which provides a biotic index with eight levels, from 0 (unpolluted) to 7 (extremely polluted) (Borja *et al.*, 2000). AMBI was applied in areas involving a large set of impacted sources (domestic water, heavy metals, submarine outfalls, drilling cuts with ester-based mud, industrial and mining waste) in accordance with community structure measures and multivariate methods in assessing anthropogenic impacts (Borja *et al.*2003, 2004; Muxika *et al.*, 2005).

The M-AMBI index is a refined and integrative formula of AMBI, designed to better define the EQS by including other metrics and parameters describing the benthic community. It combines the proportion of ‘disturbance-sensitive taxa’, through the computation of the AMBI index, species richness and diversity through the use of the Shannon-Wiener diversity index.

M-AMBI adopts a trivariate approach to integrate all parameters through the use of discriminant analysis and factorial analysis techniques to evaluate the ecological conditions using AMBI software (Borja *et al.*, 2000). It derives EQR, giving final values that express the relationship between the observed and reference conditions values. At 'High status', the reference condition may be regarded as an optimum and the EQR approaches the value of 1 while, at 'Bad status' the EQR approaches the value 0 (Muxika *et al.,* 2007). The threshold values for the M-AMBI classification are based upon the European intercalibration (Borja *et al.*, 2007, 2009): 'High' quality, >0.77; 'Good', 0.53-0.77; 'Moderate', 0.38-0.53; 'Poor', 0.20-0.38; and 'Bad', <0.20.

1.3 Regional Natural Park of Tepilora: Stagno Longu of Posada.

The Regional Natural Park of Tepilora is situated in the north-eastern of Sardinia, in the Province of Nuoro (Italy), bordering the Tyrrhenian Sea (Figure 1). Semi-natural area of almost 8000 hectares of vast forests, trails, water springs, rivers and lagoonal dunes areas. It includes very important and significant areas regarding biodiversity conservation with some unique features that cannot be found anywhere else in Sardinia (www.parcotepilora.it).

Once this area was used for cutting woods and as a pasture land till the 1980s, when 16% of the total area was reforested and provided with hiking infrastructure and fire protection services, so becoming a natural reserve. In 2005, in agreement with the Sardinian Region, the Province of Nuoro and the Sardinian Forest Authority, it was decided to established it as Regional Park, with the aim of protecting the natural resources and encouraging sustainable development of the territory (www.parcotepilora.it).

Due to its singular geodiversity, it is possible to find igneous, metamorphic and sedimentary rocks within a few kilometre from each other. Due to the high environment variability, there are a number of endemic species that include birds, plants, mammals, amphibians, reptiles and invertebrates (such as *Papilio hospiton* and *Lasiommata paramegera*) (www.parcotepilora.it). The Rio Posada delta has an associated network of coastal lakes which include the Stagno Longu lagoon (Schenk, 2010).

The lagoon is included within the Regional Park of Tepilora, it is also an Important Bird Area and part of the Natura 2000 Network (Regione Autonoma della Sardegna, 2017) and inserted within Ramsar, wetland sites of international importance (DECRETO, 2018).

The lagoon water body is conceded with Rio Posada in the north and to the sea by means of a narrow man-made cemented channel in the East site. The lagoon comprehends two sub basins with depth ranges from 0.1 to 0.8 m.

Figure 1: Location of the National Park of Tepilora and Stagno Longu (Ardau, 1998).

Stagno Longo is surrounded by a local road in the North, West and South sites. The Eastern part of the lagoon is connected with a set of canals and faces a coastal dune system and the beach of Posada on the North and the beach of San Giovanni on the South site (Figure 2).

Figure 2: Stagno Longu water basin view from satellite (Google Earth Pro).

According to the current knowledge, an area of Sardinia's Regional Natural Park of Tepilora and the territories of the Rio Posada (which includes many lake and lagoon such as Stagno Longu) have been recognized as 'Biosphere Sanctuary' in accordance with the UNESCO's Man and Biosphere programme (MAB) (MAB UNESCO, 2018). MAB was founded to support the use of sustainable and rational resources and the preservation of the biosphere with the aim of encouraging the development of formulaic balance between man and the environment (MAB UNESCO, 2018).

With this aim in mind, management bodies in Sardinia, coordinated by European, regional and provincial standards are at the search for this balance. They have set specific goals including the protection of natural and cultivated biodiversity, conservation of habitat and species and of the quality of surface and ground waters (MAB UNESCO, 2018).

1.4 Objectives

Taking the vital biological, social and economic value of coastal ecosystems into account, this research project will offer the insight needed to successfully accomplish future tasks such as ecosystem-based management, sustainable usage of resources and also effective conservation and protection. With this proposed project, the first environmental characterization of the Stagno Longu, main coastal lagoonal area within the Rio Posada basin will be performed.

The project will provide fundamental knowledge on the basic characteristics of the above-mentioned area, its benthic fauna and the current EQS. Altogether, those information will represent basic references for further monitoring programs, which will be essential to ensure sustainable management of water resources and implementation of further policy action which will be needed to improve the coherence between economic, societal and environmental goals.

During this study, macrobenthic fauna assemblages will be described, taking into account sediment composition and the following hypothesis testing will be undertaken:

H_1 = there is no significant difference in species abundance between seasons;
H_2 = there is no significant difference in species abundance between basins;
H_3 = there is no significant difference in species abundance between stations;
H_4 = there is no significant difference in the interactions within them (season, basins, stations)

1.5 References

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CHAPTER 2: Environmental characterization of the Stagno Longu of Posada, Sardinia: assessing the benthic community and Ecological Quality Status.

Cristina Vanda Orro [a, *], David Cabana [b]

[a, *] *Corresponding author. Universidad do Algarve, E-mail: a61929@ualg.pt, (C.V. Orro).*

[b] *International Marine Centre (IMC), Loc. Sa Mardini, Torregrande, 09170, Oristano, Italy.*

Abstract

Macrobenthic invertebrate assemblages were investigated at six sites in Stagno Longu, north-eastern Sardinian costal lagoon (Italy), in order to perform the first environmental characterization of the benthic fauna and to assess the ecological quality status. Despite the fact that this coastal lagoon belongs to Nature2000 sites, Ramsar list of wetlands of international importance and recently was added to the Man and Biosphere program (MAB), it still lacks specific management plan as well as environmental assessment and monitoring programs. Benthic assemblages comprised 32 taxa, of which *Streblospio sp., Neanthes nubila*, *Corophium ascherusicum* and *Scrobicularia plana* contributed the most to the abundance. Multivariate analysis showed the role of seasonal variations (higher species richness during summer season and more abundance during winter), basin's location and its relative location into the lagoon in influencing benthic assemblages composition. This study also explored the relationship between macrobenthos community and sediment composition which was found to influence abundance, diversity and richness of benthic assemblages. Sediments from the North basin of the lagoon was mostly muddy (rich in OC (organic matter content), H_2O content and fine sands) presenting high numbers of sub-surface deposit feeders (*Neanthes nubila*); the West basin comprised calcium carbonate (CC) (mostly death hard-shell of Bivalvia), gravel and clay and, dominated by filter feeders, while the East basin was mainly composed by sand and showed higher diversity of surface, sub-surface deposit feeders and filter feeders. Recently, several indices have been proposed to be used as ecological indicators in estuarine and coastal waters. The ecological quality of the Stagno Longu was assessed using biotic indices AMBI and M-AMBI which revealed the lagoon to be in a moderate and moderate to good status, respectively.

In conclusion, these results provide fundamental knowledge on the basic characteristics of the above-mentioned area, its benthic fauna and the current EQS. Altogether, those information represents basic references for further monitoring programs, which will be essential to ensure management of water resources and implementation of further policy action in order to improve the coherence between economic, societal and environmental goals.

Keywords

Benthic Macroinvertebrates; Coastal Lagoons; Mediterranean Sea; Biotic Indices; Ecological Quality Status; Sediment Composition.

Introduction

Since ancient times, Mediterranean coastal lagoons represent a source of livelihood for human settlements as they provide important ecosystem services. Their high biological productivity (Allongi, 1998) provides habitat for resident species and also functions as nursery and feeding ground for endangered and migratory species (Levin, 2001) thus sustaining important fisheries resources and recreational areas for human population. Furthermore, they contribute to climate and atmospheric regulation, disturbance prevention and water quality improvement, by decreasing the pollution loads brought by the rivers in coastal environments (Levin, 2001). Benthic macroinvertebrate communities inhabiting coastal lagoons, are composed by several taxonomic groups and they are central components of those aquatic ecosystems. Many studies have used benthic macroinvertebrate communities and described how macrobenthic biocoenosis evolution responds relatively rapidly under the influence of perturbations (such as anthropogenic and natural influences) (Ponti and Abbiati. 2004; Reizopoulou and Nicolaidou, 2007), basing their work on the publication of Pearson & Rosenberg (1978) and in the Mediterranean by Pérès& Bellan (1973). Although, such studies rarely take into account the effect of biogenic habitat variability which, it is likely to be of particular concern due to the robust association between species diversity and habitat (Hewitt, 2008). Infact, each taxon exhibits distinct traits as morphological, behavioural, reproductive and larval features depending on environmental characteristics (e.g.: water trophic status, hydro-period (Kownacki *et al.*, 2000), canopy cover and detritus inputs (Harper *et al.*, 1997)).

Physical and chemical characteristics of the water may also influence the distribution of macrobenthos (dissolved oxygen concentration, depth (Santos and Henry, 2001), conductivity, alkalinity and temperature (Melo, 2009)) as well as food availability (Sanseverino *et al.*, 1998) and interspecific trophic interactions, such as competition and predation (Walker, 1998). Other potential sources of spatial variability of benthic macroinvertebrates are geomorphological environmental characteristics (lagoon size, tidal range (water renewal), level of confinement, exposure (Guelorget *et al.*, 1983) and substrate composition (Bouchanan, 1863). Therefore, benthic macroinvertebrates composition, distribution and biodiversity are controlled by chemical and physical environmental variables which, in aquatic ecosystems, are characterized by frequent fluctuations on a daily and seasonal basis (Koutsoubas *et al.*, 2000).
Macrobenthos composition and abundance, beside their central role in marine ecosystem functioning, are a well-established target and being the most widely and reliably used biological indicators (Simboura and Zenetos, 2002; Bouchet *et al.*, 2018; Basset *et al.*, 2004). Macrobenthos are potentially considered good indicators as they relatively rapidly respond to natural and anthropic stress: (i) they include several infaunal species and are relatively sedentary, as they cannot avoid deteriorating water/sediment quality condition; (ii) they have relatively long life-spans thus, they indicate and integrate water/sediment quality condition over time, (iii) they include a broad range of taxonomic, functional and trophic groups that exhibit different tolerances to different source of disturbance, (iv) most species are sensitive to disturbances such that the communities respond fairly quickly with changes in species composition and abundance. In addition, (v) most species that compose benthic assemblages are important components of the food chain, having a role in cycling nutrients and materials between the underlying sediments and the overlying water column, and often act to transport not only nutrients, but also toxicants to the rest of the system (Dauer, 1993). For their several characteristics, macrobenthic invertebrates are commonly used as indicators for the assessment of the biological condition of coastal waterbodies and routinely used in environmental bio-monitoring studies (e.g. Grall & Glemarec 1997; Borja *et al.*, 2003; Bouchet & Sauriau 2008). It is well documented that human activities such as urban sewage, industrial activities, aquaculture and habitat destruction (Frontalini *et al.* 2009, Bouchet and Sauriau, 2008, Simboura and Zenetos, 2002) increase nutrient and organic inputs, resulting in changes of the whole ecosystem.

To protect and improve of all European surface and ground waters (European Commission, 2000) the European Water Framework Directive (WFD) established a framework which final objective was to achieve at least 'good water status' by 2015, or at least 2027 (European Commission, 2012), requiring all Member States (MS) to assess the Ecological Quality Status (EQS) for all water bodies. According to the WFD directive, the EQS is assigned through the assessment of biological, hydromorphological and physio-chemical quality elements, and determined by comparing data obtained from monitoring networks with reference (possibly undisturbed) conditions, thereby deriving an Ecological Quality Ratio (EQR). The EQR is expressed as a numerical value between zero and one, with 'High status' represented by value close to one and 'Bad status' represented by value close to zero. The Biological Quality Elements (BQE) proposed by the WFD as quality elements for the classification of coastal waters are the composition, abundance and biomass of phytoplankton, aquatic flora, benthic invertebrate fauna and fish.

In order to comply with the implementation of the WFD and accurately determine the biological integrity, there was the need of a method that incorporate biotic responses through the evaluation of all processes, from individuals to ecosystems. Therefore, numerous indices based on macrobenthos fauna have been developed for assessing the EQS in European coastal lagoons. Through the intercalibration exercise, significant differences in status classification among MS have been harmonized by comparing and adjusting the good status boundaries of large geographical units with similar water body types (Reizopolou *et al.*, 2018). For transitional waters in the Mediterranean, adopted indices were: multivariate-AMBI (M-AMBI) (Muxika *et al.,* 2007), derived from AZTI' Marine Biotic Index (AMBI) (Borja *et al.*, 2000), BO2A (Dauvin and Ruellet, 2009), INVHIMB and QAELS (Reizopolou *et al.*, 2018). For Italian water bodies, the use of M-AMBI (in association with AMBI) was selected. Those taxonomic indices rely on properties of the benthic assemblages like species richness, species diversity, abundance of the indicator species and species sensitivity to anthropogenic disturbance (Diaz *et al.*, 2004).

The marine biotic index AMBI is based on the distribution of individual abundances of the soft-bottom communities categorized into five ecological groups (Grall and Glémarec, 1997). Species were distributed in those groups according to their sensitivity to an increasing stress gradient (enrichment of organic matter) (Hily, 1984; Glémarec, 1986) which provides a biotic index with eight levels, from zero (unpolluted) to 7 (extremely polluted) (Borja *et al.*, 2000).

M-AMBI, is a refined and integrative formula designed to better define the EQS by including other metrics and parameters describing the benthic community (Muxika *et al.,* 2007). It combines the proportion of 'disturbance-sensitive taxa', through the computation of the AMBI index, species richness and diversity through the use of the Shannon-Wiener diversity index. It adopts a trivariate approach to integrate all parameters through the use of discriminant analysis and factorial analysis techniques. It derives EQR, giving final values that express the relationship between the observed and reference conditions values. At 'High status', the reference condition are considered as an optimum and the EQR approaches the value of one while, at 'Bad status' the EQR approaches the value zero (Muxika *et al.,* 2007). Although many studies were performed throughout European coastal lagoons with respect to the WFD, there are still many areas that have not received any concern, despite their ecological, biological and economic importance. Within those are the water bodies enclosed in the Regional Natural Park of Posada, Sardinia (Italy). The park presents mountainous areas and flat strips with rivers and coastal lagoons such as the Stagno Longu of Posada, which provide a wide array of regulating, provisioning and cultural ecosystem services to the region and beyond. According to the current knowledge, it belongs to the Nature2000 site (Regione Autonoma della Sardegna, 2017) and in 2007, in accordance with the UNESCO's Man and Biosphere programme (MAB), territories of the Rio Posada were added to the World Network of Biosphere. Nonetheless, Posada Regional Park lacks specific management plan as well as environmental assessment and monitoring programs.

Taking the vital ecological, biological, economic and social value of coastal ecosystems into account, the main aims of this study were: (i) to undertake the first environmental characterization of the Stagno Longu, main coastal lagoonal area within the Rio Posada basin, by describing benthic communities structure and their environmental affinities; and (ii) to assess the EQS according to the WFD providing fundamental knowledge on the basic characteristics of the above-mentioned area.

Materials And Methods

Study Area

The Stagno Longu lagoon is a poly-euhaline restricted coastal lagoon situated in the North-Eastern coast of Sardinia, Italy (40°39’N; 09°44’E), found parallel to the shoreline of the Tyrrhenian Sea. The lagoon is situated in the alluvial plain of Posada, which was deposited by the Rio Posada river and Rio Santa Caterina river, the smaller one bordering and entering the lagoon toward its north-eastern part (Figure 1).

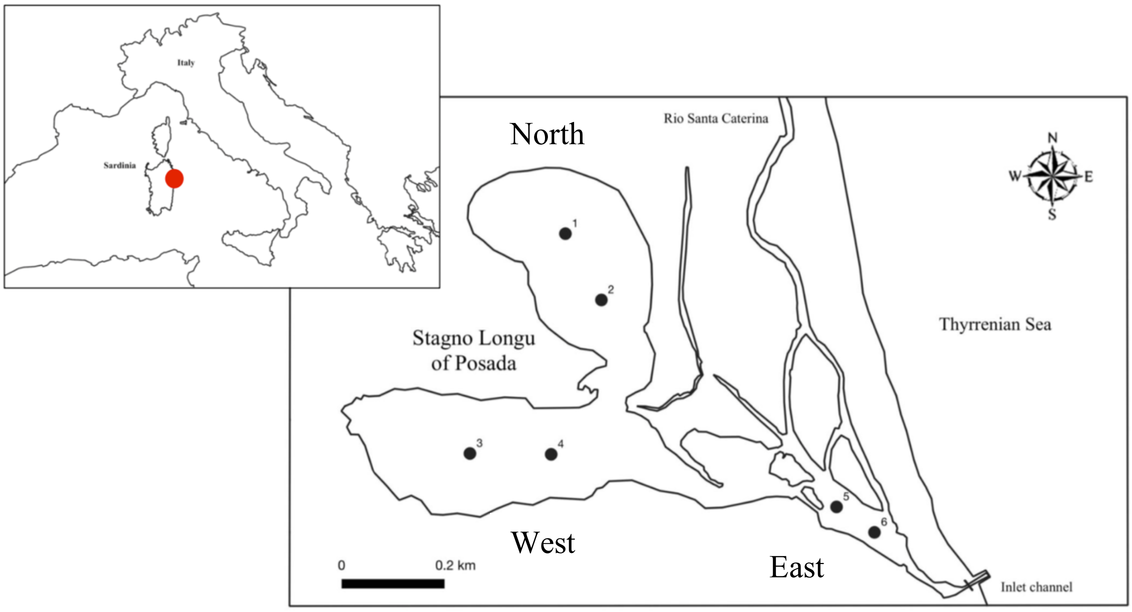

Figure 1: Map of the Stagno Longu of Posada. Sampling sites are indicated: North basin (station 1 and 2), West basin (station 3 and 4) and East basin (station 5 and 6).

Stagno Longu is included within the Regional Natural Park of Tepilora. The lagoon is part of a protected natural reserve that includes important and significant areas regarding endemic biodiversity conservation, providing a wide array of regulating, provisioning and cultural ecosystem services to the region and beyond. It belongs to the Nature2000 sites (Regione Autonoma della Sardegna, 2017) and in June 2017, the International Coordinating Council of the Man and Biosphere (MAB) has added this site to the World Network of Biosphere (MAB, UNESCO, 2018) and inserted within Ramsar, sites of international importance (DECRETO, 2018).

It has an area of ~0.27 km^2, perimeter of ~4.000 m and depth ranges from 0.1 m to 0.8 m; it presents lagoonal complexes, comprising a northern, western and eastern lagoon basins connected to the nearby sea by a narrow artificial inlet channel (approximately 10 m wide).

During the sampling campaigns and lagoon exploration, lacustrine vegetation was found to be represented by the association of *Ruppia maritima*, *Ruppia cirrhosa*, *Gracilaria* and *Enteromorpha sp.*. Along the shoreline, patches of *Chaetomorpha linum*, *Ulva intestinalis* and *Ulva compressa* meadows were identifies, whereas in the central sections of the lagoon, although small patches of the same species are found, bare sediments dominate. The Stagno Longu is also an important area for endemic and migratory birds (*Podicipediformes*, *Pelecaniformes*, *Phoenicopteriformes*, *Charadriiformes* and *Coraciformes*) ("Censimenti, 1993-1997") and renowned for its fisheries activities (i.e. *Mugilidae*, *Anguillidae* and *Moronidae*) (Schenk, 2010).

Sampling and laboratory analysis

Sampling design was established to assess spatial and temporal variations in the distribution of benthic invertebrates within the main basins (North, West and East) of the lagoon. Spatial scale was provided by two randomly selected stations at each of the main basin, considering basin's geophysical conformation based on vicinity to the water inflow (marine and fresh water sources). North basin; site with potentially lower water circulation and distant from fresh and/or marine water inputs (stations 1 and 2). West basin: site closer the main fresh water tributary (station 3 and 4). East basin: site closer to the inlet channel connected to the sea (station 5 and 6). Temporal variability was assessed by choosing opposite seasons in terms of rainfall and temperatures ranges. Benthic macroinvertebrates were collected at each station during two periods (winter and summer 2019); at each seasonal period, the sampling campaign was performed twice (winter season: January/February; summer season: May/June).

Sediment samples (three replicates) were taken for analysis of the macrobenthic community from all sampling stations by means of an Ekman-Birge (grasping area 400 cm^2) and sieved immediately through a mesh (0.5 mm) to retain the macrobenthic organisms, which were then preserved in 90% ethanol and sorted under a stereomicroscope in the laboratory.

Specimens were identified to the lowest possible taxonomic level (species level) or to the family level and counted.
Granulometry analysis were performed: organic matter content (OM) and calcium carbonate content (CC) determined on sediment samples taken using a manual corer (60cm in length, 10 cm in diameter) on a rowboat. The surface ("t", 0-2 cm) and subsurface ("b", 2-5 cm) layers were carefully sliced-off, frozen and treated as a single sample. For analysis, samples were defrozen and completely dried at 60°C.
From each sample, grain-size distribution was determined by wet sieving to separate sand (>63 µm) and silt and clay fractions (<63 µm), following procedures indicated by De Falco (2004). Samples <63 µm were analysed using a Galai CIS 1 laser particle sizer (Molinaroli *et al.*, 2000). After wet sieving, sand fraction's grain sizes were analysed by using a mechanical shaker and classified according to the Wentworth scale (Buchanan, 1984): 64-2 mm gravel, 2-0.25 mm and, 0.25-0.065 mm fine sand, 0.065-0.0039 mm silt and 0.0039-0.001 mm clay.
OM was determined by loss on ignition. OM was measured as the difference of dried sediment before and after desiccation (Dean, 1974). For each sample, CC was estimated by means of calcimeter as a percentage of calcium carbonate ($CaCO_3$ %), calculated as described by Balázs (Horváth *et al.*, 2005).At each sampling site, water temperature, salinity, dissolved oxygen in the surface water , pH and depth along the lagoon were recorded.

Data analysis

Data analysis was performed using PRIMER v6 & PERMANOVA+ software package (Clarke and Gorley, 2001). Macrobenthos community structure was analysed regarding univariate variables such as: abundance (*N*, number of individuals), number of species (*S*), species richness (*d*) and Shannon-Wiener diversity (*H'*) indices.
Similarity between stations within basins and seasons was analysed by clustering techniques on the biological matrix of 28 species per 6 sites after square root transformation of the abundance value. In order to detect the similarity between sampling stations, cluster analysis was applied to the unweighted pair group average algorithm and the Bray-Curtis Similarity Index.

SIMPER analysis was performed in order to identify the percentage contribution of the most abundant species to the overall similarity within each groups that were assessed according to results of the cluster analysis. Resemblance patterns among the sampling stations and seasons were observed using non-metric multidimensional scaling (nMDS) technique.

Furthermore, Principal Component Analysis (PCA) (correlation-based square root transformed and standardized abiotic matrix) was applied to the granulometry data. Interactions within factors were tested *a posteriori* using pair wise comparisons. Links between the distribution pattern of the macroinvertebrate assemblages and the environment variables were investigated using Global BEST test.

Variation in macrobenthic assemblages among basins and seasons were tested using a three-way crossed design PERMANOVA based on Bray-Curtis (dis)similarity measures. The design was based on Seasons (winter, summer, fixed), Basin (North, West and East, fixed) as orthogonal factors and Stations (1, 2, 3, 4, 5 and 6, random within Basins, nested within Basins).

Biotic indices

In the present study, AMBI (Borja *et al.*, 2000, 2007) and M-AMBI (Muxika *et al.*, 2007) indices were adopted in order to assess the health of the Stagno Longu of Posada.

The assignment of the identified species into one of the five ecological groups was performed on the classification proposed by Borja *et al.* (2000) for the biotic index AMBI, based upon the latest version of the AMBI software freely available at http://www.azti.es, related species list version (Borja and Muxika, 2005) and upon M-AMBI by Muxika *et al.* (2007). Monitored results were compared with reference conditions obtained from the European intercalibration (Reizopolou *et al.*, 2018) for Mediterranean transitional waters.

Results

Composition of macrobenthic assemblages

Univariate analysis

Among the macrobenthos collected, a total of 14968 individuals belonging to 31 species were collected (i.e.: 5101 Polychaeta, 5532 Crustacea and 2526 Bivalvia). In addition, 1013 Oligochaeta) and 756 Chironomidae larvae were found, but excluded as data sets were built upon benthic macroinvertebrates taxa. Crustacea accounted for 42% of all macrobenthic species, followed by Polychaeta (39%) and Bivalvia (19%). Macrobenthic abundance varied between seasons: with a total of 5368 in winter and 7791 in summer. Where, 2810 Polychaeta were present in winter and 2291 in summer; 1510 Crustacea in winter and 4022 in summer while, 1048 Bivalvia in winter and 1478 in summer.
The most common and abundant species was the *Streblospio sp.* with a wide distribution in most sampling stations (except station 1) in both seasons. Other abundant macroinvertebrates with a wide distribution (found at all stations for both seasons) were Polychaeta *Neanthes nubila*, Crustacea *Corophium ascherusicum*, *Gammarus insensibilis* and the Bivalvia *Scrobicularia plana*. The Polychaeta *Polydora hoplura*, Crustacea *Corophium sextonae* and Bivalvia *Cerastoderma edule* were also common.
Ecological indices of benthic communities (average abundance of total number of individuals (N), total number of species (S), species richness (d) and Shannon Wiener diversity index (H')) in each basin for winter and summer seasons are shown in Figure 2. N recorded in any one time (from replicates count) ranged from 5 in station 1 to 1123 in station 5, while the highest d (16) collected was found in station 6, and the lowest (only one) was found in station 1, both during summer sampling. d ranged from 1.1 in station 1 to 2.1 in station 6 during the winter season, while during summer time it ranged from 0.7, always in station 1 to 1.7 in station 6. H' varied from 0.7 in station 1 to 1.8 in station 6, this variation had not changed through seasons. Station 6, closer to the sea channel, has the greatest marine influence and also highest S and H' while restricted one such as station 1, far away from the open sea was found to be species-poor and with low diversity.

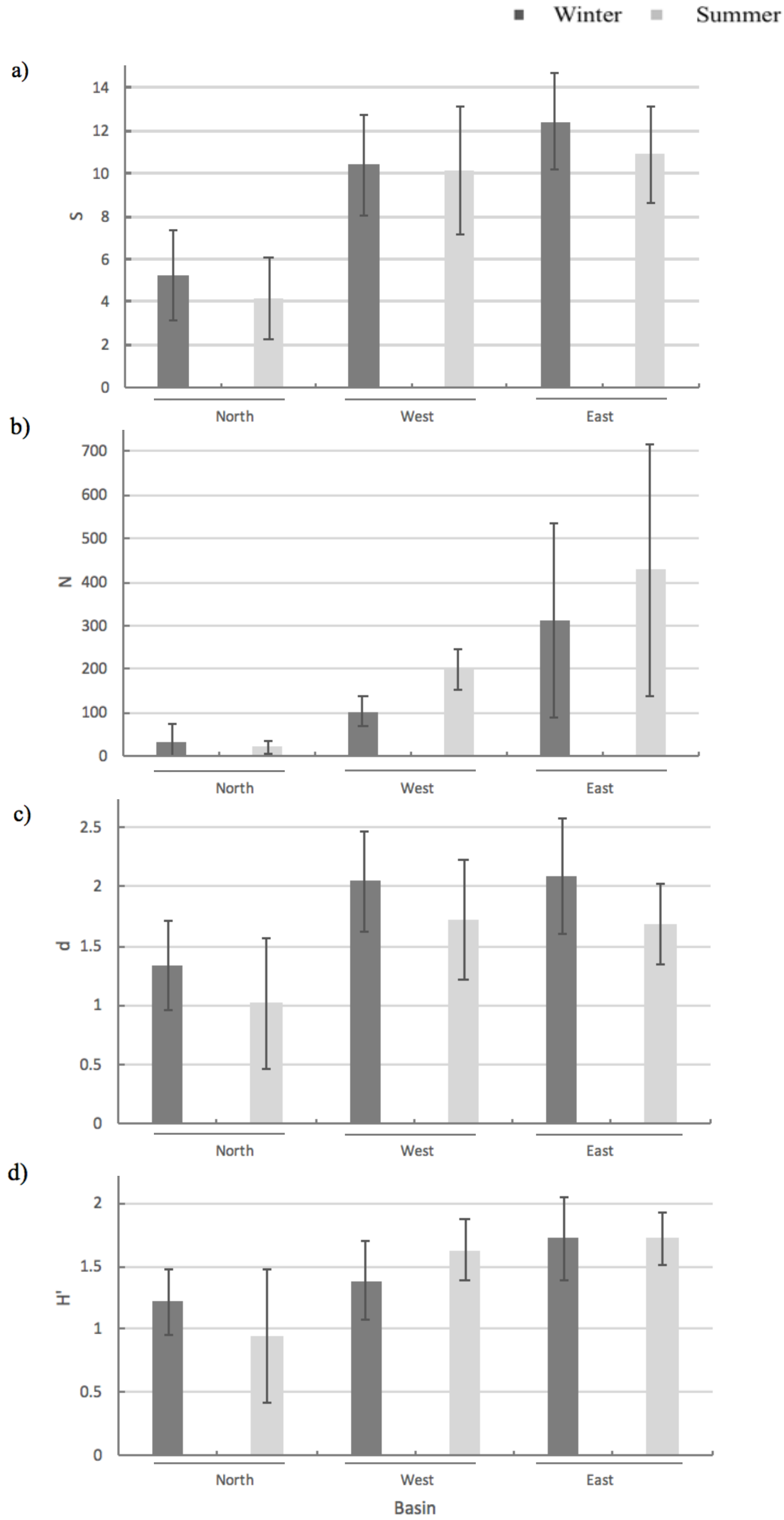

Figure 2: Ecological indices of benthic communities: a) average of total number of species (S), b) total number of individuals (N), c) species richness (d) and d) Shannon Wiener diversity index (H') for each basin for winter and summer seasons.

Multivariate Analysis

Cluster analysis ordination (Figure 3) of the total samples displayed a high similarity (at ~60%) across stations of the Stagno Longu lagoon. Some samples that showed lower similarity (station 1 in winter (W) and station 1 in summer (S)) are found along the edge of the North basin. Within such a low similarity, three groups of stations could be described: those that included a spatial heterogeneous group formed by the rest of station 1W, 1S, 2W and 2S in the remaining North basin, another composed by 3W, 3S, 4W and 4S situated on the West basin and the last one, situated at the inlet on the lagoon, East basin, composed by 5W, 5S, 6W and 6S.

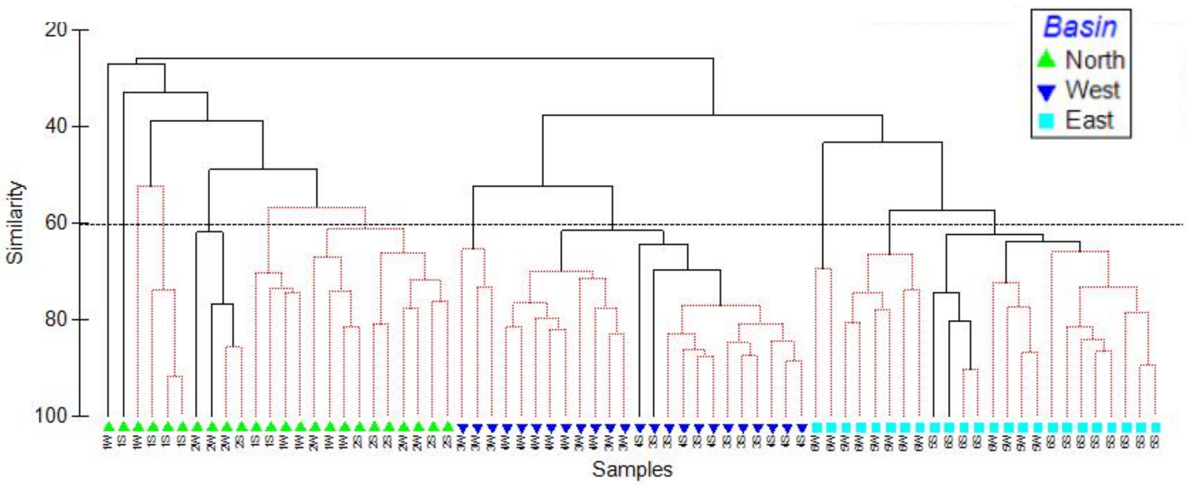

Figure 3: Cluster analysis ordination of the sampling sites: station 1, 2, 3, 4, 5 and 6 divided by major basins of the Stagno Longu lagoon for both winter (W) and summer (S) seasons.

nMDS ordination of the total samples (Figure 4) displayed a high similarity (at ~40%) across basin of the Stagno Longu lagoon as well as across seasons (W=winter, S=summer). Some samples that showed lower similarity, and appear to be outliers (station 1W and 1S) are found along the edge of the North basin. Within such a low similarity, three groups could be described: those that included a spatial heterogeneous group formed by the rest of station 1W, 1S, 2W and 2S in the remaining North basin, another composed by 3W, 3S, 4W and 4S situated on the West basin and the last one, situated at the inlet on the lagoon, East basin, composed by 5W, 5S, 6W and 6S.

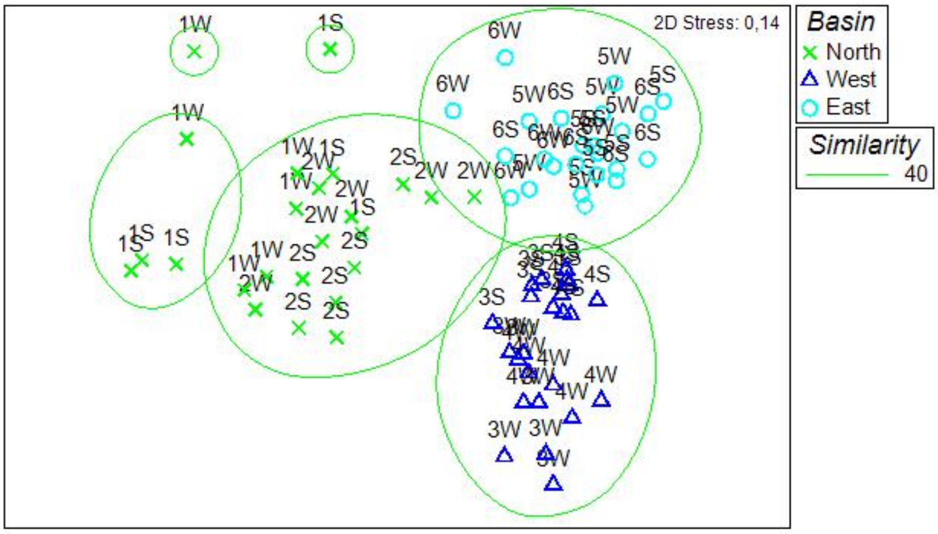

Figure 4: nMDS plot of the sampling sites: station 1, 2, 3, 4, 5 and 6 divided by major basins of the Stagno Longu lagoon for both seasons winter (W) and summer (S) (2D stress level 0.14).

SIMPER analysis based on Bray-Curtis similarity, measured with a cut off for low contribution at 80% , was used to identify the role of individual species in typifying levels for each station groups (Table 1a) and species that were most responsible for distinctions between seasons (Table 1b). Results for average similarity between seasons were 61.82% for winter and 67.08% for summer, with an average dissimilarity of 44.60% across winter and summer. Most dominant species that consistently contributed the most for both seasons were *Neanthes nubila* (19.17% for winter and 20.95% for summer) followed by *Scrobicularia plana*, *Streblospio sp.* and *Corophiu ascherusicum*, with fewer less abundant species for both seasons.

The average similarity was 48% for the North basin, 71% for the West basin, and 65% for the East basin. The higher average dissimilarity among basin were 75% from North and East basins, 74% between North and West basins and 60% between West and East basins. Discriminating species that contributed most depended on the basin location: *Neanthes nubila* in the North, *Scobicularia plana* in the West, *Streblospio sp.* and *Corophium ascherosicum* in the East.

Table 1: Summary of SIMPER analysis results: average abundance of species (% cover), their contribution to the within group similarity (Contrib. %) and the cumulative total of contribution (%) 80% cut off: a) between station groups and b) between seasons.

a) Basin	Species	Average Abundance	Contrib. %	Cum.%
North	*Neanthes nubila*	3.17	55.62	5562
	Corophium ascherusicum	2.12	24.13	79.75
	Corophium sextonae	1.25	7.82	87.57
West	*Scrobicularia plana*	8.24	34.45	34.45
	Corophium ascherusicum	3.96	12.67	47.12
	Streblospio sp.	3.57	11.72	58.84
	Heteromastus filiformis	2.02	7.75	66.6
	Capitella capitata	1.97	7.36	7395
	Corophium sextonae	2.66	7.03	80.98
East	*Streblospio sp.*	10.01	20.84	20.84
	Corophium ascherusicum	6.77	16.21	3705
	Corophium sextonae	5.78	11.09	48.14
	Cyathura carinata	4.6	10.66	58.81
	Neanthes nubila	3.75	8.98	67.79
	Malita palmata	3.48	8.92	76.71
	Scrobicularia plana	2.82	5.86	82.57

b) Season	Species	Average Abundance	Contrib. %	Cum %
Winter	*Neanthes nubila*	2.74	19.17	19.17
	Scrobicularia plana	3.69	18.48	37.65
	Streblospio sp.	4.34	12.68	50.32
	Corophium ascherusicum	2.65	11.13	61.45
	Corophium sextonae	2.12	6.91	68.36
	Melita palmata	1.93	5.58	73.94
	Gammarus insensibilis	1.75	4.32	78.26
	Capitella capitata	0.89	4.32	82.59
Summer	*Neanthes nubila*	2.93	20.95	20.95
	Corophium ascherusicum	5.91	20.71	41.66
	Streblospio sp.	5.09	14.78	56.43
	Scrobicularia plana	4.05	14.55	70.98
	Corophium sextonae	4.34	9.72	80.7

PCA ordination analysis was used to explain the variability of the community structure in relation to the similarities with granulometry composition found at each station (Figure 5). The first two components explained 83% of the total basin variability. The first axis (PC1) of the PCA ordination accounted for 44% of the variability and the second (PC2) accounted for the other 39% of the relationship between macrobenthos composition and the selected environmental variables. According to the results the main environmental variables that determined station features were H_2O content, fine sand, sand and OC (coefficients: -0.527, -0.514, -0.497 and -0.453, respectively).

In addition, PCA ordination showed three major different groups based on the sediment particles composition. Station 1 and 2 (North basin) were positively correlated with H_2O content, fine sand and OC while negative correlated to sand. In contrast, the stations 5 and 6 (East basin) were negatively correlated with these variables and positively correlated to sand. Station 3 and 4 (West) positively correlated to CC, gravel and clay and negatively correlated to silt.

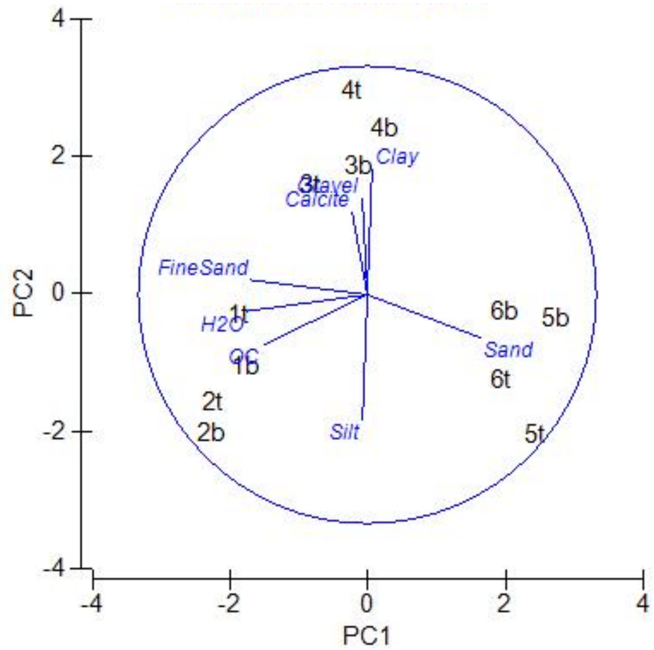

Figure 5: PCA ordination plot showing granulometry composition of sediment for each sampling station (station 1, 2, 3, 4, 5 and 6) with distinction between surface layer (0-2 cm, represented by ‘t’) and subsurface layer (2-5 cm represented by ‘b’).

Abiotic Parameters

Major hydrological parameter of water surface for all station sampled during winter and summer ([min-max] data for salinity, dissolved oxygen, temperature, pH and depth) are shown in Table 2.

Table 2: Minimum [min] and maximum [max] hydrological parameters of water surface for each station sampled for winter and summer seasons: salinity (ms/cm), dissolved oxygen concentration ([O_2] mg/l), temperature (°C), pH, depth (m).

Basins	North				West				East			
Station	1		2		3		4		5		6	
Season	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer
Salinity	12.9-46.0	13-46.6	12.5-47.8	13.6-48.1	10.4-49.8	12.2-50	6.5-50.2	13.3-49	17.7-49.7	8.8-53.3	10.3-50.8	8.9-54.7
[O_2]	10.8-11.5	7.6-8.6	11.5-12	9.1-9.2	11.4-12.7	6.5-9.2	13.2-11.7	7-9.9	12.4-11.3	8.4-11.5	11.3-12.7	8.9-11.53
Temperature	8.9-9.5	19.5-28.8	8.2-8.8	20.6-29.6	8.2-10	21.3-30.2	7.8-9.9	21.7-29.8	9.5-13	22.5-28.9	10.5-13.1	22.6-26.9
pH	7.3-7.4	8.1-8.4	7-7.7	8.1-8.4	7.2-8.1	8-8.01	7.7-8.1	8-8.1	7.4-8	8-8.3	7.3-8	8.1-8.3
Depth	0.1-0.3	0.3-0.4	0.1-0.3	0.3-0.6	0.4-0.5	0.4-0.5	0.4-0.5	0.5-0.6	0.2-0.3	0.2-0.2	0.2-0.4	0.2-0.3

Salinity values within the same stations in the North and West basins, showed minimum values lower during winter and higher during summer. On the contrary the East's stations showed the other way around. [O_2] values showed minimum values higher in winter and lower in summer, except for station 6 (closest to the channel inlet to the sea) which displayed the opposite. Temperature measures higher during summer compared to winter season while pH almost didn't vary within stations and within seasons. Depth ranged from 0.1 to 0.6 in the North basins, 0.4 to 0.6 in the West basin, and the lowest found close to the sea in the East basin, from 0.2 to 0.4.

Granulometry characteristics (percentage of sediment water content, organic content, percentage of CC; gravel, sand, fine sand, silt and clay particles) with distinction between of the sediment core layer of the surface (0-2 cm) and subsurface (2-5 cm) of the Stagno Longu lagoon are listed in Table 3. Stagno Longu lagoon complexes have different sediment characteristics depending on the basin location. Station 1 and 2 (North basin) showed higher water content (75.98-85.04%) while, station 5 and 6 (East basin) the lowest (24.70-37.50%).

In regard to different particle size, station 1 and 2 were composed mainly by fine sand (32.52-37.61%) and clay particles (27.55-30.87%) with highest values of organic content (OC) (20.25-18.09%). Station 3 and 4 (West basin) also showed high values of fine sand and clay, but in contrast station 4 had highest percentage of gravel (mostly composed by shells, 12.77-11.19%). In regard to station 5 and 6, the most abundant particles were those composed of sand (44.09-38.93%) followed by clay (32.01-26.79%) and silt (23.22-18%). These two stations had the lowest percentage of OC (2.23-1.16) and CC (2.74-1.41%).

Table 3: Granulometry analysis for each station sediment core with percentage of water content (H_2O content), organic content (OC), percentage of Calcum Carbonate (CC); gravel, sand, fine sand, silt and clay particles with distinction between surface layer (0-2 cm, represented by 't') and subsurface layer (2-5 cm represented by 'b') for each sampling station.

Station	Core Size		H_2O Content	OC	Gravel	Sand	Fine Sand	Silt	Clay	CC
1	0-2	t	79.90	11.01	1.24	11.15	37.61	19.14	30.87	5.03
	2-5	b	75.98	12.13	1.99	12.07	35.94	21.17	28.83	4.24
2	0-2	t	85.04	18.09	1.31	13.91	34.78	21.27	28.74	2.15
	2-5	b	84.44	20.25	1.98	15.50	32.52	22.45	27.55	2.08
3	0-2	t	64.43	2.29	1.77	11.41	36.82	15.10	34.91	6.26
	2-5	b	52.51	5.34	2.80	21.43	25.77	16.62	33.38	19.79
4	0-2	t	55.58	5.37	11.19	14.36	24.45	13.54	36.47	6.74
	2-5	b	48.40	4.99	12.77	18.05	19.18	15.70	34.31	7.77
5	0-2	t	31.03	1.32	1.22	43.18	5.60	23.22	26.79	2.74
	2-5	b	24.70	1.16	1.17	44.09	4.73	18.45	31.55	2.65
6	0-2	t	37.15	2.23	1.16	38.93	9.91	20.93	29.07	2.08
	2-5	b	37.50	2.09	1.33	38.93	9.74	18.00	32.01	1.41

Global BEST test was applied to study the match between multivariate among-samples abundance of the assemblage and that from environmental variables associated with it. Spearman rank correlation coefficient indicated that there was a good agreement between the distribution of macrobenthic fauna's abundance and granulometry analysis. It showed that in winter, variables such as fine sand, sand and gravel were the major variables explaining benthic fauna distribution (0.704) while in summer OC and gravel (0.871).

The PERMANOVA tests was used to test the spatial and temporal variations on the basis of the resemblance measure, using permutation methods. It revealed high spatial-temporal variability (significant differences within each factor (Seasons and Basins) and significant interaction between the factors (between Station within Basins (St(Ba)) and between Basin within Seasons (SexBa)) in the abundance of macrobenthic community (Table 4). Pairwise comparisons showed significant difference between Seasons, Basins and Basin within Seasons (Se(Ba)) ($p < 0.05$), except for the North basin.

Table 4: (a) PERMANOVA on Bray-Curtis (dis)similarities for differences in the abundance of macrobenthic assemblages. Data were square-root (x+1) transformed. (b) Pair-wise comparison results between Seasons, Basins, Station nested in Basin (St(Ba)) ans Basin within Seasons (Se(Ba)). Probabilities of relevant tests indicated in bold.

(a)	Source	df	MS	Pseudo-F	P(perm)	Unique perms	P(MC)
	Se	1	6137.2	66.721	**0.027**	9486	0.0008
	Ba	2	38026	12.186	0.0626	15	**0.0001**
	St(Ba)	3	3120.5	40.668	**0.0001**	9897	0.0001
	SexBa	2	3102.6	33.731	**0.0407**	9906	0.01
	SexSt(Ba)	3	919.82	11.988	0.2487	9902	0.2566
	Residual	60	767.3				
	Total	71					

(b)	Source	Name	t	P(perm)	Unique perms	P(MC)
	Seasons	Winter, Summer	2.583	0.0286	9446	**0.0006**
	Basins	North, West	3.5108	0.3315	3	**0.0029**
		North, East	3.2224	0.3372	3	**0.0019**
		West, East	3.9543	0.3382	3	**0.001**
	St(Ba)	North (1,2)	2.0276	**0.0037**	9946	0.0069
		West (3,4)	1.8662	**0.0049**	9956	0.0104
		East (5,6)	2.0881	**0.0021**	9950	0.0033
	Se(Ba)	North (Winter, Summer)	0.81013	0.6645	3	0.6488
		West (Winter, Summer)	3.3877	0.4981	3	**0.0158**
		East (Winter, Summer)	2.3181	0.4951	3	**0.0414**

Biotic Indices

A total of 27 species were fitted into five ecological groups based on the ATZI classification. Among these 27 species, 5 species (18.5%), 3 species (11%), 16 species (59.3%), 2 species (7.4%), one species (3.7%) were grouped as EG I, II, III, IV and V respectively as the dominant benthic macroinvertabrates species within each ecological group. AMBI values ranged between 1.7 to 3.2. Accordingly, in summer and winter all stations were classified as slightly disturbed (Figure 6) and dominated by indifferent species, EG II (those that are present in low density and are relatively constant in abundance over time) such as *Desdemona ornata*, *Idotea chelipes* and *baltica*, and/or tollerant species, EG III (tolerant to high organic enrichment), ie.: Polichaetae *Neanthes nubila*, *Streblospio sp.*, *Cyathura carinata*, *Corophium ascherosicum* and *sextonae*, Bivalvia *Scrobicularia plana* and *Cerastoderma edule*.

On the other hand, M-AMBI results values varied between 0.42 and 0.66. The ecological grade fell between good and moderate status. The results revealed that station 1, 2, 3, 4, were classified as moderate for both seasons, whereas station 5 from good in winter descended to moderate in summer and 6 remained stationary in good state (Figure 6).

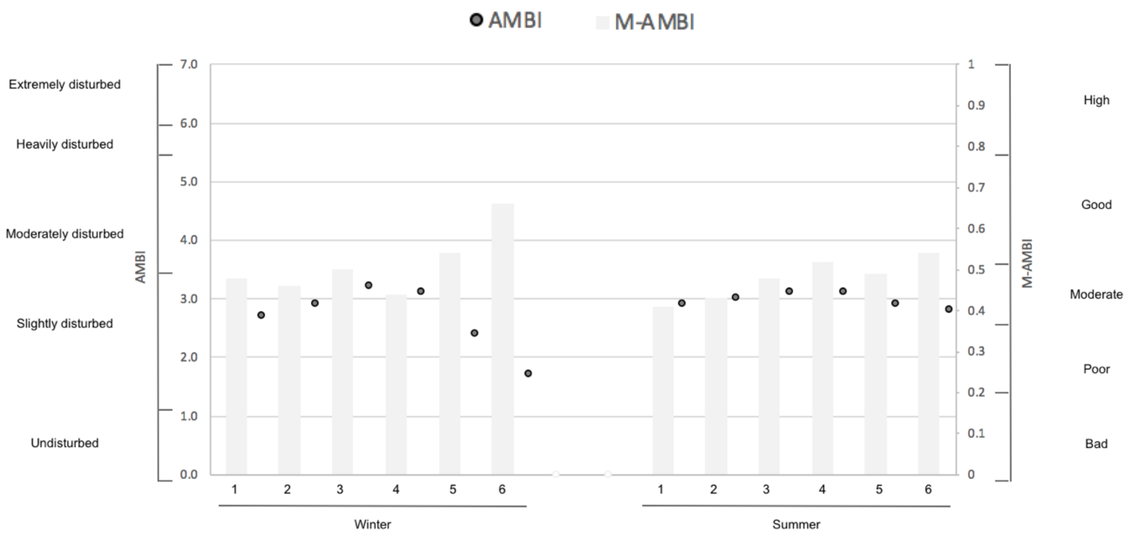

Figure 6: Spatial distribution and classification of AMBI and M-AMBI values found along each station, for winter and summer season. Station 1 and 2, North basin; station 3 and 4, West basin and station 5 and 6, East basin.

Discussion

Taxonomic structure of the benthic community of the Stagno Longu was characterized as typical of brackish water lagoons with mesohaline common fauna (Pérès, 1967), comparable to that of other Mediterranean lagoons (Reipoulou *et al*, 2004; Amanieu *et al.*, 1977; Mistri *et al.*, 2002). Despite its small size and low average depth, Stagno Longu exhibited high abundance: benthic community was numerically dominated by Crustacea, Polychaeta and Bivalvia. Overall, predominant taxa included polychaeta sub-surface deposit feeder *Neanthes nubila*, small-size surface deposit feeders *Streblospio sp.*, *Corophium ascherosicum*, *Gammarus sensibilis* and *Corophium sextonae*. Filter feeders were almost exclusively represented by hard-shelled Bivalvia *Scrobicularia plana*.

Deposit feeder *Neanthes nubila* showed to be the most abundant species located in the North basin (station 1 and 2) during both winter and summer seasons. Soft sediments (mostly muddy) with high organic matter contents were found to be favourable environment for this taxon (Bakken, 2006), as they mostly feed on detritus, algae residues and bacteria (Sayam *et al.*, 1983; Pesch *et al.*, 1988). Overall, the North basin's community showed the lowest abundance and species richness: this resembles conditions previously seen in Mediterranean studies (Reizopolou *et al.* 2004; Amanieu *et al.*, 1977; Gravina *et al.*, 1989): coastal lagoons have very variable environmental conditions due to their shallowness and restricted communication with the marine environment which results in low diversity. Levels of natural stress increases in confinement areas, which results in a decrease of species richness and an increase in abundance of few species individuals (Guelorget and Perthuisot, 1992).

The West basin showed the most variable community structure: during winter time, the inner part (station 3) was dominated by *Scrobicularia plana* while the outer part (station 4) by *Streblospio sp.*. During summer season, *Scrobicularia plana* continued to dominate over the whole basin, flanked by high numbers of *Corophium* taxa. Despite the fact that this lagoon has little connection to the sea inlet, shift in community composition and distribution may be related to tide amplitude and low depths, causing motile organisms (such as *Streblospio sp.* and *Corophium*) to be washed away/over new environments and resilient species (*Scrobicularia plana*), those that borrow into the mud to prevent being washed away, persist in the same habitat.

The East basin, the closest to the sea channel, showed the highest abundance and species richness for both seasons (Figure 2). It was characterized by a predominance of *Streblospio sp.* and Crustacea (*Corophium ascherosicum, C. sextonae, Gammarus insensibilis* and *Cyathura carinata*). Those species are entirely maritime, found in estuaries and coastal waters (Levin, 2001) but likely to colonize lagunar areas which are in immediate contact with the sea.

According to statistical analysis on macrobenthic community abundance, results showed significant difference between seasons, between basins and between stations. Significant differences were also found among the interactions between stations within basins and basins within stations. These were further confirmed by pair-wise comparison results (Table 4).

Among the measured spatial and temporal variables, basin location and sediment composition contributed the most on the structure and distribution of benthic macroinvertebrate communities. This was subjected to spatial variability of physical variables of the lagoon, primarily on the level of confinement and distance to the sea inlet.

n-MDS results, based on benthic fauna similarities, showed and confirmed that Stagno Longu lagoon was characterized by three distinct communities, highly correlated to basin's location (Figure 3). Sediment composition (for both, surface and sub-surface sediment layers) from the inlet toward the sea connection of each basin influenced the composition and structure of the benthic macrofaunal community along the lagoon. North basin, highly influenced by the presence of H_2O content, fine sand and OC (characterized by a very muddy conditions of the sediments), presented high numbers of sub-surface deposit feeders (*Neanthes nubila*) and opportunistic species such as *Streblospio sp.* and *Polydora hoplura*. Going toward the centre, the West basin composed of CC (mostly death hard-shell of Bivalvia), gravel and clay, was dominated by filter feeders and the East basin, showing the higher diversity of surface, sub-surface deposit feeders and filter feeders, was mainly composed by sand. (Figure 4).

In coastal lagoons, it is quite hard to distinguish between the role of sediment type and confinement (Reizopoulou *et al.*, 2004), since those variables are both dependent on the water circulation of the lagoon, which is the most important factor in affecting the biological organization of Mediterranean lagoons (Frisoni *et al.*, 1984). However, distinct patterns of macrobenthic assemblage distribution have been identified along a sedimentary gradient of grain size composition and organic matter content (Schlancher and Wooldridge, 1996; Bachelet *et al.*, 1996; Fresi *et al.*, 1983; Quintino and Rodriguez, 1989). In the case of Stagno Longu lagoon, the decrease in organic matter content from inner- (North basin) to outer areas

(West, East) situated close to the sea channel, was associated with dominance of species that included opportunistic species which are capable of proliferating in reduced sediments and can also tolerate toxic conditions and on the other hand, sensitive and indifferent species, mainly associated with clean sandy downstream waters (Grall and Glémarec, 1997).

An interesting fact is the seasonal shift from a preponderance of Polychaeta over Crustacea during winter season to the reverse relationship during summer season. In addition, Crustacean species (i.e.: *Corophium ascherosicum*, *Corophium sextonae* and *Melita palmata*), even if in small numbers, were found to be within those species that contributed the most for distinctions between basins, for both seasons. This can be related to the enhancement of favourable/unfavourable environmental conditions among season and it can also be related to species life history. Stagno Longu, having a relatively small spatial extent may facilitate species dispersal capabilities: most studies on micro-crustaceans have found them to be efficient disperses (Michels *et al.*, 2001; Cohen and Shurin, 2003) on the contrary, studies on polychaete found that the majority of the species tend to have limited dispersal potential (Carson *et al.*, 2006).

Regarding the spatial pattern, higher values of Shannon-Wiener diversity index were obtained at stations near the sea channel (East and West basin) and lower values at the innermost stations (North basin) (Figure 2). This change reflects the effect of habitat characteristics on biodiversity and the importance of habitat variation to ecosystem functioning in coastal areas. It is well documented that muddy environments are homogeneous sediment that generally holds lower diversity compared to heterogeneous habitats with higher habitat formers (Gray, 2002; Thrust *et al.*, 2006), which correlated with physical and chemical variables (temperature, salinity (Barnes, 1980)) and food supply (Gravina *et al.*, 1989)) of the area. Similar results were found in other Mediterranean coastal lagoons (Koutsoubas *et al.*, 2000; Reizopoulou *et al.*, 1996) were the level of confinement determined by water renewal determined the biological zonation, distribution and diversity of macrobenthic invertebrates (Guelorget and Perthuisot, 1992).

Based on the AMBI results, all stations in both seasons were classified as slightly disturbed (BI-2) whereas, M-AMBI concluded that the status of most of the stations (for winter and summer seasons) was moderate, except for station 5 which was in good status in winter but descended to moderate in summer and station 6 in both seasons which ranked to a good quality status. Of particular interest is the fact that stations in the innermost part of the lagoon showed higher values on AMBI compared to the outer ones, closer to the sea inlet. This reflect the higher organic matter content found in those areas, which accordingly are characterized by the presence of some species found in organically enriched areas, such as *Heteromastus filiformis*, *Capitella capitata* and *Polydora hoplura*.

Like other studies, there is a high significant correlation between OC in the sediment and AMBI (Muxika *et al.*, 2005; Carvalho *et al.*, 2006). Slightly divergences on AMBI and M-AMBI results may be due to lack of appropriate assignment of reference conditions; when using multivariate methods such as M-AMBI to assess the ecological quality status, there is the need to establish adequate reference conditions (Forchino *et al.*, 2011): this step is critical to correctly evaluate pressure on benthic communities within the WFD (Teixeira *et al.*, 2008). Infact, reference conditions for a water body type describe the biological elements that correspond totally, or almost totally, to undisturbed (pristine) conditions (Muxika *et al.*, 2007). In Italy, it has been quite difficult to set reference conditions, principally due to lack of information in the literature and to the particular oceanographic characteristics of the Italian Peninsula (Occhipinti-Ambrogi *et al.*, 2009). However, recent work on transitional waters in the Mediterranean Sea have harmonized and adjusted the good status boundaries through an intercalibration exercise, concluding reference condition for large geographical units that include MS with similar water bodies (Reizopolou *et al.*, 2018). In the present contribution to assess the health of Stagno Longu lagoon, the EQS obtained from the various indices concluded that, overall Stagno Longu coastal waters were moderately in good conditions.

Conclusions

This study represents the first environmental characterization and quantitative analysis of macrobenthic communities and their relationship between sediment composition across the Stagno Longu of Posada. It was demonstrated that benthic macroinvertebrates assemblages were influenced by temporal and spatial variations. In particular, sediment composition and level of confinement have been shown to have a key role in the composition and variations of the macroinvertebrate communities.

This work provides fundamental knowledge on the basic characteristics of the above-mentioned area, its benthic fauna and the current Ecological Quality Status. Altogether, those information represents basic references for further monitoring programs, which will be essential to ensure management of water resources and implementation of further policy action. In the case of Stagno Longu coastal lagoon, this study offers the insight needed to successfully accomplish future research into focusing on monitoring programs on environmental variables influence and hydrology of the area which will help to achieve future tasks such as ecosystem-based management, sustainable usage of resources and also effective conservation and protection which will be needed to improve the coherence between economic, societal and environmental goals.

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ANNEX I

Table 5: Species list of the number of individuals found in the North basin (station 1 and 2) at each sampling campaigns (1 & 2) performed in winter and summer seasons.

Season	Winter						Summer						Winter						Summer					
Sampling	1			2			1			2			1			2			1			2		
Specie name / Station	1A	1B	1C	1A	1B	1C	1A	1B	1C	1A	1B	1C	2A	2B	2C	2A	2B	2C	2A	2B	2C	2A	2B	2C
Neanthes nubila	14	5	15	5	5	2	8	4	0	7	5	4	33	29	6	12	30	8	22	8	11	32	11	12
Capitella capitata	0	0	0	0	0	0	0	0	1	0	0	0	0	1	1	0	1	0	0	0	0	0	0	0
Heteromastus filiformis	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
Polydora hoplura	1	0	0	0	0	0	0	1	0	0	0	0	3	4	2	0	7	0	1	1	2	0	0	0
Spio filicornis	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	2	0	0	0	0
Streblospio sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1	3	1	3	2	0	2	1	7
Scolelepsis cantabra	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Desdemona ornata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lekamesphaera hookeri	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cyathura carinata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Corophium ascherusicum	2	2	4	0	2	0	11	9	7	0	0	1	2	33	3	7	50	3	19	6	7	1	1	9
Corophium sextonae	0	6	6	0	0	2	8	6	3	0	0	0	5	14	0	2	31	1	8	0	0	0	0	0
Corophium orientale	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Malita palmata	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	2	10	0	1	0	0	0	0	0
Gammarus insensibilis	0	0	0	6	0	0	0	1	2	0	0	0	1	3	0	0	5	1	2	0	0	0	0	0
Upogebia pusilla	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lucifer typus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Idotea chelipes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Idotea baltica	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leptocheliidae	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Actinia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Carcinus maenas	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Scrobicularia plana	1	1	0	0	1	0	1	0	0	0	0	0	0	0	0	1	2	1	0	1	1	3	1	1
Cerastoderma edule	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Ruditapes decussatus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrobia acuta	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mytilus edulis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 6: Species list of the number of individuals found in the West basin (station 3 and 4) at each sampling campaigns (1 & 2) performed in winter and summer seasons.

Season	Winter						Summer						Winter						Summer					
Sampling	1			2			1			2			1			2			1			2		
Specie name / Station	3A	3B	3C	3A	3B	3C	3A	3B	3C	3A	3B	3C	4A	4B	4C	4A	4B	4C	4A	4B	4C	4A	4B	4C
Neanthes nubila	1	0	1	3	3	2	6	5	5	2	4	5	2	1	2	1	2	4	4	3	3	3	2	3
Capitella capitata	7	7	4	2	6	6	5	7	3	0	3	3	4	1	7	2	4	6	9	2	4	0	8	8
Heteromastus filiformis	2	2	4	6	6	9	5	2	7	3	7	2	2	3	4	3	8	5	7	5	1	1	12	2
Polydora hoplura	3	0	3	8	6	7	9	5	10	0	0	1	13	0	4	4	2	9	25	5	19	0	0	0
Spio filicornis	0	0	3	2	2	1	0	0	8	0	0	0	1	1	1	1	1	12	4	4	21	0	0	0
Streblospio sp.	1	2	3	5	2	3	29	14	14	0	27	24	15	28	31	11	15	17	19	12	29	20	11	39
Scolelepsis cantabra	0	0	0	0	1	1	1	0	0	0	0	0	0	1	1	0	0	1	2	0	0	0	0	0
Desdemona ornata	6	0	1	5	9	0	1	0	0	0	0	1	3	3	0	0	1	0	0	0	1	0	1	0
Lekamesphaera hookeri	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0
Cyathura carinata	0	0	0	0	0	0	3	1	1	0	1	2	0	0	0	0	0	0	5	11	12	16	6	10
Corophium ascherusicum	0	0	0	0	10	8	71	41	51	50	43	74	0	0	1	2	8	6	33	31	11	60	51	57
Corophium sextonae	0	1	0	5	4	5	62	31	44	7	10	19	0	0	0	0	0	0	38	49	15	14	5	9
Corophium orientale	0	0	1	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Malita palmata	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0
Gammarus insensibilis	1	0	0	0	4	2	1	0	0	0	0	0	0	1	1	0	5	1	0	2	10	0	0	0
Upogebia pusilla	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lucifer typus	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Idotea chelipes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11	0	0	0
Idotea baltica	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leptochelidae	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Actinia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Carcinus maenas	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Scrobicularia plana	21	33	47	66	60	80	79	55	82	64	46	52	78	83	71	78	46	90	95	90	116	92	77	74
Cerastoderma edule	0	0	0	2	2	5	0	2	1	0	0	1	0	0	0	1	2	1	1	0	2	0	0	0
Ruditapes decussatus	1	0	3	0	0	0	0	0	0	0	0	0	2	0	1	0	0	0	0	0	0	0	0	0
Hydrobia acuta	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mytilus edulis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 7: Species list of the number of individuals found in the East basin (station 5 and 6) at each sampling campaigns (1 & 2) performed in winter and summer seasons.

Season	Winter						Summer						Winter						Summer					
Sampling	1			2			1			2			1			2			1			2		
Specie name / Station	5A	5B	5C	5A	5B	5C	5A	5B	5C	5A	5B	5C	6A	6B	6C	6A	6B	6C	6A	6B	6C	6A	6B	6C
Neanthes nubila	7	4	12	44	35	53	7	10	17	7	18	10	1	8	4	16	7	4	25	26	16	33	19	11
Capitella capitata	1	1	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Heteromastus filiformis	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Polydora hoplura	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Spio filicornis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Streblospio sp.	55	124	62	345	498	361	108	78	305	157	199	136	0	13	1	201	38	19	81	81	29	75	132	38
Scolelepsis cantabra	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Desdemona ornata	9	24	28	62	116	67	2	5	5	0	1	0	0	3	0	5	1	0	0	0	1	0	0	0
Lekamesphaera hookeri	1	0	2	4	1	1	1	0	4	0	3	3	0	1	0	1	2	3	2	4	2	1	1	0
Cyathura carinata	5	3	4	8	10	12	22	35	206	36	67	74	1	1	0	11	2	2	53	99	33	34	35	19
Corophium ascherusicum	12	15	18	27	3	52	65	65	298	59	42	44	18	14	24	28	19	49	174	211	81	46	48	7
Corophium sextonae	8	15	17	55	8	60	57	51	204	13	12	0	11	17	23	7	0	23	193	251	92	34	42	6
Corophium orientale	0	0	1	0	0	4	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Malita palmata	32	34	20	5	3	35	0	1	0	0	4	3	72	25	30	44	18	34	10	7	7	12	3	13
Gammarus insensibilis	15	10	9	2	0	10	1	1	10	1	0	0	118	23	53	7	3	8	24	21	6	1	0	0
Upogebia pusilla	1	1	1	1	0	0	0	0	0	0	2	0	0	0	0	3	1	1	0	0	1	0	2	0
Lucifer typus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Idotea chelipes	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Idotea baltica	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0
Leptochelidae	0	0	0	0	0	1	6	0	0	0	0	0	0	0	0	1	0	2	0	0	1	0	0	0
Actinia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Carcinus maenas	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0
Scrobicularia plana	6	18	0	4	3	17	2	1	3	16	17	12	21	9	1	20	9	6	38	9	27	0	8	3
Cerastoderma edule	3	0	0	0	0	0	0	0	0	3	0	0	0	2	1	0	0	0	0	0	0	0	0	0
Ruditapes decussatus	0	6	0	1	5	0	1	1	1	0	0	0	6	0	0	6	2	1	19	12	11	0	0	0
Hydrobia acuta	0	0	0	13	22	15	33	39	70	4	3	8	0	0	0	32	22	16	41	88	44	13	1	8
Mytilus edulis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	0